Table S1: Primers used to generate SC(1-246) double- and triple-mutant constructs.

Name	**Primers**
Double Mutant Primers	
SC(1-246) 5’	5’ GAGTTATGATAGTAGTAAACATCACTGAAAAAATCACAGTGAAGGTTTTGAGGC 3’
SC(1-246) 3’	5’ GCCTCAAAACCTTCACTGTGATTTTTTCAGTGATGTTTACTACTATCATAACTC 3’
Degenerate Forward	5’ GAGAACCTGTATTTTCAG**NN**G**NN**GACAAAGGATTATAGTAAAG 3’
Degenerate Reverse	5’ CTTTACTATAATCCTTTGTC**NN**C**NN**CTGAAAATACAGGTTCTC 3’
Degenerate Forward	5’ GAGAACCTGTATTTTCAG**NN**C**NN**CACAAAGGATTATAGTAAAG 3’
Degenerate Reverse	5’ CTTTACTATAATCCTTTGTG**NN**G**NN**CTGAAAATACAGGTTCTC 3’
Degenerate Forward	5’ CTTTACTATAATCCTTTGTG**NN**G**NN**CTGAAAATACAGGTTCTC 3’
Degenerate Reverse	5’ GAGAACCTGTATTTTCAG**NN**C**NN**CACAAAGGATTATAGTAAAG 3’
SC(1-246)LV Forward	5’ AACCTGTATTTTCAGCTAGTAACAAAGGATTAT 3’
SC(1-246)TV Forward	5’ AACCTGTATTTTCAGACAGTAACAAAGGATTAT 3’
SC(1-246)VV Forward	5’ AACCTGTATTTTCAGGTAGTAACAAAGGATTAT 3’
SC(1-246)IL Forward	5’ AACCTGTATTTTCAGATACTAACAAAGGATTAT 3’
SC(1-246)IT Forward	5’ AACCTGTATTTTCAGATAACAACAAAGGATTAT 3’
SC(1-246)IA Forward	5’ AACCTGTATTTTCAGATAGCAACAAAGGATTAT 3’
SC(1-246)TP Forward	5’ GAGAACCTGTATTTTCAGACACCAACAAAGGATTATAGTAAAGAA 3’
SC(1-246) Reverse 1	5’ CTGAAAATACAGGTTCTCGTTCCATGGCAC 3’
SC(1-246) Reverse 2	5’ ATTCACTCTTGATTCTTTACTATAATCCTTTGT 3’
SC(1-246)TA Forward	5’ CCATGGAACGAGAACCTGTATTTTCAGACAGCAACAAAGGATTATAGTAAAGAATCAAGA 3’
SC(1-246)IW Forward	5’ CCATGGAACGAGAACCTGTATTTTCAGATATGGACAAAGGATTATAGTAAAGAATCAAGA 3’
SC(1-246) Reverse	5’ CTGAAAATACAGGTTCTCGTTCCATGGCAC 3’
SC(1-246)LQ Forward	5’GTGCCATGGACCGAGAACCTGTATTTTCAGCTGCAGACAAAGGATTATAGTAAAGAATCAAGAGTG 3’
SC(1-246)LQ Reverse	5’ CACTCTTGATTCTTTACTATAATCCTTTGTCTGCAGCTGAAAATACAGGTTCTCGGTCCATGGCAC 3’

