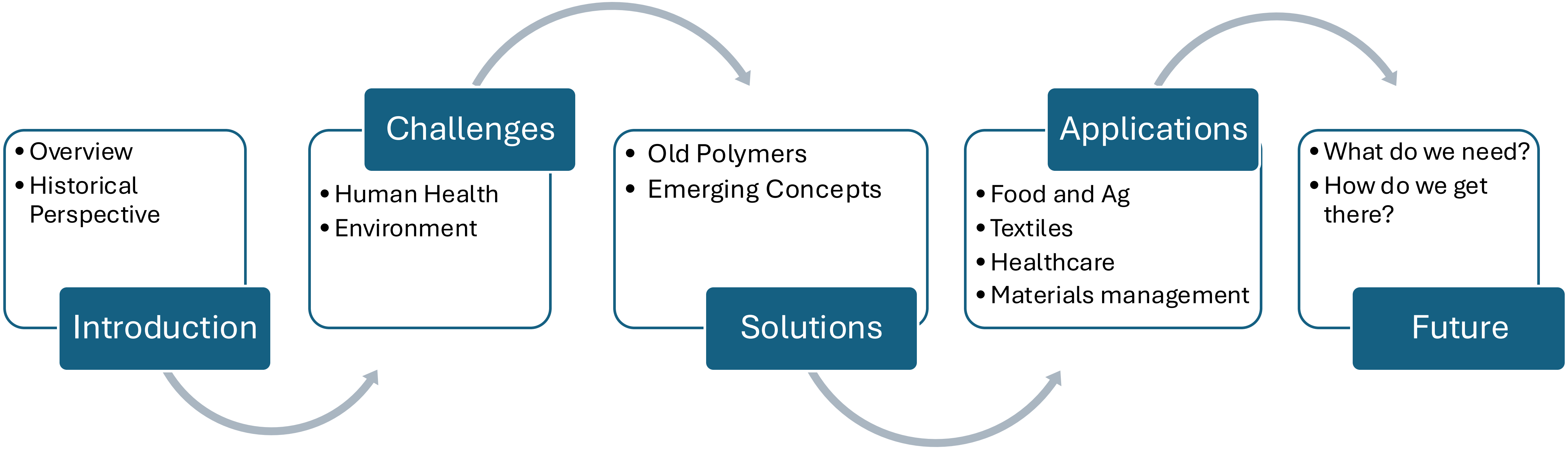
Housekeeping

1. Assignment #2 is due on Wednesday
2. Checkpoint #2 for the project is due NEXT Wednesday
3. I realize there was a minor discrepancy in the value of assignments etc. in the uploaded files-I deleted the outdated file. Any questions?

- **Class participation and attendance** (10%)
- **Homework (4)** (20
- **Exams (2)** – tak t indi %) – problem statement based, free form sed on creativity and thinking as opposed n.
- **Class project** – tea as will be provided, but the thought is that t teeth into something they are passionate a presen it could impact currently utilized materials t nprove sustai ility.

- **Class participation and attendance** (10%)
- **Homework (6)** (30%)
- **Midterm presentation** – (25%) – halfway update for the final class project. Will entail a written portion (1-2 pages) as well as a 5 minute in-class presentation with your project group. We hope that this will be helpful in ironing out issues before the final presentations.
- **Class project** – team (35%) – 20 minute presentations. Some ideas will be provided, but the thought is that the students will dig their teeth into something they are passionate about and present on how it could impact currently utilized materials to improve sustainability.

Course Overview



Course Overview

Introduction
- Overview
- Historical Perspective

Challenges
- Human Health
- Environment

Solutions
- Old Polymers
- Emerging Concepts

Applications
- Food and Ag
- Textiles
- Healthcare
- Materials management

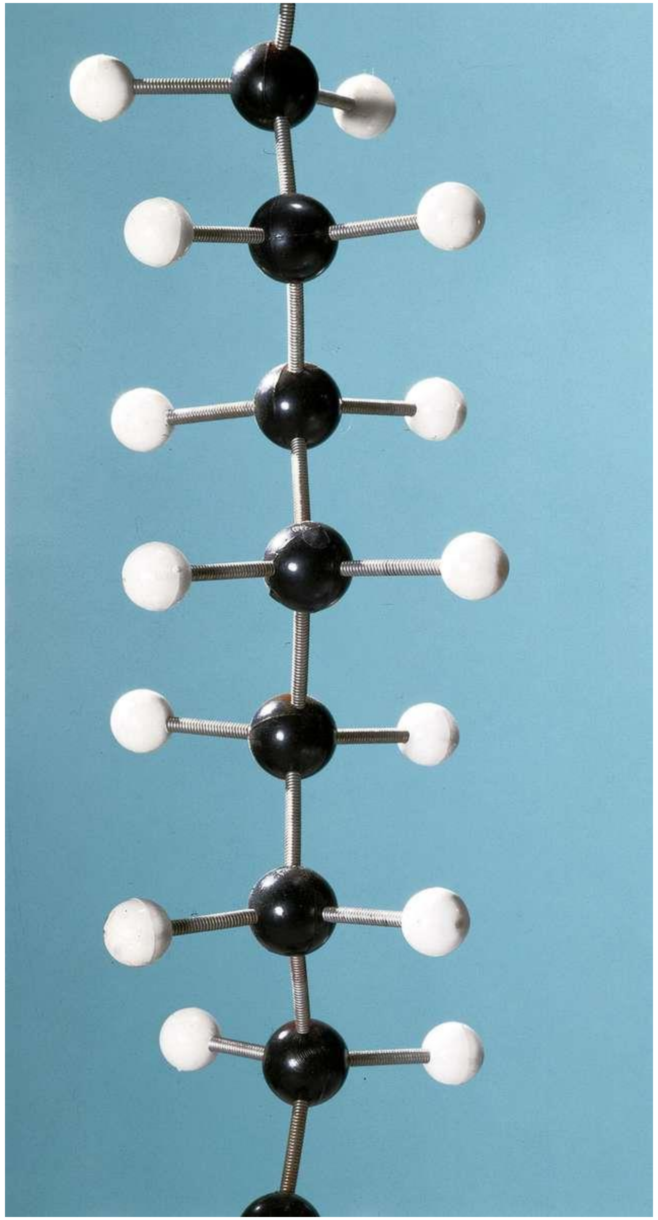
Future
- What do we need?
- How do we get there?

Learning Objectives

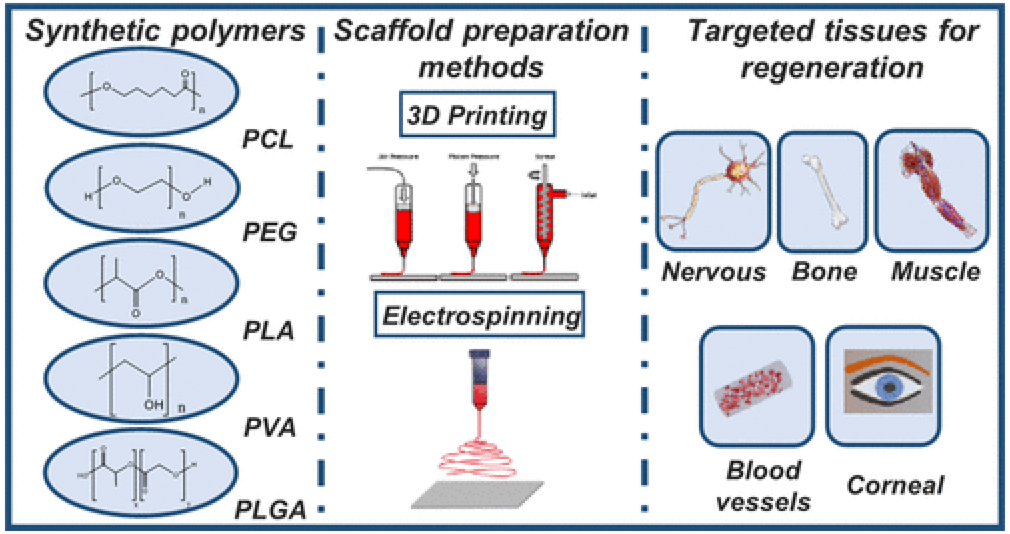
1. Understand why synthetic polymers are so useful
2. Be able to identify synthetic and natural polymers
3. Understand how incorporating synthetic elements (e.g. peptoids) into materials can alter their performance
4. Exposure to hydrogel biomaterials design (specifically using synthetic polymers/crosslinkers)

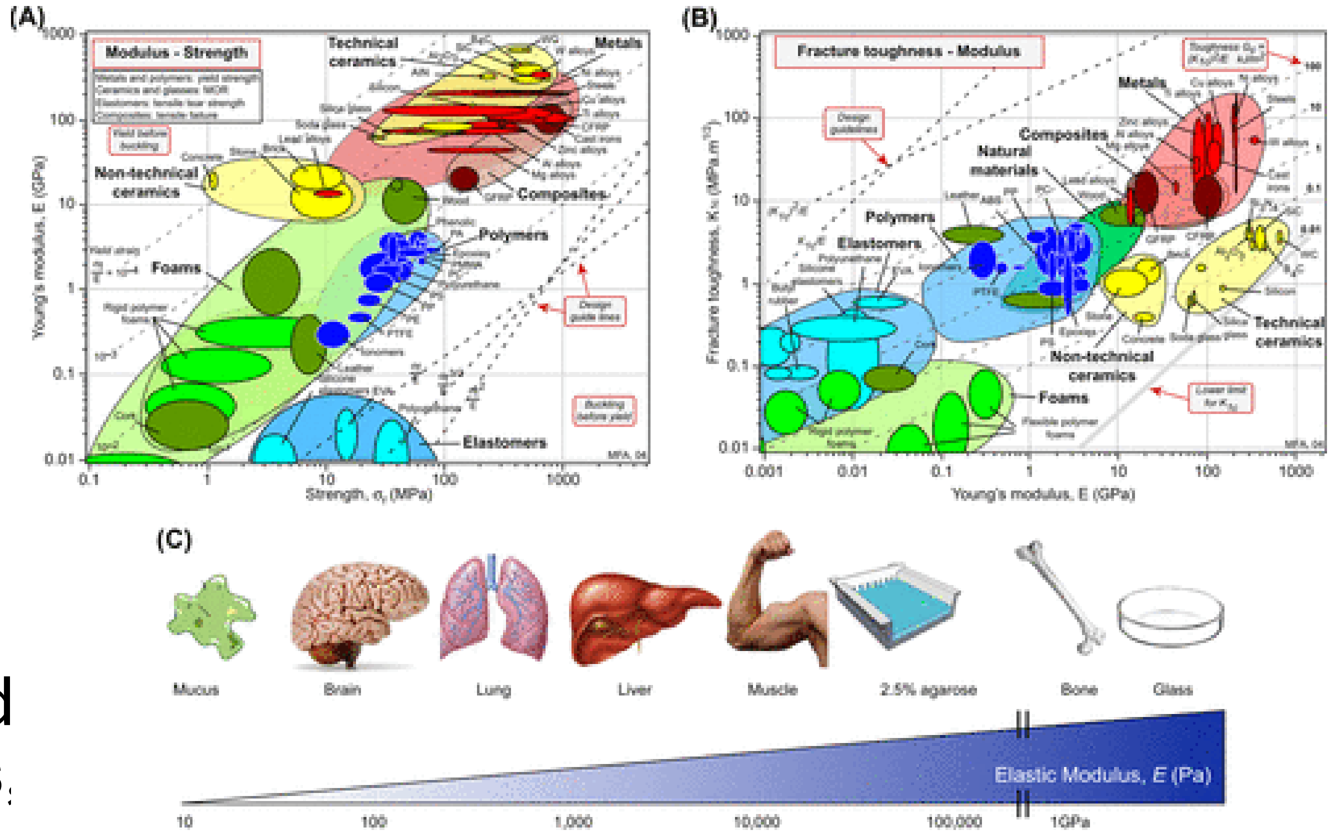
Polymers are not going anywhere

- Sealing applications (‘O’ rings, gaskets)
- Clothing, sportswear and accessories
- Packaging and containers
- Electrical and thermal insulation
- Construction and structural applications
- Paints, glues and lubricants
- Car parts (tires, bumpers, dashboards)
- Household items (kitchenware, toys)
- Medical applications (syringes, rubber gloves)
- Hygiene and healthcare (toothbrushes, shampoo)



Synthetic polymers are integral to modern science

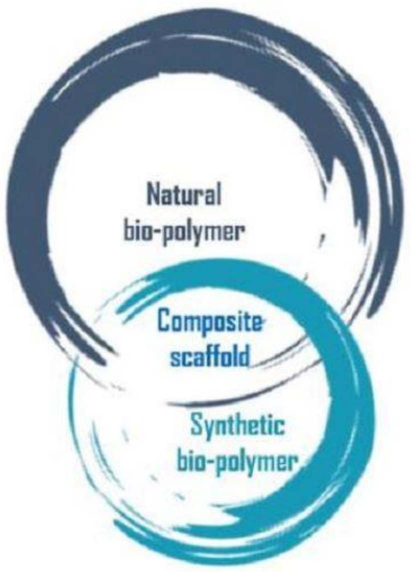


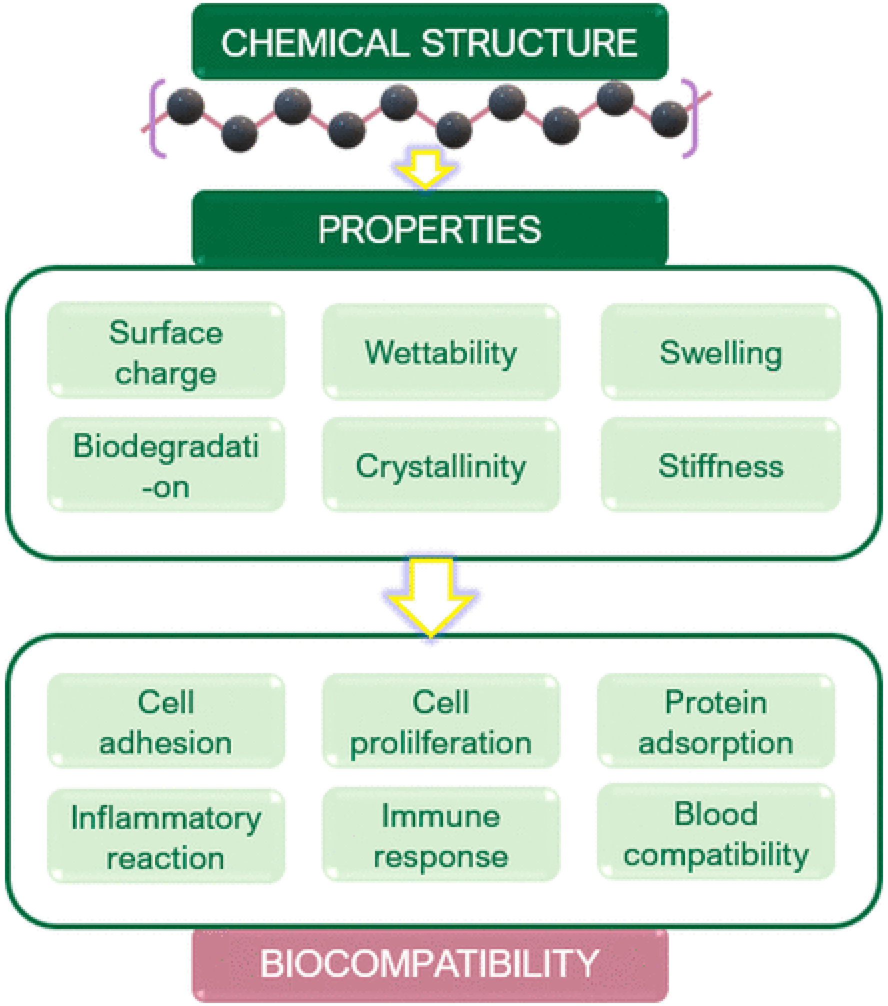


Modern applications in biomedicine (like tissue engineering) require specialized properties (strength, toughness, etc.)

Terzopoulou, Z., Zamboulis, A., et al. *Biomacromolecules* **2022** *23* (5), 1841-1863

Synthetic polymers offer unique properties



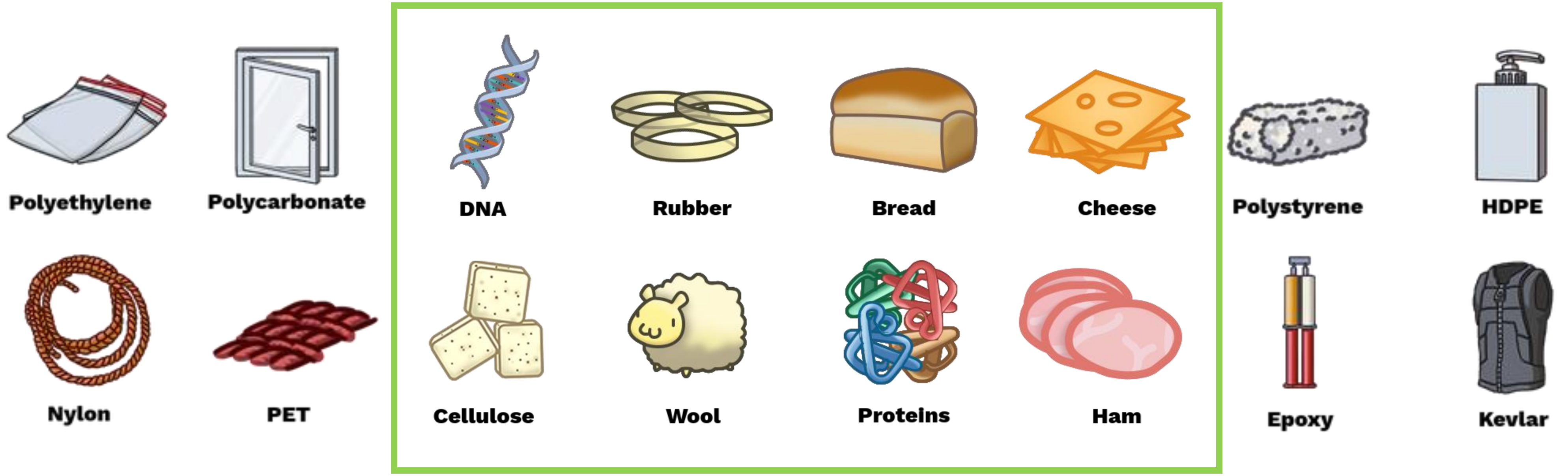


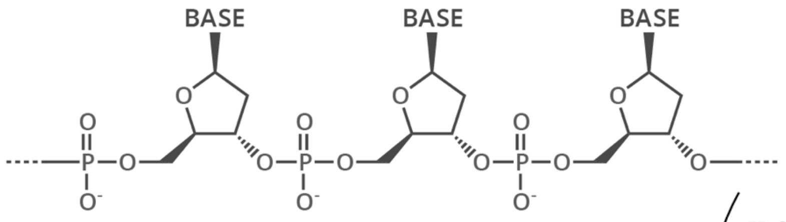
Terzopoulou, Z., Zamboulis, A., et al. *Biomacromolecules* **2022** *23* (5), 1841-1863

Other factors to consider when choosing a polymer for your application

	Advantages	Disadvantages
Natural polymers	• Inherently bioactive • Possess cell-interactive groups on their backbones • Offer better cell attachment, growth, multiplication and differentiation • Chemically benign degradation products • Elicit low immune response	• Difficult processing • Low cost effectiveness • Poor mechanical properties • Precarious outcome due to batch-to-batch variations • Insufficient mechanical strength • Hydrophilicity • Need of crosslinking to improve strength
Synthetic polymers	• High flexibility in the processing • More economical • Tunable mechanical properties • Higher mechanical strength • Better structural stability	• Lacking bioactivity • May produce intense immune response • Necessitate more modifications compared to natural polymers to impart bioactivity

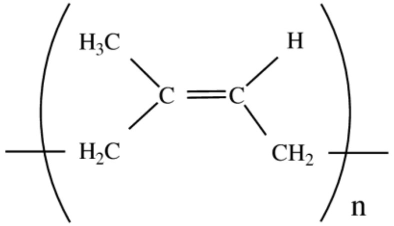
Which are natural polymers?

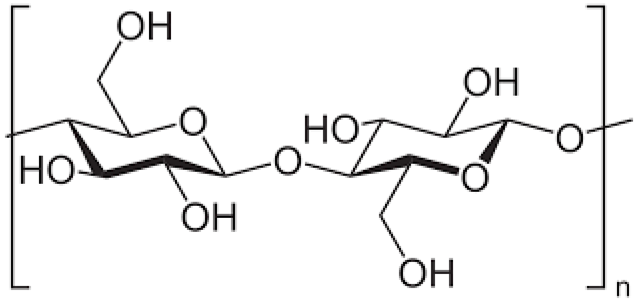




DNA

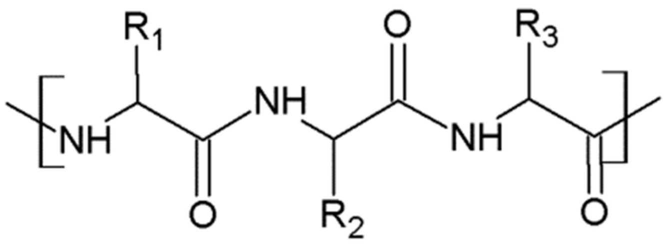
Rubber



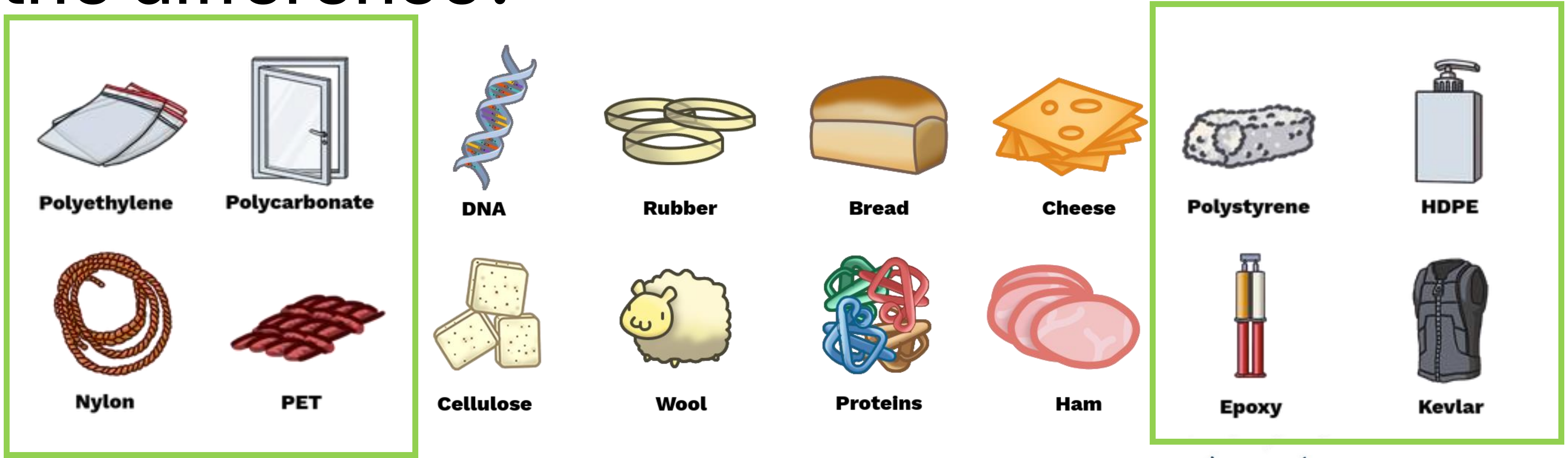


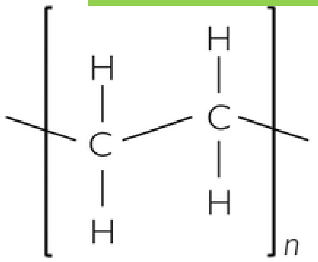
Cellulose

Protein

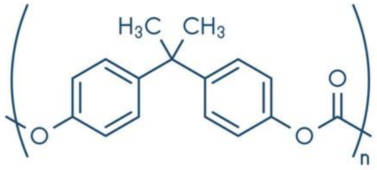


The rest are synthetic polymers…so what's the difference?

