Hydrophilic-Hydrophobic

TEL AVIV UNIVERSITY

Designing delivery systems – concepts, examples and concerns

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The good and dark sides of drug delivery

Targeting in drug delivery

Paul Ehrlich 1854-1915

The Nobel Prize in Physiology or Medicine 1908

Ilya Mechnikov, Paul Ehrlich

The Magic Bullet

Targeting

Passive

Extravasation-dependent

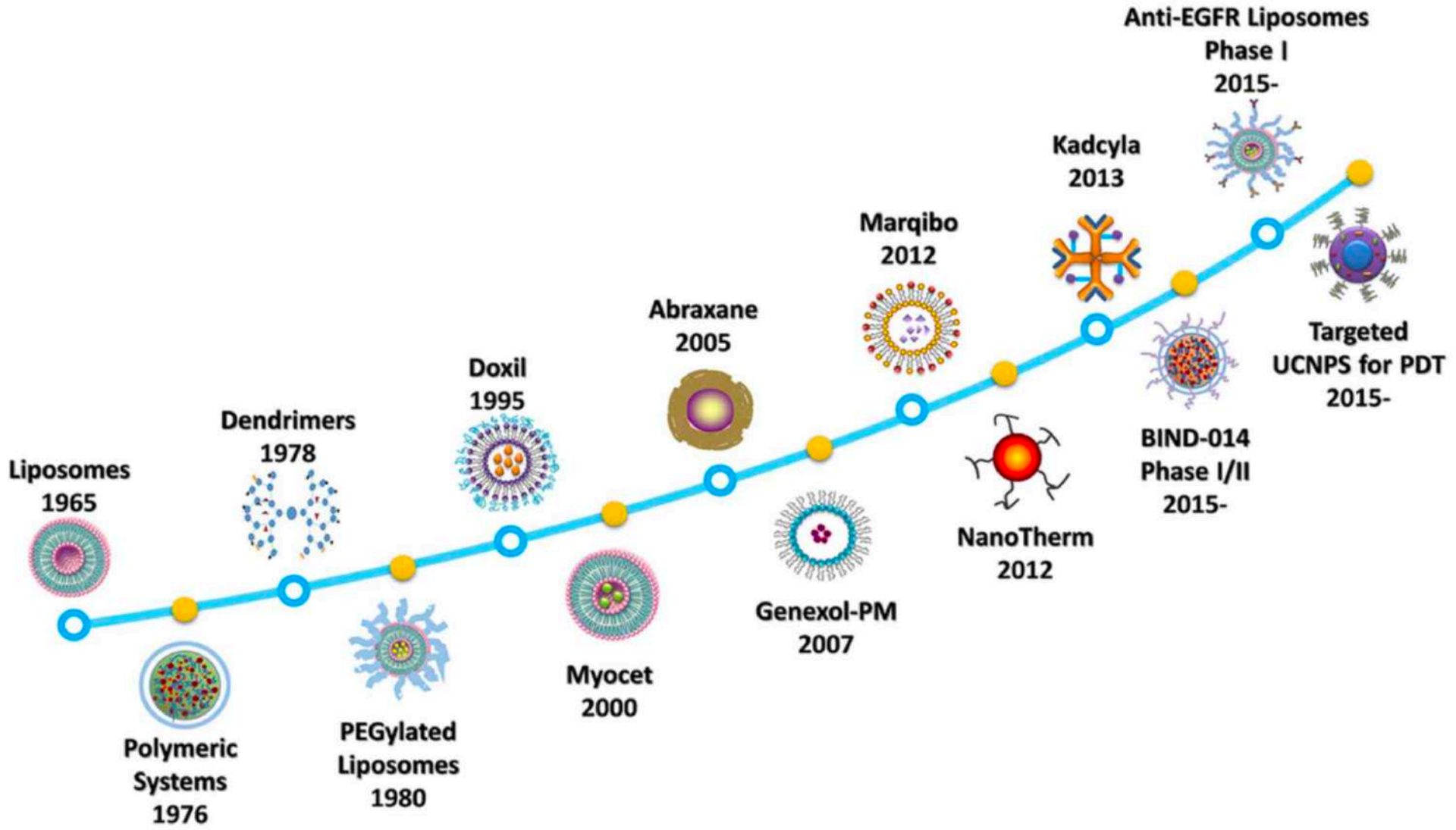
Mechanism that exploits the pathophysiological properties of disease tissues

Active

Ligand-targeted

Mechanism that exploits specific biological structures, namely receptors expressed on biomembranes

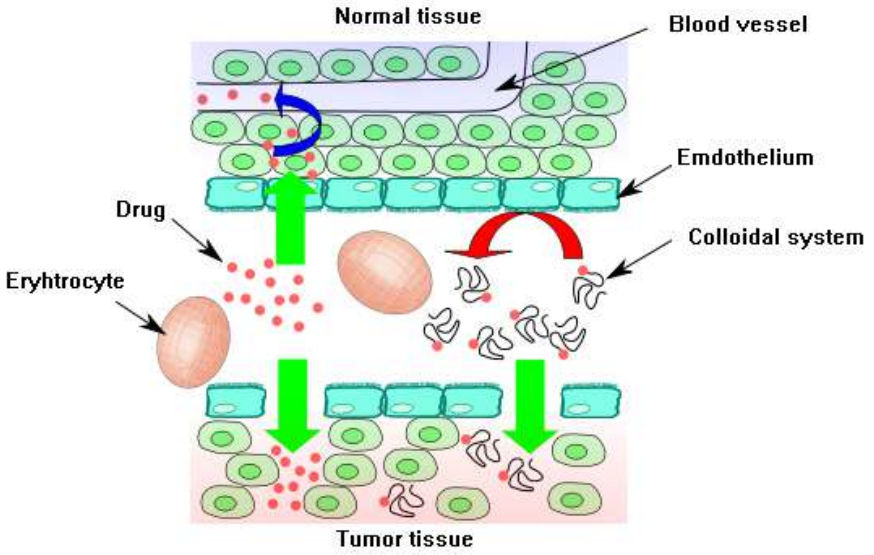
Delivery systems in use



Timeline of the development of nanomedicines
Shen and Wang et al. Oncology reports 2017
https://doi.org/10.3892/or.2017.5718

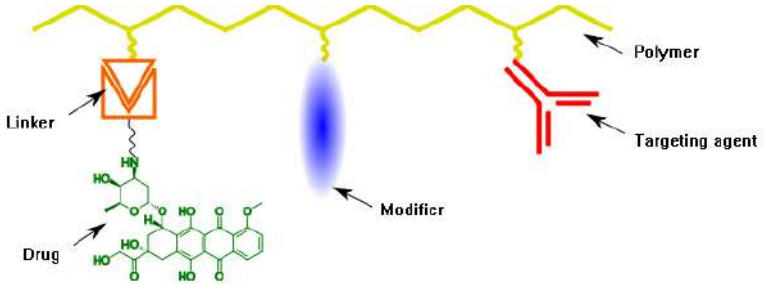
Passive and active drug delivery

Passive

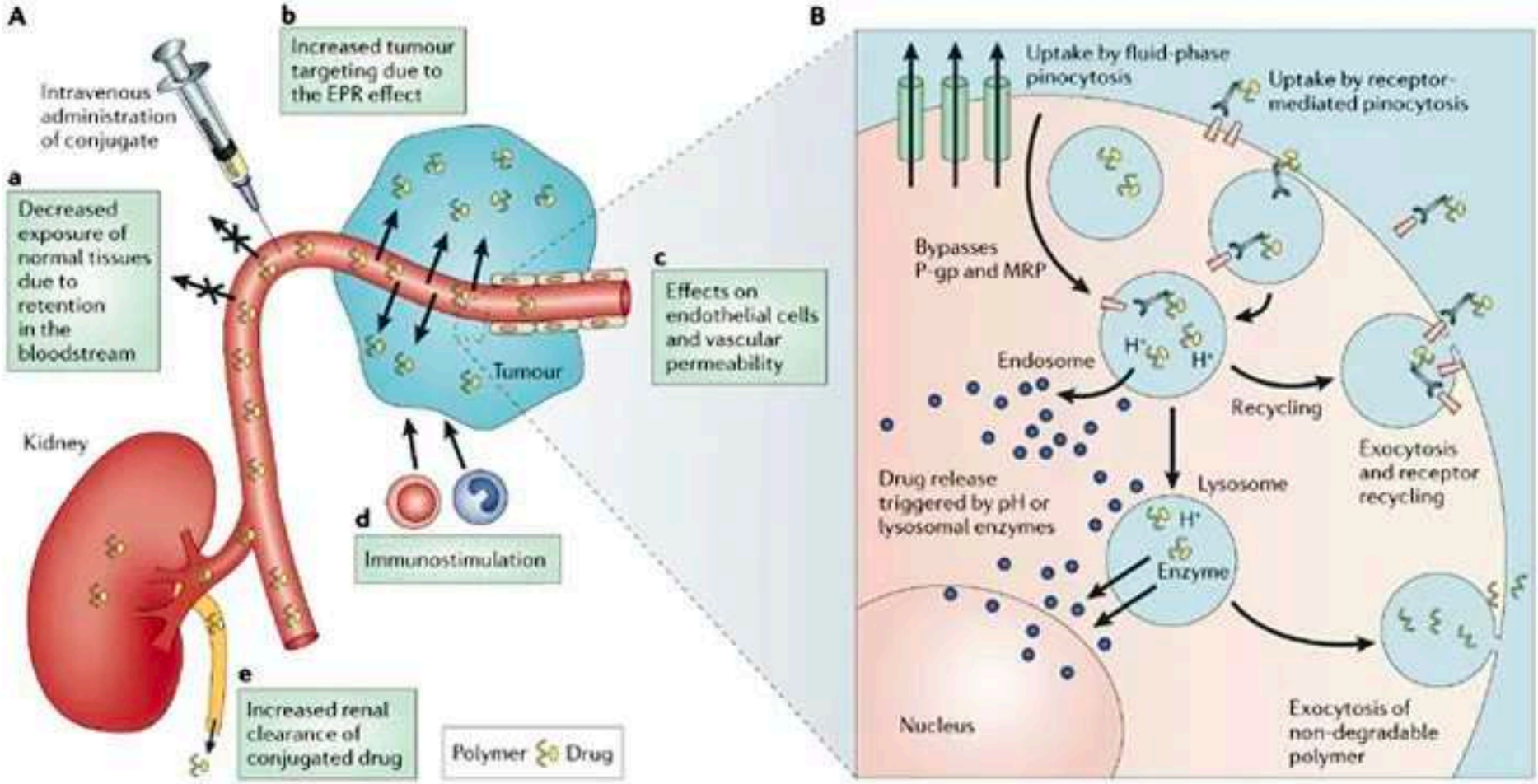


Targeting

Active



Passive drug delivery



Active targeting

Drug targeting is the inventive process of finding new medications based on the knowledge of the biological target

Bio-recognition is the ability of the drug to display therapeutical selectivity according to any mechanism which involves the specific recognition of a biological target.

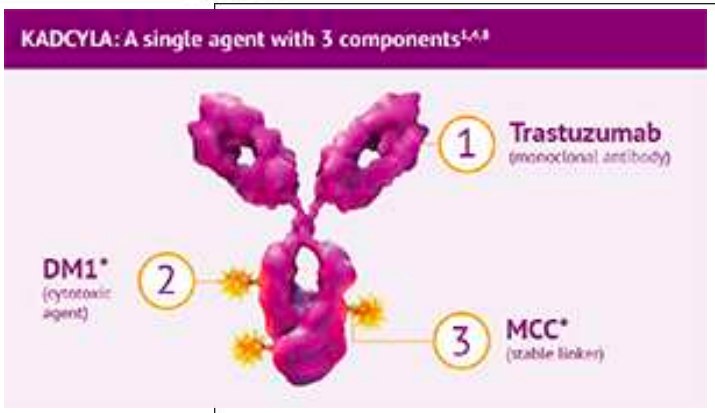
1. Recognition of the pharmacological target (drug related)

2. Accumulation in the target organ or tissue selectively and quantitatively, independent of the site and methods of its administration (carrier related).

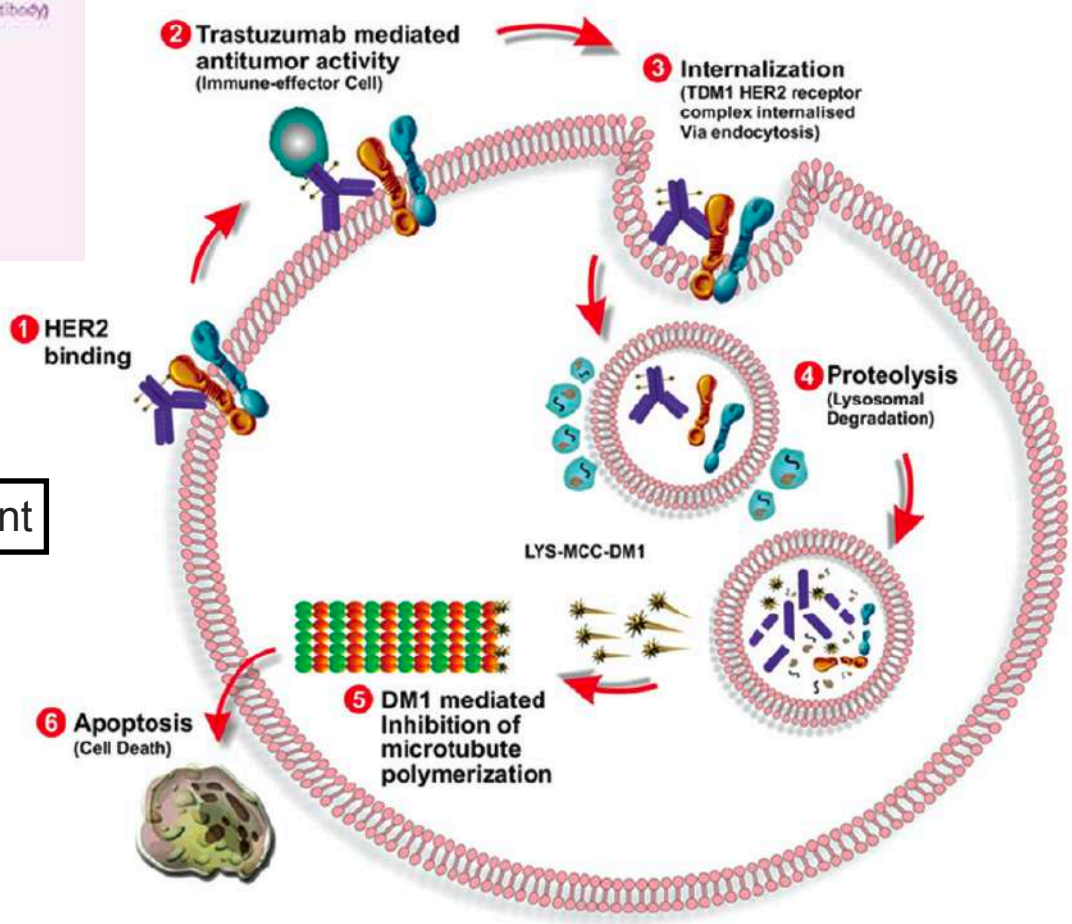
3. Exploitation of specific cell mechanisms for the cell-up-take and activation (carrier related)

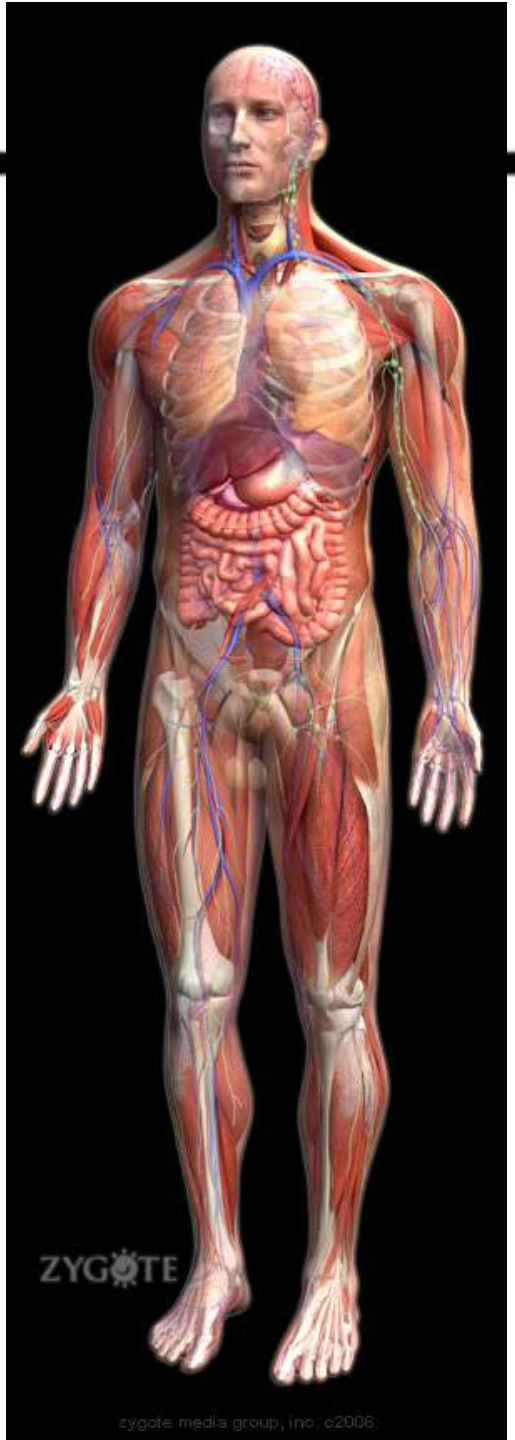
4. Exploitation of environmental conditions, namely enzyme switching of inactive molecules into active drugs (ADEPT, GDEPT, PDEPT, ATTEMPTS)

Antibody based delivery systems



$94,000 for an average treatment

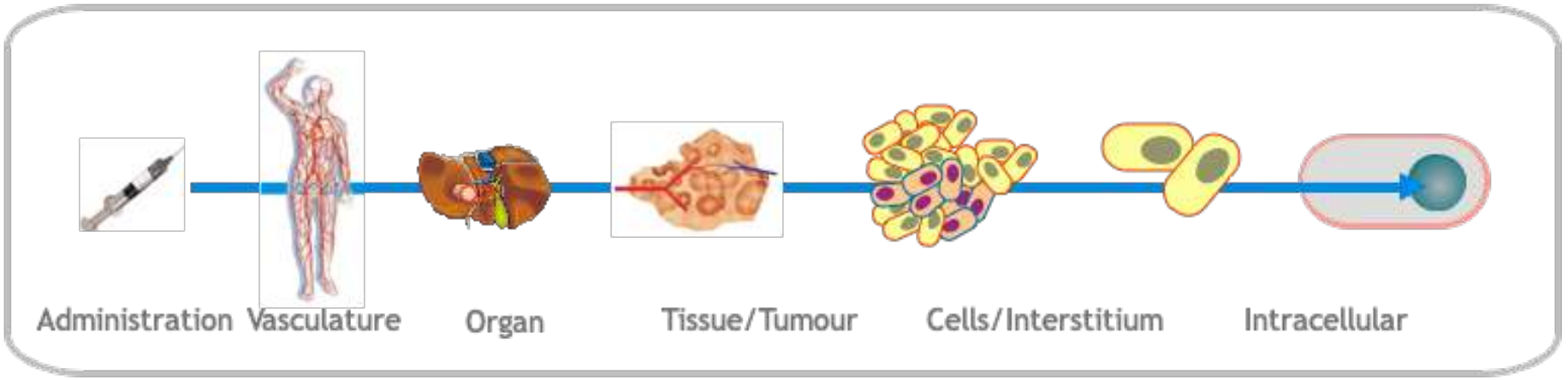




Possible routes of drug entry include:

- Enteral (GI tract)
  - oral (swallow, 50 %)
  - sublingual (under the tongue)
  - rectal
- injected parenteral (20 %)
  - intravenous
  - subcutaneous
  - intramuscular
- Other parenteral
  - inhalation (20 %)
  - transdermal

Systemic Therapeutics: Barriers



Pharmacokinetics – what the body does to the drug.

TAU

For clinical purposes it is generally accepted that a dynamic equilibrium exists between the concentration of drug at the site of action and the concentration of the drug in blood plasma.

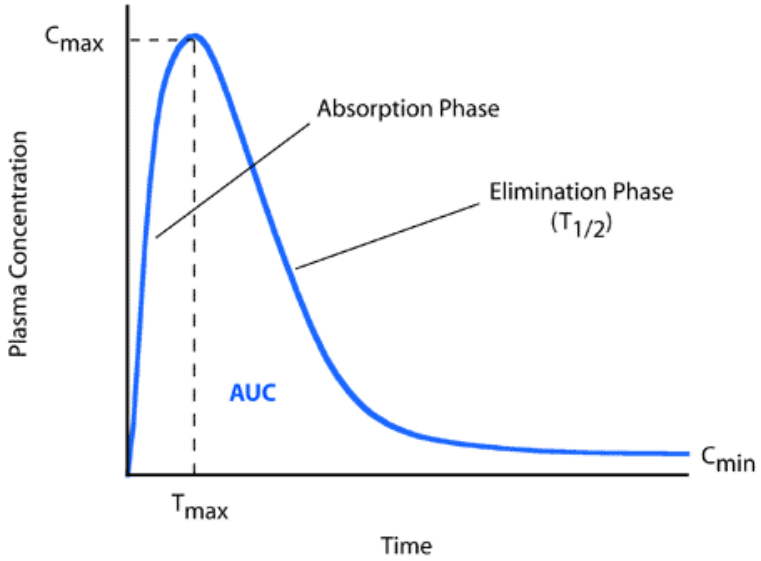
Plasma concentration is an **indirect indicator** of concentration at site of action.

Absorption

Distribution

Metabolism

Elimination



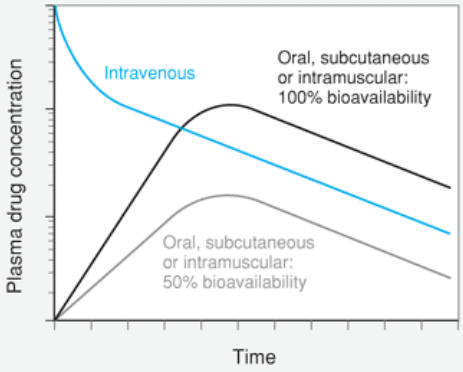
The Concentration-Time plot is the classic PK curve

AUC (Area Under the Curve) – total amount of drug passing through the body

Maximize benefit to the body by controlling the concentration-time plot.
Drug delivery – the right amount in the right place at the right time

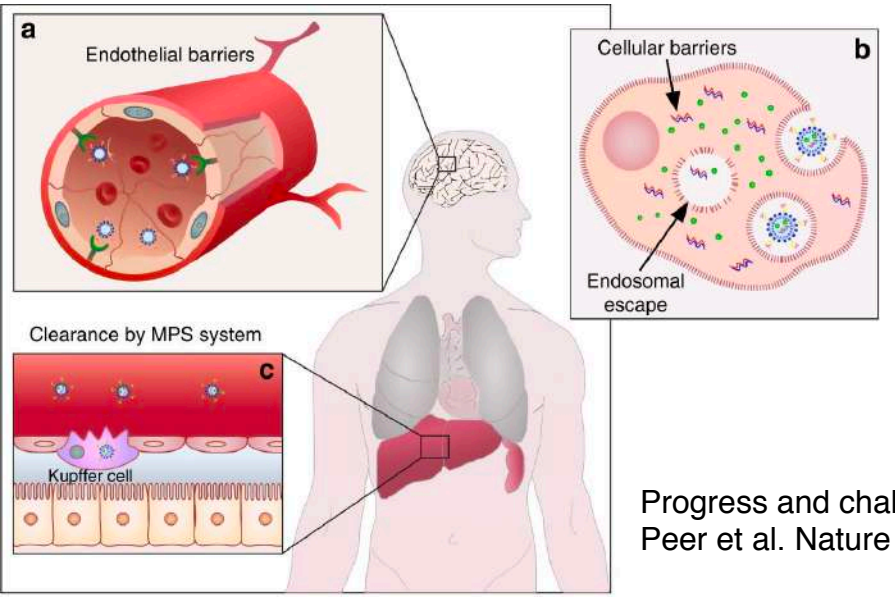
Bioavailability – how much drug gets into the body?

$$\textbf{Bioavailability} = \frac{\text{Quantity of drug reaching systemic circulation}}{\text{Quantity of drug administered}} = \frac{AUC_{route}}{AUC_{IV}}$$



- An IV administered drug is immediately available in the circulation.
- Other routes of administration (*e.g.* oral) demonstrate slower entry of drug into the blood.
- 100 % bioavailability: the total amount of drug reaching the systemic circulation will be the same for all routes of drug administration
- Non-intravenous routes will require a longer period of time to reach a maximal concentration of drug in the plasma.
- Branded versus generic drugs may be different.
- **Use Bioavailability to monitor the effectiveness of drug delivery.**

Physiological barriers to drug delivery



- The body does not know that a drug is there to do good. It sees it as a XENOBIOTIC and will try to protect itself against the drug.
- A drug must overcome physical, chemical, and biological barriers to reach its molecular and cellular sites of action.
- Most drugs must distribute from the blood into local tissues, a process that may be impeded by structures such as the blood–brain barrier.

Progress and challenges towards targeted delivery of cancer therapeutics
Peer et al. Nature Communications (2018)

What choices does a drug have when crossing a biological barrier?

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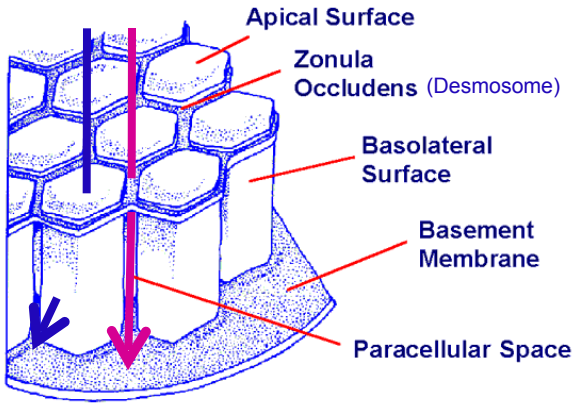
➢**Epithelium** – tissue lining the cavities and surfaces of the body – consist of one or more layers of cells and intercellular material.

➢Drug absorption is controlled by the epithelial layer – the presence of various types of cell junctions means that neighbouring cells are sealed together to form a continuous layer of cells.

➢ This aggregate of cell membranes and intercellular spaces serve as a macroscopic membrane and acts as a barrier to drug absorption.

Trans

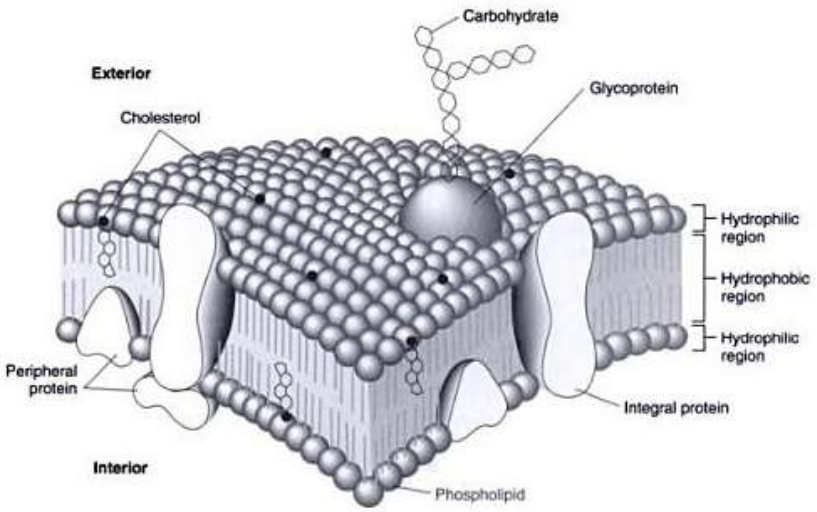


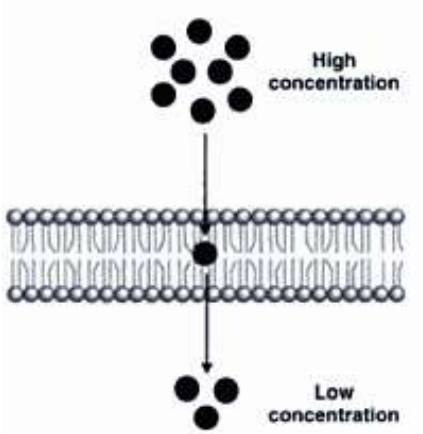
Para

Transcellular – ACROSS - the drug is taken up and then released by the cells in a two stage process working its way through the barrier – IMPORTANT route for the majority of drugs - intestinal epithelium is the major barrier for orally administered drugs.

Paracellular - BETWEEN - the drug diffuses through the tight aqueous junctions and pores between the cells – important for very hydrophilic molecules such as mannitol

Getting into and out of cells - the lipid bilayer



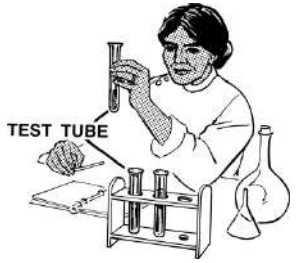


- The main function of lipid bilayer is to contain the aqueous contents cells and separate them from the aqueous exterior
- These membranes have specialized transport systems to assist the passage of nutrients/waste products/ions
- **Lipophilic molecules tend to penetrate membranes more rapidly because the cell membrane is lipid in nature**
- **They diffuse across the membrane by passive diffusion - natural tendency for molecules to move from regions of high concentration to low concentration – caused by the molecules' kinetic energy**

Lipophilic substances are attracted to, and dissolve well, in lipids/non-polar solvents (OILY).

Hydrophilic substances are attracted to, and dissolve well, in water/polar solvents (WATERY).

How do we measure a drug's lipophilicity?



