

Supplement

Factors defining effects of macromolecular crowding on protein
stability: an *in vitro/in silico* case study using cytochrome c

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Table S1

Figures S1-S4

Table S1. Corrected GuHCl concentrations as a function of different Ficoll/dextran concentrations, accounting for solvent excluded volume (described in Materials) using the crowding agent's partial specific volume (same value, 0.65 ± 0.02 ml/g, for Ficoll and the 2 dextrans) to calculate how much physical space is occupied by the polymer and not accessible to solvent and GuHCl). For 200, 300 and 400 mg/ml of Ficoll/dextran, ($1/f_{av}$) is 1.15, 1.24, and 1.35 respectively.

Dextran/Ficoll	0.5 M GuHCl	0.75 M GuHCl	1.0 M GuHCl	1.25 M GuHCl	1.5 M GuHCl	1.6 M GuHCl	1.75 M GuHCl
200 mg/ml	0.57 M	0.86 M	1.15 M	1.43 M	1.72 M	1.84 M	2.0 M
300 mg/ml	0.62 M	0.93 M	1.24 M	1.55 M	1.86 M	1.98 M	2.17 M
400 mg/ml	0.67 M	1.01 M	1.35 M	1.69 M	2.03 M	2.16 M	2.36 M

Figure S1. GuHCl-induced unfolding curves of cytochrome c in buffer (crosses) and in 300 mg/ml Ficoll70 (squares) at 20 °C, pH 7 monitored by CD changes at 220 nm. The GuHCl concentration in the case of 300 mg/ml Ficoll 70 is corrected for solvent excluded volume due to the presence of the polymer.

Figure S2. A. Trp emission (which is quenched in the folded state due to energy transfer to the heme; excitation at 285 nm) for cytochrome c as a function of increasing GuHCl concentration. Inset shows a cut through of intensity at 350 nm vs. GuHCl concentration. It is clear that the signal remains folded-like up to about 2 M GuHCl. The unfolding curve obtained by fluorescence (inset) matches the CD-detected transition shown in Figure S1, as is expected for a 2-state-like unfolding reaction. B. Visible CD of cytochrome c at 0, 200, 300, and 400 mg/ml Ficoll 70 in buffer and in 1.75 M (un-corrected concentration) GuHCl as indicated. The similarity of these spectra indicates that the environment around the heme in cytochrome c remains the same in crowding agents, as well as in 1.75 M GuHCl.

Figure S3. Difference in the probability of native heme-protein contact formation in the unfolded state of cytochrome c at $1.26 k_B T/\epsilon$ (A) between $\phi_c(\text{Ficoll 70}) = 40\%$ and bulk, (B) between $\phi_c(\text{Dumbbell70}) = 40\%$ and bulk, and (C) between $\phi_c(\text{SD70}) = 40\%$ and bulk. Contacts defined in an orange box are the ones most affected by crowding.

Figure S4. The change in the folding free energy ($\Delta\Delta G_{fu}$) by crowding at $\phi_c=40\%$ plotted as a function of the size of a crowder in angstroms. (A) The solid line is computed by Zhou's scaled particle theory (SPT) formula and the red circles are the results from computer simulations in this study. (B) The solid line is computed by Minton's SPT formula and the red circles are the results from computer simulation in this study. Free energy is in unit of $k_B T$ where k_B is the Boltzmann constant and T is temperature.

Figure S1

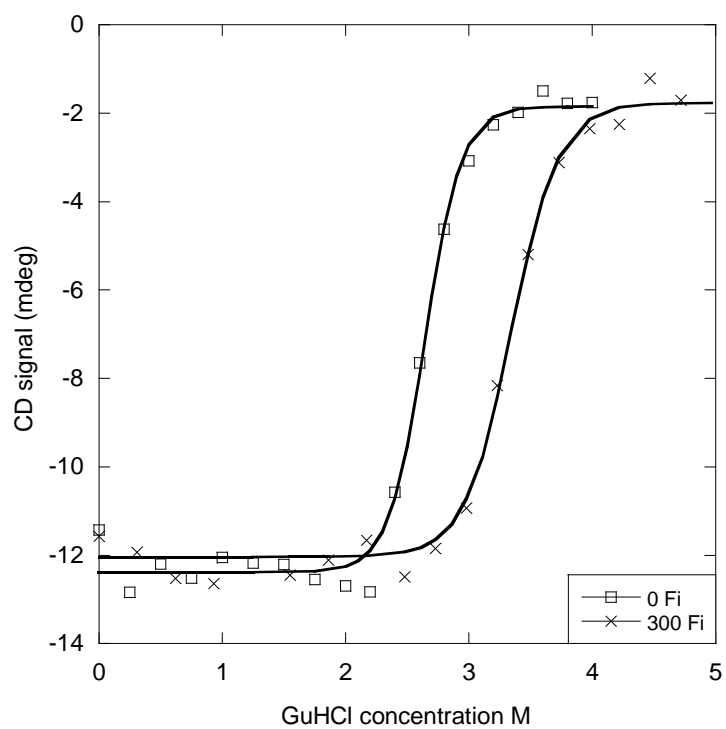
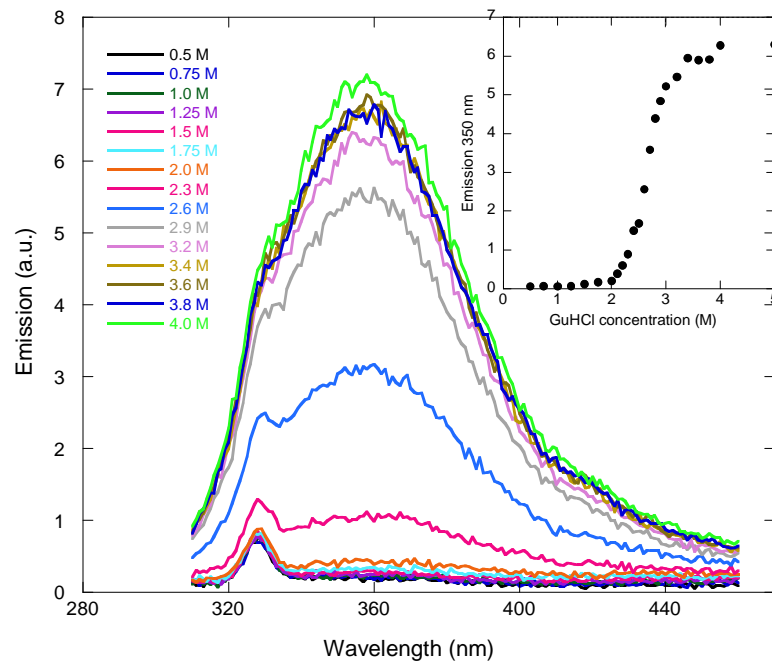


