

# Supplemental Material: Combining diffusion NMR and SANS enables precise measurements of polymer chain compression in a crowded environment

Swomitra Palit<sup>1</sup>, Lilin He<sup>2</sup>, William A. Hamilton<sup>3</sup>, Arun Yethiraj<sup>4</sup> and Anand Yethiraj<sup>1</sup>

<sup>1</sup>*Department of Physics & Physical Oceanography,*

*Memorial University, St. John's, NL, Canada A1B3X7*

<sup>2</sup>*Biology and Soft Matter Division, Neutron Sciences Directorate,*

*Oak Ridge National Laboratory, Oak Ridge, TN 37831, USA*

<sup>3</sup>*Instrument and Source Division, Neutron Sciences Directorate,*

*Oak Ridge National Laboratory, Oak Ridge, TN 37831, USA and*

<sup>4</sup>*Department of Chemistry, University of Wisconsin, Madison, Wisconsin 53706, USA*

## MATERIALS

For PFG NMR studies, we used three different molecular weights of polyethylene glycol (PEG,  $M_w = 22,000$  with  $M_w/M_n = 1.10$ ,  $M_w = 42,800$  with  $M_w/M_n = 1.18$ , and  $M_w = 132,000$  with  $M_w/M_n = 1.20$ ), purchased from Polymer Source Inc. In SANS experiments, for contrast reasons, we used deuterated PEG ( $M_w = 20,000$  with  $M_w/M_n = 1.15$ ). Deuterated PEG was also obtained from Polymer Source Inc. Ficoll®PM 70 (referred to as Ficoll70 in the text) with average molecular weight of 70,000 ( $R_c = 4.6$  nm) was purchased from Sigma Aldrich. Deuterium Oxide ( $D_2O$ , 99.9%) was purchased from Cambridge Isotope Laboratories, Inc.

## METHOD: PFG NMR

For sample preparation, the desired volume fraction of Ficoll70 was dissolved in deionized  $H_2O$ . The solution was stirred for 10 hours. For each polymer concentration the appropriate mass of (undeuterated) polyethylene glycol ( $M_w = 22,000$  with  $M_w/M_n = 1.10$ ,  $M_w = 42,800$  with  $M_w/M_n = 1.18$ , and  $M_w = 132,000$  with  $M_w/M_n = 1.20$ ) was added to 1 cm<sup>3</sup> of this solution. Each time, the solution was stirred five hours before experiment. Samples were then transferred to 5 mm outer diameter NMR tubes.

PFG NMR measurements were carried out on a Bruker Avance II 600 spectrometer equipped with a Bruker 14.08 T magnet and a Bruker diffusion Diff30 probe with a maximum Z gradient strength of 1800 G/cm (18 T/m). A stimulated echo pulse sequence was used to measure the diffusion coefficient. The gradient steps were varied and the signal for  $H_2O$ , PEG and Ficoll70 were collected as a function of gradient. The procedure for analysis of the results is described elsewhere in detail [1]. To avoid probe heating and to control sample temperature, the probe was cooled by flowing water and the temperature was maintained at

25°C.

## METHOD: SANS

Solution preparation was identical to that for PFG NMR, with the only difference that we used deuterated PEG ( $M_w = 20,000$  with  $M_w/M_n = 1.15$ , from Polymer Source Inc.), and the solutions were made in 60%:40%  $H_2O:D_2O$ . In order to check for consistency between NMR and SANS, one set of PFG NMR measurements were carried out in 60%  $H_2O$ /40%  $D_2O$  solutions.

For sample preparation, the desired volume fraction of Ficoll70 was dissolved in a solution of  $H_2O$  and  $D_2O$  with 40% in  $D_2O$ . The solution was stirred for 10 hours. An appropriate mass of deuterated polyethylene glycol was added to 1 cm<sup>3</sup> of this solution. Each time, the solution was stirred five hours before experiment.

