Comment on “Effective Long-Range Attraction between Protein Molecules in Solution Studied by Small Angle Neutron Scattering”

Liu et al. recently postulated the existence of a universal weak long-range attraction for proteins in solution [1]. This novel interaction was based on the observation of a so-called “zero-\(Q\) peak” in small-angle neutron scattering (SANS) experiments. They explicitly addressed earlier studies with lysozyme where the existence of equilibrium clusters had been demonstrated [2]. They also found a zero-\(Q\) peak for lysozyme, and their major conclusion was that this was overlooked by previous experiments [2]. Here we now reinvestigate lysozyme solutions under equilibrium cluster conditions. We show that a zero-\(Q\) peak is not an omnipresent feature of lysozyme solutions attributed to a universal long-range attraction but rather an artifact related to sample purity and solvent quality. The preparation procedures and solvent conditions [20 mM N-(2-hydroxyethyl)piperazine-N’-[(2-ethanesulfonic acid) (HEPES) buffer, \(pH = 7.8\)] are described in Ref. [2]. We use the same lysozyme as Liu et al. (Fluka, L7651) and present results from two different batches (“batch I” and “batch II”). Combined SANS and static light scattering (SLS) data of 250 mg/mL lysozyme in a \(D_2O\) buffer from batch I are shown in Fig. 1(a). The measurements were performed several days after sample preparation. A plot of the effective structure factor \(S(q)\) clearly demonstrates that there is no rising \(I(q)\) down to \(q = 0.02\) nm\(^{-1}\), i.e., a factor of 2 smaller than the lowest \(q\) reached by Liu et al. [1]. This demonstrates that a zero-\(Q\) peak is completely absent under these conditions—provided one has a high quality lysozyme sample as demonstrated below.

However, it is important to point out that for batch II we have also found conditions where \(I(q)\) is rising at low \(q\) [Fig. 1(c)]. The initial SANS and SLS measurements of samples from batch II in \(H_2O\) were performed immediately after preparation. \(I(q)\) lacks any sign of a zero-\(Q\) peak, a finding also supported by \(S(q)\) from SLS/SANS [Fig. 1(b) and inset]. However, the situation changes dramatically if we use \(D_2O\). It is known that the solubility of lysozyme in \(D_2O\) is lower than in water [3], and this reduced solvent quality has obvious consequences for the stability of lysozyme samples [Fig. 1(c)]. Although a fresh sample shows an almost flat \(I(q)\) at low \(q\), we observe a pronounced dependence on sample age. Already one day after preparation, \(I(q)\) increases considerably at low \(q\), which becomes more pronounced with time. It is worth pointing out that the measurements described in Ref. [1] were obtained after an equilibration time of a few days. This slow time-dependent process and the fact that it occurs only in samples obtained with batch II and in \(D_2O\) indicates clearly that the increasing low-\(q\) intensity is not an equilibrium feature caused by an additional long-range electrostatic attraction but related to sample purity and solvent quality.

With these results on lysozyme, we thus provide convincing evidence that the rising \(I(q)\) at low \(q\) is not due to “the existence of weak long-range attractions” which “is universal for all protein solutions” and “was overlooked in previous experiments [2]” as claimed in Ref. [1].

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