Supporting Information

De novo Design of Protein Mimics of B-DNA

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Experimental Methods

Peptide synthesis, purification, and characterization: All peptides were synthesized on Advanced ChemTech 348 Ω synthesizer (Louisville, KY) using Rink amide NovaGel™ resin (resin substitution: 0.63 mmole/g, 0.1 mmole scale synthesis) and 9-fluorenylmethoxycarbonyl (Fmoc) protection chemistry. Peptide synthesis reagents, solvents, and Fmoc-protected amino acids were purchased from Advanced ChemTech, Fisher Scientific, and Novabiochem unless otherwise noted. Side chain deprotection and peptide cleavage was performed using a trifluoroacetic acid (TFA) cleavage cocktail (TFA/Triisopropylsilane (TIPS)/H_2O, 95:3:2 or TFA/TIPS/1,2-ethanedithiol/H_2O, 95:2.5:2.5:1 for sequences bearing a cysteine residue). Peptides were precipitated in ice-cold ether, filtered, and lyophilized. Disulfide bonded peptides were obtained by air oxidation of cysteine bearing peptides. Briefly, peptides were dissolved in 0.1 M Tris·Cl buffer, pH 8.2-8.5, with 0.5 M guanidium hydrochloride (Gdn·HCl) to give a final concentration of 0.5 mg/mL. The reaction mixture was stirred overnight with vessel open to air and quenched by addition of 5% TFA. All peptides were purified by RP-HPLC on C18 columns (Grace Vydac, preparative (22 × 250 mm, 10-15 μm) and semi-preparative (10 × 250 mm, 10 μm) using linear gradients of solvent A, [water/acetonitrile/TFA (99:1:0.1)] and solvent B, [water/acetonitrile/TFA (90:10:0.07)]. The purity of peptides was verified by analytical RP-HPLC (Grace Vydac, C18 column, 4.5 × 250 mm, 5 μm). The molecular weights of the peptides were determined by MALDI TOF-MS using α-cyano-4-hydroxycinnaminic acid or sinapinic acid. Concentrations of peptide stock solutions were determined from UV-absorption of tryptophan (ε = 5,690 $M^{-1}.cm^{-1}$) and tyrosine (ε = 1,215 $M^{-1}.cm^{-1}$) residues at 280 and 276 nm, respectively in 6M Gdn·HCl, 0.01 M phosphate buffer, pH 6.5.[1]

Overexpression and purification of Ocr and Ocr99: *E. coli* strain BL21 (DE3) pLysS cells transformed with plasmids pAR2993, and pAR3790 were kind gifts from Prof. W. Studier (Brookhaven National Laboratory). Ocr and a truncated mutant of the protein lacking the C-terminal end, Ocr99, were expressed following literature protocols from plasmids pAR2993 and pAR3790, respectively.[2-4] Briefly, a single isolated colony was grown in 10 mL LB broth with 100 μg/mL ampicillin and 34 μg/mL chloramphenicol overnight using a incubator shaker at 37°C, 250 rpm. Fresh LB medium with the same antibiotic composition was inoculated with a 1:50 dilution of the overnight culture. Cells were further grown at 37 °C and 250 rpm until OD_{650} of medium reached 0.6 (typically within 2.5 h) and were induced by addition of isopropyl β-D-thiogalactoside to 0.4 mM in each flask. The cells were harvested by centrifugation using a floor centrifuge for 1 h at 9,000 g after 3 h of induction period. Cell paste was stored at −20 °C (−80 °C for longer periods of storage) if it was not lysed immediately. Cell paste was resuspended (5 mL/g of cell paste) in low salt buffer (0.3 M NH_4Cl, 20 mM Tris·Cl, 4% glycerol, pH 8.0). Benzamidine hydrochloride hydrate and phenylmethylsulfonyl fluoride were added fresh to this buffer to give a final concentration of 10 μM of each protease inhibitor. Cells were lysed while still on ice by sonication (1 minute/g of cell paste). Lysed cells were centrifuged at 20,000 g, 4 °C for 3 h. The cell debris was discarded and the supernatant was purified using three consecutive gel filtration columns to obtain pure Ocr/Ocr99.

