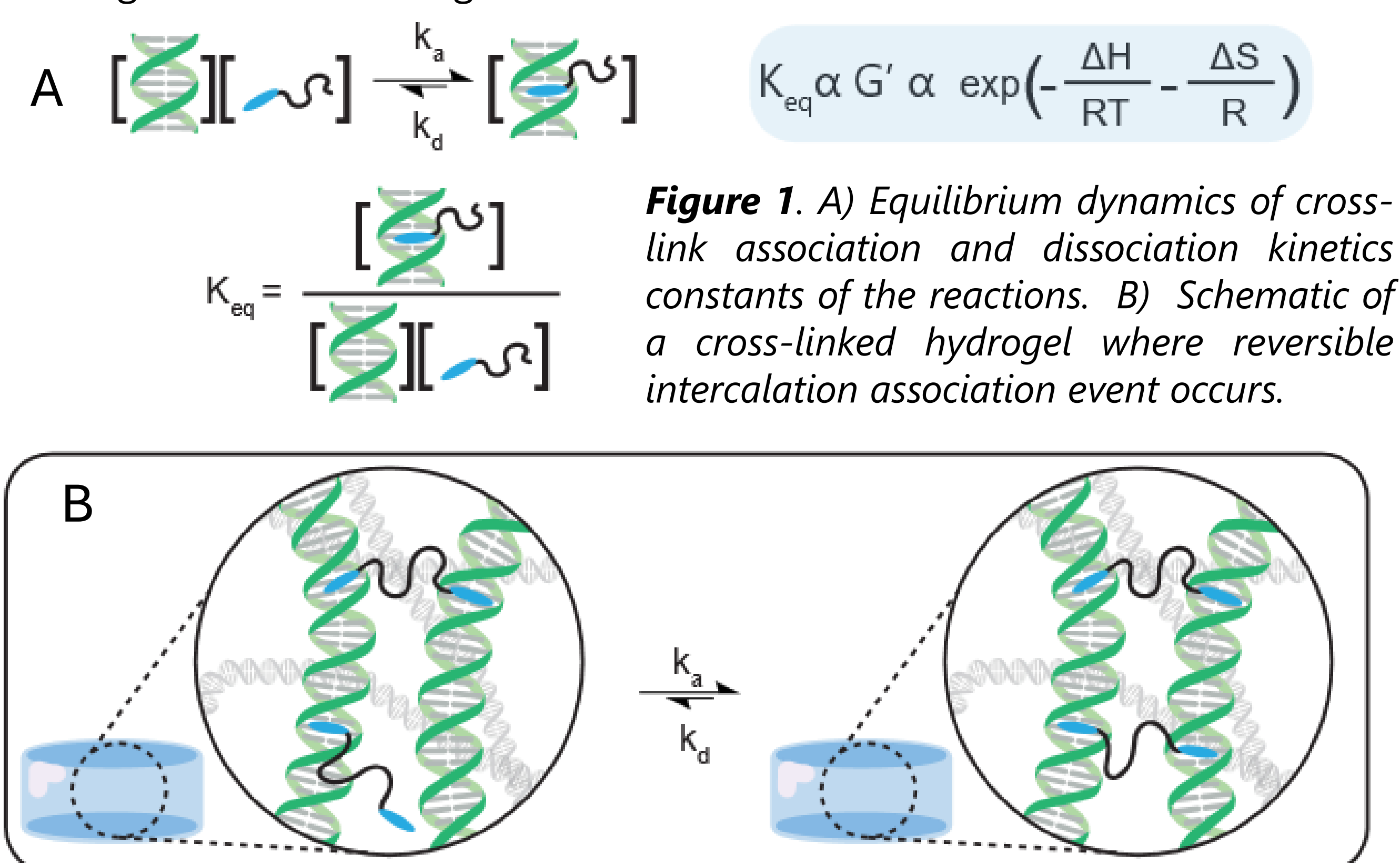


## Introduction to DNA Hydrogels

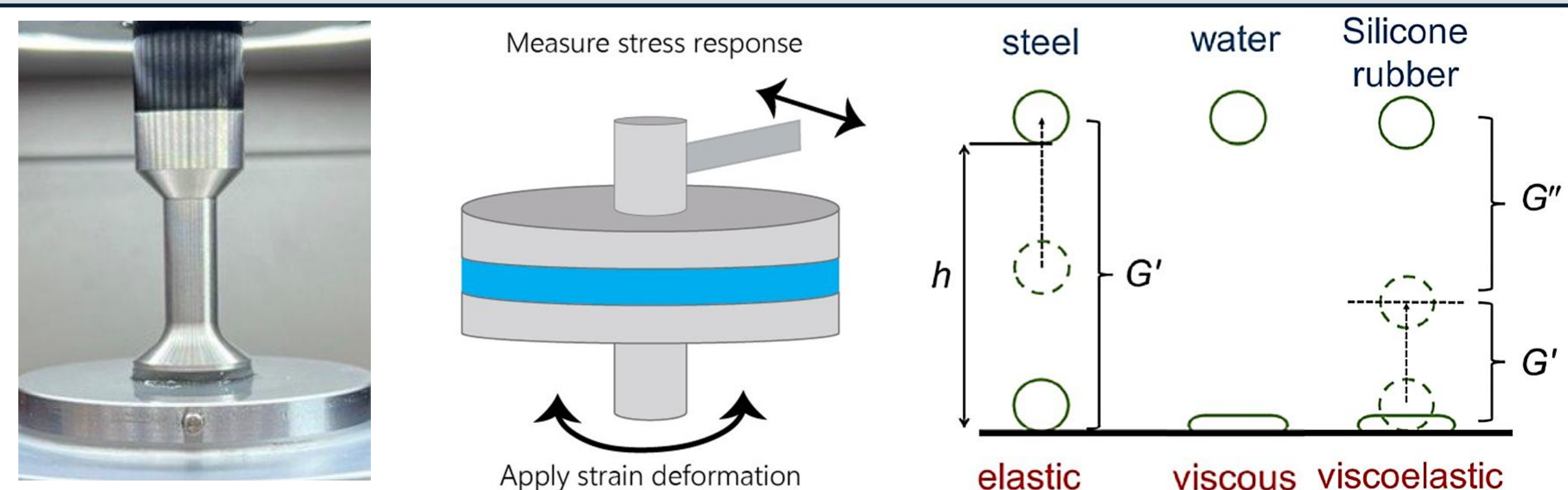
DNA intercalating supramolecular hydrogels (DISHs) are dynamic materials formed through reversible binding of small molecule intercalators to DNA. Intercalation serves as the primary cross-linking mechanism, enabling the formation of 3D networks under aqueous conditions. The thermodynamic balance of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



