Using Quantum Dots in Fluorescence Resonance Energy Transfer Studies

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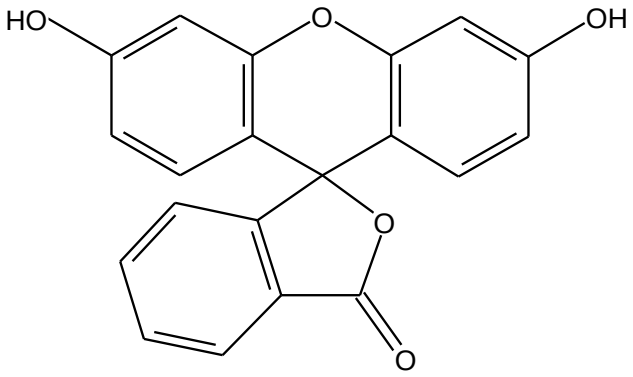
Introduction

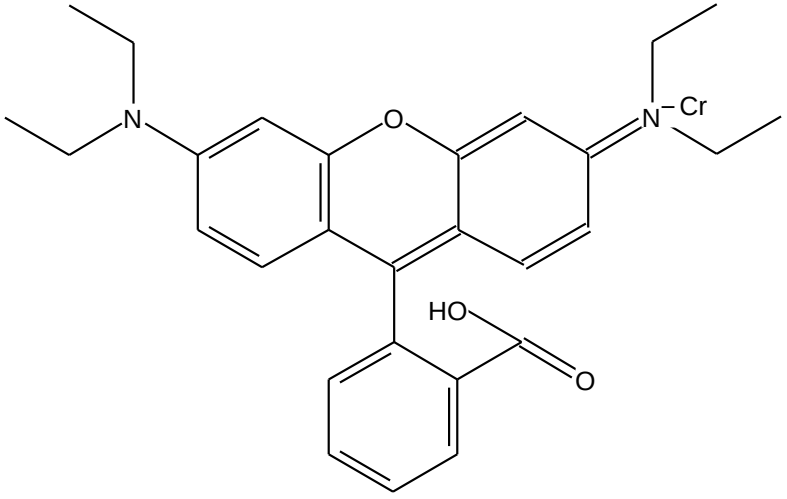
- Using organic fluorophores as labels
- A brief overview of fluorescence resonance energy transfer
- Quantum dots (QD) - features and synthesis
- Combining FRET and QDs in an immunoassay technique

Organic Fluorophores

- Examples include
  - fluorescein
  - rhodamine
  - GFP
- Some features include
  - Wide variety of fluorophores
  - Lots of techniques available for conjugation
  - GFP's allow for the expression of fluorescent proteins



Flourescein



Rhodamine

Organic Fluorophores

- Organic fluorophores have several disadvantages
  - Different fluorophores require different methods of conjugation to the target molecule
  - Narrow excitation spectra
  - Broad emission bands
  - Short lifetimes
- As a result
  - Spectral overlap occurs when different labels are used together
  - Autofluorescence cannot be ignored

Fluorescence Resonance Energy Transfer

- Spectral overlap can be used constructively
- If the emission spectra of one species overlaps absorption spectra of another energy transfer can occur
- FRET is a distance dependent interaction
- Energy is transferred *non-radiatively*
- Can be used for qualitative and quantitative studies

Fluorescence Resonance Energy Transfer

- FRET can be observed by monitoring quenching of donor fluorescence
- Observing acceptor enhancement is possible but complicated
- Important parameters in FRET
  - Förster distance (R_o) - important for lifetime calculations
  - Orientation factor (κ^2) - needed to account for anisotropy

