Supplementary Information (SI) for Polymer Chemistry.
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Supporting Information for

Sequence-defined structural transitions by calcium-responsive proteins

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I. DNA and protein sequences for RTX variants

Genes encoding each protein variant were flanked with restriction sites for directional cloning and purchased as gene fragments (Twist Bioscience). Genes were subcloned into pQE-9 using BamHI and HindIII restriction sites, bolded in the sequences below.

Block V (Wild Type) DNA sequence

CCCCGTCACCTTTGGCTTATCAGT**GGATCC**CATATGGAGCTCGGCGCTAGCGGCAGCGCACGCGA
CGATGTCCTTATCGGCGATGCGGGCGCTAACGTCCTCAACGGACTGGCTGGTAATGACGTATTAT
CAGGAGGGGCAGGTGACGATGTGTTATTAGGGGACGAAGGCAGTGATTTGCTGTCTGGGGATGCA
GGAAACGATGATCTGTTCGGTGGTCAGGGTGATGATACCTATCTGTTTGGGGTTGGTTACGGTCA
CGACACGATCTATGAGTCCGGCGGCGGCCACGATACAATCCGTATTAATGCCGGAGCAGACCAAC
TGTGGTTTGCGCGCCAGGGAAATGATCTTGAAATACGTATTCTCGGTACCGATGATGCGTTGACT
GTTCATGATTGGTATCGGGACGCTGATCATAGAGTTGAAATAATTCATGCAGCGAATCAGGCTGT
GGATCAAGCCGGTATTGAAAAACTGGTAGAGGCCATGGCCCAGTACCCGGACGAATTCACTAGTC
TCGAG**AAGCTT**AGATCTAGTGACATCTGGACGCTAAGACCG

Block V (Wild Type) protein sequence

MRGSHHHHHHGSHMELGASGSARDDVLIGDAGANVLNGLAGNDVLSGGAGDDVLLGDEGSDLLSG
DAGNDDLFGGQGDDTYLFGVGYGHDTIYESGGGHDTIRINAGADQLWFARQGNDLEIRILGTDDA
LTVHDWYRDADHRVEIIHAANQAVDQAGIEKLVEAMAQYPDEFTSLEKLN*

Global Substitution – Alanine DNA sequence

CCCCGTCACCTTTGGCTTATCAGTGGATCCCATATGGAGCTCGGCGCTAGCGGTAGTGCGCGCGC
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CTGGTGGAGCAGGAGCCGACGTTCTTCTCGGCGATGAGGGCGCCGATCTGTTATCAGGTGACGCC
GGGGCCGACGATCTGTTTGGGGGCCAGGGAGCCGATACGTACTTGTTCGGCGTGGGCTACGGTGC
GGACACCATCTATGAAAGCGGTGGAGGTGCAGATACCATTCGTATCAACGCTGGAGCAGATCAGC
TCTGGTTTGCTCGCCAGGGCAATGATCTTGAAATTCGTATATTGGGGACTGACGATGCTCTGACA
GTACATGATTGGTATAGAGATGCGGATCACCGGGTCGAAATTATTCATGCTGCAAATCAGGCAGT
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TCGAGAAGCTTAGATCTAGTGACATCTGGACGCTAAGACCG

Global Substitution – Alanine protein sequence

MRGSHHHHHHGSHMELGASGSARADVLIGDAGANVLNGLAGADVLSGGAGADVLLGDEGADLLSG
DAGADDLFGGQGADTYLFGVGYGADTIYESGGGADTIRINAGADQLWFARQGNDLEIRILGTDDA
LTVHDWYRDADHRVEIIHAANQAVDQAGIEKLVEAMAQYPDEFTSLEKLN*

Global Substitution – Histidine DNA sequence

CAATCCGCCCTCACTACAACCGGGATCCCATATGGAGCTCGGCGCTAGCGGAAGCGCTCGACACG
ACGTACTTATCGGAGACGCGGGACACAATGTCCTTAATGGCCTTGCTGGTCACGACGTGCTTAGC
GGCGGTGCAGGACATGACGTGTTATTGGGTGATGAGGGGCACGACCTGCTGTCTGGTGACGCAGG
CCATGATGACTTATTCGGTGGTCAAGGTCATGATACTTACTTGTTTGGCGTGGGCTATGGACACG
ACACGATTTACGAATCAGGCGGCGGACACGATACTATAAGAATCAATGCTGGAGCGGACCAACTG
TGGTTCGCCAGACAAGGTAATGACCTGGAGATACGCATCCTCGGTACAGACGATGCCTTAACTGT
ACATGACTGGTACCGTGATGCTGACCATCGTGTCGAGATAATACACGCCGCGAACCAAGCAGTGG
ACCAAGCTGGGATTGAGAAGTTAGTCGAGGCAATGGCTCAATACCCCGACGAATTCACTAGTCTC
GAGAAGCTTAGATCTCTACTCTGGCGTCGATGAGGGA

Global Substitution – Histidine protein sequence

MRGSHHHHHHGSHMELGASGSARHDVLIGDAGHNVLNGLAGHDVLSGGAGHDVLLGDEGHDLLSG
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LTVHDWYRDADHRVEIIHAANQAVDQAGIEKLVEAMAQYPDEFTSLEKLN*

Global Substitution – Serine DNA sequence

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GAGAAGCTTAGATCTCTACTCTGGCGTCGATGAGGGA

Global Substitution – Serine protein sequence

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LTVHDWYRDADHRVEIIHAANQAVDQAGIEKLVEAMAQYPDEFTSLEKLN*

Global Substitution – Asparagine DNA sequence

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TAACGACGATTTGTTTGGCGGGCAAGGCAATGACACTTATCTGTTCGGCGTTGGTTATGGTAATG
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GAGAAGCTTAGATCTCTACTCTGGCGTCGATGAGGGA

Global Substitution – Asparagine protein sequence

MRGSHHHHHHGSHMELGASGSARNDVLIGDAGNNVLNGLAGNDVLSGGAGNDVLLGDEGNDLLSG
DAGNDDLFGGQGNDTYLFGVGYGNDTIYESGGGNDTIRINAGADQLWFARQGNDLEIRILGTDDA
LTVHDWYRDADHRVEIIHAANQAVDQAGIEKLVEAMAQYPDEFTSLEKLN*

Global Substitution – Aspartic acid DNA Sequence

CCCCGTCACCTTTGGCTTATCAGTGGATCCCATATGGAGCTCGGCGCTAGCGGTTCTGCCAGAGA
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Global Substitution – Aspartic acid protein sequence

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DAGDDDLFGGQGDDTYLFGVGYGDDTIYESGGGDDTIRINAGADQLWFARQGNDLEIRILGTDDA
LTVHDWYRDADHRVEIIHAANQAVDQAGIEKLVEAMAQYPDEFTSLEKLN*

Global Substitution – Glutamic acid DNA sequence

CAATCCGCCCTCACTACAACCGGGATCCCATATGGAGCTCGGCGCTAGCGGTTCTGCAAGAGAAG
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GGCGGCGCGGGAGAGGACGTATTGCTCGGTGACGAGGGTGAAGACCTGCTGAGTGGTGATGCAGG
CGAGGACGATCTGTTTGGTGGACAAGGCGAGGACACATACTTGTTCGGAGTGGGGTACGGAGAGG
ACACTATCTATGAGTCTGGTGGTGGGGAAGATACAATTCGAATTAACGCAGGAGCAGACCAGTTA
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GAGAAGCTTAGATCTCTACTCTGGCGTCGATGAGGGA

Global Substitution – Glutamic acid protein sequence

MRGSHHHHHHGSHMELGASGSAREDVLIGDAGENVLNGLAGEDVLSGGAGEDVLLGDEGEDLLSG
DAGEDDLFGGQGEDTYLFGVGYGEDTIYESGGGEDTIRINAGADQLWFARQGNDLEIRILGTDDA
LTVHDWYRDADHRVEIIHAANQAVDQAGIEKLVEAMAQYPDEFTSLEKLN*

Consensus Repeat – Alanine DNA sequence

CAATCCGCCCTCACTACAACCGGGATCCCATATGGAGCTCGGCGCTAGCGGTGGAGCAGGGGCGG
ACACTTTATACGGTGGTGCCGGCGCCGATACATTGTACGGTGGTGCGGGAGCTGATACCCTGTAC
GGTGGCGCAGGTGCTGACACGTTATATGGCGGTGCTGGGGCTGACACATTGTATGGCGGTGCCGG
GGCAGACACCTTATATGGTGGTGCCGGCGCGGATACCCTTTATGGTGGCGCAGGCGCGGATACTC
TGTACGGTGGTGCTGGAGCGGACACTCTGTATATTAACGCAGGAGCAGACCAGCTGTGGTTCGCA
CGTCAAGGCAACGATCTGGAAATACGCATCCTGGGTACAGACGACGCCTTGACTGTCCACGACTG
GTATCGTGACGCGGACCATCGCGTAGAGATTATACATGCAGCAAACCAAGCGGTAGATCAAGCTG
GGATAGAGAAACTGGTCGAGGCCATGGCTCAATACCCGGATGAATTCACTAGTCTCGAGAAGCTT
AGATCTCTACTCTGGCGTCGATGAGGGA

Consensus Repeat – Alanine protein sequence

MRGSHHHHHHGSHMELGASGGAGADTLYGGAGADTLYGGAGADTLYGGAGADTLYGGAGADTLYG
GAGADTLYGGAGADTLYGGAGADTLYGGAGADTLYINAGADQLWFARQGNDLEIRILGTDDALTV
HDWYRDADHRVEIIHAANQAVDQAGIEKLVEAMAQYPDEFTSLEKLN*

Consensus Repeat – Histidine DNA sequence

CAATCCGCCCTCACTACAACCGGGATCCCATATGGAGCTCGGCGCTAGCGGTGGTGCAGGACATG
ACACACTGTACGGCGGGGCGGGTCATGATACTCTTTATGGCGGCGCCGGCCACGATACTTTGTAT
GGTGGAGCAGGCCACGACACCCTCTACGGTGGGGCTGGGCATGACACACTTTATGGCGGGGCGGG
ACACGATACTCTGTACGGCGGCGCTGGTCACGACACATTATATGGTGGCGCGGGGCATGATACTT
TATACGGTGGAGCGGGCCACGATACACTGTACATCAATGCTGGAGCTGACCAACTTTGGTTCGCG
CGCCAGGGTAACGACTTAGAGATCCGAATTTTGGGCACTGACGACGCCCTTACGGTACACGACTG
GTACCGCGACGCAGACCACCGTGTTGAGATCATCCACGCCGCCAACCAGGCAGTTGATCAAGCCG
GCATCGAGAAATTAGTCGAAGCTATGGCTCAGTACCCGGACGAATTCACTAGTCTCGAGAAGCTT
AGATCTCTACTCTGGCGTCGATGAGGGA

