Supplementary Information

The intrinsically disordered Tarp protein from chlamydia binds actin with a partially preformed helix

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Results

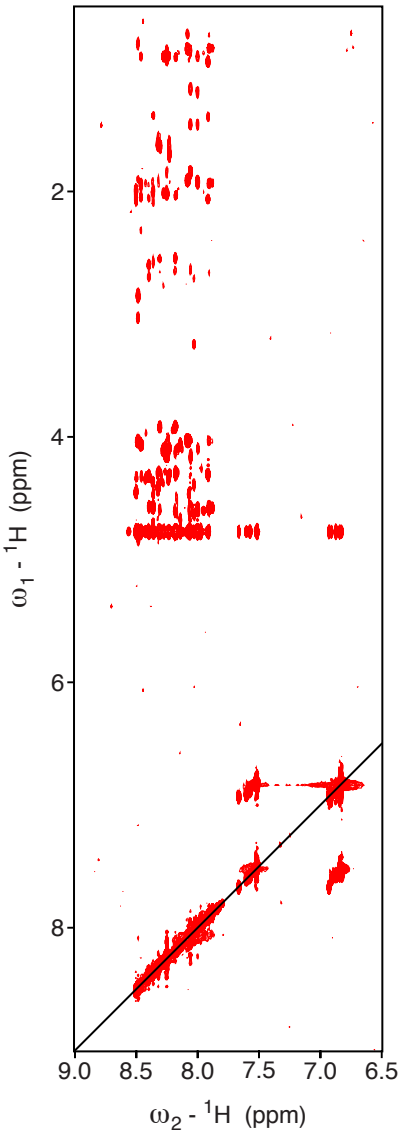


Figure S1. Two-dimensional ^{1}H-^{1}H projection of 3D [^{1}H,^{15}N]-HSQC-NOESY. The spectrum was acquired with a mixing time of 200 ms, at 800 MHz, 298 K. The full ^{15}N spectral width is projected in this image. The few crosspeaks visible correspond to intra-residue correlations. The absence of correlations between amide protons is particularly significant – the only non-diagonal peaks present in the lower half of the spectrum correspond to crosspeaks between two protons in the same glutamine or asparagine side chain.