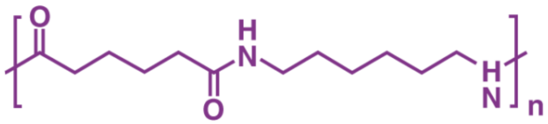




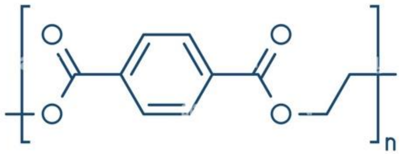
Polyethylene



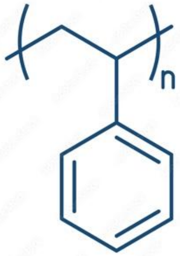
polycarbonate



Structure of Nylon – 6,6

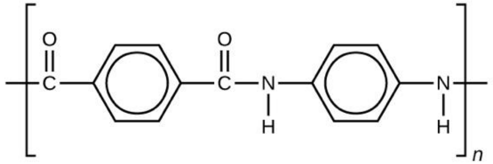


PET



polystyrene

Kevlar



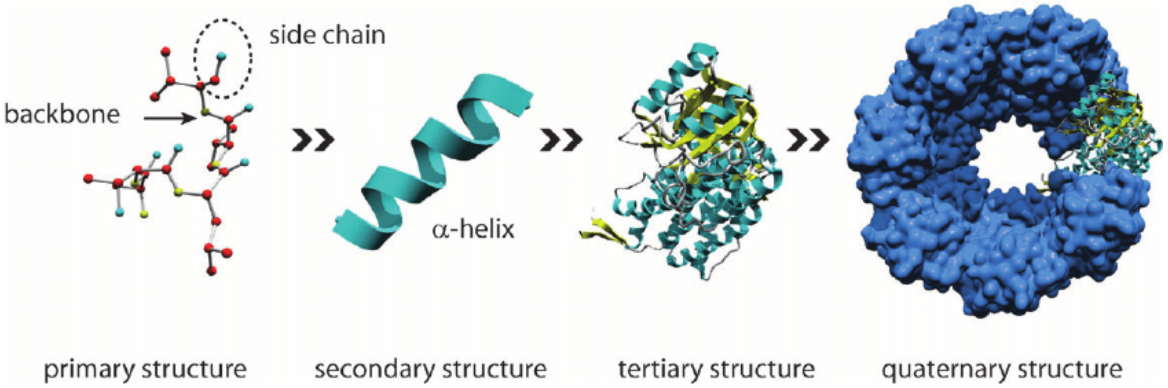

Chemistry
SYNTHETIC
POLYMERS

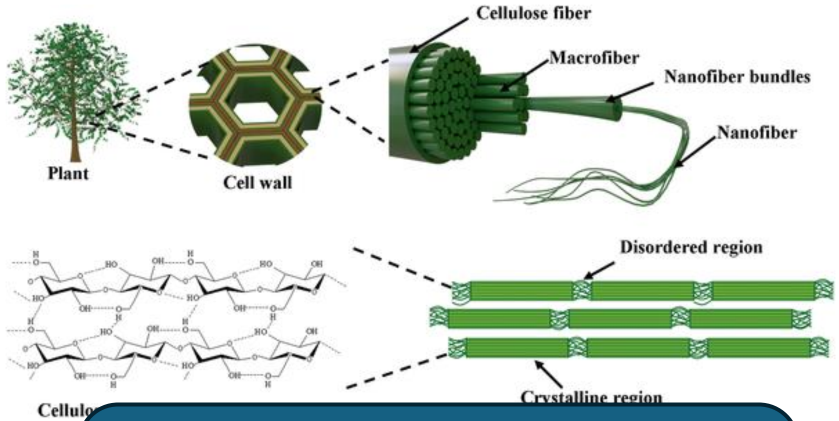
The rest are synthetic polymers…so what's the difference?

- Man-made polymers are designed to be durable and resistant to degradation.
- They are often made from synthetic materials that have strong carbon-carbon bonds, making them less susceptible to breakdown by environmental factors:
  - UV light
  - Moisture
  - Microorganisms
- Additionally, they often lack the specific chemical structures or functional groups that natural enzymes can target and degrade.

- In contrast, natural polymers are inherently biodegradable:
  - Proteins
  - nucleic acids
  - polysaccharides
- They are composed of repeating units that can be broken down by natural enzymes produced by microorganisms
- These polymers tend to have chemical structures that are more susceptible to hydrolysis and enzymatic action, leading to their degradation over time in the environment.

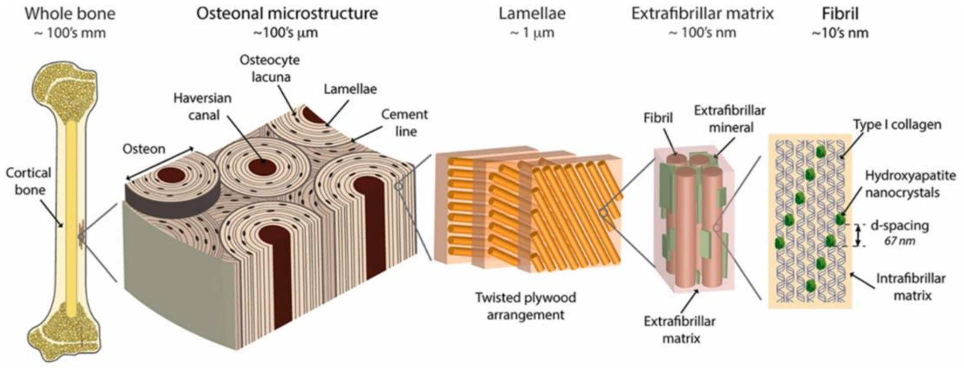
Self assembly and higher order structures

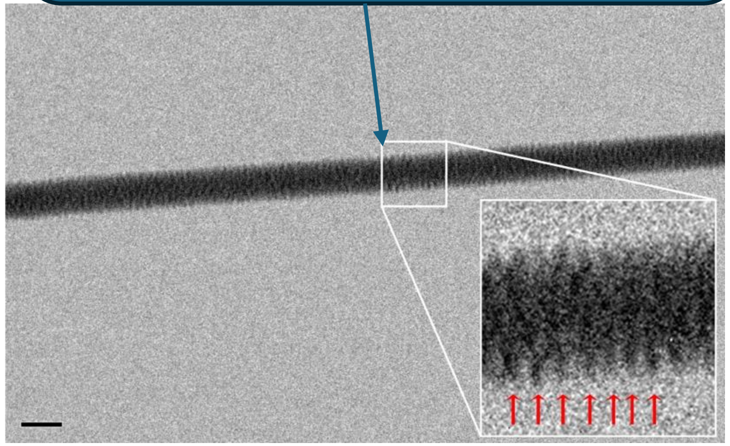




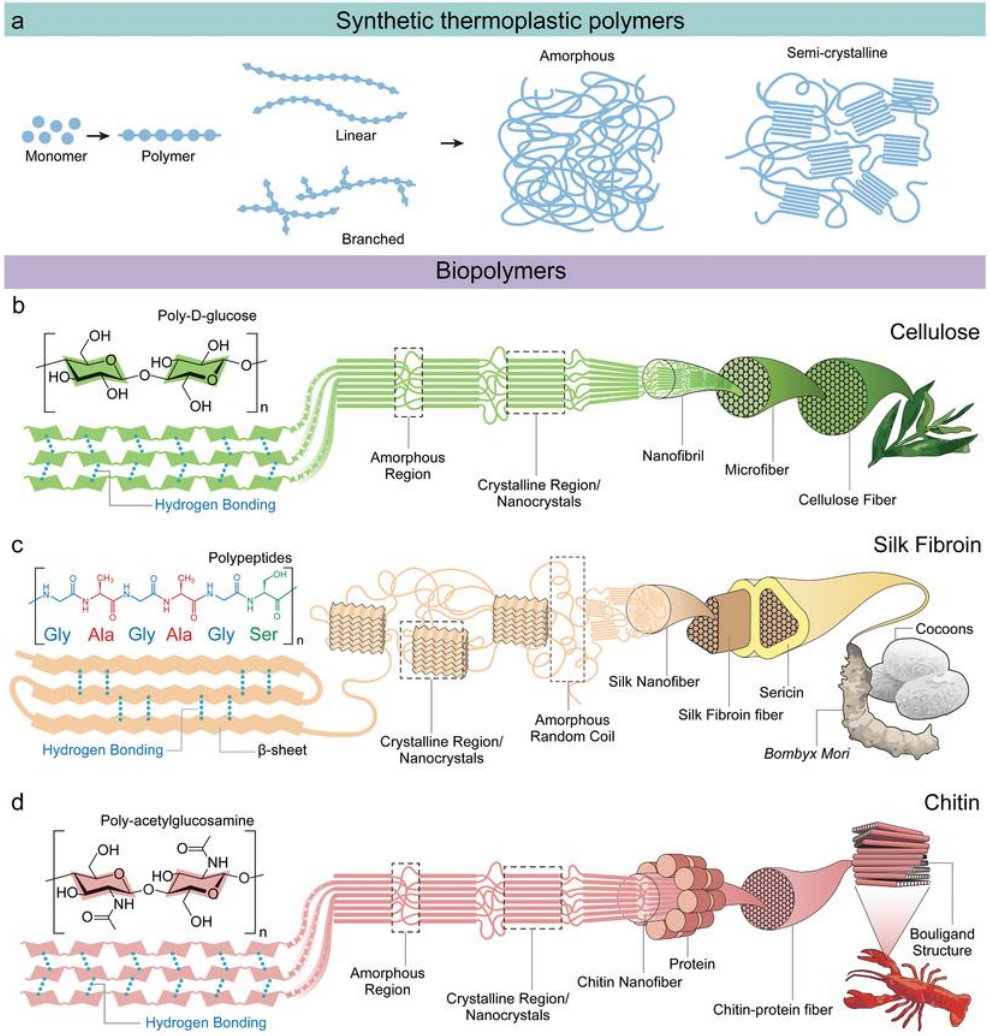
Biology is extremely good at creating comple
from relatively simple building blocks…

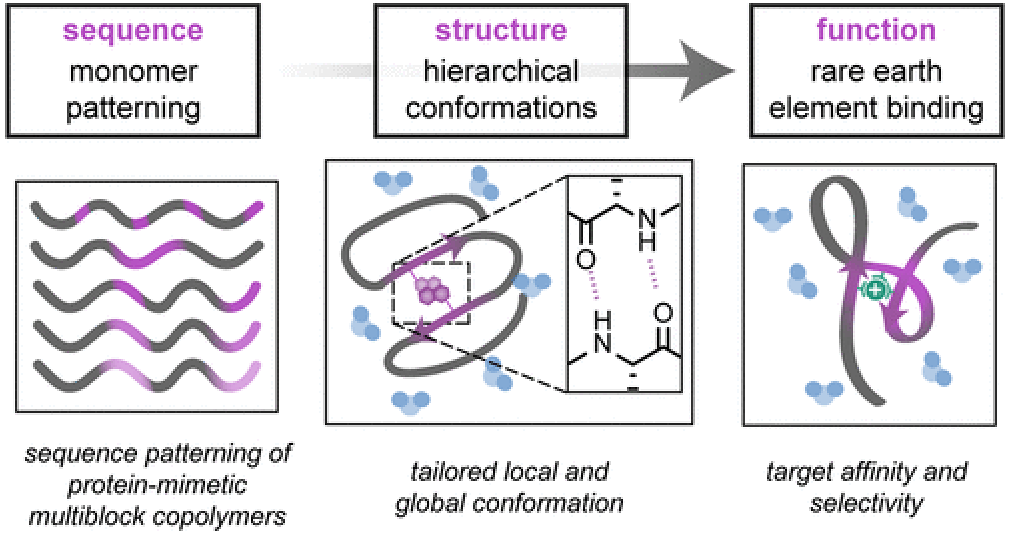
Anyone know what this is?

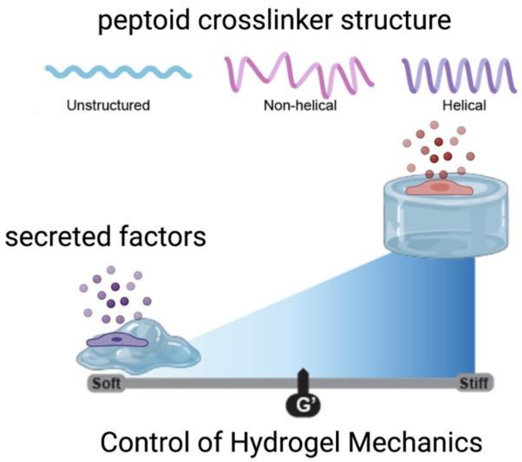


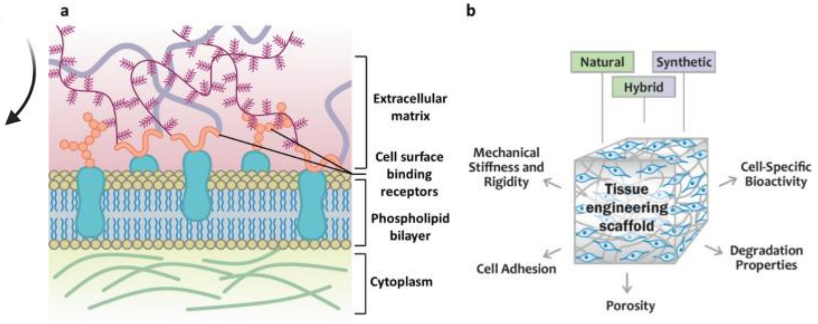


Self assembly and higher order structures

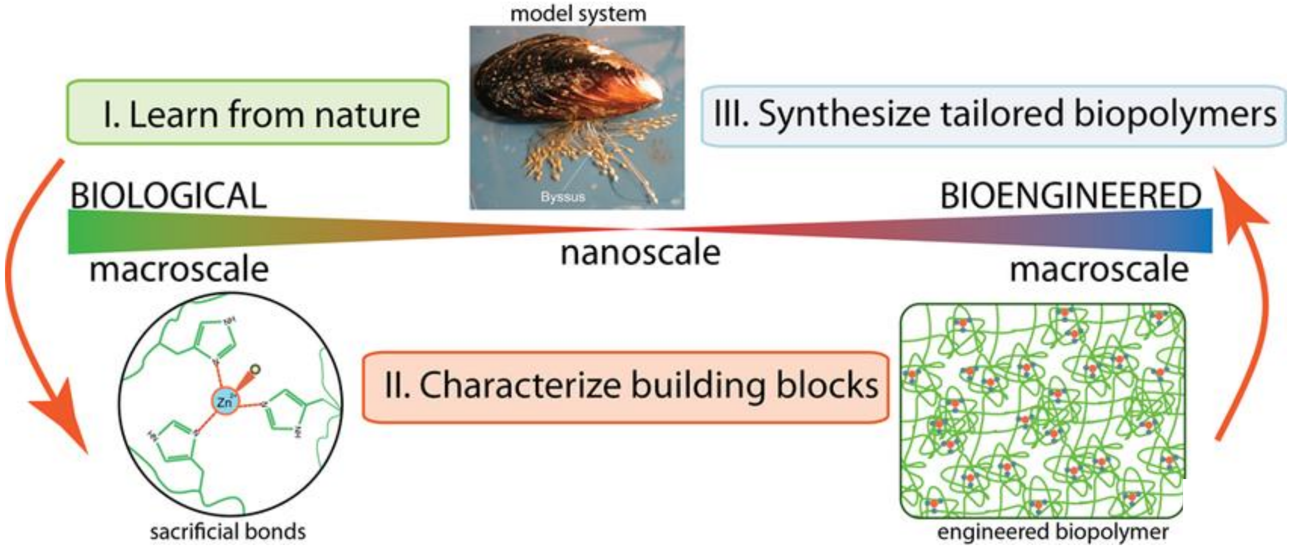






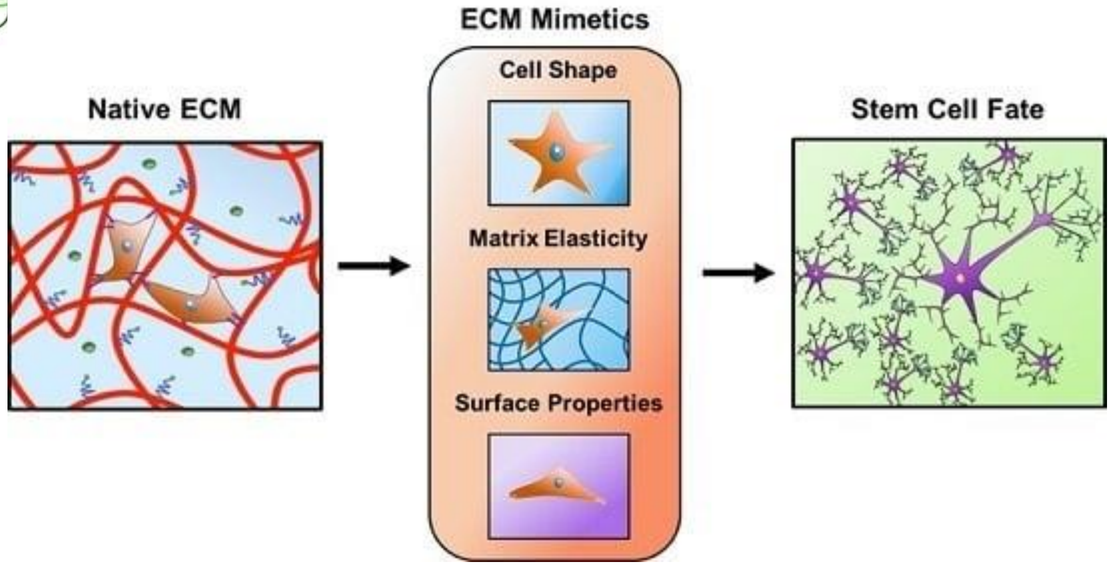


Self assembly and higher order structures

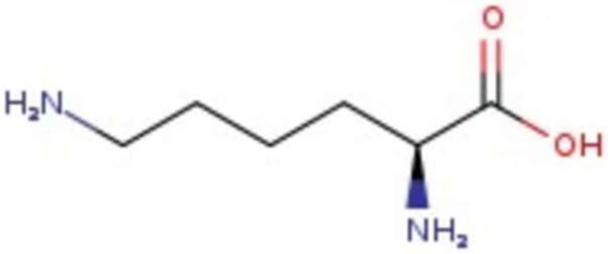


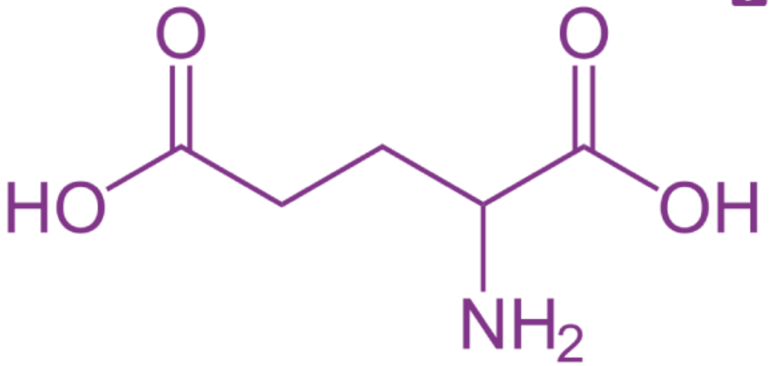
What are some aspects of natural materials that are particularly difficult to recapitulate?

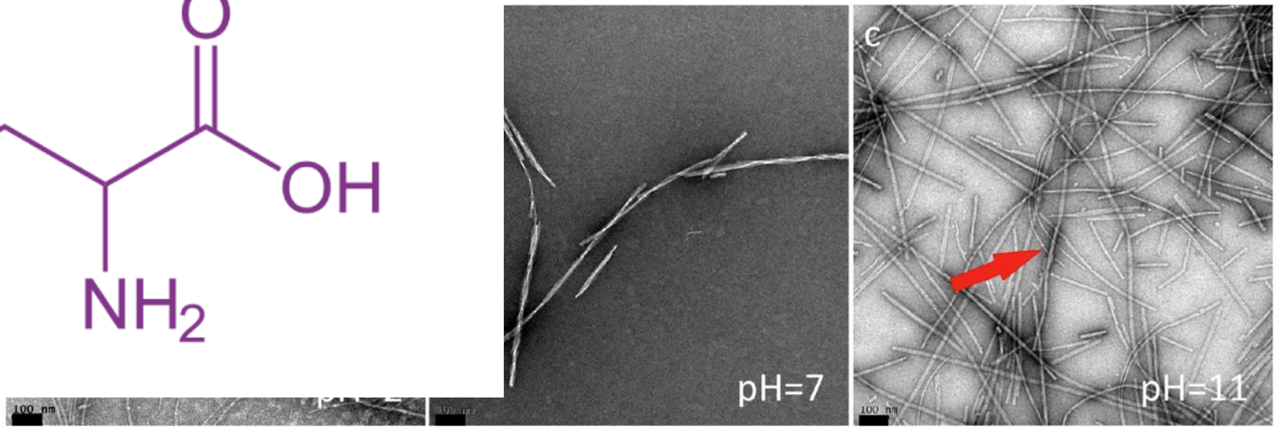
We could just use natural ECM (products like Matrigel)? Anyone know where Matrigel comes from?



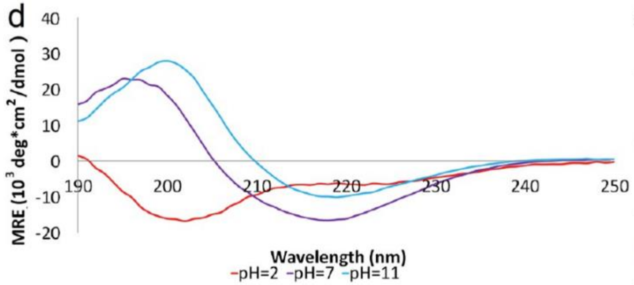
Self assembly and higher order structures







: Palm$_2$K-(EK)$_4$-OVA$_{CytoT}$



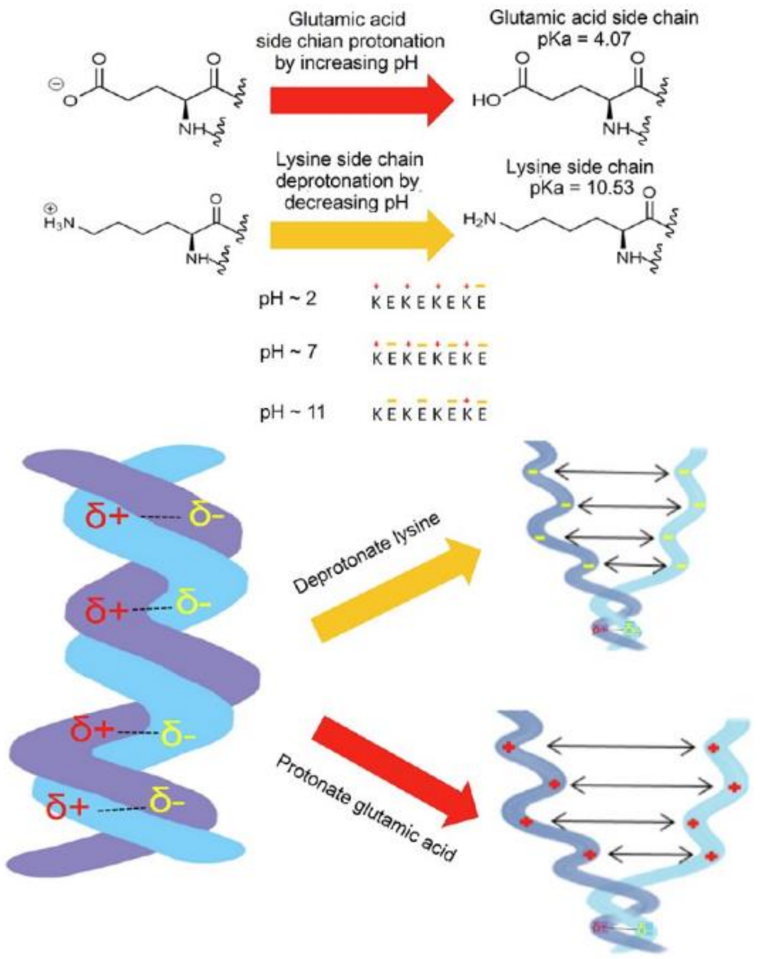
e

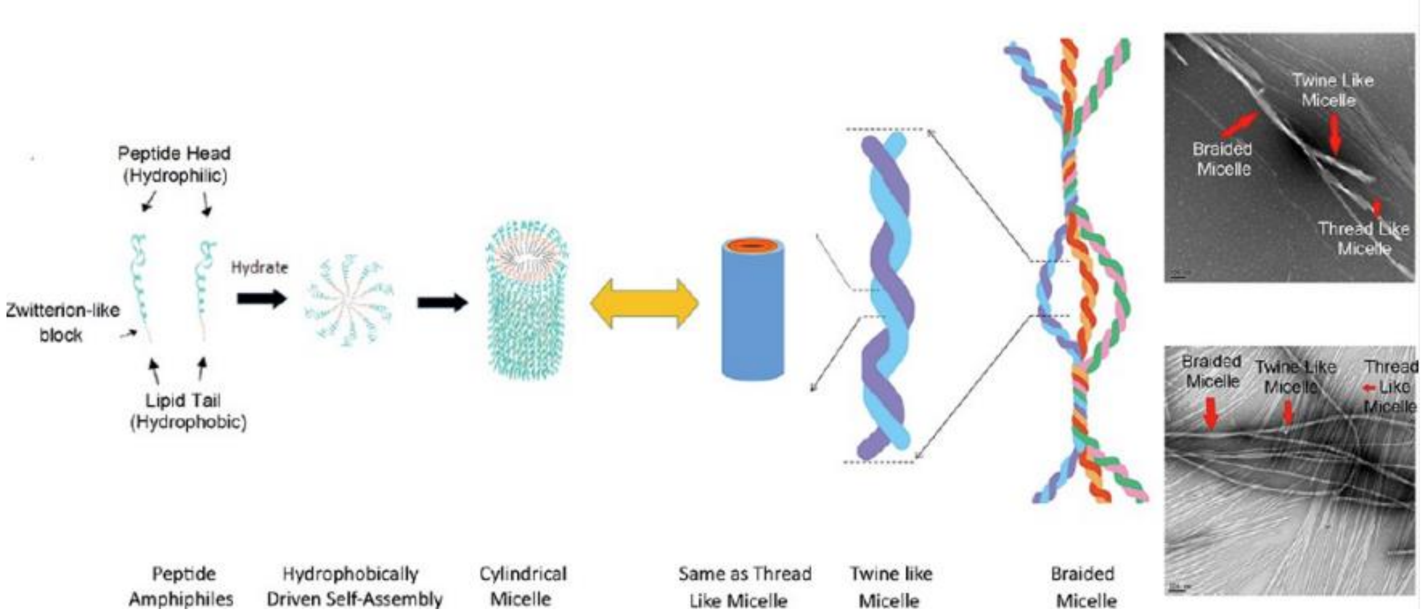
	pH=2	pH=7	pH=11
α-Helix	15.0%	0.0%	0.0%
β-Sheet	27.7%	100.0%	100.0%
Random Coil	57.3%	0.0%	0.0%

Why do you think this (KE)4 block led to higher order structure self assembly?