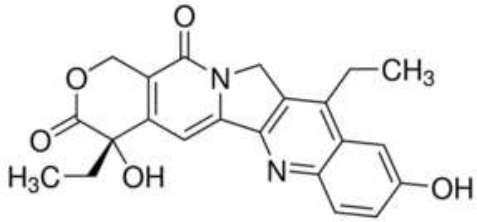
➢We can simulate the biological membrane by shaking the drug with an immiscible mixture of a lipophilic oily solvent (octanol, the lipid bilayer) and a hydrophilic solvent (water, the extra/intra-cellular fluid).

➢The partitioning between the two layers can then be calculated.

Partition Coefficient, P = [Oil] / [Aqueous]

And it is normally expressed as a log:

Log P= $\log_{10}$ (Partition Coefficient)



SN-38 **LogP** octanol/water = 2.67
[oil]/[water] = 2670

The higher the Log P value the more lipophilic the drug *i.e.* more of the drug dissolves in the oil.

Most drugs ionise and, in these cases, the pH of the medium that the drug is in will have a profound effect on the partition coefficient.

Log P

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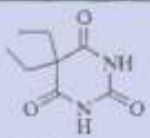
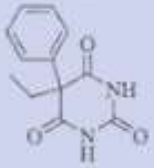
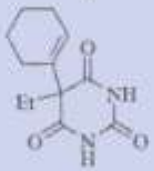
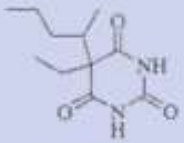
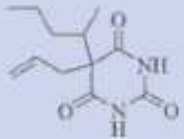
➢Values of Log P that which are too high (> 6) or too low (<-2) are associated with poor transport characteristics

➢ Very high Log P – poor aqueous solubility – remain in lipidic membrane

➢ Very low Lop P – not sufficiently lipophilic to pass through lipid membrane

Barbiturate	Structure	Partition Coeff.	% Absorbed From rat colon
Barbital		0.7	12
Phenobarbital		4.8	20
Cyclobarbital		13.9	24
Pentobarbital		28	30
Secobarbital		50.7	40

Log P

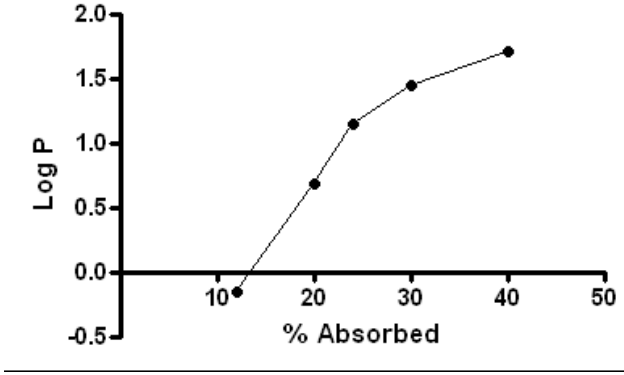
-0.15

0.68

1.14

1.44

1.71



Most drugs are acids or bases - pH has a profound effect on Log P (Log D)

For an acid that dissociates /ionizes:

$$HA \rightleftharpoons H^+ + A^-$$

The equilibrium can be driven to the right by an increase in pH. Think of it as adding OH^- to mop up the H^+ ions.

The dissociation constant, $pK_a = -\log([A^-][H^+]/[HA])$

a **pK_a <2** means that it is a **strong acid** (highly ionised)
a **pK_a >2** but <7 indicates a **weak acid** (not fully ionised)

A pK_a can be expressed similarly for protonated bases:

$$HB^+ \rightleftharpoons H^+ + B$$

So how long does it take?

❖ *Intravenous*	*< 1 minute*
❖ *Inhalation*	*2-3 minutes*
❖ *Sublingual*	*3-5 minutes*
❖ *Intramuscular*	*10-20 minutes*
❖ *Subcutaneous*	*15-30 minutes*
❖ *Rectal*	*5-30 minutes*
❖ *Oral*	*30-90 minutes*
❖ *Transdermal (topical)*	*Variable (minutes to hours)*

So what are the "demands" to become an oral available drug molecule?

Lipinski's rule of five

- No more than 5 hydrogen bond donors (the total number of nitrogen–hydrogen and oxygen–hydrogen bonds)
- No more than 10 hydrogen bond acceptors (all nitrogen or oxygen atoms)
- A molecular mass less than 500 daltons
- An octanol-water partition coefficient[6] (log *P*) that does not exceed 5

Christopher A. Lipinski

Pfizer

So why do we need drug delivery systems?

Common concerns about small drug molecules:

- Poor solubility
- Get cleared quickly
- Degradation
- Non-specific

Potential solution - make better drugs

So why do we need drug delivery systems?

TAU

Common concerns about small drug
mo
- F
- C
- D
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The challenge - small changes in the drug molecule can strongly affect its activity

So why do we need drug delivery systems?

Common concerns about small drug molecules:

- Poor solubility
- Get cleared quickly
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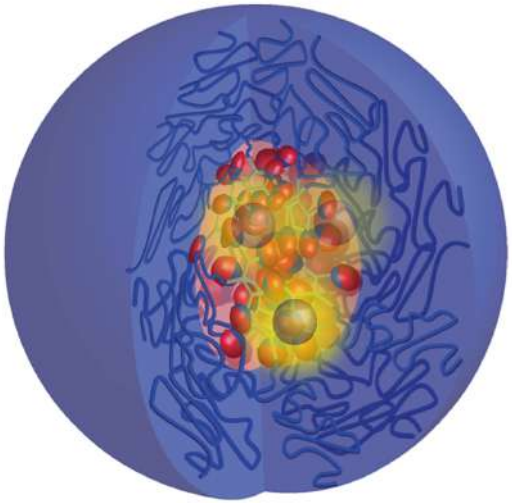


Benefits of delivery platforms:

- Improved solubility = loading capacity
- Long circulation times = structural stability
- Protects drugs from degradation = cargo stability
- Selective and efficient release = response mechanism
- Can be targeted = passive or active targeting

Delivery systems are required not only for medicine

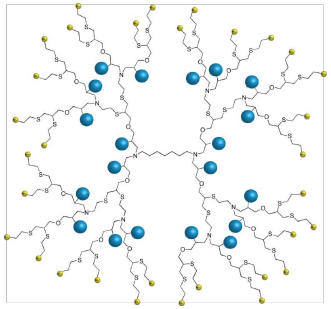
Common concerns about pesticides:

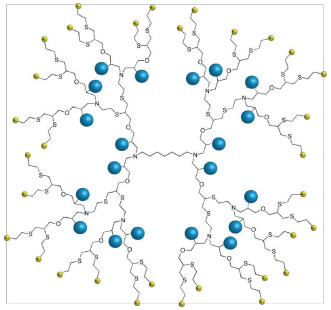
- Poor solubility
- Degradation
- Loss of activity
- Get washed quickly

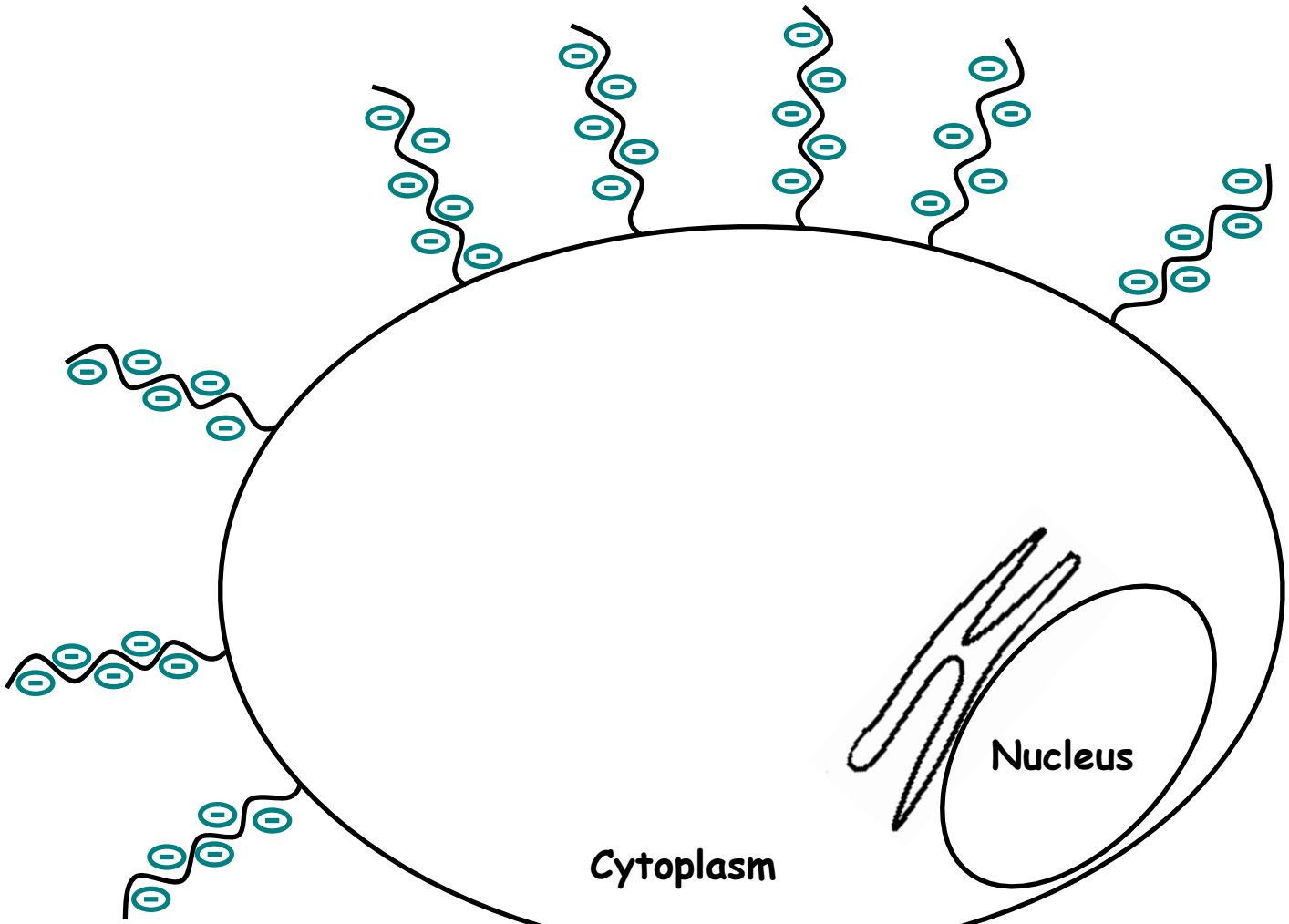
Increased amounts of applied pesticides leads to increase in both costs and danger to the environment

Polymeric delivery platforms can help to overcome these challenges

1st Requirement from Delivery Platforms: stability



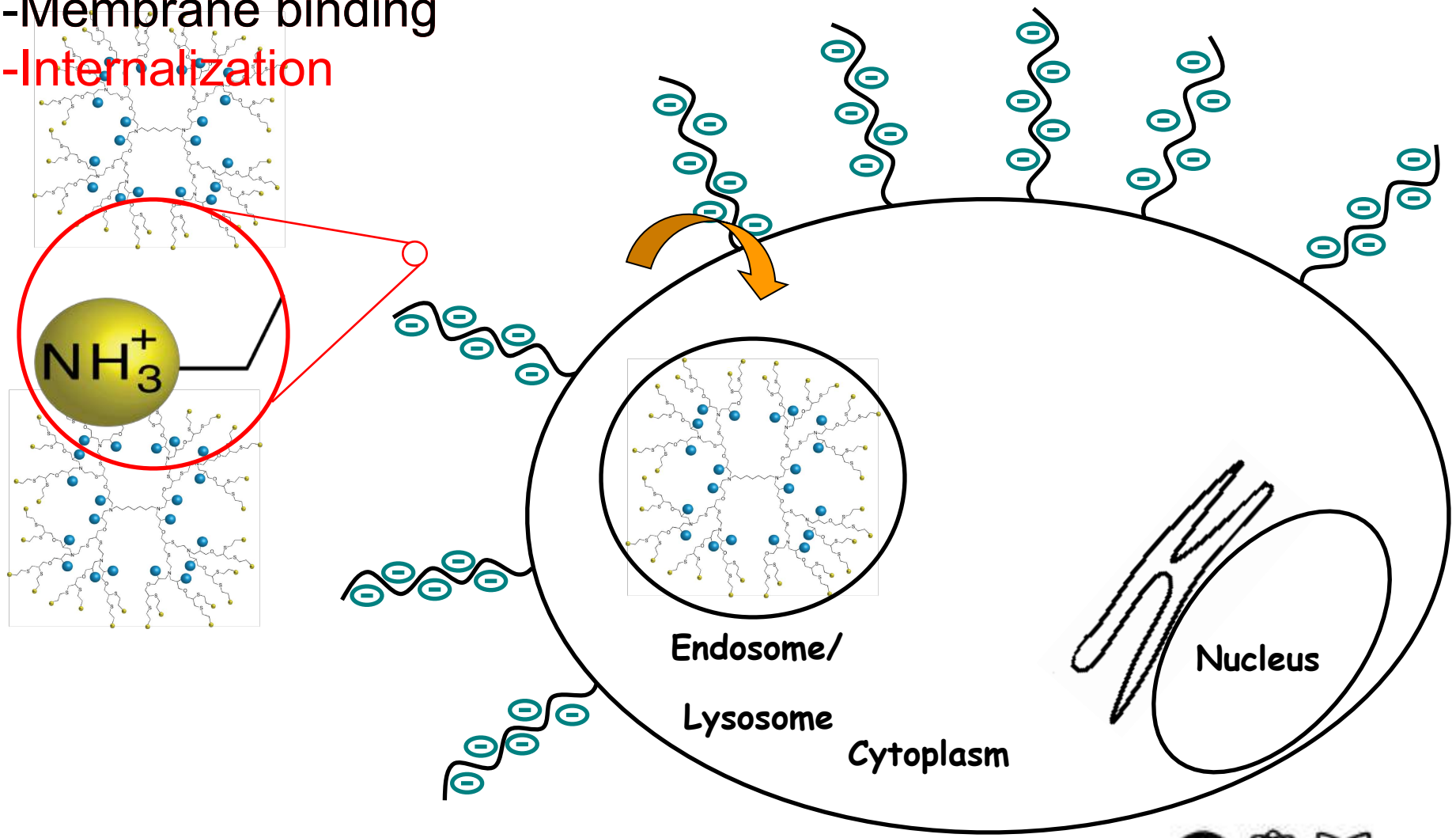




Key Requirements from Delivery Platforms

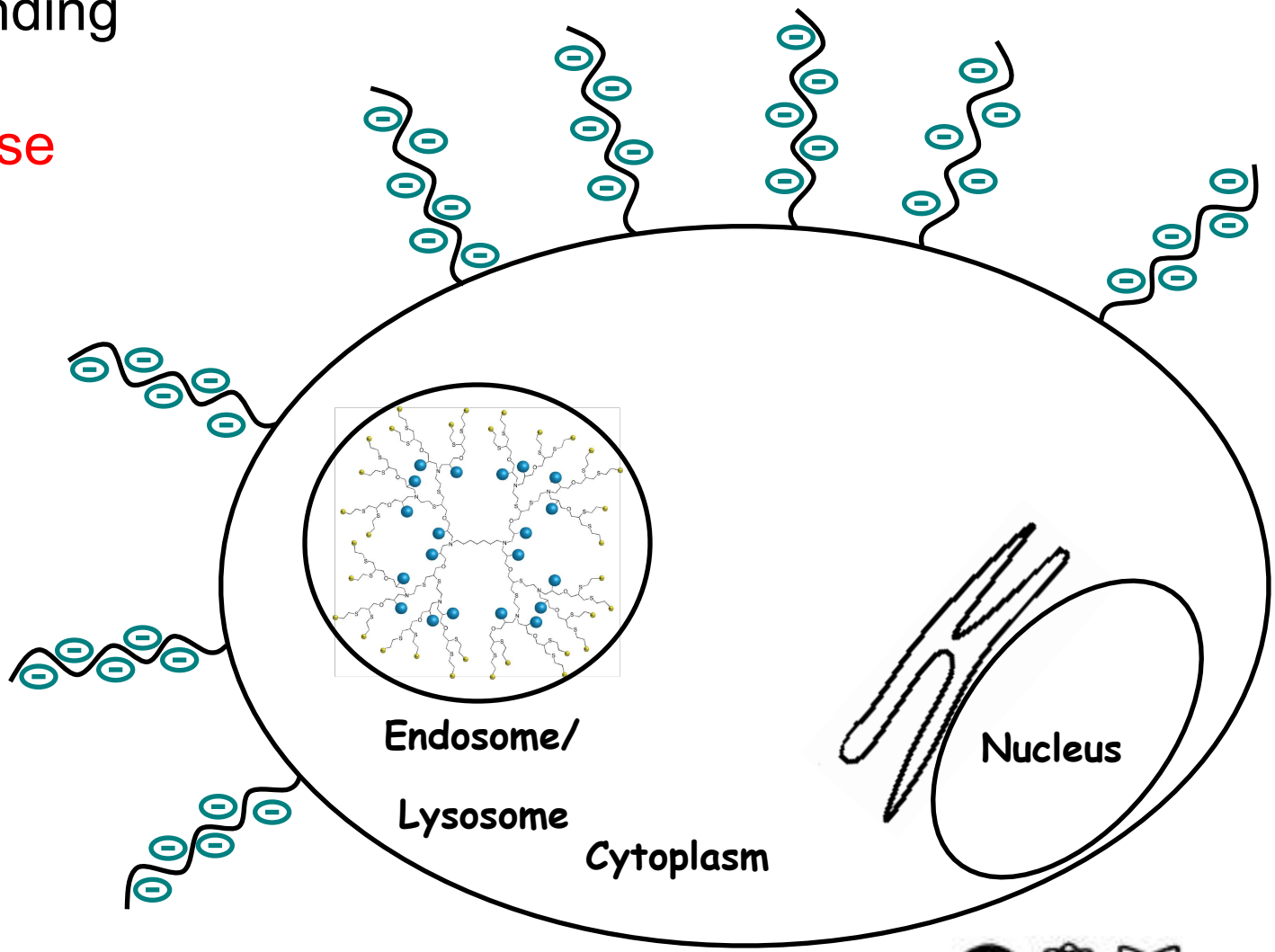
-Membrane binding

-Internalization



Key Requirements from Delivery Platforms

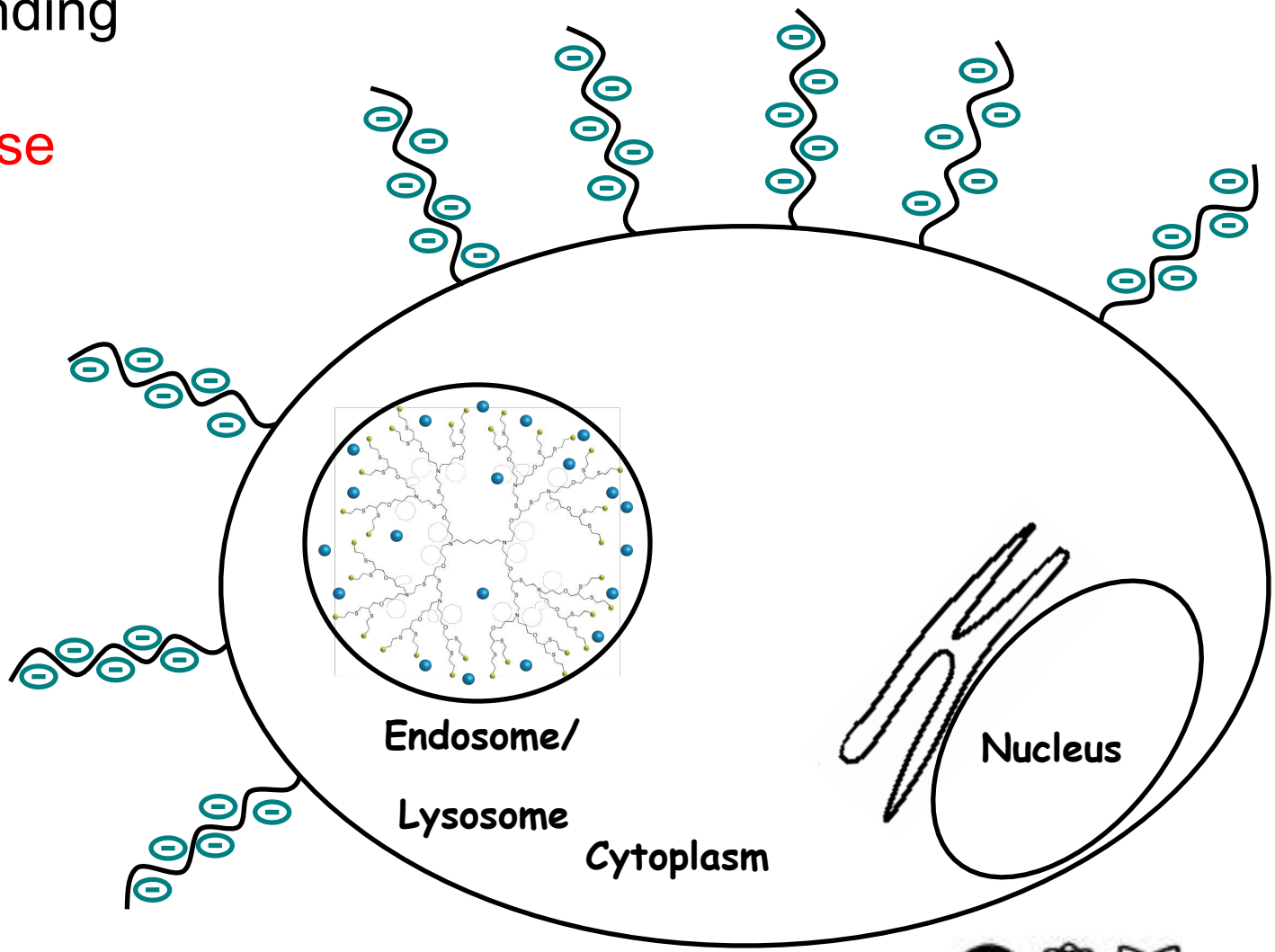
-Membrane binding
-Internalization
-Payload release



Key Requirements from Delivery Platforms

TAU

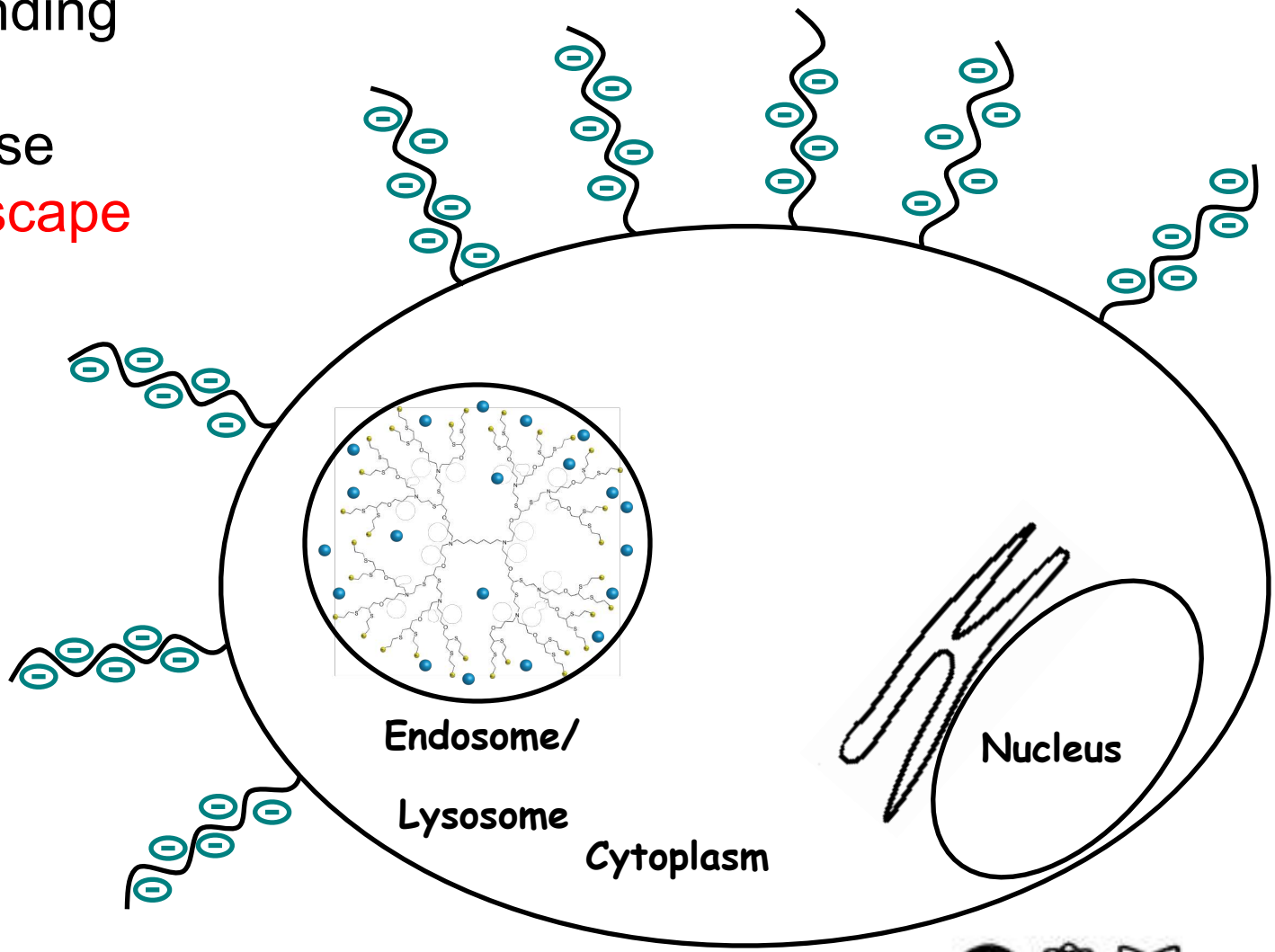
-Membrane binding
-Internalization
-Payload release



Key Requirements from Delivery Platforms

TAU

- Membrane binding
- Internalization
- Payload release
- Endosomal escape (cyto release)



Key Requirements from Delivery Platforms

TAU

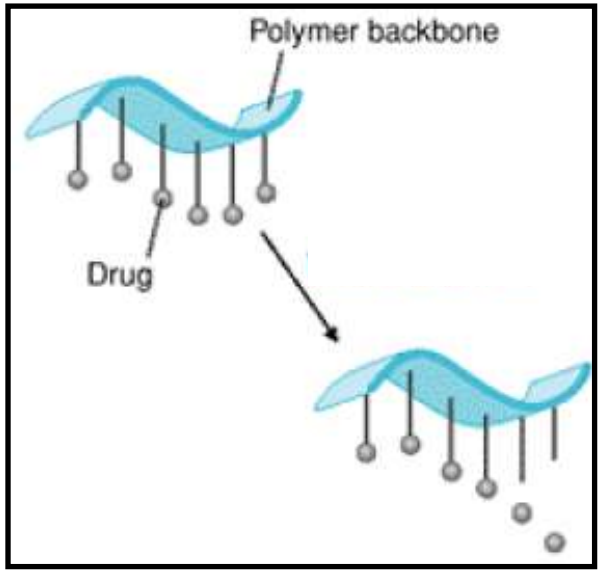
- Me
- Int
- Pa
- En
  (cy

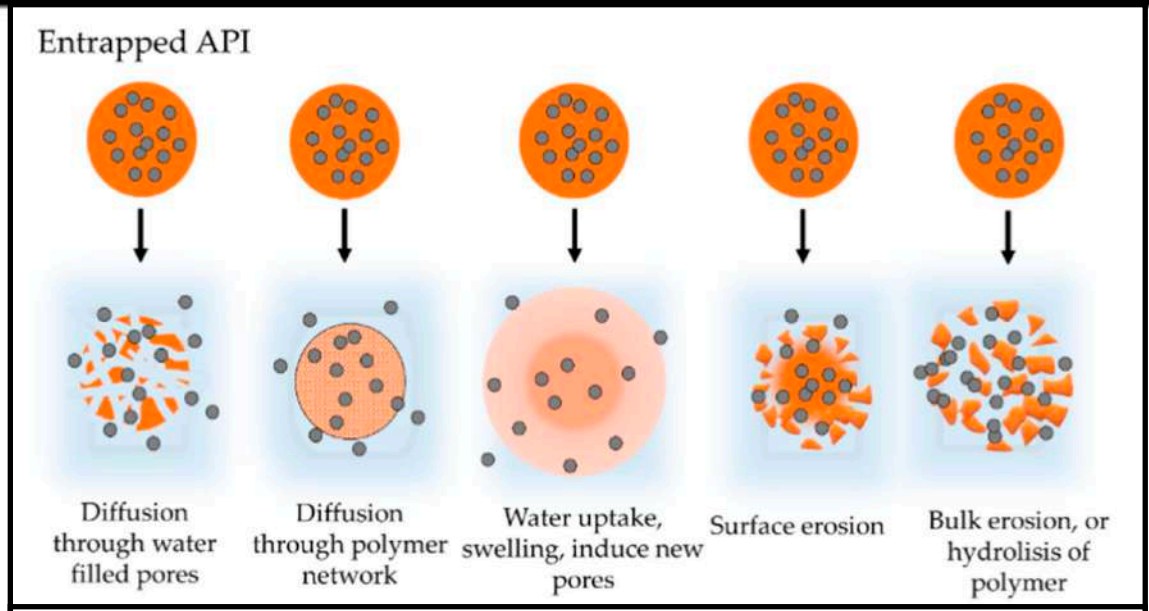
There are many challenges to address- some are similar to the free drug and some are different

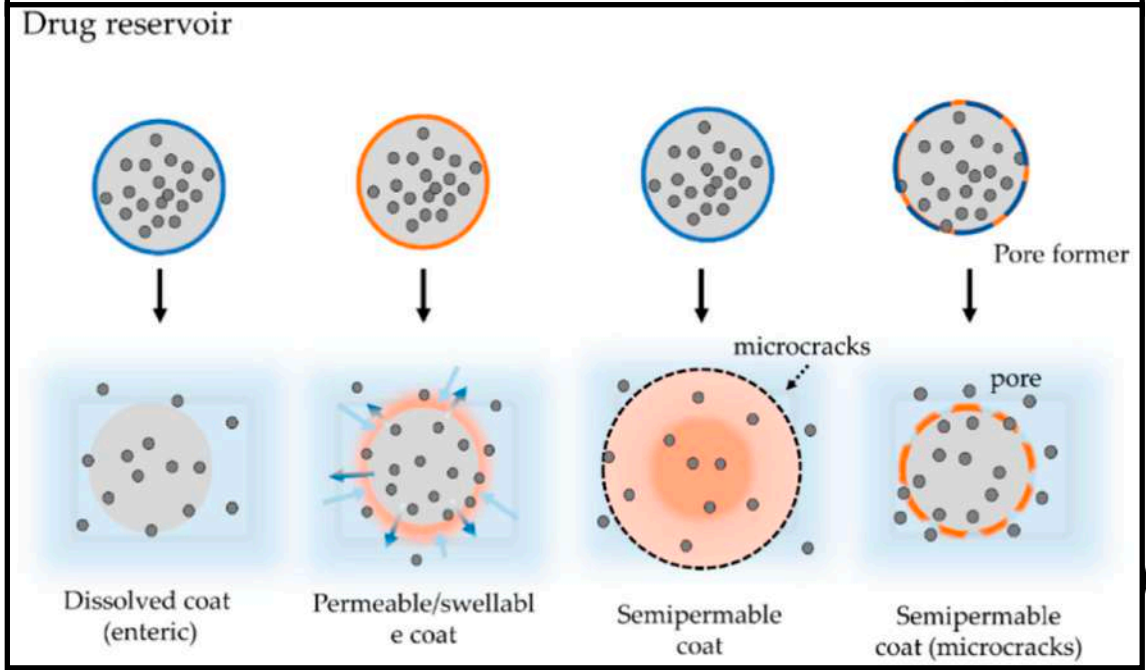
Delivery systems need to be stable and release their cargo

Drugs can be ***covalently*** or ***non-covalently*** bound to the polymeric platform

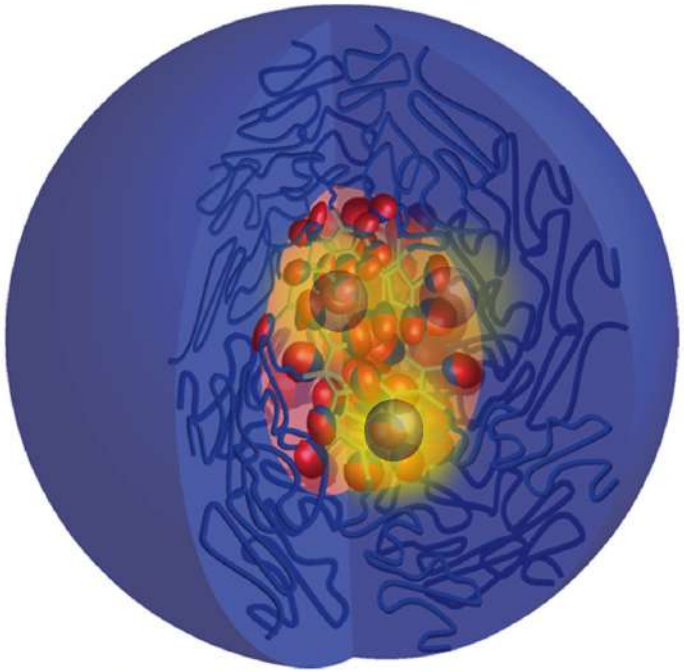
Different release mechanisms can be utilized:







Delivery systems need to be stable and release their cargo



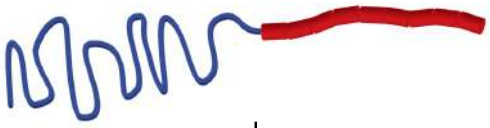
Stability

Targeting?