Fluorescence Resonance Energy Transfer

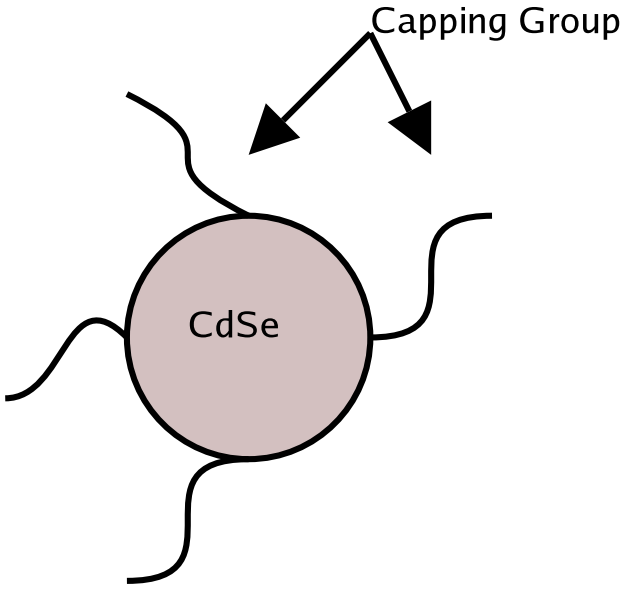
- Requirements for FRET
  - Donor and acceptor should be in close proximity
  - Acceptor absorption spectrum should overlap with the donor emission spectrum
  - Donor and acceptor transition dipoles should be approximately parallel

Quantum Dots

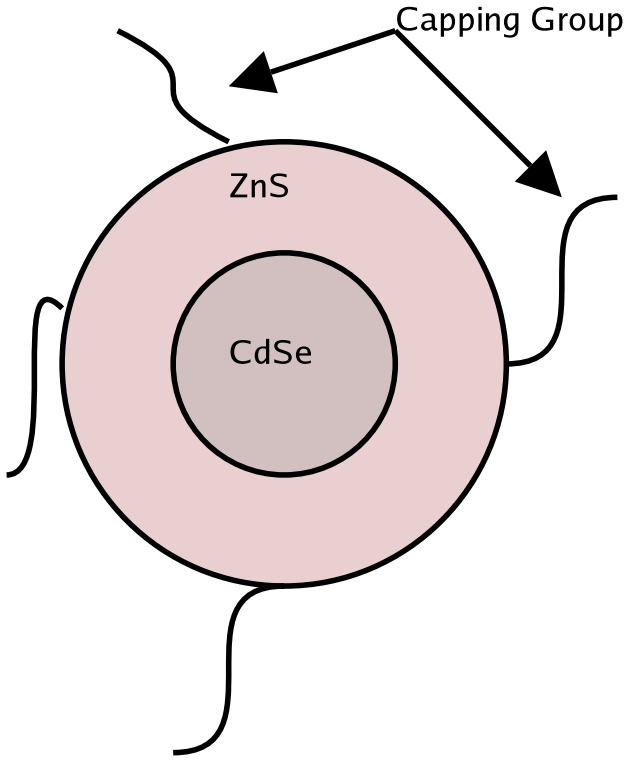
- Quantum dots have several useful features
  - High emission quantum yields
  - Sharp emission spectra
  - Chemical stability and photostability
  - Their color is easily tunable through the whole spectrum
  - Long lifetimes & shelf life

Types of Quantum Dots

- There are essentially two type of quantum dots



Simple quantum dot



Core - shell quantum dot

Synthesizing Quantum Dots

- Arrested precipitation
  - This method does not always give stable QDs and is not very scalable
- Zeolite based ion exchange
  - The zeolite structure imposes size and order constraints on the included semiconductor guest

