



Using DNA-Protein Hydrogels to Study T-cell Activation Parameters Based on Microfluidics

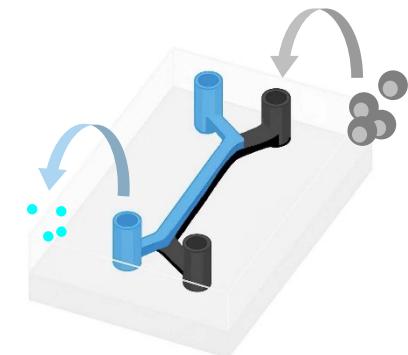
12313205 Aochen Dengzhang

12212860 Yushan Wei

12212859 Sijie Li

12313254 Ruirui Zhang

12212502 Xiangtao Shi



Content

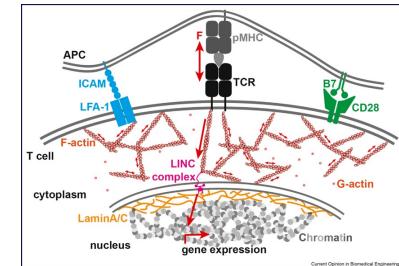
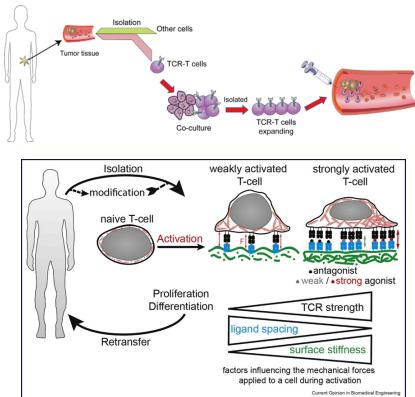


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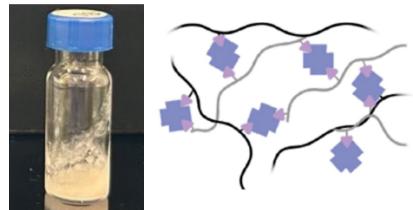
Background

Aochen Dengzhang



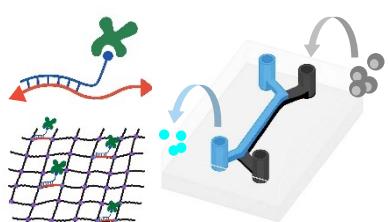
Material

Yushan Wei



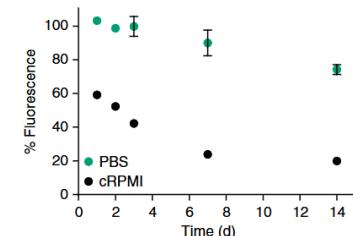
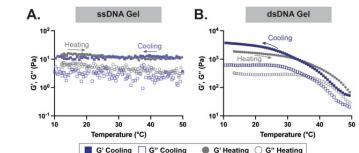
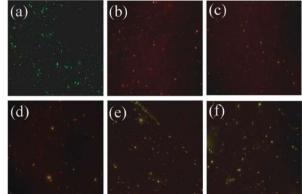
Design

Sijie Li



Characterization

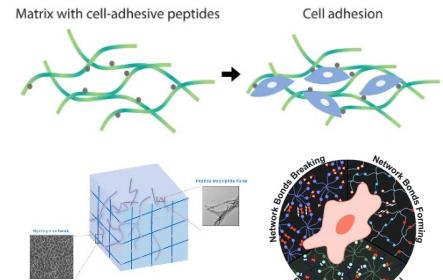
Ruirui Zhang



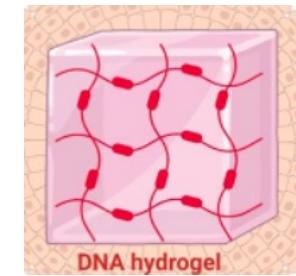
Discussion

Xiangtao Shi

Traditional Hydrogels



Hybrid DNA Hydrogels

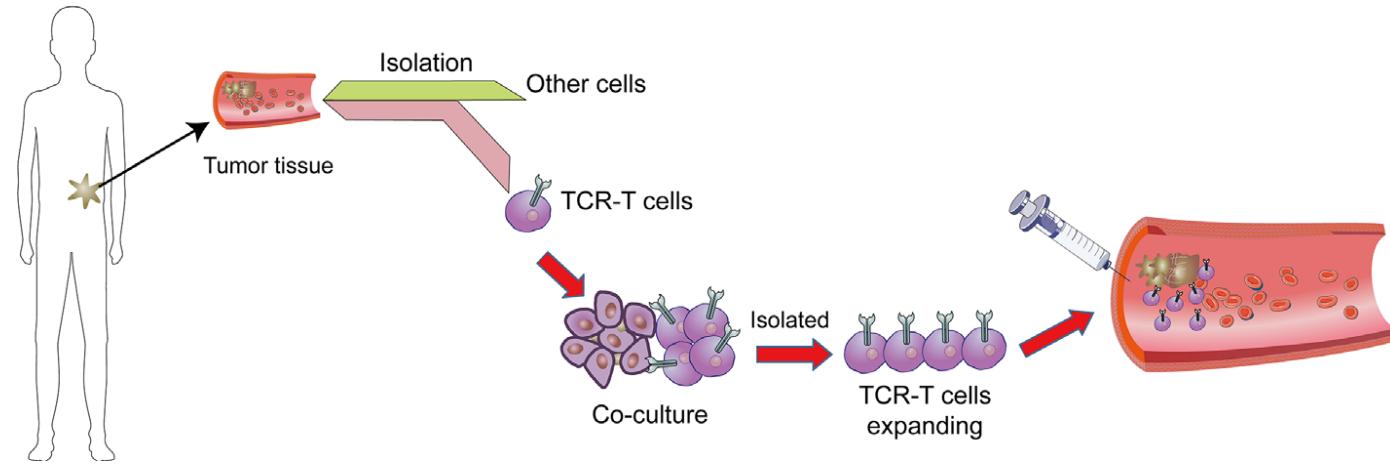


Background



➤ Three Signals for T-Cell Activation:

- *T-cell Receptor (TCR) Stimulation*
- *Costimulation*
- *Prosurvival Cytokines*



➤ In the body, these signals are provided by

antigen presenting cells (APCs).

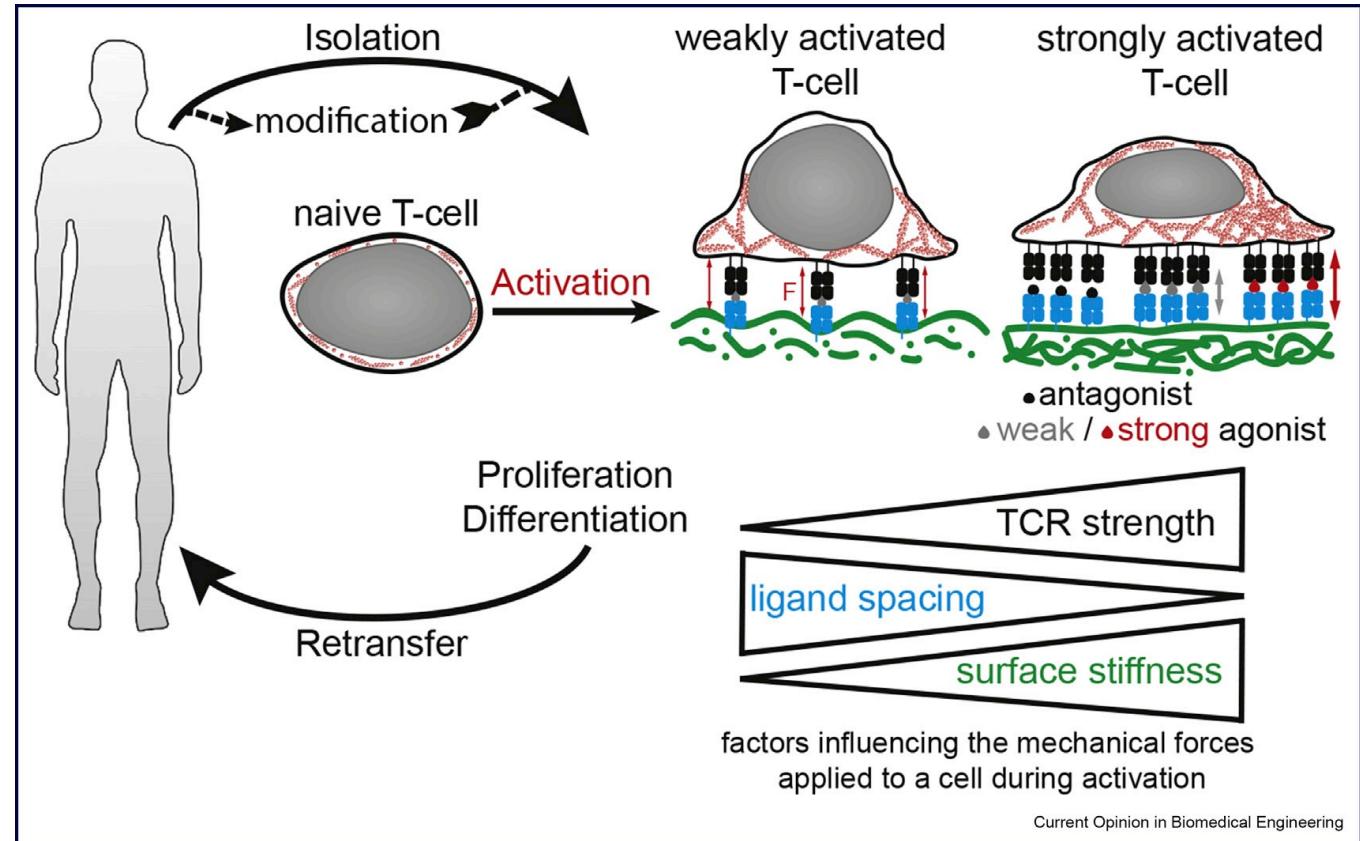
➤ In T-cell therapy, the cells need to be **activated** to exert their full therapeutic potential.