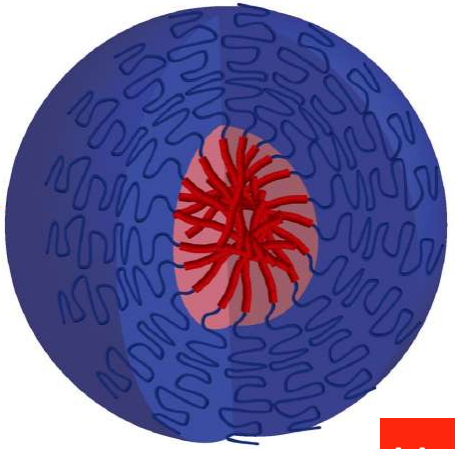
Selective release

Amphiphilic block copolymers self-assemble into nanostructures

Hydrophilic – Hydrophobic



Self assembly

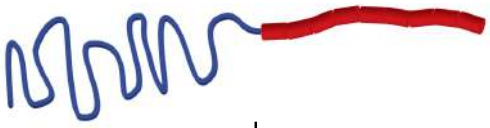


Hydrophilic

Hydrophobic

Smart polymers change their structure upon stimuli

Blue = hydrophilic
Red = hydrophobic

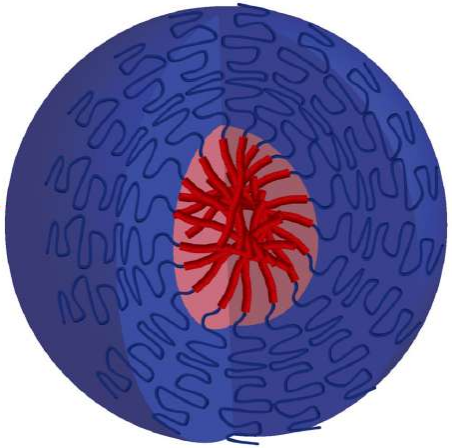


External stimuli

hydrophilic-hydrophilic

Structural change

Self assembly



Thermal Activation

Photochemical Activation

pH Activation

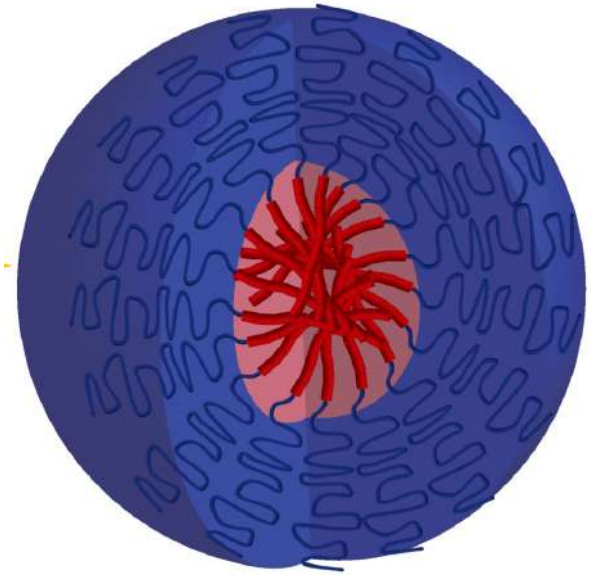
pH Responsive polymers

pH in normal tissues ~7.4

pH in tumor and inflammatory tissues ~6.5-6.9

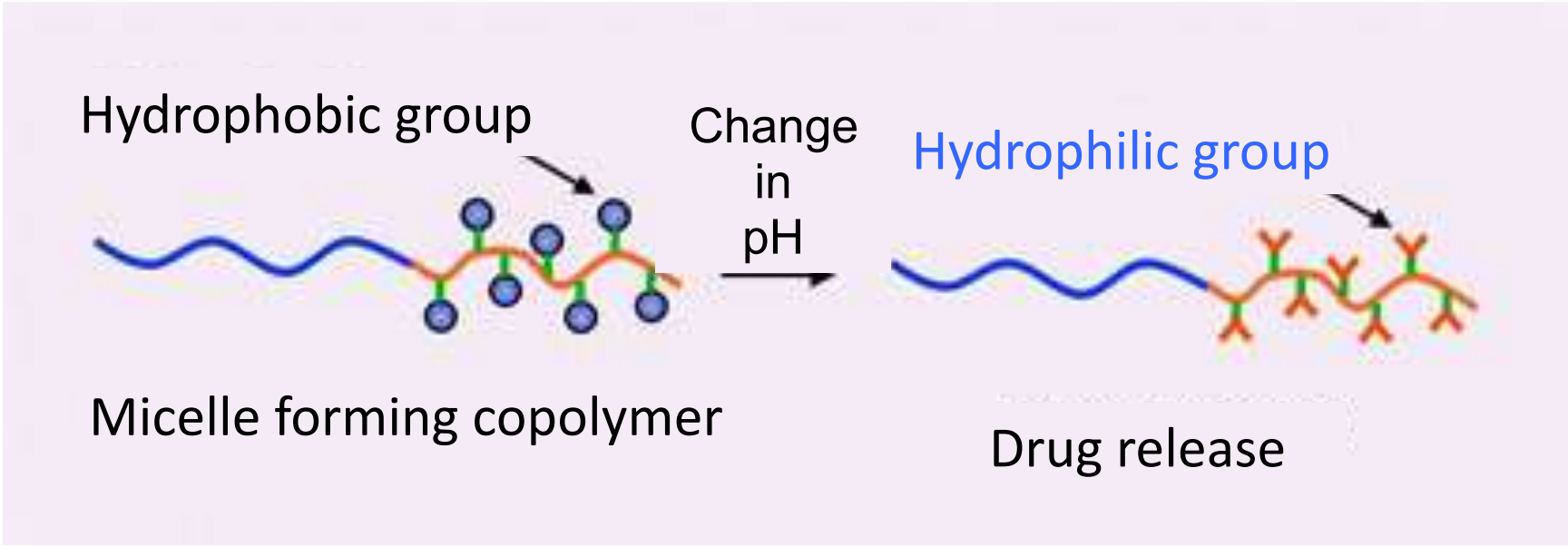
pH in endosomal and lysosomal vesicles 5–6

Hydrophilic

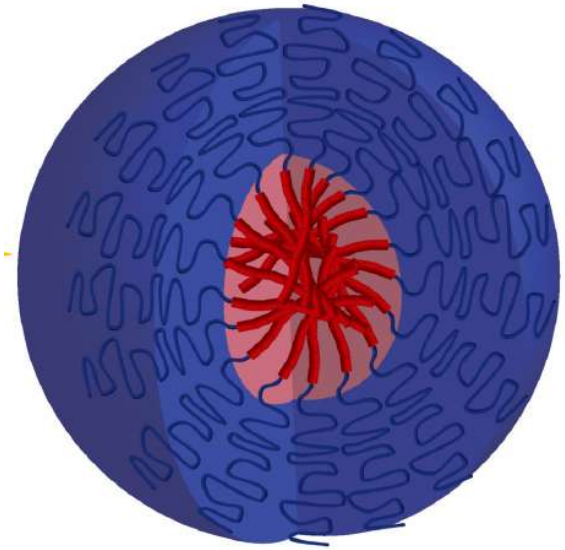


Hydrophobic

Possible responsive groups?