**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

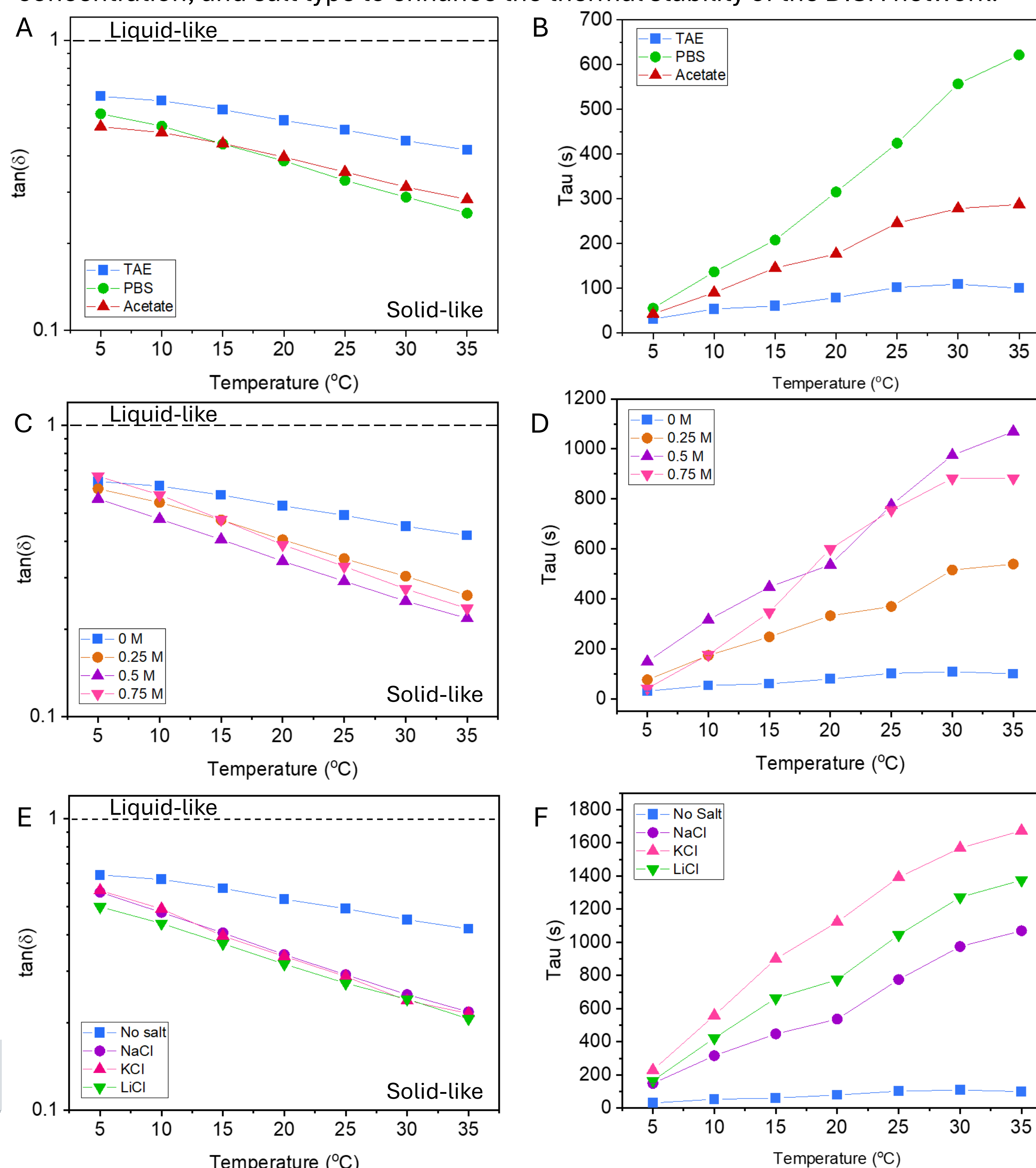


**Figure 2.** The properties of a viscoelastic material under rheological conditions.

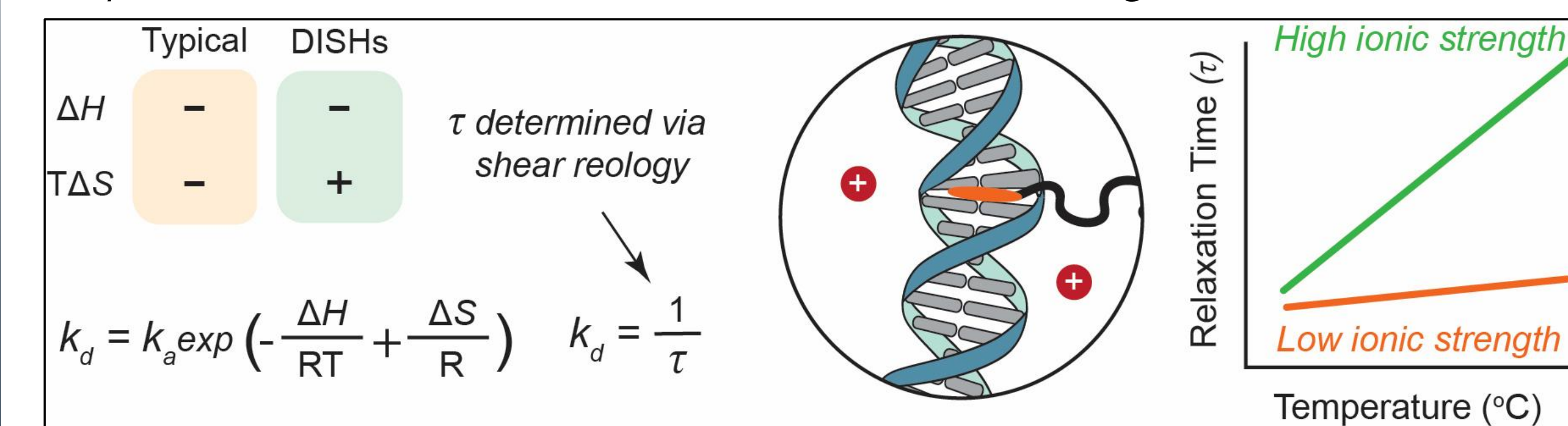
Rheology was used to characterize the viscoelastic behavior of DNA intercalating supramolecular hydrogels (DISHs) as a function of temperature, frequency, and ionic conditions. The storage modulus ( $G'$ ) reflects elastic energy storage within the network, while the loss modulus ( $G''$ ) represents 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 showed entropically-driven binding, exhibiting thermal stiffening behavior. To further explore this, we utilized