Consensus Repeat – Histidine protein sequence

MRGSHHHHHHGSHMELGASGGAGHDTLYGGAGHDTLYGGAGHDTLYGGAGHDTLYGGAGHDTLYG
GAGHDTLYGGAGHDTLYGGAGHDTLYGGAGHDTLYINAGADQLWFARQGNDLEIRILGTDDALTV
HDWYRDADHRVEIIHAANQAVDQAGIEKLVEAMAQYPDEFTSLEKLN*

Consensus Repeat – Serine DNA sequence

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CGACAAGGTAATGACTTAGAGATACGTATCCTGGGGACAGATGACGCACTTACGGTGCATGACTG
GTATAGAGACGCGGACCATCGTGTCGAGATAATACACGCGGCCAACCAGGCGGTAGACCAGGCCG
GTATTGAGAAGCTGGTCGAGGCGATGGCACAATACCCCGACGAATTCACTAGTCTCGAGAAGCTT
AGATCTCTACTCTGGCGTCGATGAGGGA

Consensus Repeat – Serine protein sequence

MRGSHHHHHHGSHMELGASGGAGSDTLYGGAGSDTLYGGAGSDTLYGGAGSDTLYGGAGSDTLYG
GAGSDTLYGGAGSDTLYGGAGSDTLYGGAGSDTLYINAGADQLWFARQGNDLEIRILGTDDALTV
HDWYRDADHRVEIIHAANQAVDQAGIEKLVEAMAQYPDEFTSLEKLN*

Consensus Repeat – Asparagine DNA sequence

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CGCCAAGGCAATGACCTCGAAATTCGTATCTTAGGGACGGACGACGCGCTCACTGTTCATGATTG
GTATCGGGATGCGGATCACCGTGTGGAAATTATTCACGCGGCTAACCAGGCTGTCGACCAAGCAG
GTATAGAGAAACTTGTGGAAGCGATGGCCCAATATCCAGATGAATTCACTAGTCTCGAGAAGCTT
AGATCTCTACTCTGGCGTCGATGAGGGA

Consensus Repeat – Asparagine protein sequence

MRGSHHHHHHGSHMELGASGGAGNDTLYGGAGNDTLYGGAGNDTLYGGAGNDTLYGGAGNDTLYG
GAGNDTLYGGAGNDTLYGGAGNDTLYGGAGNDTLYINAGADQLWFARQGNDLEIRILGTDDALTV
HDWYRDADHRVEIIHAANQAVDQAGIEKLVEAMAQYPDEFTSLEKLN*

Consensus Repeat – Aspartic acid DNA sequence

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GGCGGAGCCGGAGACGACACACTGTATGGTGGCGCCGGTGATGACACATTGTACGGTGGGGCAGG
GGACGACACACTCTACGGCGGCGCCGGCGATGATACTCTGTATGGTGGTGCGGGTGATGACACCT
TGTACGGCGGAGCCGGTGATGACACACTTTACATCAACGCTGGGGCCGACCAATTATGGTTCGCC
CGTCAGGGCAACGATTTGGAAATTAGAATCCTGGGGACCGATGATGCTCTTACTGTGCACGACTG
GTACCGGGATGCCGACCACCGTGTTGAGATTATCCATGCAGCTAATCAAGCTGTAGACCAAGCAG
GCATTGAGAAACTTGTTGAGGCCATGGCACAGTACCCAGATGAATTCACTAGTCTCGAGAAGCTT
AGATCTCTACTCTGGCGTCGATGAGGGA

Consensus Repeat – Aspartic acid protein sequence

MRGSHHHHHHGSHMELGASGGAGDDTLYGGAGDDTLYGGAGDDTLYGGAGDDTLYGGAGDDTLYG
GAGDDTLYGGAGDDTLYGGAGDDTLYGGAGDDTLYINAGADQLWFARQGNDLEIRILGTDDALTV
HDWYRDADHRVEIIHAANQAVDQAGIEKLVEAMAQYPDEFTSLEKLN*

Consensus Repeat – Glutamic acid DNA sequence

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GGTGGCGCAGGTGAAGACACATTGTATGGCGGTGCTGGCGAAGACACGCTCTATGGTGGTGCTGG
TGAGGACACGCTTTACGGTGGTGCAGGCGAGGACACCCTGTACGGTGGTGCCGGGGAAGACACTC
TTTACGGTGGAGCCGGCGAGGACACTCTTTACATAAATGCCGGCGCTGACCAGTTGTGGTTTGCG
CGCCAAGGAAATGATCTTGAAATACGCATCTTAGGAACCGACGACGCTTTAACCGTCCATGATTG
GTACCGCGACGCGGACCATCGTGTTGAGATCATTCACGCTGCGAACCAAGCTGTTGATCAAGCCG
GGATTGAGAAGCTGGTGGAAGCAATGGCCCAATACCCGGATGAATTCACTAGTCTCGAGAAGCTT
AGATCTCTACTCTGGCGTCGATGAGGGA

Consensus Repeat – Glutamic acid protein sequence

MRGSHHHHHHGSHMELGASGGAGEDTLYGGAGEDTLYGGAGEDTLYGGAGEDTLYGGAGEDTLYG
GAGEDTLYGGAGEDTLYGGAGEDTLYGGAGEDTLYINAGADQLWFARQGNDLEIRILGTDDALTV
HDWYRDADHRVEIIHAANQAVDQAGIEKLVEAMAQYPDEFTSLEKLN*

II. Table of protein properties

The protein sequence was used to calculate pI, molar extinction coefficient at 280 nm (ε_{280}), and expected molecular weight.[1] Observed molecular weight measured with matrix-assisted laser desorption/ionization time of flight mass spectrometry (MALDI-TOF MS).

Table S1. Protein variant properties

Protein Variant	**pI**	**ε_{280} (M^{-1} cm^{-1})**	**Expected Molecular Weight (kDa)**	**Observed Molecular Weight (kDa)**
Block V (Wild Type)	4.40	18450	19.1	19.1
Global Substitution				
Alanine	4.41	18450	18.7	18.7
Histidine	5.03	18450	19.3	19.3
Serine	4.41	18450	18.9	18.9
Asparagine	4.41	18450	19.1	19.1
Aspartic acid	4.12	18450	19.1	19.1
Glutamic acid	4.21	18450	19.3	19.2
Consensus Repeat				
Alanine	4.48	27390	18.1	18.1
Serine	4.48	27390	18.2	18.2
Histidine	5.25	27390	18.7	18.7
Asparagine	4.48	27390	18.5	18.5
Aspartic acid	4.13	27390	18.5	18.5
Glutamic acid	4.24	27390	18.6	18.6

[1]E. Gasteiger *et al.*, “Protein Identification and Analysis Tools on the ExPASy Server” in The Proteomics Protocols Handbook, J. M. Walker, Ed. (Humana Press, Totowa, NJ, 2005), 10.1385/1-59259-890-0:571, pp. 571-607.

III. Protein purification

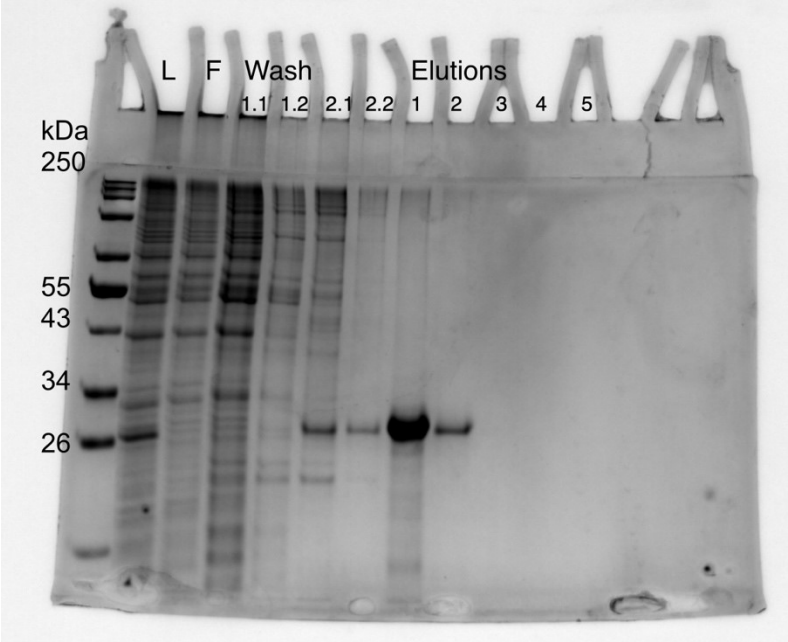

Figure S1. Representative SDS-PAGE of protein fractions from lysate (L), flowthrough (F), washes (1.1, 1.2, 2.1, 2.2), and elution (1-5) stages of Ni-NTA purification. Experimental conditions: 12% polyacrylamide, 200 V, 45 minutes, global substitution variant with asparagine.

IV. MALDI-TOF MS of RTX variants

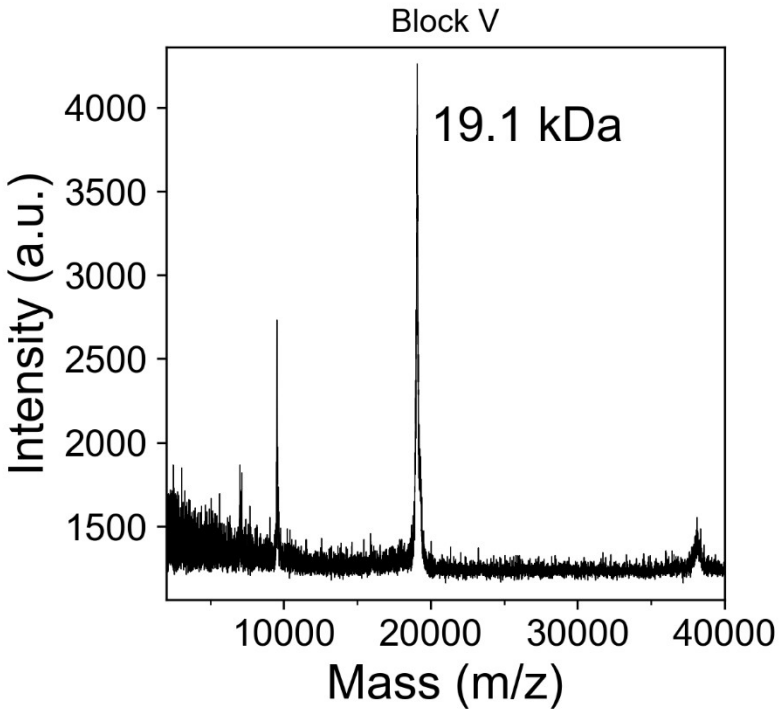

Figure S2. MALDI-TOF MS of Block V (expected 19.1 kDa).

