

**Table 2**  
Effects of crowders on protein structure.

Protein	Crowder	Crowder concentration	Experimental conditions	Observations	References
p27ID and FosAD	Dextran 9.5, 37.5 and 77 kDa Ficoll 70 kDa	0–250 g l <sup>-1</sup>	5 °C and pH 7.0	Irrespective of the size, crowding was not sufficient to induce ordered structure in intrinsically disordered but biologically active proteins.	[56]
BSA (bovine serum albumin) and HSA (human serum albumin)	Dextran 6 and 40 kDa Ficoll 70 kDa PEG 8 kDa	0–200 g l <sup>-1</sup>	pH 7.0	Dextran 6 exhibited maximum quenching of Trp fluorescence for both the proteins under all concentration of urea as compared to dextran 40. However, dextran 6 showed a maximum increase in helicity at the highest concentration of urea for HSA, whereas dextran 40 showed for BSA.	[57]
Equine skeletal muscle Myoglobin	Dextran 6, 40 and 70 kDa PEG 8 and 35 kDa Ficoll 70 BSA 66.5 kDa Lysozyme 14.3 kDa	0–400 g l <sup>-1</sup>	pH 4.0 and 7.0	In native condition, dextran 6 and PEG 8 showed significant distortion of the heme pocket whereas dextran 40, 70 and PEG 35 gave rise to little or no change. In denaturing condition, PEG 35 showed maximum heme retention (at 200 g l <sup>-1</sup> ) followed by dextran 70, 40, and then 6 (at 400 g l <sup>-1</sup> ) and PEG 8 showed bell shaped response. Ficoll 70 showed lower stabilizing effect. BSA prevented heme loss, whereas lysozyme showed heme dissociation.	[58]
BSA (bovine serum albumin) and HSA (human serum albumin)	Dextran 6, 40 and 70 kDa PEG 0.2 and 8 kDa	0–10 g l <sup>-1</sup>	pH 7.0	Dextran 40 and 70 showed fast solvation and affected HSA more than BSA in comparison to dextran 6, PEG 0.2 and 8 which affected BSA more than HSA.	[59]
HSA (human serum albumin)	Dextran 6, 40 and 70 kDa	0–175 g l <sup>-1</sup>	pH 7.0	Dextran 70 and 40 showed larger $\Delta r$ (change in inter domain distance) in comparison to dextran 6 during chemical denaturation whereas during thermal denaturation, dextran 70 showed least variation in $\Delta r$ followed by dextran 6 and then by dextran 40. Dextran 6 had the largest change in unfolding free energy followed by dextran 70 and then 40. Dextran 70 had more change in free energy of domain separation than dextran 40.	[60]
Recombinant bovine odorant binding protein (bOBP)	PEG 0.6, 4 and 12 kDa	0, 80, 150 and 300 g l <sup>-1</sup>	pH 7.8	The secondary structure was unaffected by different sizes and concentration of PEG but the tertiary structure was markedly affected at high concentration of PEG 0.6 relative to PEG 4 and 12.	[71]
<i>D. desulfuricans</i> Apoflavodoxin	Dextran 70 kDa Ficoll 70 kDa	0–400 g l <sup>-1</sup>	20 °C and pH 7.0	Rise in the secondary structure of native protein giving rise to a more folded structure.	[83]
<i>B. burgdorferi</i> VisE	Dextran 70 kDa Ficoll 70 kDa PEG 1.4 kDa	0–400 g l <sup>-1</sup>	20 °C and pH 7.0	The secondary structural content of native protein was increased. Chemically denatured (2.5 M urea) protein collapsed to spherical structure with altered secondary structure content at high concentrations of crowder.	[84]
Bovine pancreatic RNase A	Ficoll 70 kDa PEG 0.2 and 20 kDa	30 and 35% (w/v)	25 °C and pH 3.0	Unfolded RNase A became native and more compact in the presence of ficoll 70 and PEG 20 but PEG 0.2 did not significantly drive the refolding of denatured RNase A.	[127]
<i>S. cerevisiae</i> Fet3p	Ficoll 70 kDa Dextran 20 kDa	0–35% (w/v)	20 °C and pH 5.0	No significant change in the secondary structure upon addition of ficoll 70 but slight changes at high concentrations of dextran 20.	[161]

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