Annual Review of Immunology, 2009, 27, 591-619

Background



- In addition to the three well-described signals, **mechanical forces** strongly affect T-cell activation.
- There are various factors influencing the mechanical forces applied to a cell during activation:
 - **TCR Signal (pMHC)**
 - **Ligand Spacing**
 - **Stiffness of the Activating Surface**



Study Objectives



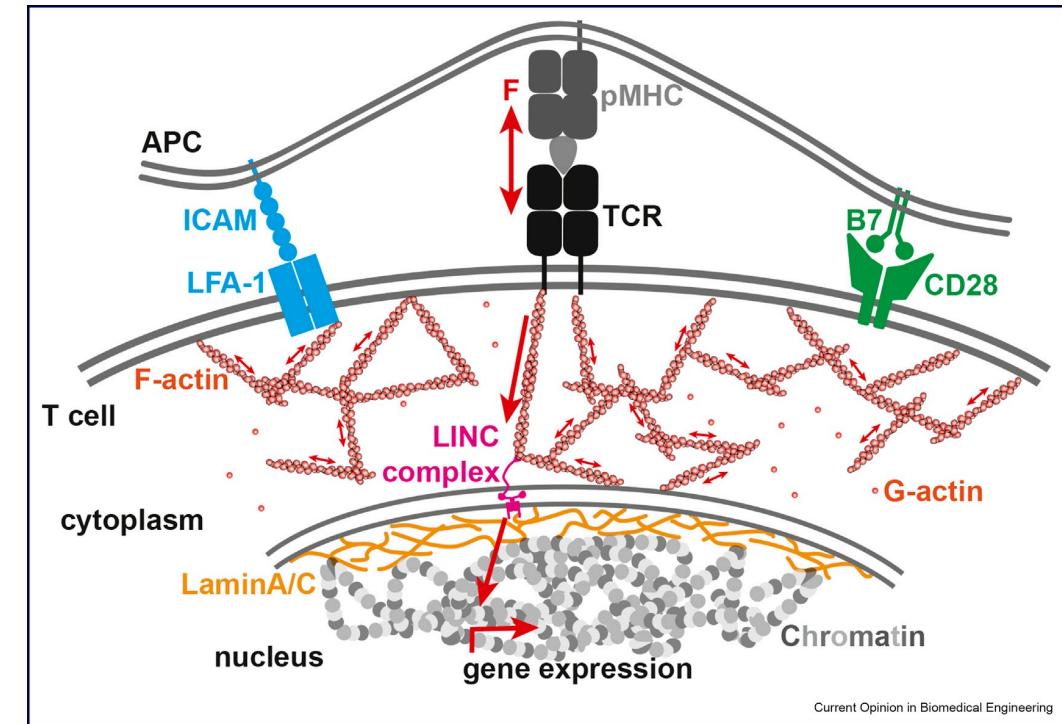
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1. Optimize T-cell Activation In Vitro

- Study optimal parameters of
 - *Antigen Density*
 - *Stiffness Effects*

2. Enable In Vivo Translation



Current Opinion in Biomedical Engineering, 2019, 10, 134–141

Content

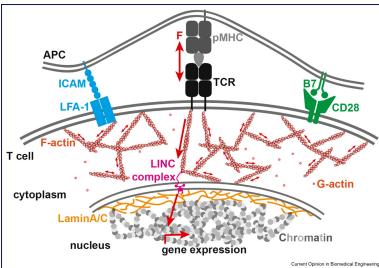
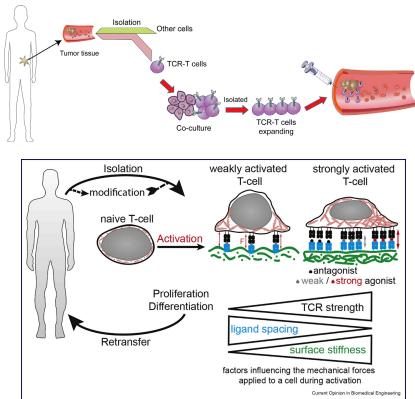


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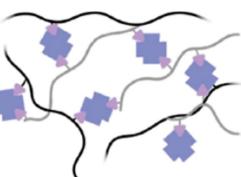
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Aochen Dengzhang



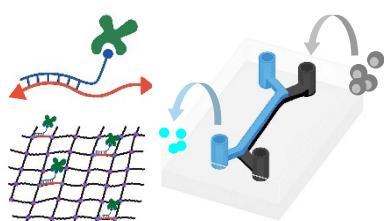
Material

Yushan Wei



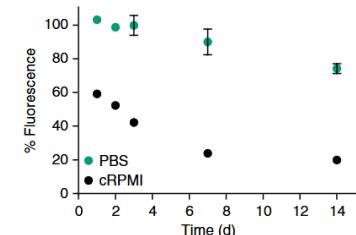
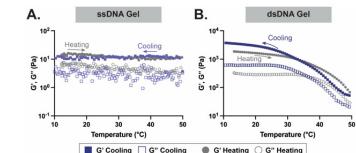
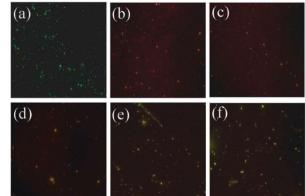
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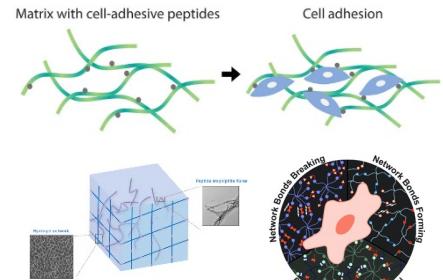
Ruirui Zhang



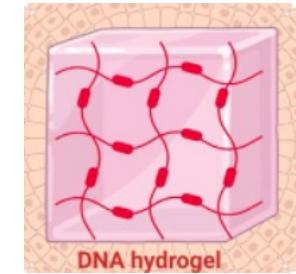
Discussion

Xiangtao Shi

Traditional Hydrogels



Hybrid DNA Hydrogels



Protein-DNA Hydrogel



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Article

Leveraging Protein–Ligand and DNA Interactions to Control Hydrogel Mechanics

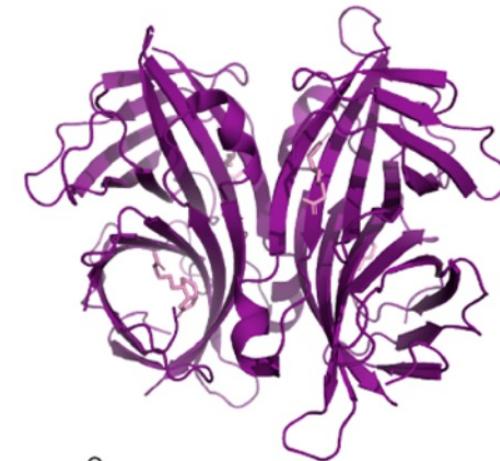
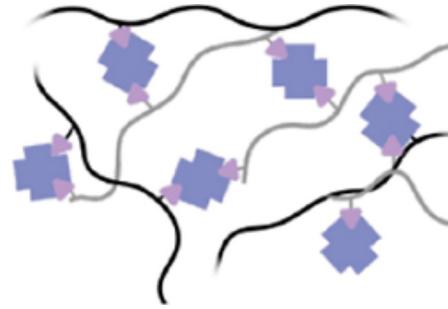
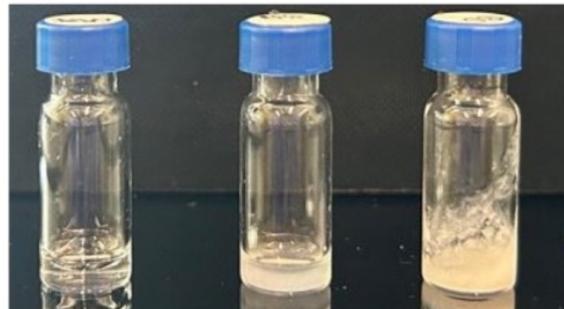
Namrata Ramani, Jeongmin Hwang, Alex J. Anderson, Jennifer Delgado, Laura Hernández-López, C. Adrian Figg, Peter H. Winegar, and Chad A. Mirkin*



Cite This: <https://doi.org/10.1021/jacs.5c03523>



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Protein-DNA Hydrogel



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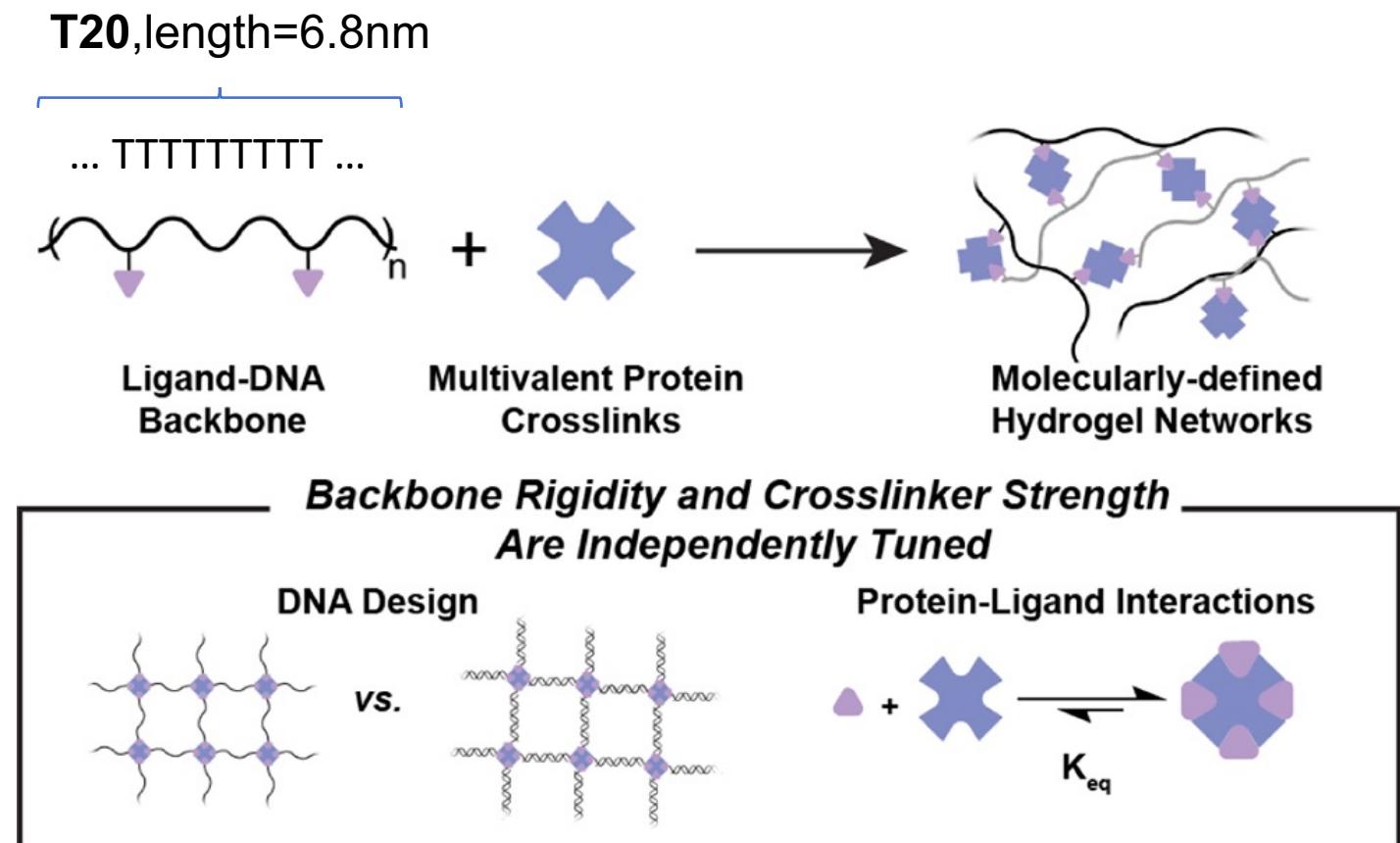
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□ The structure of protein-DNA hydrogel