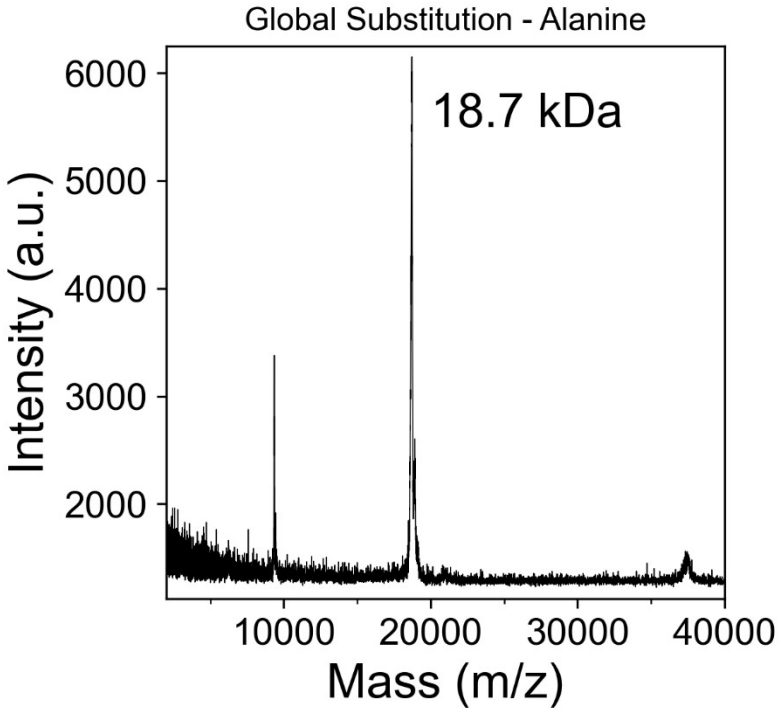

Figure S3. MALDI-TOF MS of global substitution – alanine (expected 18.7 kDa).

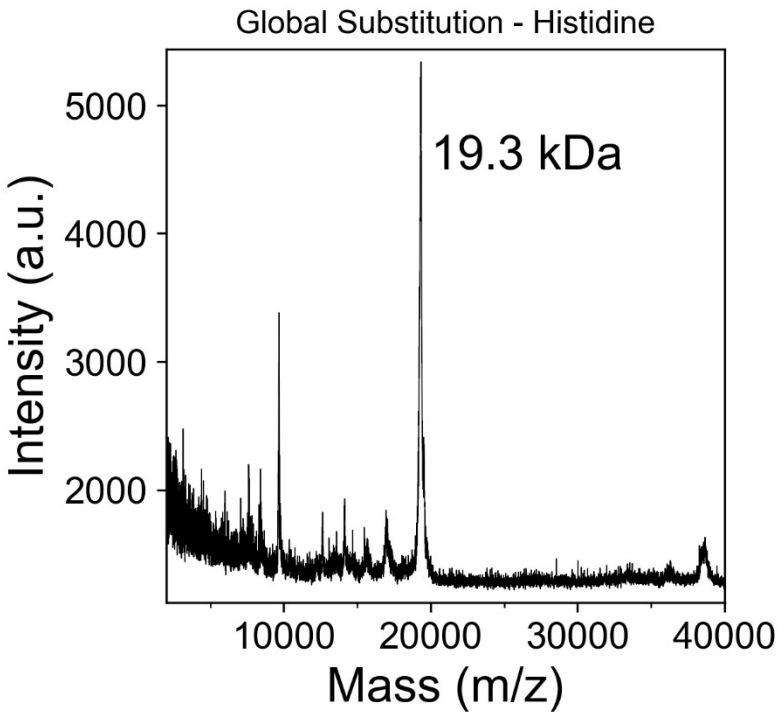

Figure S4. MALDI-TOF MS of global substitution – histidine (expected 19.3 kDa).

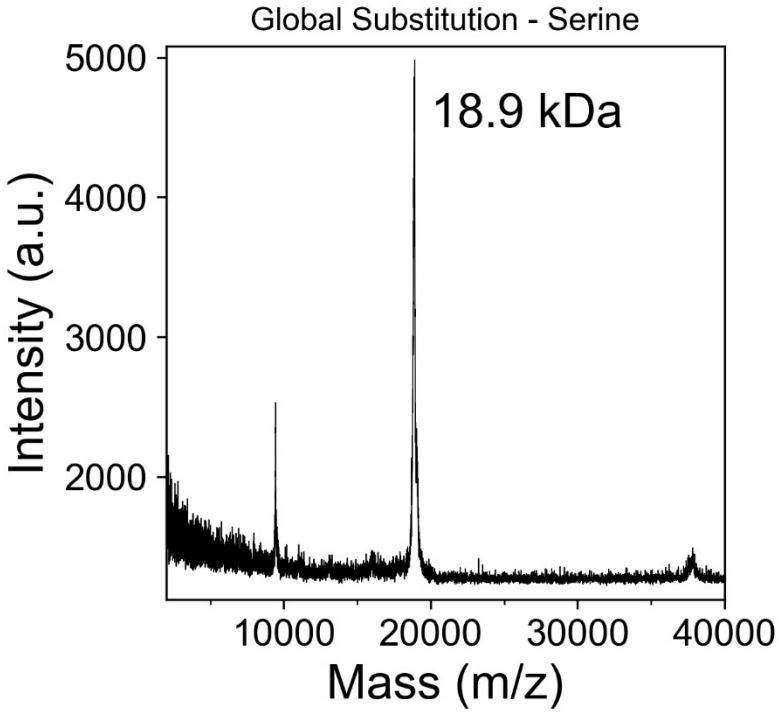

Figure S5. MALDI-TOF MS of global substitution – serine (expected 18.9 kDa).

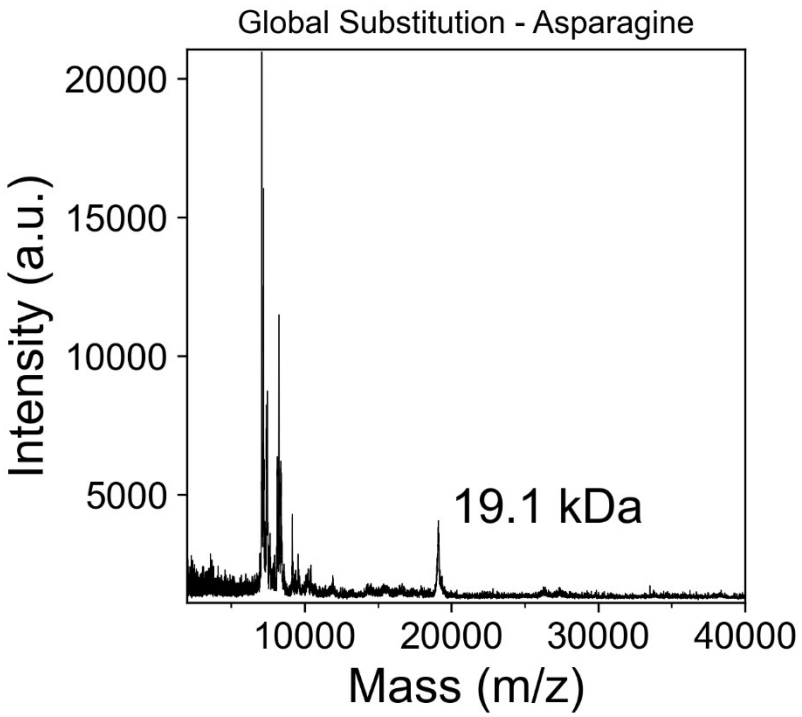

Figure S6. MALDI-TOF MS of global substitution – asparagine (expected 19.1 kDa).

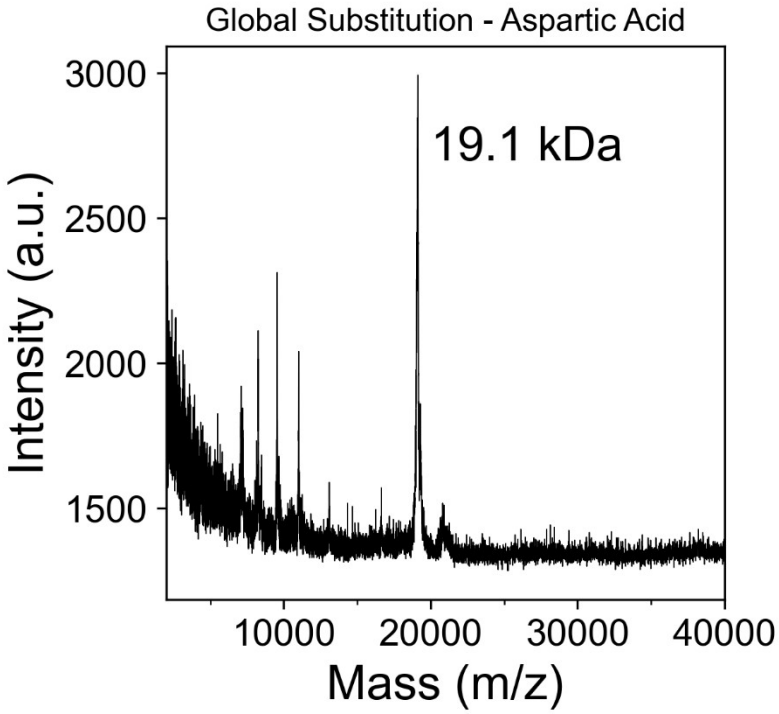

Figure S7. MALDI-TOF MS of global substitution – aspartic acid (expected 19.1 kDa).