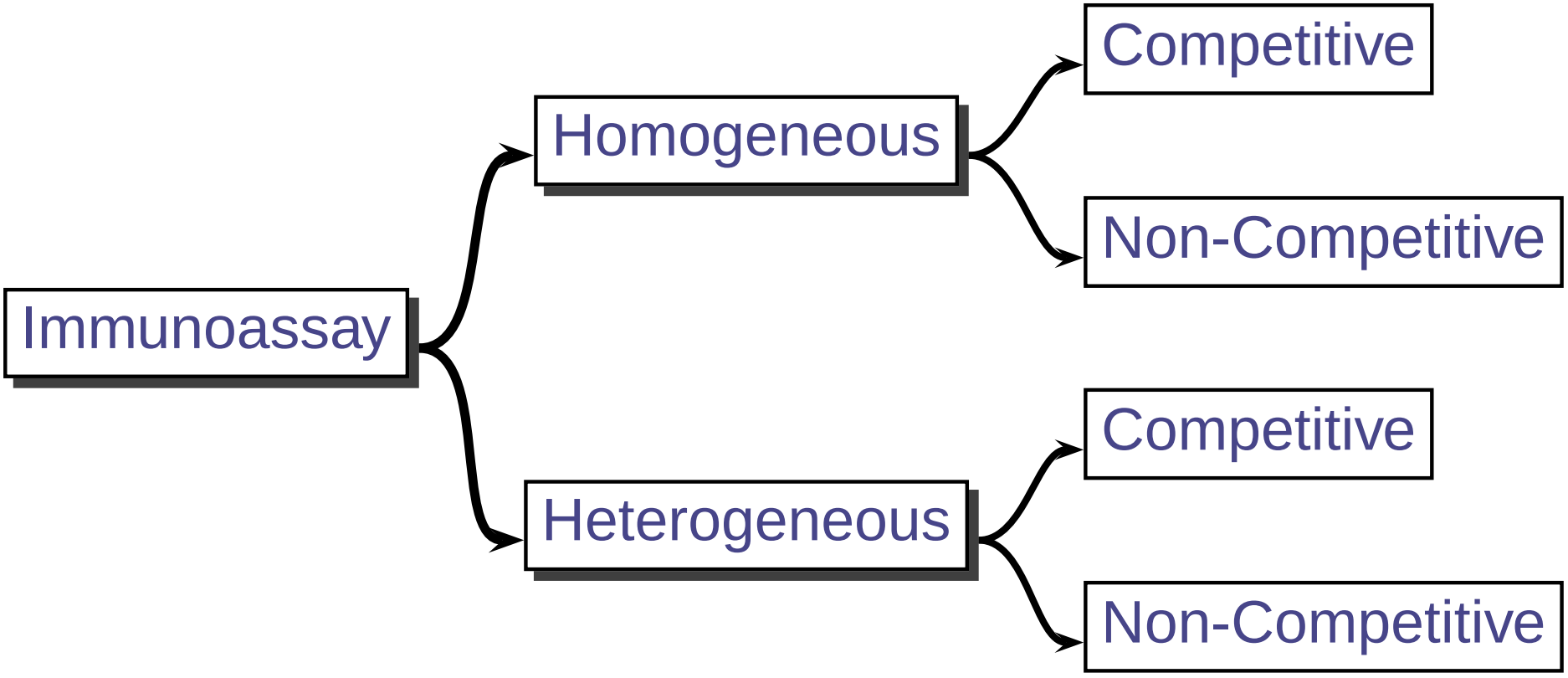
Synthesizing Quantum Dots

- Cluster fusion
  - Adding S^{-2} to CdS clusters can increase their size (inorganic polymerization)
- Thermal decomposition of organometallics
  - Synthesis and capping occurs simultaneously and gives monodisperse QDs

Capping (aka Surface Passivation)

- Capping is vital for stability, functionalization, and interactions with other molecules
- Common capping reagents are thiols and polyphosphates
  - mercaptoacetic acid
  - dihydrolipoic acid
  - trioctyl phosphine
- Features to consider
  - Surface density - too low might lead to aggregation
  - Effect of hydrolysis on the capping molecule
  - Length of capping molecules