Zhang, R, Morton, LD et al. *ACS Biomater. Sci. Eng.* 2018, 4, 7, 2330–2339

Self assembly and higher order structures

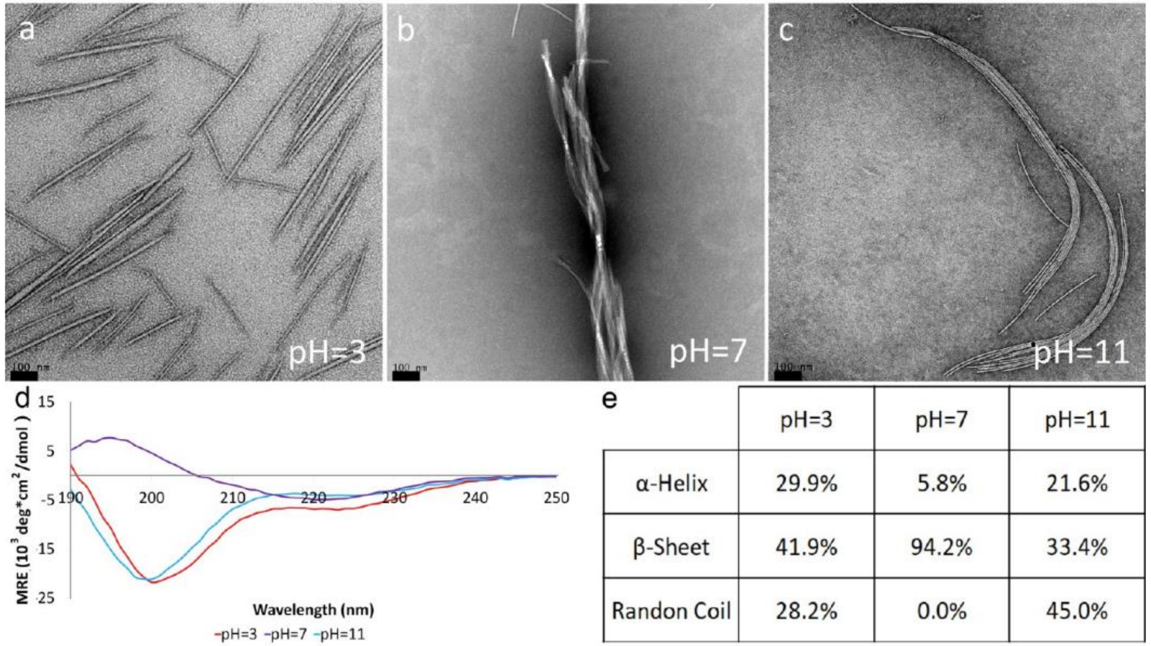




What do you think happened when we tried K8 and E8 linkers instead of the alternating (KE)4?

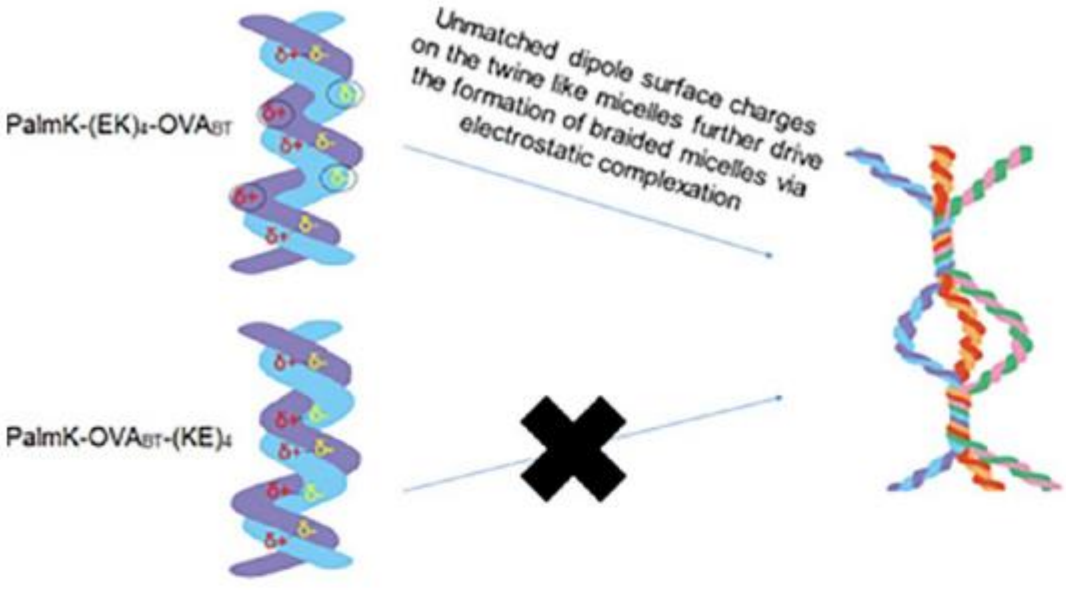
Zhang, R, Morton, LD et al. *ACS Biomater. Sci. Eng.* 2018, 4, 7, 2330–2339

Controlling hierarchical self assembly in a synthetic system



e

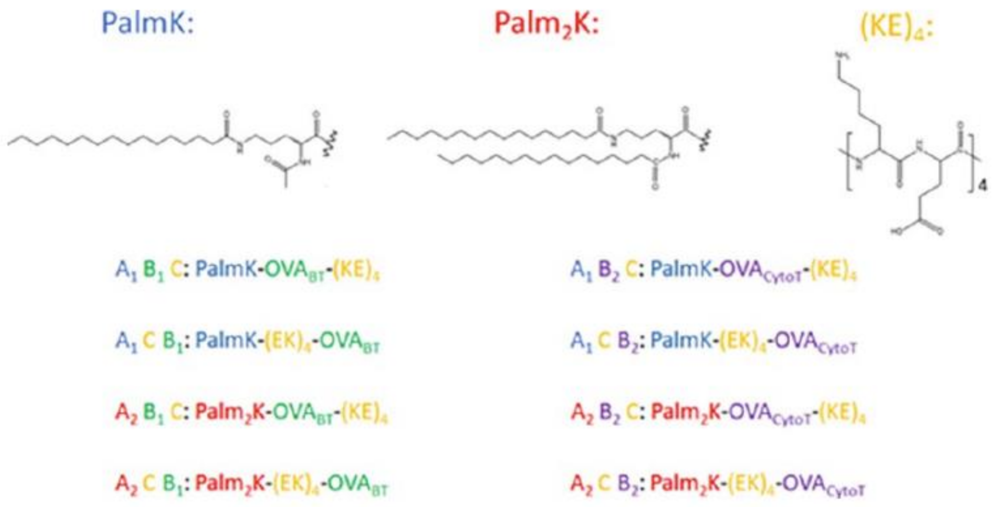
	pH=3	pH=7	pH=11
α-Helix	29.9%	5.8%	21.6%
β-Sheet	41.9%	94.2%	33.4%
Randon Coil	28.2%	0.0%	45.0%

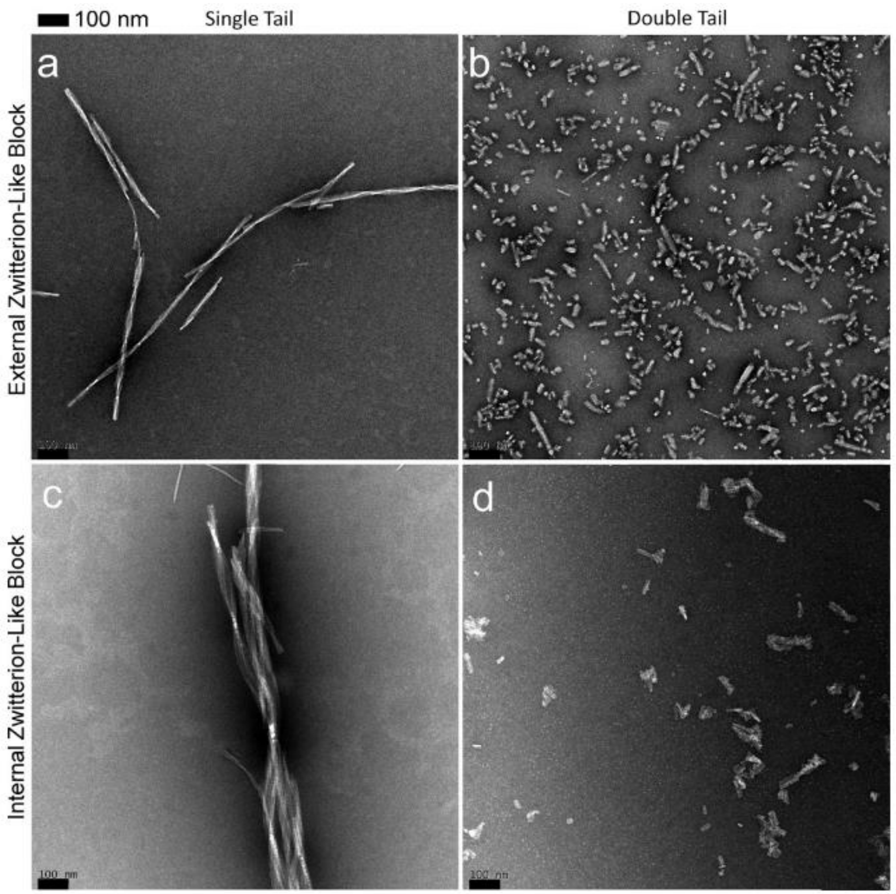


We were really surprised that changing the location of the linker prevented the formation of braided micelles… sequence definition is super important!

Zhang, R, Morton, LD et al. *ACS Biomater. Sci. Eng.* 2018, 4, 7, 2330–2339

Controlling hierarchical self assembly in a synthetic system

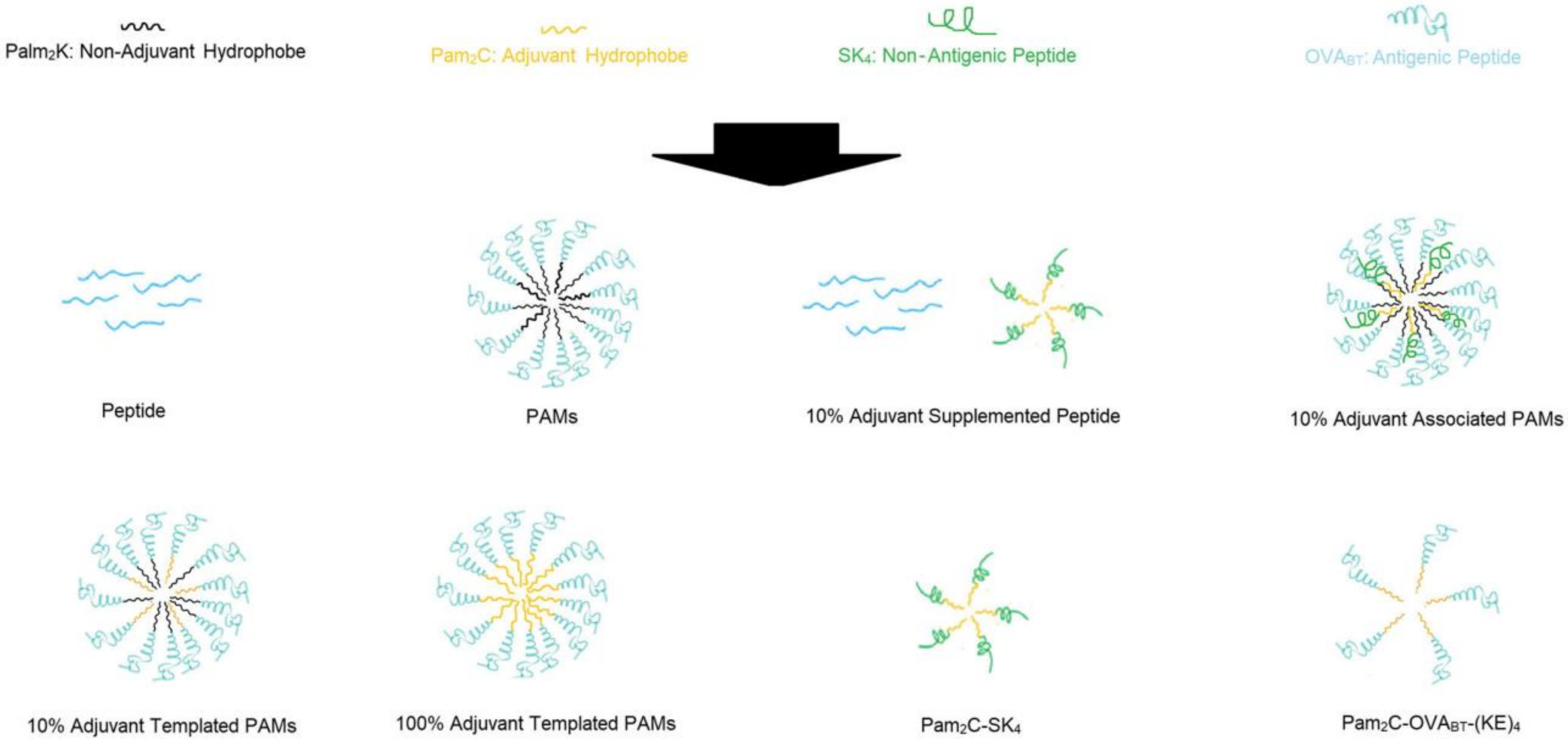




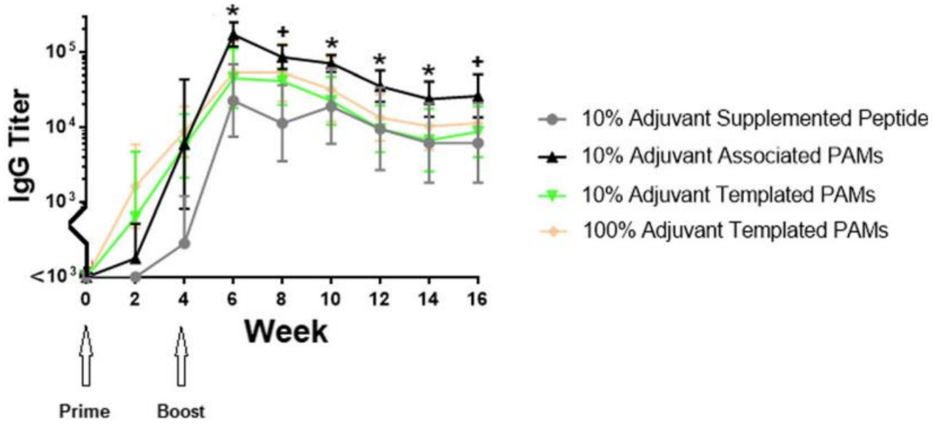
When we began this project we wanted to create synthetic vaccines. Which panel (a-d) would be best for vaccine applications?

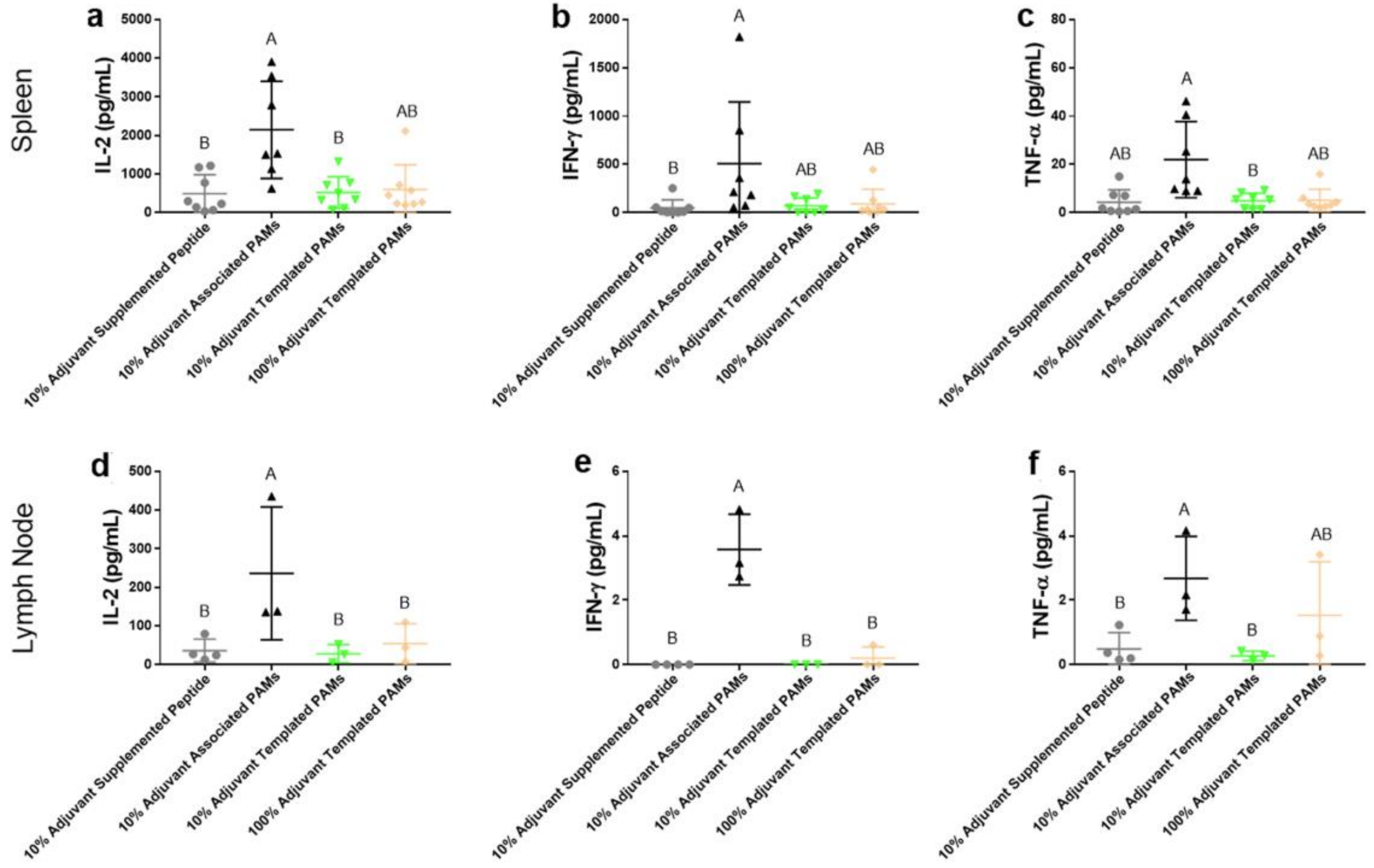
Zhang, R, Morton, LD et al. *ACS Biomater. Sci. Eng.* 2018, 4, 7, 2330–2339

Self-assembled vaccines mimic natural adjuvant display



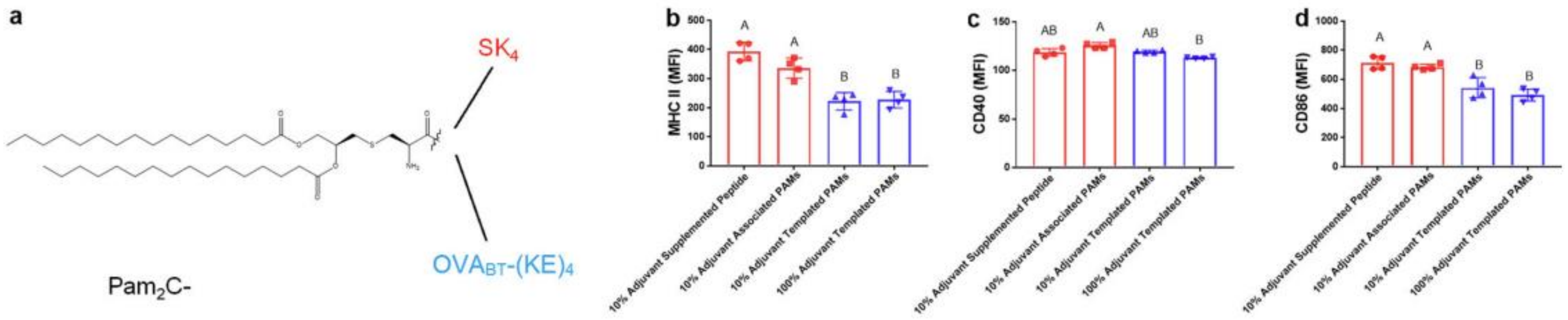
Self-assembled vaccines mimic natural adjuvant display





PAMs are great…but they aren't necessarily very sustainable. We were synthesizing these via solid phase peptide synthesis and purifying by high performance liquid chromatography. How could we have sourced these more sustainably?

Varying the peptide sequence also alters adjuvanticity



Perhaps unsurprisingly, the adjuvant we used changed things a lot…newer adjuvant designs are being developed all the time. Anyone know what technology has leaped to the forefront of these predicted sequences?

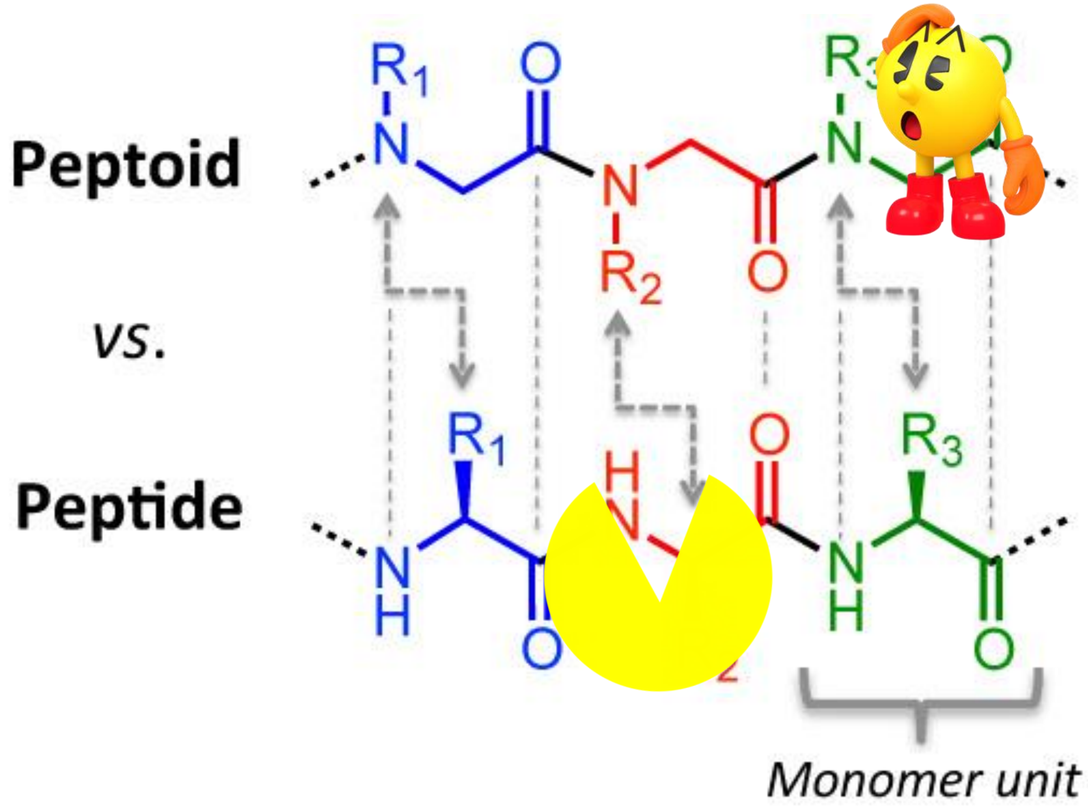
So, can't we just make degradable, synthetic polymers?

