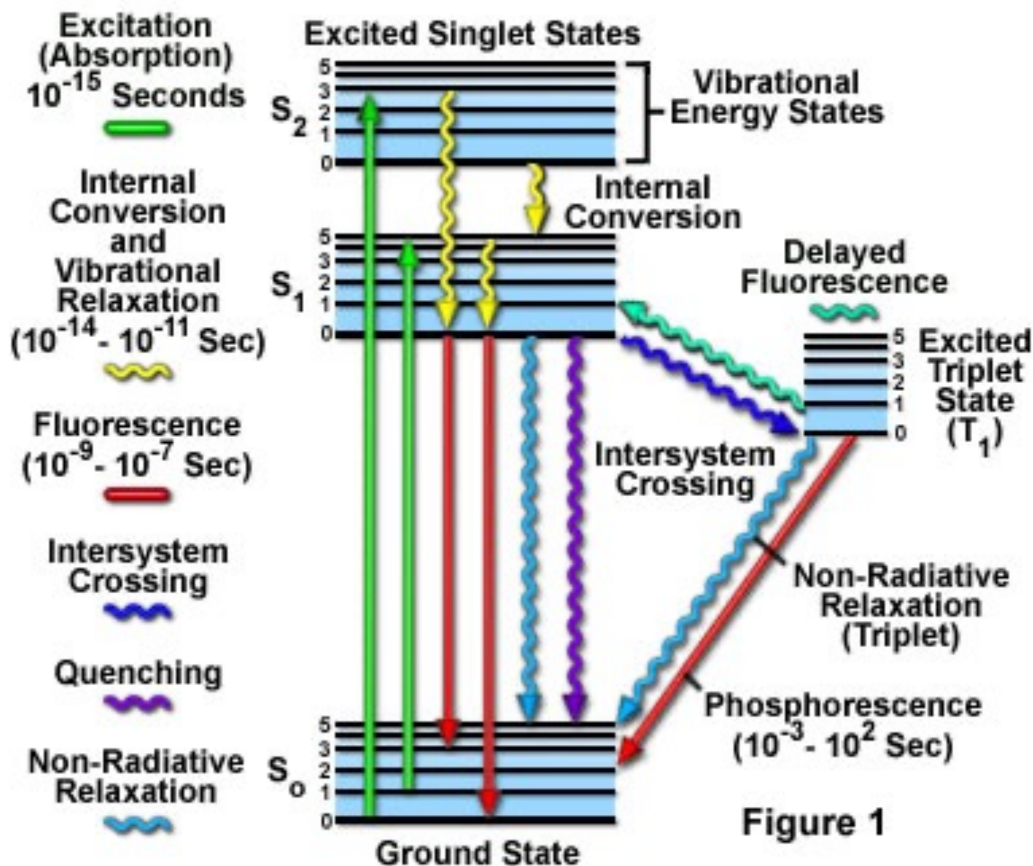
DNA Photonics

Hieu Bui and John Reif

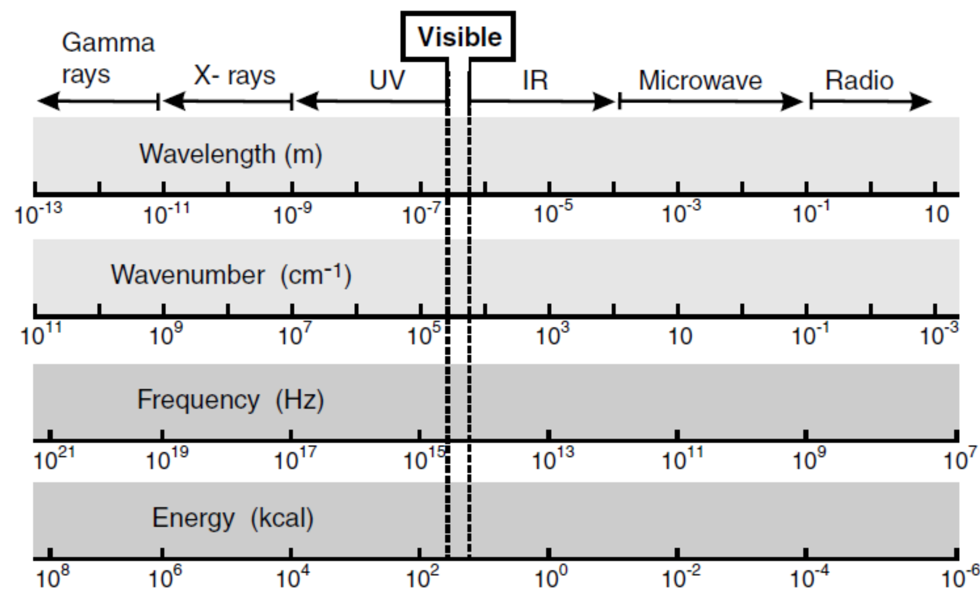
Outline

- **Fluorescent resonance energy transfer (FRET)**
- **Fluorescent Labels**
  - **Fluorophores, quantum dots**
  - **Single FRET**
    - **Molecular Beacon**
    - **DNA Tweezers**
  - **Multiple FRET**
    - **Homo-FRET**
    - **Hetero-FRET**
- **Optically-induced molecules**
  - **Photocleavable spacer**
  - **Aminopurine**
- **Plasmonics**
  - **AuNPs, AgNPs**
- **Radioisotopic Labels**

Photonic Energy: $E = h\upsilon = \frac{hc}{\lambda}$



Complicated, but thorough energy diagram, depicting all possible paths of relaxation upon excitation

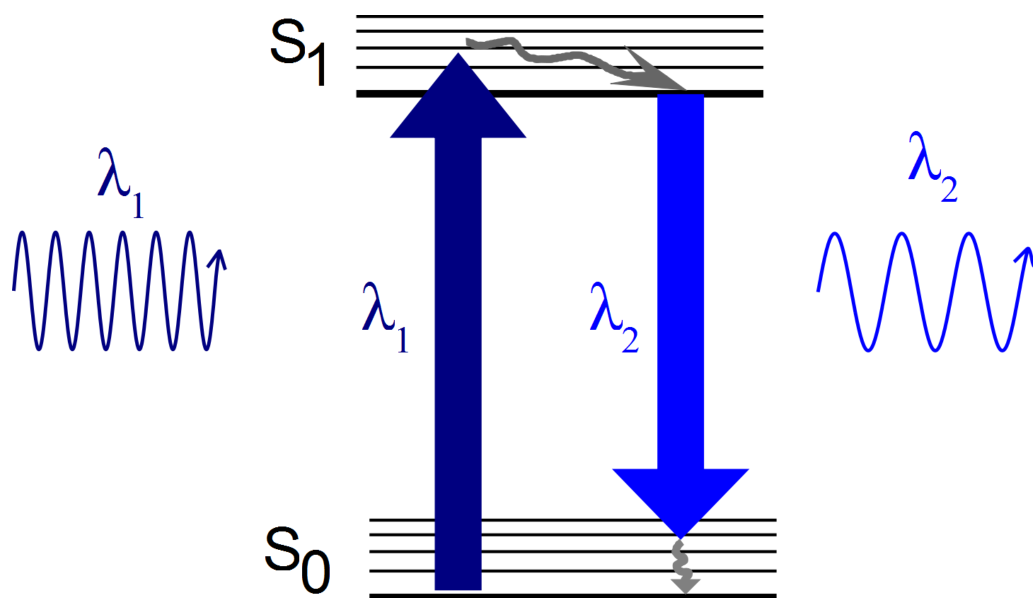


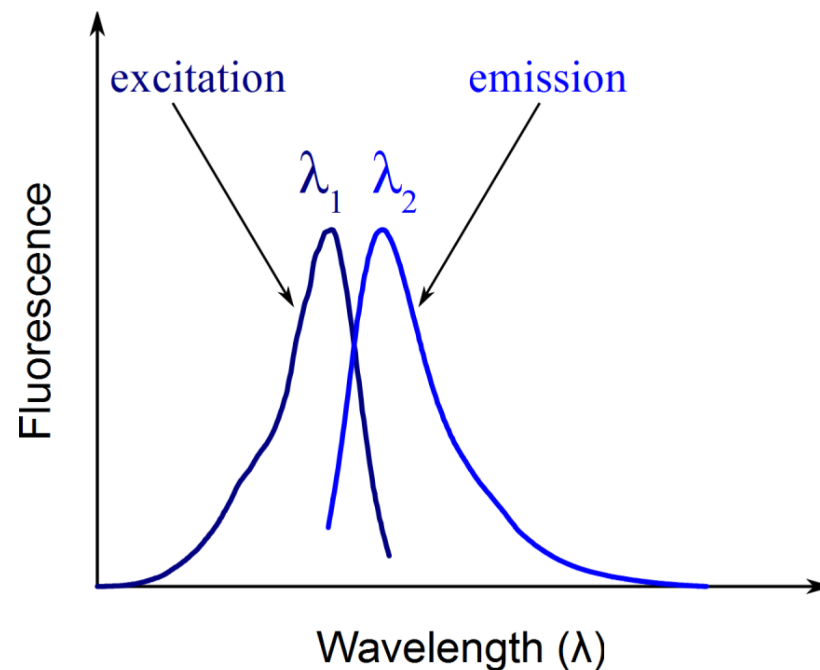
Electromagnetic spectrum

*www.neuro.fsu.edu/~dfadool/RChase2.**ppt***

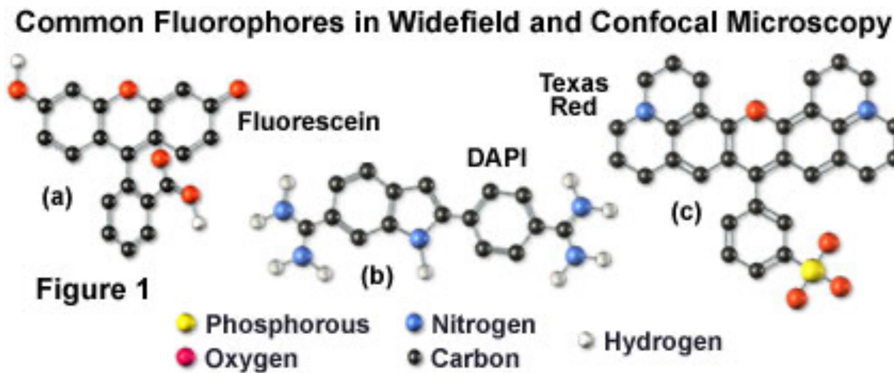
What is fluorescence?

