Supporting Information

Real-time Hydrogen/Deuterium Exchange Kinetics via Supercharged Electrospray Ionization Tandem Mass Spectrometry

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EXPERIMENTAL

ESI-HDX-MS. Mass spectra for all HDX-MS experiments were acquired using an LTQ-orbitrap hybrid mass spectrometer (Thermo Fischer Scientific, Waltham, MA, USA) equipped with a fluoranthene anion source for ETD experiments. Ions were formed using nanoelectrospray emitters prepared by pulling borosilicate capillaries (1.0 mm o.d./0.78 mm i.d., Sutter Instruments, Novato, CA, USA) to a tip inner diameter of ~1 μm with a Flaming/Brown micropipette puller (Model P-87, Sutter). A platinum wire (0.127 mm diameter, Sigma, St. Louis, MO, USA) was inserted through the capillary into the solution and electrospray was initiated and maintained by applying ~800 V to the wire relative to instrument ground. The temperature inside the instrument nanospray source was maintained at ~38 °C. For ETD, either the 7+, 10+ or 13+ charge state was isolated and subsequently reacted with fluoranthene anions for 30 ms. The resulting fragment ions were measured in the orbitrap analyzer set to 100,000 resolution for detection.

A 375 μM solution of fully deuterated bovine ubiquitin was prepared by dissolving solid protein (Sigma, St. Louis, MO, USA) in a deuterium water (Euriso-top, Saint-Aubin Cedex, France) solution containing 19 mM ammonium acetate and 0.005% D-formic acid (Cambridge Isotopes, Andover, MA, USA) and heating the solution at 68 °C for three hours. The protein was then stored at room temperature for more than one week after which time ~99% deuterium incorporation was measured using mass spectrometry. Exchange for hydrogen was initiated by diluting a 1 μL aliquot of the deuterated ubiquitin solution into 99 μL of 200 mM ammonium acetate, pH = 6.2, containing 1.0% *m*-nitrobenzyl alcohol (Sigma, St. Louis, MO, USA). The sample was mixed for a few seconds before a ~7 μL aliquot was loaded into a nanospray capillary, ESI was established, and the first ETD spectrum was acquired within approximately two minutes. The remaining sample was held in a 38 °C heatblock for the 185 and 385 minute timepoints. Mass spectra were recorded continuously for the first 12 minutes, after which time the voltage applied to the electrospray capillary was set to zero to minimize change in solution pH that can occur during ESI,[1] and the data acquisition was paused. Mass spectra were again recorded between 18 – 22 minutes and four minutes of data were acquired every ten minutes up until 92 minutes. A new nanospray capillary was loaded with a fresh 7 μL, 38 °C aliquot for each of the 182 and 380 minute timepoints. For each timepoint, two minutes of continuous acquisition were scan averaged. For example, the 10 minute timepoint was obtained by scan averaging data between 9 and 11 minutes.

The identities of the fragment ions were assigned using a m/z uncertainty constraint of ± 0.005 and Protein Prospector (http://prospector.ucsf.edu/prospector/mshome.htm) to calculate exact masses. The average extent of H/D exchange for each c or $z\bullet$ ion at a given timepoint was determined from the average mass of each fragment, calculated using the intensity-weighted isotopic abundances with and without HDX. That average extent of H/D exchange for each ion was subtracted from that of the nearest neighboring c or $z\bullet$ ion toward the termini to give either a single amide HDX timepoint value or a summed value when adjacent fragment ions were not detected. These values as a function of time were fit to exponential functions using Igor Pro 4.07 to obtain HDX rate constants.

Traveling Wave Ion Mobility Spectrometry. ESI mass spectra and arrival time distributions of individual charge states of ubiquitin were acquired using a hybrid quadrupole/ion mobility/time-of-flight instrument (Synapt™ High Definition Mass Spectrometer; Waters, Milford, MA, USA) equipped with a Z-spray ion source. Ions were formed using

nanoelectrospray emitters prepared identically as described above. Ubiquitin solutions were prepared by diluting solid protein in 20 mM ammonium acetate, pH = 7.0, to a protein concentration of 10 μM with either 0% or 1.0% *m*-NBA. The mobility cell was operated in linear ramp mode for wave height (5 – 16 V) with wave velocity = 300 m/s, and a pressure of 0.4 Torr of argon. The TOF analyzer was operated in "V" (single reflectron) mode. Mass spectra were smoothed three times using the Waters MassLynx™ software mean smoothing algorithm with a 5 unit window and arrival time distributions were smoothed three times with a 1 unit window.