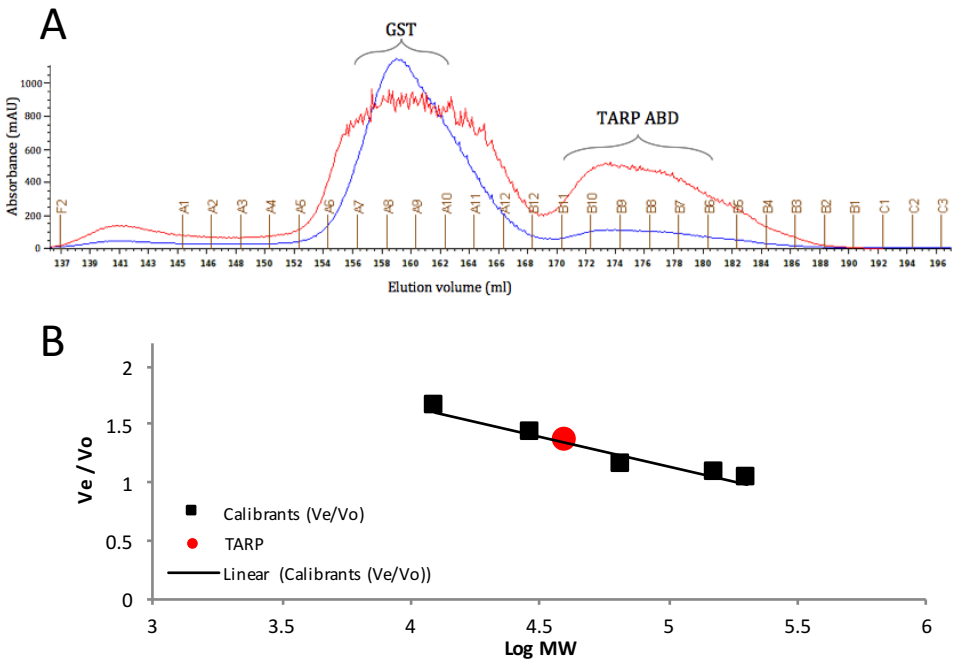


Figure S2. Size exclusion chromatography of Tarp$_{726\text{-}825}$. (**A**) Size exclusion chromatogram of a mixture of GST and Tarp$_{726\text{-}825}$ (after digestion of the GST-Tarp$_{726\text{-}825}$ fusion protein) in a 75 cm S75 Sephadex column, at 280 nm (blue) and 214 nm (red). (**B**) Standard protein calibrants (cytochrome *c*, carbonic anhydrase, bovine serum albumin, alcohol