- *Network-structured hydrogel*
- *Ligand-modified DNA strands as the backbone*
- *Proteins as crosslinking nodes*

□ Factors that can change protein-DNA hydrogel's mechanical properties

- *Protein-ligand types*
- *Temperature*
- *DNA sequences*
- *Ligand density*



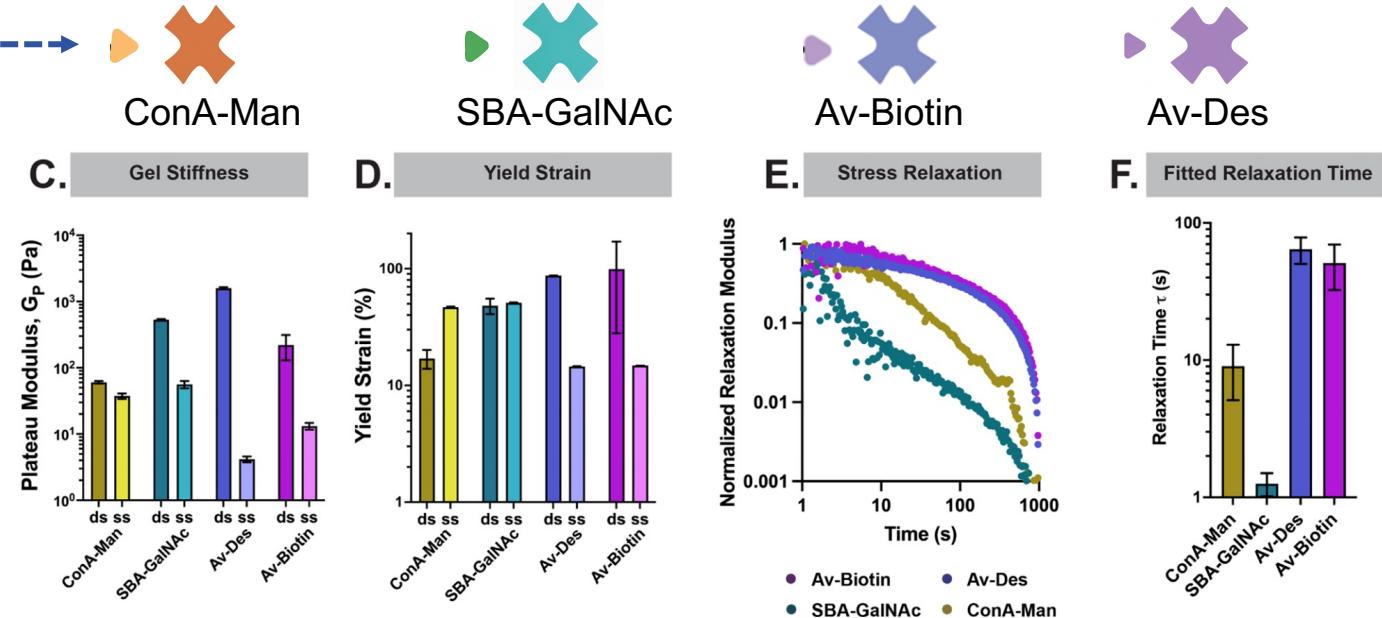
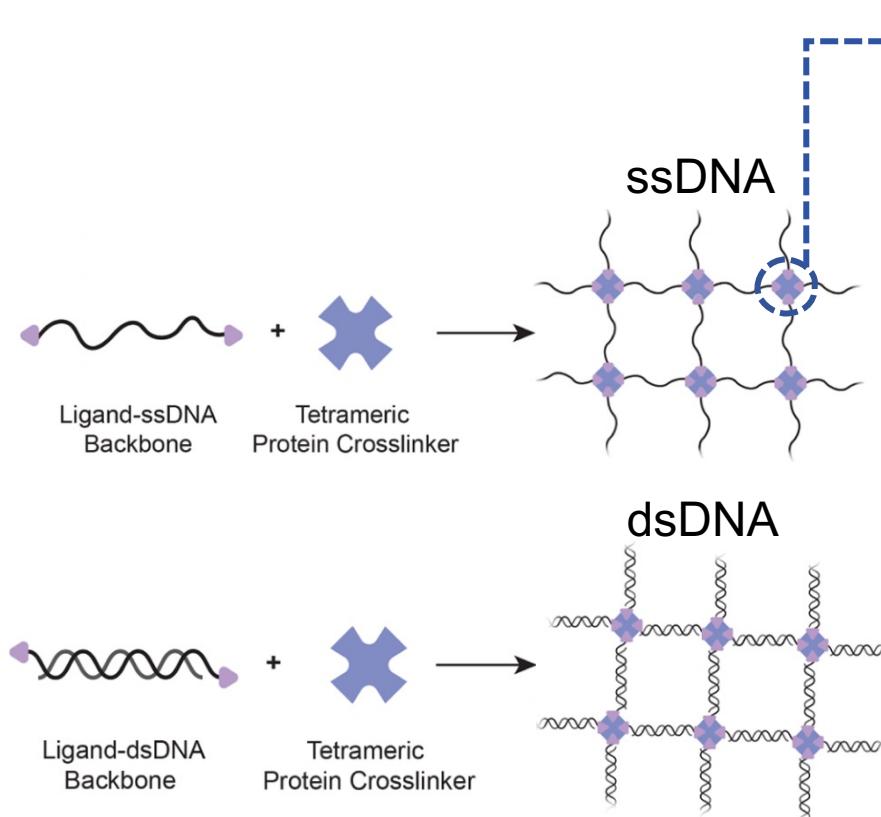
J. Am. Chem. Soc. **2025**, 147, 17293–17302

Protein-ligand Pair



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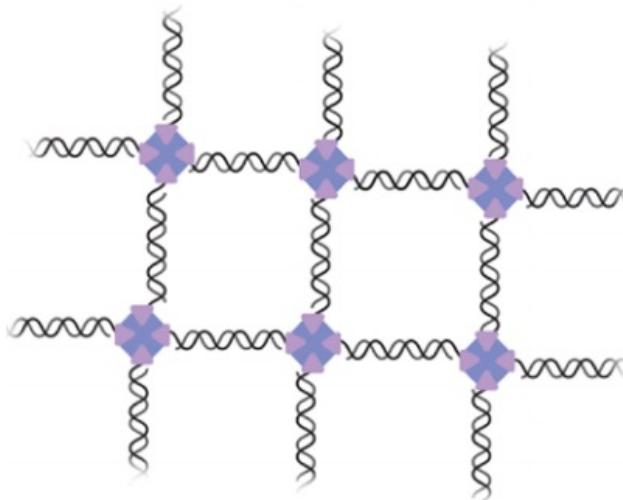
The greater the affinity between the ligand and the protein, the higher the stiffness of the hydrogel.

Temperature

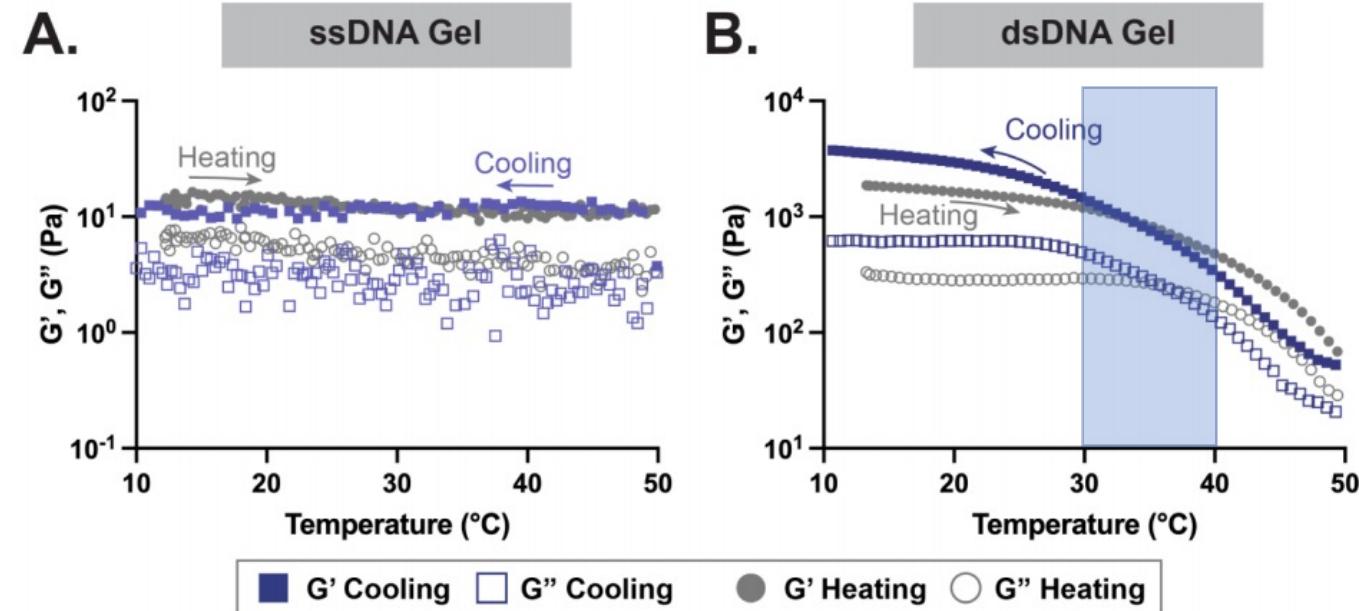


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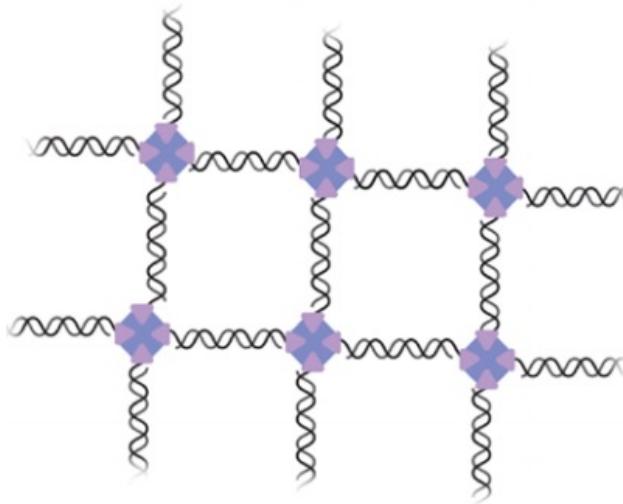
As **temperature increases**, the double strands in dsDNA tend to unwind, reducing stability and leading to a **decrease in hydrogel stiffness**.