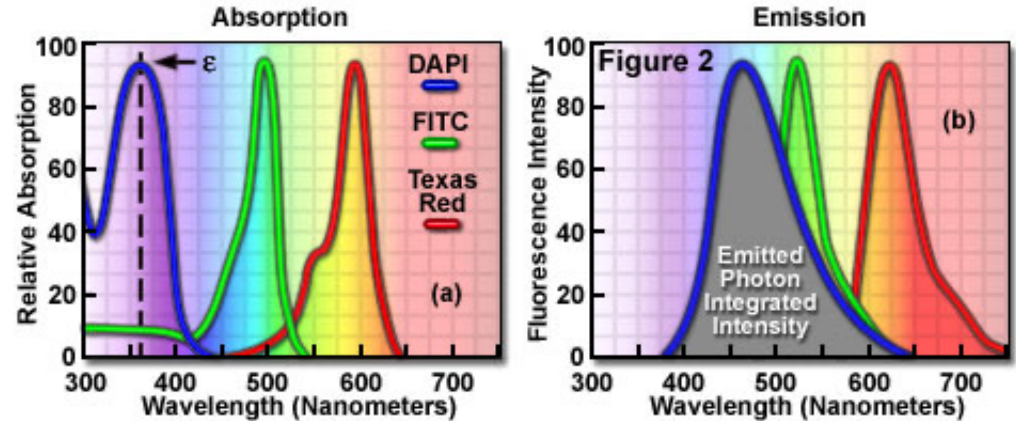
- **Emission of light/photon/electromagnetic radiation by a substance that has absorbed light/photon/electromagnetic radiation**
  - **Examples: fluorophore / chromophore, quantum dot**
- **In most cases, emitted light has a longer wavelength, and therefore lower energy, than the absorbed light.**





Examples of Fluorescence Substances





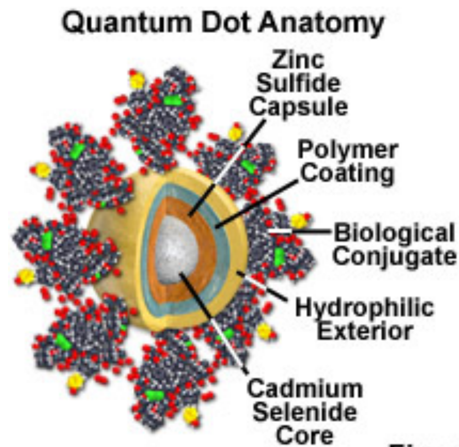
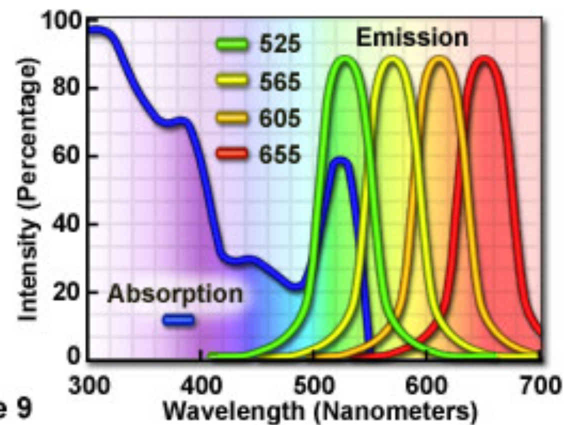


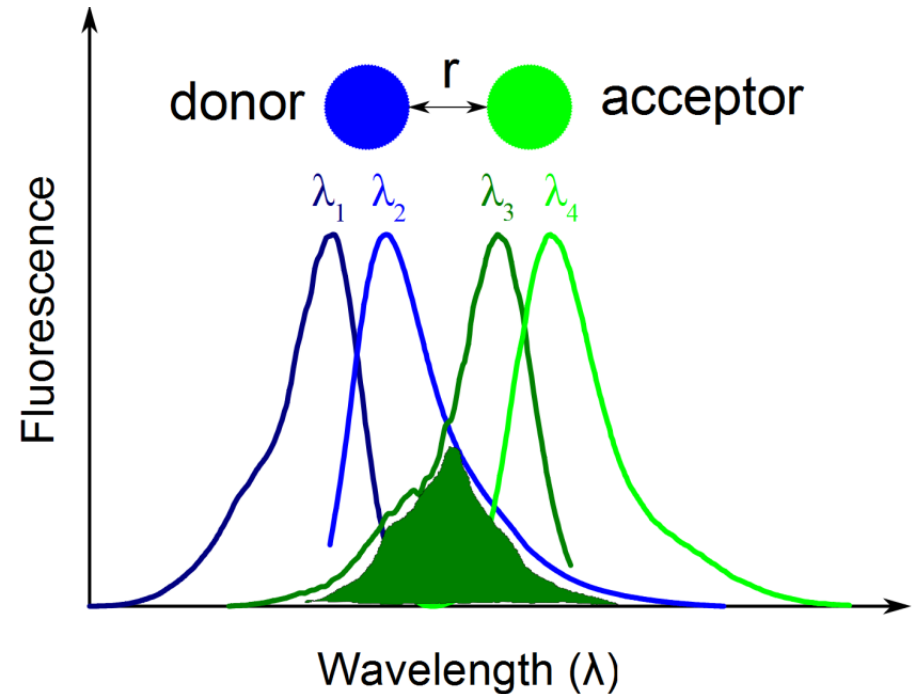
Figure 9



http://www.olympusconfocal.com/theory/fluorophoresintro.html

FRET

- **Fluorescence (Förster) resonance energy transfer (FRET):**
  - **Non-radiative coupling between two fluorophores**
    - **Spectral overlap**
    - **Inter-distance <10 nm**



$$E = \frac{1}{1+\left(\frac{r}{R_0}\right)^6}$$

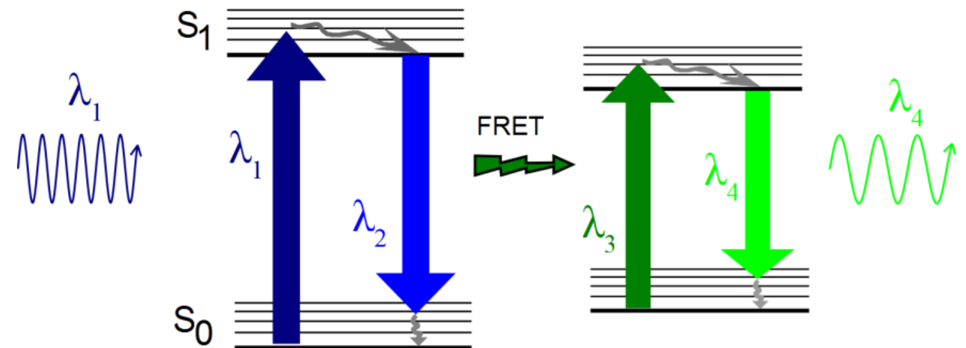
$$J = \int f_D(\lambda) \in_A (\lambda) \lambda^4 d\lambda$$

$$R_0^6 = \frac{9000 Q_0 \ln(10) \kappa_0^{\,2} J}{128 \pi^5 n^4 N_A}$$

E **: coupling efficiency**

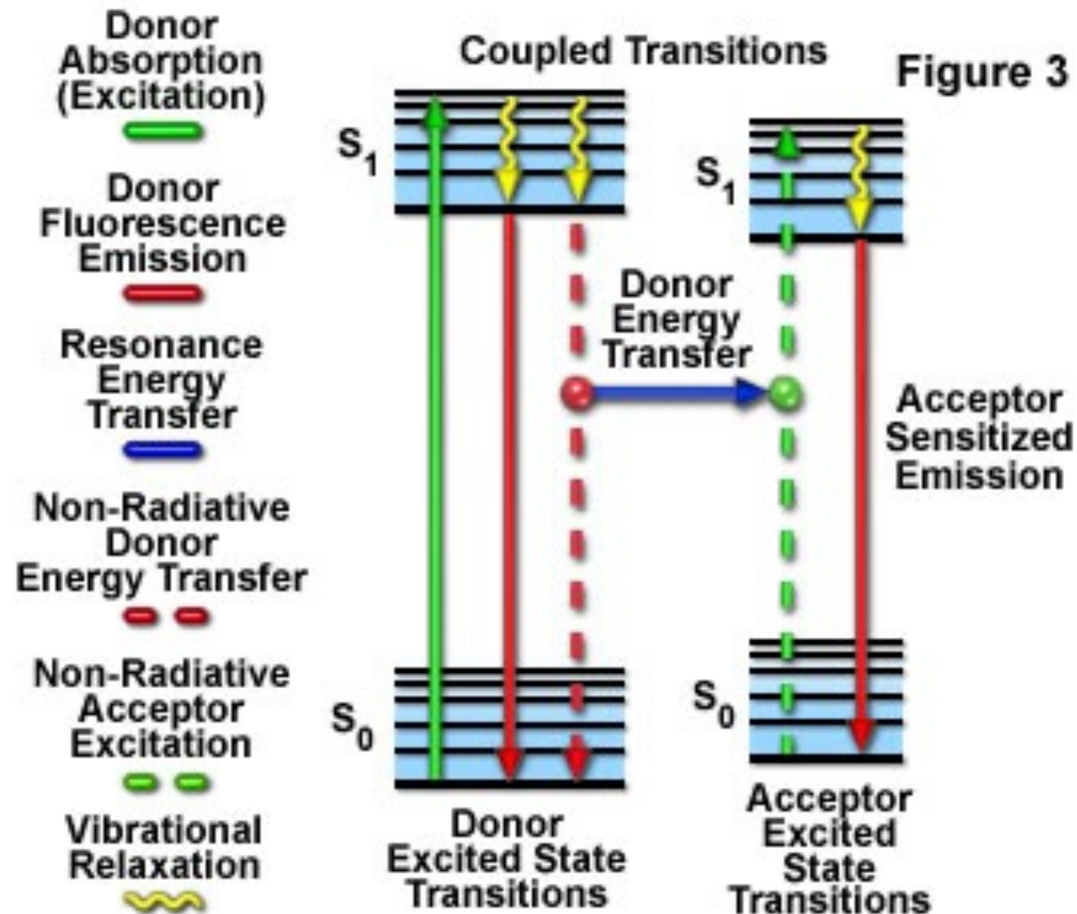
r **: distance between donor and acceptor**

