Synthesis and Characterization of Crosslinked Lysozyme-Dextran Nanogels for Drug Delivery

Kelsey DeFrates, Rowan University

University of Pennsylvania Singh Center for Nanotechnology REU Program 2017

Principal Investigators: David Eckmann, Russell Composto

Mentor: Olivia McPherson

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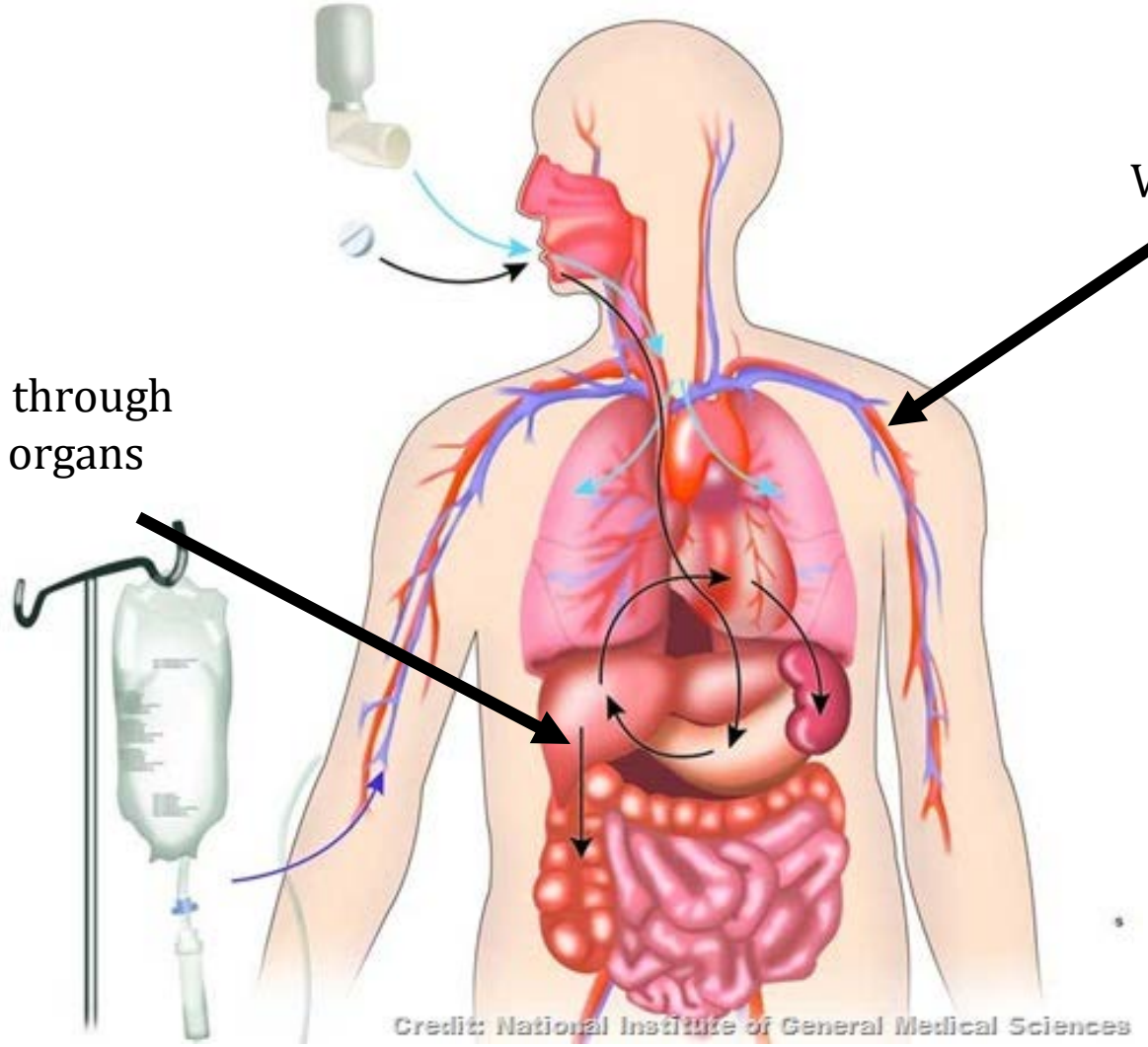
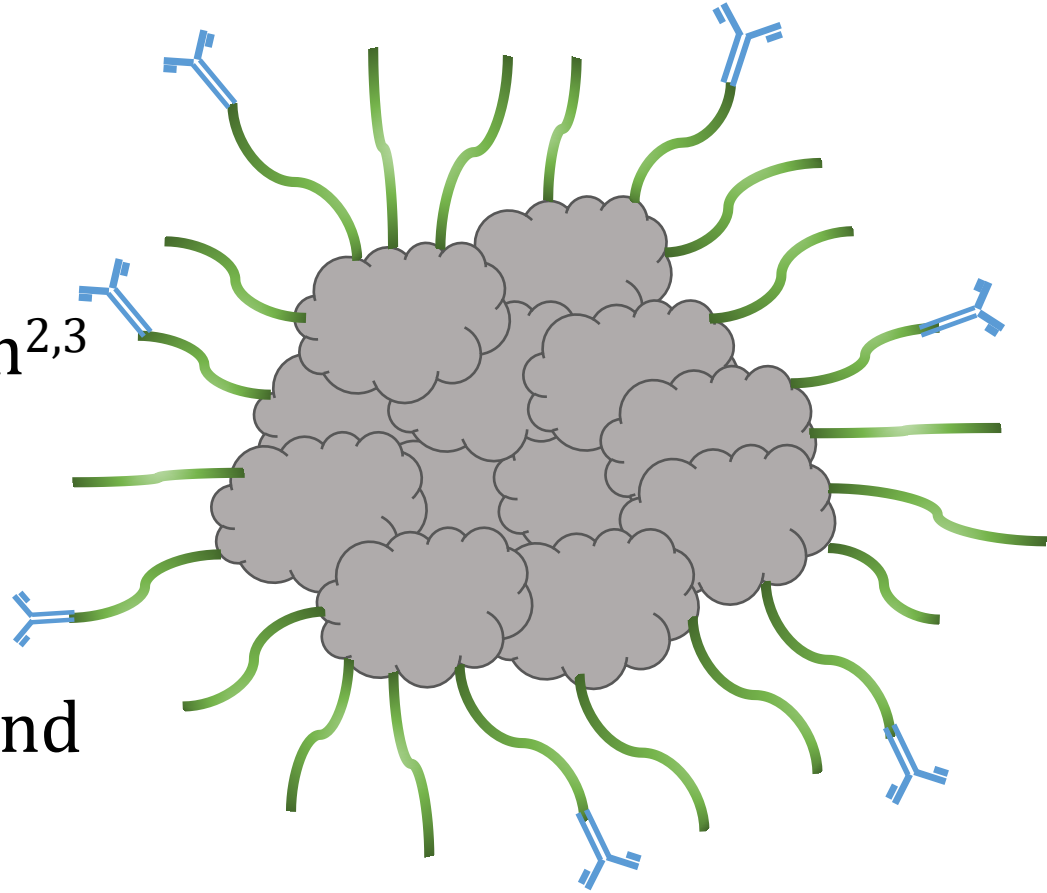
Conventional drug delivery systems can be inefficient and damaging to non-target organs[1,2].



