Supplementary Information

Oligourea helix bundle binds detergents with diverse polar head groups

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Supplementary materials and methods

Materials

Unless stated otherwise, reagents were purchased from Sigma Aldrich. C_8E_6, $C_{12}E_9$, F_6OPC, Mega-10 for CD experiment were purchased from Anatrace. High purity water (MQ H_2O) for biophysical experiments (crystallization and CD) was generated by Milli-Q apparatus (Millipore). **H1** was prepared following the reported procedures.[1]

Circular dichroism (CD)

Circular dichroism (CD) experiments were performed on a Jasco J-815 spectrometer. A quartz cell with a path length of 1 mm was used.

Ligand-dependent CD experiments were performed in aqueous solutions of foldamers at a concentration of 200 µM in the absence or the presence of detergent ligands at a concentration of 200 µM. Each sample solution of 500 µL in an Eppendorf tube was heated at 80 °C for 20 minutes, cooled to 4 °C and kept at 4 °C overnight prior to use for CD experiments. The data were recorded at 4 °C between wavelengths of 190 and 250 nm at 0.5 nm intervals at a speed of 50 nm/min with an integration time of 2 seconds.

Temperature-dependent CD experiments were performed in the same solution used for the ligand-dependent CD experiments. For these experiments, samples were heated from 4 °C to 90 °C using a gradient of 1 °C/min. The mid-point of the transition ($T_{1/2}$) values were determined by fitting temperature-dependent CD data to a simple two-state Boltzmann unfolding model using OriginPro 9.0.

CD-monitored titration of CTAB analogues into foldamers were performed in 300 µL of aqueous solutions starting from an foldamer concentration of 100 µM followed by serial addition of 4 µL of CTAB analogue solutions. The concentrations of added CTAB analogue solutions varied as 0.47 mM, 0.47 mM, 0.94 mM, 1.9 mM, 3.8 mM, 7.5 mM, 23 mM and 38 mM to adjust [Oligourea]:[Ligand] as 1:16, 1:8, 1:4, 1:2, 1:1, 2:1, 5:1 and 10:1, respectively. The data for CD-monitored titration were recorded at 20 °C between wavelengths of 190 and 250 nm at 0.5 nm intervals at a speed of 50 nm/min with an integration time of 2 seconds. The CD-monitored titration data were fitted to the Hill equation (considering the reaction as Bundle + n(Ligand) $\leftrightarrows$ Bundle(Ligand)$_n$) using OriginPro 9.0 by considering [**H1**] as 100 µM and [Ligand] as from 6.25 µM to 1 mM.

Note: Micelle formation of the ligands can also influence helical propensity of foldamers. Concentrations of the ligands for CD experiments were carefully designed to be below CMC (critical micelle concentration) values of ligands,[2] except $C_{12}E_9$. Excluding the CD analysis of the $C_{12}E_9$ case, the trend observed in the CD analysis consistently support the hypothesis that the binding of the ligand enhances the stability of the bundle complex.

Crystallography

Cocrystals of **H1** grown in the presence of various detergent-based ligands were obtained following previously reported procedures.[3] Briefly, a purified, lyophilized powder of **H1** was dissolved in MQ water to a concentration of around 10 mg/mL. 0.5 – 1 µL of this solution was then mixed with an equal volume of crystallisation reagent (listed in Table S1) and equilibrated as a hanging drop against a well solution composed of around 500 µL of crystallisation reagent. Prior to data collection crystals were typically cryoprotected in a solution composed of the crystallisation reagent supplemented with 20 – 25% glycerol and frozen in liquid nitrogen. Diffraction data were collected at the SOLEIL Synchrotron (France) for **H1**-Mega-10, **H1**-C_8E_6, **H1**-$C_{12}E_9$, **H1**-F_6OPC, and at the European Synchrotron Radiation Facility (ESRF, France) for **H1**-CTAB. The diffraction data were integrated and scaled using XDS[4] and CCP4.[5] The structures were solved by molecular replacement using previously reported X-ray structures as search models[3] using Phaser[6] from the CCP4 suite.[5] Geometric restraints for detergents were generated using PRODRG[7] with model building and restrained refinement performed in Coot[8] and Refmac5,[9] respectively. Data collection and refinement statistics can be found in Table S1. The structures have been deposited in the CCDC with accession codes listed in Table S1.