R_0 **: Förster radius**



Bernard Valeur, Molecular Fluorescence Principles and Applications (Wiley-VCH, 2002, pg. 121)

FRET



More simple, concise scheme, depicting the transfer of energy from Donor to Acceptor

www.neuro.fsu.edu/~dfadool/RChase2.ppt

FRET

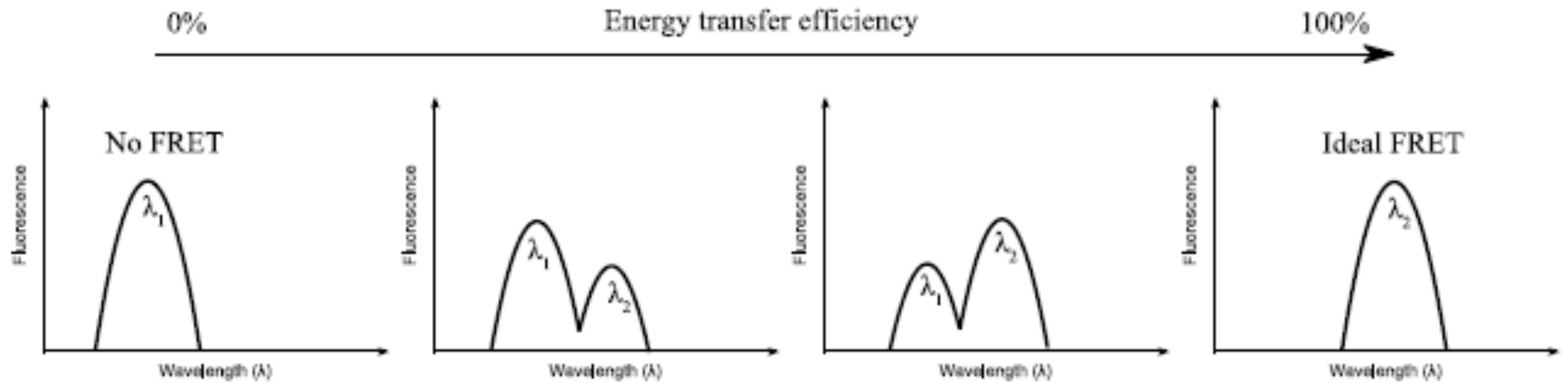


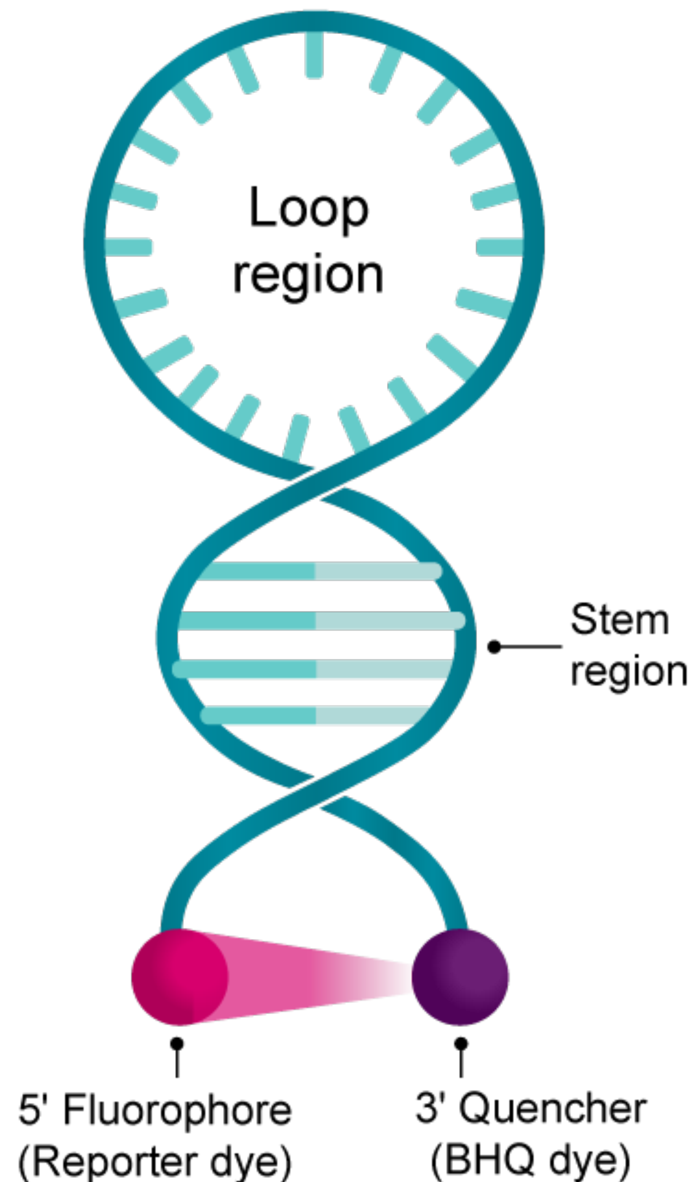
Illustration of variation in energy transfer efficiency and the corresponding fluorescence emission spectrum of the two fluorophores system.

Molecular Beacons

Molecular Beacon probes:

- These are dual-labelled probes that form a stem-loop (hairpin) structure, bringing the reporter and quencher into proximity.
- The loop region contains the sequence that hybridizes to the target sequence, while the complementary sequences at both ends of the probe form the stem.
- These probes generate fluorescence under non-hydrolytic conditions via hybridization to the target.

https://www.biosearchtech.com/

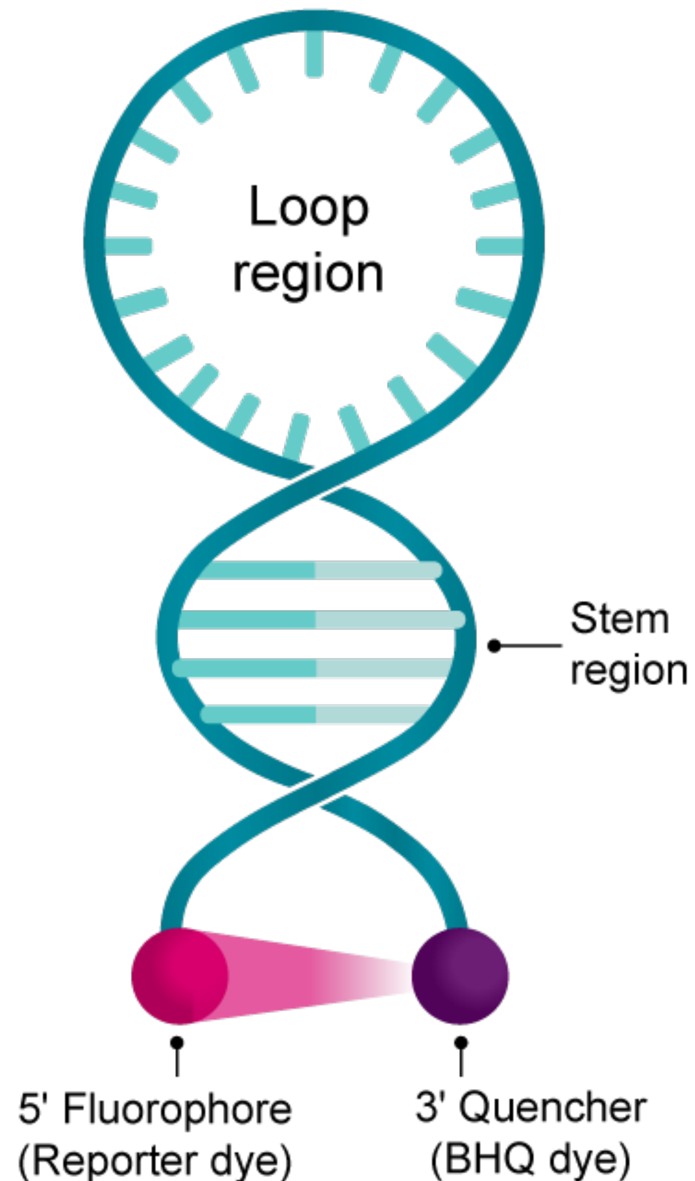


Molecular Beacons

Molecular Beacon structure:

