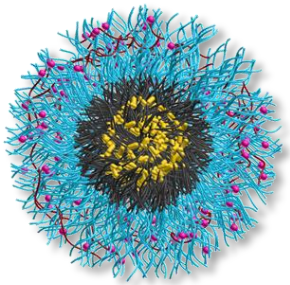
Developing Natural Product-based Polymers for Medical Applications

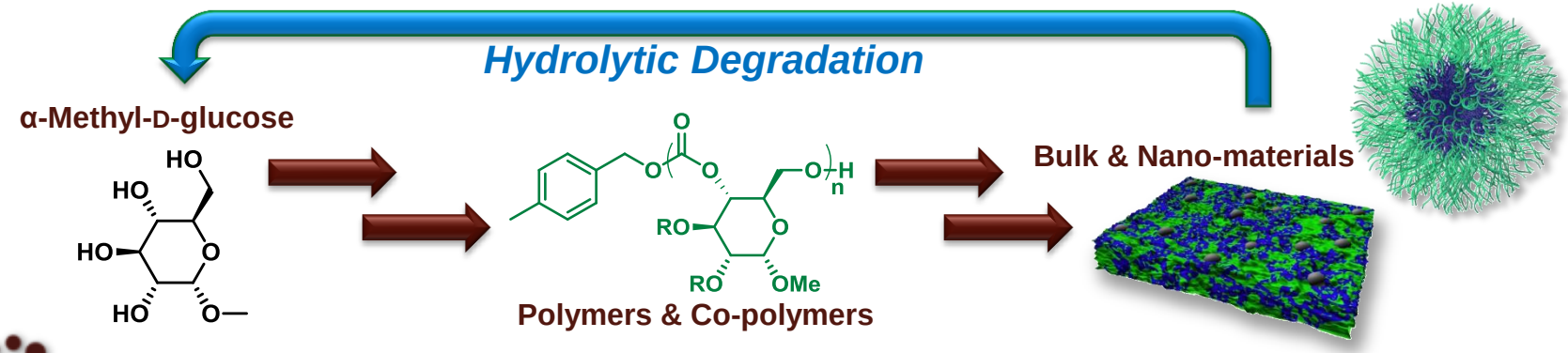
http://www.fraunhofer.de/en/.html

Tiffany P. Gustafson, PhD

Texas A&M University
Department of Chemistry

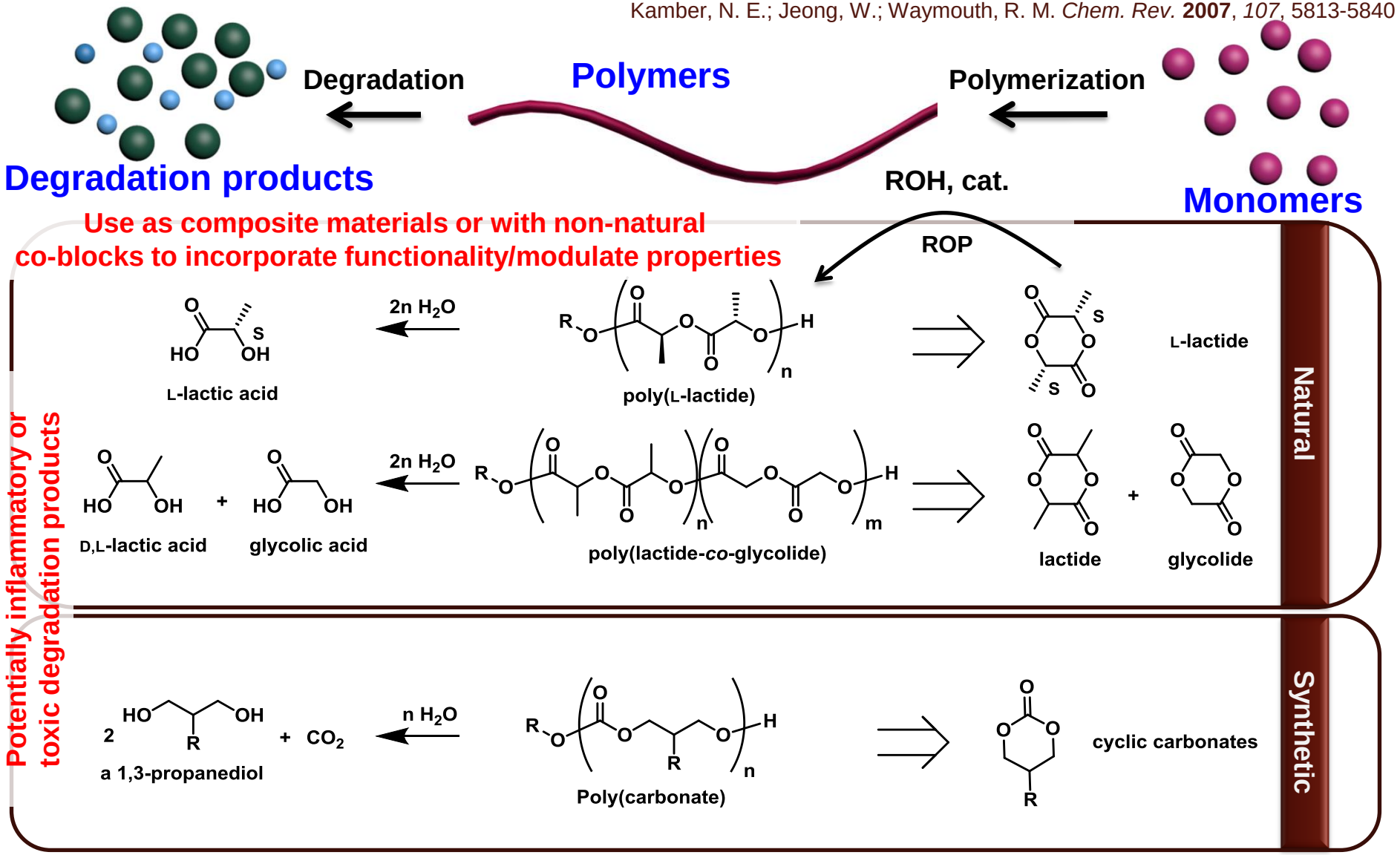


CHEM 466 - Polymer Chemistry, April 17, 2014



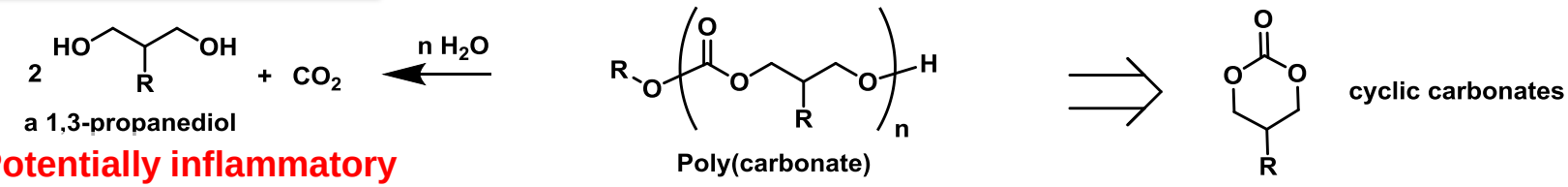
Common Hydrolytically-degradable Polymers Prepared by Ring Opening Polymerization (ROP)

Kieswetter, M. K.; Shin, E. J.; Hedrick, J. L.; Waymouth, R. M. *Macromolecules* **2010**, *43*, 2093-2107
Kamber, N. E.; Jeong, W.; Waymouth, R. M. *Chem. Rev.* **2007**, *107*, 5813-5840



Natural Product Polymers with the Functionality of Synthetic Polymers

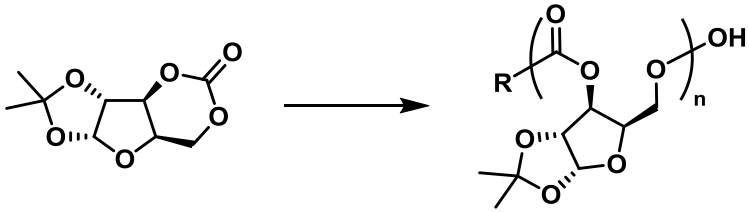
Synthetic Monomer



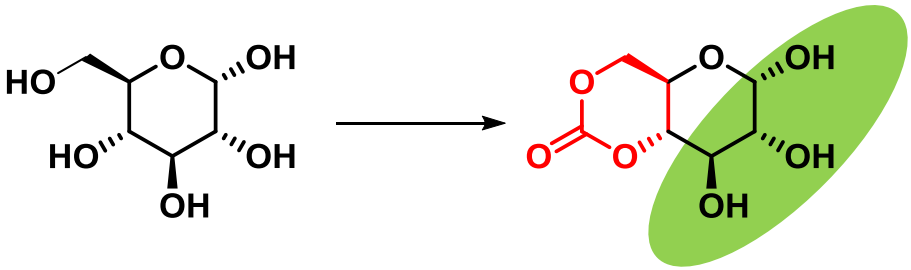
Potentially inflammatory or toxic degradation products

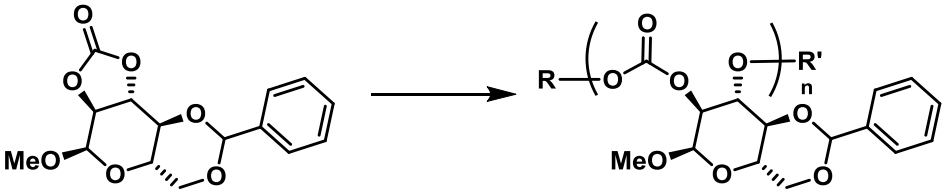
Tempelaar, S.; Mespouille, L.; Coulembier, O.; Dubois, P.; Dove, A. P., *Chem. Soc. Rev.* **2012**, *41*, 1312-1336