Yes, but as always it is a bit more complicated than that…

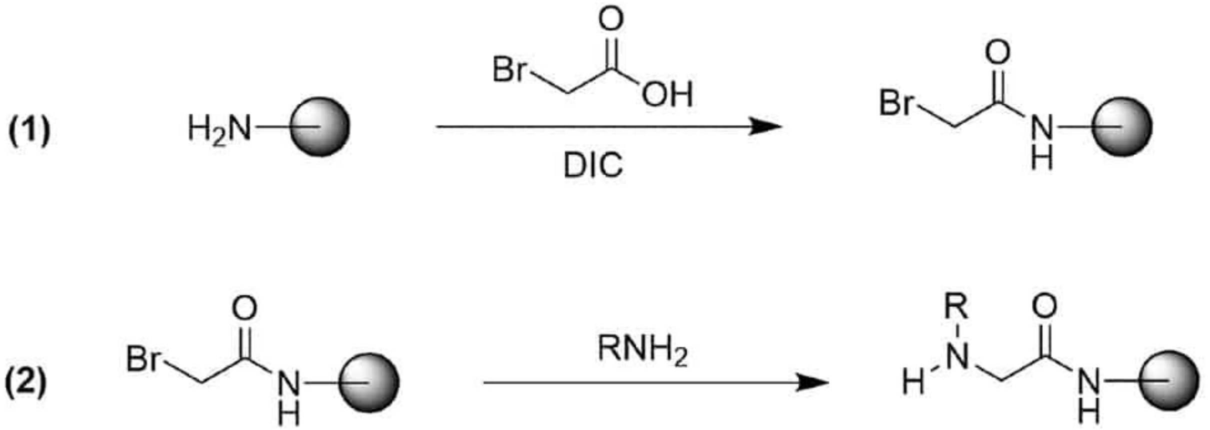
Polylactic Acid (PLA): Made from renewable resources like corn starch or sugarcane, PLA is compostable and breaks down into lactic acid, which can be metabolized by microorganisms.
Polyhydroxyalkanoates (PHAs): These are produced by bacteria and can be used as biodegradable plastics. They degrade naturally in soil and water through microbial activity.
Polycaprolactone (PCL): A synthetic aliphatic polyester that is biodegradable and is used in applications like compostable bags and biomedical devices.
Polyglycolic Acid (PGA): Often used in medical sutures, PGA is biodegradable and breaks down into glycolic acid, which is then metabolized by the body.

Reason	Explanation
Cost	Biodegradable polymers are often more expensive to produce than traditional plastics due to raw materials, production processes, and economies of scale.
Performance	Non-degradable polymers offer superior mechanical properties, such as strength, durability, and resistance to heat and chemicals, essential for many applications.
Infrastructure	Existing infrastructure for traditional plastics is extensive and well-established, requiring significant investment to transition to biodegradable polymers.
Shelf Life and Stability	Biodegradable polymers may degrade under certain conditions during storage or use, limiting their application in products requiring long shelf life or exposure to challenging environments.
Recycling Challenges	Biodegradable polymers can complicate recycling if mixed with traditional plastics, contaminating the recycled material and reducing its quality.
Consumer Awareness and Acceptance	Lack of widespread consumer awareness and acceptance of biodegradable plastics, requiring education and encouragement for adoption.
Environmental Conditions	Biodegradable polymers' effectiveness depends on specific environmental conditions like microorganisms, temperature, and moisture, which may not always be present.
Regulatory and Policy Support	Need for stronger regulatory frameworks and policy incentives to promote the use of biodegradable polymers, including legislation, subsidies, and support for research and development.

What makes synthetic polymers non-biodegradable?

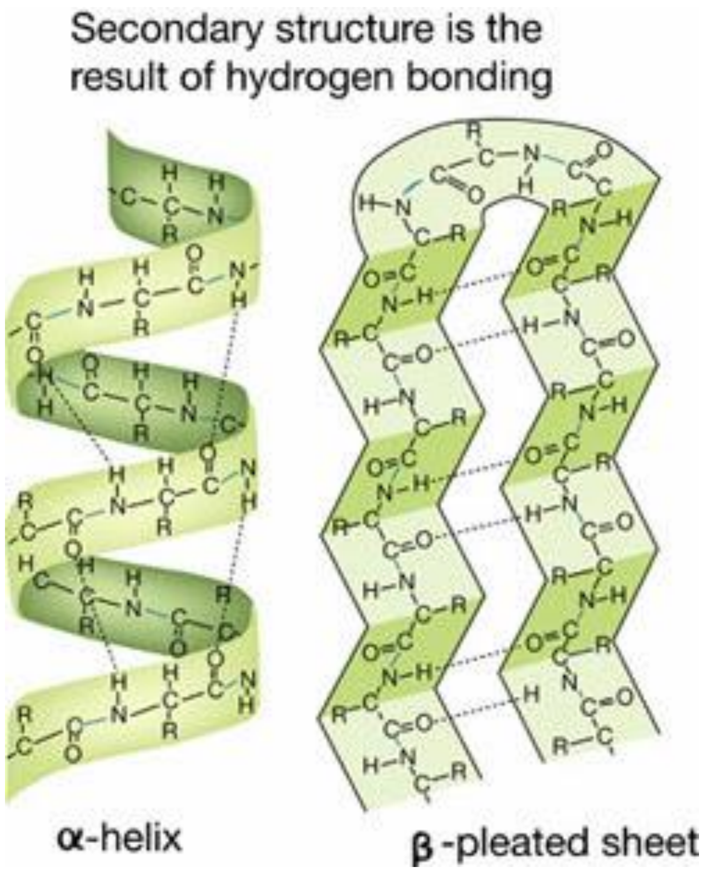


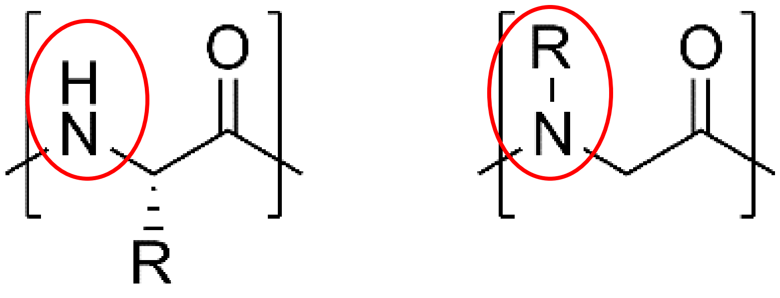
Peptides are based on the 20 amino acids (20 side chains)
Peptoids can be made from any primary amine via a submonomer synthesis, offering 100s-1000s of available side chains



Morton, LD., Hillsley, A., et al. *J. Mater. Chem. B*, 2020, **8**, 6925-6933

Higher order structures in natural polymers (i.e. proteins) is typically controlled by H-bonding



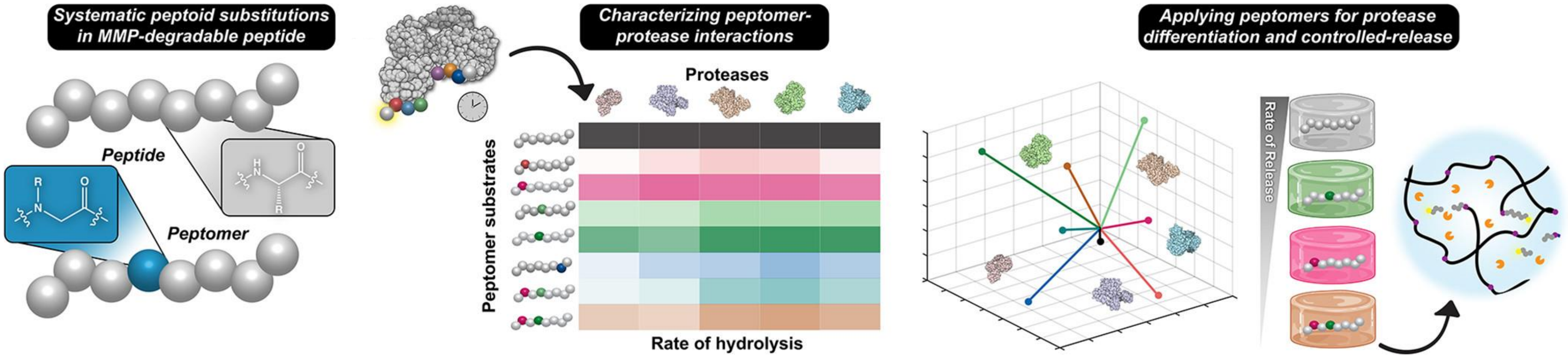


Peptide **Peptoid**

Peptoids have no backbone H-bonding…how do you think that impacts their higher order structure?

Morton, LD., Hillsley, A., et al. *J. Mater. Chem. B*, 2020, **8**, 6925-6933

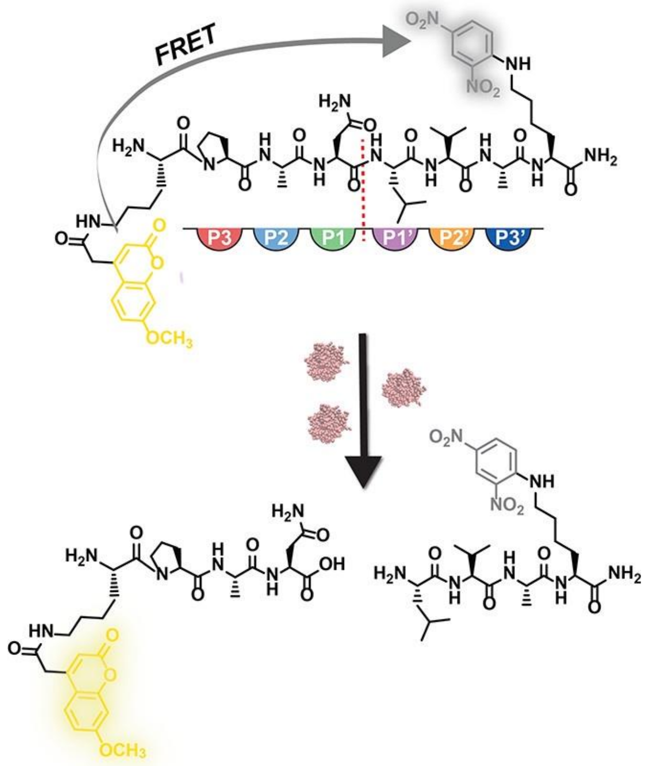
This allows peptoids to be specifically degraded based on sequence

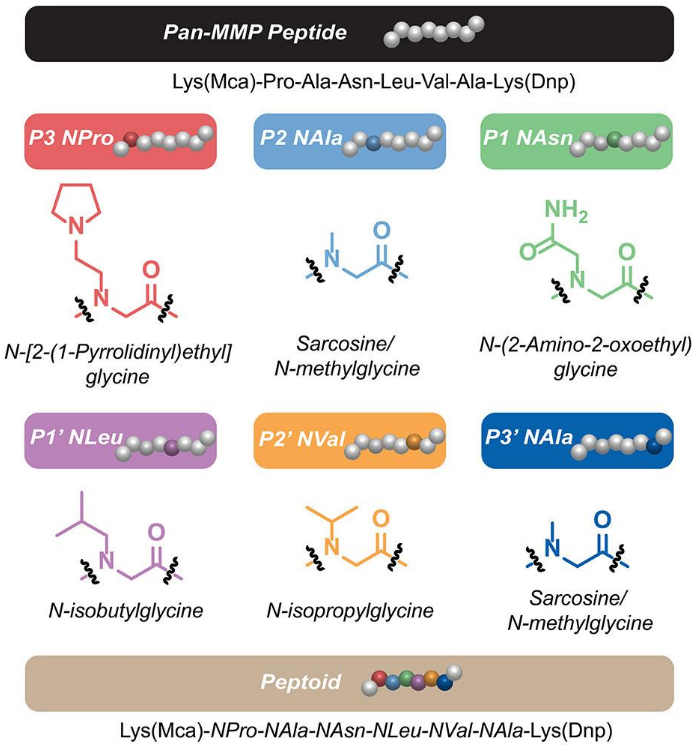


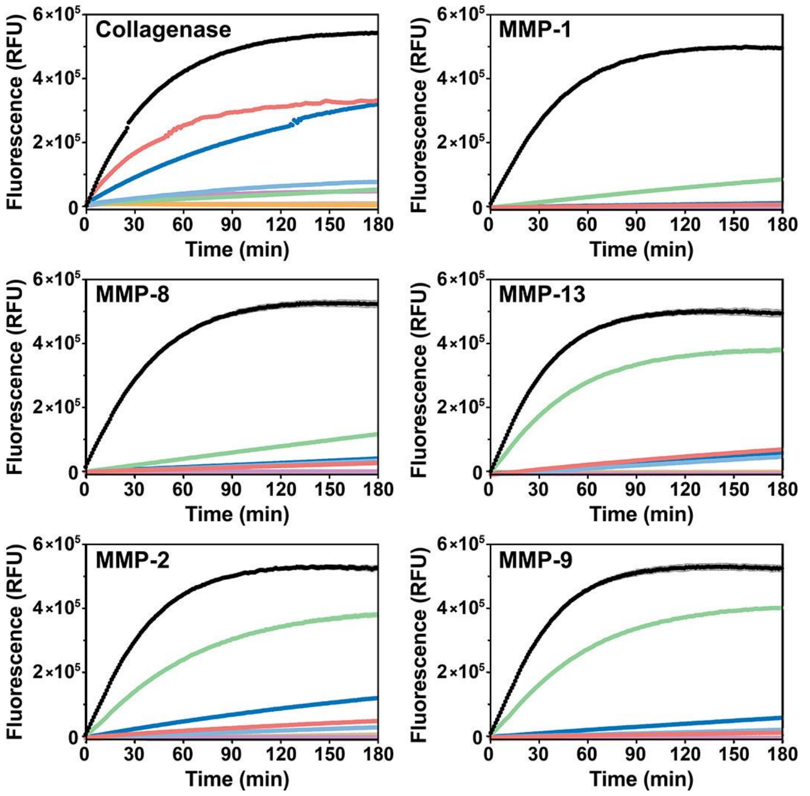
Different peptoid-peptide hybrids (peptomers) will degrade at desperate rates, allowing us to use them as:
Biosensors to determine the concentrations of a variety of proteases in vitro or in vivo
Controlled-release platforms for drug delivery and continual release by incorporating them as hydrogel crosslinkers

Austin, MJ., Schunk, H., et al. *Biomacromolecules* 2022, 23, 11, 4909–4923

Peptoid location dictates degradation rate to a variety of proteases

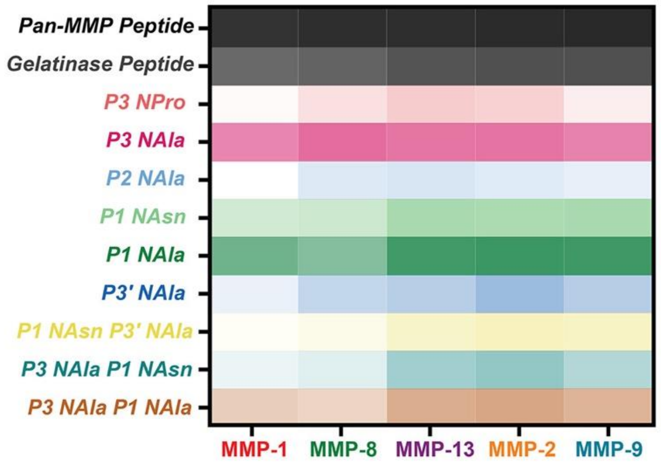




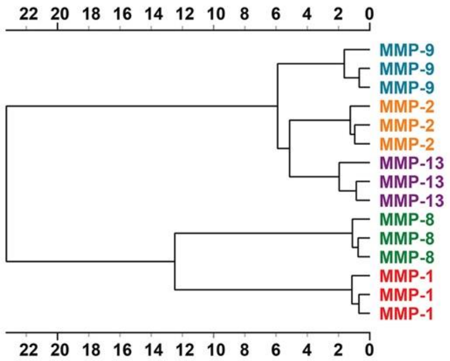


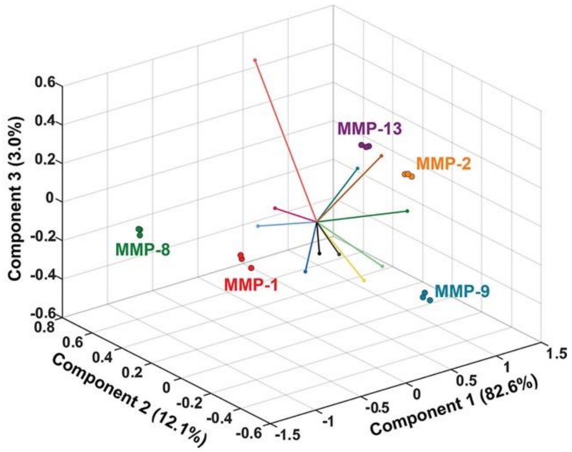
Austin, MJ., Schunk, H., et al. *Biomacromolecules* 2022, 23, 11, 4909–4923

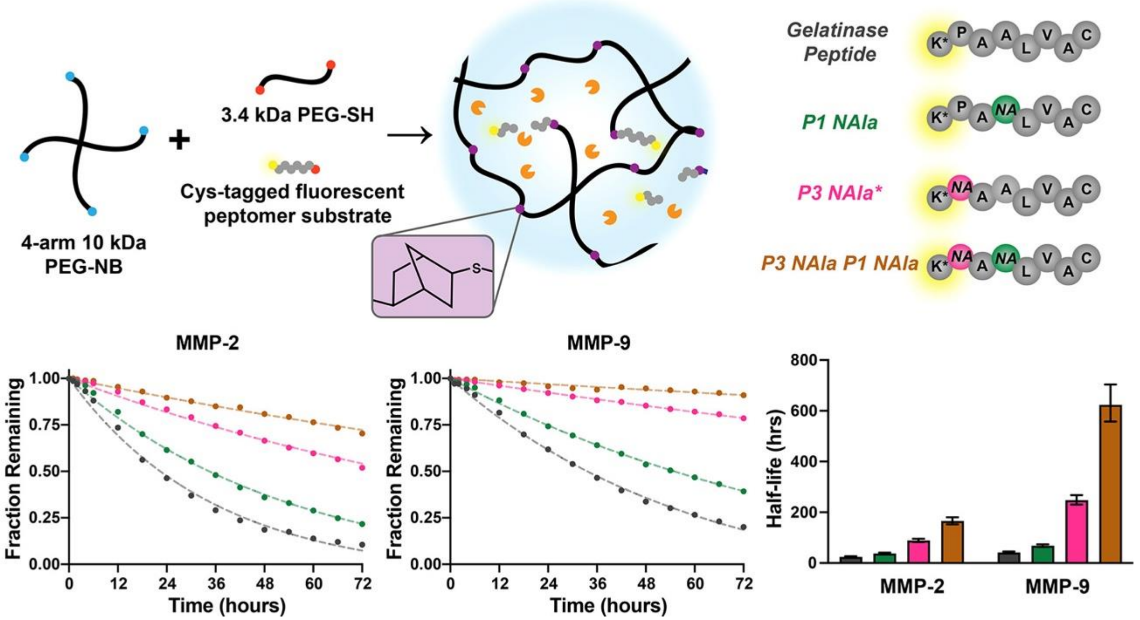
This selective degradation can be understood via machine learning to develop release platforms



Color dictates rate of cleavage of each peptomer







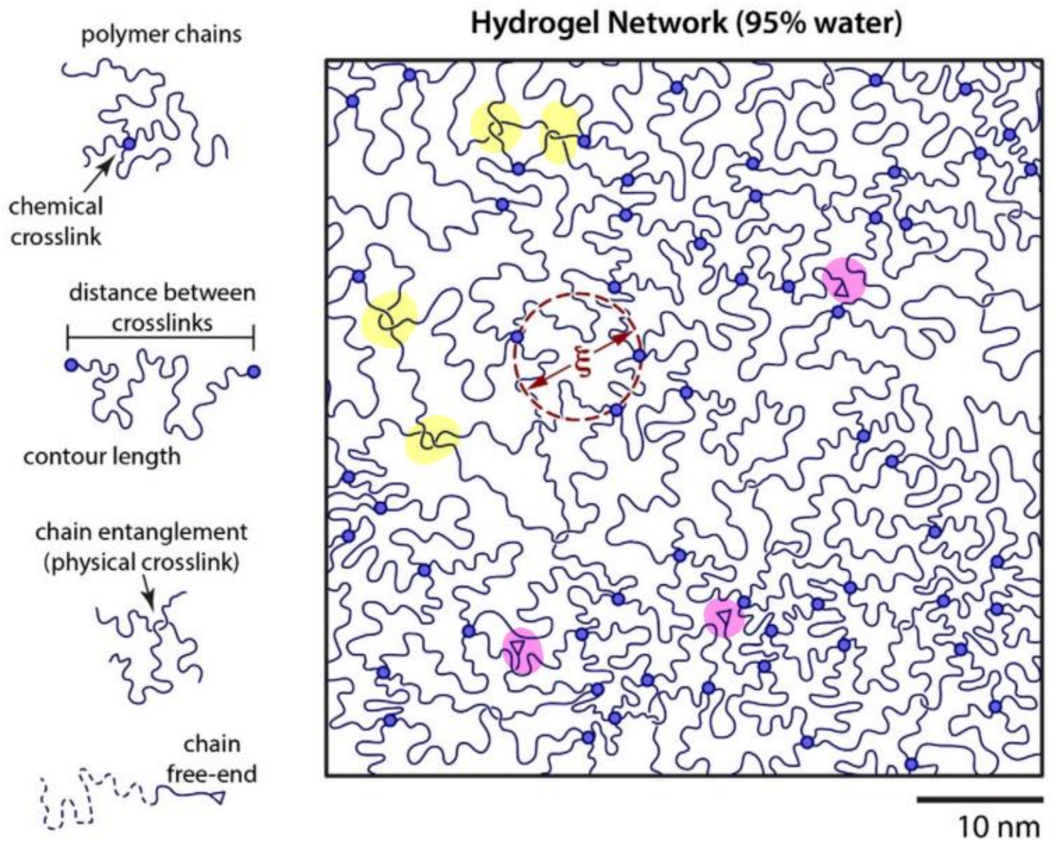
Selective cleavage can be controlled reliably with protease selection (in vivo location) and peptomer sequence

Austin, MJ., Schunk, H., et al. *Biomacromolecules* 2022, 23, 11, 4909–4923

Hydrogel Structure is based on Swollen Polymer Networks

- Typically classified by types of crosslinks:
  - Covalent
  - Physical
    - Ionic
    - Hydrophobic association
    - Hydrogen bonds
    - Guest host

Theoretical Structure:

