Supplementary Data for

Osmotic-shock induced protein destabilization in living cells and its reversal by glycine betaine

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***Running title:** Osmotic shock and protein stability*

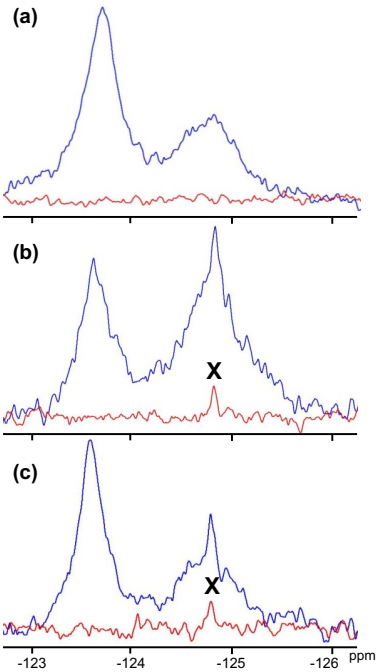

Figure S1. Supernatant controls to assess leakage at 298 K. In-cell spectra (blue) are overlaid with supernatant spectra (red) for the corresponding experiment. Normal in-cell experiment with no osmotic shock shows no leakage (a) whereas leakage of fluorine containing metabolites occurs as a result of hyperosmotic shock (b) and hyperosmotic shock in the presence of glycine betaine (c). The leaked metabolites include free 5-fluoroindole and truncated SH3.

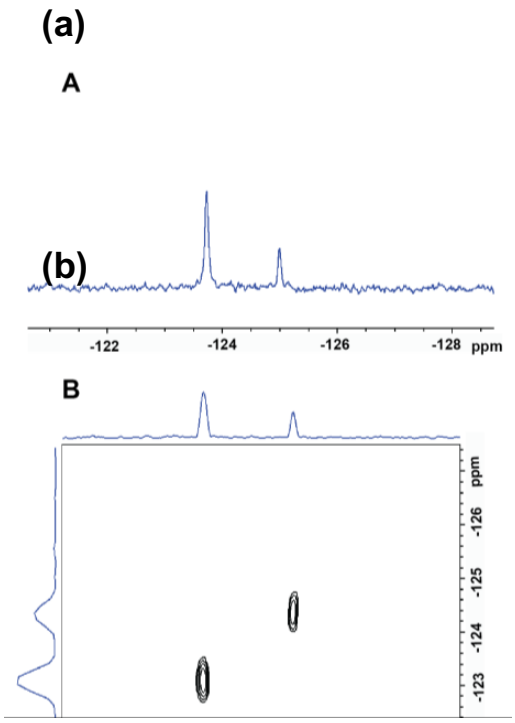

Figure S2. Truncated SH3 does not under go conformational exchange. (a) 1D ^{19}F NMR spectrum of incompletely purified SH3 in 0.4 M Na_2SO_4 at 298 K, which completely folds intact SH3 [1]. This result proves that the upfield resonance does not arise from intact SH3. (b) 2D ^{19}F homonuclear exchange spectroscopy (EXSY) at 298 K with a mixing time of 140 ms, performed as described by Smith et al. [2]. The absence of cross peaks indicates the species giving rise to the upfield peak does not fold and, therefore, is not intact SH3. Chromatography followed by mass spectroscopy (Fig. S3) show that the upfield peak arises from the truncated protein.

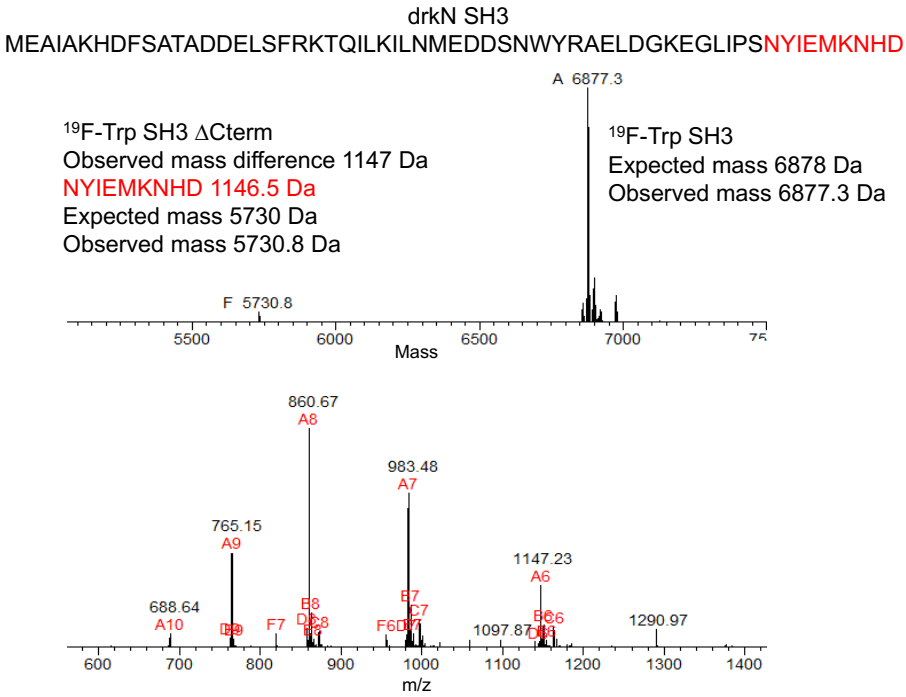

Figure S3. Mass spectrometry to identify truncated SH3. Mass spectrometry after size exclusion chromatography reveals contamination with a compound of smaller molecular weight than intact fluorine labeled SH3. The molecular weight of this species corresponds to SH3 missing its nine C-terminal residues. The bottom mass spectrum shows the detected ions by their individual m/z ratio with ions from the intact protein labeled A and those from the truncated protein labeled F. Contamination of SH3 with this truncated protein affects the apparent stability observed by NMR (Fig. S2) and can be removed *via* an additional chromatography step (See Methods).

