Engineering of an Environmentally Responsive Beta Roll Peptide for Use as a Calcium-Dependent Cross-Linking Domain for Peptide Hydrogel Formation

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Supporting Information

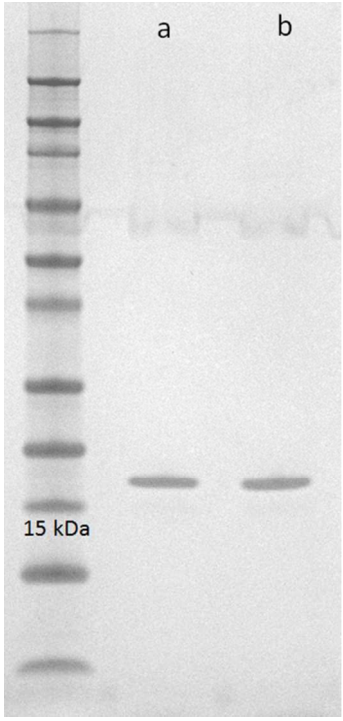

Figure S1. WT and leucine SDS-PAGE. Pure fractions of WT beta roll with the C-terminal capping region (a, 15.9 kDa) and the leucine mutant with the C-terminal cap (b, 15.9 kDa) are shown above following ion exchange chromatography.

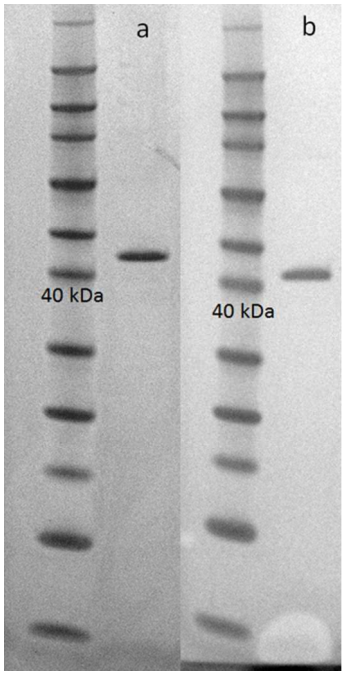

Figure S2. SDS-PAGE for HS-WT and HS-leucine. Pure fractions of HS-WT beta roll (a, 31.9 kDa) and the HS-leucine mutant (b, 31.9 kDa) are shown above following IMAC and SEC purification. Other HS fusion proteins have been shown to migrate slower during electrophoresis. [1] All constructs were verified by DNA sequencing.

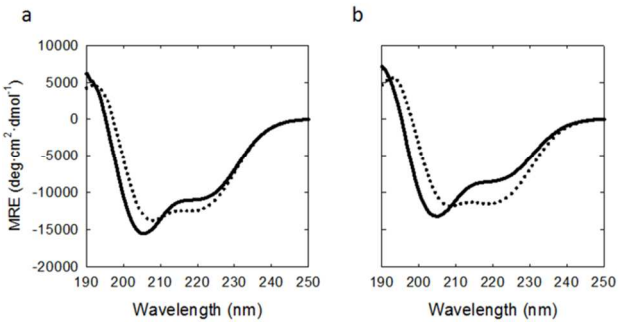

Figure S3. HS-WT and HS-Leucine beta roll CD Spectra. Purified samples of HS-WT (a) and HS-leucine beta roll were analyzed by CD spectroscopy with (···) and without (—) 10mM calcium. The spectra are dominated by the helical domain indicated by the positive peaks at 192nm and negative peaks around 205nm. An obvious shift in secondary structure is observed after the addition of calcium due to beta roll folding. All measurements were taken in triplicate in 50mM Tris pH7.5 at 25°C.