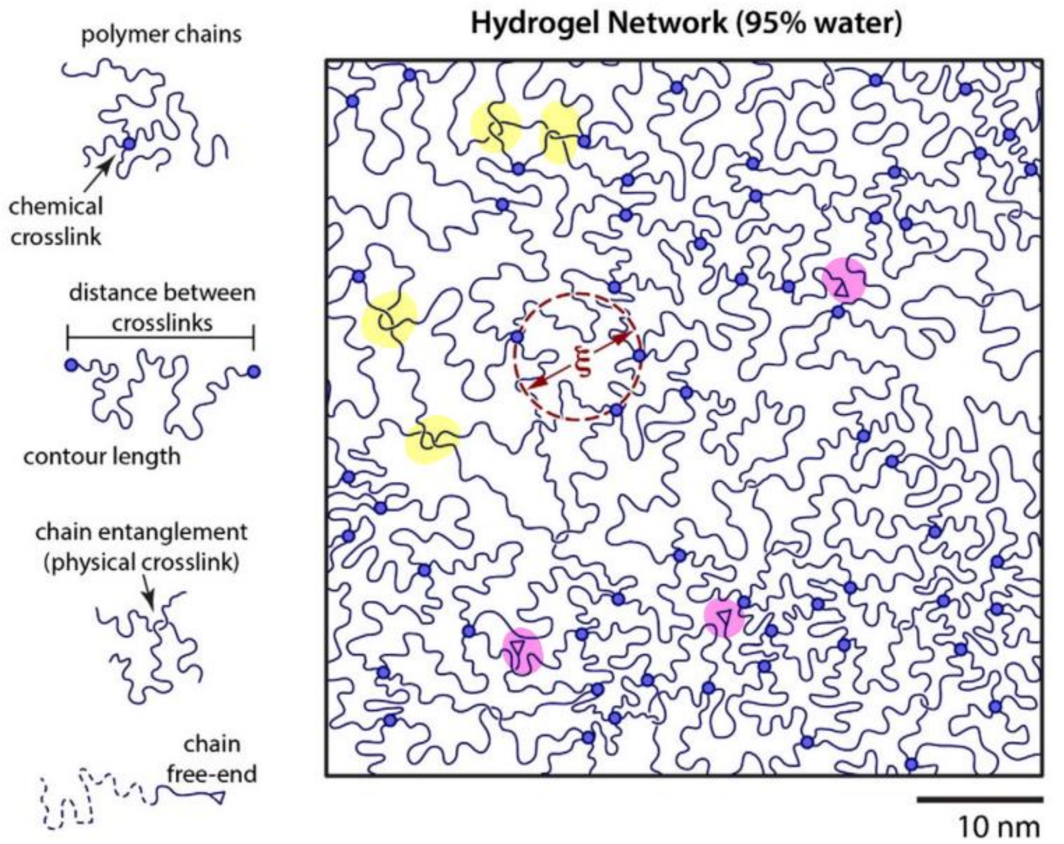


https://hydrogeldesign.org/

Hydrogel Structure is based on Swollen Polymer Networks

- ξ ~ mesh size (size of average "gap")
- Entanglement is very concentration dependent, and will lead to departure from theoretical calculations for rigidity, diffusivity, etc.
- Loop formation will also alter resulting bulk parameters

Theoretical Structure:



https://hydrogeldesign.org/

Covalent Crosslinking Mechanisms Dictate Hydrogel Fabrication

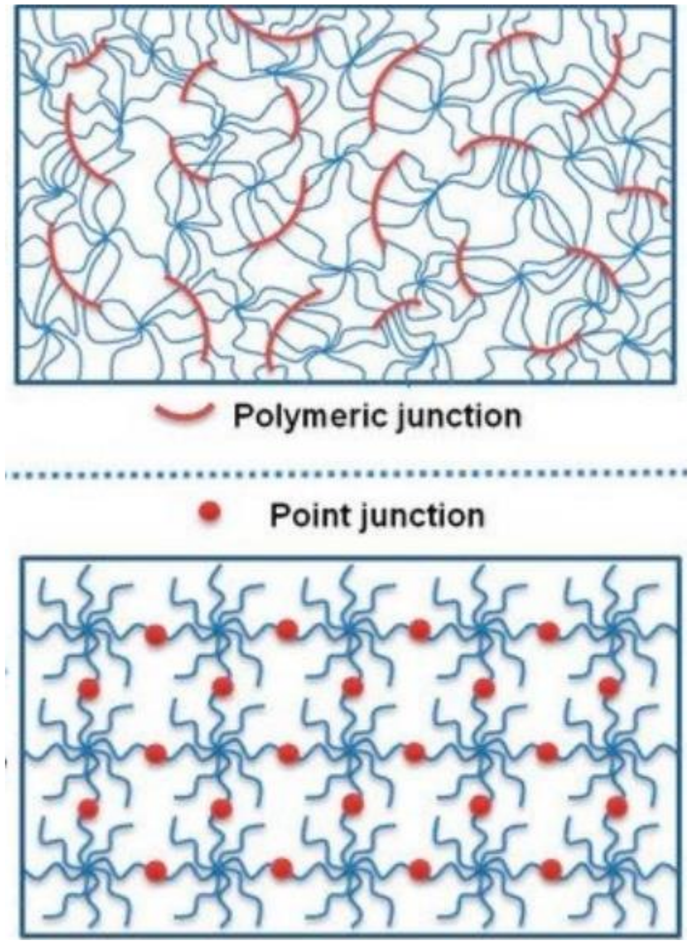
- Recall: Polymers are large molecules built of simple smaller units called monomers

Chain growth: I* + M → P* + M → P_n

➤ Polymer growth takes place between a monomer (M) and a reactive center (*)

Step growth: A-A + B-B → A-B

➤ A "condensation" reaction occurs between 2 polyfunctional molecules

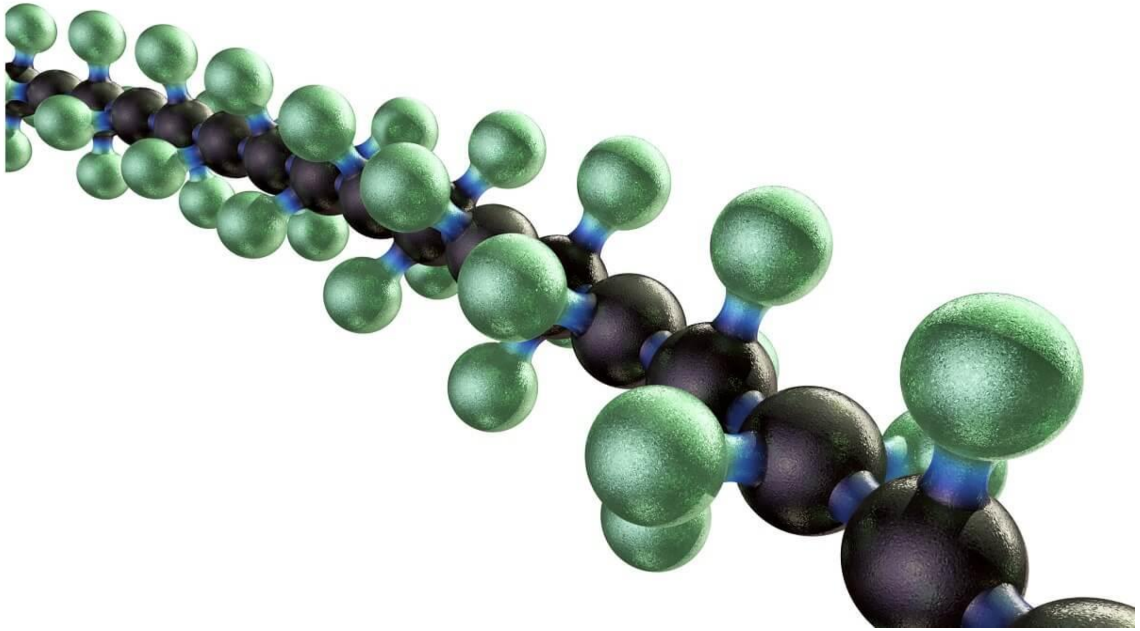


https://hydrogeldesign.org/

Polymers are the Key to Developing Hydrogels for Specific Applications

Synthetic:

- Poly(vinyl alcohol)
- Poly(lactic-co-glycolic acid)
- Poly(ethylene glycol)
- Pluronics (PEG-PPO-PEG)



https://hydrogeldesign.org/

Checkpoint:

Can you list some key hydrogel properties for bioengineering applications?

- Optically clear
- Soft, tissue-like properties
- Very high water uptake/content
- Easy Chemical Modification
- Responsive Swelling (can be "smart")
- Can be degradable
- Can be fabricated in situ (light, enzymes, temperature, salt concentration)

https://hydrogeldesign.org/ American Academy of Opthalmology

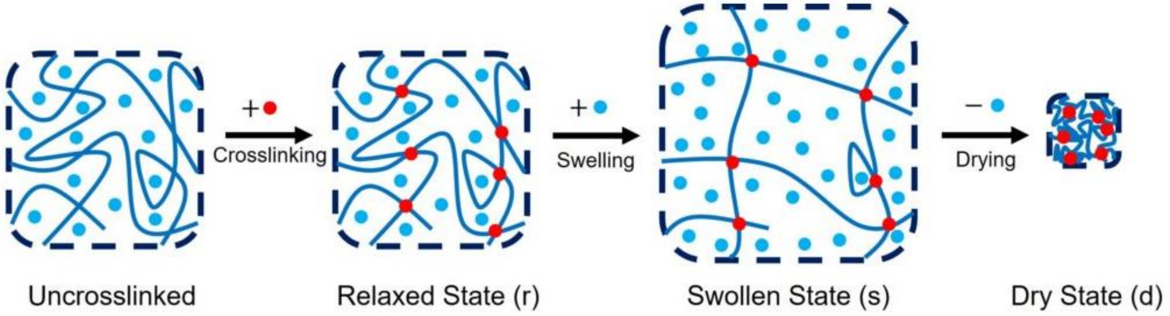
Understanding Swelling is Critical for Utilizing Hydrogels

- $\Delta G_{swell} < 0$ (spontaneous)
- $\Delta G_{swell} = \Delta G_{mix} + \Delta G_{elongation}$

Favorable interaction between polymer and solvent (negative)

Gain in entropy by mixing polymer and solvent

Loss of entropy in network chains as they are stretched



- Swelling depends on solvent quality: χ (interaction parameter)
- Can quantify and predict swelling based on thermodynamics-and relate it back to mesh size (ξ) and the molecular weight between crosslinks (M_C)

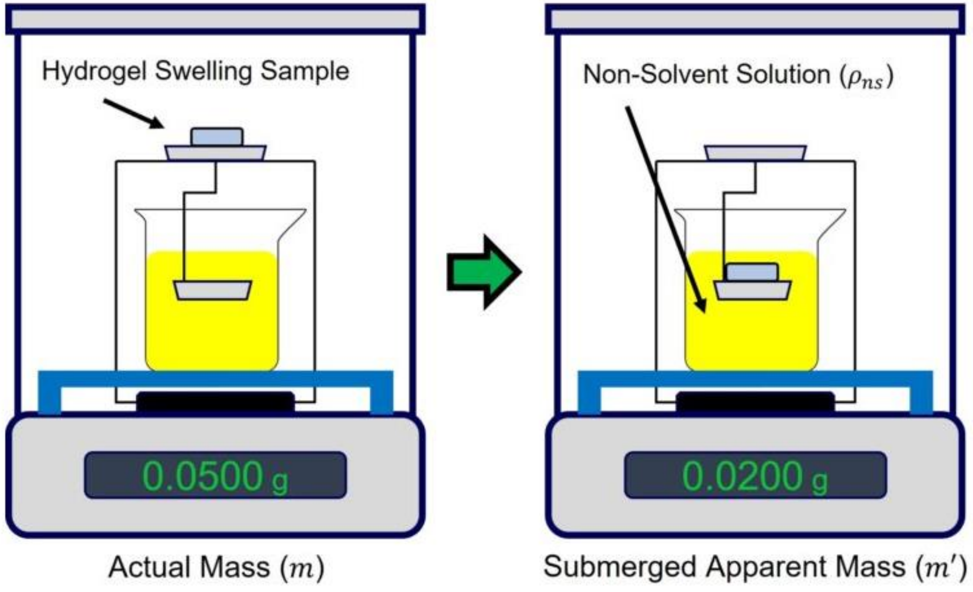
https://hydrogeldesign.org/ Richbourg et al., 2021

Understanding Swelling is Critical for Utilizing Hydrogels

Flory-Rehner and Peppas-Merrill Theory

Fun fact: I actually learned this from Peppas himself. The one from the theory ☺

By equating the free energy (to satisfy equilibrium):



$$V = \frac{m - m'}{\rho_{ns}}$$

$$\varphi_r = \frac{V_d}{V_r}$$

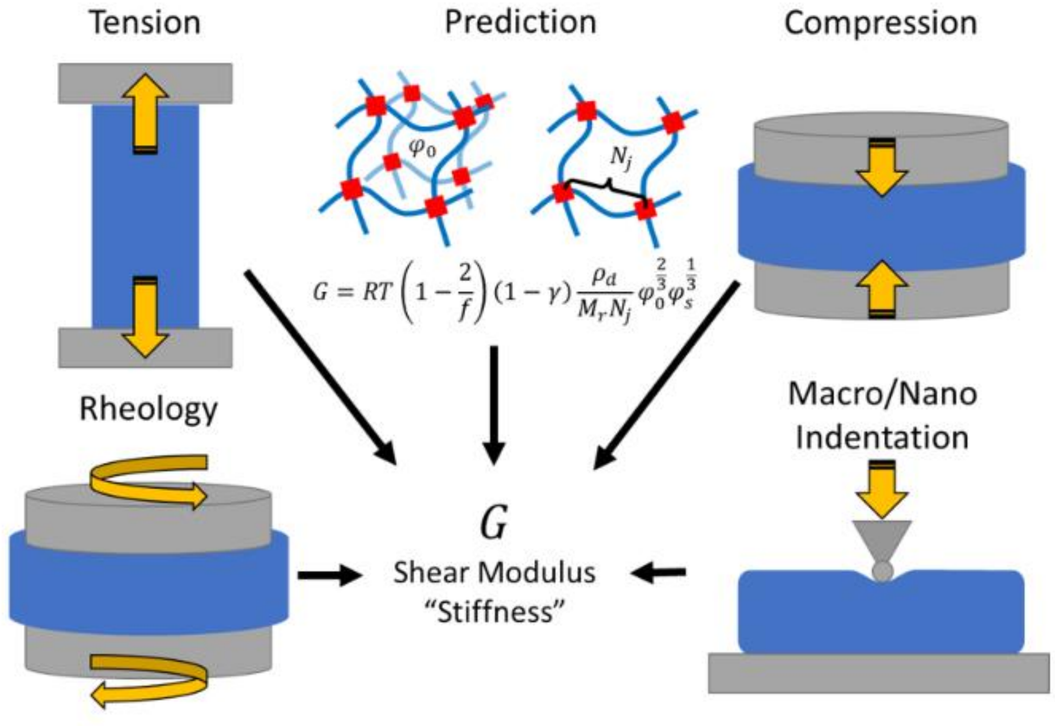
$$\varphi_s = \frac{V_d}{V_s}$$

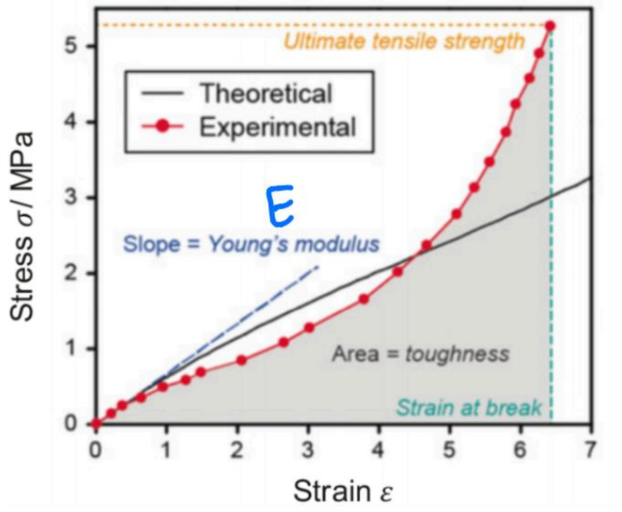
Initial Polymer Volume Fraction (ϕ_0)
Degree of Polymerization between junctions (N_j)
Junction Functionality (f)
Frequency of Chain-End Defects (γ)
Polymer-Solvent Interaction Parameter (χ)
Molecular weight of polymer repeating unit (M_r g/mol)
Dry Density of Polymer Network (ρ_d g/mL)

$\phi_s = Q^{-1}$ (the inverse of the swelling ratio)

https://hydrogeldesign.org/ Richbourg et al., 2021

Determining Young's Modulus for a Gel

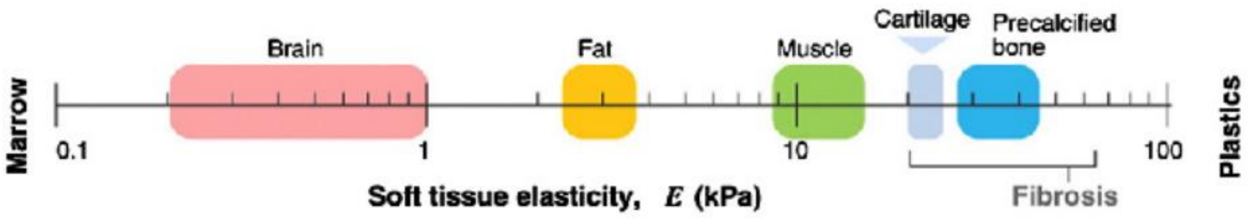


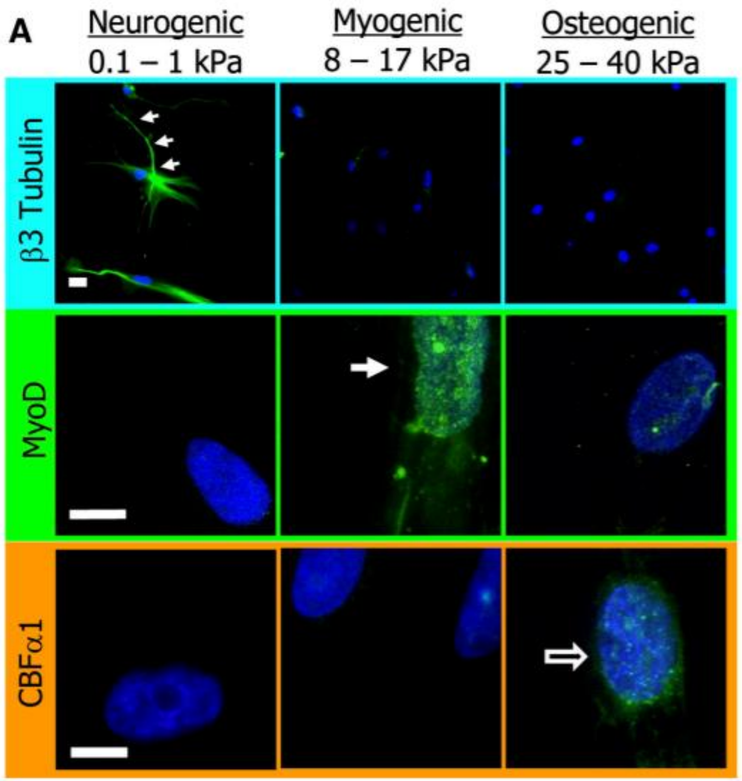


This theory is great and very useful…but it neglects some very interesting aspects of hydrogel design

https://hydrogeldesign.org/ Richbourg et al., 2021

Why Stiffness Matters: A great example from Dennis Disher's Work

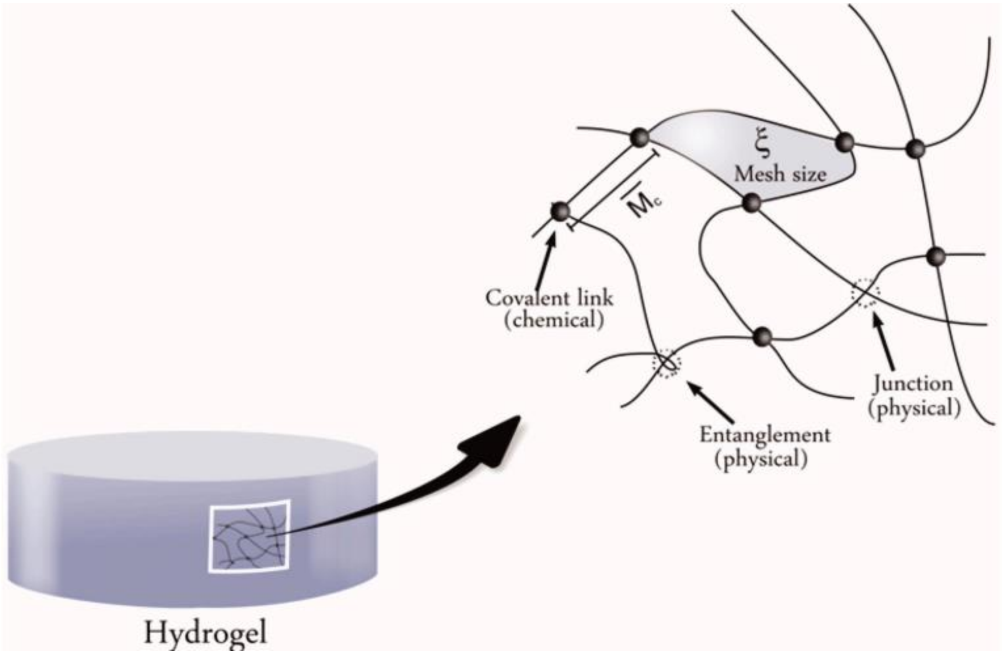




Stem cells "feel" the stiffness of their surface, influencing their differentiation pathways

Engler et al. 2006

Hydrogel Diffusivity is Dictated by Mesh Size



Flory-Rehner and Peppas-Merrill Theory

$$\xi = \phi_s^{-\frac{1}{3}} \left[\left(1 - \frac{2}{f} \right) \bar{l}^2 C_\infty \lambda N_j \right]^{\frac{1}{2}}$$

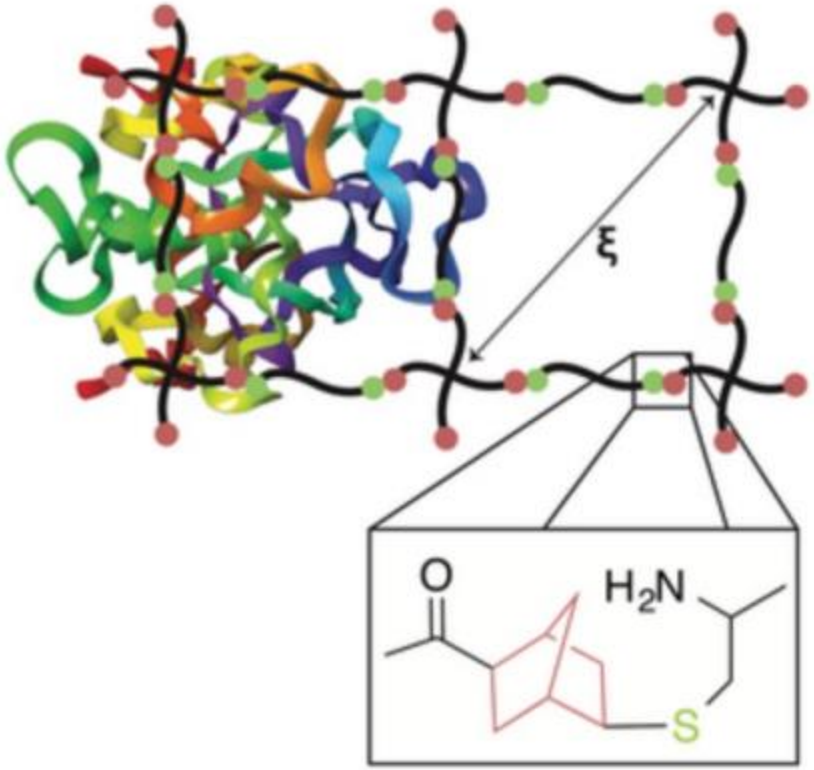
Using our newly calculated ϕ_s, as well as:

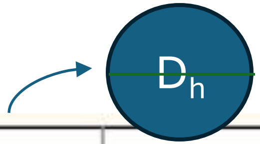
Junction Functionality (f)
The Average Bond Length in the Polymer Backbone
(l) Flory's Characteristic Ratio (C_∞)
Number of Backbone Bonds in the Polymer Repeating Unit (λ)
The degree of polymerization between junctions (N_j)

We can determine mesh size.

https://hydrogeldesign.org/ Richbourg et al., 2021 Rehmann et al., 2017

Protein Diffusivity is Modulated Through Mesh Size



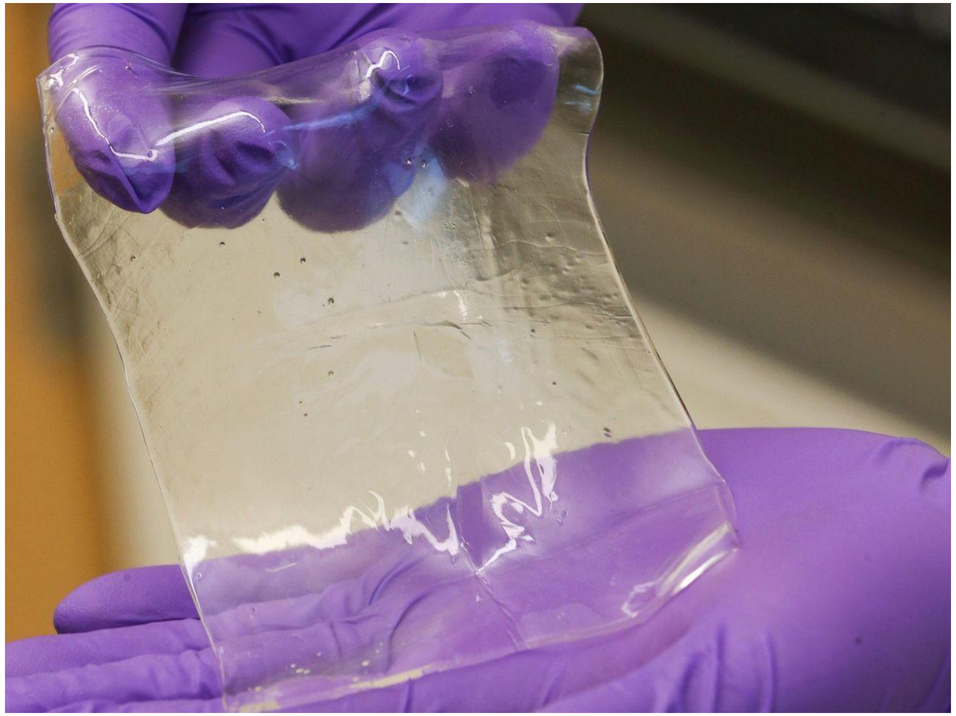


Protein	Molecular Weight	Hydrodynamic Diameter	Diffusivity in Water ($\times 10^{-8}$ cm^2/s)	Diffusivity in Hydrogel ($\times 10^{-8}$ cm^2/s)
Aprotinin	7 kDa	3.0 nm[81]	161	24.1 ± 3.8
Myoglobin	17 kDa	3.9 nm[75]	124	9.4[*]
Lactoferrin	77 kDa	6.1 nm[75]	79	2.8 ± 0.1
BSA	66 kDa	7.2 nm[75]	67	1.9 ± 0.5
Thyroglobulin	670 kDa	17.2 nm (Manufacturer)	28	< 0.1[**]

So if you want to deliver a protein of a known D_h the mesh size of the hydrogel is of paramount importance

Rehmann et al., 2017

Checkpoint 2:
Can you list some applications for hydrogels?



