SUPPORTING INFORMATION

Catch and Release: Engineered Allosterically Regulated *β*-Roll Peptides Enable On/Off Biomolecular Recognition

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Supplementary Figures

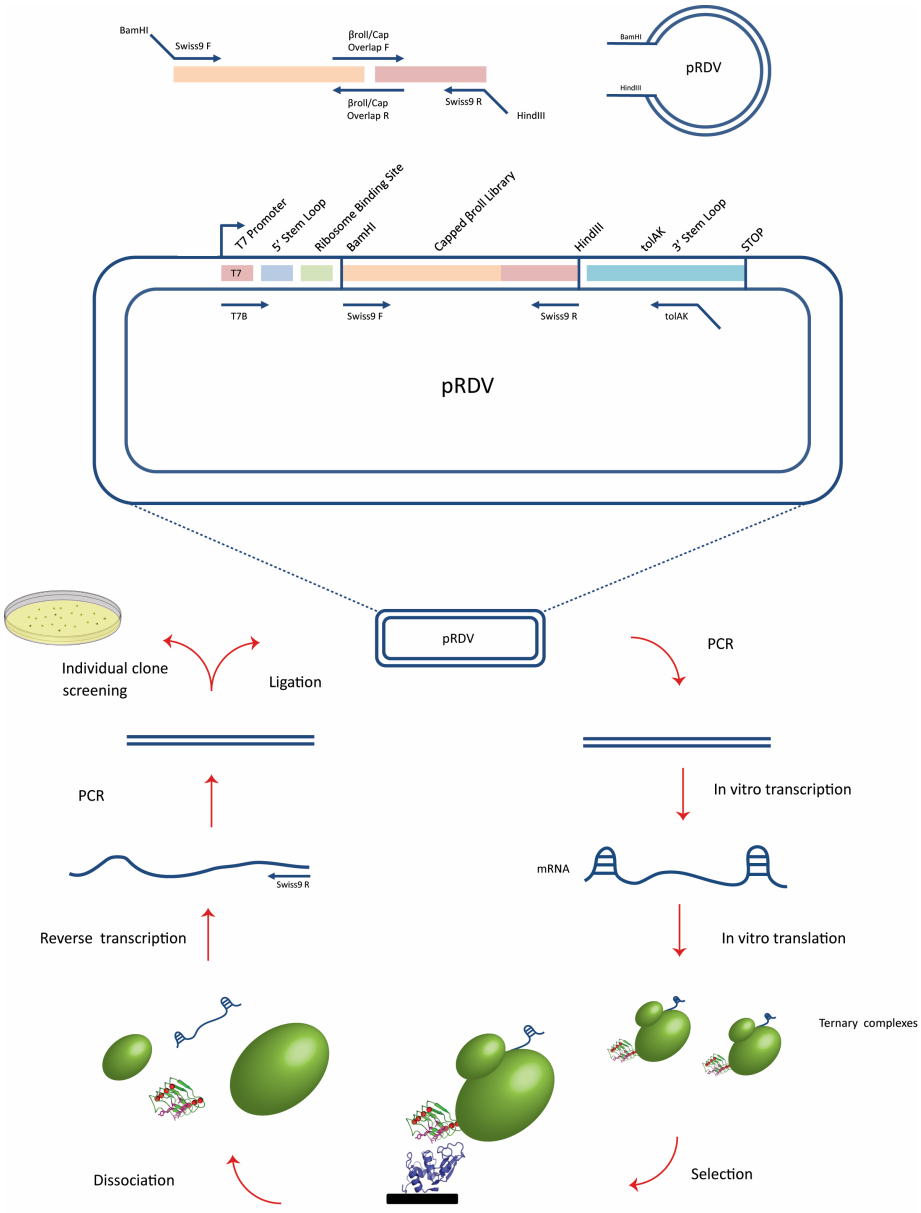

Figure S1. Schematic of the ribosome display selection method.[1] After cloning the RTX library into the ribosome display vector (pRDV), the library was transcribed and translated *in vitro*. Resulting complexes were incubated with the immobilized target. The mRNA of the bound complexes was reverse transcribed and PCR amplified to serve as the input for another round of selection.

