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Title:	Biofunctionalization of Nanoparticles
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10.February.2010

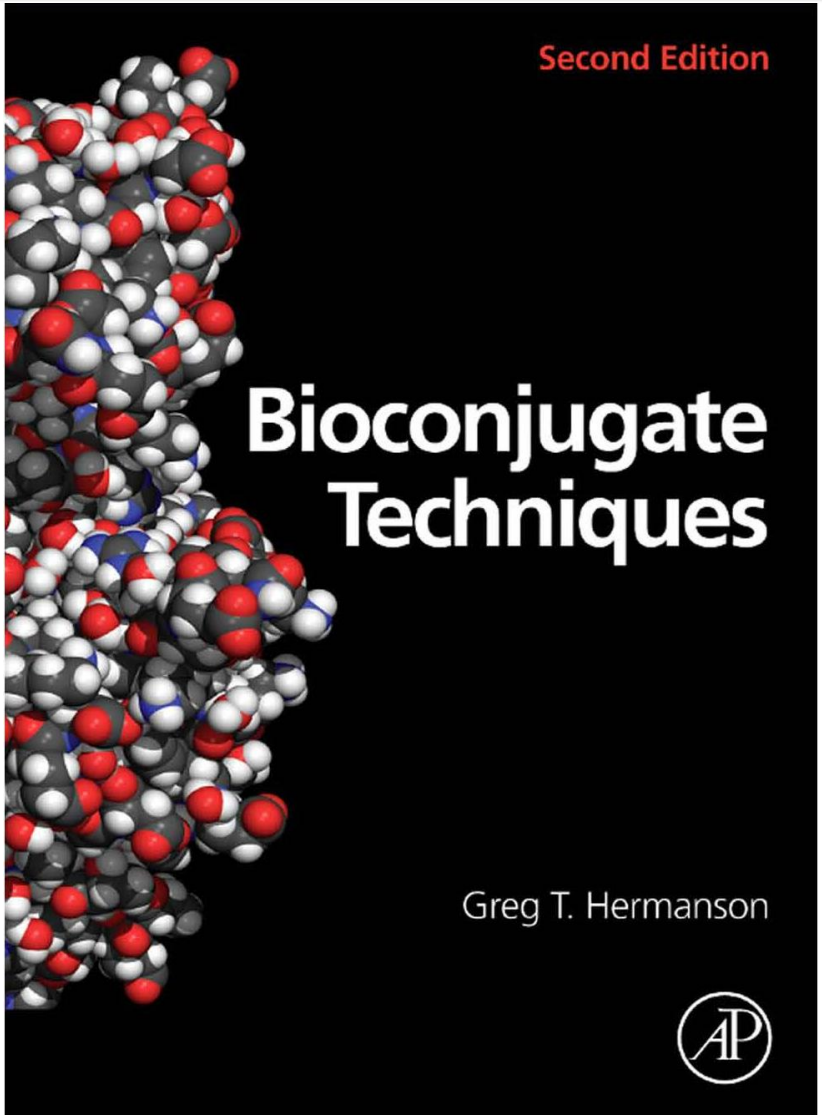
Biofunctionalization of Nanoparticles

Overview

- Adapting nanoparticles to aqueous environments
- Functionalities on particles and biomolecules
- Covalent crosslinking
- Non-covalent binding
- Case-studies

Resources

- www.piercenet.com
- Bioconjugate Techniques, 2nd Ed. Greg T. Hermanson, Published by Academic Press, Inc., 2008, 1202 pages.



Thermo of Nanoparticles

- Surface energy is important due to surface area to volume ratios
- $\Delta G_{particle} = \Delta G_{bulk} + \Delta G_{surface}$
- Colloids are thermodynamically unfavored, so surface chemistry must confer stability

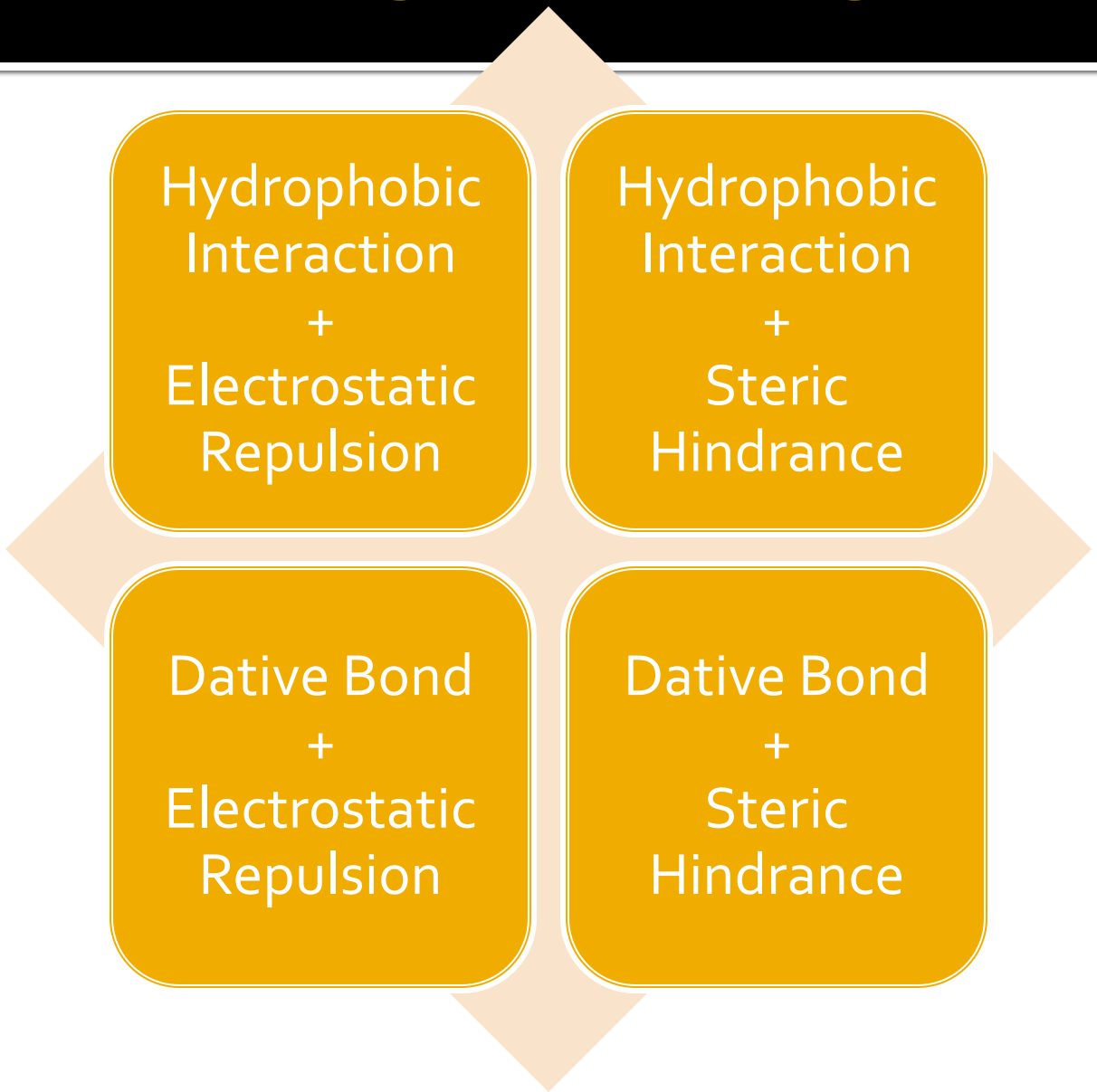
A Simple Case: Gold Nanoparticles

- Reduction of gold salt with sodium citrate in aqueous environment produces gold colloids with a negative surface charge
- Electrostatic repulsion confers water-solubility and colloidal stability

Trickier: Water insoluble particles

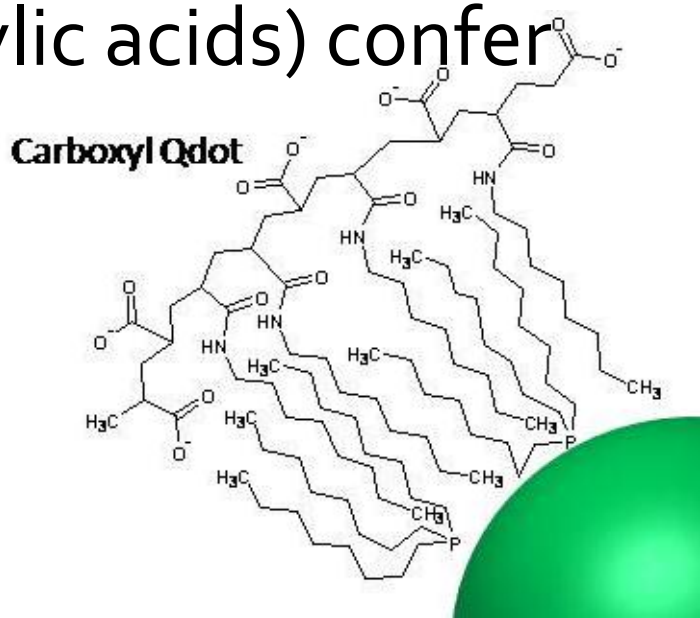
- Colloids synthesized in organic solvents
  - e.g. Quantum Dots (QDs) or Magnetic Iron Oxide Nanoparticles (MIONs)
  - Surface passivated with hydrophobic surfactants
- Stabilize via electrostatic repulsion or steric hindrance
- Attach organic coating via
  - Hydrophobic interaction
  - Dative bonds

Basic Coating Groupings



Hydrophobic Interaction + Electrostatic Repulsion

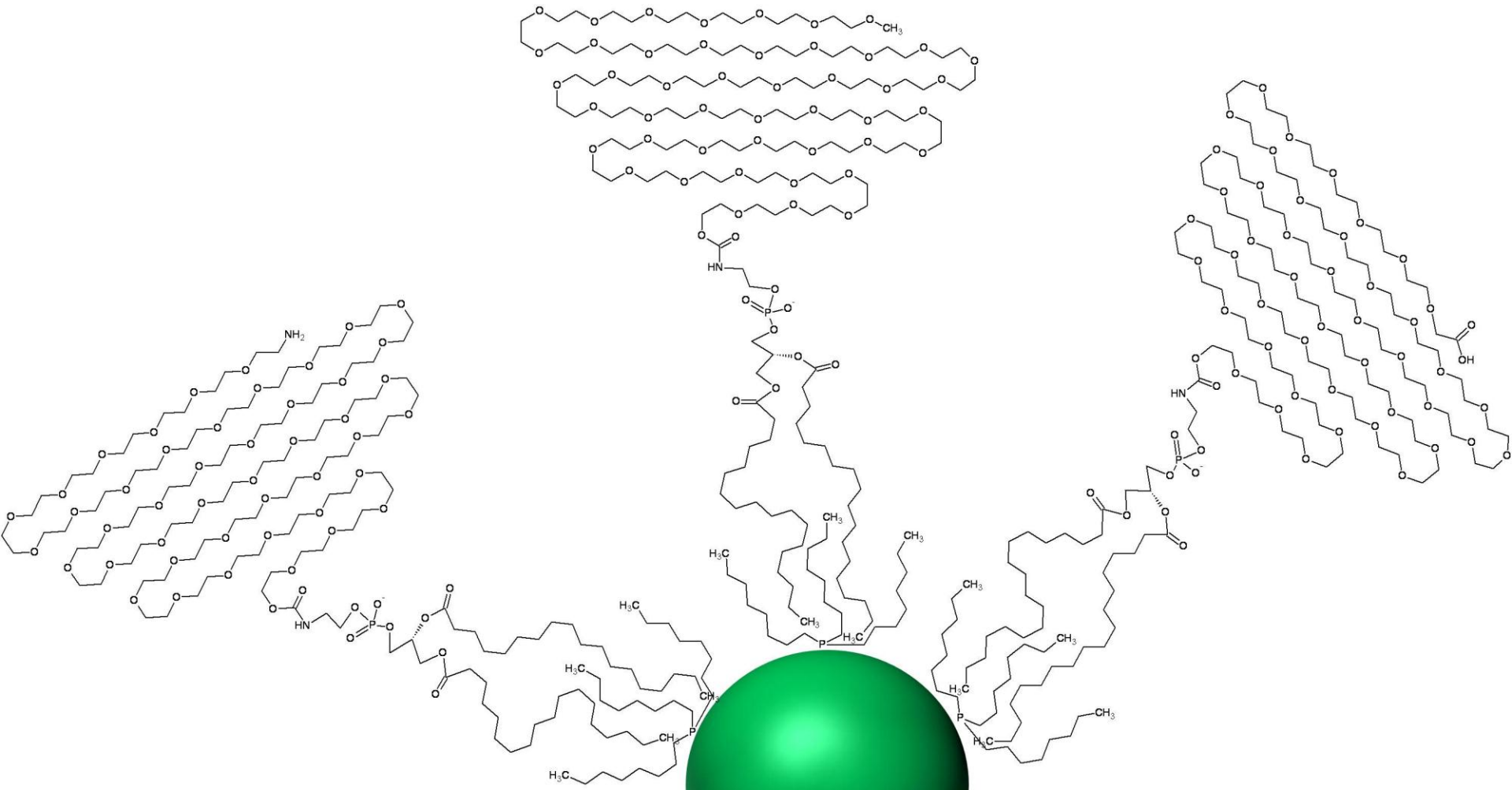
- i.e. QDs coated with amphiphilic polymers
  - Invitrogen ITK Carboxyl Qdots
- Hydrophobic portion of polymer (alkyl chains) interact with surfactant
- Charged groups (e.g. carboxylic acids) confer high surface charge density