SC(1-246)VG Forward	5’ GCCATGGACCGAGAACCTGTATTTTCAGGTCGGCACAAAGGATTATAGTAAAGAATCAAGAG 3’
SC(1-246)VG Reverse	5’ CTCTTGATTCTTTACTATAATCCTTTGTGCCGACCTGAAAATACAGGTTCTCGGTCCATGGC 3’
Triple Mutant Primers	
Degenerate Forward	5’ TGGAACGAGAACCTGTATTTTCAG**NN**C**NN**G**NN**TAAGGATTATAGTAAAGAATCAAGA 3’
Degenerate Forward	5’ TGGAACGAGAACCTGTATTTTCAG**NN**C**NN**T**NN**GAAGGATTATAGTAAAGAATCAAGA 3’
Degenerate Forward	5’ TGGAACGAGAACCTGTATTTTCAG**NN**T**NN**G**NN**CAAGGATTATAGTAAAGAATCAAGA 3’
Degenerate Forward	5’ TGGAACGAGAACCTGTATTTTCAG**NN**T**NN**C**NN**GAAGGATTATAGTAAAGAATCAAGA 3’
Degenerate Forward	5’ TGGAACGAGAACCTGTATTTTCAG**NN**G**NN**C**NN**AAAGGATTATAGTAAAGAATCAAGA 3’
Degenerate Forward	5’ TGGAACGAGAACCTGTATTTTCAG**NN**A**NN**C**NN**GAAGGATTATAGTAAAGAATCAAGA 3’
Degenerate Forward	5’ TGGAACGAGAACCTGTATTTTCAG**NN**A**NN**T**NN**GAAGGATTATAGTAAAGAATCAAGA 3’
Degenerate Forward	5’ TGGAACGAGAACCTGTATTTTCAG**NN**A**NN**T**NN**CAAGGATTATAGTAAAGAATCAAGA 3’
Degenerate Forward	5’ TGGAACGAGAACCTGTATTTTCAG**NN**T**NN**A**NN**CAAGGATTATAGTAAAGAATCAAGA 3’
Degenerate Forward	5’ TGGAACGAGAACCTGTATTTTCAG**NN**G**NN**G**NN**GAAGGATTATAGTAAAGAATCAAGA 3’
Degenerate Forward	5’ TGGAACGAGAACCTGTATTTTCAG**NN**T**NN**T**NN**TAAGGATTATAGTAAAGAATCAAGA 3’
Degenerate Forward	5’ TGGAACGAGAACCTGTATTTTCAG**NN**A**NN**A**NN**AAAGGATTATAGTAAAGAATCAAGA 3’
SC(1-246)RHW Forward	5’ GAGAACCTGTATTTTCAGAGACATTGGAAGGATTATAGTAAAGAA 3’
SC(1-246)DDY Forward	5’ GAGAACCTGTATTTTCAGGATGATTATAAGGATTATAGTAAAGAA 3’
SC(1-246) Reverse 1	5’ CTGAAAATACAGGTTCTCGTTCCATGGCACCAG 3’
SC(1-246) Reverse 2	5’ CTGAAAATACAGGTTCTCGTTCCATGGCACCAGACCAGAAGAATGATGATGATGATG 3’

Table S2: Extinction coefficients and molecular masses used to calculate the concentration of the SC(1-246) mutants. The extinction coefficient 0.936 mL mg^{-1} cm^{-1} determined for wild-type SC(1-246) was used for NH_2-terminal double mutants except for I1W2, M1W2, W1E2, A1W2, and W1A2. Extinction coefficients for these mutants were calculated by the Expasy tool, https://web.expasy.org/protparam/.

NH_2-terminal Mutants	**Extinction Coefficient** $E_{280,0.1\%}$ (mL mg^{-1} cm^{-1})	**Molecular Mass** (M_r)(Daltons)
A1K2	0.936	29145
A1S2	0.936	29091
G1A2	0.936	29061
G1G2	0.936	29047
G1H2	0.936	29127
G1P2	0.936	29087
I1W2	1.120	29232
K1A2	0.936	29312
L1P2	0.936	29143
L1T2	0.936	29147
M1E2	0.936	29193
M1K2	0.936	29139
M1L2	0.936	29177
M1W2	1.250	29250
Q1K2	0.936	29189
Q1L2	0.936	29121
R1Q2	0.936	29217
R1R2	0.936	29245
S1K2	0.936	29148
T1A2	0.936	29127
V1G2	0.936	29089
W1E2	1.25	29248
A1W2, W1A2	1.25	29190
A1T2, G1D2	0.936	29105
E1T2, N1D2	0.936	29162
L1Q2, L1K2	0.936	29174
E1S2, I1A2, I1L2, I1T2, L1V2, T1D2, T1P2, T1V2, V1V2	0.936	29149
D1D2Y3	1.02	29225
E1L2K3	1.20	29202
E1S2W3	1.27	29239
F1L2Q3	1.11	29226
G1G2G3	1.01	29008
I1I2V3	1.23	29157
R1H2W3	1.33	29310

