Exploring the Effect of pH on DNA-Intercalating Supramolecular Hydrogels

Amy Di Vito, Shaina M. Hughes, Dr. Nathan J. Oldenhuis
Department of Chemistry, University of New Hampshire, 23 Academic Way, Durham NH, 03824

Material properties of DNA hydrogels

DNA-based hydrogels are 3D polymeric cross-linked networks that swell in water. In this project, we utilized DNA as the base of our hydrogels due to its biocompatibility and ability to be cross-linked through intercalation. Intercalation is the reversible insertion of a planar, aromatic molecule in between DNA base pairs. DNA intercalating supramolecular hydrogels (DISHs) have tunable material properties that exhibit either endothermic or exothermic behavior. Through previous studies, we have determined Acridine-PEG DISHs become more elastic as temperature rises, meaning they are primarily driven by entropic forces. With this information, we wanted to further probe the impact temperature has on the elastic properties utilizing pH.

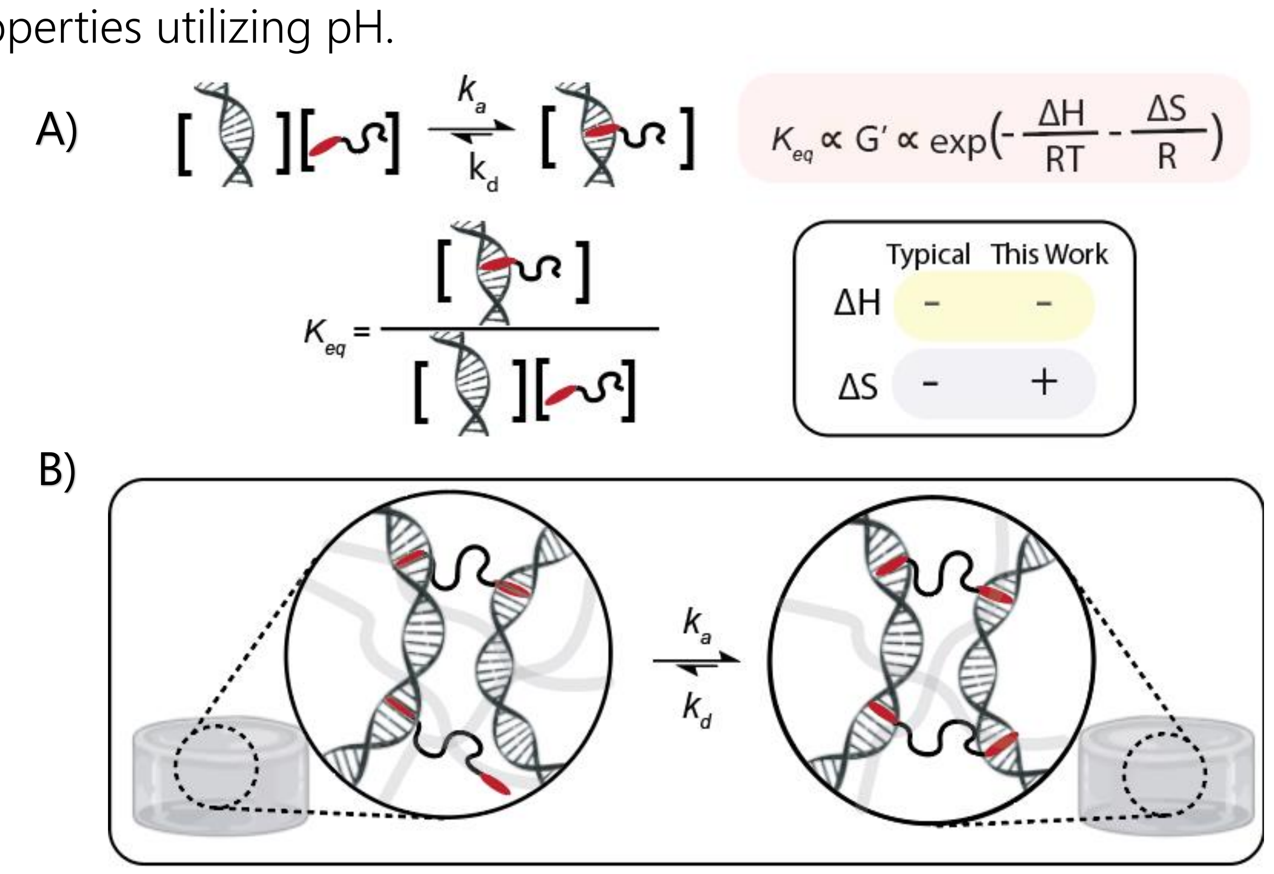


Figure 1. A) Equilibrium dynamics of cross-link association and dissociation kinetics constants of the reactions. B) Schematic of a cross-linked hydrogel where reversible intercalation association event occurs.