1. De Jong, W. H.; Borm, P. J. *International journal of nanomedicine* **2008,** 3, (2), 133-49.
2. Ferrer, M. C. *et. al. PloS one* **2014,** 9, (7), e102329. doi: 10.1371/journal.pone.0102329.

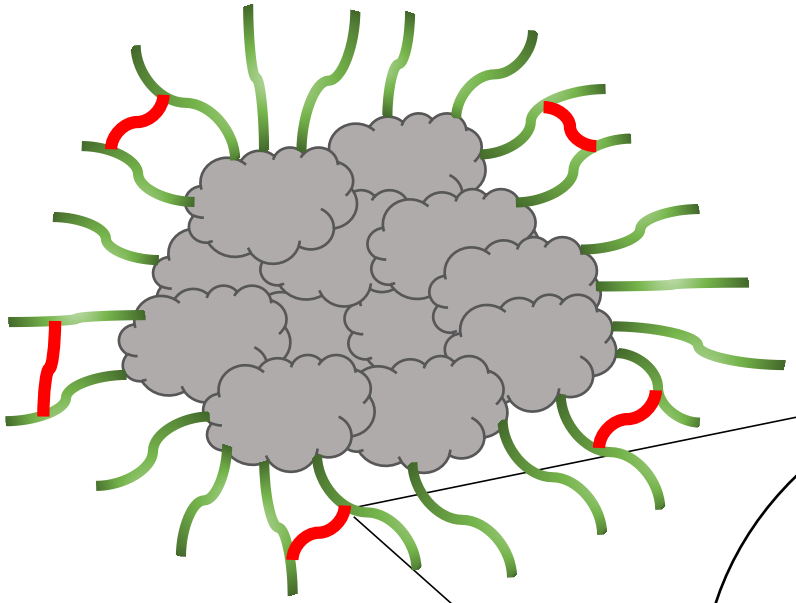
Core-Shell Nanogels: A Promising Drug Delivery Platform.

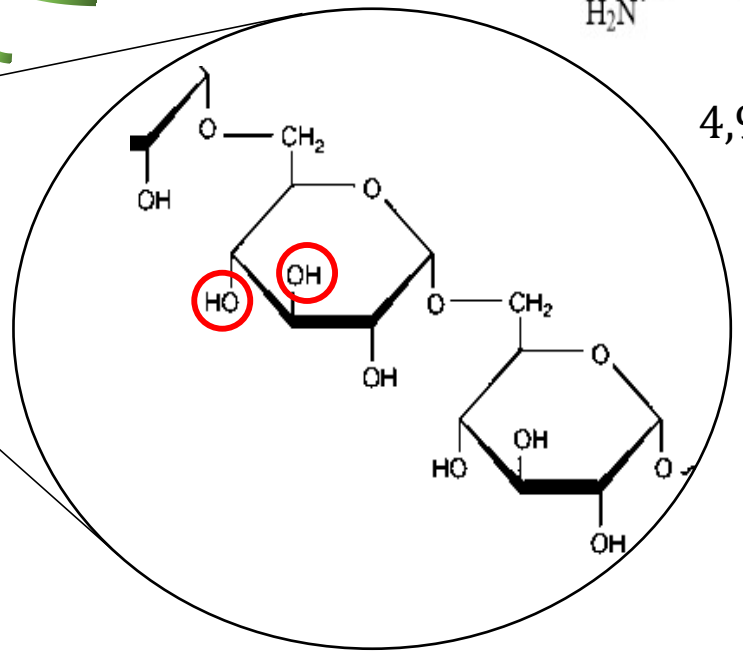
- Hydrophobic Core → Drug Loading[2,3]
  - Proteins: Lysozyme
- Hydrophilic Shell → Stabilization, Stealth[2,3]
  - Polysaccharides: Dextran
- Attachment of antibodies for targeted delivery[2,3]
- Synthesized through Maillard reaction and heat-induced gelation[4]

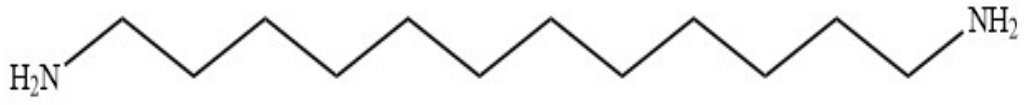


2.Ferrer, M. C. *et. al. PloS one* **2014,** 9, (7), e102329. doi: 10.1371/journal.pone.0102329
3. Li, J.; Yao, P. *Langmuir : the ACS journal of surfaces and colloids* **2009,** 25, (11), 6385-91. doi: 10.1021/la804288u
4. Li, J.; *et. al. Langmuir : the ACS journal of surfaces and colloids* **2008,** 24, (7), 3486-92. doi: 10.1021/la702785b.

