ELECTRONIC SUPPLEMENTARY INFORMATION (E.S.I.)

Liquid-Phase and Gas-Phase Investigation of Biomolecules in a Single Experiment

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ADDITIONAL RESULTS AND DISCUSSION

In order to verify the reliability of the data obtained by TDA-MS, we investigated the influence of TDA column dimensions, and the applied pressure, on the spectral pattern of myoglobin. Altering dimensions of capillary as well as the pressure applied to the inlet vial influences the flow rate of carrier fluid according to the Poiseuille equation:

$$Q = \frac{\Delta P \pi r^4}{8 \eta L} \qquad \text{(eq. 2)}$$

where r and L are the radius and the length of the capillary column, respectively, while ΔP is the pressure difference applied to the inlet vial (here: overpressure relative to the atmosphere). For example, we observed that variation of capillary diameter resulted in the changes of the MS pattern of the test protein (myoglobin). When using the capillary with ID 25 μm, the flow rate was lower than when using capillaries with greater ID (although pressure was the same, **Tab. S2**). For this reason, the retention time of the test protein in the capillary was longer. Due to the different flow rate, the protein molecules were subjected to shear stress for a longer time in the case of 25-μm capillary than in the case of capillaries with a larger ID. The protein molecules were influenced by shear stress in the capillary with ID 25 μm to a greater extent than in the capillary with ID 50 μm. This is because the retention time of the protein is longer in capillary with small ID than in capillary with large ID. For this reason, the relative abundance ratio of folded and unfolded forms of the protein was greater in the case of the thicker (50 μm) capillary than in the case of thinner capillaries (*e.g.* 25 μm). When the hydrodynamic pressure was increased to ~ 172 kPa when using the capillary with ID 25 μm, the flow rate also increased, and the ratio of folded/unfolded myoglobin was slightly raised (**Tab. S2**). While the discussion above focused on the influence of shear stress on the conformations of a protein observed in MS spectra, one should note that the flow rate of carrier liquid can affect the number of electric charges present on molecules after ionization in the gas phase. If the preference for charging folded and unfolded proteins is different, then the intensity ratios listed in **Tab. 2** may not adequately represent the real abundance ratios of these two forms in the liquid phase. Therefore, the current method cannot disentangle the influence of these factors.

EXPERIMENTAL DETAILS

Chemicals

Ammonium acetate was obtained from Riedel-de Haën (St Gallen, Switzerland). Hydrochloric acid (36.5%, v/v) was purchased from J.T. Baker (Phillipsbur, NJ, USA). Hydrofluoric acid (48%) was purchased from Union Chemical Works (Hsinchu, Taiwan). ovalbumin (chicken egg white, ~ 90%), myoglobin (horse heart, ~ 90%), sodium hydroxide, and tryptophan, were all purchased from Sigma-Aldrich (St Louis, MO, USA). Deionized water was purchased from Millipore (Taipei, Taiwan). Platinum nanoclusters were synthesized according to the published protocol (Kawasaki *et al*., *Chem. Commun*., 2010, 46, 3759).

Experimental setup

The setup shown in **Fig. 1** incorporates a home-made pressurization system which enables injection of samples, and the elution of analyte zones. Taylor dispersion analysis measurements were carried out using a UV imaging detector (ActiPix D100; Paraytec, York, UK). The outlet of the TDA capillary column (polyimide-coated fused-silica capillary; Polymicro, Phoenix, AZ, USA) was tapered and approached to the orifice of mass spectrometer (Micro-Q-TOF II Focus, Bruker Daltonics, Bremen, Germany). Analyte zones were recorded at two detection windows imaged by the active pixel sensor (ActiPix), and directed to the ion source of the mass spectrometer. When installed in the system, the capillary was initially flushed with sodium hydroxide (1 M) for 10 min at ~ 100 kPa, and rinsed with deionized water for 15 min at ~ 100 kPa. The outlet of the TDA capillary was tapered by pulling the capillary in flame, and subsequent etching in 24% hydrofluoric acid during 30 min. Deionized water was subsequently used to wash the capillary outlet. The distance between the capillary outlet and the MS inlet was 1.5 mm. The flow rate of the dry gas (N_2) was: 3.5 L min^{-1}, the voltage of ion funnel 1 was 300 V, the voltage of ion funnel 2 was 400 V, the voltage of hexapole was 400 V. The temperature of the dry gas, and the distance between the MS inlet and capillary outlet were optimized in a series of preliminary experiments before these parameters were fixed (*cf.* **Tab. S1**).

During the experiments, test samples (80 μL, 10^{-4} M) were initially loaded into glass vials (20 μL). A typical test sample was a mixture of tryptophan (10^{-5} M) and myoglobin (10^{-4}

M), at the volume ratio of 1:1. The injection was carried out by applying pressure of ~ 69 kPa to the inlet vial for 5 s. The capillary was filled with buffer before the injection. Then, the running buffer (20 mM ammonium acetate at pH 7.4) was pumped by applying pressure of ~ 100 kPa. The vial containing buffer solution was grounded by putting a grounded platinum electrode into the solution. When the samples reached the second detection window of the UV absorption detector (ActiPix), the mass spectrometer was turned on to record mass spectra of the eluting analytes. The mass spectrometer was on stand-by before the samples reached the second detection window in order to prevent the influence of electric field in front of the orifice of mass spectrometer on the migration of analytes along the capillary (*e.g.* due to formation of electroosmotic flow).

ADDITIONAL TABLES

Table S1. Typical parameters used in the proposed method.

