Published in "Physical Review Letters 96: 219801, 2006"
which should be cited to refer to this work.

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.

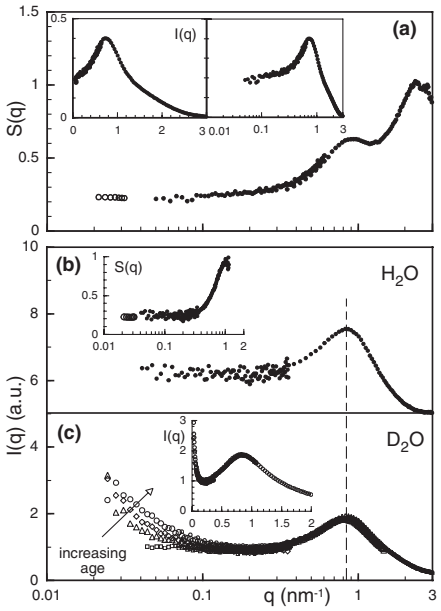

FIG. 1. SANS and SLS data on lysozyme in a 20 mM HEPES buffer. (a) $S(q)$ of a several days old 250 mg/mL sample from batch I in D_2O at 20 °C. SANS (●), SLS (○). Inset: SANS $I(q)$ in lin-lin and lin-log presentation to facilitate comparison with Fig. 4 in Ref. [1]. (b) $I(q)$ from a fresh 200 mg/mL sample from batch II at 25 °C in H_2O. Inset: $S(q)$ together with SLS data (open symbols). (c) $I(q)$ of a sample in D_2O at different times after preparation: fresh (□), one day (△), two days (◇), and five days (○) old. Inset: lin-lin plot of $I(q)$ five days after preparation. The cluster peak remains constant irrespective of batch, age, or solvent (dashed line).

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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Received 15 December 2005; published 1 June 2006
DOI: 10.1103/PhysRevLett.96.219801
PACS numbers: 87.14.Ee, 61.12.Ex

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