SANS measurements were conducted at Oak Ridge National Laboratory (ORNL) on the GP-SANS instrument [2]. Two sample to detector distances were used (1.7 m and 18.5 m) for a range of scattering vectors from 0.001 Å<sup>-1</sup> to 0.5 Å<sup>-1</sup>. The  $H_2O/D_2O$  composition points of minimum scattering intensity for Ficoll70 were determined using contrast variation Ficoll70 samples in solutions containing various  $H_2O/D_2O$  ratios. The ratio at which the scattering length densities of Ficoll70 and  $H_2O/D_2O$  were matched and therefore Ficoll70 did not contribute to the scattering signal was determined as  $60 \pm 1\%$   $H_2O$  and  $40 \pm 1\%$   $D_2O$ . Samples were loaded into quartz banjo cells mounted in temperature-controlled brass sample holders and a constant temperature of 25°C were maintained for all experiments. Scattering intensity profiles were analyzed using Igor Pro macros developed at NIST [3].

SANS data are presented as plots of the intensity of the scattered neutron beam,  $I(q)$  as a function of scattering vector  $q = (4\pi \sin \vartheta / \lambda)$ , where  $\vartheta$  is one half of the scattering angle and  $\lambda$  is the neutron wavelength. The intensity can be written as  $I(q) = I_0 P(q)$ , where  $P(q)$

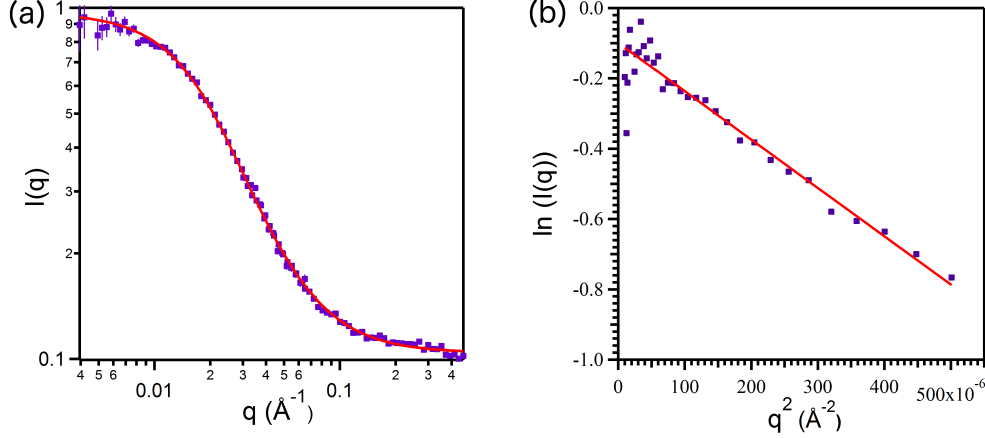


Figure S1: SANS scattering intensity  $I(q)$  vs  $q$  for a PEG/Ficoll70 mixture with a  $c_p = 0.05 \text{ g/cm}^3$  and  $\Phi_F = 0.3$ . (a) Radius of gyration,  $R_g$ , of PEG, obtained from a fit to the Debye model is  $6.9 \pm 0.96 \text{ nm}$ . (b) Guinier plot shows linearity of  $\ln(I(q))$  as a function of  $q^2$  for  $qR \leq 0.89$ , yielding  $R_g$  equals to  $6.8 \pm 1.9 \text{ nm}$ .

is the form factor which provides information on the size and shape of the scatterers. For a Gaussian polymer radius of gyration  $R_g$ , the shape factor is determined by the Debye formula,  $P(q) = \frac{2}{x^2} (e^{-x} - 1 + x)$ , where  $x = q^2 R_g^2$  and the radius of gyration of the scattering object,  $R_g$ , can be extracted from fitting the plot of  $I(q)$  vs  $q$  to the Debye model: this is shown in Figure S1(a) for a PEG/Ficoll70 mixture with a  $c_p = 0.05 \text{ g/cm}^3$  and  $\Phi_F = 0.3$ .

In the limit of very low angle or small  $q$  (the Guinier approximation) one can further write  $P(q) = \exp\left(\frac{-q^2 R_g^2}{3}\right)$ , where the radius of gyration of the scattering object,  $R_g$ , can be extracted from the slope of a plot of  $\ln(I(q))$  vs  $q^2$ . Such a fit is shown for a PEG/Ficoll70 mixture with a  $c_p = 0.05 \text{ g/cm}^3$  and  $\Phi_F = 0.3$  in Figure S1(b).

