Supplementary Material

Insights into the role of protein molecule size and structure on interfacial properties using designed sequences

Mirjana Dimitrijev Dwyer\textsuperscript{1}, Lizhong He\textsuperscript{1,2,*}, Michael James\textsuperscript{3}, Andrew Nelson\textsuperscript{3}, Anton P. J. Middelberg\textsuperscript{1}

\textsuperscript{1} Centre for Biomolecular Engineering, Australian Institute for Bioengineering and Nanotechnology and School of Chemical Engineering, The University of Queensland, St Lucia, QLD 4072, Australia

\textsuperscript{2}Department of Chemical Engineering, Monash University, Clayton Victoria 3800, Australia

\textsuperscript{3} Bragg Institute, Building 87, Australian Nuclear Science and Technology Organisation, Locked Bag 2001, Kirrawee DC, NSW 2232, Australia

* Author for correspondence (Lizhong.he@monash.edu)
DAMP1 bulk structuring (circular dichroism)

DAMP1 bulk structuring was investigated by circular dichroism (CD, Jasco 810 Spectropolarimeter, Easton, MD) using a 0.025mg/mL DAMP1 in Milli-Q water to eliminate the effect of buffer salts on the CD signal.

Figure S1. Circular dichroism profile of 0.025 mg/mL DAMP1 in milli-Q water. The lack of $\alpha$-helical or other features in this spectrum confirm DAMP1 is unstructured in bulk solution, unlike DAMP4 [1], which shows highly $\alpha$-helical bulk structuring.
Calculating d-DAMP4 deuteration extent (mass spectrometry)

To characterize deuterated DAMP4 (d-DAMP4), a Waters Quattro Micro API quadrupole mass spectrometer (Waters Corporation, Milford, MA, USA) with electrospray ionization (ESI) was used in positive ion mode. Lyophilised d-DAMP4 was resuspended in milliQ, then directly injected at a flowrate of 10μL/min. Control and data collection was performed by the Waters MassLynx software.

Manual reconstruction of the d-DAMP4 mass spectrum using the formula mass = (m/z).n – n (where n is charge number, 10 – 19 in this spectrum) for all detected peaks, then taking the average, yielded the d-DAMP4 mass of 11650Da. From the mass of h-DAMP4 (11116.5 Da) it was calculated that a total 531 of a possible 776 hydrogen atoms were replaced by deuterium atoms (68.43% total deuteration) to account for this increase in mass. Of the 776 hydrogens in the DAMP4 sequence, 193 are exchangeable (ie. bound to a nitrogen, sulphur, or oxygen atom, not a carbon), therefore the extent of deuteration on a non-exchangeable basis is 531/583 = 91.08%.

Figure S2. Mass spectrum of deuterated DAMP4. From the mass of 11650 Da, the deuteration extent was calculated to be 91.1% on a non-exchangeable hydrogen basis.
Interfacial Tension Measurements

**Figure S3.** Interfacial tension versus time for 3.2 µM DAMP4.

**Figure S4.** Interfacial tension versus time plots showing that very similar profiles are obtained with DAMP1-only as with mixtures of DAMP1 and DAMP4 (from Figure 1C) when results are compared at the same total bulk mass concentration (or molar helix equivalent).

**References**