The supernatant was first applied onto a XK 16/20 column packed with DEAE-sepharose fast flow anion exchange resin (Amersham Biosciences) pre-equilibrated with low salt buffer (0.3 M NH_4Cl, 20 mM Tris·Cl, pH 8.0) for 180 minutes with a flow rate of 1 mL/min at 4 °C. A gradient from 30 to 100 % high salt buffer (1 M NH_4Cl, 20 mM Tris·Cl, pH 8.0) was run for 1000 minutes with a flow rate of 1.0 mL/min at 4 °C. Fractions containing Ocr/Ocr99 were concentrated using Amicon-Ultra 15 centrifugal filter units with a molecular weight cut-off 5 kDa and were spun

using a swinging bucket rotor at 3,750 g and 4 °C. Concentrated samples were applied to a XK 16/70 column packed with Superdex 200 prep grade size exclusion resin (Amersham Biosciences) pre-equilibrated at 4 °C with a flow rate of 1 mL/min in low salt buffer. T he column was run under the same conditions for 5 h. Any nucleic acid contamination was removed by applying samples onto a XK 16/20 phenyl-sepharose high performance column (Amersham Biosciences). Before each run the column was pre-equilibrated 3 h in 2.4 M $(NH_4)_2SO_4$, 50 mM NH_4Cl, 20 mM Tris·Cl, pH 8.0 with a flow rate of 1 mL/min, flushed with the same buffer for 30 min with a flow rate of 0.8 mL/min. The protein was eluted using a gradient from 2.4 to 0 M $(NH_4)_2SO_4$ in 20 mM Tris·Cl, pH 8.0 for 6 h at 4 °C. Samples were concentrated using Amicon filters, dialyzed against 20 mM Tris·Cl, pH 8.0 and stored at 4 °C. The concentration of Ocr and Ocr99 stocks were determined as described above using $\varepsilon = 31,860$ $M^{-1}.cm^{-1}$ and $\varepsilon = 29,430$ $M^{-1}.cm^{-1}$, respectively at 280 nm.[5] The protein stock solutions were stored at −20 °C in low salt buffer supplemented with 50 % glycerol v/v for longer periods of time.

Analytical Ultracentrifugation: Apparent molecular masses of peptides were determined by sedimentation equilibrium on a Beckman ProteomeLab™ XL-I ultracentrifuge (Indianapolis, IN). Purified peptides were dissolved in 1× PBS (10 mM phosphate buffer, 137 mM NaCl, 2.7 mM KCl, pH 7.4) and loaded into equilibration cells at three different concentrations (10, 20, and 30 μM). The peptides were equilibrated at three rotor speeds (20,000, 35,000, and 40,000 rpm) for 18 h at 20 °C. Absorbance scans at 240, 270 and 280 nm were fit to equation (1) describing the equilibrium sedimentation of a homogeneous single ideal species:

$$Abs(r) = A' \, \exp[H \cdot M(x^2 - x_0^2)] + B \tag{1}$$

where Abs = Absorbance at radius r, A′= absorbance at reference radius x_0, H = $(1-\overline{V}\rho) \cdot \omega^2/2RT$, with $\overline{V}$ = partial specific volume of the peptide, ρ = solvent density, ω = angular velocity in radians/second, M = apparent molecular weight, E = blank absorbance. Data were fit using Igor Pro v5.03 and partial specific volumes and solution densities were calculated using the program SEDNTERP.

References

1.   C. N. Pace, F. Vajdos, L. Fee, G. Grimsley and T. Gray, *Protein Sci.*, 1995, **4**, 2411.
2.   S. S. Sturrock, D. T. F. Dryden, C. Atanasiu, J. Dornan, S. Bruce, A. Cronshaw, P. Taylor and M. D. Walkinshaw, *Acta Crystallogr., Sect. D: Biol. Crystallogr.*, 2001, **57**, 1652.
3.   J. J. Blackstock, S. U. Egelhaaf, C. Atanasiu, D. T. F. Dryden and W. C. K. Poon, *Biochemistry*, 2001, **40**, 9944.
4.   K. K. Mark and F. W. Studier, *J. Biol. Chem.*, 1981, **256**, 2573.
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Supplementary Data

Table S1. The dimer coiled coil was superimposed on B-DNA backbone.