Natural products with "built in" diol for cyclic carbonate formation



Shen, Y.; Chen, X.; Gross, R. A. *Macromolecules* **1999**, *32*, 2799-2802



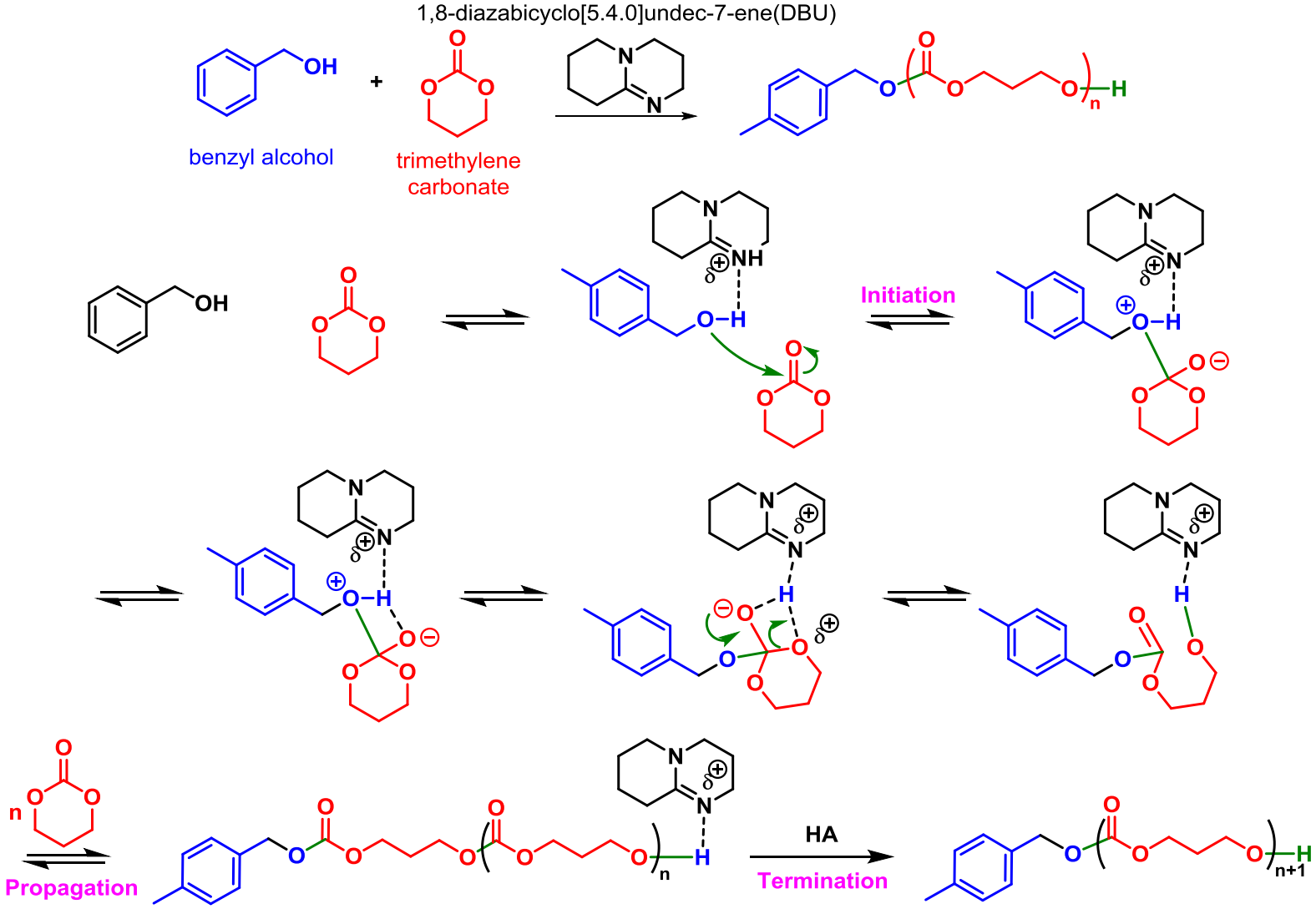


Haba, O.; Tomizuka, H.; Endo, T. *Macromolecules* **2005**, *38*, 3562-3563
Azechi, M.; Matsumoto, K.; Endo, T. *J. Polym. Sci., Part A: Polym. Chem.* **2013**, *7*, 1651-1655

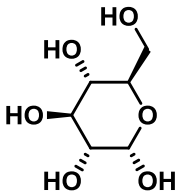
Use as functional groups OR protect to change properties/functionality

Organobase Catalyzed Ring Opening Polymerization (ROP) Initiator/Chain-End Activation

Kieswetter, M. K.; Shin, E. J.; Hedrick, J. L.; Waymouth, R. M. *Macromolecules* **2010**, *43*, 2093-2107
Kamber, N. E.; Jeong, W.; Waymouth, R. M. *Chem. Rev.* **2007**, *107*, 5813-5840

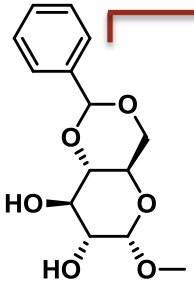


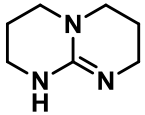
Degradable Polymers Derived from Natural Products: Organocatalyzed ROP of glucose-based cyclic carbonate



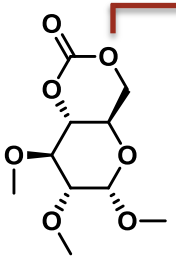
D-Glucose

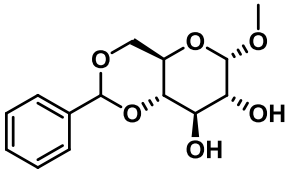
Commercially Available Starting Material





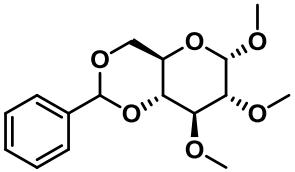
TBD = 1,5,7-Triazabicyclo[4.4.0]dec-5-ene3





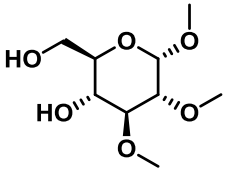
CH_3I, NaH

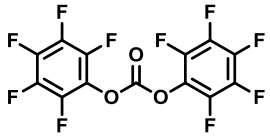
DMF, 18 h, 95%



H_2, Pd/C, conc. HCl

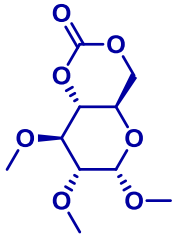
dioxane, RT, 69 h, 93%



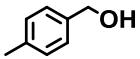


, CsF

THF, 60°C, 25 h, 36%

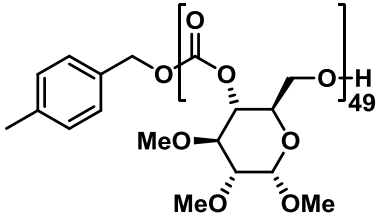


$[M]_0$ = 1 M



2 mol% TBD

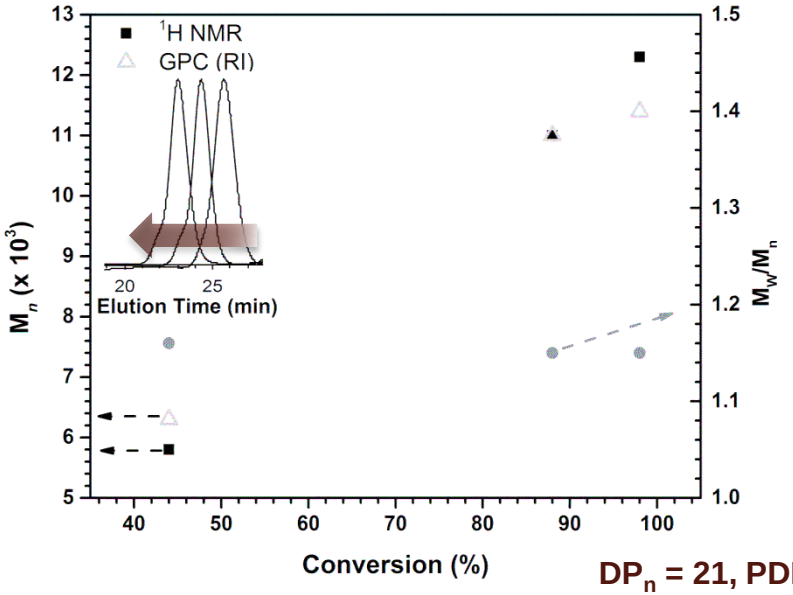
CH_2Cl_2, 6 h, 69%

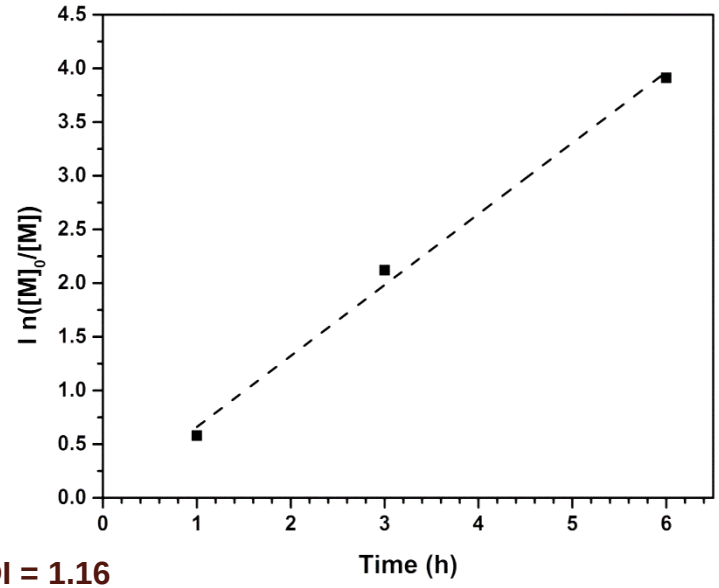


Poly(D-glucose carbonate)
PDGC

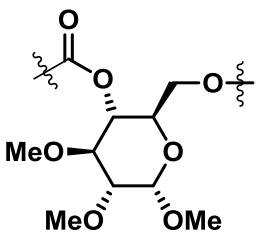
Mikami, K.; Lonnecker, A. T.; Gustafson, T. P.; Zinnel, N. F.; Pai, P.; Russell, D. H.; Wooley, K. L. *J. Am. Chem. Soc.* **2013**, *135*, 6826-6829

Degradable Polymers Derived from Natural Products: Organocatalyzed ROP of glucose-based cyclic carbonate





DP_n = 21, PDI = 1.16

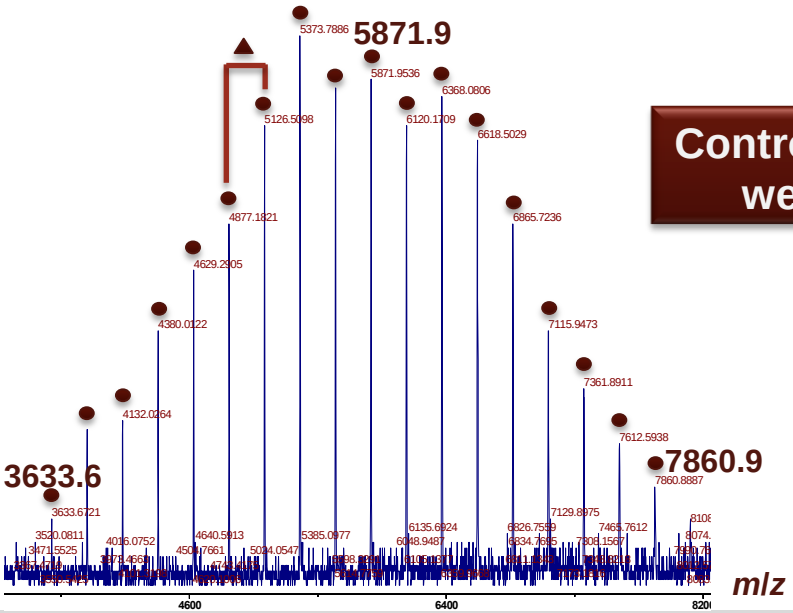


Repeating unit = 248.2 Da

▲ : difference = 248.3

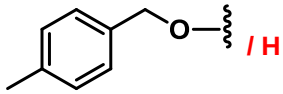
$M_{n\ laser}$ = 5390 g/mol (DP_n = 21)

