Author version of article published in *Progress in Polymer Science* **147**, 101752 (12/01/2023)

Thermoresponsive polymers: from natural proteins to amino acid based polymer synthesis

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ABSTRACT

In polymer science, thermoresponsiveness refers to macromolecular systems that display a marked and discontinuous change in their physical properties with temperature. Such smart polymers are the focus of increasing attention as they provide new solutions to many applications (*e.g.*, drug delivery, nanotechnology, tissue engineering and biotechnology). This review focuses on amino acid based polymers, mainly synthetic polypeptides that are obtained by ring-opening polymerization. These include polymers based on natural amino acids, synthetic or modified amino acids and *N*-alkylated glycine derivatives. Based on what is known about the behavior of natural proteins in response to temperature variations, this review provides a comprehensive overview of the state of the art of thermosensitive polypeptides through a detailed description i) of the structure/thermoresponsiveness relationship, ii) of the mechanisms involved at the molecular level, iii) of their possible applications both in materials science and in biomedical applications.

KEYWORDS: Protein-like polymers, ring-opening polymerization, polypeptide, thermoresponsive, LCST, phase transition, aggregation.

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LIST OF ACRONYMS:

MEG_3MA	2-(2-(2- methoxyethoxy)ethoxy)ethyl-methacrylate
$EtBzBuIm^+$	4-ethylbenzylbutylimidazolium
MEG_2MA	(2-methoxyethoxy)ethyl-methacrylate
METMASPS	[Boc-*L*-methionine-(2-methacryloylethyl)]sulfoniopropanesulfonate
HEMA	2-hydroxyethyl methacrylate
T_{CP}	cloud point – LCST behavior
T_{CU}	clearing point – UCST behavior
CuAAC	copper catalyzed alkyne-azide cycloaddition
C-UCST	coulombic interaction upper critical solution temperature
DP	degree of polymerization
DNA	deoxyribonucleic acid
ELP	elastin-like polypeptide
ΔH_{solv}	enthalpy of solution
ΔS_{solv}	entropy of solution
ΔS_{water}	entropy of the aqueous solvent
Nor	*exo/endo*-norbornenyl
ΔG_{solv}	gibbs free energy of dissolution
H-UCST	hydrogen bonding upper critical solution temperature
IDP	intrinsically disordered protein
LCST	lower critical solution temperature
NtBuNOEtG	*N*-(2-(tert-butylamino)-2-oxoethyl)-glycine
DMA	*N*,*N*-dimethylacrylamide
Lys(Z)	*N*- ε -benzyloxy-*L*-lysine
AHypOMe	*N*-acryloyl-4-trans-hydroxy-*L*-proline methyl ester
NAS	*N*-acryloyloxysuccinimide
NHS	*N*-hydroxysuccinimide
PEtOx	poly(2-ethyl-2-oxazoline)
PiPOx	poly(2-isopropyl-2-oxazoline)
P(TrpVBz)	poly(4-vinyl benzyl tryptophan)
PAProOMe	poly(acryloyl-proline methyl ester)
P(Boc-lysA)	poly(Boc-*L*-lysinylacrylamide)
PEEP	poly(ethyl ethylene phosphate)
PALG	poly(*γ*-allyl-*L*-glutamate)
PPLG	poly(*γ*-propargyl-*L*-glutamate)
PGA	poly(*L*-glutamic acid)
PSA	poly(*L*-serinyl acrylate)
PNtBuNOEtG	poly(*N*-(2-(tert-butylamino)-2-oxoethyl)-glycine)
PNAAMe	poly(*N*-acryloyl-alanine *O*-methyl ester)
PNAGMe	poly(N-acryloyl-glycine *O*-methyl ester)

PNAlG	poly(*N*-allylglycine)
PNDeG	poly(*N*-dodecylglycine)
PNEtG	poly(*N*-ethylglycine)
PNOHEtG	poly(*N*-hydroxyethylglycine)
PNIPAM	poly(N-isopropyl acrylamide)
PNPgG	poly(*N*-propargylglycine)
PNPrG	poly(*N*-propylglycine)
PEG$_x$TLG	poly(*γ*-(4-(oligoethylene glycol)-1,2,3-triazol-1-yl)-*L*-glutamate)
PEG$_x$LG	poly(*γ*-(oligoethylene glycol)-*L*-glutamate)
PMeS$^+$X$^-$PLG	poly(*γ*-3-methylthiopropyl-*L*-glutamate)
PPOPhBLG	poly(*γ*-4-(4-propargoxyphenoxycarbonyl)benzyl-*L*-glutamate)
PPOBLG	poly(*γ*-4-(propargoxycarbonyl)benzyl-*L*-glutamate)
PCMeBLG	poly(γ-4-chloro-methylbenzyl-*L*-glutamate)
PBLG	poly(γ-benzyl-*L*-glutamate)
PMLG	poly(γ-methyl-*L*-glutamate)
PDMAE	poly[2-(dimethylamino)ethyl methacrylate]
P(Boc-TrpVBz)	Poly[4-vinyl benzyl (Boc-tryptophan)]
PCys	polycysteine
PLeu	polyleucine
PLys	polylysine
PMet	polymethionine
PMVE	polymethyl vinyl ether
PVCL	poly(*N*-vinylcaprolactam)
PPG	polypropylene glycol
PPO	polypropylene oxide
PSar	polysarcosine
PTyr	polytyrosine
RAFT	reversible addition−fragmentation chain-transfer polymerization
RNA	ribonucleic acid
BOC	tert-butyloxycarbonyl protecting group
UCST	upper critical solution temperature

1. INTRODUCTION

In Nature, self-assembly and self-organization is a driving force of life at all scales, from populations to individual organisms to cells.[1] A full understanding of cellular processes must take into account the spatial distribution of key biomacromolecules, the proteins. Subcellular organization plays a crucial role in biology, and it is well known that eukaryotic cells compartmentalize their components within lipid membranes (nucleus, endoplasmic reticulum, mitochondria, etc.).[2] However, it has only recently been shown that the cell also organizes its components in a more flexible way: by using controlled phase separation

processes. Indeed, many membrane-less organelles consisting of biomacromolecules have been identified in both the nucleus and cytoplasm of living systems, implying that they offer some additional advantages as a compartmentalization strategy.[3,4] Such biomolecular condensates are formed though phase separation, in which one or more components undergo a demixing transition to form two or more coexisting phases. Understanding the principles underlying such phase separation is still a major challenge, in biology as well as in chemistry as it has consequences on (bio)chemical reactivity.[5,6] Outside the cell, water-soluble macromolecules, whether synthetic polymers or biomacromolecules (nucleic acids, proteins, etc.) are also known to undergo phase separation to form condensates (liquid-liquid to coacervates or liquid-solid to aggregates).[6] In materials, this phase separation is an essential property in most formulations that include polymers. These processes are based on the same thermodynamic principles: the starting point is a thermodynamically stable solution that is subjected to conditions that induce demixing, such as a temperature drop or the intrusion of a non-solvent.[7,8] In such processes, using synthetic but protein-like macromolecules made of amino acids opens up promising perspectives that shed light on biological phase separations and pave the way to new biomimetic approaches.[9]

In general, phase separation of polymers is a powerful tool to formulate nanomaterials or to control the fine structure of thin films in coatings. Indeed, combined with the right macromolecular engineering, phase separation (or self-assembly) can be used to prepare a wide range of materials (membrane, gels, etc.).[10,11] Recent advances in this direction have included the design of synthetic polymer structures with stimuli-responsive phase separation behavior, specifically with respect to light, pH and temperature.[12] In comparison, the phase separation induced by naturally-produced proteins serves many fundamental biological processes such as the regulation of biochemical reactions, the sequestration of toxic factors or more generally improved subcellular organization in living systems.[9]. It is interesting to note that the membrane-less organelles are not all continuously present, but assemble in response to the cell cycle, cytosol crowding or oxidative stress.[13] Although proteins are known to be key macromolecules for phase separation *in vivo*, compared to synthetic polymers the factors that govern this assembly and their effects on biomolecular chemistry remain poorly understood.

In cells, proteins achieve most biological functions through binding, catalysis, stimuli responsiveness and even as matrix components. They are assembled by ribosomal synthesis, according to a programmed genetic sequence. Proteins are derived from around 20 naturally occurring amino acids which are linked by amide bonds in the protein chain. It is generally accepted that the primary sequence of amino acids give birth to their unique properties and to their specific folding into highly organized structures.[14] Proteins are thus sequence-controlled polymers with defined length and properties: they are generally electrically charged heteropolymers composed of hydrophilic and hydrophobic residues (Fig. 1). Depending on the nature and distribution of these amino acids in the backbone, the protein presents alternating hydrophilic or hydrophobic zones that play a major role in the folding and the physicochemical behavior of the system. [15] If the hydrophobic zones are numerous and distributed randomly along the chain, the protein will fold up so as to isolate these zones in the core of the assembly while the hydrophilic zones that ensure good solubility in water remain at the periphery. This is the case of globular proteins. If the hydrophobic zones are few and segment the protein chain into homogeneous blocks, chain folding is no longer sufficient to isolate the hydrophobic zones and they will adopt a collective behavior to decrease the interaction energy with water. This is the case of oligomeric structures of some proteins (cytoskeleton, etc.). The driving force of protein self-assembly to fold or to phase separate as nano- and micrometric objects also

involves supramolecular bonding, exploiting the amphiphilic properties, the secondary structure or the coulombic interactions.[15,16] All these interactions differ in the energy values in play, the evolution of interaction energies as a function of intermolecular distances and their sensitivity to the physicochemical conditions of the environment. The overall energy input ultimately allows the formation of high energy bonds that are promoted by spatial proximity such as disulfide bridges and coordination bonding of transition metals.

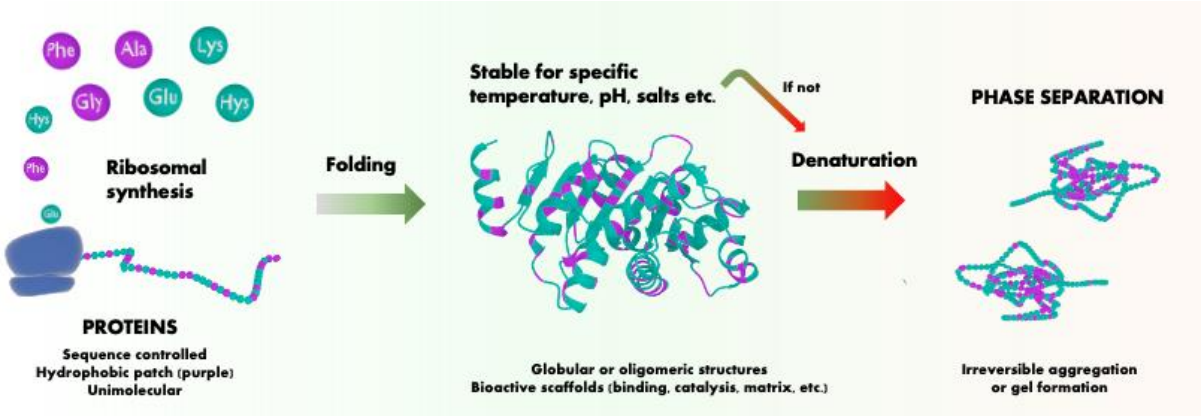


Fig. 1. Proteins and phase separation: the polymer chain represents a protein (violet, hydrophobic residues and blue, hydrophilic residues). At the end of ribosomal synthesis, hydrophobic residues (entropy) and other non-covalent interactions (enthalpy) lead to protein folding. Proteins are stable under specific conditions of pH, ionic strength, temperature, etc. If they are subjected to too strong a change in these parameters, denaturation can occur, usually resulting in irreversible phase separation of the polymer (aggregation, gel formation, etc.).

The interactions and driving forces mentioned in the previous paragraph only make sense if we consider that proteins are macromolecules that are surrounded by a very specific solvent, water. Indeed, to better understand the key role of the aqueous phase on proteins, it is interesting to highlight their folding stability. The folding of proteins depends on numerous interactions between amino acid side chains and between these chains and the solvent.[17] The three-dimensional structure of the polypeptide is stable only within a precise range of physico-chemical conditions and, outside this range, it denatures (Fig. 1). This denaturation can be induced by increasing temperature, pressure, extreme pH or the presence of salts, alcohol or surfactants in large quantities.[15,18,19] Usually, the denaturation of proteins is irreversible and is associated with aggregation of the polypeptide. Denaturation first causes the breaking of low energy bonds within the molecule, which leads to a reorganization of the chain and generally to the emergence of the more hydrophobic areas of the protein.[20,21] This decreases the protein-solvent interactions in favor of protein-protein interactions leading to the formation of aggregates at low concentration and a gel beyond a critical concentration.[22,23] In this respect, water plays a crucial role during unfolding, and aggregation processes are usually under the control of entropy.[24]

This review article first gives a detailed description of the influence of temperature on the structure and physico-chemical properties of proteins including intrinsically disordered proteins. By comparing this behavior to that of synthetic thermosensitive polymers such as PNIPAM, this article gives a full overview of how polymer chemistry can afford peptide-based polymers having physico-chemical properties that are influenced by the temperature (chemical structure, LCST, secondary structure, etc.) and how these fundamental properties impact their uses in materials sciences (gel formation, coatings, etc.).

2. PROTEINS AND TEMPERATURE

The context of temperature with proteins can refer to the metabolic reactions occurring in cells and that are induced by an increase in temperature: indeed, specific proteins are produced and involved in this case (*e.g.*, heat shock proteins) to adapt the living system to such changes. This review does not cover this topic nor the impact of this adapted metabolism on the stability of other proteins.[25] Readers are referred to specific articles for more information on this topic.[26,27]

As already mentioned in the introduction, the way and the strength with which proteins interact with water is crucial to understand their physico-chemical behavior. It can be said that proteins generally interact strongly with water, and only the hydrated form is soluble. If the available water concentration is reduced by the addition of salts or water-miscible organic solvents (*e.g.*, methanol, ethanol, or acetone), the proteins precipitate out of solution. This is why in bioconjugate chemistry it is often important to perform chemical reactions with proteins at low temperatures: at room temperature, proteins are rapidly denatured by organic molecules added to the medium that can be called precipitating agents. Changes in the environmental conditions of proteins indeed induce denaturation via different paths and any unfolding of the protein leads to aggregation. As with the addition of solvent or salts, proteins are irreversibly denatured by heat and start to aggregate when the temperature is increased. This aggregation is usually irreversible and has been used to define the folding stability of protein structures (Fig. 2).[17,28] Studies have shown that this stability depends on the protein sequence and that modification of the sequence could prolong the stability of the protein structure despite an increase in temperature (structural rigidification, increased hydrophobicity, increased protein-solvent interaction, etc.).[29] At the molecular level, when the polypeptide backbone is subjected to an increase in temperature, there is an increase in molecular motion which leads to the weakening and/or breaking of supramolecular bonding interactions (intra, intermolecular and also with the water molecules of the solvent). If some supramolecular bonds break, the structure of the protein is weakened, *i.e.*, other weaker supramolecular bonds become easier to break (Fig. 2). Denaturation by increasing temperature is therefore a process that starts quite suddenly at a critical temperature and ends at a slightly higher temperature.[15] Renaturation is sometimes possible with small proteins (ribonuclease, lysozyme) under laboratory conditions, but denaturation is most often irreversible. In general, thermal denaturation occurs when the temperature is elevated to ~45°C or higher.[30] From a thermodynamic point of view, this also highlights the difference in entropy between the folded and denatured states.[15] This difference is the main free energy component opposed to the folding, but it is generally not large enough to completely counteract the other pro-folding components (mainly the hydrophobic effect). When thermal denaturation occurs, the kinetic energy added to the system increases the configurational entropy of the denatured state much more than it increases that of the folded state. With enough heat, the entropy difference between the two states becomes large enough to overwhelm the folding components and this leads to unfolding (and phase separation). Interestingly, nonpolar interactions, which at moderate temperatures mainly arise from changes in the entropy of the aqueous solvent (ΔS_{water}), also exhibit temperature-dependent behavior.[31] At higher temperatures they are increasingly driven by changes in solvent enthalpy (although ΔS_{water} is always positive).[32] This shift from an entropy-driven process to an enthalpy-driven process is related to the heating-induced decrease in ΔS_{water}; at high temperatures, the kinetic energy of the water molecules surrounding the protein is considerably higher and weakens the hydrophobic effect.[32]

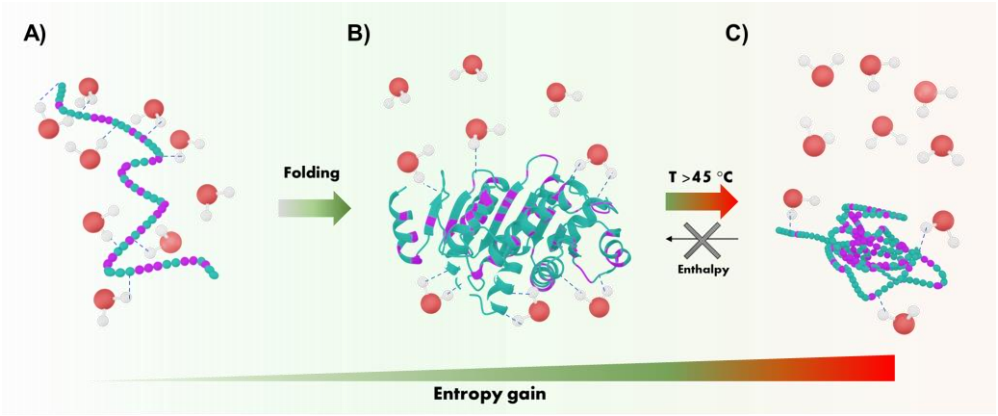


Fig. 2. Entropy and water solvation of the protein backbone: the polymer chain represents the protein (violet, hydrophobic residues and blue, hydrophilic residues) in interaction with water molecules (red and white). When the nascent polypeptide chain is hydrated (A), the polymer is unstable as hydrophobic residues (entropy) and other non-covalent interactions (enthalpy) lead to protein folding (B). Folded backbones subjected to an increase in temperature undergo phase separation (C). This phase separation is irreversible as there is a large difference in enthalpy between the polymer conformations in B and C.

3. INTRINSICALLY DISORDERED PROTEINS AND POLYMER-LIKE THERMORESPONSIVENESS

Intrinsically disordered proteins (IDPs) are a special class of proteins in biological systems that are able to induce reversible phase separations. Indeed, it has long been accepted that the primary sequence and structure of a protein (secondary, tertiary and quaternary) directly determines its function. This paradigm, supported by the models of Pauling[32] and by the folding experiments of Anfinsen[17], is true for many protein systems. For instance, enzymes are three-dimensional globular structures that facilitate the organization of binding sites into an active site that physically and chemically adapts to natural substrates so that highly efficient catalysis can take place. However, and more recently, biochemistry has identified parts of proteins, or even whole proteins, without an organized structure: these so-called "intrinsically disordered regions" vary in size, ranging from short segments of the polypeptide chain to entire proteins. IDPs are thus a class of proteins that are completely disordered. IDPs include proteins with different functions, such as DNA packaging and repair, ion transport, nuclear trafficking of proteins, and regulation of cellular processes (cell cycle, transcription, and splicing), as well as proteins involved in diverse pathologies, such as cancer, neurodegenerative and cardiovascular diseases.

The main difference between folded and disordered proteins reflects the magnitude of conformational fluctuations.[33] IDPs are often characterized by a high net charge leading to unfavorable electrostatic interactions and low hydrophobicity,[34] even if the real picture behind the proteome now seems more complex. Interestingly, net charge and charge configuration have both emerged as key features that dictate protein-protein interactions in the context of phase separation with IDPs.[35,36] In accordance the first experiments designed to discover the proteins that make up biomolecular condensates showed the predominance of IDPs.[37,38] These proteins contain relatively long coil segments, possibly interspersed with short segments of other secondary structures. Their tertiary structure is either an extended

strand or a fused globule. When these disordered segments interact with other proteins, they readily bind upon folding to a different secondary and tertiary structure; the partner protein then serves as a folding template in the condensate.[39] Even though IDP sequences are not yet fully understood, it appears that stretches of many amino acids, sometimes highly repeated, maintain an extended coil, preventing the hydrophobic interactions necessary for three-dimensional folding.

From what is known about this folding, some key features of the primary sequence that promote disorder have been identified. [40–42] The amino acid residues should:

(1) promote solvation (polar and charged residues),

(2) create intra-chain repulsion (polyelectrolytes),

(3) create steric barriers to the appearance of secondary structure (for example, amino acids like proline and glycine that disrupt helices and beta-sheets).

These features that prevent folding lead to highly soluble and non-aggregated proteins. Adding a large number of hydrophobic amino acids to these sequences results in considerable entropy gains upon binding to a partner; these segments often form fused globules (coacervates) and present a peculiar temperature response. Indeed, the hydrophobicity of IDPs is correlated with polymer-like phase separation upon temperature variation.[43] The first examples to be studied were the phenylalanine-glycine (FG) repeat regions of nuclear pore proteins that can give rise to hydrogels.[44] Later, the impact of different hydrophobic residues was explored in other protein systems such as RNA-binding proteins,[45] proteins involved in ubiquitination,[46,47] or membrane proteins.[48] In these different systems, the sequence of hydrophobicity (determined by both aromatic and aliphatic residues) is very important to maintain phase separation especially with respect to temperature change[48] or on the basis of their sensitivity to different ions, as interpreted by the Hofmeister series.[49] Overall, hydrophobic IDPs are able to form thermoresponsive phase separations such as an entropic lower critical solution temperature (LCST) phase transition (Fig. 3).[50] Such behavior is primarily explained by the fact that the bulky and apolar side chains of hydrophobic amino acids cannot interact favorably with water and thus their solvation requires an ordered hydration shell with minimal enthalpic advantage. The transfer energy of the displacement of a hydrophobic group in water (solvation energy) scales with the accessible surface area of the solvent, so larger aliphatic side chains are more hydrophobic.[51] The entropic cost of water confinement increases with temperature, which favors self-association of hydrophobic side chains at higher temperature to minimize their solvent accessible surface area, resulting in temperature-dependent coacervation or aggregation.

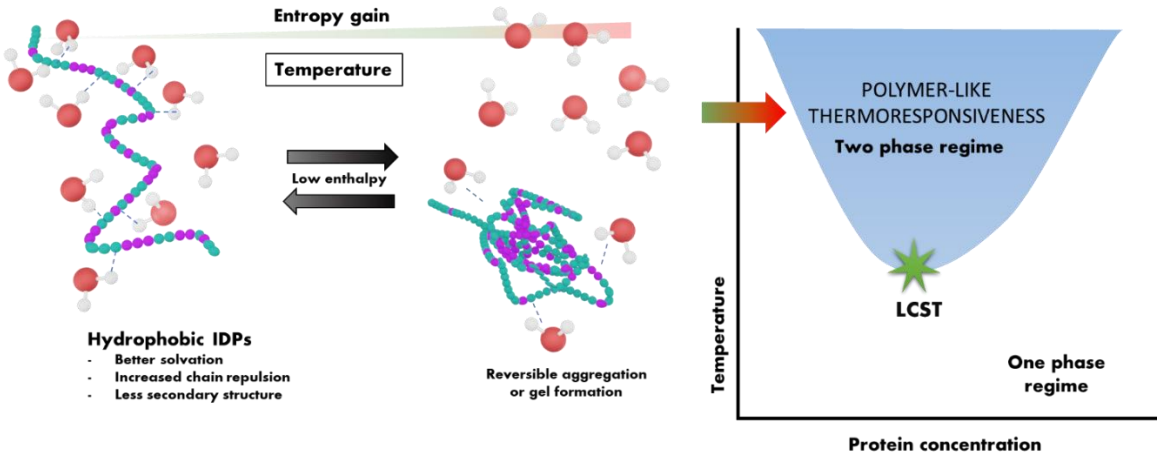


Fig. 3 Hydrophobic IDPs are LCST-polymers: the polymer chain represents an IDP (violet and blue) interacting with water molecules (red and white). This interaction is disrupted by an increase in temperature (entropy), but is easily reversible as the difference in enthalpy between the polymer conformations is small in soluble state or upon phase separation.

The role of hydrophobicity in temperature-induced phase separations has been particularly studied in the context of elastin-like polypeptides (ELPs).[52,53] ELPs are a class of recombinant IDP-like polypeptides based on -Val-Pro-Gly-Xaa-Gly- pentapeptide repeats that were originally identified in tropoelastin and in α-elastin (Xaa being Ala, Gly, or Val).[54–56] These proteins possess an LCST that can be shifted to a higher or lower temperature by varying the hydrophobicity of the "guest" residue (Xaa). The pioneering work in this field by Dan W. Urry shows a clear correlation between the solvation energy of the guest residue and the LCST.[56,57] Amino acid guest residues with an aromatic side chain have the greatest impact on the lowering of the LCST, but tyrosine shifts LCST more than phenylalanine, foiling predictions based on hydrophobicity alone and suggesting a unique chemistry conferred by the hydrogen bonding ability of the phenol group. In addition, the determinants of UCST versus LCST have been explored systematically, notably by Ashutosh Chilkoti's group.[58–60] Our group has also significantly explored ELPs, especially applying chemical modifications of ELPs scaffolds to tune their properties[61–64] or to functionalize them with saccharides and oligosaccharides,[65–68] in block copolymer structures[69–72] or to develop artificial organelles[73,74].

Overall, proteins that respond to biologically relevant factors by changing their conformation or mechanical properties are very promising building blocks for the design of smart biomaterials (*e.g.* for medicine, in nanotechnology).[75–78] Among external factors, modulating the temperature of a protein solution is an efficient strategy to induce aggregation of proteins, generally in an irreversible manner for structured proteins, but sometimes in a reversible manner as observed with IDPs and some other proteins.[79] It is now well established that recombinant or natural proteins can be used as a basis for genetic engineering to design heat-sensitive polypeptides; these are likely to receive increasing attention in industry as new classes of thermoresponsive polymers with enhanced biodegradability.

4. FROM PROTEINS TO THERMORESPONSIVE AMINO-ACID BASED POLYMERS.

Unlike most proteins, whose thermoresponsive behavior is defined by higher-order structure which can be irreversibly disrupted on denaturation, many synthetic polymers display reversible thermoresponsive behavior. This behavior is determined by the thermodynamics of solution: the balance between polymer-solvent, polymer-polymer and solvent-solvent interactions. The field of thermoresponsive polymers is enormous, and we will not attempt to review it here. Instead, the following sections briefly reviews some key concepts that have been transfered to the design of thermoresponsive amino acid based polymers.

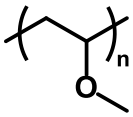
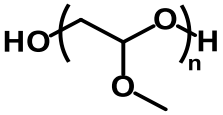
4.1 LCST and UCST Polymers

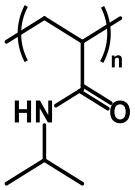
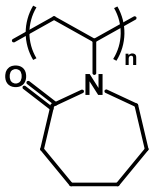
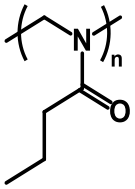
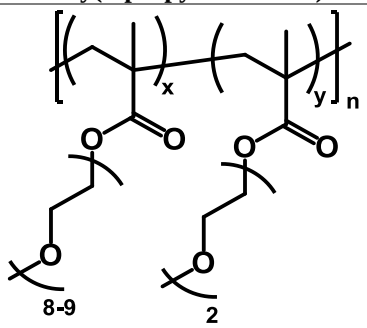
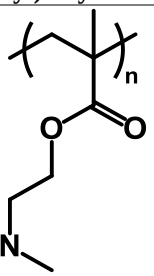
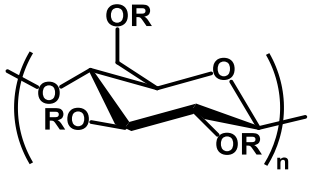
A thermoresponsive polymer, broadly defined, is one whose solution separates into polymer-rich and polymer-poor phases in response to a change in temperature. Two kinds of behavior are commonly observed, depending on whether the polymer solution phase separates on heating or on cooling. In the first case, the polymer becomes less soluble as the temperature increases, and above a certain temperature, the cloud point (T_{CP}), phase separation occurs. The minimum temperature at which phase separation occurs is known as the lower critical solution temperature (LCST) – below this temperature the polymer is completely miscible with the solvent. In the second case, the polymer becomes more soluble as the temperature increases. The maximum temperature at which polymer-rich and polymer-poor phases can coexist is known as the upper critical solution temperature (UCST) – above this temperature, the polymer is completely miscible with the solvent. In a few cases, both LCST and UCST behaviors can be experimentally observed for the same polymer-solvent combination.[80] This behavior is driven by changes in enthalpy and entropy on dissolution. Dissolution is thermodynamically favorable if the Gibbs free energy of dissolution (ΔG_{solv}) is negative (equation 1).

$$\Delta G_{solv} = \Delta H_{solv} - T\Delta S_{solv} \quad (1)$$

If the enthalpy of solution (ΔH_{solv}) is negative, but the entropy of solution (ΔS_{solv}) is positive, the solution will display LCST-type behavior, becoming soluble when T is lower than $\Delta H_{solv}/\Delta S_{solv}$. This situation is unusual for small molecules, but relatively common for polymers with the right balance of hydrophilic and hydrophobic groups, such as poly(*N*-isopropyl acrylamide) (PNIPAM). In this case, the water molecules around the polymer chains structure themselves, leading to a loss of entropy of the system. Some common examples of synthetic polymers that exhibit an LCST in water are the poly(*N*-alkyl acrylamide)s, poly(N-vinyl amides), poly(oxazoline)s, poly(oligoethylene glycol) methacrylates, hydroxypropyl cellulose[81] and other cellulose ethers (Table 1).

-TABLE 1– Selected Common LCST polymers

Polymer	Representative cloud point T_{CP} (°C)	Ref.
 **PMVE, poly(methyl vinyl ether)**	37°C for dilute solutions 25°C for concentrated solutions	[82]
 **Poly(propylene glycol)**	16°C Mw=3000 g.mol^{-1} 43°C Mw=1000 g.mol^{-1}	[83]

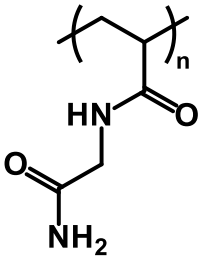
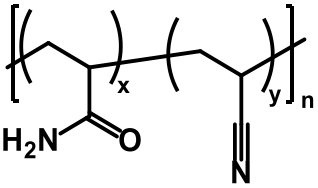
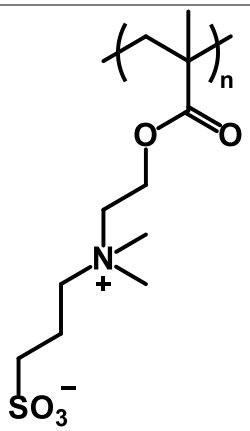
 **PNIPAM**	32°C	[84]
 **PVCL, poly(*N*-vinylcaprolactam)**	30°C – 40°C depending on Mw	[85]
 **Poly(2-propyl-oxazoline)**	25°C	[86]
 **Poly(oligo(ethylene glycol) methacrylate-*co*-2-(2-methoxyethyl)ethyl methacrylate)**	28°C – 90°C depending on composition	[87]
 **Poly(dimethylaminoethyl methacrylate)**	30°C – 80°C depending on Mw and pH	[88]
 **Hydroxypropyl cellulose (R = H or $CH_2CH(OH)CH_3$)**	45°C	[89]

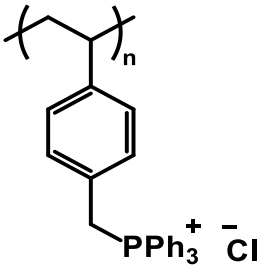
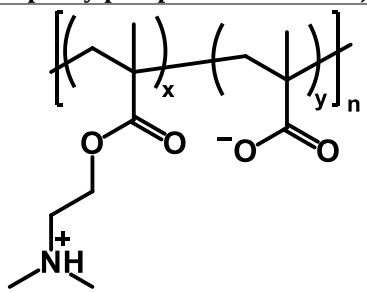
It is interesting to note that some of the most widely-used LCST polymers: poly(*N*-alkyl acrylamides)[90] and poly(oxazoline)s[86], are structural isomers of polypeptides. In these cases, the LCST behavior is driven by the balance between the polar, hydrogen-bond-forming amide groups and the hydrophobic alkyl chains. Dissolution is enthalpically favorable, but entropically unfavorable due to the structuring effect of the hydrogen bonds and the

hydrophobic substituents. The temperature at which phase separation occurs can be modulated by varying the hydrophobic substituents,[90] or by copolymerization of two or more monomers with different substituents.

The opposite case, UCST-type behavior, is enthalpy driven: this is observed in polymers that have strong polymer-polymer interactions in the solid state, such as through hydrogen bonding (H-UCST) or coulombic interactions (C-UCST). In this case, dissolution is entropically favorable – the polymer solution is less ordered than the 2-phase system – but enthalpically unfavorable, as the polymer-solvent interactions are weaker than the polymer-polymer interactions. Thus, the polymer is insoluble at lower temperatures, and becomes soluble at higher temperatures when the increase in entropy on solution ($T\Delta S_{solv}$) is sufficient to compensate for the loss of enthalpy (ΔH_{solv}). UCST behavior is less commonly observed in synthetic polymers than LCST behavior, although many synthetic polymers will display UCST-type behavior under appropriate conditions of temperature and pressure.[91] A few examples of synthetic polymers that display UCST-type behavior in water at atmospheric pressure include poly(acrylamide-co-acrylonitrile)[92], derivatives of poly(*N*-acryloyl glycinamide)[93], zwitterionic copolymers[94], poly(ampholyte)s[95] and poly(ionic liquid) copolymers[96].

