

**Supporting Information for**  
**Pluronic Additives: A Solution to Sticky Problems in Digital**  
**Microfluidics**

Vivienne N. Luk<sup>1,2</sup>, Gary CH. Mo<sup>2,3</sup>, Aaron R. Wheeler<sup>1,2,4\*</sup>

<sup>1</sup> *Department of Chemistry, University of Toronto, 80 St George St., Toronto, ON, M5S 3H6*

<sup>2</sup> *Donnelly Centre for Cellular and Biomolecular Research, 160 College St., Toronto, ON, M5S 3E1*

<sup>3</sup> *Department of Chemical Engineering and Applied Chemistry, University of Toronto, 200 College St., Toronto, ON, M5S 3E5*

<sup>4</sup> *Institute for Biomaterials and Biomedical Engineering, University of Toronto, 164 College St., Toronto, ON, M5S 3G9*

\* Corresponding Author

email: [awheeler@chem.utoronto.ca](mailto:awheeler@chem.utoronto.ca); tel: (416) 946 3864; fax: (416) 946 3865

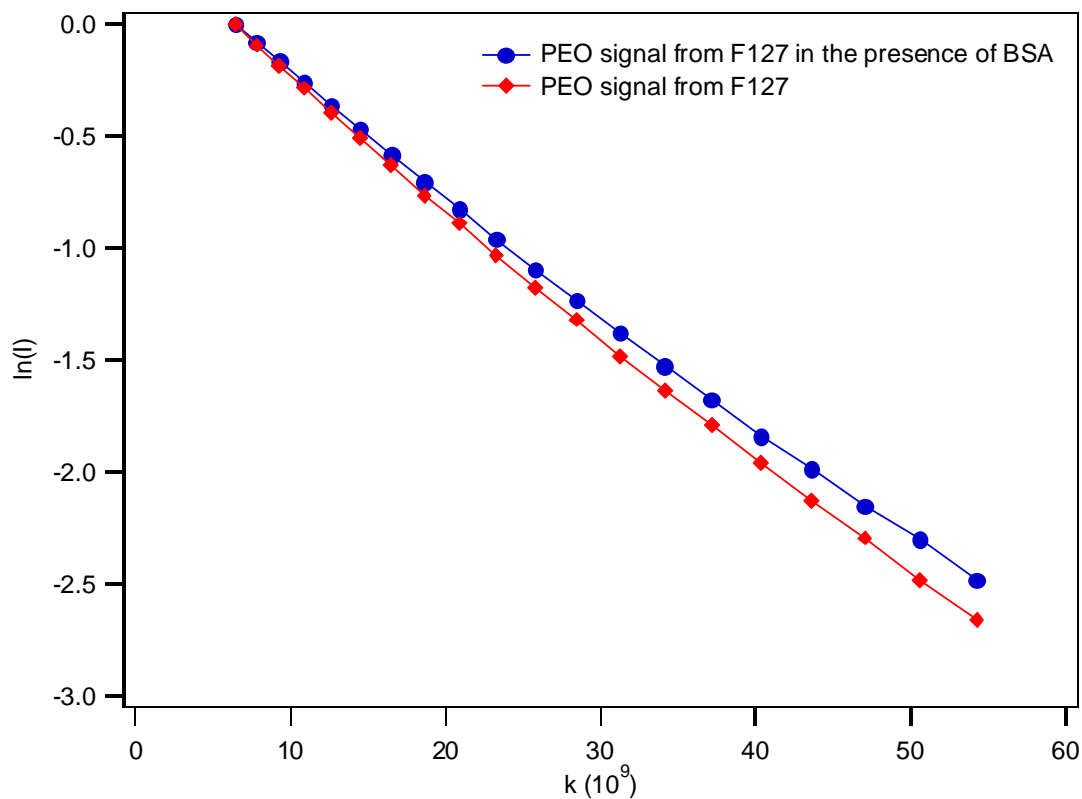
## **Experimental: NMR Investigation of Pluronic-Protein Interaction**

