Supporting Information for

Molecular Basis for Self-Assembly of a Human Host-Defense Peptide that Entraps Bacterial Pathogens

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This supporting information includes:

Supporting Experimental Section

Insturmentation. An Agilent 1200 series instrument equipped with a thermostatted column compartment set at 20 °C, and a multi-wavelength detector set at 220 and 280 nm (500 nm reference wavelength with 100 nm bandwidth), was used to perform analytical and semi-preparative high-performance liquid chromatography (HPLC). An Agilent PrepStar 218 instrument outfitted with an Agilent ProStar 325 dual-wavelength detector set at 220 and 280 nm was used to perform preparative HPLC. A Clipeus C18 column (5 μm pore, 4.6 x 250 mm, Higgins Analytical, Inc.) set at a flow rate of 1 mL/min was employed for all analytical HPLC experiments. A ZORBAX C18 column (5 μm pore, 9.4 × 250 mm, Agilent Technologies) set at a flow rate of 5 mL/min was employed for all semi-preparative HPLC purification. A Luna 100 Å C18 LC column (10 μm pore, 21.2 × 250 mm, Phenomenex) set at a flow rate of 10 mL/min was used for all preparative HPLC purification. HPLC-grade acetonitrile (MeCN) and trifluoroacetic acid (TFA) were routinely purchased from EMD and Alfa Aesar, respectively. For all HPLC runs, solvent A was 0.1% TFA/H_2O and solvent B was 0.1% TFA/MeCN.

An Agilent 1260 series LC system equipped with an Agilent 6230 TOF system housing an Agilent Jetstream ESI source was employed to perform high-resolution mass spectrometry. A Poroshell 120 EC-C18 column (2.7 μm particle size, 2.1 × 100 mm, Agilent Technologies) set at a flow rate of 0.4 mL/min was used for all LC-MS analyses. The samples were analyzed by using a solvent gradient of 5–95% B over 5 min with 0.1% formic acid/H_2O as solvent A, and 0.1% formic acid/MeCN (LC-MS grade, Sigma-Aldrich). The MS profiles were analyzed and deconvoluted by using Agilent Technologies Quantitative Analysis 2009 software version B.03.02.

All routine optical absorption measurements and the agglutination assays were performed by using a Beckman Coulter DU 800 UV-visible spectrophotometer maintained at ambient temperature. Extinction coefficients (280 nm) for native and mutant HD6 were calculated by using ExPASy ProtParam are listed in Table S1. Solution and buffer pH values

were verified by using a Mettler Toledo S20 SevenEasy pH meter or a HANNA Instruments HI 9124 pH meter equipped with a microelectrode.

Site-Directed Mutagenesis and Purification of Mutant Peptides. The HD6 mutants (Table S1) were generated by employing a modified Quick-Change site-directed mutagenesis protocol (Stratagene). The pET28b-*proHD6* plasmid was used as the template. The forward and reverse primers are listed in Table S7 and the primer pairings are listed in Table S8. For all mutagenesis reactions, the PCR conditions were 95 °C for 30 min (1x); 55 °C for 1 min and 68 °C for 17 min (25x); 4 °C hold. Following PCR amplification using PfuTurbo DNA polymerase, a 1.5-μL aliquot of *Dpn*I was added to a 50-μL PCR reaction and it was incubated at 37 °C for 1.5 h. A second 1.5-μL aliquot of *Dpn*I was then added to the PCR reaction and it was incubated at 37 °C for another 1.5 h. The *Dpn*I digests were transformed into chemically-competent *E. coli* TOP10 cells. Single colonies were selected and grown in 5 mL of LB media containing 50 μg/mL of kanamycin (37 °C, 16-18 h). The plasmids were isolated by using a QIAprep spin miniprep kit and their identities were verified by DNA sequencing. The overexpression and purification of His_6-proHD6 mutants, and preparation and purification of HD6 mutants, were conducted as described for native HD6. During the trypsin-catalyzed cleavage reaction, degradation of the 32-residue F29A product was observed by analytical HPLC following incubation times >15 min. The peptide yields ranged from 1.8 mg/mL (F2A) to 4.5 mg/mL (H27W). The HPLC retention time, and results from LC-MS for these mutants are presented in Table S2. All experiments were performed with at least two independently prepared batches of each peptide.

Circular Dichroism Spectroscopy. For all circular dichroism (CD) measurements, peptide solutions (20 μM, 300 μL) were prepared in 10 mM sodium phosphate pH 7.4 and transferred to a 1-mm path-length quartz CD cell (Hellma). The CD spectra were collected from 260-190 nm at 1-nm intervals (5 s averaging time, three independent scans per wavelength) by using an Aviv Model 202 CD spectrometer operated at room temperature. The data obtained from the three scans were averaged and the resulting averaged spectra are reported. The CD

spectra were collected from at least two independently prepared and purified samples of each peptide and in two independent trials.

Thiol Quantification Assays. The assays were conducted by modifying a reported literature protocol.[S1] To obtain a GSH standard curve, 0, 3, 6, 9, 12, or 15 μL of GSH (3 mM in 10 mM HCl) was added to Milli-Q water to achieve a volume of 15 μL and then added to 943 μL of the assay buffer (100 mM sodium phosphate, 200 μM EDTA, pH 7.0; degassed for at least 1 h by bubbling Ar). A 42-μL aliquot of 2,2'-dithiodipyridine (DTDP, 4 mM in 24 mM HCl) was added to the solution to achieve the final volume of 1 mL. The resulting solution was incubated at room temperature for 15 min and the absorbance at 341 nm was measured. The GSH standard curve was obtained by plotting corrected absorbance versus GSH concentration.

For quantifying the number of free thiol residues present in each peptide sample, peptide stock solutions were freshly prepared in Milli-Q water and the concentrations were determined by optical absorption spectroscopy. Then, aliquots of the peptide stock solutions were diluted with the assay buffer and an aliquot of DTDP was added to each sample as described above to achieve final peptide concentrations of 6 μM. The solutions were incubated at room temperature for 15 min and the absorbance at 341 nm was measured. The number of free thiol residues in each sample was determined by using the GSH standard curve. These assays were conducted with at least two independently prepared and purified samples of each peptide and in three independent trials.

Disulfide Bond Connectivity Determination. The canonical α-disulfide bond connectivity (Cys^4—Cys^{31}, Cys^6—Cys^{20}, Cys^{10}—Cys^{30}) for each of the six HD6 mutant peptides presented in this work was confirmed by employing trypsin digestion followed by four cycles of manual Edman degradation (Scheme S1, Table S3). Each HD6 mutant (1 mg) was incubated in 1.0 mL of 100 mM Tris-HCl pH 8.0, 20 mM $CaCl_2$, 1 M urea, and 0.1 mg/mL of trypsin at 37 °C. F2A, V22T, and F29A were incubated for 3 h and I25T, H27A, and H27W were incubated for 11 h before 100 μL of 6% TFA was added to quench the reaction. The digested peptides were

purified by semi-preparative HPLC (20–40% B over 20 min at 4 mL/min) and lyophilized to dryness.

Each purified peptide product containing three disulfide bonds (Scheme S3) was then subjected to four cycles of manual Edman degradation.[S2] In each cycle, the peptide was resuspended in 200 μL of 5% phenylisothiocyanate (PITC, Thermo Scientific) in 50% pyridine/H_2O, and incubated at 50 °C for 30 min. The reaction was then dried at 60 °C under vacuum, resuspended in 100 μL of anhydrous TFA, and incubated at 45 °C for 10 min. The TFA was removed by flushing with nitrogen and the remaining crude material was dried at 45 °C under vacuum. Then, 200 μL of pre-chilled 5% pyridine/H_2O was added to the crude product and the resulting cloudy solution was extracted with 250 μL of pre-chilled *n*-butyl acetate three times. The aqueous layer was dried at 60 °C under vacuum to obtain the peptide for LC-MS analysis and the next cycle of Edman degradation. The results from LC-MS are listed in Table S3.