-TABLE 2 – Examples of UCST polymers

Hydrogen bonding (H-UCST)	**Representative cloud point T_{CU} (°C)**	**Ref.**
 **Poly(*N*-acryloyl glycinamide)**	24°C	[93]
 **Poly(acrylamide-*co*-acrylonitrile)**	6°C – 60°C depending on composition	[92]
Coulombic (C-UCST)	**Representative cloud point T_{CU} (°C)**	**Ref.**
	33°C	[97]

Poly(2-(*N*-3-sulfopropyl-*N*,*N*-dimethyl ammonium)ethyl methacrylate)		
 **Poly(4-vinylbenzyl triphenylphosphonium chloride)**	38°C in 475 mM NaCl	[98]
 **Poly(2-dimethylaminoethyl methacrylate-*co*-methacrylic acid)**	25°C in 50% ethanol/water	[95]

Both LCST and UCST behavior are sensitive to changes in the nature of the solvent, as well as the structure of the polymer. Changes to the solvent, such as the addition of salts, neutral solutes such as sugars, or cosolvents, can have a dramatic effect on the thermoresponsive behavior. This is especially relevant for biological applications, as results obtained in distilled water may not be applicable to physiological conditions. In general, increasing the ionic strength of a solution results in a decrease in LCST, although the effect of different salts varies depending on their position in the Hofmeister series.[99–102] In some cases, addition of chaotropic salts such as sodium thiocyanate may even increase the LCST of the polymer solution (Fig. 4). The effect on UCST behavior can be very complex: in general increasing the ionic strength of the solution reduces the UCST for zwitterionic copolymers,[103] but specific interactions between the polymer and counterions may lead to the development of UCST behavior where none existed before, as in the case of poly(4-vinylbenzyltriphenyl phosphonium chloride), which shows UCST behavior only in the presence of additional chloride anions.[98] The complex interactions between counterions and UCST polymers have been reviewed by *Niskanen* and *Tenhu*.[104]

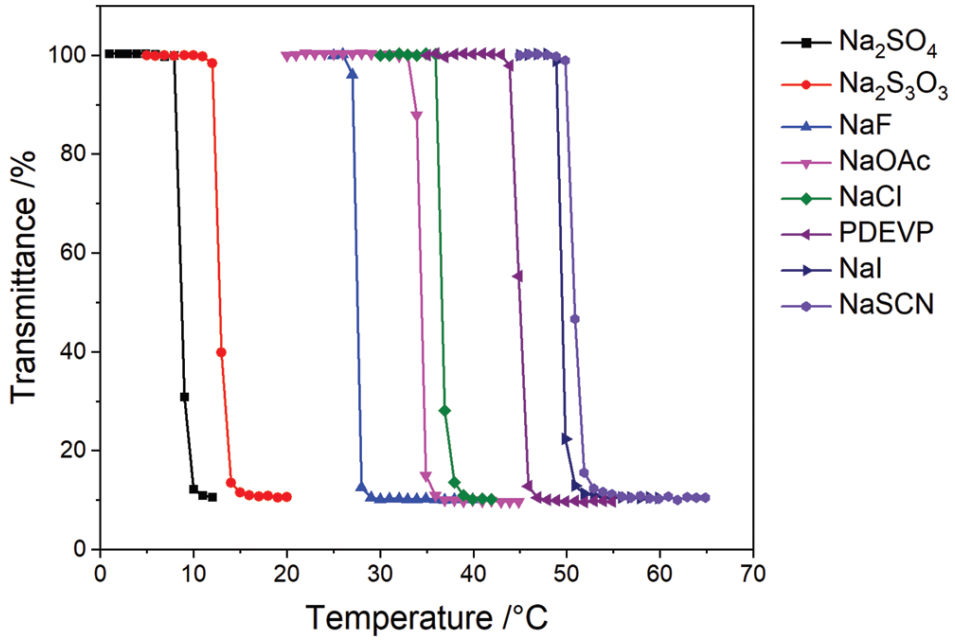


Fig. 4. Transmittance curves during the heating scans (cooling scans not shown for better readability) with poly(diethyl vinyl-phosphonate) PDEVP and different sodium salts of the Hofmeister series. The cloud point was determined at a 10% decrease in transmittance for 2.5 wt% aqueous solutions with a salt concentration of 500 mM of the respective salt (NaSCN, NaI, NaCl, NaF, NaOAc, $Na_2S_2O_3$ and Na_2SO_4) (heating/cooling rate=1°Cmin^{-1}, holding time=0.05 min). Figure adapted from [104].

Changes in the structure of the polymer can also have a strong effect. The LCST or UCST of many thermoresponsive polymers is dependent on their molar masses, with a higher molar mass leading to a reduced LCST or a higher UCST. UCST polymers in particular frequently show strong dependence on molar mass as polymer-polymer interactions are stronger for higher molar mass polymers.[104] Many LCST polymers, including PNIPAM, are relatively insensitive to changes in molecular weight, however. For low molecular weight polymers, the end groups can also have a strong effect on the solubility behavior, with hydrophilic end groups increasing solubility, while hydrophobic groups decrease it (Fig. 5). [82,105,106] A similar effect is observed in block copolymers, with the addition of a hydrophilic block to an LCST-type polymer leading to an increase in the cloud point at a specific concentration.[106]

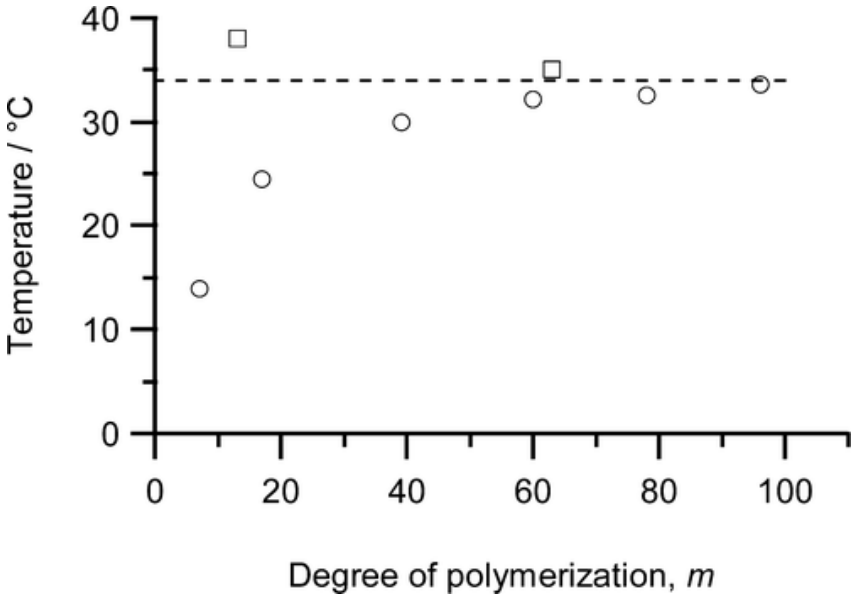


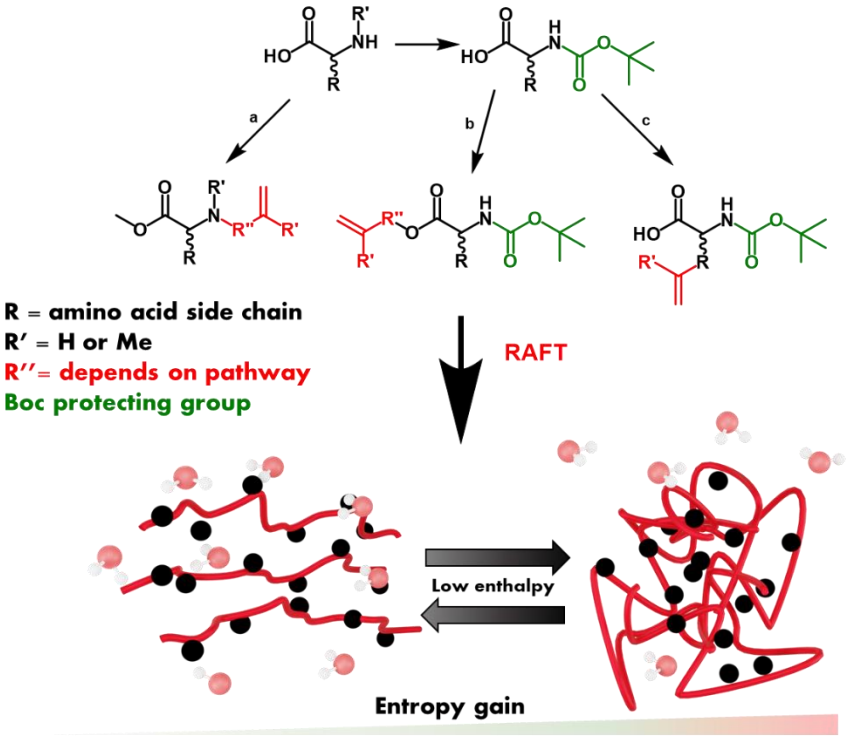
Fig. 5. Cloud points (1 wt %) for $C_{12}NIPAM_m$ (in D_2O) as a function of the degree of polymerization, m. Circles are $C_{12}NIPAM_m$ and squares are PNIPAM oligomers with no hydrophobic group attached. Figure adapted from [105] ($C_{12}NIPAM_m$) with data from [107] (PNIPAM oligomers without hydrophobic end groups).

Copolymerization is another approach to tune thermoresponsive behavior. Incorporation of hydrophilic or hydrophobic monomers into a polymer that shows LCST behavior in water will result in an increase or decrease, respectively, of the LCST. This approach can be used to tailor LCST behavior over a wide temperature range, as exemplified by the poly(oligoethylene glycol methacrylate)s.[87] In some cases, copolymerization can lead to the emergence of thermoresponsive behavior exhibited by neither homopolymer, as in the case of copolymers of acrylamide and acrylonitrile, which exhibit UCST behavior that can be adjusted from 6 to 60°C by varying the copolymer composition.[92]

It should be noted that the exact position of a cloud point depends on the technique used to measure it: turbidimetry, dynamic light scattering and differential scanning calorimetry being the most commonly used techniques.[108] The dispersity of the polymer may also play a role, as highly disperse polymers will undergo fractionation as they precipitate, leading to broader transitions. For these reasons, it is sometimes difficult to compare cloud point values between different studies.[84] Considering that turbidimetry is currently the most widely used technique for cloud point determination, *Zhang* and coauthors have provided recommendations in a recent tutorial review .[108]

4.2 Amino-acid based vinyl polymers

Thermoresponsive materials with improved biocompatibility and biodegradability are highly desirable, especially in the biomedical sector, and a simple way to achieve this is to combine synthetic thermoresponsive polymers with peptidic structures. In addition, materials that incorporate amino acids can possess novel properties not easily found in other synthetic polymers. This strategy is developed here through a selection of representative examples (conjugates, grafting from, grafting onto, etc.). In this direction, reversible deactivation radical polymerization of vinyl monomers has been largely used to prepare a wide range of polymers bearing amino acids on their side chains.[109] By transforming amino acids to vinyl esters, (meth)acrylates, vinyl carbamates and (meth)acrylamides, RAFT polymerization can be used to produce polymers bearing the amino acid's pendant side chain.[110] Scheme 1 summarizes the 3 methods to prepare such vinyl monomers: by implementing an *N*-alkylation or amidation of the amino-acid (a), by making a peptide coupling with the carboxylic acid function (b) or by preparing a conjugate using the side chain of the amino acid (c). Upon polymerization, the polymers thus retain the chiral center of the amino-acid unit, and either display the native side chain of the amino acid (pathway a and b in scheme 1) or can behave as weak electrolytes via the amino-acid's carboxylic acid group (pathway c in scheme 1). Some of the many examples that can be found in the literature have proven to be thermoresponsive by using specific amino acids to functionalize the side chain of water soluble polymethacrylamides.



Scheme 1. Possible synthesis pathways to introduce polymerizable double bonds units on amino-acids. Pathway a, b and c showcase respectively the addition on the amine group of the amino-acid, the carboxylic acid group with *N*-Boc protection and on the side chain of the amino acid with *N*-Boc protection of a vinyl monomer. The R'' group (a) will be C=O and for (b) $CO_2CH_2CH_2$ and once polymerized via RAFT these polymers may express thermoresponsive behavior.

Representative examples can be found in the works of *Endo*'s group. They first prepared poly(acryloyl-proline methyl ester) (PAProOMe) by combining *N*-acryloylation and RAFT polymerization.[111] (pathway a in Scheme 1). This polymer was known to show LCST behavior at around 14°C [112] but the use of RAFT techniques allowed the preparation of well-defined polymers. This design introduces a hydrophilic amide bond between the amine of the proline and the acrylic backbone to counterbalance the hydrophobic side chain of the proline units. The equilibrium between these two behaviors gives rise to the LCST behavior. The much more hydrophilic non-methylated counterparts of the same polymers were not thermoresponsive. The LCST could be further tuned by copolymerizing the proline-containing monomer with more hydrophilic monomers such as *N*,*N*-dimethylacrylamide (DMA) or *N*-acryloyl-4-trans-hydroxy-*L*-proline methyl ester (AHypOMe).[113] With this new design, *Endo* and coauthors were able to tune the LCST to a temperature up to 44°C by increasing the DMA content and up to 55°C by increasing the AHypOMe content. (Table 3) The macromolecular engineering of such polymers was studied further, and they have been shown to be highly versatile in more complex polymeric systems.[109,114]

Alanine-based acrylamides have also been investigated in a similar context using amidation (pathway a in Scheme 1).[115,116] *Yu et al.* were able to vary the transition behavior by changing the substituent on the alanine's carboxylic acid (Table 3). Changing from acrylamides to methacrylamides resulted in lower transition temperatures, while all polymers' behavior was dependent on the molar masses. The chirality of the amino acid had little effect on the thermoresponsiveness. On the other hand, the free carboxylic counterpart showed UCST

behavior at low pH.[117] This was due to strong polymer-polymer hydrogen bonding (H-UCST) due to the protonated carboxylic acid functions.

While it provides neither a side chain nor a chiral center, glycine-based methyl ester acrylamides were also shown to produce thermoresponsive polymers. Poly(*N*-acryloyl-Glycine *O*-methyl ester) (PNAGMe) synthesized via pathway a (Scheme 1) displayed an LCST around 72°C.[117,118]. Copolymers of this monomer with the previously discussed alanine-based acrylates, gave polymers with LCSTs between those of the two homopolymers.[116,117] (Table 3) Interestingly, glycinamide-based polymers showed reverse thermogelling behavior.[119] Gels formed by poly(*N*-acryloyl-glycinamide) PNAGA at room temperature became soluble on heating to around 30°C, (Table 3) and the polymers showed H-UCST behavior at lower concentrations.[120] The UCST behavior in dilute solution was disrupted by ionic contaminants that bonded with the amide groups.[121] Remarkably, this UCST behavior was hysteretic with heating and cooling cycles that had not previously been reported.(Table 3) Other polymers based on asparaginamide (modified asparagine) also showed UCST behavior. These polymers bearing two amide moieties (PNAAAM) (Table 3) showed similar hysteretic behavior to that of PNAGA.[122]

Poly(*N*-acryloyl-*L*-valine *N′*-methylamide) (PAVMA) obtained from a valine derivative also showed LCST-like behavior.[123] These polymers presented low transition temperatures around 8°C for all molar masses. (Table 3) This was due to the high hydrophobicity presented by the valine side chain. *Bose* and coauthors also used methionine and introduced an acrylic unit by *C*-protecting the carboxylic acid with concomitant Boc protection on the amine group. (pathway b in Scheme 1) They further modified the sulfur bearing side chain to produce [Boc-*L*-methionine-(2-methacryloylethyl)]sulfoniopropanesulfonate (METMASPS) zwitterionic monomers that were later polymerized by radical polymerization. (Table 3) Both monomer and polymer exhibited thermoresponsive C-UCST behavior influenced by the pH and the presence of anions. The monomer was insoluble at room temperature near its isoelectric point (pH=4.8 - 6.8) and exhibits a UCST type transition at 57°C due to the weakening of the electrostatic attraction between opposing charges. Upon polymerization, the macromolecule was soluble at all temperatures and pH values in aqueous solutions. Nonetheless, the addition of certain anions aggregated the polymer and allowed for a UCST-type transition in agreement with the properties of the monomer units (T_{CU} between 15°C and 65°C varying with the molar masses and the concentration of the anions).The authors also observed UCST behavior in the presence of citrate and acetate anions at higher pH levels (around 8), and demonstrated that the thermoresponsive behavior of such zwitterionic polymers could be tuned by both changing the pH and the salt.[124]

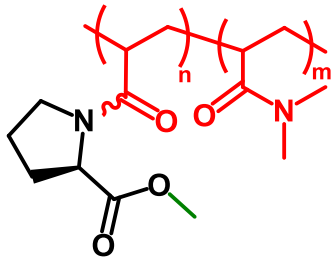
By modifying the side chain of *N*-Boc protected lysine (pathway c in Scheme 1), *Dinda* and coauthors also formed poly(Boc-*L*-lysinylacrylamide) [P(Boc-lysA)] displaying UCST behavior.[125] This behavior was only observed in mixtures of water and organic solvents (DMF, DMSO and MeOH) because of the hydrophobic Boc protecting group. They thus determined a minimal water content to form aggregates at room temperature that become soluble upon heating. Increasing the water content led to a corresponding increase in transition temperature until a maximum content where the polymers became insoluble in all conditions. (Table 3) This behavior was attributed to the polymer-organic solvent dipole-dipole interaction that is much more stable than the water-protic organic solvent hydrogen bonding. As expected, both water content and polymer length increased the T_{CU}. The same researchers were also able to induce an LCST type property in these polymers by adding large organic cations or ionic liquids to the solution. For instance, by solubilizing the polymers in aqueous solution at pH 8.5

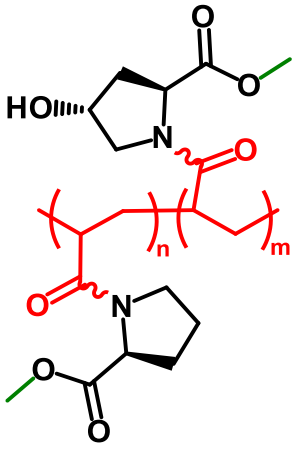
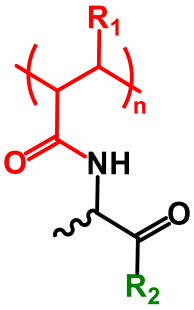
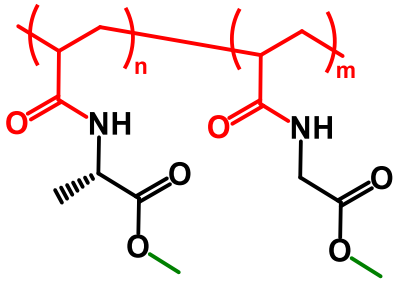
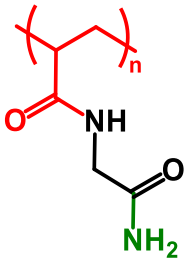
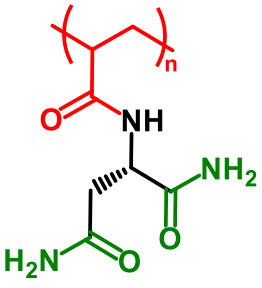
and above, the carboxylate groups of the lysine units formed complexes with counterions such as Bu_4N^+, Bu_4P^+ or $EtBzBuIm^+$ (from 4-ethylbenzylbutylimidazolium) to afford thermoresponsiveness. (Table 3) This behavior was attributed to the increasing hydrophobic interactions between the large organic groups of the counter ions at increasing temperature, in agreement with the fact that longer chains showed lower T_{CP} values since there were more hydrophobic interactions. [125] The LCST could thus be varied over a large temperature range (40°C – 80°C) by changing the polymer concentration, the type of salt and its concentration. Overall, by adding lysine units to the side chain of a thermoresponsive polyacrylamide, the authors were able to tune both the UCST and LCST behaviors.

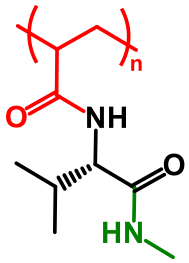
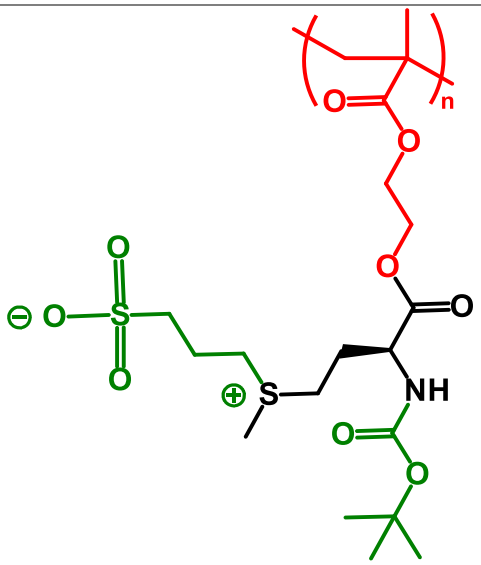
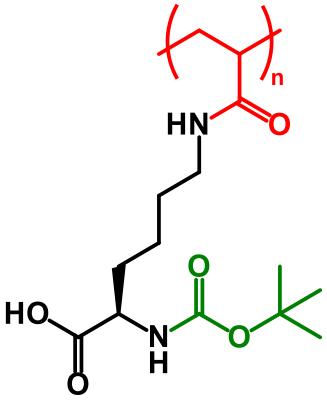
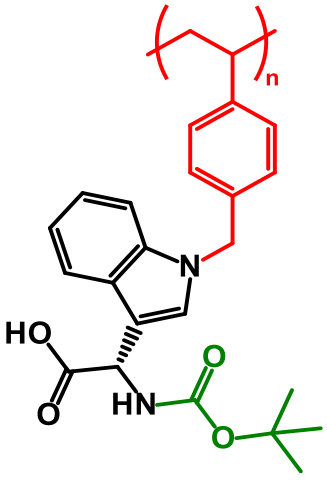
Very similar results, including UCST behavior in mixtures of water and organic solvents and LCST behavior in the presence of bulky hydrophobic cations, were observed by the *Mandal* group in polymers of 4-vinylbenzyl (Boc-tryptophan). Boc-protected tryptophan was functionalized with 4-vinyl-benzyl on its side chain amine (pathway c in Scheme 1) and subsequently polymerized.[126] After Boc deprotection, the zwitterionic polymers PTrpVBz showed C-UCST behavior that could be tuned by changing the pH (due to the protonation state of both the amine and carboxylic acid of tryptophan). Thermoresponsive behavior was observed at lower pH where the ammonium and carboxylic acid moieties are more prevalent than the carboxylate ones.[126] (Table 3) At higher pH, the polymer was completely soluble.

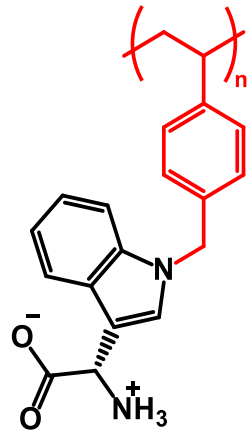
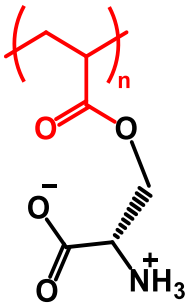
Using the alcohol on the side chain of serine (pathway c in Scheme 1) it was also possible to prepare thermoresponsive polyacrylates. (Table 3) Given the zwitterionic nature near its isoelectric point, the electrostatic attraction between counterions of poly(*L*-serinyl acrylate) (PSA) formed aggregates at low temperature. This interaction could be disrupted at higher temperatures giving reversible C-UCST behavior.[127]

-TABLE 3 – Structures and thermoresponsive properties of amino-acid modified vinyl polymers

Polymer structure (in red vinyl polymer backbone, in black the amino-acid and in green protecting groups and/or modifications to the amino-acid)	**LCST T_{CP} (°C) and conditions**	**UCST T_{CU} (°C) and conditions**	**Ref.**
 **PAProOMe and copolymers with DMA**	14°C – 44°C n:m = 1:0 –1:1	-	[111]

 **PAHypOMe-*co*-PAProOMe**	14°C – 55°C n:m = 1:0 – 0:1	-	[113]
 R_1=H or CH_3 R_2=OH, iPA, OMe, OEt or OiPr **P(M)A(*D,L*)AlaR_2**	14°C – 38°C depending on R_1 or R_2 and Dp	45°C – 55°C if R_1 = H and R_2 = OH and pH=2	[115][117]
 **PNAAMe-*co*-PNAGMe**	17°C – 72°C n:m = 1:0 – 0:1	-	[116–118]
 **PNAGA**	-	Gel to sol 27°C – 32°C depending on Mw and concentration	[119]
		22°C – 23°C upon heating and 7°C – 10°C upon cooling depending on temperature ramp rate	[120,121]
 **PNAAAM**	-	4°C – 28°C depending on concentration and Mw with varying hysteresis	[122]

 **PAVMA**	7°C – 10°C	-	[123]
 **PMETMASPS**	-	15°C – 65°C pH=3.5 with SO_4^{2-} or $H_2PO_4^-$ pH=8 with Ct^{3-} or Ac^-	[124]
 **PBoc-LysA**	At pH = 8.5 (carboxylate form) 60.8°C in the presence of Bu_4NBr 54.4°C in the presence of Bu_4PBr 46.7°C in the presence of Bu_4NCl 43.4°C in the presence of EtBzBuImBr	Values at maximum amount of water 36.7°C in MeOH:H_2O (72:38) 45.6°C in DMF:H_2O (55:45) 72.5°C in DMSO:H_2O (72:38)	[125]
 **PBocTrpVBz**	At pH = 8.5 (carboxylate form) 58°C in the presence of Bu_4NI 56°C in the presence of Bu_4NBr 51°C in the presence of Bu_4NCl	Values at maximum amount of water 58°C in MeOH:H_2O (82:18) 72°C in DMSO:H_2O (63:27) 70°C in DMF:H_2O (53:37)	[126]

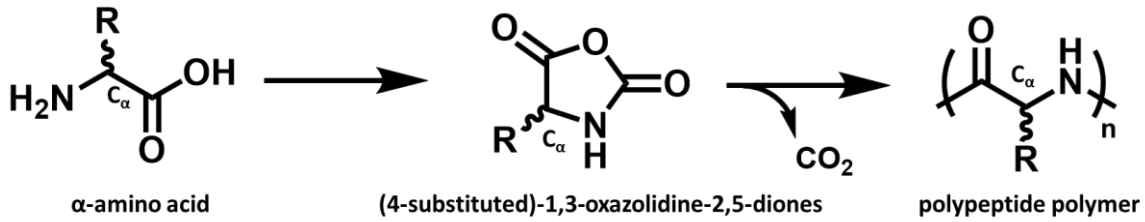
 **PTrpVBz at its isoelectric point (pH = 4.5)**	-	38°C – 65°C pH = 1.50 – 1.95		
 **PAS at its isoelectric point (pH = 2.85)**	-	18°C – 80°C depending on Mw, concentration and salts	[127]	

These polymers of vinyl monomers that have been conjugated to amino acids show that thermoresponsiveness is particularly influenced by the presence of peptide/amide bond linkages. It is also interesting to consider combinations of true polypeptide backbones with thermoresponsive polymers. Moving one step closer to proteins, polymer chemistry makes it easy to prepare amino acid-based structures that have fully peptidic backbones. For the last two decades, the study of these polymers has revealed a relationship between structure and thermoresponsiveness that allows a better understanding of the thermoresponsiveness of proteins and enables the development of new classes of thermoresponsive polymers that integrate the advantages of the biomimetic backbones (degradation, etc) with the processability of synthetic polymers (gels, etc.). Yet in order to do so, one must first master the polymerization technique which allows the preparation of peptidic polymers. The ways to obtain such materials will be discussed briefly in the following section.

4.3 Synthetic polypeptides by ring-opening polymerization: combining protein and synthetic polymer features in the same backbone

While the use of proteins for material design holds tremendous promise in many applications, polyaminoacid backbones are not always easy to prepare.[128] Approaches that use genetic engineering or synthetic biology are quite promising but require significant development of biological platforms thus still restricting their scale and scope. Moreover, they have not been very useful to prepare materials at large scale due to their poor stability and high cost of production.[129] Indeed, proteins inspire the use of amino acids as monomers in synthetic analogues called polypeptides, which are much easier to produce by a simple chemical process while also being biocompatible and biodegradable. In this context, polymer chemistry is the most economical and efficient route to synthetic polypeptides, combining

advantageous features of synthetic polymers (solubility, processability, rubber elasticity, etc.) with those of natural proteins (secondary structure, functionality, biocompatibility, etc.).[130] Amino acids are very difficult to polymerize in their native form. A common and efficient way to afford polypeptides by polymerization is to cyclize the amino acid to their corresponding (4-substituted)-1,3-oxazolidine-2,5-diones (Scheme 2), more commonly known as *N*-carboxyanhydrides (NCAs).[131]



Scheme 2. Schematic representation of the transformation of an α-amino acid into its corresponding *N*-carboxyanhydride. These monomers can be ring-opened, releasing CO_2 and producing polypeptide polymers.

These cyclic molecules are more active due to the high electrophilicity of the 5-position carbonyl and the acidic nature of the nitrogen proton. Moreover, the liberation of CO_2 on ring opening results in a significant increase in entropy on polymerization, making these monomers highly unstable under ambient conditions. Thus, they must be handled and used under inert conditions, either under a Schlenk line with N_2 flow or argon protection, or in a glovebox. The ring-opening polymerization of NCAs involves the simplest reagents, and allows the preparation of polymers made of amino acids in both good yield and large quantity.[132] Historically, the limitations of NCA polymerization have been (1) the purification of the monomers, which usually had to be achieved by crystallization; (2) the presence of side reactions that can significantly reduce molecular weight control. These two limitations have now been fully overcome. First, several catalysts/strategies have been developed in recent years to avoid side reactions during the polymerization process, which allows precise control of the molecular weight. Second, the purification of often non-crystalline NCA monomers can be efficiently achieved by other methods, such as filtration. The living ROP of NCAs has specifically proved very useful in the preparation of synthetic copolymers with very narrow dispersity.[133] Peptidic polymers have been available for many decades, but have been used primarily as inert materials to develop biocompatible matrices. However, the synthesis of peptidic polymers has progressed significantly over the last 10 years, and it is now possible to prepare polypeptides via different living processes, allowing the introduction of numerous functionalities including ones that are useful for thermoresponsiveness.[132] These preparations are easily extended to other macromolecular engineering technologies. Furthermore, these polymers are being synthesized in solvents not previously studied such as aqueous media.[134] In this context, as simplified analogues of proteins, polypeptides are easier to prepare and are promising candidates for new applications such as nanomedicine or bioprinting. Compared to natural proteins, synthetic polypeptides are simple macromolecules in which an amino acid is repeated many times, but they have a similar tendency to adopt ordered secondary conformations such as α-helices or β-sheets, a property that is sought-after in polymer science.[135] For instance, polypeptide polymers offer a unique way to direct nanoscale structure formation through intermolecular and/or intramolecular interactions and therefore, polypeptide polymers have found significant interest in a variety of biomedical applications.[136] Furthermore, the chemical structure of the entire macromolecule (and in

particular the polymer backbone) is similar to the chemical structure of proteins. The polymers retain the degradation properties of their natural models. Unlike synthetic polymers made by radical polymerization processes, polypeptides are inherently biodegradable and could improve polymer sustainability.[137]

Moving a step beyond the approaches developed in section 4.2, synthetic thermoresponsive polymers have been conjugated to polypeptides or proteins. Indeed, several studies have combined sequence-controlled peptidic structures and even proteins to synthetic polymers to confer thermoresponsiveness. The reader can refer to many examples in the literature that cover these different topics.[138] In the following sections, we will focus on peptidic polymers prepared via polymer chemistry, mainly by the ROP of NCAs. First the macromolecular engineering techniques employed to conjugate the thermoresponsive polymers to the polypeptide block will be described. These include grafting polypeptides onto thermoresponsive polymers (section 5.3) and the preparation of block copolymers using grafting-from approaches with thermoresponsive macroinitiators: indeed, the ROP of NCAs is highly compatible with other classical polymerization techniques (section 5.3 and 5.4).

4.4 Grafted copolymers

In this section, thermoresponsive polymers obtained by grafting polypeptides onto synthetic polymer backbones will be discussed. (Fig. 6)

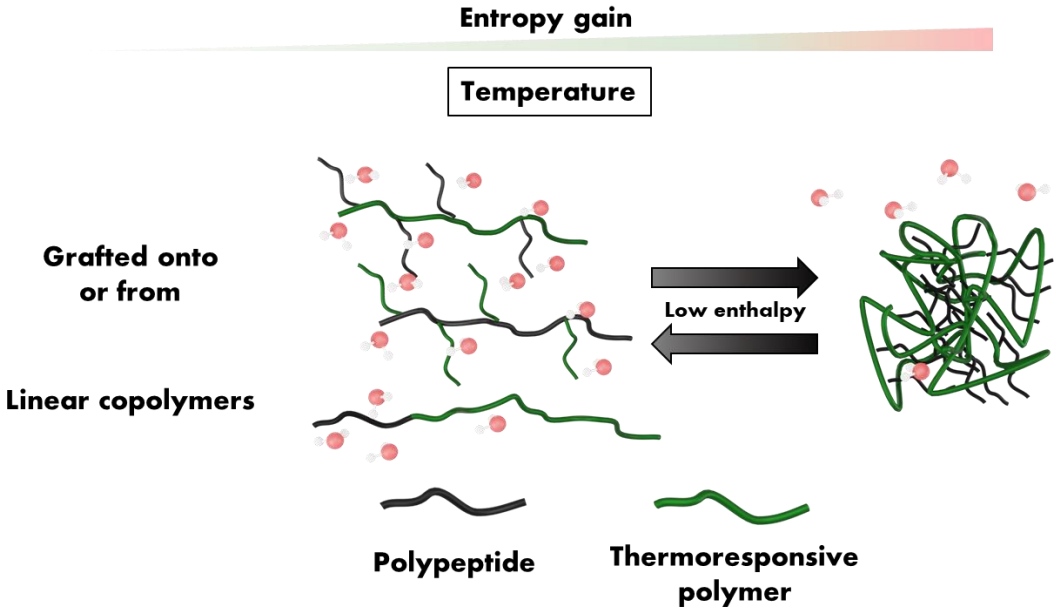


Fig. 6. Two macromolecular engineering techniques to bestow thermoresponsiveness on polypeptides. The first approach (top): grafted copolymers. This can be done in two ways: 1- grafting polypeptides from (using NCA polymerization) or onto a thermoresponsive polymer backbone; 2- grafting a thermoresponsive polymer from or onto the reactive side chains of a polypeptide. The second approach (bottom): diblock copolymers with one polypeptide block and one thermoresponsive polymer block. This approach can be carried out sequentially, with one block serving as the macroinitiator for the other, or by conjugating both blocks after polymerization.