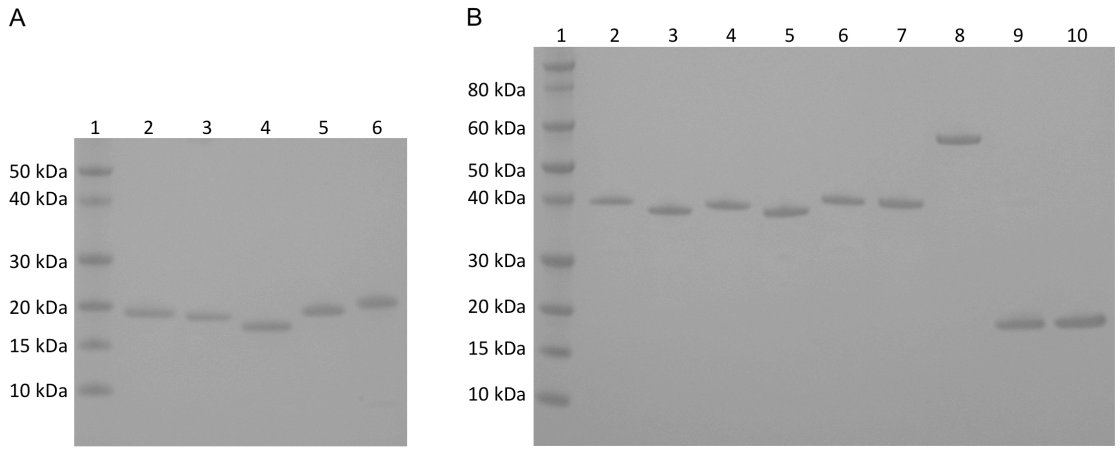

Figure S2. SDS-PAGE analysis of WT and mutant peptides. (A) (1) Protein ladder. (2) WT β-roll: 15.9 kDa. (3) P101: 16 kDa. (4) PN206: 16.0 kDa. (5) PN406: 15.8 kDa. (6) PN715: 15.9 kDa. (B) (1) Protein ladder. (2) WT-WT: 32.7 kDa (3) PN206-PN406: 32.7 kDa. (4) PN406-PN406: 32.6 kDa. (5) PN406-PN206: 32.7 kDa. (6) PN406-PN715: 32.5 kDa. (7) PN715-PN406: 32.5 kDa. (8) PN406-PN406-PN406: 49.2 kDa. (9) WT/PN406: 16.0 kDa. (10) PN406/PN406: 15.9 kDa. We have previously shown that β-rolls run artificially large on SDS-PAGE.[2] In addition, peptide hydrophobicity has been shown to result in gel shifting, which can cause the differences in the apparent molecular weights of different mutants.[3]