Design of the synthetic gene for preproHD6

A synthetic gene for human preproHD6 was ordered from DNA 2.0 and optimized for *E. coli* codon usage. The synthetic gene was designed to include a N-terminal *Nde*I restriction site (N-terminal Met residue encoded by the *Nde*I site) and a C-terminal stop codon followed by a *Xho*I restriction site. It contains an N-terminal TEV protease cleavage site (ENLYFQG) followed by the 19-residue signal peptide sequence ("pre") and 81-residue proHD6. The TEV cleavage sequence and the "pre" region were not utilized in this work. The synthetic gene was obtained in pJ201 from DNA 2.0 and this pJ201-*preproHD6* was employed as a template for PCR amplification of proHD6.

In the nucleotide and amino acid sequences below, the sequences corresponding to the TEV site is in italics and the *Nde*I and *Xho*I codons and corresponding amino acid are in bold.

NdeI-TEV-preproHD6-STOP-**XhoI**

***E. coli* optimized nucleotide sequence:**

CATATG*GAGAATCTGTATTTCCAGGGT*CGCACCCTCACGATCCTCACCGCCGTCCTGCTG
GTAGCCCTCCAGGCTAAGGCCGAGCCGCTGCAAGCAGAGGACGATCCGCTGCAGGCAAA
AGCATACGAAGCGGACGCGCAGGAACAACGCGGTGCGAACGATCAAGACTTTGCGGTTA
GCTTCGCGGAGGATGCTAGCAGCTCTCTGCGTGCGCTGGGTAGCACGCGTGCATTCACTT
GCCACTGCCGTCGTAGCTGTTACTCCACCGAATATAGCTATGGCACCTGCACGGTGATGG
GCATTAATCATCGCTTTTGTTGTCTGTAA**CTCGAG**

Translated sequence for NdeI-TEV-preproHD6-STOP-**XhoI:**

H M *E N L Y F Q G* R T L T I L T A V L L V A L Q A K A E P L Q A E D D P L Q A K A Y E A D
A Q E Q R G A N D Q D F A V S F A E D A S S S L R A L G S T R A F T C H C R R S C Y S T
E Y S Y G T C T V M G I N H R F C C L

Table S1. Amino acid sequences of peptides employed in this work.[a]

Peptide	Amino Acid Sequence
HD6	AFTCHCRRSCYSTEYSYGTCTVMGINHRFCCL
F2A	AATCHCRRSCYSTEYSYGTCTVMGINHRFCCL
V22T	AFTCHCRRSCYSTEYSYGTCTTMGINHRFCCL
I25T	AFTCHCRRSCYSTEYSYGTCTVMGTNHRFCCL
H27A	AFTCHCRRSCYSTEYSYGTCTVMGINARFCCL
H27W	AFTCHCRRSCYSTEYSYGTCTVMGINWRFCCL
F29A	AFTCHCRRSCYSTEYSYGTCTVMGINHRACCL

[a] Mutations are colored in red.

Table S2. Characterization of peptides employed in this work.

Peptide[a]	Retention Time (min)[b]	Free Thiol[c]	Calculated *m/z*	Observed *m/z*	Yield (mg/L culture)	ε_{280} ($M^{-1}cm^{-1}$)[d]
HD6	16.9	0.23 ± 0.08	3705.5	3705.5	1.9	4845
F2A	15.7	0.05 ± 0.06	3629.5	3629.1	1.8	4845
V22T	15.8	0.10 ± 0.04	3707.5	3707.5	2.5	4845
I25T	16.3	0.21 ± 0.05	3693.4	3693.5	4.1	4845
H27A	17.1	0.16 ± 0.12	3642.2	3641.8	2.6	4845
H27W	17.4	0.07 ± 0.06	3754.5	3754.5	4.5	10345
F29A	16.3	0.06 ± 0.09	3629.5	3629.1	4.3	4845
His_6-proHD6	21.8	n.d.[e]	11124	11124	26.3	6335
His_6-proF2A	20.2	n.d.[e]	11048	11048	36.6	6335
His_6-proV22T	20.6	n.d.[e]	11126	11126	31.0	6335
His_6-proI25T	20.7	n.d.[e]	11112	11112	33.2	6335
His_6-proH27A	20.8	n.d.[e]	11059	11059	31.4	6335
His_6-proH27W	20.5	n.d.[e]	11173	11173	23.4	11835
His_6-proF29A	20.2	n.d.[e]	11048	11048	35.6	6335

[a] See Table S1 for amino acid sequences. The mutant peptides are all oxidized. [b] Retention times determined by using analytical RP-HPLC on a C18 column and a gradient of 10–60% B over 30 min at 1 mL/min. [c] Free thiol content determined by using the DTDP assay (mean ± SDM, n ≥ 3). [d] Extinction coefficients at 280 nm were calculated by using the on-line resource ExPASy ProtParam. [e] n.d. = not determined.

Table S3. MS analysis of peptides after each Edman degradation cycle.

Peptide	No. of Edman Degradation Cycles	Molecular Formula	Calculated *m/z*	Observed *m/z*
F2A	0	$C_{144}H_{216}N_{42}O_{47}S_7$	3509.4	3509.2
	1	$C_{129}H_{197}N_{39}O_{43}S_7$	3204.3	3204.3
	2	$C_{120}H_{184}N_{36}O_{40}S_5$	2929.2	2929.3
	3	$C_{108}H_{160}N_{34}O_{34}S_6$	2669.0	2669.0
	4	$C_{92}H_{142}N_{30}O_{30}S_3$	2243.0	2242.9
V22T	0	$C_{149}H_{218}N_{42}O_{48}S_7$	3587.4	3587.2
	1	$C_{134}H_{199}N_{39}O_{44}S_7$	3282.3	3282.4
	2	$C_{119}H_{182}N_{36}O_{41}S_5$	2931.2	2931.3
	3	$C_{107}H_{158}N_{34}O_{35}S_6$	2671.0	2671.1
	4	$C_{91}H_{140}N_{30}O_{31}S_3$	2245.0	2244.9
I25T	0	$C_{148}H_{216}N_{42}O_{48}S_7$	3573.3	3573.1
	1	$C_{133}H_{197}N_{39}O_{44}S_7$	3268.2	3268.4
	2	$C_{118}H_{180}N_{36}O_{41}S_5$	2917.2	2917.1
	3	$C_{106}H_{156}N_{34}O_{35}S_6$	2657.0	2657.1
	4	$C_{90}H_{138}N_{30}O_{31}S_3$	2230.9	2230.7
H27A	0	$C_{147}H_{218}N_{40}O_{47}S_7$	3519.4	3519.4
	1	$C_{132}H_{199}N_{37}O_{43}S_7$	3214.3	3214.1
	2	$C_{117}H_{182}N_{34}O_{40}S_5$	2863.2	2863.1
	3	$C_{105}H_{158}N_{32}O_{34}S_6$	2603.0	2602.9
	4	$C_{89}H_{140}N_{28}O_{30}S_3$	2176.9	2176.9
H27W	0	$C_{155}H_{223}N_{41}O_{47}S_7$	3634.4	3634.2
	1	$C_{140}H_{204}N_{38}O_{43}S_7$	3329.3	3329.8
	2	$C_{125}H_{187}N_{35}O_{40}S_5$	2978.2	2977.9
	3	$C_{113}H_{163}N_{33}O_{34}S_6$	2718.0	2718.0
	4	$C_{97}H_{145}N_{29}O_{30}S_3$	2292.0	2292.0
F29A	0	$C_{144}H_{216}N_{42}O_{47}S_7$	3509.4	3509.3
	1	$C_{135}H_{201}N_{39}O_{43}S_7$	3280.3	3280.4
	2	$C_{120}H_{184}N_{36}O_{40}S_5$	2929.2	2929.3
	3	$C_{108}H_{160}N_{34}O_{34}S_6$	2669.0	2669.1
	4	$C_{92}H_{142}N_{30}O_{30}S_3$	2243.0	2242.9

