

Do equilibrium clusters exist in concentrated lysozyme solutions?

In contrast to our previous findings (1), Shukla *et al.* (2) claim the absence of clusters in concentrated lysozyme solutions. This claim is based on scattering data that show no significant differences from our data, where conditions are similar. Our sample preparation and measurements are thus comparable and both our conclusions based on virtually identical data. Nevertheless, the existence of clusters is refuted because, first, “the interference peak . . . displays a clear trend toward higher q -values with increasing protein concentration” and, second, “is adequately and consistently described by the form and structure factors of individual lysozyme particles using an interaction potential involving short-range attraction and long-range repulsion” (conclusions in ref. 2). However, we both observe the usual shift in peak position at low concentrations, where the well separated molecules experience the long-range repulsion, and, crucially, we both also find an essentially concentration-independent position for concentrated solutions (1, 2) where the closer molecules overcome the repulsive barrier and the short-range attraction induces clusters. [Note that they do not compare data

from concentrated solutions in their article (2) but only in supporting information (SI) Fig. S9.] Furthermore, fits of form and structure factors result in values for the pair potential but cannot provide direct information on the presence of monomers and/or clusters. Nonetheless, for a potential with similar values as determined by Shukla *et al.* (2), MD simulations have confirmed the presence of clusters (3). Therefore, their data (2) actually confirm our earlier data and interpretation (1) that the peak position is concentration-independent and clusters are present in lysozyme solutions, which, as stated in both of our titles, are concentrated.

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2. Shukla A, *et al.* (2008) Absence of equilibrium cluster phase in concentrated lysozyme solutions. *Proc Natl Acad Sci USA* 105:5075–5080.
3. Cardinaux F, Stradner A, Schurtenberger P, Sciortino F, Zaccarelli E (2007) Modeling equilibrium clusters in lysozyme solutions. *Europhys Lett* 77:48004.

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The authors declare no conflict of interest.

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