Light-tunable Hydrogels

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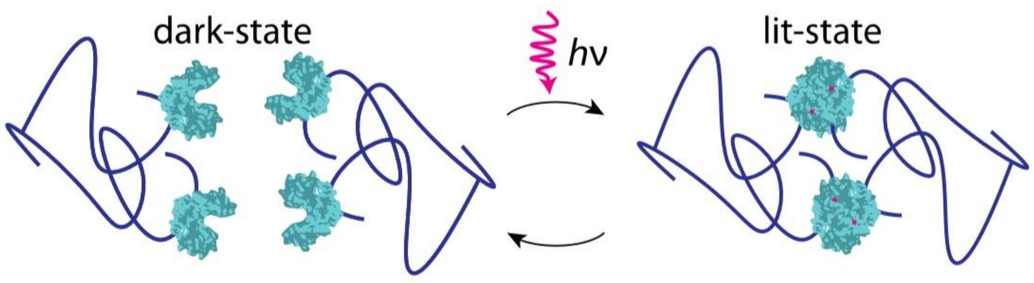
Introduction

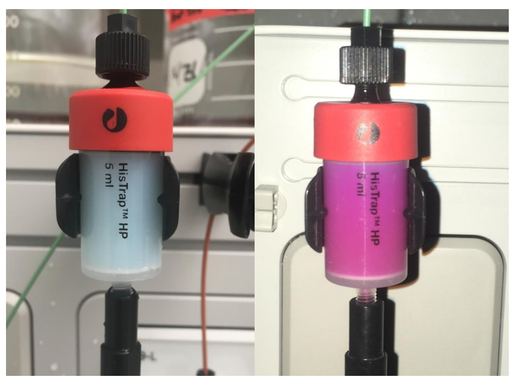
- Hydrogels are multifunctional, water-filled structures made up of cross-linked polymer chains. Cells are able to grow on hydrogels because they can mimic the extracellular matrix. This makes them useful for tissue engineering, wound healing, and cell culture.
- If we had spatial and reversible control over a gel's stiffness, we would be able to further mimic the extracellular matrix.
- Attaching photo-switchable proteins (opto-proteins) to the polymers in a hydrogel would allow the density of cross-links to be controlled by light.
- Opto-proteins have the unique ability to change shape and bind to each other when exposed to a certain wavelength of light and unbind and return to their original shape in the dark

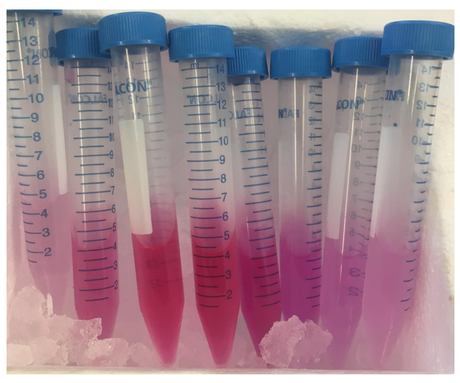


Above: visualization of how opto-proteins work

Methods

- A plasmid containing an arabinose promoter and genes for the opto-protein Cry2, fluorescent reporter mCherry, and a SNAP-tag was created, transformed into TOP10 *E. Coli* cells.
- Opto-protein was purified from the *E. Coli* using fast protein liquid chromatography (FPLC)

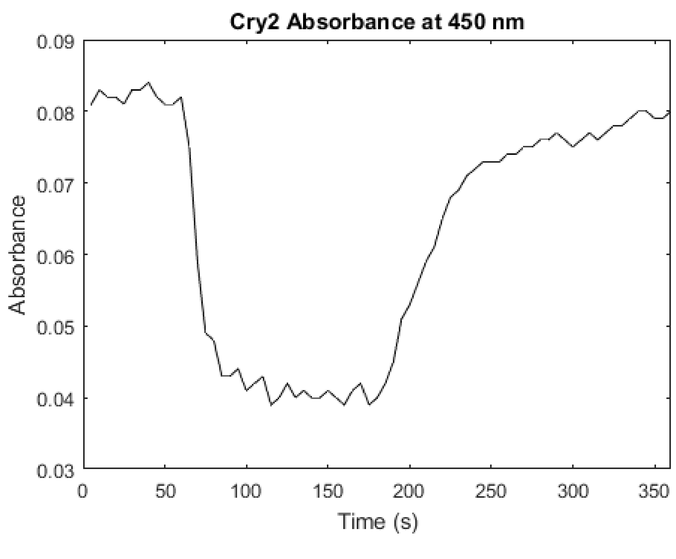




Left: HiTrap Column in FPLC before and during purification. Column became saturated with protein as indicated by mCherry fluorescent reporter. Right: Fractions of purified Cry2.

- The photo-switching ability was tested by monitoring the absorbance at 450 nm with the presence and absence of blue light.

Results



Purified Cry2 is photo-switchable

Figure 1: Cry2 absorbance at 450 nm over time. When exposed to a blue LED from 60 to 180 seconds, absorbance dramatically decreases, indicating that the protein has transformed to its lit-state. Absorbance quickly rises back up after LED is removed.