Table S4. Calculated sedimentation coefficients of F2A.[a]

Concentration (μM)	$S_{20,w}$ (S)	D (F)	MW (kDa)	Partial Specific Volume (mL/g)
40[b]	0.798	12.9	5.06	0.6973
100[b]	0.846	14.7	4.63	0.6973
100[b]	0.833	15.4	4.42	0.6973
160[b]	0.847	15.9	4.36	0.6973
160[b]	0.887	16.8	4.24	0.6973
50[c]	0.796	10.6	6.04	0.6973
50[c]	0.762	10.7	5.69	0.6973
100[c]	0.790	12.9	4.92	0.6973
100[c]	0.792	11.6	5.54	0.6973
160[c]	0.808	15.1	4.29	0.6973
160[c]	0.783	13.8	4.56	0.6973

[a] The temperature was 20 °C. Data were obtained by analysis with the dc/dt method implemented in DCDT+ using 26-34 scans with 50 kDa diffusion broadening maximum. Sedimentation coefficients are $s_{20,w}$ values, adjusted with a solvent density (ρ) of 0.99967 g/mL, and a solvent viscosity (η) of 1.0061 cP. Viscosity units are in centipoise (cP) (1 Poise = 1 g · cm^{-1} · s^{-1}). Sedimentation coefficients are in Svedbergs (1 Svedberg = 100 fs = 1 × 10^{-13} s). Diffusion coefficients correspond to the best-fit molecular mass in Fick units (1 Fick = 1 × 10^{-7} cm^2/s). [b] The samples were in 10 mM sodium phosphate pH 7.4. [c] The samples were in 10 mM Tris-maleate pH 6.4.

Table S5. Calculated sedimentation coefficients of F29A.[a]

Concentration (μM)	$S_{20,w}$ (S)	D (F)	MW (kDa)	Partial Specific Volume (mL/g)
40[b]	1.242	16.4	6.19	0.6973
40[b]	1.154	15.4	6.00	0.6973
100[b]	1.271	13.5	7.56	0.6973
100[b]	1.232	14.1	7.02	0.6973
160[b]	1.284	14.4	7.31	0.6973
160[b]	1.239	13.8	7.18	0.6973
50[c]	1.161	14.5	6.42	0.6973
50[c]	1.125	11.0	8.21	0.6973
100[c]	1.272	14.7	7.01	0.6973
100[c]	1.215	12.7	7.66	0.6973
160[c]	1.175	13.4	7.01	0.6973
160[c]	1.147	12.0	7.64	0.6973

[a] The temperature was 20 °C. Data were obtained by analysis with the dc/dt method implemented in DCDT+ using 26-34 scans with 50 kDa diffusion broadening maximum. Sedimentation coefficients are $s_{20,w}$ values, adjusted with a solvent density (ρ) of 0.99967 g/mL, and a solvent viscosity (η) of 1.0061 cP. Viscosity units are in centipoise (cP) (1 Poise = 1 g · cm^{-1} · s^{-1}). Sedimentation coefficients are in Svedbergs (1 Svedberg = 100 fs = 1 × 10^{-13} s). Diffusion coefficients correspond to the best-fit molecular mass in Fick units (1 Fick = 1 × 10^{-7} cm^2/s). [b] The samples were in 10 mM sodium phosphate pH 7.4. [c] The samples were in 10 mM Tris-maleate pH 6.4.

Table S6. Sedimentation coefficient calculations using HYDROPRO.[S3]

Species	$S_{20,w}$ (S)	Partial Specific Volume (mL/g)[a]
HD6 monomer	0.746	0.6994
HD6 dimer	1.17	0.6994
HD6 tetramer	1.83	0.6994

[a] Monomer, dimer, tetramer $\bar{v}$ values of HD6 were estimated by SEDNTERP.[S4]

Table S7. Primers employed for subcloning and site-directed mutagenesis.[a]

Primer	Sequence
HD6	5'-GAATTCCATATGGAGCCGCTGCAAGCAG-3'
HD6-R	5'-GATCCTCGAG**TTA**CAGACAACAAAAGCGATG-3'
F2A	5'-GTAGCACGCGTGCAGCGACTTGCCACTGC-3'
F2A-R	5'-GCAGTGGCAAGTCGCTGCACGCGTGCTAC-3'
V22T	5'-GCTATGGCACCTGCACGACCATGGGCATTAATC-3'
V22T-R	5'-GATTAATGCCCATGGTCGTGCAGGTGCCATAGC-3'
I25T	5'-CACGGTGATGGGCACCAATCATGCGTTTTG-3'
I25T-R	5'-CAAAAGCGATGATTGGTGCCCATCACCGTG-3'
H27A	5'-GGTGATGGGCATTAATGCGCGCTTTTGTTGTCTG-3'
H27A-R	5'-CAGACAACAAAAGCGCGCATTAATGCCCATCACC-3'
H27W	5'-GGTGATGGGCATTAATTGGCGCTTTTGTTGTCTG-3'
H27W-R	5'-CAGACAACAAAAGCGCCAATTAATGCCCATCACC-3'
F29A	5'-GCATTAATCATCGCGCGTGTTGTCTG**TAA**CTCG-3'
F92A-R	5'-CGAG**TTA**CAGACAACACGCGCGATGATTAATGC-3'

[a] Underlined black codons indicate restriction sites. Bold codons indicate stop codons. The codons containing the mutations are underlined and highlighted in red.

Table S8. Templates and primer pairings employed in site-directed mutagenesis.

Template	Product	Primers Used
pET28b-proHD6	pET28b-proHD6(F2A)	F2A, F2A-R
pET28b-proHD6	pET28b-proHD6(V22T)	V22T, V22T-R
pET28b-proHD6	pET28b-proHD6(I25T)	I25T, I25T-R
pET28b-proHD6	pET28b-proHD6(H27A)	H27A, H27A-R
pET28b-proHD6	pET28b-proHD6(H27W)	H27W, H27W-R
pET28b-proHD6	pET28b-proHD6(F29A)	F29A, F29A-R

Scheme S1. Determination of disulfide bond connectivity of F2A by employing trypsin digestion and manual Edman degradation. This step-by-step summary applies for all HD6 mutants analyzed in this work. The calculated and observed masses of all mutant HD6 are listed in Table S3.

```
HNP1    ACYCRIPACIAGERRYGTCIYQGRLWAFCC
HNP2     CYCRIPACIAGERRYGTCIYQGRLWAFCC
HNP3    DCYCRIPACIAGERRYGTCIYQGRLWAFCC
HNP4    VCSCRLVFCRRTELRVGNCLIGGVSFTYCCTRVD
HD5    ATCYCRTGRCATRESLSGVCEISGRLYRLCCR
HD6   AFTCHCRR-SCYSTEYSYGTCTVMGINHRFCCL
```

Figure S1. Amino acid sequence alignment of human α-defensins with the hydrophobic residues labeled in red. The sequences of human neutrophil α-defensins (HNP1-4) and human Paneth cell α-defensins (HD5 and HD6) are aligned in a single-letter notation using the Cys[I] residue as the reference point. A dash ("-") in the HD6 sequence is used to maintained alignment of all Cys residues.