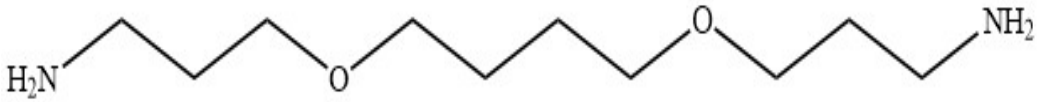
Stiffness of nanogels influences cell binding[5]. Crosslinking of shell provides mechanism for controlling nanoparticle stiffness.



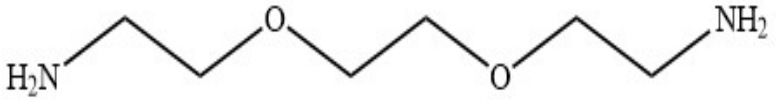




1, 12 – Diaminododecane (DAD)



4,9 – Dioxa – 1, 12 – dodecanediamine (DODD)



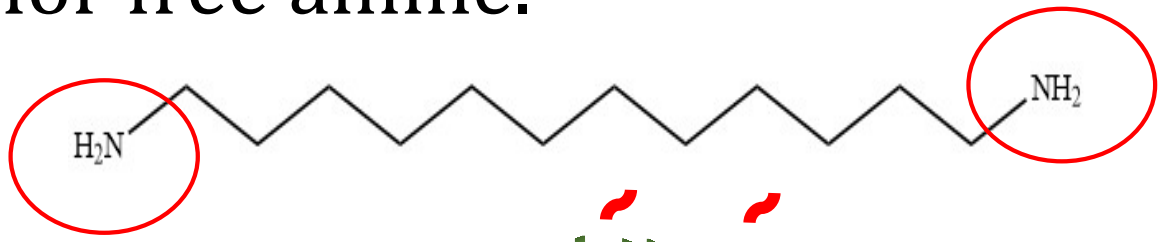
2, 2’ - (Ethylenedioxy)bis(ethylamine) (EOD)

5. Anselmo, A. C.; Mitragotri, S. *Advanced Drug Delivery Reviews* **2017,** 108, 51-67. doi: 10.1016/j.addr.2016.01.007.

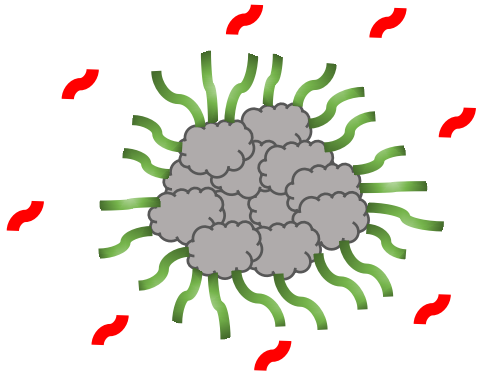
Table 1: Hydrodynamic diameters and PDI of LDNG before and after oxidation and crosslinking obtained through DLS.

	Batch 1			Batch 2		
Sample	Hydrodynamic Diameter (nm)	Percent Change	PDI	Hydrodynamic Diameter (nm)	Percent Change	PDI
LDNG	259.7	--	0.061	242.7	--	0.048
Oxidized LDNG	332.1	+27.9%	0.290	257.6	+6.14%	0.066
LDNG with EOD	347.3	+4.58%	0.312	375.6	+45.81%	0.185
LDNG with DODD	292.3	-12.0%	0.063	402.5	+56.25%	0.119
LDNG with DAD (2.5 mg/mL)	364.4	+9.73%	0.503	518.5	+101.28%	0.222
LDNG with DAD (0.25 mg/mL)	311.7	-6.14%	0.147	500.8	+94.41%	0.257

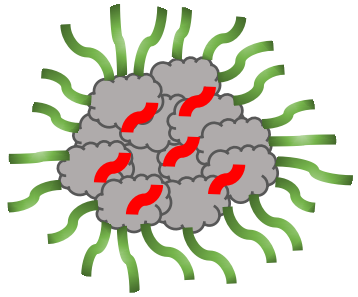
Nanogel crosslinking verified through ninhydrin assay, a colorimetric test for free amine.



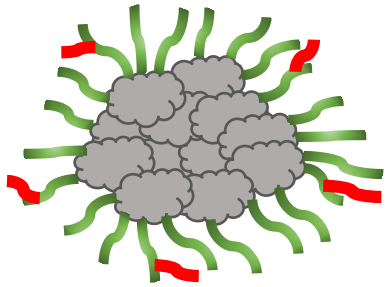
1. Crosslinker remains unreacted



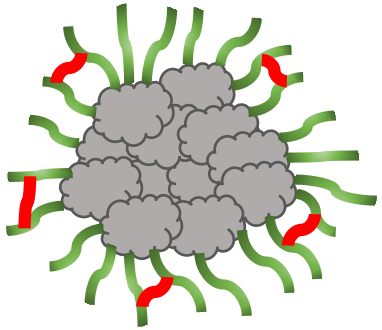
2. Crosslinker is internalized by hydrophobic core



