SUPPORTING INFORMATION

Concentrated solutions of single-chain nanoparticles: A simple model for intrinsically disordered proteins under crowding conditions

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I. MODEL AND SIMULATION DETAILS

We used a simple bead-spring model for the polymer precursors, based on the well-known Kremer-Grest model [1]. Precursors were constructed as linear chains of $N = 200$ monomers. A 40 % of them were selected as functional reactive monomers. These were randomly distributed along the chain contour, with the only constraint that consecutive functional monomers were not permitted (preventing trivial cross-linking events). The non-bonded interactions between the monomers were modelled by a purely repulsive Lennard-Jones (LJ) potential:

$$V_{\rm LJ}(r) = 4\epsilon[(\sigma/r)^{12} - (\sigma/r)^6 + 1/4], \tag{1}$$

which was cut-off at $r_{\rm c} = 2^{1/6}\sigma$. We set $\epsilon = \sigma = 1$ as units of energy and distance respectively. Interactions between mutually bonded monomers were modelled by a FENE potential [1]:

$$V_{\rm FENE}(r) = -\epsilon K_{\rm F} R_0^2 \ln\left[1 - (r/R_0\sigma)^2\right], \tag{2}$$

with $K_{\rm F} = 15$ and $R_0 = 1.5$. The use of the LJ and FENE potentials prevents strong fluctuations of the bonds and guarantees chain uncrossability [1]. After equilibration of the precursor conformations, intramolecular cross-linking was activated. Two functional monomers formed a bond when their mutual distance was smaller than $r_{\rm b} < 1.3\sigma$ and they remained bonded (through the FENE potential) for the rest of the simulation, i.e., cross-linking was irreversible. Each functional monomer participated in just one cross-linking event, i.e., two functional monomers at mutual distance $r < r_{\rm b}$ did not form a bond if at least one of them was already bonded to another functional monomer. The precursors were coupled to the same thermal bath but did not interact with each other, i.e, cross-linking was purely intramolecular by construction. With this ingredient and the used purely repulsive non-bonded interactions, the simulated model provides a generic description of SCNP synthesis at high dilution and good solvent conditions. Further details of the model and cross-linking procedure can be found in Ref. [2].

Cross-linking was completed in all cases, i.e., all functional groups formed a bond with another one. A total of 200 SCNPs were constructed by the former procedure, and they were used for the simulations of the solutions, where the intermolecular interactions were switched on. Thus, all the SCNPs (with a total of $N_{\rm mon} = 40000$ monomers) were initially inserted in a cubic box of size L, with periodic boundary conditions and monomer density

$\rho = N_{\rm mon}/L^3 = 0.002$. This density is, both for the SCNPs and the linear precursors, orders of magnitude below the overlap concentration (see manuscript) and is considered as 'infinite dilution' $\rho \to 0$. The minimum distance between any two SCNPs in the initial box was chosen to prevent contact and eventual concatenation of rings of different SCNPs. Once the box at $\rho \to 0$ was constructed and equilibrated, it was very slowly compressed (in order to prevent concatenation) and equilibrated at different selected densities. Equilibration runs extended over sufficiently long times so that each macromolecule diffused a distance of several times its diameter, to achieve full decorrelation of its initial conformation. The equilibrated boxes were used for acquisition runs to characterize the conformational properties of the SCNPs. The selected densities covered the whole range from infinite dilution to $\rho = 0.85$, which qualitatively corresponds to melt density [1]. For comparison we also performed simulations of solutions of the linear precursors in the same concentration range. Moreover we simulated a mixture of SCNPs diluted in a linear matrix at $\rho = 0.85$ for direct comparison with the SANS experiments. All the simulations (including the cross-linking runs) were performed under Langevin dynamics (see details in Ref. [2]) at temperature $T = 1$. Equilibration and acquisition runs typically extended over 10^8 time steps. A home-made code was used for the cross-linking simulations. The simulations of the solutions were performed by using the GROMACS 4.6.5 package [3].

