Supporting Information

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SI Materials and Methods

PAGE Analysis. Gel electrophoresis was performed with SDS present at 0.1% (wt/vol) in the running buffer (3.5 mM) and lithium dodecyl sulfate (LDS) at 2% in the loading buffer used to solubilize and load 1–3 μg of each hairpin. The SDS and LDS concentrations in the running and loading buffer, respectively, each exceed by at least approximately 1.5× the CMC of SDS in the running buffer (measured as 2.1 ± 0.1 mM in 1× MES running buffer via pyrene fluorescence). The Mark-12 MW standards used for gel calibration consist of a reduced and unstained mixture of 12 SDS-solubilized globular proteins: myosin (200 kDa); β-galactosidase (116.3 kDa); phosphorylase B (97.4 kDa); BSA (66.3 kDa); glutamic dehydrogenase (55.4 kDa); lactate dehydrogenase (36.5 kDa); carbonic anhydrase (31.0 kDa); trypsin inhibitor (21.5 kDa); lysozyme (14.4 kDa); aprotinin (6.0 kDa); insulin B chain (3.5 kDa); and insulin A chain (2.5 kDa). The migration positions of the 6.0 kDa–55.4 kDa standards were used for MW estimation. Gel shifts were calculated as follows: dMW = (apparent MW-theoretical MW)/(theoretical MW) × 100%.

Size-Exclusion Chromatography (SEC). At constant temperature, the amount of SDS bound by membrane proteins saturates once detergent concentrations exceed the CMC of SDS in the buffer system under consideration [reviewed in (1)]. The SDS concentration in mobile phase buffer (≈10 mM) exceeds by approximately 5 times its reported CMC under identical buffer conditions [1.99 mM, (2)] and was thus expected to produce saturation loading. Because of reports indicating that silica gel columns can bind SDS (3), flow-through from the column was assayed for SDS concentration before sample injection; detergent concentration of flow-through matched that of reservoir mobile phase after 2 column volumes. Initial experiments where hairpins were dissolved in excess SDS in the mobile-phase buffer were not feasible due to overlap between protein peak elution and elution of excess SDS. Utilization of the MES electrophoresis running buffer as a mobile phase also proved to be of limited feasibility due to high background absorbance of the buffer components at 280 nm and incompatibility of buffer components with protein quantitation assays.

The following standards were used for SEC-S2000 calibration: blue dextran (>2,000 kDa; used for void volume); conalbumin (75.0 kDa); ovalbumin (43.0 kDa); carbonic anhydrase (29.0 kDa); RNase A (13.7 kDa); aprotinin (6.5 kDa); and insulin B chain (3.5 kDa). Each protein standard was dissolved in mobile-phase buffer with 5% (vol/vol) β-mercaptoethanol, boiled 5 min, then incubated overnight at room temperature, after which 0.4 mg of each was applied individually to the equilibrated SEC-S2000 column as described above. Calibration curves were prepared according to GE Healthcare by plotting the gel-phase distribution coefficient (K_{av}) vs. the logarithm of formula MW using the equation: $K_{av} = (V_e/V_o)/(V_c\text{-}V_o)$, where V_o is column void volume, V_e is elution volume, and V_c is geometric column volume calculated from column dimensions. V_o and V_e were estimated from the flow rate and retention time of blue dextran and protein standards, respectively. The column was calibrated three times using the entire set of abovementioned standards, and K_{av} values averaged. The logarithmic relationship between mean K_{av} and the molecular weight of each standard was fit to a second-order polynomial ($R^2 = 0.997$), and the equation of the line of best fit was used to estimate hairpin and glycophorin apparent MW values.

SDS and Protein Determination. For SDS determination, 20 μL of 1/5 diluted fraction was added to 280 μL of 10 mM Na phosphate, pH 7 and mixed with 2.5 mL of 0.0024% (w/v) aqueous methylene blue in an 18 × 250 mm glass test tube. Each aliquot was extracted with 10 mL HPLC-grade chloroform (Caledon) and the absorbance of the organic layer at 655 nm was measured. Standard absorbance curves were prepared by chloroform extraction of 0–30 μg SDS in 300 μL of 10 mM Na phosphate, pH 7. Comparison of standard curves indicated that the error in the assay was approximately ± 1 μg.

1. Imamura T (2006) in *Encyclopedia of Surface and Colloid Science*, ed Somasundaran P (Taylor & Francis, New York), pp 5251–5263.
2. Fuguet E, Rafols C, Roses M, Bosch E (2005) Critical micelle concentrations of surfactants in aqueous buffered and unbuffered systems. *Anal Chim Acta* 548:95–100.
3. Moller JV, le Maire M (1993) Detergent binding as a measure of hydrophobic surface area of integral membrane proteins. *J Biol Chem* 268:18659–18672.