dehydrogenase, and β-amylase; black squares) were used to calibrate the S75 column across a molecular weight range of 12.4 kDa to 200 kDa. The relationship between molecular weight (MW), elution volume (Ve), and void volume (Vo) was used to determine that Tarp$_{726\text{-}825}$ (red circle) elutes from the column with an apparent molecular weight of ~ 40 kDa.

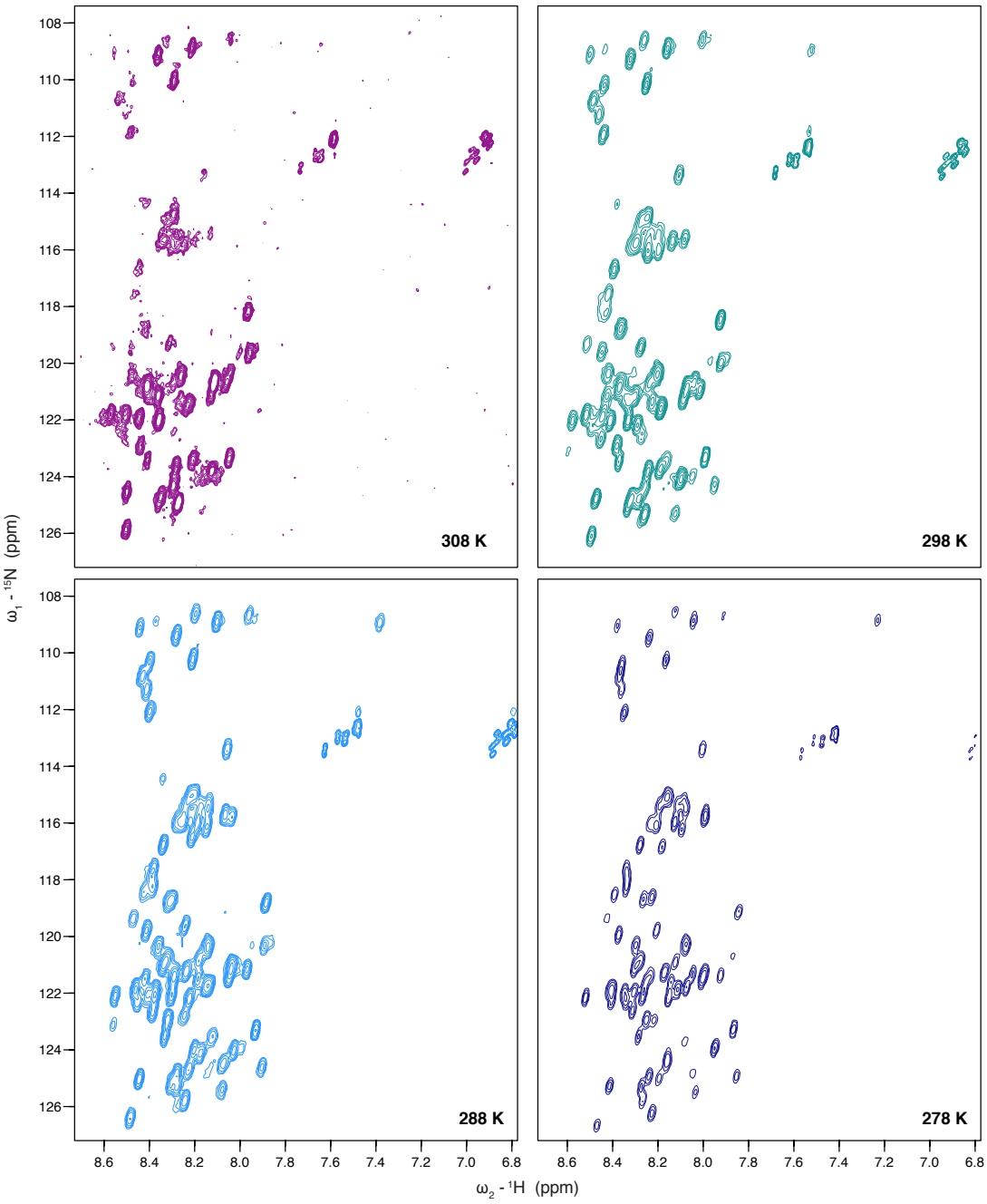
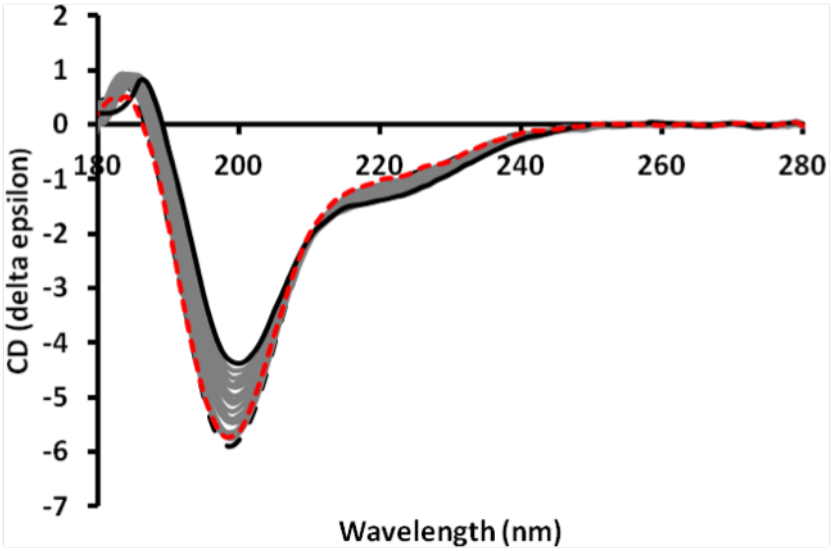