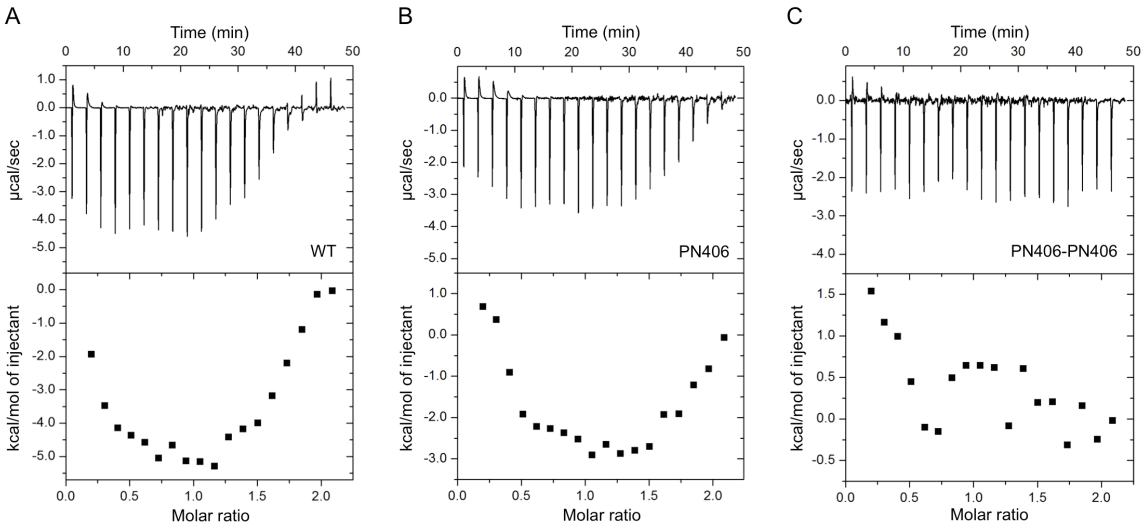

Figure S3. Exemplary ITC analysis of (A) wild-type β-roll, (B) PN406 and (C) PN406-PN406 in the presence of 10 mM $MgCl_2$.

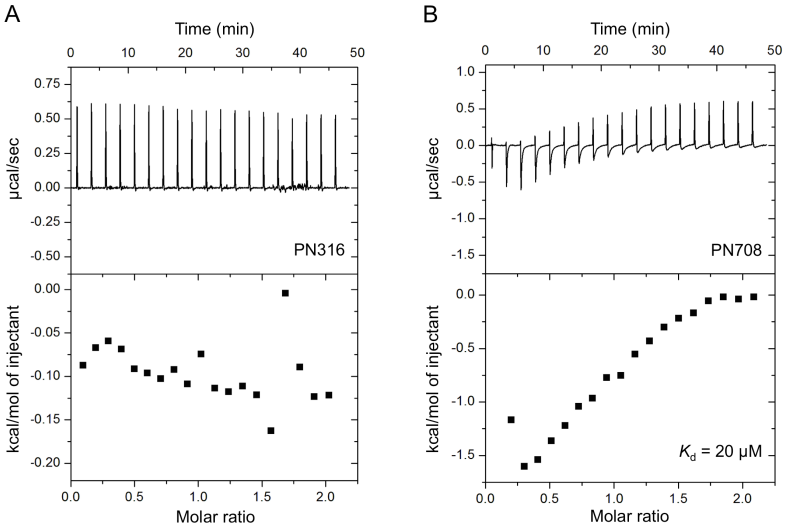

Figure S4. Exemplary ITC analysis of PN316 and PN708 in the presence of 10 mM $CaCl_2$. (A) PN316 did not demonstrate an affinity for the target. (B) PN708 bound lysozyme with affinity of the same order compared to wild-type β-roll.

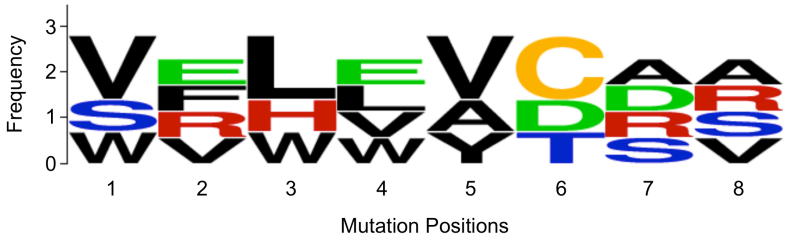

Figure S5. Amino acid frequencies among the single RTX mutants (P101, PN206, PN406 and PN715) at the randomized positions.

