SUPPLEMENTARY INFORMATION

Ranaspumin-2: structure and function of a surfactant protein from the foam nests of a tropical frog

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Author contributions: AC and MWK devised and managed the project; CDM prepared and characterized the protein and, with BOS, determined the NMR structure; IRRAS was done by AM and AB, and neutron reflection by XZ and JRL; AC proposed the clam-shell unfolding model and wrote the paper, in collaboration with other authors.

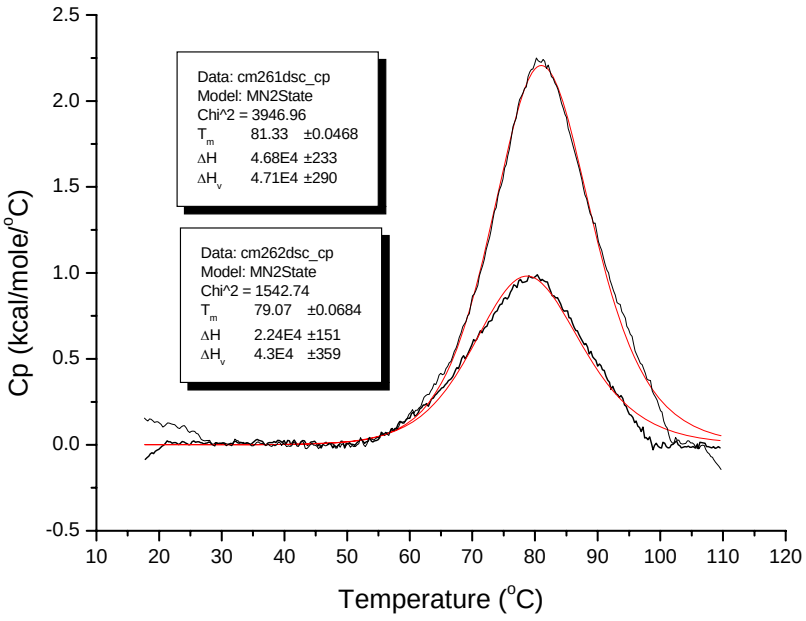

Figure S1: DSC of thermal unfolding of recombinant Rsn-2 in 20mM phosphate buffer, pH 7. The upper trace shows the cooperative endothermic response typical of a cooperative 2-state unfolding transition of a monomeric protein. The lower trace is a re-scan after cooling *in situ*, showing partial refolding of the protein even after heating to 110 °C. The ratio of van't Hoff to calorimetric enthalpies ($\Delta H_{VH}/\Delta H_{cal}$ = 1.01 for first scan) is consistent with cooperative 2-state unfolding of a protein monomer under these conditions.

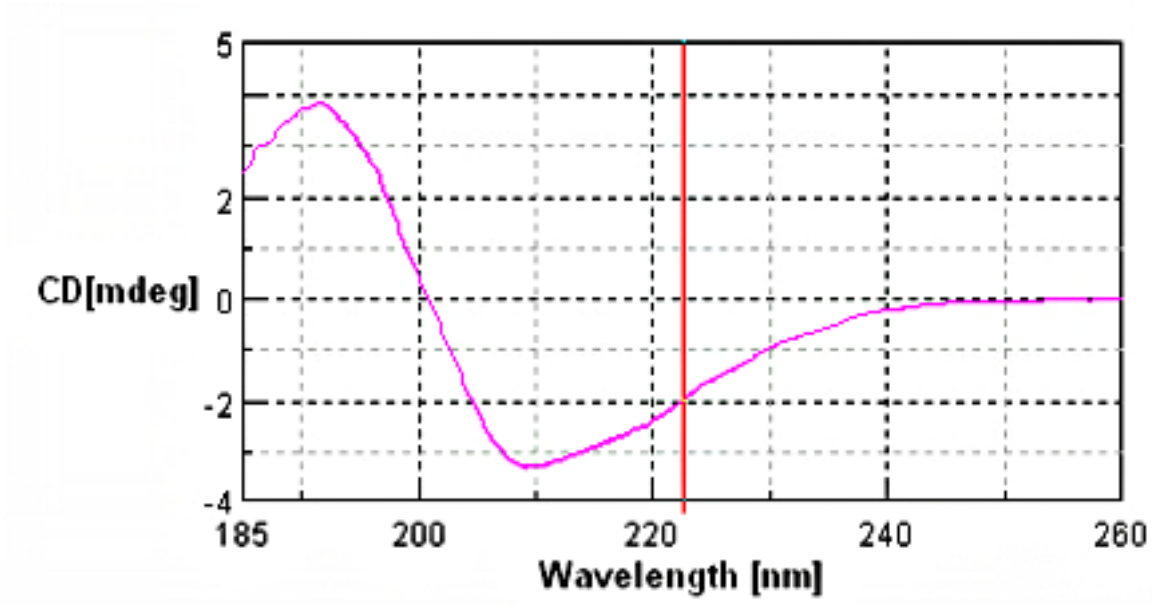

Figure S2: Far-UV circular dichroism (CD) spectrum of recombinant Rsn-2 in 20mM phosphate buffer, pH 7.