Figure S2

A.



B.

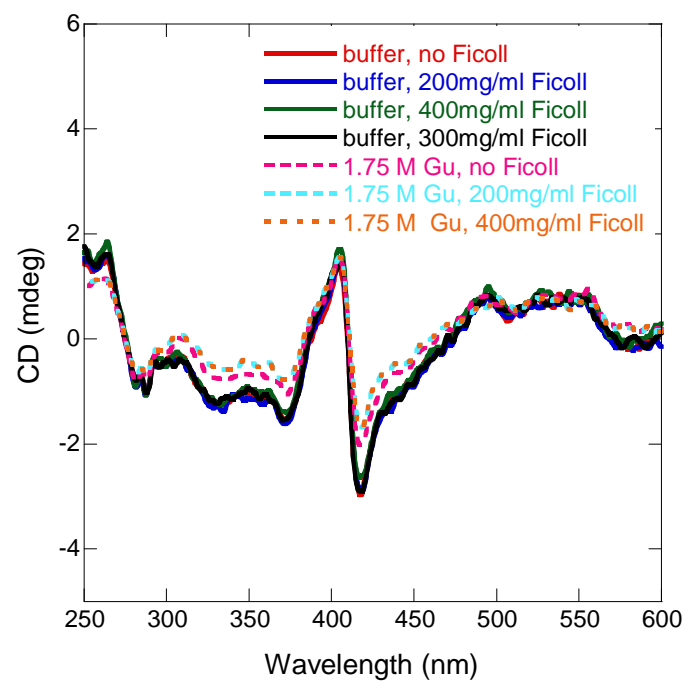


Figure S3

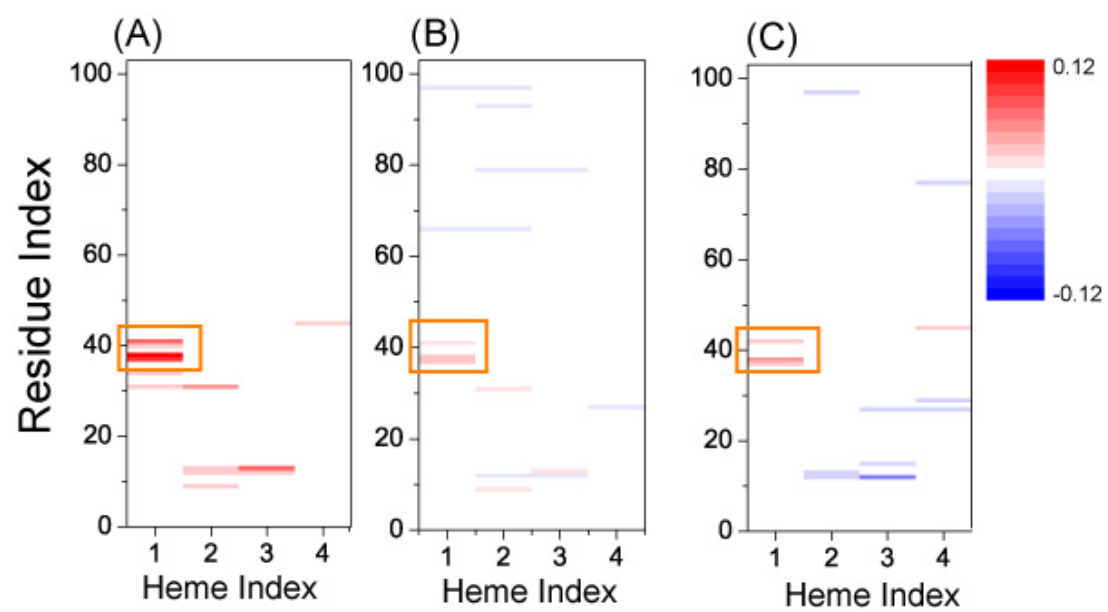


Figure S4