RESULTS AND DISCUSSION

Supercharged ESI-TWIMS. The ESI-MS and TWIMS arrival time distributions for each charge state formed with and without *m*-NBA are shown in Figure S-1. For the 0% *m*-NBA solution, charge states ranging from 4+ to 7+ are formed. A small abundance (~3%) of 9+ and 7+ dimer is also observed. With 1% *m*-NBA, the maximum charge state increased from 7+ to 10+, and the average charge increased from 5.5+ to 7.4+. The dimer abundance is significantly lower (<0.6%) indicating that *m*-NBA decreases the dimer stability. The TWIMS reduced arrival times, estimated by multiplying the centroid of a given peak by the charge,[2] increase from 32.2 ms for the most abundant 4+ conformer, or family of unresolved conformations, to 47.2 ms for the most abundant 6+ conformer, or family of unresolved conformations. These distributions extend to longer arrival times, indicating that some partially unfolded conformers are present as well. In contrast, the data for the 7+ indicates that the majority of the ions are significantly unfolded (reduced arrival time = 62.0 ms) and that even the minor population of more compact ions (reduced arrival time = 53.4 ms) are slightly more unfolded than the major compact conformer for the 6+. The arrival time distributions for the 8+ – 10+ charge states, which are only formed from solutions containing 1.0% *m*-NBA, each consist of a single peak that shifts to slightly longer reduced arrival times with

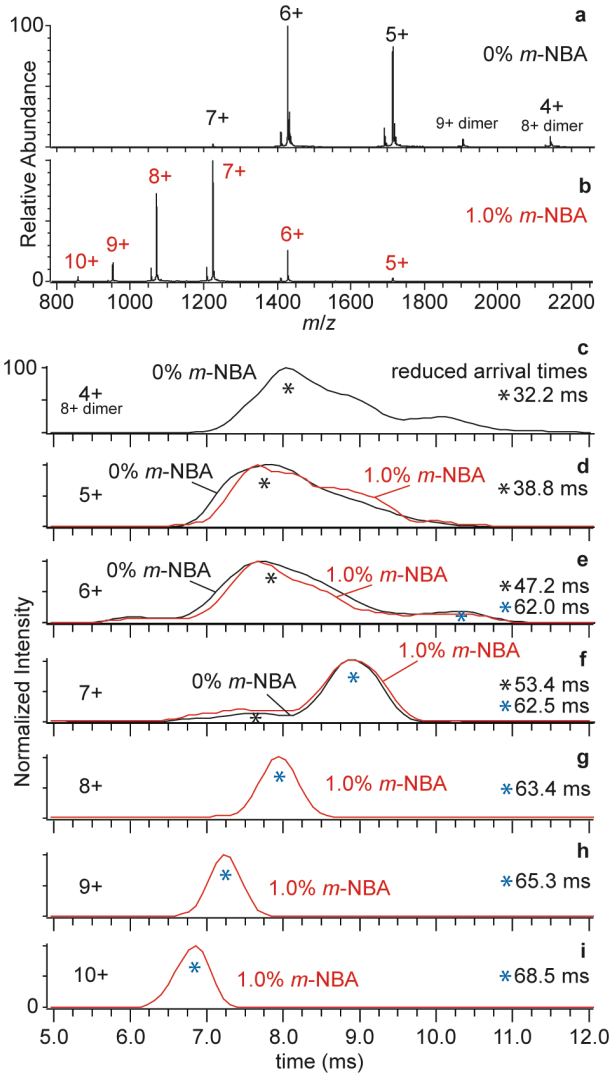

Figure S-1. Nanoelectrospray mass spectra of aqueous solutions containing 10 μM ubiquitin and 20 mM ammonium acetate with (a) 0% and (b) 1% *m*-NBA. The corresponding TWIMS arrival time distributions (c–i) are overlaid by charge state. Reduced arrival times were estimated by multiplying the charge state by the centroid of the peak, indicated with a * in the figure.

increasing charge state, consistent with a single conformer, or family of unresolved conformations, with cross sections indicating significant unfolding.