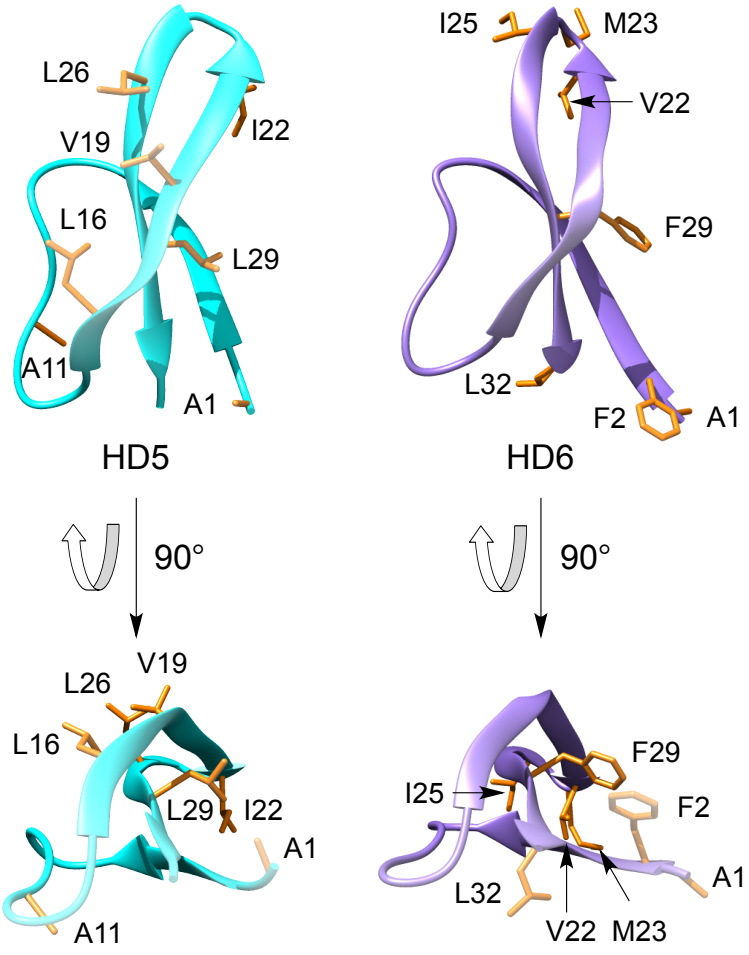

Figure S2. Previously reported X-ray crystal structures of HD5 (PDB: 1ZMP, cyan)[S5] and HD6 (PDB: 1ZMQ, purple)[S5] showing hydrophobic residues in orange.

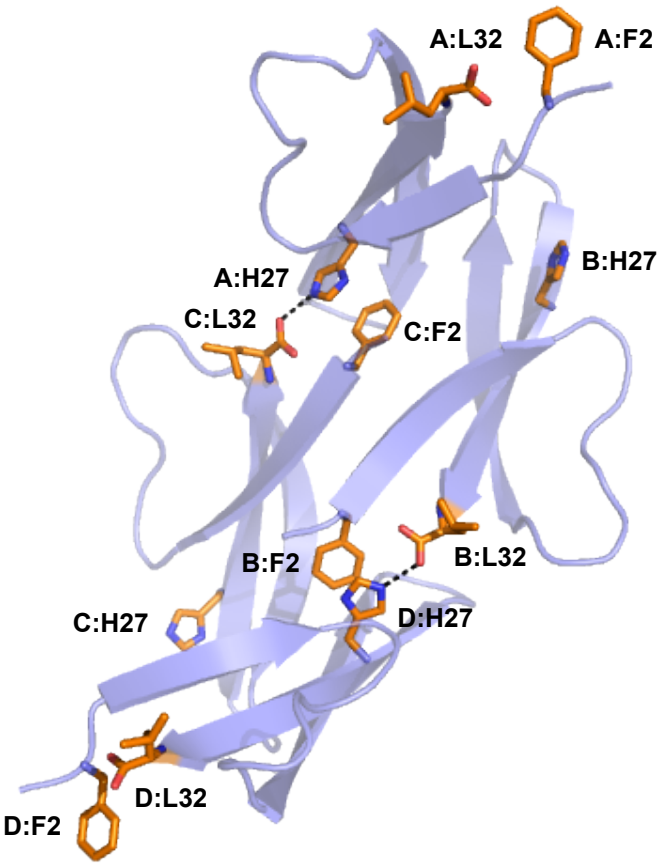

Figure S3. Previously reported X-ray crystal structure of HD6 (PDB: 1ZMQ)[S5] with F2, H27, and L32 shown. Individual HD6 monomers are labeled A-D. The electrostatic interaction between H27 of one monomer and carboxylate of a C-terminus of another monomer is indicated by a dashed line.

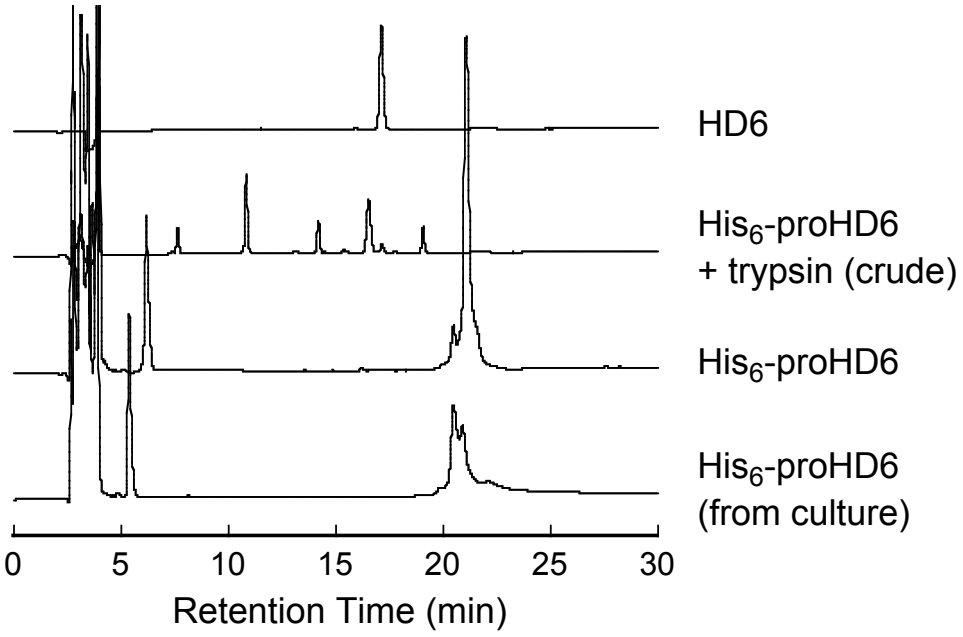

Figure S4. Analytical HPLC traces from a representative overexpression of His_6-proHD6 and purification of HD6 (30 μM x 80 μL). Absorbance at 220 nm was monitored with a reference wavelength of 500 nm. Method: 10–60% B over 30 min at 1 mL/min.