The most studied polypeptide, poly(*L*-glutamic acid) (PGA) has been grafted several times with LCST-type polymers.[139] *Jing*'s group prepared similar copolymers by partially grafting PNIPAM onto PGA that was functionalized with *N*-hydroxysuccinimide (NHS).[140] The latter served as coupling intermediate to graft with amino-semitelechelic PNIPAM to obtain PGA-*co*-P(GA-*g*-PNIPAM) (Table 4). These polymers showed LCST behavior at neutral pH

at around 35°C, consistent with the PNIPAM block (5-7). However, when the pH was lowered, the LCST first disappeared (pH=4.5) then reappeared around pH=4.2 with a T_{CP} around 25°C. This was attributed to the protonation of the glutamic acid side chains that confers a more hydrophobic nature to the whole polymer. In another study, *Zhao* and co-workers developed thermo-responsive micelles of poly(NIPAM-*co*-*N*-acryloyloxysuccinimide) grafted with PBLG.[141] On precipitation in DMF/water these copolymers assembled in either spherical or worm-like micelles. The authors first copolymerized NIPAM and *N*-acryloyloxysuccinimide (NAS) via RAFT. They then reacted the NAS group with a protected diamine giving the corresponding acrylamide while expelling *N*-hydroxysuccinimde. The further deprotection of the amine allowed for initiating points for the ROP of BLG NCA chains affording P(NIPAM-*co*-NAS)-*g*-PBLG (Table 4). These copolymers expressed LCST behavior at 36.3°C consistent with that of the thermoresponsive PNIPAM block. The morphology of the assemblies was tuned by the blocks' respective lengths and by the functionalization of the PBLG. In similar work, *Chen* and co-workers developed cross-linked micelles based on PGA grafted with PNIPAM and 2-hydroxyethyl methacrylate (HEMA). This polymer self-assembled above the LCST of PNIPAM but nanomaterials were only observed at higher pH values as the pH-induced coil-to-helix transition of PGA at low pH caused the polymer to aggregate and destabilized the micelle formation. To counterbalance this effect, the micelles were further cross-linked by the free radical polymerization of HEMA units to form PGA-*co*-(PGA-*g*-PNIPAM)-*co*-(PGA-*g*-PHEMA) . (Table 4) The resulting micelles were responsive to both temperature and pH.[142] These examples show the advantages of combining the polypeptide backbone with thermoresponsive synthetic polymers for further drug delivery applications.

Another polypeptide widely used to prepare conjugates with thermoresponsive polymers is poly(*L*-cysteine). For instance, *Mandal* and co-workers synthesized polycysteine-*graft*-poly(2-isopropyl-2-oxazoline) (PCys-*g*-PiPOx) (Table 4) and studied its thermoresponsive behavior.[143] The group used propargylated *L*-cysteine *N*-carboxyanhydride to produce the corresponding polypeptide by ROP. In a second step, using azide end-functional poly(2-isopropyl-2-oxazoline), they used the copper(I) catalyzed alkyne–azide 'click' reaction to produce the corresponding grafted polycysteines. A higher T_{CP} was observed at higher molar masses of polyoxazoline, in agreement with the thermoresponsive behavior of the homopolymer. On the other hand, higher molar masses of PCys grafted with the same PiPOx length exhibited lower transition temperatures due to the increased hydrophobicity. In the same direction, by keeping the same length of polypeptide backbone, longer PiPOx showed lower transition temperatures than their shorter counterparts. Much like classical LCST expressing polymers, the phase diagram of these graft copolymers showed the typical U-shape transition between the soluble and biphasic mixture with respect to concentration in H_2O. Furthermore, these amphiphilic copolymers self-assembled to form "composite" vesicles. Changing the polypeptide to poly(*L*-tyrosine), *Mandal* and co-workers synthesized polytyrosine-*graft*-poly(2-ethyl-2-oxazoline) (PTyr-*g*-PEtOx) (Table 4) that displayed LCST at T_{CP} = 64°C – 68°C.[144] The same behavior as in the case of the polycysteine-*g*-polyoxazoline was observed: higher molar masses of PTyr showed lower T_{CP} due to higher hydrophobicity and lower overall content of LCST exhibiting polymer. The LCST could be further decreased by increasing the concentration and/or the DP of PEtOx.

All these examples of grafted copolymers were designed to self-assemble into different nanoobjects, driven by the polypeptide. Indeed, the choice of polypeptides was not only driven by their biodegradable nature and the self-assembly was significantly influenced and tuned by the polypeptide block, either because they were hydrophobic (PBLG), or they formed insoluble

secondary structures (PGA, PCys and PTyr). The self-assembly promoted dye and drug encapsulation, while the thermoresponsiveness allowed on demand drug release by heating.

4.5 Diblock copolymers

Polypeptides have also been used to prepare di-block copolymers where one block is an LCST or UCST polymer. In such designs, the polypeptide block can be prepared via the ROP of NCA using a grafting from approach using a thermoresponsive macroinitiator to allow easy workup. (Fig. 6)

Rao and coauthors investigated this grafting-from approach by using monoamine-terminated PNIPAM as a macroinitiator for the polymerization of BLG NCA.[145] After deprotection, the resulting PNIPAM-*b*-PGA (Table 4) formed micelles in response to changes in both pH and temperature, properties conferred by the PNIPAM and the polypeptide block respectively. At room temperature and basic pH, both blocks were soluble but by reducing the pH to more acidic conditions the carboxylate side chains were protonated, inducing micellization driven by formation of insoluble PGA helices. On the other hand, while the PGA was in its soluble form, PNIPAM was aggregated by heating above its T_{CP} (around 32°C) forming the reverse micelle with a shell of PGA. (Fig. 7) This was further tuned by only partially deprotecting the BLG monomer units. Retaining some hydrophobic benzyl groups allowed thermoresponsiveness to be obtained at other pH values and at lower temperatures.[146] (Table 4) *Mezzenga*'s group performed a study with similar polymers but changed the macromolecular engineering to a triblock structure. Using PNIPAM with amines at both chain ends, BLG NCA was polymerized on both sides in a single step and then deprotected to afford the corresponding PGA blocks. This design allowed for hydrogel formation, showcasing the versatility of this type of approach to prepare thermoresponsive materials.[147] Following similar methodologies, *Huang* and coauthors used γ-propargyl-*L*-glutamate (PLG) NCA and diamine end-capped polypropylene glycol (PPG) to produce triblock copolymers (PPLG-*b*-PPG-*b*-PPLG). The pendant alkynes were further grafted with azido-PEG units and the triblock copolymers formed micelles.[148] *Zhao* et al. extended this principle to replace the PGA block by a polylysine (PLys) backbone.[149] In this case, PNIPAM with a terminal amine function was used as a macroinitiator for *N'*-benzyloxy-*L*-lysine NCA (Lys(*Z*)-NCA) and the deprotected polymer (PLys-*b*-PNIPAM) (Table 4) presented a T_{CP} around 32-34°C consistent with PNIPAM behavior. This diblock copolymer was also pH-responsive due to the free amine side chains of PLys, owing for self-assembly interplay with temperature and pH, much like in the case with PGA block but in a higher pH range.

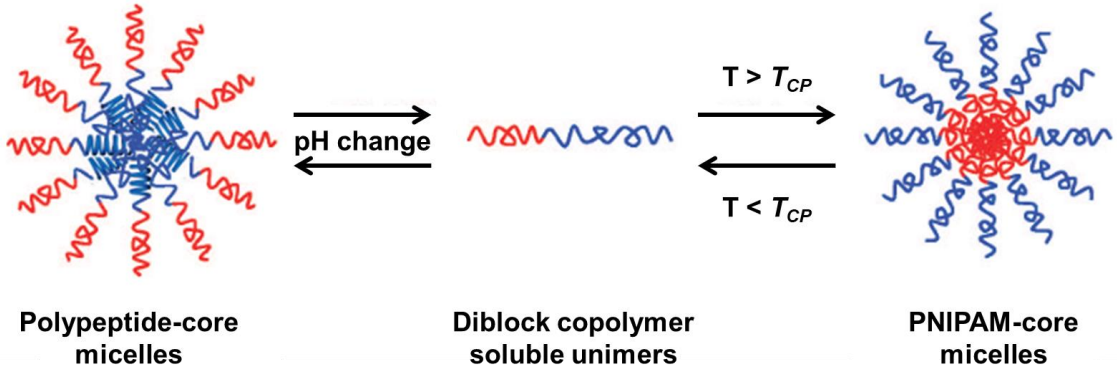


Fig. 7. pH and thermoresponsive diblock copolymers based on polypeptides (in blue) and PNIPAM (in red). The water-soluble polypeptides undergo a secondary structure change when the pH is changed (left) and become insoluble forming the core of the micelles. In the case of PGA, the carboxylate groups (at high pH) allow for

solubilization, and the carboxylic acid groups (at low pH) induce formation of the insoluble α-helix. Inversely, PLys has pendant hydrophilic ammonium groups at low pH that, when deprotonated, fold the PLys into the hydrophobic micelle core. The PNIPAM block undergoes dehydration upon heating (right) above its T_{CP} forming the hydrophobic core of the micelles. This Fig. was adapted from [145]

A grafting from approach was also achieved from the polypeptide block. *Zhang*'s group used this alternative strategy by polymerizing NIPAM from a PBLG macro chain transfer agent . (Table 4) After deprotection, the polymers were water soluble and responsive to both pH and temperature as previously described.[150] *Mokrus* and coauthors have controlled the morphology of nanomaterials made of such copolymers by modifying the PGA side chains using different saccharides (P(SacLG)-*b*-PNIPAM) . (Table 4) Interestingly, particles with a saccharide corona were smaller than their sugar-free counterparts and the LCST was slightly shifted towards higher temperatures. The authors claimed the formation of polymersomes upon saccharide grafting and the T_{CP} could be further tuned by changing the substitution degree, the size of the saccharide, and its hydrophilicity. Incorporating such saccharides was also useful for further cellular recognition *in vivo*.[151]

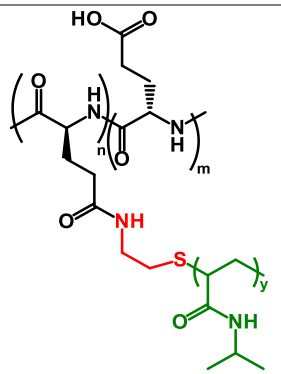
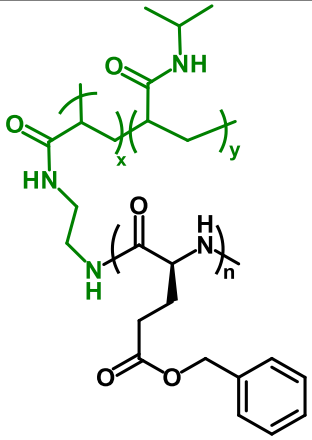
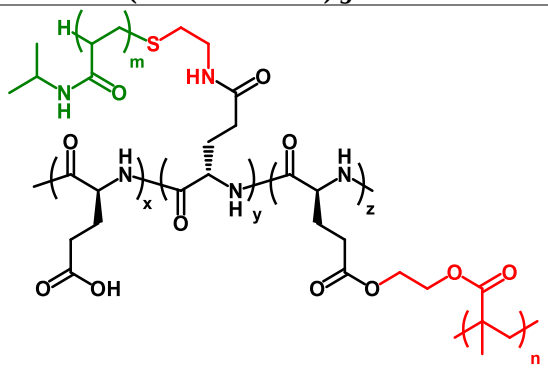
Using another design with acrylates, *Lecommandoux* and coauthors developed polymeric micelles or polymersomes from poly[2-(dimethylamino)ethyl methacrylate]-*block*-poly(glutamic acid) (PDMAE-*b*-PGA). (Table 4)[152] These polymers were prepared by copper catalyzed alkyne-azide cycloaddition (CuAAc) click reaction between ω-azido and ω-propargyl functionalized polymers. By taking advantage of the LCST behavior of PDMAE around 40°C it was possible to form polymersomes by self-assembly above pH 11 making them both pH and temperature responsive. The LCST could also be increased with higher PGA length as it adds hydrophilicity to the system. With other monomers, *Zhang* and coauthors also developed thermoresponsive nanomaterials by polymerizing BLG NCA with amine end-functionalized poly(ethyl ethylene phosphate) (PEEP) forming poly(γ-benzyl-*L*-glutamate)-*block*-poly(ethyl ethylene phosphate) (PBLG-*b*-PEEP) . (Table 4)[153] Upon self-assembly, the micellar solutions presented LCST behavior that decreased with high concentration, molar mass content and NaCl concentration but were not pH responsive. Poly(γ-glutamic acid) was also explored in this design,[154] but these will not be discussed here as these polymers were produced by bacterial fermentation and not by synthetic routes.

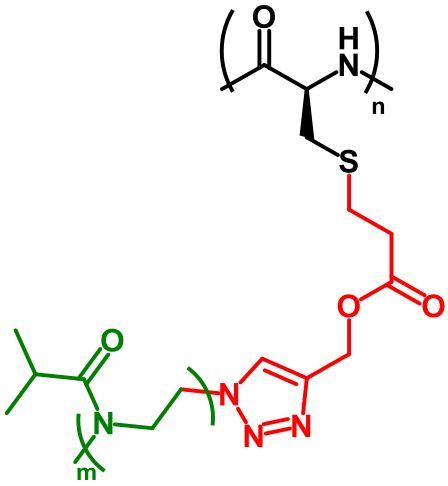
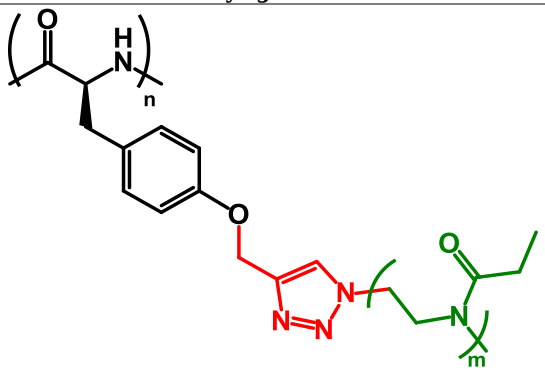
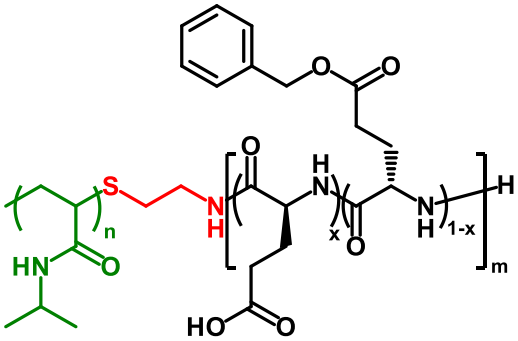
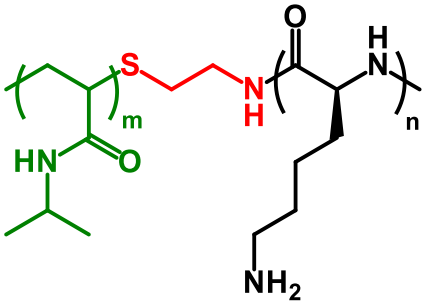
Beyond nanomaterials, and taking advantage of the tendency of α-helix and β-sheet forming peptides, [155–157] or polypeptides from NCAs [158,159] to form hydrogels, copolymer design was also achieved to make them thermoresponsive.[160] For instance *Zhu* and coauthors synthesized poly(ethylene glycol)-*block*-poly(*O*-benzyl-*L*-threonine) (PEG-*b*-P(OTre)) (Table 4) copolymers that were able to form hydrogels at low temperatures (5°C) at high copolymer loading (above 12%).[161,162] This was attributable to the formation of *β*-sheet nanofibers that stabilized this structure as verified by FTIR and circular dichroism (CD). As the temperature rose, these secondary structures destabilized and formed more spherical nanoparticles that transitioned into a "sol" state. On further heating, the thermoresponsive behavior of PEG allowed for the formation of a gel due to its dehydration. The low temperature gel formation was highly dependent on the length of the threonine block as low DP polymers do not form stable secondary structures.[161] Replacing the *L*-threonine monomer units by *L*-tyrosine resulted in thermogelling block copolymers with a transition around body temperature . (Table 4)[163] In this case, the authors polymerized benzyl-protected tyrosine NCA from a PEG inititator and subsequently deprotected to obtain the desired copolymer. Interestingly, a study suggested that soluble compositions could thermogel after prolonged treatment at temperatures lower than the critical one. This means that gelation could also be kinetically

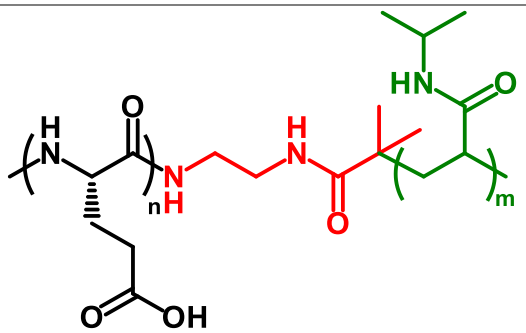
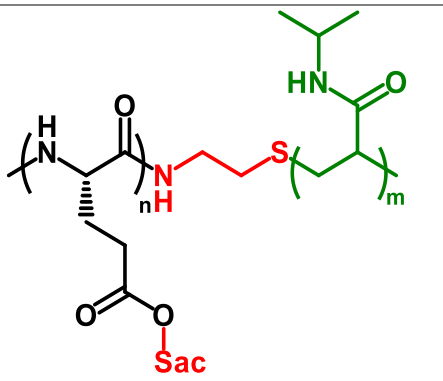
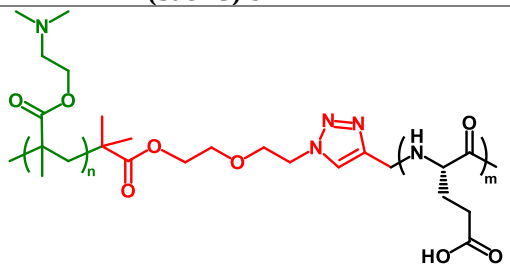
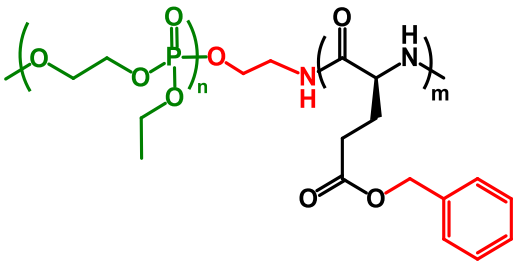
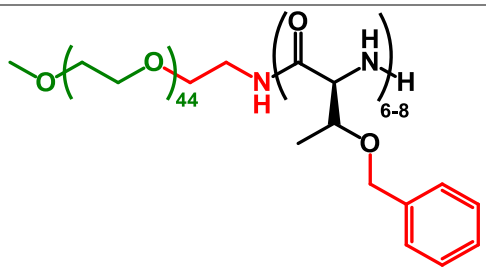
controlled and there is no clear connection between the secondary structure shift and the thermoresponsivness. *Hao*'s group combined the same PEG block with poly(*L*-valine), PVal, to produce thermogelling copolymers. The formation of β-sheet filaments was induced by the PVal segment and contributed to the formation of thermoresponsive hydrogels.[164] Other amino acids were later added to this design to implement other properties. For example, by sequentially polymerizing Lys(Z)-NCA and Valine NCA from a PEG macroinitiator, thermoresponsive hydrogels have been designed with pH responsiveness.[165] (Table 4) Terpolymerizing glycine, alanine and isoleucine NCAs from PEG-NH_2 macroinitiator also afforded hydrogels.[166] These copolymers had much longer polypeptidic blocks (Table 4) than other examples while showing similar thermogelling behavior. The polypeptide block has a higher β-sheet content at high temperature, and its gelation temperature is dependent on the copolymer composition. It is notable that the thermogelling behavior was not reversible on cooling and sonication was necessary to retrieve soluble polymers that could be readily thermogelled. These hydrogels were further tested for drug loading/release triggered by enzymatic degradation.[166]

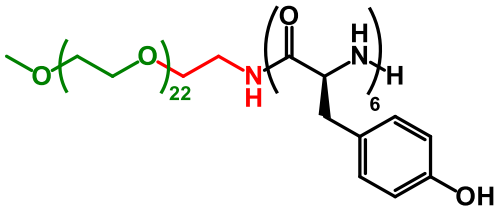
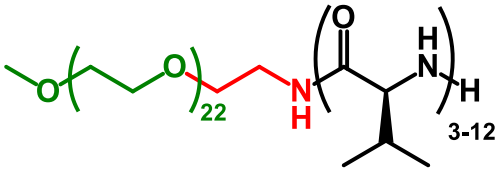
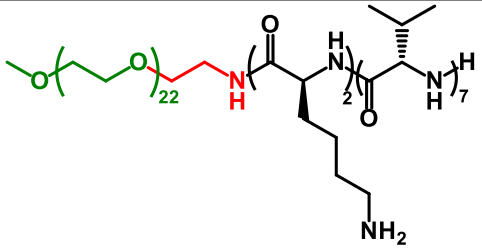
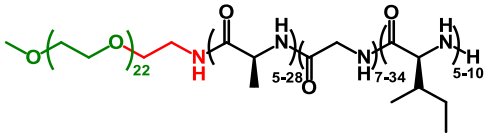
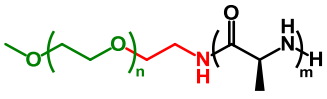
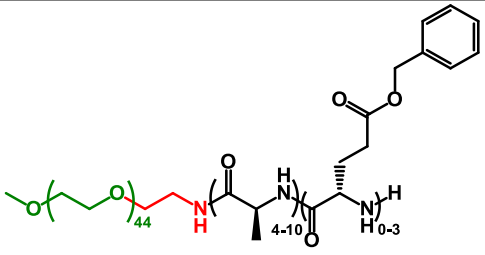
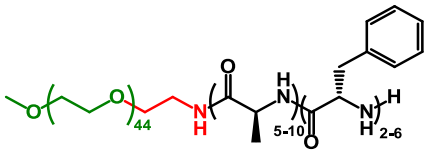
Polymerizing *L*-alanine NCA alone from PEG macroinitiator has also proven useful in such applications (Table 4). [167,168] The thermal behavior of the block copolymers could easily be tuned by end capping with different hydrophobic moieties and changing the lengths of the blocks.[169,170] The thermal behavior was further tuned by copolymerizing Ala NCA with more hydrophobic BLG NCA. (Table 4) In this case, both nano-objects and hydrogels were obtained and studied. Block copolymers of PEG and alanine-co-phenylalanine have also been studied and formed thermoresponsive organogels in chloroform.[171] Similar behavior was also observed in aqueous solutions, where the copolymers (Table 4) underwent a sol to gel transition on heating.[172] The key role of β-sheets was showcased in this design by introducing *D*-Alanine monomer units to disrupt the secondary structure. This considerably increased the transition temperatures due to the resulting increased solubility.[173] However, the loss of secondary structure had little effect on the hydrogels' mechanical properties. Thermogelling behavior was also obtained with PGA backbones. *Chen*'s team prepared a series of alkyl glutamates and ring opened the corresponding NCAs using PEG-NH_2.[174,175] They showed that shorter alkyl chains like methyl and ethyl had lower transition temperatures than longer ones like n-propyl and butyl. (Table 4) This was explained by the easier β-sheet formation for the monomer units with the smaller alkyl chains. It was also shown that the alkyne group on PPLG's pendant chain could be used for clicking moieties that could form similar thermogelling polymers. In this way extra functionality could be added to the polymer for example by adding bioactive moieties like sugars or biotin[176] (Table 4) Another non-natural amino acid known to give β-sheet secondary structures gave similar results.[177] Triblock copolymers of oligomers of allylglycine and PEG showed thermogelling behavior even at high temperatures. (Table 4) Beyond β-sheets, helical polypeptides were less effective at promoting hydrogel formation in response to changes in temperature. PEG-*b*-peptides formed gels at room temperature and had modulus change at higher temperature. In the examples above, although the PEG block is thermoresponsive, the behavior is arguably due to the peptide block that changes its conformation upon heating. Nonetheless, the thermal behavior of such secondary structures is not yet proven, and the behavior might stem from the interaction between the PEG, the peptidic backbone's secondary structure and the solvent.[160] Indeed, given the high copolymer loading, it seems this behavior is consistent with the known thermosensitivity of PEG.[178]

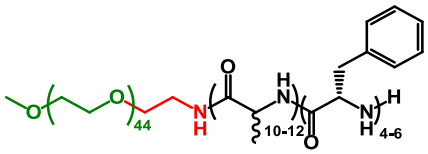
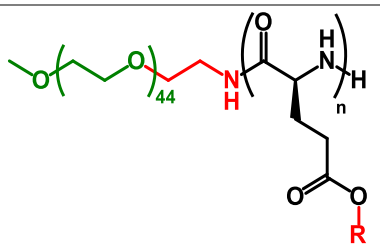
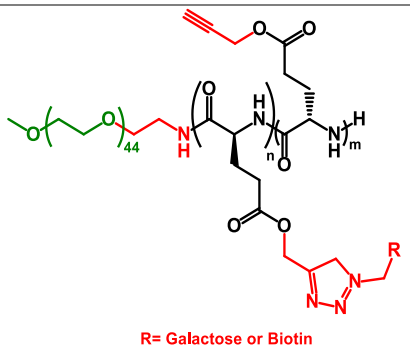
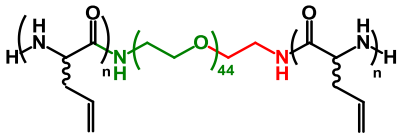
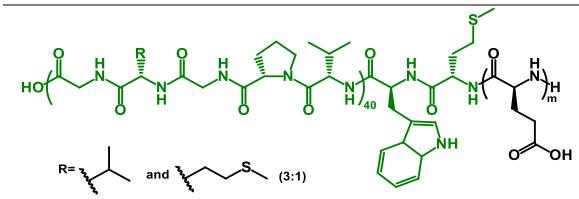
-TABLE 4 – Structures and thermoresponsive properties of graft or block copolymers between polypeptides and thermoresponsive polymers

Polymer structure (in green: thermoresponsive polymer, in black polypeptide polymer and in red linkers and/or modifications)	Amino acid	LCST T_{CP}(°C) and conditions	UCST T_{CU}(°C) and conditions	Ref
 **PGA-*co*-P(GA-*g*-PNIPAM)**	glutamic acid	25°C – 35°C Depending on pH and Mw	-	[140]
 **P(NIPAM-*co*-NAS)-*g*-PBLG**	glutamic acid	35.5°C	-	[141]
 **PGA-*co*-(PGA-*g*-PNIPAM)-*co*-(PGA-*g*-PHEMA).**	glutamic acid	32°C – 37°C	-	[142]

 **PCys-*g*-PiPOx**	cysteine	36°C – 50°C depending on Mw and concentration	-	[143]
 **PTyr-*g*-PiPOx**	tyrosine	64°C – 68°C depending on Mw and concentration	-	[144]
 **PNIPAM-*b*-PGA and PNIPAM-*b*-P(GA-*co*-BLG)**	glutamic acid	If x = 1 32°C If x < 1 21°C – 40°C depending on pH and x values	-	[145,146]
 **PNIPAM-*b*-PLys**	lysine	32°C – 36°C depending on PLys length	-	[149]

 **PGA-*b*-PNIPAM**	glutamic acid	32°C – 39°C depending on pH	-	[150]
 Sac = saccharides like glucose or maltose **P(Sac**LG**)-*b*-PNIPAM**	glutamic acid	32°C – 39°C depending on the grafted saccharides	-	[151]
 **PDMAE-*b*-PGA**	glutamic acid	36°C – 48°C depending on PGA length and pH	-	[152]
 **PEEP-*b*-PBLG**	glutamic acid	29°C – 37°C depending on MW, salt and concentration	-	[153]
 **PEG-*b*-PBLT**	threonine	Sol to Gel 58°C – 64°C depending on Mw and the concentration	Gel to Sol 7°C – 10°C depending on the Threonine units	[161] [162]

 **PEG-*b*-PTyr**	tyrosine	Sol to Gel 50°C – 25°C depending on concentration	-	[163]
 **PEG-*b*-PVal**	valine	Sol to gel 58°C – 64°C depending on Mw and the concentration	-	[164]
 **PEG-*b*-PLys-*b*-PVal**	lysine and valine	Sol to gel 25°C – 65°C depending on the concentration and pH	-	[165]
 **PEG-*b*-P(Ala-*co*-Gly-*co*-Ile)**	alanine, glycine and isoleucine	Sol to gel 18°C – 90°C depending on copolymer composition and concentration	-	[166]
 **PEG-*b*-PAla**	alanine	Sol to gel 5°C – 40°C depending on concentration and Mw	-	[167,169,170]
 **PEG-*b*-P(Ala-*co*-BLG)**	alanine and glutamic acid	Gel contraction with increasing temperature. No clear transition.	zzz-	[167]
 **PEG-*b*-P(Ala-*co*-Phe)**	alanine and phenylalanine	Sol to gel 10°C - 40°C depending on the weight fraction and molecular weight	-	[172,173]

 **PEG-*b*-P(*D*,*L*-Ala-*co*-Phe)**	*D*,*L*-alanine and phenylalanine	Sol to gel 20°C - 80°C depending on the weight fraction and D:L ratio	-	[173]
 **PEG-*b*-PalkLG**	glutamic acid	Sol to gel 11°C – 65°C depending on R=Me>Et>iPr>Bu	-	[174,175]
 **PEG-*b*-P(PLG-*co*-P(Gala or Biotin)G)**	glutamic acid	Sol to gel 20°C - 30°C broad transition	-	[176]
 **PAG-*b*-PEG-*b*-PAG**	allylglycine	Sol to gel 80°C - 96°C depending on concentration and molecular weight	-	[177]
 **ELP-*b*-PGA**	glutamic acid	45°C – 70°C depending on Mw and concentration	-	[179]

Peptide polymers have unique biomimetic properties. The use of polypeptides coupled to classical thermoresponsive polymers is a common approach to prepare thermoresponsive polypeptides. In these polymers, the polypeptide brings an additional contribution, notably thanks to its stimuli-responsive conformational rigidity (nanomaterials, gels, etc.). This design seems to be of great interest in many applications, especially in nanomedicine. To go further in the biomimicry, emerging approaches show an interest in replacing classical synthetic polymers by peptidic polymers in order to produce even more biocompatibility. For example, ELPs have recently been used as macroinitiators to polymerize BLG-NCA, producing diblock copolymers (Table 4) with only amino acids in the backbone.[179] When deprotected the copolymer was water soluble and upon heating the ELP collapsed due to the formation of β-turns. This thermal transition was influenced by the length of the polyglutamate block, having a higher T_{CP} and a broader transition than the native ELPs. Based on this example, the

remainder of the review presents how ring-opening polymerization can also be used to prepare polypeptide polymers with a backbone composed entirely of amino acids.

5. THERMORESPONSIVE POLYPEPTIDES AND PEPTIDOMIMETICS

At the crossroads between synthetic polymers and natural proteins such as IDPs, polypeptide polymers can themselves be thermoresponsive. The different examples and structures associated with this property are described in detail in this final section of the review which shows how different structural leverages can be put in place to achieve this property (side chain modification, peptoidic backbones and copolymerization).

5.1 Thermoresponsiveness through polypeptide side chain modification

The simplest way to make a polypeptide backbone thermoresponsive, as with conventional thermoresponsive polymers, is to functionalize the side chains of the amino-acid repeating units by introducing functionalities (ethylene glycol units, etc.) that are known to provide this property. This approach therefore involves the modification of the polypeptide backbone on the side chain, a modification that can be achieved following two different approaches (Scheme 3):

a) synthesizing amino acids with the desired side chain before making the NCAs and polymerizing them.

b) modifying the polymer using post-polymerization chemistry to introduce the stimuli responsive group.

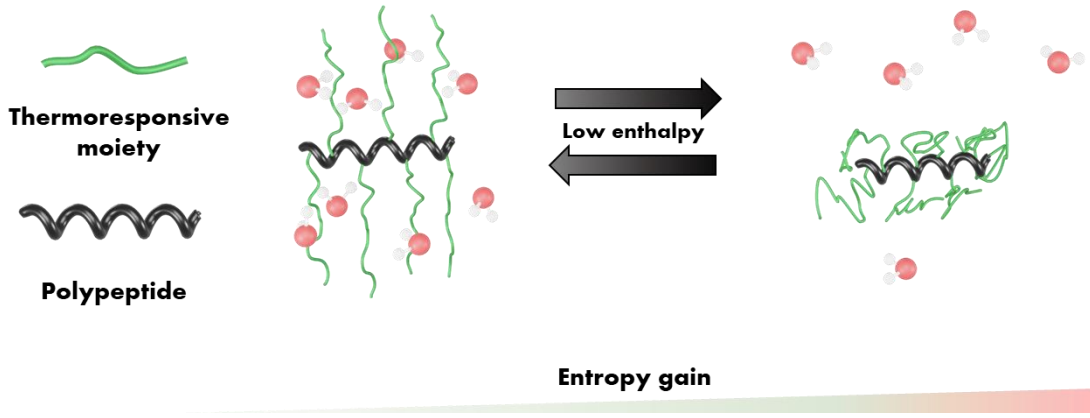
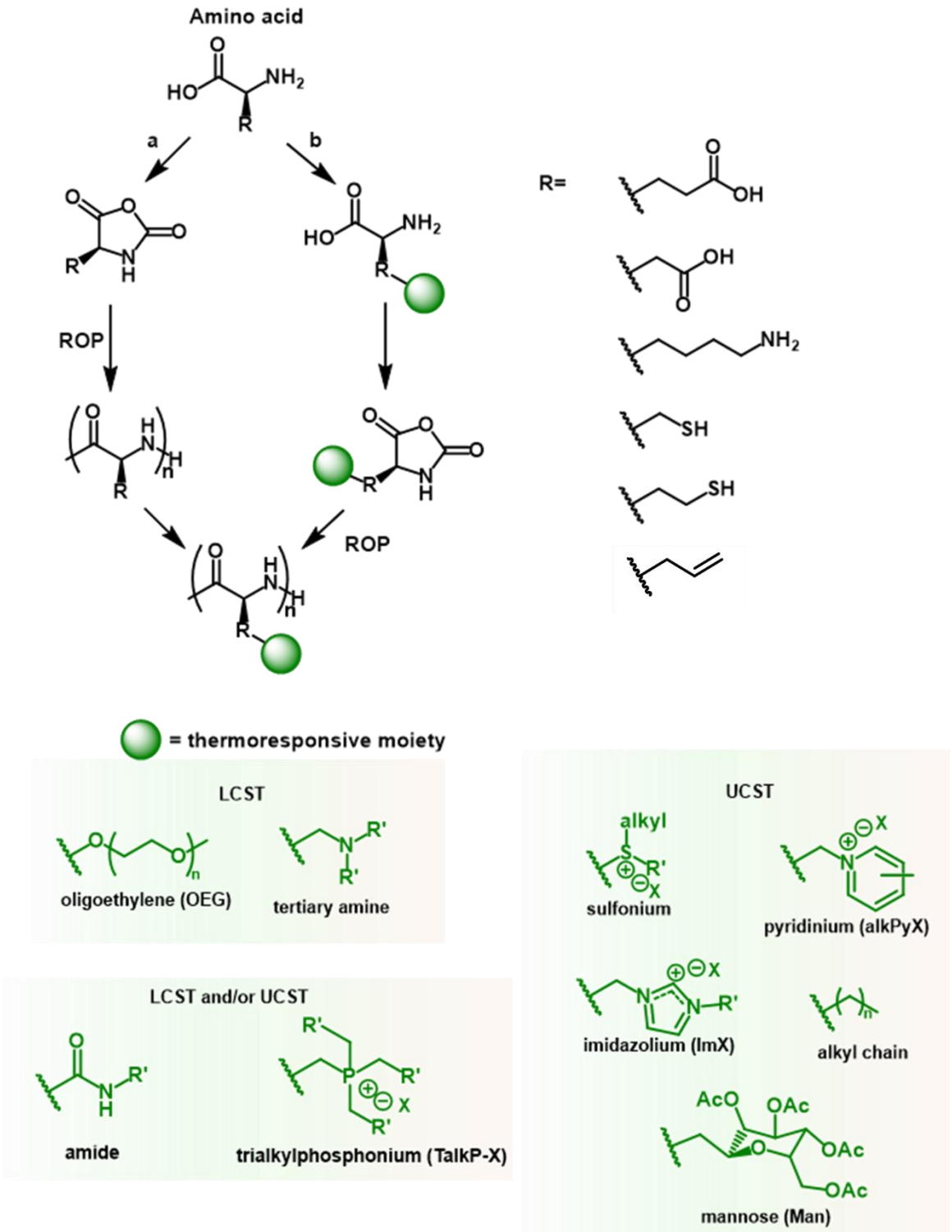


Fig. 8. Side-chain modified polypeptides and their thermoresponsive behavior. The stimuli responsiveness stems from the functions introduced on the side chain. The LCST/UCST is expressed depending on their entropy and water (red and white balls) solvation interactions at different temperatures. The polypeptide is confined and surrounded by dehydrated LCST/UCST side-chains at maximum entropy. The side-chains rehydrate as entropy decreases.