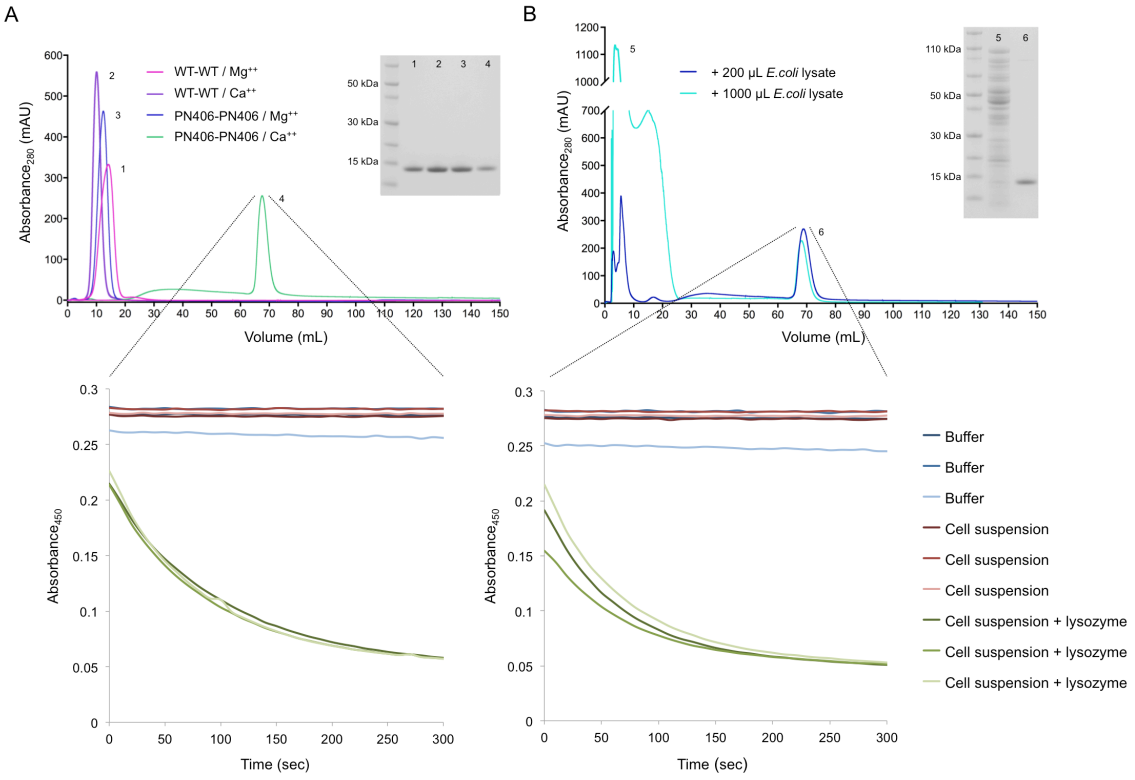

Figure S6. Activity assay of the eluted lysozyme. (A) Lysozyme eluted off the MBP-PN406-PN406/Ca^{++} column. The decrease in the absorbance at 450 nm indicates the activity of the enzyme. (B) Lysozyme eluted off the MBP-PN406-PN406/Ca^{++} column, which was co-loaded with *E. coli* crude cell lysate. The decrease in the absorbance at 450 nm indicates the activity of the enzyme.

Supplementary Tables

Table S1. Sequencing results of the positive selections.

Mutant	Amino acids at randomized positions	Frequency
P101	W F L E A T D A	4/6
P105	L Y R Q A T D A	1/6
P106	V P E G S P V P	1/6

Table S2. Sequencing results of the positive/negative selections

Mutant	Amino acids at the randomized positions
PN101	G M G W G N V W
PN105	P T S P R E H S
PN112	V L G V Q D Q A
PN118	A R A V A D T A
PN206	V R W W V C S R
PN211	W A P W R G C R
PN301	G I L V P G R H
PN307	G Q L R T H P A
PN311	V E R A E R T V
PN312	R F S R R R P R
PN316	A R R V E R T V
PN402	S C A D P P A A
PN406	S V L L V D R V
PN505	C E R K G M A P V
PN508	N Q A P E D N L
PN603	C W R Q P S R R
PN605	G R P R A W A G
PN607	V C R W R H P C
PN610	G C A G G R P R
PN612	H R A R C S A H
PN613	S E T P P R Q V
PN614	E S L L C S G G
PN616	R A A T A P P R
PN619	R M P A P P T A
PN621	W F L E R S A P
PN702	S N A Q V G S D
PN703	W M R M R H R G
PN708	R R G V T D R A
PN710	T W R T R A T P
PN711	C L W R N T T P
PN714	R E Q R P A R R
PN715	V E H V Y C A S
PN721	G P W G T T H A
PN722	W V V W T P D I