Supplementary figures

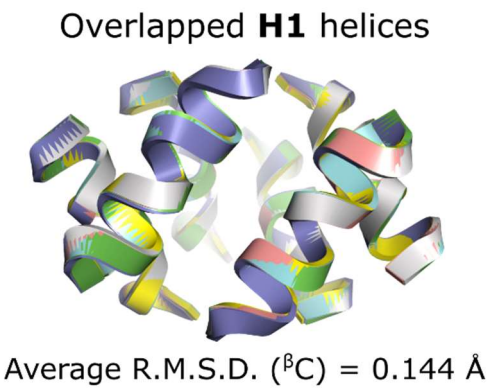

Figure S1. Overlay of **H1** 6-helix bundles from crystal structures of **H1**-ligand complexes. Average R.M.S.D. for $^{\beta}$C is 0.144 Å.

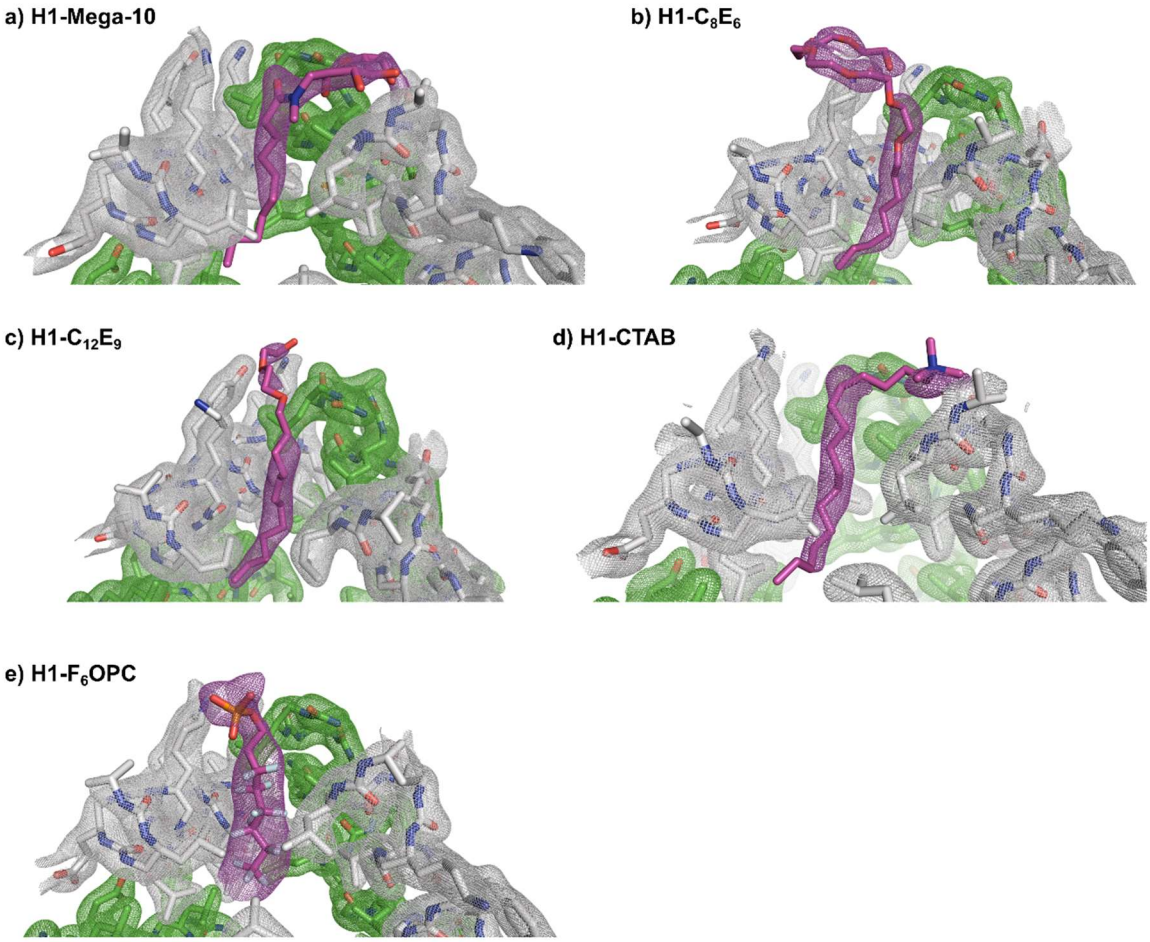

Figure S2. Electron density maps ($2mF_o - DF_c$) at a σ level of 1.0 of the **H1**-ligand complexes focused on the ligand colored in magenta (ligands: (a) Mega-10, (b) C_8E_6, (c) $C_{12}E_9$, (d) CTAB, and (e) F_6OPC)