Parameter	Value
Injection pressure	~ 69 kPa
Run pressure	~ 10-103 kPa
Capillary diameter	50 × 365 µm (ID × OD)
Injection volume	~ 60 nL
Detection wavelength	200 ± 10 nm
Dry gas temperature	180 °C
Distance between MS orifice and capillary outlet	1.5 mm
Mass range	180-3000

Table S2. Ratios of peak intensities corresponding to the folded and the unfolded forms of myoglobin obtained with different capillary ID and pressure. Capillary length: 90 cm.

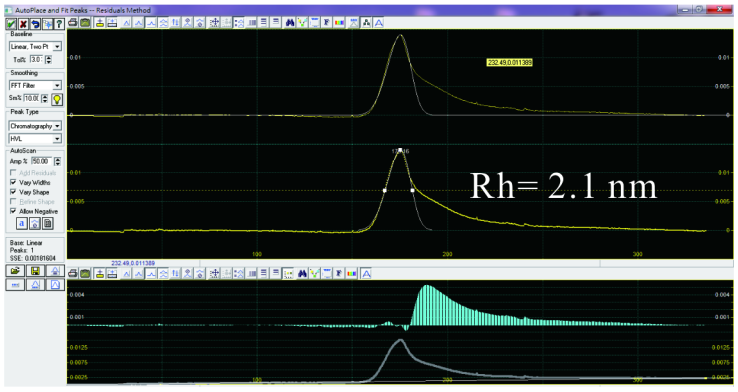
Capillary ID / μm	Pressure / kPa	Flow rate / μL min^{-1}	Velocity / cm min^{-1}	Folded/unfolded* $I_p(1952)/I_p(1884)$
25	~ 69	0.06	13.34	2.47 ± 0.43**
25	~ 172	0.16	33.60	3.46 ± 0.20**
50	~ 69	0.74	37.50	22.70 ± 8.23**

* Ratio of peak intensities (I_p) at *m/z* 1952 and *m/z* 1884, corresponding to the folded and the unfolded protein forms, respectively.

** Absolute deviation ($n = 2$).

ADDITIONAL FIGURES

A.



B.

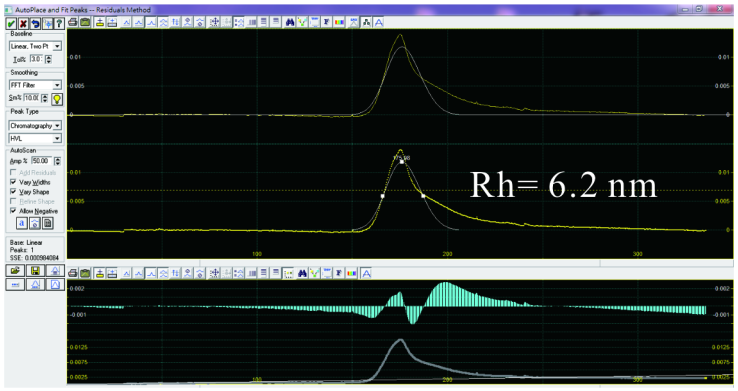


Figure S1. The influence of curve fitting to the peak of myoglobin (using PeakFit software, ver. 4.12, 1999-2003; Seasolve Software, San Jose, CA, USA) on the apparent hydrodynamic radius (R_h): (A) correct fitting with deconvolution of the main peak and the tail; (B) incorrect fitting which leads to erroneous R_h value.

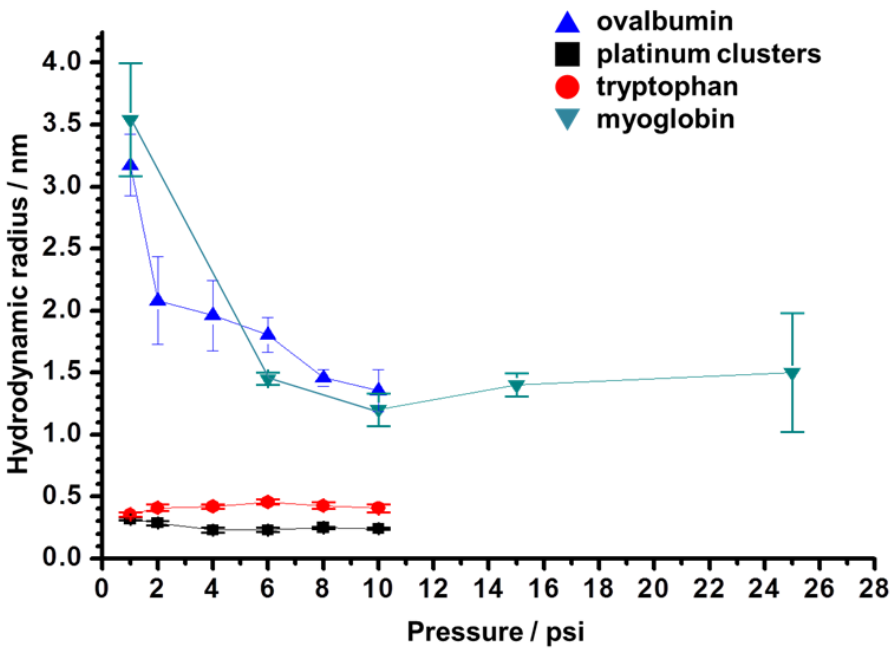


Figure S2. Influence of hydrodynamic pressure on the apparent hydrodynamic radii measured by Taylor dispersion analysis. The samples contained: platinum nanoclusters (1 mg mL^{-1}), tryptophan (10^{-4} M), ovalbumin (10^{-4} M) and myoglobin (10^{-4} M). In the case of platinum nanoclusters, tryptophan and ovalbumin, the results were obtained in triplicate. In the case of myoglobin the results were obtained in duplicate. The error bars correspond to spread and standard deviation in the case of myoglobin and other samples, respectively. Note that this experiment was carried out with a simplified version of this setup (without MS).