However, the thermoresponsive behavior of the polypeptides is often influenced by the conformation of the polypeptide backbone. PGA and PLys have been the two most studied polypeptides in the literature to achieve the modification of the lateral chains as developed in the next two sections of the review.



Scheme 3. Two synthesis pathways can be used to introduce thermoresponsive behavior to polypeptide backbones. Pathway a corresponds to the addition of a thermoresponsive moiety to an amino acid while pathway b relies on post-polymerization functionalization of polypeptides with a thermoresponsive moiety. The amino acids used all have side group functionalities (from top to bottom): glutamic acids, aspartic acid, lysine, cysteine, methionine and allylglycine. The thermoresponsive moieties confer either LCST, UCST or both behaviors. In the last case the behaviors may be observed on the same polymer or separately on different polymers.

5.1.1 Polyglutamate:

As with polymers obtained by radical polymerization processes, modification with oligoethylene glycol units (OEG) is an effective means to introduce thermosensitivity.[110] Grafting OEG onto polyglutamate derivatives has for instance provided easy access to tunable and versatile thermoresponsive polypeptides. In a representative example, *Li* and co-authors used OEG units to modify the lateral chain of glutamic acid before NCA synthesis (strategy a in Scheme 3) and successfully polymerized these new monomers by ROP to afford poly(*γ*-(oligoethylene glycol)-*L*-glutamate) PEG_xLG (Table 5).[180,181] The authors reported that grafting a single ethylene glycol unit produced non water-soluble polymers, while grafting di- or tri-ethyleneglycol units afforded water-soluble ones. These last polymers presented LCST-type behavior with a T_{CP} of around 32°C and 57°C for $PEG_2LG$and PEG_3LG, respectively, showing that increasing the ethylene glycol side chain length increased the T_{CP}. Nevertheless, the PEG_2LG LCST behavior was not fully reversible and the transition occurred over a broad range of temperatures. The authors attributed this behavior to the secondary structure of the polypeptide backbone which in the case of PEG_2LGshowed a high content of *β*-strand that increased with time and use. In marked contrast, PEG_3LG showed 100% helicity in water over the whole range of temperatures. This hypothesis corroborated the β-sheet secondary structure of the insoluble polymers and copolymers containing EG_1-Glu units. Overall, only the *α*-helical conformation of the polypeptide backbone allowed for a regular OEG shell and solubilization in water. Upon heating, this outer shell collapsed due to the decreasing interaction with the water thus inducing aggregation. The importance of the *α*-helix was further proven by synthesizing a copolymer containing *L*- and *D*- glutamate units that adopted a random coiled disordered state in water with no LCST behavior. OEG containing polypeptides were also used to formulate hydrogels when the ROP was achieved from a PEG block as macroinitiator.[182,183] *Dong and Liao* followed the same synthetic strategy and synthesized PEG_2LG(Table 5) with different polymerization degrees, DP = 29-135.[184] The different polymers presented LCST in the range of T_{CP} = 34 - 36°C, with irreversible liquid crystal transitions above 100°C that have been extensively studied for this class of polymers.[185,186] Interestingly, when molar masses were increased, the polypeptides adopted different morphologies, following the order spherical > micelles > worm-like micelles, a behavior attributable to an *α*-helix to β-sheet transition. However, all polypeptides showed irreversible aggregation after several heating and cooling cycles as the α-helical structures were irreversibly transformed into non-soluble β-sheets.[180]. To counterbalance β-sheet formation, *Klok*'s group prepared brush-like copolymers based on this design. In this case, the thermal behavior of the polymers was retained as the helicity was maintained due to the close packing of the helices.[187] Copolymerization proved to be another good solution to counterbalance β-sheet formation. *Yu* and coauthors showed that introducing alanine units in a molar ratio between 15% and 31% preserved the T_{CP} in a similar temperature range while maintaining the helical structure. (Table 5) These copolymers displayed a slight hysteresis in their thermoresponsive behavior and PEG was also used as macroinitiator to promote thermogelling properties.[188] Copolymerization with leucine monomer units provided similar results. The copolymers possessed a first block of pure PEG_2LG and a second block containing a mixture of leucine and EG_1LG or EG_2LG units. (Table 5) The length and composition of each block as well as the monomer distribution in the second one determined water solubility, LCST and thermogelling behavior.[189]

Chen and co-workers developed libraries of grafted thermoresponsive PGA using another interesting strategy, by clicking azido-OEG onto previously polymerized PPLG to produce

poly(γ-(4-(oligoethylene glycol)-1,2,3-triazol-1-yl)-*L*-glutamate) $PEG_{2-3}TLG$. (Table 5)[190] In contrast to the copolymers produced by *Chen* et al., all the polymers showed α-helical secondary structure independent of the molecular weight and the nature of the grafted OEG. They thus studied the effect of polymerization degree and grafted units' nature on the T_{CP}, obtaining transitions in a range of 22-74°C. The LCST was further tuned by using salts and by changing the polymer concentration. The polypeptide backbones were nontoxic and were susceptible to enzymatic degradation, in contrast to classical LCST polymers. *Heise*'s group also clicked OEG units and saccharides units concomitantly on the pendant chains and reported similar thermoresponsiveness with the glycopolypeptides.[191] (Table 5) Similarly, azido-OEG clicked polymers were developed with slightly longer alkyl chains in the linker.[192] (Table 5) The authors showed that incorporating BLG units in the backbone could be used to tune the LCST behavior. This last study showed that higher BLG content was correlated with less helical structure in the copolymers which induced lower transition temperatures. This is also consistent with the increased hydrophobicity of BLG units compared those bearing OEG.

Thermoresponsive OEG-polypeptides have also been designed by *Tang* and co-authors using poly(γ-4-(propargoxycarbonyl)benzyl-*L*-glutamate) (PPOBLG) or poly(γ-4-(4-propargoxyphenoxycarbonyl)benzyl-*L*-glutamate) (PPOPhBLG) and click chemistry to afford various grafted copolymers bearing OEG_{3-7} (Table 5) and different hydrophobic linking groups. [193,194] Polymers with longer OEG chains were soluble in water and presented LCST behaviors influenced by the hydrophobic nature of the different linkers, the polymer concentration or the addition of salts. The authors also showed that all synthesized polymers displayed UCST behavior in alcohol-water mixtures in a temperature range of T_{CP} = 45-70°C depending on the alcohol type and concentration. The authors further grafted azidohexanal onto the polymer backbone and crosslinked it using 1,6-hexanediamine. This polymer demonstrated stable behavior in the range of pH = 2.0-5.8 with an LCST around 27°C. Due to the labile imine bond, however, when the pH was higher than 6.15 the LCST completely disappeared, and the polymer was completely soluble

Xu and coauthors developed similar charged, helical poly(γ-4-azidomethylbenzyl-*L*-glutamate) based polymers. Similarly to the previous studies, they clicked alkyne-terminated OEG_7 onto the pendant chains to afford thermoresponsive side chains. The results for this polymer were consistent with the other reported polymers, showing the variety of possible methods to induce thermoresponsivity to glutamate-based polymers with OEG side chains. The authors then modified the polymers further by alkylating the triazole ring to form an alkyltriazolium salt. Different alkyl substituents conferred different LCST behavior to the polymers, but only polymers having BF_4^- as counterions showed LCST behavior in aqueous media. (Table 5).[195] The cloud point could be decreased by increasing the polymer concentration or adding $NaBF_4$. To further tune the LCST, the alkyl side chain was elongated, decreasing the transition temperature by adding more hydrophobicity.

Using thiol-yne chemistry *Li* and co-authors reported the use of PPLG and Y-shaped OEG pendants to confer LCST.[196] (Table 5) These polymers retained helical secondary structure at temperatures lower than T_{CP}. Interestingly, polymers lost all thermal behavior when the thiol group was oxidized to a sulfone or even further to a sulfoxide. The same authors further reported multi-responsive copolypeptides by using the copolymer poly(γ-propargyl-*L*-glutamate-*co*-γ-benzyl-*L*-glutamate). Orthogonal deprotection of the benzyl groups conferred pH responsiveness, while thermoresponsive properties were obtained by conjugation of the pendant propargyl group to thiol-terminated triethylene glycol monomethyl ether (OEG_3) (Table 5). The random copolymers showed an LCST influenced by the pH and the overall

charge of the polypeptide: at higher pH, the LCST could not be observed as the carboxylate groups made the copolymer highly soluble.[197] Furthermore, the helical structure of these polymers, when present, persisted for up to 10 heating/cooling cycles. Using thioether linkages, *Li* and co-authors developed a series of LCST-type poly(*γ*-allyl-*L*-glutamate) (PALG) that were functionalized with S-OEG (Table 5).[198] Varying the number of EG repeating units from three to four led to an increase in LCST of 26°C at DP = 125 due to the increase in hydrophilicity. Increasing molar mass also led to a small decrease in LCST: for DP = 68 T_{CP} = 7.6°C, and DP = 125 T_{CP} = 5.5°C. The thioether linkage further allows the polymers to be oxidation responsive. Once oxidized to sulfoxide or sulfone, they lost the LCST feature because of the increased hydrophilicity. *Tang*'s group reported the synthesis of PEG_7TSPOBLG with a longer hydrophobic spacer (Table 5) having an LCST. This thermoresponsive behavior was only weakly dependent on the polymer concentration but was readily tuned with the molecular weight and the oxidation state of the thioether linkage.[199] The same group prepared thermoresponsive polymers based on polyglutamate bearing azobenzene and S-OEG pendant groups PEG_7S^{OX}AzoTPLG.[200] (Table 5) These polymers exhibited UCST type behavior in EtOH/water that could be tuned by light irradiation due to cis-trans isomerization of the azobenzene. The oxidized polymer presented a less pronounced UCST in the solvent mixture and an LCST around 67°C in water at 1%wt concentration but the behavior was not completely reversible.

Following a similar methodological approach, *Heise* and coauthors synthesized block copolymers based on benzyl-*L*-glutamate and *tert*-butyl-*L*-glutamate monomer units. After selective deprotection of the *tert*-butyl groups, the glutamic acid blocks were functionalized with OEG that conferred LCST properties.[201] All the polymers adopted helical structures stabilized by the OEG pendant groups but only the copolymer with the lowest repeating blocks $((BG)_5$-$(EG_{10}LG)_5)_4$ (Table 5), demonstrated an LCST at 60°C.

Y-shaped pendant OEG groups grafted onto poly(γ-4-(allyloxycarbonyl)benzyl-*L*-glutamate) (PAOBLG) backbone also showed LCST behavior close to body temperature.[202] PAOBLG (Table 5) bearing diethyleneglycol units presented an LCST at 37.5°C while increasing the number of EG units led to an increase of around 9°C per unit of the LCST. Interestingly, these Y-shaped decorated polypeptides showed LCST at much higher concentrations than their linear counterparts allowing for finer tuning of the temperatures. As the authors showcased for example, a transition close to body temperature could easily be targeted using these strategies.

Tang and coworkers developed star-shaped thermoresponsive polypeptides. In this design, triblock polymers were prepared from two OEG-bearing glutamate blocks around a central PPG (Table 5) .[203] The copolymers demonstrated LCST behavior, forming nanomaterials at temperatures of 43.7 and 17.6°C for OEG_2 and OEG_4 respectively. Cell viability was demonstrated at 0.5 and 1 mg /mL for OEG_2 and OEG_4 respectively. Also, the polymers were partially degraded by proteinase K after 12 hours as demonstrated by SEC chromatography.

Overall, the use of OEG grafted on glutamate backbones provides tunable thermoresponsive polypeptides. This responsiveness arises from the presence of the side chains: when the OEG chains get dehydrated at higher temperatures, the aggregation is enhanced by the stabilization of water-insoluble secondary structures of the polyglutamate backbone. These thermoresponsive building blocks have been used to formulate copolymers for different applications. For example, surgical adhesives have been developed by copolymerizing EG_2LG NCA with *L*-DOPA, *L*-arginine, *L*-cysteine, and ε-*N*-acryloyl-*L*-lysine based NCAs.[204] Another study used a thermoresponsive block (PEO-b-PPO-b-PEO) instead of the glutamate

monomers to produce similar results.[205] The T_{CP} was in both cases tuned around 37°C and the adhesives have shown promising results *in vivo*.

Other works approach the preparation of functionalized polyglutamate by first synthesizing the functional monomers. *Tang*'s team modified glutamic acid on its side chain with a trithiocarbonate moiety bearing different lengths of OEG. The corresponding NCAs were successfully polymerized to produce the thermoresponsive polymers. (Table 5) These polypeptides showed LCST behavior in the temperature range of 23-55°C with helicity controlled by the backbone length.[206]

Grafting other thermoresponsive groups than OEG has also been investigated. Chen and co-authors developed a PPLG grafted with (2-methoxyethoxy)ethyl-methacrylate (MEO_2MA) or 2-(2-(2- methoxyethoxy)ethoxy)ethyl-methacrylate (MEO_3MA) by click chemistry. (Table 5) The LCST could be tuned through the co-grafting of both MEO_2MA and MEO_3MA with different DPs. Adding more MEO_3MA led to a gradual decrease in the α-helical conformation due to steric hindrance. Yet all copolymers showed LCST behavior in the range of 19.9-40.8°C, showing a linear correlation between the T_{CP} and the MEO_3MA content in physiological saline. [207]

3-Methylthiopropyl glutamate NCAs were also used to prepare LCST polypeptides via post-polymerization grafting.[208] Upon ROP, the polymer was functionalized by alkylation of the methylthio group giving the corresponding sulfonium species ($PMeS^+X^-PLG$) (Table 5). This alkylation led to thermoresponsiveness in alcoholic solutions depending on the counterion. In particular, the authors introduced an alkyne moiety that was further functionalized using CuAAC click chemistry to introduce ethylammonium side chains. (Table 5) This function allowed the polypeptides to be water soluble at pH<7.4 where the ammonium was protonated while at higher pH the polymers showed UCST-type behavior.[208]

Introducing imidazolium side chains also led to thermoresponsive behavior. For this purpose, the same group polymerized (chloropropoxycarbonyl)-benzyl-*L*-glutamate NCA and subsequently substituted the chlorine with an azide group.[209] The latter was easily clicked with 3-butyl-1-propargylimidazolium bromide to obtain the final polypeptide (Table 5). Changing the counteranion produced UCST behavior in both EtOH and water. It was also possible to start similar synthesis from poly(allyl-*L*-glutamate) by modifying it to poly(γ-2,3-dibromopropyl-*L*-glutamate) by bromination of the allyl.[210] The bromide units could be transformed into azides that were further clicked with moieties bearing the imidazolium units (Table 5), The resulting doubly-functionalized polymers had transition temperatures 5°C lower than their mono-functionalized counterparts.

A thermo- and light-responsive modified polyglutamate was developed by adding 1-butylimidazolium pendant groups to a triethylene glycol/azobenzene side chain. The resulting polypeptides still exhibited UCST in ethanol and water/ethanol mixture, while one polymer exhibited UCST in aqueous NaI solution. The transition temperature of the produced $PPTAzoBuIm^+X^-LG$ (Table 5) increased with increasing NaI concentration in the range of 10°C to 90°C.[211] Incorporating this polymer in hydrogels allowed for better drug release, which could be tuned by temperature and UV irradiation of the azobenzene linkage.

Tang and coworkers also tested other functional groups: adding amide functions produced UCST behavior via the formation of hydrogen bonding networks.[212] Similarly, the addition of azidoalkanes was at the origin of UCST behaviors in alcohols and water/ethanol mixtures . (Table 5)[213] All these polypeptides adopted helical structures while some of the results

obtained with these classes of polypeptides suggested possible applications in the formulation of hydrogels for drug delivery.[214,215]

In another study, grafting γ-4-chloromethylbenzyl-*L*-glutamate with imidazolium salts produced many different UCST-type polymers. By changing the alkyl group of the imidazolium moiety and the counter ion, the UCST could be tuned from 25°C to 55°C. All polymers showed a helical structure at all temperatures. The choice of the counter anion and the hydrophobic side chain thus played the major role in stabilizing the interaction with water and the phase transition. Poly(γ-4-chloro-methylbenzyl-*L*-glutamate) (PCMeBLG) was also functionnalized with 1-butylimidazolium-X^- ($PBuIm^+X^-$MeBLG) (Table 5) pendant groups to exhibit UCST behavior.[216] These polymers showed highly helical structures that were not disrupted by changes in temperature. In a subsequent study they obtained similar results with pendant pyridinium groups.[217] (Table 5) Only pyridinium groups with methyl substituents and in the presence of BF_4^- exhibited this behavior in both MeOH and water. The presence and the position of the methyl on the aromatic ring provided new leverage to modify the UCST. In another study, the same authors showcased similar results by functionalizing poly(γ-6-chlorohexyl-*L*-glutamate) to give $PPTBuIm^+BF_4^-$LG . (Table 5)[218]

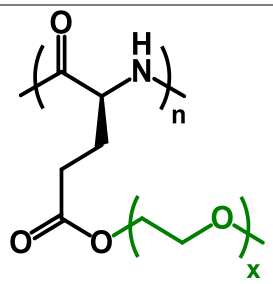
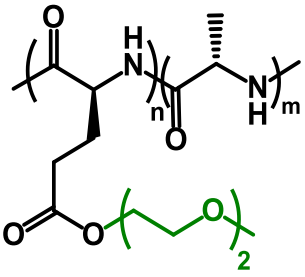
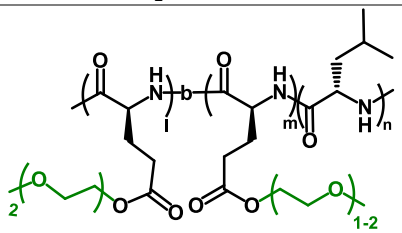
The same group showed the possibility of conferring antibacterial and UCST activity on the same backbone.[199] Modified PBLG having both a thioether bond and chlorine endgroup was used to produce a polypeptide with pendant imidazolium groups. Helical $PBuIm^+BF_4^-$ SPOBLG (Table 5) demonstrated a UCST close to the body temperature and inhibited growth of *S. aureus* (6 mg/mL) thanks to the hydrophobic spacer combined with the ionic moieties present. The UCST could be tuned through oxidation of the thioether by the addition of H_2O_2 while maintaining the antibacterial activity.

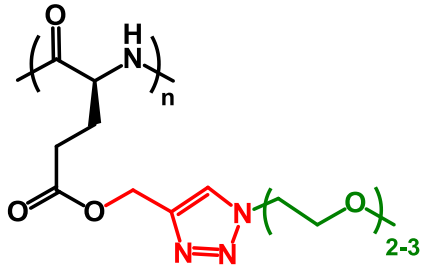
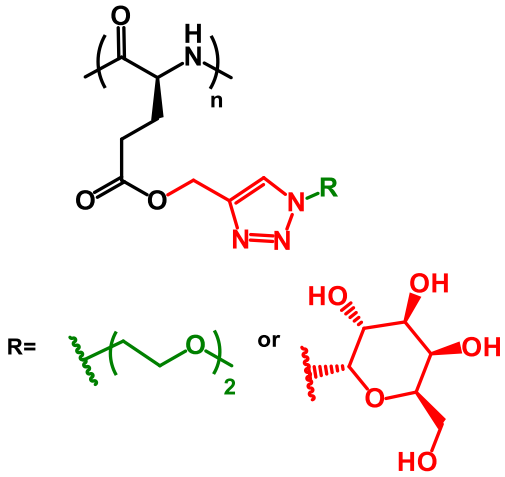
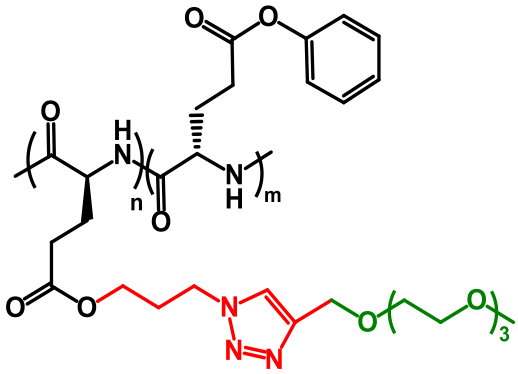
Poly(γ-propargyl-*L*-glutamate) has also been coupled with less common moieties such as (dialkylamino)alkyl substituents. Polymers with an ethyl linker and bearing (diethylamino)ethyl (DEA), (diisopropylamino)ethyl (DPA) (cyclopentylamino)ethyl (PR), (cyclohexylamino)ethyl (PD) and (cycloheptylamino)ethyl (HEA) side chains showed dual thermo- and pH-responsiveness . (Table 5)[219] These polymers bearing more hydrophobic moieties underwent aggregation when the pH was increased due to the ammonium group deprotonation of the side chain. Thermal aggregation was only observed in the case of the more hydrophobic side chains. Thus, the polymers with the most hydrophilic side chains were neither pH nor thermoresponsive and stayed soluble in all the studied conditions. This was counterbalanced by using more hydrophobic (longer or bulkier) linkers than the ethyl group: PGA bearing (dimethylamino)isopropyl (ipDMA) and (dimethylamino)-2-methylpropyl (mpDMA) on its side chain showed dual responsiveness while their ethyl linker counterparts didn't. These pH responsive polymers showed an LCST at specific pH values. The T_{CP} was increased by decreasing the pH, whilst keeping the value between the pKa and the pH transition point. This proved harder for more hydrophobic moieties such as DPA and HEA with a very narrow usable pH range. Such findings exploring different side chains while changing the linker lengths, the hydrophobicity, the number of carbons, and their dispositions provided a wide range of thermoresponsive behavior. The same group further developed thermogelling polymers by coupling the polypeptide to a PEG block, [220] and γ-PGA also produced an LCST.[221] Yet doing so with the α-glutamate produced unexpected UCST behavior.[222] The authors partially functionalized PGA with different amines. (Table 5) The remaining free carboxylic groups and the newly formed amide bonds on the side chains created a hydrogen bond network that was disrupted at higher temperatures. The phenomena showed hysteresis during the cooling part. These organized aggregates at lower temperatures were favored by the

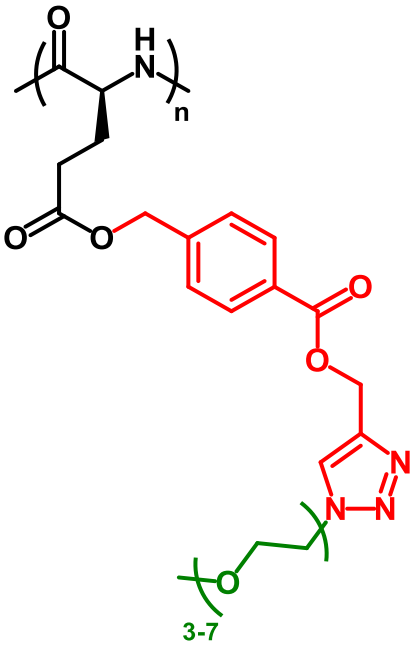
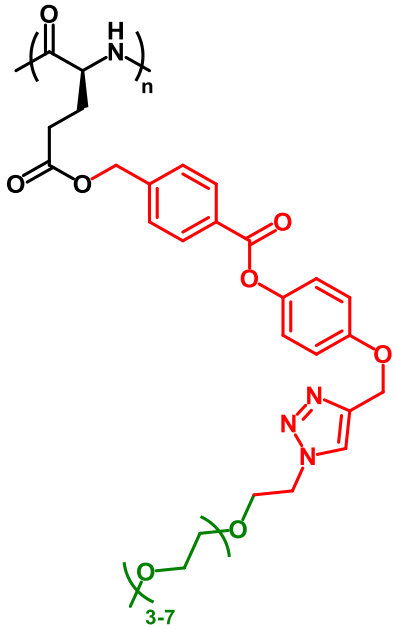
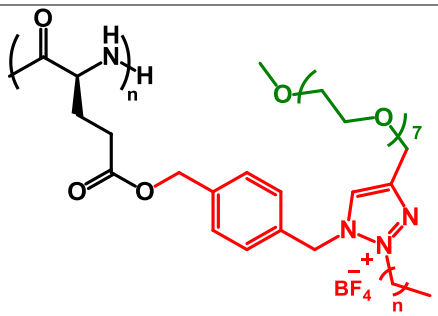
α-helical structure of the copolymers. This was corroborated by the absence of thermal behavior for racemic analogues.[222]

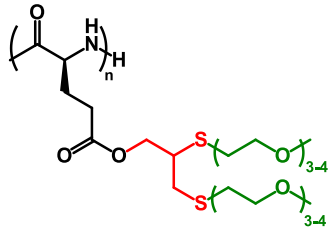
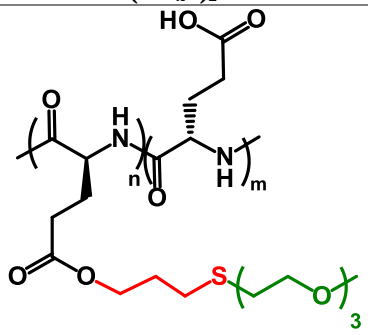
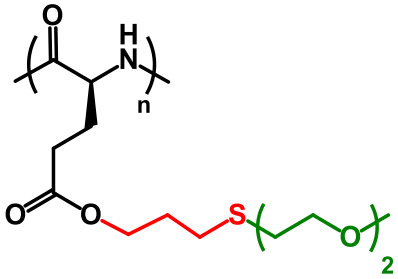
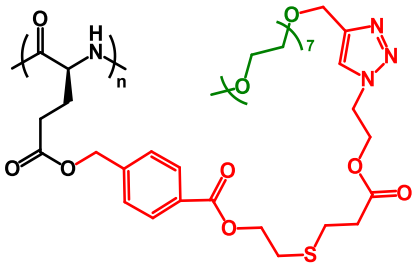
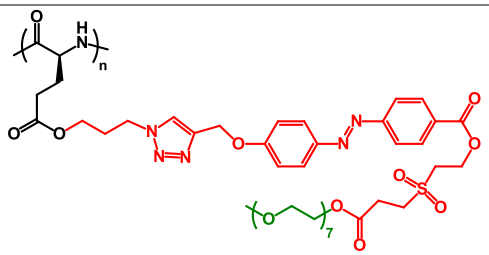
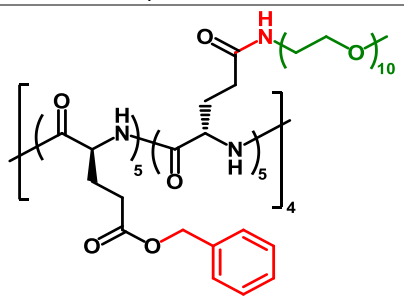
Another original thermoresponsive moiety showed UCST behavior in EtOH/water mixtures when introduced on glutamate side chain. By incorporating iodide as a counterion to a triazolium moiety bearing a hydrophobic group, the resulting polypeptide exhibited thermoresponsiveness . (Table 5)[223] Introducing mannopyranoside and/or biphenyl pendant groups had similar effect . (Table 5)[224] These glycopolypeptides showed UCST behavior in higher water contents than the previously discussed counterparts. Finally, another group of polymers based on polyglutamate backbone displayed both UCST and LCST. *Tang*'s group grafted polyglutamate with diethylene glycol and tributyl phosphonium iodide PBu3P+X-DEGLG . (Table 5)[225] The polymer with DP = 80 showed an LCST near 42°C and UCST behavior above that temperature in aqueous solutions of 2 mg/mL and 0.01 mol/L NaI. Also, they observed that a shorter polymer (DP = 48) showed an LCST of 32°C, a difference of 10°C in comparison to DP 80 at the same conditions. They further developed a large set of polymers displaying different thermoresponsive behavior depending on the linker, the counter ion and the phosphonium substituents (Table 5). [225]

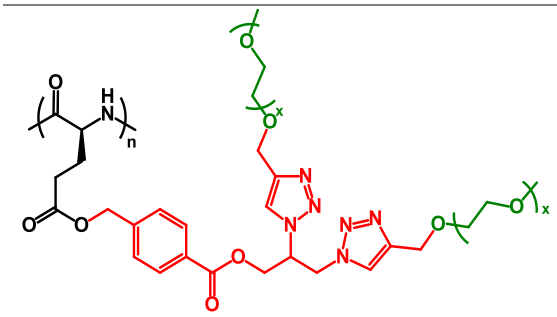
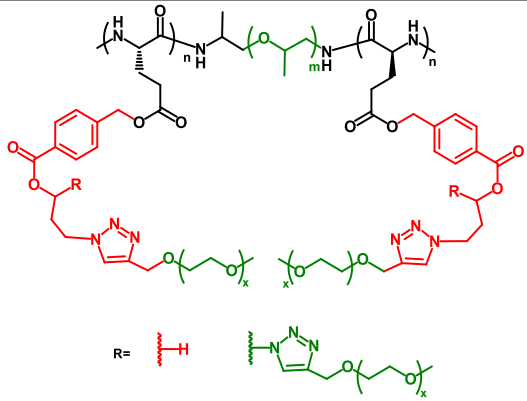
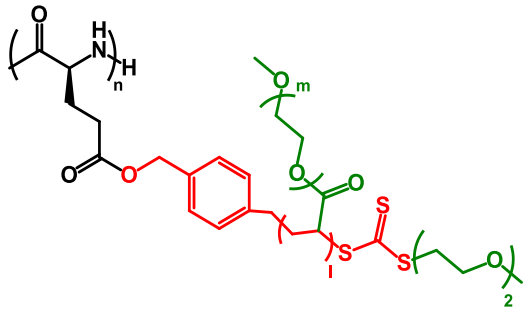
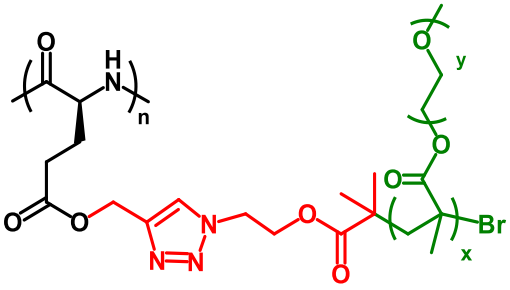
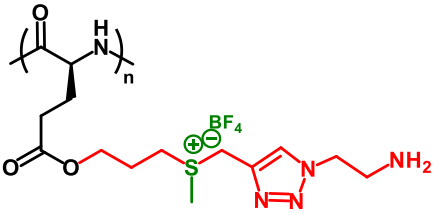
-TABLE 5 – Structures and thermoresponsive properties of polyglutamate based polypeptides with thermoresponsive side chain modifications

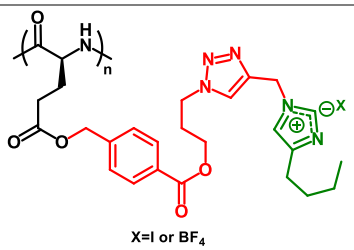
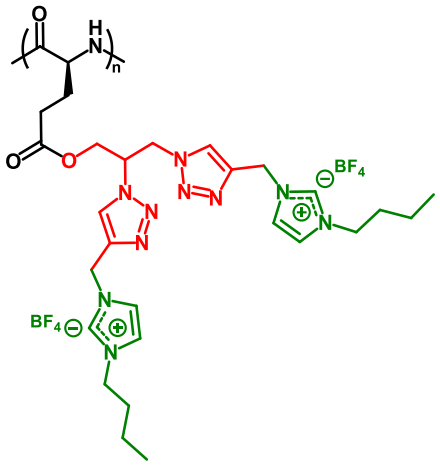
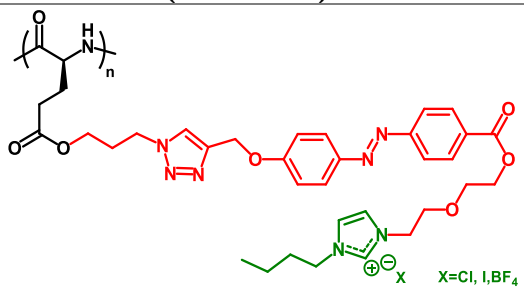
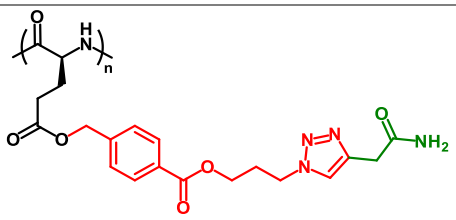
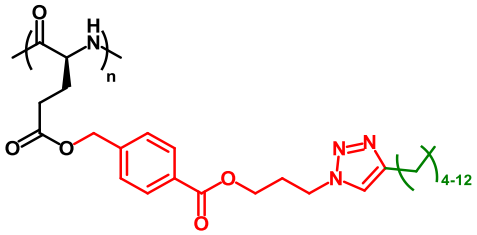
Polymer structure (in green: thermoresponsive modification, in black polypeptide polymer and in red linkers and/or modifications	**LCST T_{CP} (°C) and conditions**	**UCST T_{CU} (°C) and conditions**	**Ref**
 **PEG_xLG**	32°C – 57°C depending on EG length	-	[180, 181]
	34°C – 36°C x=2, depending on Mw	-	[184]
 **PEG_2LG-*co*-PAla**	27°C – 52°C depending on overall Mw and n:m ratio	-	[188]
 **PEG_2LG-*b*-$PEG_{1-2}LG$-*co*-PLeu**	20°C – 40°C depending on Mw and copolymer composition and structure	-	[189]