Introducing Mismatches

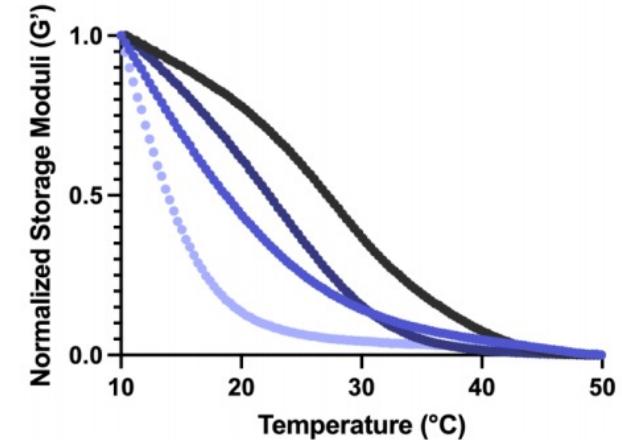


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Complementary DNA Strand Design			
●	A20		AAAAAAAAAA
●	$T_m = 48.8 \text{ } ^\circ\text{C}$		
●	Mismatch 1 (M1)		AAAAAGAAAA
●	$T_m = 41.4 \text{ } ^\circ\text{C}$		
●	Mismatch 2 (M2)		AAAGAAAAGAA
●	$T_m = 33.0 \text{ } ^\circ\text{C}$		
●	Mismatch 3 (M3)		AGAAGGAAGA
●	$T_m = 26.3 \text{ } ^\circ\text{C}$		



Replacing some A bases in the DNA strand with G reduces the binding strength of the double-stranded DNA. The **more mismatched bases**, the **lower the stiffness** of the hydrogel.

Content

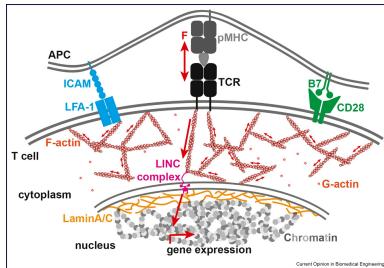
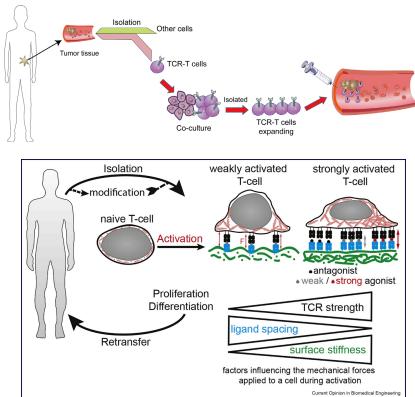


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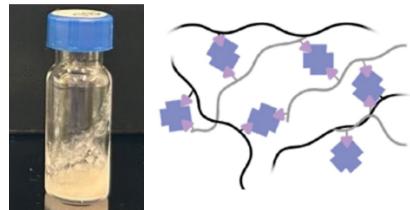
Background

Aochen Dengzhang



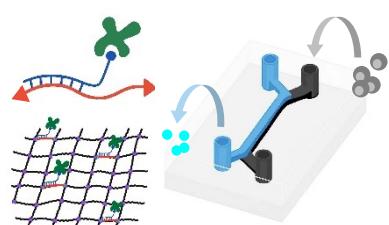
Material

Yushan Wei



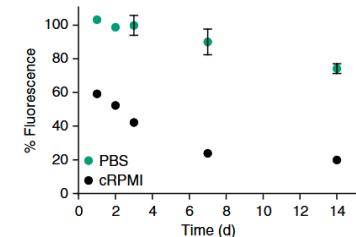
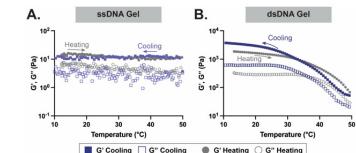
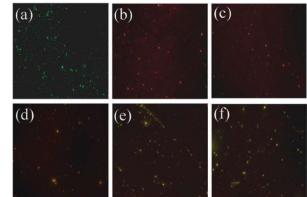
Design

Sijie Li



Characterization

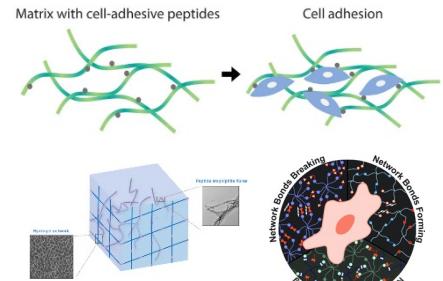
Ruirui Zhang



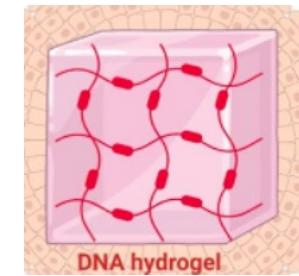
Discussion

Xiangtao Shi

Traditional Hydrogels



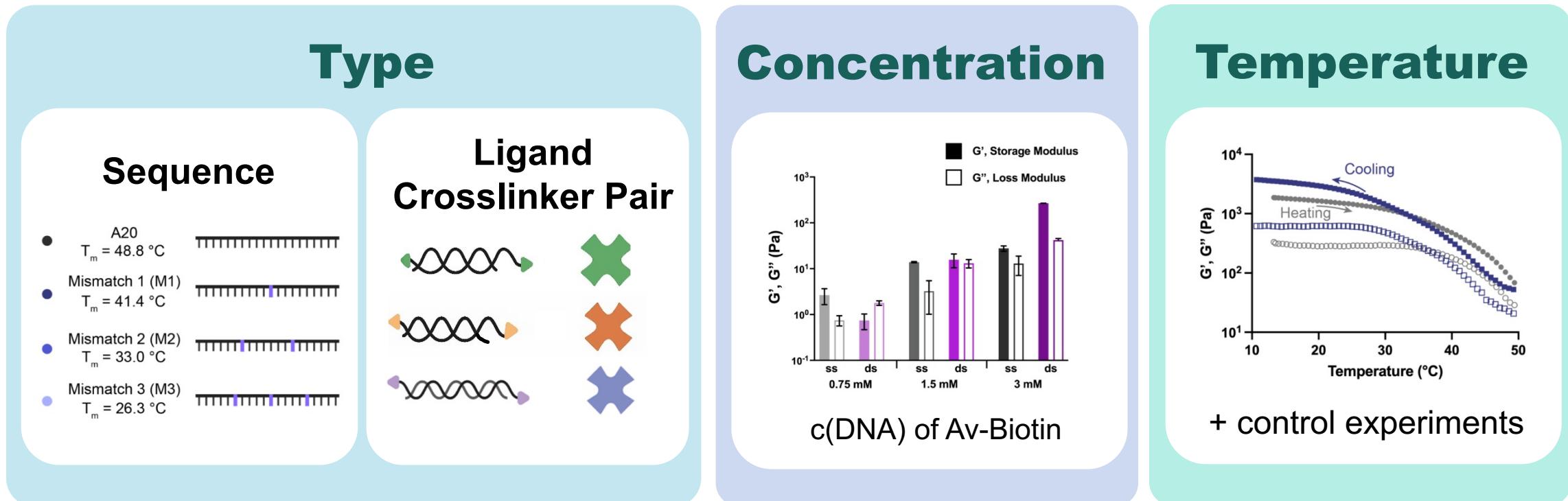
Hybrid DNA Hydrogels



Hydrogel Design



➤ Hydrogel Stiffness (G' , G'')

