# Designing DNA Intercalating Supramolecular Hydrogels with Tunable Structural Properties

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## Introduction to DNA Hydrogels

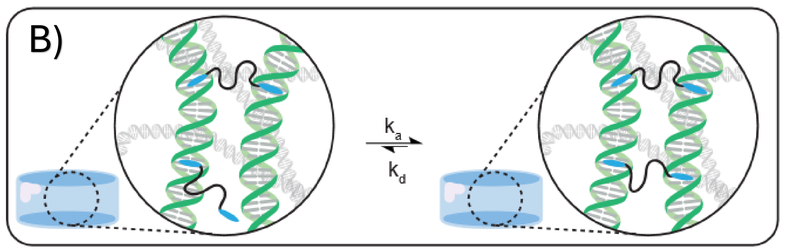
DNA intercalating supramolecular hydrogels (DISHs) are dynamic materials formed through reversible binding of small molecule intercalators to DNA. Intercalation serves as the primary cross-linking mechanism, enabling the formation of 3D networks under aqueous conditions. The thermodynamic balance of enthalpic (ΔH) and entropic (ΔS) contributions governs network formation and material properties, allowing hydrogel behavior to be tuned through crosslinker design.

A) $[\text{DNA}][\text{DNA}] \underset{k_d}{\overset{k_a}{\rightleftharpoons}} [\text{DNA}]$

$$K_{eq} \alpha\, G' \alpha\, \exp\left(-\frac{\Delta H}{RT} - \frac{\Delta S}{R}\right)$$

$$K_{eq} = \frac{[\text{DNA}]}{[\text{DNA}][\text{DNA}]}$$

***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.*




Intercalation is the reversible insertion of planar aromatic molecules between DNA base pairs and serves as the primary cross-linking mechanism in these materials. The balance of enthalpic and entropic contributions determines binding affinity and, in turn, network structure and mechanical response. By selecting intercalators with distinct thermodynamic signatures, gelation behavior can be tuned, enabling control over the thermal and mechanical properties of the resulting hydrogel.

## Rheology

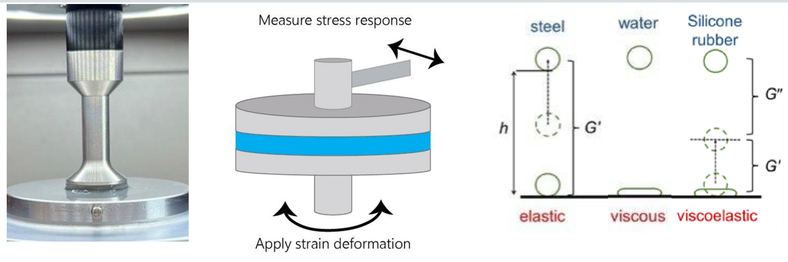



***Figure 2.*** *The properties of a viscoelastic material under rheological conditions.*

Rheology was used to characterize the viscoelastic behavior of DNA intercalating supramolecular hydrogels (DISHs) as a function of temperature, frequency, and ionic conditions. The storage modulus (G') reflects elastic energy storage within the network, while the loss modulus (G") represents viscous energy dissipation. The relative contributions of G' and G" provide insight into network structure and dynamics, enabling direct assessment of how intercalation thermodynamics influences hydrogel mechanics.

## Entropically-driven Gelation of Acridine DISHs

Acridine-based DISHs exhibit entropy-driven binding that produces thermal stiffening. Systematic variation of buffer, salt concentration, and ion type was used to directly tune network stability and mechanical response.