Hydrophobic Interaction + Steric Hinderance

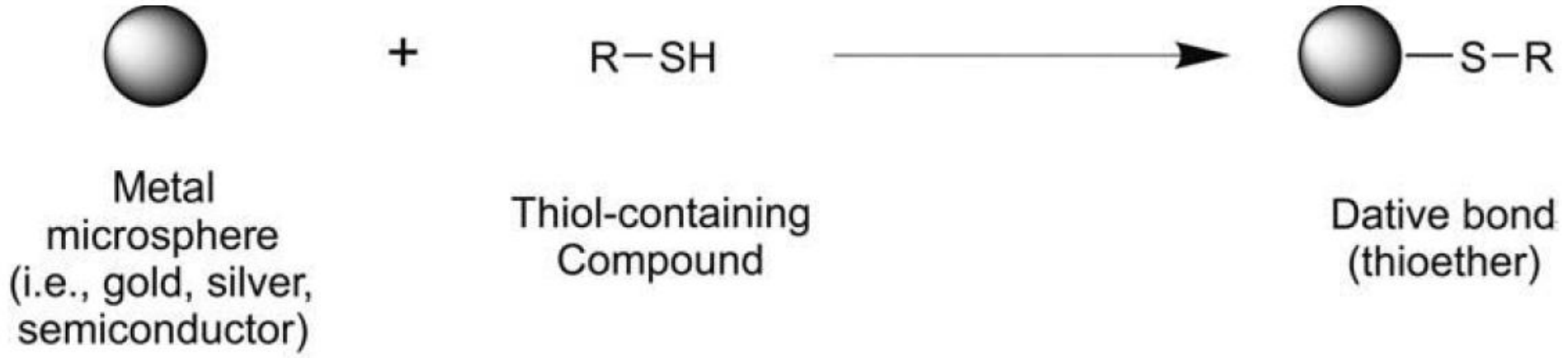
- e.g. amphiphilic coating containing PEG
  - Invitrogen ITK Amino-PEG Qdots
  - Lipid-PEG coated QDs, MIONs
- Hydrophobic portion (alkyl chains or lipid) interact with surfactant
- Hydrophilic PEG confers water stability and reduces non-specific interactions through steric hindrance

Hydrophobic Interaction + Steric Hinderance



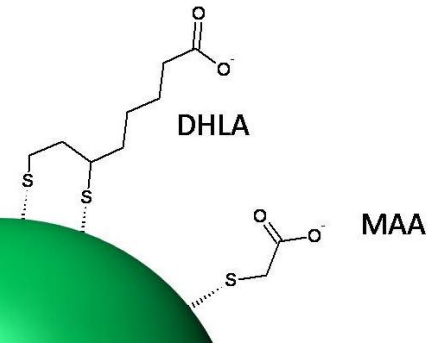
Dative Bond

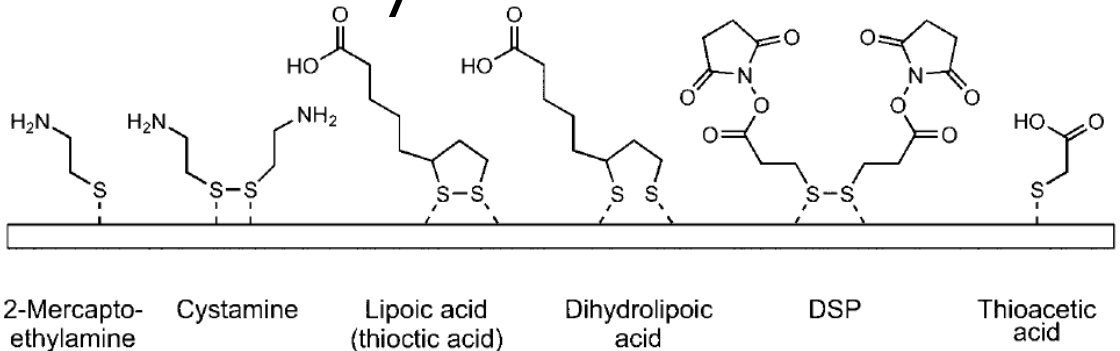
- aka coordinated covalent bond
- Two electrons come from single atom, rather than two atoms each sharing one electron
- e.g. free electron pair on sulfur atom of thiols



Dative Bond + Electrostatic Repulsion

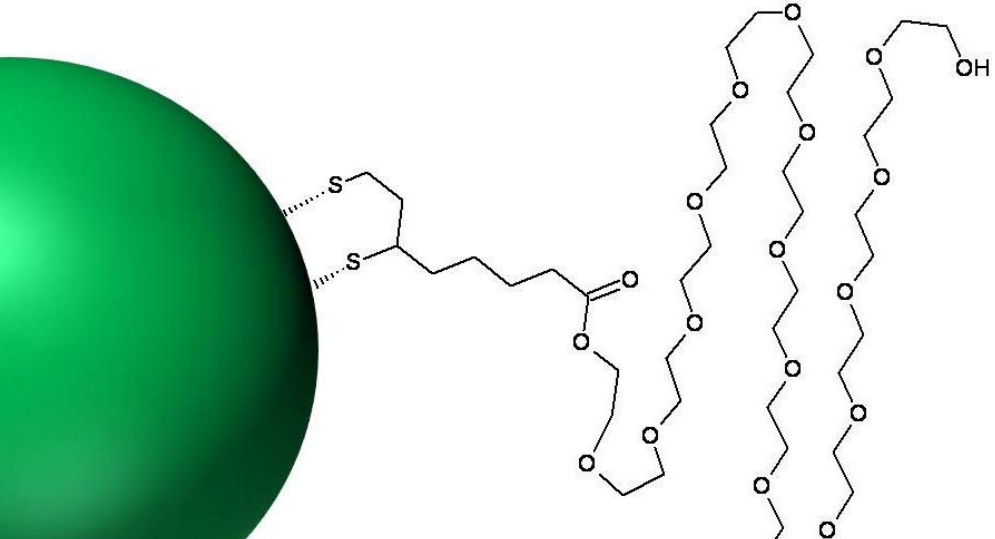
- Thiols + charged group
- Hydrophobic surfactant removed from surface
- Thiol binds to surface of particle (gold, QD)
- Charged group confers water solubility
- Small molecule → thin coating
- Dithiols improve stability over time

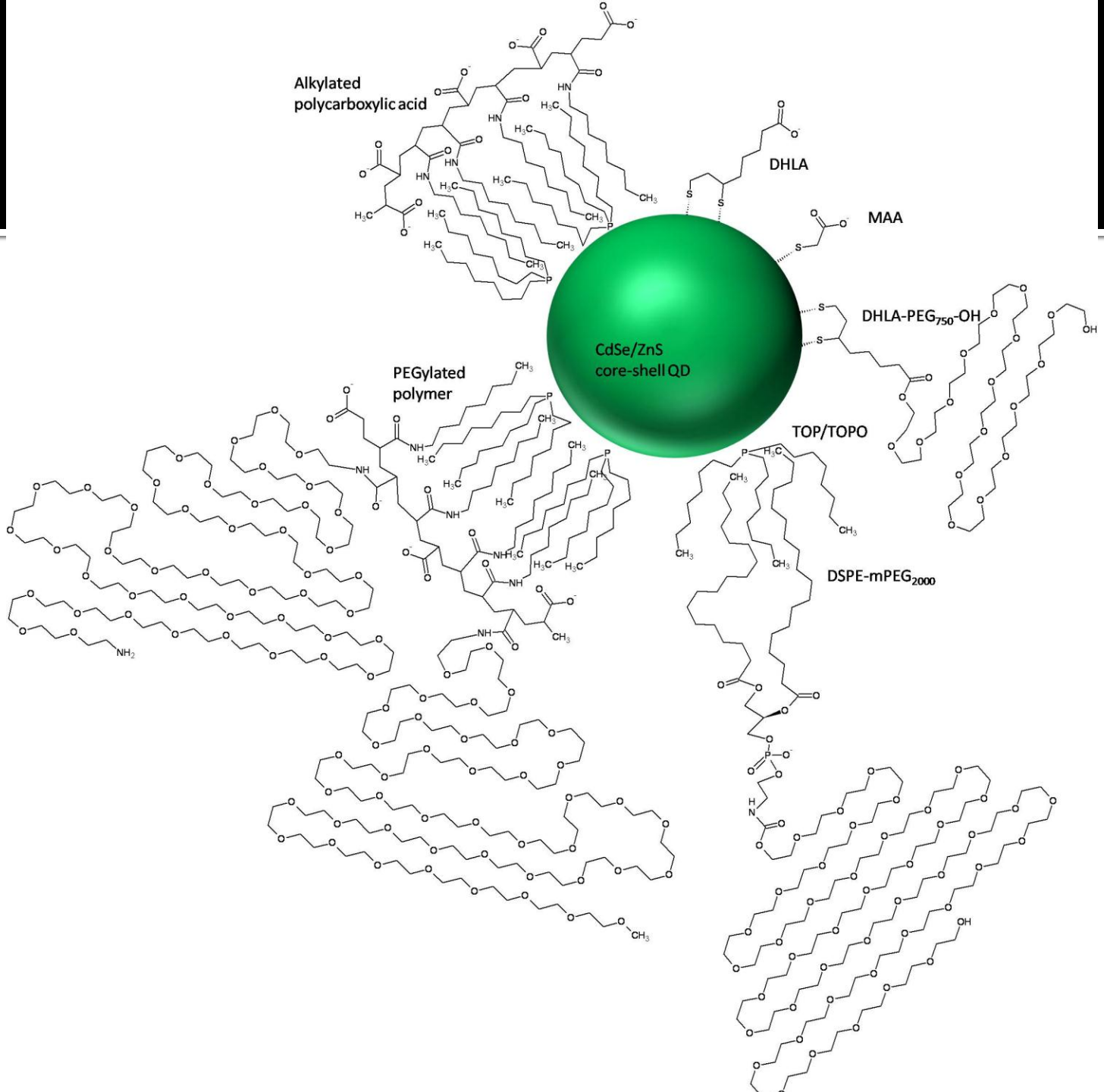




Dative Bond + Steric Hindrance

- PEGylated thiols/thiolated PEGs
- Relatively thin coating and reduced non-specific binding
- Heterobifunctional PEGs result in a PEG coating with another functional group




