Supplementary Information (SI) for Analyst.

Supporting Information for: Measuring the hydrodynamic radii of peptides and proteins with an unmodified LC-ESI-MS instrument operating in a Taylor dispersion regime

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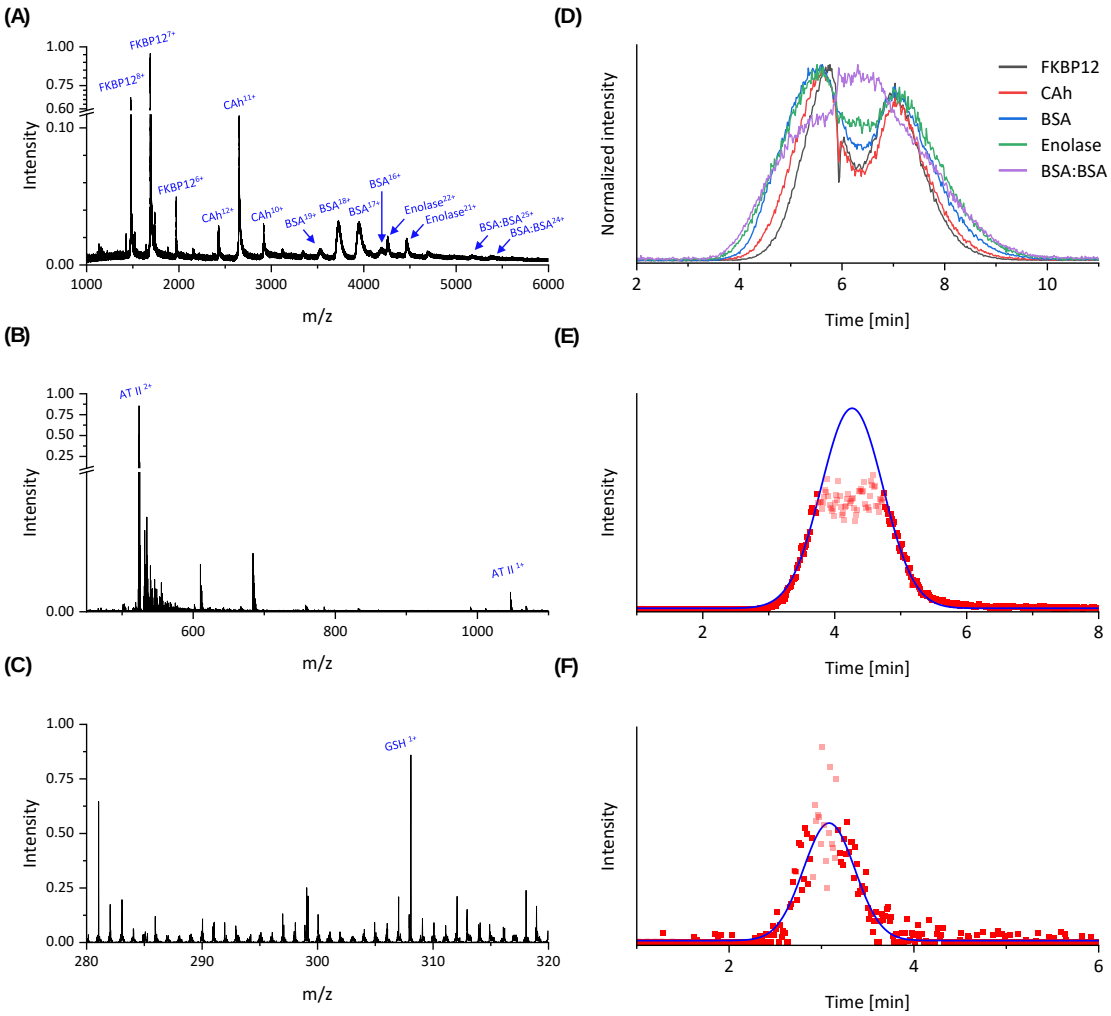

Fig. S1. (A) HYDRAULIC-MS spectrum of a mixture of FKBP12, carbonic anhydrase, BSA monomer, enolase dimer, and BSA dimer. Separate measurements were performed for **(B)** angiotensin II and **(C)** glutathione. **(D)** Overlay of summed and normalized EICs of all charge states of each protein in (A). Corresponding Gaussian fits for R_h calculation are shown in Fig. 2B in the main text. Gaussian fits of the summed EICs of all charge states of the peptides **(E)** angiotensin II and **(F)** glutathione. The flow rate was 15 µL/min. Corresponding R_h values are shown in Fig. 2C in the main text and are listed in Table S2.

