DIFFUSION OF POLYMERS UNDER CROWDING BY COMPLEX BIOMOLECULES AS STUDIED BY PULSE GRADIENT NMR

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ABSTRACT

Nonspecific interactions of macromolecules in the cell interior lead to a phenomenon known as macromolecular crowding. Interactions between a molecule of interest and the many molecules in the cell interior can modify its characteristics. For example, crowding can cause changes in biomolecules including protein structure, enzyme kinetics and protein-protein interactions. The effect of crowding is of concern because these biological molecules are normally studied in dilute solution, rather than their real biological environment inside crowded cells. Thus, it is important to understand the behavior of such biomolecules in the presence of proteins, enzymes and DNA as the crowders.

Traditionally, biophysical studies of crowding have used simple crowding agents like dextran and. In order to move beyond simple crowding agents, we will measure the translational diffusion of polyethylene glycol (PEG), using pulsed gradient NMR, in the presence of a more realistic crowder, cell lysate, the mixture resulting when cells are sheared open. These results will provide a better understanding of complex crowding systems such as the interior of real cells. Also, the work will provide insight into parameters to optimize to obtain quality data of proteins via in-cell NMR.

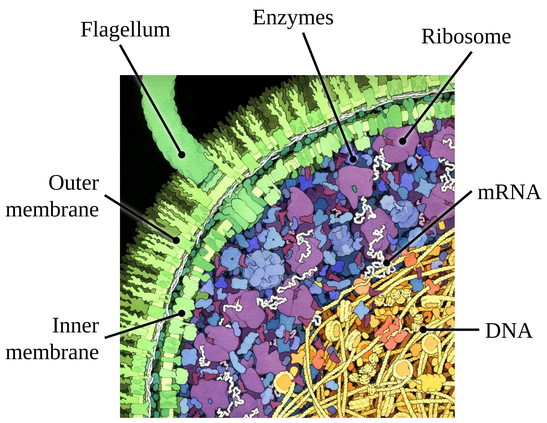

Figure 1. Representation of the crowded environment inside a small region of an *E. Coli* cell. The DNA is shown in yellow, the cytoplasm in blue and purple, and the cell wall in green. Image from David Goodsell.

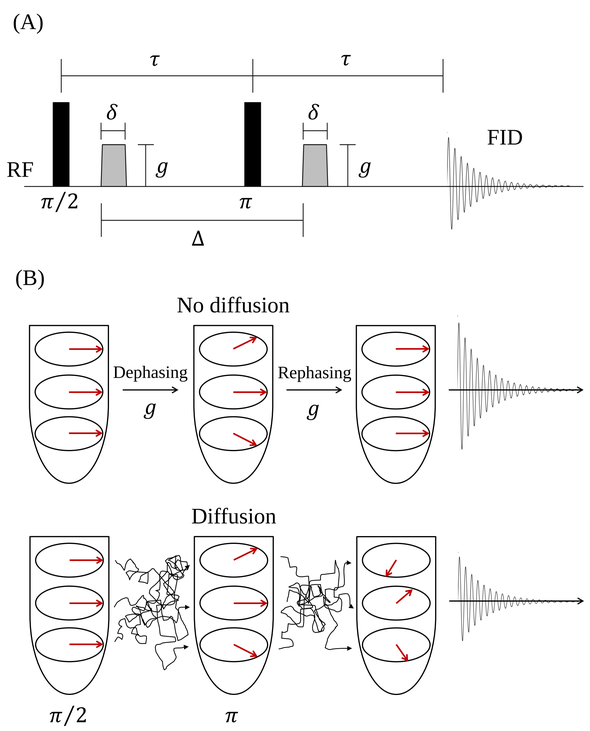

Figure 2. ^{1}H Pulse Field Gradient Spin Echo NMR. The echo appears at 2τ. (A) After each pulse ($\pi/2$ and π) there are two magnetic field gradients (g) of duration δ and a time of separation between gradients Δ. (B) Illustration of the magnetization vector. The first gradient dephases the magnetization, while the second gradient refocuses it. For molecules that diffuse more, the total magnetization after refocusing is less than for molecules that diffuse less, which means the total signal will have a lower amplitude.

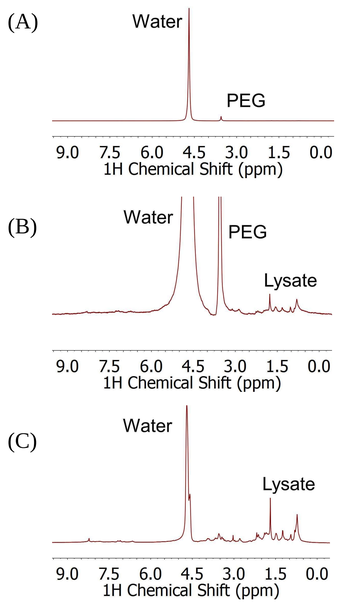

Figure 3. ^{1}H NMR Spectra (A) Polyethylene glycol ($0.03\ g/ml$) (PEG) in 90 mg/ml cell lysate. (B) Magnified plot of PEG in cell lysate; the intensity of lysate peaks is small compared to PEG and water. (C) Cell lysate only spectrum, using presaturation technique for water suppression during the NMR experiment.

