Table 1
Effects of crowders on protein stability.

Protein	Crowder	Crowder concentration	Experimental conditions	Observations	References
FKBP (FK-506 binding protein)	Dextran 6, 10, 20, 40, 70, 100 and 150 kDa Ficoll 70 kDa	0–200 g l^{-1}	21.5 °C and pH 6.5	Maximum stabilization at 40 kDa (100 g l^{-1}).The difference in free energy change, $\Delta\Delta G$ (ΔG_{D40}[a] − ΔG_{buffer}[b]) = 0.59 kcal mol^{-1}, where ΔG_{D40} = 5.35 kcal mol^{-1} and ΔG_{buffer} = 4.76 kcal mol^{-1}.	[30]
ε and θ subunits of the *E. coli* DNA polymerase III holoenzyme (Pol III)	Dextran 6, 40, 70, 100 and 150 kDa Ficoll 70 kDa	100 g l^{-1}	15 °C and pH 7.6	Increase in binding constant of ε186 and θ subunits in the presence of dextran but it diminished with increasing size of dextran suggesting decrease in stabilizing effect. The binding constants in buffer and dextran 6 were 1.4 and 7.5 μM^{-1}, respectively.	[50]
Horse heart cytochrome *c*	Dextran 40 and 70 kDa Ficoll 70 kDa	0–400 g l^{-1}	20 °C and pH 7.0	Dextran 40 was more stabilizing than dextran 70 in the presence of GdmCl. $\Delta\Delta G_{ND}$ (the protein stability change due to crowding) from the simulations increased as the crowder size decreased. The changes in T_m were 5–10 °C in the case of ficoll 70 and dextran 70 and 10–20 °C for dextran 40, respectively.	[51]
HSA (human serum albumin)	Dextran 6, 40 and 70 kDa	0–175 g l^{-1}	pH 7.0	Dextran 6 had the largest value of difference in urea-induced unfolding free energy change ($\Delta\Delta G^{\circ}$ = 12 kJ mol^{-1}) followed by dextran 70 ($\Delta\Delta G^{\circ}$ = 9.6 kJ mol^{-1}) and dextran 40 ($\Delta\Delta G^{\circ}$ = 5.2 kJ mol^{-1}) at 150 g l^{-1}.	[60]
P. aeruginosa apoazurin	Dextran 20, 40 and 70 kDa	0–300 g l^{-1}	20 °C and pH 7.0	The size of the dextran did not matter for its contribution in the stability of apoazurin. The unfolding free energy changes (ΔG_D°) were 36 and 40.2 kJ mol^{-1} in buffer and in all the three crowders (at 300 g l^{-1}), respectively.	[52]
A. vinelandii apoflavodoxin	Dextran 6, 20 and 70 kDa	40% (w/v)	pH 6.0	Comparable increase in the T_m of apoflavodoxin unfolding in all the sizes of dextran. The changes in T_m (ΔT_m = $T_{m,crowder}$[c] − $T_{m,\ buffer}$[d]) were 4.2, 3.8 and 4.2 °C for dextran 6, 20 and 70, respectively.	[53]
Equine skeletal muscle Myoglobin	Dextran 40 and 70 kDa Ficoll 70 kDa	0–200 g l^{-1}	pH 7.0	Both the sizes of dextran showed slight variation than ficoll 70 with urea concentration in terms of T_m. The values of T_m decreased from 85.2 to 83.4 °C in 0 M urea and from 58.2 to 56.2 °C in 5 M urea, respectively in the case of ficoll 70. However, dextran 40 showed higher average fluorescence lifetime indicating greater Trp-heme separation than the other crowder.	[55]
c-MYC i-motif	PEG 0.2, 0.4, 1, 2, 4 and 8 kDa	20% and 40% (w/w)	pH 4.0–7.0	The T_m was dependent on pH and the molecular mass of PEG. At pH values above 4.5, the stabilization of the i-motif was increased with the increasing size of PEG. The maximum changes in T_m (ΔT_m = $T^{c}_{m,\ PEG}$ − $T_{m,\ buffer}$[d]) were 15.8 °C for PEG 2 (20% w/w) and 27.4 °C for PEG 8 (40% w/w), respectively at pH 6.0.	[54]
Recombinant bovine odorant binding protein (bOBP)	PEG 0.6, 4 and 12 kDa	0, 80, 150 and 300 g l^{-1}	pH 7.8	PEG 0.6 possessed more pronounced stabilizing effects than PEG 4 and 12 at moderate concentration, whereas at higher concentration, both PEG 0.6 and 4 stabilized more than PEG 12. The unfolding free energies (G°_{N-D}) were −7.7, −9.9, −8.3 and −9.1 kJ mol^{-1} for buffer, PEG 0.6 (300 g l^{-1}), 4 and 12 (150 g l^{-1}), respectively.	[71]

[a] ΔG_{D40} represents ΔG in the presence of dextran 40.
[b] ΔG_{buffer} represents ΔG in the absence of crowding agents.
[c] $T_{m,\ crowder}$ and $T_{m,\ PEG}$ represent T_m in the presence of any crowding agent and PEG, respectively.
[d] $T_{m,\ buffer}$ represents T_m in the absence of crowding agents.

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