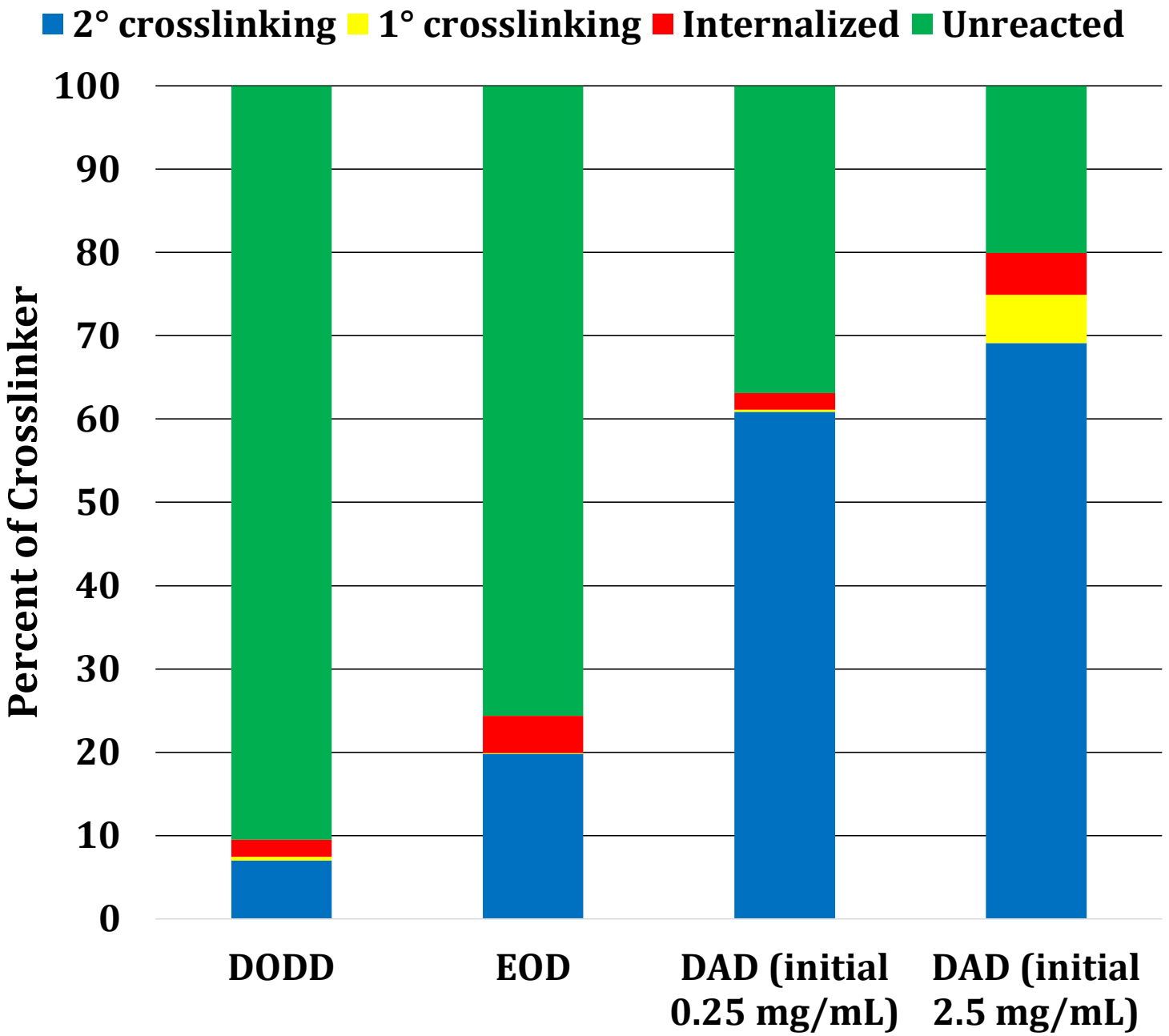
3. 1° crosslinking

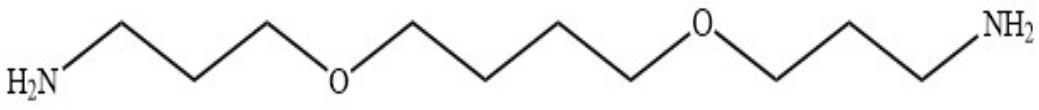


4. 2° crosslinking

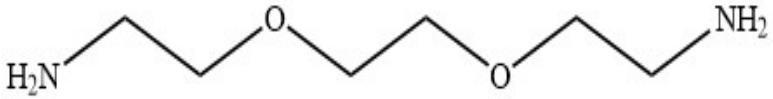


Percent of crosslinker found in each “destination” determined by ninhydrin assay.