	Contin	Selcon 3	Solution Structure
Helix	11%	17%	14%
Strand	36%	35%	35%
Turn	21%	19%	19%
Unordered	32%	29%	32%

Table S1: Comparison of secondary structure content of Rsn-2 in solution estimated from Contin or Selcon 3 analysis of CD spectra, or from the NMR structure.

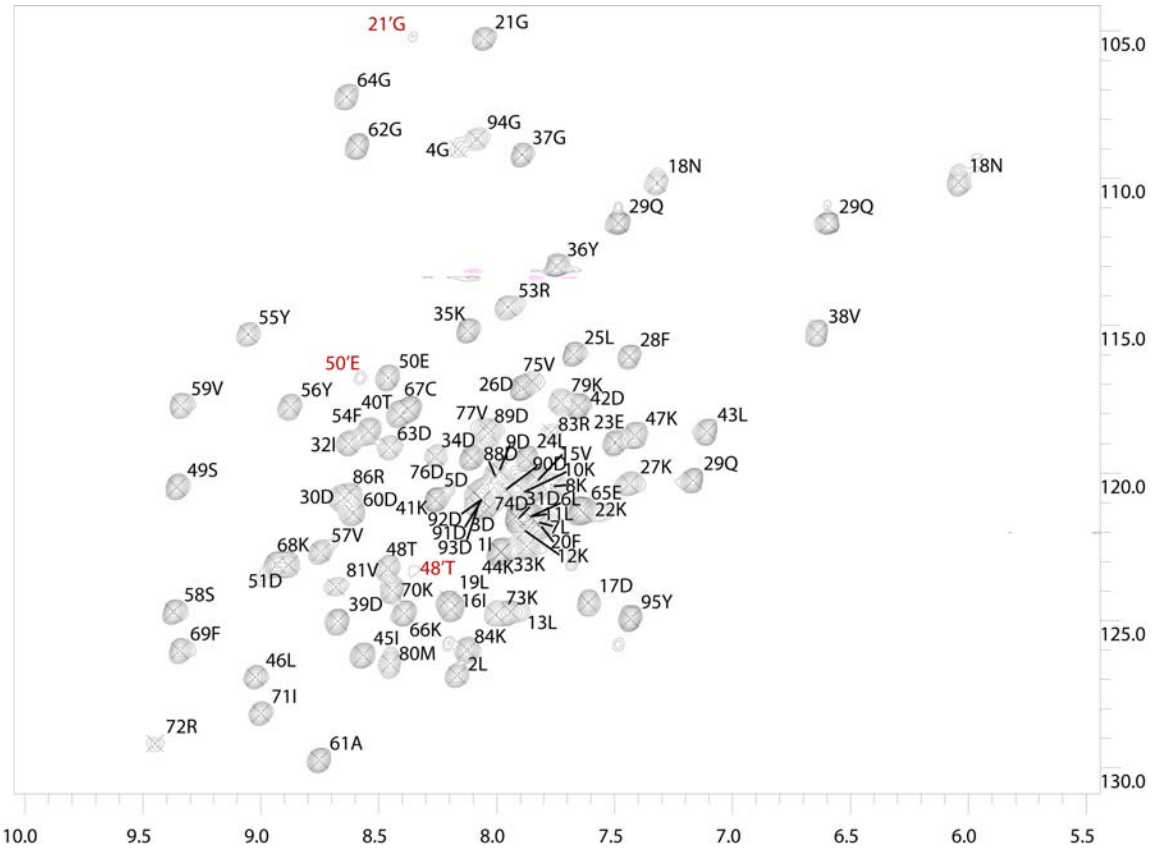

Figure S3: ^{15}N-HSQC of Rsn-2 recorded at 14.1 T, 308 K, 20 mM NaPi pH 7, 50 mM NaCl. The backbone and sidechain resonance assignments are indicated and the peaks corresponding to minor conformations of selected residues are highlighted in red. (Note added in proof: residue numbers shown here should be incremented by one to match PDB entries.)