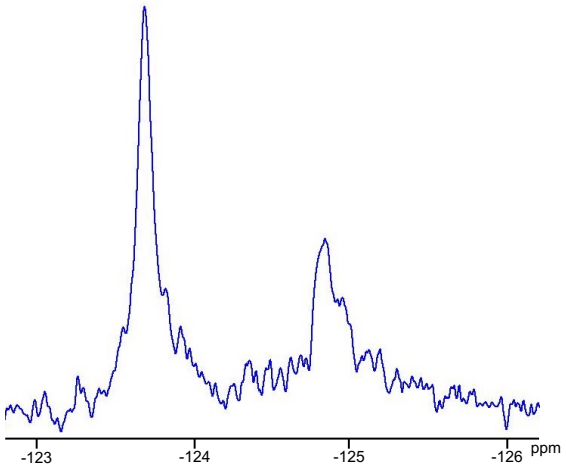

Figure S4. ^{19}F NMR spectrum of an uncentrifuged lysate sample in D_2O at 298 K. The increased concentration of macromolecules in the non-clarified lysate results in more attractive interactions with SH3, leading to a larger fraction of SH3 in the unfolded form, which appears as a shoulder on the right of the X_{lys} peak. Both peaks are also broader near the base compared to a clarified lysate spectrum (Figure 2C).

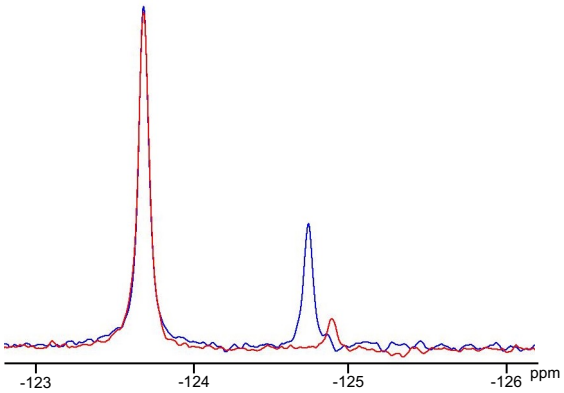

Figure S5. ^{19}F NMR spectra of purified SH3 in H_2O buffer (blue) and D_2O buffer (red) at 298 K. The average free energy of unfolding for SH3 is 0.63 ± 0.03 kcal/mol in H_2O buffer and 1.5 ± 0.1 kcal/mol in D_2O buffer. The D_2O spectrum was normalized to the position and intensity of the folded (downfield) resonance in H_2O.

Table S1. Free energies of unfolding ($\Delta G_U^{\circ\prime}$) from raw and deconvoluted spectra with uncertainties (the standard deviation of the mean from three trials) at 298 K

Condition	$\Delta G_U^{\circ\prime}$ (kcal/mol) Raw Spectra	$\Delta G_U^{\circ\prime}$ (kcal/mol) Deconvoluted Spectra
Buffer		
D_2O, pH 7.8	1.5 ± 0.1	1.6 ± 0.1
D_2O + 0.3 M NaCl	1.7 ± 0.2	1.8 ± 0.2
D_2O + 0.3 M NaCl + 1 mM glycine betaine	1.8 ± 0.1	1.9 ± 0.1
H_2O, pH 7.8	0.63 ± 0.03	0.67 ± 0.03
H_2O, pH 7.8, 0.68 molal glycine betaine	1.1 ± 0.1	1.1 ± 0.1
Cells		
D_2O	1.6 ± 0.2	1.3 ± 0.3
D_2O + 0.3 M NaCl	0.54 ± 0.08	0.44 ± 0.03
D_2O + 0.3 M NaCl + 1 mM glycine betaine	1.5 ± 0.2	1.5 ± 0.4

Table S2. Changes in the free energies of unfolding ($\Delta\Delta G_U^{\circ\prime}$) at 298 K (Condition-Buffer).

Condition	$\boldsymbol{\Delta\Delta G^{\circ\prime}_{U,298K}}$ **(kcal/mol) Raw Spectra**
D_2O, pH 7.8	
Buffer + 0.3 M NaCl	0.2 ± 0.2
Buffer + 0.3 M NaCl + 1 mM betaine	0.3 ± 0.1
Cells	0.1 ± 0.2
Cells + 0.3 M NaCl	-1.0 ± 0.1
Cells + 0.3 M NaCl + 1 mM glycine betaine	0 ± 0.2
H_2O, pH 7.8	
Buffer + 0.68 molal glycine betaine	0.50 ± 0.1

References

[1] Zhang O., Forman-Kay J.D. Structural characterization of folded and unfolded states of an SH3 domain in equilibrium in aqueous buffer. Biochemistry 34 (1995) 6784-6794.
[2] Smith A.E., Zhou L.Z., Gorensek A.H., Senske M., Pielak G.J. In-cell thermodynamics and a new role for protein surfaces. Proc. Natl. Acad. Sci. USA 113 (2016) 1725-1730.