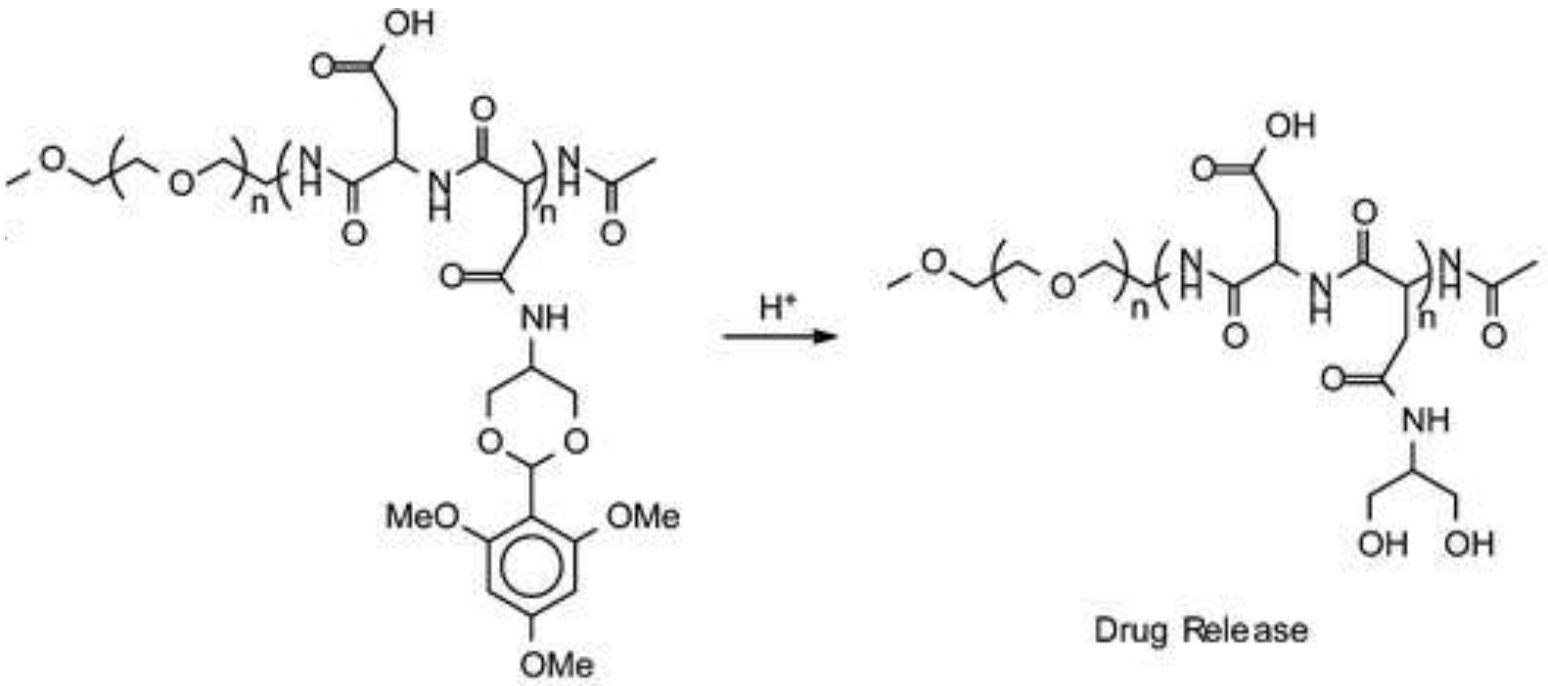


Hydrophilic



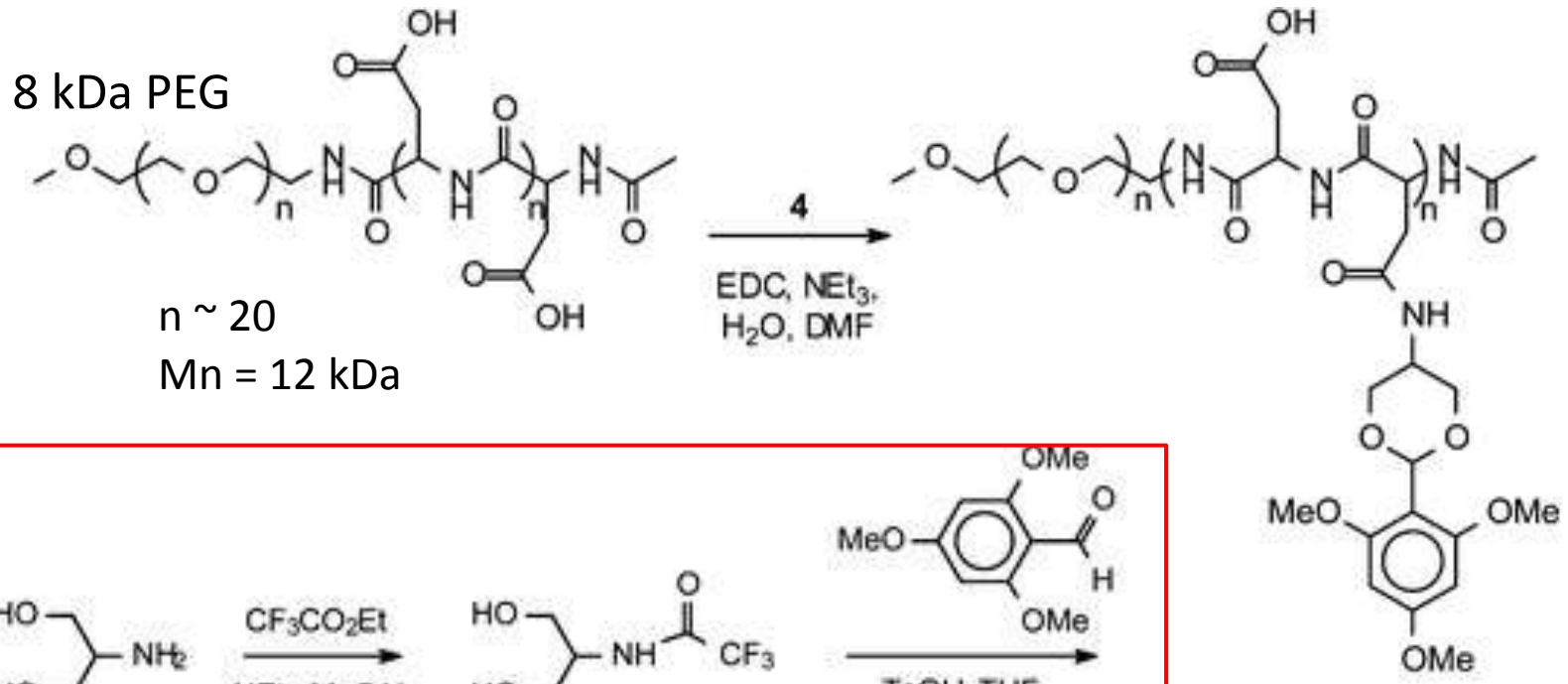
Hydrophobic

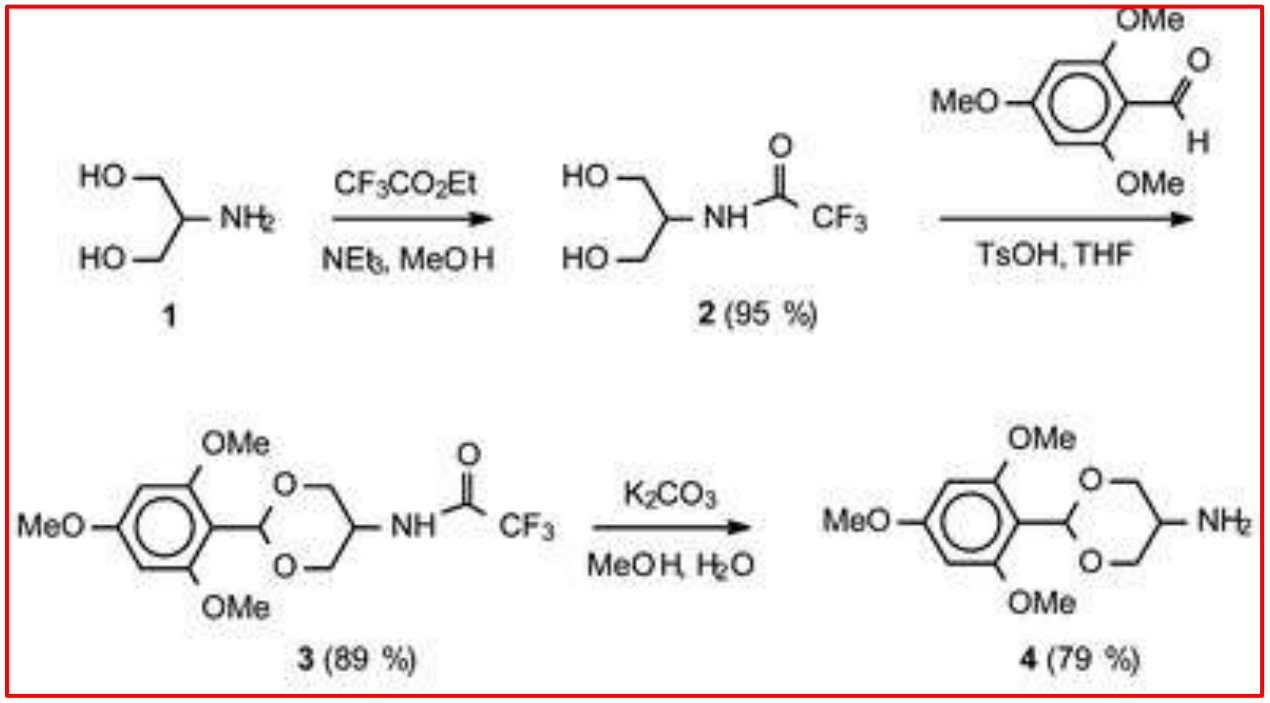
Acetal based pH responsive polymers



pH Responsive polymers:
side chain functionalization

Acetal based pH responsive polymers

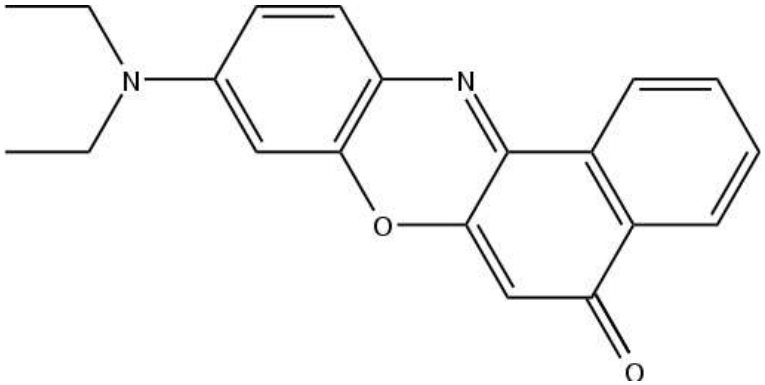


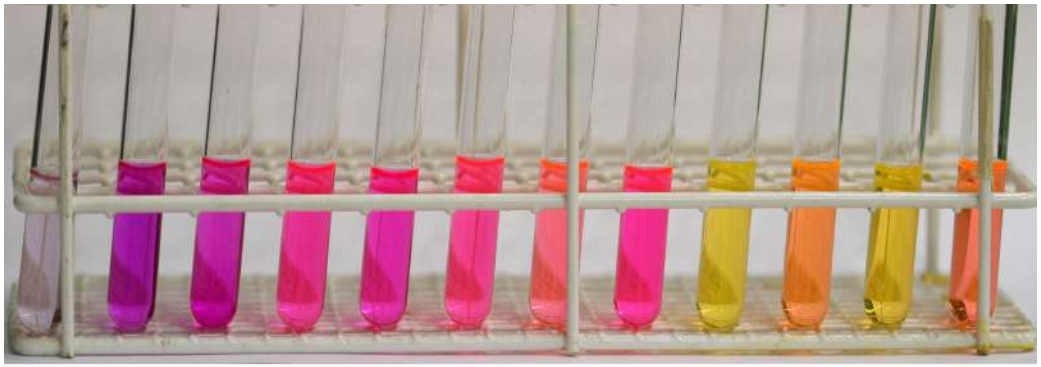


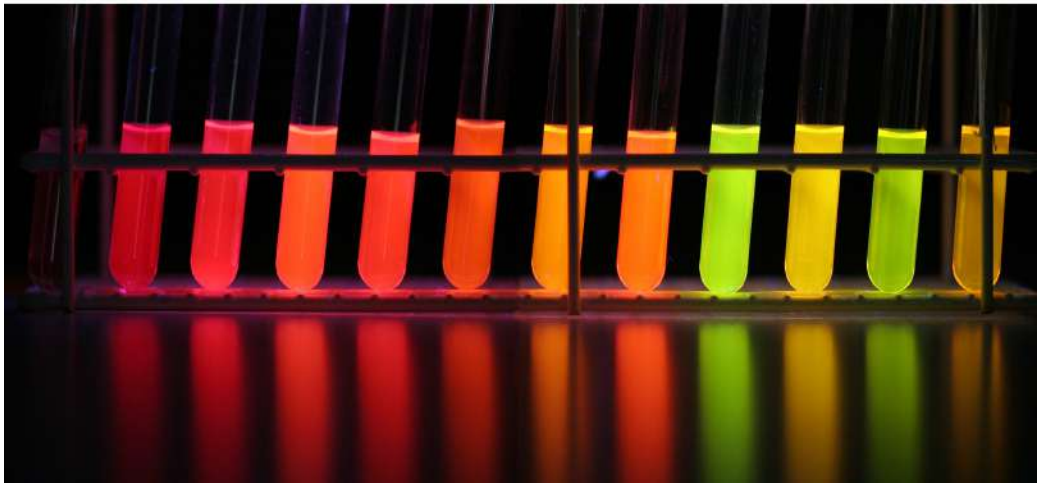
~ 30 % of carboxylic acids were coupled

Nile Red “Turns On” in Hydrophobic Environments

Nile Red

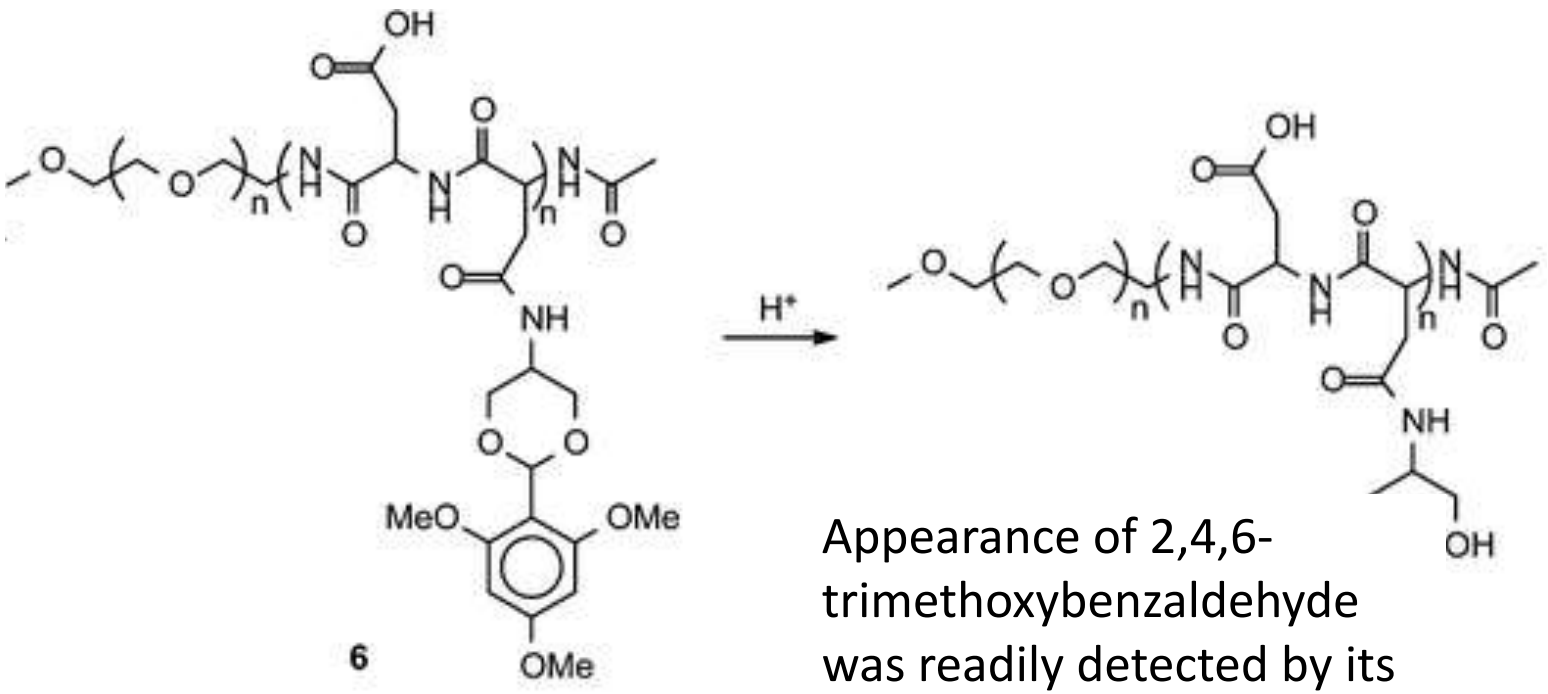






H_2O → Toluene

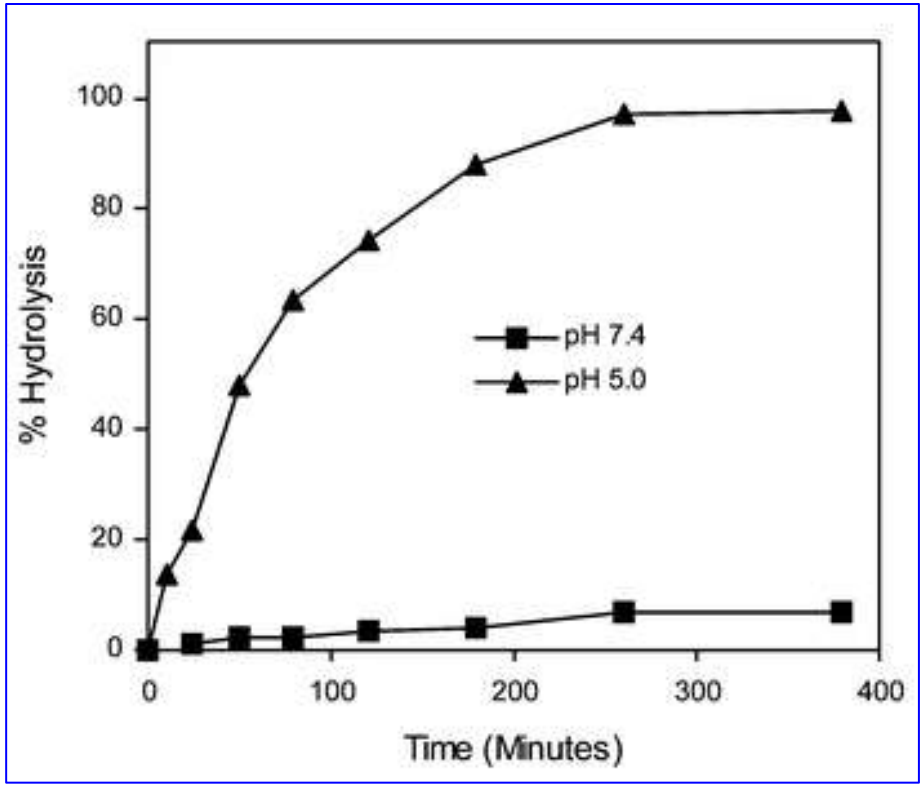
Acetal based pH responsive polymers

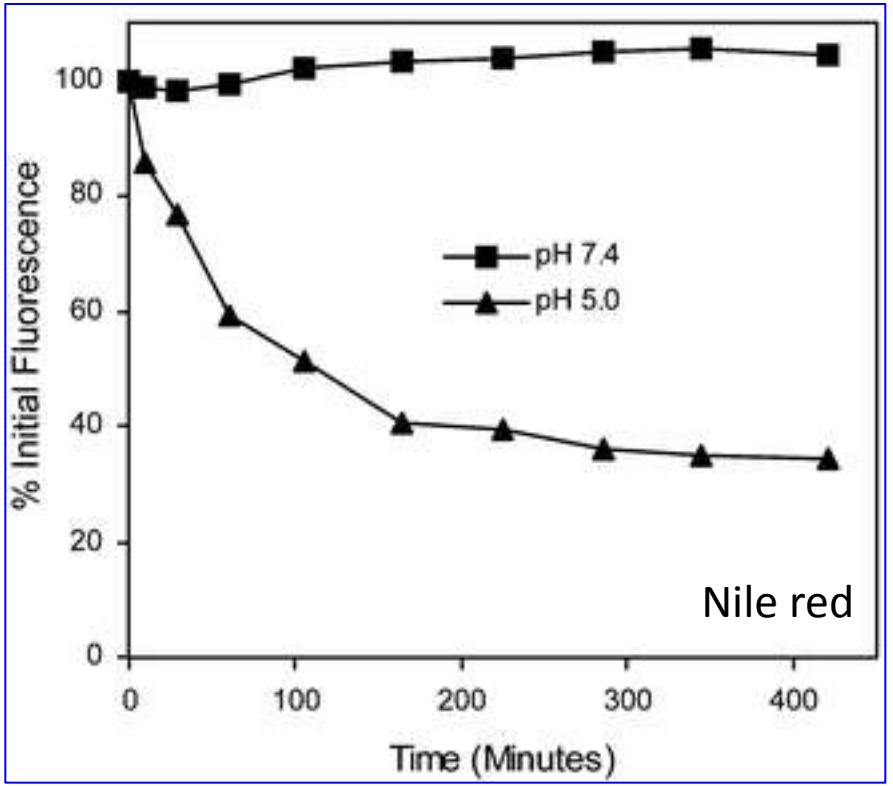


CMC = 0.34 mg mL^{-1}

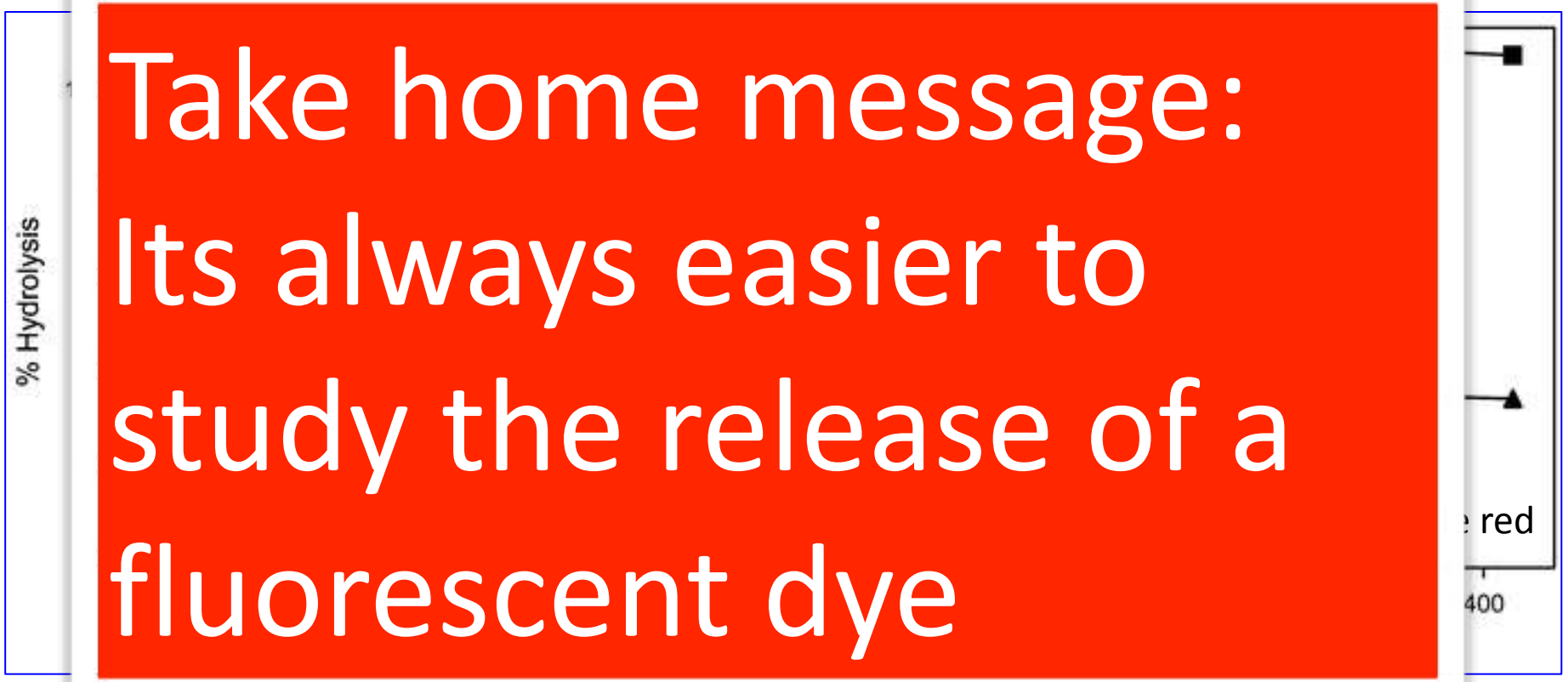
Appearance of 2,4,6-trimethoxybenzaldehyde was readily detected by its absorbance at 292 nm

Acetal based pH responsive polymers



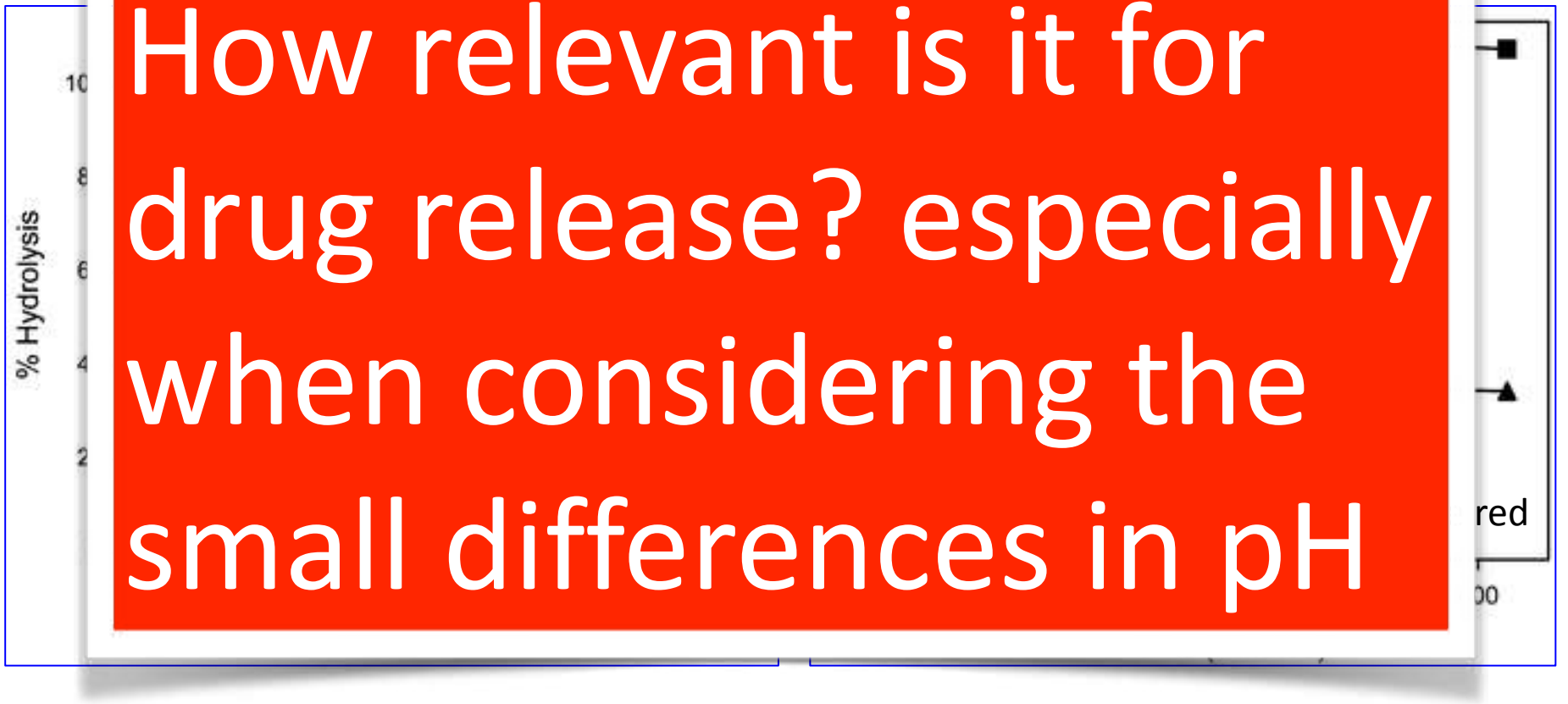


Acetal based pH responsive polymers



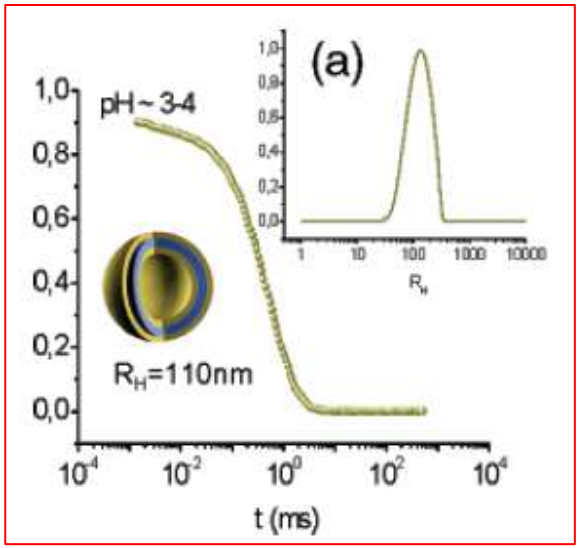
Take home message:
Its always easier to study the release of a fluorescent dye

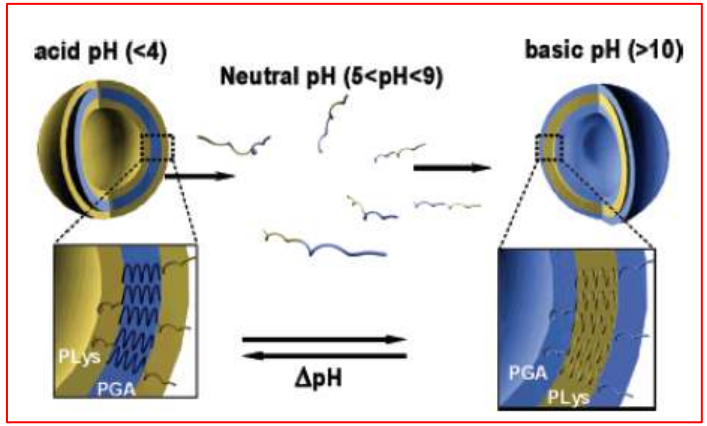
Acetal based pH responsive polymers

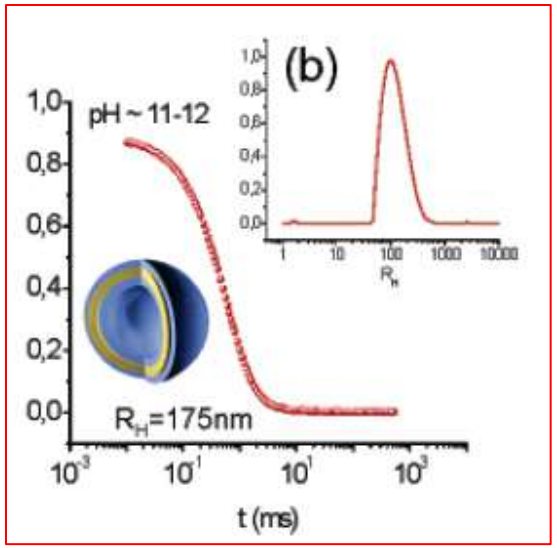


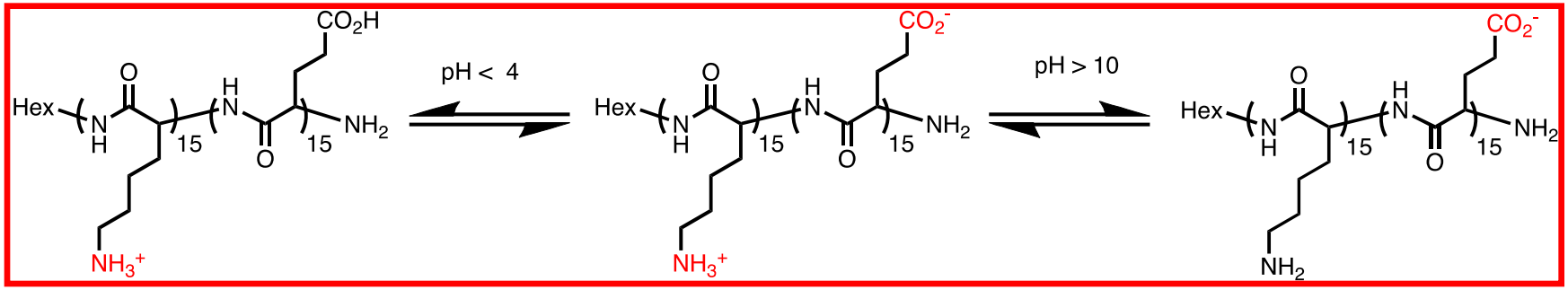
How relevant is it for drug release? especially when considering the small differences in pH

pH Responsive peptide diblock copolymers



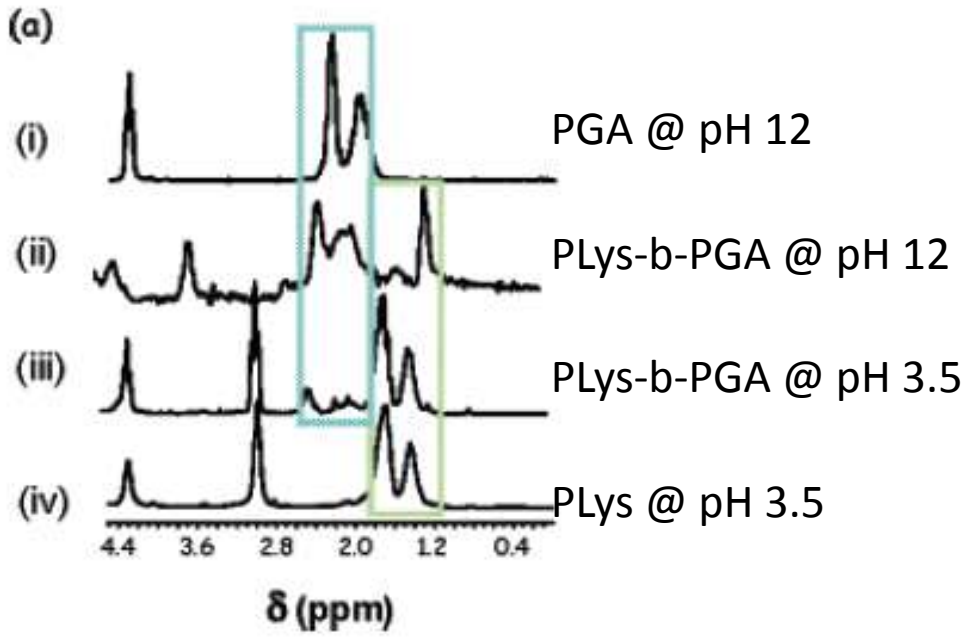






S. Lecommandoux, *JACS*. **2005**, 127, 2026.

pH Responsive peptide diblock copolymers



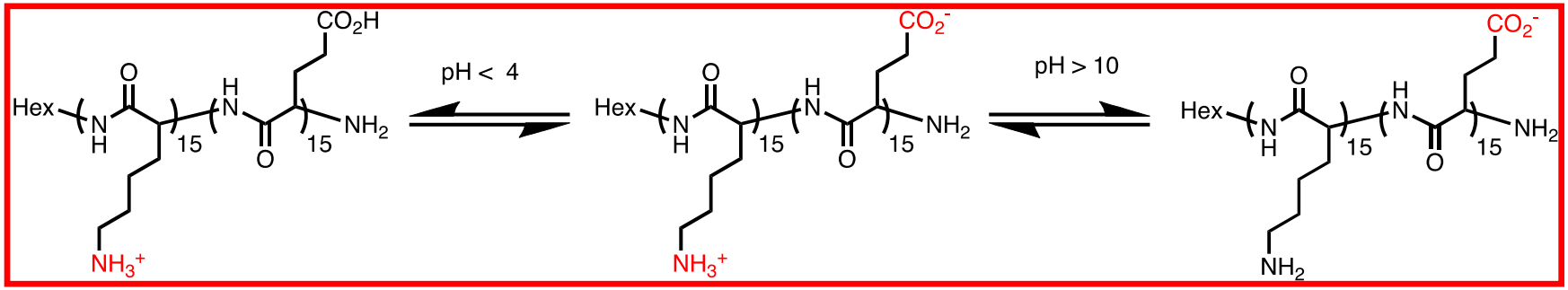
PGA @ pH 12

PLys-b-PGA @ pH 12

PLys-b-PGA @ pH 3.5

PLys @ pH 3.5

Sebastien Lecommandoux
Laboratoire de Chimie des Polymères Organiques (LCPO) @ U Bordeaux, CNRS, and Bordeaux National Polytechnic Institute.



S. Lecommandoux, *JACS*. **2005**, 127, 2026.

pH Responsive peptide diblock copolymers

(a)

(i)

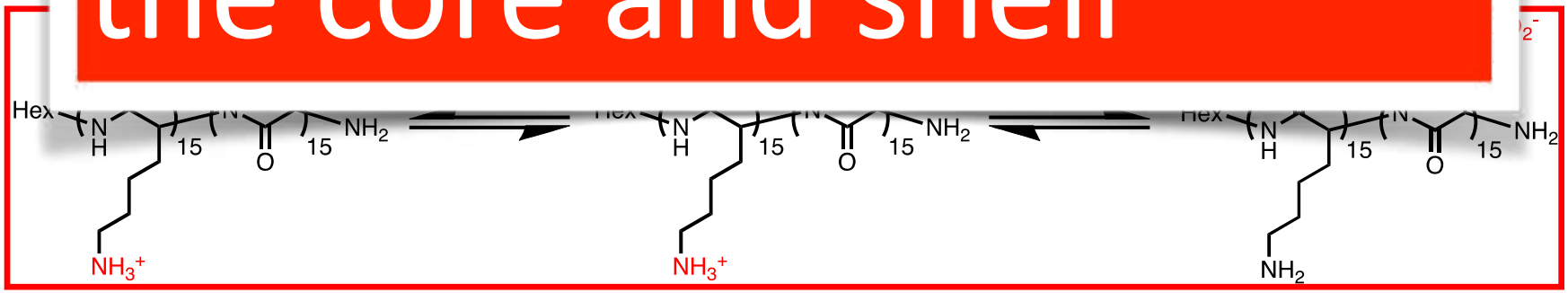
(ii)

(iii

(iv

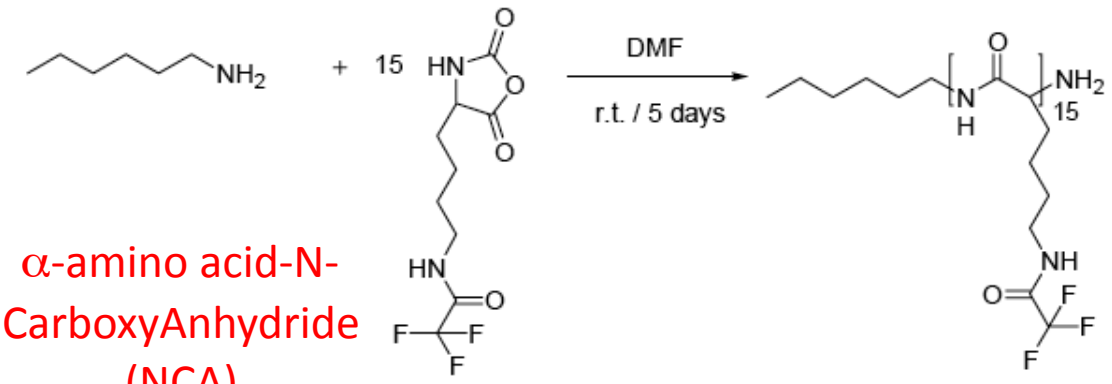
PGA @ pH 12

NMR can be used to distinguish between the core and shell

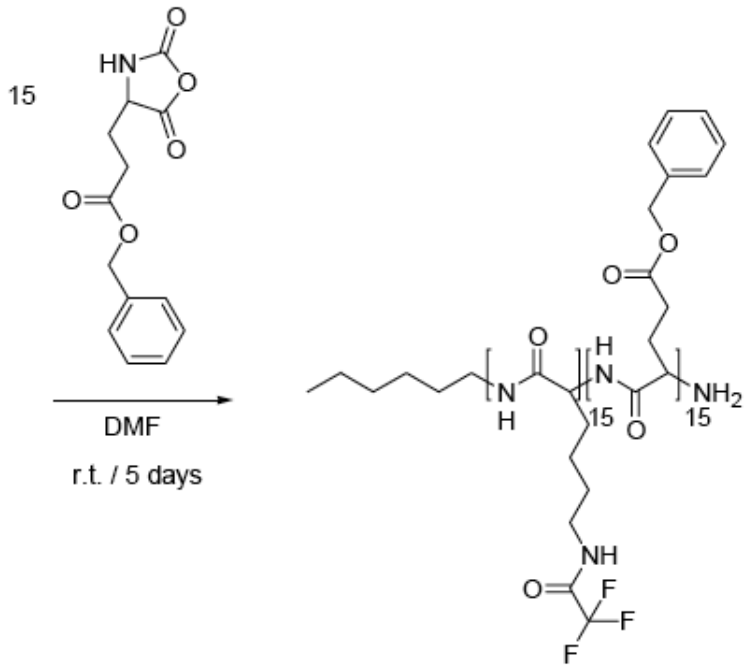


S. Lecommandoux, *JACS*. **2005**, 127, 2026.

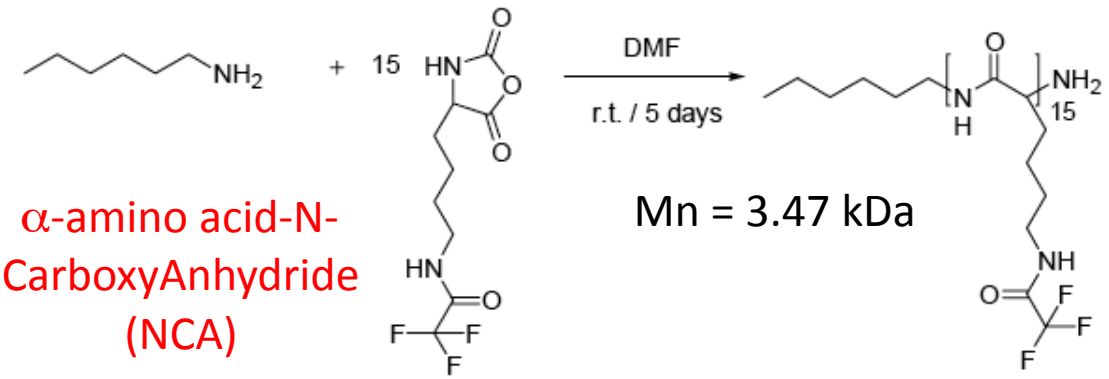
NCA Synthesis of diblock copolypeptides



α-amino acid-N-CarboxyAnhydride (NCA)

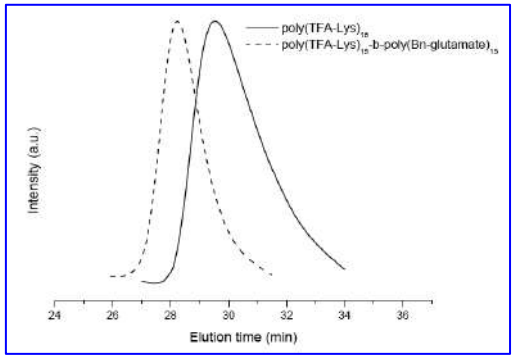


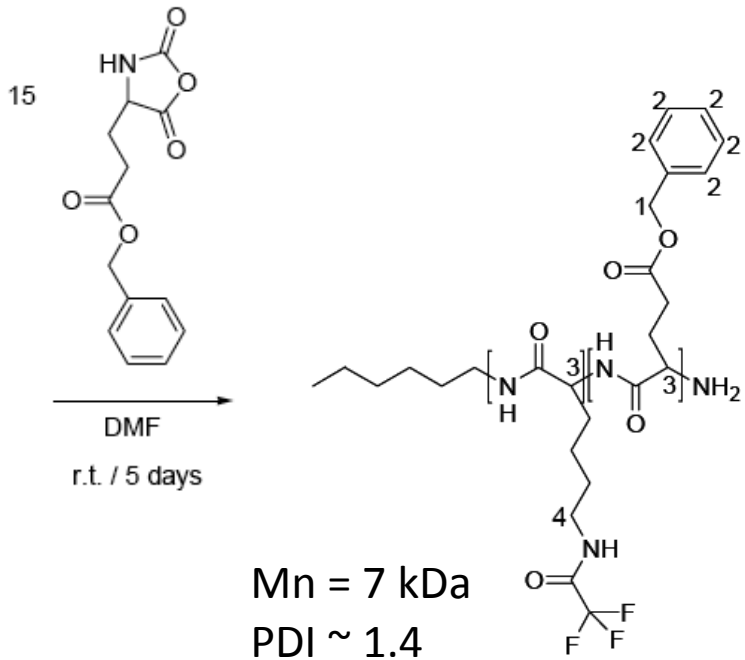
Synthesis of diblock copolypeptides



α-amino acid-N-CarboxyAnhydride (NCA)