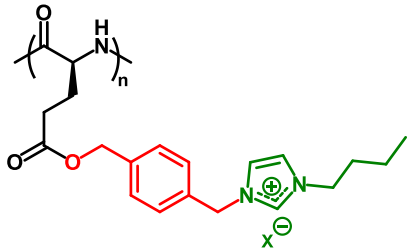
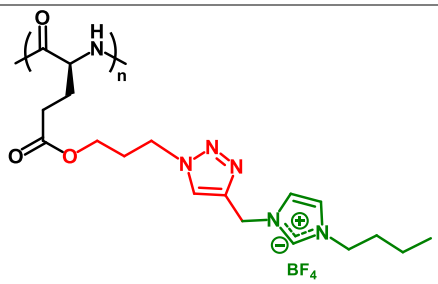
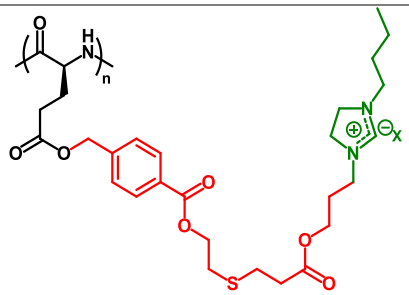
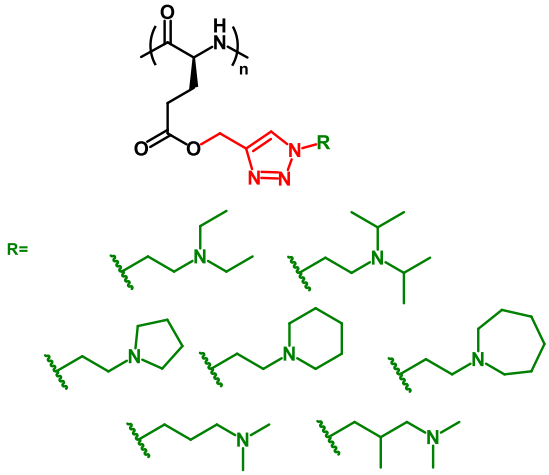
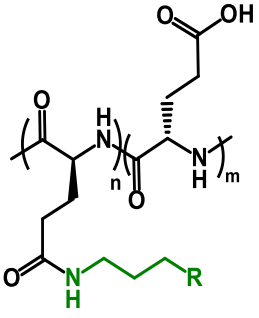
 **PEG$_{2\text{-}3}$TMe**LG	22°C – 72°C depending on Mw and concentration	-	[190]
 **PEG$_{2\text{-}3}$TMeLG-*co*-PTGalTMeLG**	18°C – 85°C depending on galactose content	-	[191]
 **PEG$_3$TPLG-*co*-PBLG**	22°C – 53°C depending on BLG content	-	[192]

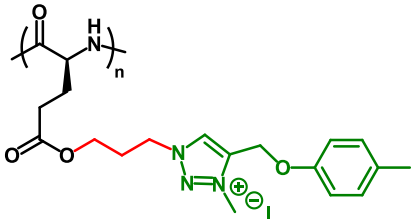
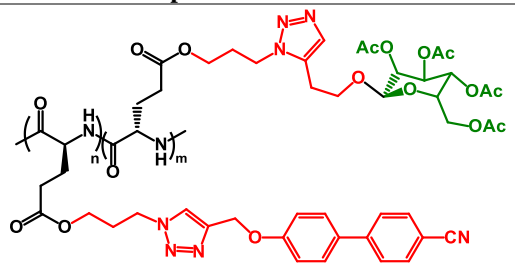
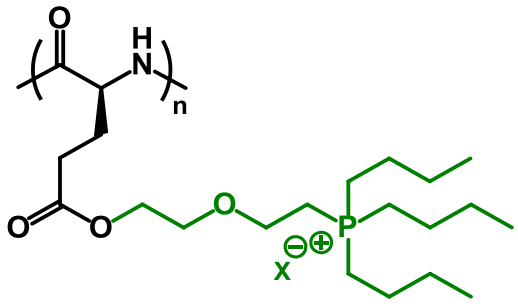
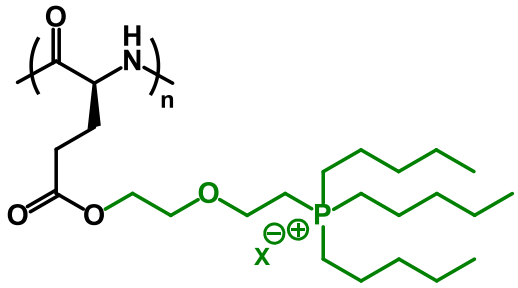
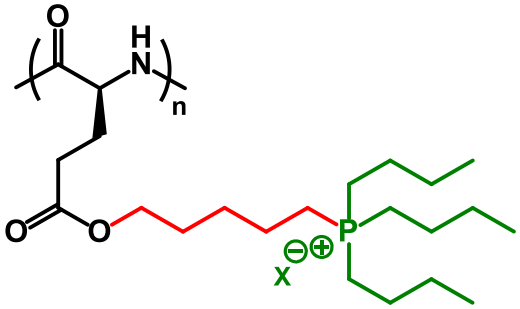
 **PEG$_x$TPOBLG**	10°C – 45°C depending on pH	-	[193, 194]
 **PEG$_x$TPOPhBLG**	35°C – 70°C depending on salt, and alcohol concentration	In alcohol/water mixtures 40°C - 85°C	[194]
 **PEG$_7$TAlk$^+$BF$_4^-$MeBLG**	40°C – 75°C depending on concentration, salt, and alkyl chain	-	[195]

 **P(EG$_x$S)$_2$LG**	20°C – 48°C depending on Mw and OEG length	-	[196]
 **PGA-*co*-PEG$_x$SPLG**	25°C – 55°C depending on pH	-	[197]
 **PEG$_2$SPLG**	10°C – 49°C	-	[198]
 **PEG$_7$TSPOBLG**	15°C – 60°C depending on oxidation degree of the thiol group	-	[199]
 **PEG$_7$S^{OX}AzoTPLG**	67°C irreversible	5°C - 10°C in EtOH/H_2O	[200]
	60°C	-	[201]

$((BG)_5$-$(EG_{10}LG)_5)_4$			
 **$P(TEGx)_2POBLG$**	37.5°C	-	[202]
 **$PEG_xTPOBLG$-*b*-PPrOH-*b*-$PEG_xTPOBLG$**	43.7°C		[203]
 **PEG_2S_3MeBLG**	23°C – 55°C depending on l,m and n values	-	[206]
 **$P(MEO_{2\text{-}3}MA)_nTMeLG$**	19.9°C - 40.8°C depending on Mw	-	[207]
 **$PNTMeS^+BF_4^-PLG$**	-	10°C – 60°C pH=7.42 – 7.80	[208]

 **PBuIm⁺X⁻TPOBLG**	-	5°C – 45°C depending on the Mw and counterion	[209]
 **P(BuIm⁺BF₄⁻T)₂LG**	-	15°C – 30°C depending on concentration	[210]
 **PBuIm⁺X⁻AzoTPLG**	-	in EtOH/H_2O and in aqueous conditions 10°C – 90°C depending on concentration and counterion	[211]
 **PAmTPOBLG**	-	25°C – 55°C depending on functionality and concentration	[212]
 **PAlkTPPOBLG**	-	in EtOH/H_2O 40°C – 75°C depending on alkyl chain and water content	[213]

 **PBuIm$^+$X$^-$MeBLG**	-	40°C – 70°C depending on counterion	[216]
 **PBuIm$^+$BF$_4^-$TPLG**	-	21°C – 35°C depending on concentration	[218]
 **PBuIm$^+$BF$_4^-$ SPOBLG**	-	35°C – 70°C depending on concentration and counterion	[199]
 **PAlkAmTMeLG**	17°C – 65°C depending on pH and sidechain modification	-	[219]
 **PAlkLGAm-*co*-PGA**	-	25°C – 50°C depending on copolymer composition and pH	[222]

 **PpTolTMe$^+$I$^-$PLG**	-	in EtOH/H_2O 10°C – 45°C depending on Mw and concentration	[223]
 **PbiPhTPLG-*co*-PManTPLG**	-	in EtOH/H_2O and MeOH/H_2O 10°C – 50°C depending on copolymer composition and concentration	[224]
 **PBu$_3$P$^+$X$^-$DEGLG**	-	X=I in H_2O 30°C – 40°C < T_{CU} if X=BF_4 only T_{CU} = 10°C – 90°C depending on the molecular weight and concentrations of polymer and salts, T_{CP} and T_{CU} increase with Mw	
 **PPe$_3$P$^+$X$^-$DEGLG**	-	X=I or BF_4 in PBS T_{CU} = 20°C – 65°C	[225]
 **PBu$_3$P$^+$X$^-$PeLG**	-	X=I or BF_4 in PBS T_{CU} = 65°C – 90°C	

Glutamate-based polypeptides present a wide variety of approaches to introduce thermosensitivity. The discussion in this review and Table 5 illustrates this point well showing LCST and UCST behavior, sometimes with the same polymer and with transition temperatures ranging from 10°C to 90°C. Side chain substituents and the surrounding environment play a major role in the behavior, and solvent type, concentration, and ionic strength are parameters that contribute greatly to the thermosensitivity. These biocompatible thermoresistant polymers

have shown promise for biomedical applications.[226] Besides polyglutamates, other polypeptide backbones bearing reactive side chains have also been studied intensively, as presented in the following paragraphs.

5.1.2 Polylysine

The amine groups on the side chains of poly(*L*-lysine) (PLys) provide another approach towards thermoresponsive polypeptides. *Tsitsilianis* and coauthors have for instance used the aza-Michael addition reaction to graft *N*-isopropylacrylamide monomer units on the pendant amines of PLys. (Table 6) The grafting density was tuned to retain some unprotected free amines that give the polymers pH-responsiveness. Low levels of grafting (around 29%mol) induced an LCST at a temperature around 54°C, but when heated further resulted in a gel due to an α-helix to β-sheet transition of PLys. Increasing the NIPAM ratio to 69% mol decreased extensively the LCST to a temperature of 32°C at pH levels from 5 to 11 (close to that of homo-PNIPAM) and no gel was formed due to disruption of the PLys secondary structure. Adding further NIPAM substituents further decreased the transition temperature. At lower pH the amines were completely protonated and no LCST was observed due to the increased hydrophilicity of the charged moieties. This could be counterbalanced by the addition of salts, and a cloud point was observed at pH as low as 8.8 in the presence of 2 M NaCl. [227]

Another example involving PLys backbone involved the polymerization of (*Boc*-NH-oxyacetoacetate)-Lys-NCA. After deprotection, the polymer was grafted by imine linkage of (3,4,5-oligoethyleneoxide) benzaldehyde with first- and second-generation dendrimers. (Table 6) The polymers demonstrated LCST similar to that of the dendrimers alone in the range of 31-36°C. The LCST was further decreased by increasing the length of the polypeptide chains whilst keeping a similar dendrimer content. Grafting the dendrimers to the side chain amines of the polypeptides allowed α-helical secondary structuring even at pH levels < pKa where normally PLys is in a random coil conformation. The dehydration of the dendrimers above the T_{CP} induced the helicity as seen by circular dichroism. These thermoresponsive copolymers were further modified on the lysine side chain with phenylboronic acid capable of reacting with catechol moieties. This thermo-switchable detector was applied to the detection of bioactive dopamine.[228]

5.1.3 Polycysteine

Li et al. developed a thermoresponsive polymer based on polycysteine with $OEG_{2\text{-}4}$-disulfide bond side chain. PEG_xSSCys predominantly adopted β-sheet conformations. Upon heating, these polymers formed aggregates that were not soluble upon cooling the samples, conferring an irreversible LCST behavior. Adding DMF to the aggregated solutions only swelled the aggregates suggesting that chemical crosslinking through intermolecular disulfide interchange had taken place. Using a $PEG\text{-}NH_2$ macroinitiator to polymerize EG_4SSCys NCA gave an irreversibly thermogelling polymer in dilute solution[229] However, introducing thioether linkages led to reversible LCST behavior and oxidative responsiveness as demonstrated by implementing the ROP of S-(2-(2-methoxyethoxy)ethoxy) iso-butyrate-*L*-cysteine using a $PEG_{45}\text{-}NH_2$ macroinitiator.[230][231] The LCST behavior was influenced by the concentration as well as the oxidation state of the thioether linkage and high molar mass polymers were insoluble in water unless oxidized to sulfoxide or sulfones. While lower molecular weight polymers saw their T_{CP} increase with increasing oxidation, no LCST was observed with the high molar masses upon oxidation. Oxidation shifted the secondary structure to more random conformations and this behavior was used to create oxidation-responsive micelles.[230][231]

Deming and Kramer also investigated the thermoresponsive behavior of other poly(S-EG_4-*L*-homocysteine). The group synthesized the modified NCAs and used them to produce homopolypeptides. (Table 6) The polypeptides remained in a helical form indicating that the ethylene glycol units induced the thermal stimuli response.[232] Using a different strategy, the same group developed a series of thermoresponsive polypeptides based on homopoly(cysteine) that was further alkylated with oligoethylene glycol and then demethylated.[233] (Table 6) During this study the group was able to finely tune the LCST by changing the end chain moieties of the OEG side chain as well as the linking groups to the backbone. Using more hydrophilic moieties increased the aggregation temperature while oxidation completely solubilized the polymers. The same authors also noticed that the length of the ethylene glycol side chains promoted an increase of the LCST by around 20°C per EG residue.

Similarly to the imidazolium bearing glutamates discussed earlier, [209,210] cysteines decorated with such moieties showed UCST behavior. (Table 6)[234] The strategy used was similar to the one discussed in section 6.1.1. Finally, trialkylphosphonium groups have also been added to polycysteine side chains. In this case only UCST behavior was observed in contrast to polyglutamate backbones(see 6.1.1).[235] PCys bearing bromine was functionalized with triphenyl phosphine producing the charged polymer. (Table 6) UCST behavior was observed in the presence of certain anions that aggregated the polymer at low temperatures.

5.1.4 Other examples

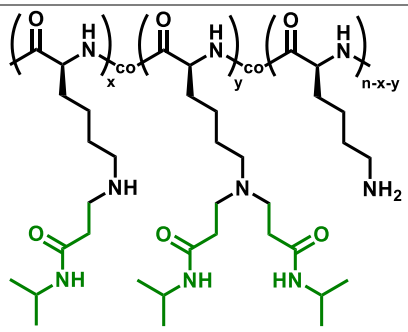
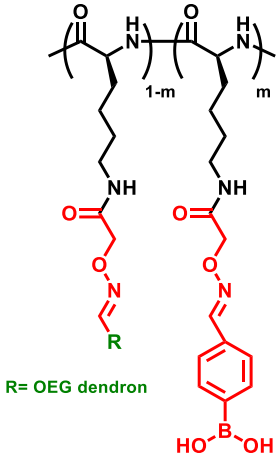
More indirect approaches to stimuli-responsive thiol-bearing polypeptides have also been developed. *Deming* and coauthors first modified the side chain of polyallylglycine by combining epoxidation with ring-opening by 2-(2-methoxyethoxy)ethanethiol to form a thioether linkage. The resulting polymer had an alpha helical structure and exhibited an LCST with a cloud point of 40°C. Other water-soluble thiols were also used to functionalize the polymer. Most of the resulting polymers showed water solubility and helical conformation but only 2-(2-Methoxyethoxy)ethane-1-thiol modified poly(5,6-epoxy-L-norleucine), (PEG_2SEnLeu) was thermoresponsive. (Table 6) This suggested that the temperature-driven transition depended not only on the helical form but also on the nature of the modification. Other OEG groups did not allow for the same thermoresponsive behavior, revealing very little flexibility in this class of polymer. Oxidizing the thioether linkage to a sulfoxide decreased considerably its helical form, and broke the thermoresponsive behavior.[236]

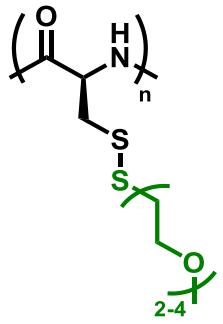
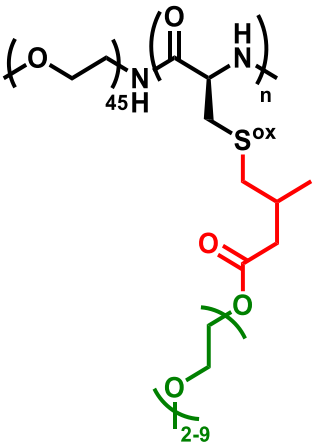
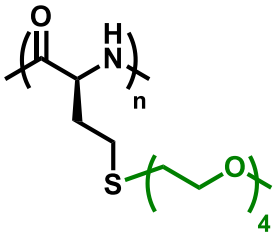
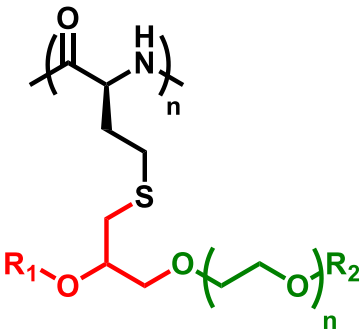
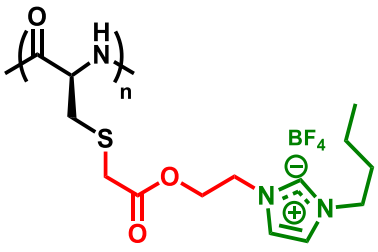
Methionine NCA also provided an interesting approach in this context. Upon ROP, *Mandal et al.* induced the cationic form of polymethionine (PMet) by reaction of 2-nitrobenzyl bromide at the thioether linkage. (Table 6)[237] By changing the counterions, the polypeptides exhibited UCST behavior that was influenced by the concentration as well as the nature of the salts. These cationic polymers were bound to DNA and were further photoreleased via light induced cleavage of the 2-nitrobenzyl moiety.[237] *Ouchi* et al. synthesized a polymer constituted of *α*-aspartate-glycolic acid through bulk polymerization. The resulting polydepsipeptides were then alkylated with *N*-isopropylamine by amide formation on the side chain of the aspartic acid. (Table 6)[238] The polymers possessed LCST behavior at a temperature of 29°C (5%wt) due to the introduction of hydrophobic *N*-isopropylamine as pendant groups that helped to regulate the hydrophobic/hydrophilic balance.

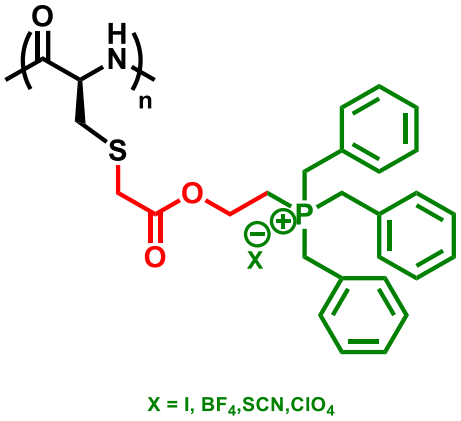
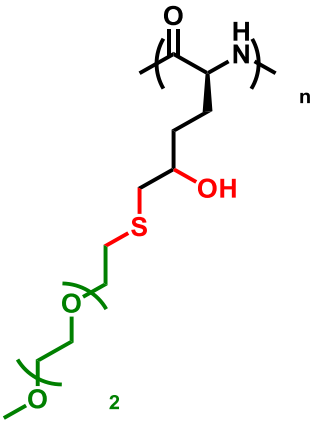
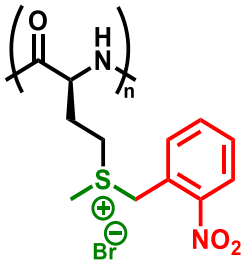
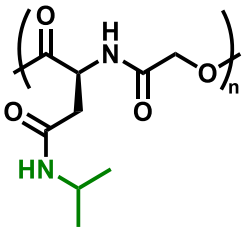
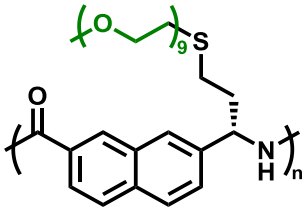
As other example of peptidomimetic structure, *Onitsuka* and coauthors investigated the thermoresponsiveness of a polypeptide made of non-natural amino acids containing aromatic spacers between peptide bonds (forming what is known as arylopeptides). These unusual peptides displayed both soluble and non-soluble helical secondary structures and thus promoted a LCST behavior. [239]

Finally, we mention here a specific approach to impart a classical polypeptide with thermoresponsiveness. *Deming* and co-workers developed a block copolypeptide with LCST behavior when mixed with metal complexes to achieve nano objects with magnetic properties.[240] Complexes of $[Fe^{II}(p\text{- }pi)_2(NCS)_2]$ with a copolypeptide based on glutamate and leucine ($PGlu_{254}$-*b*-$PLeu_7$) demonstrated LCST behavior at 60°C. Temperatures below 60°C promoted solubilization and formation of the low-spin-state iron (II) complex while higher temperatures decreased solubility and promoted the transition to high-spin complexes. This work showed that hydrophobic additives can afford LCST in a polymer that does not present it usually.

-TABLE 6 – Structures and thermoresponsive properties of different polypeptides with thermoresponsive side chain modifications based on different amino-acids. The amino acid constituting the backbone is indicated.

Polymer structure (in green: thermoresponsive modification, in black polypeptide polymer and in red linkers and/or modifications	**Amino acid**	**LCST T_{CP} (°C)/UCST T_{CU} (°C) and conditions**	**Ref**
 **P(NIPAM)Lys**	lysine	LCST 18°C – 65°C depending on copolymer composition and and pH)	[227]
 **PEG_xLys dendronized**	lysine	31°C - 36°C depending on Mw	[228]

 **PEG$_x$SSCys**	cysteine	LCST 40°C – 50°C depending on EG length. Irreversible	[229]
 **PEG-*b*-PEG$_x$*iso*BuCys**	cysteine	LCST 45°C – 70°C Depending on the oxidation state and OEG length	[230,231]
 **PEG4hCys**	homocysteine	LCST 22°C – 52°C depending on salt concentration	[232]
 **R1, 2 = H, Met, Ac, Et** **PEGxhCys**	homocysteine	LCST 20°C – 80°C depending on EG length and oxidation degree	[233]
 **PBuIm$^+$BF$_4^-$Cys**	cysteine	UCST 5°C – 45°C depending on salt type and concentration	[234]

 **$PPPh_3$Cys**	cysteine	UCST 25°C – 60°C depending on anion	[235]
 **PEG_2SEnLeu**	homoallylglycine	LCST 40°C	[236]
 **PNBMeS$^+$Br$^-$Met**	methionine	UCST 21°C – 60°C depending on concentration	[237]
 **P(isoPrAsn-*alt*-Glc)**	aspartic acid	LCST 27°C	[238]
 **Oligoetherated arylopeptide**	2,6-naphthalene methionine	LCST 60°C	[239]

Section 6.1 provides examples illustrating the endless possibilities that chemical modification of side chains can bring to polypeptides to make them thermosensitive. Based on these pioneering works, one can imagine many designs to introduce sufficient hydrophobicity and control to limit secondary structuring, two key parameters also found with IDPs that make them thermoresponsive in living systems. Regarding polypeptides derived from ROP, most methodologies that have been developed involve numerous steps and tedious chemical modifications and purifications. Like IDPs, thermosensitivity from the polypeptide backbone of unmodified amino acids is also possible and is discussed in the last part of this review.

5.2 Thermoresponsiveness induced by *N*-alkylation of polypeptide backbone and inherently thermoresponsive polypeptides.

Moving again one step closer to thermosensitive proteins such as IDPs, another strategy has been to design polymers whose main chain actively participates in the phase separation. In this context, one family of glycine-derived polymers stands out, the polypeptoids. These polymers are the topic of the first section of this last part of the review. In the second part, polymers prepared from natural monomers that can achieve phase separation are also reviewed. This includes proline-based polymers and the use of NCA copolymerization as a means of mimicking the distribution introduced by the natural protein sequence.

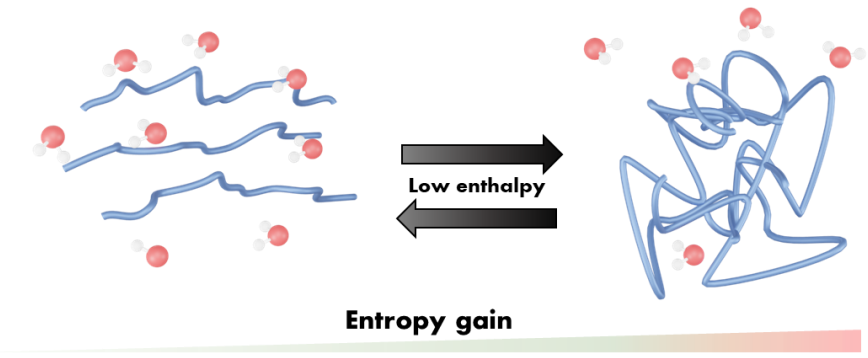


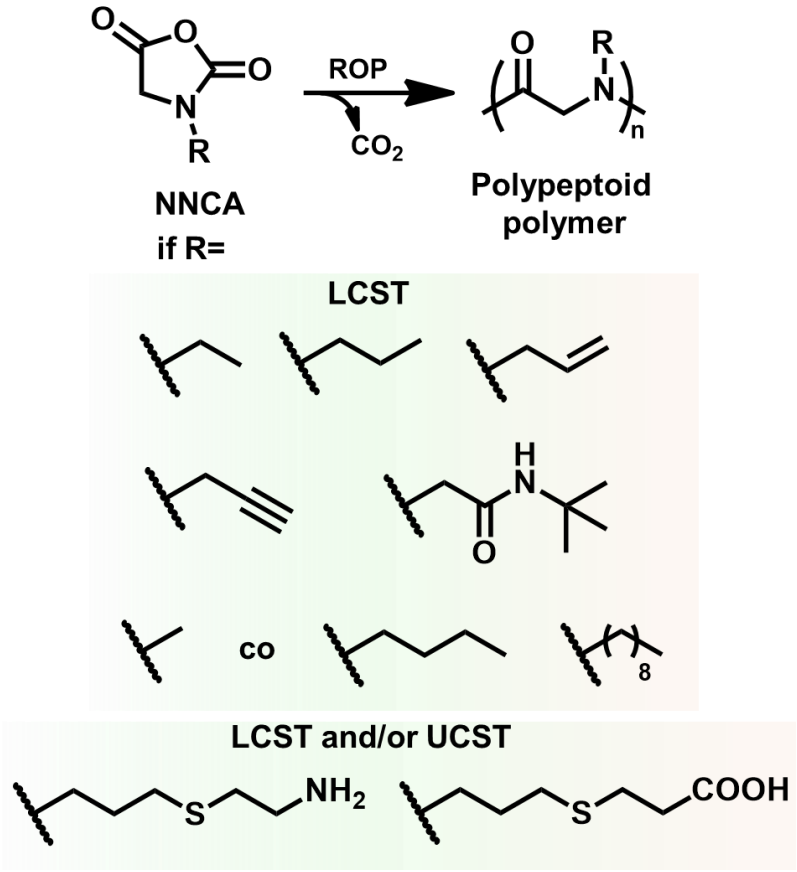
Fig. 9. Amino-acid based polymers (blue coils) water (red and white balls) solvation displaying intrinsic LCST/UCST behavior. An increase in entropy drives the dehydration of the polypeptide into aggregates that is reversible as the polymer chains are not strongly stabilized by enthalpic contribution (like in IDPs).

5.2.1 Polypeptoids and other peptidomimetic polymers

Peptoid polymers, also called poly(*N*-substituted-glycines) or polypeptoids are *N*-alkylated analogs of synthetic polypeptide polymers (Scheme 4): they are polypeptides with an *N*-substituted amide bond. The physicochemical properties of polypeptoids can be tailored by *N*-alkylation, enabling control over (1) the hydrophilic to lipophilic balance,[241] (2) the charge characteristics,[242] (3) the backbone conformation,[243] (4) the solubility,[244] and (5) the thermal and crystallization properties.[245,246] The poly(*N*-alkylated glycine) backbone can be prepared by ROP of *N*-alkylated-*N*-carboxyanhydrides (NNCA).[247] Peptoid polymers are known to exhibit UCST or LCST depending on their structure and their environment. The thermoresponsive behavior can be tuned by varying the molar mass, architecture, concentration, and composition. The presence of a tertiary amide in the backbone is the key reason explaining their innate thermoresponsiveness in comparison to their non-

alkylated counterparts and this behavior stems from the cis-trans interplay of the peptoid backbone.

To better understand the thermoresponsiveness, the influence of *N*-alkylation using various aliphatic side chains was investigated by *Schlaad* and coauthors. They prepared a series of polypeptoids based on *N*-substituted glycines with alkyl side chains containing 1 to 4 carbons: (Table 7) *N*-methylglycine (NMeG), *N*-ethylglycine (NEtG), *N*-propylglycine (NPrG), *N*-allylglycine (NAlG), *N*-isopropylglycine (NiPrG), *N*-propargylglycine (NPgG), *N*-butyl (NBuG) and *N*-isobutylglycine (NiBuG).[248] Interestingly, poly(NMeG) and poly(NEtG) were soluble in water even at 40% concentration whereas the polymers with C4 side chains (PNPgG, PNBuG and PNiBuG) were insoluble in water. Polymers with C3 side chains showed cloud point temperatures that increased in this manner *N*-propyl (T_{CP} = 15−25°C) < *N*-allyl (T_{CP} = 27-54°C) < *N*-isopropyl (T_{CP} = 47−58°C). The authors also observed a significant decrease in the T_{CP} of PNAlG as the concentration was increased. This was attributed to a better hydration of the shorter chains compared to the hydration of the end group.



Scheme 4. Schematic representation of the ring opening polymerization of N-alkylated N-carboxyanhydrides (NNCA) to obtain polypeptoid polymers. The choice of the side chain (R group) can confer thermoresponsive behavior innate to the polypeptoid backbone. Other thermoresponsive moieties can be added to confer the property to polypeptoid and are also illustrated in the following section.

As for the previous strategies developed with polypeptides, peptoid polymers have been also functionalized with thermoresponsive moieties to better control the thermoresponsiveness. *Sun* and coauthors for instance investigated the influence of thio-diethylene glycol(S-EG_2) or thio-triethylene glycol (S-EG_3) *N*-alkylated side chains introduced onto a poly(propargylglycine) backbone through thiol-yne grafting . (Table 7)[249] Interestingly, the

T_{CP} increased with molar mass for both -EG_2 and -EG_3 modified polymers. The same authors also found that NaCl did not influence the T_{CP} but increasing the polymer concentration slightly decreased the T_{CP} from 26°C (1 mg/mL) to 22°C (2 mg/mL). However, no further change was observed even at 8 mg/mL. The influence of the *N*-alkylated side chain was also studied in a design of peptoid polymers containing charged groups. For that study, *Sun* and coauthors prepared polymers bearing (-NH_2) or (-COOH) moieties that were grafted onto PNAlG and PNPgG backbone by thiol-ene/yne chemistry using cysteamine (-NH_2 groups) or mercaptoacetic acid (-COOH groups) . (Table 7)[250] The COOH containing polymers showed UCST behavior while the NH_2 containing polymers presented LCST behavior. In both cases, the thermoresponsiveness was influenced by the pH. The COOH containing polymers presented UCST behavior with T_{CP} in the temperature range of 30 - 65°C at pH = 4.2, and at a concentration of 0.5 mg/mL. The UCST was tunable by modulating the number of COOH moieties, as polymers with one or two carboxylic acids on their *N*-subsitution presented for the same molar mass a T_{CP} of 30 or 65°C, respectively. This shed light on the role of −COOH induced interaction with water molecules as confirmed in the study by monitoring the band at ~3400 cm^{-1}(*ν*OH) using FTIR. This band was significantly reduced at higher temperature, revealing the appearance of hydrogen bonding between the polymer and the aqueous solvent. On the other hand, PNAlG having an amine on its side chain displayed LCST behavior at pH = 13.2 with T_{CP} between 25 and 54°C and at higher DP the T_{CP} was decreased. For both polymers (NH_2 and COOH) the LCST or UCST was only observed in a small pH window where the ionizable moieties are neutral. Another study of the same group used similar click grafting to modify polypeptoids using thiolated groups to prepare PNPgG functionalized with 80% of S-EG_2 and 20% of S-EG_3 . (Table 7) At this specific ratio, the copolymers displayed LCST behavior at T_{CP} = 36.5°C, a convenient temperature for further biomedical applications.[251] The authors also studied the redox response by oxidizing the thioether bond into sulfoxide or sulfone using H_2O_2. It was found that the polymers lost their LCST behavior by becoming more water soluble, and this behavior was reversible upon reduction with thioglycolic acid as T_{CP} = 50°C and 64°C were found, after one or two heating-cooling cycles, respectively.

In another work, *Ling* and coauthors reported the preparation of PNMeG-*co*-PNBuG from *N*-substituted glycine *N*-thiocarboxyanhydrides (NTAs) with tunable LCST properties . (Table 7)[252] By controlling the molecular weight and the content of NMeG it was possible to tune the T_{CP} from 27 to 71°C. They also reported the salting effect that decreased the T_{CP} by the addition of Na_2SO_4, NaCl, and NaBr. Investigating the effect of charged copolymers more closely, *Sun* and coauthors also evaluated the influence of the *N*-alkylated side-chain copolymers constituted of NAlG and *N*-alkylglycine with different carbon chain lengths (Table 7) .[253] The copolymers were post-functionalized through thiol-ene chemistry affording copolymers with -NH_2 or -COOH groups, and another series of copolymers was prepared by grafting onto 2-methoxyethanethiol (SH-EG) as a comonomer instead of hydrophobic groups. (Table 7) The copolymers bearing -COOH and -EG presented UCST behavior in water as previously reported for the fully COOH-grafted homopolymers.[250] The T_{CP} in this class of copolymers was dependent on concentration, and -EG content: higher concentration and lower content of -EG increased the T_{CP}. Moreover, the salt content did not affect the T_{CP}, and the architecture suppressed the UCST as they observed in copolymers based on poly(*N*-propargylglycine). Interestingly, the copolymers bearing *N*-octyl side chains ($PNAG_{49}$-COOH-*r*-$PNOG_{14}$) presented UCST in methanol at 2 mg/mL with a T_{CP} = 48°C whereas *N*-butyl or *N*-hexyl copolymers were soluble in methanol even at 10 mg/mL. Further study of the content of PNOG in the random copolymers bearing COOH indicated that the highest NOG content gave

the highest T_{CP} (i.e. PNAG$_{49}$-COOH-*r*-PNOG$_{44}$ T_{CP}=53°C). The copolymers bearing -NH$_2$ and -EG groups did not exhibit any thermal response as they remained water soluble at all pH values. In marked contrast, the copolymers with *N*-alkylated hydrophobic groups presented LCST behavior. Copolymers having *N*-hexyl side chains (PNAG$_{42}$-NH$_2$-r-PNHG$_{10}$) for instance displayed T_{CP} in the range of pH values = 9.8 - 13 that dropped from 43.5 to 29.3°C at 2 mg/mL. The T_{CP} was tuned by changing the *N*-alkylated side chain length (using similar composition in random copolymers): the T_{CP} decreased from 55 to 39°C when changing from *N*-butyl to *N*-octyl side chains. Moreover, the study demonstrated that tuning the monomer composition modulated the LCST: the T_{CP} decreased from 43.5 to 31°C as *N*-hexyl content increased from 20% to 50%, respectively. Adding a third building block fully changed the physicochemical properties as *Zhang* and coauthors demonstrated by preparing polypeptoid terpolymers (Table 7) with sol-gel transitions (T_{gel}) induced by the high solubility of NMeG.[254] The copolymers constituted of *N*-allylglycine, NMeG and *N*-dodecylglycine monomer units presented sol-gel transitions when increasing temperature in the range of T_{gel} = 26°C – 60°C. The study also demonstrated that the Young modulus could be tuned from 6 to 762 Pa by varying the composition and the concentration of copolymers. These mechanical properties allowed the hydrogels to be used as biomaterials in tissue engineering to promote chondrogenesis.