Phosphorous Atom	Residue	Distance(Å)[a]	Side Chain Atom	Heptad Position
11B	GLU 22A	2.987	C_δ	g
10B	GLU 22A	4.936	C_δ	g
8B	TYR 17A	4.860	O_η	b
7B	LEU 13A	3.855	C_γ	e
6B	LYS 8B	1.620	N_ζ	e
5B	GLU 11B	0.402	C_δ	c
4B	GLU 10B	1.058	C_δ	b
3B	GLU 6B	6.114	C_δ	e
12A	GLN 4B	2.227	C_γ	c
11A	GLU 6A	4.936	C_δ	e
10A	GLU 10A	3.508	C_δ	b
8A	GLU 11A	3.009	C_δ	c
7A	LYS 15A	2.745	N_ζ	g
6A	GLU 20B	1.994	C_δ	e
5A	ASN 21B	2.362	C_γ	f
4A	GLU 22B	3.914	C_δ	g
3A	LYS 27A	4.864	N_ζ	e

[a]The closest atom on the side chain of selected amino acid was paired with the closest phosphorous atom on the B-DNA backbone. An rms fit of 3.765 Å was obtained.

Table S2. DM1 superimposed on B-DNA backbone.

Phosphorous Atom	Residue	Distance(Å)[a]	Heptad Position
12B	GLU 6B	0.817	g
11B	ASP 7B	1.634	g
10B	GLU 11B	2.363	b
7B	GLU 20A	3.131	e
6B	ASP 21A	1.671	e
5B	GLU 22A	3.294	c
10A	GLU 27A	2.803	b
8A	GLU 18B	1.260	c
7A	ASP 17B	2.536	g
4A	GLU 11A	2.333	g
3A	GLU 10A	2.224	e

[a] An rms fit of 2.309 Å was obtained. Shown are the distances between the C_γ (Asp) or C_δ (Glu) and phosphorous atoms.

Table S3. DM2 superimposed on B-DNA backbone.

Phosphorous Atom	Residue	Distance(Å)[a]	Heptad Position
12B	GLU 6B	0.684	g
11B	ASP 7B	1.658	g
10B	GLU 11B	2.201	b
7B	GLU 20A	3.229	e
6B	ASP 21A	1.526	e
5B	GLU 22A	3.272	c
10A	GLU 27A	1.883	b
8A	GLU 18B	1.482	c
7A	ASP 17B	2.465	g
4A	GLU 11A	2.252	g
3A	GLU 10A	2.210	e

