

# Translational and Rotational Diffusion of Globular Protein in Concentrated Polymer Solution

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## Introduction

The transport of proteins in polymer solutions and gels plays an important role in many applications and processes, such as enzymatic degradation of filter cake in oil industry, protein drug delivery in tissue, bioseparation and etc<sup>1-5</sup>. The protein in the polymer matrix undergoes two types of motions: translational diffusion, which is the center of mass motion in a macroscopic scale and rotational diffusion, which is the local dynamics in a microscopic scale. Many techniques have been explored to measure translational diffusion and a variety of theoretical models have been developed. In contrast, less work has been found to study rotational diffusion of proteins in polymer solutions both experimentally and theoretically. Our idea is to label the protein with <sup>19</sup>F, which can differentiate protein from polymer background in NMR spectrum. Translational and rotational diffusion of proteins will be studied by Pulsed Field Gradient <sup>19</sup>F-NMR and T<sub>1</sub> and T<sub>2</sub> relaxation of <sup>19</sup>F.

## Experiment

### 1. PFG-NMR experiment

Pulsed field gradient <sup>19</sup>F-NMR experiment was conducted on Varian NMR500 at 25 ± 0.1°C. Diffusion experiment was performed with Dbppste pulse sequence. The gradient of up to 78G/cm was used. The duration of pulse was controlled to be 1ms to 2ms for various samples and diffusion delay was in the range of 50ms to 100ms. Radio frequency pulse widths used for a 90° nutation angle were calibrated for each sample before carrying out diffusion measurement. To improve the signal to noise ratio, long accumulation time was used to ensure the signal to noise ratio above 10:1. The number of scans was between 64 to 1024. Typically, 5 data points at varying gradient current were taken for each sample. The diffusion coefficient were obtained by a nonlinear, weighted, least squares fit of the NMR signal to  $A = A_0 \exp[-(\gamma I g_c \delta)^2 D(\Delta - \delta/3)]$ .

### 2. T<sub>1</sub> and T<sub>2</sub> relaxation study

Fluorine T<sub>1</sub> and T<sub>2</sub> relaxation study were also conducted on the same NMR apparatus for diffusion measurement at 25 ± 0.1°C. Std1h and cpmgt2 sequences were used for T<sub>1</sub> and T<sub>2</sub> measurement, respectively. The relaxation of fluorine signal at -76ppm was studied up to 16s for T<sub>1</sub> and 70ms for T<sub>2</sub>. Radio frequency pulse widths used for a 90° nutation angle were calibrated for each sample before carrying out diffusion measurement. To improve the signal to noise ratio, long accumulation time were used to ensure the signal to noise ratio above 10:1. The number of scans was between 64 to 1024. 8 data points at various decay time were measured and the decayed signal intensity was fit to  $A/A_0 = 1 - \exp(-t/T_1)$  and  $A/A_0 = \exp[-t/T_2]$  by a nonlinear, weighted, least squares method.

## Results and Discussion

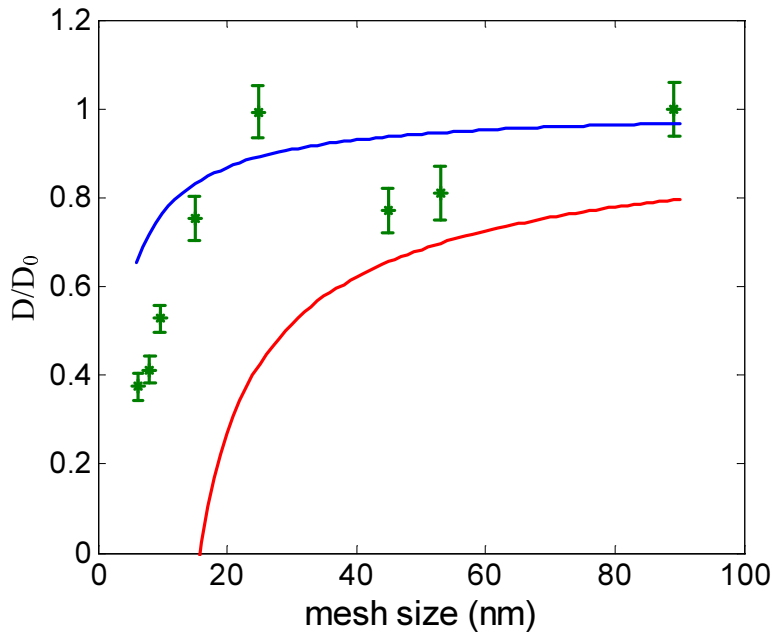
### 1. Translational diffusion of ovalbumin in Hydroxylpropyl Guar