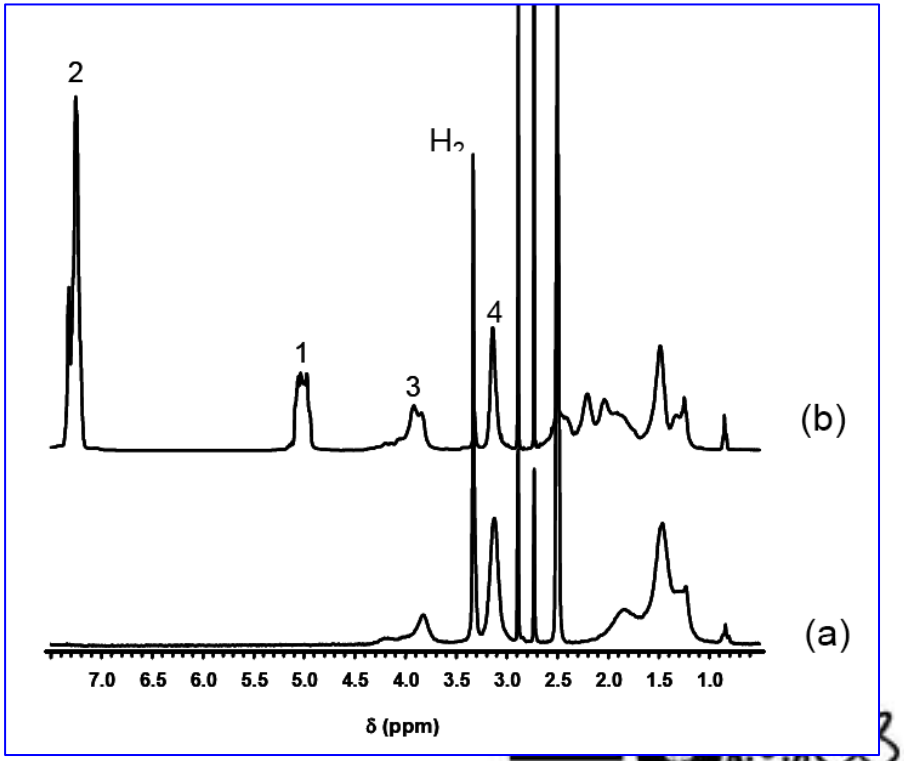
Mn = 3.47 kDa



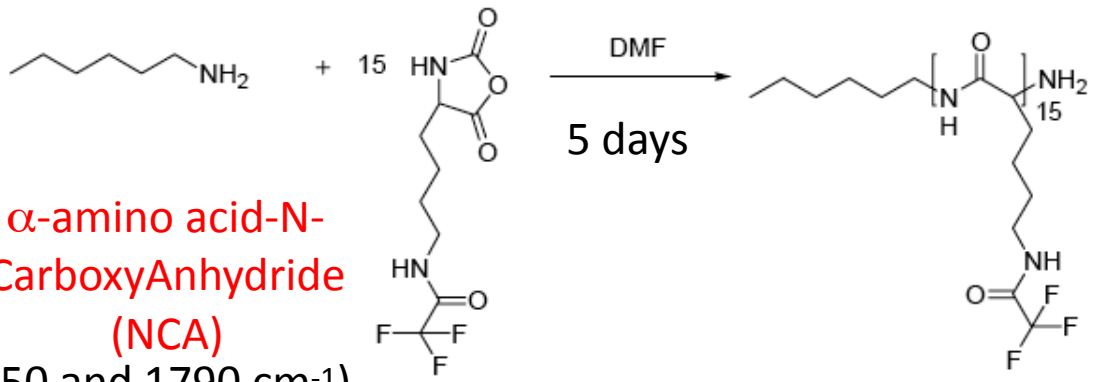


Mn = 7 kDa

PDI ~ 1.4

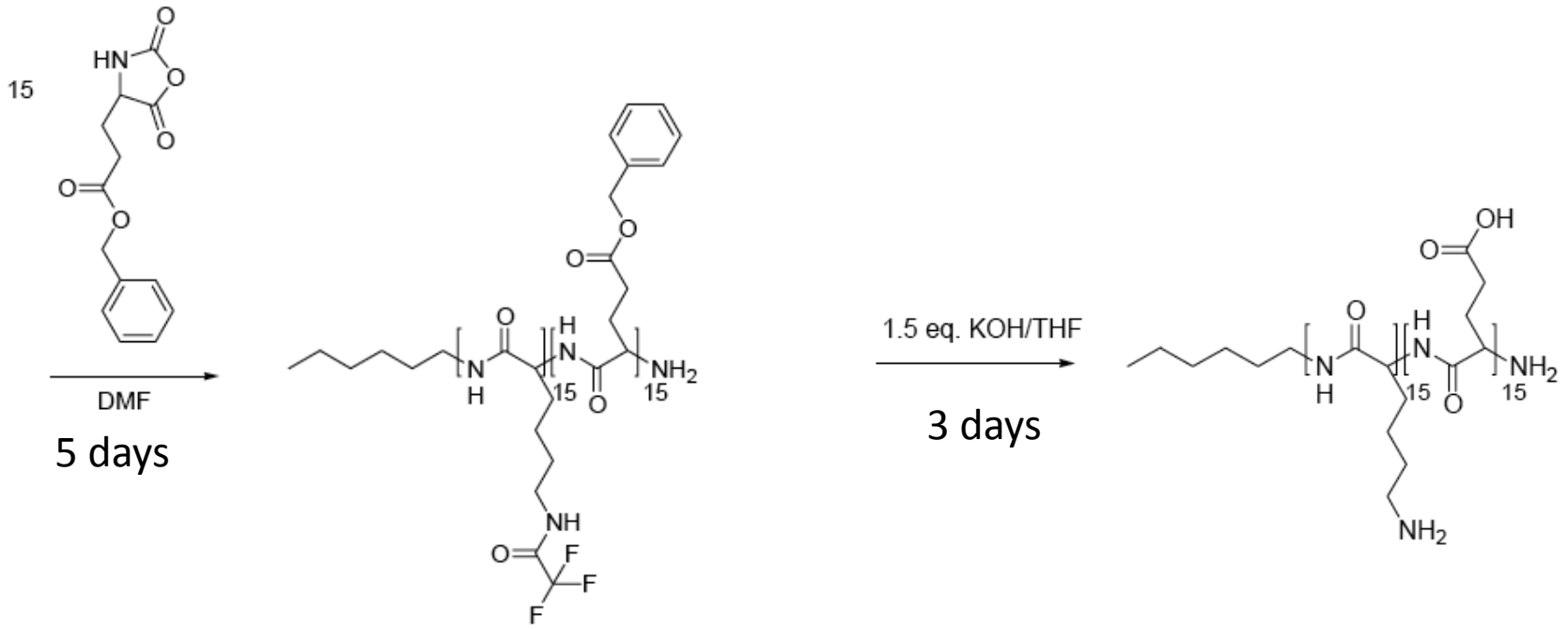


Synthesis of diblock copolypeptides



α-amino acid-N-CarboxyAnhydride (NCA)
(1850 and 1790 cm^{-1})

Relatively long reaction times



NCA polymers

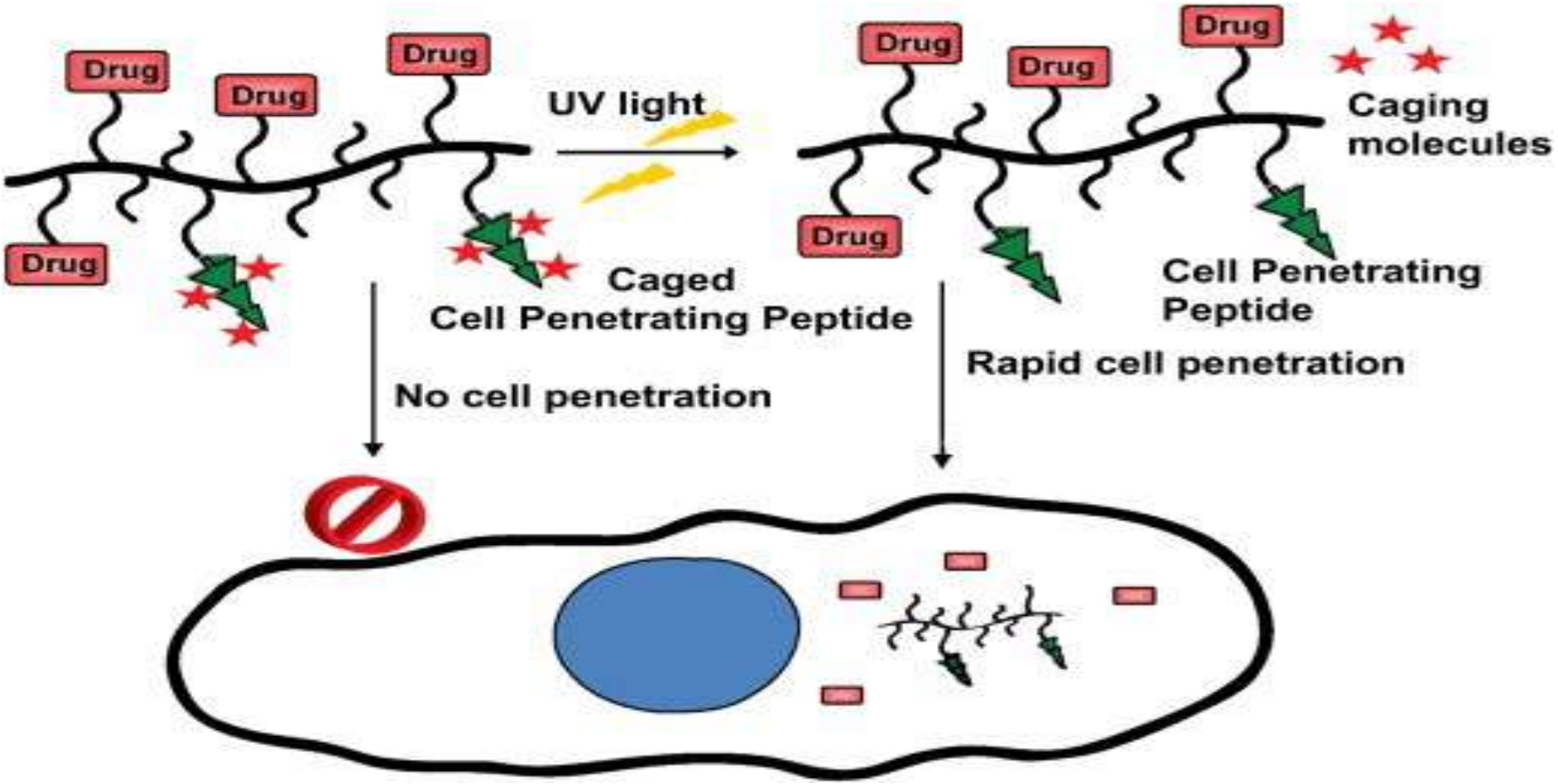
Advantages:

- Bio-mimietic
- Degradable (sometimes)
- Rich morphologies (α-helix, β-sheet, random-coil)

Disadvantages:

- Requires high purity of NCA monomers
- Very sensitive to impurities (e.g. water)
- Rather slow polymerization
- Limited monomers (functional group limitations)

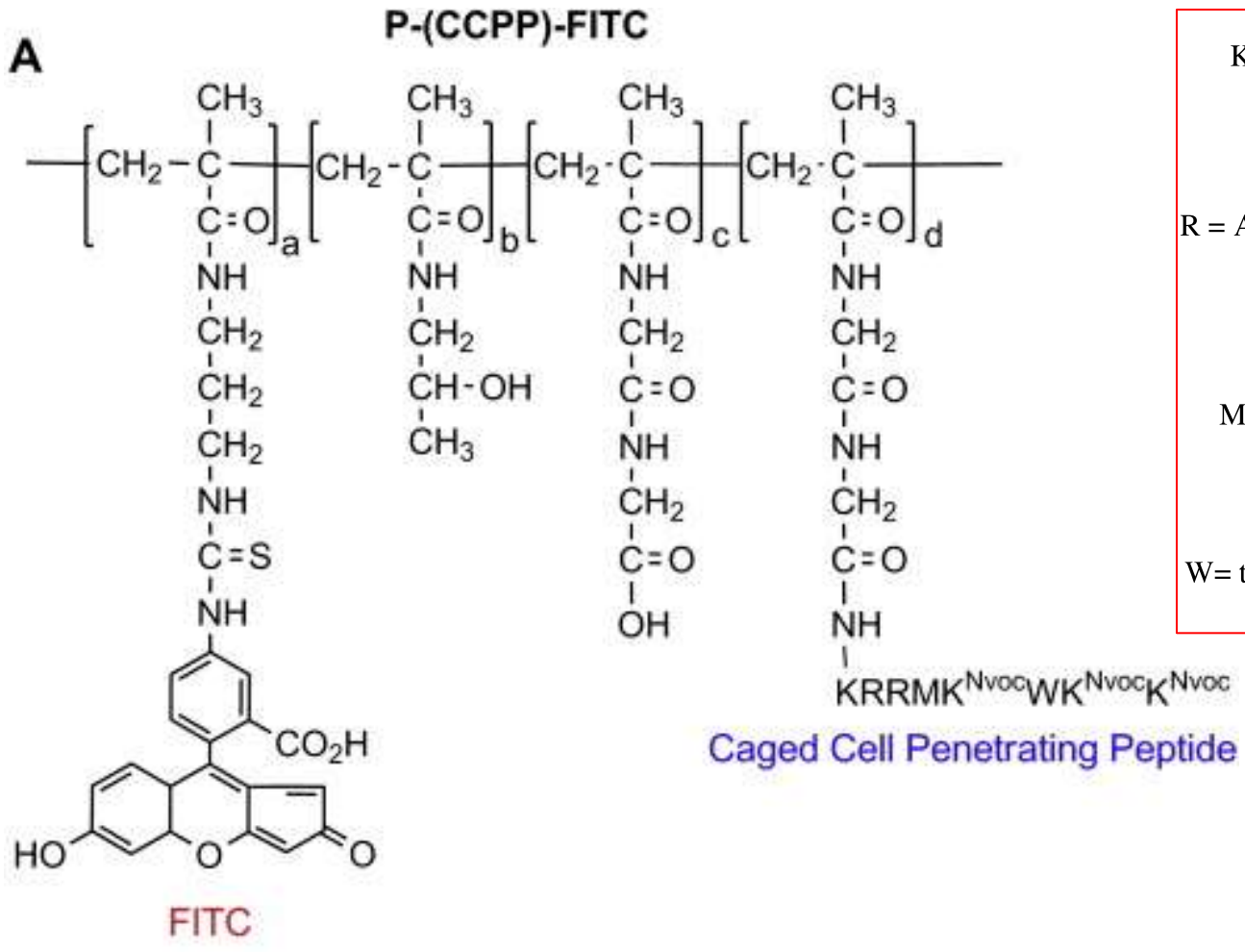
Photo Responsive Polymeric DDS

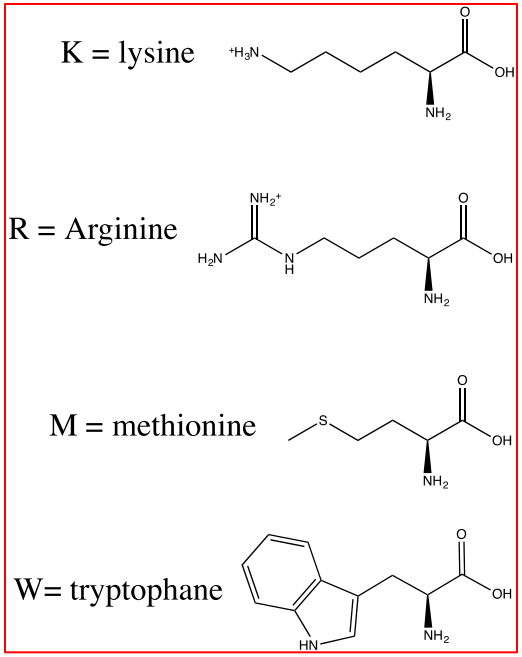


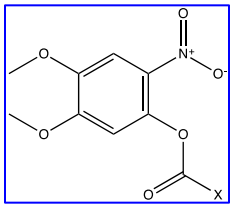
Y. Shamay , L. Adar , G. Ashkenasy , A. David *Biomaterials*, 32, **2011**, 1377 – 1386.

Photo Responsive Polymeric DDS

TAU

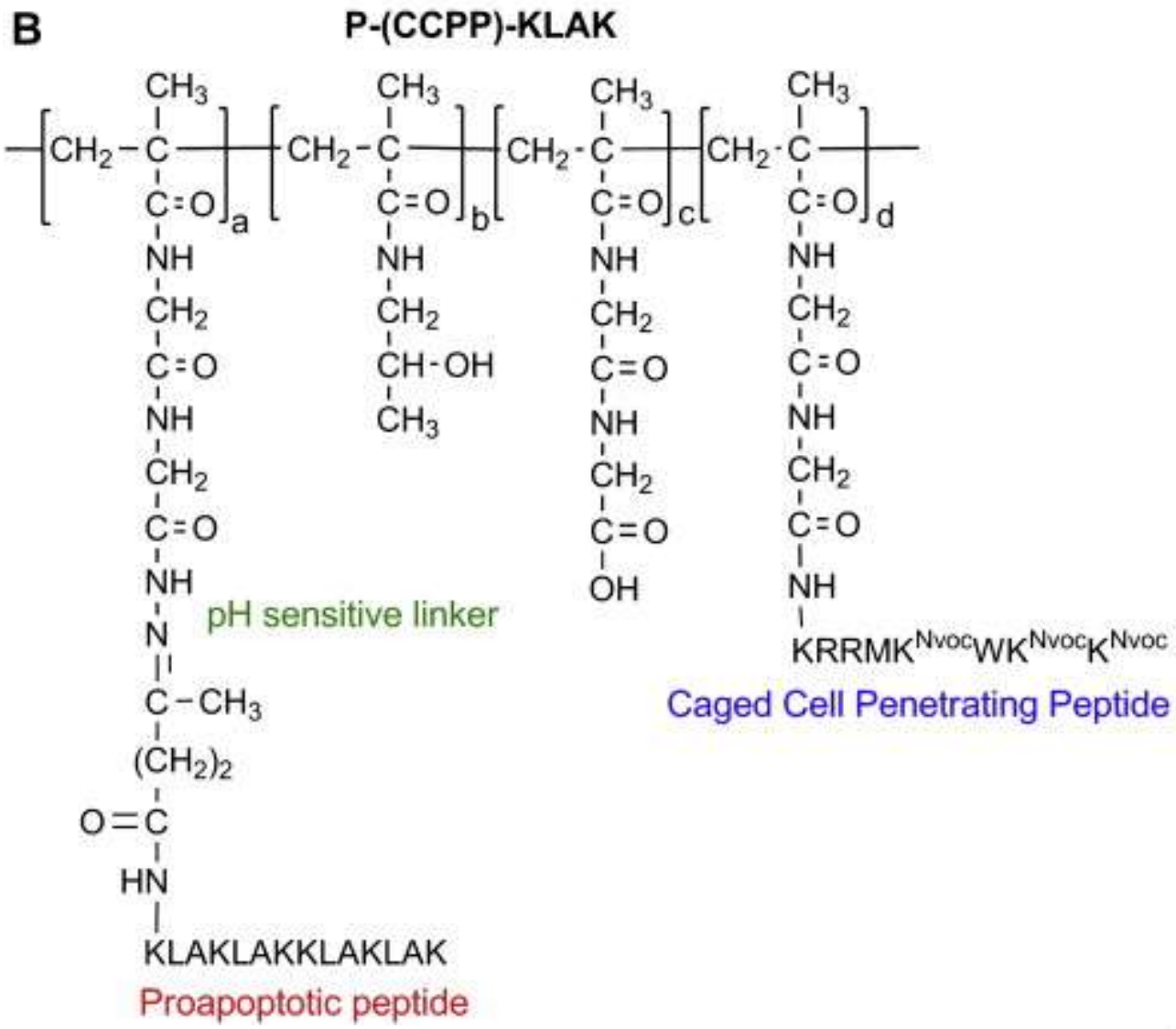




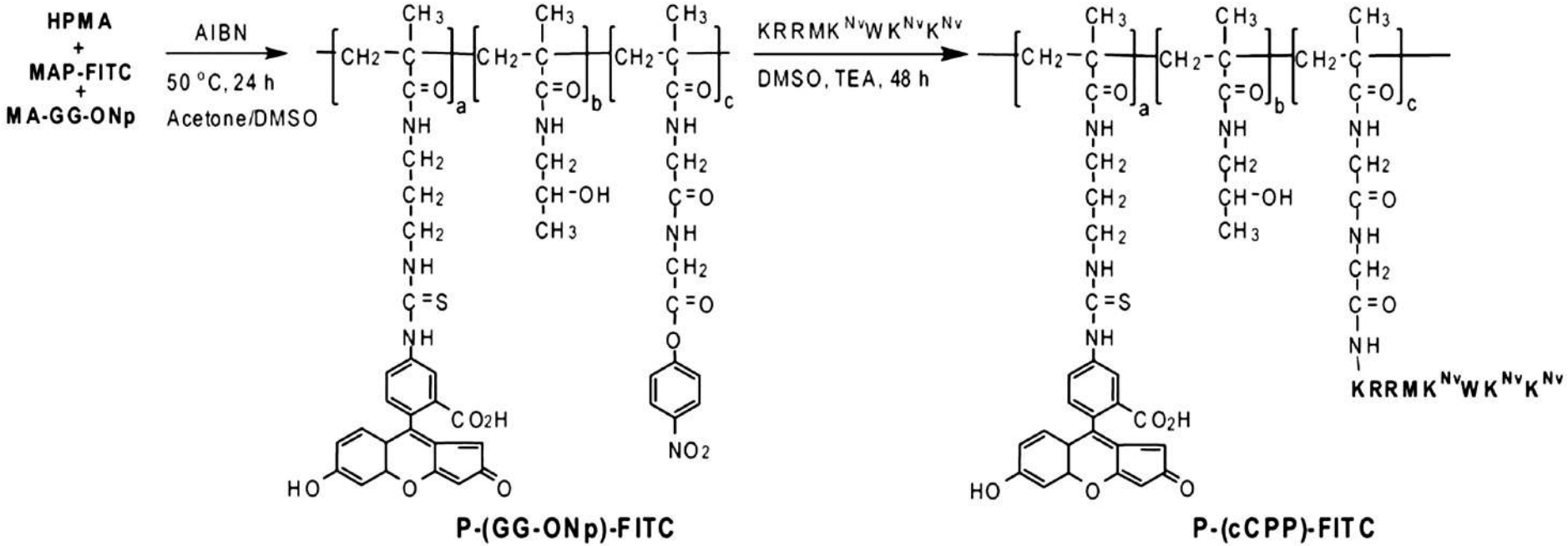


HPMA: N-(2-hydroxypropyl)-methacrylamide

Photo Responsive Polymeric DDS



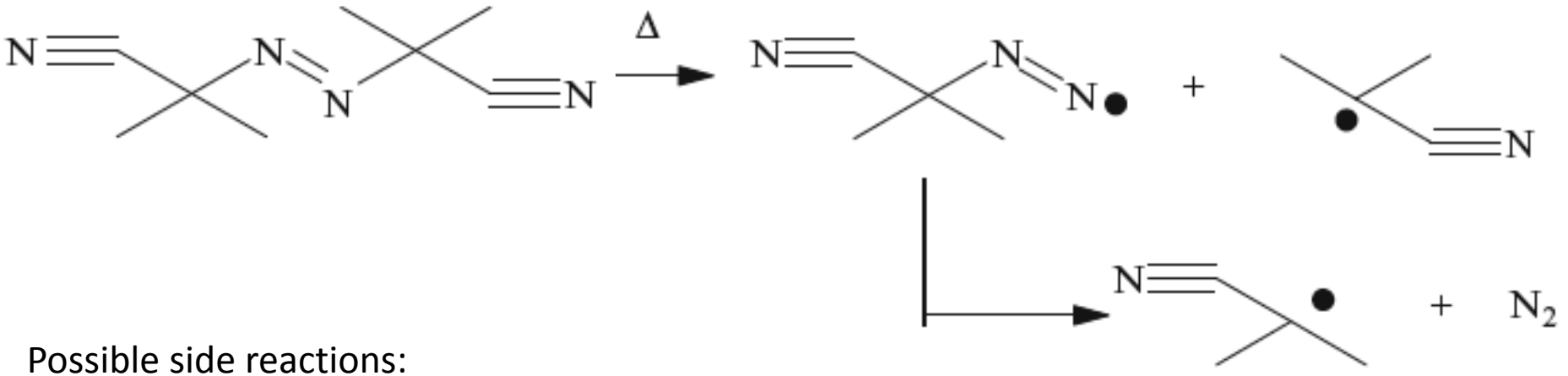
Free Radical Polymerization



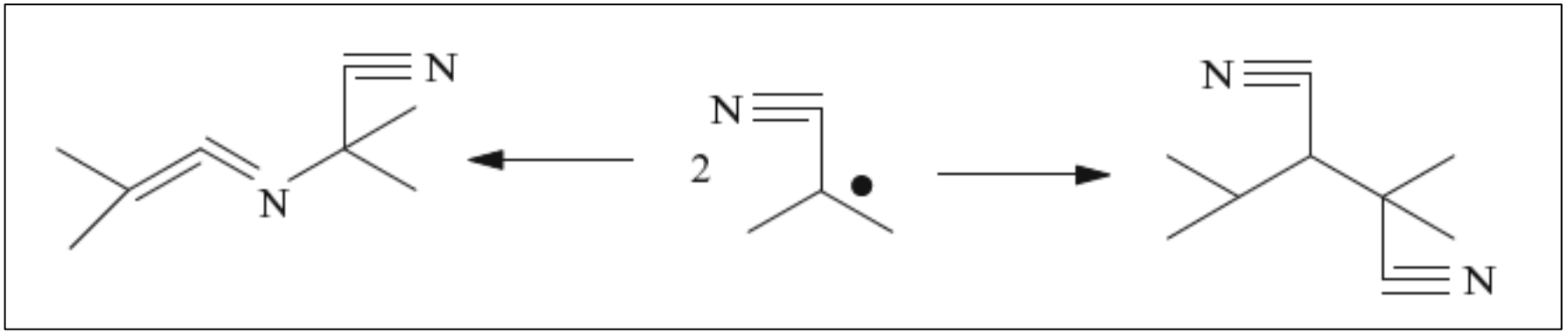
HPMA: N-(2-hydroxypropyl)-methacrylamide

Thermal radical initiators: AIBN

$$R\text{-}N{=}N\text{-}R \xrightarrow{\Delta} 2R\bullet + N_2$$

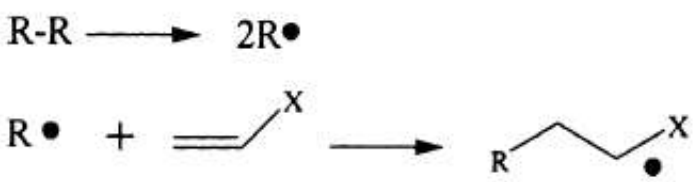


Possible side reactions:

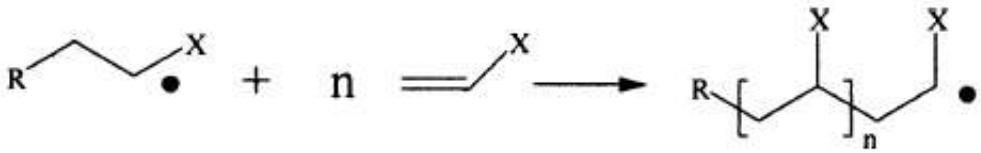


Free-radical chain-growth polymerization

1. Initiation

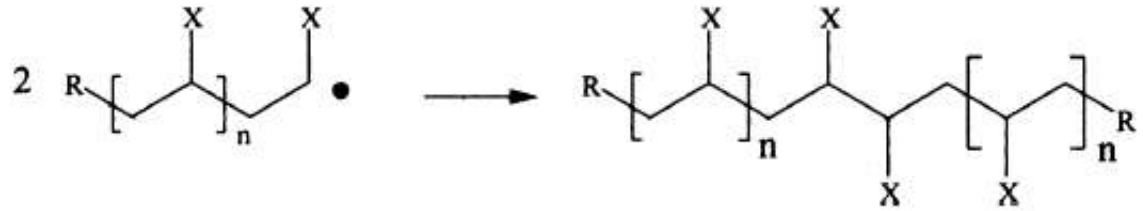


2. Propagation

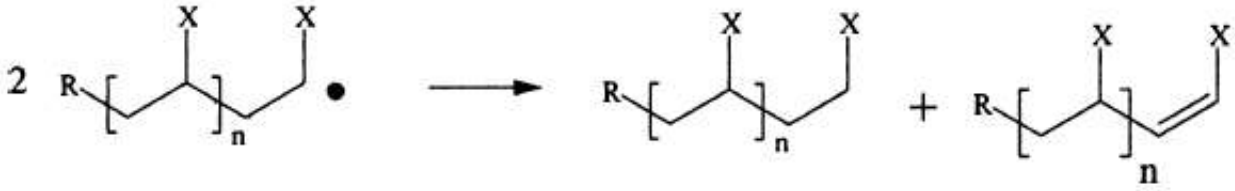


3. Termination

a. By combination



b. By disproportionation



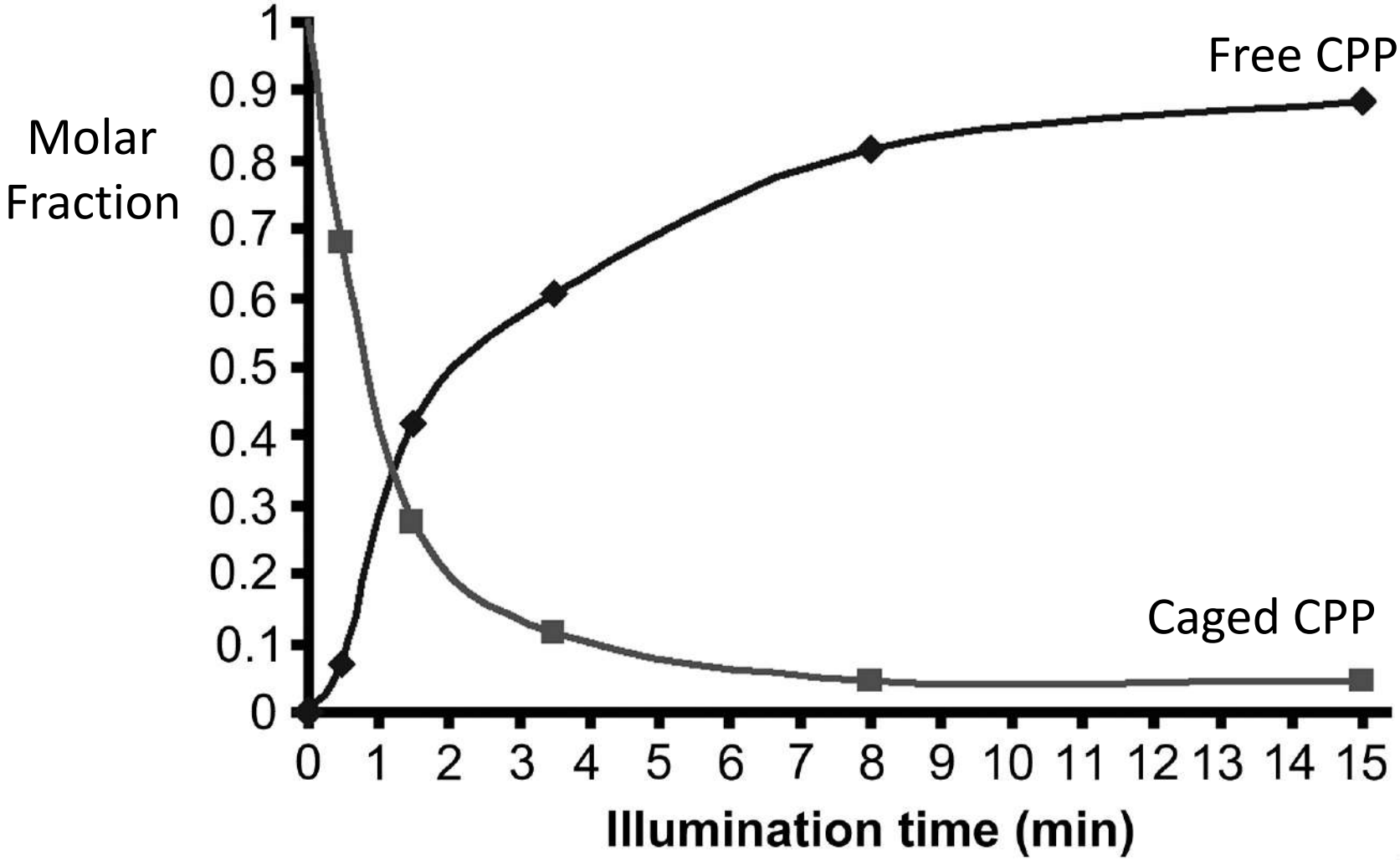
c. By transfer

Free Radical Polymerization

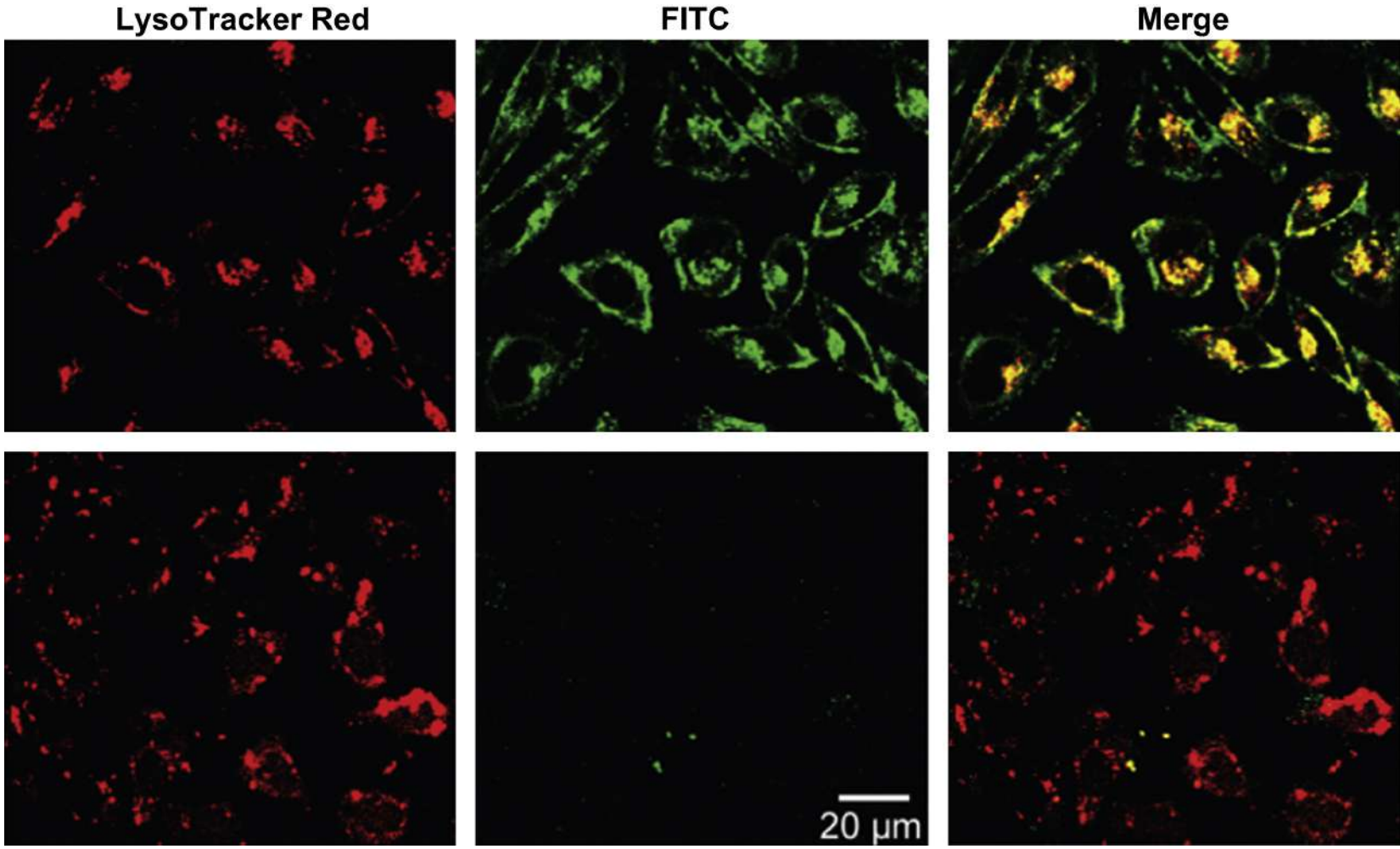
HPMA conjugate	%Mol Opn/CO_2H	%Mol cCPP	# of peptides/ polymer chain
HPMA-(GG-ONp)-FITC	8.3	0	0
HPMA-(cCPP)-FITC	7.3	1	2
HPMA-FITC	8.3	0	0