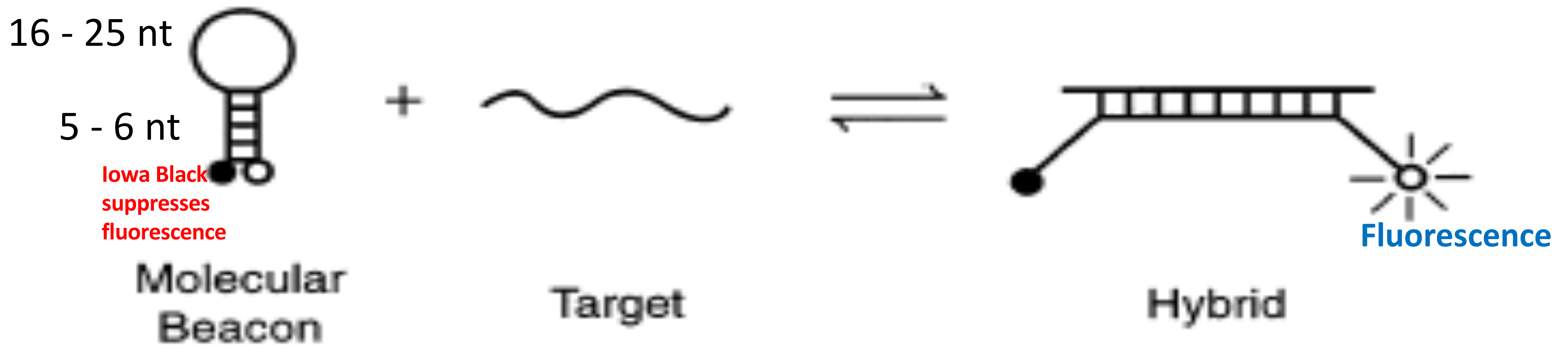
- **5′ fluorophore:** The reporter dye emits fluorescence when the probe is linearised and hybridised to the target, separating the dye and quencher.
- **3′ quencher:** When the probe is in its hairpin structure, the proximity of the quencher to the reporter prevents fluorescence emission.
- **Stem:** The stem is a double-stranded region formed by binding the complementary sequences (5-7 nt) at both ends of the probe.
- **Loop:** The loop is a 18-30 nt sequence that is complementary to the target sequence.

https://www.biosearchtech.com/

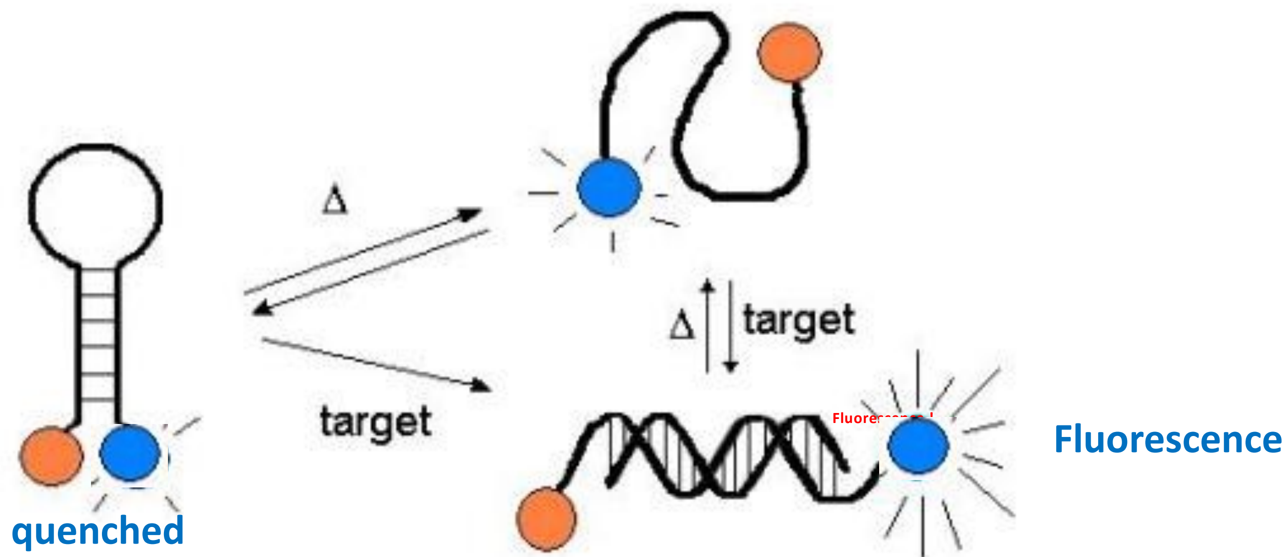


Single FRET

Principle of Operation of Molecular Beacons



Suppressor



http://test.isof.cnr.it/ppage/capob/thiof.html

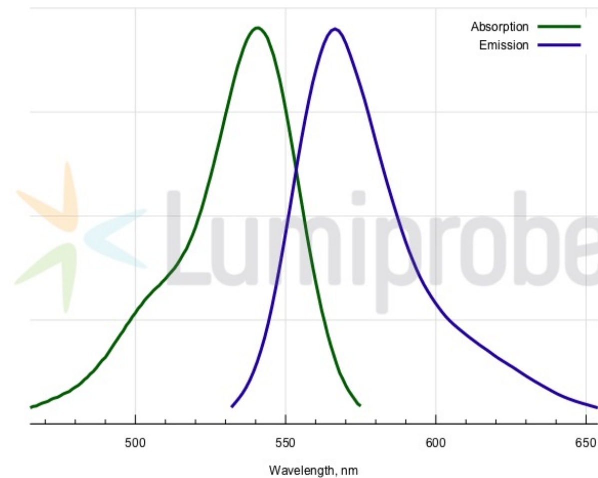
www.phy.ohiou.edu/~lbcao/reference_files/P6.ppt

FRET using 5' TAMRA and 3' TET pair:

5' TAMRA Absorption & Emission:

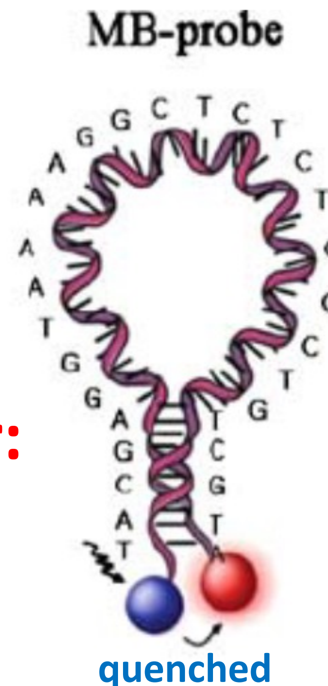
Is a fluorescent compound with:

- **an excitation peak at 552 nm**
- **an emission peak at 578 nm**



3' TET Quencher

Molecular Beacon using 5' TAMRA and 3' TET pair:



Fluorescence

Sang-Hoon Lee

Example of Use of FRET: Yurke-Tuberfield DNA Tweezers

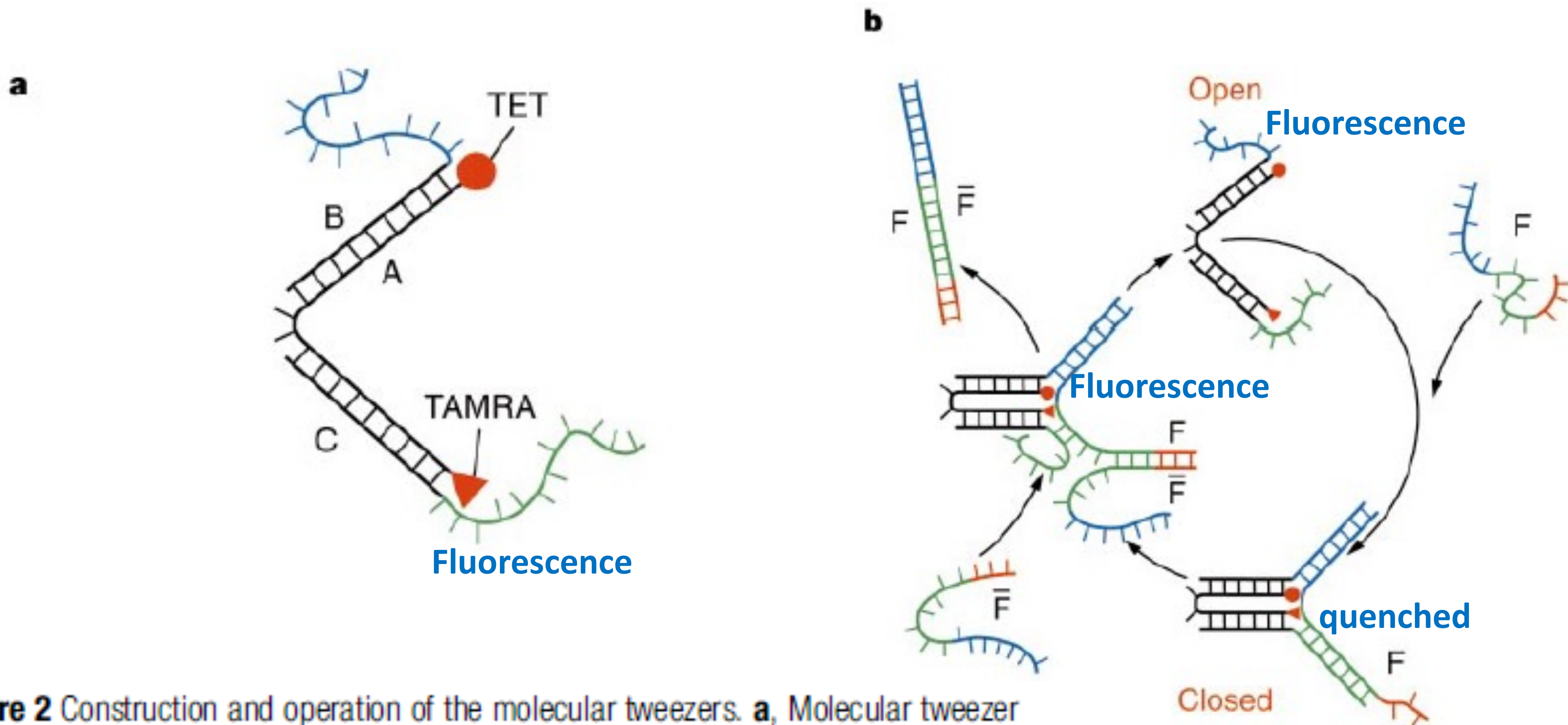
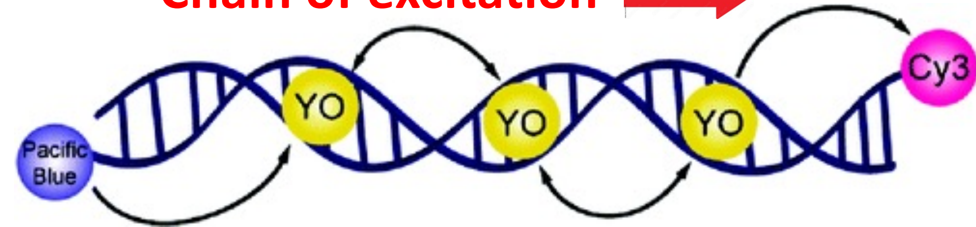