Table S3. Selected mutants of the positive/negative selections

Mutant	Amino acids at the randomized positions
PN101	G M G W G N V W
PN112	V L G V Q D Q A
PN118	A R A V A D T A
PN206	V R W W V C S R
PN311	V E R A E R T V
PN316	A R R V E R T V
PN406	S V L L V D R V
PN614	E S L L C S G G
PN708	R R G V T D R A
PN715	V E H V Y C A S

Table S4. The changes in the Gibbs free energy upon interaction with lysozyme

Peptide	ΔG (kcal/mol) *
WT	-6.2 ± 0.3
PN101	-7.5 ± 0.2
PN206	-7.2 ± 0.4
PN406	-7.7 ± 0.2
PN715	-7.2 ± 0.6
WT-WT	-8.1 ± 0.5
PN206-PN406	-8.6 ± 0.3
PN406-PN206	-9.5 ± 0.5
PN406-PN406	-10.1 ± 0.8
PN406-PN715	-8.0 ± 0.1
PN715-PN406	-8.2 ± 0.2
PN406-PN406-PN406	-9.4 ± 0.3
PN406/PN406	-7.5 ± 0.1
WT/PN406	-6.2 ± 0.3

* The values are reported as mean ± SD (n=3).

Table S5. PCR primers for cloning experiments

Cloning Experiment	Primer	Sequence
Library Design	Swiss9 Forward	5’ TCGCGGCCCAGCCGGCCATGGCGGGTTCTGCACGCG ACGATGTGCTGATCGGCGACGCGGGTGCGAATNNKCTGN NKGGCCTGGCTGGTAACGACGTCTTGTCTGGTGGTGCGGG CGATGATNNKCTGNNKGGTGACGAGGGCTCCGATCTGCT GAGCGGTGATGCCGGCAACGAC 3’
	Swiss9 Reverse	5’ TTCGGCCCCCGAGGCCCCGCCACGGATCGTGTCATG GCCACCACCGGACTCMNNAATMNNGTCGTGACCATAACC AACACCGAACAGGTAGGTATCGTCGCCCTGACCGCCMNN CAAMNNGTCGTTGCCGGCATCACCGCTCAGCAGATCGGA GCCCTCGTCACC 3’
	β-roll/Cap Overlap Forward	5’ GTGGCCATGACACGATCCGTATCAACGC GGGGGCGGACCA 3’
	β-roll/Cap Overlap Reverse	5’ TGGTCCGCCCCCGCGTTGATACGGATCG TGTCATGGCCAC 3’
RTX Library into pRDV	Swiss9 pRDV Forward	5' AATAATGGATCCGGTTCTGCACGCGACGATGTGC 3'
	Swiss9 pRDV Reverse	5' TAATAAAAGCTTGTCCGGATACTGCGCCATTGCCTC 3'
RTX Library for *in vitro* transcription and translation	T7B	5’ ATACGAAATTAATACGACTCACTATAGGGAGAC CACAACGG 3’
	tolAK	5’ CCGCACACCAGTAAGGTGTGCGGTTTCAGTTG CCGCTTTCTTTCT 3’
P101, PN206, PN406 and PN715 into pMAL-c4e-intein	Forward	5’ AATAATGGTACCGGGTTCTGCACGCGACGATGTGC 3’
	Reverse	5’ TAATAAAAGCTTTTAGTCCGGATACTGCGCCATTGCC 3’
WT-WT into pMAL-c4e-intein	$Forward_1$	5’ ATTATAGGTACCGGGCAGCGCG 3’
	$Reverse_1$	5’ TTAAATAAGCTTGTCCGGGTATTGTGCCATTGCTTC 3’
	$Forward_2$	5’ ATTATAAAGCTTGGCGGTGGCGGTAGCGGCGGTGGCG GTTCTGGCAGCGCGCGTGATGAC 3’
	$Reverse_2$	5’ TTAAATAAGCTTTTAGTCCGGGTATTGTGCCATT 3’
Concatemer cloning into pMAL-c4e-	$Forward_1$	5’ ATTATAGGTACCGGGTTCTGCACGCG 3’
	$Reverse_1$	5’ TTAAATAAGCTTGTCCGGATACTGCGCCATTGCC 3’
	$Forward_2$	5’ ATTATAAAGCTTGGCGGTGGCGGTAGCGGCGGTGGC