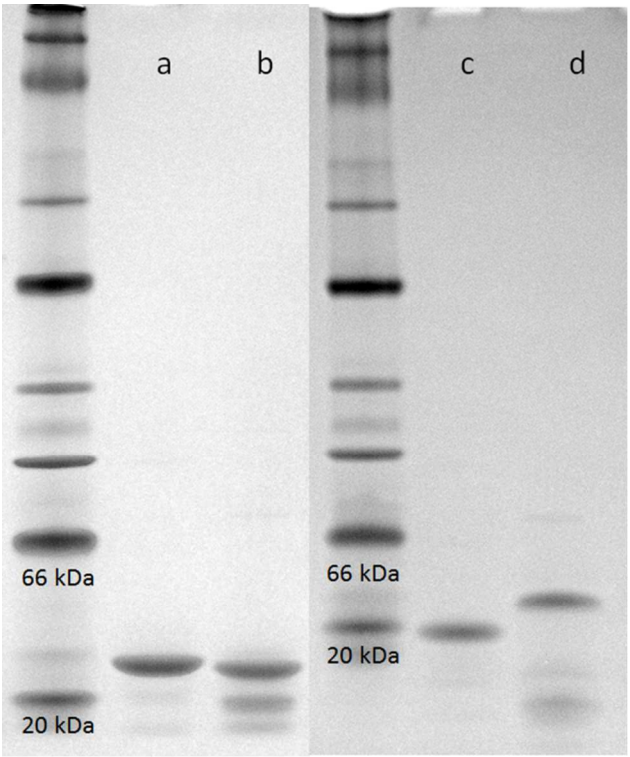

Figure S4. WT and leucine native PAGE. WT (a) and leucine (b) beta roll domains were run on 4-16% bis-Tris native gels (Invitrogen). WT (c) and leucine (d) fractions were then run in buffer supplemented with 5mM calcium. Purity of the samples was ensured by SDS-PAGE (Figure S1). While these results are not precisely quantitative, there is a clear perturbation in migration observed following calcium addition.

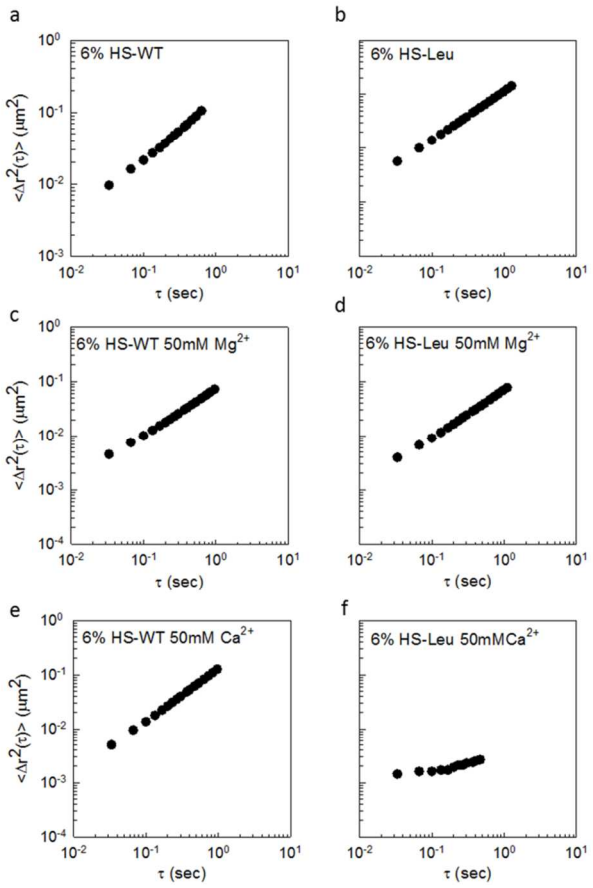

Figure S5. HS-WT and HS-leucine microrheology. The calculated mean square displacements for HS-WT and HS-leucine are given with respect to time in buffer (a-b), 50mM magnesium (c-d), and 50mM calcium (e-f), respectively. All samples appear viscous except 6% HS-leucine in 50mM calcium (f), which appears elastic.

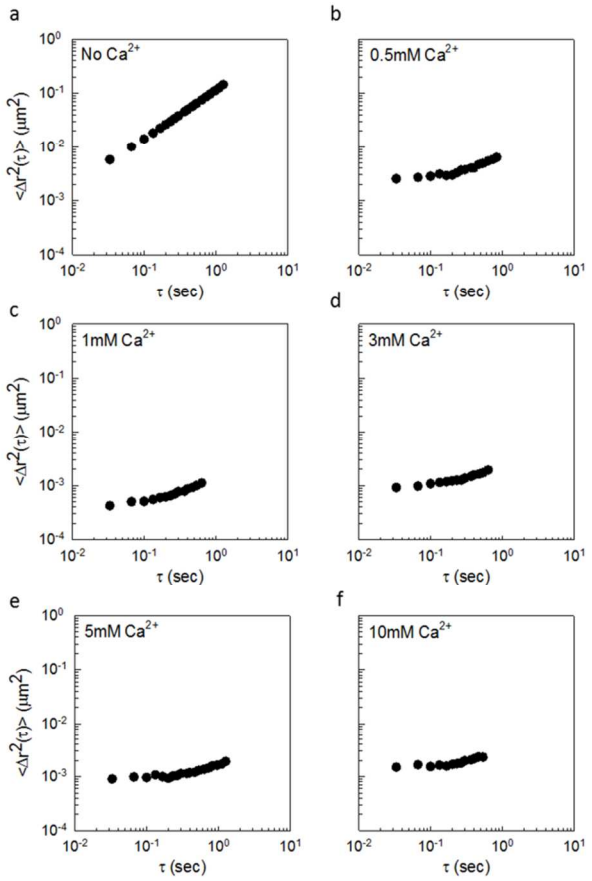

Figure S6. HS-leucine microrheology in response to calcium. The calculated mean square displacements for HS-leucine samples are given with respect to time at various calcium concentrations.