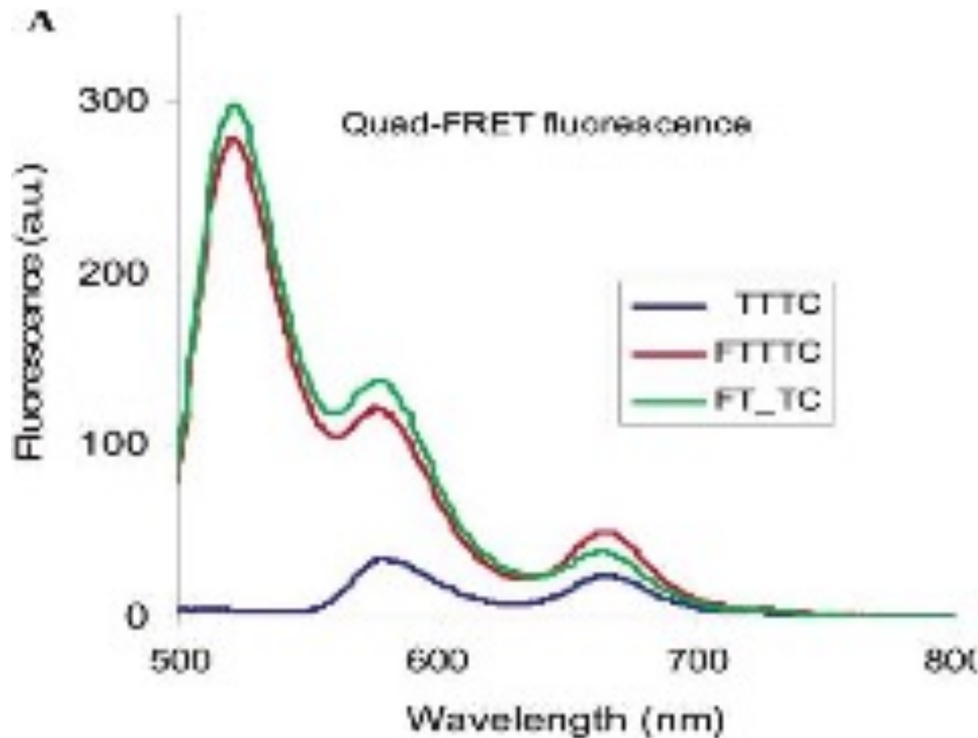
Figure 2 Construction and operation of the molecular tweezers. **a**, Molecular tweezer structure formed by hybridization of oligonucleotide strands A, B and C. **b**, Closing and opening the molecular tweezers. Closing strand F hybridizes with the dangling ends of strands B and C (shown in blue and green) to pull the tweezers closed. Hybridization with the overhang section of F (red) allows $\bar{F}$ strand to remove F from the tweezers, forming a double-stranded waste product F$\bar{F}$ and allowing the tweezers to open. Complementary sections of B, C, F and $\bar{F}$ that hybridize to close and open the tweezers are coloured as in Fig. 1.

http://www.nature.com/nature/journal/v406/n6796/abs/406605a0.html

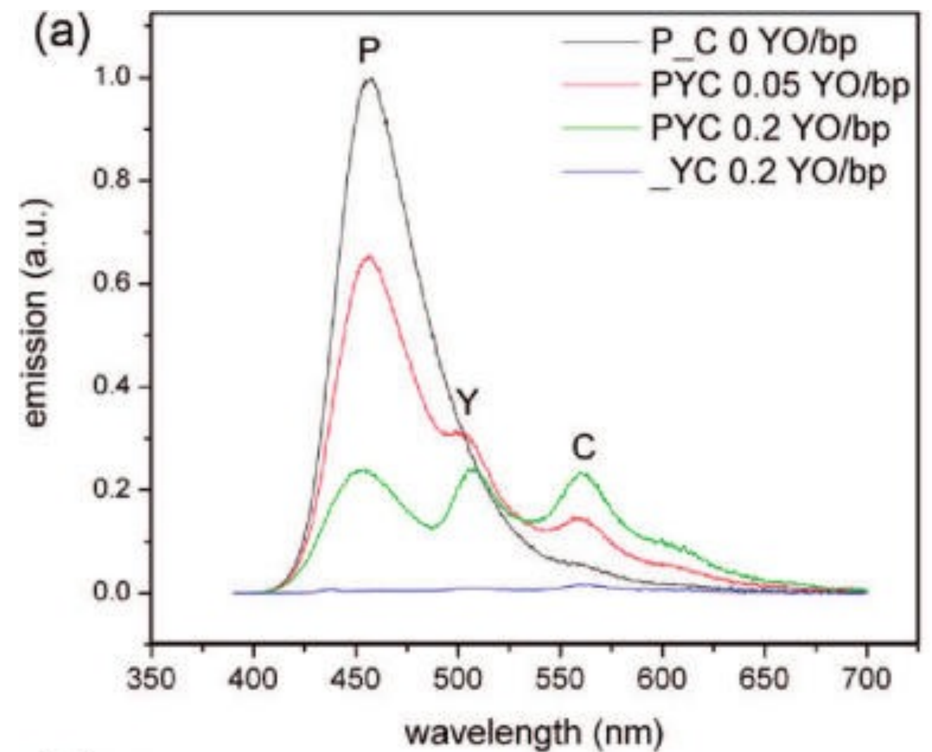
Examples of Use of Multiple FRET:

Iowa Black suppresses blue fluorescence



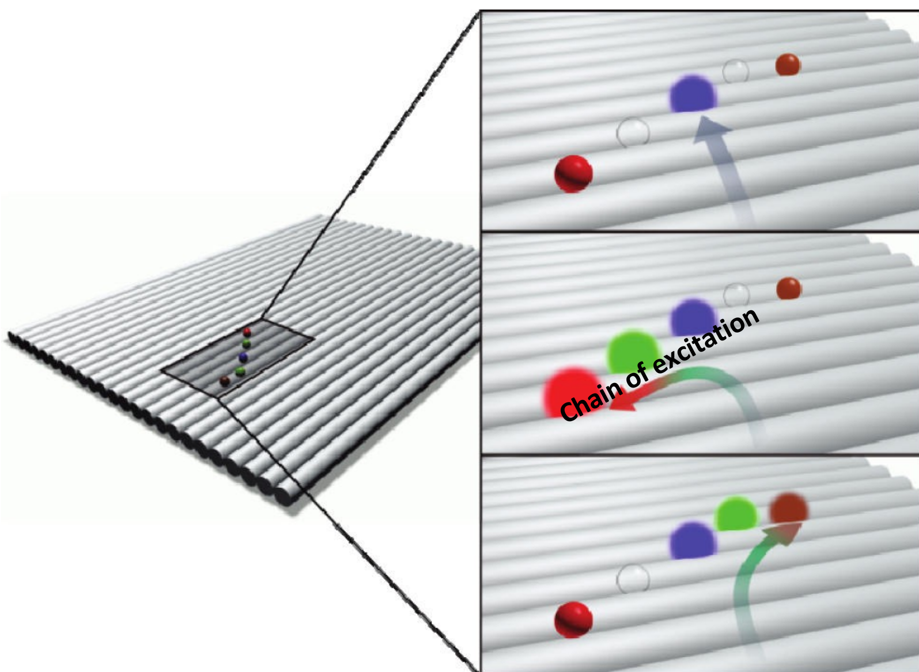


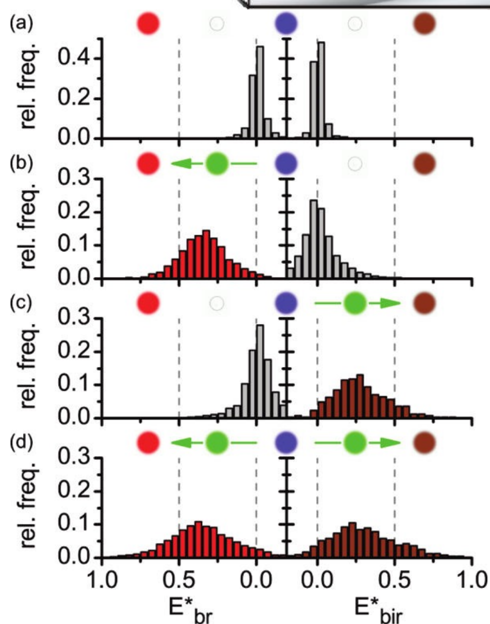
Vyawahare et al Nano Letters 2004, 4, 1035