Application - Immunoassay

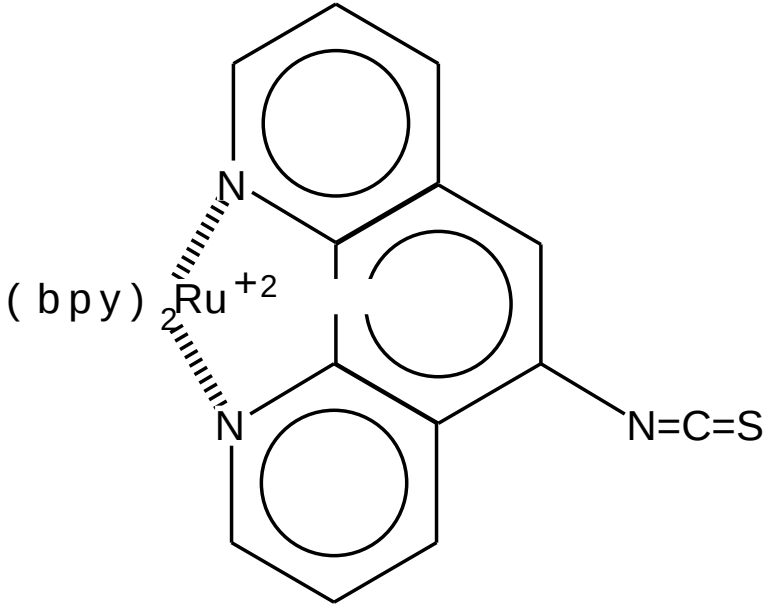


FRET & Immunoassay

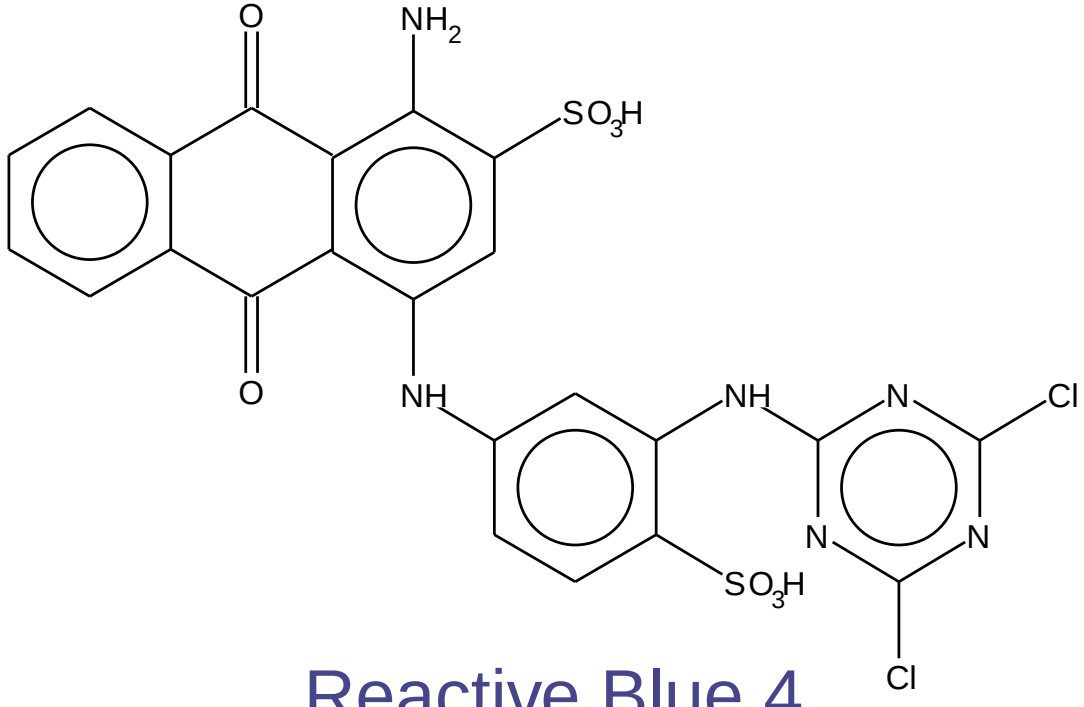
- This assay technique uses FRET to study tagged antibody - antigen interactions
- The assay is homogenous
- Inorganic fluorophores have been used to improve the technique