In this study, we are exploring the impact of pH on Acridine-based DISHs. As the pKa of acridine is 5.3, by lowering the pH of our system we can explore the impact of electrostatic interactions between acridine and the phosphate backbone on the hydrogel thermodynamics. At pH 8, interactions are likely dominated by π-π stacking and hydrophobic effects. At pH 5, ionic bonding will give more control over gel strength and dynamics.

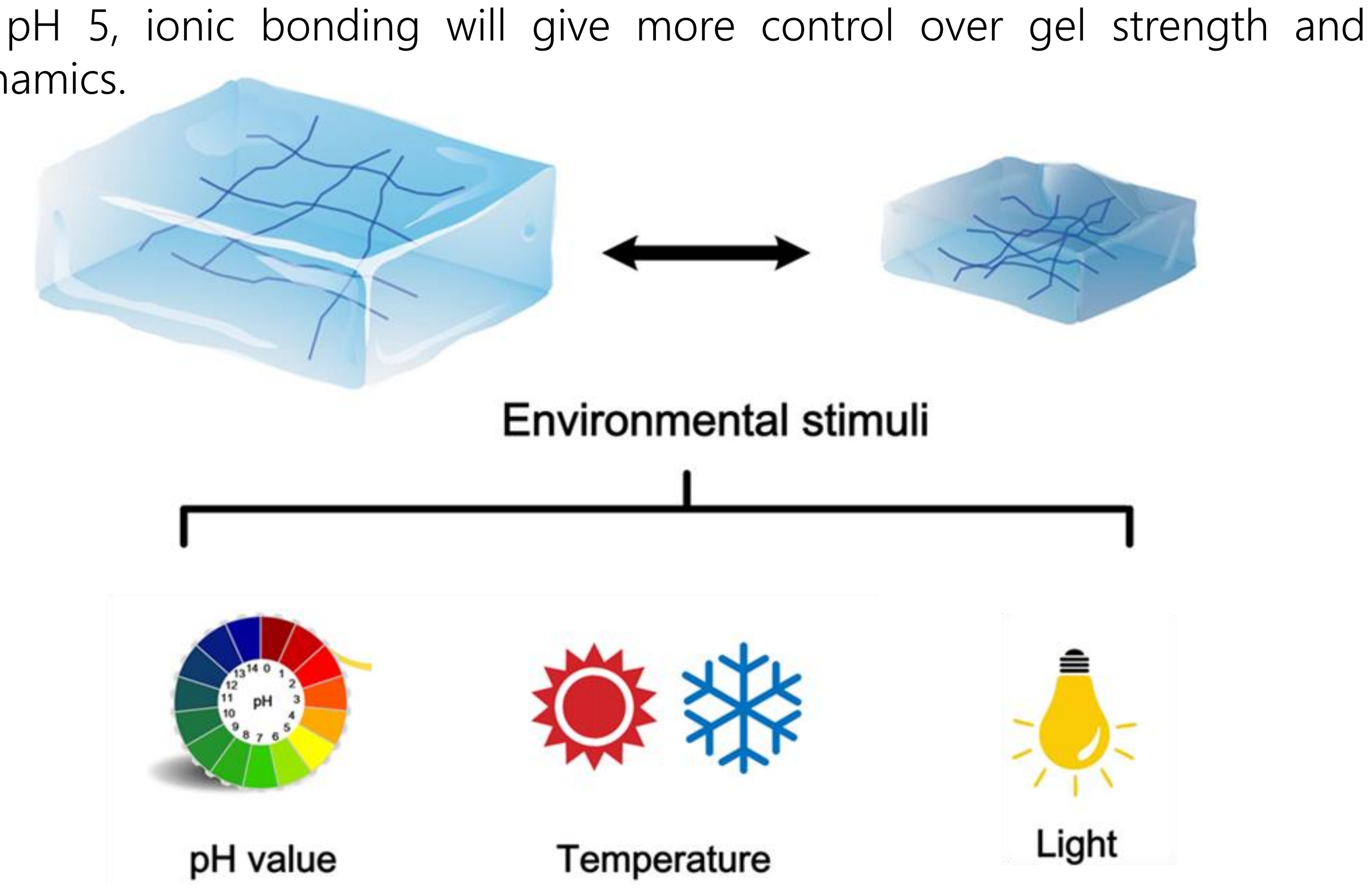
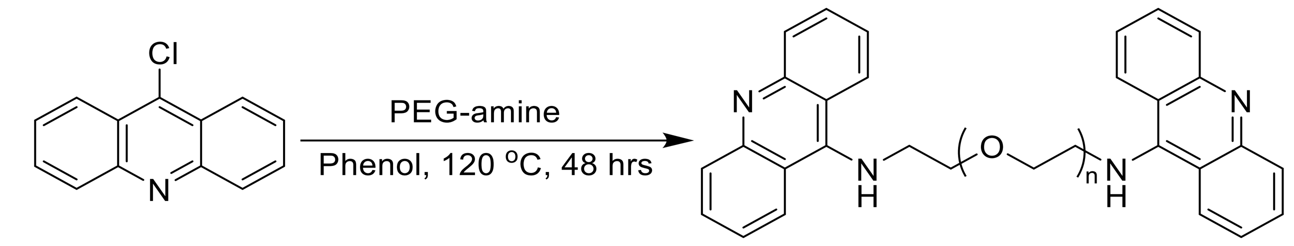