Alkylated
polycarboxylic acid
DHLA
MAA
DHLA-PEG750-OH
CdSe/ZnS
core-shell QD
PEGylated
polymer
TOP/TOPO
DSPE-mPEG2000

Electrostatically-stabilized QDs

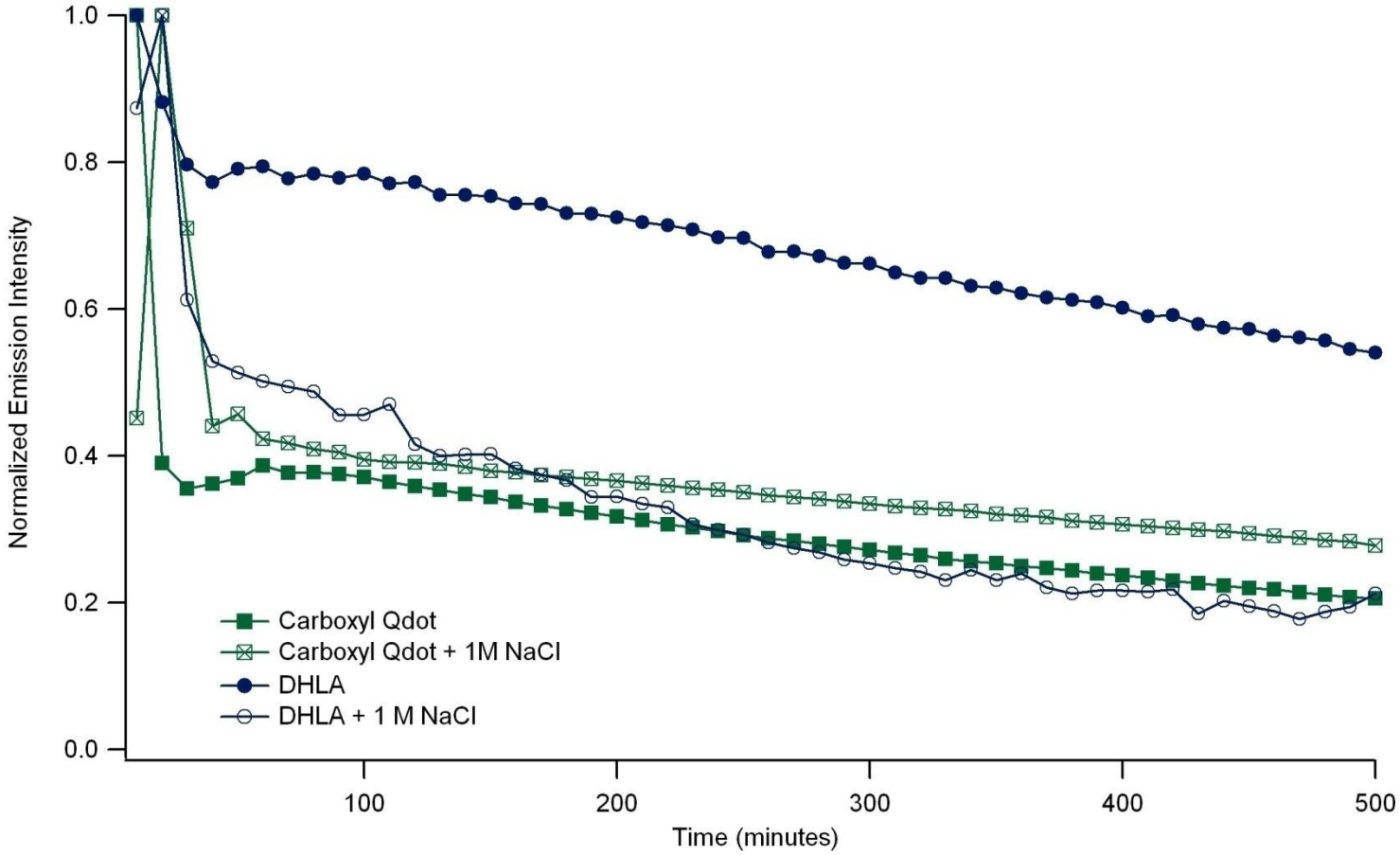
Dative Bond

- Smallest possible coating
- QDs: lower QY (~0.1-0.2)
- Sensitive to pH, salt concentration

Hydrophibic Interaction

- Larger particle size
- QDs: higher QY (~0.8)
- Better environmental stability; some steric hindrance

QDs in high salt buffer



Sterically-stabilized QDs

Dative Bond

- Thinner coating
- QDs: lower QY (~0.1-0.2)
- Prevents non-specific interactions

Hydrophibic Interaction

- Large particle size
- QDs: higher QY (~0.8)
- Prevents non-specific interactions

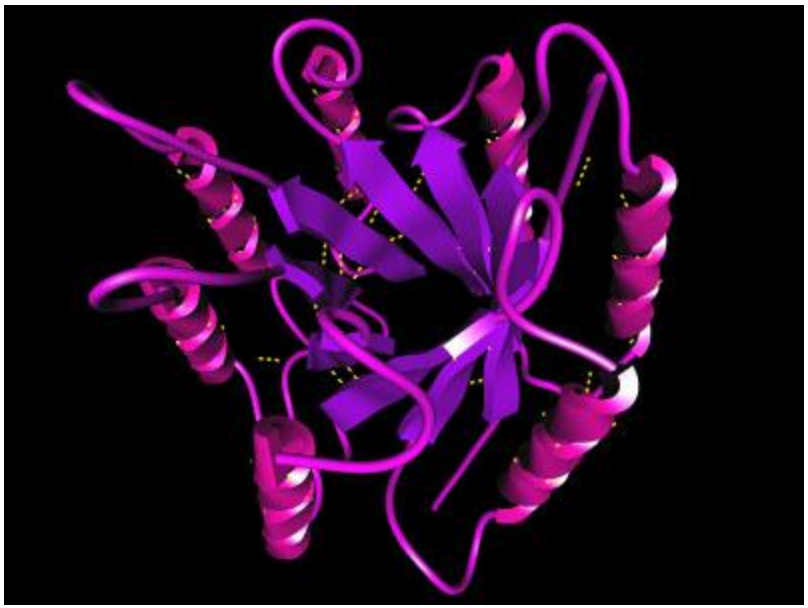
Functional groups on particle surface

- Coating dependent!
- Electrostatic: COO^-, NH_3^+
- Steric: just about anything…
  - COO^-, NH_3^+ , biotin, streptavidin, maleimide, …

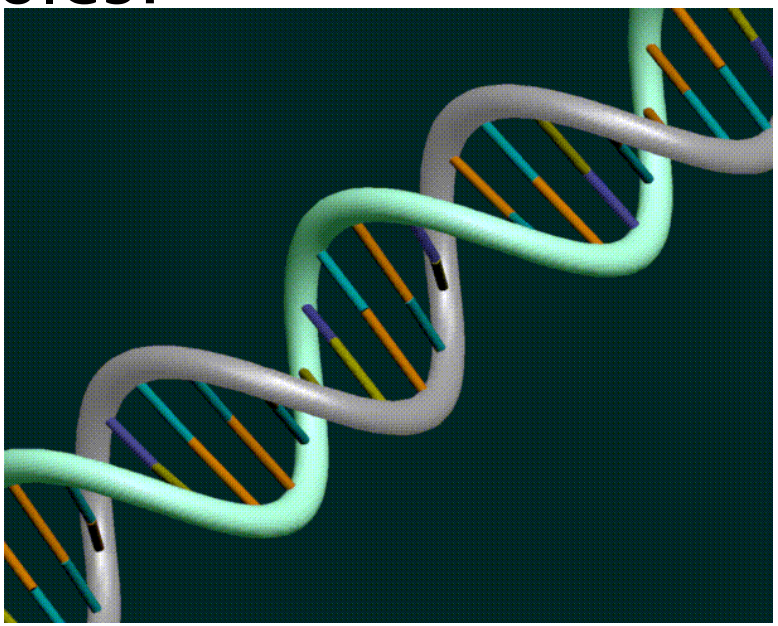
- What kinds of bio-friendly bonds can we make with these functional groups?

Biochemistry Review…

- Biofunctionalization involves the attachment of biomolecules to the nanoparticles…
- What functional groups are available on the most common biomolecules?



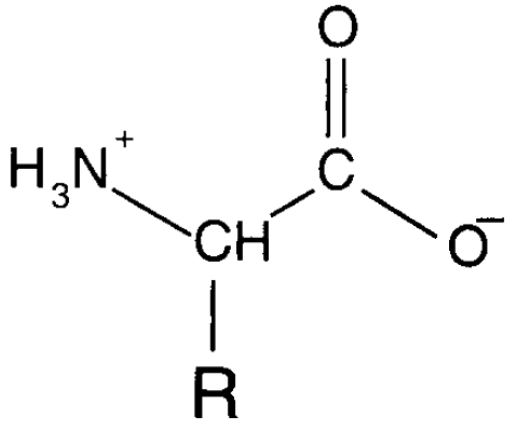
vis.lbl.gov/Research/ProteinShop/index.html



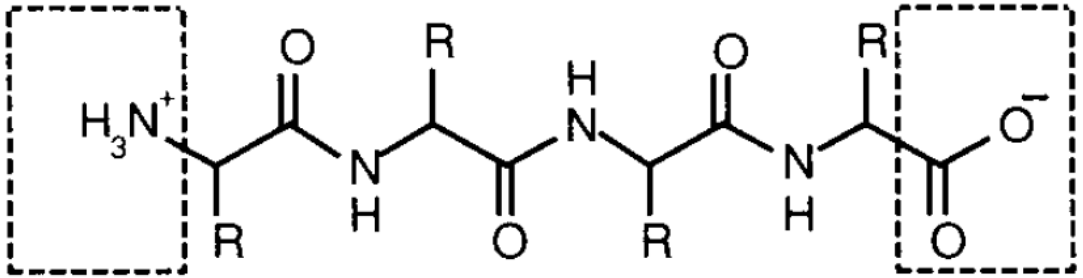
http://www.csb.yale.edu/userguides/graphics/ribbons/help/dna_rgb.html

Primary Structure of Proteins

- Polymer of amino acids
  - 20 natural monomers
    - Non-polar (hydrophobic)
    - Polar
    - Charged