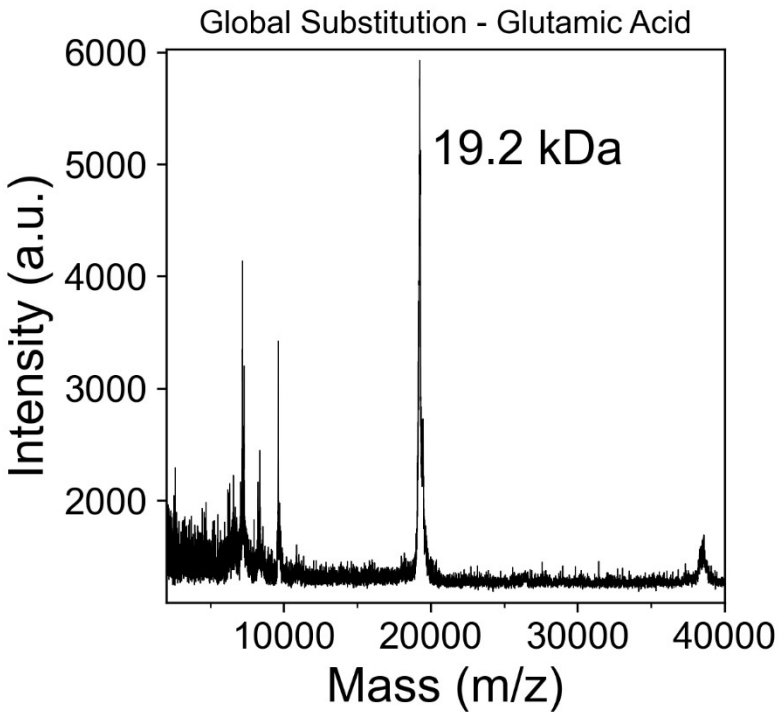

Figure S8. MALDI-TOF MS of global substitution – glutamic acid (expected 19.2 kDa).

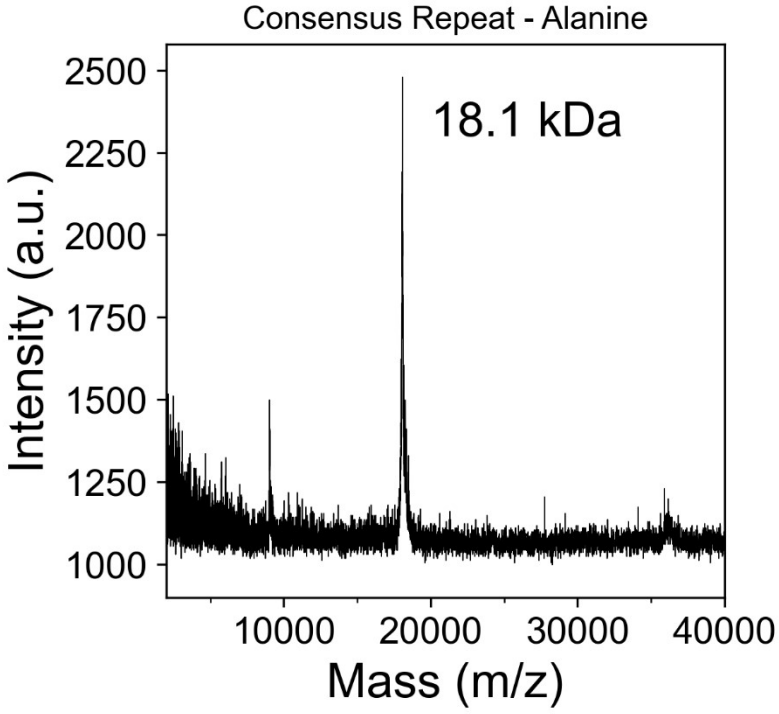

Figure S9. MALDI-TOF MS of consensus repeat – alanine (expected 18.1 kDa).

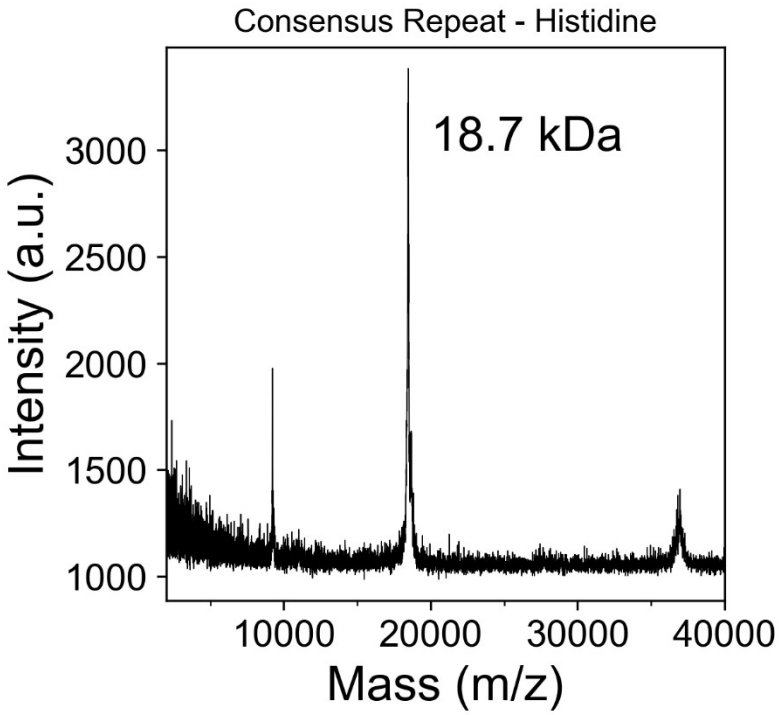

Figure S10. MALDI-TOF MS of consensus repeat – histidine (expected 18.7 kDa).

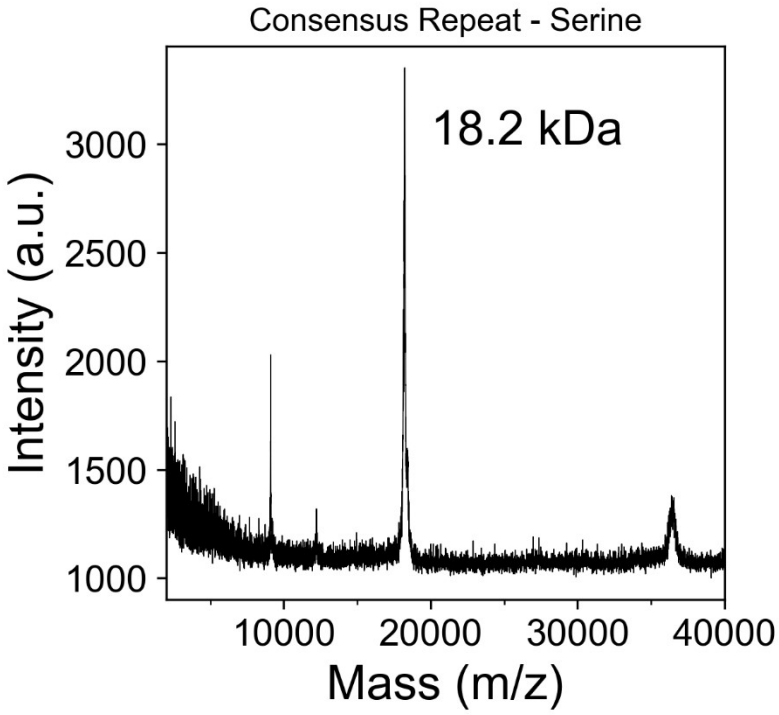

Figure S11. MALDI-TOF MS of consensus repeat – serine (expected 18.2 kDa).

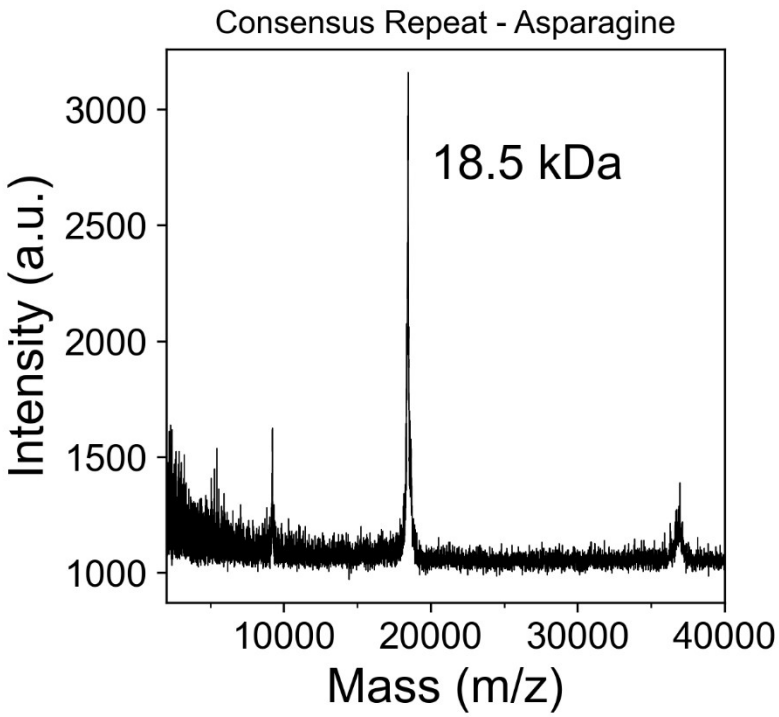

Figure S12. MALDI-TOF MS of consensus repeat – asparagine (expected 18.5 kDa).

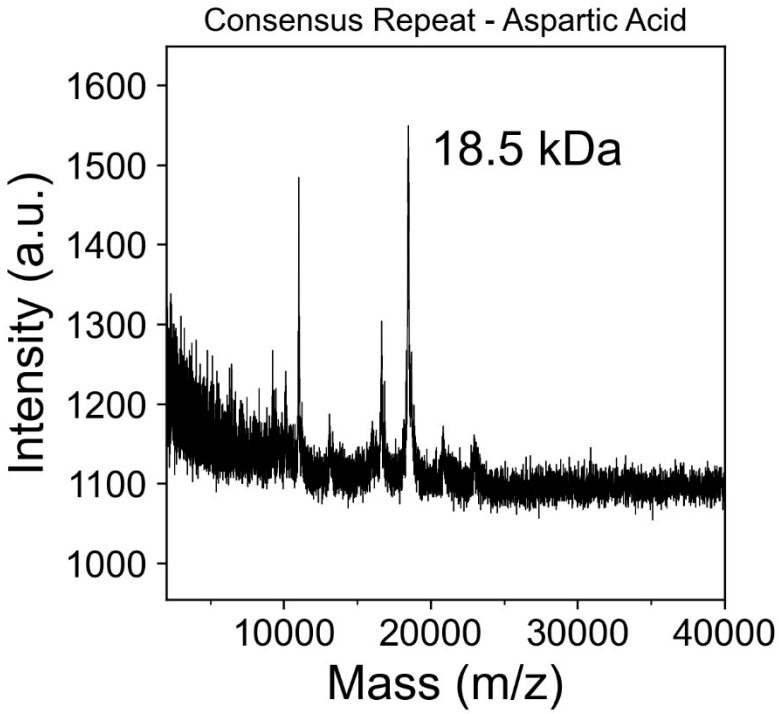

Figure S13. MALDI-TOF MS of consensus repeat – aspartic acid (expected 18.5 kDa).

