

Supplementary Material

In situ pulmonary mucus hydration assay using rotational and translational diffusion of gold nanorods with PS-OCT

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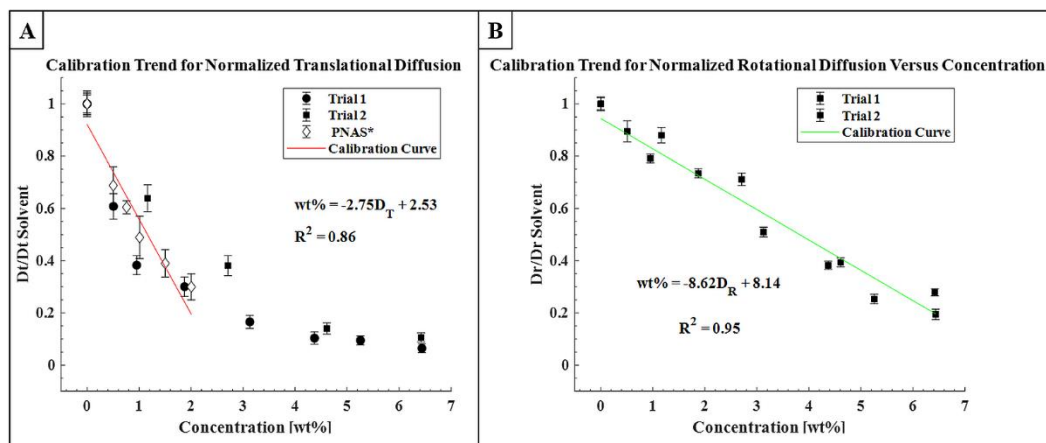
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In this supplemental document, we provide context on the linear regressions used to determine trendlines 3.2. Additionally, we provide visuals for the statistical significance of results presented in Figure 2 and Figure 3 of the main text.

1 Linear Regression for trendline determination

Linear regression analysis was applied to the translational diffusions in mucus trial 1 samples, trial 2 samples, and our previously published translational diffusion findings in stationary mucus [1]. This approach aimed to extract trendlines that could establish a correlation between translational diffusion dependence and the prediction of mucus concentration. Considering the absence of an existing model for this specific relationship, we determined that a linear regression could effectively characterize the range from 0 to 2 wt%. In contrast, for rotational diffusion, the model was deemed suitable for concentrations up to 6.5 wt%.

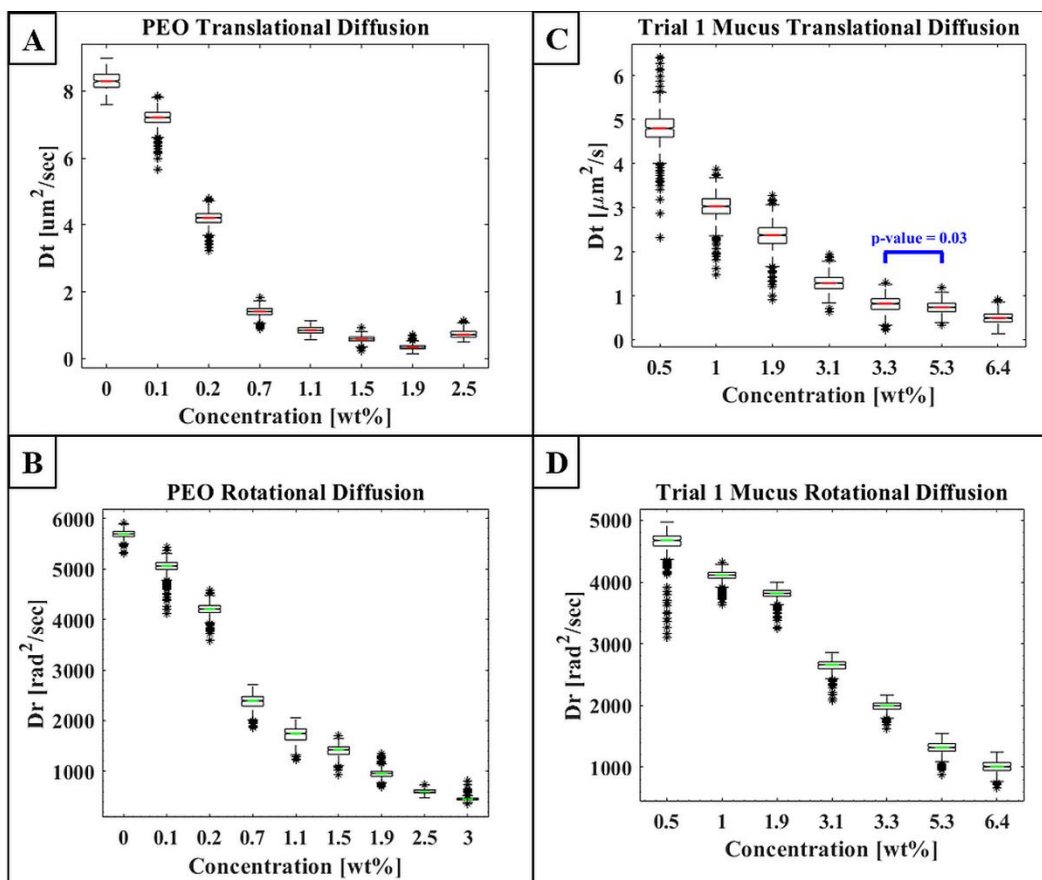


Supplemental Figure 1: (A) Translational diffusion coefficients, normalized by translational diffusion in solvent, are plotted versus mucus concentration. A linear regression is performed over 0 – 2 wt%. * [1] (B) Rotational diffusion coefficients, normalized by translational diffusion in solvent, are plotted versus mucus concentration. A linear regression is performed over 0 – 6.5 wt%.

2 Statistical significances between sample concentration diffusion rates

In assessing the statistical significance across all concentration samples, we employed a one-way ANOVA test. The results revealed a notable distinction between both the translational and

rotational diffusion coefficients, supported by nearly negligible p-values. Supplemental Figure 2 showcases the distinction of each concentration for both translational diffusion and rotational diffusion.



Supplemental Figure 2: Box and whisker plots for PEO samples' translational (A) and rotational (B) diffusion coefficients over a range of concentrations. Box and whisker plots for mucus trial 1 samples' translational (C) and rotational (D) diffusion coefficients over a range of concentrations. The only neighboring sample comparison with a p-value above 0.001 is marked in (C). Red and green lines show the median diffusion coefficient.

References

- [1] R. K. Chhetri et al., "Probing biological nanotopology via diffusion of weakly constrained plasmonic nanorods with optical coherence tomography," *Proceedings of the National Academy of Sciences of the United States of America* 111(41), E4289–E4297, National Academy of Sciences (2014) [doi:10.1073/pnas.1409321111].