Copolymer architecture is also an important parameter influencing the thermoresponsiveness of copolypeptoids as *Zhang* and coauthors demonstrated when they prepared cyclic and linear copolymers of *N*-ethylglycine (NEtG) and *N*-butyl glycine (NBuG).[255] (Table 7) The LCST that the authors observed was influenced mainly by the *N*-ethylglycine content, and the architecture having values in the range of T_{CP}=20°C - 60°C. Polymers with a higher content of NEG presented higher T_{CP} attributed to the decrease of hydrophobic NBuG side chain content. Moreover, cyclic polymers presented a 4°C lower T_{CP} than linear polymers demonstrating the influence of the architecture on the thermoresponsive behavior. In another work, LCST was also studied using bottle brush polymers with poly(norbornene) as the main backbone and polypeptoids as pendant chains.[256] (Table 7) The polypeptoids were prepared via ROP using various ratios of NEtG and NBuG initiated by norbornene-functional amines. The resulting norbornene-functional macromonomers were polymerized by ROMP using Grubb's catalyst. The LCST of the bottlebrush copolymers ranged from 25°C to 80°C. Two parameters influenced the T_{CP}: the NEtG content and the salt concentration. Whereas higher content of NETG promoted a higher T_{CP}, the higher salt concentration decreased the T_{CP}.

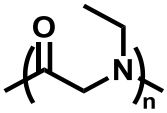
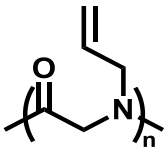
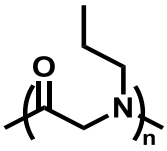
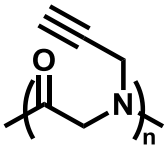
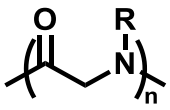
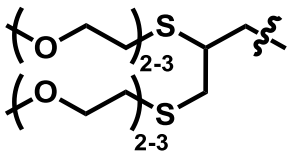
The LCST behavior of polypeptoids was used by *Akiyoshi* and coauthors to prepare maltriose-*b*-poly(*N*-propylglycine) having DP = 17 that displayed T_{CP} = 26°C at 10 mg/mL.[257] (Table 7) The influence of concentration was studied demonstrating that at 1 mg/mL T_{CP} increased to 43°C. This LCST behavior was exploited to prepare permeable particles by incubating the copolymers above T_{CP} to promote self-assembly into bilayer micelles. A fluorescence screening test demonstrated that only rhodamine G, which possesses cationic functional groups, permeates into the micelles due to the interaction with the negatively charged surface of the micelles. *Sun* and coauthors also designed nanomaterials with thermoresponsive polypeptoids. These authors prepared block copolymers with *N*-allyl and *N*-octyl glycine monomer units and functionalized with cysteamine (PNPrSAlG-*co*-PNOcG).(Table 7) [258] The copolymers displayed LCST and assembled into nanoparticles at RT. The LCST behavior was pH dependent with a T_{CP} of 47.5 and 50.4°C at pH 9.5 and 10.5, respectively. The copolymers were fully soluble at lower pH and insoluble at higher pH. Interestingly, the polymers incubated in water for 24 h (pH = 8) self-assemble into fiber-like micelles whereas

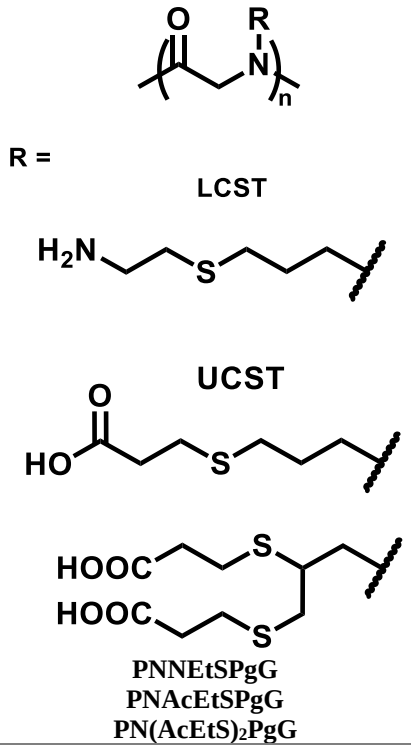
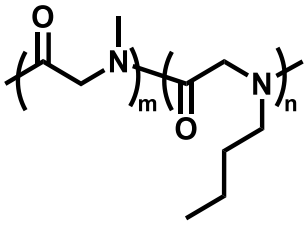
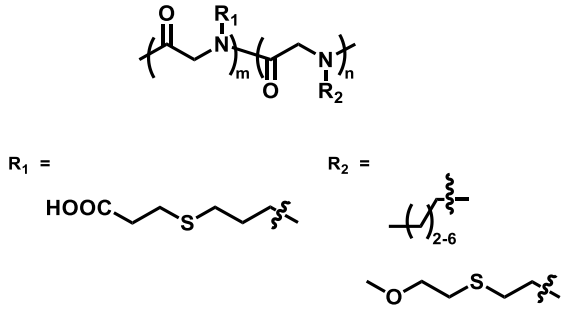
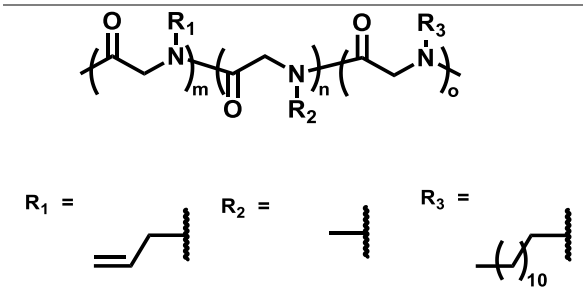
lowering the pH to 6.5 promotes the formation of spherical micelles. This behavior was attributed to differences in the protonation of NH_2 groups, which was corroborated through zeta potential measurements. Taking advantage of the LCST properties found in PNMeG-*co*-PNBuG) copolymers (Table 7), *Zirbs* et al. prepared another kind of nanomaterial: Fe_3O_4 magnetic nanoparticles.[259] The solution containing the copolymer-coated nanoparticles presented T_{CP} in the range of 33°C - 58°C at 3 mg/mL, and the suspension became homogenous after cooling. Inorganic coated nanomaterials were also designed by *Zhang* and coauthors using silicate surfaces with nanoneedles decorated with PNEtG-*co*-PNBuG copolymers.[260] The needles collapsed when the temperature was increased, as corroborated by *in situ* atomic force microscopy (AFM) in liquid from 8.2 nm to around 2 nm when they decreased the temperature from 60°C to 25°C.

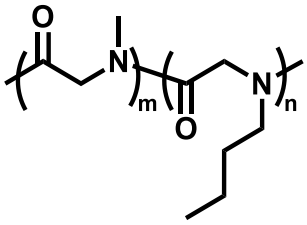
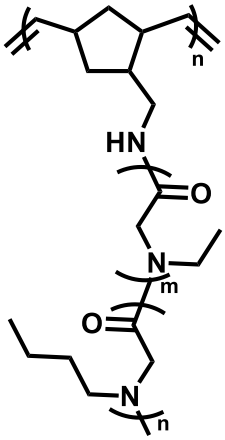
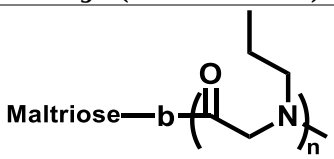
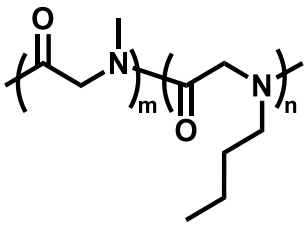
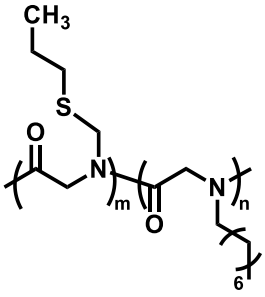
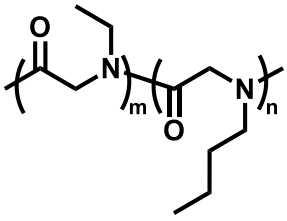
An interesting strategy towards thermoresponsive polypeptoids is to combine polypeptides with poly(*N*-alkylated glycines). *Fu* and coauthors reported the preparation of such poly(peptide-peptoid)s from coROP of NCAs and NNCAs.[261] Copolymers of γ-propargyl-glutamate and *N*-octylglycine were later modified with thiolated triethyleneglycol (PPLG-g-$EG_3)_{58}$-*b*-$PNOcG_9$ (Table 7), and presented T_{CP} = 35°C. Moreover, the copolymers self-assembled into worm-like nanoparticles when they were incubated for a longer time at 90°C.
Using another synthetic strategy, polypeptide-polypeptoids with LCST behavior have been prepared through the Ugi reaction.[262] The alternating polymers of glycine and *N*-(2-(*tert*-butylamino)-2-oxoethyl)-glycine (NtBuNOEtG) (Table 7) presented T_{CP} = 22°C at 10 mg/mL that increased to 27°C at 2 mg/mL demonstrating concentration-dependence. The authors also prepared a copolymer with PEG_{2k} that increased in turbidity starting at 35°C at 10 mg/mL. In another work mixing polypeptides and polypeptoids, *Koyama* investigated alternating copolymers of valine and *N*-alkylated glycines, including *N*-hydroxyethylglycine (NOHEtG), NPrG, and NMeG side chains that were prepared through the same Ugi reaction.(Table 7) [263] The copolymers presented UCST behavior having T_{CP} from 15 to 25°C. The alternating copolymers presented similar behavior with a slight increase in the transition temperatures by decreasing the *N*-alkylated side chain similar to that previously observed by *Schlaad*.[248] Similar work was done using the Ugi methodology by preparing three LCST polymers: an alternating copolymer of glycine and NtBuNOEtG; PNtBuNOEtG; and poly(-(2-(*tert*-butylamino)-2-oxoethyl)4-amidohydroxybutyrate. (Table 7) [264] The LCST behavior at 2 mg/mL was related to the polymer structure: when glycine was incorporated in the backbone the T_{CP} was 26°C, while removing it from the backbone and modifying the *N*-alkylated glycine for a longer carbon chain (amidobutyrate) gave a T_{CP} = 20°C, and including a hydroxy group (4-amidohydroxybutyrate) reduced it further. They also studied the incorporation of other substituents in the *N*-alkyl position (such as *N*-(2-(*tert*-butylamino)-2-oxo(1-isopropyl), *N*-(2-(cyclohexyl-amino)-2-oxoethyl, *N*-(2-(pentyl-amino)-2-oxoethyl ethyl but the polymers were not water-soluble demonstrating the importance of the appropriate structure, through the hydrophobicity of side chains. Later, *Koyama* et al. used the same polymerization technique to produce alternating copolymers of glycine and modified *N* and *C* alkylated glycines.[265] This polymerization technique differs from the ROP of NCAs as it is possible to control the sequence of the produced polymers. Yet the control over the polymerization in these cases was not always observed (in this study all produced polymers had dispersities higher than 1.5). By introducing an alkane chain on the C_α and either a propyl or a hydroxyethyl substituent on the amine, the different copolymers exhibited UCST, LCST or UCST and LCST simultaneously. (Table 7) This thermoresponsiveness was explained by the alternating hydrophilic (native glycine) and hydrophobic (N and C alkylated) units. The behavior could be tuned by varying the side chains. Polymers bearing hydroxyethyl moieties on the amine and short alkane chains

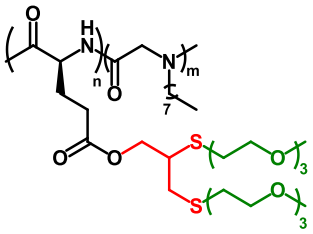
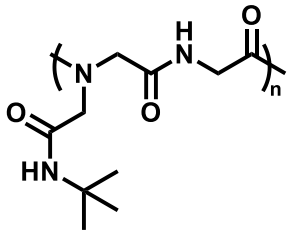
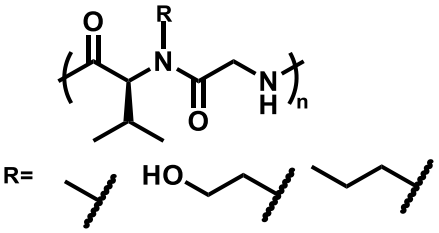
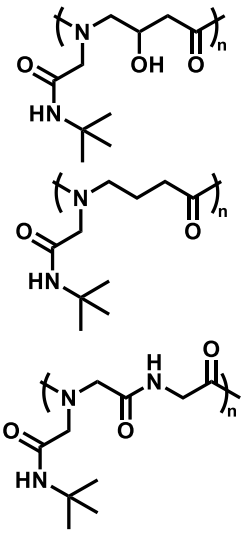
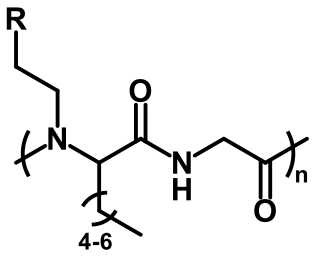
on the α-carbon showed UCST behavior (40°C), but by increasing the alkane chain from a pentane to a hexane an LCST also appeared around 60°C. The final polymer being *N*-propylated and having a pentane as a side chain in the hydrophobic comonomer showed only an LCST at about 25°C. These behaviors were explained by the side chain hydrophobicity/hydrophilicity. The authors' hypothesis is that the pendant pentane chain is too short to interact with the neighboring hydrophobic moieties giving the UCST behavior. On the other hand, the longer alkane favors intrachain hydrophobic interaction and thus an LCST can appear. The polymer bearing the propyl group and the pentane chain only shows an LCST due to the increased hydrophobicity. Furthermore, the authors used these thermoresponsive peptides as crosslinking agents in stimuli-responsive hydrogels by adding reactive side chains.

-TABLE 7 – Structures and thermoresponsive properties of different polypeptoïd based polymers.

Polymer structure	Amino acid	LCST T_{CP}(°C) and conditions	UCST T_{CU}(°C) and conditions	Ref.
 **PNEtG**	*N*-ethylglycine, *N*-allylglycine, *N*-propylglycine, *N*-propargylglycine,	15°C – 58°C depending on the C-length	-	[248]
 **PNAlG**				
 **PNPrG**				
 **PNPgG**				
	*N*-propargylglycine	22°C – 58°C depending on EG length, MW and concentration	-	[249]
 **PN(EG$_x$S)$_2$G**		36.5°C – 50°C -90°C depending on the EG length and architechture	-	[251]
	N-allylglycine or *N*-propargylglycine	20°C – 70°C pH dependant	30°C – 65°C pH dependant	[250]

 **PNNEtSPgG** **PNAcEtSPgG** **PN(AcEtS)$_2$PgG**				
 **PSar-*co*-PNBuG**	*N*-butylglycine, sarcosine	27°C – 71°C depending on Sar content, MW, and salt	-	[252]
 **PNAcEtSPrG-*co*-PNAlkG-*co*-PNEGSAlG**	*N*-allyl, *N*-butylglycine, *N*-hexylglycine, *N*-octylglycine	30°C – 50°C depending on pH, content of hydrophobic side chain and pH	-	[253]
 **PSar-*co*-PNAlG-*co*-PNDeG**	sarcosine, *N*-dodecylglycine, *N*-allylglycine	Sol to gel 26°C – 60°C depending on copolymer composition	-	[254]

 **PSar-*co*-PNBuG linear or cyclic**	sarcosine, *N*-butylglycine	20°C – 60°C depending on copolymer composition and topology	-	[255] [266]
 **PNor-*g*-P(NEtG-*co*-NBuG)**	*N*-ethylglycine, *N*-butylglycine	25°C – 80°C depending on copolymer composition	-	[256]
 **Maltriose-*b*-PNPrG**	*N*-propylglycine	26°C – 43°C depending on concentration	-	[257]
 **PNMeG-*co*-PNBuG magnetic nanoparticles**	sarcosine, *N*-butylglycine	33°C – 58°C depending on copolymer composition	-	[259]
 **PNPrSAlG-*co*-PNOcG**	*N*-allylglycine, *N*-octylglycine	40°C – 60°C depending on pH	-	[258]
 **PNEtG-*co*-PNBuG**	*N*-butylglycine and *N*-ethylglycine	20°C – 90°C	-	[260]

 **P(EG$_3$S)$_2$LG-*co*-PNOcG**	glutamate and *N*-octylglycine	35°C	-	[261]
 **PGly-*co*-PNtBuNOEtG**	glycine, *N*-(2-(tert-butylamino)-2-oxoethyl)-glycine	20°C – 35°C depending on the concentration	-	[262]
 **PVal-*co*-PNOHEtG-*co*-PNPrG**	valine, *N*-hydroxyethyl glycine, *N*-propylglycine, *N*-methylglycine	-	15°C – 25°C depending on the side chain	[263]
	alternating copolymers and gammapeptoids	13°C – 26°C depending on the structure	-	[264]
 **R=OH or CH$_3$**	alternating copolymers	60°C - 70°C Depending on the structure	30°C – 70°C Depending on the structure	[265]

5.2.2 Natural amino acid based

Surprisingly, while the IDPs show that natural polypeptides can show LCST behavior, there are few examples of synthetic polypeptide polymers with thermoresponsive behavior made from natural amino acids. Among these amino acids, proline was found to be effective for promoting thermoresponsiveness. Fundamentally, proline bridges the gap between polypeptides and polypeptoids. This amino acid possesses both the tertiary amide and the chiral center of these two classes of polymers. This combination induces secondary structures, a water-soluble extended helix known as PLPII induced by the amide bond when in its trans conformation, a more compact, highly insoluble PLPI structure when the cis conformation is predominant. This last structure predominates in organic environments and explains the insolubility of polyproline in water at higher temperatures.[267] First results were obtained with small proline oligomers containing up to 10 units which evidenced thermoresponsive shifts associated with their conformational changes.[268–270] Later, in a series of papers *Oka* and co-authors developed homopolymers and random copolymers of proline with higher molar masses using diphenylphosphoryl azide coupled with a base (triethylamine) to polymerize the amino acids by condensation. The authors showed that oligoprolines with approximately 20 repeating units displayed an LCST at temperatures ranging from 50°C to 65°C depending on the concentration (5-20 mg/ml).[271] Nonetheless, their results did not show secondary structure dependent behavior. They then added different comonomers to observe the influence on thermoresponsiveness. This was done by preparing a dipeptide of proline and a second amino acid that was then copolymerized with proline using the DPPA technique mentioned above. Substituting some proline units with *O*-benzylhydroxyproline reduced the T_{CP} by 5°C even with only 1% of hydrophobic monomer content. Using the same strategy, copolymers incorporating small amounts of glycine or alanine allowed further modification of the LCST.[272] Copolymers with low amounts (<12%) of glycine showed slightly higher T_{CP} compared to the homopolymer but allowed the oligomers (Mn<2.0kg/mol) to be soluble at concentrations as high as 160 mg/ml. This was attributed to the more hydrophilic nature of glycine compared to proline. Yet adding a more hydrophobic comonomer such as alanine had similar effect. Oligomers containing 3 or 6% of alanine also showed slightly higher T_{CP}. The same researchers successfully prepared other oligomers with higher alanine contents (up to 17%) that had even higher T_{CP} of around 73°C at 50 mg/ml.[273] The authors showed that all examples had a PLPII secondary structure at all temperatures but it should be noted that in these measurements the CD spectroscopy was done at much lower concentration than the turbidimetry. Our group has recently showcased the thermoresponsive behavior of much higher molar masses of PLP.[267] It was shown that polyprolines prepared via aqueous NCA ROP[274] with more than 20 repeating units displayed hysteretic LCST. (Fig. 10) We showed that the secondary structure induced by the chirality of the monomer units is at the origin of this behavior. CD measurements proved the shift from the highly soluble PLPII conformation to an intermediate insoluble conformation. Further analyses via FTIR proved the presence of both cis and trans amide conformations providing evidence that temperature-induced changes in the secondary structure were responsible for the reversibly hysteretic LCST.

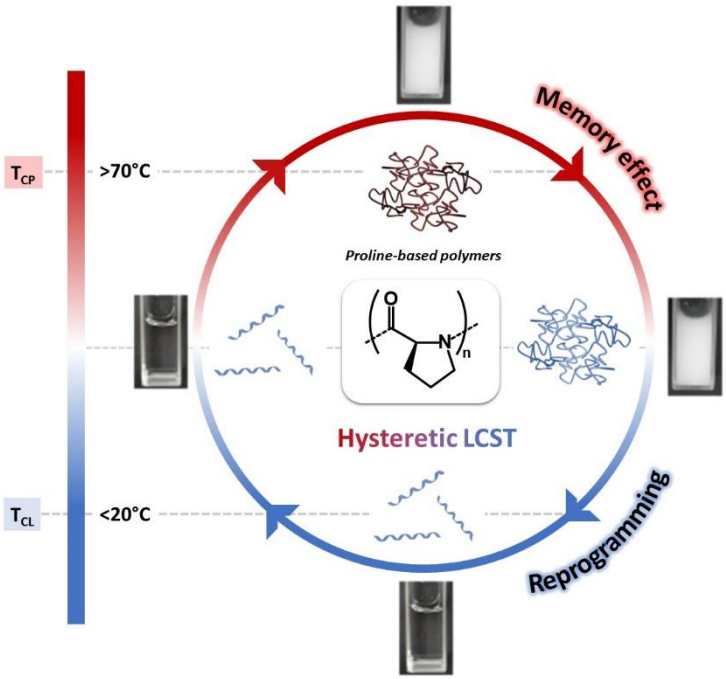


Fig. 10. Homopolyproline is soluble in aqueous solution at room temperature when in the PLP II conformation. Upon heating to temperatures above 70°C the polymer aggregates and stays insoluble upon cooling. This hysteretic LCST can thus be used to memorize the thermal history of the sample. The polymer can then be resolubilized and the process reprogrammed by cooling at lower temperatures. Fig. adapted from [267].

Another interesting example of thermoresponsive polypeptides based on natural amino acids was published by *Koyama* and coauthors using copolymers containing valine and glycine units.[275] Using the ROP of the corresponding NCAs, they produced both block and random polypeptides with molar masses of 3.4kDa and 3.3kDa respectively. Both copolymers exhibited similar LCST at a temperature around 80°C. To better understand the effect of the sequence on thermoresponsiveness, they prepared an alternating peptide using a three component polycondensation. Unexpectedly, this new copolymer expressed a UCST at low temperatures, independent on the chiral nature of the backbone. This behavior was tuned by the *N*-alkylation of the valine units.[263] These examples clearly highlight the importance of the primary structure of the peptide backbone on the thermoresponsiveness and the limitations brought in this direction by the ROP of NCA. In other studies, polymers bearing ureido side functions have displayed UCST behavior due to their hydrogen bonding possibilities.[276] For instance, copolymers based on ornithine and citrulline showed interesting UCST behavior.[277] In particular, random copolymers based on ornithine and citrulline prepared with varying molecular weights and monomer compositions express UCST behavior in a large range of temperatures (5°C – 40°C).[278] The authors evidenced the importance of the α-helical secondary structure in the formation of such aggregates at low temperatures. Racemic copolymers still showed UCST behavior but formed coacervate droplets at lower temperatures

and not aggregates. The ureido was at the origin of temperature-disrupted hydrogen bonds necessary for such behavior.[279] The UCST could be altered by alkylation of the ureido group.[280] These examples were obtained by modifying poly(*L*-ornithine) hydrobromide. Yet it is also possible to obtain benzyl-protected polyornithine by ROP of its NCA (*Z*-Orn NCA). Subsequent deprotection provides a homopolymer with pendant amine functions that were functionalized with ureido groups giving the copolymers.[281] UCST polypeptides were also achieved by copolymerizing *Z*-Orn NCA with Z-Lys NCA. These copolymers showed further versatility in controlling the UCST behavior thanks to the lysine units. Nonetheless, the secondary structuring of these copolymers did not allow for reversibility, as the formation of β-sheet like structures stabilized irreversible large scale aggregation.[282] UCST polypeptides have shown great promise as they are stable in physiological conditions, are both enzymatically[281] and biodegradable[278] and can also promote cell adhesion coatings[280] and controlled delivery of active molecules[282].

6. CONCLUSIONS AND PERSPECTIVES

Understanding the fundamental principles that govern the solubility of proteins in water, particularly as a function of temperature, can be a key source of inspiration for the rational design of thermosensitive polymers. In biology, proteins react to biologically relevant factors by modifying their conformation and three-dimensional structure, resulting in changes in solubility or mechanical properties. Among external factors, temperature modulation is an efficient strategy to induce protein aggregation, generally irreversibly for structured proteins, but sometimes reversibly, as observed with IDPs. It is now well established that recombinant or natural proteins can be used as the basis for genetic engineering to design thermoresponsive polypeptides. These are likely to receive increasing attention from industry as new classes of polymers with improved biodegradability.

Genetically engineered IDPs such as ELPs represent an excellent example of how simplified protein sequences can give rise to desired and tunable functionality. ELPs indeed exhibit LCST behavior similar to the behavior found in some synthetic polymers and are promising in many applications. The polymeric counterparts to these simplified genetically engineered structures are not yet very popular. Nevertheless, among the different approaches presented in this review, synthetic polypeptides appear as ideal candidates for the elaboration of biomimetic and biodegradable thermosensitive polymers. This class of polymers can be readily used in biomedical applications due to its similarity to protein structures. In this perspective, there are still many challenges to better mimic proteins, and also to refine their thermoresponsive behavior.

In this review, we have comprehensively presented the thermosensitive behavior of peptide polymers, explaining the similarities between these structures and naturally thermosensitive proteins. Interestingly, the control of hydration-dehydration of the polymer backbone or side chains is the main factor that governs the thermosensitivity process. Concerning proteins, the changes are mainly controlled by the hydration of the hydrophobic parts of the backbone, a factor that is intimately related to the amino acid sequence and to their folding behavior. With synthetic polypeptides, the thermosensitivity depends mostly on the hydration of side chains and/or the polymer chain by simply controlling the hydration of amide bonds as with other synthetic polymers. Compared to proteins, the impact of the distribution of monomer units or the influence of the polymer folding (secondary structure) are still poorly studied and understood parameters. All in all, the introduction of amino acids in the backbone of

thermoresponsive polymers shows promise in their use in biomedical applications. Active substance encapsulation and controlled release, drug loaded hydrogels and antibacterial coatings are all prime examples that come out in the literature and have been reviewed here. The biocompatibility of amino-acid based polymers as well as their biodegradability makes them an interesting novel class of thermoresponsive polymers. We believe that in the future, as this field progresses, these polymers may well replace classical thermoresponsive polymers in other applications.

Looking further, we believe there are many opportunities to better control primary sequence and its consequences on the secondary structures and consequently the thermoresponsive behavior of synthetic polypeptides. This requires first to develop synthetic methods and processes allowing for the design of sequence-controlled polypeptide backbones. Recent development in catalysis and flow chemistry processes may solve some of these issues. In addition, recent synthetic developments allowing for high molar mass polyproline have demonstrated that even with rather simple homopolymer structures, such correlation between secondary structure/folding and solubility could provide new thermosensitive materials with a super-hysteresis that provides a new perspective and paradigm. Another opportunity concerns the elaboration of polypeptide polymers and materials at the interface between the "classical research fields" of protein engineering and synthetic polypeptides. Such biohybrid systems could probably take advantage of the best in these two independent fields, solving some independent challenges and providing original and potentially unexpected properties. We believe this research area will grow significantly in the coming years, offering new tools for (bio)materials science, catalysis, protein engineering, biochemistry, pharmaceutics, etc. at academic and industrial levels.

ACKNOWLEDGMENT

PSA received support from CONACYT (scholarship holder no. 548662). This work was supported by the French National Agency for Research (ANR): grant no. ANR-17-CE07-0039-01. MB received support from The French Ministry of Higher Education, Research and Innovation (Ministère de l'Enseignement Supérieur, de la Recherche et de l'Innovation, MESRI).

REFERENCES

[1] Saha T, Galic M. Self-organization across scales: from molecules to organisms. Philos Trans R Soc B Biol Sci 2018;373:20170113. https://doi.org/10.1098/rstb.2017.0113.

[2] Heald R, Cohen-Fix O. Morphology and function of membrane-bound organelles. Curr Opin Cell Biol 2014;26:79–86. https://doi.org/10.1016/j.ceb.2013.10.006.

[3] Hyman AA, Weber CA, Jülicher F. Liquid-Liquid Phase Separation in Biology. Annu Rev Cell Dev Biol 2014;30:39–58. https://doi.org/10.1146/annurev-cellbio-100913-013325.

[4] Banani SF, Lee HO, Hyman AA, Rosen MK. Biomolecular condensates: organizers of cellular biochemistry. Nat Rev Mol Cell Biol 2017;18:285–98. https://doi.org/10.1038/nrm.2017.7.

[5] Perry SL. Phase separation: Bridging polymer physics and biology. Curr Opin Colloid Interface Sci 2019;39:86–97. https://doi.org/10.1016/j.cocis.2019.01.007.

[6] Keating CD. Aqueous Phase Separation as a Possible Route to Compartmentalization of

Biological Molecules. Acc Chem Res 2012;45:2114–24. https://doi.org/10.1021/ar200294y.

[7] Walter H, Johansson G. Partitioning in aqueous two-phase systems: An overview. Anal Biochem 1986;155:215–42. https://doi.org/10.1016/0003-2697(86)90431-8.

[8] Albertsson P-Å. Partition of Cell Particles and Macromolecules in Polymer Two-Phase Systems, 1970, p. 309–41. https://doi.org/10.1016/S0065-3233(08)60244-2.

[9] Abbas M, Lipiński WP, Wang J, Spruijt E. Peptide-based coacervates as biomimetic protocells. Chem Soc Rev 2021;50:3690–705. https://doi.org/10.1039/D0CS00307G.

[10] Walheim S, Böltau M, Mlynek J, Krausch G, Steiner U. Structure Formation via Polymer Demixing in Spin-Cast Films. Macromolecules 1997;30:4995–5003. https://doi.org/10.1021/ma9619288.

[11] Darling SB. Directing the self-assembly of block copolymers. Prog Polym Sci 2007;32:1152–204. https://doi.org/10.1016/j.progpolymsci.2007.05.004.

[12] GIL E, HUDSON S. Stimuli-reponsive polymers and their bioconjugates. Prog Polym Sci 2004;29:1173–222. https://doi.org/10.1016/j.progpolymsci.2004.08.003.

[13] André AAM, Spruijt E. Liquid–Liquid Phase Separation in Crowded Environments. Int J Mol Sci 2020;21:5908. https://doi.org/10.3390/ijms21165908.

[14] Kessel A, Ben-Tal N. Introduction to Proteins. Chapman and Hall/CRC; 2018. https://doi.org/10.1201/9781315113876.

[15] Buxbaum E. Fundamentals of Protein Structure and Function. Cham: Springer International Publishing; 2015. https://doi.org/10.1007/978-3-319-19920-7.

[16] Musacchio A. On the role of phase separation in the biogenesis of membraneless compartments. EMBO J 2022;41. https://doi.org/10.15252/embj.2021109952.

[17] Anfinsen CB. Principles that Govern the Folding of Protein Chains. Science (80-) 1973;181:223–30. https://doi.org/10.1126/science.181.4096.223.

[18] Pace CN, Shirley BA, McNutt M, Gajiwala K. Forces contributing to the conformational stability of proteins. FASEB J 1996;10:75–83. https://doi.org/10.1096/fasebj.10.1.8566551.

[19] Shortle D. The denatured state (the other half of the folding equation) and its role in protein stability. FASEB J 1996;10:27–34. https://doi.org/10.1096/fasebj.10.1.8566543.

[20] Seckler R, Jaenicke R. Protein folding and protein refolding. FASEB J 1992;6:2545–52. https://doi.org/10.1096/fasebj.6.8.1592207.

[21] England JL, Haran G. Role of Solvation Effects in Protein Denaturation: From Thermodynamics to Single Molecules and Back. Annu Rev Phys Chem 2011;62:257–77. https://doi.org/10.1146/annurev-physchem-032210-103531.

[22] Erik van der Linden, Foegeding EA. Gelation. Mod. Biopolym. Sci., Elsevier; 2009, p. 29–91. https://doi.org/10.1016/B978-0-12-374195-0.00002-1.

[23] Renkema JM., Lakemond CM., de Jongh HH., Gruppen H, van Vliet T. The effect of pH on heat denaturation and gel forming properties of soy proteins. J Biotechnol 2000;79:223–30. https://doi.org/10.1016/S0168-1656(00)00239-X.

[24] Fisicaro E, Compari C, Braibanti A. Entropy/enthalpy compensation: hydrophobic effect, micelles and protein complexes. Phys Chem Chem Phys 2004;6:4156. https://doi.org/10.1039/b404327h.

[25] Somero GN. Proteins and Temperature. Annu Rev Physiol 1995;57:43–68. https://doi.org/10.1146/annurev.ph.57.030195.000355.

[26] Park C-J, Seo Y-S. Heat Shock Proteins: A Review of the Molecular Chaperones for Plant Immunity. Plant Pathol J 2015;31:323–33. https://doi.org/10.5423/PPJ.RW.08.2015.0150.

[27] Calderwood SK. Heat shock proteins and cancer: intracellular chaperones or extracellular signalling ligands? Philos Trans R Soc B Biol Sci 2018;373:20160524. https://doi.org/10.1098/rstb.2016.0524.

[28] Anfinsen CB. The formation and stabilization of protein structure. Biochem J 1972;128:737–49. https://doi.org/10.1042/bj1280737.

[29] Steipe B. Consensus-Based Engineering of Protein Stability: From Intrabodies to Thermostable Enzymes, 2004, p. 176–86. https://doi.org/10.1016/S0076-6879(04)88016-9.

[30] BISCHOF JC. Thermal Stability of Proteins. Ann N Y Acad Sci 2005;1066:12–33. https://doi.org/10.1196/annals.1363.003.