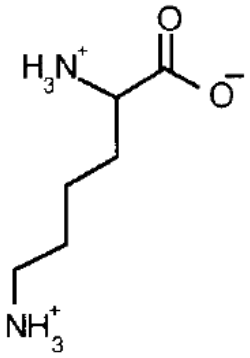


N-Terminal

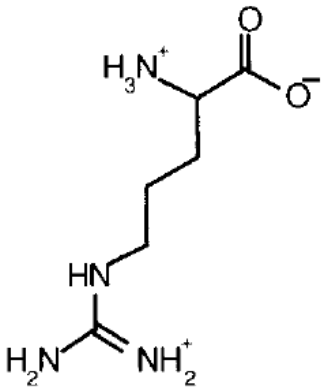


Polypeptide

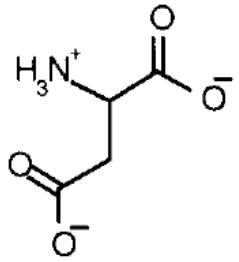
Notable Amino Acids



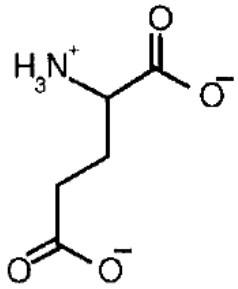
Lysine



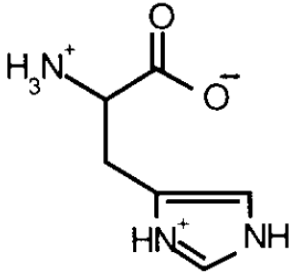
Arginine



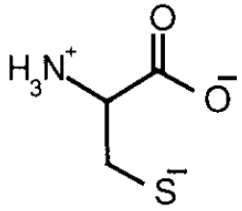
Aspartic Acid



Glutamic Acid



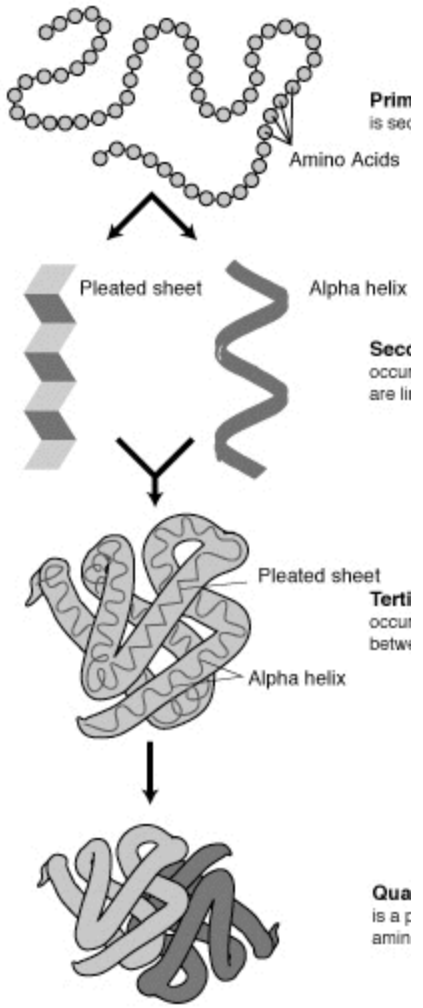
Histidine



Cysteine

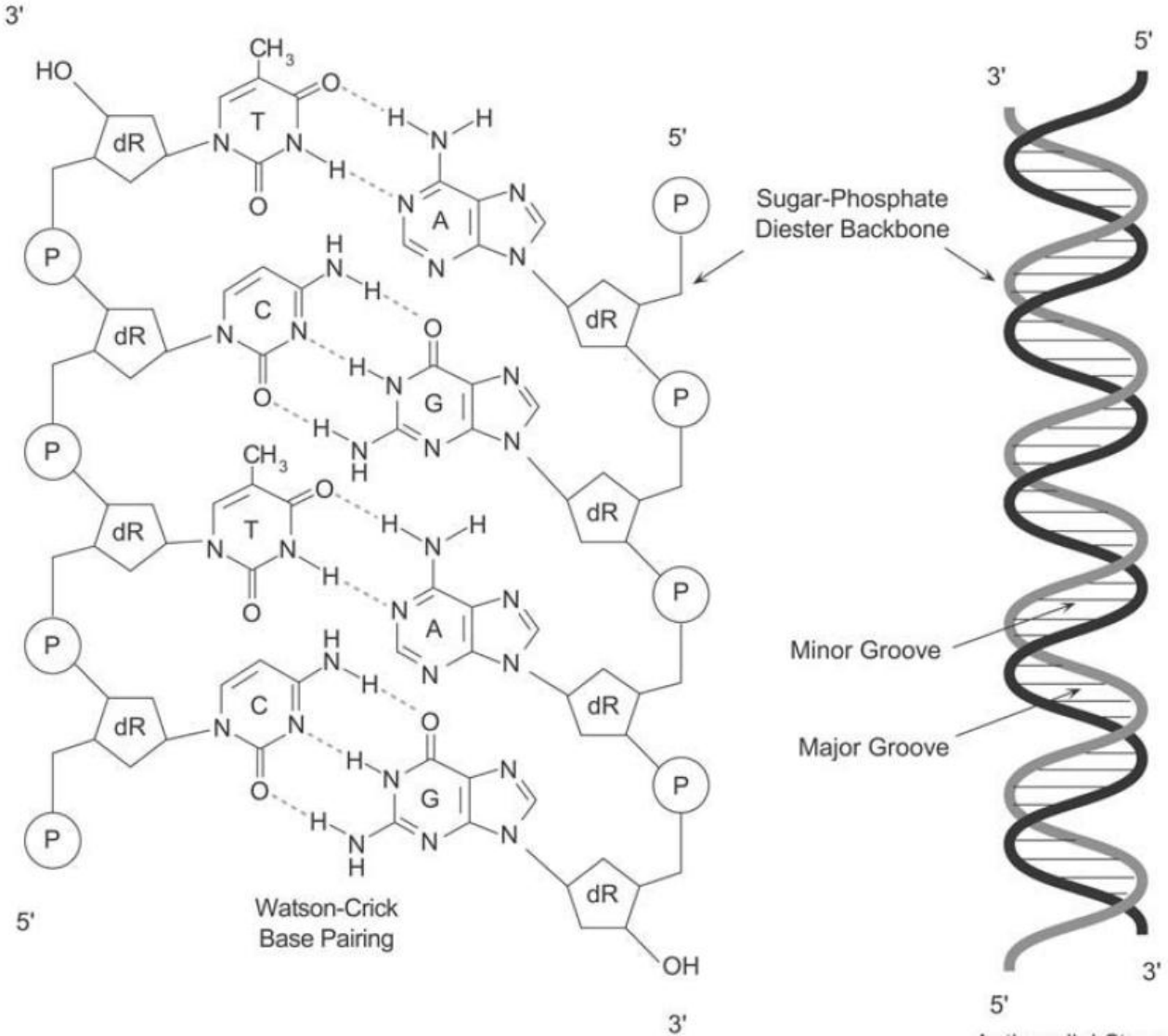
Protein Secondary Structure

- 2˚ structure:α-helices and β-sheets
  - Stabilized through hydrogen bonding
- 3 ˚ structure: folded proteins
  - Hydrophobic interactions ,salt bridges, and disulfide bonding further stabilize structure
- 4 ˚ structure: multiple subunits



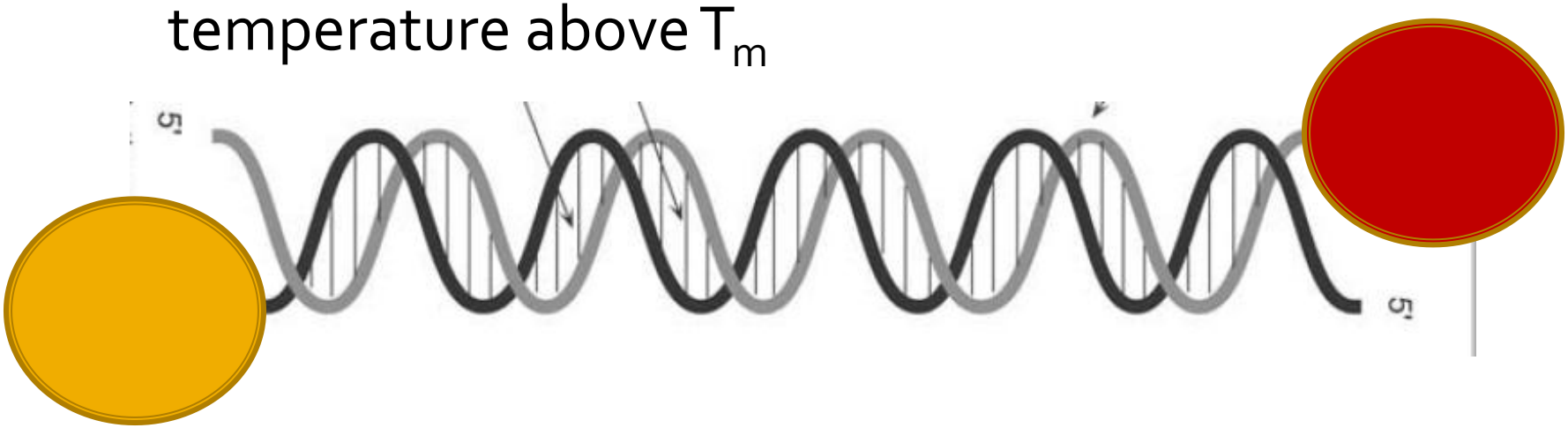
http://en.wikipedia.org/wiki/Protein_structure

Nucleic Acid Structure



Utilizing Complimentary DNA

- Nucleic acid hybridization can be used to reversibly bind two components
  - Strength of interaction depends on length and G/C content of complimentary sequence
  - Disrupt hydrogen bonding with high salt or raising temperature above T_m

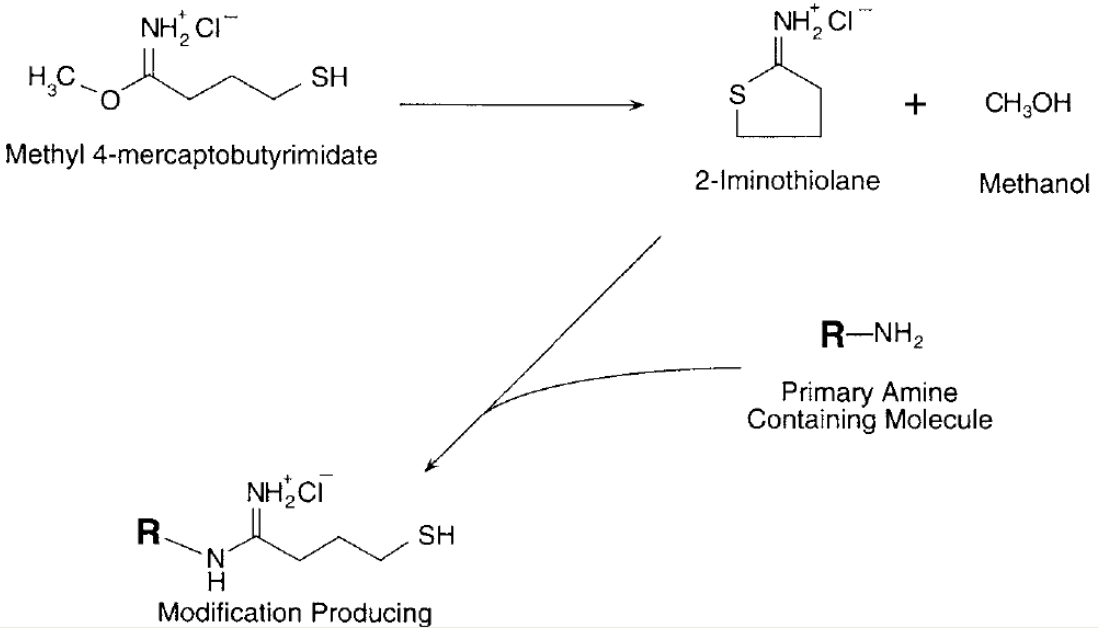


Functionalized NA Sequences

- No amine, carboxyl, or thiol groups intrinsic to nucleic acid structures
- Modified NAs can be incorporated into oligomer synthesis…
  - e.g. Integrated DNA Technologies (www.idtdna.com)
  - Incorporate amines, thiols, or biotin with various linker lengths

Converting Functional Groups

- Create functional groups to suit the application
- Extra step, but could improve control of downstream reactions



Linking functional groups

- Covalent bonds
  - Zero-length crosslinkers
  - Homobifunctional crosslinkers
  - Heterobifunctional crosslinkers