Table S2: NMR and refinement statistics for Rsn-2

	Protein
NMR distance and dihedral constraints	
Distance constraints	
Total NOE	2322
Ambiguous	999
Unambiguous	
Intra-residue	727
Sequential ($\|i-j\|=1$)	250
Medium-range ($\|i-j\|<4$)	110
Long-range ($\|i-j\|>5$)	236
Hydrogen bonds	17
Structure statistics	
Violations (mean and s.d.)	
Distance constraints (Å)	$0.061\pm1.95\times10^{-3}$
Violations per structure (>0.5 Å)	4.24±1.36
Max. distance constraint violation (Å)	0.94
Deviations from idealized geometry	
Bond lengths (Å)	$0.0458\pm1.28\times10^{-4}$
Bond angles (º)	$0.610\pm1.98\times10^{-2}$
Impropers (º)	$1.92\pm1.12\times10^{-1}$
Dihedral angle constraints (º)	42.1±0.234
Average r.m.s.d. to the unbiased mean structure** (Å)	
Heavy	1.24
Backbone	0.79
Ramachandran plot (% residues in)	
Most favoured	66.1
Additionally allowed	26.8
Generously allowed	4.4
Disallowed	2.8

** r.m.s. deviation to the unbiased mean structure was calculated among 25 refined structures for residues 17-87.

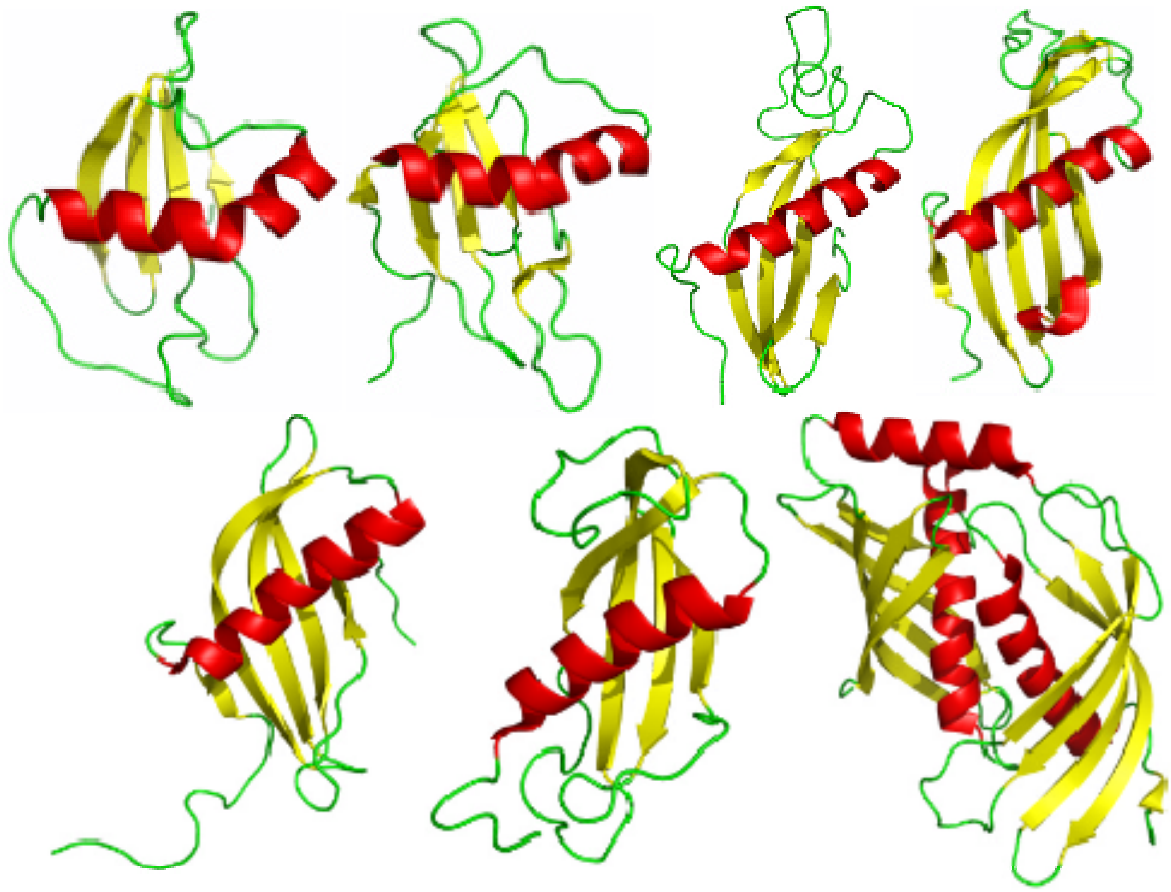

Figure S4: Comparison of Rsn-2 (top left) with structurally similar proteins from the Protein Data Base. From left to right, top to bottom: Rsn-2, human cystatin (stefin) A (1GD3), chicken egg white cystatin (1A67), human cystatin D (1RN7), rice oryzacystatin (1EQK), pig protegrin precursor (1N5H), and mouse latexin (1WNH).

From:

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