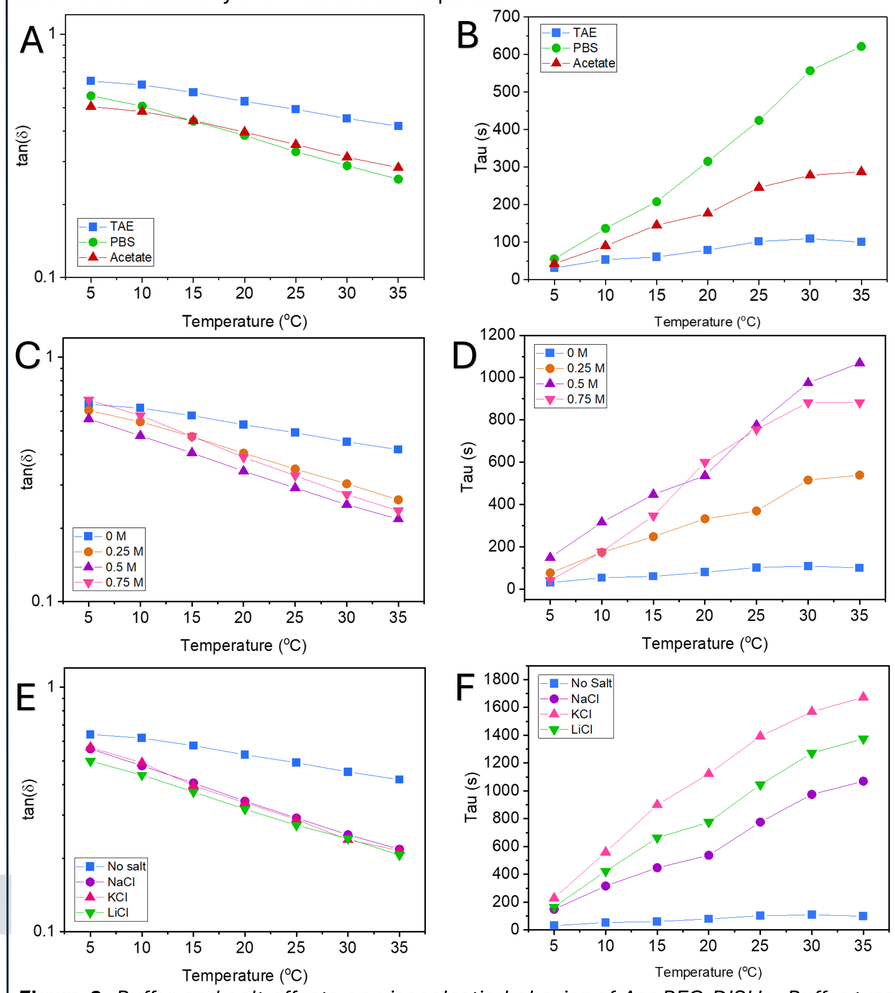



***Figure 3.*** *Buffer and salt effects on viscoelastic behavior of Acr-PEG DISHs. Buffer type (A,B), salt concentration (C,D), and salt type (E,F) were varied. (A,C,E) Tan(δ) as a function of temperature at 1 rad/s. Increasing ionic strength enhances elasticity at elevated temperatures. (B,D,F) Relaxation time (τ) obtained from Kohlrausch–Williams–Watts fits. τ increases with temperature and ionic strength.*

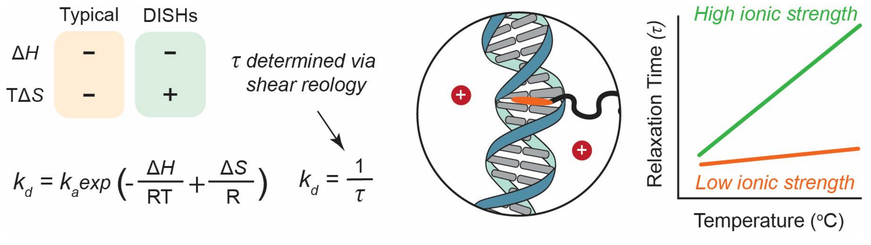



**Figure 4. I***onic strength and ion type modulate the thermodynamics of acridine-based DISHs, increasing relaxation time (τ) and enhancing network elasticity at elevated temperatures.*

## Photo Cross-linking of Psoralen DISHs

Psoralen-based DISHs exhibit dynamic behavior including shear thinning and self healing. Upon UVA exposure, psoralen undergoes a [2+2] cycloaddition with thymine, enabling covalent cross linking. Here, this photoreaction is used to convert dynamic networks into mechanically robust DNA hydrogels for cell culture.

