LETTER

Response to Harve *et al*: Effects on protein folding speed and shape despite possible size changes in Ficoll 70

The letter by Harve *et al*. (1) highlights the importance of characterizing the synthetic agents used to mimic in vitro the crowded cell. They report light scattering data that indicate that Ficoll 70 (Fc) decreases in size above 50 mg/mL. However, Fc may change shape at higher concentrations, and, if so, the DLS radius calculation will break down. We found a linear increase in the logarithm of the protein-folding rate constant as a function of Fc from 0 to 100 mg/mL, with data points taken every 25 mg/mL (2); thus, the effect of increasing the number of Fc molecules, regardless of possible size reduction, is to facilitate faster protein folding, which is also in accord with theoretical predictions. We have specifically addressed the issue of size and shape changes of synthetic crowding agents in a separate study (3). Harve *et al*. (1) suggest that the structure of Fc changes with concentration based on far-UV CD. However, their CD data below 210 nm are characteristic of noise. Inspection of the data above 210 nm reveals little change in the CD signal as a function of Fc concentration. The major point of our study is not the kinetic effect, but the surprising finding of dramatic shape changes in an asymmetrical protein as a function of Fc and chemical/thermal perturbation. Nonetheless, the idea proposed by Harve *et al*. (1) of combining different crowding agents to represent the in vivo scenario is excellent.

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1. Harve KS, Vigneshwar R, Rajagopalan R, Raghunath M (2008) Macromolecular crowding in vitro as means of emulating cellular interiors: When less might be more. *Proc Natl Acad Sci USA* 10.1073/pnas.0810077106
2. Homouz D, Perham M, Samiotakis A, Cheung MS, Wittung-Stafshede P (2008) Crowded, cell-like environment induces shape changes in aspherical protein. *Proc Natl Acad Sci USA* 105:11754–11759.
3. Homouz D, Stagg L, Wittung-Stafshede P, Cheung M (2008) Macromolecular crowding modulates folding mechanism of α/β protein apoflavodoxin. *Biophys J*, in press.

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The author declares no conflict of interest.

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