II. EXPERIMENTAL DETAILS

The SCNPs were obtained through Michael addition-mediated multidirectional self-assembly of individual polymeric chains (precursors) at room temperature in tetrahydrofuran, by following the procedure reported in Ref. [4]. As precursors, linear copolymers of methyl methacrylate (MMA) and (2-acetoacetoxy)ethyl methacrylate (AEMA) P($MMA_{0.63}$-co-$AEMA_{0.37}$) were used. Ethylene glycol diacrylate (Sigma-Aldrich) acted as intrachain cross-linking agent. The molecular weight of the SCNPs was 91.7 kg/mol with $M_w/M_n = 1.07$ as determined by gel permeation chromatography.

Deuterated PEO (Polymer Source, Inc.) had $M_w = 96$ kg/mol with $M_w/M_n = 1.08$. The proper amounts of SCNPs (0.0168 g) and dPEO (0.4032 g) were dissolved in chloroform. Once the mixture was completely dissolved, it was precipitated in diethyl ether. The precipitated product was gradually heated up to 80°C under vacuum to completely remove

possibly trapped solvent molecules. For the solutions, dDMF (Sigma-Aldrich) was used as solvent.

The SANS experiments on the solutions were conducted at room temperature at the KWS-2 instrument at the Forschungs-Neutronenquelle Heinz Maier-Leibnitz in Garching (Germany). With an incident wavelength $\lambda = 5.27$ Å, three sample-detector distances (SDD) were used: 1.15, 5.76 and 19.76 m. Experiments on the nano-composite with dPEO were performed at 100°C, i. e., in the molten state of dPEO, at the SANS-1 instrument at SINQ, Paul Scherrer Institute, Villigen (Switzerland). In this case, measurements with $\lambda = 12$ Å (SDD = 18 m) and 6 Å (SDD = 8 and 2 m) were combined. All samples were filling Hellma Quarz cells of 2 mm thickness.