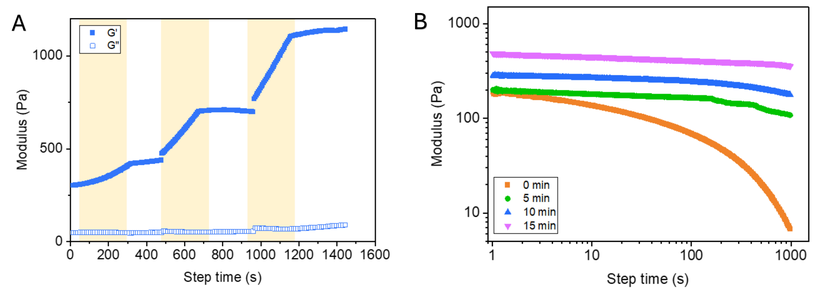



***Figure 4.*** *UVA induced photo cross linking drives modular stiffening and transition to permanent network formation. (A) Time sweep showing increasing storage modulus (G') during intermittent UV exposure (shaded regions). (B) Stress relaxation profiles demonstrate reduced relaxation with increasing UV exposure.*

## 2D Cell Culture of Psoralen DISHs

Human dermal fibroblasts (HDFs) were cultured on fibronectin-coated psoralen DISHs. Cells adhered and spread over time, demonstrating that the hydrogels support cell attachment and proliferation.

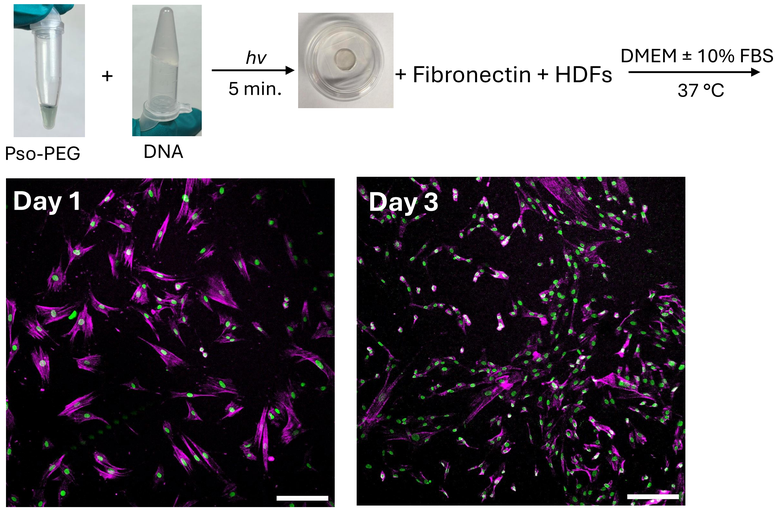



**Figure 5.** *Confocal fluorescence micrographs of HDFs cultured on Pso-PEG DISHs at Day 1 and Day 3. Cells exhibit adhesion and spreading over time. Nuclei stained with anti-lamin B1 and actin with phalloidin 647. Scale bar: 200 μm.*

## Future Work

We will also continue to probe the psoralen-based DISHs to improve cell adhesion and begin degradation studies to confirm our network is biodegradable.

## Acknowledgements

Thank you to the UNH chemistry department, Wynter Paiva, Matthew Currier, Patrick Strobel, Lola Fadairo, Zahraa Albeshir, Noelle Honan, Sam Ashooh, Lauren Barnes, Pearson Franz, Kaelin Marshall, Laura Hills, Pat Stone.

https://www.natolab.com/

References

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