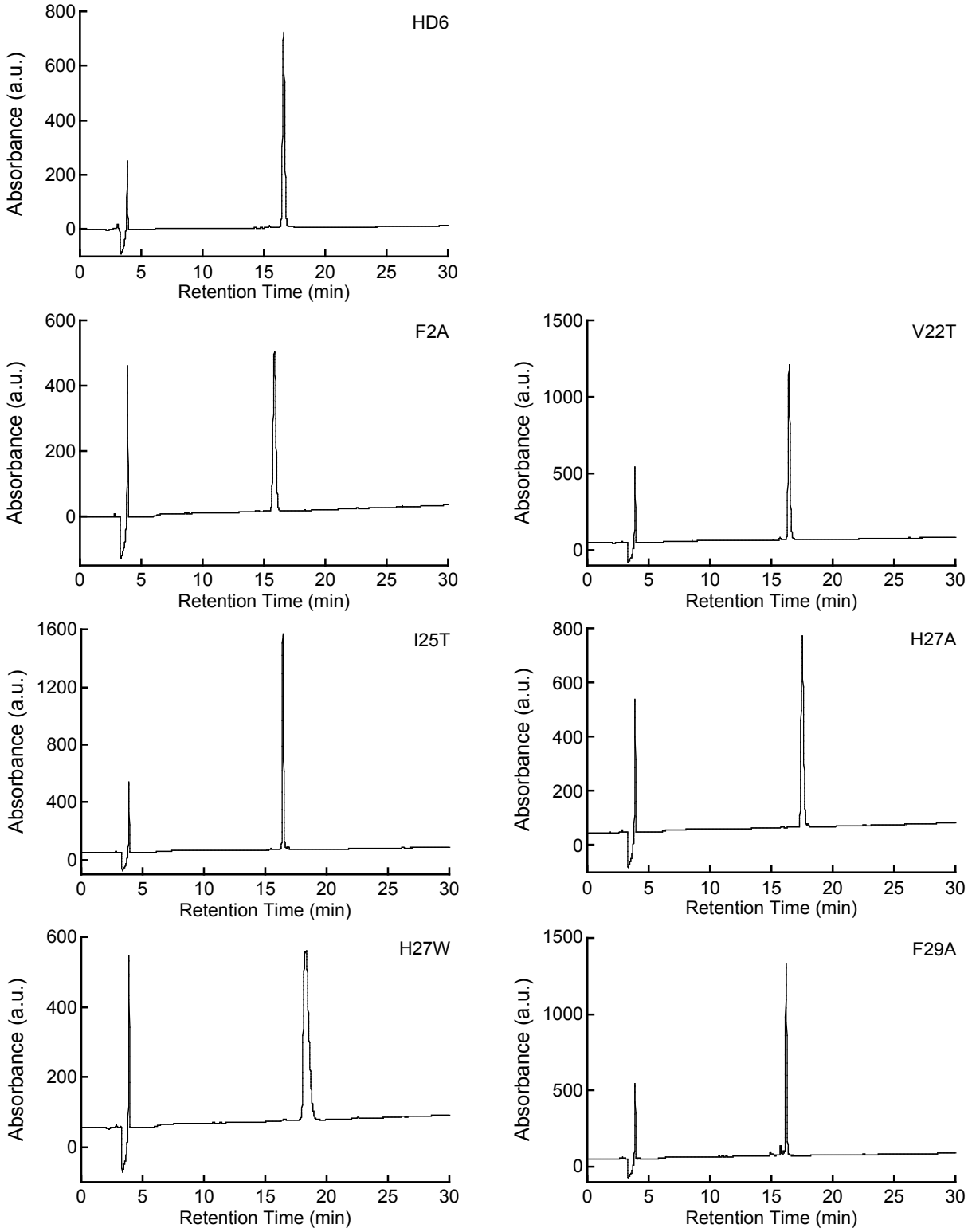

Figure S5. Analytical HPLC traces of native and mutant HD6 (30 μM x 80 μL). Absorbance at 220 nm was monitored with a reference wavelength of 500 nm. Method: 10–60% B over 30 min at 1 mL/min.

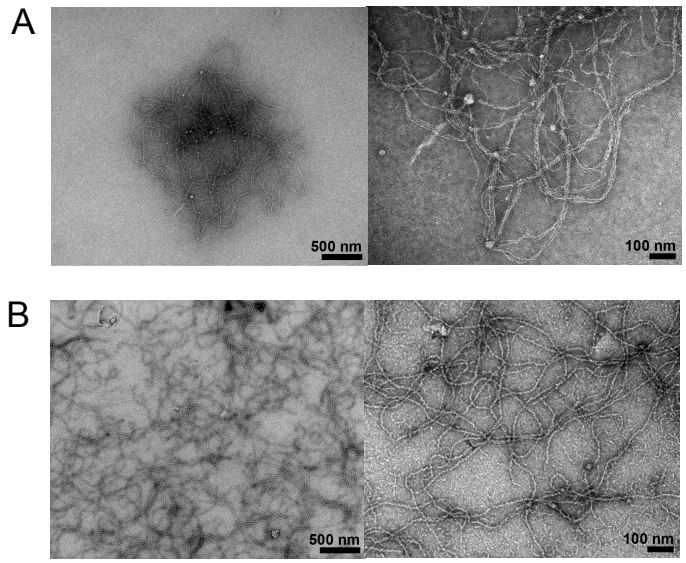

Figure S6. Transmission electron micrographs of HD6 at pH 7.4 (10 mM sodium phosphate). (A) 2 μM HD6 and (B) 20 μM HD6 at magnification of 30,000x (left) and 98,000x (right).

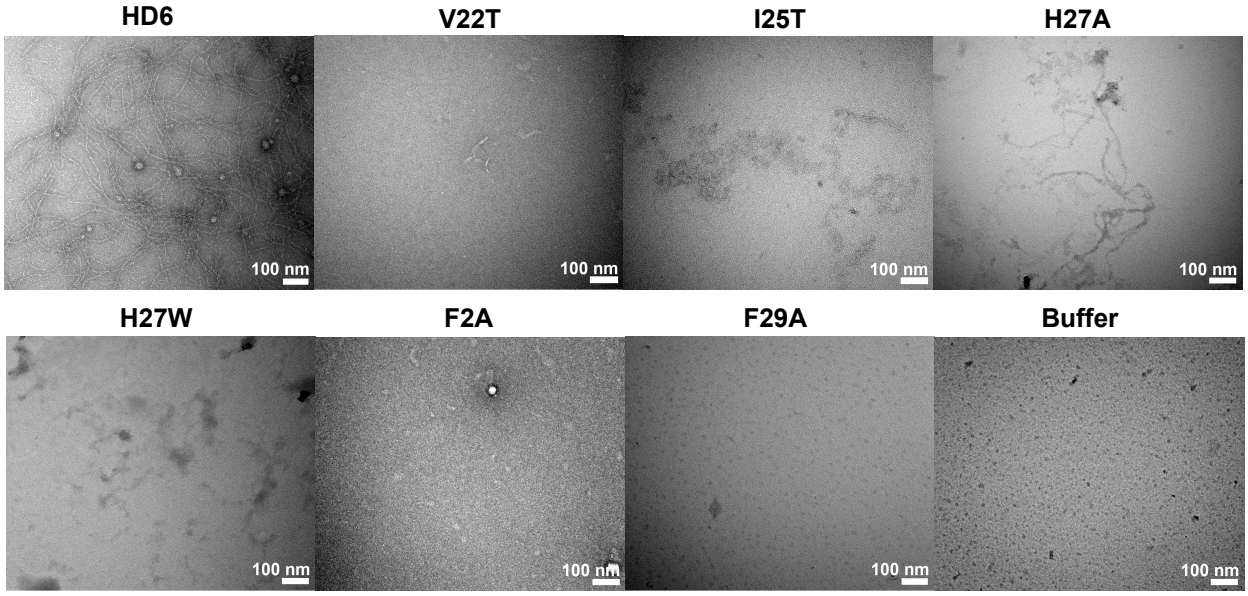

Figure S7. Transmission electron micrographs of native and mutant HD6. The peptide concentrations were 20 μM and all samples were incubated in 10 mM Tris-maleate pH 6.4 for at least 2 h.

