COMMUNICATION

Oligourea helix bundle binds detergents with diverse polar head groups

Received 00th January 20xx,
Accepted 00th January 20xx

DOI: 10.1039/x0xx00000x

Sung Hyun Yoo,[a,b] Jérémie Buratto,[a] Arup Roy,[a] Morgane Pasco,[a] Karolina Pulka-Ziach,[c] Gavin W. Collie*[d] and Gilles Guichard*[a]

Here we report a series of crystal structures (and accompanying biophysical data) of an array of diverse detergent guests bound to an oligourea foldamer helix bundle. These results significantly increase our structural and chemical understanding of aqueous guest recognition by oligourea foldamers and will aid the design of further functionalised oligourea-based self-assemblies.

Introduction

There is currently significant interest in the design and synthesis of oligomers able to mimic (and build upon) the folding and functions of natural polymers such as peptides and nucleic acids.[1–3] Such molecules, termed foldamers,[1,4] are expected to find application in a broad range of areas including biosensing, biomedicine and as functional nanomaterials.[5–15] One particular area of focus within the foldamer field, and the subject of this current work, involves the design of aqueous self-assembling systems with the ultimate goal of creating functional, protein-like architectures with tuneable properties such as selective substrate recognition or catalysis.[16–20] Recently, we have reported the ability of amphiphilic oligourea foldamers to self-assemble in aqueous conditions into precise, protein-like helix bundles,[21–23] and furthermore, that one of these assemblies is able to adaptively bind (that is, conformationally rearrange upon ligand binding) a series of alkyl glycosides.[24]

Here, we set out to explore (and expand) the repertoire of lipidic guests able to be recognised by the oligourea helix bundle, as step towards adding further functionality to the system, such as selective guest recognition. Crystallographic screening of a broad set of chemically diverse detergents resulted in the identification of five additional chemotypes able to adaptively bind to the oligourea helix bundle, with circular dichroism studies providing insight into the contribution of the lipidic tails in the binding process. These findings increase our understanding of aqueous encapsulation by artificial folded self-assemblies and provide further design principles that will hopefully aid the creation of improved oligourea-based assemblies with bespoke properties such as selective ligand recognition or catalysis.

Results and Discussion

We have previously reported the ability of an amphiphilic oligourea foldamer (termed **H1**) to self-assemble into a stable six-helix bundle in aqueous conditions (Figure 1).[21] It was then shown that this foldamer helix bundle is able to recognise and encapsulate simple primary alcohols, and further, to be able to adaptively bind a series of n-alkyl glycosides (Figure 1c).[23,24] In an effort to explore whether detergent-type molecules with non-glycosidic polar head groups (including ionic, zwitterionic and non-ionic) could be recognised by and bind to the **H1** helix bundle, we screened a set of commercially available detergents,[25] by X-ray crystallography. Detergents were screened by co-crystallisation with **H1** using previously reported crystallisation conditions and procedures.[24]

These efforts yielded five new crystal structures, revealing ligand-complexes for **H1** bound by detergents with a diverse array of polar head groups including a linear saccharide (Mega-10), oligo ethylene glycols (C_8E_6 and $C_{12}E_9$), trimethyl ammonium bromide (CTAB) and a phospho zwitterion (F_6OPC) (Figure 1d – h). All five complexes reveal **H1** to form a six-helix bundle highly isomorphous to that reported previously for the **H1**-OGP complex[24] (R.M.S.D. of alignments = 0.144 Å) (Figure

[a] Univ. Bordeaux, CNRS, Bordeaux INP, CBMN, UMR 5248, IECB, 2 rue Robert Escarpit, 33607, Pessac, France.
E-mail: g.guichard@iecb.u-bordeaux.fr
[b] Present address: Department of Chemistry, Chonnam National University, Gwangju 61186, Republic of Korea
[c] Faculty of Chemistry, University of Warsaw, 02093 Warsaw, Poland.
[d] Discovery Sciences, R&D, AstraZeneca, Cambridge CB4 0WG, U.K.
E-mail: gavin.collie@astrazeneca.com

†Electronic Supplementary Information (ESI) available: See DOI: 10.1039/x0xx00000x