FRET & Immunoassay

- Youn et al.[1] have used a Ru complex and Reactive Blue 4 as donor/acceptor to assay HSA and AHA



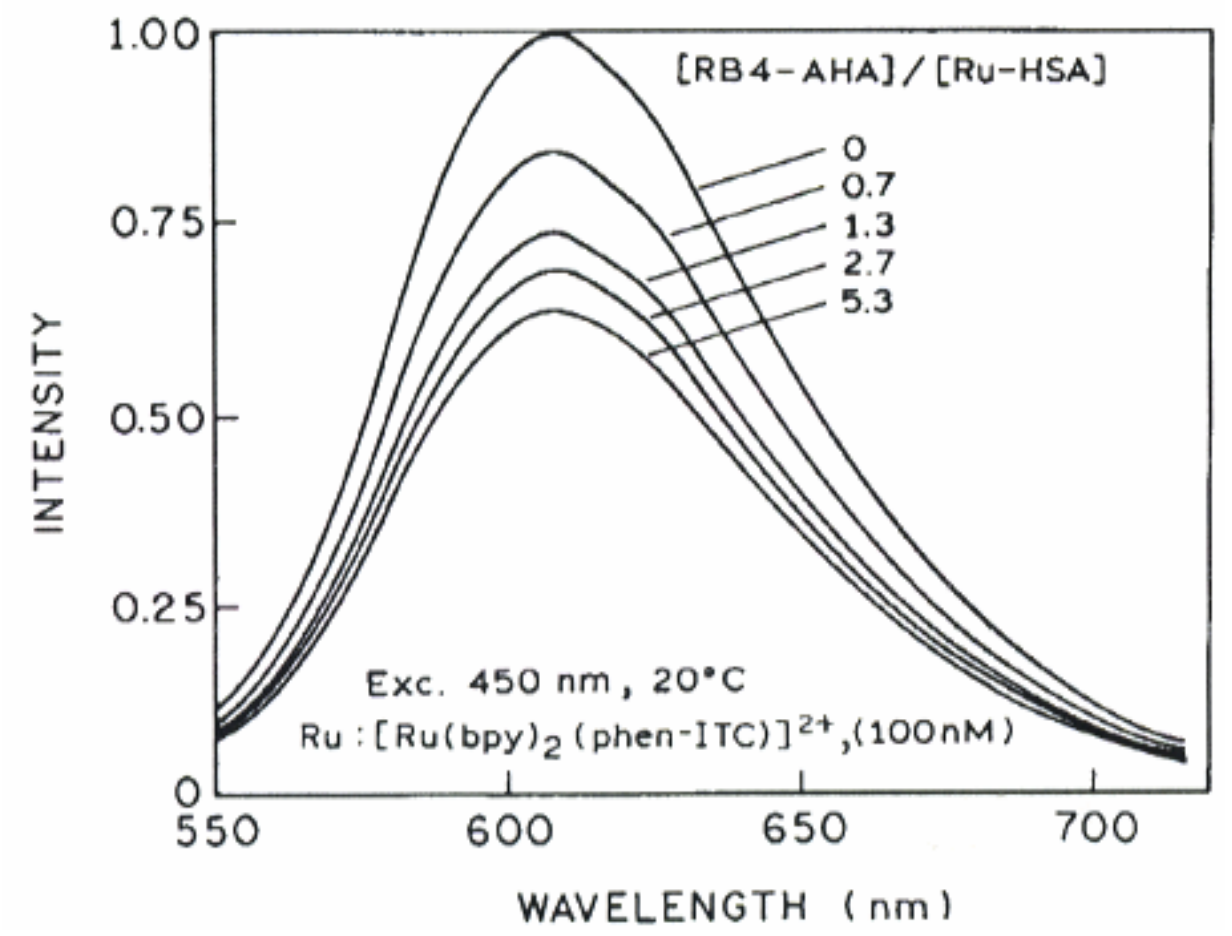
Ru Complex



Reactive Blue 4

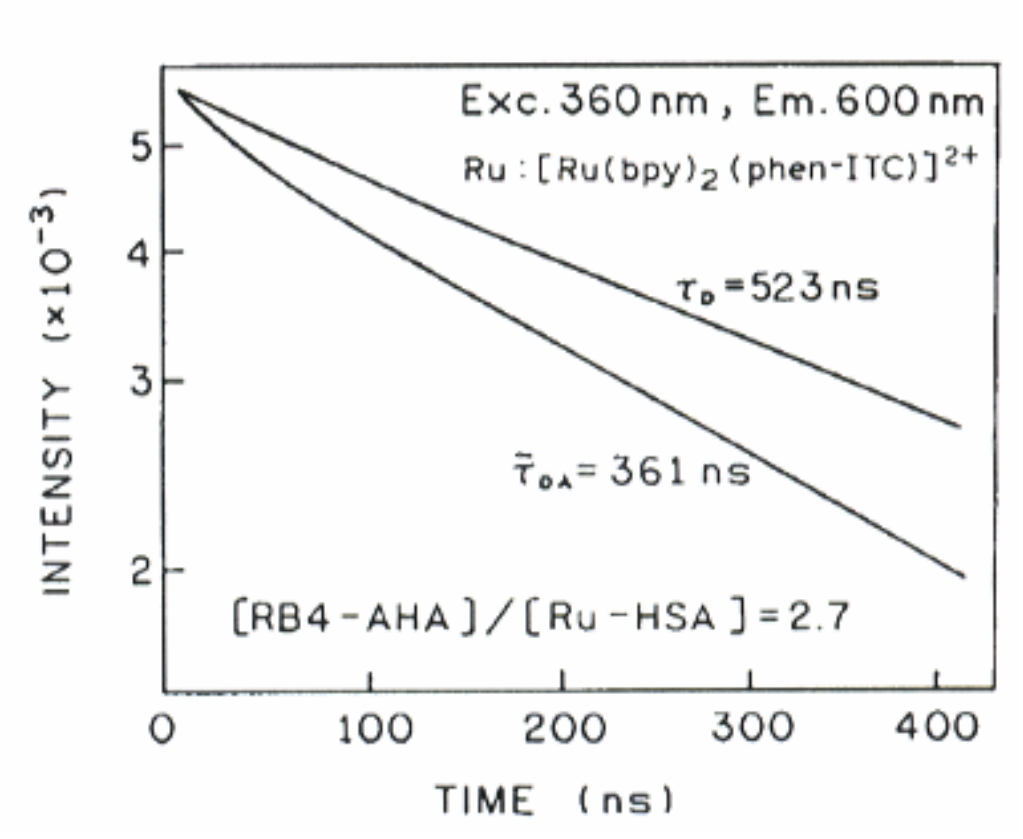
[1] Youn et al., *Anal. Biochem.*, **1995**, *232*, 24-30

FRET & Immunoassay



Emission spectra of Ru-labelled HSA in the presence of different molar equivalants of RB4-labelled AHA

FRET & Immunoassay



Decay of intensity of Ru-labelled HSA in the absence and presence of RB4-labelled AHA

FRET & Immunoassay

- The inorganic fluorophore approach used by Youn has a few problems
  - Synthesis of the Ru complex is time consuming
  - The complex itself is expensive
  - Quantum yield of the complex is relatively low
  - RB4 is prone to hydrophobic interactions
- Solution? Quantum Dots!