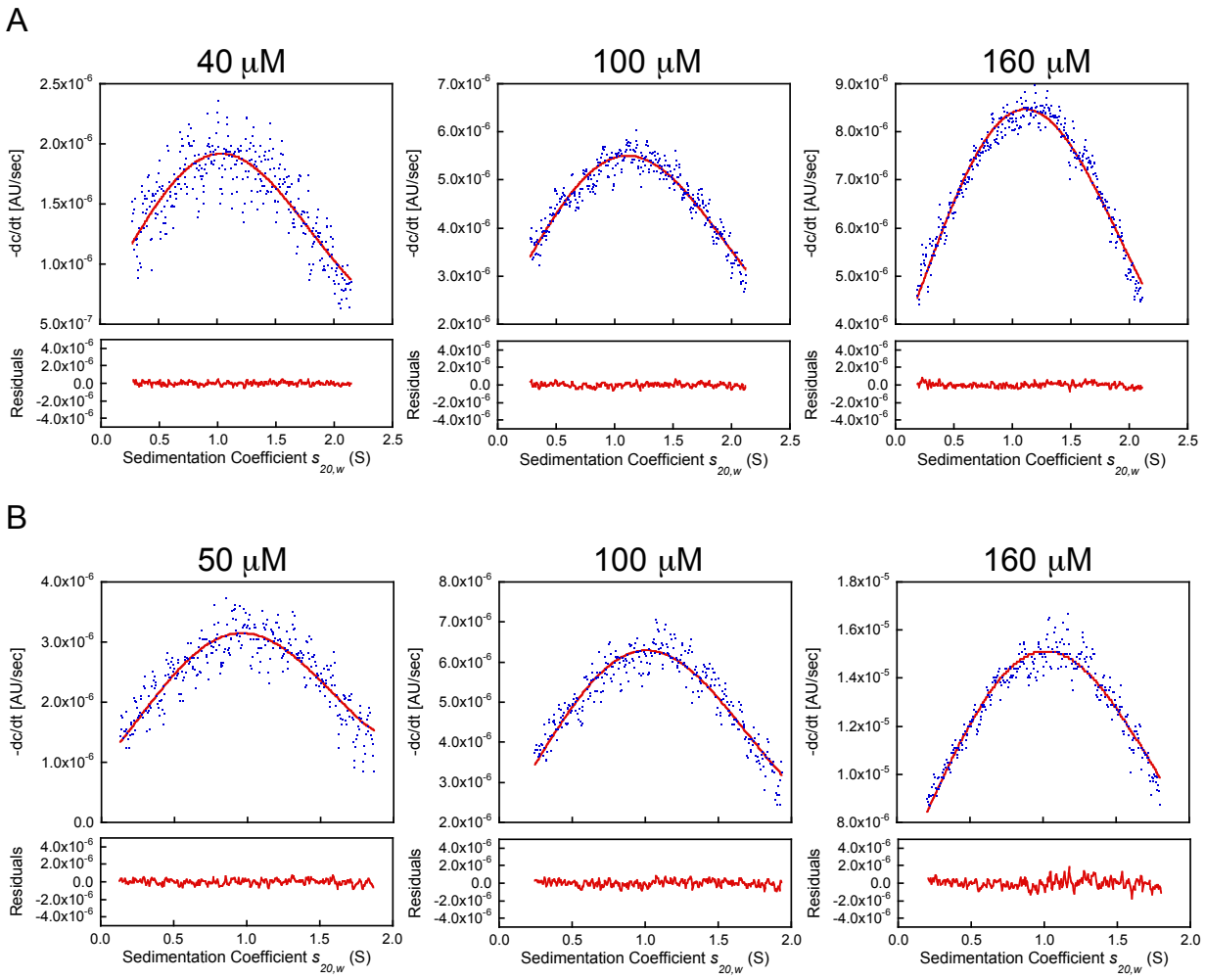

Figure S8. Sedimentation velocity experiments of F2A in (A) 10 mM sodium phosphate pH 7.4 and (B) 10 mM Tris-maleate pH 6.4 shown as single Gaussian fits (red line) of –dc/dt data (blue dots) measured with UV absorbance at 280 nm. The peptide concentrations ranged from 40 μM to 160 μM. The summaries of the fits are provided in Table S3.