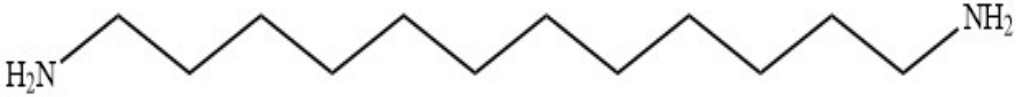




DODD

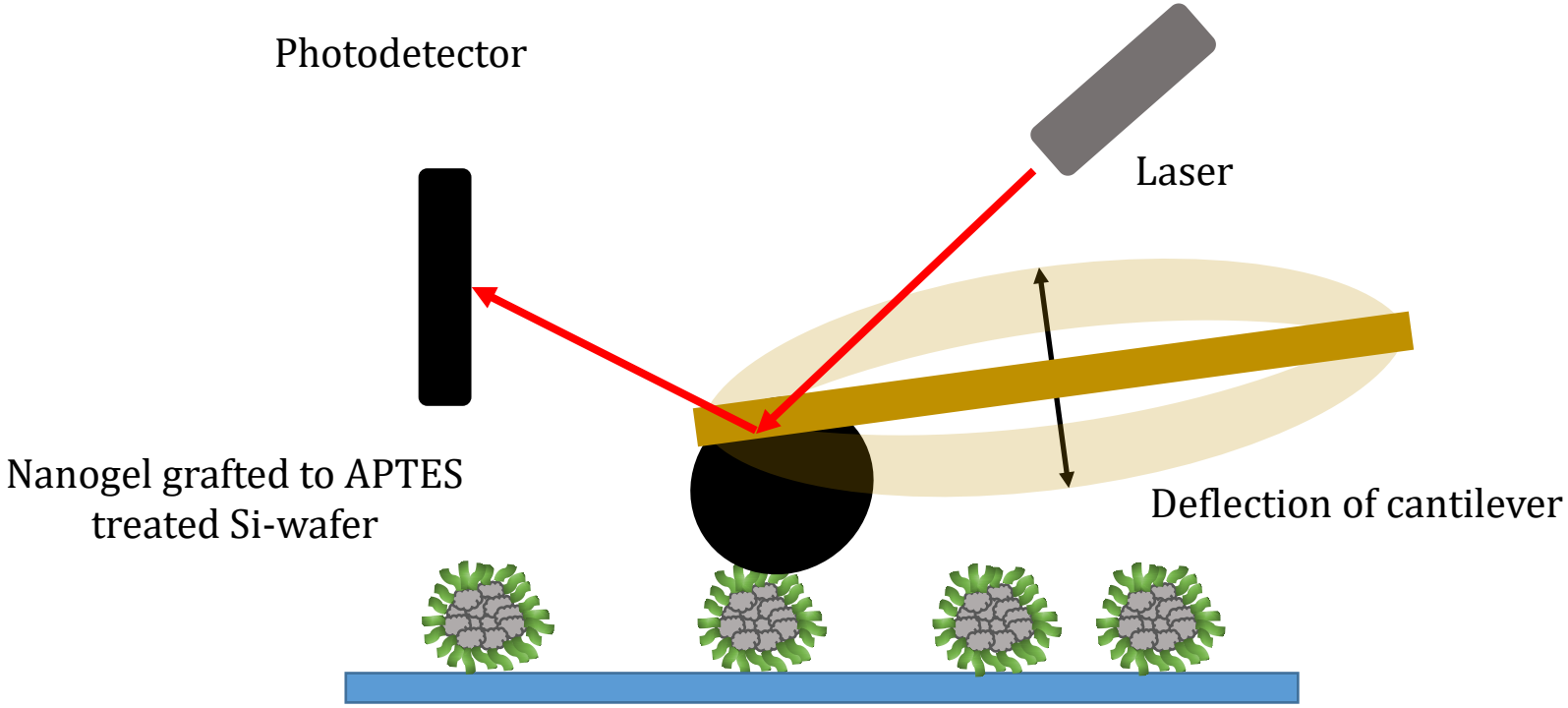


EOD



DAD

Nanogels can be imaged with AFM. Single nanogels can also be indented to obtain elastic modulus.

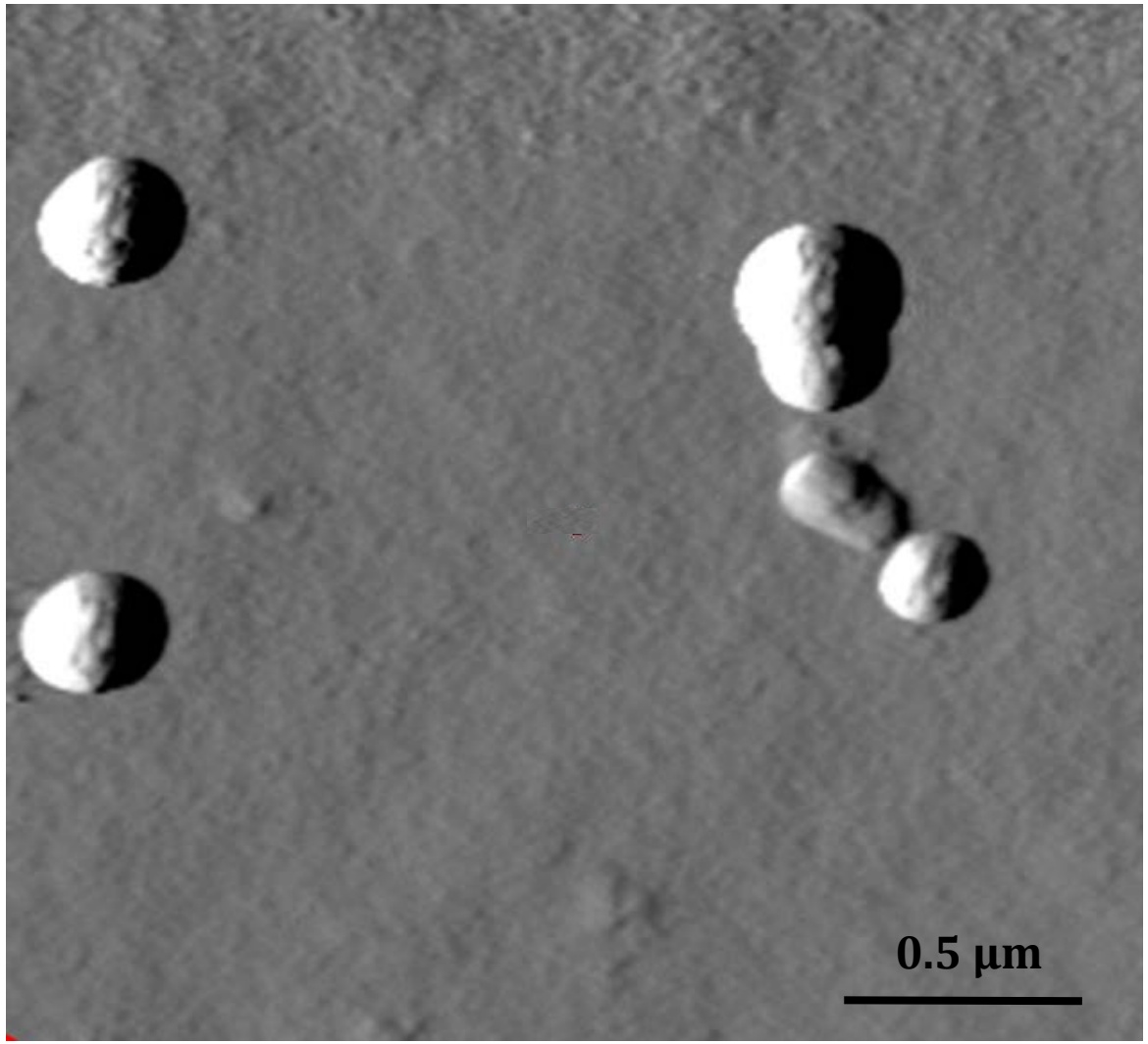


Hertz Model[6]