Figure 2. *Exploring the impact of pH, temperature, and light on hydrogel properties.*

Synthesis of Acridine-PEG



Reaction Scheme 1*. 1-step synthesis of Acridine-PEG.*

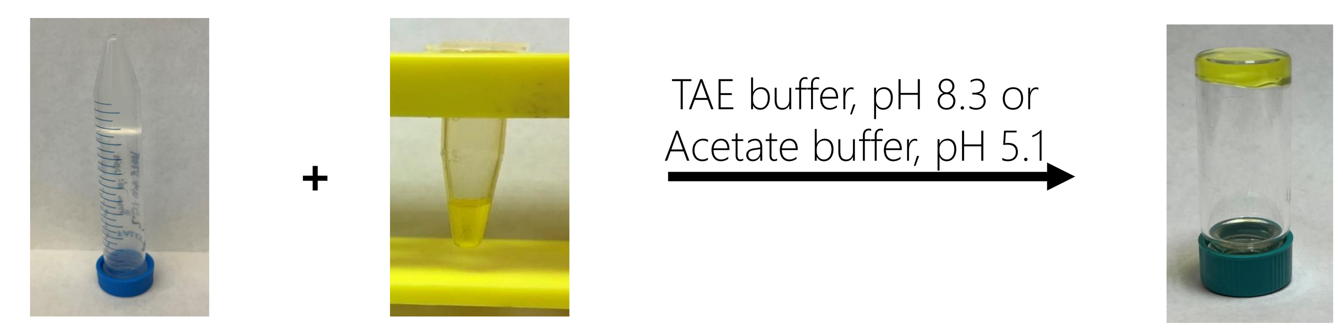


Figure 3*. Formation of the Acr-PEG DISH. The bifunctional cross-linkers were mixed with commercially available salmon milt DNA in TAE or Acetate buffer to obtain a final composition of 5 wt. % DNA and 2 mM cross-linker.*