$M_{n\ NMR}$ = 7600 g/mol (DP_n = 30)



Controlled polymerization with well-defined end groups

● : end group =

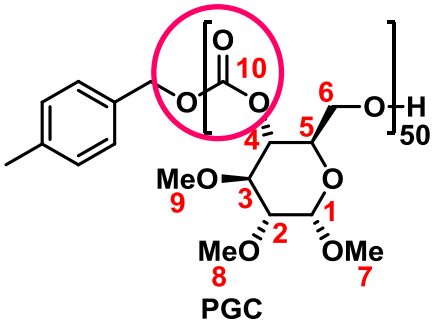


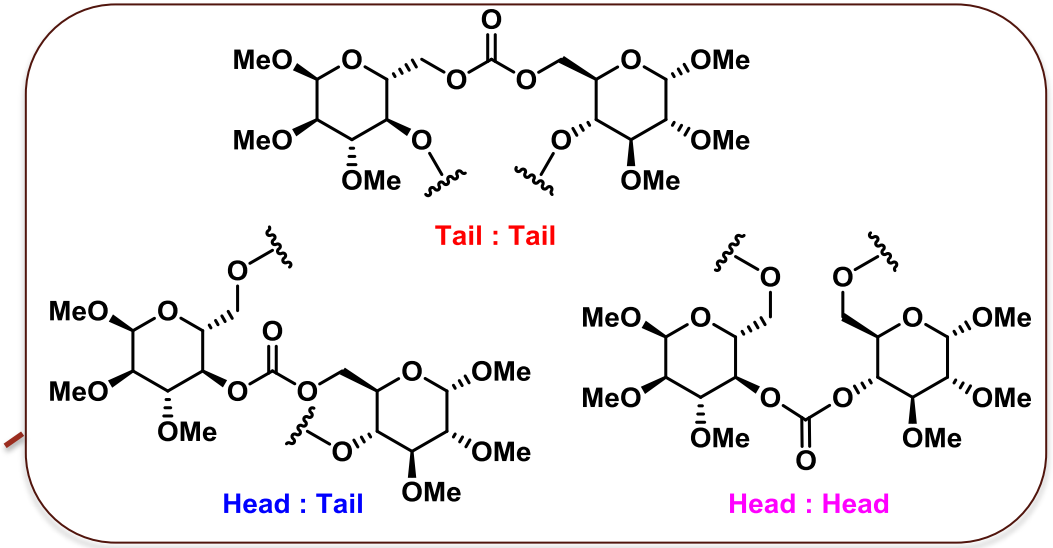
K^+ Adduct

Controlled ROP Gives PGC with Regiorandom Propagation

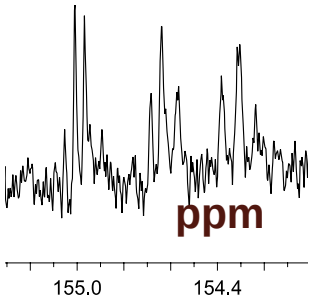
Regiorandom propagation was confirmed by ESI tandem MS analysis by electron transfer dissociation (ETD)

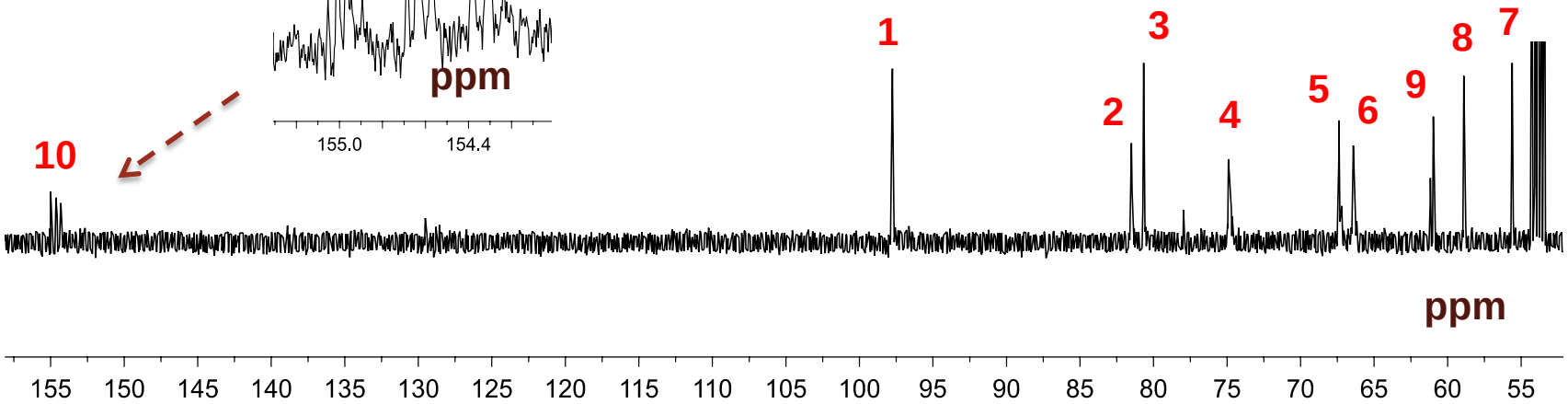
^{13}C NMR (CD_2Cl_2, 125 MHz)