Features of a Quantum Dot Immunoassay

- Why would quantum dots help?
  - Easy to synthesize
  - QDs can be used as both donor & acceptor
  - They can be made resistant to bleaching
  - High quantum yields lead to a larger R_o
  - Lower chances of intramolecular energy transfer
  - Allows for the possibility of simultaneous assay of multiple antigen - antibody pairs

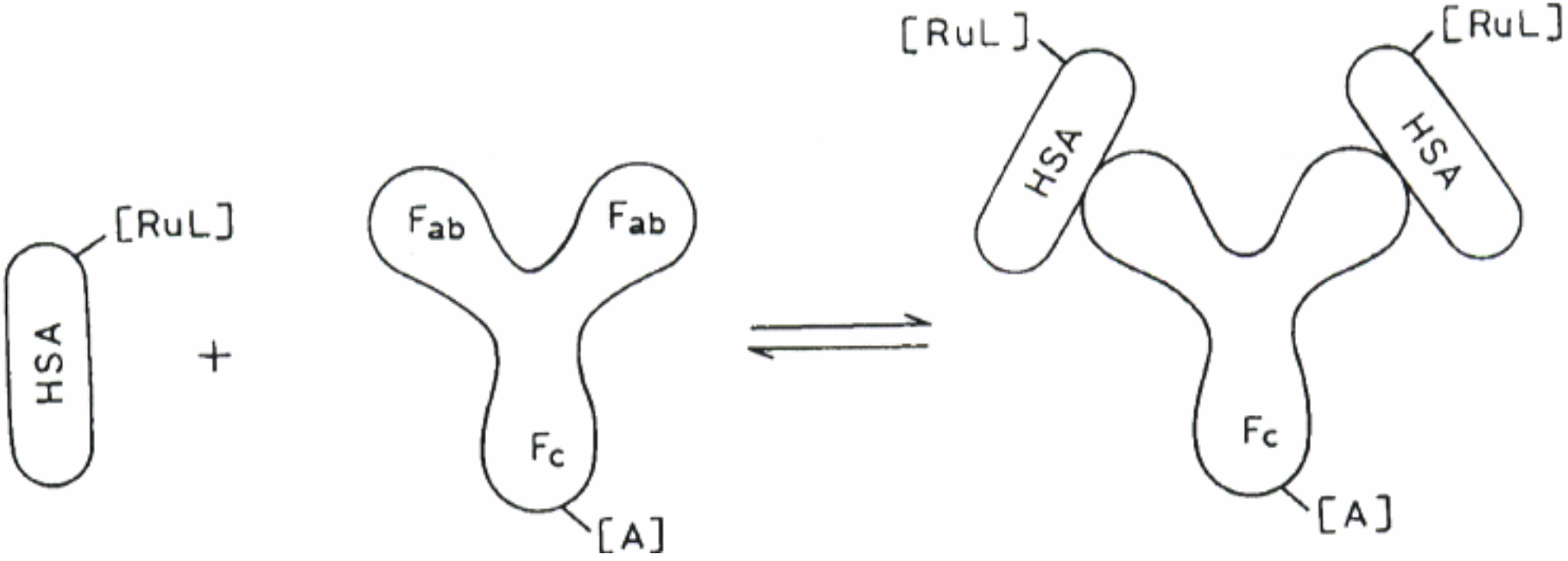
Overview of the Proposal

- Prepare core shell QDs
  - higher stability
  - larger band gap
- Cap the QDs
- Conjugate QDs with antigen and antibody
  - EDC condensation
- Mix the tagged antigen and antibody and monitor quenching of donor fluorescence

What Does the Assay Give Us?