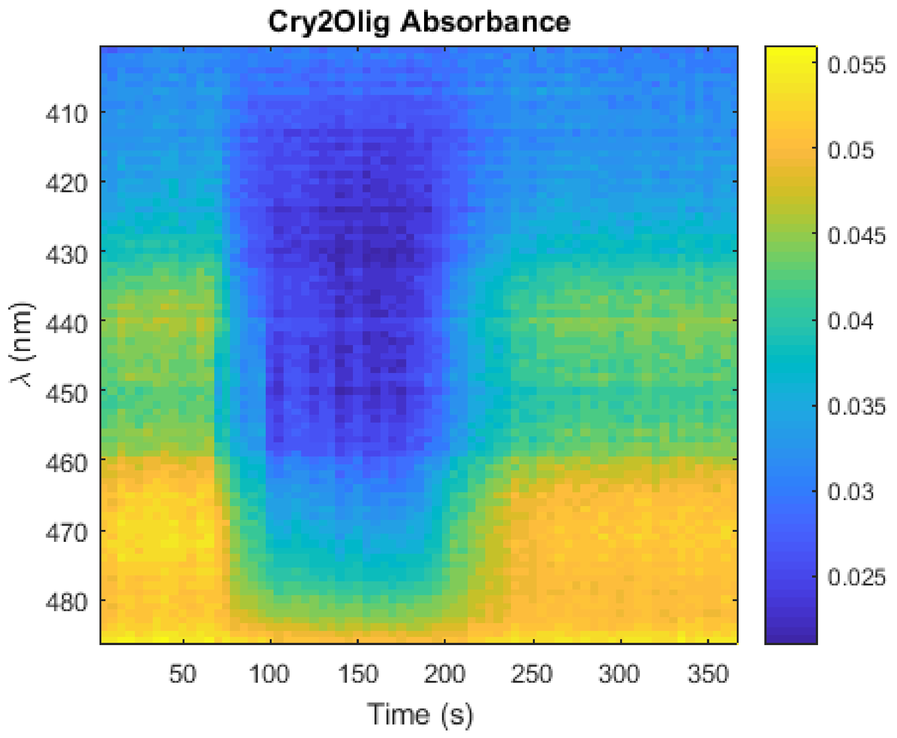


Figure 2: Cry2 absorbance measured over time at 400 to 490 nm. Exposed to a blue LED from 60 to 180 seconds.

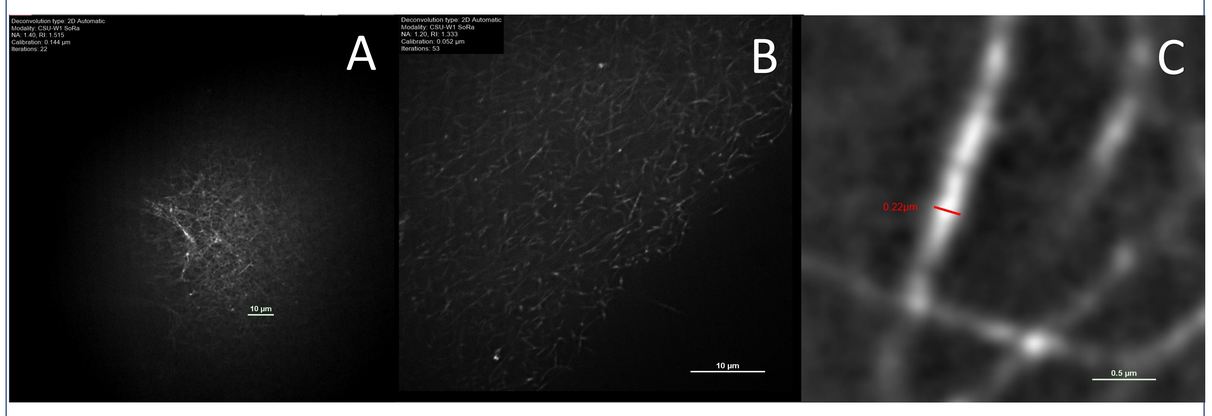
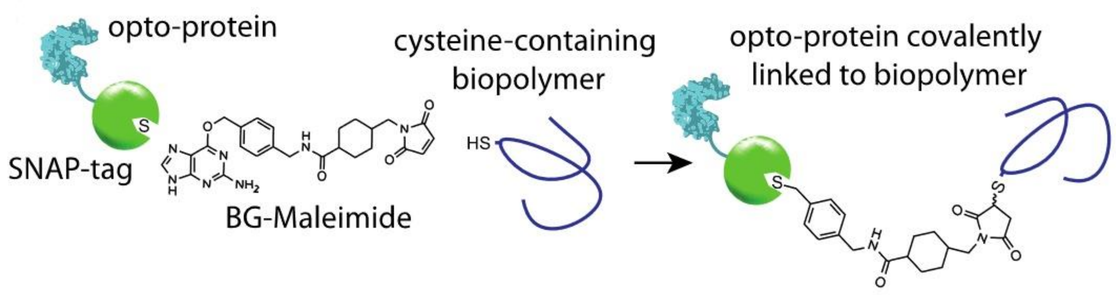


Figure 3: Increasing magnification of a 2 mg/mL collagen gel tagged with Alexa dye with a maleimide.

Future Directions

- To connect to opto-proteins to the collagen fibers, BG-maleimide will be used to covalently link the SNAP-tag on the opto-protein to cysteines in the collagen



- We will further classify the gel's properties using atomic force microscopy (AFM) and microrheology.
- This system can be applied to other components of the extracellular cellular matrix and a variety of different opto-proteins can be used.

Acknowledgements

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