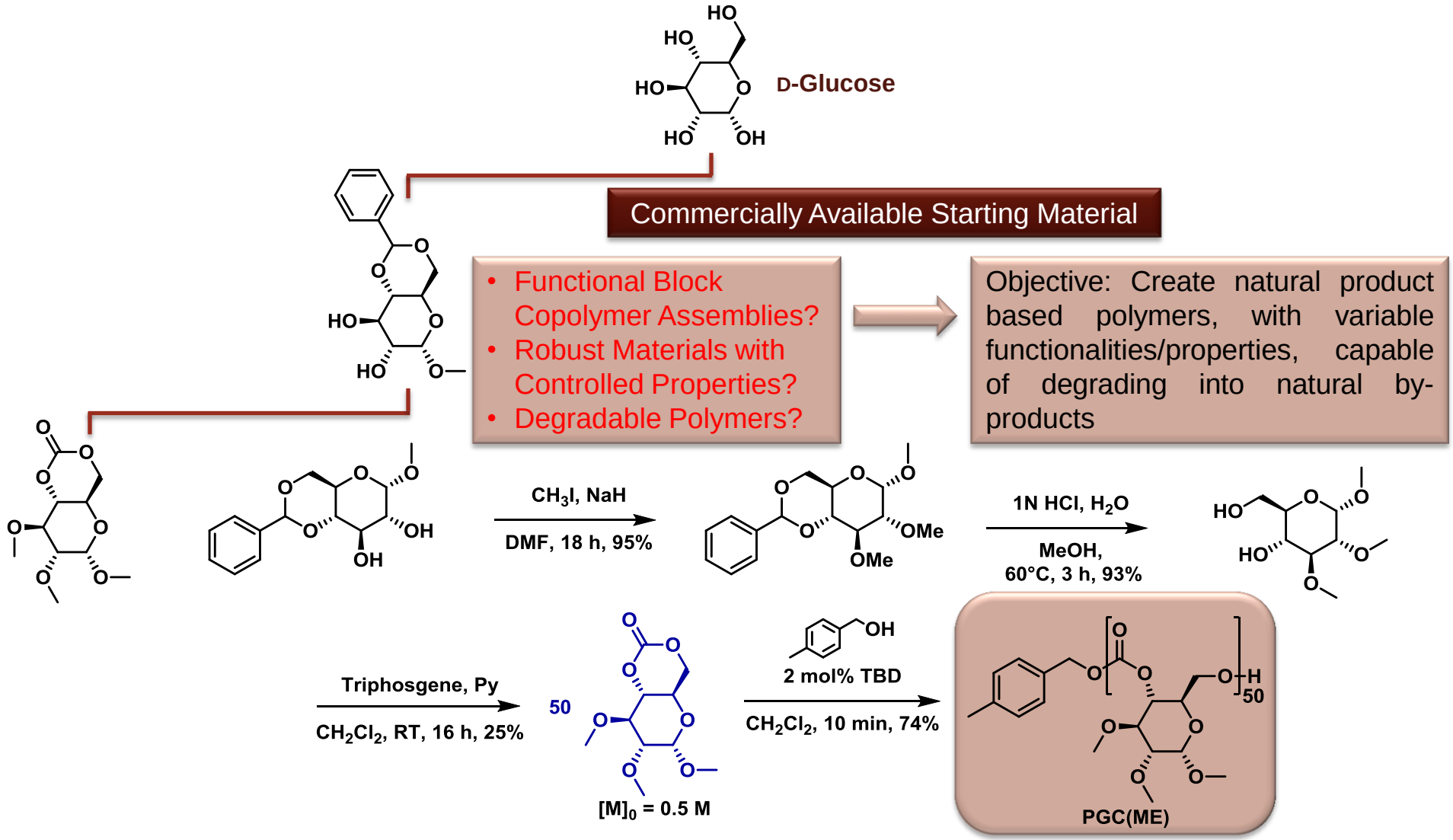


Regiorandom



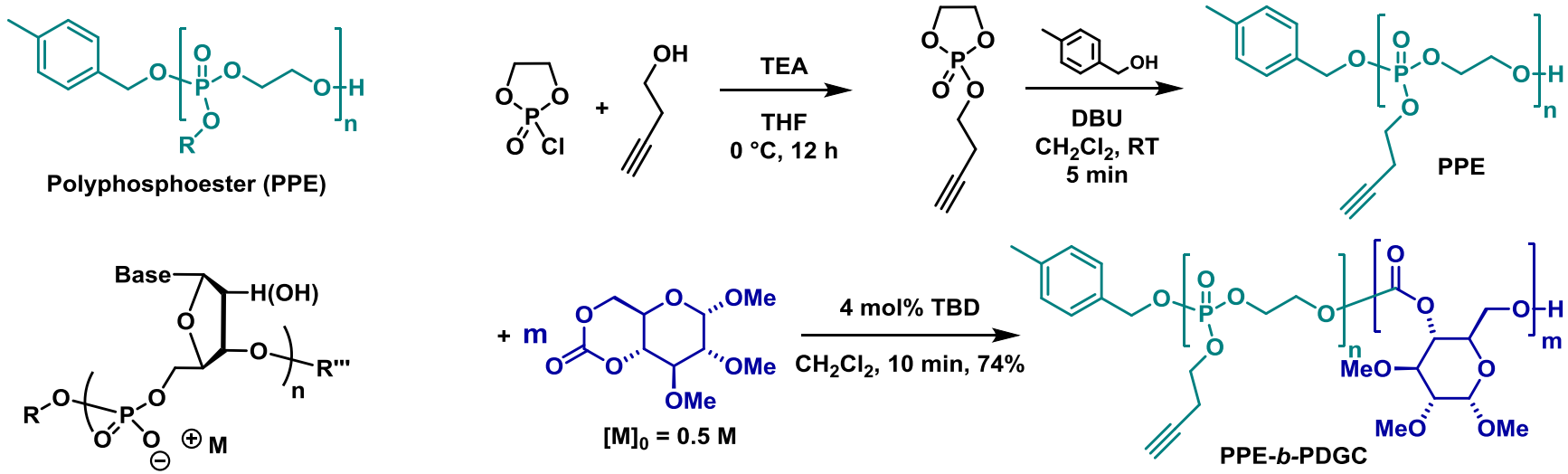


Degradable Polymers Derived from Natural Products for Nanomedicine



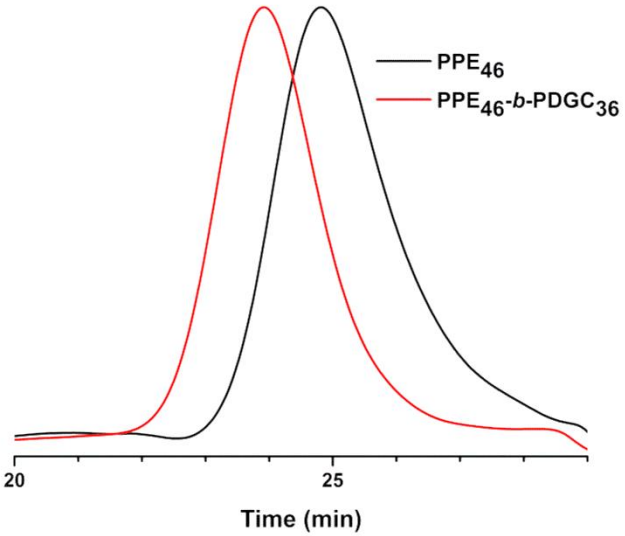
Gustafson, T.P.; Lonnecker, A. T.; Heo, G. S.; Zhang, S.; Dove, A. P.; Wooley, K. L. *Biomacromolecules*, **2013**, *14*, 3346–3353

Macroinitiation of Glucose Monomer Generates PPE-*b*-PDGC Block Copolymer



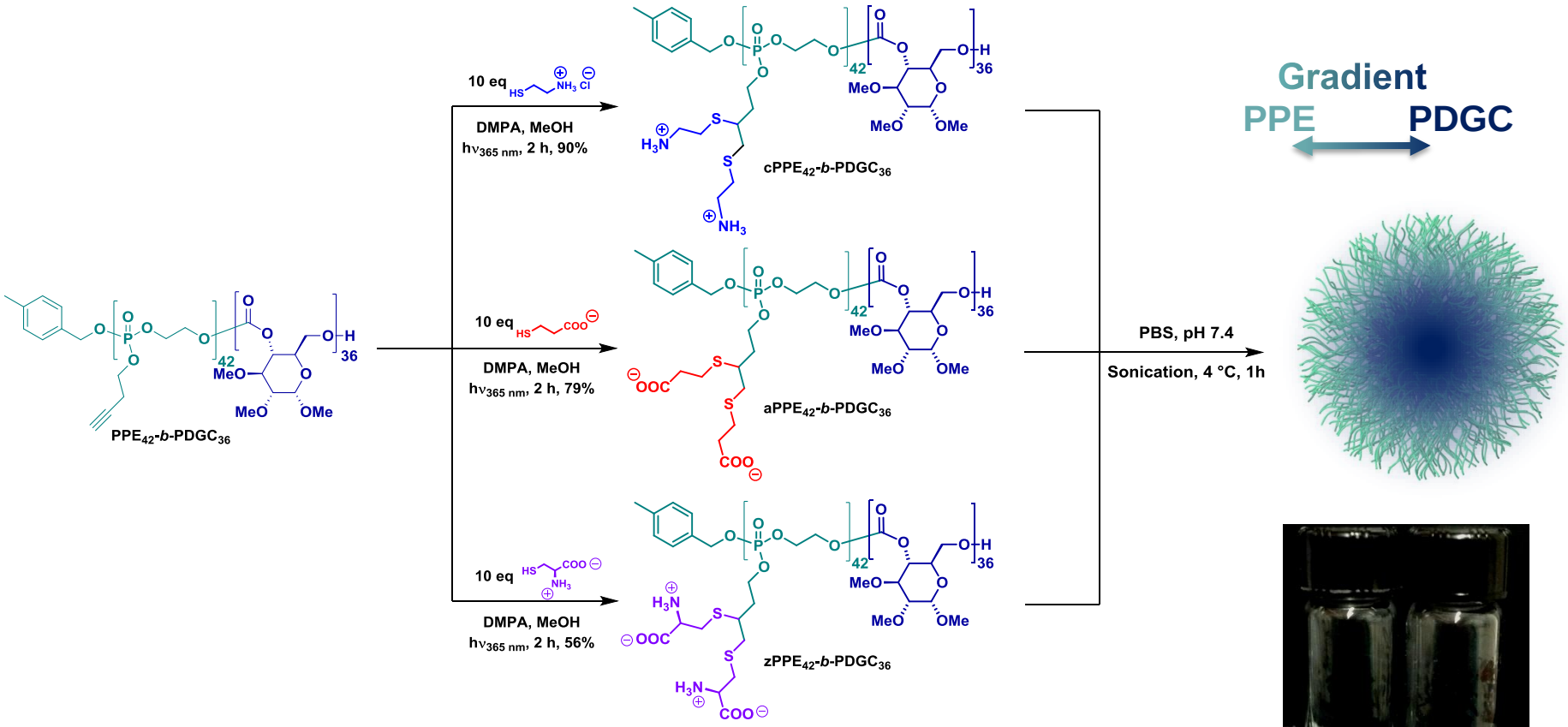
Phosphodiesters constitute the backbone of DNA, RNA

Polymer	Conv. (%)[a]	PDI	M_n (NMR, Da)	T_g (ºC)
$PDGC_{46}$	98	1.13	11500	123
PPE_{42}	96	1.12	7500	-36
PPE_{42}-*b*-$PDGC_{36}$	85	1.10	16400	5
PPE_{42} + $PDGC_{46}$	--	--	--	- 37 / 95



Gustafson, T. P.; Lonnecker, A. T.; Heo, G. S.; Zhang, S.; Dove, A. P.; Wooley, K. L. *Biomacromolecules* **2013**, *14*, 3346-3353
Zhang, S.; Li, A.; Zou, J.; Lin, L. Y.; Wooley, K. L. *ACS Macro Lett.* **2012**, *1*, 328-333

Supramolecular Self-assembly: Potential temperature sensitive phase behavior?

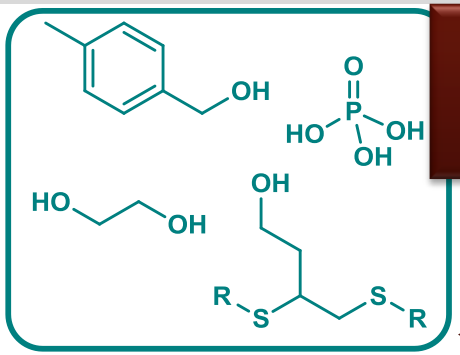


Functionalized polymers do not undergo phase segregation

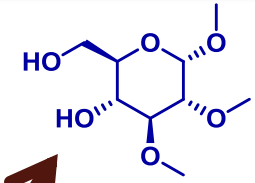
LCST

- **Functional PPE_{42}-*b*-$PDGC_{36}$ precipitates from aqueous solution upon warming from 4 °C to room temp**

Improving Materials and Degradation Characteristics

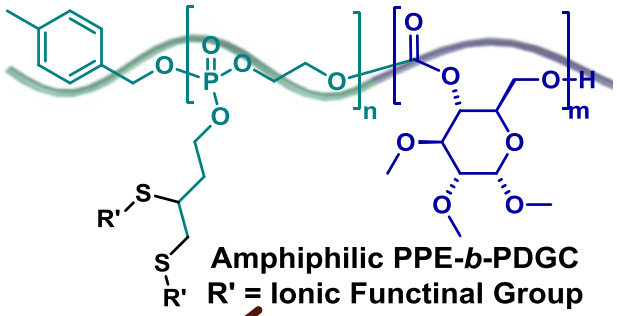


Ethylene glycol is concerning for medical applications

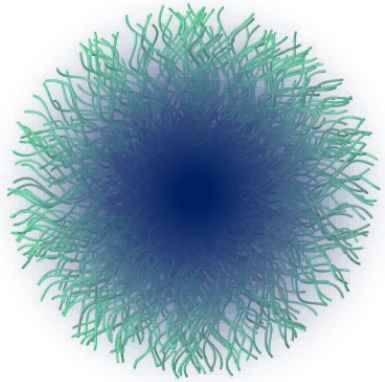


CO_2

Hydrolytic Degradation



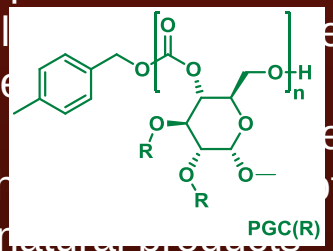
Amphiphilic PPE-*b*-PDGC
R' = Ionic Functinal Group



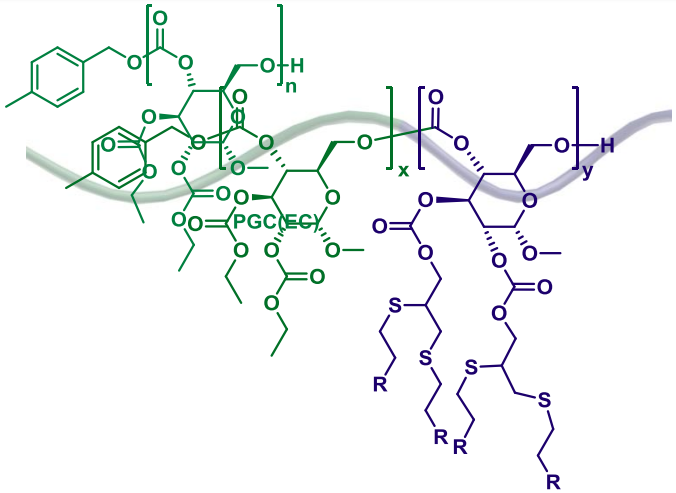
LCST

Strategies:

1) Develop robust materials with controlled properties *via* modification of the 2,3-glucose protecting groups
2) Design full e poly(saccharide
3) Replace the e linkages with th f bioresorbable n



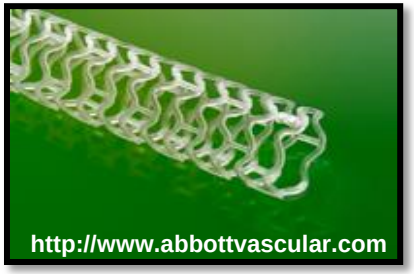
Fully degradable poly(saccharide)s



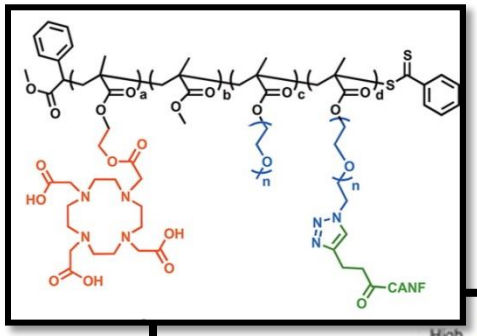
From Polymer Design to Applications: Degradable polymers with potential for use in medical applications

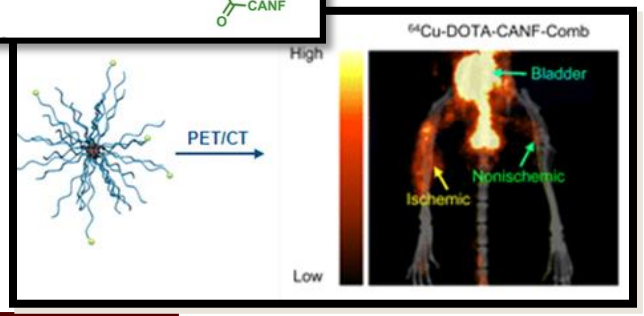
- Implantable Devices
- Therapeutics
  - Nanomaterials for Drug Deliver
- Diagnostics and Imaging
  - Improving diagnostics – contrast agents
  - Following and characterizing nanotherapeutics *in vitro* and *in vivo*

OsteoFab Patient Specific Cranial Device
Oxford Performace Materials (PEKK)

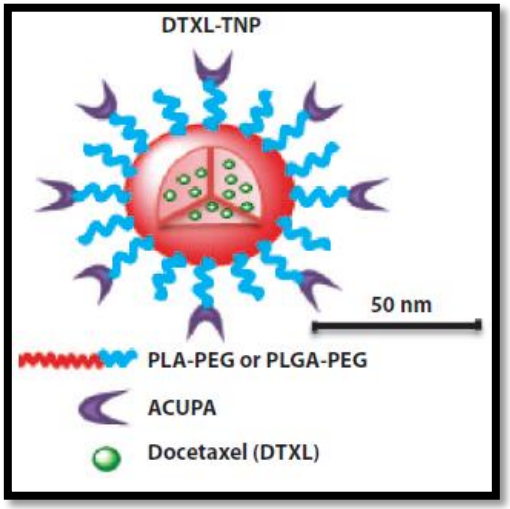


Absorb Bioresorbable Vascular Scaffold System
Abbott (PLLA & PDLLA + Everolimus)





Detection of angiogenesis
Liu, Y. *et al*. *J. Nucl. Med.* **2011**, *52*, 1956-1963

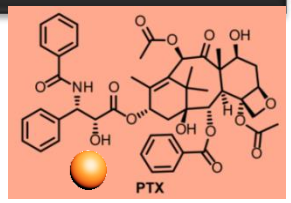


BIND Therapeutics
PMSA targeted (NSCLC, prostate)
Hrkach, J. *et al*. *Sci. Transl. Med.* **2012**, *4*, 128ra39

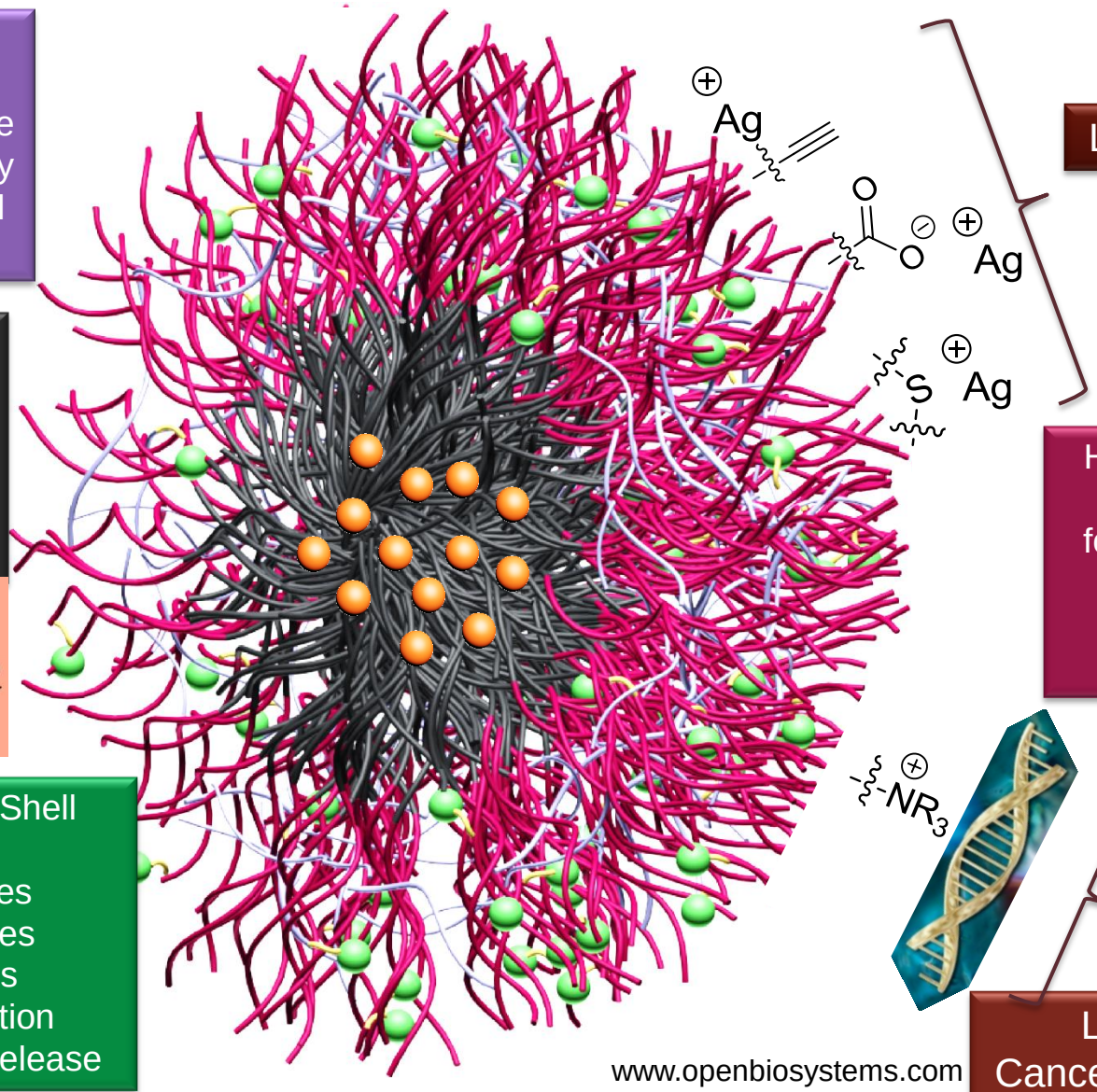
Shell Crosslinked Knedel-like (SCK) Nanoparticles for Improved Therapeutics Delivery

Shell Crosslinks for:
- Structural stability
- Gating of guest release
- Addition of functionality
- Alteration of biological properties

Hydrophobic Core For:
- Packaging of therapeutics
- Determination of particle shape, rigidity, *etc.*



Functionalities in the Shell and/or Core:
- Radiolabeling sites
- Fluorescent probes
- Targeting ligands
- Control biodistribution
- Controlled Payload Release