Average Mw . 23000 Da, PDI 1.42, FITC loading 1.8 %Mol

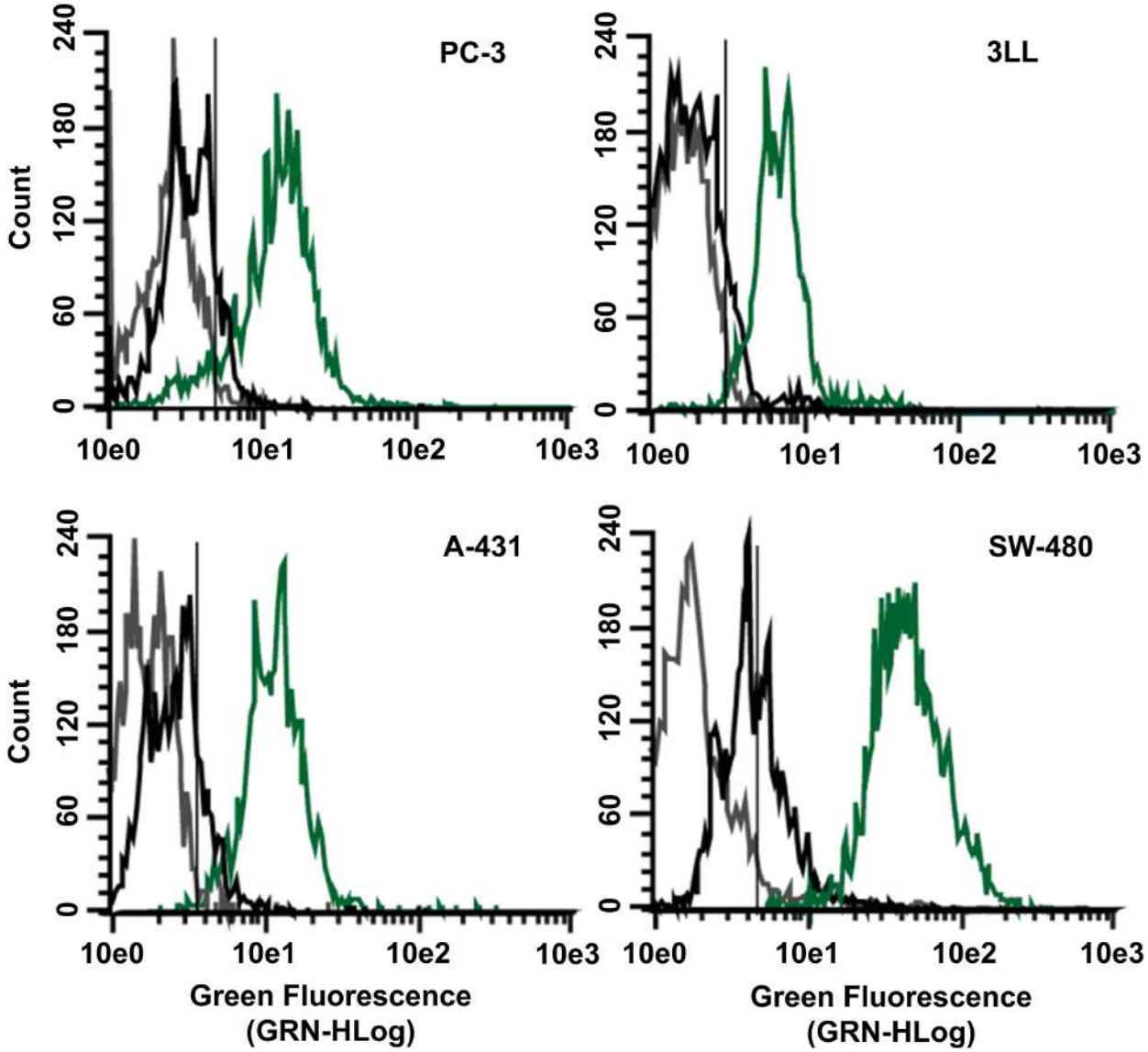
Measuring Photoresponse



Light Induced Cell Internalization



Light Induced Cell Internalization



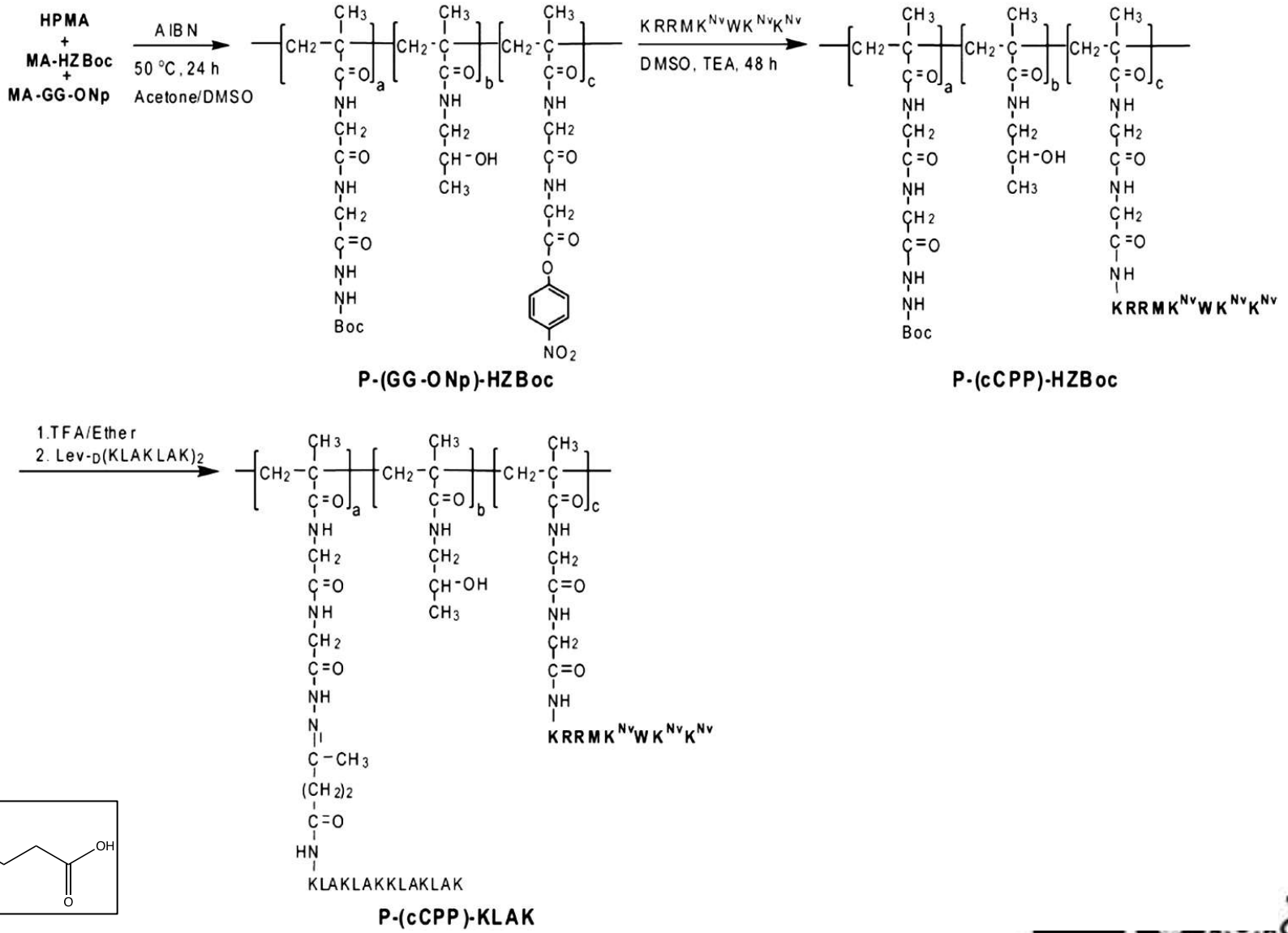
CPP + hν

CPP – hν

No CPP

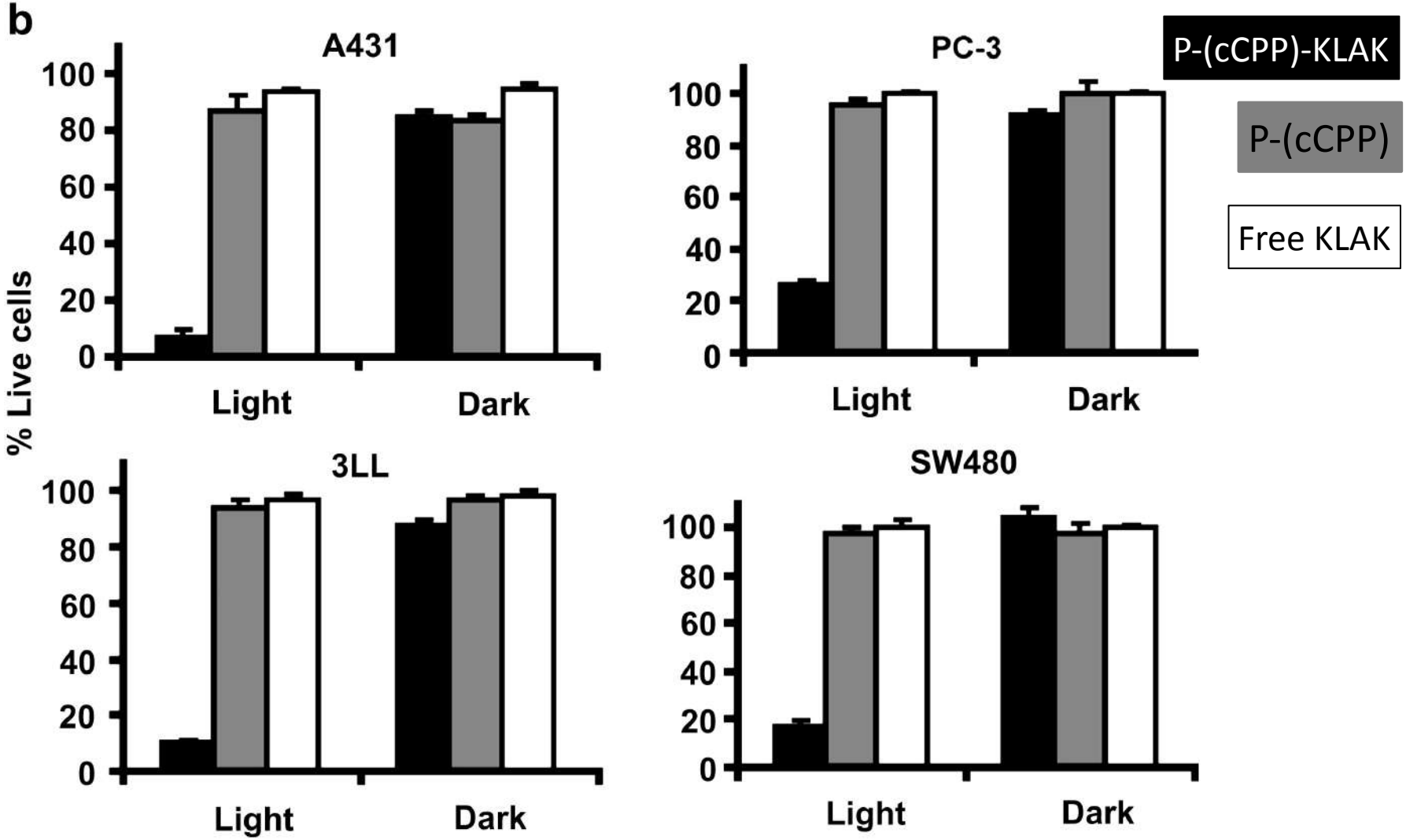
FACS: Fluorescence-activated cell sorting

Free Radical Polymerization



Levulinic acid

Light Induced Cell Toxicity of P-(cCPP)-KLAK

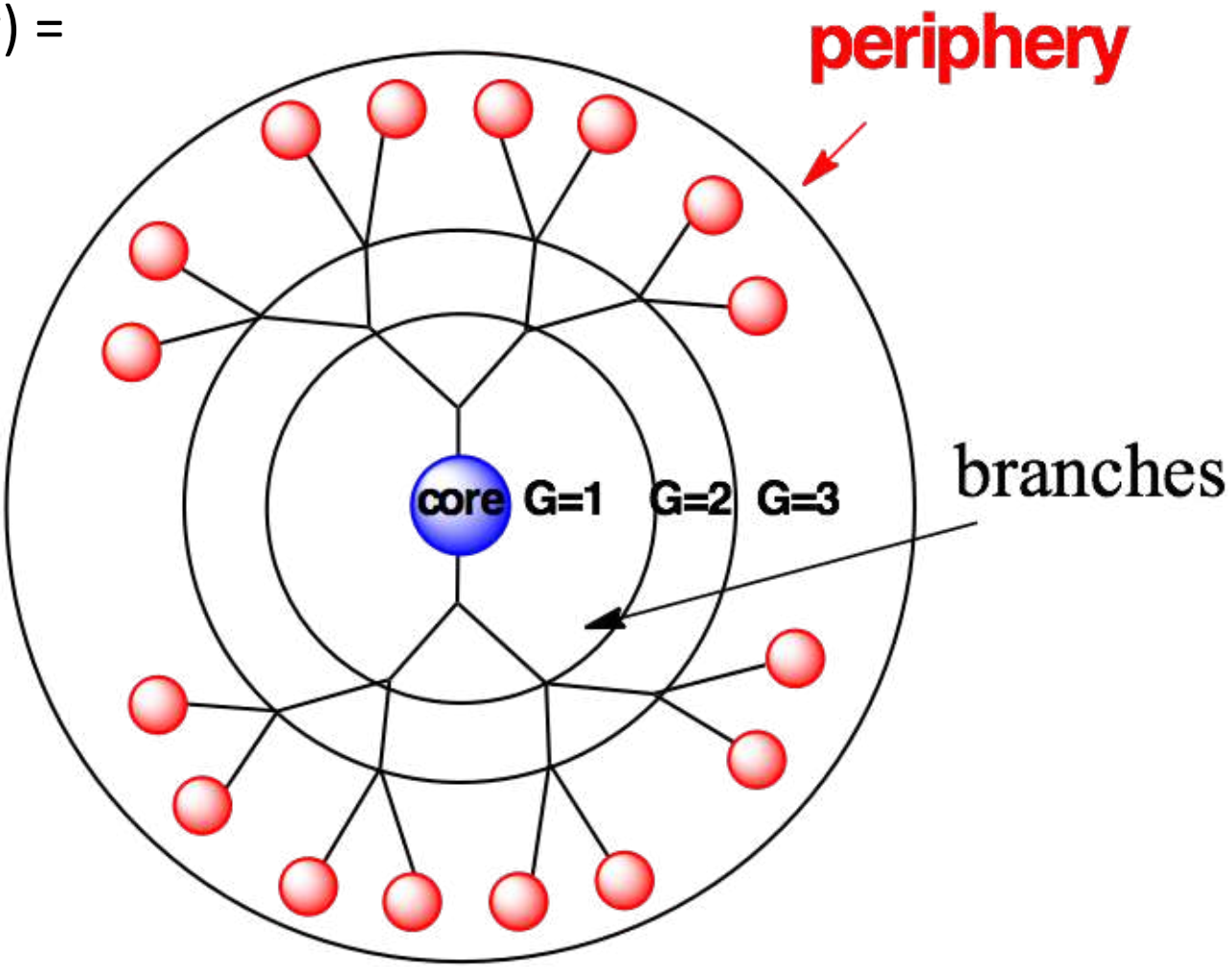


Light Induced Cell Toxicity of P-(cCPP)-KLAK

There is a need to develop photo-cleavable groups that can be removed by longer wavelengths

Dendrimers are Structurally Perfect Branched Polymers

- Dendron (δενδρον) = tree (Greek)
- Defined structure
- High number of end-groups
- Inner cavities



The pioneers of dendrimer chemistry

TAU

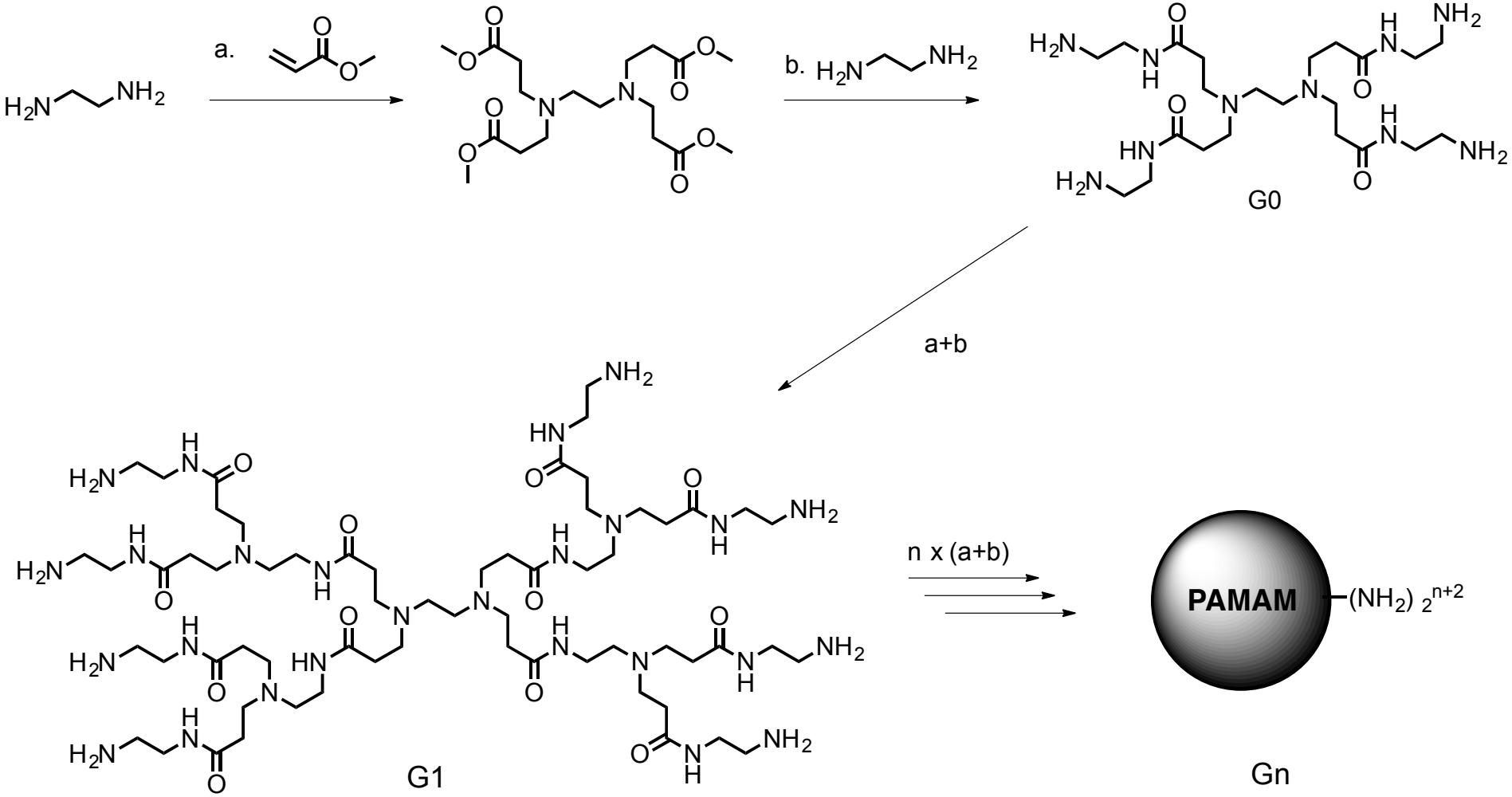
D. Tomalia
1985:PAMAM dendrimers

F. Vogtel
1st Dendrimer synthesis
1978

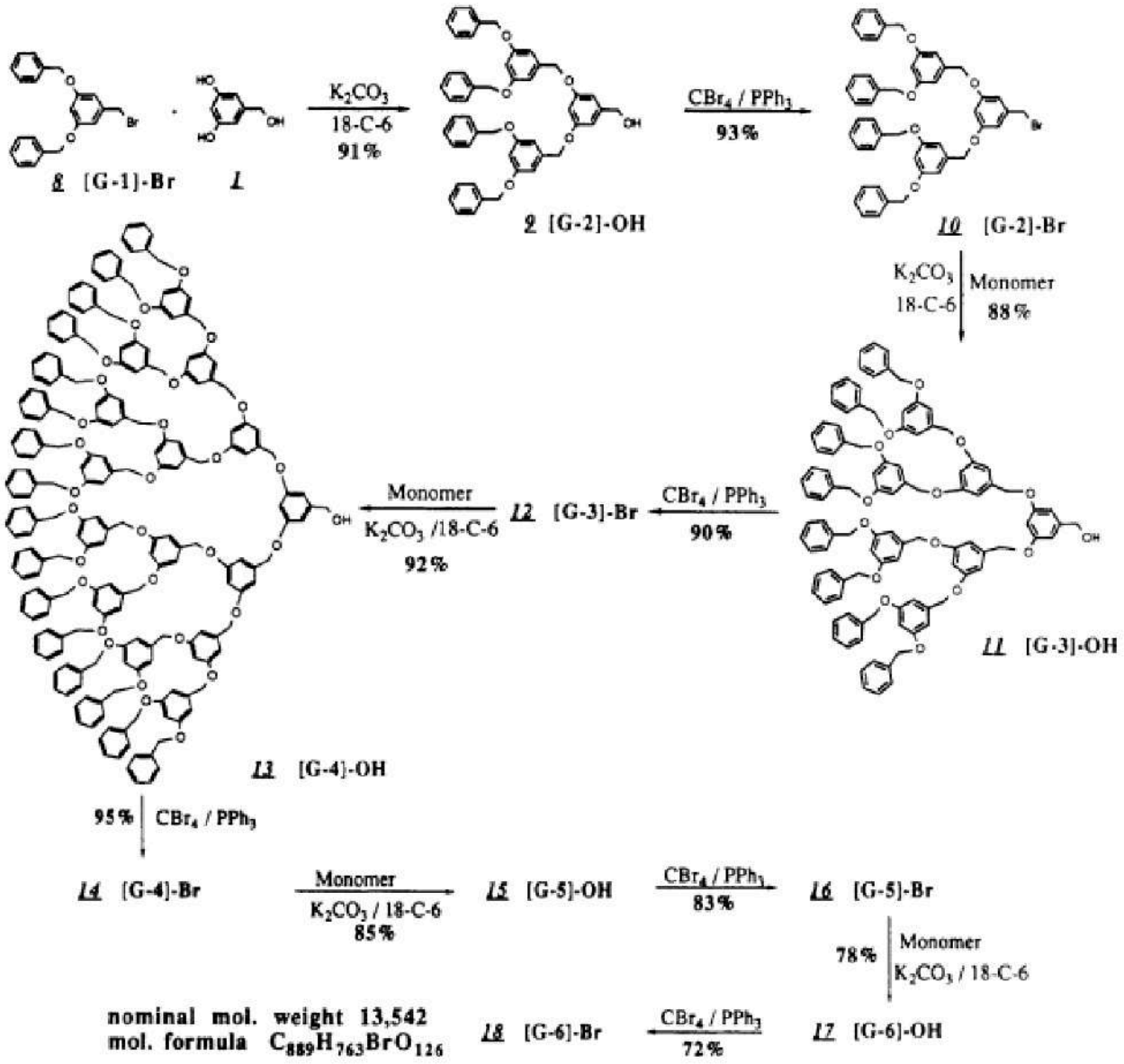
G. R.Newkome
Another dendrimers pioneer
1985

J. M. J. Frechet and C. J. Hawker
1990: Convergent synthesis

Dendrimers Synthesis through a Divergent Approach



Dendrimers Synthesis through a Convergent Approach

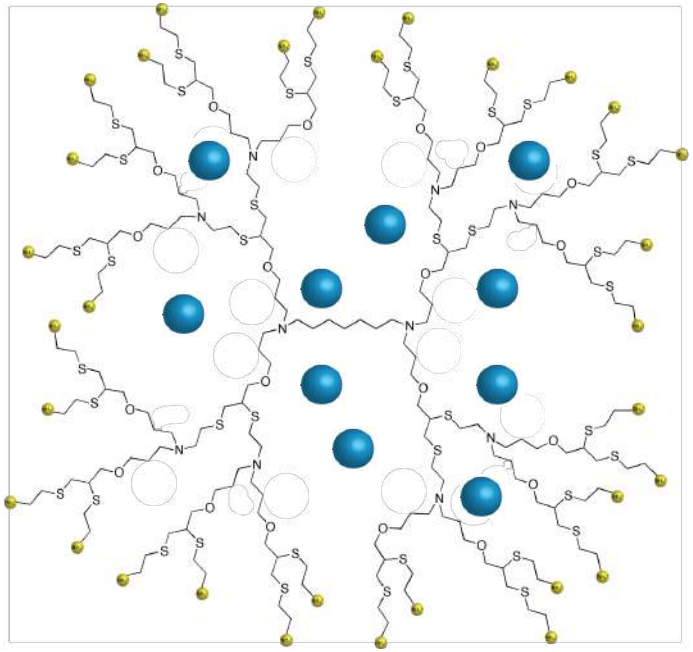


Dendrimers are Structurally Perfect Branched Polymers

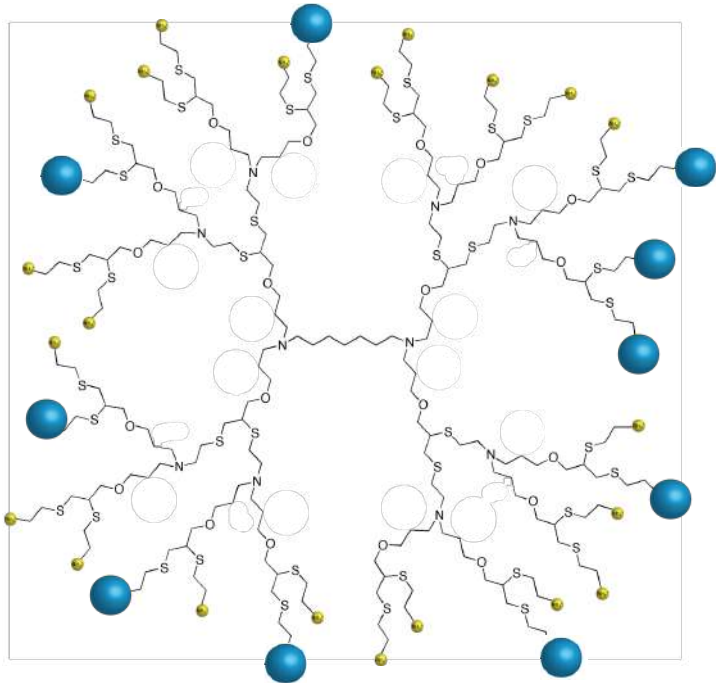
Dendrimers offer high molecular precision (but often require tedious synthesis and purification)