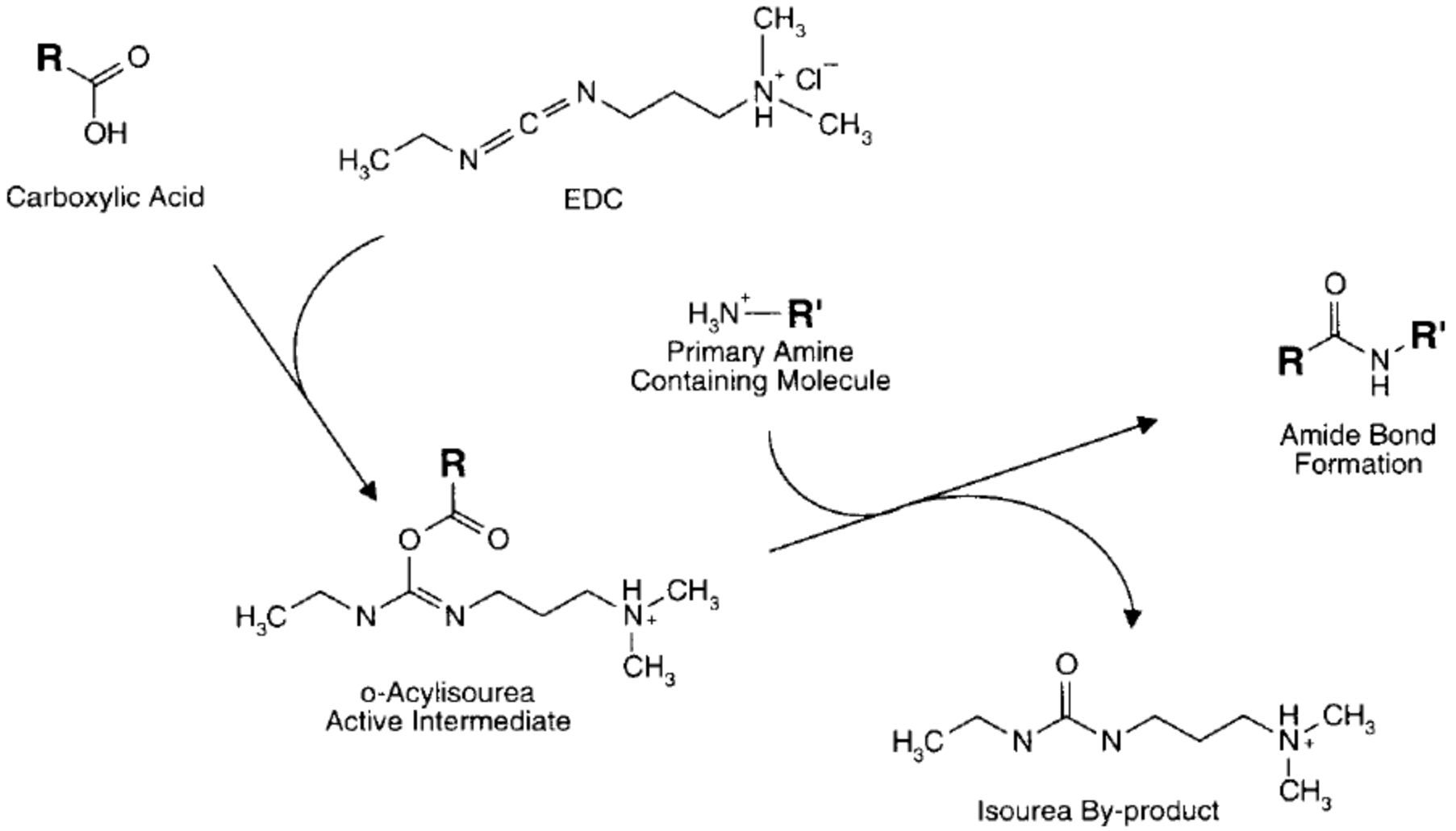
Zero-length Crosslinkers

- Two compounds linked without the addition of extra atoms– no linker atoms
- Reagents added to mediate the reaction by forming active intermediates

Carbodiimides

- Link amines with carboxylates or phosphates
  - Two protein molecules
  - Protein + peptide
  - Protein + oligonucleotide
  - Biomolecule + particle surface
  - Protein/peptide/oligonucleotide/particle + small molecule
- Water-soluble and water-insoluble varieties

Most Common: EDC



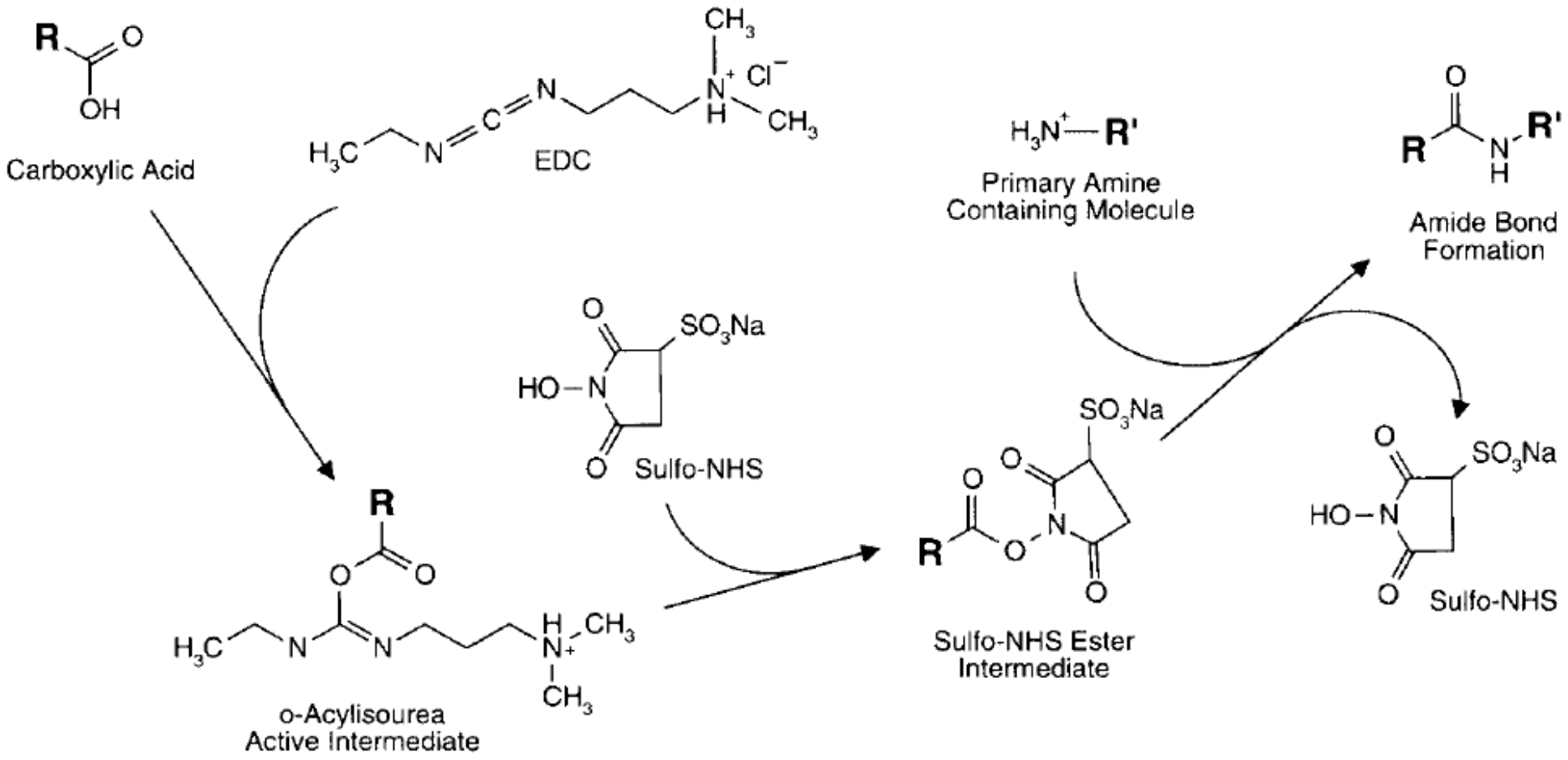
Most Common: EDC

- Reaction most favored at pH 4.5-7.5
- Hydrolysis occurs faster at lower pH
- If mix Protein A + EDC…
  - Polymerization of the protein! Proteins contain both carboxylates and amines
- Avoid buffers with carboxylates and amines
  - TRIS
- Active intermediate reacts slowly with amines and is prone to hydrolysis…

Improving Reaction Efficiency

- Sulfo-NHS – water soluble reagent that improves the solubility and stability of the active intermediate
- Same reaction product as with EDC alone, but improved efficiency

Improving Reaction Efficiency



One-Step Reaction

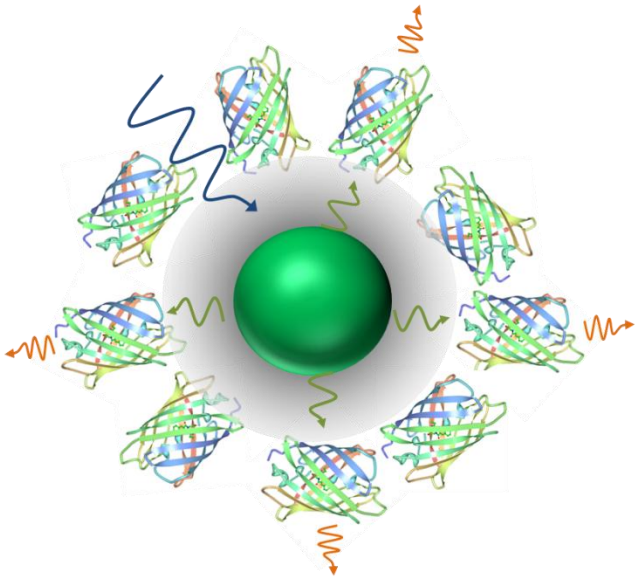
- Carboxyl QDs + Protein + EDC + S-NHS
  - Mixed and reacted for 2 hrs at R.T. or O/N at 4˚C
  - Byproducts and excess reagents/unbound protein removed with a centrifugal filtration device
- Pros: straight-forward, minimal manipulation of the particles, efficient
- Cons: Polymerization of the protein on and off the particle

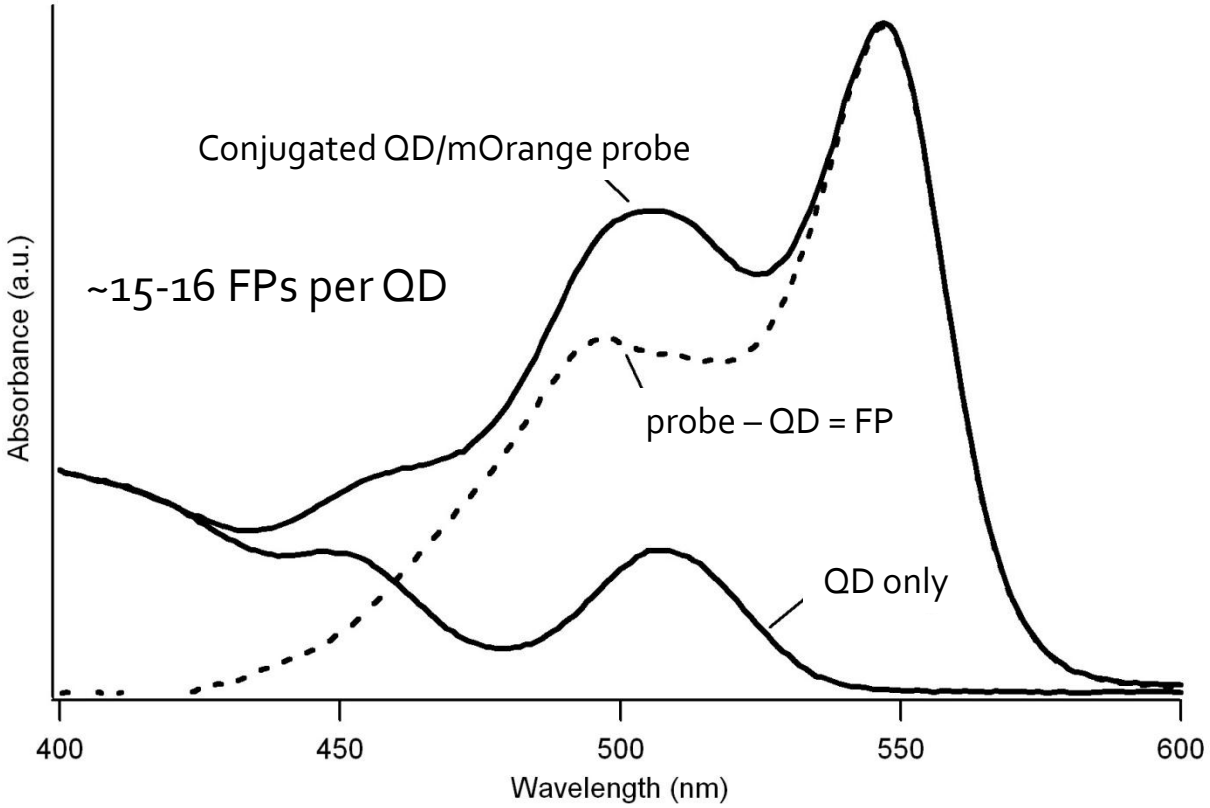
Two-step Reaction

- Carboxyl QDs + EDC + S-NHS
  - Carboxylate activated; excess EDC then quenched with β-mercaptoethanol
  - Nanoparticle prep could be cleaned up prior to second step or protein can be added directly
    - Why clean up first?
- Pros: Eliminates protein polymerization
- Cons: More susceptible to particle aggregation