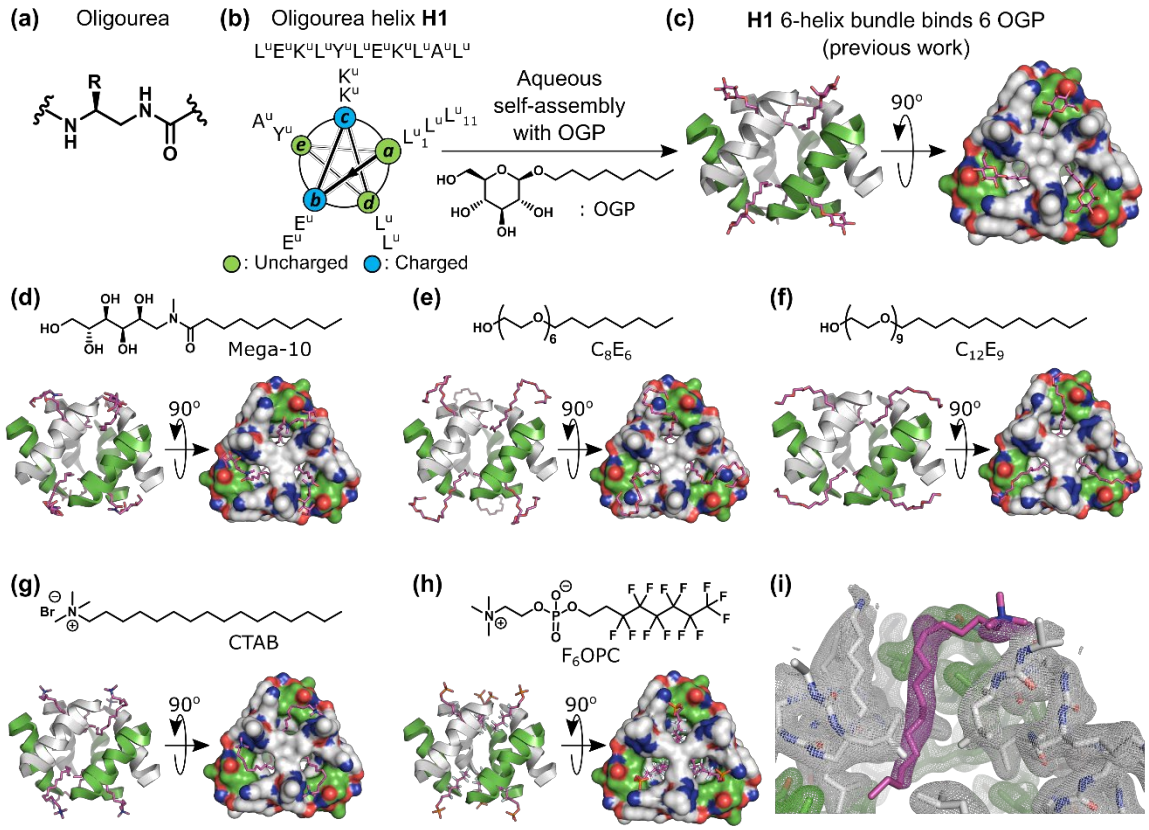


Figure 1 (a) Chemical structure of the oligourea motif. (b) Sequence of amphiphilic oligourea foldamer **H1** and its pentad repeat structure. (c – h) Crystal structures of the **H1** 6-helix bundle bound by six ligand molecules (i.e. **H1**-Ligand complex) (ligands: (c) OGP[24], (d) Mega-10, (e) C_8E_6, (f) $C_{12}E_9$, (g) CTAB and (h) F_6OPC). **H1** helices (in cartoon (left) or in surface (right) models) are colored green and white for clarity. Ligands are shown as stick models with carbons coloured magenta. Hydrogens are omitted for clarity. Nitrogen, Oxygen, Fluorine and Phosphorus are coloured blue, red, cyan and orange, respectively. (i) Electron density map ($2mF_o - DF_c$) at a σ level of 1.0 of the **H1**-CTAB complex focused on the CTAB.

S1). Each complex involves six **H1** helices associating primarily through hydrophobic side chains to form a hydrophobic core, with six guest detergent molecules binding per six-helix bundle. Similar to **H1**-OGP, the binding of all guest molecules to **H1** resulted in a bundle with a more compressed shape compared to apo-**H1**. Curiously, while the electron density for the lipid tails of the detergents (which bind to the hydrophobic interior of the bundles) is of sufficient strength to allow these regions to be modelled, the polar head groups are noticeably less stable (Figure 1i and S2), suggesting none of the five detergents form stable polar interactions with the bundle, and thus implying the primary driving force for host-ligand binding to be the hydrophobic effect.

