The ESI device was microfabricated using photolithography technique on silicon wafer. The device has a mixing moving stage.

### EXPERIMENTAL

Spraying conditions: To study the solution-phase H/D exchange of proteins and peptides, it is important to have a stable spray during the course of H/D exchange reaction. Different spraying angles (SA) between microfabricated device outlet capillary and spraying solvent outlet capillary were examined. Figure 1 shows the stability of spray for ubiquitin in water when SA is 90-degree (in the same horizontal plane).

**Figure 1** Demonstration of spray stability for ubiquitin under water/ubiquitin solution simulated conditions (one inlet of microfluidic device (see Figure 1) was used to deliver ubiquitin solution in water and the second inlet delivered pure water into the spraying chamber). 3.0% Aɑ in methanol solution was used as the spraying “partner”.

**Figure 2** The observed mass spectral patterns for [ubiquitin + 7H] after the solution-phase H/D exchange of ubiquitin in (a) 1% D₂O and (b) CH₃OD/O/H₂O acid (95:4.5:0.5) at different flow rates. A 1% D₂O in H₂O was used as the deuterating solvent. The spraying “partner” was CH₃OD/O/H₂O acid (95:4.5:0.5). Red dotted vertical lines are positioned at the same m/z value to use as a guide.

**Figure 3** Demonstration of deuteration level of ubiquitin dissolved in 10% D₂O and ubiquitin dissolved in CH₃OD/O/H₂O acid (95:4.5:0.5) at different flow rates. Relative intensity of [ubiquitin + 7H] and [ubiquitin + 8H] is shown using equation 1.

**Figure 4** Determination of deuterium levels of ubiquitin dissolved in 10% D₂O and ubiquitin dissolved in CH₃OD/O/H₂O acid (95:4.5:0.5) at different flow rates. Relative intensity of [ubiquitin + 7H] and [ubiquitin + 8H] is shown using equation 1.

**Figure 5** Determination of deuterium levels of ubiquitin dissolved in 10% D₂O and ubiquitin dissolved in CH₃OD/O/H₂O acid (95:4.5:0.5) at different flow rates. Relative intensity of [ubiquitin + 7H] and [ubiquitin + 8H] is shown using equation 1.

**Figure 6** The observed mass spectral patterns for [ubiquitin + 8H] after the solution-phase H/D exchange of ubiquitin in (a) 1% D₂O and (b) CH₃OD/O/H₂O acid (95:4.5:0.5) at different flow rates. A 1% D₂O in H₂O was used as the deuterating solvent. The spraying “partner” was CH₃OD/O/H₂O acid (95:4.5:0.5). Red dotted vertical lines are positioned at the same m/z value to use as a guide.

### CONCLUSIONS

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