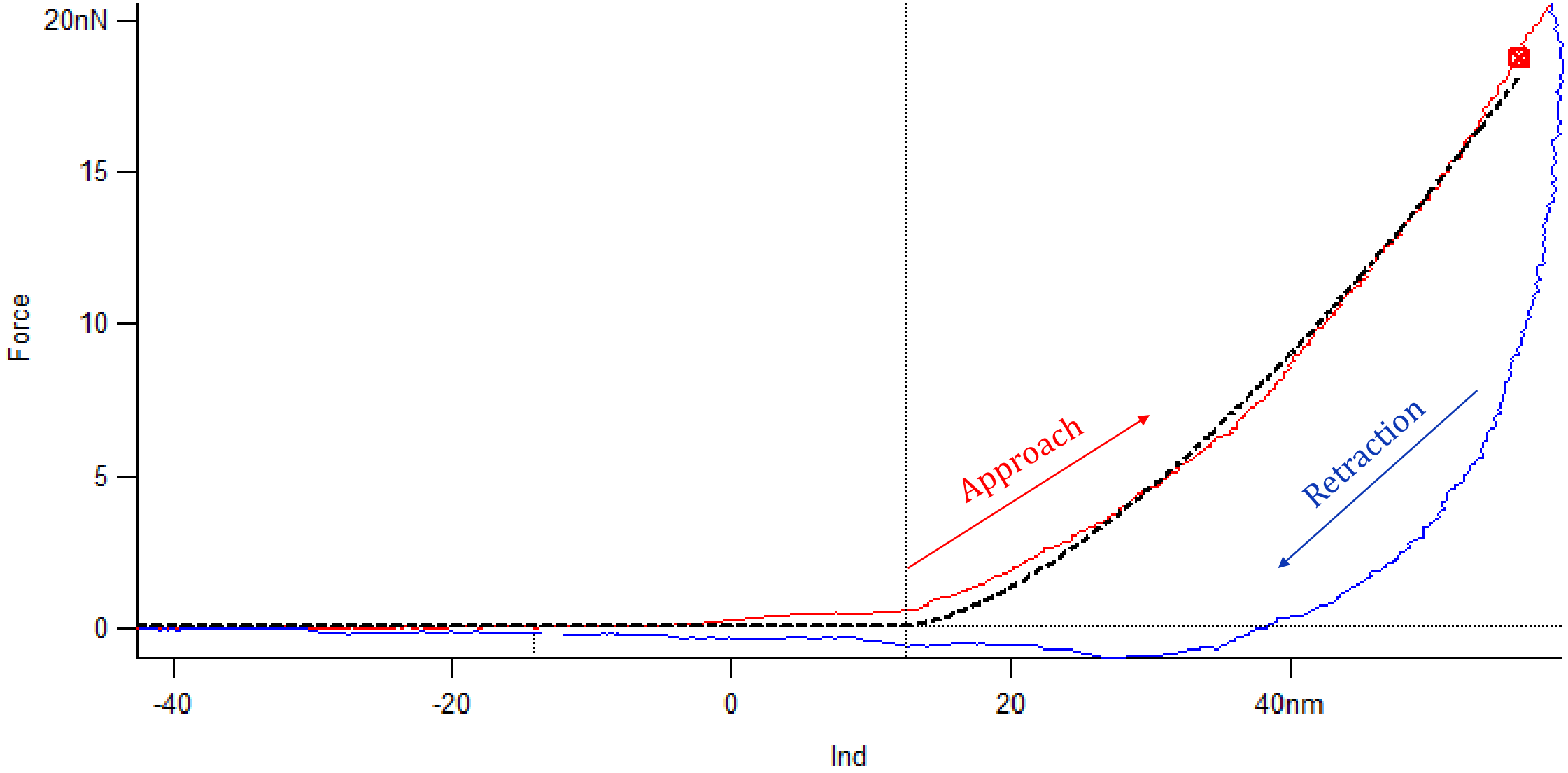
$$d^3 = \frac{9}{16}\frac{(1-\nu)^2}{RE^2}F_o^2$$

d = indentation
F_o = load force
R = radius of probe
ν = Poisson Ratio (0.3)
E = Elastic Modulus