According to effective medium theory, the background polymer solution is treated as homogeneous Brinkman fluid with hydrodynamic screening length  $\xi$ . Under this assumption, the translational and rotational diffusion coefficients were calculated by Solomentsev<sup>6</sup>, who showed that

$$\frac{D}{D_0} = \frac{1}{1 + R/\xi + 1/9(R/\xi)^2} \quad \text{Eq. 1}$$

$$\frac{D_r}{D_{r_0}} = \frac{1 + R/\xi}{1 + R/\xi + 1/3(R/\xi)^2} \quad \text{Eq. 2}$$

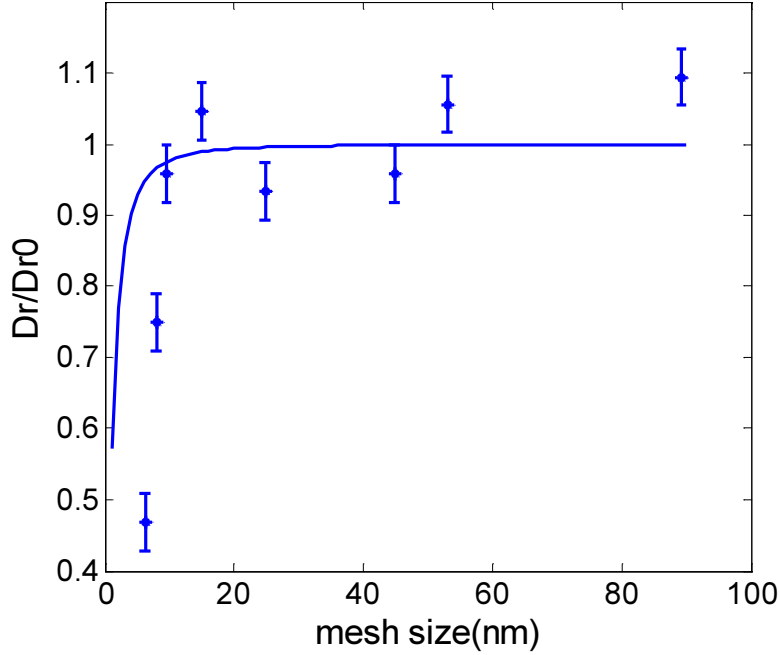
In Figure 1, we compare our experimental data with the diffusion coefficient calculated from effective medium theory (blue line) and the confined diffusion of a single particle inside a cylindrical pore<sup>7</sup>(red line).



**Figure 1** Translational diffusion coefficient as a function of mesh size of HPG solution. Compare experimental results with effective media theory. The protein radius used in this calculation is 3nm. Blue line is from effective medium theory, green points are from experimental measurement and red line is from confined diffusion of particle in cylinder

### 2. Rotational diffusion of ovalbumin in Hydroxylpropyl Guar

Rotational correlation time is a nice indicator for the overall rotational diffusivity of macromolecule.  $T_1$  and  $T_2$  are both polynomial functions of correlation time  $\tau_c$ <sup>8</sup>. By measuring  $T_1$  and  $T_2$ , rotational correlation time and rotational diffusion coefficient can be calculated. The effective medium predictions calculated from Equation 2 with  $R=3\text{nm}$  are compared with experimental measurement of rotational diffusion coefficient in Figure 2.



