Supporting Information

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Phage Display of Selenopeptides

Cloning vectors. The sequence of the phage cloning vector M13KE (New England Biolabs) is available at http://www.neb.com/neb/tech/nucleotide_seq_maps/sequences/m13ke.seq. The pMal-pIII protein expression vector (GENBANK accession #AF031088) is available from the authors upon request.

Oligonucleotide inserts. Oligonucleotides were synthesized by the phosphoramidite method by the Organic Synthesis Division of New England Biolabs. The inserts were as follows, with Acc65I and EagI restriction sites in bold; \( M=A \) or \( C \); \( N=A, C, T \) or \( G \).

Library insert: 5'-CATGTTTCGGCGGATTTGGTGCGAGACCTGCAACCGAMNNMNNMNATCAMNNMNNMNMMNNNAGAGTGAGAATAGAAGAGGTACC CGGG-3'
fdh SECIS control insert: 5'-CATGTTTCCGGCCGTACCGACCGATTTGGTGCGAGACCTGCAACCGGATGAGCGTACACACCCGAGAATAGAAAGGTACC CGGGCATG-3'

Duplex extension primer (NEB product #8101): 5'-CATGCCCCGGGTACCTTC TATTCTC-3'

Phage library construction. To construct the phage display library and the fdh SECIS control, the oligonucleotide inserts listed above were synthesized, gel-purified, and annealed to the duplex extension primer. The duplex was extended with dNTPs and Klenow fragment, digested with Acc65I and EagI, gel-purified and ligated into Acc65I/EagI digested phage cloning vector M13KE.
The ligation products were electroporated into *E. coli* ER2537 (F’ lacI<sup>+</sup> Δ(lacZ)M15 proA<sup>B+</sup>/fhuA2 supE thi Δ(lac-proAB)Δ(hsdMS-mcrB)5 and plated with 100 µL of a late log-phase ER2537 culture in 3 mL of agarose top on LB agar plates with 210 µM IPTG and 98 µM Xgal. The agarose top contained 10 g/L tryptone (Difco), 5 g/L yeast extract (Difco), 86 mM sodium chloride, 5 mM magnesium chloride, and 7 g/L agarose (American Bioanalytical) supplemented with 2 µM sodium selenite. The M13KE vector carries the lacZα fragment, resulting in characteristic blue plaques when plated with an α-complementing strain on X-gal medium. After a 16 h 37° C incubation, blue plaques were selected and the individual clones were amplified in early log-phase cultures of ER2537 supplemented with 2 µM sodium selenite. Sequencing templates were prepared by ethanol precipitation of phage DNA from 4 M sodium iodide. Phage clones were stored at 4° C in a 150 mM Tris pH 7.4, 50 mM sodium chloride, 100 µM DTT buffer with 0.02% sodium azide. Automated DNA sequencing was performed on a PE-ABD 377 or 373 instrument using Dye-Deoxy<sup>™</sup> terminator chemistry (PE Applied Biosystems) with the -96 gIII sequencing primer (NEB product #1259, 5’-CCCTCATAGTTAGCGTAACG-3’).

**pMal-pIII fusion protein expression.** The library inserts from individual phage clones were amplified by PCR from ~100 ng of sequencing template DNA using the duplex extension primer and the -96 gIII sequencing primer. The amplified products were gel-purified and digested with Acc65I and EagI. The digested insert was gel-purified and ligated into Acc65I/EagI digested pMal-pIII protein expression vector. The ligation products were electroporated into ER2537, plated on LB with 100 µg/mL ampicillin, and analyzed by restriction
mapping and automated DNA sequencing. The pMal-pIII fusion proteins were expressed in ER2537 and purified as previously described. For N-terminal protein sequencing, proteins were subjected to electrophoresis and electroblotted according to the procedure of Matsudaira, with modifications as previously described. The membrane was stained with Coomassie blue R-250 and the protein band of approximately 46 kDa was excised and subjected to sequential degradation on a PE-Biosystems 494A Protein/Peptide Sequencer using standard gas-phase cycles.

References

Figure S-1. HPLC PTH analysis, displayed in subtractive mode, of the fusion of the fdh SECIS with the maltose binding protein. The expected sequence was SARVSecHGPSV. a. Cycle 3 subtracted from cycle 4. b. Cycle 4 subtracted from cycle 5. c. Cycle 5 subtracted from cycle 6.
Clone 6 plaque count and size as a function of supplemental sodium selenite concentration in plating medium. Visible plaques were counted without magnification, and plaque diameter was measured under 7-fold magnification. Error bars represent ±1 standard deviation. Averages were based on triplicate platings, with ten plaques measured per plate.