Current Strategies for Loading Dendritic Carriers

Encapsulation
(non-covalent)

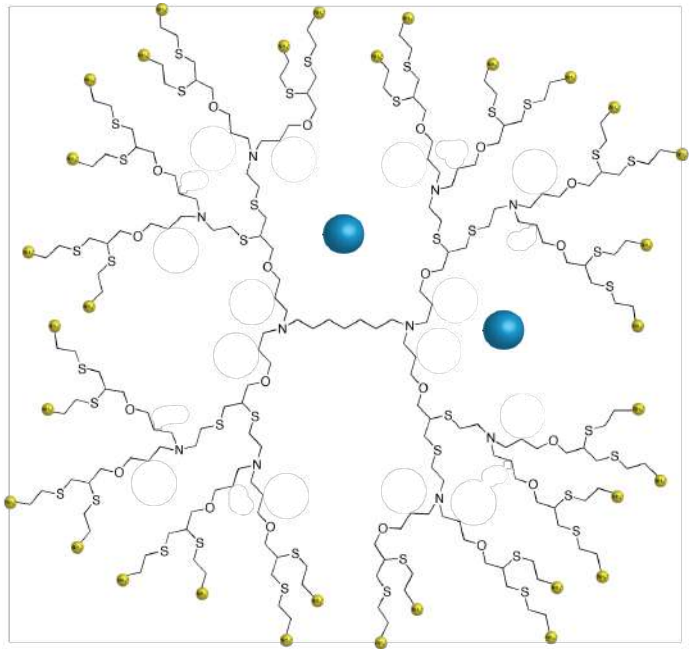


Surface loading:
(covalent binding)



Encapsulation of Cargo Molecules

Encapsulation
(non-covalent)



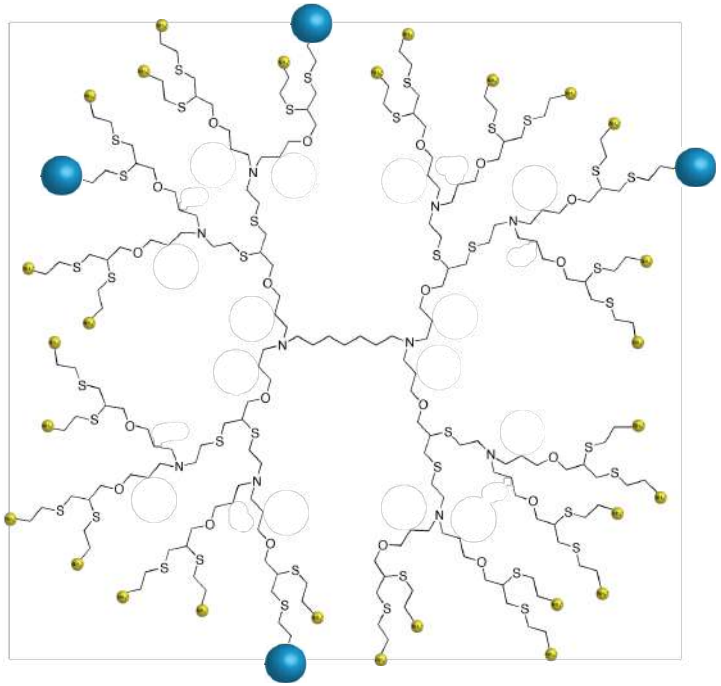
- Hard to control the stability of the complex
- Usually low number of encapsulated molecules

Covalent Loading Allows Controlling Carrier Stability

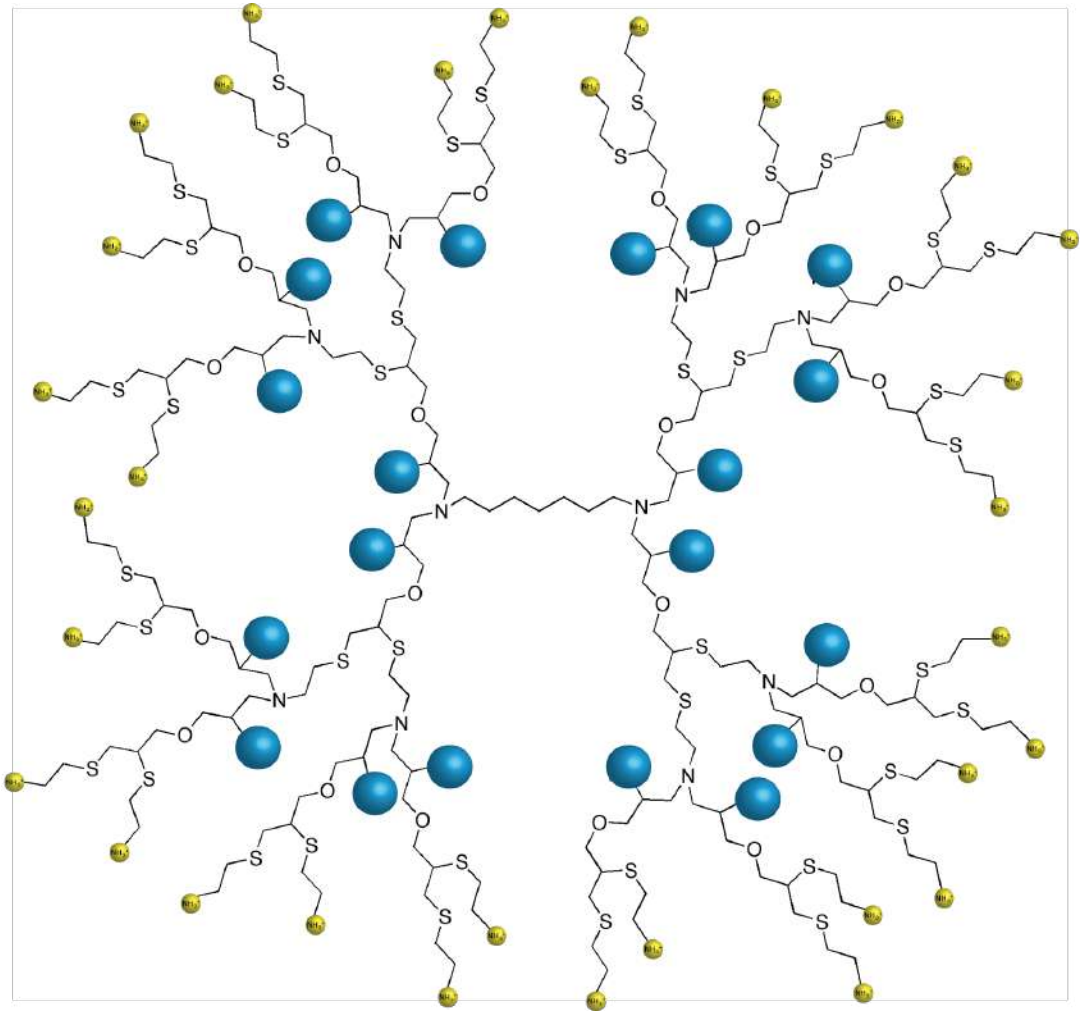
- Changes the surface properties of the dendrimer
- Limited and polydisperse number of attached molecules

Surface loading:

(covalent binding)



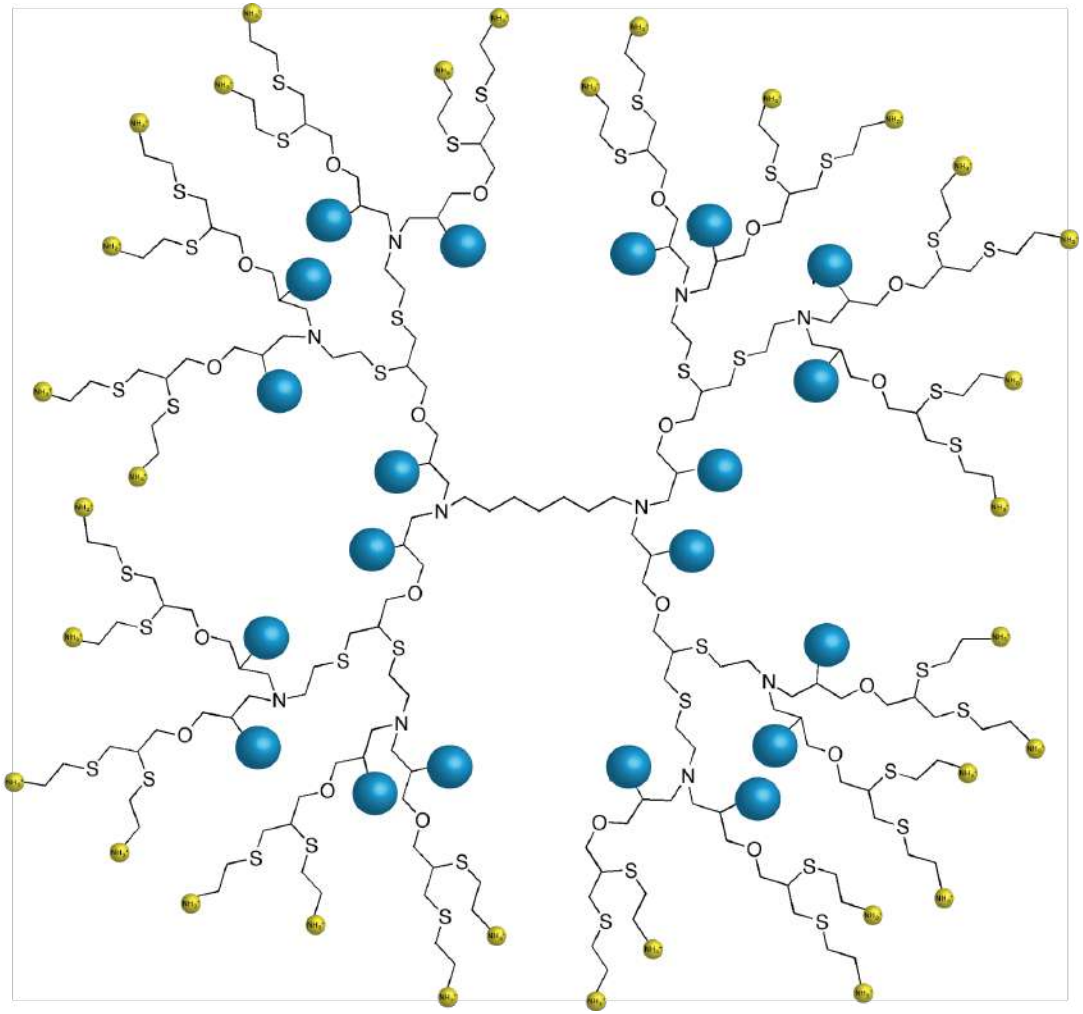
Internally Functionalized Dendrimers



Surface functionality
and
shielded payload
(encapsulation)

Stability and
stoichiometric control
(covalent-binding)

Internally Functionalized Dendrimers



Examples of internally functionalized dendrimers:

Severac *Tet. Lett.* ***2004***
Freeman *J.Org.Chem.* ***2000***
Dichtel *Org. Lett* ***2005***
Antoni *Angew. Chem.****2009***
Kang *Chem. Commun.* ***2010***

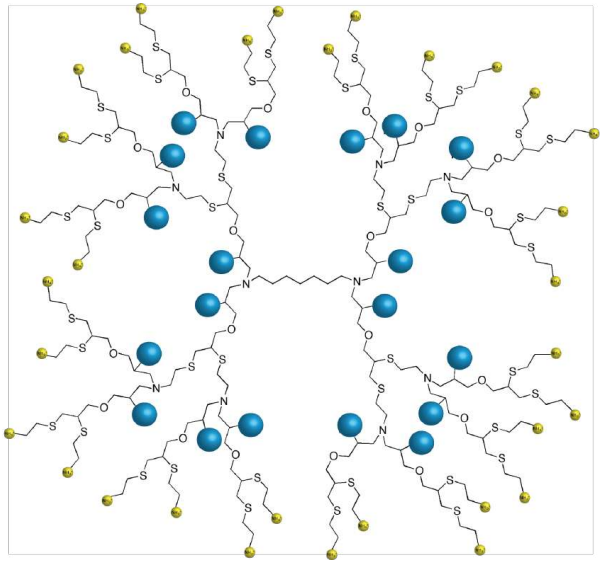
Synthetic Guidelines

-High water solubility

-Orthogonal functionalities

-High loading

-Facile synthesis
- •Cheap monomers
- •Few steps
- •Simple purification



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-High water solubility

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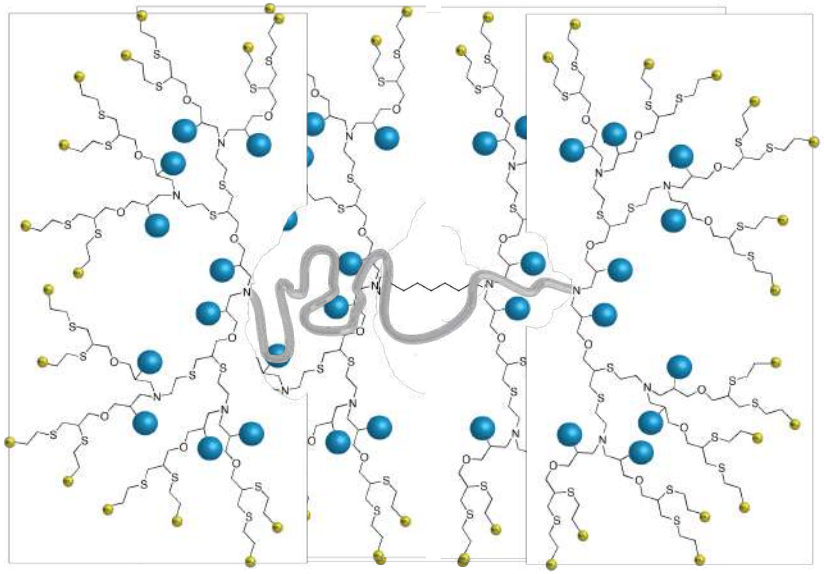
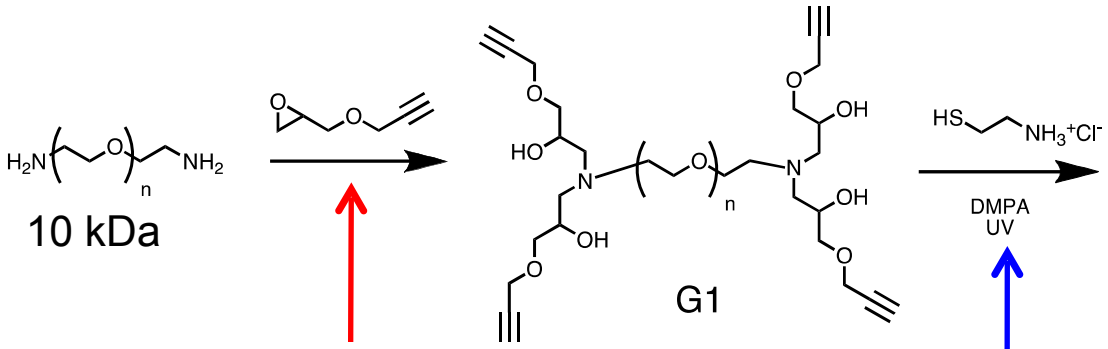


PEG core
- •Increases water solubility
- •Simple Purification: precipitation or dialysis

Amine-epoxy and Thiol-yne: Generation in Each Step



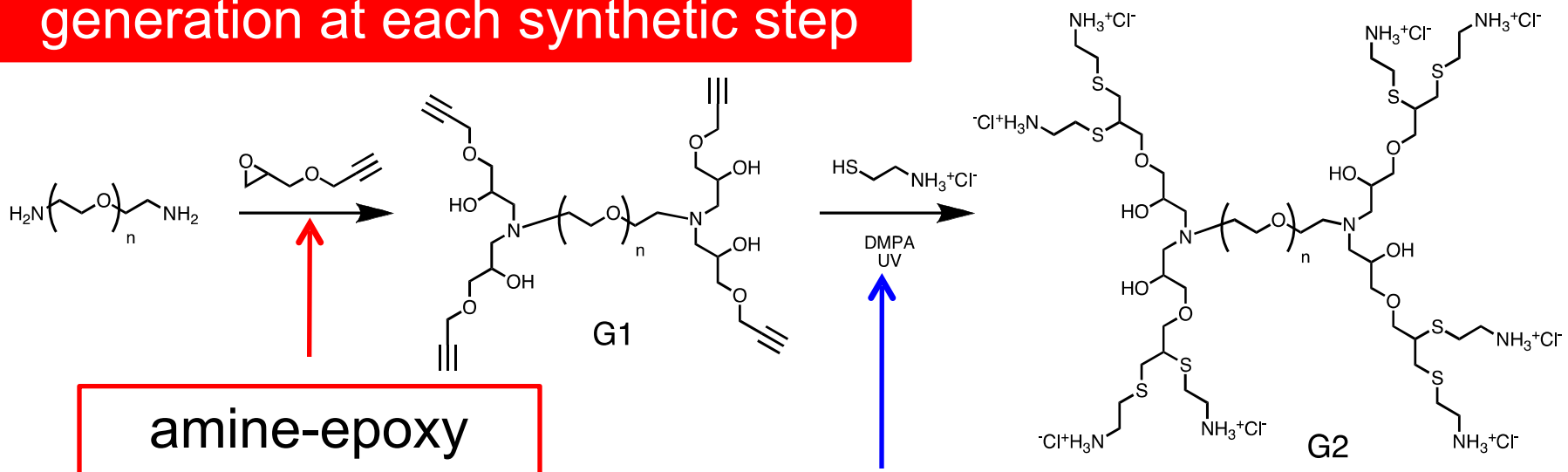
amine-epoxy reaction

thiol-yne reaction

Hydroxyl groups for further functionalization

Amine-epoxy and Thiol-yne: Generation in Each Step

Accelerated growth: generation at each synthetic step

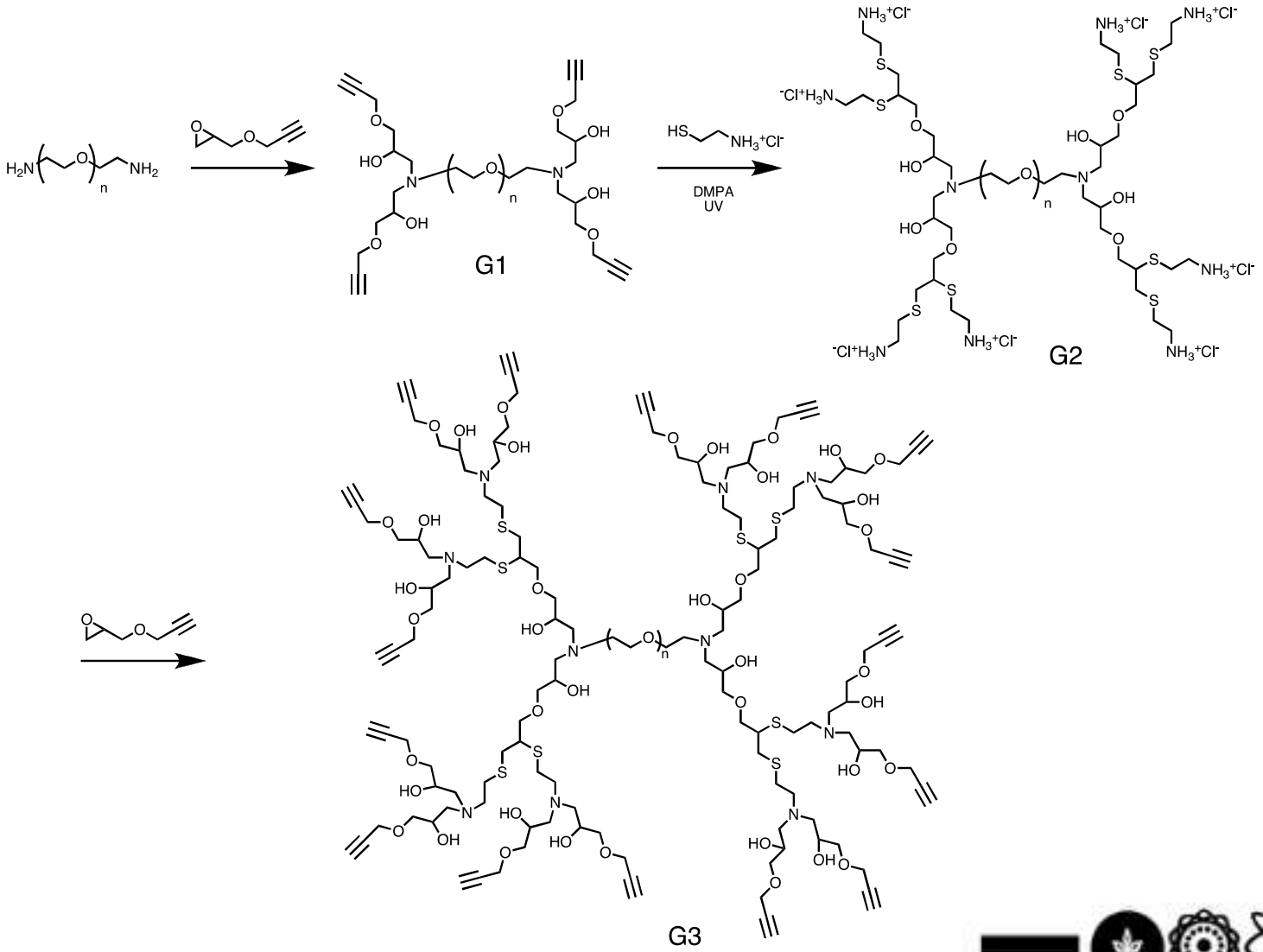


amine-epoxy reaction

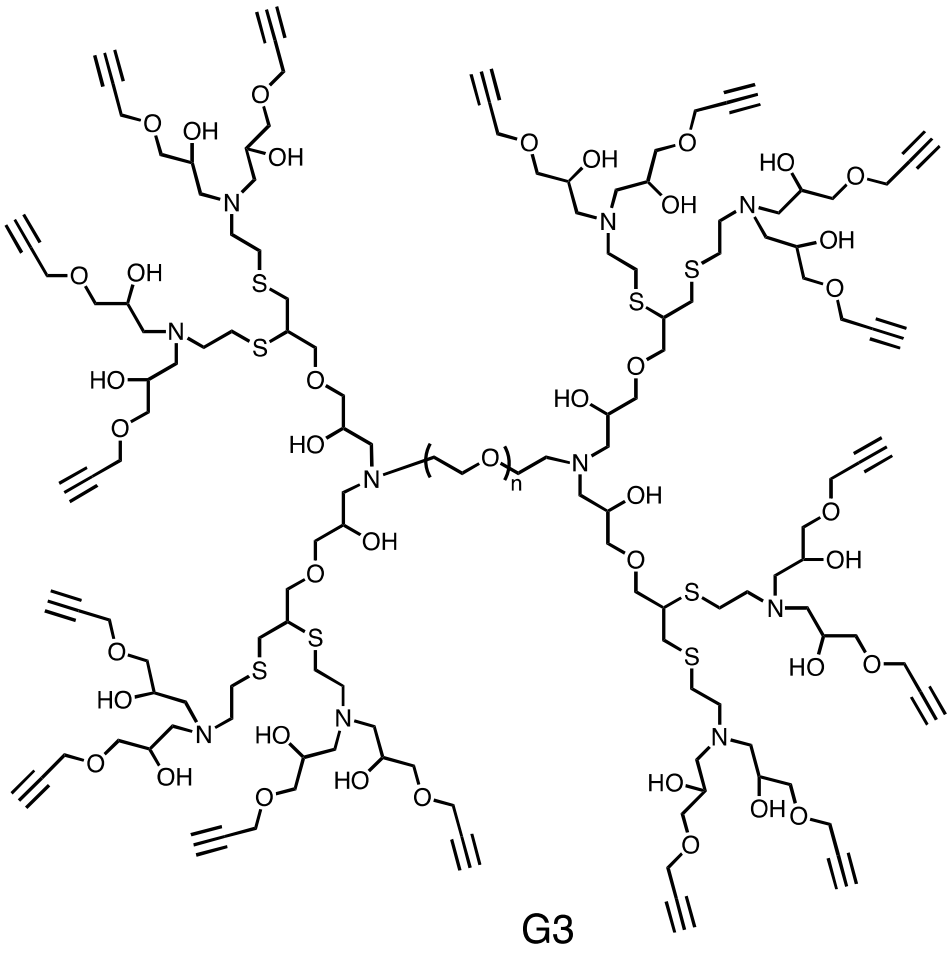
thiol-yne reaction

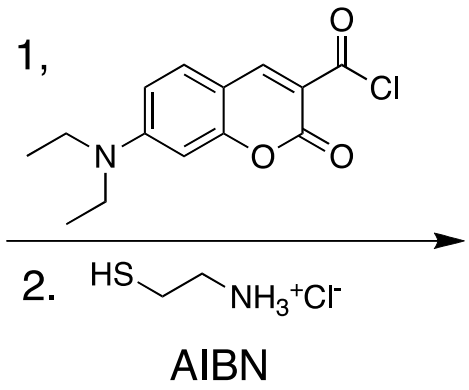
Hydroxyl groups for further functionalization

Polymer Supported AB_2/CD_2 Dendrimer Synthesis

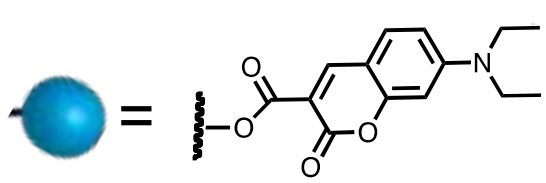


Loading is Followed by Surface Functionalization



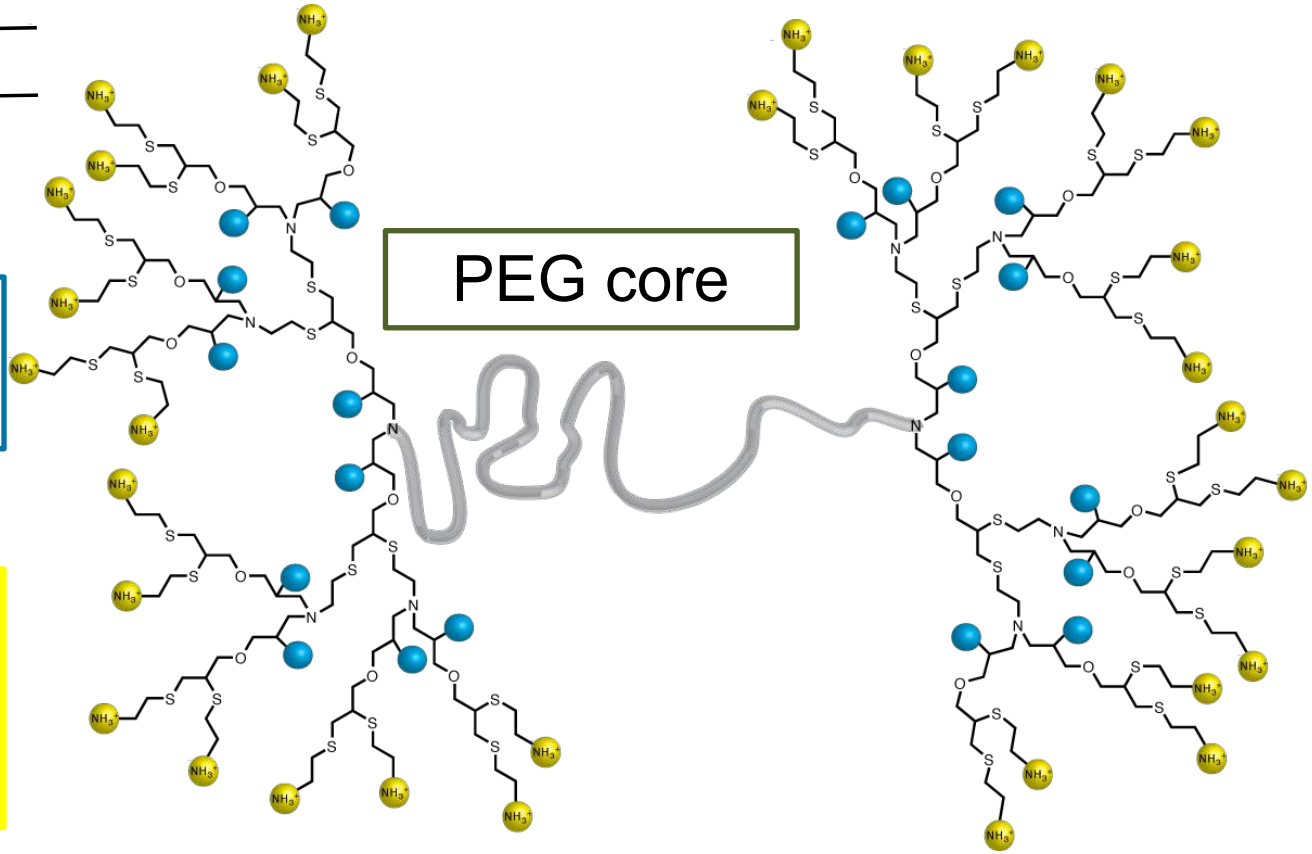


High Loading: 20 Dyes per Dendrimer (~25wt.%)