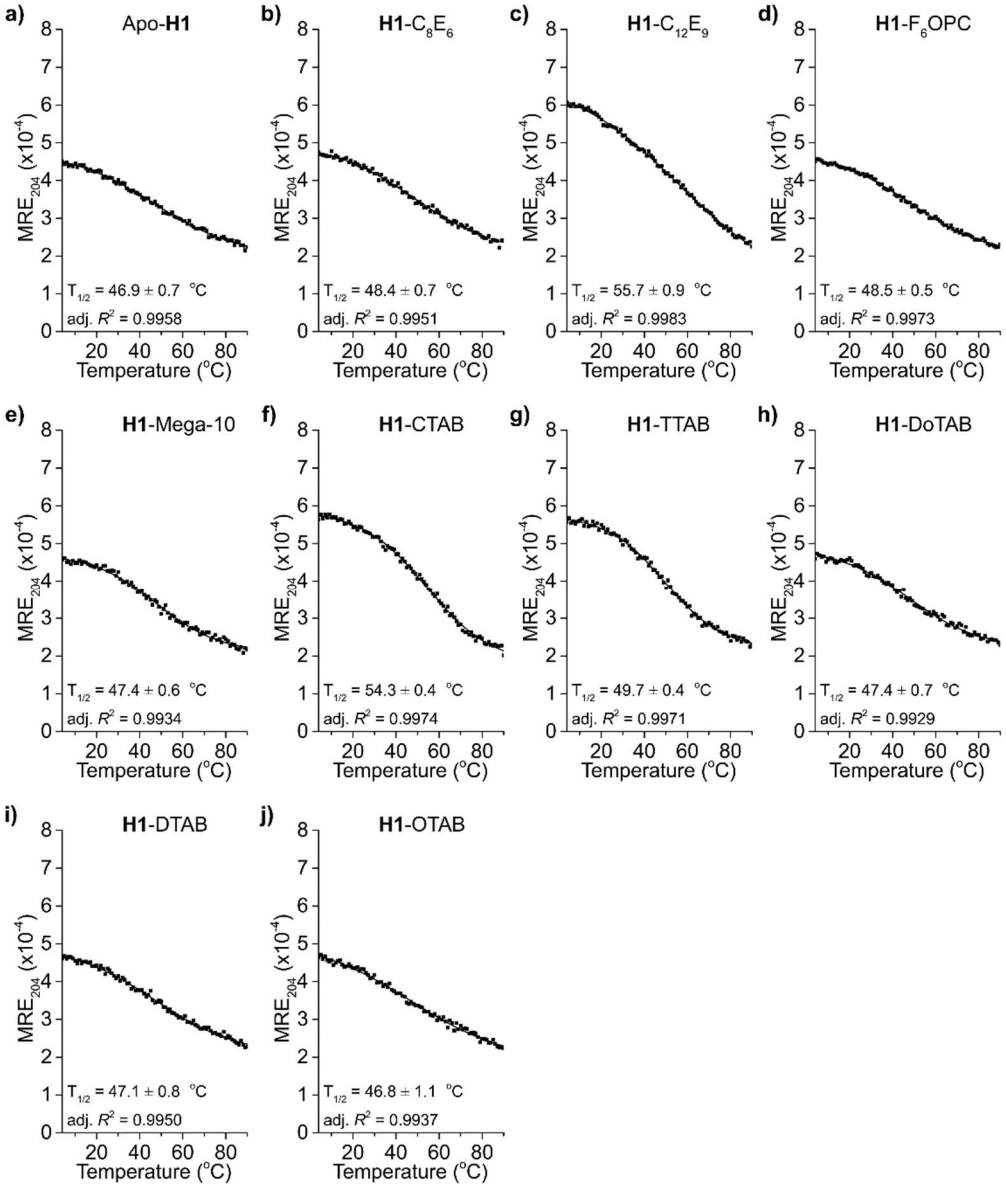

Figure S3. CD-monitored variable-temperature experiment data of **H1** at a concentration of 200 µM (a) in the absence and (b – j) in the presence of ligands at a concentration of 200 µM (1 equiv. to **H1**). Lines represent simple two-state Boltzmann unfolding model fits.