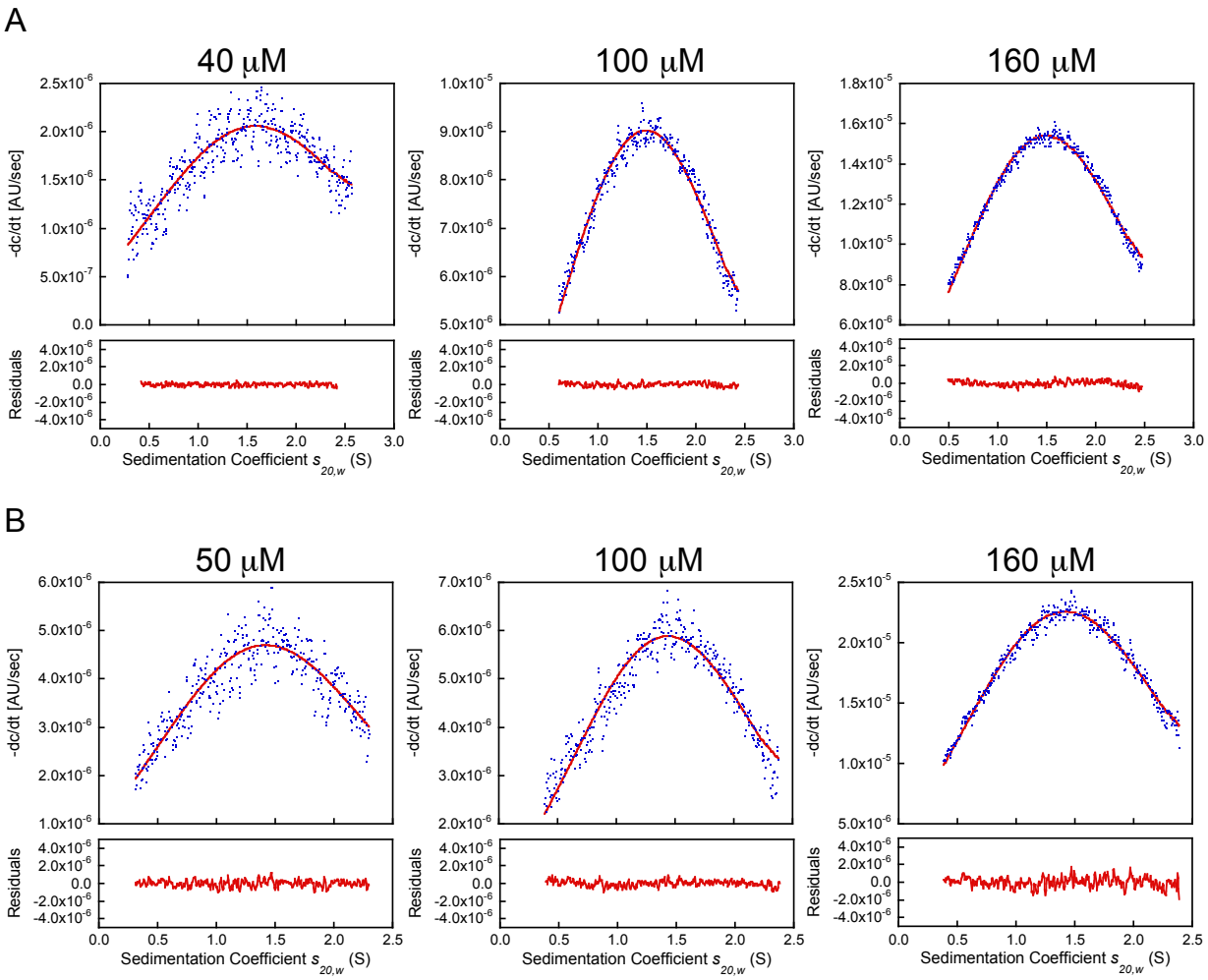

Figure S9. Sedimentation velocity experiments of F29A in (A) 10 mM sodium phosphate pH 7.4 and (B) 10 mM Tris-maleate pH 6.4 shown as single Gaussian fits (red line) of –dc/dt data (blue dots) measured with UV absorbance at 280 nm. The peptide concentrations ranged from 50 μM to 160 μM. The summaries of the fits are provided in Table S4.

The fits of the –dc/dt data shown in this work were obtained by using a one-species model. We also fit the data from the 160 μM sample of F29A with a two-species model, and this analysis did not improve the residuals when compared to the one-species model. In total, the Gaussian fits suggest that F29A at higher concentraitons primarily exists as a speices with S ~ 1.2. There may be a low abundance of a higher-order oligomer.

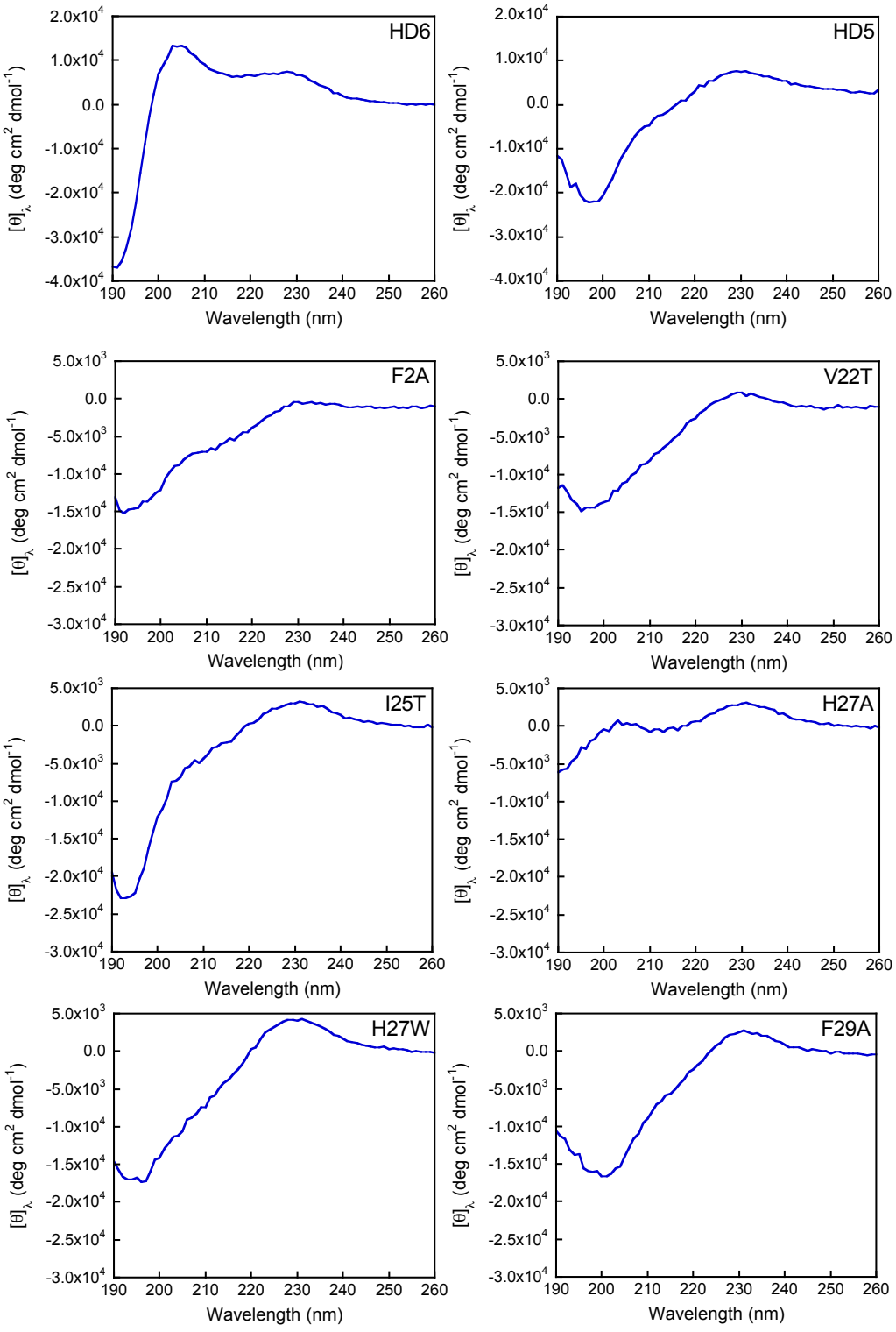

Figure S10. CD spectra of HD5 and native and mutant HD6 at pH 7.4 in 10 mM sodium phosphate. The peptide concentrations were 20 μM.