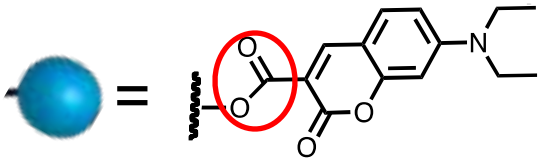
20 Internal coumarins

32 NH_3^+ surface groups: internalization



High Loading: 20 Dyes per Dendrimer (~25wt.%)

Enzymatic cleavage site



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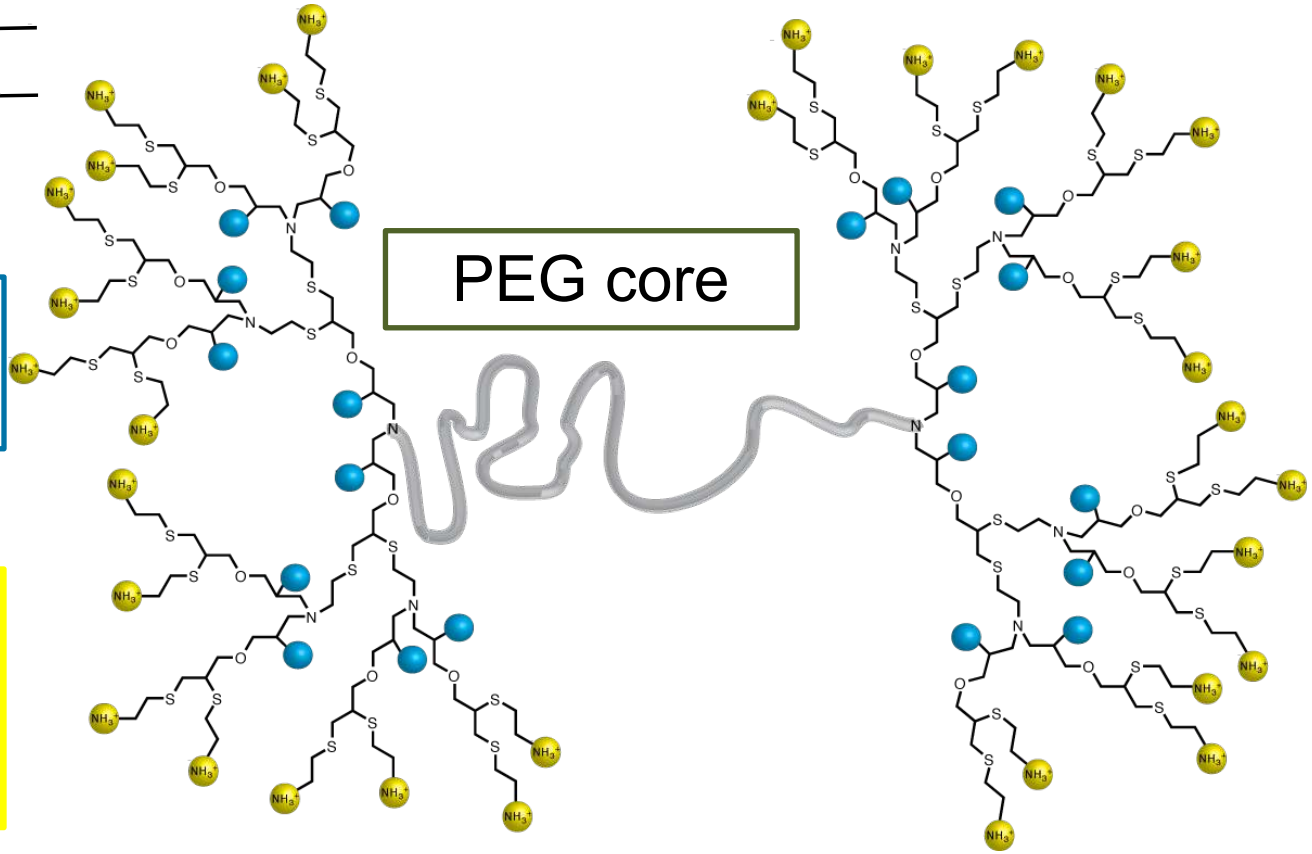

Quenched Fluorescence for the Loaded Dendrimer

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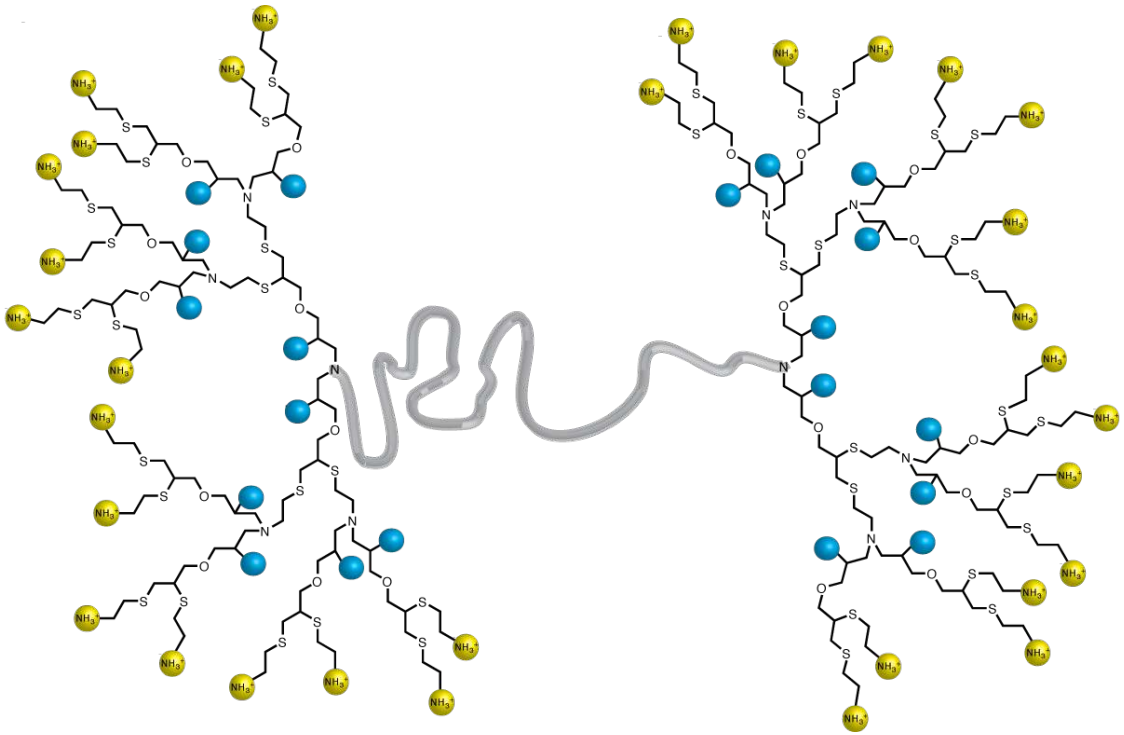
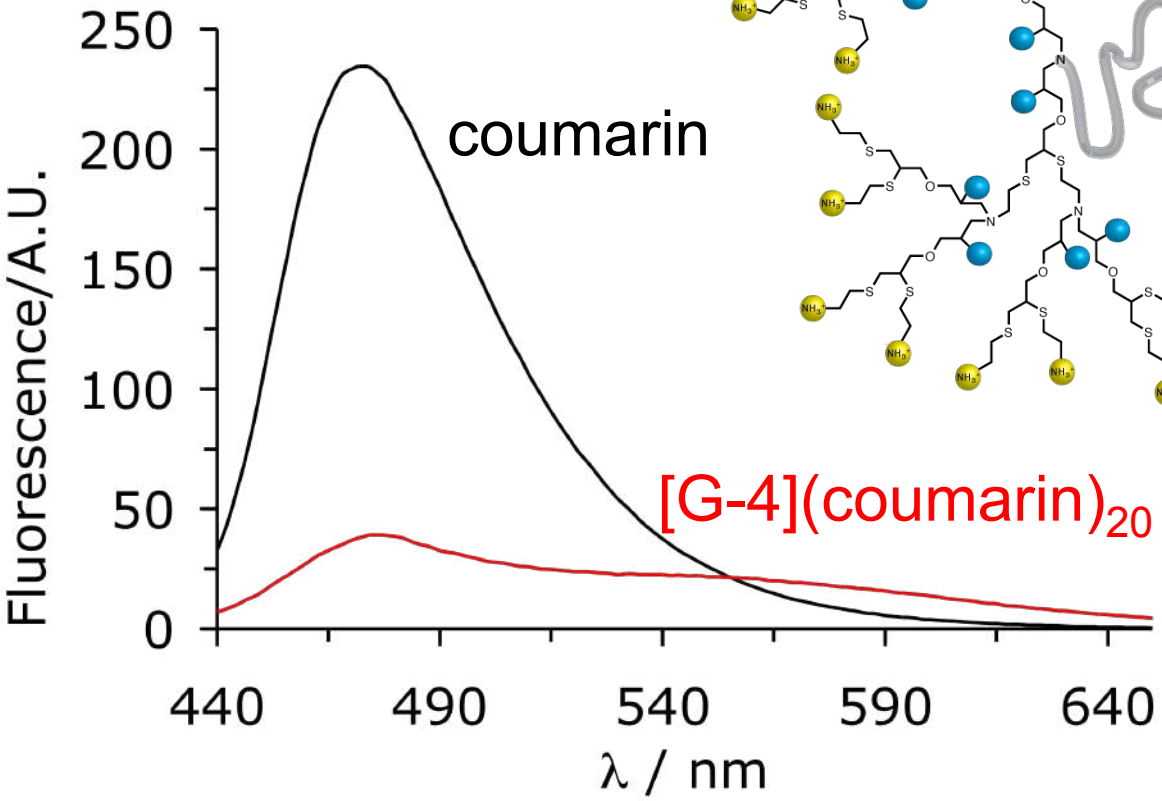
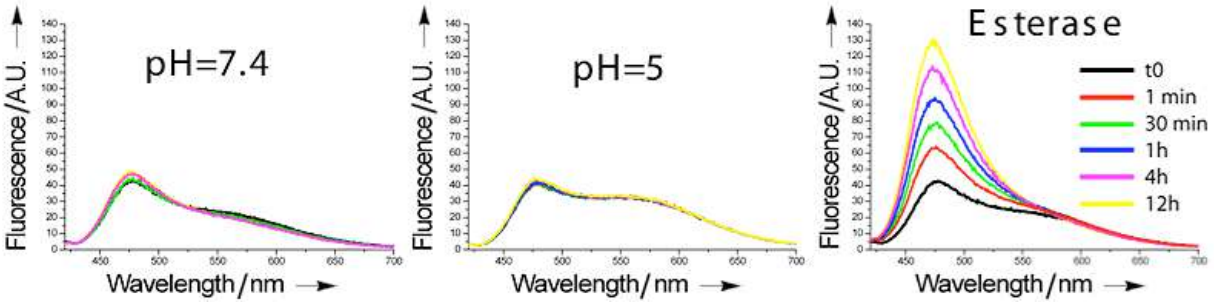
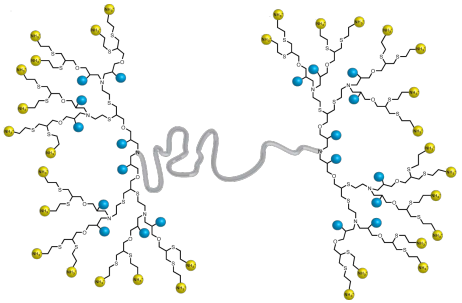
Quenched fluorescence



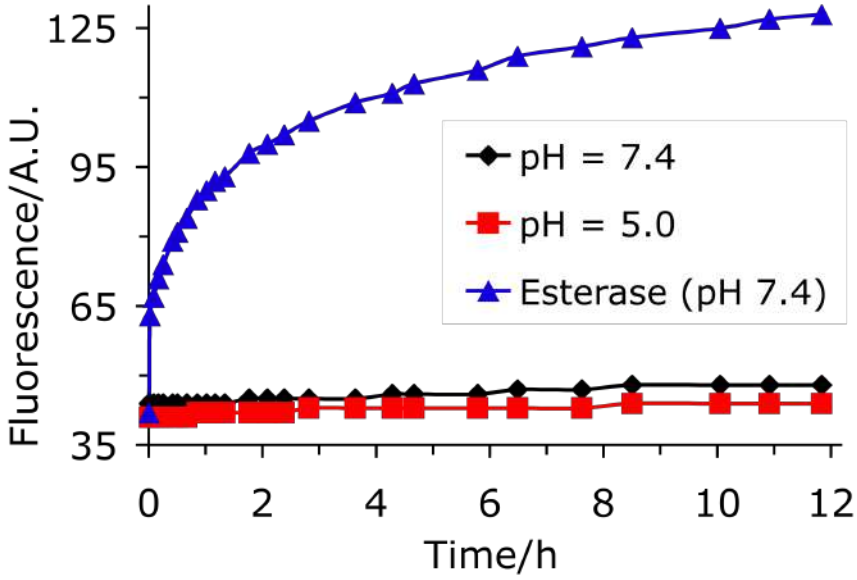


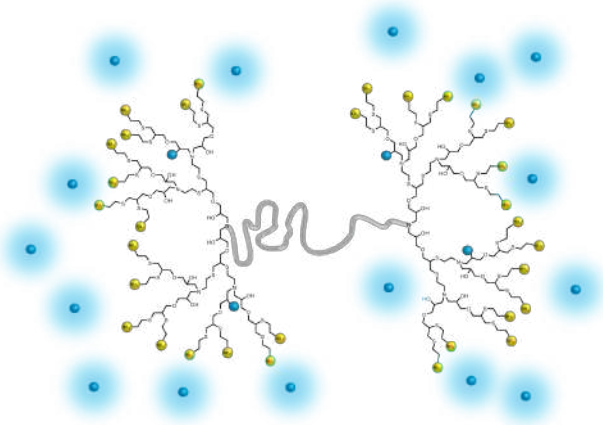
Dyes are Accessible and can be Cleaved by Esterase





Enzymatic cleavage





It is important to track all components of the delivery system

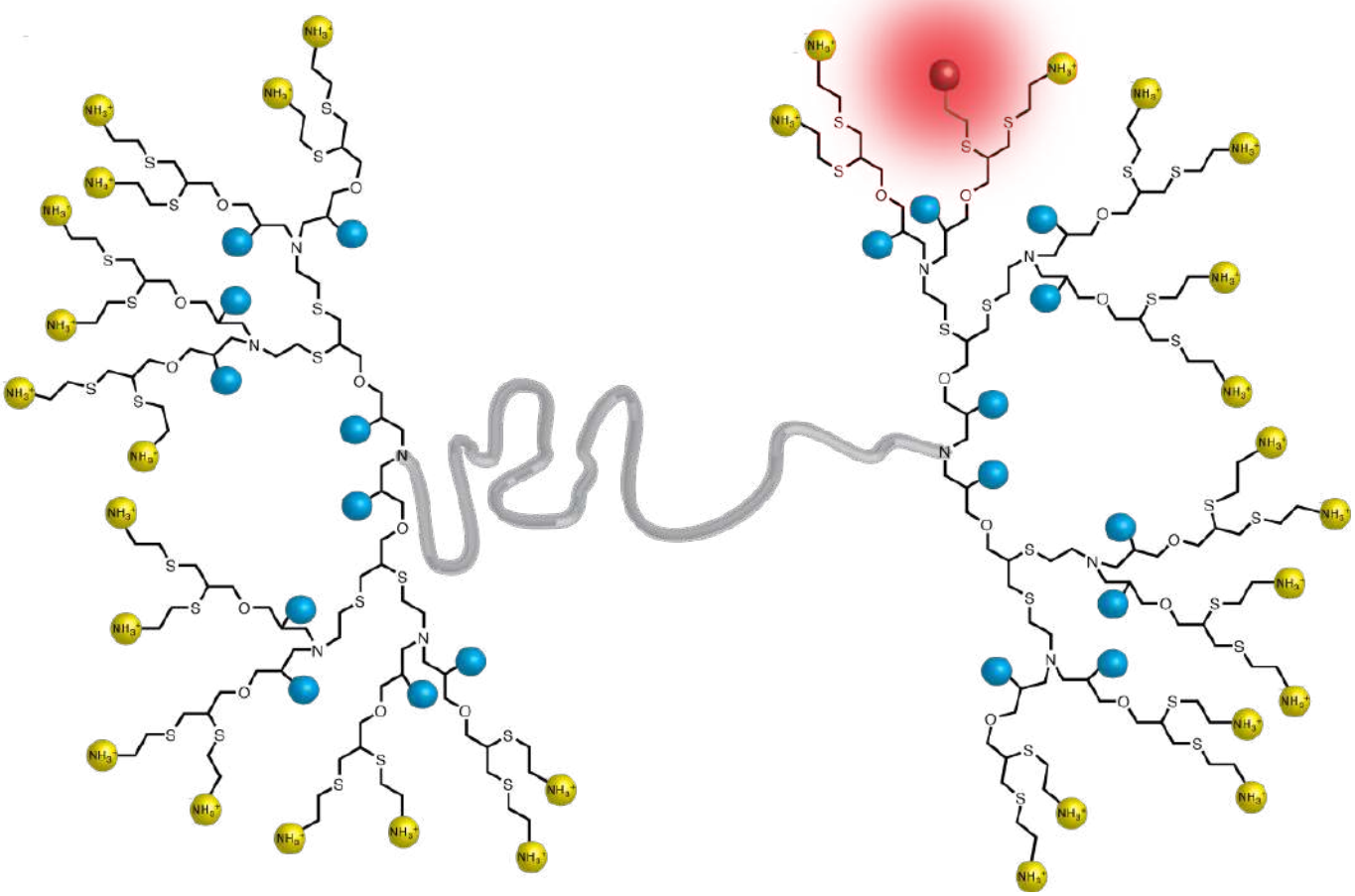
Dual tracking

What is The Fate of The Carrier?

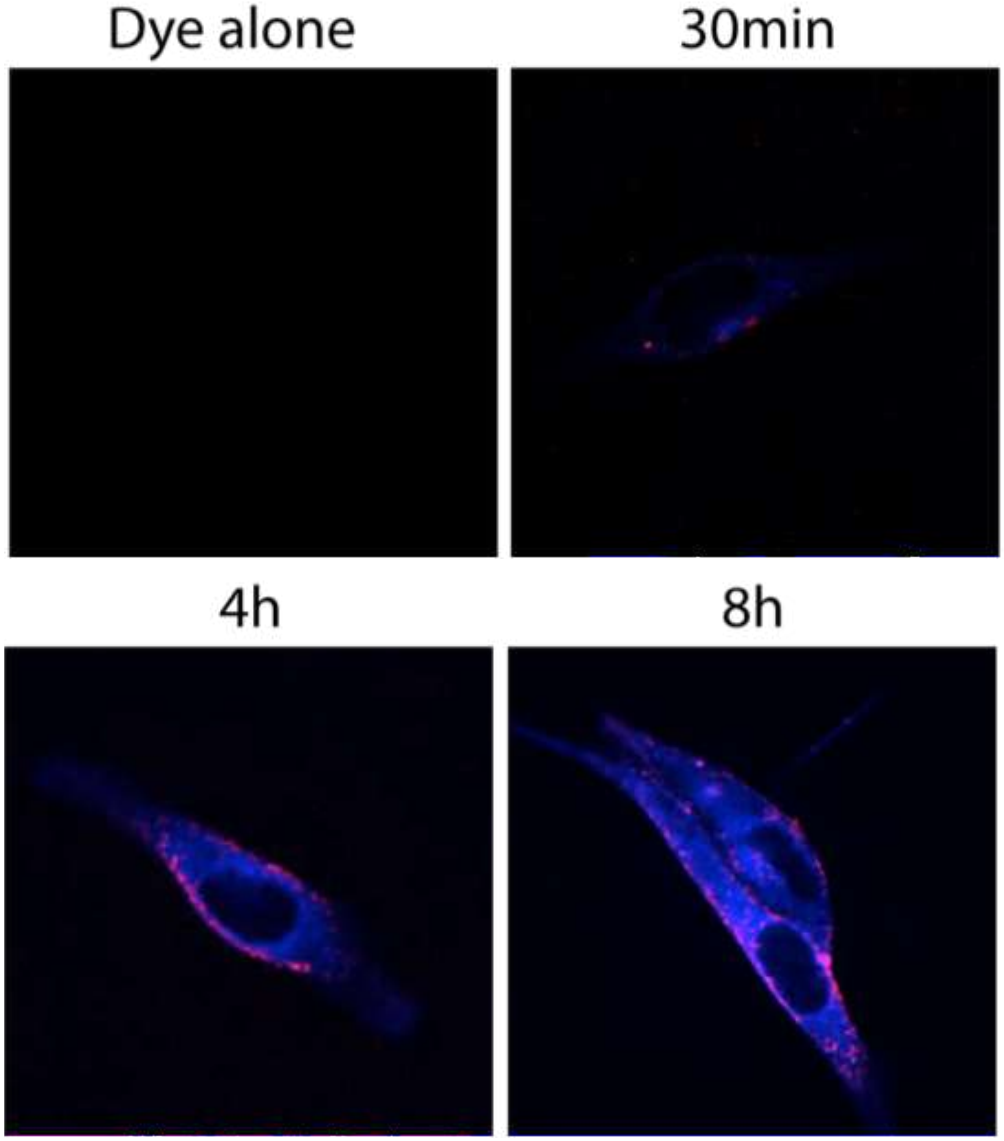
Alexa647 - trackable scalffold

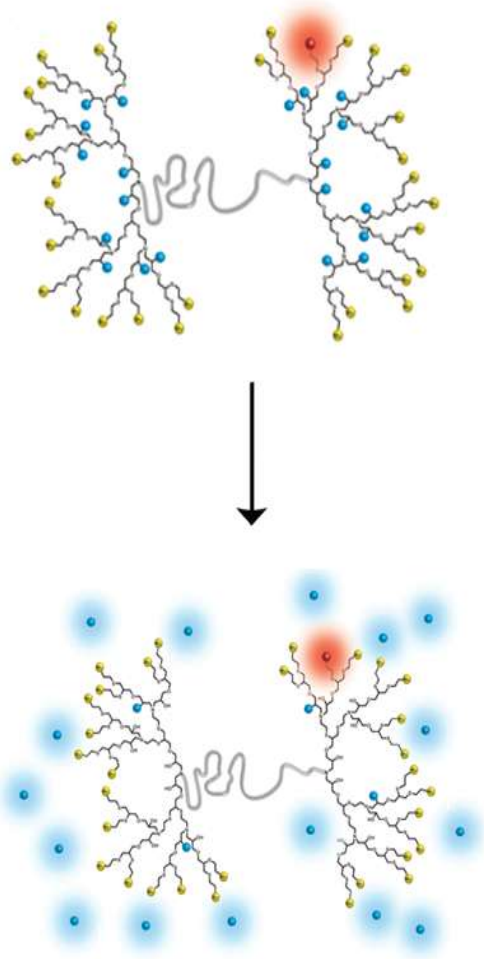
Surface = Cell internalization

Internal groups = dyes



Cell Internalization and Accumulated Dye Release





Amir *Angew. Chem.* ***2011***

Tracking both Dendrimer and Payload

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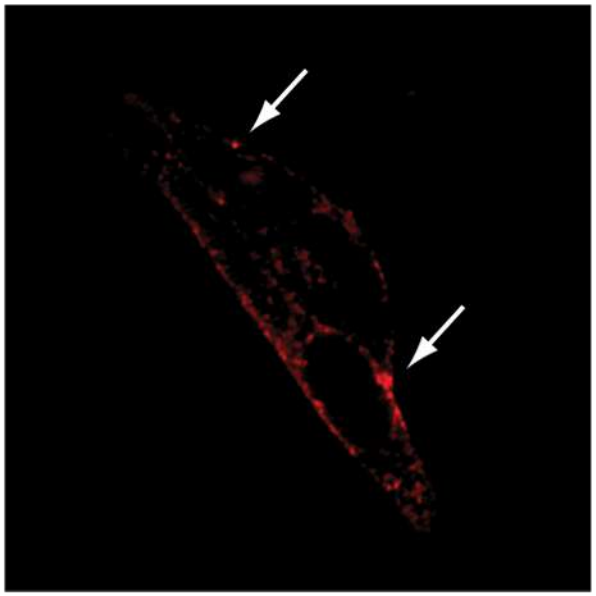
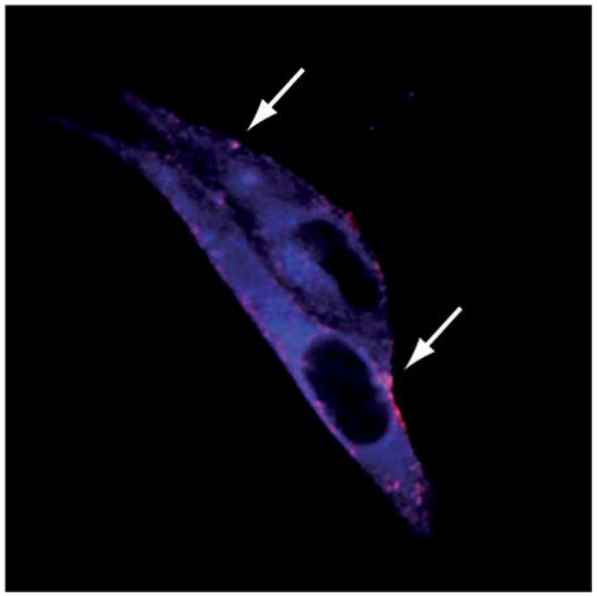
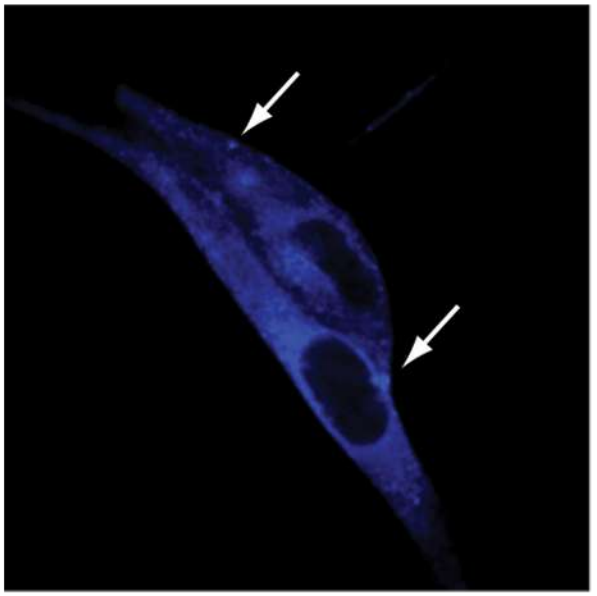
Red Channel (Alexa647) Dendrimer

Merged Channels

Blue Channel (Coumarin) Payload







Release of dyes from endocytic vesicles to the cytoplasm

Tracking both Dendrimer and Payload

Seeing a dye inside the cell doesn't always mean that the polymeric carrier is there too

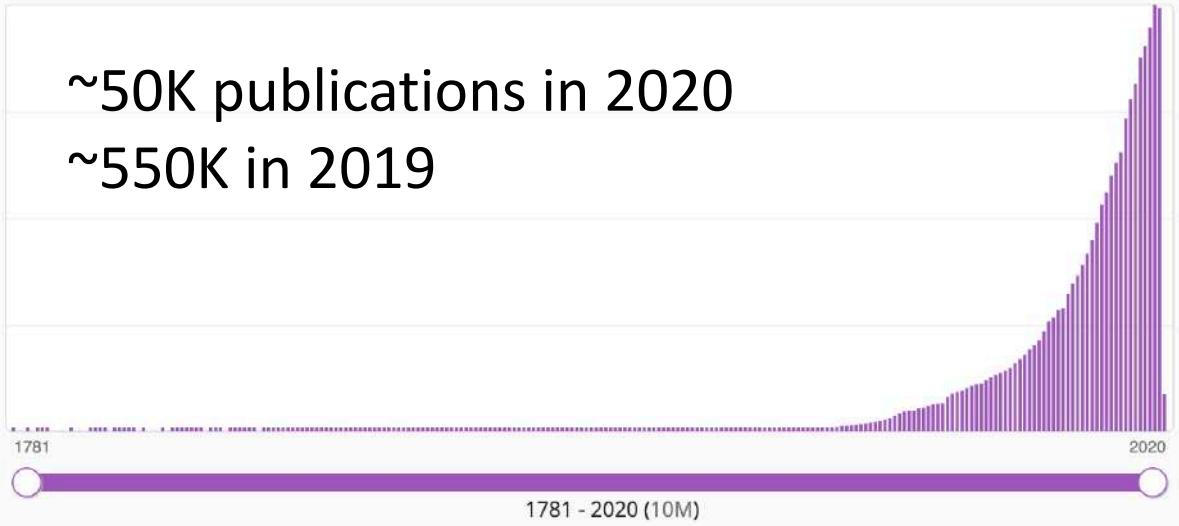
So where are we going to?

Publication Year

Searching for Delivery systems in SciFinder

~50K publications in 2020

~550K in 2019



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Does Nanomedicine Have a Delivery Problem?

Experts debate a controversial paper that suggests delivery efficiencies for cancer nanomedicines are low and not improving.

Michael Torrice

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Publication Date: July 15, 2016
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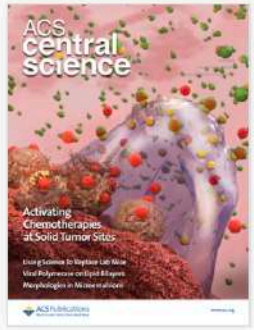

ACS Central Science

So where are we going to?

TAU

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Think together of the challenges

RETURN TO

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