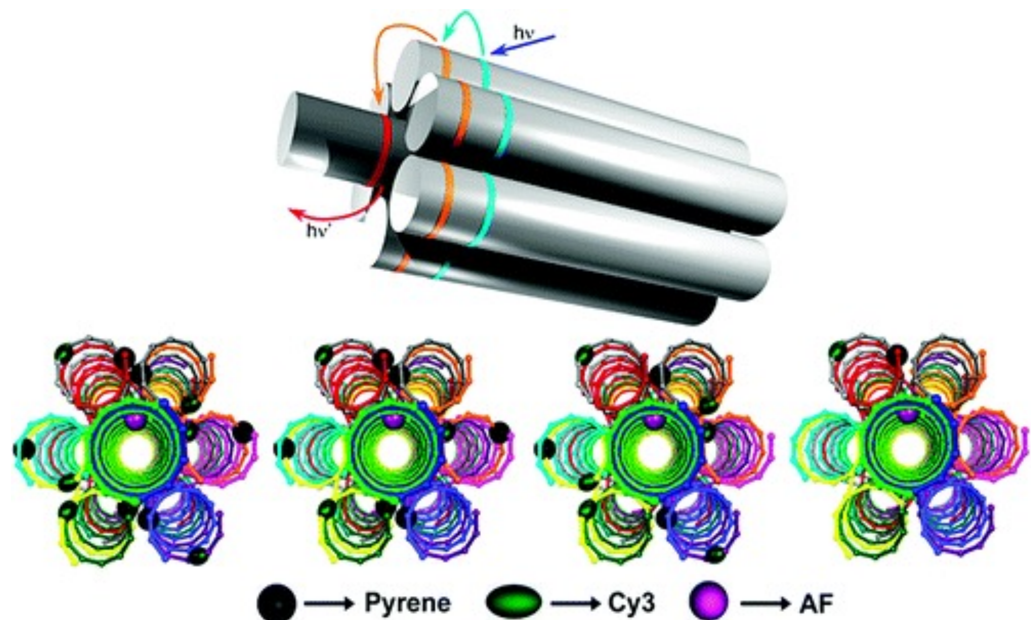
Hannestad et al JACS 2008 130 15889

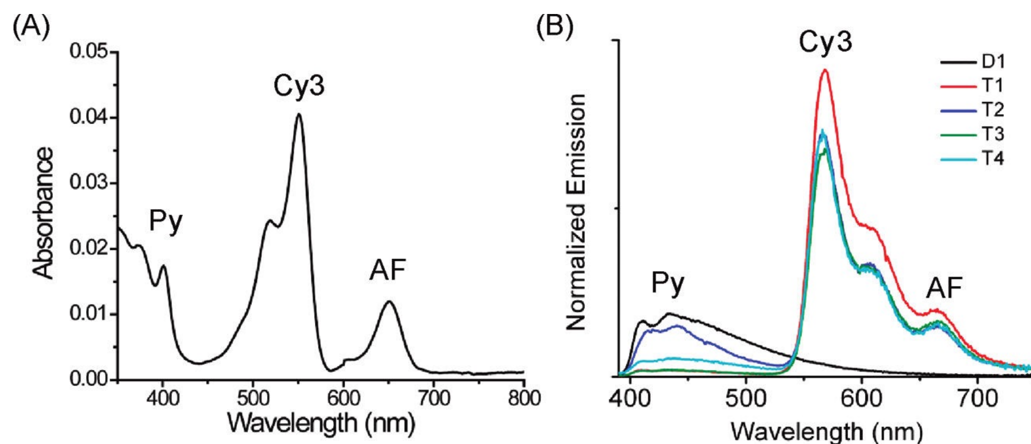
Examples of Use of Multiple FRET:





Stein et al JACS 2011, 133, 4193

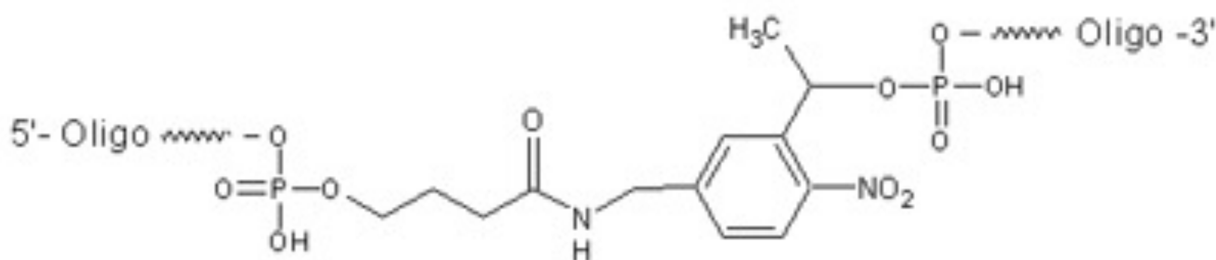




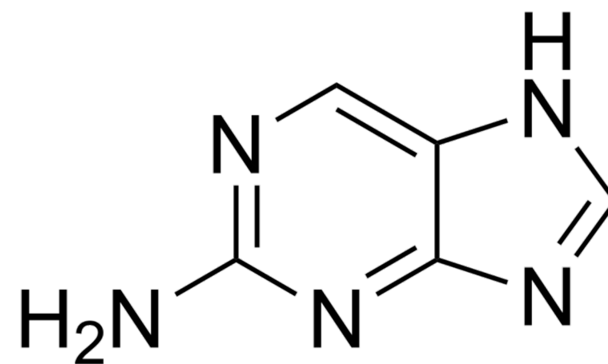
Dutta et al JACS 2011 133 11985

Example Optical-induced Molecules:

- **Photocleavable spacer:**
  - **Conformation changes under UV irradiation**



- **Aminopurine:**
  - **Fluorescent molecule**



http://www.genelink.com/newsite/products/images/modificationimages/PC-Spacer-(photocleavable).gif
http://en.wikipedia.org/wiki/2-Aminopurine

What is Plasmonics?

The science of **plasmonics** is dealing with generation, manipulation, and detection of surface plasmon polaritons (SPPs).

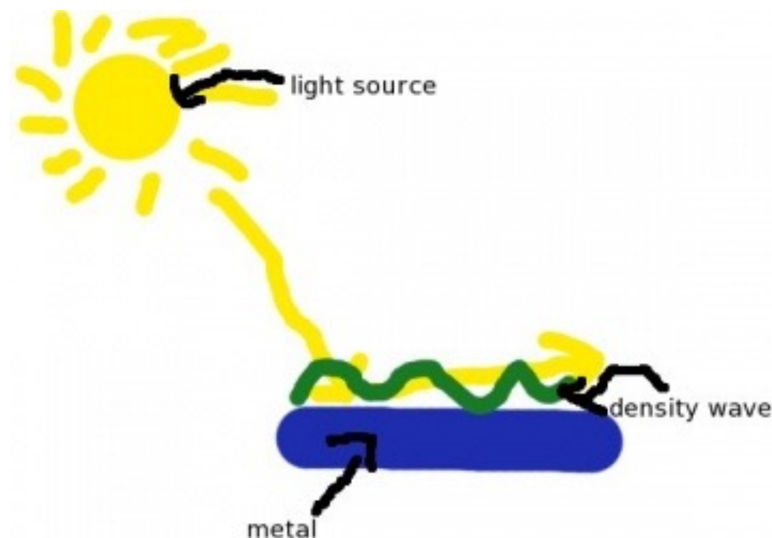
- SPP: Quasi-particle due to coupling of light and surface plasmon (SP).
- SP: electron oscillation wave at metal surfaces.

Min Qiu, Royal Institute of Technology (KTH) Electrum 229, 164 40 Kista, Sweden

Plasmonics:

- **a new branch photonics studying the interaction of light with matter in nanoscale metallic structures.**

Surface plasmons are waves that propagate along the surface of a conductor. By altering the structure of a metal's surface, the properties of surface plasmons—in particular their interaction with light—can be tailored, which offers the potential for developing new types of photonic device. This could lead to miniaturized photonic circuits with length scales that are much smaller than those currently achieved. Surface plasmons are being explored for their potential in subwavelength optics, data storage, light generation, microscopy and bio-photonics.



- **There is a light source, generally a laser.**
- **There is a piece of metal, for example a fabricated metal nano-structure.**
- **The light hits this metal, creating a density wave.**
- **This metal now has an electron density distribution.**
- **This electron density distribution frequency is a similar frequency to optics.**

http://mycompwiki.com/index.php?title=Plasmonics_Final_Report

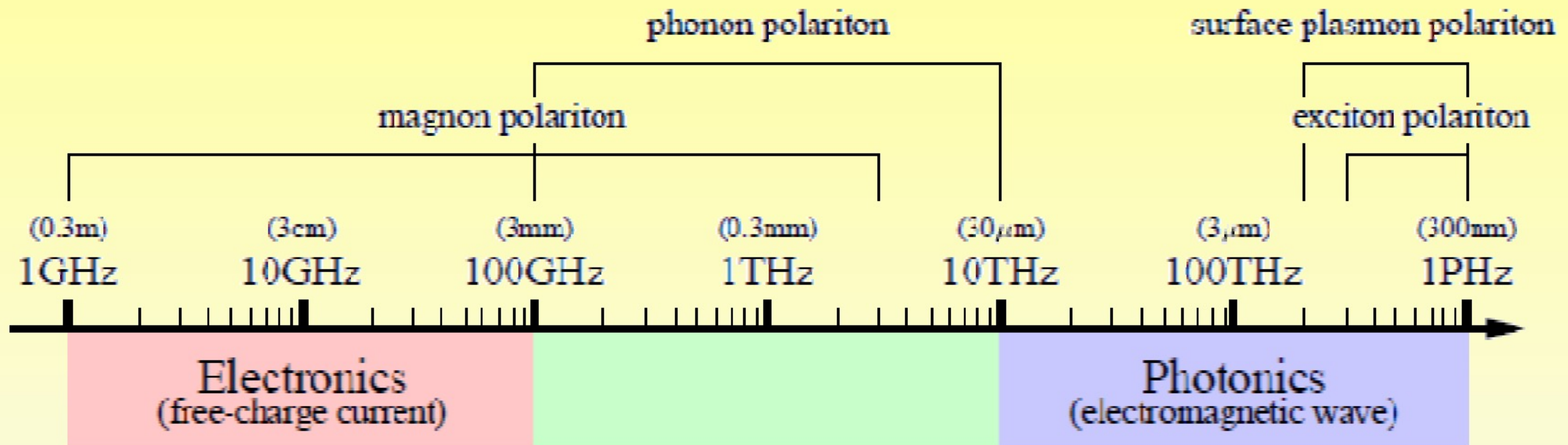
Example Optical-induced Molecules: Surface-Plasmon Excitation in the Lycurgus Cup

The Lycurgus cup is on display at the British Museum in London

- **The Lycurgus cup, when illuminated from outside, appears green.**
- **However, when Illuminated from within, it glows red.**
- **The glass contains metal nanoparticles, gold and silver, which give it these unusual optical properties.**
- **The underlying physical phenomenon for this is called surface-plasmon excitation.**

http://www.nanowerk.com/spotlight/spotid=2295.php

Frequency Spectra: Electronics vs Photonics



The frequency spectra of polaritonics (shown in the diagram as magnon polariton, phonon polariton, exciton polariton, and surface plasmon polariton) can cover both those of conventional electronics and photonics, as well as the frequency gap between the two.