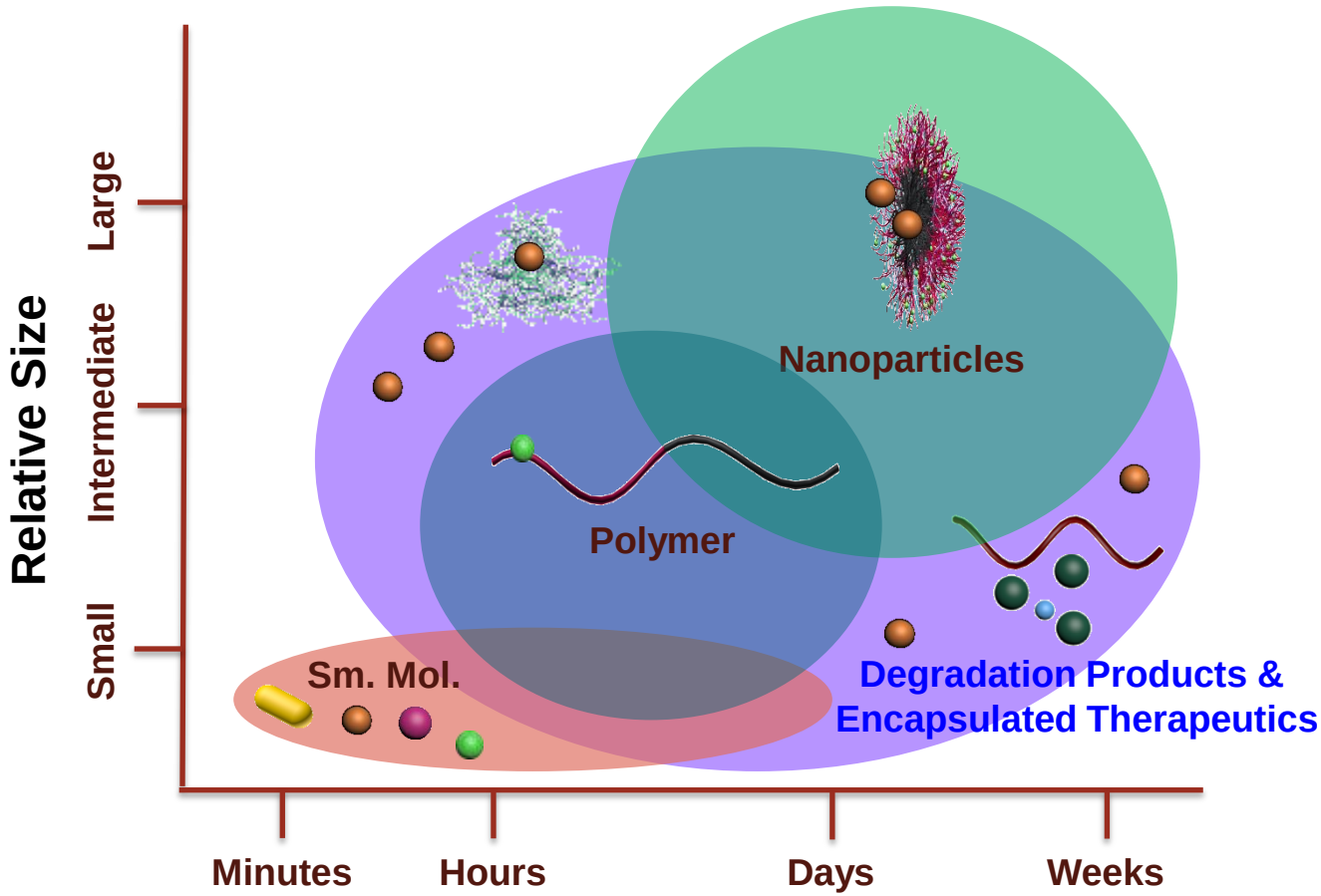
Lung Infections

Hydrophilic Shell (-, +, neutral) for Packaging of:
- Ag^+
- DNA
- siRNA

Lung Injury
Cancer Therapeutics

www.openbiosystems.com

Advantages of Nanotherapeutics for Drug Delivery and Imaging



Rate of Extravasation and Clearance
(Also dependent up route of administration)

Polymeric Nanoparticles for Treatment of Lung Metastasis of Osteosarcoma

Osteosarcoma:

- Peak incidence in adolescence (<5 to ~40 years)
- Most commonly in the *distal femur* or the *proximal tibia*
- High fatality rate
  - 70% survival at 5 years
  - < 30% with metastasis
- Most common site of metastasis is the lung
  - 40% present with overt metastasis
  - 90% estimated to have micrometastatic disease at diagnosis

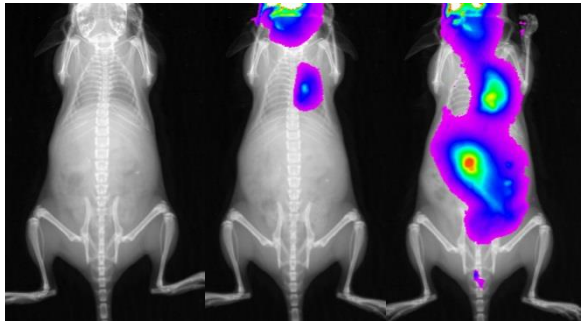
Treatment:

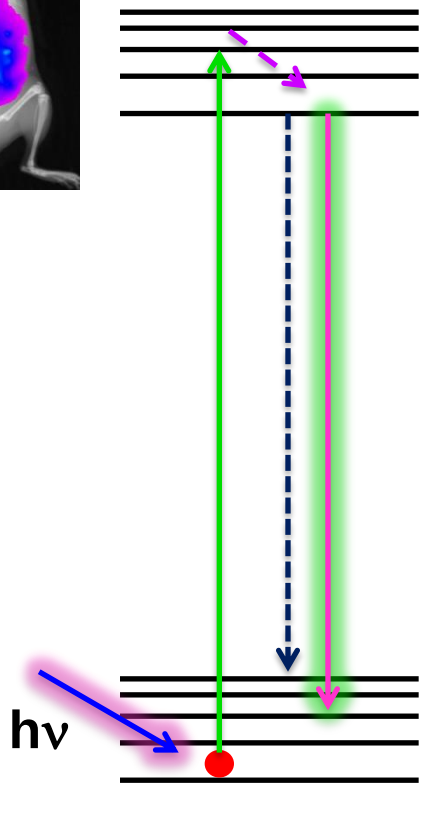
- Combination chemotherapy and surgical removal of the tumor (primary tumor)
- Chemotherapy to eliminate micrometastatic disease
- Unresectible recurrent disease is uniformly fatal

"…drugs delivered to the respiratory tract in liposomal formulation resulted in high pulmonary drug concentration, reduced systemic toxicity, and reduced dosage requirements compared with parenteral and oral administration."

Koshkina, N. V.; Kleinerman, E. S.; Waldrep, C.; Jia, S.-F.; Worth, L. L.; Gilbert, B. D.; Knight, V. *Clin. Cancer Res.* **2000**, *6*, 2876-2880

Fluorescent Labels: Why are they needed and what are we observing?

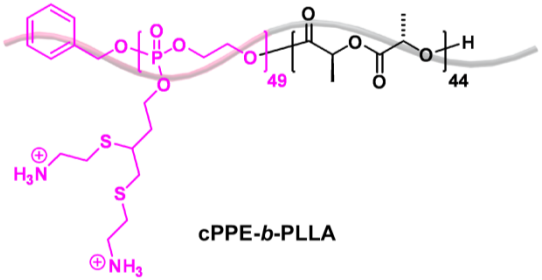


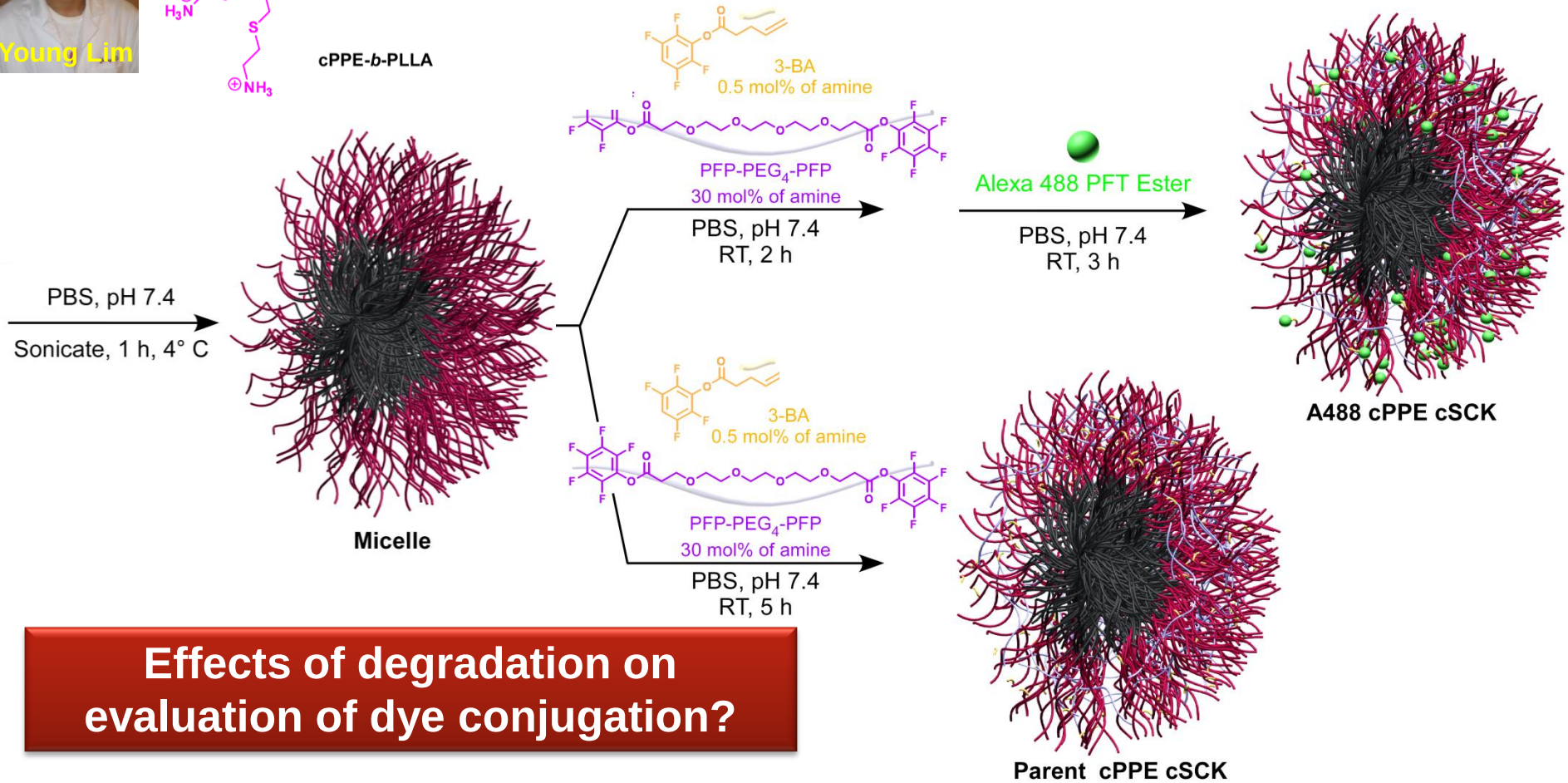


1. Light absorption (excitation)
   Photon absorption
2. Relaxation & internal conversion
3. Non-radiative decay
   No observed emission
4. Radiative decay (emission)
   Photon emission observed

Fluorescent Labeling of Degradable Polymeric Materials

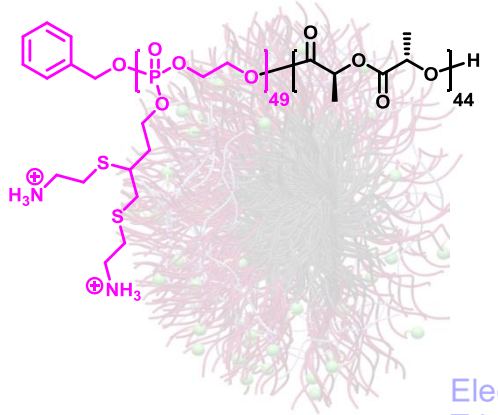
Lim, Y. H.; Heo, G. S.; Cho, S.; Wooley, K. L. *ACS Macro Lett.* **2013**, *2*, 785-789
Gustafson, T. P.; Lim, Y. H.; Flores, J. A.; Heo, G. S.; Zhang, F.; Zhang, S.; Samarajeewa, S.; Raymond, J. E.; Wooley, K. L. *Langmuir* **2014**, *30*, 631-641