### SCALING FORM FOR THE OSMOTIC PRESSURE

We present a short discussion of polymer scaling following Cohen et al [4], which shows that the osmotic pressure of neutral flexible polymer in solution may be written down as a phenomenological sum of scalings as a function of the polymer concentration, enabling perfect collapse of two very dense datasets of rather dissimilar polymers (PEG in water and poly- $\alpha$ -methylstyrene in toluene). The concentration is scaled with respect to a crossover concentration  $c^\# = \alpha^{-4/5} c^* = \alpha^{-4/5} N^{-4/5} / \bar{V}$ , where  $c^*$  is the overlap concentration, and for PEG,  $\alpha = 0.49 \pm 0.01$ ,

and the partial specific volume  $\bar{V} = 0.825 \text{ cm}^3/\text{g}$ . For the polymer in the Le Coeur *et al.* SANS study [5] ( $M_w = 18,000$ ), the degree of polymerization for PEG is  $N = (M_w - M_{\text{end}})/M_m$ ; with end-group and monomer molecular weight  $M_{\text{end}} = 18.02$  and  $M_m = 44.05$  for PEG,  $N = 408$ . In terms of the normalized osmotic pressure  $\tilde{\Pi} = \Pi/(RT/M_m \bar{V})$ , the scaling form is

$$\Pi^{\text{scale}} = \tilde{\Pi} N^{9/5} \alpha^{4/5} = \left(\frac{c_p}{c^\#}\right) + \left(\frac{c_p}{c^\#}\right)^{9/4}. \quad (1)$$

In this form, crossover occurs at  $c^{\text{scale}} \equiv c_p/c^\# = 1$ . For PEG with molecular weight 18,000, the scaled concentration  $c^\# = \alpha^{-4/5} N^{-4/5} / \bar{V} \sim 0.02 \text{ g/cm}^3$ , which is related by a constant factor of 1.77 to the overlap concentration  $c^* = 0.01 \text{ g/cm}^3$ . It can be seen that the expected dilute limit (look in the main manuscript for Figure 1, dotted blue line) occurs for  $c^{\text{scale}} < 0.2$  (actual polymer concentrations  $c_p < 0.002 \text{ g/cm}^3$ ) and the semi-dilute regime (look in the main manuscript for Figure 1, dashed red line) for  $c^{\text{scale}} > 6$  ( $c_p > 0.1 \text{ g/cm}^3$ ). The SANS experiments in this work are carried out in the range  $0.001 \text{ g/cm}^3 < c_p < 0.03 \text{ g/cm}^3$ , corresponding to  $0.05 < c^{\text{scale}} < 1.5$ . All except the  $c_p = 0.001 \text{ g/cm}^3$  experiment are thus completely in the cross-over regime. The PFG NMR results are carried out over a wider range,  $0.0003 \text{ g/cm}^3 < c_p < 0.04 \text{ g/cm}^3$ , corresponding to  $0.015 < c^{\text{scale}} < 2$ .