In order to investigate this further we performed circular dichroism (CD) experiments for all detergents for which we had obtained crystal structures. CD spectra recorded for **H1** (at a concentration of 200 µM) in the presence of 200 µM of each detergent revealed C_8E_6, Mega-10 and F_6OPC to exert a negligible effect on the helicity of **H1** (as indicated by a peak at 204 nm), with CTAB and $C_{12}E_9$ significantly increasing the helicity of **H1** (Figure 2a and Table S2). CD-monitored variable-temperature experiments revealed a similar trend, with $T_{1/2}$ increases (relative to apo-**H1** (i.e. **H1** in the absence of guests)) in the range of 0.5 – 1.6 °C for C_8E_6, Mega-10 and F_6OPC, and 8.8 °C and 7.4 °C for $C_{12}E_9$ and CTAB, respectively (Figure 2b, S3 and Table S2). Interestingly, the largest increase in $T_{1/2}$ was observed for the detergents with the longest lipid tails (CTAB and $C_{12}E_9$), further indicating hydrophobic effect to be the primary force driving the **H1**-detergent binding process.

In order to further explore the influence of the length of the detergent lipid tail on binding, we obtained and studied analogues of CTAB with alkyl tails composed of 14 (TTAB), 12 (DoTAB), 10 (DTAB) and 8 carbons (OTAB). CD-monitored addition of these analogues into solutions of **H1** (at equimolar concentrations) revealed a clear trend: that increasing the length of the alkyl chain increases bundle stability, as measured by changes (increases) in % folding (Figure 3a and Table 1), with % folding increases in the range of 3 – 4 % for OTAB, DTAB and DoTAB, and of 24 % and 29 % for TTAB and CTAB, respectively. CD-monitored variable-temperature studies revealed a similar trend, with increases in alkyl chain length increasing thermal stability of the helix bundle (Figure 3b). With the aim of assessing the **H1**-detergent binding properties in more detail, OTAB, DTAB, DoTAB, TTAB and CTAB were sequentially titrated into solutions of apo-**H1** (Figure S4). Hill plot fitting of the CD-monitored titration data revealed a good fit for the **H1**-TTAB and **H1**-CTAB combinations. For the two well-fitted combinations, we extracted [L]1/2 – i.e. the ligand concentration required to occupy half of the binding sites – by assuming that the receptor state is the 6-helix bundle. $[L]_{1/2}$ values of 7.78 mM and 0.25 mM were determined for TTAB and CTAB, respectively (Figure S4). The $[L]_{1/2}$ value determined for

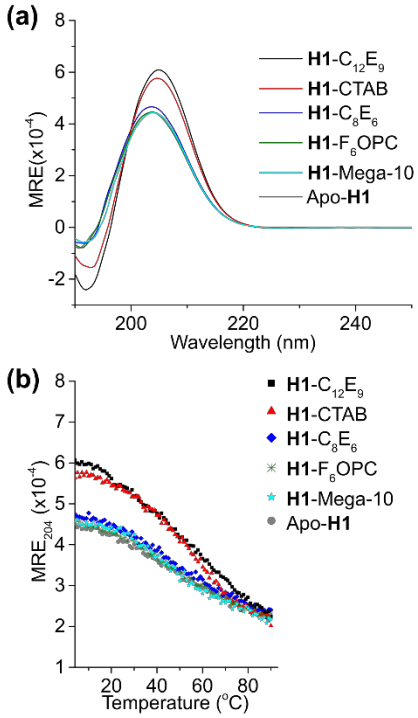


Figure 2 (a) CD spectra and (b) temperature-dependent CD spectra of **H1** ([**H1**] = 200 μM) in the absence (i.e. apo-**H1**) or presence of ligands ([Ligand] = 200 μM). MRE, molar residual ellipticity (deg cm^2 dmol^{-1} residue^{-1}); MRE_{204}, MRE at 204 nm.