strategic selection of buffer, salt concentration, and salt type to enhance the thermal stability of the DISH network.



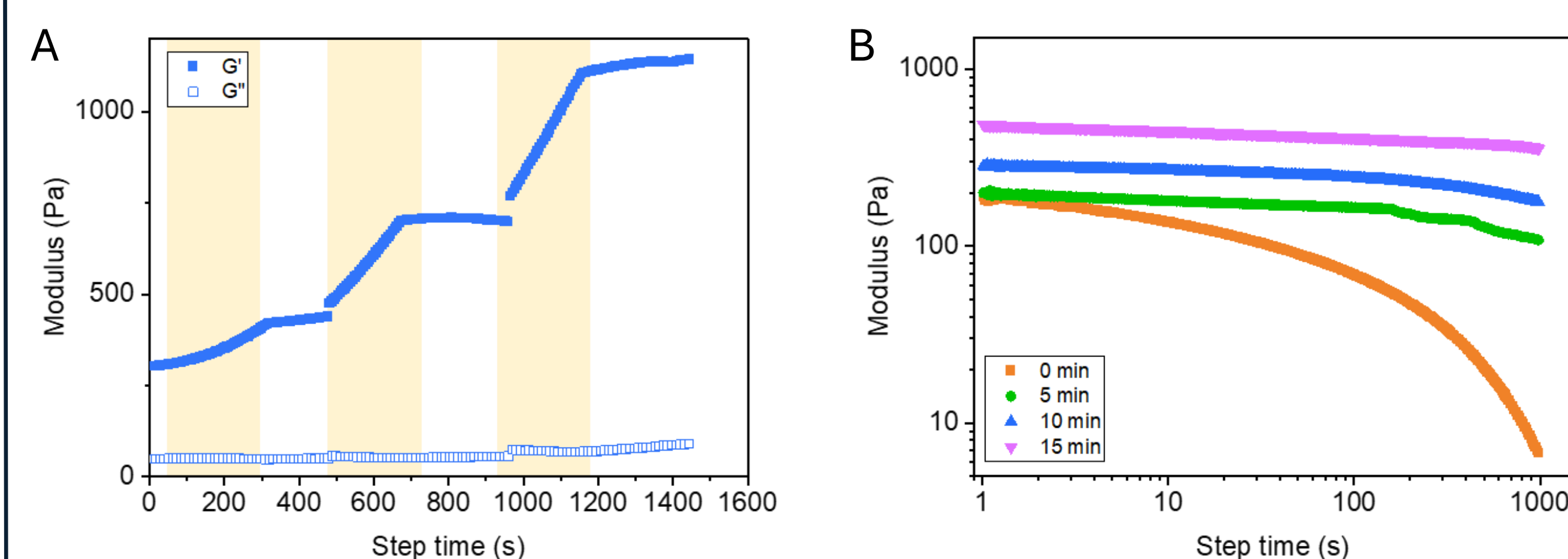
**Figure 3.** Buffer and salt effects on viscoelastic behavior of Acr-PEG DISHs under varying conditions: buffer type (A,B), salt concentration (C,D), and salt type (E,F). (A,C,E)  $\tan(\delta)$  as a function of temperature at 1 rad/s. Across all systems, increasing ionic strength led to enhanced elasticity at elevated temperatures. (B,D,F) Relaxation time ( $\tau$ ) was calculated from the Kohlrausch-William-Watts function stress relaxation fit. As temperature increased,  $\tau$  increased with enhanced ionic strength.