[31] Privalov PL, Gill SJ. Stability of Protein Structure and Hydrophobic Interaction, 1988, p. 191–234. https://doi.org/10.1016/S0065-3233(08)60377-0.

[32] Lesk AM. Hydrophobicity—getting into hot water. Biophys Chem 2003;105:179–82. https://doi.org/10.1016/S0301-4622(03)00086-3.

[33] Dill KA, Chan HS. From Levinthal to pathways to funnels. Nat Struct Mol Biol 1997;4:10–9. https://doi.org/10.1038/nsb0197-10.

[34] Uversky VN, Gillespie JR, Fink AL. Why are ?natively unfolded? proteins unstructured under physiologic conditions? Proteins Struct Funct Genet 2000;41:415–27. https://doi.org/10.1002/1097-0134(20001115)41:3<415::AID-PROT130>3.0.CO;2-7.

[35] Mao AH, Crick SL, Vitalis A, Chicoine CL, Pappu R V. Net charge per residue modulates conformational ensembles of intrinsically disordered proteins. Proc Natl Acad Sci 2010;107:8183–8. https://doi.org/10.1073/pnas.0911107107.

[36] Müller-Späth S, Soranno A, Hirschfeld V, Hofmann H, Rüegger S, Reymond L, et al. Charge interactions can dominate the dimensions of intrinsically disordered proteins. Proc Natl Acad Sci 2010;107:14609–14. https://doi.org/10.1073/pnas.1001743107.

[37] Martin EW, Mittag T. Relationship of Sequence and Phase Separation in Protein Low-Complexity Regions. Biochemistry 2018;57:2478–87. https://doi.org/10.1021/acs.biochem.8b00008.

[38] Posey AE, Holehouse AS, Pappu R V. Phase Separation of Intrinsically Disordered Proteins, 2018, p. 1–30. https://doi.org/10.1016/bs.mie.2018.09.035.

[39] Martin EW, Holehouse AS. Intrinsically disordered protein regions and phase separation: sequence determinants of assembly or lack thereof. Emerg Top Life Sci 2020;4:307–29. https://doi.org/10.1042/ETLS20190164.

[40] Imai K, Mitaku S. Mechanisms of secondary structure breakers in soluble proteins. Biophysics (Oxf) 2005;1:55–65. https://doi.org/10.2142/biophysics.1.55.

[41] Radivojac P, Iakoucheva LM, Oldfield CJ, Obradovic Z, Uversky VN, Dunker AK. Intrinsic Disorder and Functional Proteomics. Biophys J 2007;92:1439–56. https://doi.org/10.1529/biophysj.106.094045.

[42] Chou PY, Fasman GD. Prediction of the Secondary Structure of Proteins from their

Amino Acid Sequence, 2006, p. 45–148. https://doi.org/10.1002/9780470122921.ch2.

[43] Garanger E, MacEwan SR, Sandre O, Brûlet A, Bataille L, Chilkoti A, et al. Structural Evolution of a Stimulus-Responsive Diblock Polypeptide Micelle by Temperature Tunable Compaction of its Core. Macromolecules 2015;48:6617–27. https://doi.org/10.1021/acs.macromol.5b01371.

[44] Frey S, Rees R, Schünemann J, Ng SC, Fünfgeld K, Huyton T, et al. Surface Properties Determining Passage Rates of Proteins through Nuclear Pores. Cell 2018;174:202-217.e9. https://doi.org/10.1016/j.cell.2018.05.045.

[45] Riback JA, Katanski CD, Kear-Scott JL, Pilipenko E V., Rojek AE, Sosnick TR, et al. Stress-Triggered Phase Separation Is an Adaptive, Evolutionarily Tuned Response. Cell 2017;168:1028-1040.e19. https://doi.org/10.1016/j.cell.2017.02.027.

[46] Dao TP, Kolaitis R-M, Kim HJ, O'Donovan K, Martyniak B, Colicino E, et al. Ubiquitin Modulates Liquid-Liquid Phase Separation of UBQLN2 via Disruption of Multivalent Interactions. Mol Cell 2018;69:965-978.e6. https://doi.org/10.1016/j.molcel.2018.02.004.

[47] Yang Y, Jones HB, Dao TP, Castañeda CA. Single Amino Acid Substitutions in Stickers, but Not Spacers, Substantially Alter UBQLN2 Phase Transitions and Dense Phase Material Properties. J Phys Chem B 2019;123:3618–29. https://doi.org/10.1021/acs.jpcb.9b01024.

[48] Pak CW, Kosno M, Holehouse AS, Padrick SB, Mittal A, Ali R, et al. Sequence Determinants of Intracellular Phase Separation by Complex Coacervation of a Disordered Protein. Mol Cell 2016;63:72–85. https://doi.org/10.1016/j.molcel.2016.05.042.

[49] Murthy AC, Dignon GL, Kan Y, Zerze GH, Parekh SH, Mittal J, et al. Molecular interactions underlying liquid−liquid phase separation of the FUS low-complexity domain. Nat Struct Mol Biol 2019;26:637–48. https://doi.org/10.1038/s41594-019-0250-x.

[50] Ruff KM, Roberts S, Chilkoti A, Pappu R V. Advances in Understanding Stimulus-Responsive Phase Behavior of Intrinsically Disordered Protein Polymers. J Mol Biol 2018;430:4619–35. https://doi.org/10.1016/j.jmb.2018.06.031.

[51] Auton M, Bolen DW. Additive Transfer Free Energies of the Peptide Backbone Unit That Are Independent of the Model Compound and the Choice of Concentration Scale. Biochemistry 2004;43:1329–42. https://doi.org/10.1021/bi035908r.

[52] Roberts S, Dzuricky M, Chilkoti A. Elastin-like polypeptides as models of intrinsically disordered proteins. FEBS Lett 2015;589:2477–86. https://doi.org/10.1016/j.febslet.2015.08.029.

[53] Garanger E, Lecommandoux S. Emerging opportunities in bioconjugates of Elastin-like polypeptides with synthetic or natural polymers. Adv Drug Deliv Rev 2022;191:114589. https://doi.org/10.1016/j.addr.2022.114589.

[54] Foster JA, Bruenger E, Gray WR, Sandberg LB. Isolation and Amino Acid Sequences of Tropoelastin Peptides. J Biol Chem 1973;248:2876–9. https://doi.org/10.1016/S0021-9258(19)44088-X.

[55] GRAY WR, SANDBERG LB, FOSTER JA. Molecular Model for Elastin Structure and Function. Nature 1973;246:461–6. https://doi.org/10.1038/246461a0.

[56] Vrhovski B, Weiss AS. Biochemistry of tropoelastin. Eur J Biochem 1998;258:1–18. https://doi.org/10.1046/j.1432-1327.1998.2580001.x.

[57] Zhao B, Li NK, Yingling YG, Hall CK. LCST Behavior is Manifested in a Single Molecule: Elastin-Like polypeptide (VPGVG) n. Biomacromolecules 2016;17:111–8. https://doi.org/10.1021/acs.biomac.5b01235.

[58] Dzuricky M, Rogers BA, Shahid A, Cremer PS, Chilkoti A. De novo engineering of intracellular condensates using artificial disordered proteins. Nat Chem 2020;12:814–25. https://doi.org/10.1038/s41557-020-0511-7.

[59] Garcia Quiroz F, Li NK, Roberts S, Weber P, Dzuricky M, Weitzhandler I, et al. Intrinsically disordered proteins access a range of hysteretic phase separation behaviors. Sci Adv 2019;5. https://doi.org/10.1126/sciadv.aax5177.

[60] Quiroz FG, Chilkoti A. Sequence heuristics to encode phase behaviour in intrinsically disordered protein polymers. Nat Mater 2015;14:1164–71. https://doi.org/10.1038/nmat4418.

[61] Petitdemange R, Garanger E, Bataille L, Bathany K, Garbay B, Deming TJ, et al. Tuning Thermoresponsive Properties of Cationic Elastin-like Polypeptides by Varying Counterions and Side-Chains. Bioconjug Chem 2017;28:1403–12. https://doi.org/10.1021/acs.bioconjchem.7b00082.

[62] Petitdemange R, Garanger E, Bataille L, Dieryck W, Bathany K, Garbay B, et al. Selective Tuning of Elastin-like Polypeptide Properties via Methionine Oxidation. Biomacromolecules 2017;18:544–50. https://doi.org/10.1021/acs.biomac.6b01696.

[63] Rosselin M, Xiao Y, Belhomme L, Lecommandoux S, Garanger E. Expanding the Toolbox of Chemoselective Modifications of Protein-Like Polymers at Methionine Residues. ACS Macro Lett 2019;8:1648–53. https://doi.org/10.1021/acsmacrolett.9b00862.

[64] Ibrahimova V, Zhao H, Ibarboure E, Garanger E, Lecommandoux S. Thermosensitive Vesicles from Chemically Encoded Lipid-Grafted Elastin-like Polypeptides. Angew Chemie - Int Ed 2021;60:15036–40. https://doi.org/10.1002/anie.202102807.

[65] Xiao Y, Chinoy ZS, Pecastaings G, Bathany K, Garanger E, Lecommandoux S. Design of Polysaccharide-b-Elastin-Like Polypeptide Bioconjugates and Their Thermoresponsive Self-Assembly. Biomacromolecules 2020;21:114–25. https://doi.org/10.1021/acs.biomac.9b01058.

[66] Rosselin M, Chinoy ZS, Bravo-Anaya LM, Lecommandoux S, Garanger E. Multivalent Elastin-Like Glycopolypeptides: Subtle Chemical Structure Modifications with High Impact on Lectin Binding Affinity. ACS Macro Lett 2021;10:65–70. https://doi.org/10.1021/acsmacrolett.0c00775.

[67] Anaya LMB, Petitdemange R, Rosselin M, Ibarboure E, Garbay B, Garanger E, et al. Design of Thermoresponsive Elastin-Like Glycopolypeptides for Selective Lectin Binding and Sorting. Biomacromolecules 2021;22:76–85. https://doi.org/10.1021/acs.biomac.0c00374.

[68] Levêque M, Xiao Y, Durand L, Massé L, Garanger E, Lecommandoux S. Aqueous synthesis and self-assembly of bioactive and thermo-responsive HA- b -ELP bioconjugates. Biomater Sci 2022;10:6365–76. https://doi.org/10.1039/D2BM01149B.

[69] Le Fer G, Portes D, Goudounet G, Guigner JM, Garanger E, Lecommandoux S. Design and self-assembly of PBLG-: B -ELP hybrid diblock copolymers based on synthetic and elastin-like polypeptides. Org Biomol Chem 2017;15:10095–104. https://doi.org/10.1039/c7ob01945a.

[70] Dai M, Georgilis E, Goudounet G, Garbay B, Pille J, Van Hest JCM, et al. Refining the

design of diblock elastin-like polypeptides for self-assembly into nanoparticles. Polymers (Basel) 2021;13:1–17. https://doi.org/10.3390/polym13091470.

[71] Dai M, Goudounet G, Zhao H, Garbay B, Garanger E, Pecastaings G, et al. Thermosensitive Hybrid Elastin-like Polypeptide-Based ABC Triblock Hydrogel. Macromolecules 2021;54:327–40. https://doi.org/10.1021/acs.macromol.0c01744.

[72] Bravo-Anaya LM, Rosselgong J, Fernández-Solís KG, Xiao Y, Vax A, Ibarboure E, et al. Coupling of RAFT polymerization and chemoselective post-modifications of elastin-like polypeptides for the synthesis of gene delivery hybrid vectors. Polym Chem 2021;12:226–41. https://doi.org/10.1039/D0PY01293A.

[73] Zhao H, Ibrahimova V, Garanger E, Lecommandoux S. Dynamic Spatial Formation and Distribution of Intrinsically Disordered Protein Droplets in Macromolecularly Crowded Protocells. Angew Chemie - Int Ed 2020;59:11028–36. https://doi.org/10.1002/anie.202001868.

[74] Zhao H, Ibarboure E, Ibrahimova V, Xiao Y, Garanger E, Lecommandoux S. Spatiotemporal Dynamic Assembly/Disassembly of Organelle-Mimics Based on Intrinsically Disordered Protein-Polymer Conjugates. Adv Sci 2021;8:2102508. https://doi.org/10.1002/advs.202102508.

[75] Bian Q, Fu L, Li H. Engineering shape memory and morphing protein hydrogels based on protein unfolding and folding. Nat Commun 2022;13:137. https://doi.org/10.1038/s41467-021-27744-0.

[76] Wei G, Su Z, Reynolds NP, Arosio P, Hamley IW, Gazit E, et al. Self-assembling peptide and protein amyloids: from structure to tailored function in nanotechnology. Chem Soc Rev 2017;46:4661–708. https://doi.org/10.1039/C6CS00542J.

[77] Giaveri S, Schmitt AM, Roset Julià L, Scamarcio V, Murello A, Cheng S, et al. Nature-Inspired Circular-Economy Recycling for Proteins: Proof of Concept. Adv Mater 2021;33:2104581. https://doi.org/10.1002/adma.202104581.

[78] Silva NHCS, Vilela C, Marrucho IM, Freire CSR, Pascoal Neto C, Silvestre AJD. Protein-based materials: from sources to innovative sustainable materials for biomedical applications. J Mater Chem B 2014;2:3715. https://doi.org/10.1039/c4tb00168k.

[79] Cao Y, Adamcik J, Diener M, Kumita JR, Mezzenga R. Different Folding States from the Same Protein Sequence Determine Reversible vs Irreversible Amyloid Fate. J Am Chem Soc 2021;143:11473–81. https://doi.org/10.1021/jacs.1c03392.

[80] Clark EA, Lipson JEG. LCST and UCST behavior in polymer solutions and blends. Polymer (Guildf) 2012;53:536–45. https://doi.org/10.1016/j.polymer.2011.11.045.

[81] Arai K, Shikata T. Hydration/Dehydration Behavior of Cellulose Ethers in Aqueous Solution. Macromolecules 2017;50:5920–8. https://doi.org/10.1021/acs.macromol.7b00848.

[82] Van Durme K, Van Mele B, Bernaerts K V., Verdonck B, Du Prez FE. End-group modified poly(methyl vinyl ether): Characterization and LCST demixing behavior in water. J Polym Sci Part B Polym Phys 2006;44:461–9. https://doi.org/10.1002/polb.20710.

[83] Dai S, Tam KC. Isothermal Titration Calorimetric Studies on the Temperature Dependence of Binding Interactions between Poly(propylene glycol)s and Sodium Dodecyl Sulfate. Langmuir 2004;20:2177–83. https://doi.org/10.1021/la0357559.

[84] Halperin A, Kröger M, Winnik FM. Poly(N -isopropylacrylamide) Phase Diagrams: Fifty Years of Research. Angew Chemie Int Ed 2015;54:15342–67.

https://doi.org/10.1002/anie.201506663.

[85] Meeussen F, Nies E, Berghmans H, Verbrugghe S, Goethals E, Du Prez F. Phase behaviour of poly(N -vinyl caprolactam) in water. Polymer (Guildf) 2000;41:8597–602. https://doi.org/10.1016/S0032-3861(00)00255-X.

[86] Hoogenboom R, Thijs HML, Jochems MJHC, van Lankvelt BM, Fijten MWM, Schubert US. Tuning the LCST of poly(2-oxazoline)s by varying composition and molecular weight: alternatives to poly(N-isopropylacrylamide)? Chem Commun 2008:5758. https://doi.org/10.1039/b813140f.

[87] Lutz J-F, Hoth A. Preparation of Ideal PEG Analogues with a Tunable Thermosensitivity by Controlled Radical Copolymerization of 2-(2-Methoxyethoxy)ethyl Methacrylate and Oligo(ethylene glycol) Methacrylate. Macromolecules 2006;39:893–6. https://doi.org/10.1021/ma0517042.

[88] Plamper FA, Ruppel M, Schmalz A, Borisov O, Ballauff M, Müller AHE. Tuning the Thermoresponsive Properties of Weak Polyelectrolytes: Aqueous Solutions of Star-Shaped and Linear Poly(N,N -dimethylaminoethyl Methacrylate). Macromolecules 2007;40:8361–6. https://doi.org/10.1021/ma071203b.

[89] Gosecki M, Setälä H, Virtanen T, Ryan AJ. A facile method to control the phase behavior of hydroxypropyl cellulose. Carbohydr Polym 2021;251:117015. https://doi.org/10.1016/j.carbpol.2020.117015.

[90] Liu HY, Zhu XX. Lower critical solution temperatures of N -substituted acrylamide copolymers in aqueous solutions. Polymer (Guildf) 1999;40:6985–90. https://doi.org/10.1016/S0032-3861(98)00858-1.

[91] Seuring J, Agarwal S. Polymers with Upper Critical Solution Temperature in Aqueous Solution: Unexpected Properties from Known Building Blocks. ACS Macro Lett 2013;2:597–600. https://doi.org/10.1021/mz400227y.

[92] Seuring J, Agarwal S. First Example of a Universal and Cost-Effective Approach: Polymers with Tunable Upper Critical Solution Temperature in Water and Electrolyte Solution. Macromolecules 2012;45:3910–8. https://doi.org/10.1021/ma300355k.

[93] Xu Z, Liu W. Poly(N -acryloyl glycinamide): a fascinating polymer that exhibits a range of properties from UCST to high-strength hydrogels. Chem Commun 2018;54:10540–53. https://doi.org/10.1039/C8CC04614J.

[94] Blackman LD, Gunatillake PA, Cass P, Locock KES. An introduction to zwitterionic polymer behavior and applications in solution and at surfaces. Chem Soc Rev 2019;48:757–70. https://doi.org/10.1039/C8CS00508G.

[95] Zhang Q, Hoogenboom R. UCST behavior of polyampholytes based on stoichiometric RAFT copolymerization of cationic and anionic monomers. Chem Commun 2015;51:70–3. https://doi.org/10.1039/C4CC07930B.

[96] Banerjee P, Jana S, Mandal TK. Coulomb interaction-driven UCST in poly(ionic liquid) random copolymers. Eur Polym J 2020;133:109747. https://doi.org/10.1016/j.eurpolymj.2020.109747.

[97] Schulz DN, Peiffer DG, Agarwal PK, Larabee J, Kaladas JJ, Soni L, et al. Phase behaviour and solution properties of sulphobetaine polymers. Polymer (Guildf) 1986;27:1734–42. https://doi.org/10.1016/0032-3861(86)90269-7.

[98] Biswas Y, Maji T, Dule M, Mandal TK. Tunable doubly responsive UCST-type phosphonium poly(ionic liquid): a thermosensitive dispersant for carbon nanotubes. Polym Chem 2016;7:867–77. https://doi.org/10.1039/C5PY01574J.

[99] Wenisch SE, Schaffer A, Rieger B. Effect of Hofmeister Salts on the LCST of Poly(diethyl vinylphosphonate) and Poly(2-vinylpyridine- block- diethyl vinylphosphonate). Macromol Chem Phys 2022;223:2200063. https://doi.org/10.1002/macp.202200063.

[100] Bloksma MM, Bakker DJ, Weber C, Hoogenboom R, Schubert US. The Effect of Hofmeister Salts on the LCST Transition of Poly(2-oxazoline)s with Varying Hydrophilicity. Macromol Rapid Commun 2010;31:724–8. https://doi.org/10.1002/marc.200900843.

[101] Zhang Y, Furyk S, Bergbreiter DE, Cremer PS. Specific Ion Effects on the Water Solubility of Macromolecules: PNIPAM and the Hofmeister Series. J Am Chem Soc 2005;127:14505–10. https://doi.org/10.1021/ja0546424.

[102] Zajforoushan Moghaddam S, Thormann E. Hofmeister Effect on PNIPAM in Bulk and at an Interface: Surface Partitioning of Weakly Hydrated Anions. Langmuir 2017;33:4806–15. https://doi.org/10.1021/acs.langmuir.7b00953.

[103] Wang J-T, Wang L, Ji X, Liu L, Zhao H. Synthesis of Zwitterionic Diblock Copolymers with Cleavable Biotin Groups at the Junction Points and Fabrication of Bioconjugates by Biotin–Streptavidin Coupling. Macromolecules 2017;50:2284–95. https://doi.org/10.1021/acs.macromol.6b02665.

[104] Niskanen J, Tenhu H. How to manipulate the upper critical solution temperature (UCST)? Polym Chem 2017;8:220–32. https://doi.org/10.1039/c6py01612j.

[105] FitzGerald PA, Gupta S, Wood K, Perrier S, Warr GG. Temperature- and pH-Responsive Micelles with Collapsible Poly(N -isopropylacrylamide) Headgroups. Langmuir 2014;30:7986–92. https://doi.org/10.1021/la501861t.

[106] Strandman S, Zhu XX. Thermo-responsive block copolymers with multiple phase transition temperatures in aqueous solutions. Prog Polym Sci 2015;42:154–76. https://doi.org/10.1016/j.progpolymsci.2014.10.008.

[107] Adelsberger J, Kulkarni A, Jain A, Wang W, Bivigou-Koumba AM, Busch P, et al. Thermoresponsive PS- b -PNIPAM- b -PS Micelles: Aggregation Behavior, Segmental Dynamics, and Thermal Response. Macromolecules 2010;43:2490–501. https://doi.org/10.1021/ma902714p.

[108] Zhang Q, Weber C, Schubert US, Hoogenboom R. Thermoresponsive polymers with lower critical solution temperature: from fundamental aspects and measuring techniques to recommended turbidimetry conditions. Mater Horizons 2017;4:109–16. https://doi.org/10.1039/C7MH00016B.

[109] Mori H, Endo T. Amino-acid-based block copolymers by RAFT polymerization. Macromol Rapid Commun 2012;33:1090–107. https://doi.org/10.1002/marc.201100887.

[110] O'Reilly RK. Using controlled radical polymerisation techniques for the synthesis of functional polymers containing amino acidmoieties. Polym Int 2010;59:568–73. https://doi.org/10.1002/pi.2830.

[111] Mori H, Iwaya H, Nagai A, Endo T. Controlled synthesis of thermoresponsive polymers derived from L -proline via RAFT polymerization. ChemComm 2005:4872–4. https://doi.org/10.1039/b509212d.

[112] Safranj A, Yoshida M, Omichi H, Katakai R. Surfactant effect on the inverse volume phase transition of a polymer with amino acid side chains. Langmuir 1993;9:3338–40. https://doi.org/10.1021/la00036a003.

[113] Mori H, Kato I, Matsuyama M, Endo T. RAFT polymerization of acrylamides containing proline and hydroxyproline moiety: Controlled synthesis of water-soluble and thermoresponsive polymers. Macromolecules 2008;41:5604–15. https://doi.org/10.1021/ma800181h.

[114] Yamano T, Higashi N, Koga T. Precisely Synthesized Sequence-Controlled Amino Acid–Derived Vinyl Polymers: New Insights into Thermo-Responsive Polymer Design. Macromol Rapid Commun 2020;41:1–8. https://doi.org/10.1002/marc.201900550.

[115] Yu D, Luo C, Fu W, Li Z. New thermal-responsive polymers based on alanine and (meth)acryl amides. Polym Chem 2014;5:4561–8. https://doi.org/10.1039/c4py00480a.

[116] Higashi N, Hirata A, Nishimura S nosuke, Koga T. Thermo-responsive polymer brushes on glass plate prepared from a new class of amino acid-derived vinyl monomers and their applications in cell-sheet engineering. Colloids Surfaces B Biointerfaces 2017;159:39–46. https://doi.org/10.1016/j.colsurfb.2017.07.068.

[117] Higashi N, Sonoda R, Koga T. Thermo-responsive amino acid-based vinyl polymers showing widely tunable LCST/UCST behavior in water. RSC Adv 2015;5:67652–7. https://doi.org/10.1039/c5ra13009c.

[118] Nishimura SN, Higashi N, Koga T. A novel thermo-responsive multiblock architecture composed of a sequential peptide and an amino acid-derived vinyl polymer: Toward protein-mimicking single-chain folding. Chem Commun 2019;55:1498–501. https://doi.org/10.1039/c8cc09051c.

[119] Glatzel S, Badi N, Päch M, Laschewsky A, Lutz JF. Well-defined synthetic polymers with a protein-like gelation behavior in water. Chem Commun 2010;46:4517–9. https://doi.org/10.1039/c0cc00038h.

[120] Seuring J, Agarwal S. Non-ionic homo- and copolymers with H-donor and H-acceptor units with an UCST in water. Macromol Chem Phys 2010;211:2109–17. https://doi.org/10.1002/macp.201000147.

[121] Seuring J, Bayer FM, Huber K, Agarwal S. Upper critical solution temperature of poly(N -acryloyl glycinamide) in water: A concealed property. Macromolecules 2012;45:374–84. https://doi.org/10.1021/ma202059t.

[122] Glatzel S, Laschewsky A, Lutz JF. Well-defined uncharged polymers with a sharp UCST in water and in physiological milieu. Macromolecules 2011;44:413–5. https://doi.org/10.1021/ma102677k.

[123] Liu Z, Hu J, Sun J, He G, Li Y, Zhang G. Preparation of thermoresponsive polymers bearing amino acid diamide derivatives via RAFT polymerization. J Polym Sci Part A Polym Chem 2010;48:3573–86. https://doi.org/10.1002/pola.24137.

[124] Maji T, Banerjee S, Bose A, Mandal TK. A stimuli-responsive methionine-based zwitterionic methacryloyl sulfonium sulfonate monomer and the corresponding antifouling polymer with tunable thermosensitivity. Polym Chem 2017;8:3164–76. https://doi.org/10.1039/c7py00460e.

[125] Dinda P, Anas M, Banerjee P, Mandal TK. Dual Thermoresponsive Boc-Lysine-Based Acryl Polymer: RAFT Kinetics and Anti-Protein-Fouling of Its Zwitterionic Form. Macromolecules 2022;55:4011–24. https://doi.org/10.1021/acs.macromol.2c00633.

[126] Jana S, Anas M, Maji T, Banerjee S, Mandal TK. Tryptophan-based styryl homopolymer and polyzwitterions with solvent-induced UCST, ion-induced LCST and pH-induced UCST. Polym Chem 2019;10:526–38. https://doi.org/10.1039/c8py01512k.

[127] Maji T, Banerjee S, Biswas Y, Mandal TK. Dual-Stimuli-Responsive l -Serine-Based

Zwitterionic UCST-Type Polymer with Tunable Thermosensitivity. Macromolecules 2015;48:4957–66. https://doi.org/10.1021/acs.macromol.5b01099.

[128] Cheng J, Deming TJ. Synthesis of Polypeptides by Ring-Opening Polymerization of a-Amino Acid N-Carboxyanhydrides. 2012.

[129] Lowik DWPM, Leunissen EHP, Heuvel M Van Den, Hansen MB, Hest JCMV. Stimulus responsive peptide based materials. Chem Soc Rev 2010;39:3394–412. https://doi.org/10.1039/b914342b.

[130] Song Z, Tan Z, Cheng J. Recent Advances and Future Perspectives of Synthetic Polypeptides from N -Carboxyanhydrides. Macromolecules 2019;52:8521–39. https://doi.org/10.1021/acs.macromol.9b01450.

[131] Kricheldorf HR. Polypeptides and 100 Years of Chemistry of α-Amino AcidN-Carboxyanhydrides. Angew Chemie Int Ed 2006;45:5752–84. https://doi.org/10.1002/anie.200600693.

[132] Rasines Mazo A, Allison-Logan S, Karimi F, Chan NJ-A, Qiu W, Duan W, et al. Ring opening polymerization of α-amino acids: advances in synthesis, architecture and applications of polypeptides and their hybrids. Chem Soc Rev 2020;49:4737–834. https://doi.org/10.1039/C9CS00738E.

[133] Deming TJ. Facile synthesis of block copolypeptides of defined architecture. Nature 1997;390:386–9. https://doi.org/10.1038/37084.

[134] Grazon C, Salas-Ambrosio P, Ibarboure E, Buol A, Garanger E, Grinstaff MW, et al. Aqueous Ring-Opening Polymerization-Induced Self-Assembly (ROPISA) of N-Carboxyanhydrides. Angew Chemie - Int Ed 2020;59:622–6. https://doi.org/10.1002/anie.201912028.

[135] Bonduelle C. Secondary structures of synthetic polypeptide polymers. Polym Chem 2018;9:1517–29. https://doi.org/10.1039/C7PY01725A.

[136] Carlsen A, Lecommandoux S. Self-assembly of polypeptide-based block copolymer amphiphiles. Curr Opin Colloid Interface Sci 2009;14:329–39. https://doi.org/10.1016/j.cocis.2009.04.007.

[137] Nguyen TP, Easley AD, Kang N, Khan S, Lim SM, Rezenom YH, et al. Polypeptide organic radical batteries. Nature 2021;593:61–6. https://doi.org/10.1038/s41586-021-03399-1.

[138] Krishna OD, Kiick KL. Protein- and peptide-modified synthetic polymeric biomaterials. Biopolymers 2010;94:32–48. https://doi.org/10.1002/bip.21333.

[139] Hoogenboom R, Schlaad H. Thermoresponsive poly(2-oxazoline)s, polypeptoids, and polypeptides. Polym Chem 2017;8:24–40. https://doi.org/10.1039/C6PY01320A.

[140] He C, Zhao C, Guo X, Guo Z, Chen X, Zhuang X, et al. Novel temperature- and pH-responsive graft copolymers composed of poly(L-glutamic acid) and poly(N-isopropylacrylamide). J Polym Sci Part A Polym Chem 2008;46:4140–50. https://doi.org/10.1002/pola.22760.

[141] Wei Z, Zhu S, Zhao H. Brush macromolecules with thermo-sensitive coil backbones and pendant polypeptide side chains: Synthesis, self-assembly and functionalization. Polym Chem 2015;6:1316–24. https://doi.org/10.1039/c4py01268b.

[142] Zhao C, He P, Xiao C, Gao X, Zhuang X, Chen X. Synthesis of temperature and pH-responsive crosslinked micelles from polypeptide-based graft copolymer. J Colloid Interface Sci 2011;359:436–42. https://doi.org/10.1016/j.jcis.2011.04.037.

[143] Anas M, Jana S, Mandal TK. Vesicular assemblies of thermoresponsive amphiphilic polypeptide copolymers for guest encapsulation and release. Polym Chem 2020;11:2889–903. https://doi.org/10.1039/d0py00135j.

[144] Bose A, Jana S, Saha A, Mandal TK. Amphiphilic polypeptide-polyoxazoline graft copolymer conjugate with tunable thermoresponsiveness: Synthesis and self-assembly into various micellar structures in aqueous and nonaqueous media. Polymer (Guildf) 2017;110:12–24. https://doi.org/10.1016/j.polymer.2016.12.068.

[145] Rao J, Luo Z, Ge Z, Liu H, Liu S. "Schizophrenic" Micellization Associated with Coil-to-Helix Transitions Based on Polypeptide Hybrid Double Hydrophilic Rod–Coil Diblock Copolymer. Biomacromolecules 2007;8:3871–8. https://doi.org/10.1021/bm700830b.

[146] He C, Zhao C, Chen X, Guo Z, Zhuang X, Jing X. Novel pH- and Temperature-Responsive Block Copolymers with Tunable pH-Responsive Range. Macromol Rapid Commun 2008;29:490–7. https://doi.org/10.1002/marc.200700721.

[147] Sánchez-Ferrer A, Kotharangannagari VK, Ruokolainen J, Mezzenga R. Thermo-responsive peptide-based triblock copolymer hydrogels. Soft Matter 2013;9:4304–11. https://doi.org/10.1039/c3sm27690b.

[148] Huang Y, Luo W, Ye G. Synthesis, characterization, conformation and self-assembly behavior of polypeptide-based brush with oligo (ethylene glycol) side chains. J Mol Struct 2015;1081:274–80. https://doi.org/10.1016/j.molstruc.2014.10.045.

[149] Zhao C, Zhuang X, He C, Chen X, Jing X. Synthesis of novel thermo- and pH-responsive poly(L-lysine)-based copolymer and its micellization in water. Macromol Rapid Commun 2008;29:1810–6. https://doi.org/10.1002/marc.200800494.

[150] Zhang X, Li J, Li W, Zhang A. Synthesis and characterization of thermo- and pH-responsive double-hydrophilic diblock copolypeptides. Biomacromolecules 2007;8:3557–67. https://doi.org/10.1021/bm700729t.

[151] Mokrus M, Menzel H. Thermoresponsive Glycopolypeptide Containing Block Copolymers, Particle Formation, and Lectin Interaction. Macromol Biosci 2022;22. https://doi.org/10.1002/mabi.202100518.

[152] Agut W, Brûlet A, Schatz C, Taton D, Lecommandoux S. PH and temperature responsive polymeric micelles and polymersomes by self-assembly of poly[2-(dimethylamino)ethyl methacrylate]- b -poly(glutamic acid) double hydrophilic block copolymers. Langmuir 2010;26:10546–54. https://doi.org/10.1021/la1005693.

[153] Wu Q, Zhou D, Kang R, Tang X, Yang Q, Song X, et al. Synthesis and self-assembly of thermoresponsive amphiphilic biodegradable polypeptide/poly(ethyl ethylene phosphate) block copolymers. Chem - An Asian J 2014;9:2850–8. https://doi.org/10.1002/asia.201402524.

[154] Tachibana Y, Kurisawa M, Uyama H, Kobayashi S. Thermo- and pH-Responsive Biodegradable Poly(α- N -substituted γ-glutamine)s. Biomacromolecules 2003;4:1132–4. https://doi.org/10.1021/bm034123y.

[155] De Leon Rodriguez LM, Hemar Y, Cornish J, Brimble MA. Structure–mechanical property correlations of hydrogel forming β-sheet peptides. Chem Soc Rev 2016;45:4797–824. https://doi.org/10.1039/C5CS00941C.

[156] Kopeček J, Yang J. Peptide-directed self-assembly of hydrogels. Acta Biomater 2009;5:805–16. https://doi.org/10.1016/j.actbio.2008.10.001.

[157] Chen J, Zou X. Self-assemble peptide biomaterials and their biomedical applications.

Bioact Mater 2019;4:120–31. https://doi.org/10.1016/j.bioactmat.2019.01.002.

[158] Zhou X, Li Z. Advances and Biomedical Applications of Polypeptide Hydrogels Derived from α-Amino Acid N-Carboxyanhydride (NCA) Polymerizations. Adv Healthc Mater 2018;7:1–18. https://doi.org/10.1002/adhm.201800020.

[159] Cai L, Liu S, Guo J, Jia YG. Polypeptide-based self-healing hydrogels: Design and biomedical applications. Acta Biomater 2020;113:84–100. https://doi.org/10.1016/j.actbio.2020.07.001.

[160] He X, Fan J, Wooley KL. Stimuli-Triggered Sol-Gel Transitions of Polypeptides Derived from α-Amino Acid N -Carboxyanhydride (NCA) Polymerizations. Chem - An Asian J 2016;11:437–47. https://doi.org/10.1002/asia.201500957.