Tissue engineering

Cell manufacturing

Drug/cell delivery

Disease models

Agriculture

Consumer products (diapers, hair gel)

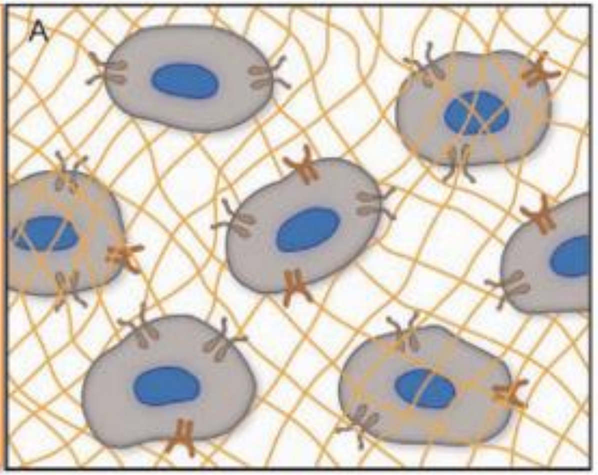
Heat sinks for electronics

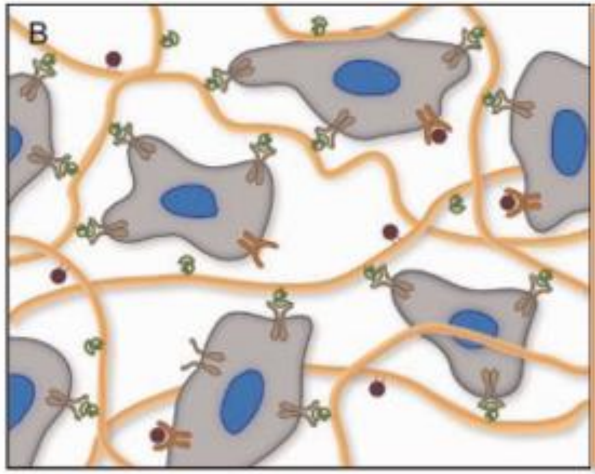
Flexible electronics


Saving irrigation
water and
protecting crops
from droughts
Biohydrogel

Synthetic Hydrogels

- Diverse backbones
- Chemically defined
- Top-down design
- Bioinert
- Traditionally static

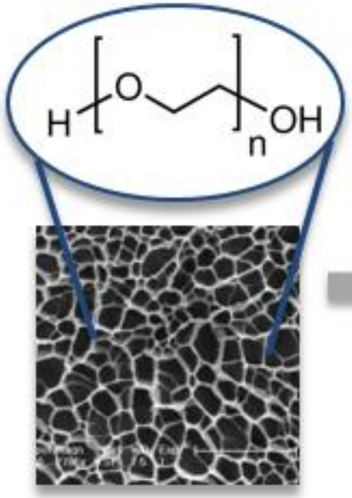


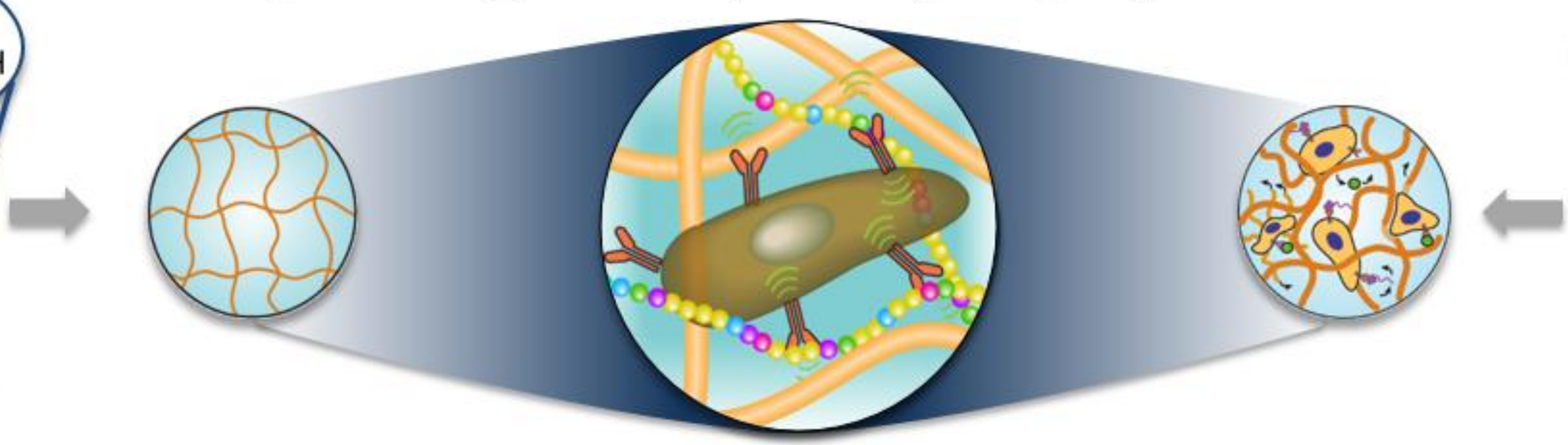


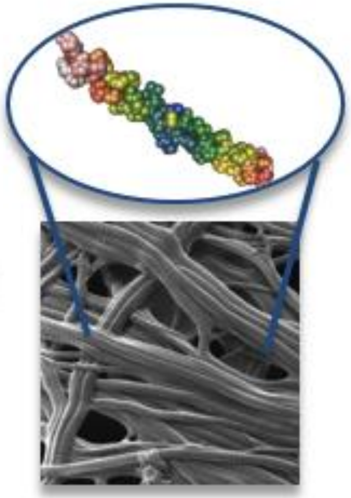
Biological ECM

- Heterogeneous
- Tissue dependent
- Self-assembled
- Bioactive
- Dynamic

Engineering bio-inspired hydrogel platforms

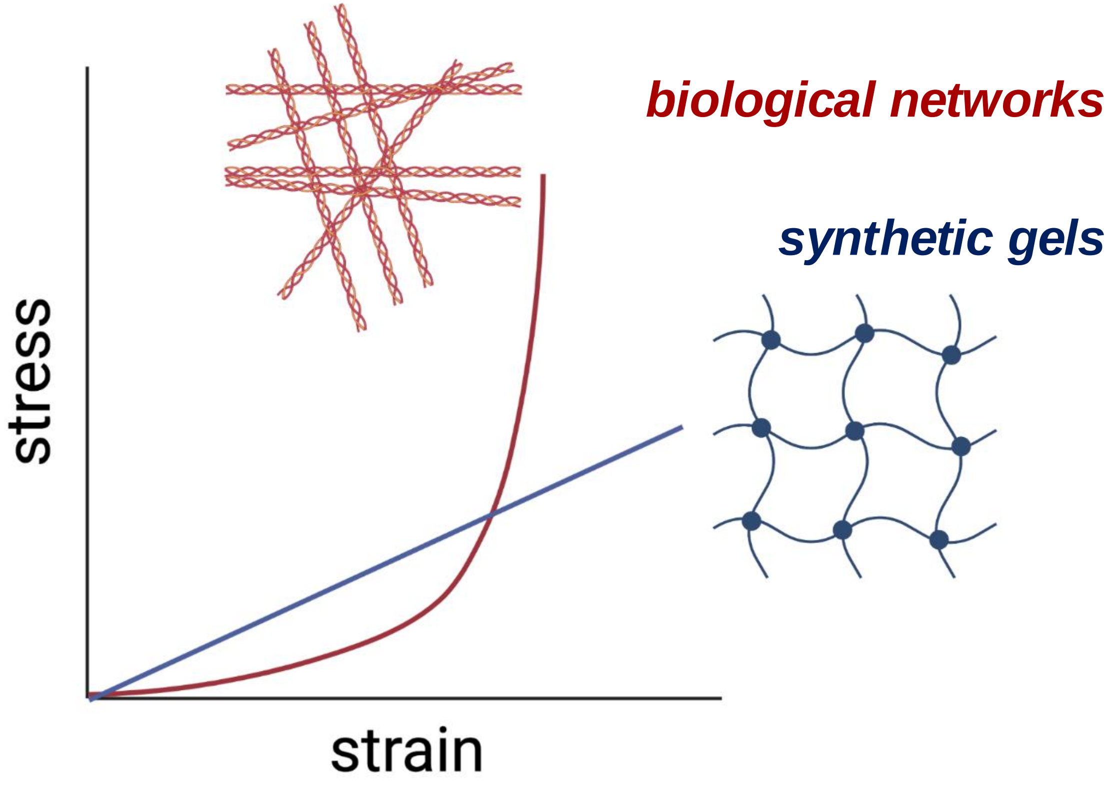






Tibbitt, M. W. et al, 2009

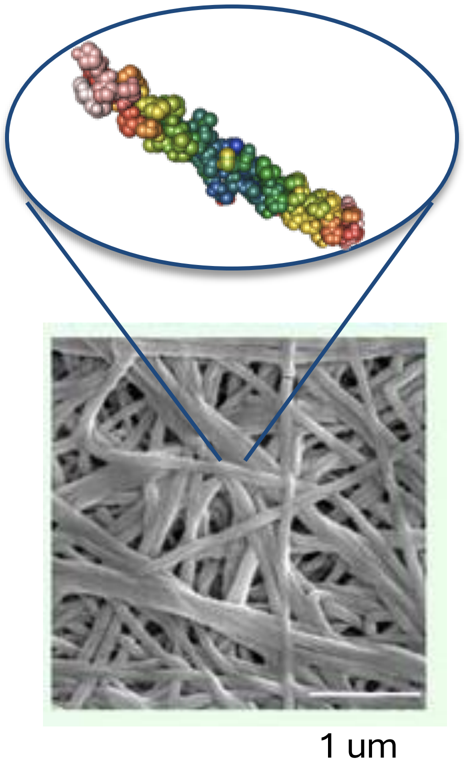
Synthetic gels do not replicate multiscale mechanics of ECM



Biological networks have nonlinear mechanics

Nonlinear mechanics arises from chain shape

Biopolymers have hierarchical structure



Collagen

Table 1 Persistence lengths of some common polymers[a]

Polymer	Persistence length
F-Actin	17 μm
Single-walled carbon nanotubes	10 μm
Double stranded DNA	50 nm
Collagen	20 nm
Alginate	15 nm
Hyaluronic acid	4 nm
Poly(3-hexylthiophene)	2 nm
Polystyrene	0.3 nm

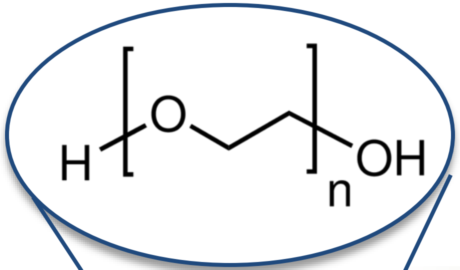
[a] Note that persistence lengths given here are measured with different techniques and in different conditions.

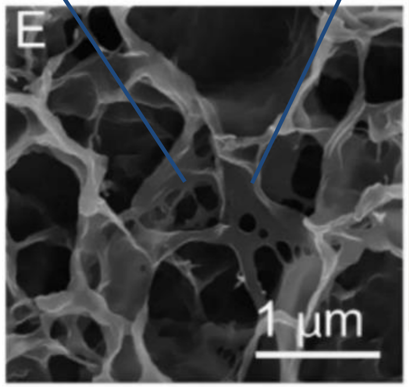
Biopolymers

↓

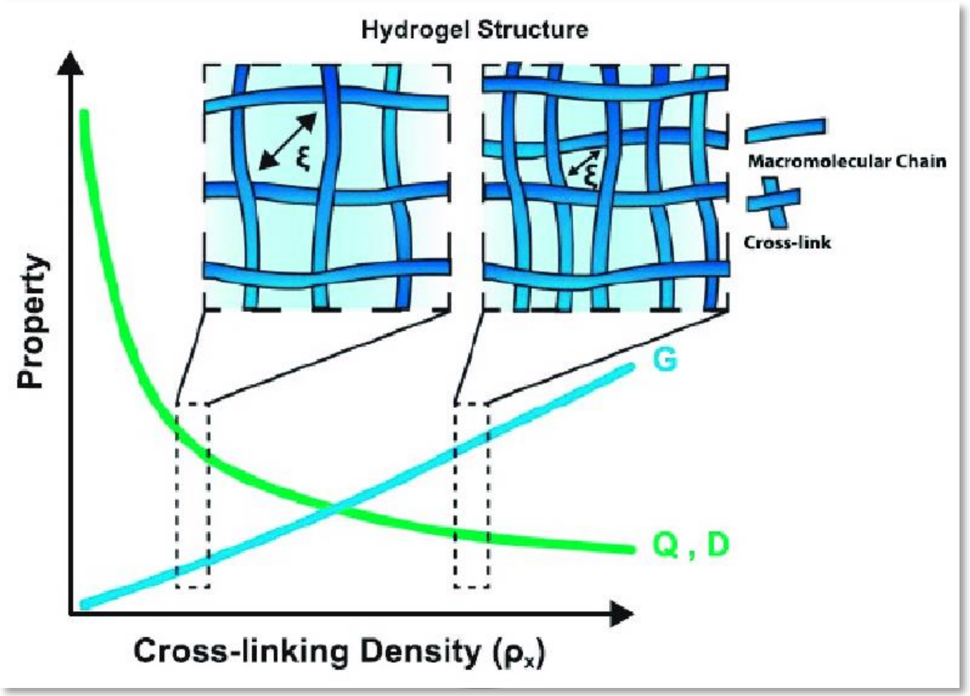
Synthetic Polymers

Chain shape does not traditionally contribute to synthetic hydrogel mechanics





PEG Hydrogel

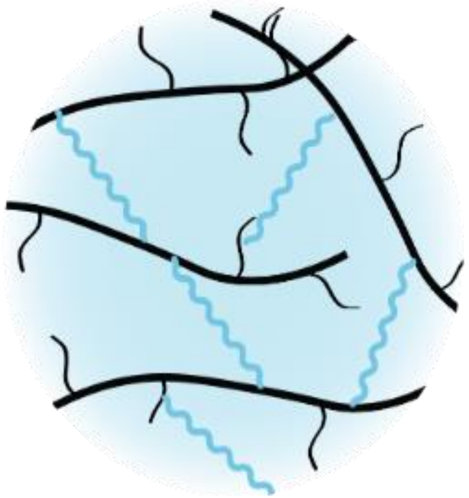


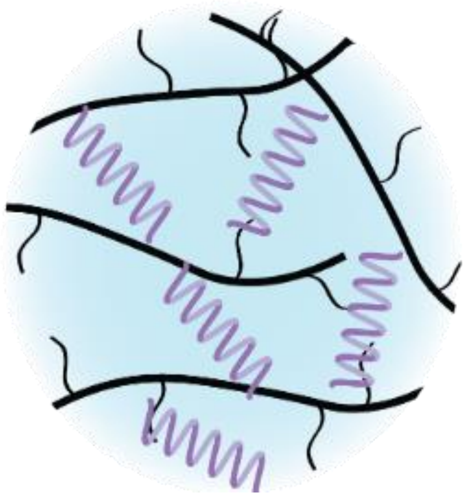
Crosslinking density/ network connectivity are linked to storage modulus

Hard to separate storage modulus from changes in mesh size/ swelling ratio

Hypothesis: Chain structure can be leveraged to yield tunable hydrogel stiffness independent of network connectivity

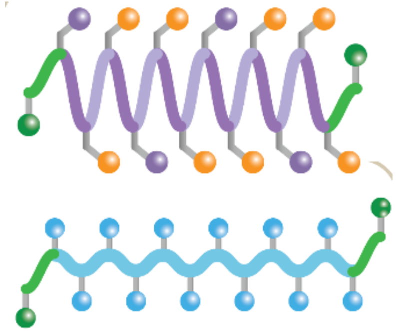
Soft





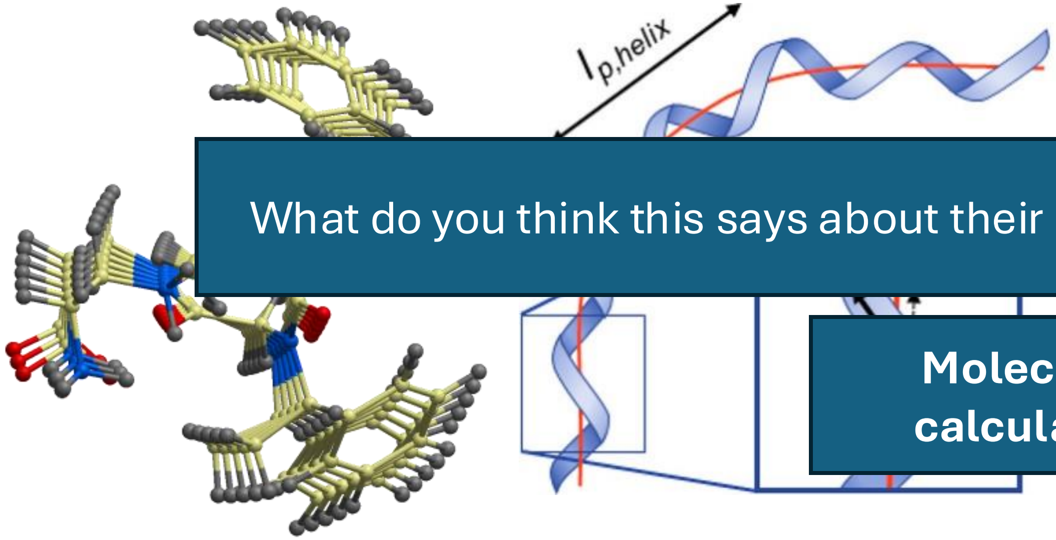
Stiff

Gel Macromer (NorHA)

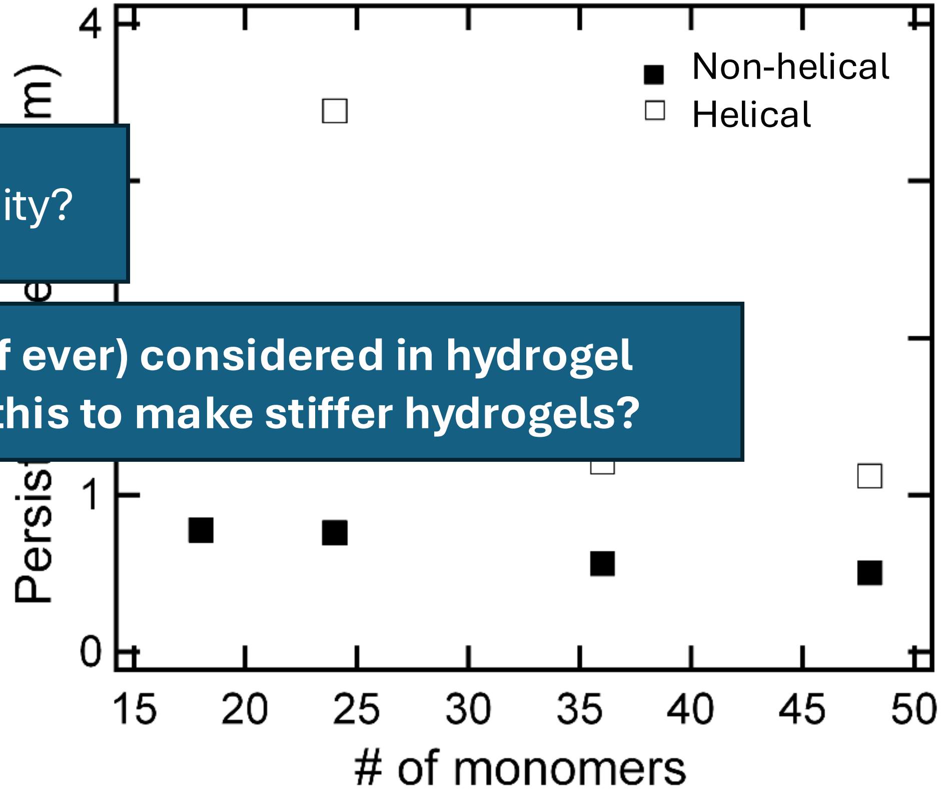


Helical/non-helical peptoid cross-linkers

Peptoid Secondary Structrue Impacts Persistence Length



What do you think this says about their relative molecular rigidity?

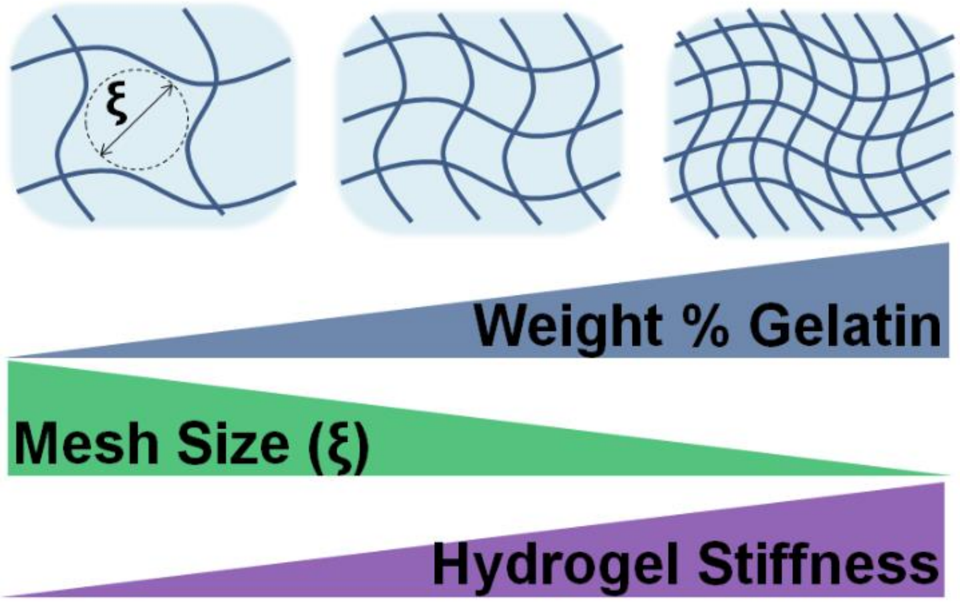


Molecular rigidity is rarely (if ever) considered in hydrogel calculations. Could we use this to make stiffer hydrogels?

- 100% chiral aromatics on one face
- Hexameric or trimeric repeat motif

Soft Matter, 2012,8, 3673-3680 *Macromolecules* 2018, 51, 5, 2089-2098

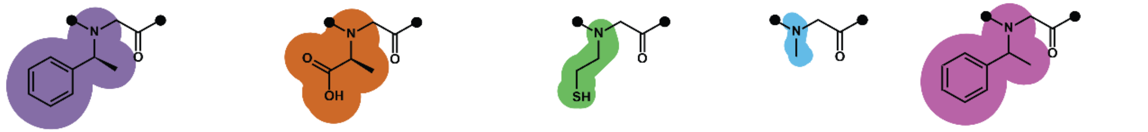
Designing Peptoid Crosslinkers for Hydrogel Fabrication



Classically, hydrogel stiffness is intrinsically coupled to mesh size (via crosslink density or polymer concentration)
But we can address this using specialty synthetic polymers like peptoids.