Rheology on pH 5 and pH 8 Acridine-PEG

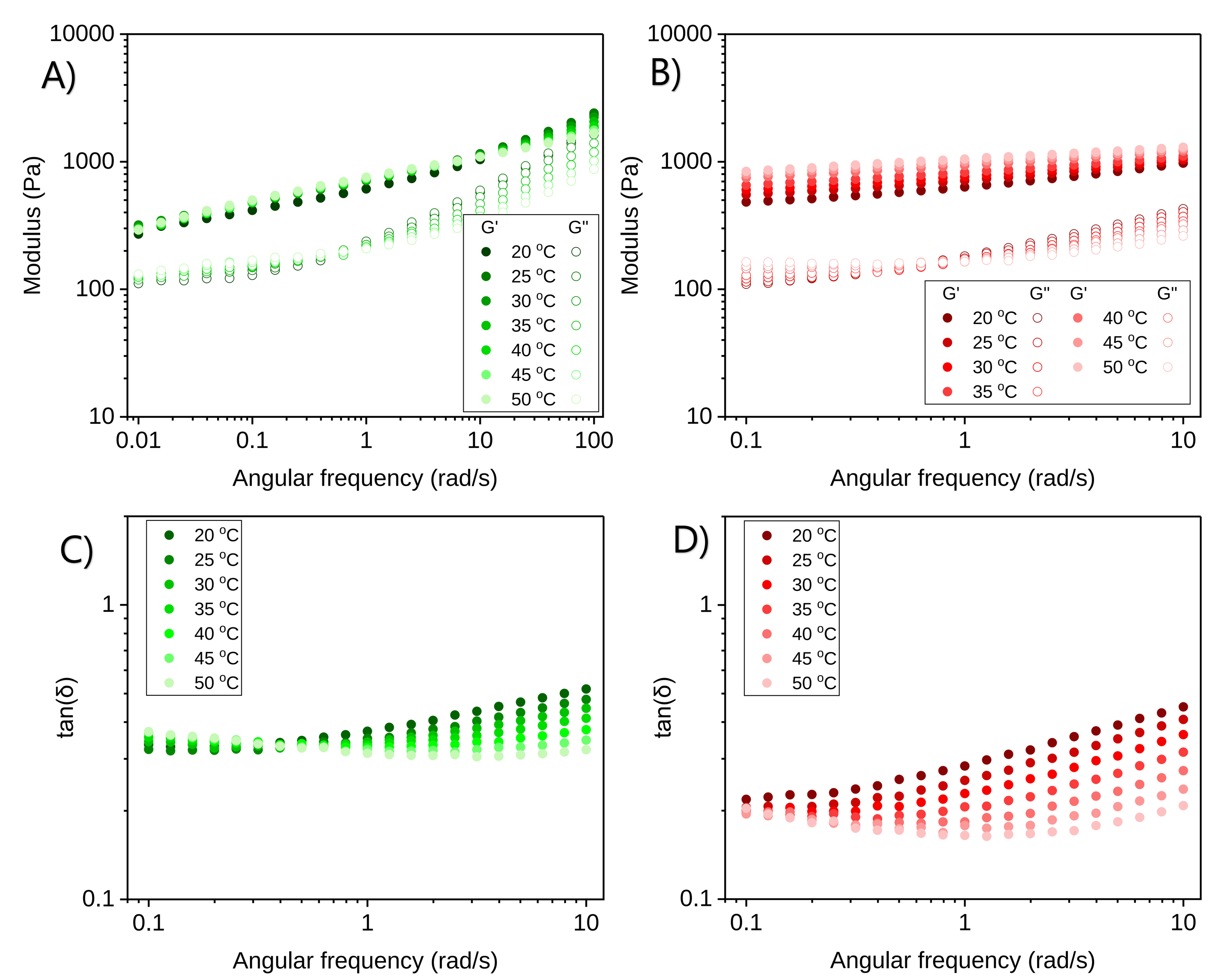


Figure 4*. a) Frequency sweep of Acridine-PEG hydrogel at pH 8 shows dominant elastic behavior (G' > G") and moderate thermal sensitivity across 20–50 °C. b) Frequency sweep of Acridine-PEG hydrogel at pH 5 shows dominant elastic behavior and thermal stiffening across 20-50 °C. c)Tan delta plot of Acr-PEG at pH 8 shows limited thermal impact on frequency response. d) Tan delta graph of pH 5 Acr-PEG shows prevalent thermal impact on frequency response.*

When comparing the impact of pH on Acr-PEG DISH behavior, we saw a clear increase in elasticity across all temperatures when Acridine was protonated at pH 5. In the pH 8 studies, the DISH showed less temperature-dependent behavior in comparison to pH 5, indicating adding electrostatic interactions with the phosphate backbone enhance the elastic-like response of the material.