intein		GGTTCTGGTTCTGCACGCGACGATGTG 3’
	$Reverse_2$	5’ TTAAATAAGCTTTTAGTCCGGATACTGCGCC 3’
PN406 into pMAL-c4e-intein-PN406-PN406	Forward	5’ ATTATAGGTACCGGGTTCTGCACGCG 3’
	Reverse	5’ TTAAATGGTACCGGAGAACCGCCACCGCCGCTACCG CCACCGCCGTCCGGATACTGCGCCATTGC 3’
WT-WT in fusion with MBP	$Forward_1$	5’ ATTATACTCGGGGGCAGCGCGCGTGATGAC 3’
	$Reverse_1$	5’ TTAAATGAATTCGTCCGGGTATTGTGCCATTGCTTCA 3’
	$Forward_2$	5’ ATTATAGAATTCGGCGGTGGCGGTAGCGGCGGTGGC GGTTCTGGCAGCGCGCGTGATGAC 3’
	$Reverse_2$	5’ TTAAATGGATCCTTAGTCCGGGTATTGTGCCATTGCT 3’
PN406-PN406 in fusion with MBP	Forward	5’ ATTATACTCGGGGGTTCTGCACGCGACGATGTG 3’
	Reverse	5’ TTAAATGAATTCTTAGTCCGGATACTGCGCCATTGC 3’

Table S6. PCR primers for site-directed mutagenesis experiments

Construct	Primer	Sequence
WT/PN406	1	5’ CGCGCGTGATGACTCGCTGGTCGGCGACGCAGG 3’
	2	5’ GCGGGCAACGACTTGCTGTTAGGCGGCGCTGGC 3’
	3	5’ CGGGCAGGGCGATGATAGGTATCTGTTCGGGGT 3’
	4	5’ GAGGGCTCGGACGTGCTCGACGGCGATGCGGG 3’
	5	5’ CGCGCGTGATGACTCGCTGGTCGGCGACGCAGG 3’
	6	5’ GGCGGGCAGGGCGATGATAGGTATGTGTTCGGGGT 3’
PN406/PN406	1	5’ GGGTTCTGCACGCGACGATTCGCTGGTCGGCGACGC 3’
	2	5’ GGCTGGTAACGACCTCTTGTTAGGTGGTGCGGGCG 3’
	3	5’ GACGAGGGCTCCGATGTGCTGGACGGTGATGCCGG 3’
	4	5’ CGGTCAGGGCGACGATAGGTACCTGTTCGGTG 3’
	5	5’ GGTCAGGGCGACGATAGGTACGTGTTCGGTGTTGG 3’
	6	5’ GGCTGGTAACGACCTCTTGTTAGGTGGTGCGGGCG 3’