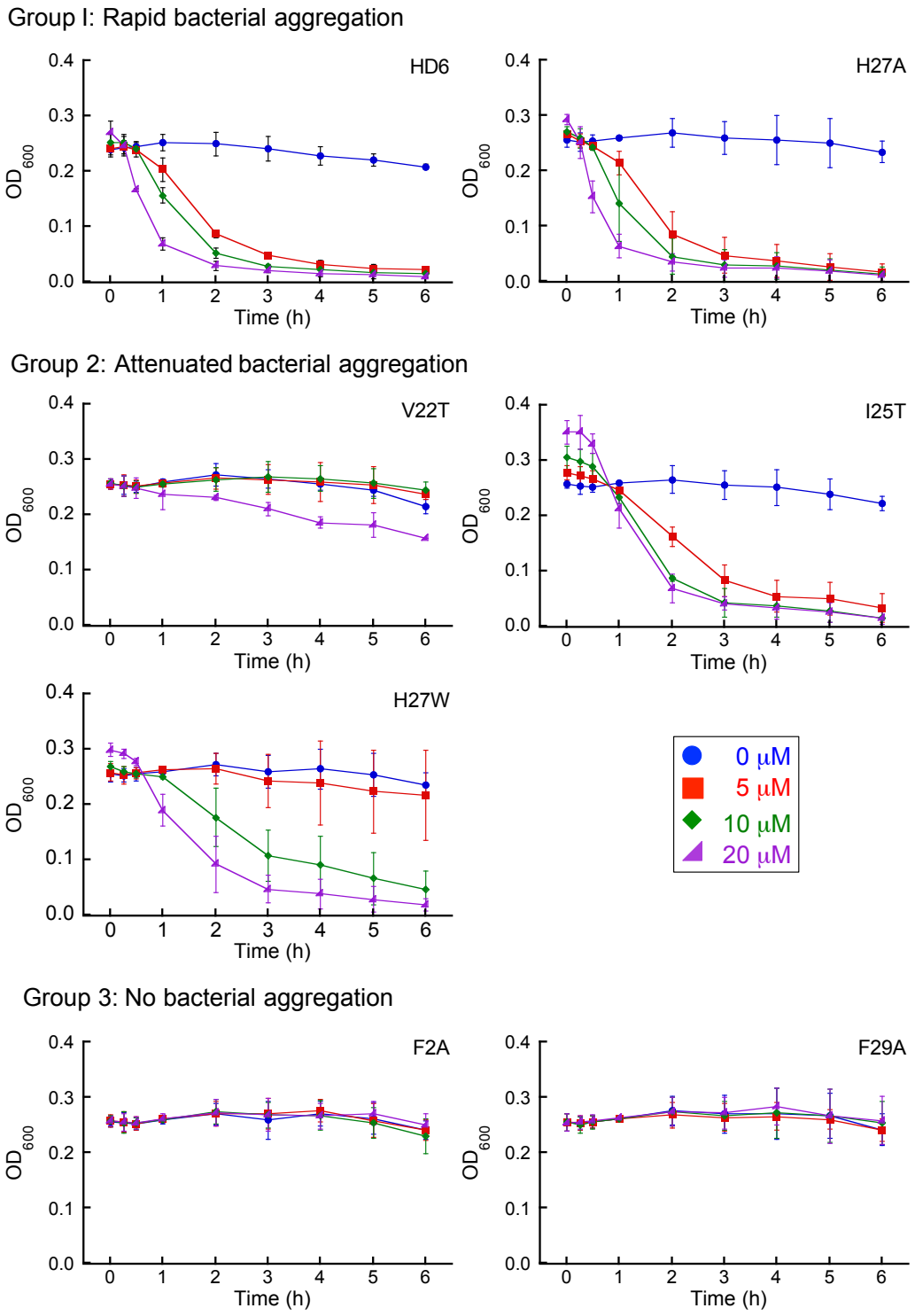

Figure S11. Agglutination assays of *L. monocytogenes* ATCC 19115 incubated with 0-20 μM HD6 or mutant (mean ± SDM, n = 3). Plots of HD6, F2A, H27A, and F29A correspond to Figure 4A and are shown here to facilitate comparison.

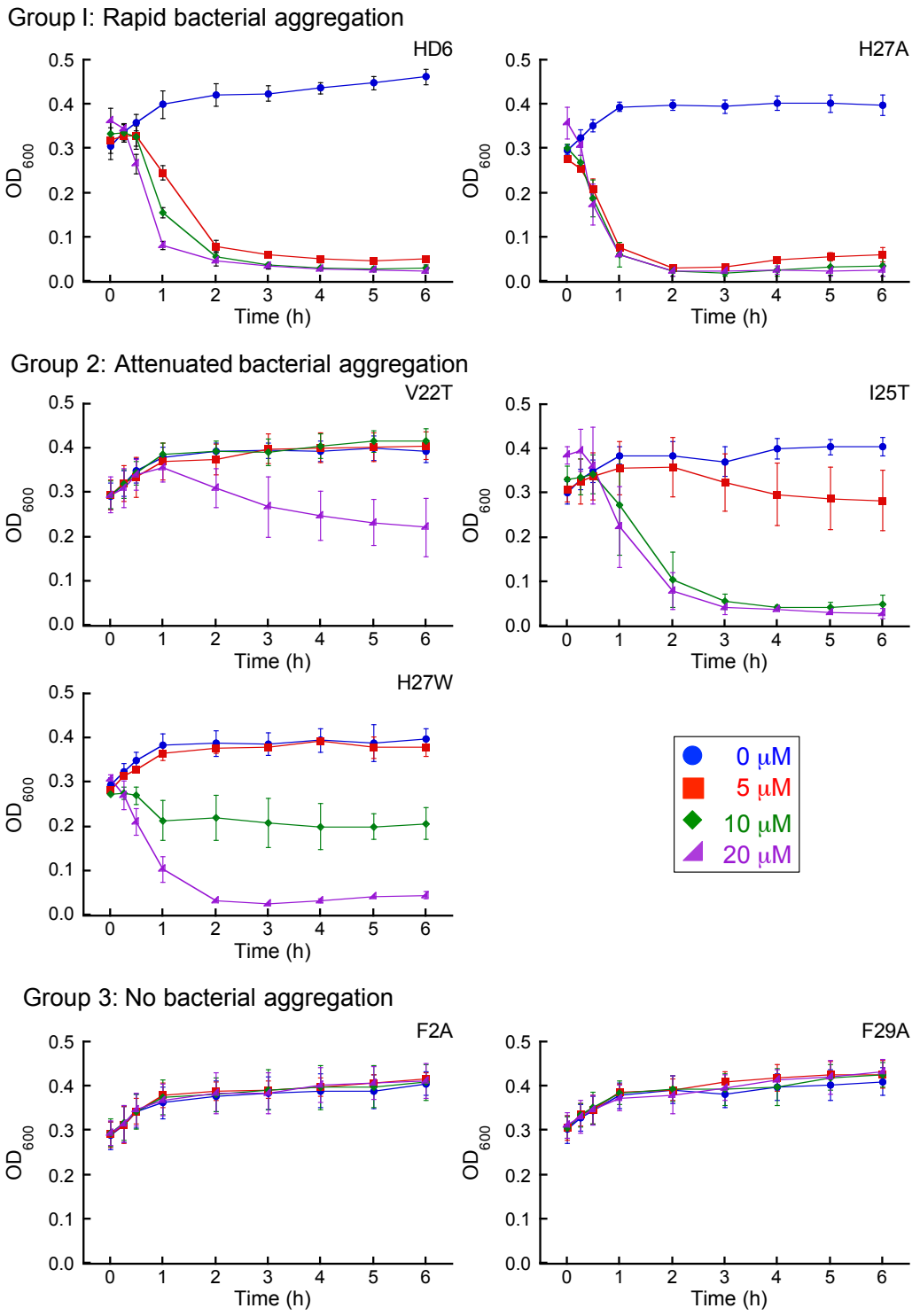

Figure S12. Agglutination assays of *E. coli* ATCC 25922 incubated with 0-20 μM HD6 or mutant (mean ± SDM, n = 3). Plots of HD6, F2A, H27A, and F29A correspond to Figure 4A and are shown here to facilitate comparison.

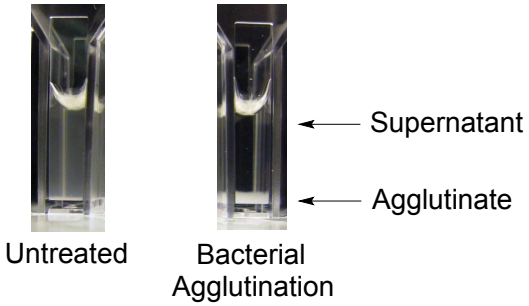

Figure S13. Representative image of an untreated *L. monocytogenes* culture and a culture after incubation with 20 μM HD6 for 6 h at 4t. The mixture is separated into two phases: supernatant and agglutinate as indicated in the figure.

Supporting References

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