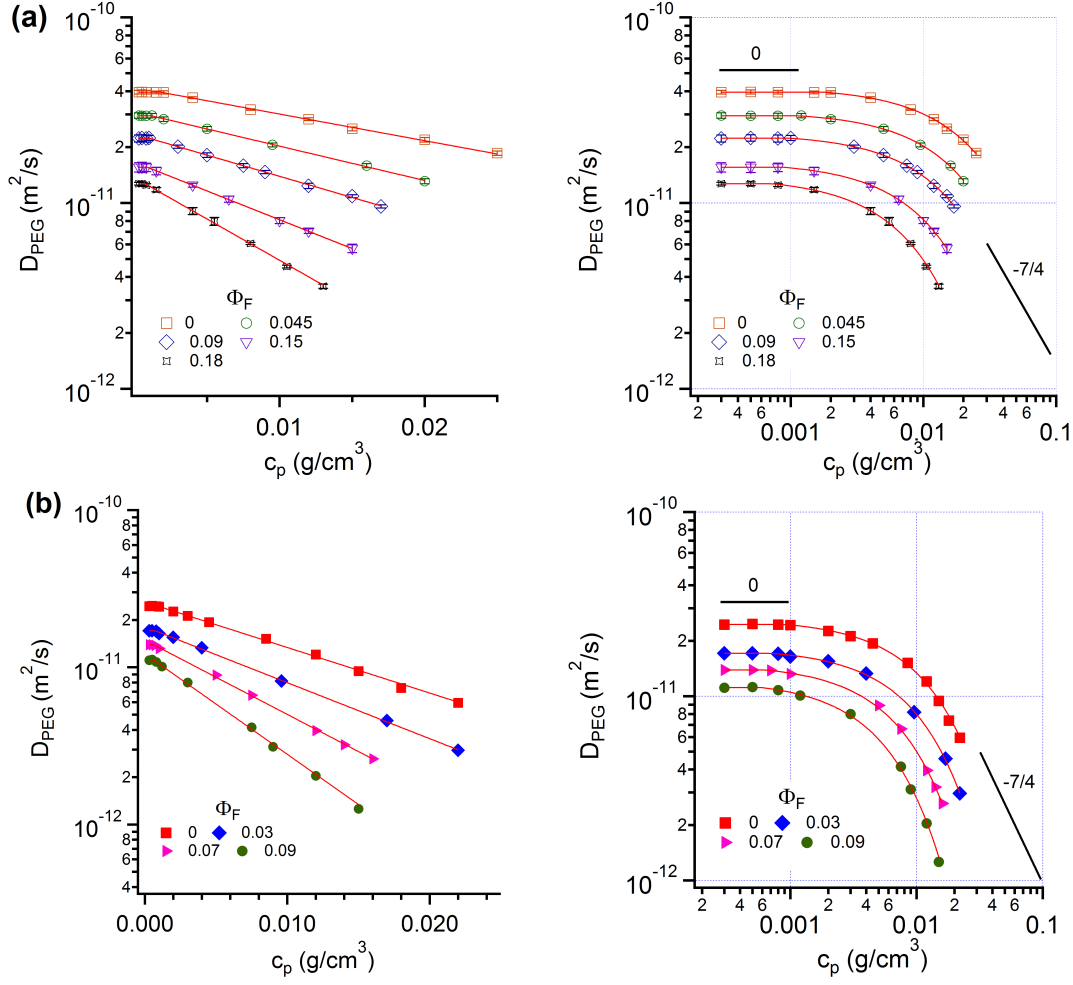


Figure S2: Diffusion coefficient shown (left) on log-linear and (right) on log-log scale for (a) PEG ( $M_w = 42,800$ ) and (b) PEG ( $M_w = 132,000$ ) in water as a function of polymer concentration  $c_p$ . Results are shown in the absence of the crowder, Ficoll70, as well as for several Ficoll70 volume fractions  $\Phi_F$ . Similar to shorter-chain PEG ( $M_w = 22,000$ ), a good fit to pure exponential behaviour is possible, *in all cases*, above a characteristic PEG concentration  $c^*$ , with an extrapolated value  $D^*$ . Below  $c^*$ , a plateau is observed at  $D(0, \Phi_F)$ . A power-law fit (e.g., of  $-7/4$ ) is not possible, but is a plausible asymptote.

### DIFFUSION MEASUREMENTS FOR HIGHER-MOLECULAR-WEIGHT POLYMERS

For completeness, we plot  $D_{\text{PEG}}$  vs.  $c_p$  for the two longer-chain (higher molecular-weight) polymers here. Figure S2 shows that the two higher-molecular-weight polyethylene glycol,  $M_w = 42,800$  and  $M_w = 132,000$ , both exhibit a trend that is similar to that of the short PEG chain ( $M_w = 22,000$ ). For low  $c_p$ , there is a plateau value for each crowder packing fraction, that indicates  $D_{\text{PEG}} \sim c^0$ . There is also a characteristic polymer concentration  $c^*$ , above which the dynamics depends exponentially on polymer concentration. Callaghan *et al.* [6] had found power law scal-

ing,  $D_{\text{PEG}} \sim c^{-7/4}$ , for longer polymers (but not for short-chain polymers). While our results are clearly not consistent with a power law, such a power law at higher concentrations (not physically accessible due to phase separation) cannot be ruled out.

