

Supporting Information

Protein design. The consensus sequence was taken as those residues with the highest global propensity at each position of the TPR motif (1). Two additional features added to the design are an N-terminal Gly-Ser before the first TPR (2–4), and a C-terminal “solvating” helix following the final TPR (5, 6).

Cloning of CTPRan proteins. The gene encoding for the original CTPR1 protein was inserted into the pGAT2 cloning vector (7) between BamHI and HindIII using PCR amplification and ligation. This cloning step changed the GNS- N-cap sequence of CTPR1 to GS-. Site-directed mutagenesis was used to introduce a new BglII restriction site within the CTPR1 gene, after the second helix of the TPR, changing the protein sequence from -PNN- to -PRS-. pGAT2 containing the so-modified CTPR1 gene was digested under two regimes: BamHI plus XbaI, and BglII plus XbaI. Two products are generated from each reaction regime (because the pGAT2 has a unique XbaI digestion site situated between the Lac operator and T7 gene RBS). The restriction products were then purified to obtain DNA corresponding to the whole CTPR1 gene plus a small part of the pGAT2 vector (from regime 1) and the TPR sequence, minus the C-terminal solvating helix, and part of the pGAT2 vector (from regime 2). These were ligated together, and since digestion with BamHI and BglII restriction enzymes gives identical sticky ends, generated the pGAT2 vector and the gene encoding the new CTPRa2. By changing the digestion products that are ligated, genes corresponding to CTPRa1, 2, 3, 4, 5, 6, 8, and 10 were constructed.

Protein purification. An 8-repeat CTPR was first purified as a GST-fusion protein using the pGAT2 vector. Protein was purified by glutathione affinity chromatography, thrombin cleavage, and gel filtration. However, this strategy resulted in additional cleavage by thrombin, and the

loss of the solvating helix. We therefore re-cloned the CTPRa-series into the vector pProEx-HTB (Invitrogen) and purified the CTPRa proteins as His-tag fusions, from which the His tag was removed by TEV-protease cleavage (1). All proteins, used for the thermodynamic measurements that we present, were purified in this latter fashion.

Crystallization and structure determination We obtained crystals of the 8-repeat CTPR that had been purified by the GST-fusion/thrombin cleavage route. Nanospray-ESI-TOF mass spectroscopy of the crystals showed that this material was missing the C-terminal solvation helix, as a consequence of the thrombin cleavage described above. We have therefore named this protein CTPRa8*. Serendipitously, CTPRa8* crystallized in an end-to-end fashion, which would not have been possible with the solvating helix present. Thus, the structures of CTPR2, CTPR3, and CTPRa8* have been solved from different crystal forms in different space groups. Nevertheless, the arrangement of helices in CTPR2 and CTPR3, which possess the solvating helix (1), and CTPRa8*, which does not, are superimposable (Kajander *et al.*, manuscript in preparation.)

CTPRa8* was crystallized from 100 mM NaAc pH 4.6-5.5, 25% MPD, and 20 mM CaCl₂. For phasing, 20 mM CaCl₂ was replaced by 5 mM CdCl₂ at pH 5.0. Crystals from the CdCl₂ solutions grew in space group P4₁2₁2 with unit cell dimensions of $a = 54.3 \text{ \AA}$ and $c = 71.8 \text{ \AA}$. The data was processed using HKL2000 (8) and the structure was solved at 2.05 Å resolution using Cd²⁺ single-wavelength anomalous diffraction data with SOLVE (9) and refined to current R-factors of R_{work}/R_{free} = 19.8/24.1% with REFMAC (10). Coordinates and structure factors for the crystal structure of CTPRa8* will be deposited in the PDB.

Circular dichroism. GuHCl-induced denaturation was performed with an automatic titrator (Microlab 500 series), which mixed two stock solutions, each with a protein concentration of 3 μM. The “folded” stock solution was in 50 mM phosphate pH 6.5, 150 mM NaCl. The

“unfolded” stock solution was in 50 mM phosphate pH 6.5, 150 mM NaCl, 7 M GuHCl. At each titration point, solutions were mixed, such that the concentration of GuHCl was increased by 0.1 M while the protein concentration remained constant. After equilibration for 45 min, CD measurements were carried out using an Aviv Model 215 CD spectrophotometer to determine the ellipticity at 222 nm.

Data fitting The partition function for a N -helix CTPR was taken to be $Z_N = e^{(N+1)J} e^{-NH} [(1 - g_-)g_+^{N+1} - (1 - g_+)g_-^{N+1}] / (g_+ - g_-)$, with $g_{\pm} = e^H (\cosh H \pm \sqrt{\sinh^2 H + e^{-4J}})$ (11). MATHEMATICA (12) was used to analytically calculate the magnetization, $m = d \log Z / dH$, thus yielding the fraction-folded, $f = (1 + m) / 2$, as a function of J and $H = \frac{1}{2} m_1 (x - x_c)$. This expression, in turn, was incorporated into a YORICK (13) non-linear least-mean-squares fitting routine, implementing the Marquardt algorithm (14), and fit to the experimental data.

References and Notes

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