- Simple detection of an antigen
- Binding kinetics
- Spatial details of the binding process
- Quantitative estimation of antigen concentration

Tagged Antigen - Antibody Interaction



Interaction of tagged HSA and AHA molecules[a]

[a] Youn et al., *Anal. Biochem.*, **1995**, *232*, 24-30

Possible Problems

- Förster distances are not well known for QDs.
- The orientation factor must be considered
  - For freely rotating fluorophores $< \kappa^2 >= 2/3$ can be assumed to be true. This is not so for this setup.
  - Possible methods include using longer capping groups or estimating κ^2 using the depolarization method[a]
- Multiple labelling of the donor on the antigen must be controlled

[a] Haas et al., *Biochemistry*, **1978**, *17*, 5064

Conclusion

- Quantum dots are a source of inexpensive and easily configurable fluorescent labels
- The use of quantum dots in FRET studies provides many advantages over organic fluorophores
- The combination of quantum dots and FRET results in an immunoassay technique whose features include
  - high speed
  - low cost
  - high throughput
  - multiple applications

Extra Information

Förster Distance

- Förster distance is defined by

$$R_o^6 = 8.785 \times 10^{-5} \frac{\kappa^2 \phi_D J}{n^4}$$

- where
  - κ^2 is the orientation factor
  - ϕ_D is the quantum yield of the donor
  - n is the refractive index of the medium
  - J is the nomalized overlap inetgral

Distance Measurements

- Donor - acceptor separation, R, can be determined by

$$E = \frac{R_o^6}{R_o^6 + R^6}$$

- E is the transfer efficiency and can be determined from fluorescence intensities or lifetimes

$$E = 1 - \frac{F_{da}}{F_d}$$

$$E = 1 - \frac{\tau_{da}}{\tau_d}$$

FRET & Antigen Concentration

- Let F_d be the donor fluorescence intensity
- Let the antigen - antibody interaction be represented by the equation

$$A_g + A_b \overset{k_1}{\rightleftharpoons} A_{gb}$$

- The rate of formation of the complex is given by

$$r_{bind} = k_{-1}[A_g][A_b]$$

- The rate of fluorescence quenching of the donor is given by

$$r_{quench} = -\frac{dF_d}{dt}$$

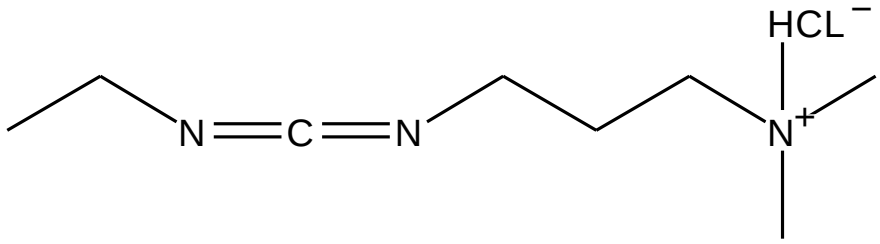
FRET & Antigen Concentration

- We assume that FRET is the only cause of donor quenching and that the donor acceptor distance is constant through the observation period
- Hence we can say that

$$
\begin{aligned}
r_{quench} &\propto r_{bind} \\
-\frac{dF_d}{dt} &\propto k_{-1}[A_g][A_b] \\
-\frac{dF_d}{dt} &= Ck_{-1}[A_g][A_b]
\end{aligned}
$$

where C could be determined from standardized concentrations

EDC Condensation



- EDC = 1-ethyl-3-(3-dimethylaminopropyl) carbodiimide
- Catalyzes the formation of amide bonds between carboxylic acids or phosphates and amines
- The derivative reacts readily with a nucleophile
- N hydroxy succinimide can be used to improve yields
- Commonly used during peptide synthesis

How Many Attached?

- We assume the protein and QD are spherical
- Protein is close packed and steric affects are ignored
- The number of proteins that can be attached is given by

$$N = 0.65 \left(\frac{R_2^3 - R_1^3}{R_p^3} \right)$$

- R_1 is the radius of the QD, R_P is the radius of the protein and $R_2 \sim R_1 + 2R_p$