The log-log plot shows that there is no power law scaling in the crossover regime, even for longer-chain polymers. While we do not observe the  $(-7/4)$  power law reported by Callaghan *et al.* [6] for longer-chain polymers, it is likely because our measurements are *not* carried out in the semi-dilute (entangled) regime, but instead in the crossover regime. Indeed, even the experimental results shown by Callaghan *et al.* for shorter-chain polymers are consistent with an expo-

nential concentration dependence.

### COMPARISON OF SANS RADIUS OF GYRATION RESULTS

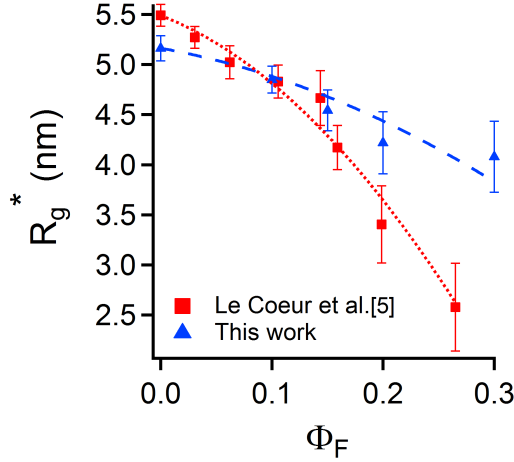


Figure S3: Comparison of  $R_g^*$ , the radius of gyration, obtained by linear extrapolation to zero  $c_p$  from the crossover regime, as a function of Ficoll70 volume fraction  $\Phi_F$ .

We compare the SANS results from this work with those from previous SANS experiments [5], for the PEG-Ficoll70 system with  $\lambda \sim 1$ . In plotting the data from Le Coeur et al., we have converted their mass fractions into volume fractions using the partial specific volume of Ficoll70, which is  $0.67 \text{ cm}^3/\text{g}$  [7]. The true  $R_g(0, \Phi_F)$  is obtained by linear extrapolation to  $c^*$ , as reported in the main manuscript. However, in order to aid comparison with the previously reported SANS results, we carry out a linear extrapolation to zero  $c_p$  of our results of  $R_g(c_p, \Phi_F)$  vs  $c_p$  at each  $\Phi_F$ . Our results show a weaker dependence on  $\Phi_F$  as compared to the ones in the work of Ref. [5].

### THE VALIDITY OF USING DIFFUSION MEASUREMENTS TO ESTIMATE THE OVERLAP CONCENTRATION

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. For pure polymer, in the absence of crowder, one can obtain the hydrodynamic radius  $R_H$  from diffusion measurements and the Stokes-

Einstein relation in the dilute regime for diffusion:  $c_p \leq c_{\text{Diff}}^* = 0.005 \text{ g/cm}^3$ . From  $R_H$ , we can obtain a radius of gyration  $R_{g,\text{Diff}} = kR_H$  in the dilute limit ( $k = 1.24$  from renormalization group calculations and  $1.16$  from past experiments; given the experimental uncertainties we use  $k = 1.2 \pm 0.04$  [8]). Using this,  $R_{g,\text{Diff}}^2$  is plotted (Figure 4 in the main manuscript) for  $c_p \leq c_{\text{Diff}}^*$  (random errors indicated by the error bars and systematic uncertainties indicated by blue shading).

From the SANS measurements carried out above  $c^*$ , at concentrations  $0.005 \leq c_p \leq 0.03 \text{ g/cm}^3$ , we get the radius of gyration:  $R_{g,\text{SANS}}$ . Observing the decrease, we test the scaling expected for semi-dilute polymer solutions above  $c^*$ :  $R_g^2 \sim c_p^{-0.23}$  [9]. While there are not many data points, it is to be noted (see Figure 4 in the main manuscript) that the fit is a one-parameter fit to the pre-factor of the  $-0.23$  power law. It is thus a better fit than the piecewise linear fit employed in Figure 5 of the manuscript, but the resulting radius of gyration is not significantly different, given the uncertainties.

In Figure 4 (main manuscript), the self-diffusion and SANS measurements appear to converge at  $c_{\text{Diff}}^*$ , suggesting that this concentration is also not far from the thermodynamic overlap concentration  $c^*$ , within the uncertainties.

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