Building better biomaterials: New sources, chemistry, and applications

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*New(ish) lab: Started in Sept. 2020

University of New Hampshire

natolab
biologically derived and bioinspired materials

Bioreactor production of pDNA

Problem: DNA nanotechnology is often limited to < 1 mg scale due to cost and environmental constraints during synthesis and purification. This prevents many DNA technologies from transitioning between the micro and macro scale.

Solution: Using bioreactor based fermentation we can produce up to 1 g / L of pDNA shifting DNA's role from expensive biologic to commodity polymer.

Applikon eZ 2 connect 3 and 7 L fermentation system allows for extensive optimization of growth profiles to maximize yield.

~500 g wet cell paste from a single bioreactor run

~ 1 g crude pDNA precipitated in isoproponol

at > 5 mg/mL pDNA makes a physical gel

$129 per batch / best yield to date: 1.1 g pure pUC20T YFP

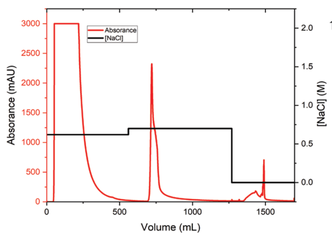
Students: Wynter Paiva, Rachel Achong, DK Alakwe

RNAase free purification

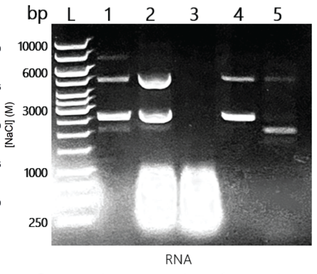
Problem: Alkaline lysis, filtration, and precipitation are used to remove cell debris, proteins, and chromosomal DNA. Further purification to remove RNA is necessary, but large scale treatment with RNAase becomes impractical.

Solution: Modern anion exchange chromatography is readily scalable and can separate RNA from DNA using a sodium chloride gradient as the eluent.

Modified AEX system with 255 mL column and 90 mL injection loop



Chromatograph showing successful separation of RNA from pDNA.

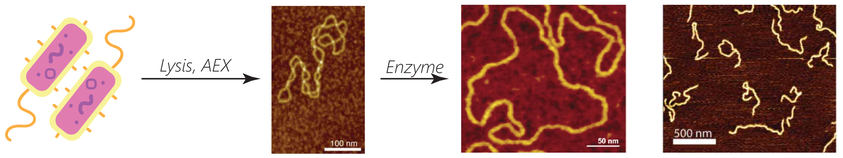


Gel showing separation of open circular and supercooled DNA from RNA impurities

Inexpensive scalable purifications enable application

Students: Wynter Paiva, DK Alakwe

Unimolecular topologically defined polymers



pUC is natively negatively supercoiled

Nickase creates open circle

restriction enzyme creates linear

50 - 250 mg of pure unimolecular starting materials available

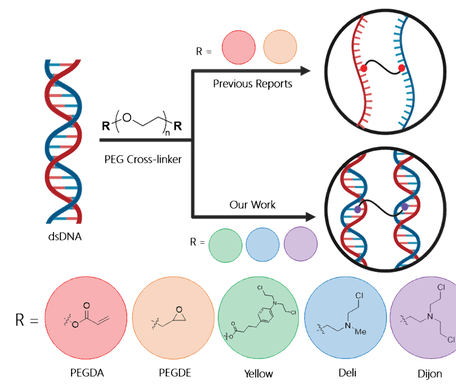
Students: Wynter Paiva, DK Alakwe, Kelsey MacCallum

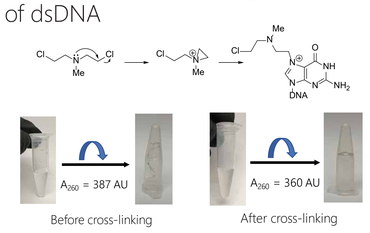
images: Doyle et al. Soft matter 2014, 10, 9721

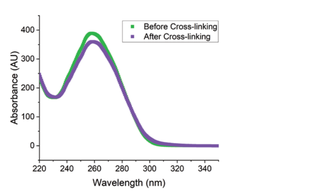
Pure dsDNA materials

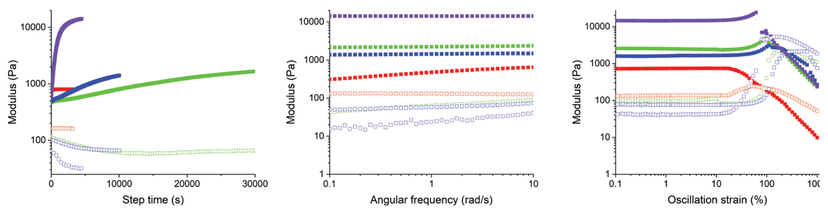
Problem: Current DNA materials do not leverage the polymeric nature of dsDNA due to either short sequences below the persistence length of DNA or chemical cross-linking methods which denature DNA.