Figure S3. [^{1}H,^{15}N]-HSQC spectra of Tarp$_{726\text{-}825}$ at varying temperatures. Top left, 308 K; top right, 298 K; bottom left, 288 K, bottom right, 278 K. No new peaks potentially corresponding to residues between 808 and 825 were observed.



Secondary Structure	20 °C (%)	85 °C (%)	Δ (%)
α-helix	5 ± 2	7 ± 2	2
β-strand	14 ± 2	19 ± 2	5
Disordered	68 ± 2	58 ± 6	-10

Figure S4. Thermal melt synchrotron radiation circular dichroism (SRCD) spectroscopy of $Tarp_{726-825}$. Top: SRCD spectra of $Tarp_{726-825}$ measured at 20 °C (dashed black line), and in successively increasing 5 °C increments (solid grey lines) up to 85 °C (solid black line), and a final spectrum after return to 20 °C (dashed red line). **Bottom:** The calculated secondary structure content of Tarp at 20 and 85 °C. Values are the average plus or minus standard deviation from three algorithms (as described in Methods).

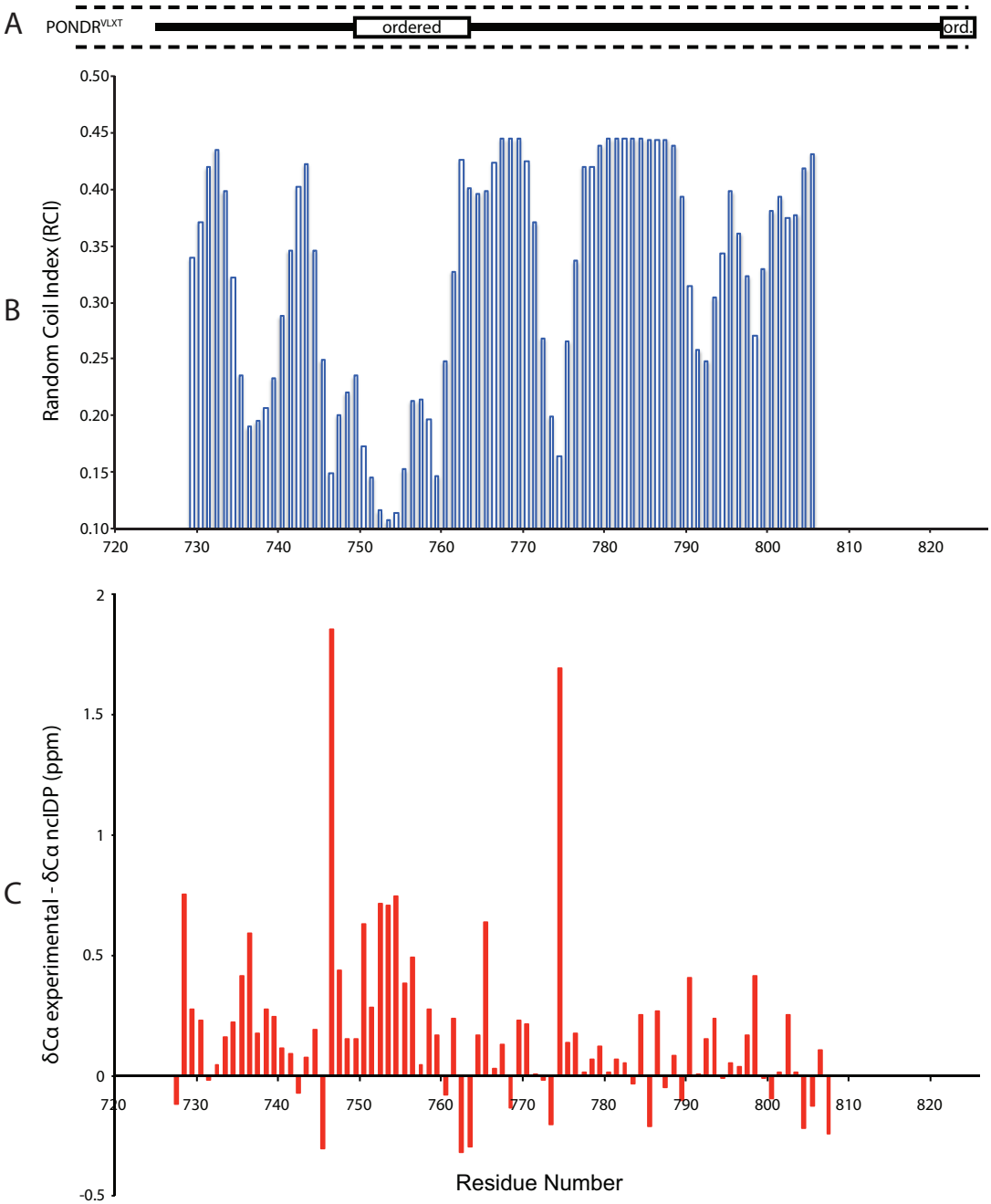


Figure S5. Order and secondary structure. (**A**) PONDR-VLXT[1,2] *in silico* disorder prediction (default parameters) for the $Tarp_{726-825}$ amino acid sequence. (**B**) The random coil index value as calculated by the RCI webserver[3] using the $Tarp_{726-825}$ backbone chemical shifts with default parameters and Wishart random coil values[4]. (**C**) Secondary neighbor-corrected Cα chemical shifts, calculated using Tamiola *et al*. random coil values for intrinsically disordered proteins[5].