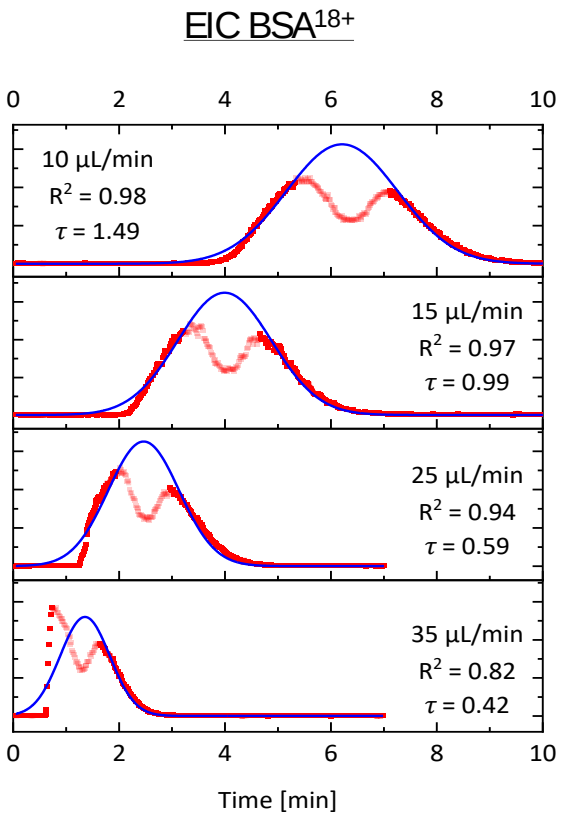

Fig. S2. EIC of BSA (18+ charge state) at flow rates of 10, 15, 25, and 35 µL/min. R^2 values of Gaussian fits at different flow rates show that higher flow rates are not suitable for R_h calculations using HYDRAULIC-MS. The τ value gives an indication of whether a molecule of a certain size at a certain flow rate is within the Taylor regime and therefore whether R_h measurements are possible with this method. Molecules are inside the Taylor regime at $\tau > 1.25$ and outside the Taylor regime at $\tau < 0.37$.[1,2]

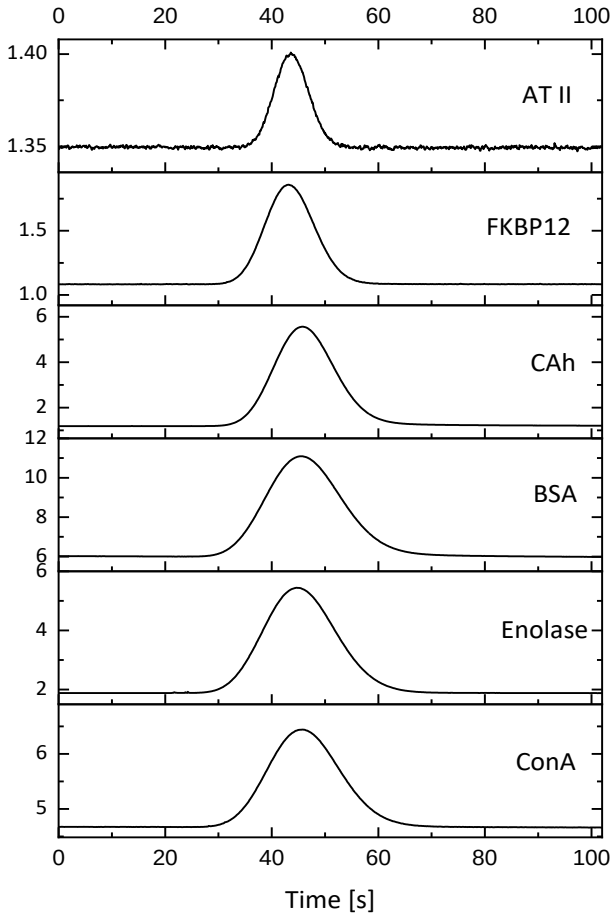

Fig. S3. Fluorescence traces from Fida 1 experiments. Vertical axis shows fluorescence intensity (RFU). R_h values were calculated using Fida Software V3.0. All values are listed in Table S2.

Temperature [°C]	Sample buffer	Running buffer	t_R [min]	η [mPa * s^{-1}]
25	H_2O	H_2O	0.655 ± 0.003	0.890
25	200 mM AmAc	10 mM AmAc	0.647 ± 0.003	0.880 ± 0.006
25	200 mM AmAc	200 mM AmAc	0.674 ± 0.002	0.916 ± 0.005
22	H_2O	H_2O	0.693 ± 0.003	0.955
22	200 mM AmAc	10 mM AmAc	0.691 ± 0.005	0.953 ± 0.008
22	200 mM AmAc	200 mM AmAc	0.718 ± 0.003	0.989 ± 0.006

Table S1. Measured elution times of angiotensin II (AT II) using FIDA fluorescence detection with different ammonium acetate (AmAc) concentrations at 25 and 22 °C. The values represent the mean with standard deviation from duplicate measurements. The retention time of AT II dissolved in H_2O together with running buffer H_2O was used as a reference to calculate the following viscosities. Viscosities were calculated using equation (6) with error propagation.

Sample	Mass [kDa]	R_h (Control) [nm]		R_h (HYDRAULIC-MS) [nm]	CCS [Å^2]
		FIDA 1	Calculated		
GSH	0.3		0.56*a	0.51	165*b
AT II	1.0	0.97		1.11	355*c
FKBP12	11.8	1.91		2.12	1429
CAh	29.2	2.62		2.57	2303
BSA	66.5	4.00	3.55*d	3.29	4104
BSA (Dimer)	133.0		4.77*d	4.56	-
Enolase	93.1	3.81		3.88	5077
ConA	25.5	3.69	2.45*e	3.38	2084
ConA (Dimer)	51.0		3.3*e	3.04	3313
ConA (Tetramer)	102.0		4.3*e	4.19	5331

Table S2. Overview of all used and measured hydrodynamic radii and CCS values of the most abundant charge state with rounded measured protein mass. References of data points marked with an asterisk – *a: calculated based on structure with PDB ID: 1PKW [3]; *b: value from reference [4]; *c value from reference [5]; *d: calculated based on structure with PDB ID: 4F5S [6]; *e values from reference [7].

Protein	Charge state	CCS [Å^2]
FKBP12	6+	1279
	7+	**1429**
	8+	1497
CAh	10+	2209
	11+	**2303**
	12+	2437
BSA	16+	4019
	17+	**4104**
Enolase	19+	5010
	20+	**5077**
ConA	**10+**	**2084**
	11+	2204
ConA (Dimer)	14+	3230
	15+	**3313**
ConA (Tetramer)	20+	5309
	21+	**5331**

Table S3. Measured CCS values of all present charge states. Bold highlighted charge state with CCS shows marks the most abundant peak, used for calibrations in the main text.

References

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