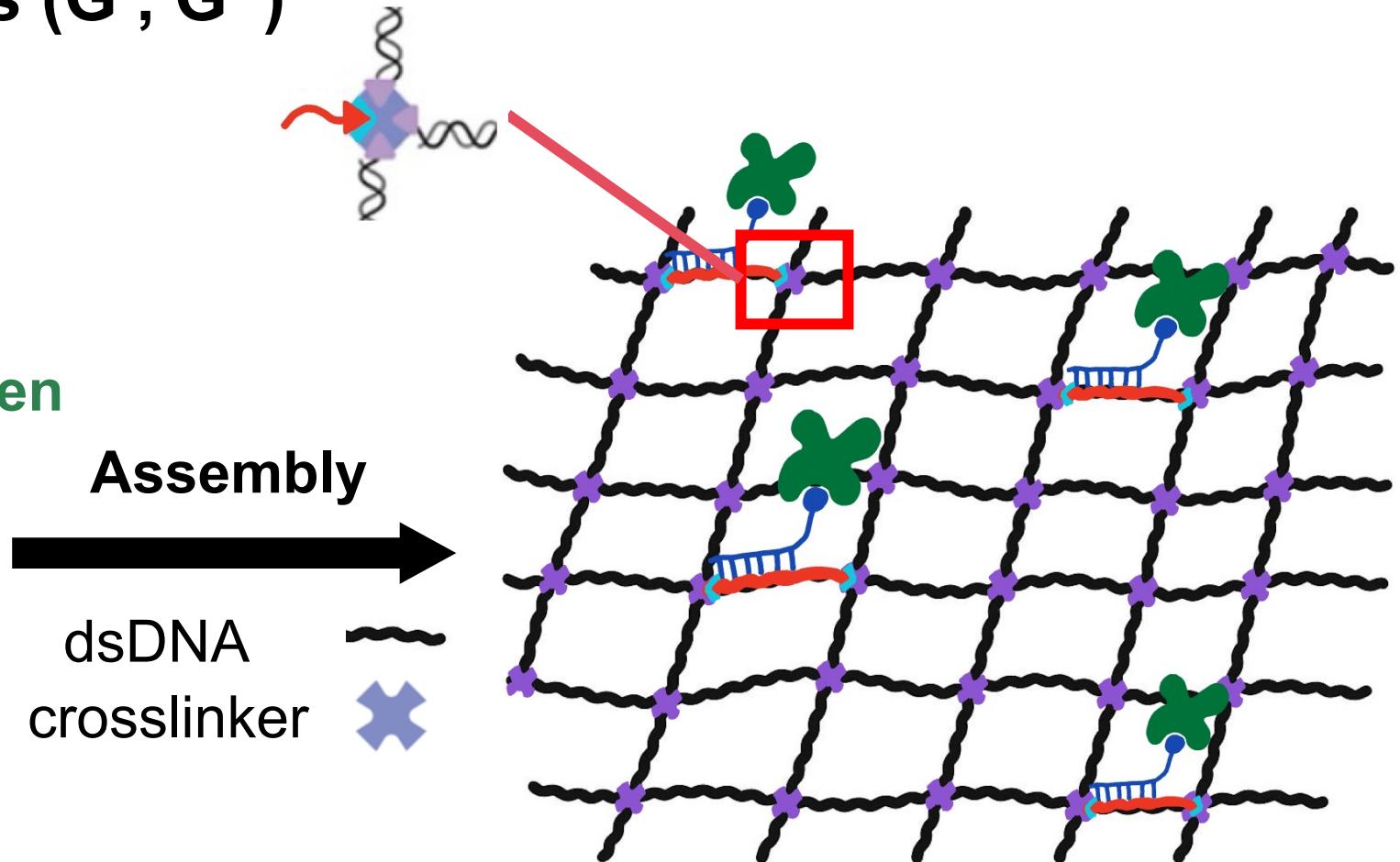
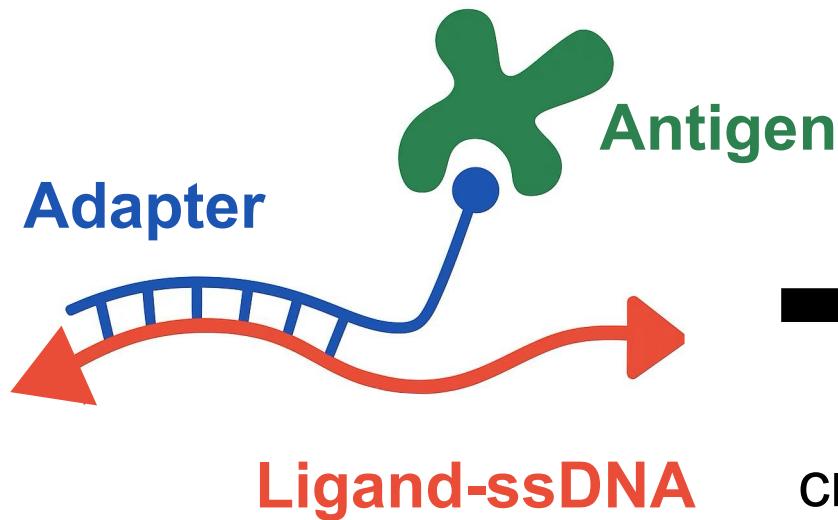


➤ Antigen Density

Hydrogel Design



- Hydrogel Stiffness (G' , G'')
- Antigen Density



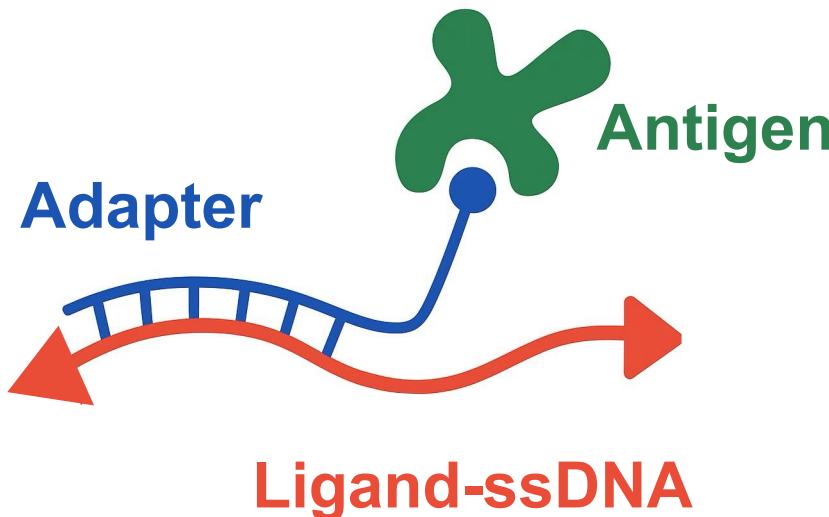
Hydrogel Design



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- Hydrogel Stiffness (G' , G'')
- Antigen Density



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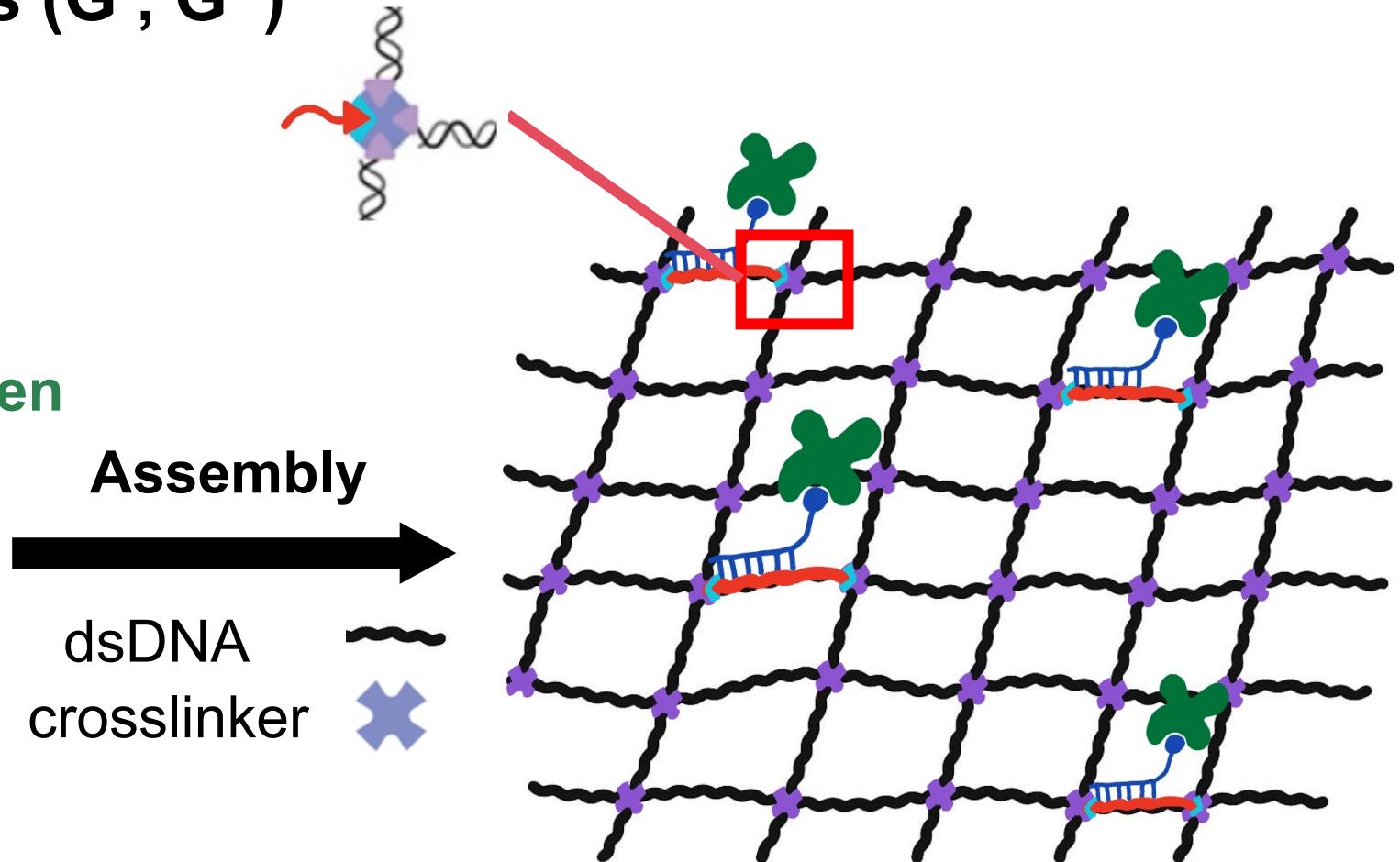
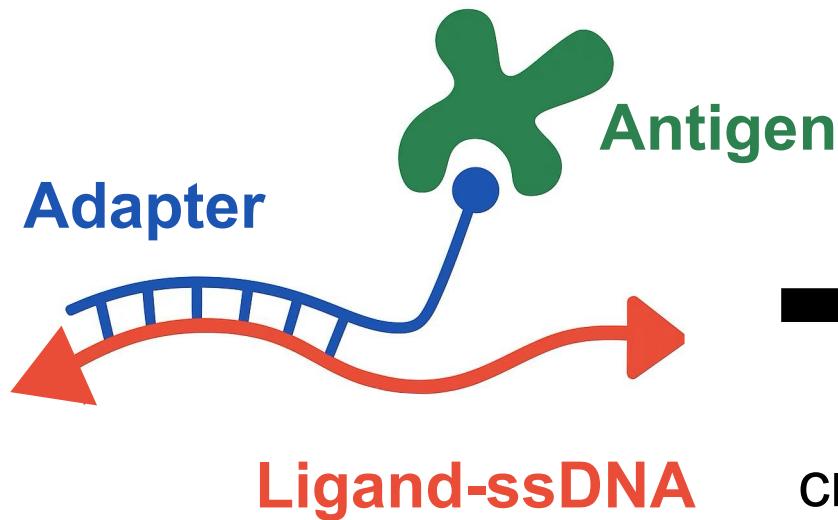
Oligo Design & Handling OligoAnalyzer™ Tool Analyzer definitions