[a] An rms fit of 2.190 Å was obtained. Shown are the distances between the C_γ (Asp) or C_δ (Glu) and phosphorous atoms.
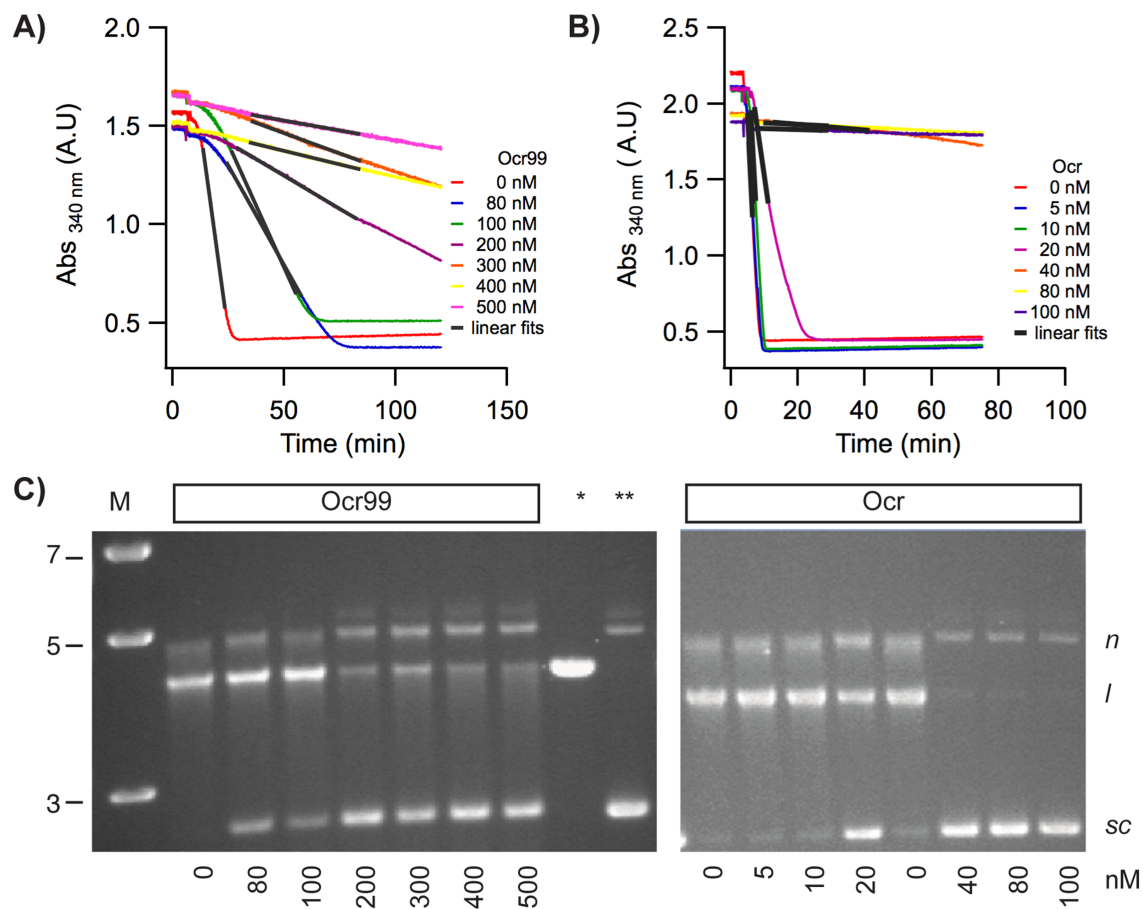
Figure S1. Sample ATPase plots showing linear fits to determine reaction rates for varying concentrations of A) Ocr99 and B) Ocr. C) Reaction mixtures from the ATPase assay were analyzed by agarose gel electrophoresis to monitor DNA cleavage. All reactions were initiated by addition of scDNA following a pre-incubation of all other components, including EcoR124I, Ocr99, and Ocr. [scDNA] = 5 nM, [EcoR124I] = 5 nM. The sizes of the DNA molecular weight marker (M) are in kilobase-pairs. *scDNA digested in the absence of peptide inhibitor. **scDNA alone. N, nicked circular DNA; l, linear and sc, negatively supercoiled DNA.
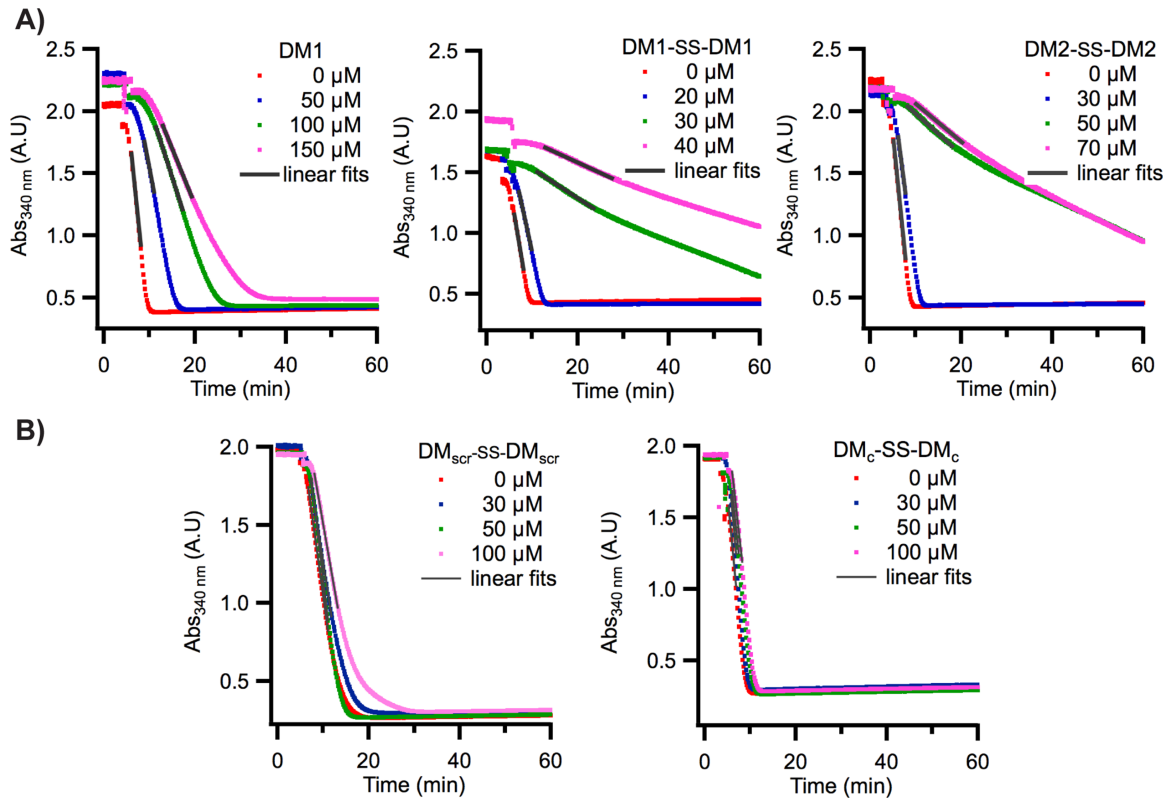
Figure S2. Sample ATPase plots showing linear fits to determine reaction rates in the presence of varying concentrations of A) Designed peptides DM1, DM1-SS-DM1, and DM2-SS-DM2 and B) peptide controls DM_{scr}-SS-DM_{scr} and DM_c-SS-DM_c.
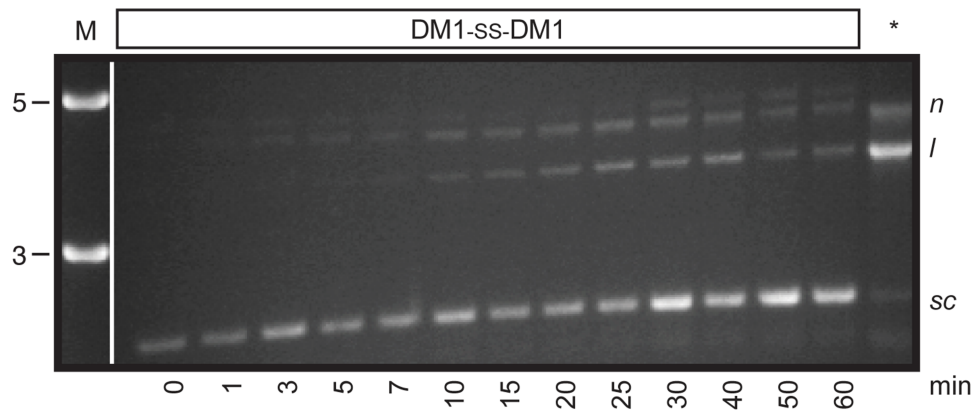