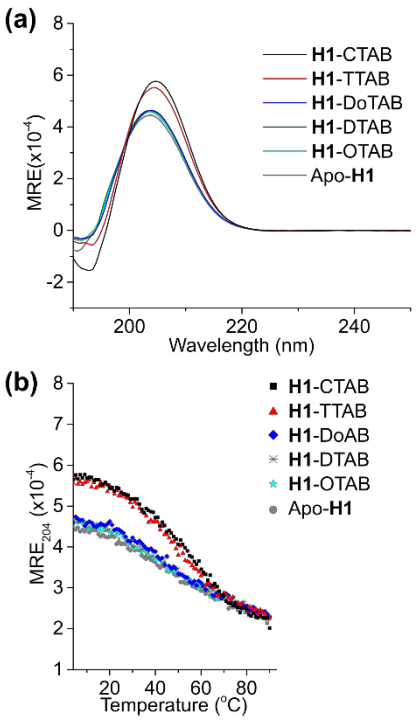


Figure 3 (a) CD spectra and (b) temperature-dependent CD spectra of **H1** ([**H1**] = 200 μM) in the absence (i.e. apo-**H1**) or presence of ligands ([Ligand] = 200 μM).

CTAB (and previously for F_6OM[24]) indicates that oligourea helix bundles such as **H1** are amenable to bind detergent guests with promising affinity, but through a mechanism which yet appears to be based largely if not exclusively on the hydrophobic components of the guests and bundle (interior). We thus anticipate that through rational engineering and optimisation of the **H1** bundle – focussing on the currently under-exploited (with respect to guest binding) solvent-exposed residues of the bundle – we will be able to further increase the affinity, and potentially even selectivity, of host-guest binding of this aqueous foldamer helix-bundle.

Conclusions

In conclusion, we have demonstrated detergents of diverse polar chemotypes (a linear saccharide, oligo ethylene glycols, a quaternary ammonium, and a phospho zwitterion) can adaptively bind to the **H1** oligourea helix bundle in water. Investigation of a series of detergents displaying a shared polar chemotype but varying alkyl tails suggested the primary driving force for the binding to be the hydrophobic effect, which is consistent with our previous observation of oligourea-sequence dependent binding for the homologous system.[24] Further studies exploiting structure-activity relationship-based optimization of the aqueous foldamer helix-bundle assembly may lead to the creation of protein mimics with tailor-made recognition properties for amphipathic guests with selectivity for distinct polar head groups and carbon chains.

Conflicts of interest

The authors declare no conflict of interest.

Table 1 Biophysical analysis based on CD experiments of **H1**-CTAB analogue complexes.

Ligand	% increase in folding[a]	Temperature-dependent	
		$T_{1/2}$ (°C)[b]	adj. R^2
No ligand	-	46.9	0.9958
OTAB	3	46.8	0.9937
DTAB	4	47.1	0.9950
DoTAB	4	47.4	0.9929
TTAB	24	49.7	0.9971
CTAB	29	54.3	0.9974

[a] % increase in folding values (% increase of MRE_{204} of interest versus MRE_{204} of **H1** alone) at a ligand concentration of 200 μM and **H1** concentration of 200 μM. [b] The midpoint of the transition ($T_{1/2}$) values was estimated by fitting temperature-dependent CD data to a simple two-state Boltzmann unfolding model using OriginPro 9.0 (See Figure S3).

Acknowledgements

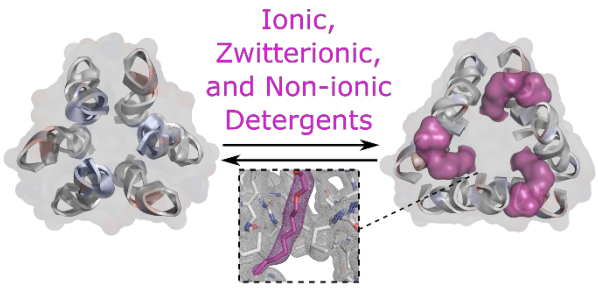
This work was supported in part by the Agence Nationale de la Recherche (ANR) grants ANR-17-CE07-0020. Post-doctoral fellowships to S.H.Y. from IdEx Bordeaux (ANR-10-IDEX-03-02), a program of the French government managed by the ANR, from ANR-17-CE07-0020, and from Basic Science Research Program through the National Research Foundation of Korea (NRF) funded by the Ministry of Education (2021R1A6A3A03040187) are gratefully acknowledged. A Marie Skłodowska-Curie postdoctoral fellowship (H2020-MSCA-IF-2014, project #661247) to A.R. is also gratefully acknowledged. We thank the SOLEIL and ESRF synchrotrons for providing access to data collection facilities. This work has benefited from the facilities and expertise of IECB Biophysical and Structural Chemistry platform (BPCS), CNRS UAR3033, Inserm US001, Univ. Bordeaux