CRISPR Genome Editing UNAFold Tool

Hydrogel Design



- Hydrogel Stiffness (G' , G'')
- Antigen Density

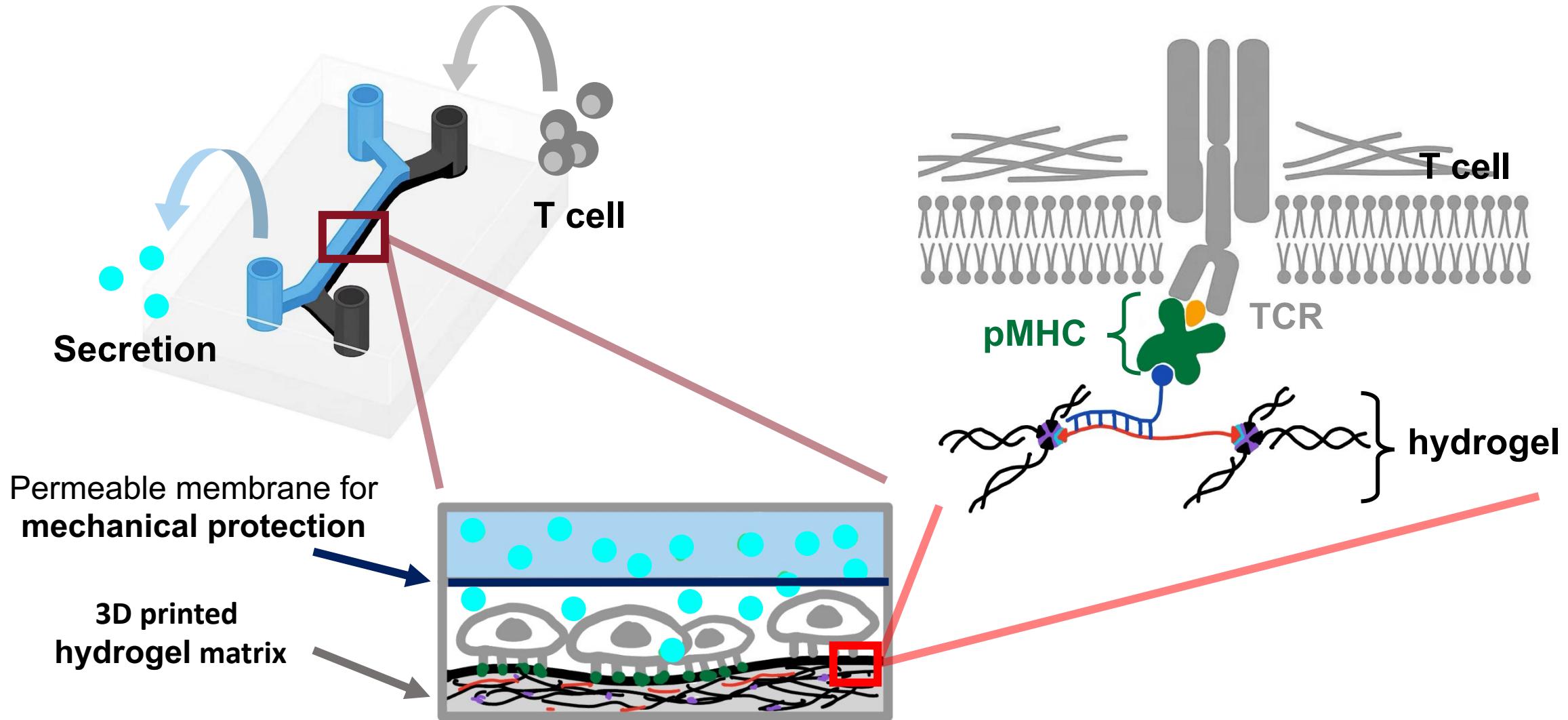


Microfluidic Design



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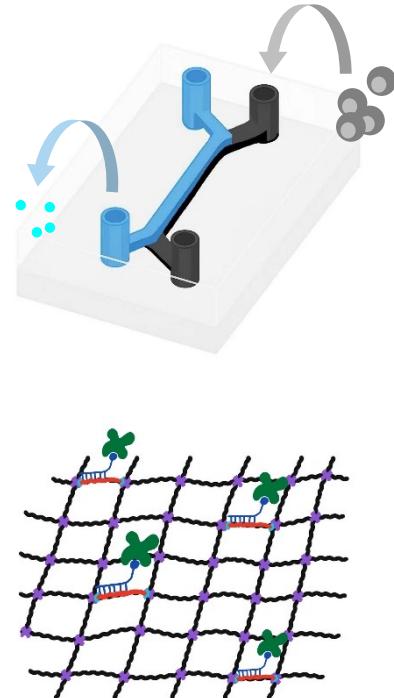


Microfluidic Design



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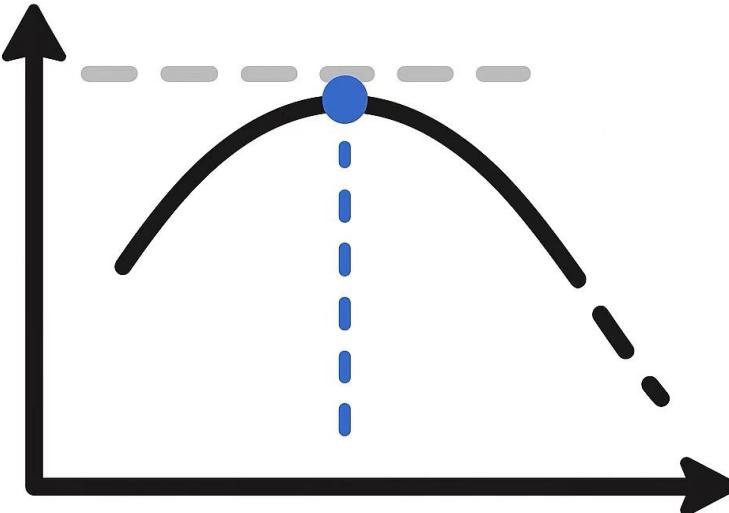


Quantitatively
measure



T cell activity

- Proliferation rate
- Cytokine production
- ...



- Hydrogel Stiffness
- Antigen Density

Content

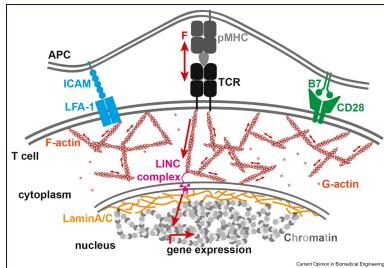
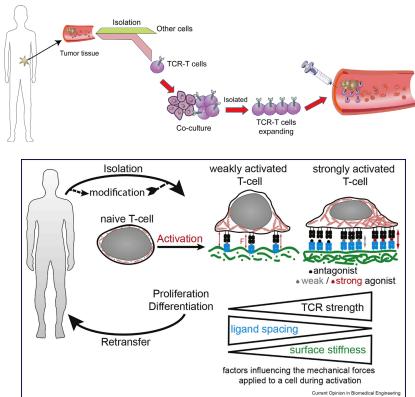


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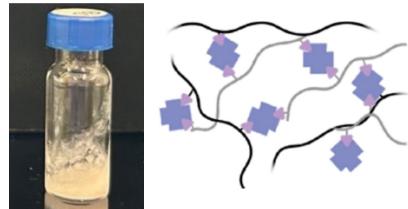
Background

Aochen Dengzhang



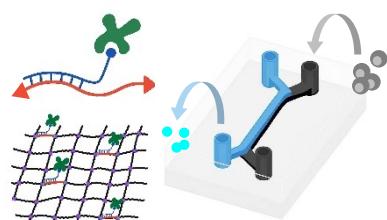
Material

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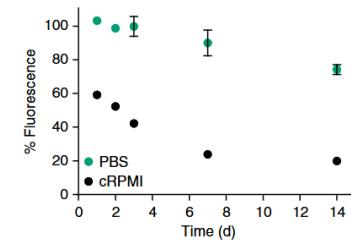
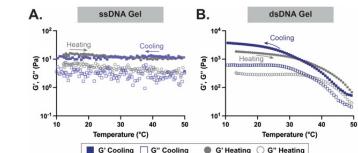
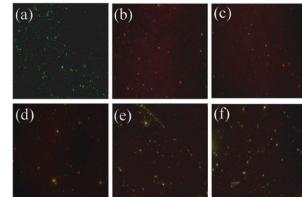
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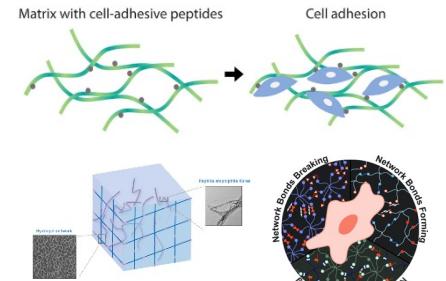
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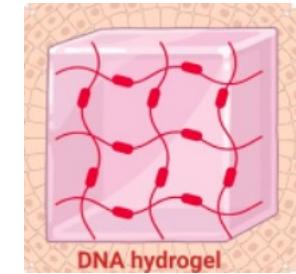
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Hybrid DNA Hydrogels



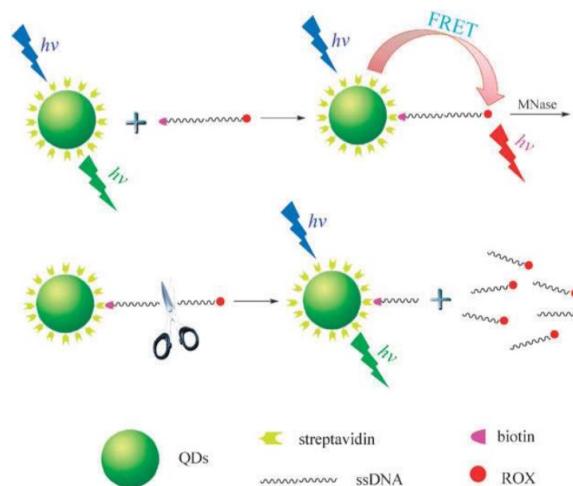
Antigen Density



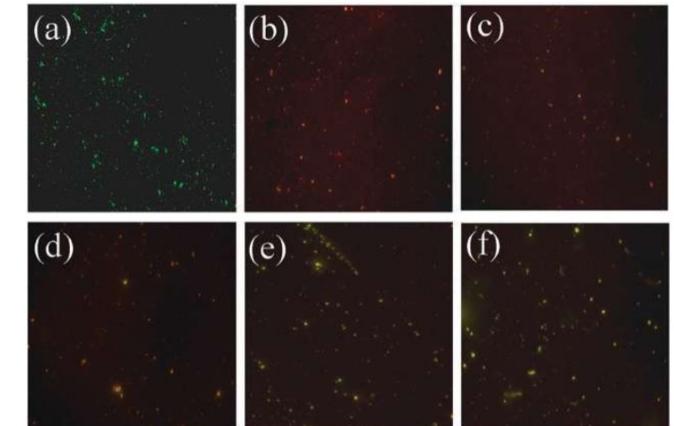
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- Self-test:
 - Verify the density of the antigen
 - conjugation of DNA or proteins and antigen
- Characterization methods:
 - Quantum dots - FRET
- Measurand:
 - The degree of binding between antigen and DNA or proteins



Scheme 1 The principle of MNase detection using our QDs–ssDNA–fluorescent dye conjugate bioprobes.



