

Supporting Information for:

**Thermal analysis of a mixture of ribosomal proteins by vT-ESI-MS:
towards a parallel approach for characterizing the *stabilitome***

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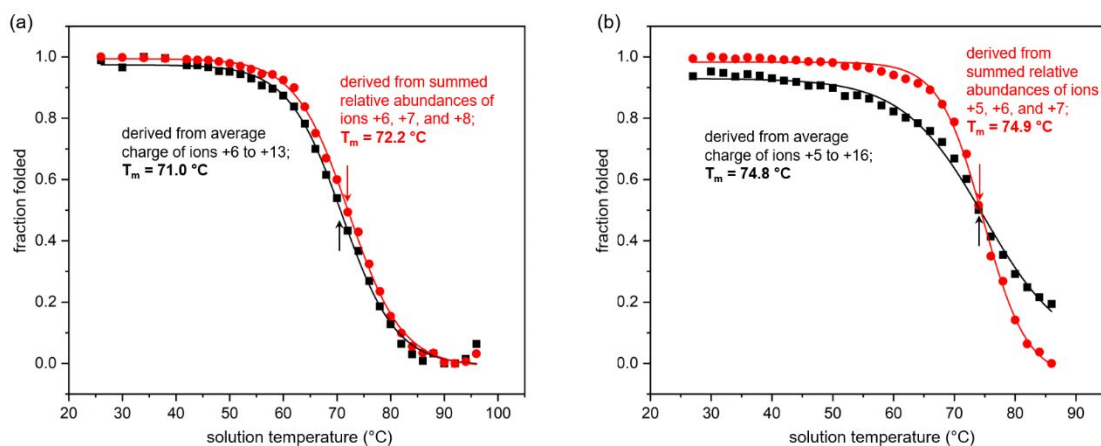


Figure S1. Fraction of folded protein as a function of solution temperature for ubiquitin in water at pH = 3 (a) and cytochrome *c* in 20 mM ammonium acetate at pH 6.8 (b); the fraction folded is calculated either by the weighted average charge state of all ions (black squares) or by tracking the signal loss of native ions with temperature (red circles). Each curve is fit with a two-state transition to obtain the melting temperature depicted by black or red arrows. See Supp. Reference 1 for details. The T_m value we obtained in (b) is slightly lower than a literature reported value (~ 80 °C),² consistent with a decreased thermal stability at pH 6.8 versus 7.4, respectively. Uncertainties are (a) ± 2.0 °C ($n = 3$)¹ and (b) ± 2.0 °C ($n = 3$).

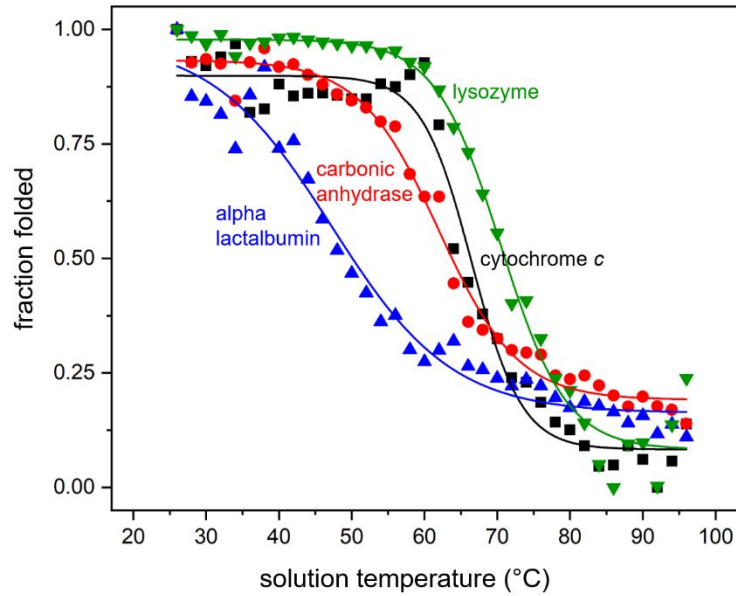


Figure S2. Representative stability curves (fraction folded versus solution temperature) for proteins in a simple mixture (20 mM ammonium acetate at pH = 6.8): alpha lactalbumin (blue triangles), carbonic anhydrase (red circles), cytochrome *c* (black squares), and lysozyme (green upside-down triangles). Average T_m values from three replicate analyses are as follows: cytochrome *c* $T_m = 72.1 \pm 8.3$ °C (See Ref. 2); carbonic anhydrase $T_m = 60.3 \pm 4.8$ °C (Lit. 64 °C);³ lysozyme $T_m = 75.2 \pm 6.2$ °C (Lit. 72 °C);⁴ alpha lactalbumin $T_m = 51.0 \pm 12.4$ °C (Lit. T_m reported to be between 50 – 60 °C)⁵. Values reported as average (\pm s.d.), $n = 3$ measurements.

Table S1. Identified proteins and associated melting temperatures.

Protein ^a	Measured mass ^b	Calculated mass ^c	$\Delta m_{(\text{exp-seq})}$	T_m (°C) ^d	Lit. T_m (°C) ^e
L9	15,770.5 (3.0)	15,769	1.5	34.0 (0.1), 35.0 (0.3)	---
L25	10,693.5 (1.3)	10,693	0.5	43.8 (0.1), 43.8 (0.4)	---
L29	7,272.2 (0.7)	7,273	-0.8	56.6 (3.6), 55.6 (1.8)	---
Ribosome Associated Initiation Factor A	12,653.0 (0.5)	12,653	0.0	83.6 (0.3), 85.4 (1.2)	---
L11	14,872.2 (0.8)	14,875	-2.8	37.7 (1.0), 35.2 (0.5)	---
L30	6,409.9 (0.2)	6,410	-0.1	37.9 (0.1), 42.8 (0.8)	---
Ribosome Hibernation Promotion Factor	10,750.1 (1.0)	10,750	0.1	74.8 (0.7), 76.9 (1.8)	---
Enolase (monomer)	45,649 (5.0)	45,655	6.0	50.9 (0.4), 56.0 (0.6)	54.6
Malate Dehydrogenase (monomer)	32,335 (4.0)	32,337	2.0	54.1 (0.3), 56.3 (0.3)	52.8

^a All found in top 20 protein hits identified by LC-MS

^b Deconvoluted using at least two charge states with single decimal precision. Uncertainty in deconvoluted mass reported in parenthesis.

^c Values correspond to the isotopically averaged mass taken from Uniprot.

^d Two biological replicates are reported. The value in parenthesis represents the uncertainty associated with a sigmoidal fit over the relevant temperature range

^e Values taken from the *Meltome atlas*: <http://meltomeatlas.proteomics.wzw.tum.de>⁶

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