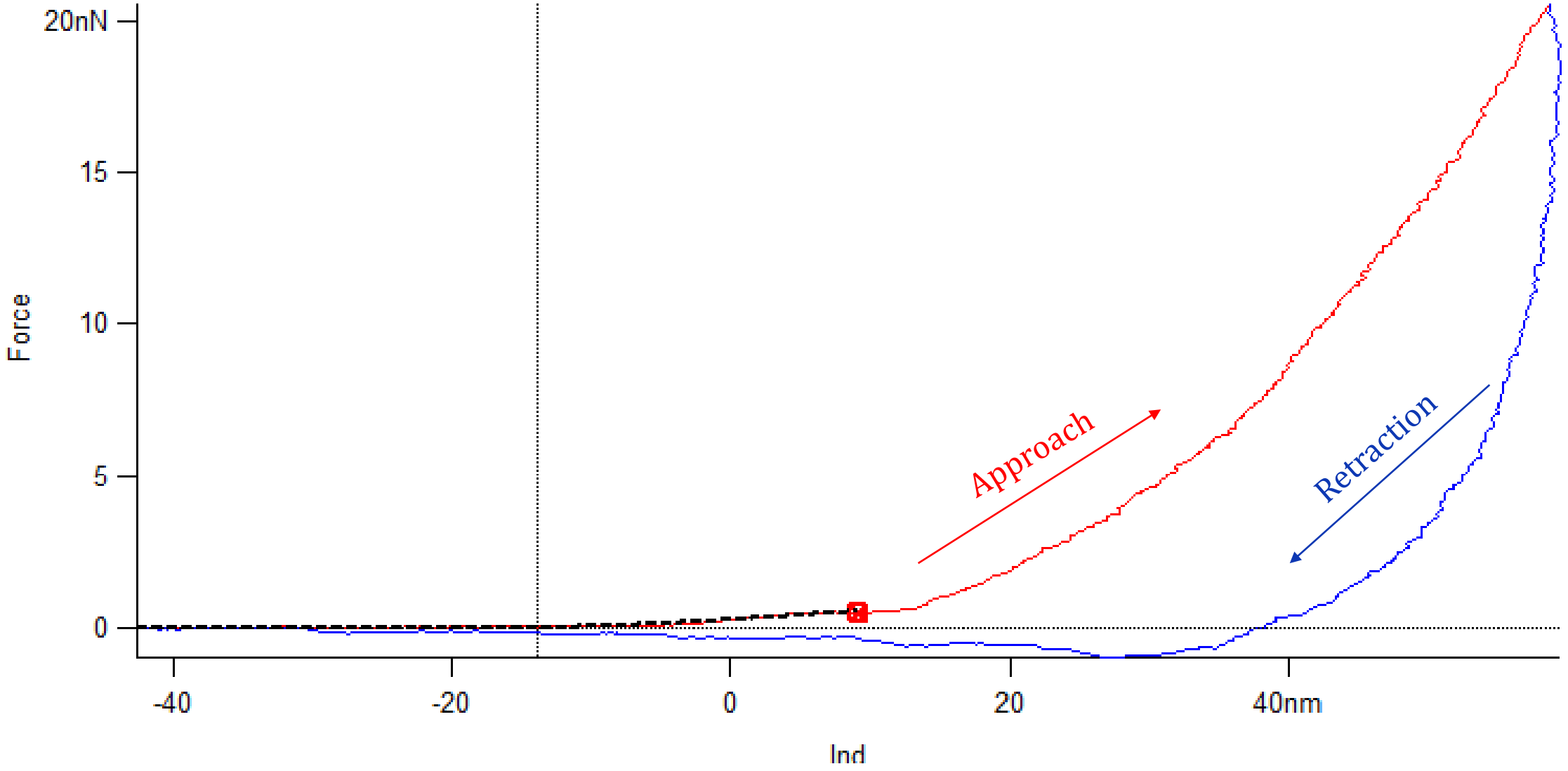
6. Radmacher, M.; *et. al. Langmuir : the ACS journal of surfaces and colloids* **1994,** 10, (10), 3809-3814. doi: 10.1021/la00022a068.



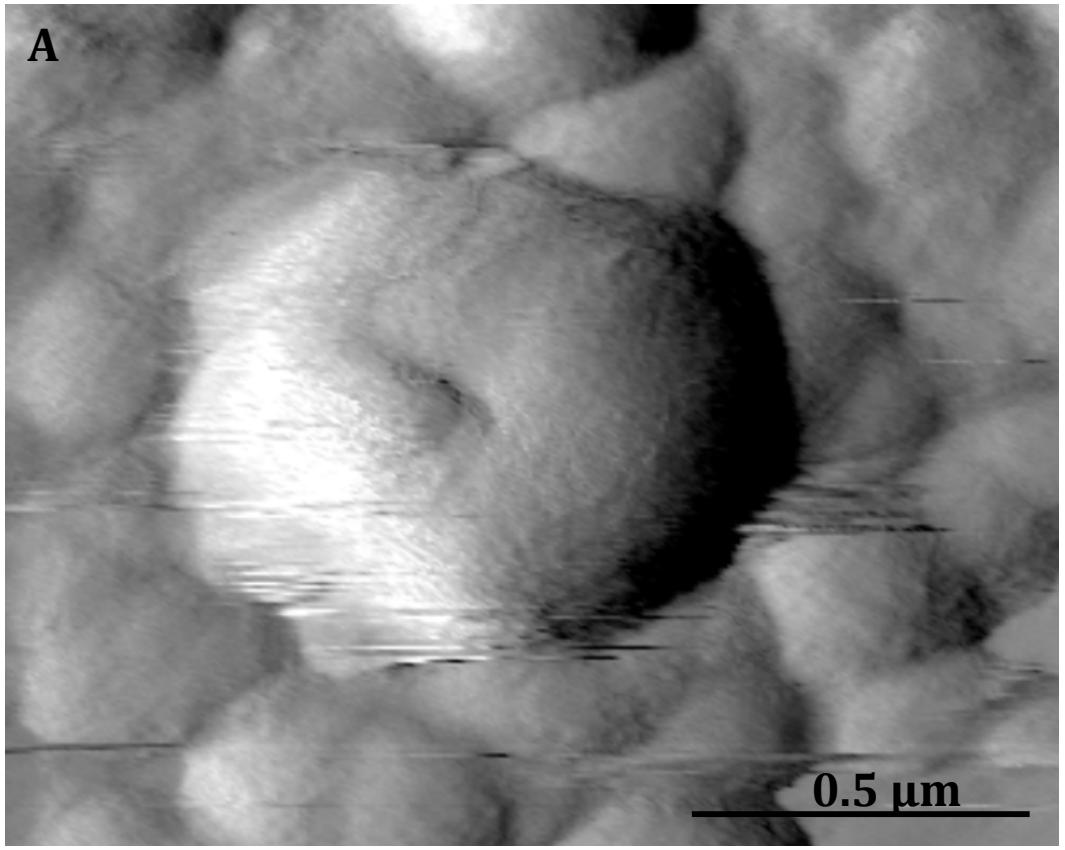
AFM topographical images of smaller nanogels taken with cantilever with silica probe reveal nanogels successfully grafted to APTES treated surfaces. Average nanogels measure approximately 200 nm in diameter and 30 nm in height.