pH Sensor Construction

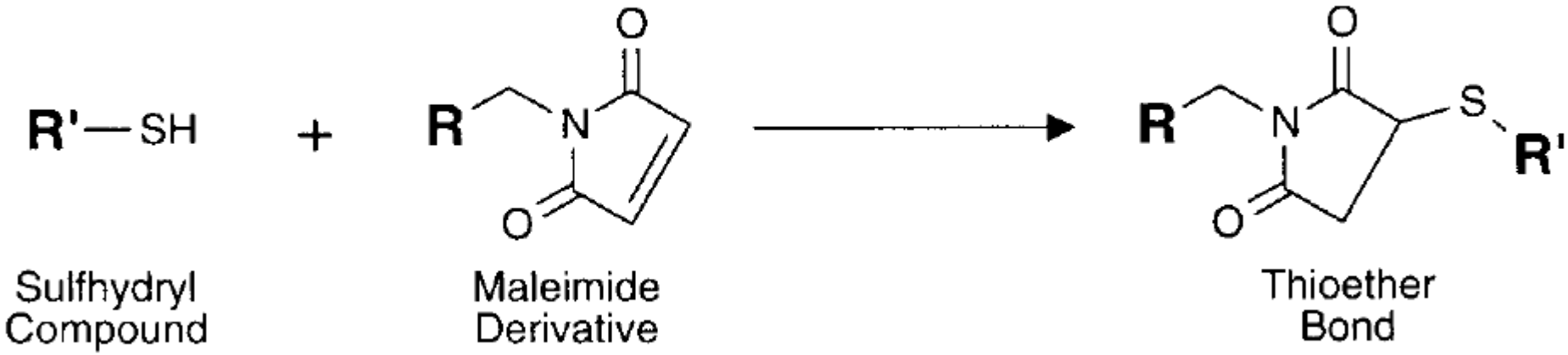
- EDC chemistry to covalently bind proteins to 525 nm Carboxyl Qdots





Thiols react with maleimide

- Maleimide reacts specifically with thiols at a pH range of 6.5-7.5
  - Reactivity with amines increases at higher pH
- Competing reaction: hydrolysis of maleimide
  - faster at higher pH



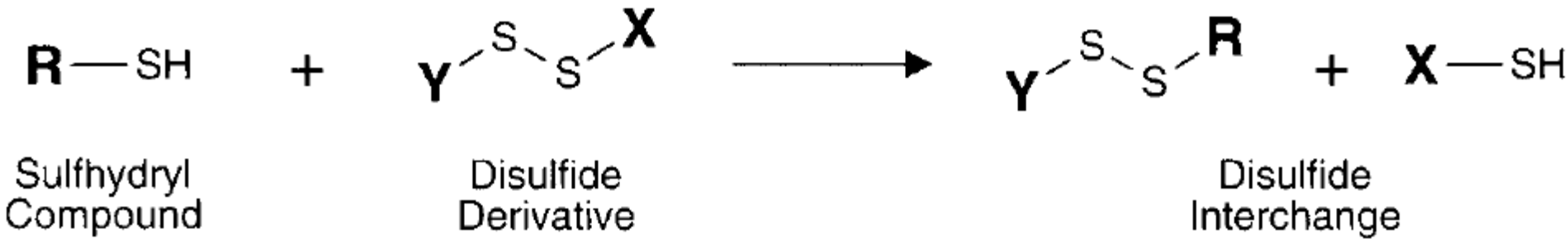
Maleimides on Nanoparticles

- Lipid-PEG-maleimide from Avanti used to coat MIONs
- Peptidic ligand for cell surface receptor VCAM1 with terminal cysteine synthesized
- MIONs + peptide = MRI imaging agent that can be targeted to vascular endothelial cells that overexpress VCAM1 due to cardiac disease

Disulfide Exchange

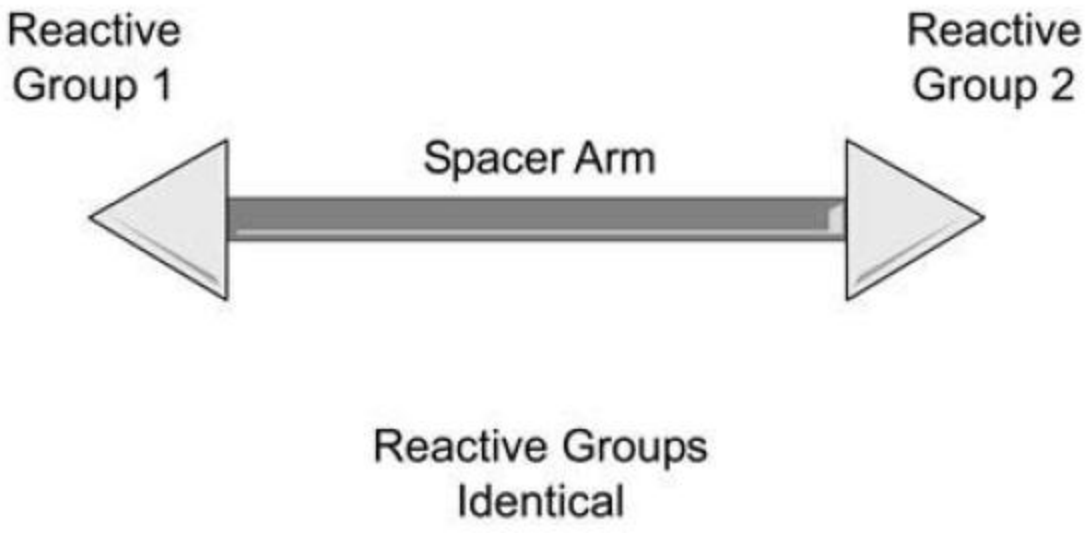
- Reversible reaction
  - Disulfide reducing reagents include DTT and TCEP
- Occurs over a wide range of conditions

- Allows for release of a compound for analysis or *in vivo* for bioactive agents



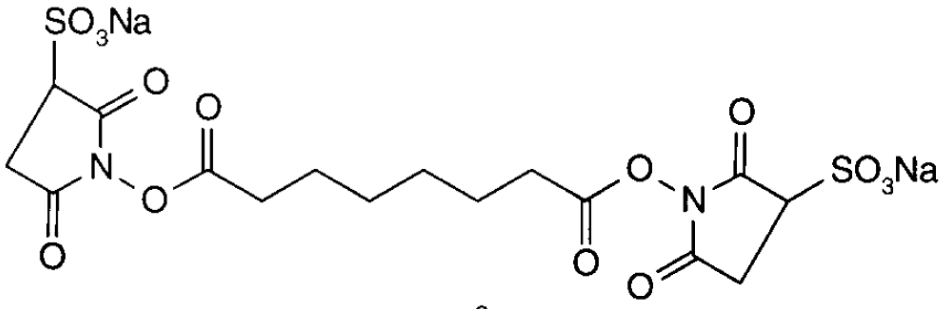
Homobifunctional Crosslinkers

- 2 identical reactive groups located on opposite ends of an organic spacer arm
  - e.g. bind protein amines to other protein amines

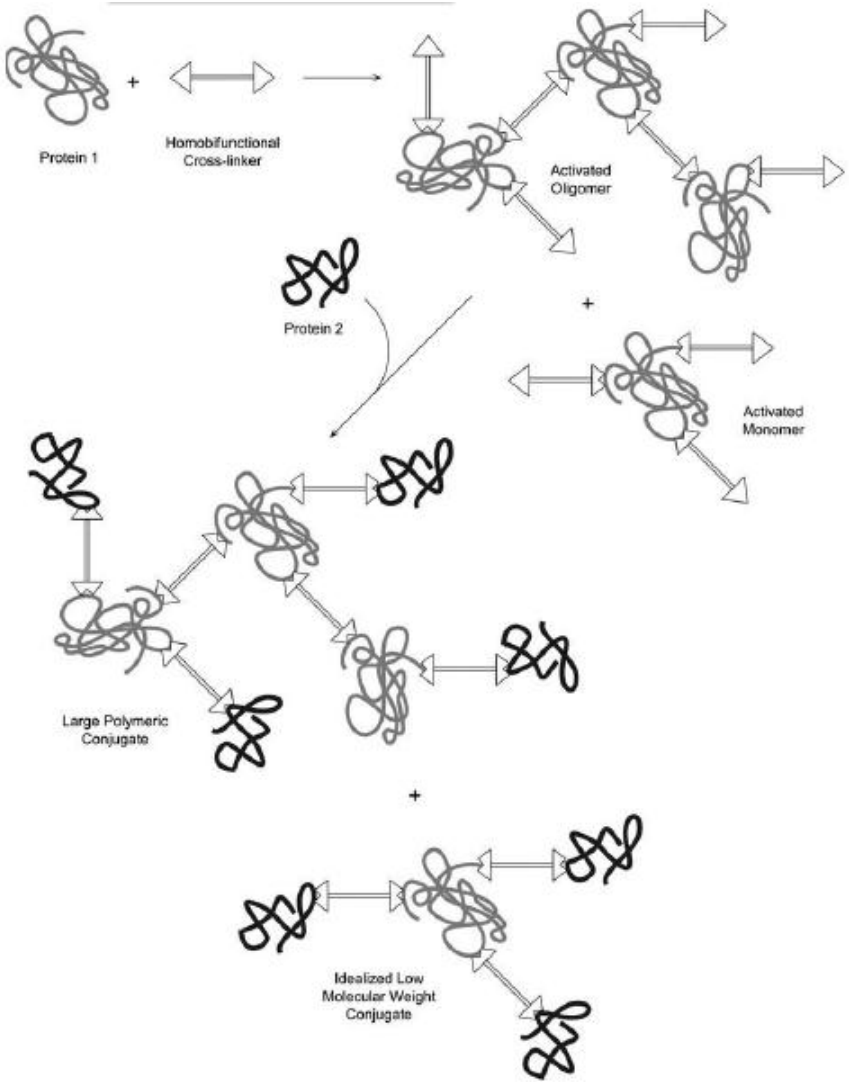


Homobifunctional Crosslinkers

- Little control over reaction products…
- Protein A + Protein B + homobifunctional NHS ester