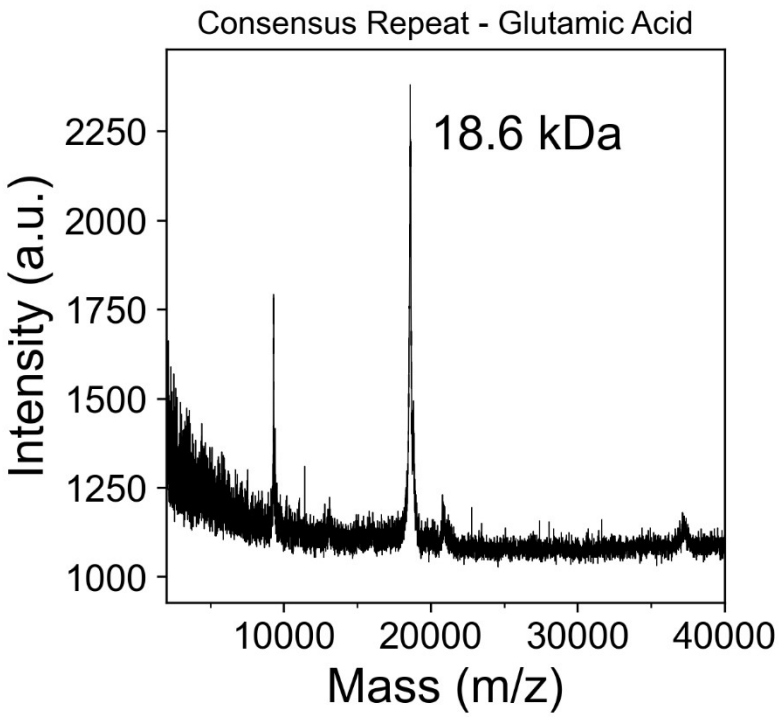

Figure S14. MALDI-TOF MS of consensus repeat – glutamic acid (expected 18.6 kDa).

V. Equations used to calculate protein concentration, mean residue ellipticity, and secondary structure comparisons

Protein concentration measurements. The concentration of protein solutions was calculated from solution absorbance at 280 nm as measured with a NanoDrop One C Spectrometer. Protein solution concentration is calculated using the Beer–Lambert law:

$$A = \varepsilon l c$$

where A is the absorbance, ε is the molar extinction coefficient of the protein in units of M^{-1} cm^{-1}, l is the optical path length in cm, and c is the protein concentration in M.

The molar extinction coefficient at 280 nm for each protein variant is estimated using the following equation:

$$\varepsilon_{protein} = N_{Tyrosine}\varepsilon_{Tyrosine} + N_{Tryptopha}\ \ \varepsilon_{Tryptophan} + N_{Cystine}\varepsilon_{Cystine}$$

where $N_{Tyrosine}$, $N_{Tryptophan}$, and $N_{Cystine}$ are the number of residues of each amino acid in the protein sequence, and the extinction coefficients of each amino acid are as follows: $\varepsilon_{Tyrosine}$ = 1490 M^{-1} cm^{-1}, $\varepsilon_{Tryptophan}$= 5500 M^{-1} cm^{-1}, and $\varepsilon_{Cystine}$ = 125 M^{-1} cm^{-1}.

Mean residue ellipticity calculations. Ellipticity measured by circular dichroism was converted to mean residue ellipticity (MRE) to facilitate the comparison of protein samples with different concentrations and protein variants with different numbers of residues. MRE is calculated using the following equation:

$$[\theta] = 100\ \theta/(clN)$$

where $[\theta]$ is MRE in deg cm^2 $dmol^{-1}$, θ is ellipticity in degrees, c is the protein concentration in M, l is the path length of the cuvette in cm, and N is the number of residues in the protein.

Secondary structure comparisons. The relative percent change of a structural component (i.e. the relative percent increase in sheet content from 0 mM $CaCl_2$ to 100 mM $CaCl_2$) is calculated using the following equation:

$$Z = \left(\frac{X - X_0}{X_0}\right) \times 100\%$$

where X is the final structural content, X_0 is the starting structural content, and Z is the relative percent change.

Similarly, a comparison of a structural component between two variants (i.e. the relative higher sheet content in a consensus repeat variant compared to Block V) is calculated using the following equation:

$$Z = \left(\frac{X_2 - X_1}{X_1}\right) \times 100\%$$

where X_2 is the variant structural content, X_1 is the reference structural content (typically Block V), and Z is the relative percent difference.

VI. Circular Dichroism Replicates

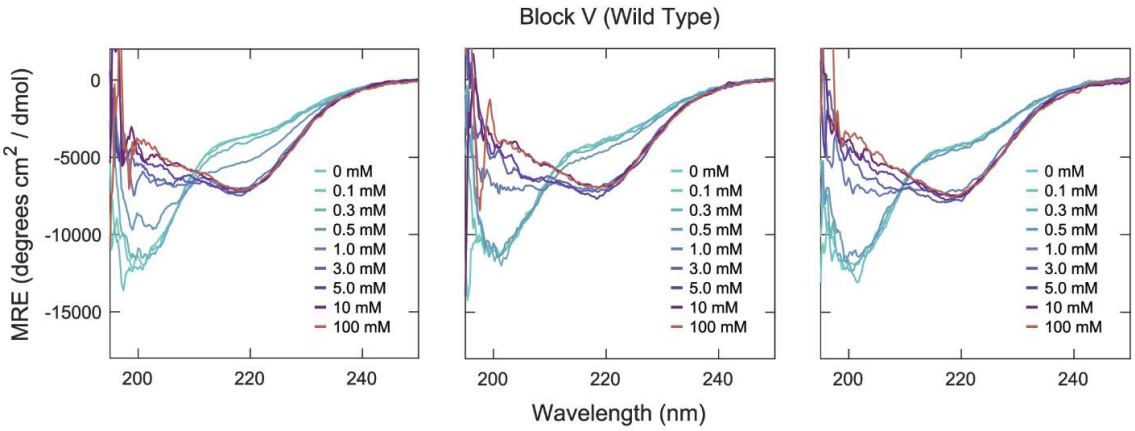

Figure S15. Triplicate circular dichroism measurements of Block V (Wild Type).

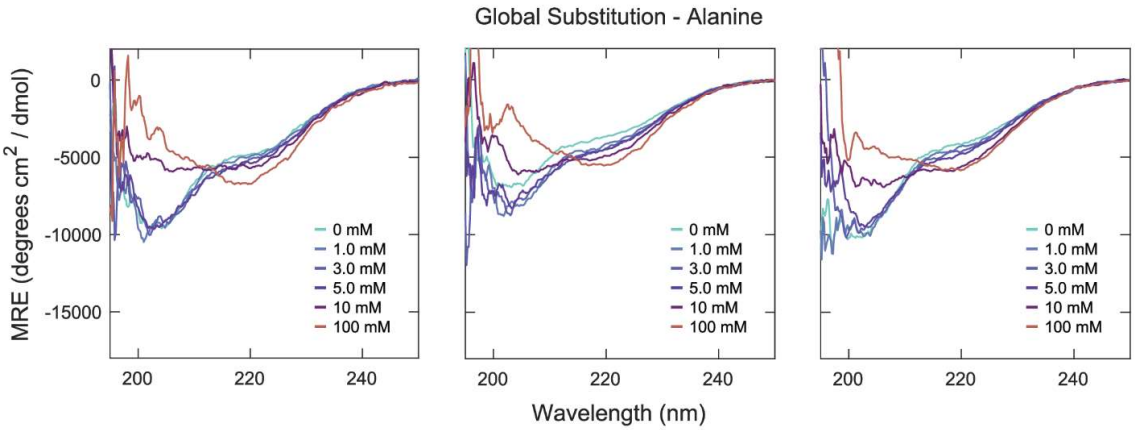

Figure S16. Triplicate circular dichroism measurements of global substitution – alanine.

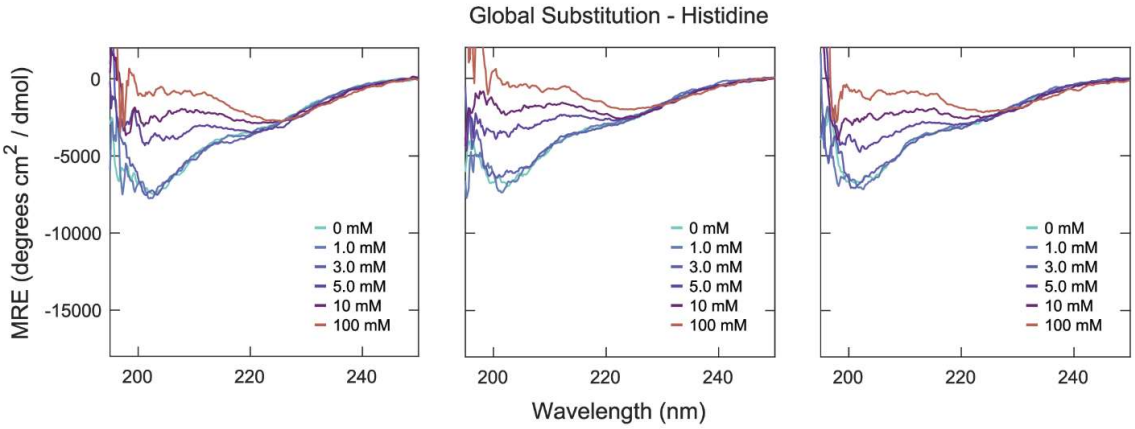

Figure S17. Triplicate circular dichroism measurements of global substitution – histidine.