Subunits:

Nspe	***Nsce***	***Nthe***	**Sar**	***Nmb***
N-(*S*-1-phenylethyl) glycine	*N*-(*S*-1-carboxyethyl) glycine	*N*-2-(thioethyl) glycine	*N*-methyl glycine	*N*-(±-1-phenylethyl) glycine



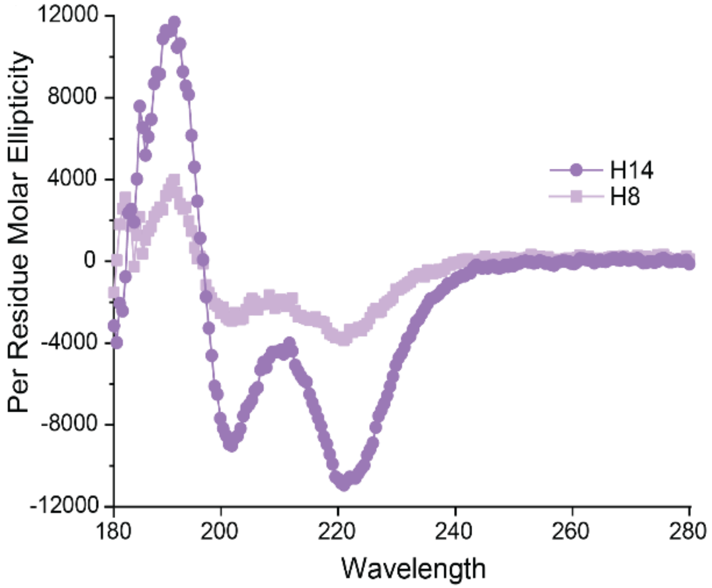
Peptoid:

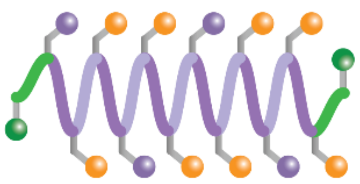
	Name	*N* (total monomers)	*Sequence*
Helical	**H8**	8	*N*the(*N*spe*N*sce*N*sce)$_2$*N*the
	H14	14	*N*the(*N*spe*N*sce*N*sce)$_4$*N*the
Non-Helical	**N8**	8	*N*the(*N*mb*N*sce*N*sce)$_2$*N*the
	N14	14	*N*the(*N*mb*N*sce*N*sce)$_4$*N*the
Unstructured	**U8**	8	Cys(SarSarSar)$_2$Cys
	U14	14	Cys(SarSarSar)$_4$Cys

Morton et al. 2020, Morton et al. 2022

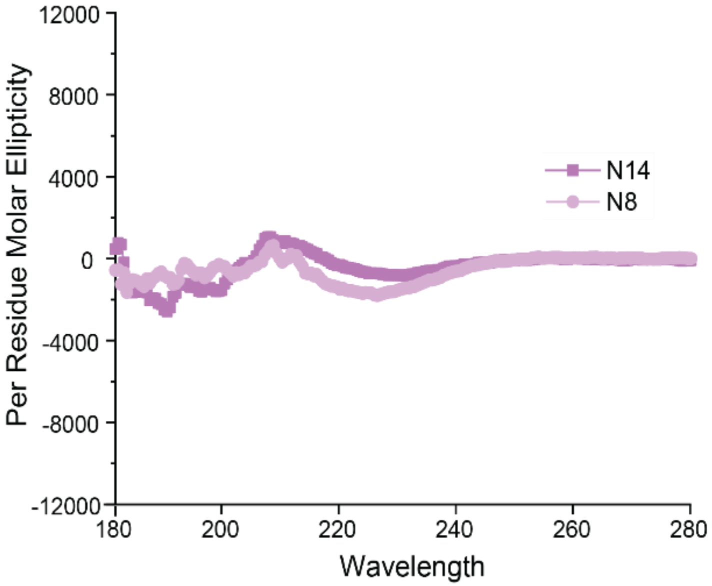
Circular Dichroism Indicates Peptoid Secondary Structure

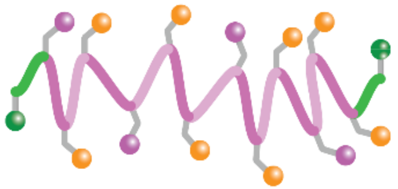
Helical



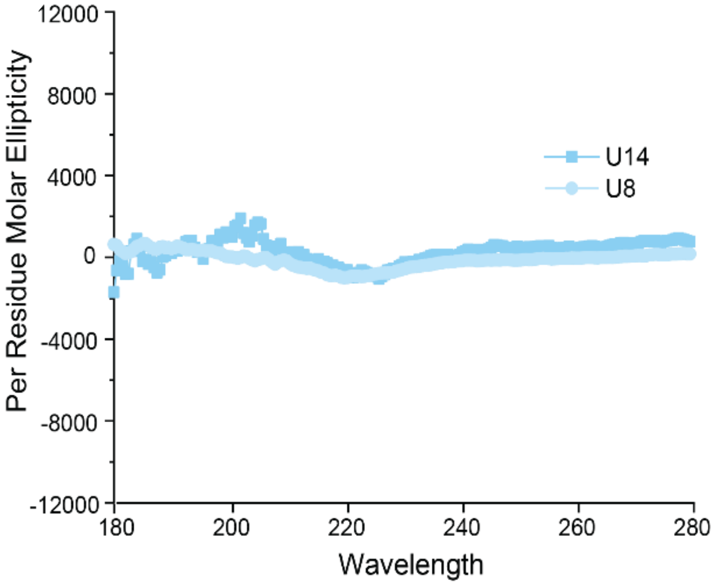


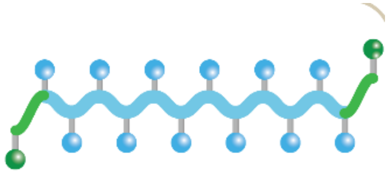
Non-Helical





Unstructured

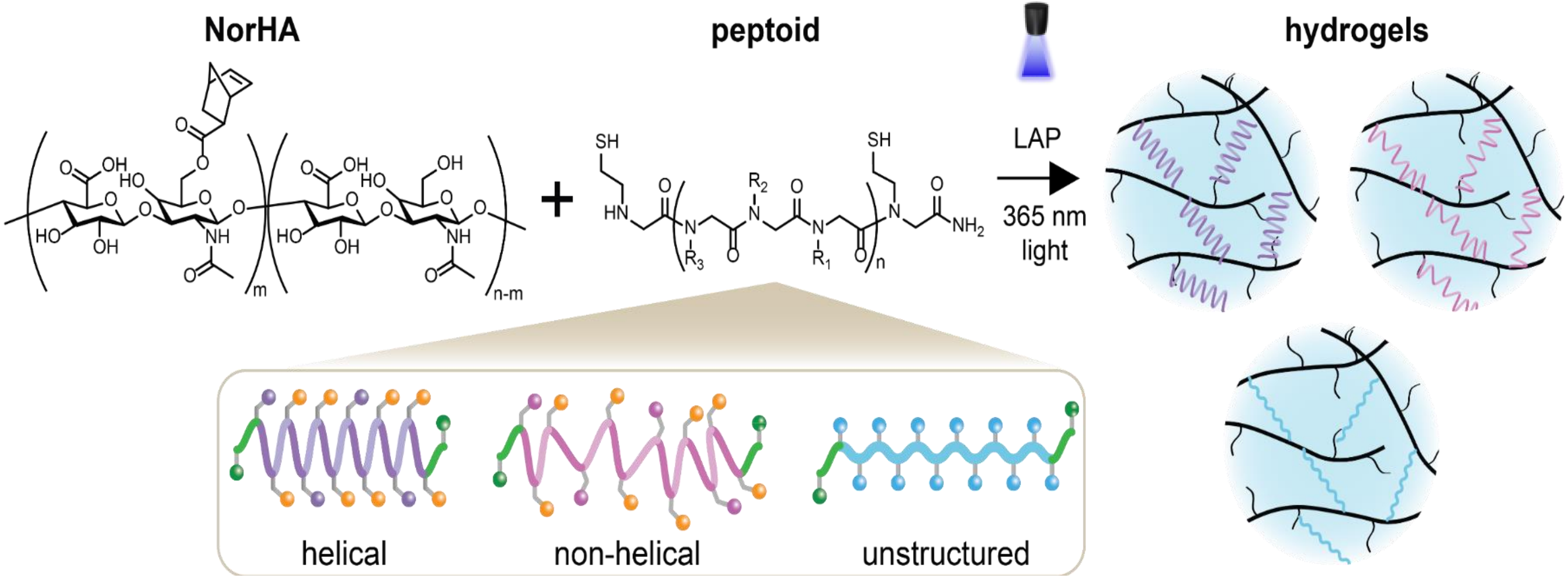




Morton et al. 2020, Morton et al. 2022

Peptoids Are Amenable to Photoinitiated Crosslinking

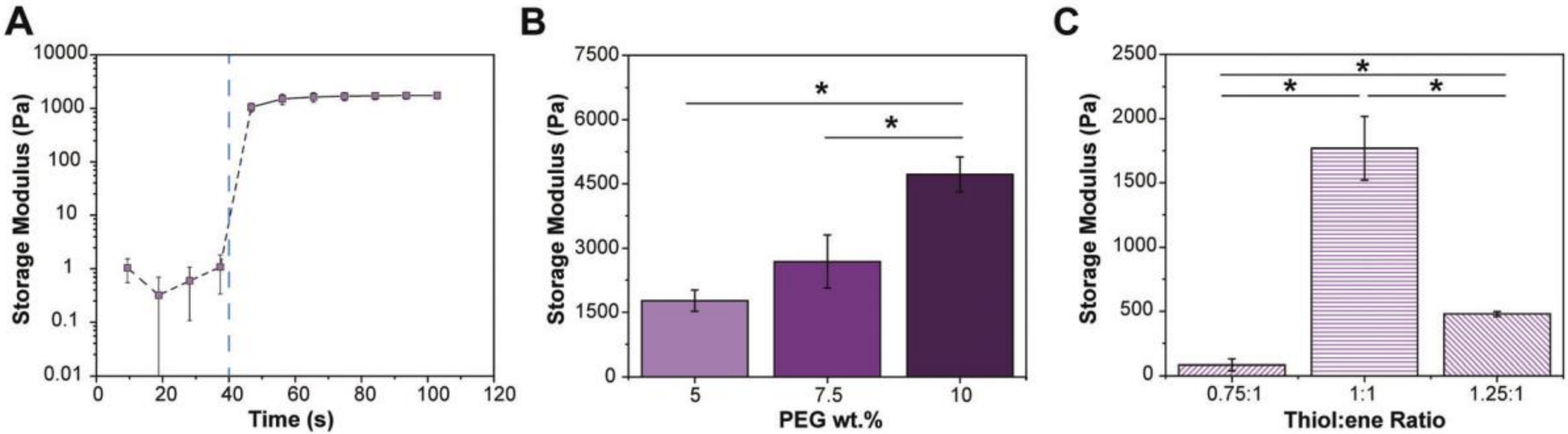
Norbornene-functionalized Hyaluronic Acid



Irradiation with light leads to bond formation and crosslinked network

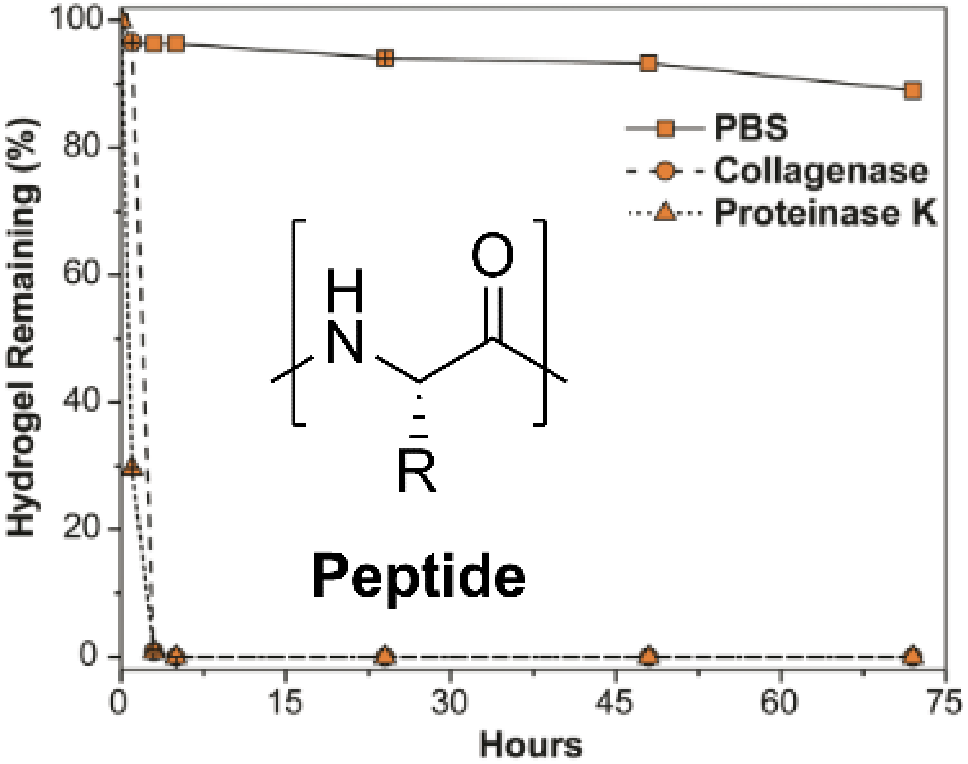
Morton et al. 2020, Morton et al. 2022

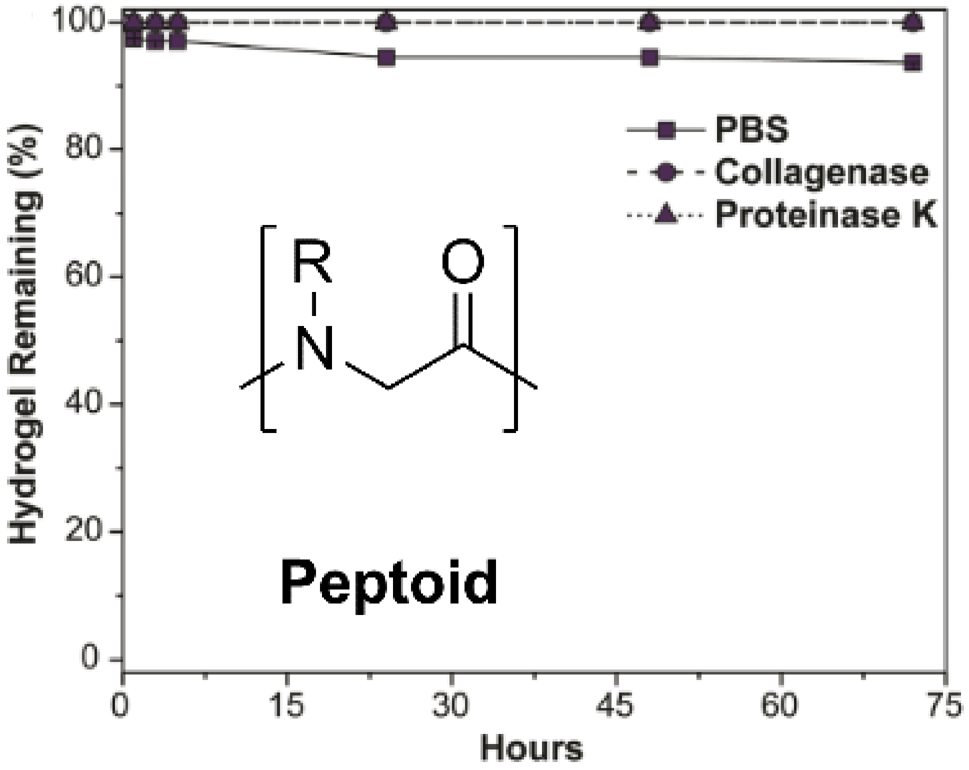
Peptoid crosslinkers work basically identically to peptide controls



Morton et al. 2020

Changing the location of the R group prevents enzymes from cleaving peptoids

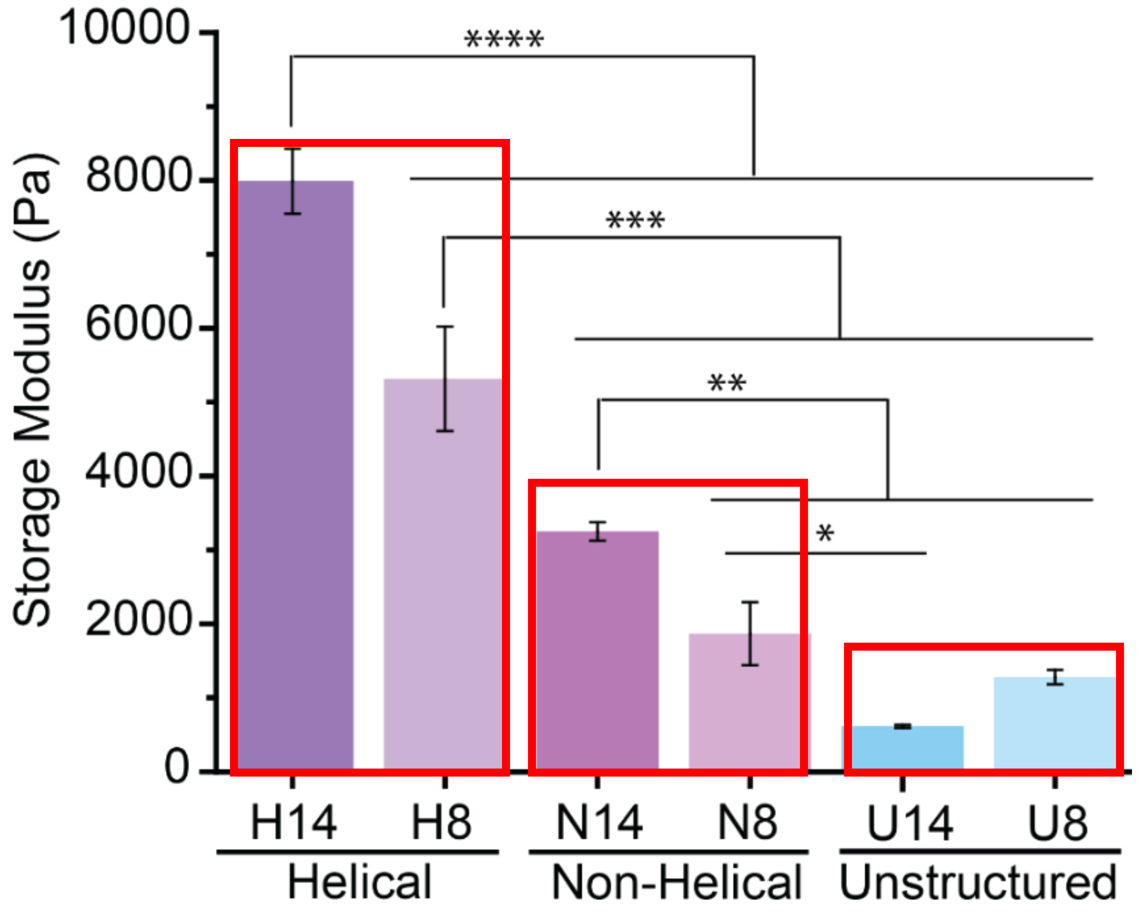




Morton, LD., Hillsley, A., et al. *J. Mater. Chem. B*, 2020, **8**, 6925-6933

Peptoid Length and Secondary Structure Modulate Bulk Hydrogel Stiffness

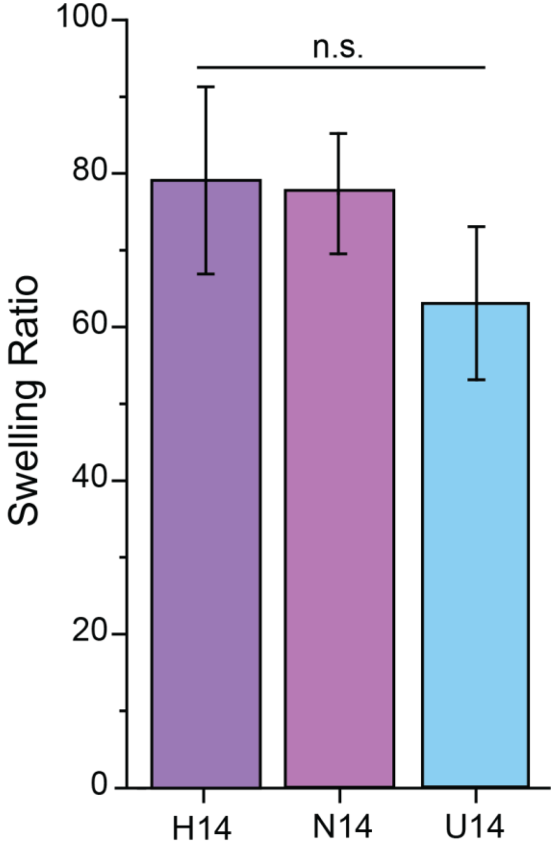
- Helical peptoids result in stiffer hydrogels
- Longer helical and non-helical peptoids increase stiffness, counter to rubber elasticity theory
- The unstructured peptoids restore the expected trend



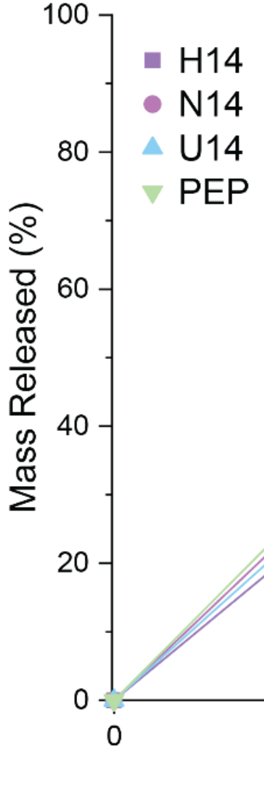
Morton 2022

Maintaining Network Connectivity Allows for other Hydrogel Properties to be Held Constant