Chem. Commun., 2008, 5990–5992

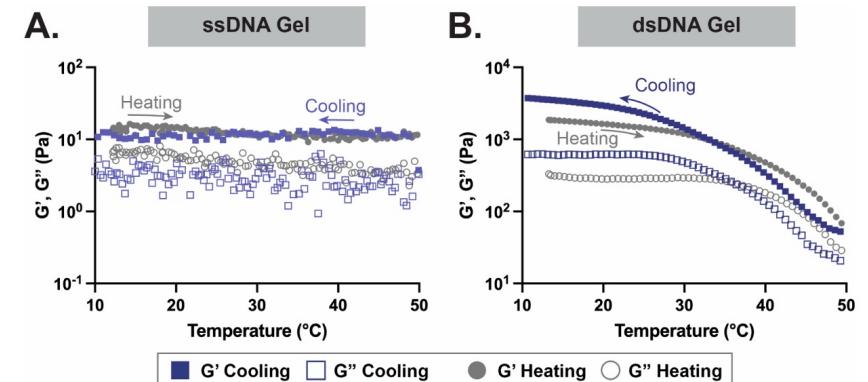
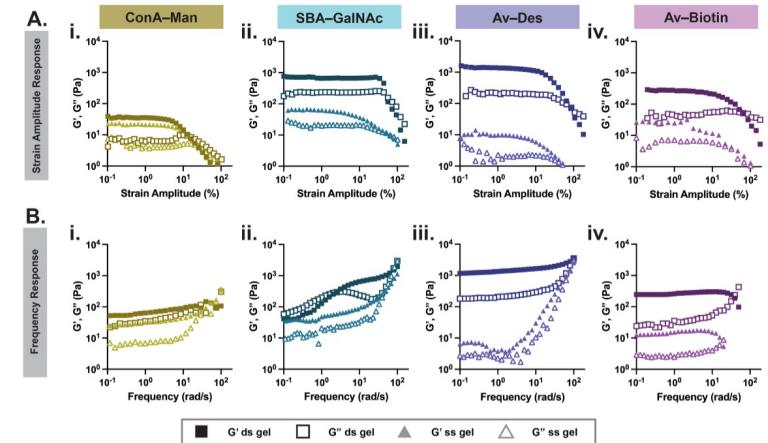
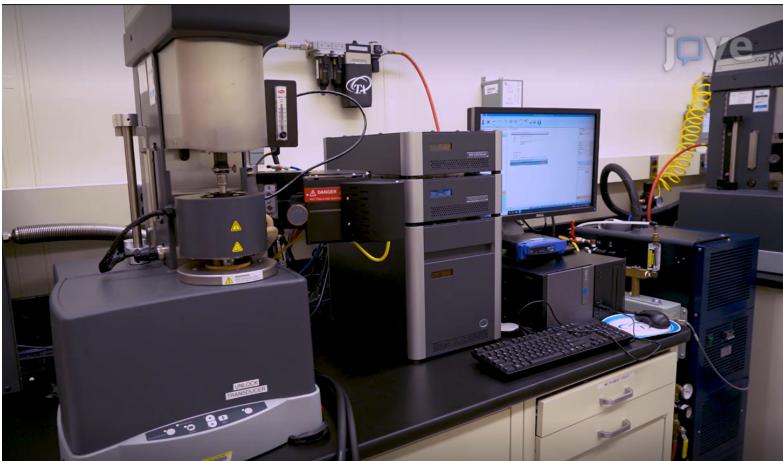
Hydrogel Stiffness



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- Independent variable:
Stiffness of Hydrogel
 - Storage modulus (G') and loss modulus (G'')
 - T, type of protein-ligand interactions and type of DNA
- Characterization methods:
Temperature-scanning rheology

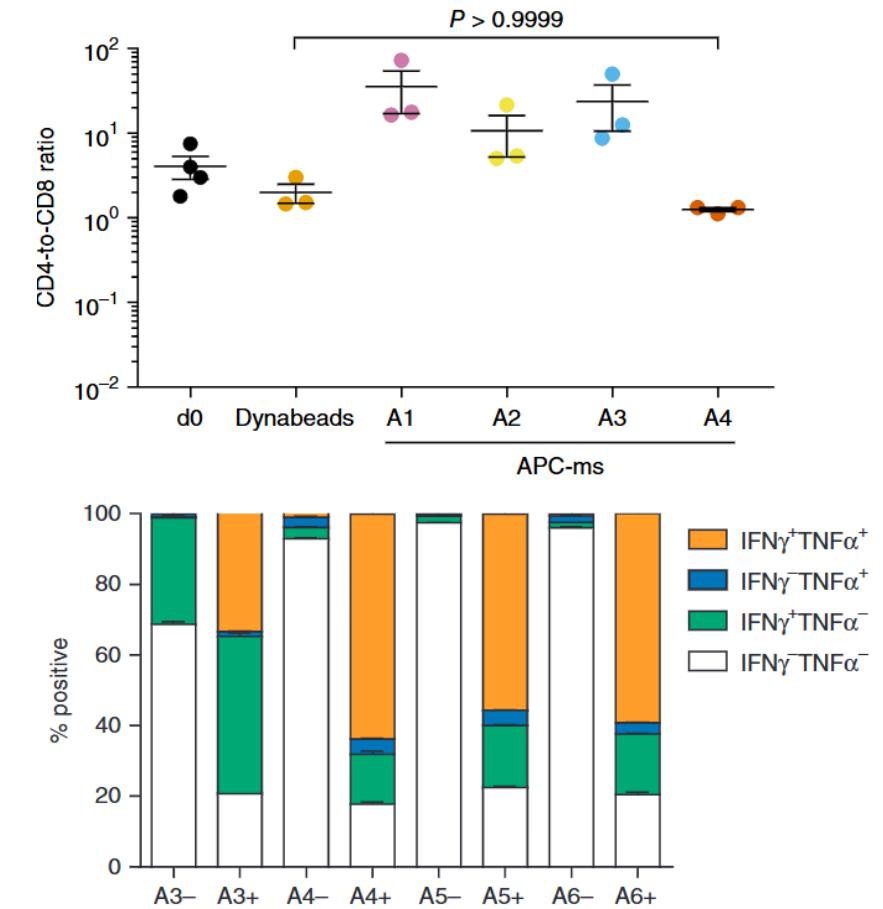


J. Am. Chem. Soc. 2025, 147, 17293–17302

T Cell Activity



- Dependent variable: activity of T cells
 - Proliferation rate and cytotoxicity
- Characterization methods:
Trypan blue dye exclusion test and flow cytometry



Nature Biotechnology, 2018, 36(2):160 - 169

Content

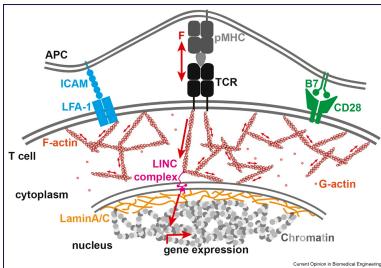
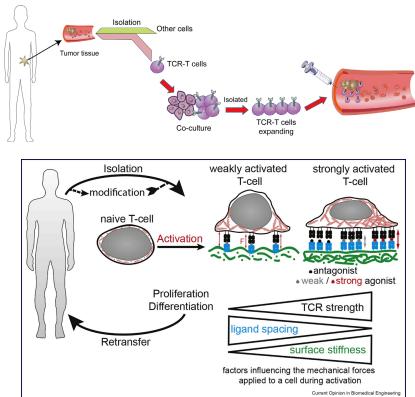


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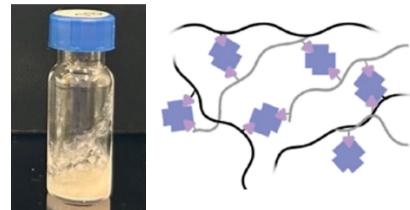
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Aochen Dengzhang



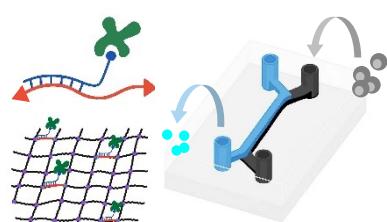
Material

Yushan Wei



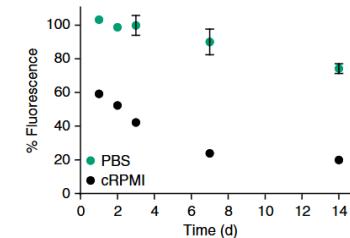
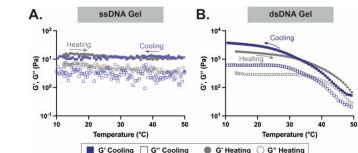
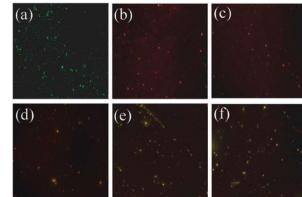
Design