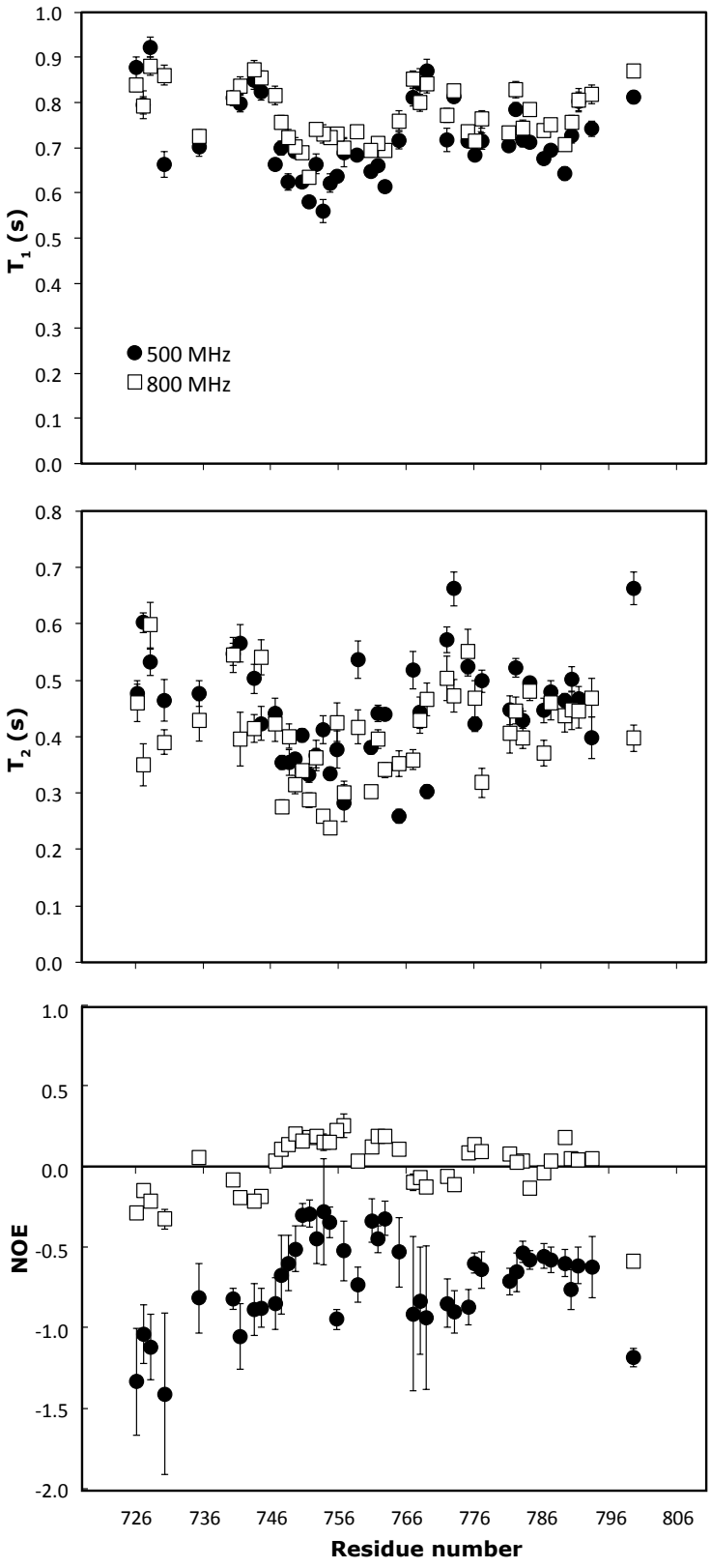


Figure S6. ^{15}N relaxation parameters. T_1, T_2 and heteronuclear NOE were measured for $Tarp_{726-825}$ at 500 (black circles) and 800 MHz (white squares), at 298 K, in G-actin buffer, pH 7.5. Error bars correspond to the fitting errors for each parameter.