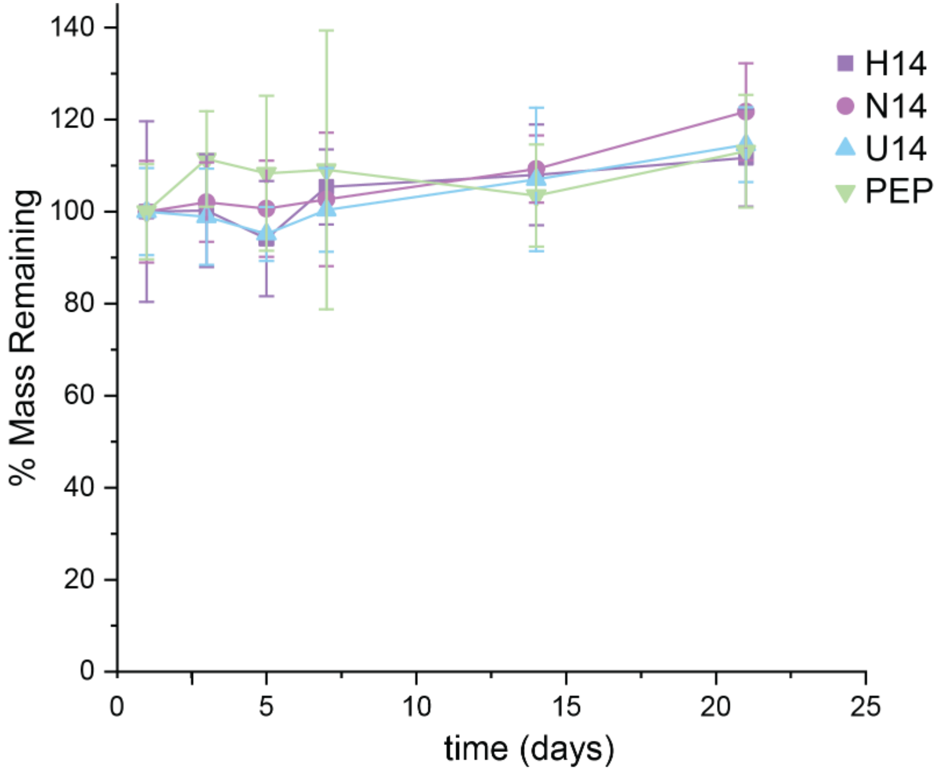
Swelling in water



Permeability to 40 kDa Dextran-FITC

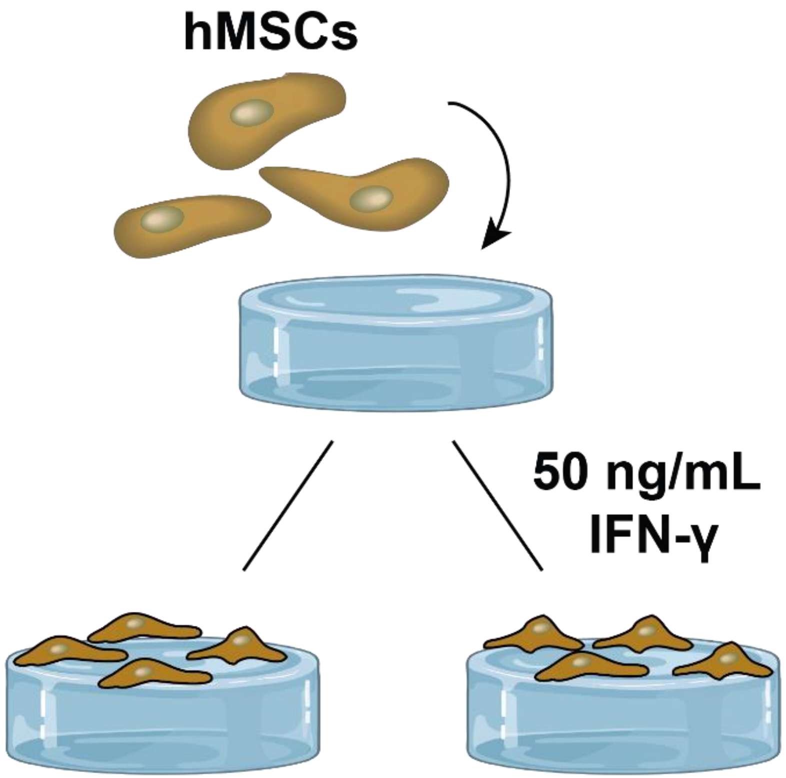


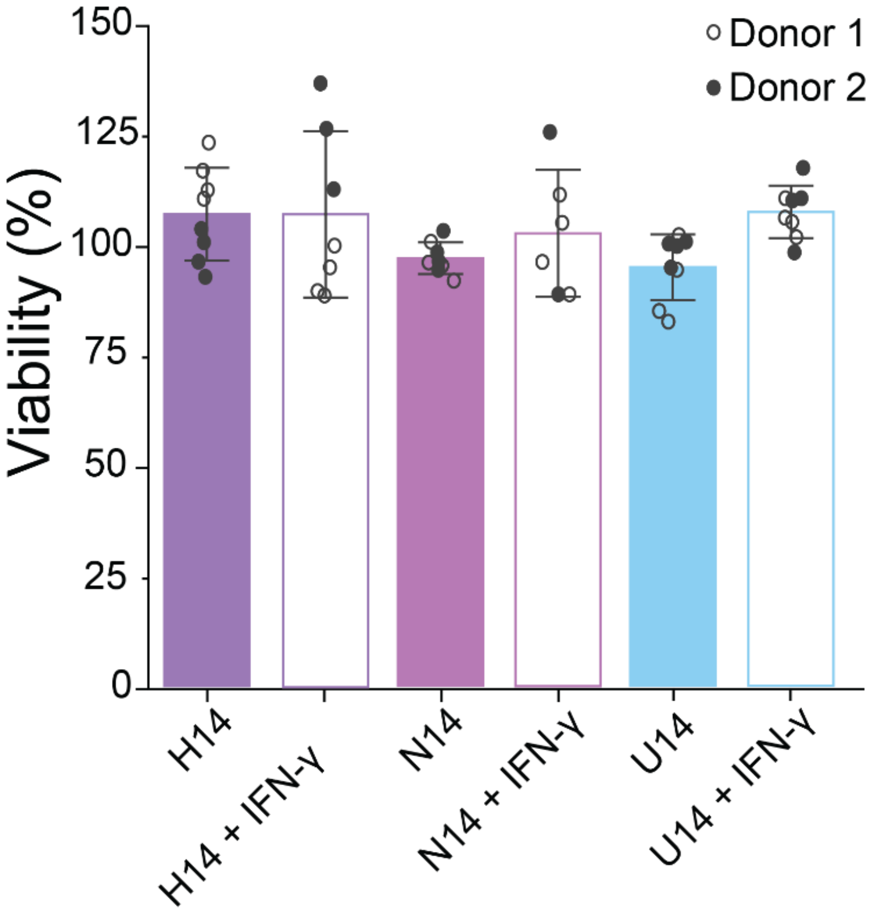
Degradation in the presence of Collagenase



Morton 2022

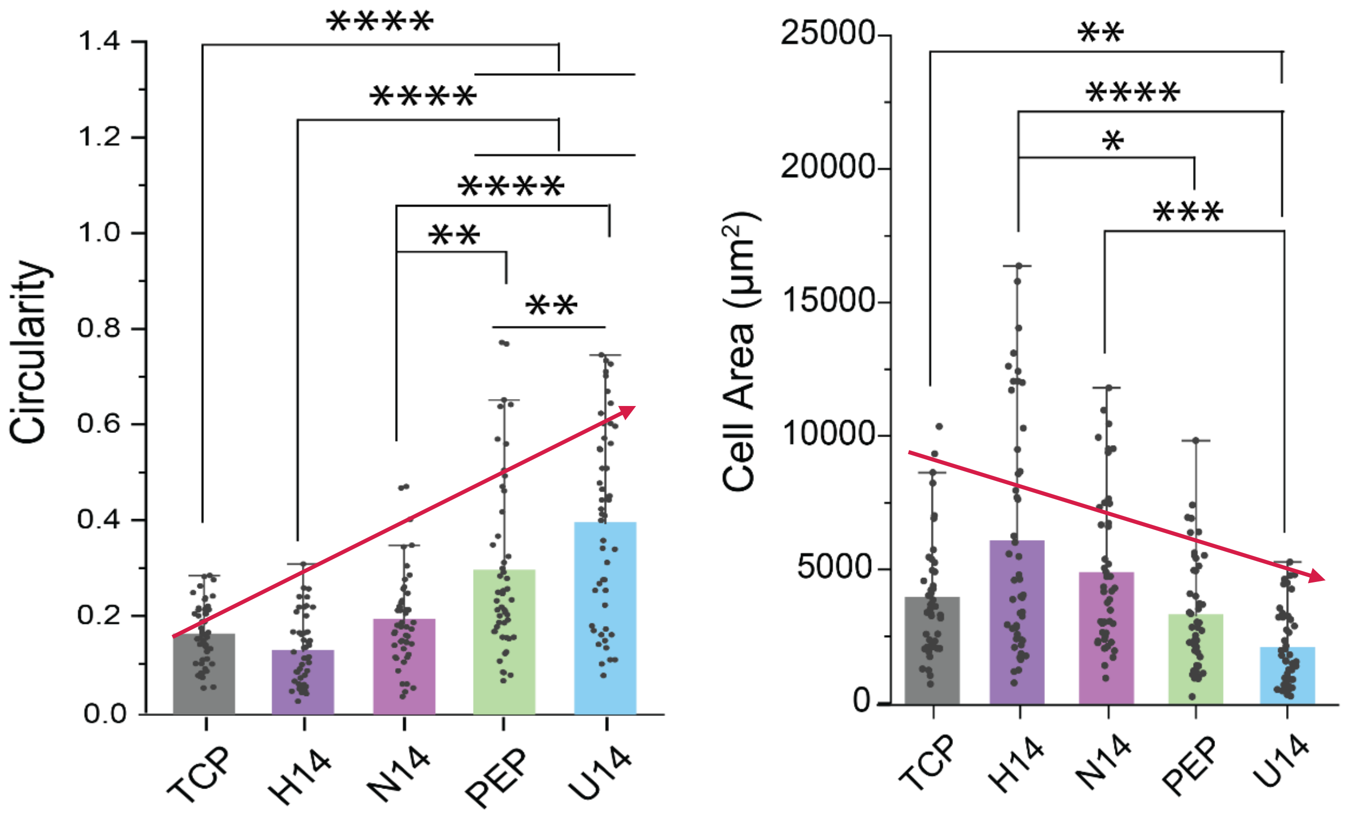
All NorHA Hydrogels were Viable Cell Culture Platforms for hMSCs

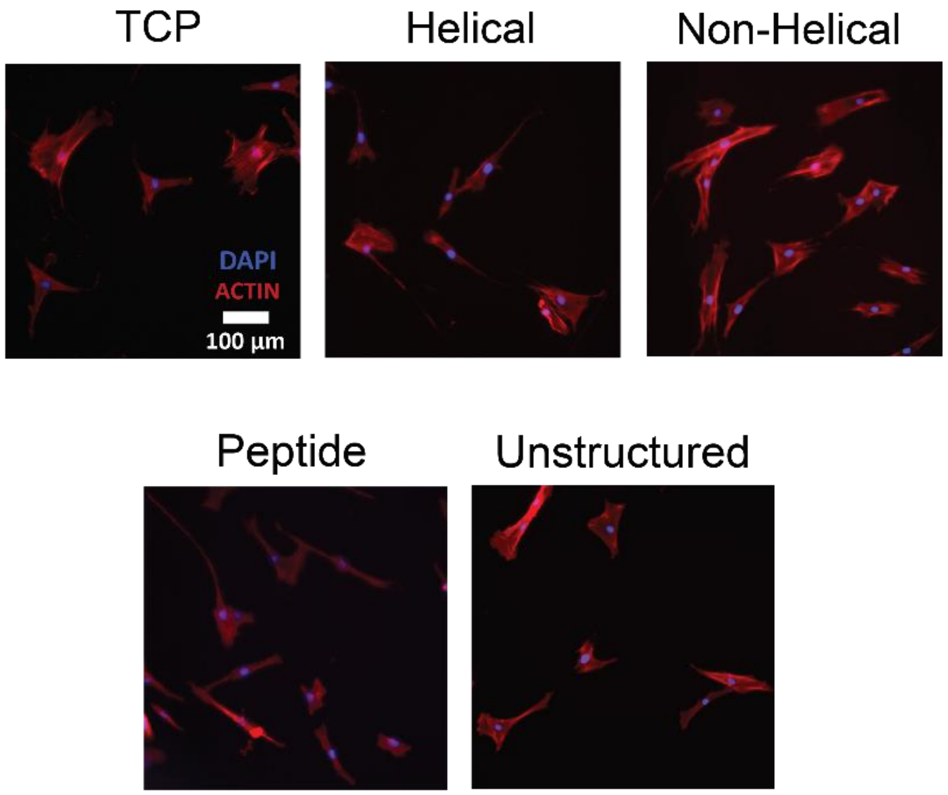




Morton 2022

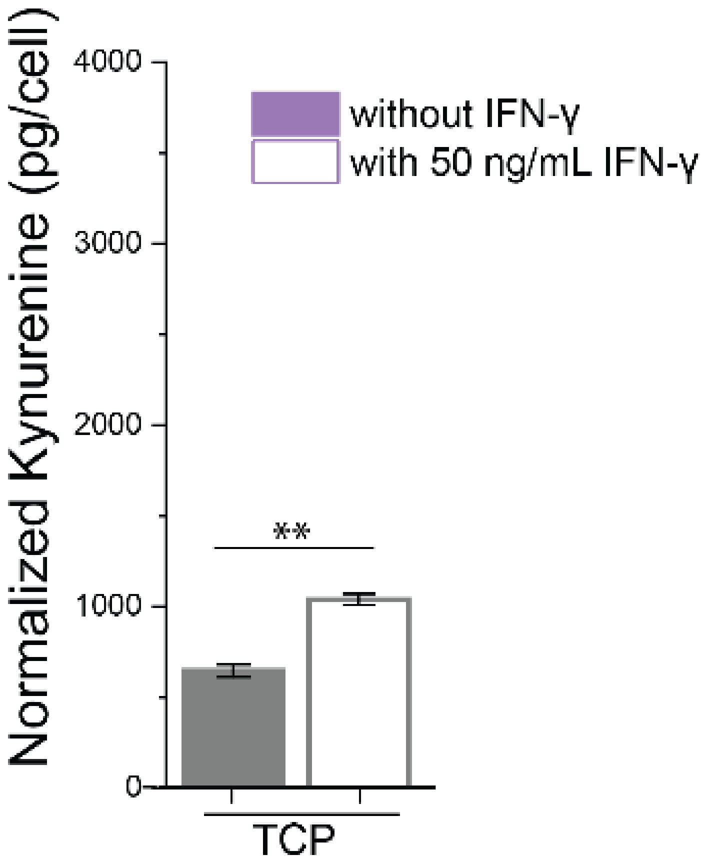
Stiffer Substrates Result in Increased Cell Spreading and Less Circular Cells





Morton 2022

Softer Substrates increase hMSC Immunomodulatory Potential



- IFN-γ was supplemented into the cell culture medium for this IDO study
- IFN-γ increases IDO expression on TCP
- Significant increases were seen with each softer substrate

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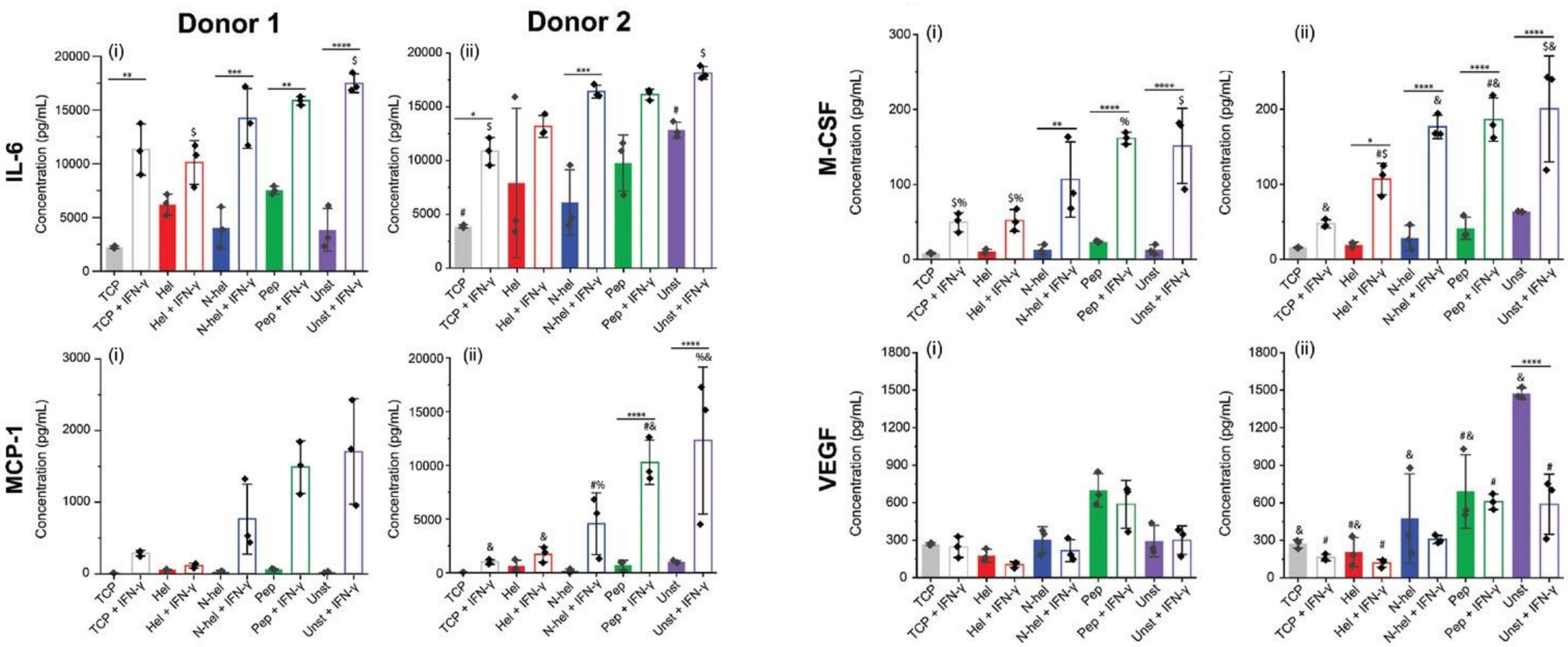
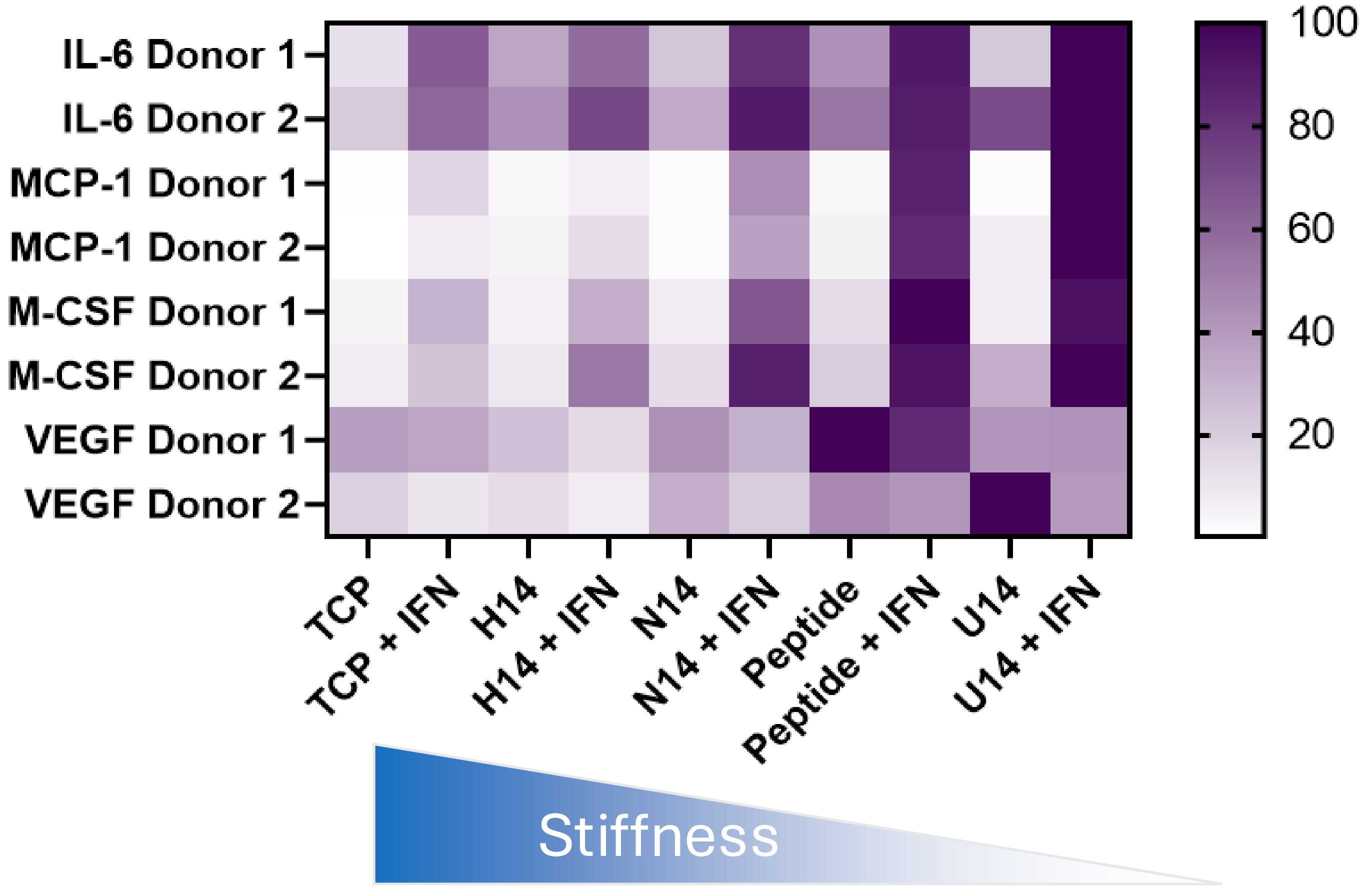
Hydrogels with comparable mesh sizes result in significantly different cellular outcomes



L.D. Morton, D.A. Castilla-Casadiego, et al. *Macromol. Biosci.* **2024**, 2400111.

Softer Substrates increase secretion of immunoregulatory and regenerative cytokines

- Soft substrates report higher values of:
  - Interleukin 6
  - Monocyte chemoattractant protein-1
  - Macrophage colony-stimulating factor
  - Vascular endothelial growth factor
- Interferon gamma is critical in upregulating secretion



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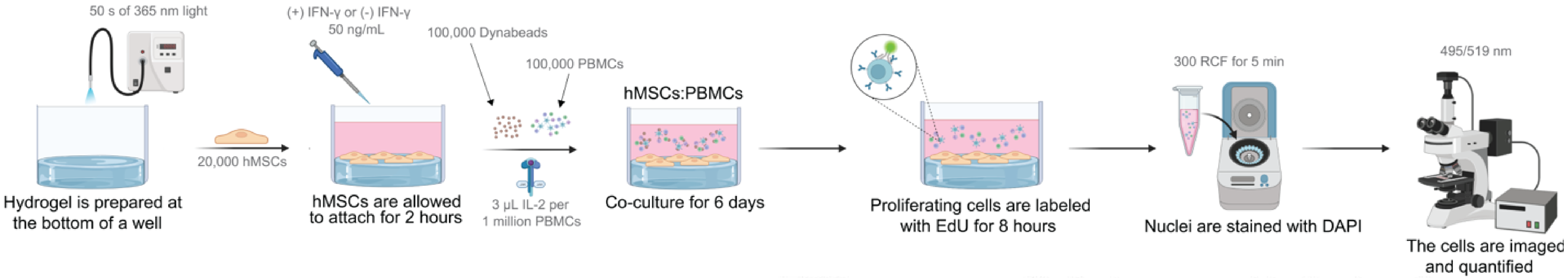
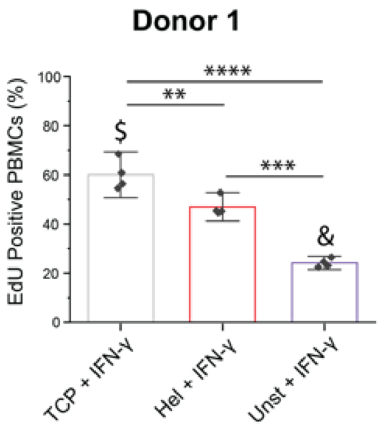
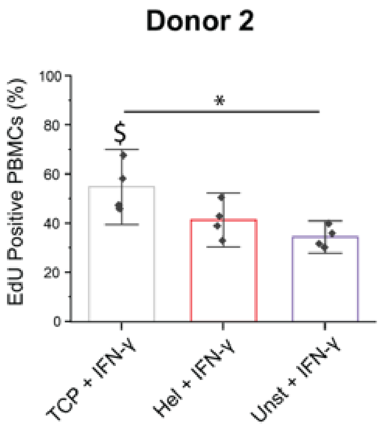
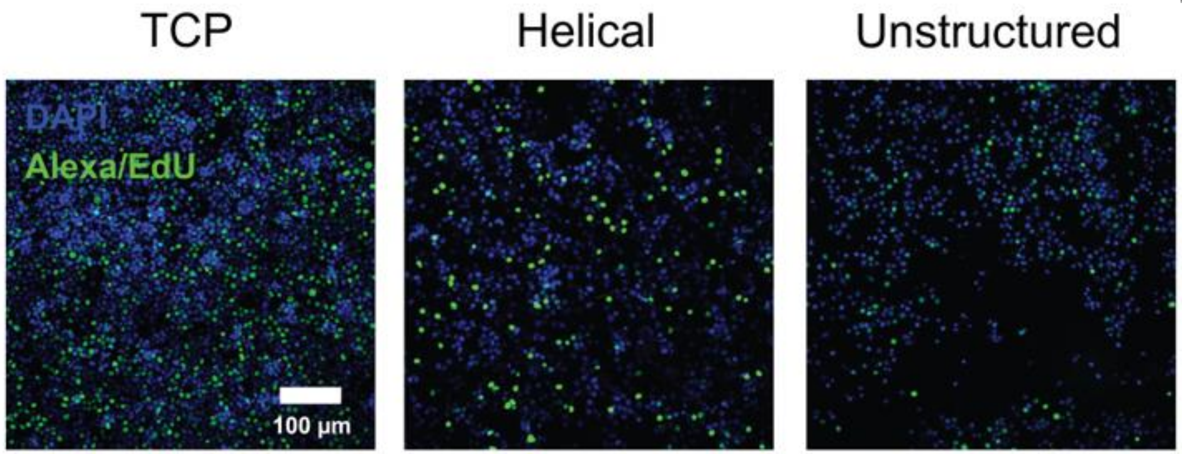
Softer Substrates Reduce the Proliferation of PBMCs









OK—so synthetic polymers are still important, but how do we know if they are sustainable?

- We want to be good scientists and engineers.
- That means we cannot just look at something and guess if it is sustainable or not.
- We need full life cycle analyses (LCA)
- Some things sound sustainable, but end up being as bad, or WORSE, than synthetics.