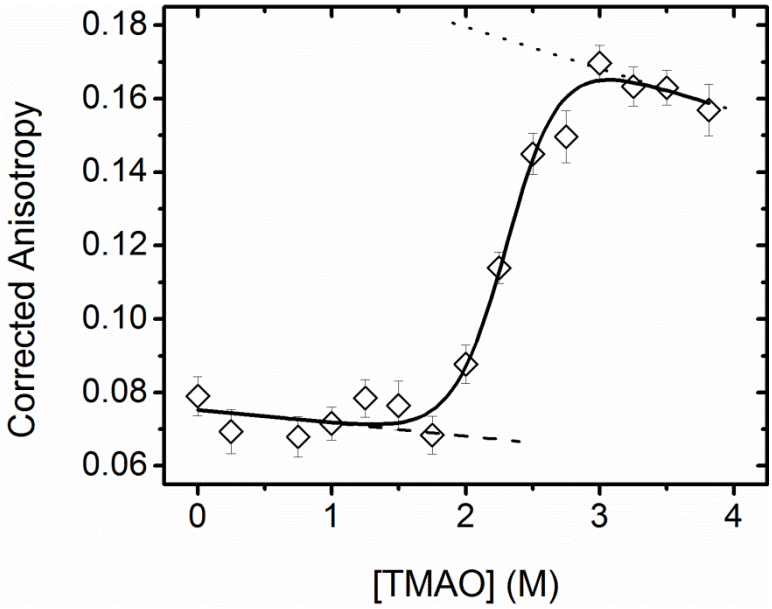
Supporting Information



Supplementary Figure 1. TMAO-induced two-state folding of α-synuclein monitored by fluorescence anisotropy. Experiments were carried out with 20 nM protein labeled with Alexa 594 at residue position 7 in $\alpha\beta\gamma$ buffer (pH 10.5 ± 0.1; 25°C). The solid curve shows non-linear least squares fit of the data to a two-state linear extrapolation model (LEM).[1] The pre- and post- transition baselines of the transition are shown as dashed and dotted lines respectively. The LEM-derived $\Delta G^{o}_{U\rightarrow T}$ and m-value parameters for the TMAO induced folding from background corrected fluorescence anisotropy data are 6.91 ± 1.60 kcal-mol^{-1} and -2.99 ± 0.72 kcal-mol^{-1}M^{-1}, respectively. These values are within experimental error limits the same as the thermodynamic parameters obtained from the single-molecule FRET and far-UV CD spectroscopy titration data presented in the main text (*i.e.,* $\Delta G^{o}_{U\rightarrow T}$: 5.95 ± 0.8 kcal-mol^{-1} and m-value: -2.82 ± 0.38 kcal-mol^{-1}M^{-1}).

Supplementary Materials and Methods

Chemicals and reagents: All experiments were carried out in αβγ buffer (0.2 M sodium chloride (NaCl), 10 mM sodium acetate (CH_3COONa), 10 mM monosodium phosphate (NaH_2PO_4), 10 mM glycine (NH_2CH_2COOH)) at pH 10.5 ± 0.1, unless otherwise stated. All chemicals used were either analytical or reagent grade, and were spectroscopically silent to ensure a high signal-to-noise ratio. Background fluorescence from TMAO was minimized by treatment with activated carbon and mixed-bed resin. Concentrations of individual TMAO stocks (in αβγ buffer) were calculated from refractive indices of respective solutions using relationships described previously.[2] Individual stock solutions were stored at -20°C and thawed prior to experiments.

Protein expression, purification and labeling: α-Synuclein was expressed and purified as described previously.[3] Single and dual Cys mutants were prepared using QuickChange site-directed mutagenesis (Stratagene, Garden Grove, CA). Labeling was carried out using previously described protocols.[4] Labeled proteins were purified by HPLC and checked by MALDI-TOF mass spectrometry. Individual protein concentrations were determined spectrophotometrically using the Edelhoch method.[3, 5]

Ensemble spectroscopy: Changes in the α-helical contents of α-synuclein were monitored as a function of SDS or TMAO by recording residue ellipticities at 222 nm using an Aviv model 62DS/202SF CD spectrometer equipped with a Peltier automated temperature control unit. All CD experiments were carried out with a wavelength bandwidth of 1 nm, a minimum averaging time of 5 s, and a temperature dead band of 0.1 °C using a 0.1 cm pathlength cuvette and 20 μM protein in αβγ buffer. Mean residue ellipticities were calculated using relationships described previously.[3] For the thermal unfolding experiments, a scan rate of 1.1°C/min was used with an averaging time of 15 s. Fluorescence anisotropy experiments were carried out with 20 nM Alexa 594-labeled α-synuclein at residue position 7 using an ISS PC1 photon-counting spectrofluorometer equipped with a Peltier automated temperature control unit. Anisotropy measurements were collected using excitation and emission wavelengths of 589 nm and 618 nm, respectively. All measurements were corrected for solvent contributions. All constant temperature ensemble experiments were carried out at 25°C.

Single-molecule FRET: Single-molecule FRET experiments were performed at room temperature using a confocal microscope setup described previously.[4] Donor and acceptor fluorescence were recorded at sub-nM concentrations of Alexa 488/594 labeled α-synuclein. The binning time for individual experiments was 500 μs. A 20 μM background of wild type unlabeled α-synuclein was used for SDS experiments. The leakage of donor emission into the acceptor channel (5%) and acceptor emission due to direct excitation (5%) were taken into account. A lower threshold of 50 and upper threshold of 200 counts were used, where threshold is the sum of signals from the donor and acceptor channels, within the binning time. FRET efficiencies (E_{FRET}) were calculated from the corrected donor (I_D) and acceptor (I_A) fluorescence intensities as

$$E_{\mathrm{FRET}} = \frac{I_{\mathrm{A}}}{I_{\mathrm{A}} + \gamma I_{\mathrm{D}}}$$

A value of unity was used for γ as supported by our previous study.[4] FRET efficiency histograms were generated and the distributions were fitted with Gaussian functions in MATLAB 7.5 (The MathWorks Inc., Natick, MA) using relationships described previously.[4, 6]

References

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