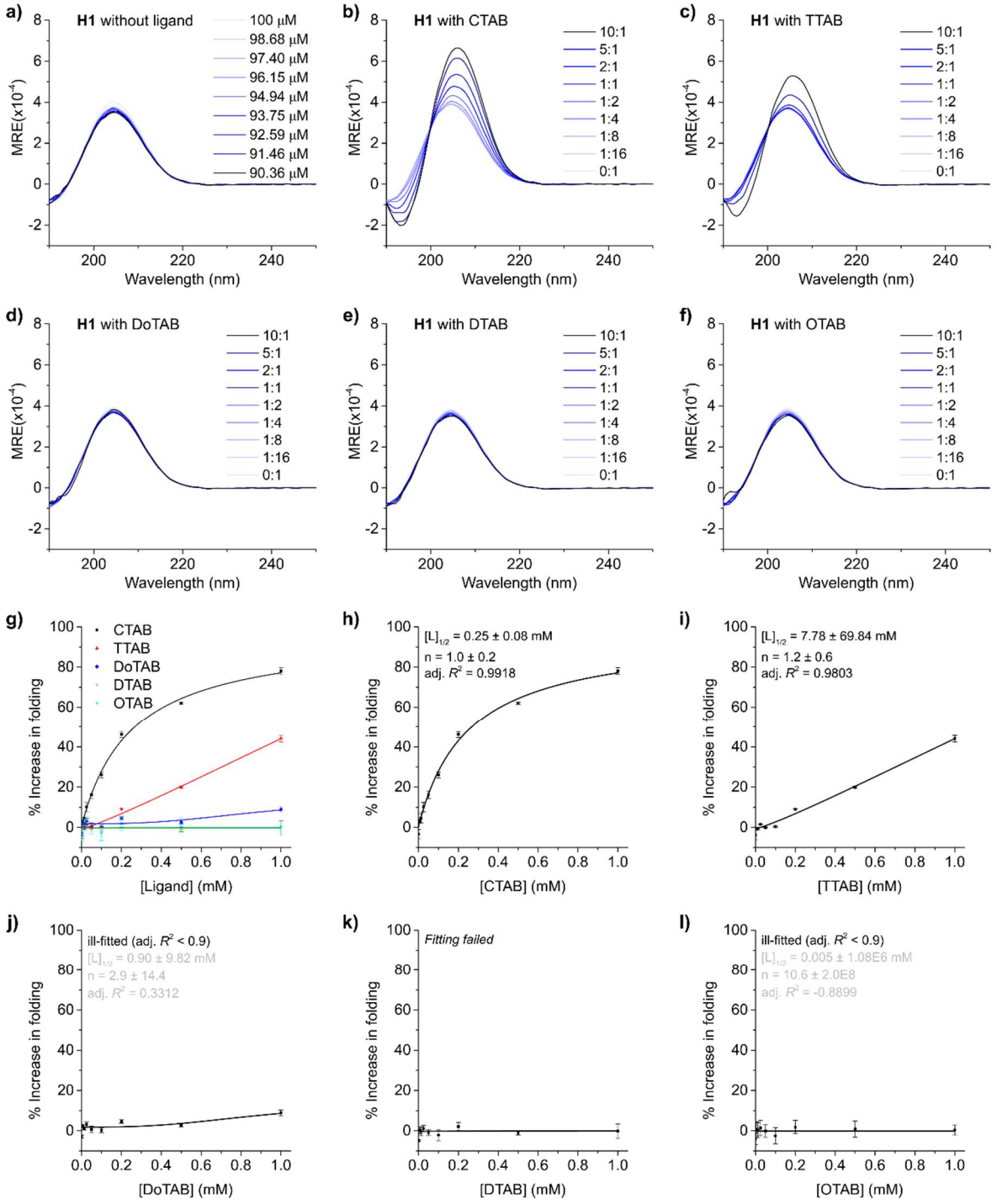

Figure S4. CD-monitored titration of CTAB analogues into **H1**. 4 µL of CTAB analogue solutions ((a) water as control, (b) CTAB, (c) TTAB, (d) DoTAB, (e) DTAB and (f) OTAB) were sequentially added to 300 µL of **H1** at a concentration of 100 µM. Ratios in (b – f) correspond to the molar ratio between CTAB analogues and **H1**. CD curves with ligands are averaged values of triplicate measurements. (g – l) Hill plot fitting of CD-monitored titration data. $[L]_{1/2}$ represents the ligand concentration needed to occupy half of the binding sites. n represents the Hill coefficient. Fitted data with adj. $R^2 < 0.9$ have not been considered and are indicated in light grey for information only. Error bars are the standard deviation of three experiments.

Supplementary tables

Table S1. Data collection and refinement statistics for X-ray crystal structures of **H1** cocrystallised with various detergents

Detergent	CTAB	C_8E_6	$C_{12}E_9$	F_6OPC	Mega-10
Crystallisation reagent	100 mM NaCl, 13 mM CTAB	100 mM NaCl, 100 mM C_8E_6	100mM NaCl, 0.5 mM $C_{12}E_9$	100mM NaCl, 22 mM F_6OPC	100mM NaCl, 70 mM Mega-10
Data collection					
Space group	$I2_13$	$I4_132$	$I4_132$	$I4_132$	$I4_132$
a, b, c (Å)	74.15, 74.15, 74.15	73.977, 73.977, 73.977	73.760, 73.760, 73.760	75.04, 75.04, 75.04	73.863, 73.863, 73.863
α, β, γ (°)	90, 90, 90	90, 90, 90	90, 90, 90	90, 90, 90	90, 90, 90