Solution: Develop new covalent and non-covalent cross-linking strategies which can leverage the interesting polymeric properties of dsDNA







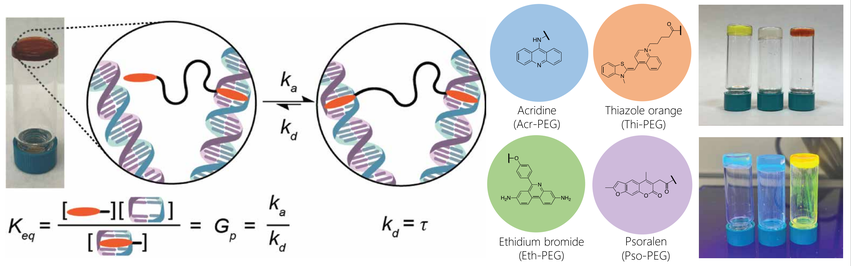


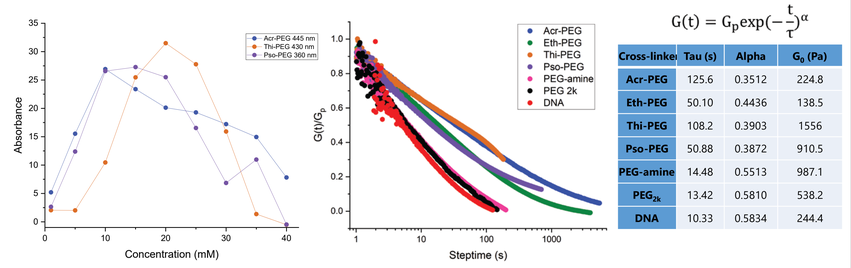
works in serum, selective for DNA, rapid cross-linking

Students: Wynter Paiva, DK Alakwe

Supramolecular cross-linkers

In this work, we leverage dsDNA intercalation to manipulate the material properties of gel. We use rheology to study the thermodynamic behavior of our hydrogels





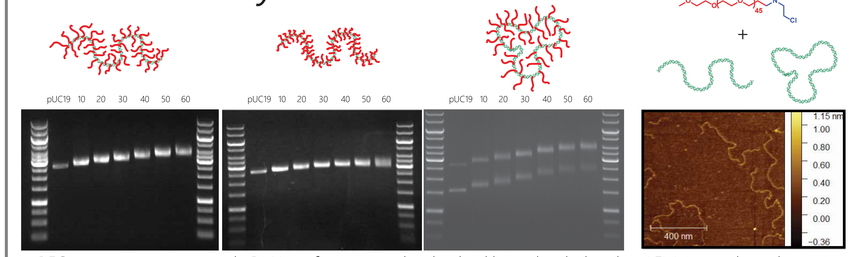
$$G(t) = G_p \exp\left(-\left(\frac{t}{\tau}\right)^{\alpha}\right)$$

Cross-linker	Tau (s)	Alpha	G_0 (Pa)
Acr-PEG	125.6	0.3512	224.8
Eth-PEG	50.10	0.4436	138.5
Thi-PEG	108.2	0.3903	1556
Pso-PEG	50.88	0.3872	910.5
PEG-amine	14.48	0.5513	987.1
PEG_{2k}	13.42	0.5810	538.2
DNA	10.33	0.5834	244.4

control thermodynamic and viscoelastic properties

Students: Shaina Hughes, Dr. Aylin Aykanat, Nick Pierini

Cyclic DNA bottle brushes



mPEG-mustargens react with DNA to form unimolecular backbone bottle brush polymers with controlled topology. Bright bands represent 5, 3.5, and 1 kbp. Left to right: lpUC19 + mPEG 2k, lpUC19 + mPEG 750, scpUC19 + mPEG 2k. Eq on top

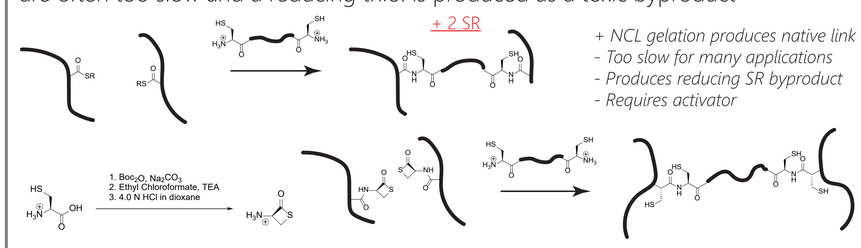
AFM is used to determine persistence length and estimate graft density.

topologically controllable, unimolecular, biodegradable backbones

Students: Nick Pierini

ß-Thiolactone for traceless ligation

Native chemical ligation (NCL) produces a native peptide bond from a thioester and a N-terminal cysteine. This method would be ideal for forming gels, but the kinetics are often too slow and a reducing thiol is produced as a toxic byproduct



+ NCL gelation produces native link
- Too slow for many applications
- Produces reducing SR byproduct
- Requires activator

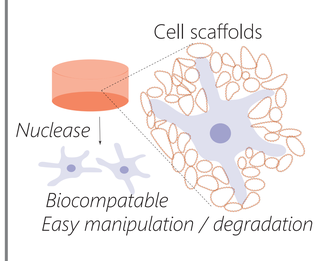
ß-thiolactone increases reaction rate through strain and does not produce a SR byproduct.

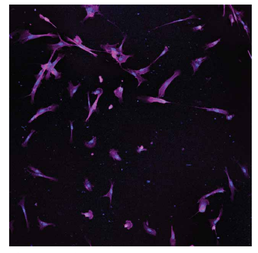
No activation is required and complete gelation is observed in less than 5 minutes. Only native cysteines are produced and the reaction will proceed in buffered solution in the presence of other proteins.

rapid, efficient, bioorthogonal cross-linking for cell scaffolds

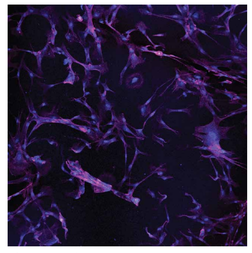
Students: Matt Currier, Lola Fadairo

Cells in DNA Gels





day 1 MCF-7



day 4 MCF-7

orthogonal manipulation and release of cells

Students: Tran Truong

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