Notes and references

1 S. H. Gellman, *Acc. Chem. Res.*, 1998, **31**, 173–180.
2 P. E. Nielsen, *Acc. Chem. Res.*, 1999, **32**, 624–630.
3 J. Saarbach, P. M. Sabale and N. Winssinger, *Curr. Opin. Chem. Biol.*, 2019, **52**, 112–124.
4 D. J. Hill, M. J. Mio, R. B. Prince, T. S. Hughes and J. S. Moore, *Chem. Rev.*, 2001, **101**, 3893–4011.
5 S. Hecht and I. Huc, *Foldamers: Structure, Properties, and Applications*, WILEY-VCH, Weinheim, 2007.
6 M. Pasco, C. Dolain and G. Guichard, in *Comprehensive Supramolecular Chemistry II*, ed. J. L. Atwood, Elsevier, Amsterdam, 2nd edn, 2017, vol. 5, pp. 89–125.
7 G. Guichard and I. Huc, *Chem. Commun.*, 2011, **47**, 5933–5941.
8 Y. Ferrand and I. Huc, *Acc. Chem. Res.*, 2018, **51**, 970–977.
9 S. H. Yoo and H.-S. Lee, *Acc. Chem. Res.*, 2017, **50**, 832–841.
10 C. Mayer, M. M. Müller, S. H. Gellman and D. Hilvert, *Angew. Chem. Int. Ed.*, 2014, **53**, 6978–6981.
11 Z. C. Girvin, M. K. Andrews, X. Liu and S. H. Gellman, *Science*, 2019, **366**, 1528–1531.
12 Z. C. Girvin and S. H. Gellman, *J. Am. Chem. Soc.*, 2020, **142**, 17211–17223.
13 G. Fittolani, T. Tyrikos-Ergas, A. Poveda, Y. Yu, N. Yadav, P. H. Seeberger, J. Jiménez-Barbero and M. Delbianco, *Nat. Chem.*, 2023, **15**, 1461–1469.
14 M. P. Del Borgo, A. I. Mechler, D. Traore, C. Forsyth, J. A. Wilce, M. C. J. Wilce, M.-I. Aguilar and P. Perlmutter, *Angew. Chem. Int. Ed.*, 2013, **52**, 8266–8270.
15 P. Sang, Y. Shi, B. Huang, S. Xue, T. Odom and J. Cai, *Acc. Chem. Res.*, 2020, **53**, 2425–2442.
16 W. S. Horne and T. N. Grossmann, *Nat. Chem.*, 2020, **12**, 331–337.
17 P. S. P. Wang and A. Schepartz, *Chem. Commun.*, 2016, **52**, 7420–7432.
18 W. S. Horne, J. L. Price, J. L. Keck and S. H. Gellman, *J. Am. Chem. Soc.*, 2007, **129**, 4178–4180.
19 S. De, B. Chi, T. Granier, T. Qi, V. Maurizot and I. Huc, *Nat. Chem.*, 2018, **10**, 51–57.
20 P. Kumar, N. G. Paterson, J. Clayden and D. N. Woolfson, *Nature*, 2022, **607**, 387–392.
21 G. W. Collie, K. Pulka-Ziach, C. M. Lombardo, J. Fremaux, F. Rosu, M. Decossas, L. Mauran, O. Lambert, V. Gabelica, C. D. Mackereth and G. Guichard, *Nat. Chem.*, 2015, **7**, 871–878.
22 C. M. Lombardo, G. W. Collie, K. Pulka-Ziach, F. Rosu, V. Gabelica, C. D. Mackereth and G. Guichard, *J. Am. Chem. Soc.*, 2016, **138**, 10522–10530.
23 G. W. Collie, R. Bailly, K. Pulka-Ziach, C. M. Lombardo, L. Mauran, N. Taib-Maamar, J. Dessolin, C. D. Mackereth and G. Guichard, *J. Am. Chem. Soc.*, 2017, **139**, 6128–6137.
24 S. H. Yoo, J. Buratto, A. Roy, E. Morvan, M. Pasco, K. Pulka-Ziach, C. M. Lombardo, F. Rosu, V. Gabelica, C. D. Mackereth, G. W. Collie and G. Guichard, *J. Am. Chem. Soc.*, 2022, **144**, 15988–15998.
25 Crystallisation screening was performed using HR2-406 detergent screen HT supplied by Hampton Research.

COMMUNICATION

Table of Contents (graphics and text)



Detergents bearing diverse polar head groups including ionic, zwitterionic and non-ionic can adaptively bind to oligourea helix bundle through hydrophobic effect in water.