**Figure 2 Rotational diffusion coefficient as a function of mesh size of HPG solution. Compare experimental results with effective media theory**

In Daniel's work<sup>9</sup>, they proposed that the individual protein molecule is exposed to the full cosolvent (polymer) concentration, but feels only a fraction of its hydrodynamic interaction.

$$\frac{\tau_{rot}}{\tau_{rot_0}} = \left( \frac{\eta}{\eta_0} \right)^q \quad \text{Eq. 3}$$

A power law like Equation 3 best fits their experimental data.  $q$  is the parameter that characterizes the reduction of hydrodynamic interaction felt by the protein. Here we introduce this power law approach into our discussion. When  $R \ll \xi$ , the tracer particle only feels the solvent's viscosity and  $\frac{\tau_{rot}}{\tau_{rot_0}}$  is a weak function of  $\xi$ . When  $R$  is comparable to  $\xi$  or much smaller than  $\xi$ , the tracer feels a fraction of hydrodynamic interaction of the polymer solution and the power law as Equation 3 is applicable. In Figure 3, by fitting the experimental data in this range to the power law, we find the reduction factor  $q$  is 0.31, which compares well with the  $q$  value in Daniel's work<sup>9</sup>.

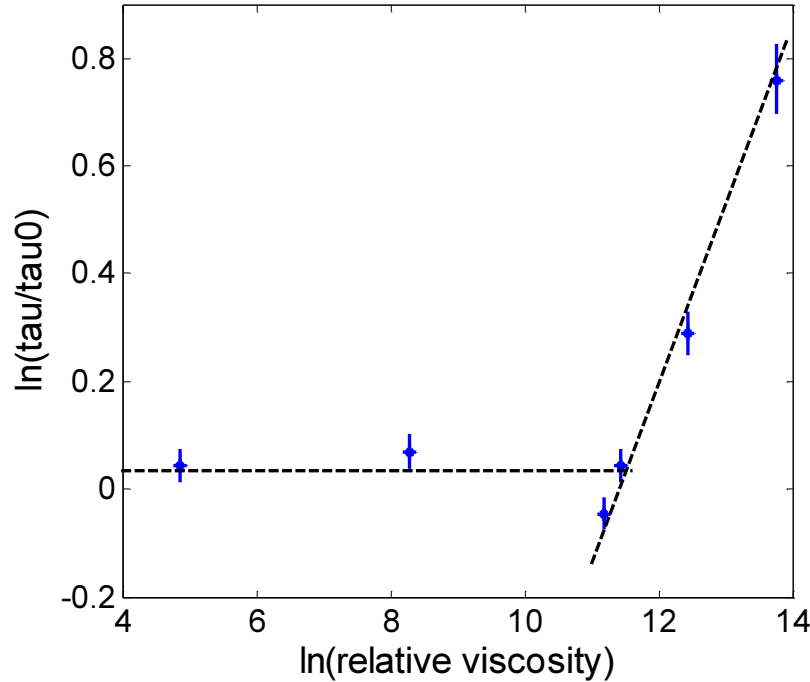


Figure 3 Rotational correlation time as a function of relative viscosity

## Conclusions

We presented experimental data for translational and rotational diffusion coefficient of protein in hydroxypropyl Guar solution by  $^{19}\text{F}$  NMR and experimental results were compared with Stokes Einstein Equation and effective medium theory. The strong deviation of translational diffusion coefficient from the Stokes Einstein Equation indicates that the in the length scale of protein size the protein molecule feels inhomogeneity of the matrix polymer. Rotational diffusion coefficient is found to stay relatively constant when the tracer size is much smaller than the mesh size of polymer matrix. When the protein size is comparable to the mesh size of polymer solution, proteins only feel a fraction of the hydrodynamic interaction of polymer matrix and the relationship between the rotational diffusion coefficient and macroscopic viscosity obeys power law. Scaling approach is taken to explain the relationship between translational diffusion and rotational diffusion. In low polymer concentration range (free rotation), rotational diffusion is not hindered by polymer matrix, while translational diffusion is slowed down much due to the existence of polymer chains. In the high polymer concentration range (constrained rotation), both diffusion processes are significantly impeded by polymer matrix and coupled together in the stretched exponential form. We propose hopping mechanism dominates the translational diffusion process. This also explains why the rotational diffusion acts as the activation energy term in the coupling function. In the future, we will further quantify the translational diffusion and rotational diffusion behavior as well as the relationship of these two in the transition range between “free” rotation and “constrained” rotation.

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**Note: We don't include all the details here, but a more complete version of this paper is being prepared to submit as a journal paper and is available upon request.**

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