**Figure 4.** Acridine-peg DISHs have unique entropically driven behaviors that can be tuned with an increase in ionic strength.

## Photo Cross-linking of Psoralen DISHs

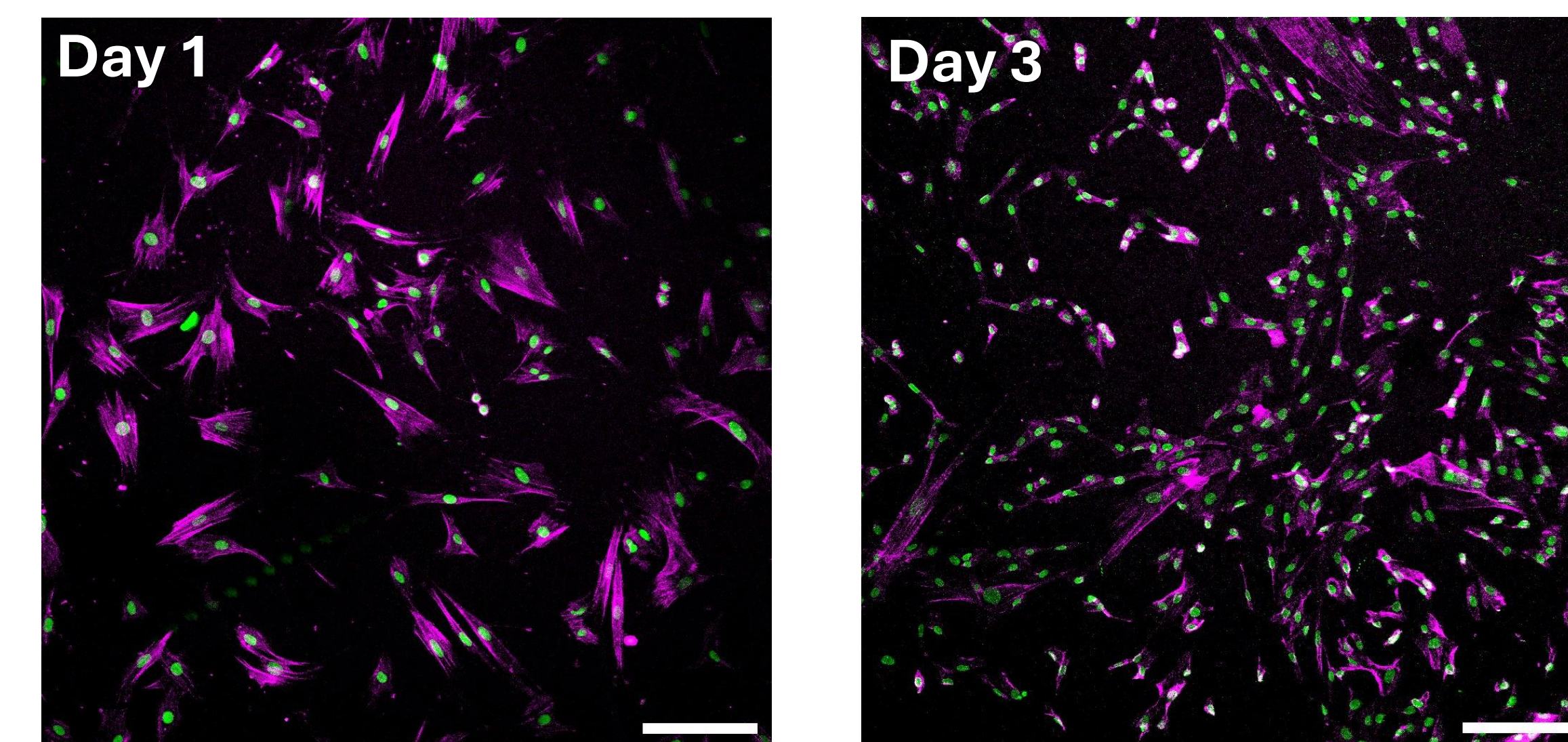
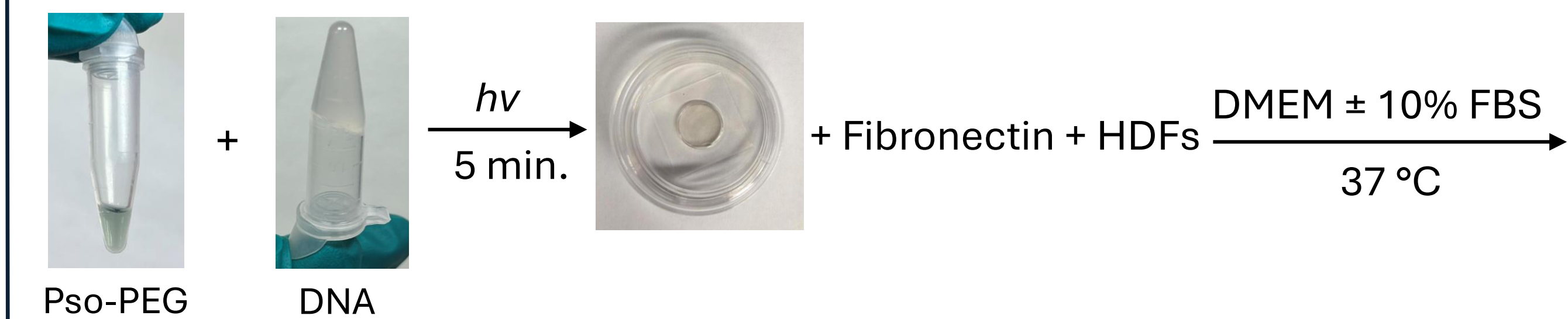
Psoralen-based DISHs exhibit dynamic behavior including shear thinning and self healing. Upon UVA exposure, psoralen undergoes a [2+2] cycloaddition with thymine, enabling covalent cross linking. Here, this photoreaction is used to convert dynamic networks into mechanically robust DNA hydrogels for cell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



**Figure 5.** Modular stiffening and transition to permanent network formation via UVA-induced photo-crosslinking. A) Time-sweep rheology showing the storage modulus ( $G'$ ) and loss modulus ( $G''$ ) during intermittent UVA exposure (shaded regions). B) Stress relaxation of Pso-PEG DISHs at various UV exposure intervals.

## 2D Cell Culture of Psoralen DISHs

Human Dermal Fibroblasts (HDFs) were seeded on the fibronectin-coated psoralen DISH to promote enhanced cell proliferation. The cells were able to adhere and spread, indicating the DNA-based hydrogel provides a cytocompatibility environment that supports the proliferation of primary human cells



**Figure 6.** 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  $\mu\text{m}$ .

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