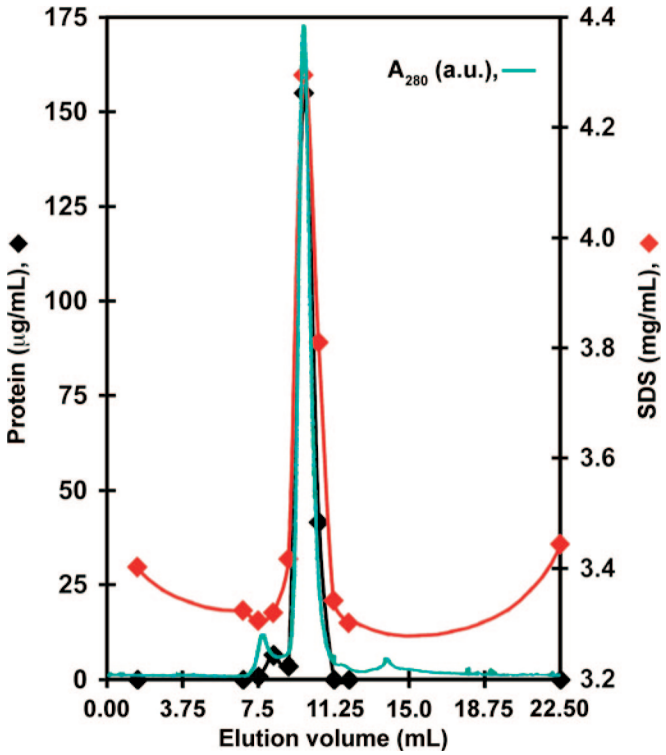

Fig. S1. Elution profile of WT TM3/4 and its SDS-binding level. Profiles for all other hairpins were essentially identical. The absorbance at 280 nm (*green*, arbitrary units), protein concentration (*black*, μg/ml), and SDS concentration (*red*, mg/mL) profiles are shown. Each point corresponds to an individual fraction. The volume of the fractions collected was 0.75 mL. All hairpins eluted in the 9.00–9.75-mL fraction (denoted the peak fraction).

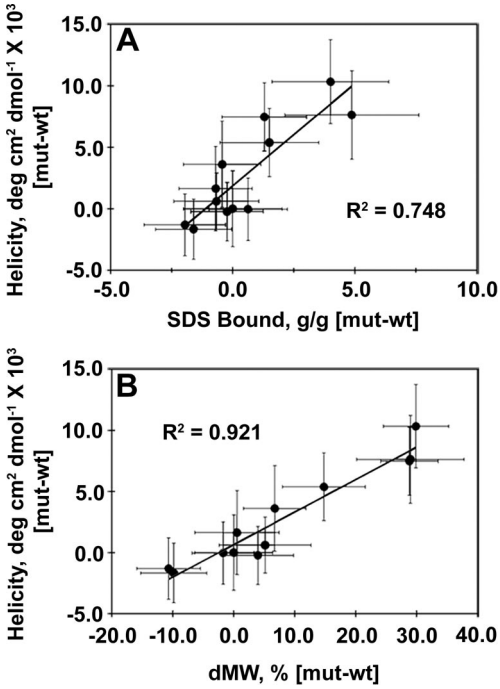

Fig. S2. Correlation of hairpin α-helicity with SDS binding and PAGE mobility. The relationships between helicity and bound SDS (*A*), and helicity and gel shift (*B*) are shown. The correlation coefficient (R^2) of the best fit line to each plot is given. Trendline *P* values are 0.003 and < 0.001, respectively. The variations in mutant-WT values were propagated from the standard deviations of each mean using standard formulae.

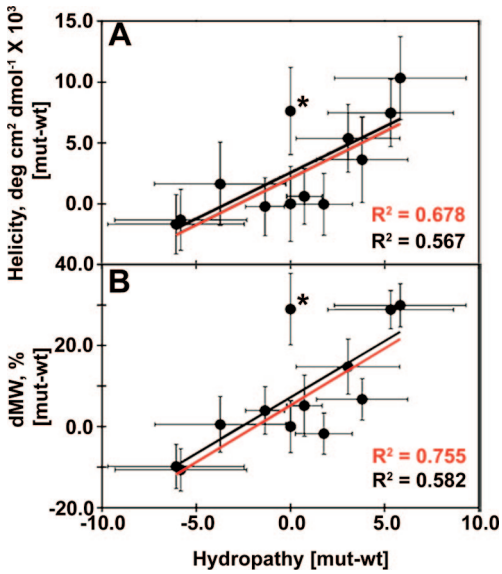

Fig. S3. Correlation of hairpin helicity and gel shift with hydropathy. Plots of mutation-dependent changes in helicity (*A*) and gel shift (*B*) vs. hydropathy changes are shown. Positive hydropathy values indicate increased apolarity and negative hydropathy values increased hydrophilicity. The E217S/S222E mutant is marked with an asterisk. Correlation coefficients (R^2) of the best fit line to each plot in the absence of E217S/S222E (red) or with all mutants (black) are shown. Trendline *P* values in the absence of E217S/S222E are 0.002 and < 0.001, respectively.