

**Supporting Information** for “A new approach to characterization of the higher order structure of disulfide-containing proteins using hydrogen/deuterium exchange and top-down mass spectrometry” by Guanbo Wang and Igor A. Kaltashov

Representative examples of isotopic distributions of fragment ions that have (Suppl. Figure 1) and have not (Suppl. Figure 2) been used to calculate the deuterium occupancy at individual backbone amides of  $\beta 2m$  in top-down HDX MS measurements.

**Supplementary Figure 1:** Isotopic distributions of  $c_{24}^{4+}$  fragment ions derived from  $\beta 2m$  undergoing H/D exchange in solution as described in **Experimental Section** for  $\beta 2m^*$  (top trace) and  $\beta 2m$  representing the end-point of the H/D exchange reactions as described in **Experimental Section** for  $\beta 2m^{**}$  (bottom trace). High resolving power of FT ICR MS allows a distinction to be made between the isotopic peaks corresponding to  $c_{24}^{4+}$  (unlabeled peaks) and other fragments (labeled with circles). Once the contributions from interfering fragment ions are removed, the isotopic distribution of  $c_{24}^{4+}$  displays a shape that can be described with a binomial distribution as shown on the graph with a dotted line. The centroid of this curve is used to calculate the deuterium content of  $c_{24}^{4+}$ .

**Supplementary Figure 2:** Isotopic distributions of  $z_{44}^{5+}$  fragment ions derived from  $\beta 2m$  undergoing H/D exchange in solution as described in **Experimental Section** for  $\beta 2m^*$  (top trace) and  $\beta 2m$  representing the end-point of the H/D exchange reactions as described in **Experimental Section** for  $\beta 2m^{**}$  (bottom trace). High resolving power of FT ICR MS allows a distinction to be made between the isotopic peaks corresponding to  $z_{44}^{5+}$  (labeled with stars) and other fragments (unlabeled peaks). However, even when the contributions from interfering fragment ions are

removed, the isotopic distribution of  $z_{44}^{5+}$  remains very uneven and cannot be described with a simple binomial distribution. As a result, this fragment ion was not used to calculate deuterium distribution across  $\beta$ 2m backbone amides.