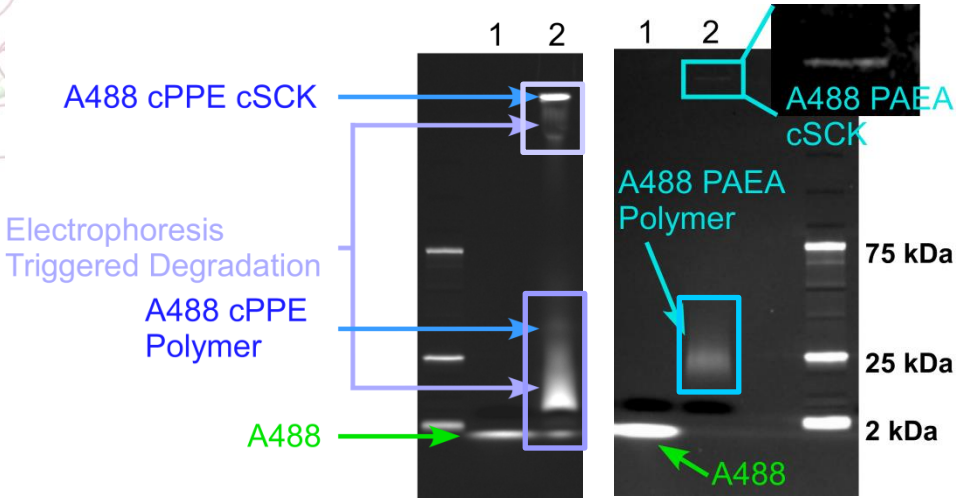


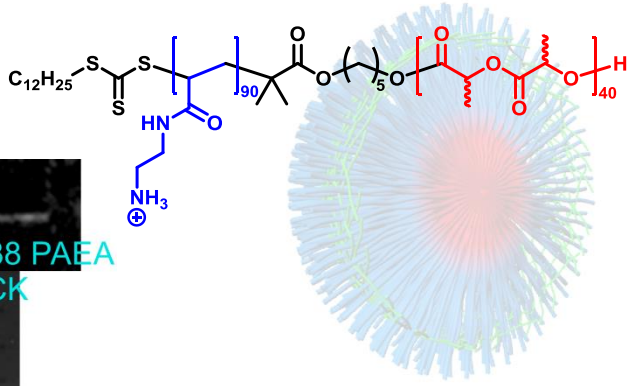
Effects of degradation on evaluation of dye conjugation?

Electrophoretic Analysis (SDS-PAGE): Effects of polymer degradation when characterizing fluorophore conjugation

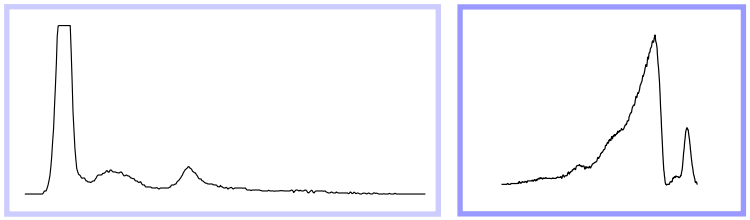


A488 cPPE cSCK
Degradable
30% crosslinking

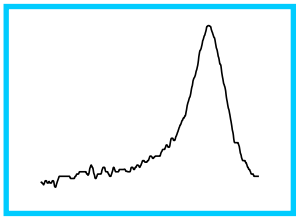




A488 PAEA cSCK
Non-degradable
5% crosslinking



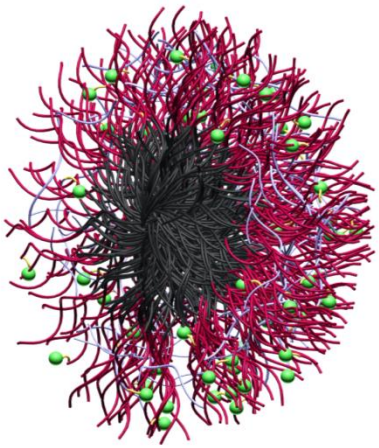
Band profiles due to electrophoresis
Triggered degradation



Band profile due to polydispersity

Samarajeewa, S.; Ibricevic, A.; Gunsten, S. P.; Shrestha, R.; Elsabahy, M.; Brody, S. L.; Wooley, K. L. *Biomacromolecules* **2013**, *14*, 1018-1027

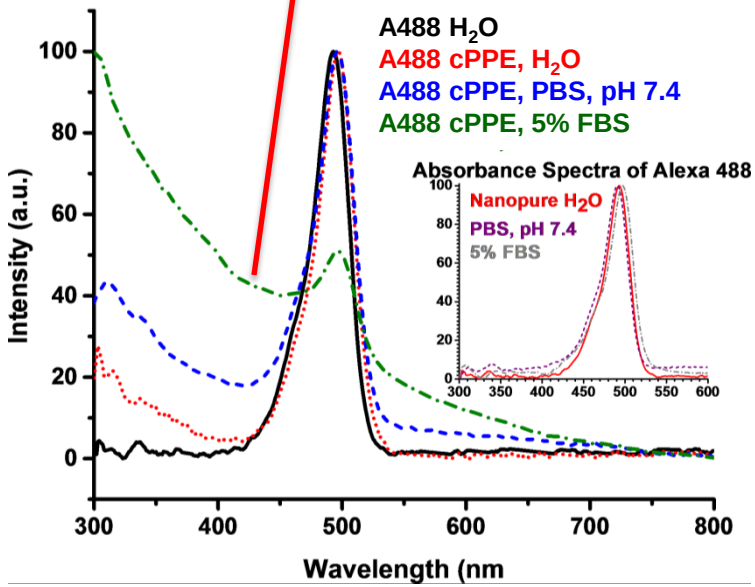
UV/Vis Absorption Suffers from Scattering, Limiting Utility for Quantitative Assessment



A488 cPPE cSCK

Solvent	Sample Type	Sample	Φ_{FL}
Nanopure H_2O	Dye	A488	0.92*
	Degradable	A488 cPPE	0.53
	Non-degradable	A488 PAEA	0.28
PBS pH 7.4	Dye	A488	-
	Degradable	A488 cPPE	0.36
	Non-degradable	A488 PAEA	-
5% FBS in H_2O	Dye	A488	-
	Degradable	A488 cPPE	1.93
	Non-degradable	A488 PAEA	-

*The Molecular Probes Handbook, Fluorescence quantum yields



Steady State Spectroscopy for Assessing Dye-cSCK Conjugation

Emission Anisotropy, *r*

- **Tumbling** or state change & **size determination** is provided thru Polarized ex./em.
- Provide information on dynamic behavior: molecular orientation & rotational diffusion
- Measure the avg. angular displacement during the time between excitation and emission.

$$r = r_0 \frac{\theta}{\theta + \tau}$$

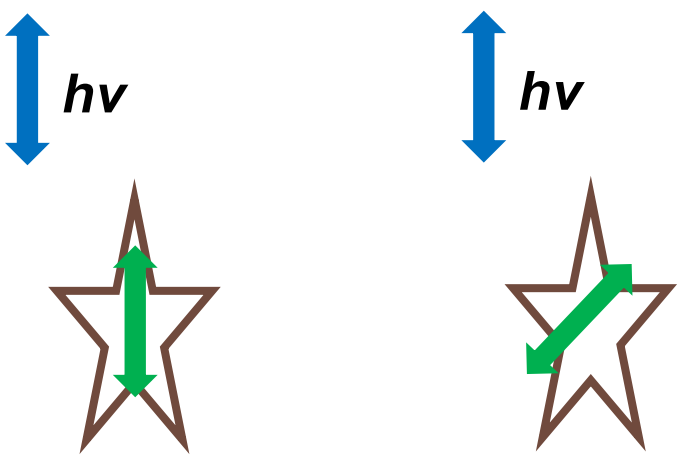
θ ← Rotational correlation time

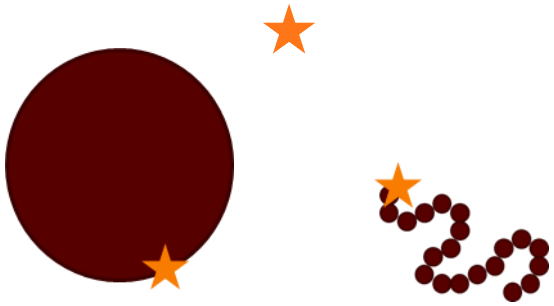
τ ← Lifetime

$$\theta = \frac{\eta V}{RT}$$

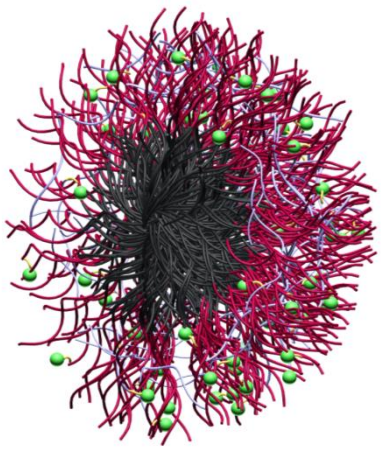
0.4 = high anisotropy
0.0 = low anisotropy

r ↑ = Slower θ
r ↓ = Faster θ





Steady State Spectroscopy: Some methods offer more reliability than others



A488 cPPE cSCK

Steady State Anisotropy

$$r = r_0 \frac{\theta}{\theta + \tau}$$

θ ← Rotational correlation time

τ ← Lifetime

$$\theta = \frac{\eta V}{RT}$$

r ↑ = Slower θ
r ↓ = Faster θ

It's not only about confirming conjugation: How to obtain information on environmental interactions?