Comparison to NMR Data.

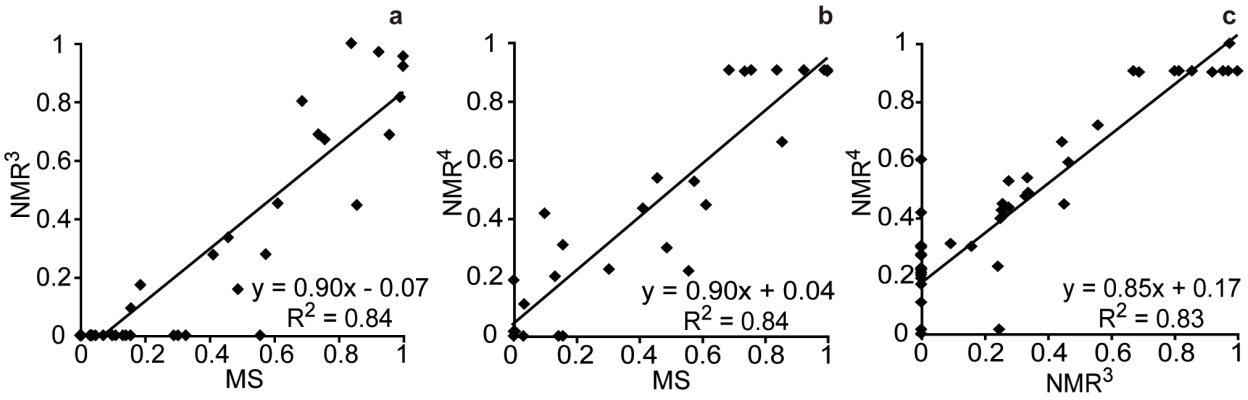

Figure S-2. "Relative Protection" values compared in plots of (a) MS versus NMR^3, (b) MS versus NMR^4, and (c) NMR^3 versus NMR^4. "Relative Protection" is calculated as the (negative) logarithm of the exchange rate constants normalized within a data set.

Table S-1. Amide HDX rate constants obtained using ESI supercharging coupled to top-down MS/MS compared to rate constants previously measured using NMR.

