Supporting Information

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SI Text

Sample Preparation. Protein solutions of 10–50 mg/ml concentration were made by using 10 mM maleic acid at pH 2.5. To prepare each film, a 1.5-μl drop of a protein solution was deposited onto a standard glass microscope slide and was left to dry in air at room temperature for ≈30 min until it had formed a film. A second drop of 1.5 μl of the solution was then deposited on top of the film and also left to dry. The films were further dried when put into the purged sample compartment of the experiment (<0.1% humidity).

Procedure for the Peak Intensity Measurements. The intensity on each peak was recorded over a period of 120 s, with 5 s per point. An average signal was taken from these measurements, and the deduced standard deviations ensured that the conditions corresponded to the photon-counting regime. To minimize the photon-counting nonlinearity, the absolute signal was constrained at <100 photons per second by using calibrated neutral density filters. The incident number of photons was then calculated from the detected number of photons and corrected for nonlinearity.

The "measured ratios" were calculated as follows:

$$R_{\mathrm{AA}} = \sqrt{\frac{S_{AA} - S_{Off\text{-}res}}{S_{Ref} - S_{Off\text{-}res}}} \qquad [1]$$

where S_{AA} is the average signal measured for an amino acid peak, $S_{\mathrm{Off\text{-}res}}$ is the average background signal (off resonance), and S_{Ref} is the average signal of the reference peak.

Protein Differentiation Overlap Integrals: Definition of Distinguishability. For a protein Px, and a peak corresponding to an amino acid AA*n*, the distribution of expected ratios is described as follows:

$$f_{Px}^{(AAn)}(R) = \frac{1}{SD_{AAn}} e^{-\frac{(R - Rc_{AAn})^2}{2SD_{AAn}^2}} \qquad [2]$$

where $Rc_{\mathrm{AA}n}$ is the average expected ratio for the amino acid n, deduced from the measured ratio using the linear fit and $SD_{\mathrm{AA}n}$ (the average reproducibility standard deviation for amino acid n).

The overlap integral between proteins Px and Py, taking into account n peaks, is given by:

$$I_{Px-Py}^{(nPeaks)} = \int f_{Px}^{(AA1)}(R) f_{Py}^{(AA1)}(R)dR \times \int f_{Px}^{(AA2)}(R) f_{Py}^{(AA2)}(R)dR \times \int f_{Px}^{(AA3)}(R f_{Py}^{(AA3)}(RdR \times \ldots \times \int f_{Px}^{(AAn)}(R) f_{Py}^{(AAn)}(R)dR. \qquad [3]$$

This overlap integral is normalized relative to the diagonal value: $I_{\mathrm{Px-Px}}^{(n\ \mathrm{Peaks})}$.