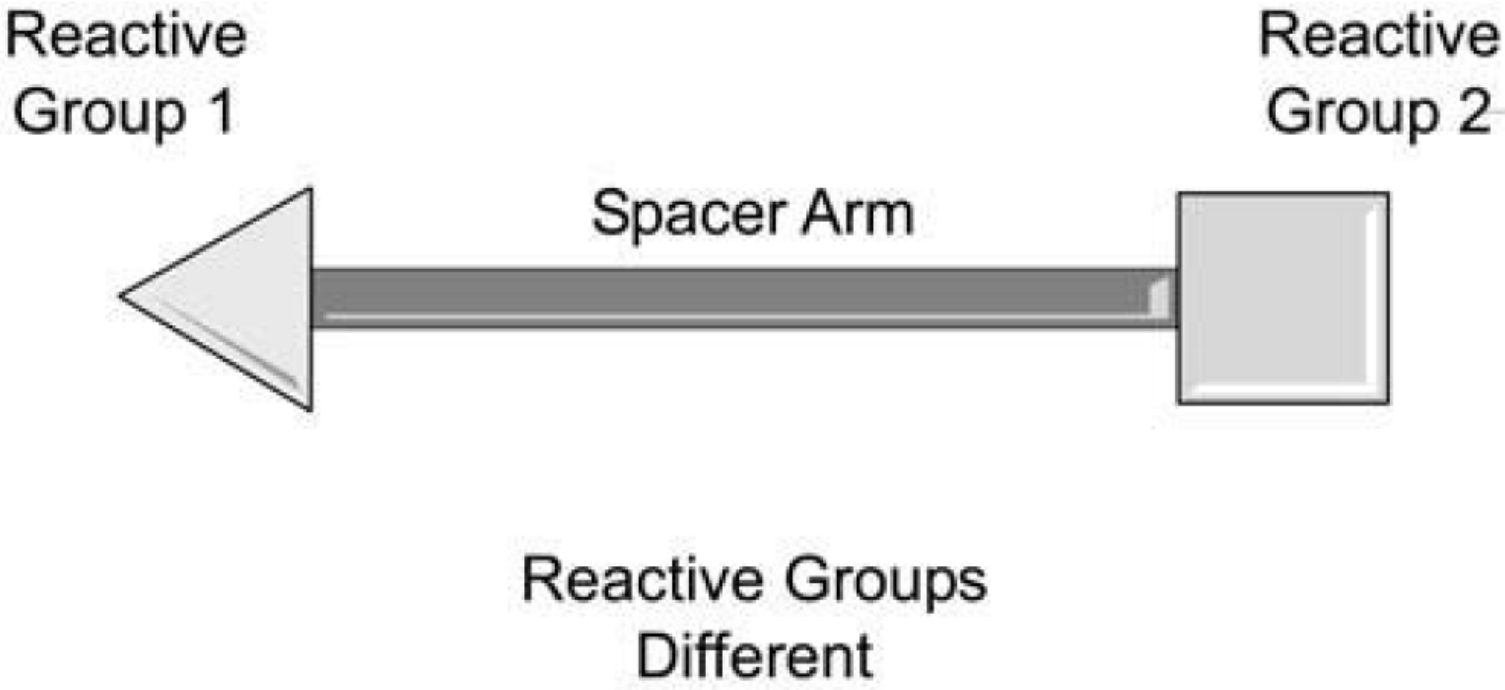


BS^3
Bis(sulfosuccinimidyl)suberate
Water Soluble
MW 572.43
11.4 Å

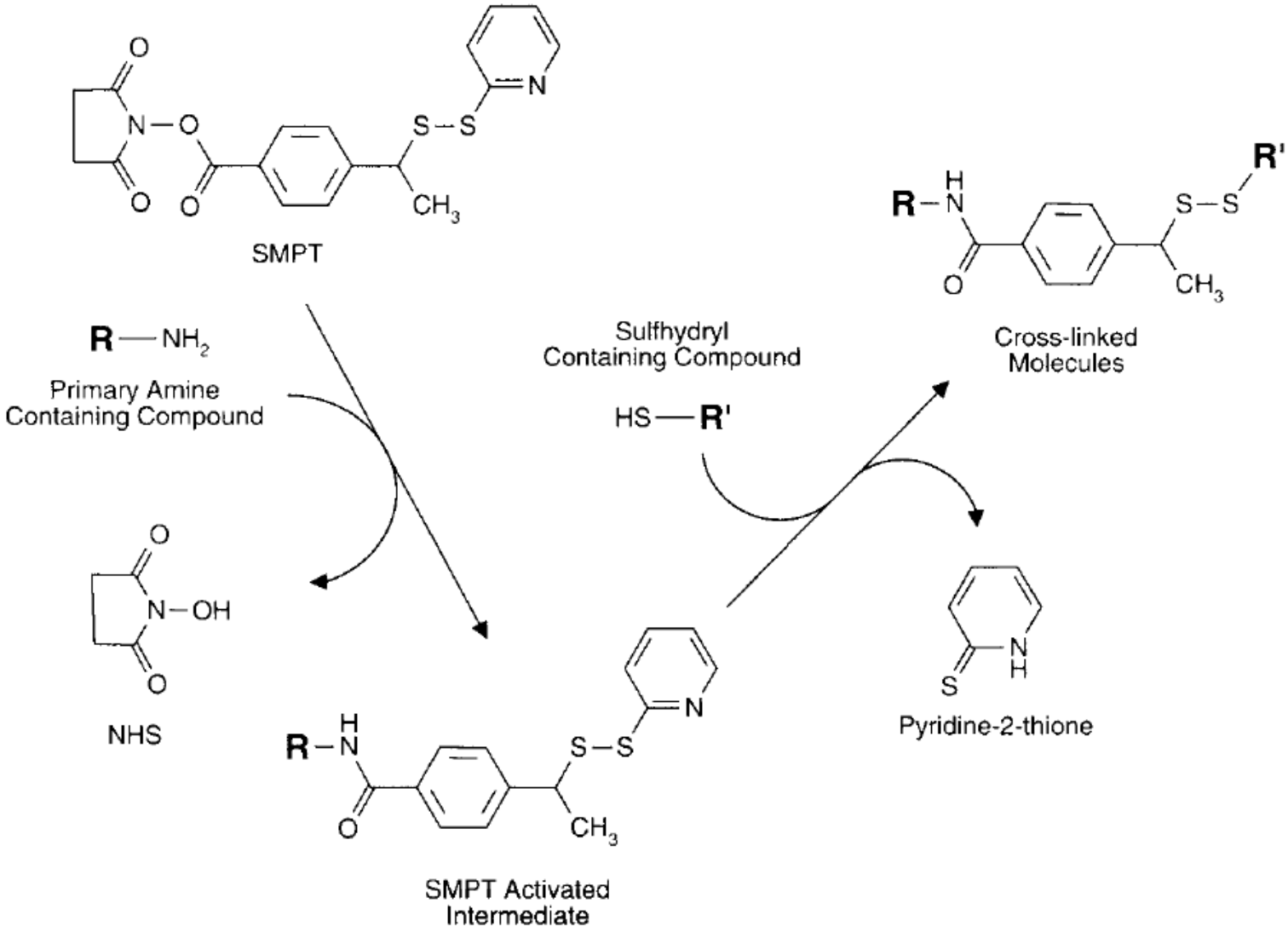


Heterobifunctinal Crosslinkers

- 2 different reactive groups located on opposite ends of an organic spacer arm
  - e.g. bind protein amines to other peptidyl thiols

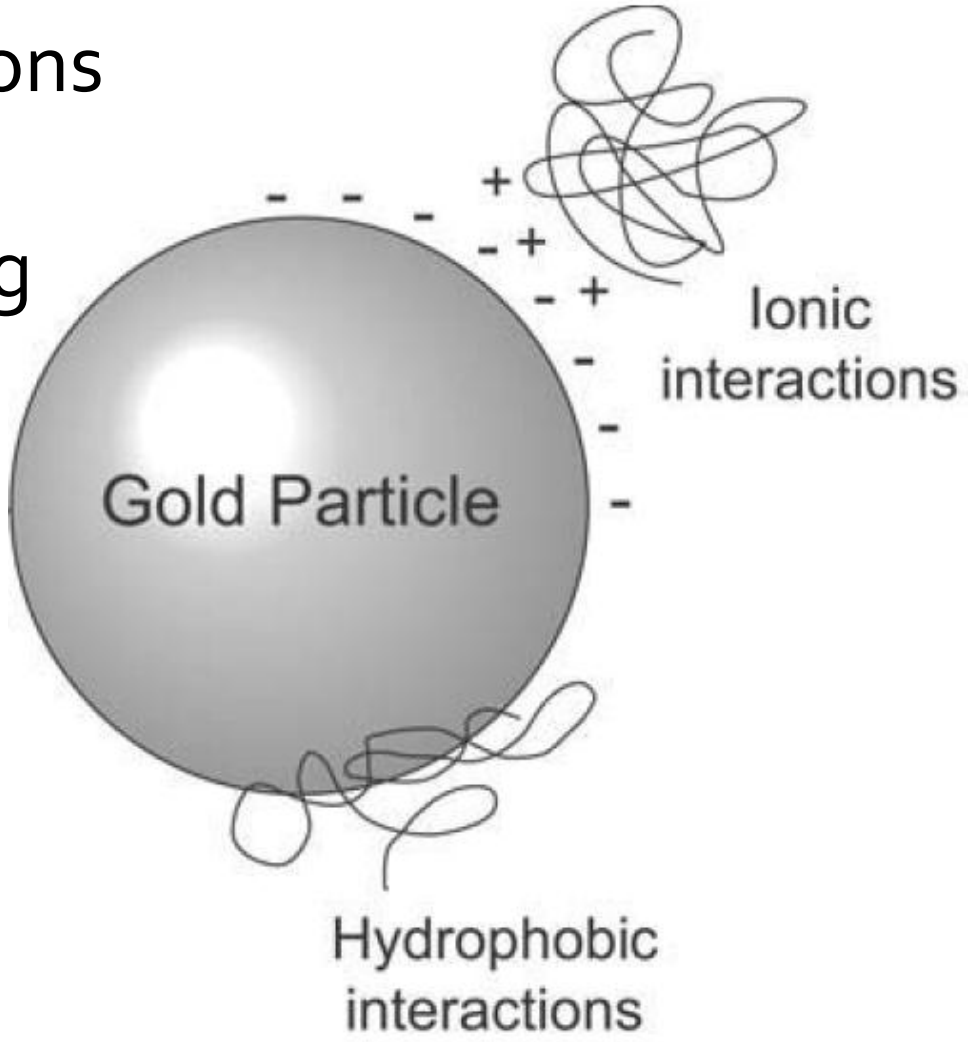


Heterobifunctional Crosslinker



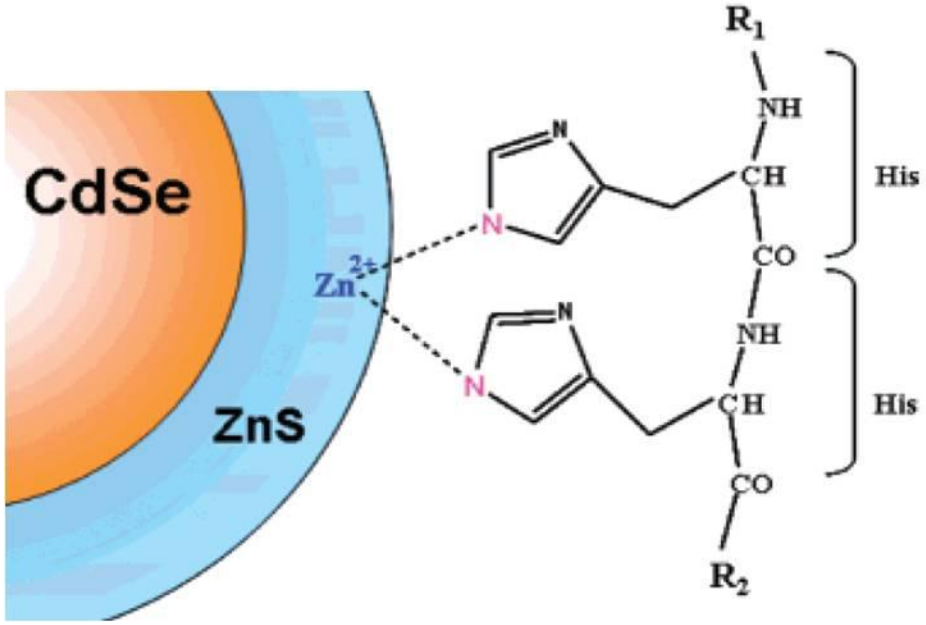
Non-covalent Biofunctionalization

- Hydrophobic interations
- Ionic interactions
- Affinity-based binding
- His-tag coordination



Polyhistidine Coordination

- If QD surface accessible, his-tag coordinates to Zn^{2+} in ZnS shell
- Assembly demonstrated with DHLA QDs[a] and carboxyl-functionalized lipid-PEG QDs[b]



[a]Sapsford et al. (2007). *J Phys Chem C* 111(31):11528 – 11538. [b]Dennis and Bao (2008). *Nano Letters* 8(5):1439-1445.