Figure S3. DNA cleavage assay as a function of time in the presence of DM1-SS-DM1. Reactions were initiated by addition of scDNA and DNA cleavage by EcoR124I was followed at 0° C over 60 min by agarose gel analysis. [scDNA] = 5 nM, [EcoR124I] = 5 nM, and [DM1-SS-DM1] = 40 µM. The size of the DNA molecular weight marker (M) is in kilo-base-pairs. *scDNA digested in the absence of peptide inhibitor. N, nicked circular DNA; l, linear and sc, negatively supercoiled DNA.
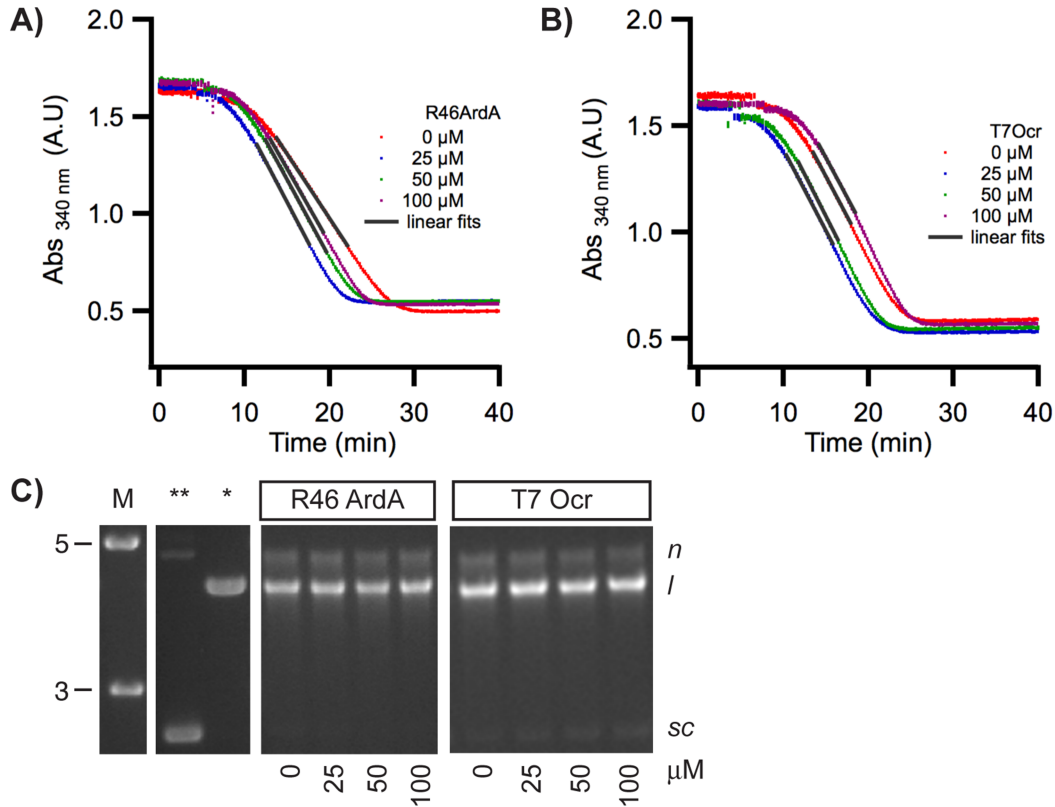
Figure S4. Sample ATPase plots showing linear fits to determine reaction rates in the presence of varying concentrations of A) R46 ArdA and B) T7 Ocr. C) Reaction mixtures from the ATPase assay were analyzed by agarose gel electrophoresis to monitor DNA cleavage. All reactions were initiated by addition of scDNA following a pre-incubation of all other components, including EcoR124I, T7 Ocr, and R46 ArdA. [scDNA] = 5 nM, [EcoR124I] = 5 nM. The sizes of the DNA molecular weight marker (M) are in kilobase-pairs. *scDNA digested in the absence of peptide inhibitor. **scDNA alone. N, nicked circular DNA; l, linear and sc, negatively supercoiled DNA.