NMR spectra were recorded on an Infinity 500 MHz NMR spectrometer (Varian Inc., Palo Alto, CA) using a 5 mm double resonance liquid probe at  $25 \pm 0.5^\circ\text{C}$ . Sample solution concentrations were 0.08% w/v F127 in TrisHCl buffer, with and without 1 mg/mL BSA.  $^1\text{H}$  NMR diffusion measurements were performed at 499.78 MHz using the stimulated echo (STE) pulsed field gradient (PFG) procedure, with 5 ms gradient pulse duration and variable gradient pulse amplitude (0.595, 0.655, 0.714, 0.774, 0.833, 0.893, 0.952, 1.012, 1.071, 1.131, 1.190, 1.250, 1.309, 1.369, 1.428, 1.488, 1.547, 1.607, 1.666, 1.726 T/m). The field gradient pulses were applied along the longitudinal ( $z$ ) direction exclusively. Typical acquisition parameters were: a  $90^\circ$  pulse length of 16  $\mu\text{s}$ , a spin echo delay of 10 ms, a recycle delay of 5 s, a spectral width of 10 kHz and a 4000 point data set. The radio frequency pulses were cycled according to the procedure outlined by Fauth et al.<sup>1</sup> to remove unwanted echoes. For each gradient amplitude, 16 scans were acquired for sufficient S/N ( $>10:1$ ). Spectra were processed with an exponential multiplication equivalent to 5 Hz line broadening prior to Fourier transformation, and were referenced to tetramethylsilane (TMS). Gradient strength was calibrated from the known diffusion coefficient of deuterated water (HDO) at  $25^\circ\text{C}$ . The  $^1\text{H}$  resonance for PEO units in F127 was assigned as 3.3 ppm.

## **Discussion: NMR investigation of Pluronic-Protein Interaction**

Pulsed Field Gradient NMR (PFG-NMR) is a well-established method used to study molecular diffusion in isotropic liquids,<sup>2-5</sup> and can be applied to examine the diffusion of Pluronic in aqueous solutions. This method can be used to determine the

presence of non-specific interactions between polymers and proteins as a function of the diffusant's (i.e., the polymer's) hydrodynamic radius. The natural logarithm of the normalized stimulated echo intensity decay,  $I$ , of F127 is shown as a function of  $k$  in Figure 1. Here,  $k = (\gamma\delta g)^2(\Delta - \delta/3)$ , where  $\gamma$  is the magnetogyric ratio of the nucleus under investigation (for  $^1\text{H}$ ,  $\gamma = 42.576 \text{ MHz/T}$ ),  $\delta$  is the gradient duration (5 ms),  $g$  is the gradient amplitude (see above), and  $\Delta$  is the diffusion time (400 ms). The slope of the plot is proportional to the diffusion coefficient of Pluronic F127. The diffusion coefficient of Pluronic F127 was determined in the presence and absence of BSA to be  $5.22 \times 10^{-11} \text{ m}^2\text{s}^{-1}$  and  $5.58 \times 10^{-11} \text{ m}^2\text{s}^{-1}$ , respectively. Thus, the diffusion coefficient of F127 is relatively constant, regardless of the presence of BSA, which suggests that there is not much interaction between the two species. In this case, the difference between the diffusion coefficients with and without BSA is 6.7%, which indicates the interaction between the two species is limited, and may simply be a result of the increase in the solution viscosity caused by the addition of proteins.



**Figure S1.** Diffusion of Pluronic 127 with (circle markers) and without (diamond marker) BSA in TrisHCl buffer. Plot of intensity decay (<sup>1</sup>H resonance of the PEO units in F127) as a function of  $k$ , where  $k = (\gamma \delta g)^2 (\Delta - \delta / 3)$ . Best fit line for free diffusion of F127 (red line) is  $y = -5.58 \times 10^{-11}x + 0.30$  ( $R^2 = 0.9984$ ) and for diffusion of F127 in the presence of BSA (blue line) is  $y = -5.22 \times 10^{-11}x + 0.29$  ( $R^2 = 0.9983$ ). The slope of the graph is proportional to diffusion coefficient:  $D_{F127} = -5.58 \times 10^{-11}$  and  $D_{F127 \text{ (with BSA)}} = -5.22 \times 10^{-11}$ .

## References

We thank Ronald Soong and Dr. Peter MacDonald for access to the 500 MHz NMR and assistance collecting and interpreting data.

## References

1. Fauth, J.-M.; Schweiger, A.; Braunschweiler, L.; Forrer, J.; Ernst, R. R., Elimination of Unwanted Echoes and Reduction of Dead Lime in Three-Pulse Electron Spin-Echo Spectroscopy. *J. Magn. Reson.* **1986**, 66, 74-85.
2. Stilbs, P., Fourier Transform Pulse-Gradient Spin-Echo Studies of Molecular Diffusion. *Prog. Nucl. Magn. Res. Sp.* **1987**, 19, 1-45.
3. Karger, J.; Pfeifer, H.; Heink, W., Principles and Applications of Self-Diffusion Measurements by Nuclear Magnetic Resonance. *Adv. Magn. Opt. Reson.* **1988**, 12, 1-89.
4. Price, W. S., Pulse-Field Gradient Nuclear Magnetic Resonance as a Tool for Studying Translational Diffusion: Part 1. Basic Theory. *Concept. Magn. Reson.* **1997**, 9, 299-336.
5. Price, W. S., Pulse-Field Gradient Nuclear Magnetic Resonance as a Tool for Studying Translational Diffusion Part 2. Experimental Aspects. *Concept. Magn. Reson.* **1998**, 10, 197-237.