[161] Zhu H, Liu Y, Gu D, Rao Z, Li Y, Hao J. Dual thermoresponsive mPEG-b-poly(O-benzyl-L-threonine acid) hydrogel based on β-sheet nano-structural disassembly and PEG dehydration. Polymer (Guildf) 2021;226:123841. https://doi.org/10.1016/j.polymer.2021.123841.

[162] Zhu H, Gu D, Rao Z, Li Y, Liu Y, Hao J. Design of gel-to-sol UCST transition peptides by controlling polypeptide β-sheet nanostructures. Polym J 2021;53:943–9. https://doi.org/10.1038/s41428-021-00490-2.

[163] Huang J, Hastings CL, Duffy GP, Kelly HM, Raeburn J, Adams DJ, et al. Supramolecular hydrogels with reverse thermal gelation properties from (Oligo)tyrosine containing block copolymers. Biomacromolecules 2013;14:200–6. https://doi.org/10.1021/bm301629f.

[164] Zhu H, Zhu M, Shuai S, Zhao C, Liu Y, Liu Y, et al. Effect of polypeptide block length on nano-assembly morphology and thermo-sensitivity of methyl poly (ethylene glycol)-poly (L-valine) copolymer aqueous solutions. J Sol-Gel Sci Technol 2019;92:618–27. https://doi.org/10.1007/s10971-019-05095-z.

[165] Zhao S, Zhu H, Chen Z, Shuai S, Zhang N, Liu Y, et al. Preparation and properties of a temperature- and pH- responsive polypeptide hydrogel. Mater Res Express 2019;6. https://doi.org/10.1088/2053-1591/ab253e.

[166] Fan J, Li R, Wang H, He X, Nguyen TP, Letteri RA, et al. Multi-responsive polypeptide hydrogels derived from: N -carboxyanhydride terpolymerizations for delivery of nonsteroidal anti-inflammatory drugs. Org Biomol Chem 2017;15:5145–54. https://doi.org/10.1039/c7ob00931c.

[167] Zhang Y, Song H, Zhang H, Huang P, Liu J, Chu L, et al. Fine tuning the assembly and gel behaviors of PEGylated polypeptide conjugates by the copolymerization of l-alanine and γ-benzyl-l-glutamate N-carboxyanhydrides. J Polym Sci Part A Polym Chem 2017;55:1512–23. https://doi.org/10.1002/pola.28516.

[168] Choi BG, Park MH, Cho SH, Joo MK, Oh HJ, Kim EH, et al. In situ thermal gelling polypeptide for chondrocytes 3D culture. Biomaterials 2010;31:9266–72. https://doi.org/10.1016/j.biomaterials.2010.08.067.

[169] Choi YY, Jang JH, Park MH, Choi BG, Chi B, Jeong B. Block length affects secondary structure, nanoassembly and thermosensitivity of poly(ethylene glycol)-poly(l-alanine) block copolymers. J Mater Chem 2010;20:3416–21. https://doi.org/10.1039/b922956f.

[170] Kim JY, Park MH, Joo MK, Lee SY, Jeong B. End groups adjusting the molecular nano-assembly pattern and thermal gelation of polypeptide block copolymer aqueous solution. Macromolecules 2009;42:3147–51. https://doi.org/10.1021/ma900341m.

[171] Jeong Y, Joo MK, Sohn YS, Jeong B. Reverse thermal organogels. Adv Mater

2007;19:3947–50. https://doi.org/10.1002/adma.200700149.

[172] Jeong Y, Joo MK, Bahk KH, Choi YY, Kim HT, Kim WK, et al. Enzymatically degradable temperature-sensitive polypeptide as a new in-situ gelling biomaterial. J Control Release 2009;137:25–30. https://doi.org/10.1016/j.jconrel.2009.03.008.

[173] Joo MK, Ko DY, Jeong SJ, Park MH, Shinde UP, Jeong B. Incorporation of d-alanine into poly(ethylene glycol) and l-poly(alanine-co-phenylalanine) block copolymers affects their nanoassemblies and enzymatic degradation. Soft Matter 2013;9:8014–22. https://doi.org/10.1039/c3sm51272j.

[174] Cheng Y, He C, Xiao C, Ding J, Zhuang X, Huang Y, et al. Decisive role of hydrophobic side groups of polypeptides in thermosensitive gelation. Biomacromolecules 2012;13:2053–9. https://doi.org/10.1021/bm3004308.

[175] Shimokuri T, Kaneko T, Akashi M. Specific thermosensitive volume change of biopolymer gels derived from propylated poly(γ-glutamate)s. J Polym Sci Part A Polym Chem 2004;42:4492–501. https://doi.org/10.1002/pola.20384.

[176] Cheng Y, He C, Xiao C, Ding J, Cui H, Zhuang X, et al. Versatile biofunctionalization of polypeptide-based thermosensitive hydrogels via click chemistry. Biomacromolecules 2013;14:468–75. https://doi.org/10.1021/bm3017059.

[177] He X, Fan J, Zhang F, Li R, Pollack KA, Raymond JE, et al. Multi-responsive hydrogels derived from the self-assembly of tethered allyl-functionalized racemic oligopeptides. J Mater Chem B 2014;2:8123–30. https://doi.org/10.1039/c4tb00909f.

[178] Shi J, Yu L, Ding J. PEG-based thermosensitive and biodegradable hydrogels. Acta Biomater 2021;128:42–59. https://doi.org/10.1016/j.actbio.2021.04.009.

[179] Le Fer G, Wirotius AL, Brûlet A, Garanger E, Lecommandoux S. Self-Assembly of Stimuli-Responsive Biohybrid Synthetic- b-Recombinant Block Copolypeptides. Biomacromolecules 2019;20:254–72. https://doi.org/10.1021/acs.biomac.8b01390.

[180] Chen C, Wang Z, Li Z. Thermoresponsive polypeptides from pegylated poly-l-glutamates. Biomacromolecules 2011;12:2859–63. https://doi.org/10.1021/bm200849m.

[181] Zhang S, Chen C, Li Z. Effects of molecular weight on thermal responsive property of pegylated poly-l-glutamates. Chinese J Polym Sci (English Ed 2013;31:201–10. https://doi.org/10.1007/s10118-013-1218-7.

[182] Shen J, Chen C, Fu W, Shi L, Li Z. Conformation-specific self-assembly of thermo-responsive Poly(ethylene glycol)- b -polypeptide Diblock Copolymer. Langmuir 2013;29:6271–8. https://doi.org/10.1021/la401095s.

[183] Zhang S, Fu W, Li Z. Supramolecular hydrogels assembled from nonionic poly(ethylene glycol)-b-polypeptide diblocks containing OEGylated poly-l-glutamate. Polym Chem 2014;5:3346–51. https://doi.org/10.1039/c4py00016a.

[184] Liao Y, Dong CM. Synthesis, conformation transition, liquid crystal phase, and self-assembled morphology of thermosensitive homopolypeptide. J Polym Sci Part A Polym Chem 2012;50:1834–43. https://doi.org/10.1002/pola.25953.

[185] Liu D, Yuan Q, Ling Y, Tang H. Synthesis and Properties of Side-Chain Liquid Crystalline Polypeptides Bearing Various Alkyl Spacers and Oligo-Ethylene-Glycol Tails. Macromol Chem Phys 2015;216:2270–8. https://doi.org/10.1002/macp.201500260.

[186] Yuan Q lin, Liu W jun, Deng Y, Ling Y, Tang H yu. Synthesis, characterization and

phase behaviors of polypeptides bearing biphenyl mesogens and oligo-ethylene-glycol tails. Chinese J Polym Sci (English Ed 2015;33:1150–61. https://doi.org/10.1007/s10118-015-1665-4.

[187] Shen Y, Desseaux S, Aden B, Lokitz BS, Kilbey SM, Li Z, et al. Shape-persistent, thermoresponsive polypeptide brushes prepared by vapor deposition surface-initiated ring-opening polymerization of α-amino acid N-carboxyanhydrides. Macromolecules 2015;48:2399–406. https://doi.org/10.1021/acs.macromol.5b00017.

[188] Yu L, Fu W, Li Z. Tuning the phase transition temperature of thermal-responsive OEGylated poly-L-glutamate via random copolymerization with L-alanine. Soft Matter 2015;11:545–50. https://doi.org/10.1039/c4sm02270j.

[189] Zhang S, Alvarez DJ, Sofroniew M V., Deming TJ. Design and Synthesis of Nonionic Copolypeptide Hydrogels with Reversible Thermoresponsive and Tunable Physical Properties. Biomacromolecules 2015;16:1331–40. https://doi.org/10.1021/acs.biomac.5b00124.

[190] Cheng Y, He C, Xiao C, Ding J, Zhuang X, Chen X. Versatile synthesis of temperature-sensitive polypeptides by click grafting of oligo(ethylene glycol). Polym Chem 2011;2:2627. https://doi.org/10.1039/c1py00281c.

[191] Kapetanakis A, Heise A. Thermoresponsive glycopolypeptides with temperature controlled selective lectin binding properties. Eur Polym J 2015;69:483–9. https://doi.org/10.1016/j.eurpolymj.2015.01.033.

[192] Yang Y, Wu Y, Li R, Ling Y, Tang H. Water-Soluble Copolypeptides with Oligo-Ethylene-Glycol and Benzyl Pendants: Synthesis, Characterization, and Thermoresponsive Properties. Aust J Chem 2016;69:112–8. https://doi.org/10.1071/CH15276.

[193] Zhou C, Shi Z, Xu F, Ling Y, Tang H. Preparation and properties of thermo- and pH-responsive polypeptide bearing OEG and aldehyde pendants. Colloid Polym Sci 2020;298:1293–302. https://doi.org/10.1007/s00396-020-04712-6.

[194] Ge C, Liu W, Ling Y, Tang H. Synthesis and thermoresponsive properties of OEGylated polypeptide with a LCST at body temperature in water and with a UCST in alcohol or ethanol/water solvent mixture. J Polym Sci Part A Polym Chem 2018;56:163–73. https://doi.org/10.1002/pola.28868.

[195] Xu Y, Zhu M, Li M, Ling Y, Tang H. Synthesis and LCST-type phase behavior of water-soluble polypeptide with Y-shaped and charged side-chains. Polym Chem 2016;7:1922–30. https://doi.org/10.1039/c5py01991e.

[196] Fu X, Ma Y, Sun J, Li Z. Biodegradable thermal- and redox-responsive poly(l-glutamate) with Y-shaped oligo(ethylene glycol) side-chain and tunable phase transition temperature. RSC Adv 2016;6:70243–50. https://doi.org/10.1039/c6ra17427b.

[197] Meng F, Fu X, Ni Y, Sun J, Li Z. Biomimetic polypeptides with reversible pH-dependent thermal responsive property. Polymer (Guildf) 2017;118:173–9. https://doi.org/10.1016/j.polymer.2017.04.059.

[198] Fu X hui, Ma Y nan, Sun J, Li Z bo. Thermal and redox dual responsive poly(L-glutamate) with oligo(ethylene glycol) side-chains. Chinese J Polym Sci (English Ed 2016;34:1436–47. https://doi.org/10.1007/s10118-016-1861-x.

[199] Liang C, Wang X, Zhou R, Shi H, Yan S, Ling Y, et al. Thermo- and oxidation-responsive homopolypeptide: Synthesis, stimuli-responsive property and antimicrobial activity. Polym Chem 2019;10:2190–202. https://doi.org/10.1039/c8py01735b.

[200] Zhang L, Zhao L, Ling Y, Tang H. Unusual light-tunable thermoresponsive behavior of OEGylated homopolypeptide with azobenzene and thioether spacers. Eur Polym J 2019;111:38–42. https://doi.org/10.1016/j.eurpolymj.2018.12.013.

[201] Lavilla C, Byrne M, Heise A. Block-Sequence-Specific Polypeptides from α-Amino Acid N-Carboxyanhydrides: Synthesis and Influence on Polypeptide Properties. Macromolecules 2016;49:2942–7. https://doi.org/10.1021/acs.macromol.6b00498.

[202] He X, Zhou R, Ge C, Ling Y, Luan S, Tang H. OEGylated polypeptide bearing Y-Shaped pendants with a LCST close to body temperature: Synthesis and thermoresponsive properties. Eur Polym J 2019;112:547–54. https://doi.org/10.1016/j.eurpolymj.2018.10.011.

[203] He X, Wang X, Zhou R, Lu H, Luan S, Tang H. Synthesis and Thermoresponsive Properties of Biocompatible and Biodegradable Triblock Copolymers Bearing Linear or Y-Shaped OEG Pendants. Macromol Chem Phys 2020;221:1–11. https://doi.org/10.1002/macp.201900421.

[204] Lu D, Wang H, Li T, Li Y, Wang X, Niu P, et al. Versatile Surgical Adhesive and Hemostatic Materials: Synthesis, Properties, and Application of Thermoresponsive Polypeptides. Chem Mater 2017;29:5493–503. https://doi.org/10.1021/acs.chemmater.7b00255.

[205] Lu D, Wang H, Li T, Li Y, Dou F, Sun S, et al. Mussel-Inspired Thermoresponsive Polypeptide-Pluronic Copolymers for Versatile Surgical Adhesives and Hemostasis. ACS Appl Mater Interfaces 2017;9:16756–66. https://doi.org/10.1021/acsami.6b16575.

[206] Jiang Y, Wang S, Zhang X, Tao Y, Wang X. Controlled metal-free polymerization toward well-defined thermoresponsive polypeptides by polymerization at low temperature. J Polym Sci Part A Polym Chem 2016;54:2618–24. https://doi.org/10.1002/pola.28140.

[207] Ding J, Zhao L, Li D, Xiao C, Zhuang X, Chen X. Thermo-responsive "hairy-rod" polypeptides for smart antitumor drug delivery. Polym Chem 2013;4:3345–56. https://doi.org/10.1039/c3py00144j.

[208] Ge C, Zhao L, Ling Y, Tang H. Thermo and pH dual responsive polypeptides derived from "clickable" poly(γ-3-methylthiopropyl-l-glutamate). Polym Chem 2017;8:1895–905. https://doi.org/10.1039/c7py00170c.

[209] Shi Z, Zhang X, Wang X, Yang F, Yu Z, Ling Y, et al. Synthesis and Properties of Mono- or Diamine-Initiated Imidazolium-Based Cationic Polypeptides. Biomacromolecules 2020;21:3468–78. https://doi.org/10.1021/acs.biomac.0c00953.

[210] Ge C, Liu S, Liang C, Ling Y, Tang H. Synthesis and UCST-type phase behavior of α-helical polypeptides with Y-shaped and imidazolium pendants. Polym Chem 2016;7:5978–87. https://doi.org/10.1039/c6py01287f.

[211] Zhao L, Zhang L, Zheng Z, Ling Y, Tang H. Synthesis and Properties of UCST-Type Thermo- and Light-Responsive Homopolypeptides with Azobenzene Spacers and Imidazolium Pendants. Macromol Chem Phys 2019;220:1–13. https://doi.org/10.1002/macp.201900061.

[212] Huang L, Sun L, Zhou C, Ling Y, Lu H, Luan S, et al. Preparation and Properties of UCST-Type Thermoresponsive Polypeptide Bearing Amide Pendants. Macromol Chem Phys 2020;221:1–9. https://doi.org/10.1002/macp.201900549.

[213] Liu W, Zhu M, Xiao J, Ling Y, Tang H. Synthesis and UCST-type phase behavior of polypeptide with alkyl side-chains in alcohol or ethanol/water solvent mixtures. J Polym

Sci Part A Polym Chem 2016;54:3425–35. https://doi.org/10.1002/pola.28230.

[214] Ge C, Ling Y, Yan S, Luan S, Zhang H, Tang H. Preparation and mechanical properties of strong and tough poly (vinyl alcohol)-polypeptide double-network hydrogels. Eur Polym J 2018;99:504–10. https://doi.org/10.1016/j.eurpolymj.2018.01.005.

[215] Zheng Z, Zhang L, Ling Y, Tang H. Triblock copolymers containing UCST polypeptide and poly(propylene glycol): Synthesis, thermoresponsive properties, and modification of PVA hydrogel. Eur Polym J 2019;115:244–50. https://doi.org/10.1016/j.eurpolymj.2019.03.034.

[216] Deng Y, Xu Y, Wang X, Yuan Q, Ling Y, Tang H. Water-soluble thermoresponsive α-helical polypeptide with an upper critical solution temperature: Synthesis, characterization, and thermoresponsive phase transition behaviors. Macromol Rapid Commun 2015;36:453–8. https://doi.org/10.1002/marc.201400625.

[217] Liu S, Ge C, Ling Y, Tang H. Preparation and UCST-type phase behaviours of poly(γ-4-methylbenzyl-L-glutamate) pyridinium tetrafluoroborate conjugates in methanol or water. Aust J Chem 2017;70:245–51. https://doi.org/10.1071/CH16344.

[218] Wu Y, Wang X, Ling Y, Tang H. Preparation and thermoresponsive properties of helical polypeptides bearing pyridinium salts. RSC Adv 2015;5:40772–8. https://doi.org/10.1039/c5ra04541j.

[219] Xiao C, Cheng Y, Zhang Y, Ding J, He C, Zhuang X, et al. Side chain impacts on pH- and thermo-responsiveness of tertiary amine functionalized polypeptides. J Polym Sci Part A Polym Chem 2014;52:671–9. https://doi.org/10.1002/pola.27048.

[220] Lin Z, Ding J, Chen X, He C. pH- and Temperature-responsive Hydrogels Based on Tertiary Amine-modified Polypeptides for Stimuli-responsive Drug Delivery. Chem - An Asian J Asian J 2023. https://doi.org/10.1002/asia.202300021.

[221] Shimokuri T, Kaneko T, Serizawa T, Akashi M. Preparation and thermosensitivity of naturally occurring polypeptide poly(γ-glutamic acid) derivatives modified by propyl groups. Macromol Biosci 2004;4:407–11. https://doi.org/10.1002/mabi.200300073.

[222] Liu Y, Zhao C, Chen C. Chirality-Governed UCST Behavior in Polypeptides. Macromolecules 2022;55:3801–10. https://doi.org/10.1021/acs.macromol.2c00291.

[223] Li M, Xu Y, Liu T, Li Y, Ling Y, Tang H. Preparation and Thermoresponsive Properties of UCST-Type Polypeptide Bearing p -Tolyl Pendants and 3-Methyl-1,2,3-triazolium Linkages in Methanol or Ethanol/Water Solvent Mixtures. Macromol Chem Phys 2017;218:1700006. https://doi.org/10.1002/macp.201700006.

[224] Li M, Wang X, Xu Y, Ling Y, Tang H. Preparation of glycopolypeptides bearing mannose moieties and biphenyl pendants and their upper-critical-solution-temperature-type thermoresponsive properties in alcohol/water solvent mixtures. Polym Int 2016;65:1493–500. https://doi.org/10.1002/pi.5259.

[225] Li M, He X, Ling Y, Tang H. Dual thermoresponsive homopolypeptide with LCST-type linkages and UCST-type pendants: Synthesis, characterization, and thermoresponsive properties. Polymer (Guildf) 2017;132:264–72. https://doi.org/10.1016/j.polymer.2017.11.016.

[226] Ji Y, Zhu M, Gong Y, Tang H, Li J, Cao Y. Thermoresponsive Polymers with Lower Critical Solution Temperature- or Upper Critical Solution Temperature-Type Phase Behaviour Do Not Induce Toxicity to Human Endothelial Cells. Basic Clin Pharmacol Toxicol 2017;120:79–85. https://doi.org/10.1111/bcpt.12643.

[227] Stamou A, Iatrou H, Tsitsilianis C. NIPAm-Based Modification of Poly(L-lysine): A

pH-Dependent LCST-Type Thermo-Responsive Biodegradable Polymer. Polymers (Basel) 2022;14. https://doi.org/10.3390/polym14040802.

[228] Yan J, Liu K, Li W, Shi H, Zhang A. Thermoresponsive Dendronized Polypeptides Showing Switchable Recognition to Catechols. Macromolecules 2016;49:510–7. https://doi.org/10.1021/acs.macromol.5b02259.

[229] Ma Y, Fu X, Shen Y, Fu W, Li Z. Irreversible Low Critical Solution Temperature Behaviors of Thermal-responsive OEGylated Poly(L-cysteine) Containing Disulfide Bonds. Macromolecules 2014;47:4684–9. https://doi.org/10.1021/ma501104s.

[230] Fu X, Shen Y, Fu W, Li Z. Thermoresponsive oligo(ethylene glycol) functionalized poly- l -cysteine. Macromolecules 2013;46:3753–60. https://doi.org/10.1021/ma400678w.

[231] Fu X, Ma Y, Shen Y, Fu W, Li Z. Oxidation-responsive OEGylated poly-l-cysteine and solution properties studies. Biomacromolecules 2014;15:1055–61. https://doi.org/10.1021/bm5000554.

[232] Kramer JR, Deming TJ. Multimodal switching of conformation and solubility in homocysteine derived polypeptides. J Am Chem Soc 2014;136:5547–50. https://doi.org/10.1021/ja500372u.

[233] Gharakhanian EG, Deming TJ. Role of Side-Chain Molecular Features in Tuning Lower Critical Solution Temperatures (LCSTs) of Oligoethylene Glycol Modified Polypeptides. J Phys Chem B 2016;120:6096–101. https://doi.org/10.1021/acs.jpcb.6b02065.

[234] Xiao J, Li M, Liu W, Li Y, Ling Y, Tang H. Synthesis and thermoresponsive properties of poly(L-cysteine)s bearing imidazolium salts. Eur Polym J 2017;88:340–8. https://doi.org/10.1016/j.eurpolymj.2017.01.036.

[235] Anas M, Dinda P, Kar M, Mandal TK. Anion-induced thermoresponsiveness in cationic polycysteine and DNA binding. Polym Chem 2021;12:6329–43. https://doi.org/10.1039/d1py01187a.

[236] Perlin P, Scott WA, Deming TJ. Modification of Poly(5,6-epoxy-l-norleucine) Gives Functional Polypeptides with Alternative Side-Chain Linkages. Biomacromolecules 2020;21:126–32. https://doi.org/10.1021/acs.biomac.9b01065.

[237] Jana S, Biswas Y, Mandal TK. Methionine-based cationic polypeptide/polypeptide block copolymer with triple-stimuli responsiveness: DNA polyplexation and phototriggered release. Polym Chem 2018;9:1869–84. https://doi.org/10.1039/c8py00178b.

[238] Ohya Y, Toyohara M, Sasakawa M, Arimura H, Ouchi T. Thermosensitive biodegradable polydepsipeptide. Macromol Biosci 2005;5:273–6. https://doi.org/10.1002/mabi.200400221.

[239] Ishido Y, Kanbayashi N, Okamura T aki, Onitsuka K. Conformational Switch of Arylopeptide: Helix–Helix Transition Based on Side Chain Solvation. Macromol Rapid Commun 2021;42:1–8. https://doi.org/10.1002/marc.202100250.

[240] Tsubasa A, Otsuka S, Maekawa T, Takano R, Sakurai S, Deming TJ, et al. Development of hybrid diblock copolypeptide amphiphile/magnetic metal complexes and their spin crossover with lower-critical-solution-temperature(LCST)-type transition. Polymer (Guildf) 2017;128:347–55. https://doi.org/10.1016/j.polymer.2016.12.079.

[241] Fetsch C, Gaitzsch J, Messager L, Battaglia G, Luxenhofer R. Self-Assembly of Amphiphilic Block Copolypeptoids – Micelles, Worms and Polymersomes. Sci Rep

2016;6:33491. https://doi.org/10.1038/srep33491.

[242] Sternhagen GL, Gupta S, Zhang Y, John V, Schneider GJ, Zhang D. Solution Self-Assemblies of Sequence-Defined Ionic Peptoid Block Copolymers. J Am Chem Soc 2018;140:4100–9. https://doi.org/10.1021/jacs.8b00461.

[243] Guo L, Li J, Brown Z, Ghale K, Zhang D. Synthesis and characterization of cyclic and linear helical poly(α-peptoid)s by N-heterocyclic carbene-mediated ring-opening polymerizations of N-substituted N-carboxyanhydrides. Biopolymers 2011;96:596–603. https://doi.org/10.1002/bip.21597.

[244] Fetsch C, Grossmann A, Holz L, Nawroth JF, Luxenhofer R. Polypeptoids from N -Substituted Glycine N -Carboxyanhydrides: Hydrophilic, Hydrophobic, and Amphiphilic Polymers with Poisson Distribution. Macromolecules 2011;44:6746–58. https://doi.org/10.1021/ma201015y.

[245] Fetsch C, Luxenhofer R. Thermal Properties of Aliphatic Polypeptoids. Polymers (Basel) 2013;5:112–27. https://doi.org/10.3390/polym5010112.

[246] Lee C-U, Li A, Ghale K, Zhang D. Crystallization and Melting Behaviors of Cyclic and Linear Polypeptoids with Alkyl Side Chains. Macromolecules 2013;46:8213–23. https://doi.org/10.1021/ma401067f.

[247] Chan BA, Xuan S, Li A, Simpson JM, Sternhagen GL, Yu T, et al. Polypeptoid polymers: Synthesis, characterization, and properties. Biopolymers 2018;109. https://doi.org/10.1002/bip.23070.

[248] Robinson JW, Secker C, Weidner S, Schlaad H. Thermoresponsive Poly(N -C3 glycine)s. Macromolecules 2013;46:580–7. https://doi.org/10.1021/ma302412v.

[249] Tian J, Sun J, Li Z. Biomimetic pegylated polypeptoids with thermoresponsive properties. Polymer (Guildf) 2018;138:132–8. https://doi.org/10.1016/j.polymer.2018.01.034.

[250] Xing C, Shi Z, Tian J, Sun J, Li Z. Charge-Determined LCST/UCST Behavior in Ionic Polypeptoids. Biomacromolecules 2018;19:2109–16. https://doi.org/10.1021/acs.biomac.8b00240.

[251] Fu X, Tian J, Li Z, Sun J, Li Z. Dual-responsive pegylated polypeptoids with tunable cloud point temperatures. Biopolymers 2019;110. https://doi.org/10.1002/bip.23243.

[252] Tao X, Du J, Wang Y, Ling J. Polypeptoids with tunable cloud point temperatures synthesized from N-substituted glycine N-thiocarboxyanhydrides. Polym Chem 2015;6:3164–74. https://doi.org/10.1039/c5py00191a.

[253] Fu X, Xing C, Sun J. Tunable LCST/UCST-Type Polypeptoids and Their Structure–Property Relationship. Biomacromolecules 2020;21:4980–8. https://doi.org/10.1021/acs.biomac.0c01177.

[254] Xuan S, Lee CU, Chen C, Doyle AB, Zhang Y, Guo L, et al. Thermoreversible and Injectable ABC Polypeptoid Hydrogels: Controlling the Hydrogel Properties through Molecular Design. Chem Mater 2016;28:727–37. https://doi.org/10.1021/acs.chemmater.5b03528.

[255] Lahasky SH, Hu X, Zhang D. Thermoresponsive poly(α-peptoid)s: Tuning the cloud point temperatures by composition and architecture. ACS Macro Lett 2012;1:580–4. https://doi.org/10.1021/mz300017y.

[256] Lahasky SH, Lu L, Huberty WA, Cao J, Guo L, Garno JC, et al. Synthesis and characterization of thermo-responsive polypeptoid bottlebrushes. Polym Chem

2014;5:1418–26. https://doi.org/10.1039/c3py01356a.

[257] Okuno Y, Nishimura T, Sasaki Y, Akiyoshi K. Thermoresponsive Carbohydrate- b-Polypeptoid Polymer Vesicles with Selective Solute Permeability and Permeable Factors for Solutes. Biomacromolecules 2021;22:3099–106. https://doi.org/10.1021/acs.biomac.1c00530.

[258] Li R, Ding X, Ding J, Sun J. Tunable Nanostructure Assembled from Dual-Responsive Crystalline Block Copolypeptoids. ACS Appl Polym Mater 2022:4–10. https://doi.org/10.1021/acsapm.2c00387.

[259] Kurzhals S, Pretzner B, Reimhult E, Zirbs R. Thermoresponsive Polypeptoid-Coated Superparamagnetic Iron Oxide Nanoparticles by Surface-Initiated Polymerization. Macromol Chem Phys 2017;218:1700116. https://doi.org/10.1002/macp.201700116.

[260] Lu L, Lahasky SH, McCandless GT, Zhang D, Garno JC. Thermoresponsive Behavior of Polypeptoid Nanostructures Investigated with Heated Atomic Force Microscopy: Implications toward the Development of Smart Coatings for Surface-Based Sensors. ACS Appl Nano Mater 2019;2:7617–25. https://doi.org/10.1021/acsanm.9b01715.

[261] Li R, Ni Y, Liu D, Li Z, Cheng Y, Xia M, et al. Synthesis and Characterization of Thermoresponsive Polypeptide/polypeptoid Block Copolymers. Gaodeng Xuexiao Huaxue Xuebao/Chemical J Chinese Univ 2021;42:850–6. https://doi.org/10.7503/cjcu20200651.

[262] Al Samad A, De Winter J, Gerbaux P, Jérôme C, Debuigne A. Unique alternating peptide-peptoid copolymers from dipeptides via a Ugi reaction in water. Chem Commun 2017;53:12240–3. https://doi.org/10.1039/c7cc06463b.

[263] Ihsan A Bin, Nargis M, Koyama Y. Effects of the hydrophilic-lipophilic balance of alternating peptides on self-assembly and thermo-responsive behaviors. Int J Mol Sci 2019;20:1–11. https://doi.org/10.3390/ijms20184604.

[264] Stiernet P, Couturaud B, Bertrand V, Eppe G, De Winter J, Debuigne A. Ugi four-component polymerization of amino acid derivatives: A combinatorial tool for the design of polypeptoids. Polym Chem 2021;12:2141–51. https://doi.org/10.1039/d1py00109d.

[265] Komuro N, Nakajima N, Hamada M, Koyama Y. Design and synthesis of amphiphilic alternating peptides with lower critical solution temperature behaviors. Polym J 2022;54:903–12. https://doi.org/10.1038/s41428-022-00639-7.

[266] Guo L, Lahasky SH, Ghale K, Zhang D. N-heterocyclic carbene-mediated zwitterionic polymerization of N-substituted N-carboxyanhydrides toward poly(α-peptoid)s: Kinetic, mechanism, and architectural control. J Am Chem Soc 2012;134:9163–71. https://doi.org/10.1021/ja210842b.

[267] Badreldin M, Le Scouarnec R, Lecommandoux S, Harrisson S, Bonduelle C. Memory Effect in Thermoresponsive Proline-based Polymers. Angew Chemie Int Ed 2022;61. https://doi.org/10.1002/anie.202209530.

[268] Dukor RK, Keiderling TA. Mutarotation studies of poly-L-proline using FTIR, electronic and vibrational circular dichroism. Biospectroscopy 1998;2:83–100. https://doi.org/10.1002/(SICI)1520-6343(1996)2:2<83::AID-BSPY2>3.0.CO;2-T.

[269] Kakinoki S, Hirano Y, Oka M. On the stability of polyproline-I and II structures of proline oligopeptides. Polym Bull 2005;53:109–15. https://doi.org/10.1007/s00289-004-0317-6.

[270] Chen F, Zhang X, Li W, Liu K, Guo Y, Yan J, et al. Thermoresponsive oligoprolines.

Soft Matter 2012;8:4869–72. https://doi.org/10.1039/c2sm25451d.

[271] Kitamura M, Yamauchi T, Oka M, Hayashi T. Thermosensitive property of poly(Hyp(Bzl)-co-Pro). Polym Bull 2003;51:143–50. https://doi.org/10.1007/s00289-003-0207-3.

[272] Kitamura M, Kakinoki S, Hirano Y, Oka M. Thermosensitive properties of poly(proline)-based polypeptide having an amino-acid of low hydrophobicity. Polym Bull 2005;54:303–10. https://doi.org/10.1007/s00289-005-0384-3.

[273] Kakinoki S, Kitamura M, Yuge M, Furuta M, Oka M, Hirano Y, et al. Solution property and irradiation effect of Random copolypeptides composed of ala and pro residues. Polym Bull 2007;58:393–400. https://doi.org/10.1007/s00289-006-0666-4.

[274] Hu Y, Tian Z-Y, Xiong W, Wang D, Zhao R, Xie Y, et al. Water-assisted and protein-initiated fast and controlled ring-opening polymerization of proline N -carboxyanhydride. Natl Sci Rev 2022;9. https://doi.org/10.1093/nsr/nwac033.

[275] Ihsan A Bin, Koyama Y. Impact of polypeptide sequence on thermal properties for diblock, random, and alternating copolymers containing a stoichiometric mixture of glycine and valine. Polymer (Guildf) 2019;161:197–204. https://doi.org/10.1016/j.polymer.2018.12.021.

[276] Seuring J, Agarwal S. Polymers with upper critical solution temperature in aqueous solution. Macromol Rapid Commun 2012;33:1898–920. https://doi.org/10.1002/marc.201200433.

[277] Shimada N, Ino H, Maie K, Nakayama M, Kano A, Maruyama A. Ureido-derivatized polymers based on both poly(allylurea) and poly(l -citrulline) exhibit UCST-type phase transition behavior under physiologically relevant conditions. Biomacromolecules 2011;12:3418–22. https://doi.org/10.1021/bm2010752.

[278] Kuroyanagi S, Shimada N, Fujii S, Furuta T, Harada A, Sakurai K, et al. Highly Ordered Polypeptide with UCST Phase Separation Behavior. J Am Chem Soc 2019;141:1261–8. https://doi.org/10.1021/jacs.8b10168.

[279] Fujii S, Kuroyanagi S, Shimada N, Matsuno J, Lee JH, Takahashi R, et al. Bundling Process of Citrulline Polypeptides upon UCST-Type Phase Separation. J Phys Chem B 2020;124:4036–43. https://doi.org/10.1021/acs.jpcb.0c00934.

[280] Xue X, Thiagarajan L, Dixon JE, Saunders BR, Shakesheff KM, Alexander C. Post-Modified Polypeptides with UCST-Type Behavior for Control of Cell Attachment in Physiological Conditions. Materials (Basel) 2018;11:95. https://doi.org/10.3390/ma11010095.

[281] Zhou Q, Palanisamy A, Albright V, Sukhishvili SA. Enzymatically degradable star polypeptides with tunable UCST transitions in solution and within layer-by-layer films. Polym Chem 2018;9:4979–83. https://doi.org/10.1039/c8py00939b.

[282] Palanisamy A, Albright V, Sukhishvili SA. Upper Critical Solution Temperature Layer-by-Layer Films of Polyamino acid-Based Micelles with Rapid, On-Demand Release Capability. Chem Mater 2017;29:9084–94. https://doi.org/10.1021/acs.chemmater.7b02748.

GRAPHICAL ABSTRACT