Table S7. Protein primary sequences

Construct	Sequence *
WT	GSARDDVLIGDAGANVLNGLAGNDVLSGGAGDDVLLGDEGSDLLSGDAGNDDL FGGQGDDTYLFGVGYGHDTIYESGGGHDTIRINAGADQLWFARQGNDLEIRILGT DDALTVHDWYRDADHRVEIIHAANQAVDQAGIEKLVEAMAQYPD
P101	GSARDDVLIGDAGANWLFGLAGNDVLSGGAGDDLLEGDEGSDLLSGDAGNDAL TGGQGDDTYLFGVGYGHDDIAESGGGHDTIRINAGADQLWFARQGNDLEIRILGT DDALTVHDWYRDADHRVEIIHAANQAVDQAGIEKLVEAMAQYPD
PN206	GSARDDVLIGDAGANVLRGLAGNDVLSGGAGDDWLWGDEGSDLLSGDAGNDV LCGGQGDDTYLFGVGYGHDSIRESGGGHDTIRINAGADQLWFARQGNDLEIRILG TDDALTVHDWYRDADHRVEIIHAANQAVDQAGIEKLVEAMAQYPD
PN406	GSARDDVLIGDAGANSLVGLAGNDVLSGGAGDDLLLGDEGSDLLSGDAGNDVLD GGQGDDTYLFGVGYGHDRIVESGGGHDTIRINAGADQLWFARQGNDLEIRILGTD DALTVHDWYRDADHRVEIIHAANQAVDQAGIEKLVEAMAQYPD
PN715	GSARDDVLIGDAGANVLEGLAGNDVLSGGAGDDHLVGDEGSDLLSGDAGNDYL CGGQGDDTYLFGVGYGHDAISESGGGHDTIRINAGADQLWFARQGNDLEIRILGT DDALTVHDWYRDADHRVEIIHAANQAVDQAGIEKLVEAMAQYPD
WT-WT	GSARDDVLIGDAGANVLNGLAGNDVLSGGAGDDVLLGDEGSDLLSGDAGNDDL FGGQGDDTYLFGVGYGHDTIYESGGGHDTIRINAGADQLWFARQGNDLEIRILGT DDALTVHDWYRDADHRVEIIHAANQAVDQAGIEKLVEAMAQYPDKLGGGGSGG GGSGSARDDVLIGDAGANVLNGLAGNDVLSGGAGDDVLLGDEGSDLLSGDAGN DDLFGGQGDDTYLFGVGYGHDTIYESGGGHDTIRINAGADQLWFARQGNDLEIRI LGTDDALTVHDWYRDADHRVEIIHAANQAVDQAGIEKLVEAMAQYPD
PN206-PN406	GSARDDVLIGDAGANVLRGLAGNDVLSGGAGDDWLWGDEGSDLLSGDAGNDV LCGGQGDDTYLFGVGYGHDSIRESGGGHDTIRINAGADQLWFARQGNDLEIRILG TDDALTVHDWYRDADHRVEIIHAANQAVDQAGIEKLVEAMAQYPDKLGGGGSG GGGSGSARDDVLIGDAGANSLVGLAGNDVLSGGAGDDLLLGDEGSDLLSGDAGN DVLDGGQGDDTYLFGVGYGHDRIVESGGGHDTIRINAGADQLWFARQGNDLEIRI LGTDDALTVHDWYRDADHRVEIIHAANQAVDQAGIEKLVEAMAQYPD
PN406-PN206	GSARDDVLIGDAGANSLVGLAGNDVLSGGAGDDLLLGDEGSDLLSGDAGNDVLD GGQGDDTYLFGVGYGHDRIVESGGGHDTIRINAGADQLWFARQGNDLEIRILGTD DALTVHDWYRDADHRVEIIHAANQAVDQAGIEKLVEAMAQYPDKLGGGGSGGG GSGSARDDVLIGDAGANVLRGLAGNDVLSGGAGDDWLWGDEGSDLLSGDAGN DVLCGGQGDDTYLFGVGYGHDSIRESGGGHDTIRINAGADQLWFARQGNDLEIRI LGTDDALTVHDWYRDADHRVEIIHAANQAVDQAGIEKLVEAMAQYPD
PN406-PN406	GSARDDVLIGDAGANSLVGLAGNDVLSGGAGDDLLLGDEGSDLLSGDAGNDVLD GGQGDDTYLFGVGYGHDRIVESGGGHDTIRINAGADQLWFARQGNDLEIRILGTD DALTVHDWYRDADHRVEIIHAANQAVDQAGIEKLVEAMAQYPDKLGGGGSGGG GSGSARDDVLIGDAGANSLVGLAGNDVLSGGAGDDLLLGDEGSDLLSGDAGNDV LDGGQGDDTYLFGVGYGHDRIVESGGGHDTIRINAGADQLWFARQGNDLEIRILG TDDALTVHDWYRDADHRVEIIHAANQAVDQAGIEKLVEAMAQYPD