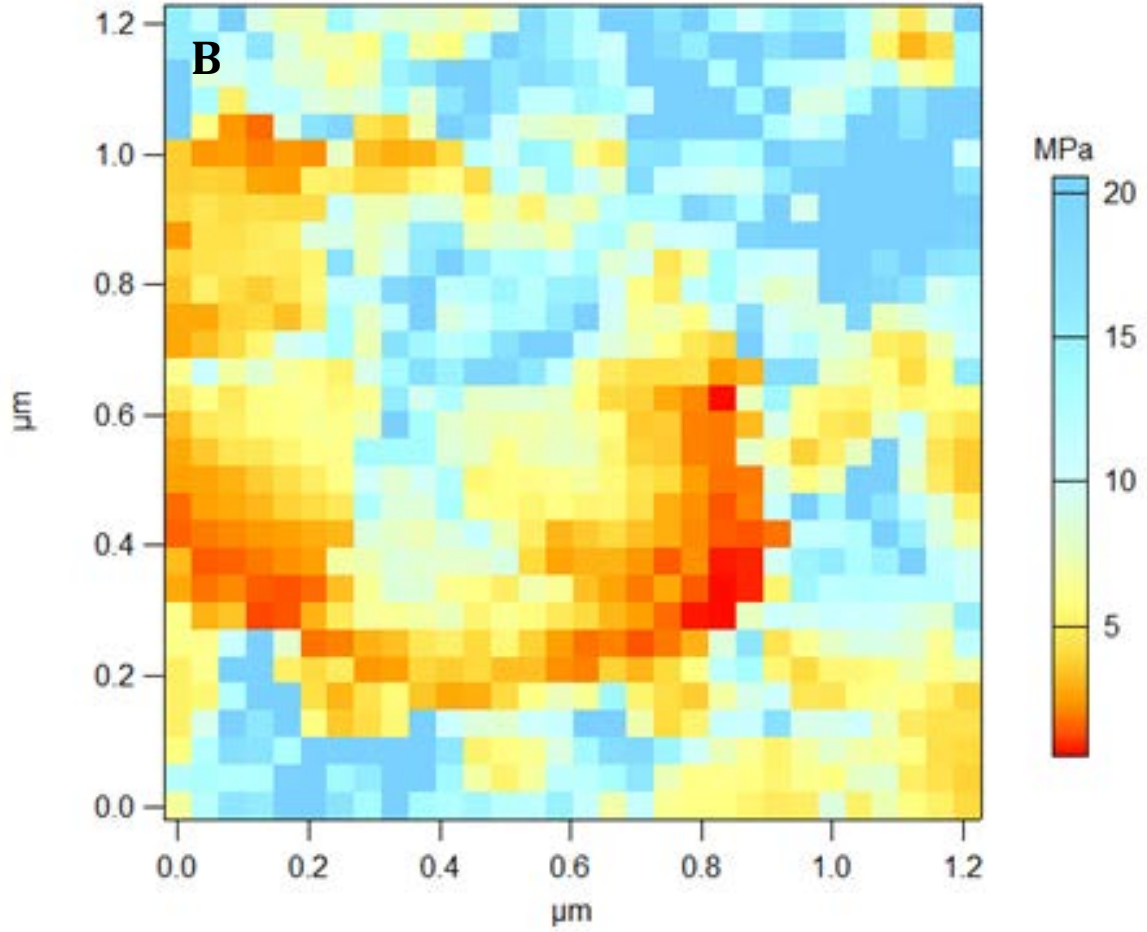


Force-indentation curve for nanogels is shown. Fitting the entire curve with Hertz model (black) gives modulus of 1.70 MPa, but neglects less steep region of curve.



Fitting of 15% of curve captures shallow region to give modulus of 137.31 KPa, suggesting a two-component system





Support for a two-component system is seen in elastic modulus map (B) of aggregate nanogels (A). Aggregate measures approximately 1 µm in diameter and 150 nm in height. Lower modulus influenced by dextran (KPa)[7,8],while higher by lysozyme core (0.5 GPa)[9].

7. Kühner, M.; Sackmann, E. *Langmuir : the ACS journal of surfaces and colloids* **1996,** 12, (20), 4866-4876. doi: 10.1021/la960282+.
8. Liu, Y.; Chan-Park, M. B. *Biomaterials* **2009,** 30, (2), 196-207. doi: 10.1016/j.biomaterials.2008.09.041.
9. Radmacher, M.; *et. al. Langmuir : the ACS journal of surfaces and colloids* **1994,** 10, (10), 3809-3814. doi:10.1021/la00022a068.

Conclusions and Future Work

- LDNG crosslinking with three different molecules
  - influences size of nanogels
  - is dependent on initial concentration and type of crosslinker used
- AFM demonstrates
  - success of LDNG grafting
  - deformability of LDNG
- Nanoindentation measurements suggest two-component system
  - Softer region modulus in 100 KPa range
  - Harder region in single MPa range
  - Establishes framework for mechanical analysis of LDNG
- Next step will involve applying this to crosslinked LDNG

Thank you…

Dr. David Eckmann and Dr. Russell Composto for their guidance on this project

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Singh Center for Nanotechnology REU Program at the University of Pennsylvania

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