Residue	MS k (min^{-1})	1SD	NMR[3] k (min^{-1})	NMR[4] k (min^{-1})	Residue	MS k (min^{-1})	1SD	NMR[3] k (min^{-1})	NMR[4] k (min^{-1})
M	≥1.2	1.6E-1			D	>1.4		>1.1E-2	>1.68
Q	≥1.2	1.6E-1	>1.1E-2	>2.03	Q^{40}	2.3E-1	1.5E-1	>1.1E-2	1.31E-1
I	3.5E-3	1.0E-3	1.1E-6	<3.83E-5	Q	7.6E-2	5.5E-2	2.2E-3	5.37E-2
F	3.7E-3	2.0E-3	3.2E-6	<3.63E-5	R	1.8E-2	*	8.8E-4	1.67E-2
V	<3.5E-3		8.0E-7	<3.63E-5	L	1.8E-2	*	>1.1E-2	
K	8.3E-3‡	1.3E-3	1.3E-4	6.93E-4	I	<3.5E-3		3.5E-6	
T	7.4E-1	2.1E-1	>1.1E-2		F	1.9E-1	1.7E-1	4.0E-4	6.69E-3
L	>1.4		>1.1E-2	>1.68	A	>1.4		>1.1E-2	
T	1.2	1.2	>1.1E-2		G	2.5E-1	1.8E-1	>1.1E-2	
G^{10}	1.4	2.8	>1.1E-2	>2.03	K	>1.4		1.1E-2	5.16E-2
K	7.9E-1	4.4E-1	>1.1E-2	1.32E-2	Q	>1.4		>1.1E-2	>2.03
T	5.6E-1	1.7E-1	>1.1E-2	>2.03	L^{50}	>1.4		3.7E-4	5.75E-3
I	2.0E-2	4.0E-3	1.0E-4	1.63E-3	E	>1.4		>1.1E-2	1.39E-1
T	>1.4		>1.1E-2	>1.68	D	1.2E-2	6.6E-3	>1.1E-2	>2.03
L	9.2E-3	6.3E-3	5.1E-7	<3.63E-5	G	2.0E-1	8.8E-2	>1.1E-2	
E	>1.4		>1.1E-2		R	1.2E-1	4.4E-2	6.8E-4	1.06E-2
V	2.3E-2	1.8E-2	3.7E-6	<3.63E-5	T	3.6E-2	1.2E-2	1.2E-4	9.31E-3
E	4.5E-2	1.1E-2	6.7E-4	3.54E-3	L	1.5E-2	4.0E-3	1.3E-5	<3.63E-5
P	Proline		>1.1E-2		S	>1.4		>1.1E-2	1.59E-1
S^{20}	6.1E-1	3.8E-1	>1.1E-2	>2.03	D	5.0E-2	*	>1.1E-2	5.55E-2
D	4.5E-3	8.4E-3	1.2E-5		Y	5.0E-2	*	8.1E-5	
T	9.1E-2	6.6E-2	3.8E-4	3.08E-3	N^{60}	9.3E-1	4.2E-1	>1.1E-2	
I	7.8E-3	4.7E-3	4.1E-5	3.45E-4	I	4.5E-1	5.7E-2	8.2E-4	9.16E-3
E	>1.4		>1.1E-2		E	>1.4		>1.1E-2	7.43E-2
N	1.5E-1	8.2E-2	8.3E-4	1.19E-2	K	>1.4		>1.1E-2	>2.03
V	<3.5E-3		2.1E-6	<3.63E-5	E	>1.4		>1.1E-2	
K	1.4E-2	*	6.6E-7	1.16E-5	S	>1.4		>1.1E-2	2.04E-1
A	1.4E-2	*	8.4E-5		T	6.5E-1	1.9E-1	>1.1E-2	1.74E-1
K	1.7E-2	9.0E-3	1.1E-5	<3.83E-5	L	<3.5E-3		9.4E-4	1.23E-1
I^{30}	5.5E-3	3.5E-3	6.9E-7	<3.63E-5	H	1.0E-1	*	>1.1E-2	7.88E-2
Q	4.6E-1	2.7E-1	1.9E-3		L	1.0E-1	*	9.1E-4	>1.68
D	5.0E-2	2.6E-2	>1.1E-2	1.40E-1	V^{70}	>1.4		1.0E-4	1.44E-3
K	1.2	1.7	>1.1E-2	5.46E-1	L	1.1E-2	2.0E-3	>1.1E-2	
E	5.6E-1	3.1E-1	4.2E-3	4.80E-2	R	≥1.1	2.3E-1	>1.1E-2	
G	1.7E-1	5.7E-2	>1.1E-2	2.61E-1	L	≥1.1	2.3E-1	>1.1E-2	
I	4.0E-3	1.2E-2	7.8E-4	1.46E-2	R	1.1	1.1E-1	>1.1E-2	
P	Proline		>1.1E-2		G	≥1.4	1.3E-1	>1.1E-2	
P	Proline		>1.1E-2		G	≥1.4	1.3E-1	>1.1E-2	>2.03

Error in the rate constants are reported as one standard deviation calculated in the Igor Pro 4.07 fitting algorithm. Summed rate constants are indicated by boxes around the contributing residues. Where the kinetics are such that two rates are indistinguishable, the data was fit to a single exponential and is indicated with a * in the 1SD field. Rates from reference [3] were back-calculated from published protection factors according to the method described in [5]. The assignment of Lys6‡ as the slower rate and Thr7 the faster rate can be inferred from the rate constants of the adjacent residues, respectively.

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Note: References (3) and (4) here are references (56) and (57) in the main article.