Sijie Li



Characterization

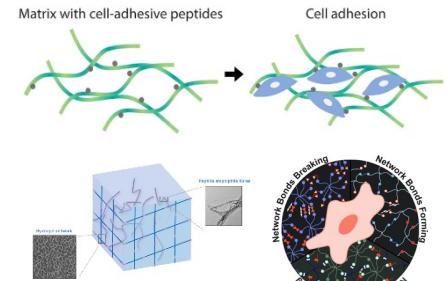
Ruirui Zhang



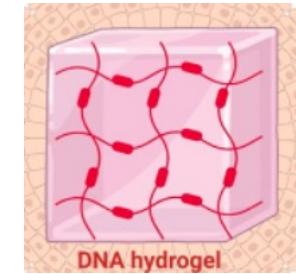
Discussion

Xiangtao Shi

Traditional Hydrogels



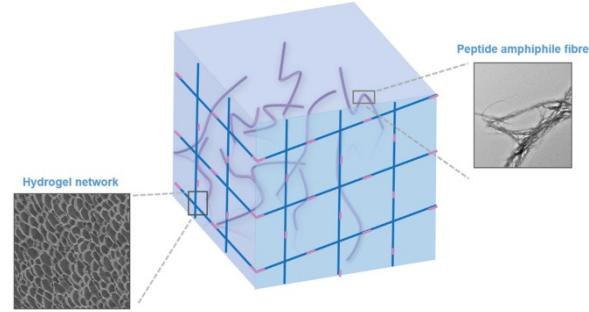
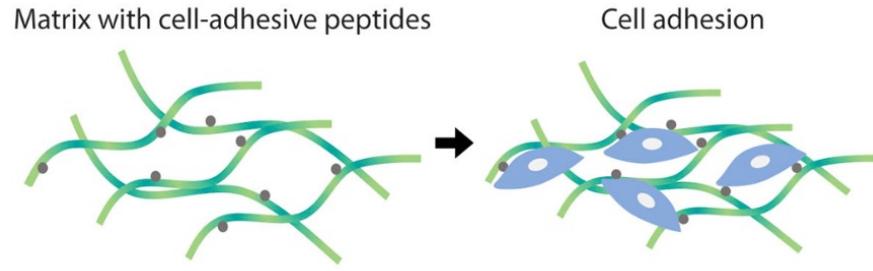
Hybrid DNA Hydrogels



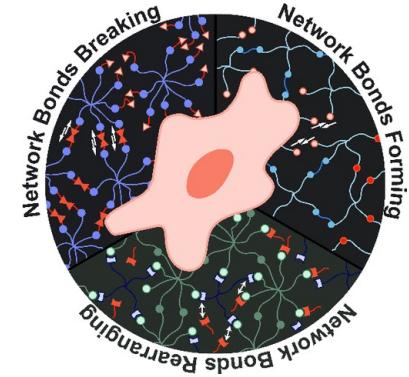
Discussion



Low batch-to-batch reproducibility



Low bioactivity



□ Polysaccharides hydrogels

- Alginate
- Chitosan
- Agarose
- Hyaluronic Acid (HA)
- ...

□ Proteins derived hydrogels

- Collagen
- Gelatin
- Elastin
- Fibrin
- ...

□ Synthetic hydrogels

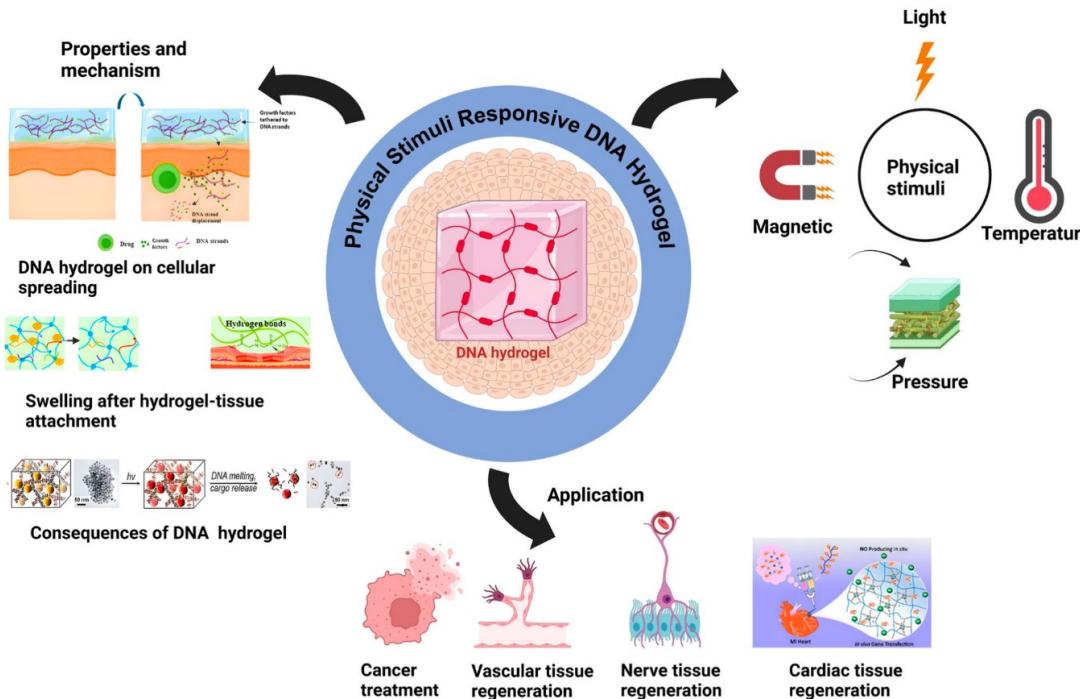
- Polyethylene glycol (PEG)
- Polyvinyl alcohol (PVA)
- Polyethylene oxide (PEO)
- Poly (acrylamide) (PAM)
- ...

Discussion



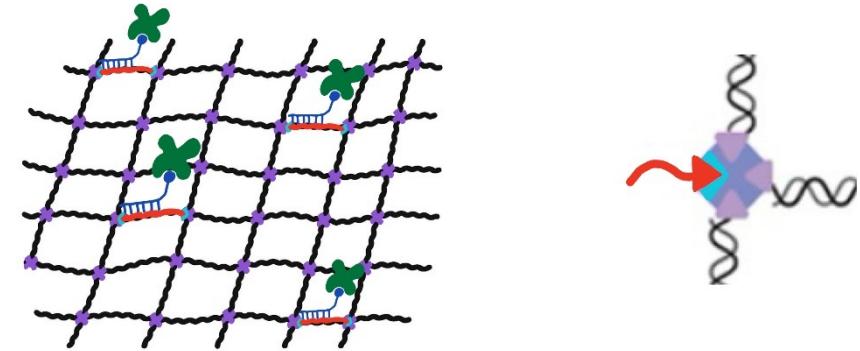
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Department of Biomedical Engineering



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- DNA hydrogels
 - Base pairing
 - PCR amplification
 - Synthetic biology of DNA



- Hybrid Biomolecular hydrogels
- Cross-linking behavior and backbone flexibility
- Can be independently tuned to control hydrogel viscoelasticity

- Hydrogel backbone: ligand-functionalized oligonucleotides
- Cross-links: multivalent protein–ligand interactions

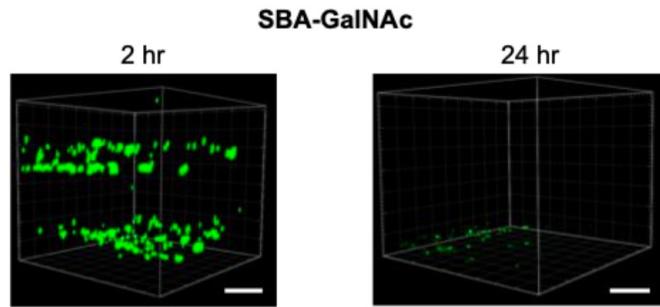
Discussion



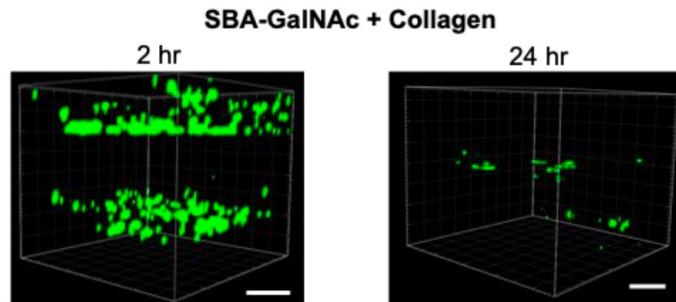
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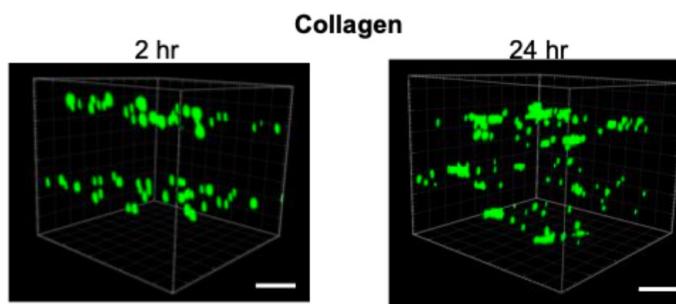
A.



B.



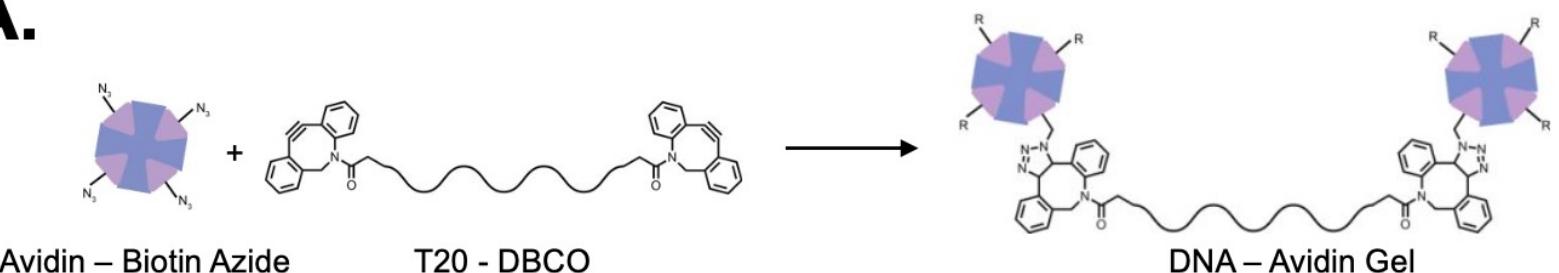
C.



Challenges

- High cost for clinical application
- Need thermally stable proteins
- ssDNA is vulnerable to DNase from cells
- Hydrogel degradation may affect the experiment
- Temperature changes might impact T cell behavior

A.



Acknowledgement



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We would like to express our gratitude to:

- **Professor Jiang Xingyu** for his valuable guidance and feedback.
- Our classmates for their constructive discussions.
- The authors of cited works whose research contributed to our understanding.

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