Comment on "Combining Diffusion NMR and Small-Angle Neutron Scattering Enables Precise Measurements of Polymer Chain Compression in a Crowded Environment"

In a recent Letter, Palit *et al.* [1] evaluated, by NMR and small-angle neutron scattering (SANS), the magnitude of a polymer chain compression due to macromolecular crowding. They intended to show that our previous measurements by SANS [2,3] strongly overestimate this compression. The radius of gyration (R_g) of the chain shows a significant reduction of roughly 50% after the addition of $\Phi = 40\%$ of Ficoll, a highly ramified polysaccharid used as crowder. This level of compression is slightly higher than predicted by theory [4] and simulations [5], but much more than that observed experimentally on disordered proteins [6] and predicted by scaling arguments and simulations [7]. Palit *et al.* [1] intended to show that (1) polyethylene glycol (PEG) solutions are in the crossover regime between the dilute and the semidilute states (above $c^\star$), and (2) as R_g does not change in the dilute regime, its value measured by SANS should correspond to the value at $c^\star$. They conclude that the linear extrapolation to zero PEG concentration used to evaluate the R_g of one chain is not valid and leads to an overestimation of the compression.

The purpose of this Comment is to show that (1) is based on a new definition of the $c^\star$ leading to values an order of magnitude smaller than when using the standard definition, and point (2), although the R_g should not vary below $c^\star$, SANS measurements give concentration-dependent apparent R_g. The real R_g should be extrapolated to $c = 0$.

(1) We agree that for $\Phi = 0$, $c^\star$ is not sharply defined (see, e.g., Ref. [8]). $c^\star(\Phi)$ is even poorly defined. However, as it is clearly noticed by the authors in the Supplemental Material, "It is not clear, *a priori*, if the concentration above which one observed the onset of hydrodynamic coupling has anything to do with the thermodynamic overlap concentration." At $\Phi = 0$, the usual definition of $c^\star$ is $c^\star \simeq 3M/4\pi Rg^3$. Figure 1 shows the overlap concentration computed from R_g of PEG from the literature, our results, and those of Palit *et al.* (open circles). Their values of $c^\star$ are an order of magnitude below all others. We think that a new definition of the commonly used $c^\star$ by Palit *et al.* should be better justified.

(2) The authors state, "Since the self-diffusion coefficient is unchanging in the polymer-dilute regime, R_g must therefore also be constant." We agree that R_g should be little dependent on the concentration in the dilute regime but SANS measures correlation. Scattered intensity $I(Q)$ includes two terms: an intramolecular term $F(Q)$ describing the correlation between scattering centers belonging to the same chain, and an intermolecular term (correlation of two scattering centers belonging to two distinct molecules). If the chain R_g does not change with concentration in the dilute regime, $F(Q)$ remains constant but the distinct terms do not [9,10]. If $I(Q)$ is refined by a single chain form factor as done by us [2,3] and Palit *et al.* [1], it results in an apparent radius of gyration $R_g(c_p)$ that is concentration dependent. The real R_g is deduced from extrapolation to zero concentration.

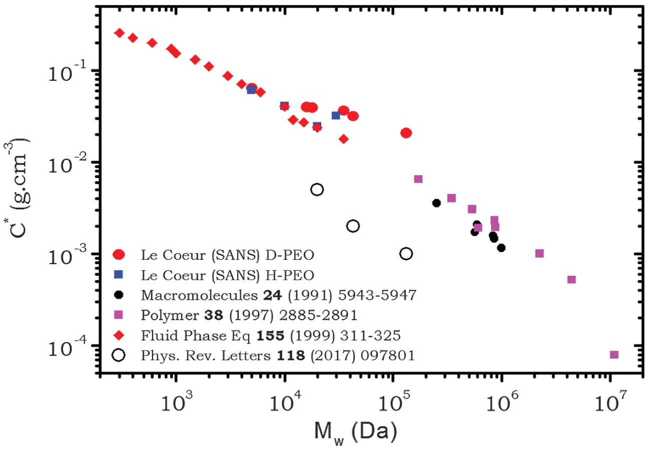

FIG. 1. $c^\star(\phi = 0)$ computed from the results found in the literature (see legend). The results obtained by Palit *et al.* [1] are plotted by black open circles.

To conclude, we show that in their Letter, Palit *et al.* have (1) used a new definition of $c^\star$ that leads to concentrations that are an order of magnitude lower than usually defined in textbooks, and (2) their claim that the radius of gyration measured by SANS is independent of concentration is not correct. Finally, we would like to point out that hydrodynamic measurements are probably not the most appropriate to study coil sizes under a crowded environment. Crowder could indeed induce a screening of the hydrodynamic interactions and the transition of the dynamics from Zimm like, with D_s related to hydrodynamic radius ($D_s \sim 1/R_h$) to a Rouse-like regime where D_s depends on coil mass only ($D_s \sim 1/M$).

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