pH-dependent thermal response

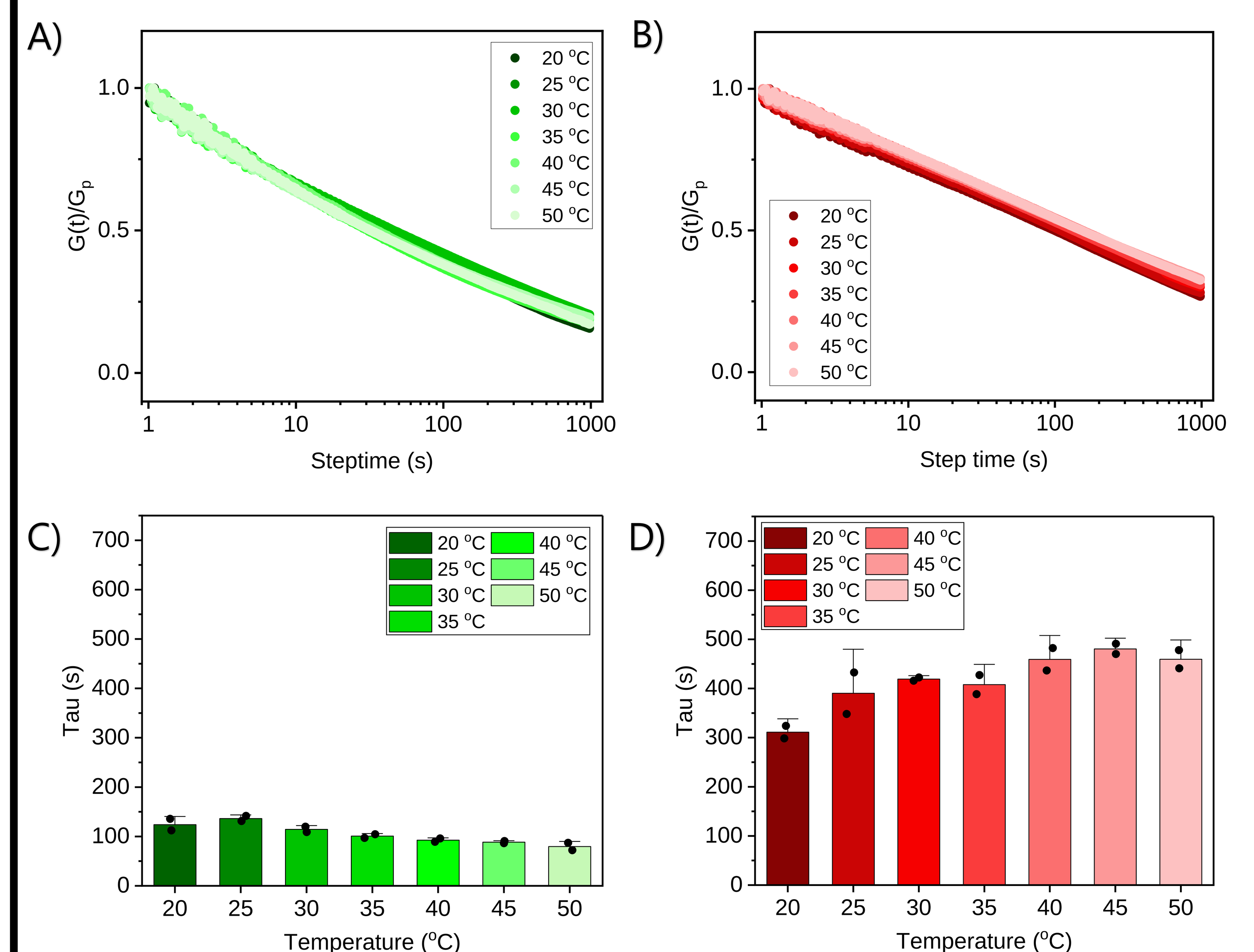


Figure 5*. a) Stress relaxation curve of Acr-PEG DISH at pH 8 normalized to the Kohlrausch-William-Watts function. b) Stress relaxation curve of Acr-PEG DISH at pH 5 normalized to the Kohlrausch-William-Watts function. c) Relaxation time constant (τ) bar graph of Acr-PEG at pH 8 shows invariant/softening behavior to increasing temperature. d) Relaxation time constant (τ) bar graph of Acr-PEG at pH 5 shows stiffening behavior to increasing temperature.*

Future Work

We will continue our studies across a wider range of pHs to better understand the impact electrostatic interactions have on the gel behavior. We will synthesize 4-arm Acridine-PEG to increase the interaction with DNA.

Buffer	pH Range
Acetate	3.6-5.6
PIPES	6.1-7.5
TRIS	7.0-9.0
TAE	7.3-8.3

Acknowledgments

Thank you to the UNH chemistry department, Wynter Paiva, Matthew Currier, Tran Truong, Patrick Strobel, Lola Fadairo, Zahraa Albeshir, Noelle Honan, Sam Ashooh, Lauren Barnes, Madigan Jennison Henderson, Pearson Franz, Kaelin Marshall, Laura Hills, and Dylan Sager. This research is funded by NSF Career 2340569.

References

Chemixgu. Viscosity of Adhesives | Chemix Guru. Chemix Guru Ltd.
Omar, J.; Ponsford, D.; Dreiss, C. A.; Lee, T.; Loh, X. J. Supramolecular Hydrogels: Design Strategies and Contemporary Biomedical Applications. Chemistry – An Asian Journal 2022, 17 (9).
DNA-Intercalating Supramolecular Hydrogels for Tunable Thermal and Viscoelastic Properties https://onlinelibrary.wiley.com/doi/10.1002/anie.202411115