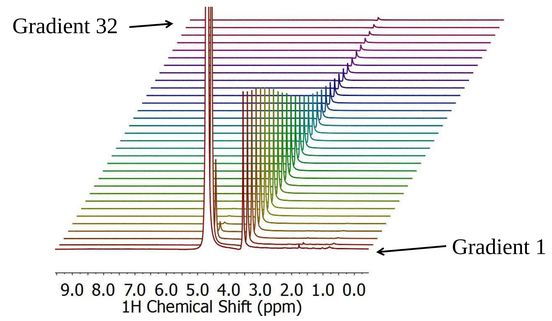

Figure 4. ^{1}H diffusion NMR spectra with increasing gradients. The water peak decays faster than the PEG peak.

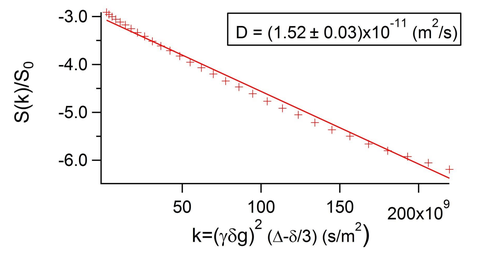

Figure 5. Log scale of the attenuated signal $S(k)/S_0$ versus the gradient strength parameter (k) for $0.03\ g/ml$ PEG in 90 mg/ml cell lysate measured via ^{1}H diffusion NMR. Linear fit gives self-diffusion coefficient value. Signal appears to have a bi-exponential component.

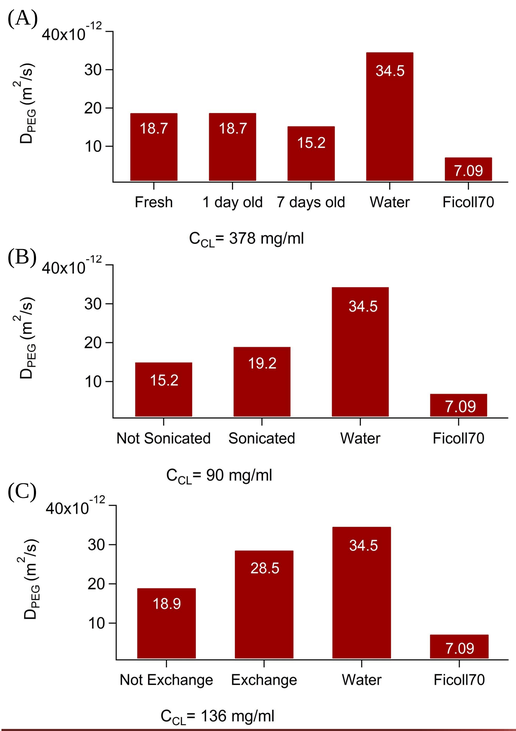

Figure 6. Self-diffusion coefficient for $0.03\ g/ml$ PEG (M_W = 22 000) in different concentrations of Cell Lysate (C_{CL}) compared with water and 10% ($149\ mg/ml$) Ficoll70 (M_W = 70 000). (A) Sample was stored at -20°C between measurements. (B) 3 pulses of 30 seconds each sonication for Cell Lysate. (C) Reduction of negatively charged lysate components (e.g. DNA) via anionic exchange. Ficoll70 data was obtained from [1]. Numbers in bar are diffusion coefficients.

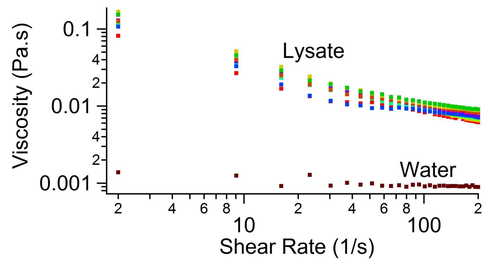

Figure 7. Log scale of Viscosity for Cell lysate sonicated at a concentration $C_{CL} = 182\ mg/ml$ versus the Shear Rate using a rheometer with a cone plate of 5 mm diameter and 1° angle.

SUMMARY

- PEG polymer diffuses more slowly in a lysate medium compared to water.
- Sonication breaks long DNA chains, as a consequence PEG diffuses faster compared to non-sonicated lysate.
- Anionic exchange procedure reduces crowded environment extracting DNA and lipids from the medium.
- In rheology measurements, cell lysate appears to have a shear thinning behavior.

FUTURE WORK

- Characterization of Cell Lysate *E.Coli* JM109.
- Study of Intrinsically Disordered Proteins as new tracer in the crowded environment.

REFERENCE

[1] S. Palit, L. He, W. A. Hamilton, A. Yethiraj, and A. Yethiraj, *J. Chem. Phys.* **147**, 114902 (2017).

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