Solvent	Sample Type	Sample	*r*
Nanopure H_2O	Dye	A488	0.015 ± 0.003
	Degradable	A488 cPPE	0.114 ± 0.004
	Non-degradable	A488 PAEA	0.129 ± 0.006
PBS pH 7.4	Dye	A488	0.013 ± 0.002
	Degradable	A488 cPPE	0.089 ± 0.002
	Non-degradable	A488 PAEA	0.132 ± 0.008
5% FBS in H_2O	Dye	A488	0.259 ± 0.006
	Degradable	A488 cPPE	0.185 ± 0.005
	Non-degradable	A488 PAEA	0.217 ± 0.012

r = steady state anisotropy λ_{ex} = 430 nm, λ_{em} = 580 nm

Time Domain Spectroscopy for Assessing Dye-cSCK Conjugation

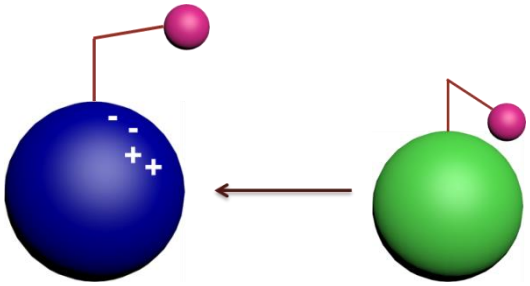
Excited State Lifetime

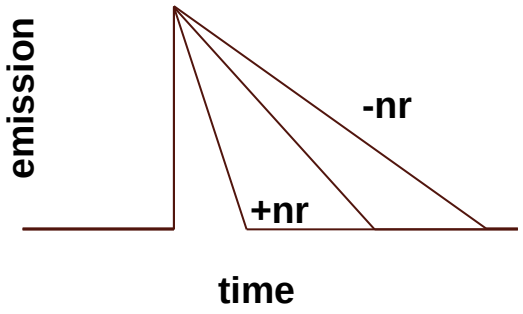
Radiative processes:

a) Intrinsic building blocks (dye)
b) Macromolecular (dye-polymer or dye-protein conjugates)
c) Supramolecular (dye-nanoparticle conjugates)

Non-radiative processes (environmental interactions):

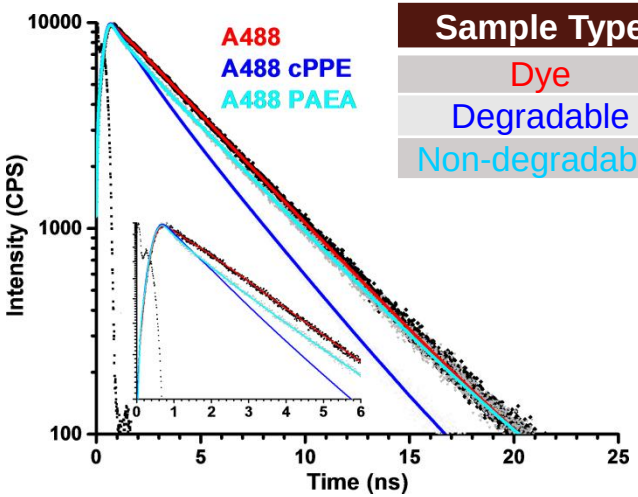
1) Solvent
2) Collisions
3) Aggregation



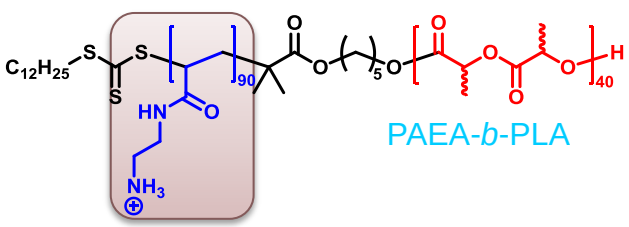


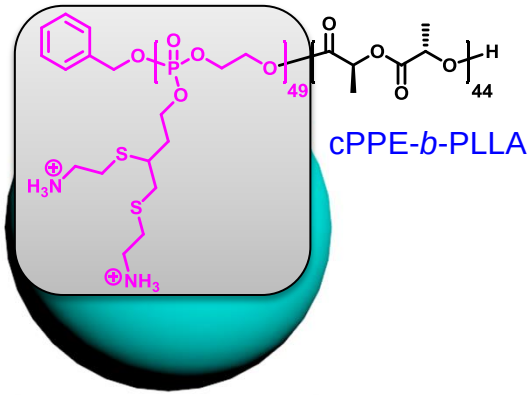
Shell Chemistry/Ionic Character is Accountable for Divergence in Decay Profiles/Lifetimes

Fluorescence lifetime in water:



Sample Type	Sample	τ_1 (ns)	τ_2 (ns)	τ_i (ns)
Dye	A488	-	4.22 (100 %)	4.22
Degradable	A488 cPPE	1.15 (27 %)	3.28 (70 %)	3.04
Non-degradable	A488 PAEA	1.05 (30 %)	4.15 (73 %)	3.86
		Polymer interaction	Solvent interaction	





Possible explanations for divergence:

1) Size/volume:

2) Shell Rigidity: PAEA-*b*-PLA T_g = 48 °C *vs.* cPPE-*b*-PLLA T_g = -7.49 °C

3) Different shell character:
   1) Average intrinsic dipole moment
   2) Ammonium density
   3) Charge density

Steady state anisotropy trends rule out size and rigidity

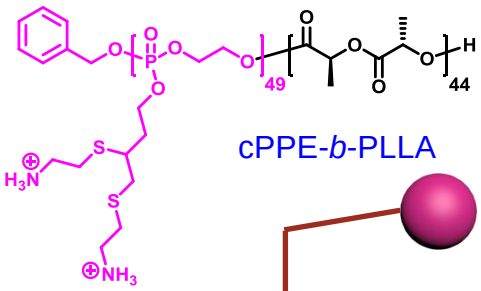
Fluorescence Lifetime Changes Indicates Increased Polymer-solvent Interactions for Degradable cPPE

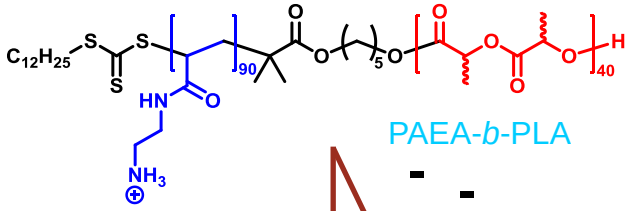
Solvent	Sample Type	Sample	τ_1 (ns)	τ_i (ns)
H_2O	Dye	A488	4.22 (100 %)	4.22
PBS pH 7.4	Dye	A488	4.08 (100 %)	4.08
H_2O	Degradable	A488 cPPE	1.15 (27%)	3.04
PBS pH 7.4	Degradable	A488 cPPE	1.42 (28 %)	3.26
H_2O	Non-degradable	A488 PAEA	1.05 (30 %)	3.86
PBS pH 7.4	Non-degradable	A488 PAEA	0.79 (21 %)	4.06

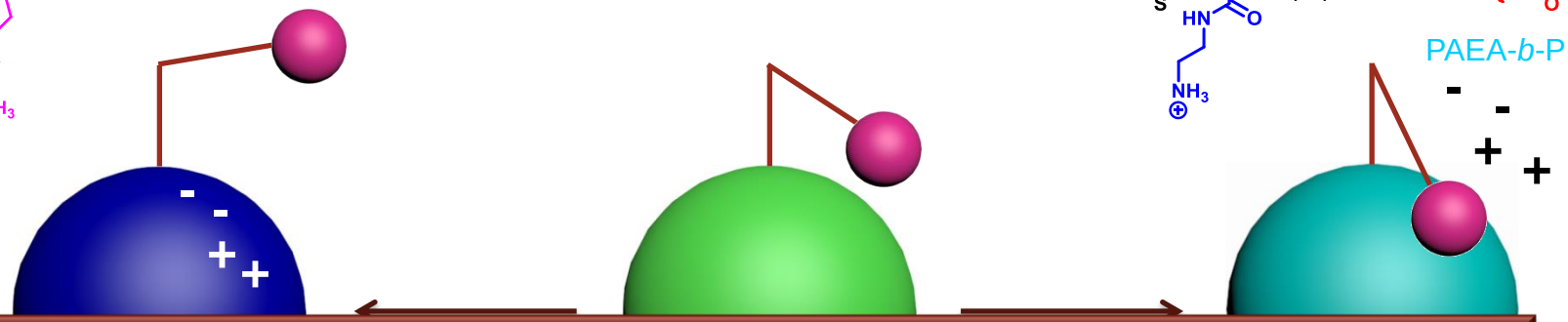
Lifetime Trends in PBS:

1) ↑ in τ_1 for A488 cPPE
2) ↓ in τ_1 for A488 PAEA
3) Rotational correlation time (θ):
   a. A488 cPPE ↑ 46%
   b. A488 PAEA ↑ 4%

$$\theta = \frac{\eta V}{RT}$$







- An understanding the spectral signature-structure-function relationship provides a fundamental understanding of nanomaterials before the complexity of drug delivery and biology become a factors

Polymer-based Nanotherapeutics

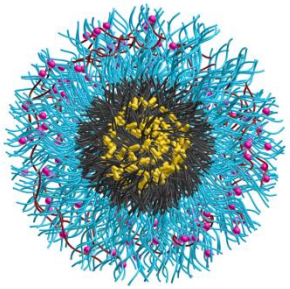
Intelligent synthetic design offers unique opportunities to improve upon traditional synthetic materials; natural product-based materials may offer increased biocompatibility, control of degradation products, *etc.*

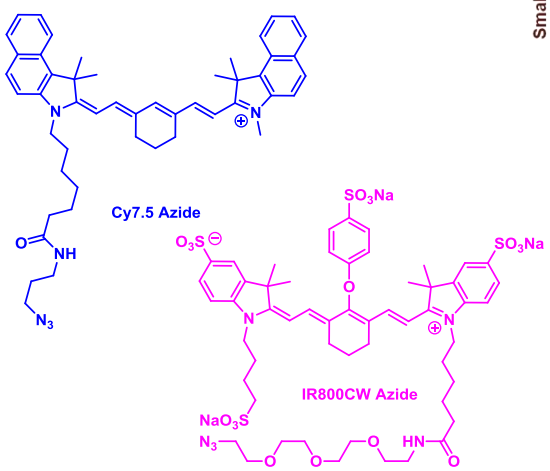
Importance of evaluating polymeric nanomaterials as therapeutics and imaging agents:

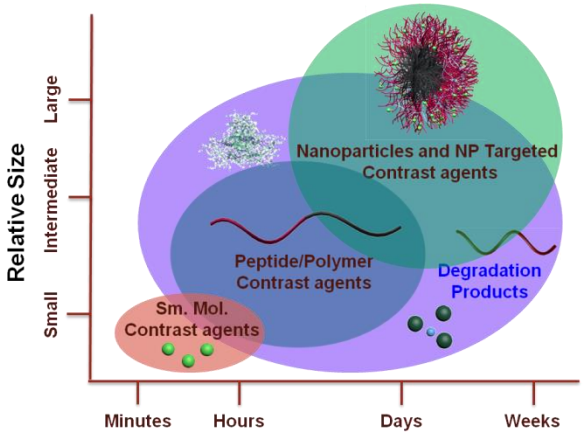
What is the goal?

How to evaluate polymeric materials to ensure they work in a consistent and reproducible manner

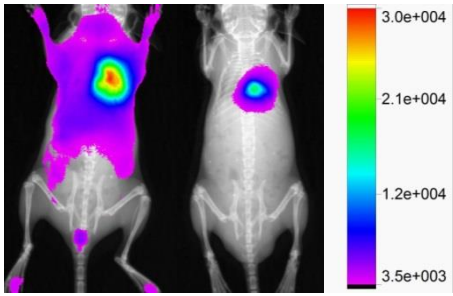
How can polymer/nanoparticle design impact observations during *in vivo* studies?







Rate of Extravasation and Clearance



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