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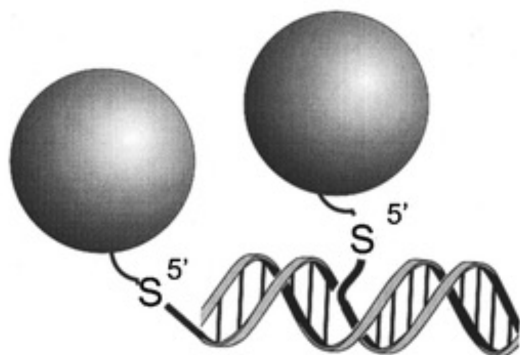
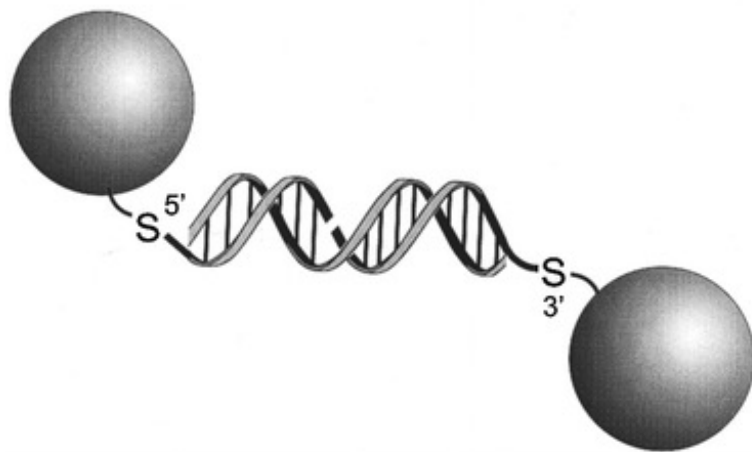
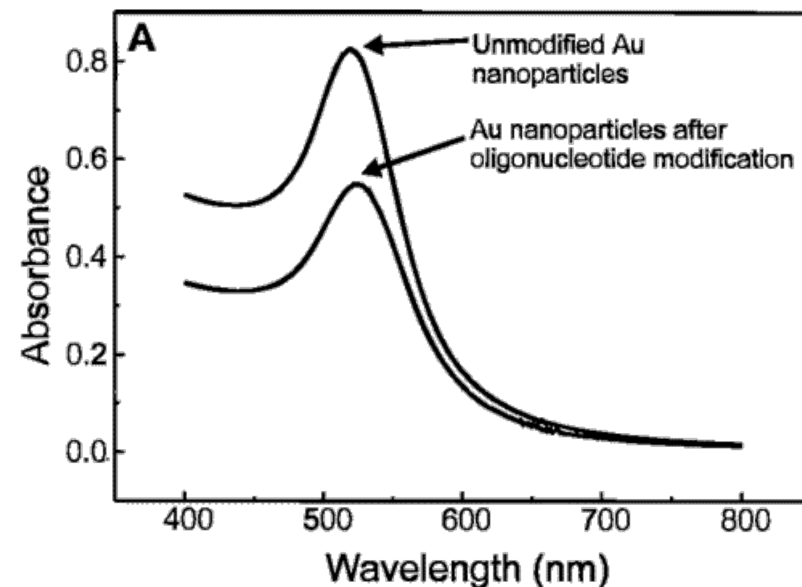
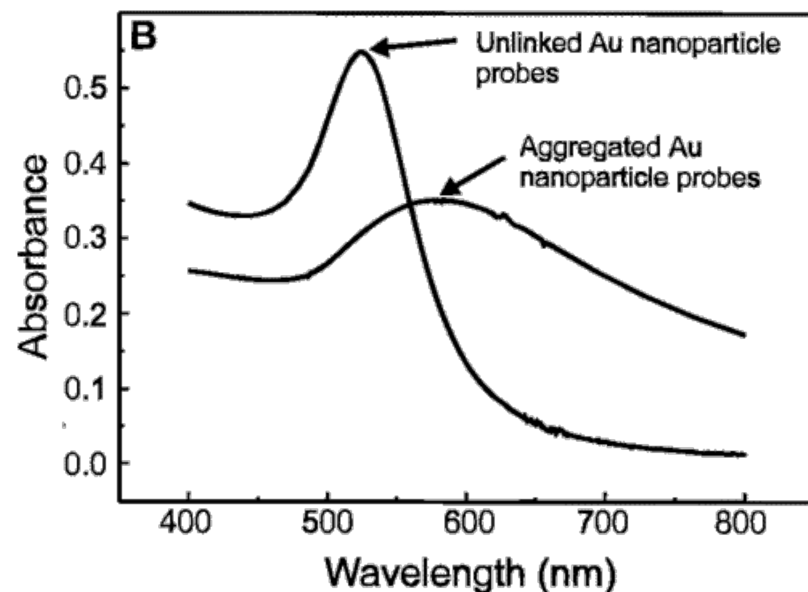
Tuning Optical Properties of Gold Nanoparticles (AuNPs)









Storhoff et al JACS 1998 120 1959

LETTER

doi:10.1038/nature10889

DNA-based self-assembly of chiral plasmonic nanostructures with tailored optical response

Anton Kuzyk[1]*†, Robert Schreiber[2]*, Zhiyuan Fan[3], Günther Pardatscher[1], Eva-Maria Roller[2], Alexander Högele[2], Friedrich C. Simmel[1], Alexander O. Govorov[3] & Tim Liedl[2]

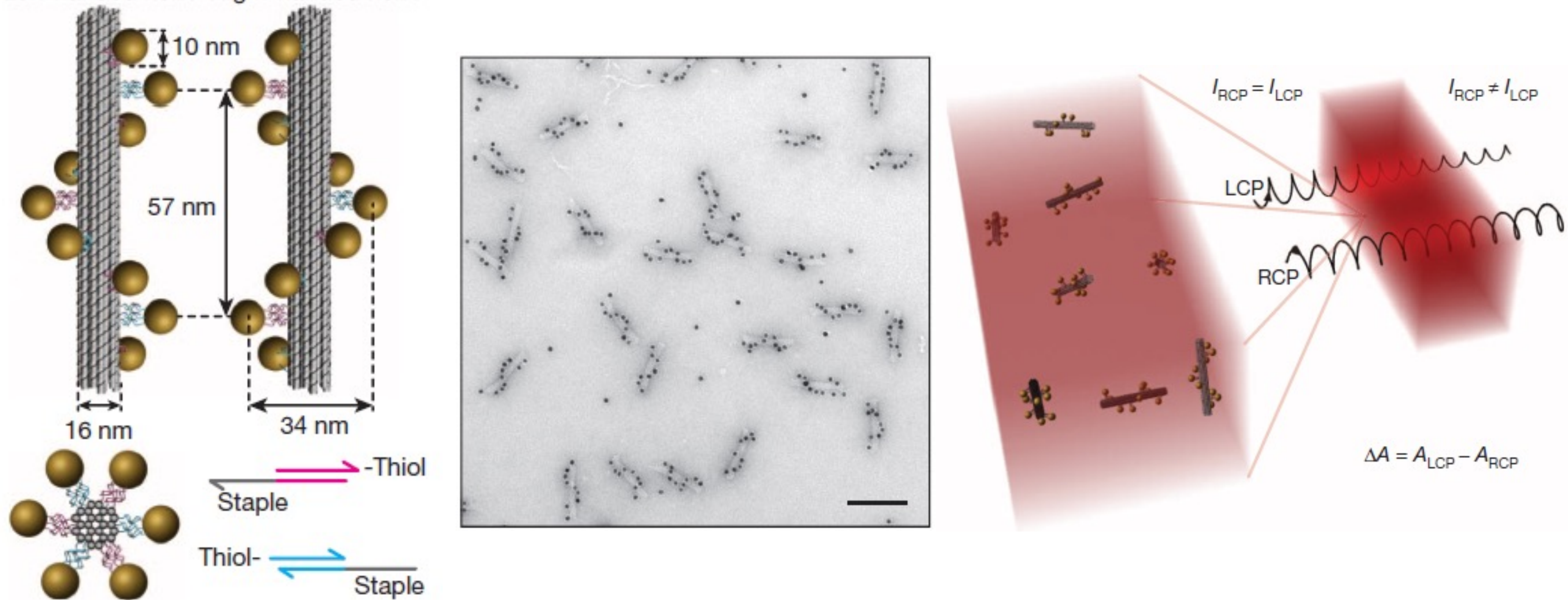
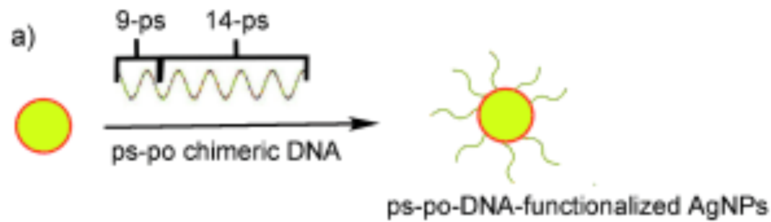
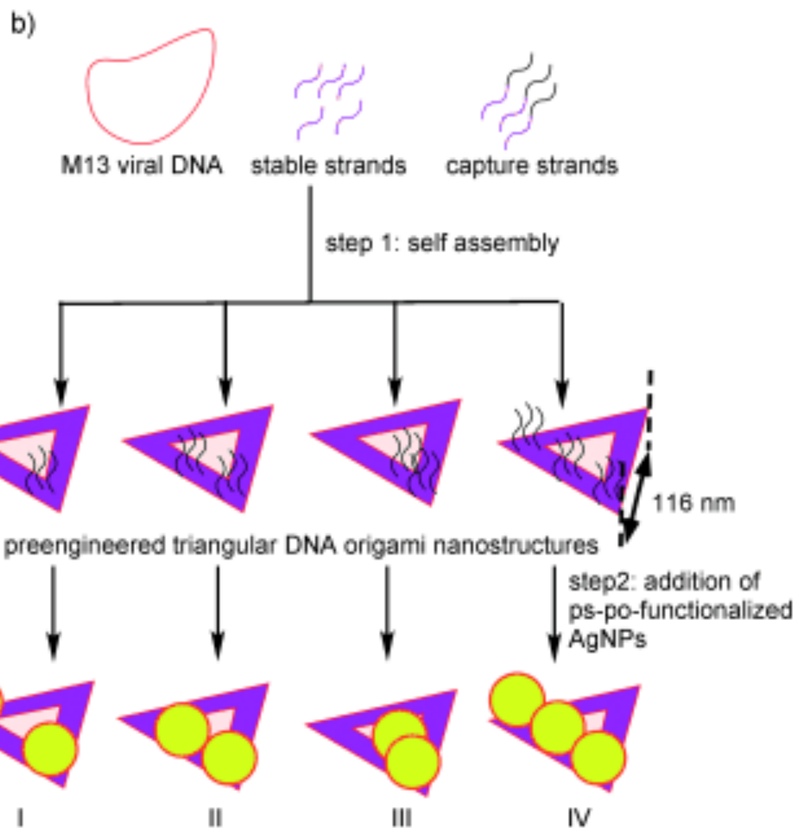
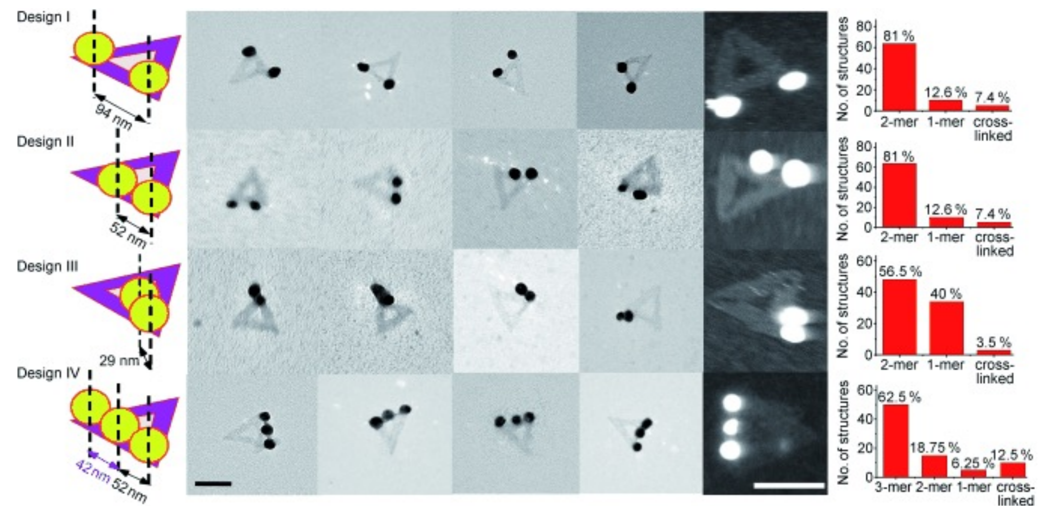