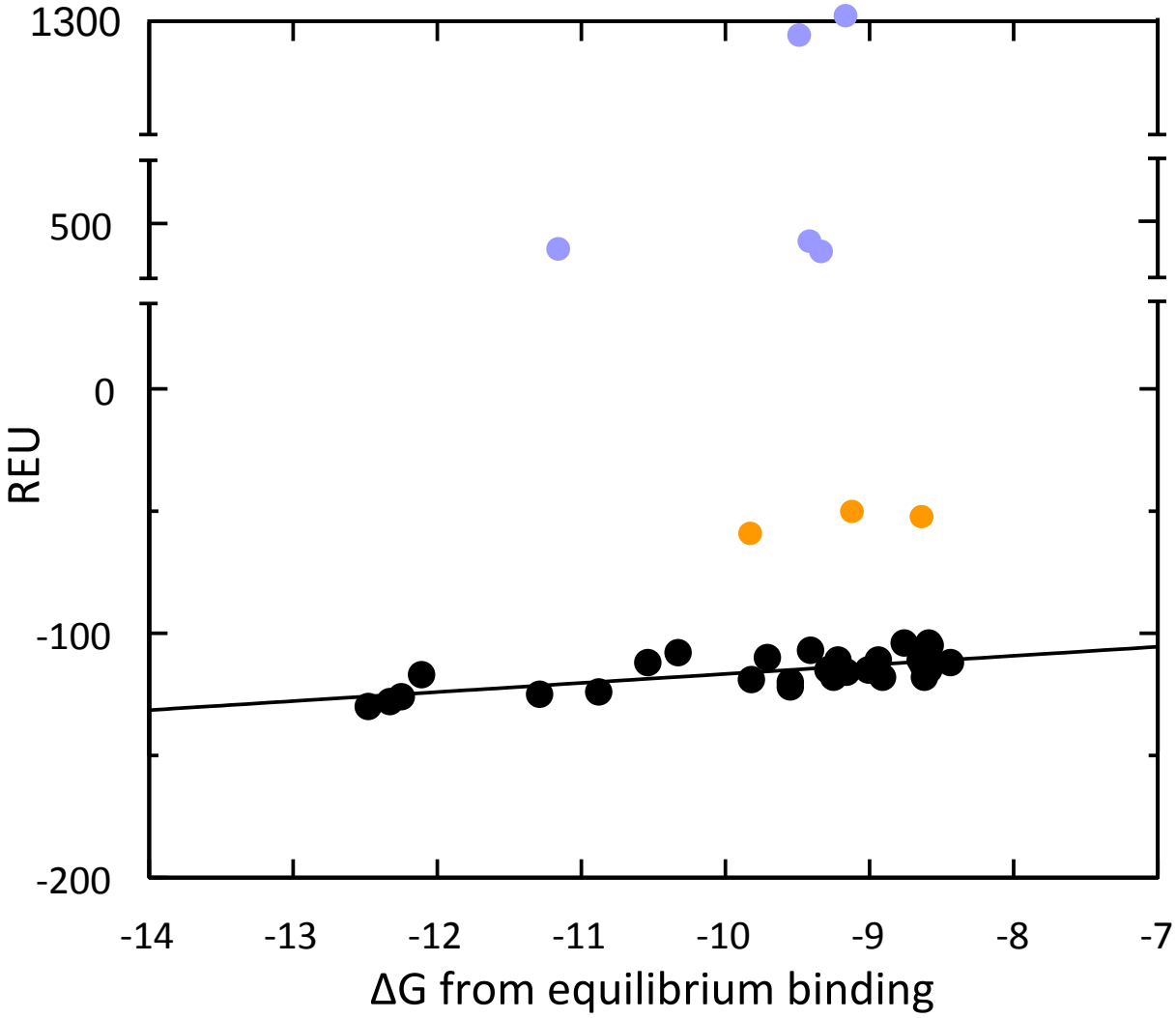

Figure S1: Correlation between REU and ΔG values calculated from equilibrium binding, expanded to include theoretical outliers with Trp (purple) or Pro (orange) in the NH_2-termini.