Advantages of His-tag Binding

- Same tag used for protein purification (IMAC) and bioconjugation
- nM affinities comparable to some antibody-antigen interactions
- No purification necessary (no byproducts or excess reactive species)
- Controllable molar ratios
- Fast– binding equilibrium reached within 15 minutes

Disadvantages of His-tag binding

- Non-covalent
- Possibly disrupted in acidic environment, by excess of imidazole or other coordinating agents
- May not withstand rigorous purification
- Only feasible when M^{2+} ions accessible
  - Particle surface accessible because of thin coating and/or defects
  - Ni^{2+}-NTA bound to surface, presenting ions

Streptavidin-biotin

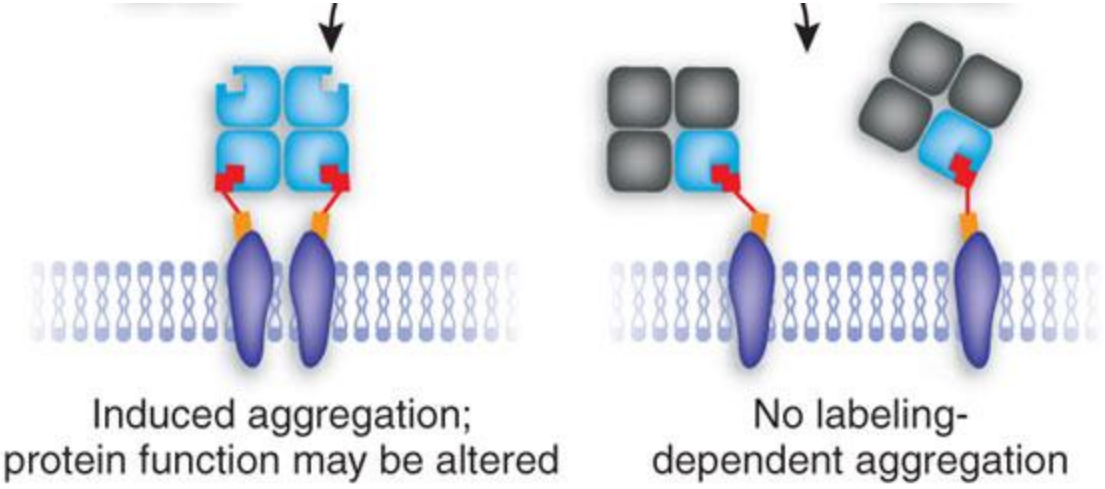
- Streptavidin: tetrameric protein with binding pockets for the small molecule biotin
- Commercially-available streptavidin-coated nanoparticles, e.g. Qdots from Invitrogen
- Biotin is a common modification for custom peptide and nucleotide syntheses
- Biotinylation kits enable in-house biotinylation of biomolecules
- Genetically engineered tags for enzymatic biotinylation of recombinant proteins

Streptavidin-biotin

- Pros: Shake-and-bake chemistry, extremely high affinity, easily accessible reagents, very specific reaction
- Cons: addition of large protein to nanoparticle surface increases size of particle, some applications require minimized distances between components (FRET)

Streptavidin-biotin

- Issue: four binding sites can lead to aggregation of biotinylated protein
- Solution: engineered monovalent Streptavidin



Nature Methods **3,** 247 - 248 (2006)

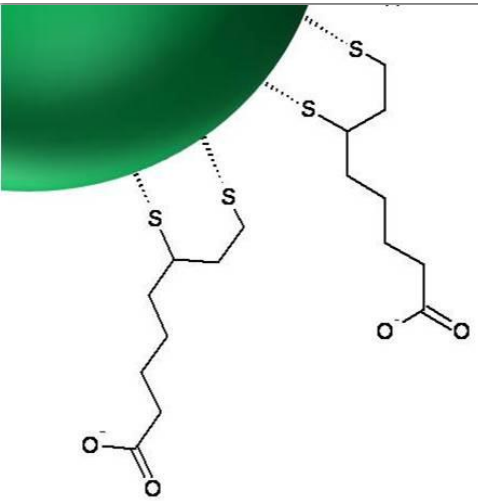
Biofunctionalization with Peptides

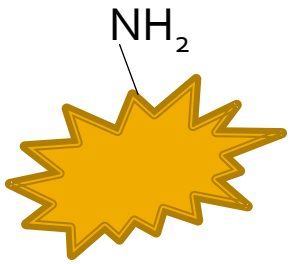
- Cell Penetrating Peptides (CPPs)
  - short peptide sequences
  - carrying particles across cell membrane
- Other sequences act like "zip codes"
  - NLS– nuclear localization sequence
- Peptides may act as ligands for cell surface receptors
  - e.g. Used to target nanoparticles to tumors

Biofunctionalization with Peptides

- Particle with maleimide functionality…
  - What amino acid(s) could mediate binding?
- Particle with carboxyl groups on surface…
  - What amino acid(s) could mediate binding?
- Particle with amino groups on surface…
  - What amino acid(s) could mediate binding?

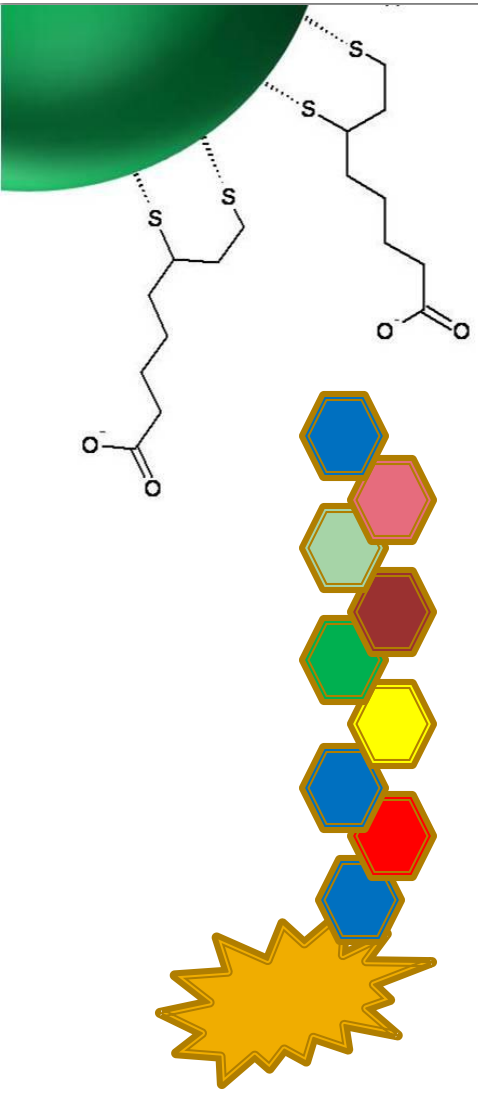
Scenario





- DHLA-coated QD + amine-functionalized dye
- EDC-based reaction– why or why not?

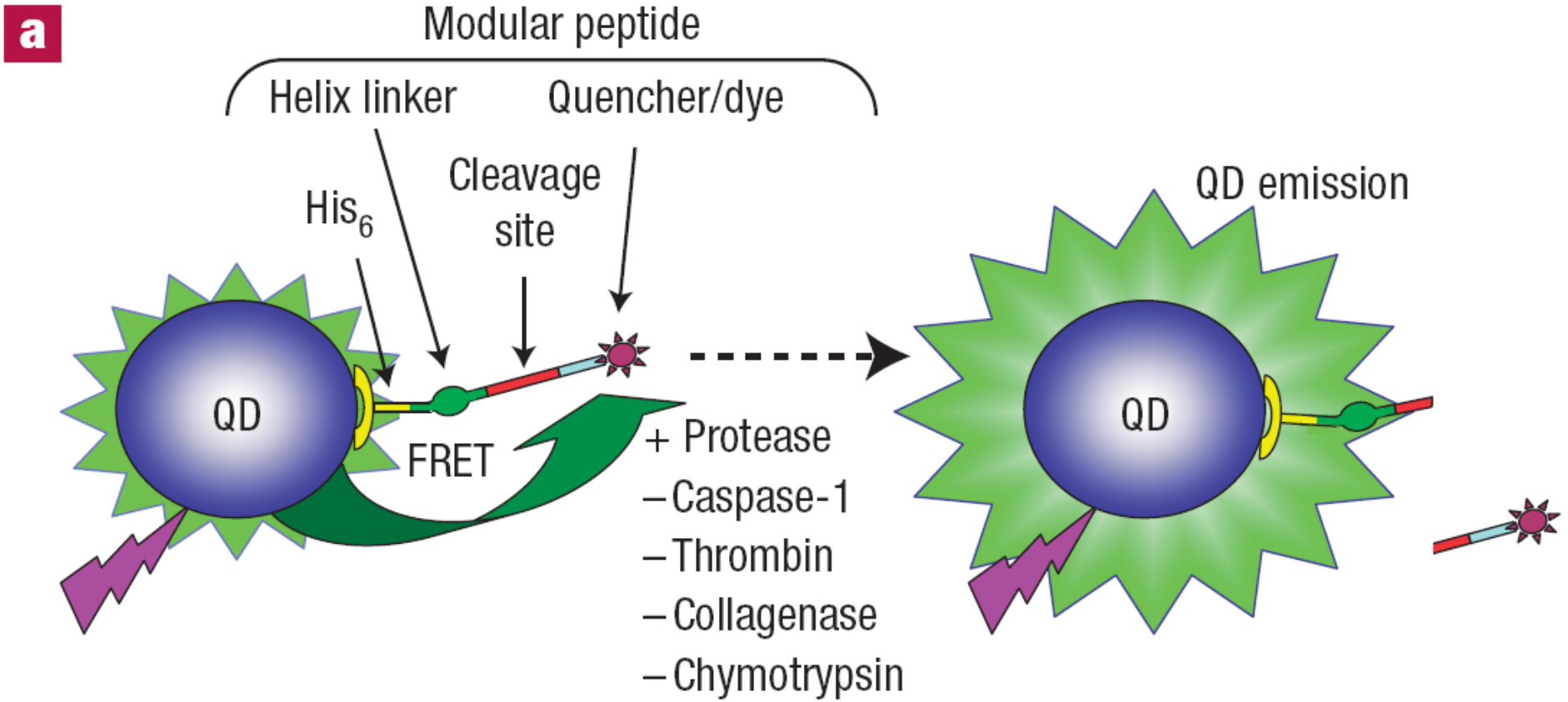
Scenario



- DHLA-coated QD + dye-labeled polypeptide
- EDC-based reaction– why or why not?
- Alternative binding method?

- DHLA-PEG coated QD + dye-labeled polypeptide?

His-tagged binding of peptide to QD



a: *Nature Materials* 5 (2006) 581-589;

Further Considerations

- Linker or no linker?
- Characterizing biofunctinalized nanoparticles
- Maintaining functionality of the biomolecule