III. REPRESENTATIVE FORM FACTORS

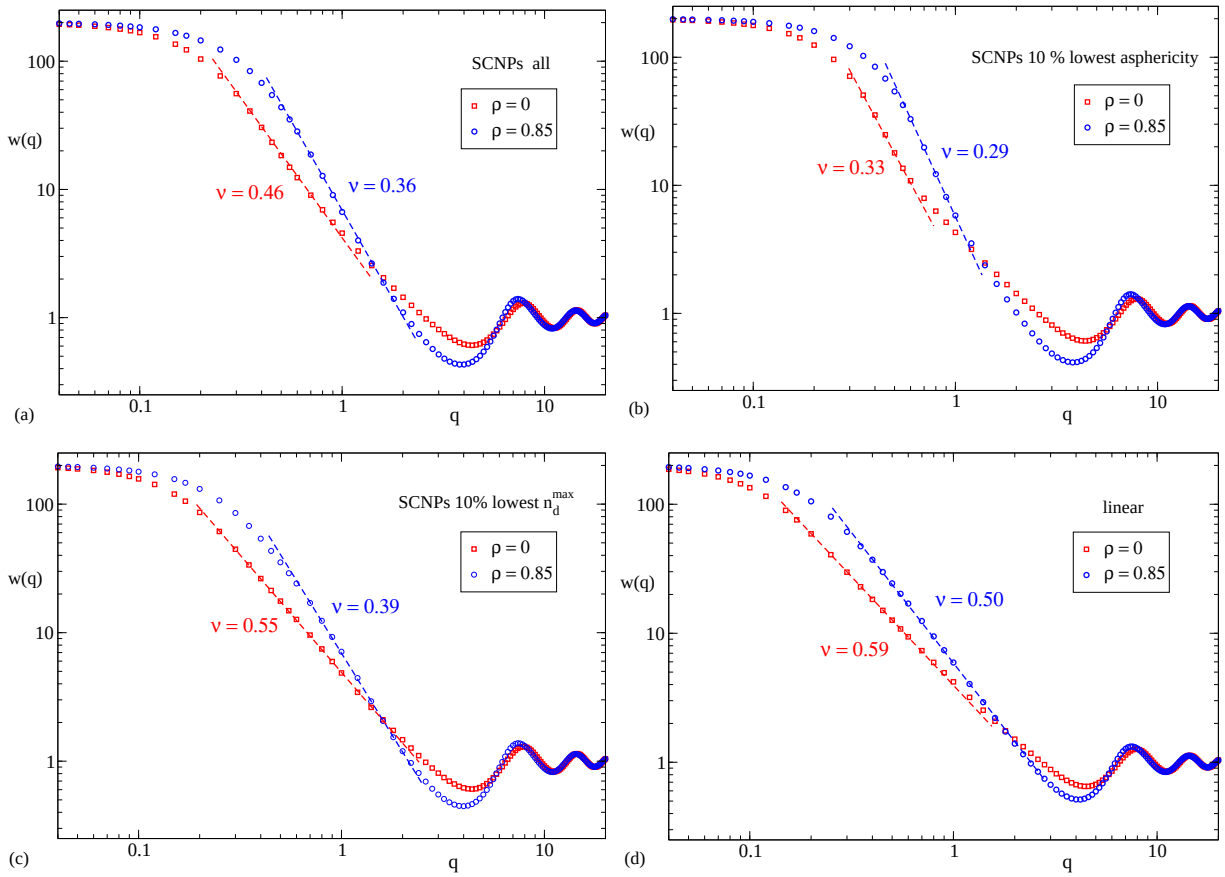
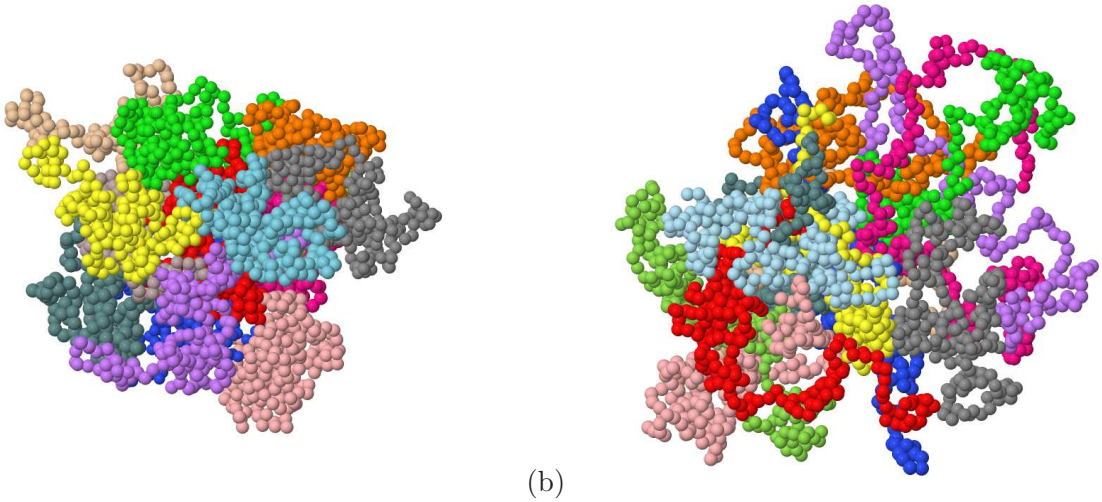


Fig. S1. Form factors at density $\rho = 0$ (red squares) and $\rho = 0.85$ (blue circles). Dashed lines are fits to power-laws $w(q) \sim q^{-1/\nu}$ in the regime $\langle R_g^2 \rangle^{-1/2} \lesssim q \lesssim b^{-1}$ ($0.2 \lesssim q \lesssim 1$ in all cases). The ν-exponents are indicated. Different panels show results averaged for all the SCNPs (a), and for the 10 % of SCNPs with the lowest asphericity (most ordered SCNPs, (b)) and the lowest n_d^{max} (most disordered SCNPs, (c)). For comparison, we include data for the linear precursors (d).

IV. SNAPSHOTS OF CONCENTRATED SOLUTIONS



(a) (b)

Fig. S2. Representative snapshots of a concentrated solution of SCNPs (a) and linear chains (b), both at monomer density $\rho = 0.3$. In both panels we represent a selected macromolecule and its 12 nearest neighbors (in terms of the distance between the macromolecular centers-of-mass). All beads in a same macromolecule are depicted in the same color. Different macromolecules are depicted in different colors.

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