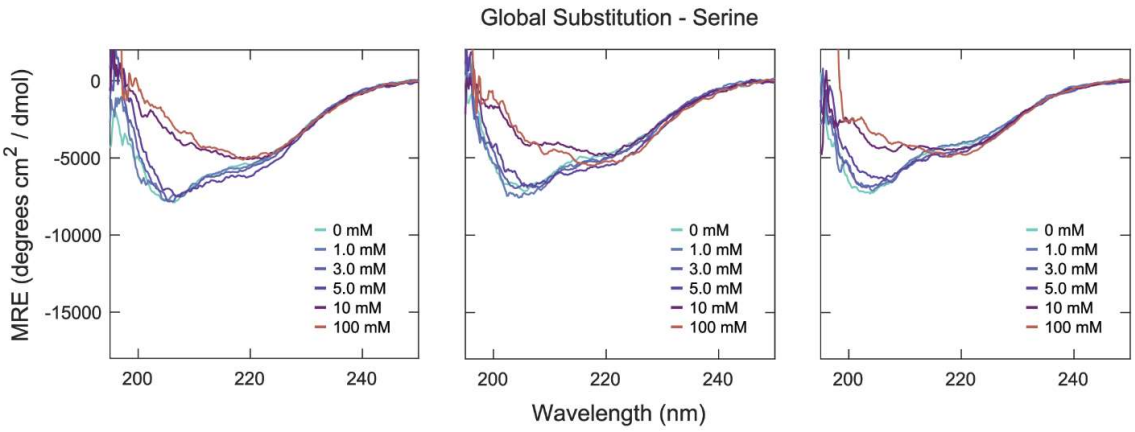

Figure S18. Triplicate circular dichroism measurements of global substitution – serine.

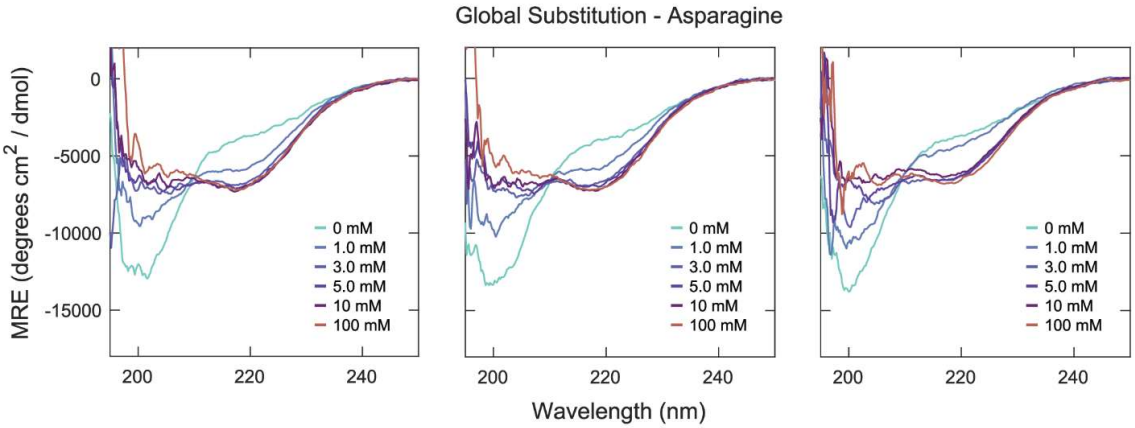

Figure S19. Triplicate circular dichroism measurements of global substitution – asparagine.

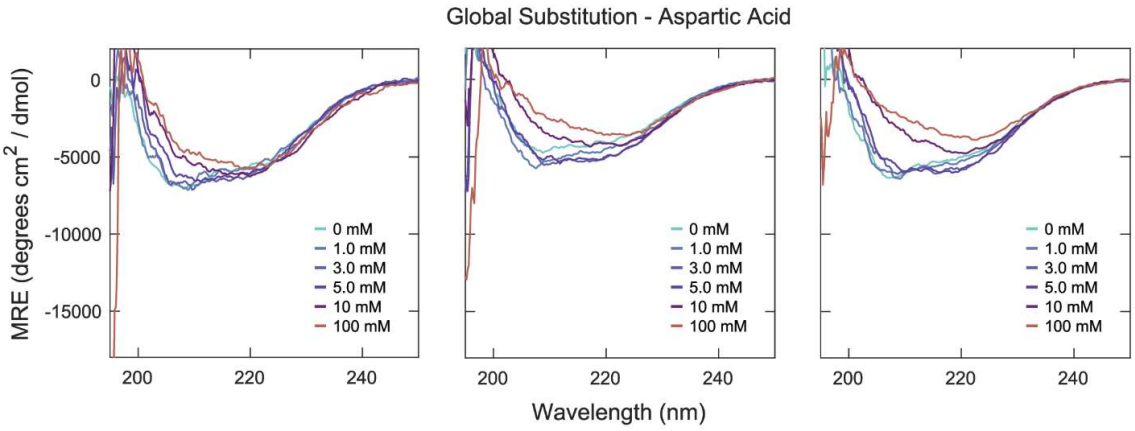

Figure S20. Triplicate circular dichroism measurements of global substitution – aspartic acid.

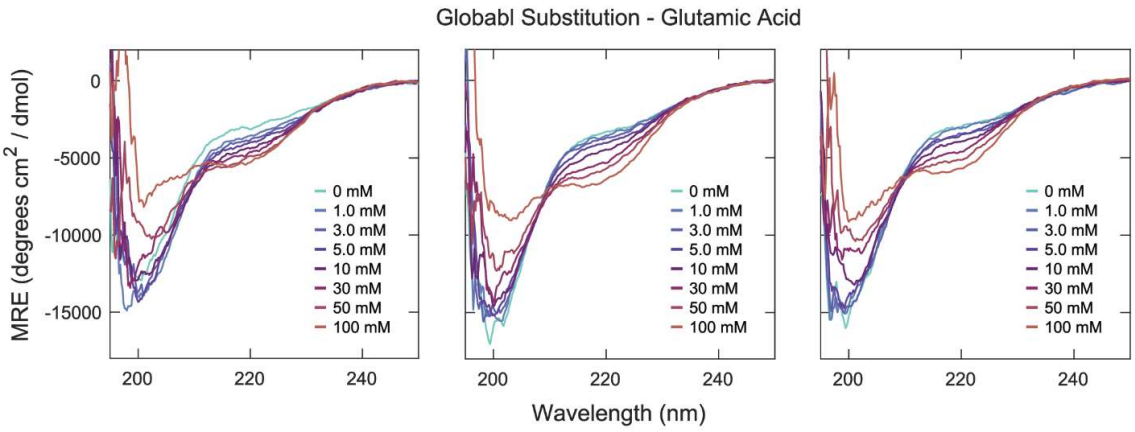

Figure S21. Triplicate circular dichroism measurements of global substitution – glutamic acid.

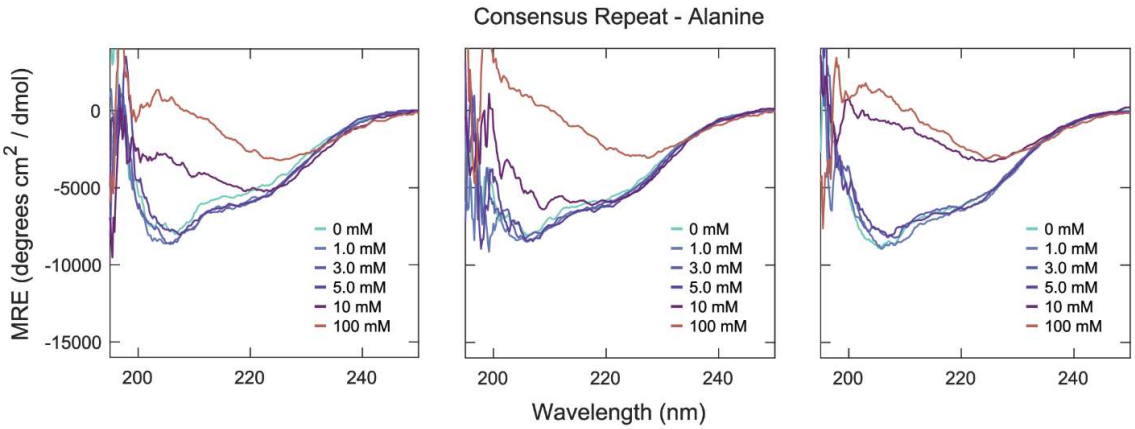

Figure S22. Triplicate circular dichroism measurements of consensus repeat – alanine.

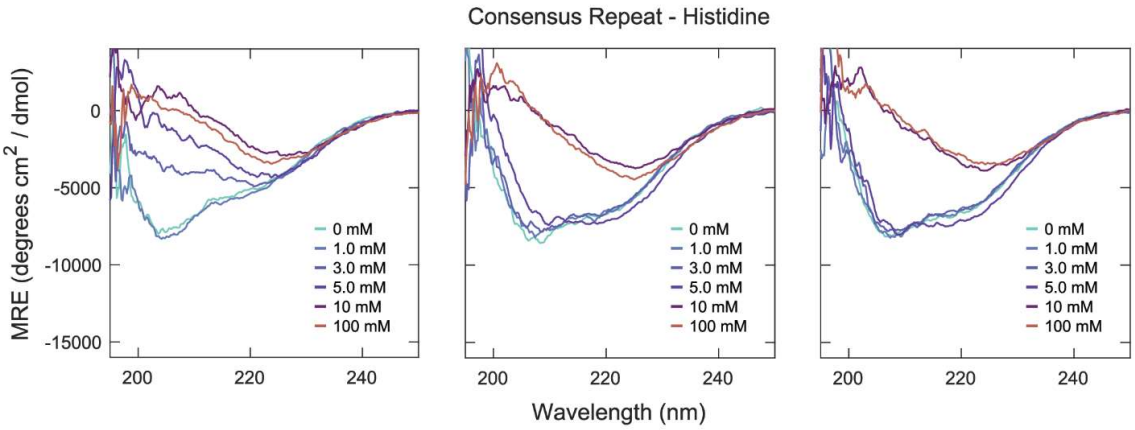

Figure S23. Triplicate circular dichroism measurements of consensus repeat – histidine.