Beta Roll Construct Primary Sequences

Wild Type Beta Roll with C-terminal Cap (15.9 kDa)

GSARDDVLIGDAGANVLNGLAGNDVLSGGAGDDVLLGDEGSDLLSGDAGNDDLFGGQGDDTYLFGVGYGH
DTIYESGGGHDTIRINAGADQLWFARQGNDLEIRILGTDDALTVHDWYRDADHRVEIIHAANQAVDQAGI
EKLVEAMAQYPD

Leucine Beta Roll with C-terminal Cap (15.9 kDa)

GSARDDVLIGDAGANLLLGLAGNDVLSGGAGDDLLLGDEGSDLLSGDAGNDLLLGGQGDDTYLFGVGYGH
DLILESGGGHDTIRINAGADQLWFARQGNDLEIRILGTDDALTVHDWYRDADHRVEIIHAANQAVDQAGI
EKLVEAMAQYPD

HS-Wild Type Beta Roll with C-terminal Cap (31.9 kDa)

RGSHHHHHHGSDDDDKWASGDLENEVAQLEREVRSLEDEAAELEQKVSRLKNEIEDLKAEIGDHVAPRDT
SYRDPMGAGAGAGPEGAGAGAGPEGAGAGAGPEGAGAGAGPEGAGAGAGPEGAGAGAGPEGAGAGAGPEG
AGAGAGPEGAGAGAGPEGAGAGAGPEGARMHGGSARDDVLIGDAGANVLNGLAGNDVLSGGAGDDVLLGD
EGSDLLSGDAGNDDLFGGQGDDTYLFGVGYGHDTIYESGGGHDTIRINAGADQLWFARQGNDLEIRILGT
DDALTVHDWYRDADHRVEIIHAANQAVDQAGIEKLVEAMAQYPD

HS- Leucine Beta Roll with C-terminal Cap (31.9 kDa)

RGSHHHHHHGSDDDDKWASGDLENEVAQLEREVRSLEDEAAELEQKVSRLKNEIEDLKAEIGDHVAPRDT
SYRDPMGAGAGAGPEGAGAGAGPEGAGAGAGPEGAGAGAGPEGAGAGAGPEGAGAGAGPEGAGAGAGPEG
AGAGAGPEGAGAGAGPEGAGAGAGPEGARMHGGSARDDVLIGDAGANLLLGLAGNDVLSGGAGDDLLLGD
EGSDLLSGDAGNDLLLGGQGDDTYLFGVGYGHDLILESGGGHDTIRINAGADQLWFARQGNDLEIRILGT
DDALTVHDWYRDADHRVEIIHAANQAVDQAGIEKLVEAMAQYPD

Oligonucleotide Sequences

PCR Primers

Intein for cloning into pMAL

	Restriction Site	Sequence
Forward	SacI	5’-ccc**gagctc**gagctcgaacaacaacaaca-3’
Reverse	KpnI	5’-ccc**ggtacc**ggacagttgtgtacaacaa-3’

Wild Type Beta Roll with C-terminal cap for cloning into pMAL-intein

	Restriction Site	Sequence
Forward	KpnI	5’-atatc**ggtacc**gggcagcgcgcgtgatgacg-3’
Reverse	HindIII	5’-gcggcg**aagctt**ttagtccgggtattgtgccattgcttcaac-3’

Leucine Beta Roll with C-terminal cap for cloning into pMAL-intein

	Restriction Site	Sequence
Forward	KpnI	5’-atatc**ggtacc**gggctcggcccgtgacgac-3’
Reverse	HindIII	5’-gcggcg**aagctt**ttagtccgggtattgtgccattgcttcaac-3’

Wild Type Beta Roll with C-terminal cap for cloning into modified pQE9

	Restriction Site	Sequence
Forward	SphI	5’-aaa**gcatgc**gaggcagcgcgcgtga-3’
Reverse	SpeI	5’-aaa**actagt**gtccggatactgcgcca-3’

Leucine Beta Roll with C-terminal cap for cloning into modified pQE9

	Restriction Site	Sequence
Forward	SphI	5’-aaa**gcatgc**atggaggctcggcccg-3’
Reverse	SpeI	5’-cccccc**actagt**ttagtccgggtatt-3’

Leucine Beta Roll Oligonucleotides

	Sequence
Forward	5'ggctcggcccgtgacgacgtgctgattggtgacgctggtgctaacctgctgctgggtctggctggtaacgatgttctgtctggcggtgcaggcgatgacctgctgctgggcgatgaaggtagcgacctgctgtctggcgatgctggtaacgac-3'
Reverse	5'acgaatcgtatcgtggccaccgccactttccagaatcagatcatgaccgtagccgacaccaaacaggtaggtgtcatcaccctgaccgcccagcagcaggtcgttaccagcatcgccagacagcaggtcgctaccttcatcgcc-3'

References

1. Wheeldon, I. R.; Gallaway, J. W.; Barton, S. C.; Banta, S. *Proc. Natl. Acad. Sci.* **2008**, *105*, 15275.