Resolution (Å)	37.07 – 1.87 (1.99 – 1.87)	52.31 – 1.80 (1.91 – 1.80)	52.16 – 1.70 (1.80 – 1.70)	53.06 – 1.70 (1.80 – 1.70)	30.15 – 1.80 (1.91 – 1.80)
R_{meas} (%)	5.7 (81.6)	8.4 (441.8)	5.0 (512.8)	5.7 (440.1)	7.0 (535.5)
I / σ	23.19 (2.65)	32.05 (1.12)	45.21 (0.79)	44.11 (1.30)	38.84 (0.84)
Reflections (total)	62748	258045	302008	314600	248791
Reflections (unique)	5741	3437	4039	4235	3417
Completeness (%)	100	100	100	100	99.9
Redundancy	10.9	75.1	74.8	74.3	72.81
Refinement					
Resolution (Å)	52.43 – 1.87	52.31 – 1.80	52.16 – 1.70	53.06 – 1.70	30.15 – 1.80
R_{work} / R_{free} (%)	22.51 / 24.69	19.33 / 21.06	19.64 / 22.54	20.31 / 20.26	19.22 / 25.41
Atoms	304	149	141	148	146
Waters	20	12	9	9	11
Overall B-factor (Å^2)	43.27	58.58	63.25	58.89	62.96
R.m.s. deviations					
Bond-lengths (Å)	0.016	0.016	0.015	0.0213	0.0132
Bond-angles (°)	1.877	1.961	1.797	1.7465	2.0770
CCDC code	2292246	2298232	2298233	2298234	2298235

Table S2. Biophysical analysis based on CD experiments of **H1**-ligand complexes.

Ligand	% increase in folding[a]	Temperature-dependent	
		$T_{1/2}$ (°C)[b]	adj. R^2
No ligand	-	46.9	0.9958
Mega-10	0	47.4	0.9934
F_6OPC	0	48.5	0.9973
C_8E_6	5	48.4	0.9951
$C_{12}E_9$	35	55.7	0.9983
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 0.2 mM and **H1** concentration of 0.2 mM.

[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).

Supplementary references

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