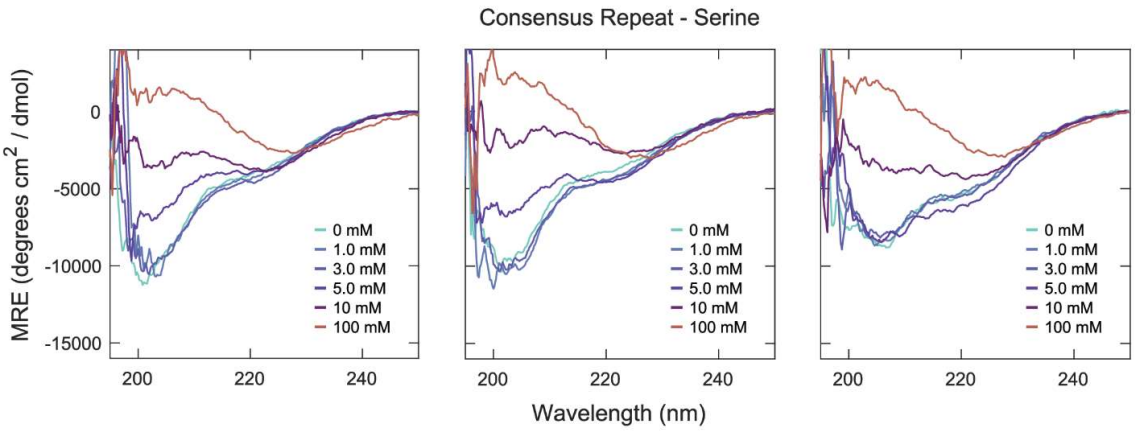

Figure S24. Triplicate circular dichroism measurements of consensus repeat – serine.

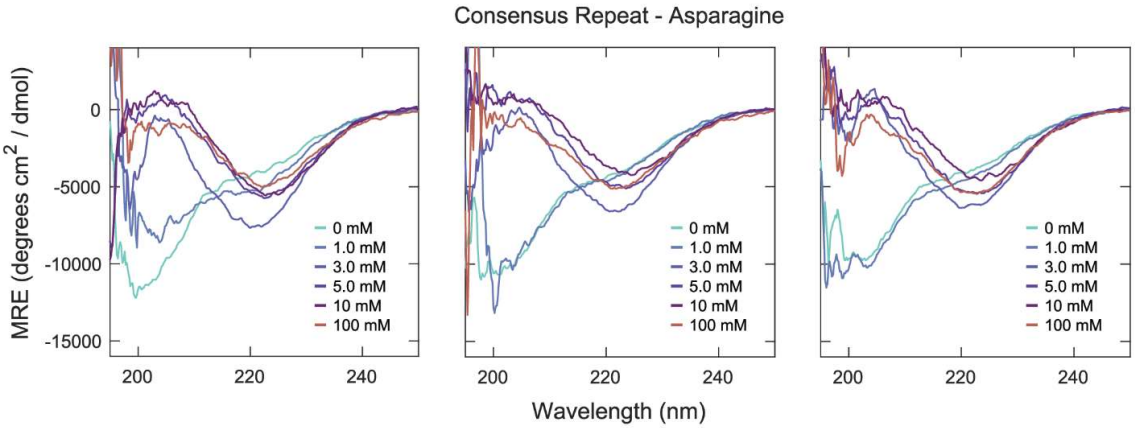

Figure S25. Triplicate circular dichroism measurements of consensus repeat – asparagine.

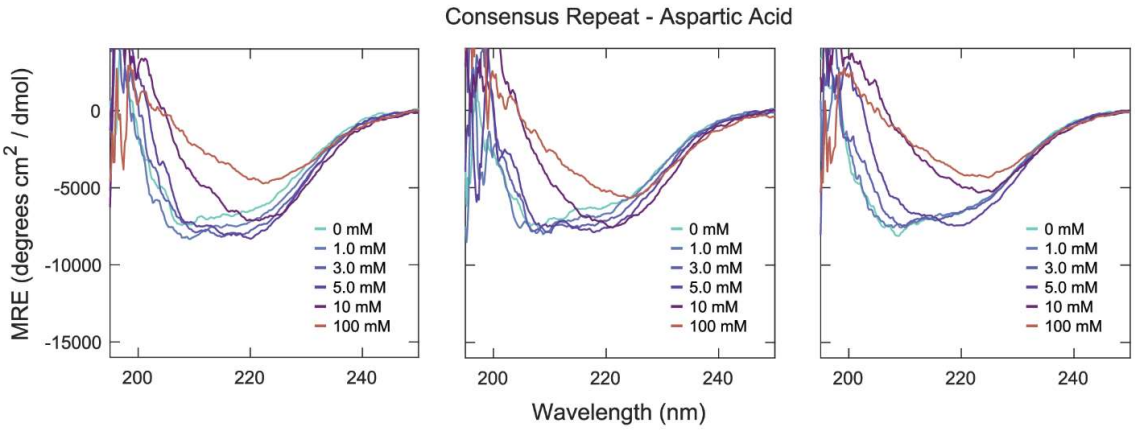

Figure S26. Triplicate circular dichroism measurements of consensus repeat – aspartic acid.

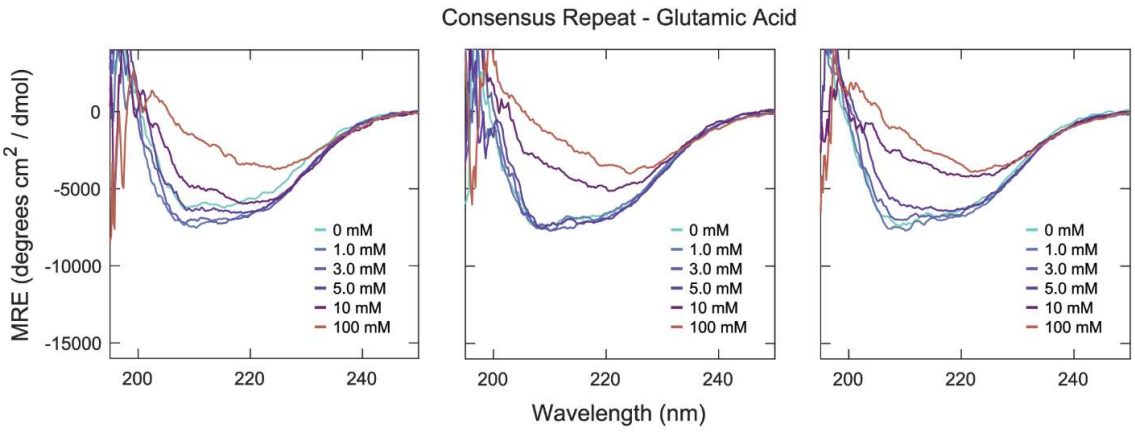

Figure S27. Triplicate circular dichroism measurements of consensus repeat – glutamic acid.

VII. Circular Dichroism Time Studies

To assess the equilibration of structural changes upon mixing with calcium, time-dependent circular dichroism spectroscopy was conducted using two conditions that would be most sensitive to slow folding dynamics:

- Block V in 1 mM $CaCl_2$, slightly above its K_d of 0.67 mM
- Global substitution with glutamic acid in 50 mM $CaCl_2$, slightly above its K_d of 11 mM

In both conditions, circular dichroism spectra indicated weaker structural signals than those observed at higher $CaCl_2$ concentrations. We sought to investigate whether longer incubation with lower $CaCl_2$ concentration would lead to conformational changes observed at higher $CaCl_2$ concentrations. Time-dependent circular dichroism spectra were acquired at 15-minute intervals over the course of 60 minutes. In both cases, circular dichroism spectra did not exhibit significant time-dependent changes for 60 minutes after mixing with $CaCl_2$.

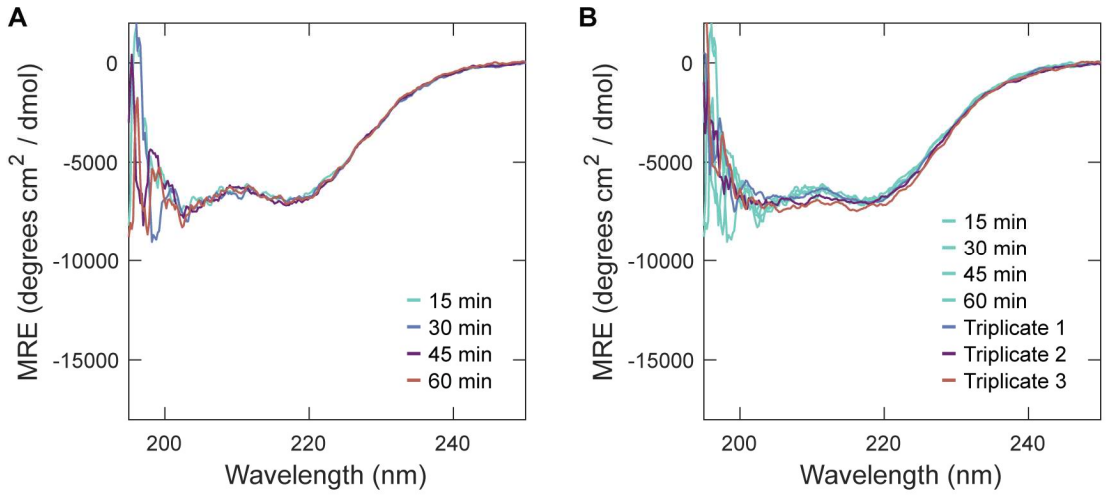

Figure S28. Time-dependent circular dichroism of Block V (wild type) in 1 mM $CaCl_2$. A) Circular dichroism spectra taken at 15-minute intervals after adding $CaCl_2$. B) Comparison between time-dependent circular dichroism and triplicate spectra at 1 mM $CaCl_2$ (**Figure S14**).

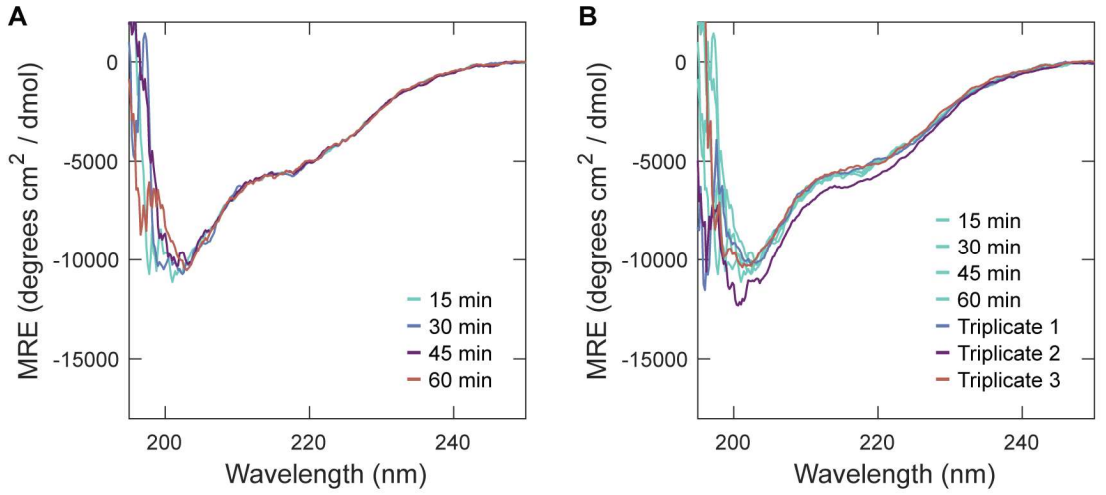

Figure S29. Time-dependent circular dichroism of global substitution – glutamic acid in 50 mM $CaCl_2$. A) Circular dichroism spectra taken at 15-minute intervals after adding $CaCl_2$. B) Comparison between time-dependent circular dichroism and triplicate spectra at 50 mM $CaCl_2$ (**Figure S20**).