Table S4. Summary of inhibitory action of Ocr and Ocr99.

Inhibitor	Residues	Charge[a]	ATPase_{50}[b] (µM)	scDNA_{50}[c] (µM)
Ocr	117	−28	0.017 ± 0.001	0.0235 ± 0.0031
Ocr99	99	−18	0.186 ± 0.004	0.172 ± 0.011

[a] Total net charge of the monomer. [b] Peptide concentration at which 50% of ATPase activitiy of the enzyme is inhibited. ATPase_{50} values were calculated by fitting a straight line to the average relative rate vs. peptide concentration data. [c] Peptide concentration at which 50% of total DNA is scDNA. scDNA_{50} was determined by fitting %scDNA vs. peptide concentration obtained from densitometric analysis of agarose gels. The data is represented as mean±SEM.

Sequence of Ocr: >1S7Z:A|PDBID|CHAIN|SEQUENCE:
MAMSNMTYNNVFDHAYEMLKENIRYDDIRDTDDLHDAIHMAADNAVPHYYADIFSVMASEGIDLEFEDSGLMPDT
KDVIRILQARIYEQLTIDLWEDAEDLLNEYLEEVEEYEEDEE

Sequence of Ocr99: >1S7Z:A|PDBID|CHAIN|SEQUENCE:
MAMSNMTYNNVFDHAYEMLKENIRYDDIRDTDDLHDAIHMAADNAVPHYYADIFSVMASEGIDLEFEDSGLMPDT
KDVIRILQARIYEQLTIDLWEDA

Table S5. Helical content of DMs in 50 mM borate and 1.5M $(NH_4)_2SO_4$ buffers.

Peptide	Concentration (μM)	% Helicity 50 mM Borate	% Helicity 1.5 M $(NH_4)_2SO_4$
DM1	50	9.0	15.5
DM1-SS-DM1	50	9.6	39.6
DM2-SS-DM2	50	10.1	76.2
DM_{scr}-SS-DM_{scr}	50	5.5	11.7
DM_c-SS-DM_c	50	3.7	7.8
Ocr99	25	15.8	52.5

Table S6. Summary of average fitted molecular weight of peptides using analytical equilibrium sedimentation.

Peptide	Formula MW (Da)	Concentration (μM)	Average fitted MW (Da)	Ratio	Oligomeric State
DM1	3519.8	10	3583.2 ± 269.2	1.02	Monomer
		20	5073.9 ± 110.3	1.44	Monomer-dimer
		30	4599.2 ± 48.8	1.31	Monomer-dimer
DM1-SS-DM1	7121.6	10	6782.6 ± 236.7	0.95	Monomer
		20	8833.9 ± 459	1.22	Monomer
DM2-SS-DM2	7235.6	10	8403.4 ± 101.3	1.16	Monomer
		20	8600.8 ± 60.4	1.26	Monomer
		30	9085.3 ± 193.5	1.19	Monomer