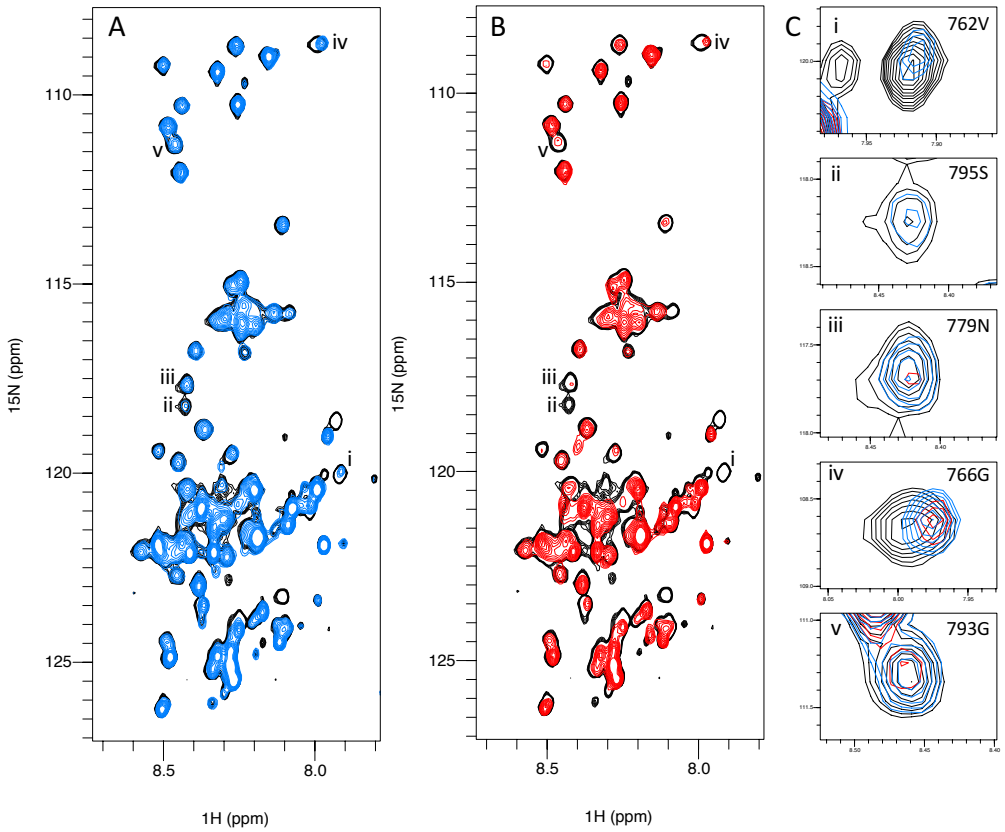


Figure S7. Binding of Tarp$_{726\text{-}825}$ to actin observed by NMR spectroscopy. (**A**) and (**B**) The central region of [^{1}H-^{15}N]-HSQC Tarp$_{726\text{-}825}$ spectra in the absence of actin (black), in the presence of 0.8:1 actin:Tarp ratio (blue) and of 2:1 actin:Tarp ratio (red). A few examples of peaks with significant decreases in intensity between 0.8:1 and 2:1 actin:Tarp ratios are highlighted with roman numerals. (**C**) Enlarged view of the peaks highlighted in (**A**) and (**B**). Spectra were scaled according to the peak intensity of residues remaining from the fusion tag.

ITC fitted parameters	
n	1.00 ± 0.06
K_d	102 ± 33 nM
ΔH	–16.10 ± 0.71 kcal mol^{-1}
–TΔS	6.56 ± 0.91 kcal mol^{-1}
ΔG	–9.54 ± 0.21 kcal mol^{-1}

Table S1. Tarp:actin binding parameters. Parameters are shown as average and standard deviation of four independent experiments.

	Tarp:actin	WAVE2:actin
K_d (experimental)	102 nM	52 nM[6]
K_d [P]	3.4 μM	440 nM
ΔG (experimental)	-9.54 kcal.mol^{-1}	unknown
ΔG [P]	-7.5 kcal.mol^{-1}	-8.7 kcal.mol^{-1}
FOLDX[7] Interaction energy	-12.16 kcal.mol^{-1}	-13.98 kcal.mol^{-1}
Interfacial contacts (ICs) [P]		
ICs charged-charged	2	6
ICs charged-polar	3	2
ICs charged-apolar	12	16
ICs polar-polar	1	0
ICs polar-apolar	10	10
ICs apolar-apolar	32	21
Non Interacting Surface (NIS)[P]		
%NIS charged	29.68	30.47
%NIS apolar	42.05	40.14

Table S2. Modeled and measured thermodynamic parameters for the Tarp and WAVE2, G-actin interaction. Parameters denoted [P] were calculated using the PRODIGY webserver[8].

References

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