VIII. Results from deconvolution of circular dichroism data with CDPro

The results from the CDSSTR, CONTIN/LL, and SELCON3 methods were normalized and averaged to facilitate quantitative comparisons. For the reference set used (SPD48), CONTIN/LL performs best overall, and best for the distorted α-helix, regular β-sheet, turn, and unordered structures. SELCON3 performs best for regular α-helix, and CDSSTR performs best for distorted β-sheet.[2] Averaging these methods improves the overall reliability. Regular and distorted helix and sheet components were combined to quantify total helix and sheet content. Data presented are the averages of CDPro results from triplicate circular dichroism measurements.

[2]N. Sreerama, R. W. Woody, Estimation of Protein Secondary Structure from Circular Dichroism Spectra: Comparison of CONTIN, SELCON, and CDSSTR Methods with an Expanded Reference Set. *Analytical Biochemistry* **287**, 252-260 (2000).

Table S2. CDPro results

Protein Variant	$CaCl_2$ (mM)	Method	α_R	α_D	β_R	β_D	T	U
Block V	0	CONTIN/LL	0.026	0.045	0.110	0.065	0.111	0.643
		SELCON3	0.032	0.054	0.113	0.072	0.134	0.569
		CDSSTR	0.013	0.034	0.118	0.070	0.128	0.626
	0.1	CONTIN/LL	0.026	0.045	0.111	0.064	0.107	0.647
		SELCON3	0.032	0.051	0.109	0.070	0.132	0.583
		CDSSTR	0.015	0.032	0.108	0.066	0.115	0.653
	0.3	CONTIN/LL	0.027	0.045	0.115	0.066	0.114	0.631
		SELCON3	0.031	0.049	0.106	0.067	0.123	0.602
		CDSSTR	0.016	0.033	0.113	0.068	0.126	0.636
	0.5	CONTIN/LL	0.027	0.040	0.124	0.071	0.138	0.060
		SELCON3	0.031	0.046	0.102	0.067	0.126	0.614
		CDSSTR	0.019	0.033	0.111	0.071	0.131	0.627
	1	CONTIN/LL	0.033	0.059	0.156	0.097	0.186	0.467
		SELCON3	0.044	0.059	0.144	0.092	0.181	0.490
		CDSSTR	0.015	0.035	0.163	0.101	0.198	0.485
	3	CONTIN/LL	0.035	0.058	0.171	0.101	0.195	0.440
		SELCON3	0.047	0.060	0.152	0.096	0.192	0.473
		CDSSTR	0.014	0.039	0.171	0.105	0.207	0.459
	5	CONTIN/LL	0.037	0.061	0.169	0.101	0.196	0.438
		SELCON3	0.048	0.061	0.146	0.096	0.186	0.473
		CDSSTR	0.018	0.035	0.166	0.106	0.214	0.455
	10	CONTIN/LL	0.032	0.053	0.167	0.104	0.206	0.438
		SELCON3	0.033	0.058	0.167	0.105	0.192	0.468
		CDSSTR	0.016	0.030	0.176	0.109	0.219	0.445
	100	CONTIN/LL	0.035	0.058	0.172	0.104	0.198	0.433
		SELCON3	0.042	0.060	0.163	0.102	0.195	0.455
		CDSSTR	0.018	0.033	0.175	0.107	0.216	0.448

Protein Variant	$CaCl_2$ (mM)	Method	α_R	α_D	β_R	β_D	T	U
Global Substitution								
Alanine	0	CONTIN/LL	0.028	0.043	0.140	0.076	0.141	0.571
		SELCON3	0.030	0.063	0.162	0.090	0.165	0.477
		CDSSTR	0.020	0.039	0.156	0.087	0.174	0.516
	100	CONTIN/LL	0.036	0.049	0.166	0.094	0.200	0.456
		SELCON3	0.046	0.052	0.149	0.097	0.177	0.462
		CDSSTR	0.013	0.035	0.172	0.102	0.199	0.476
Histidine	0	CONTIN/LL	0.014	0.043	0.218	0.098	0.126	0.501
		SELCON3	0.018	0.046	0.118	0.061	0.032	0.627
		CDSSTR	0.010	0.033	0.193	0.104	0.187	0.469
	100	CONTIN/LL	0.001	0.035	0.236	0.109	0.156	0.463
		SELCON3	0.057	0.050	0.277	0.126	0.182	0.226
		CDSSTR	0.009	0.015	0.187	0.092	0.148	0.536
Serine	0	CONTIN/LL	0.048	0.064	0.123	0.080	0.146	0.518
		SELCON3	0.069	0.131	0.187	0.118	0.163	0.343
		CDSSTR	0.032	0.045	0.161	0.082	0.157	0.515
	100	CONTIN/LL	0.045	0.029	0.119	0.088	0.220	0.497
		SELCON3	0.055	0.070	0.154	0.099	0.175	0.406
		CDSSTR	0.016	0.037	0.186	0.101	0.189	0.468
Asparagine	0	CONTIN/LL	0.025	0.044	0.101	0.062	0.102	0.667
		SELCON3	0.029	0.044	0.088	0.058	0.104	0.649
		CDSSTR	0.014	0.031	0.093	0.059	0.109	0.682
	100	CONTIN/LL	0.032	0.057	0.167	0.099	0.192	0.454
		SELCON3	0.042	0.056	0.150	0.096	0.185	0.485
		CDSSTR	0.016	0.035	0.173	0.106	0.206	0.461
Aspartic acid	0	CONTIN/LL	0.056	0.071	0.182	0.097	0.176	0.419
		SELCON3	0.053	0.118	0.209	0.119	0.163	0.318
		CDSSTR	0.035	0.058	0.186	0.086	0.175	0.460
	100	CONTIN/LL	0.036	0.054	0.204	0.104	0.190	0.411
		SELCON3	0.047	0.084	0.233	0.165	0.157	0.313
		CDSSTR	0.019	0.042	0.177	0.097	0.174	0.483
Glutamic acid	0	CONTIN/LL	0.016	0.040	0.091	0.055	0.085	0.714
		SELCON3	0.033	0.053	0.095	0.063	0.113	0.623
		CDSSTR	0.097	0.027	0.096	0.059	0.109	0.681
	100	CONTIN/LL	0.017	0.019	0.118	0.065	0.135	0.646
		SELCON3	0.036	0.050	0.133	0.085	0.157	0.520
		CDSSTR	0.011	0.032	0.150	0.089	0.170	0.540

Protein Variant	$CaCl_2$ (mM)	Method	α_R	α_D	β_R	β_D	T	U
Consensus Repeat								
Alanine	0	CONTIN/LL	0.058	0.072	0.147	0.083	0.156	0.483
		SELCON3	0.058	0.074	0.174	0.096	0.181	0.405
		CDSSTR	0.048	0.058	0.141	0.082	0.162	0.500
	100	CONTIN/LL	0.006	0.036	0.224	0.105	0.158	0.472
		SELCON3	0.003	0.037	0.236	0.132	0.254	0.382
		CDSSTR	0.004	0.021	0.197	0.102	0.165	0.498
Histidine	0	CONTIN/LL	0.077	0.083	0.142	0.085	0.170	0.442
		SELCON3	0.071	0.084	0.187	0.103	0.196	0.345
		CDSSTR	0.070	0.072	0.139	0.084	0.167	0.460
	100	CONTIN/LL	0.016	0.042	0.219	0.105	0.167	0.451
		SELCON3	0.006	0.052	0.201	0.101	0.194	0.284
		CDSSTR	0.011	0.024	0.197	0.101	0.171	0.487
Serine	0	CONTIN/LL	0.034	0.052	0.132	0.077	0.127	0.579
		SELCON3	0.029	0.043	0.110	0.074	0.155	0.581
		CDSSTR	0.022	0.042	0.126	0.074	0.145	0.585
	100	CONTIN/LL	0.007	0.035	0.227	0.110	0.159	0.469
		SELCON3	0.006	0.032	0.204	0.109	0.194	0.258
		CDSSTR	0.006	0.016	0.201	0.103	0.165	0.495
Asparagine	0	CONTIN/LL	0.029	0.044	0.121	0.070	0.133	0.603
		SELCON3	0.029	0.047	0.110	0.070	0.132	0.597
		CDSSTR	0.021	0.035	0.123	0.072	0.136	0.601
	100	CONTIN/LL	0.018	0.037	0.182	0.101	0.174	0.489
		SELCON3	0.049	0.066	0.200	0.185	0.203	0.307
		CDSSTR	0.007	0.027	0.196	0.100	0.183	0.482
Aspartic acid	0	CONTIN/LL	0.085	0.086	0.150	0.092	0.183	0.404
		SELCON3	0.076	0.085	0.197	0.106	0.207	0.302
		CDSSTR	0.081	0.081	0.145	0.089	0.178	0.420
	100	CONTIN/LL	0.034	0.046	0.197	0.103	0.187	0.433
		SELCON3	0.077	0.095	0.162	0.152	0.203	0.258
		CDSSTR	0.018	0.031	0.191	0.100	0.187	0.469
Glutamic acid	0	CONTIN/LL	0.082	0.084	0.168	0.097	0.193	0.376
		SELCON3	0.075	0.089	0.194	0.109	0.207	0.287
		CDSSTR	0.080	0.082	0.167	0.093	0.181	0.389
	100	CONTIN/LL	0.025	0.047	0.221	0.108	0.174	0.425
		SELCON3	0.004	0.043	0.192	0.098	0.204	0.321
		CDSSTR	0.013	0.028	0.190	0.100	0.176	0.485

α_R, regular α-helix; α_D, distorted α-helix; β_R, regular β-sheet; β_D, distorted β-sheet; T, turns; U, unordered