PN406-PN715	GSARDDVLIGDAGANSLVGLAGNDVLSGGAGDDLLLGDEGSDLLSGDAGNDVLD GGQGDDTYLFGVGYGHDRIVESGGGHDTIRINAGADQLWFARQGNDLEIRILGTD DALTVHDWYRDADHRVEIIHAANQAVDQAGIEKLVEAMAQYPDKLGGGGSGGG GSGSARDDVLIGDAGANVLEGLAGNDVLSGGAGDDHLVGDEGSDLLSGDAGND YLCGGQGDDTYLFGVGYGHDAISESGGGHDTIRINAGADQLWFARQGNDLEIRIL GTDDALTVHDWYRDADHRVEIIHAANQAVDQAGIEKLVEAMAQYPD
PN715-PN406	GSARDDVLIGDAGANVLEGLAGNDVLSGGAGDDHLVGDEGSDLLSGDAGNDYL CGGQGDDTYLFGVGYGHDAISESGGGHDTIRINAGADQLWFARQGNDLEIRILGT DDALTVHDWYRDADHRVEIIHAANQAVDQAGIEKLVEAMAQYPDKLGGGGSGG GGSGSARDDVLIGDAGANSLVGLAGNDVLSGGAGDDLLLGDEGSDLLSGDAGND VLDGGQGDDTYLFGVGYGHDRIVESGGGHDTIRINAGADQLWFARQGNDLEIRIL GTDDALTVHDWYRDADHRVEIIHAANQAVDQAGIEKLVEAMAQYPD
PN406-PN406-PN406	GSARDDVLIGDAGANVLEGLAGNDVLSGGAGDDHLVGDEGSDLLSGDAGNDYL CGGQGDDTYLFGVGYGHDAISESGGGHDTIRINAGADQLWFARQGNDLEIRILGT DDALTVHDWYRDADHRVEIIHAANQAVDQAGIEKLVEAMAQYPDGGGGSGGGG SGTGSARDDVLIGDAGANSLVGLAGNDVLSGGAGDDLLLGDEGSDLLSGDAGND VLDGGQGDDTYLFGVGYGHDRIVESGGGHDTIRINAGADQLWFARQGNDLEIRIL GTDDALTVHDWYRDADHRVEIIHAANQAVDQAGIEKLVEAMAQYPDKLGGGGS GGGGSGSARDDVLIGDAGANSLVGLAGNDVLSGGAGDDLLLGDEGSDLLSGDAG NDVLDGGQGDDTYLFGVGYGHDRIVESGGGHDTIRINAGADQLWFARQGNDLEI RILGTDDALTVHDWYRDADHRVEIIHAANQAVDQAGIEKLVEAMAQYPD
WT/PN406	GSARDDSLVGDAGANVLNGLAGNDLLLGGAGDDVLLGDEGSDVLDGDAGNDDL FGGQGDDRYVFGVGYGHDTIYESGGGHDTIRINAGADQLWFARQGNDLEIRILGT DDALTVHDWYRDADHRVEIIHAANQAVDQAGIEKLVEAMAQYPD
PN406/PN406	GSARDDSLVGDAGANSLVGLAGNDLLLGGAGDDLLLGDEGSDVLDGDAGNDVL DGGQGDDRYVFGVGYGHDRIVESGGGHDTIRINAGADQLWFARQGNDLEIRILGT DDALTVHDWYRDADHRVEIIHAANQAVDQAGIEKLVEAMAQYPD

* Blue and black residues represent the β-roll domain and the capping group respectively. Red residues represent the residues on the β-roll faces. Light gray residues represent the linker.

References

(1) Dreier, B., and Plückthun, A. (2012) In *Ribosome Display and Related Technologies: Methods and Protocols,* pp 261–286, Springer, New York.

(2) Dooley, K., Bulutoglu, B., and Banta, S. (2014) Doubling the cross-linking interface of a rationally designed beta roll peptide for calcium-dependent proteinaceous hydrogel formation. *Biomacromolecules 15,* 3617–3624.

(3) Shi, Y., Mowery, R. A., Ashley, J., Hentz, M., Ramirez, A. J., Bilgicer, B., Slunt-Brown, H., Borchelt, D. R., and Shaw, B. F. (2012) Abnormal SDS-PAGE migration of cytosolic proteins can identify domains and mechanisms that control surfactant binding. *Protein Science 21*, 1197–1209.