Figure 1 | Assembly of DNA origami gold nanoparticle helices and principle of circular dichroism. **a**, Left- and right-handed nanohelices (diameter 34 nm, helical pitch 57 nm) are formed by nine gold nanoparticles each of diameter 10 nm that are attached to the surface of DNA origami 24-helix bundles (each of diameter 16 nm). Each attachment site consists of three 15-nucleotide-long single-stranded extensions of staple oligonucleotides. Gold nanoparticles carry multiple thiol-modified DNA strands, which are complementary to these staple extensions. Nanoparticles and 24-helix bundles are mixed for assembly and the resulting constructs are gel-purified. **b**, TEM image of assembled left-handed gold nanohelices (scale bar, 100 nm). Analysis of the TEM data yields a 98% success rate for directed attachment of nanoparticles. **c**, Circular dichroism is measured as the difference in absorbance $\Delta A = A_{LCP} - A_{RCP}$ of left-hand-circularly polarized (LCP) and right-hand-circularly polarized (RCP) light as a function of wavelength. CD measurements were performed with a CD spectrometer on samples in cuvettes of optical path length 3 mm.

Silver-Nanoparticle Architectures





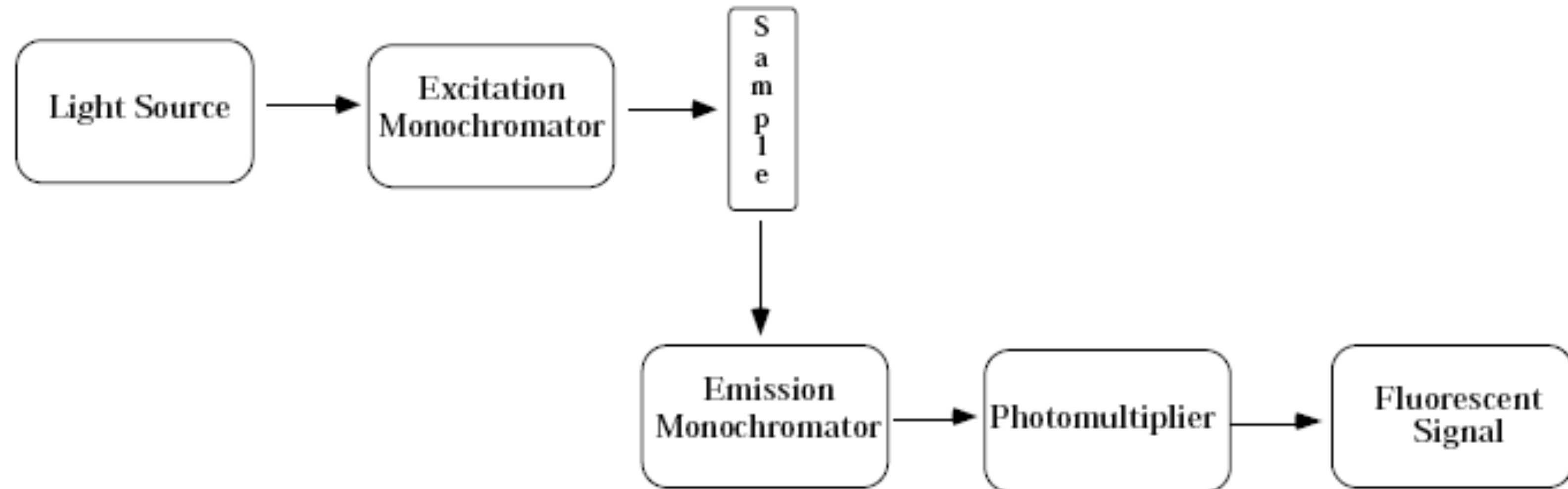


Angewandte Chemie International Edition
Volume 49, Issue 15, pages 2700-2704, 16 MAR 2010 DOI: 10.1002/anie.201000330
http://onlinelibrary.wiley.com/doi/10.1002/anie.201000330/full#fig1

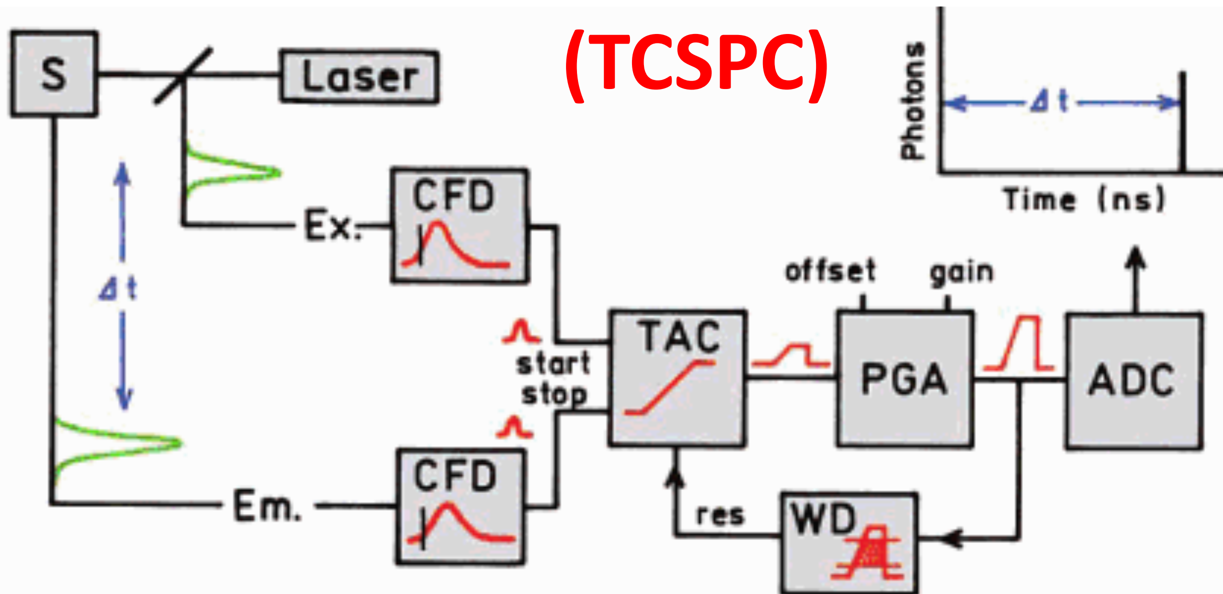
Fluorescence Detection techniques

- **Ensemble Fluorescence Spectroscopy**
- **Time-Correlated Single-Photon Counting (TCSPC)**
- **Total Internal Reflection (TIRF)**

Ensemble FRET detection



Time-Correlated Single-Photon Counting (TCSPC)



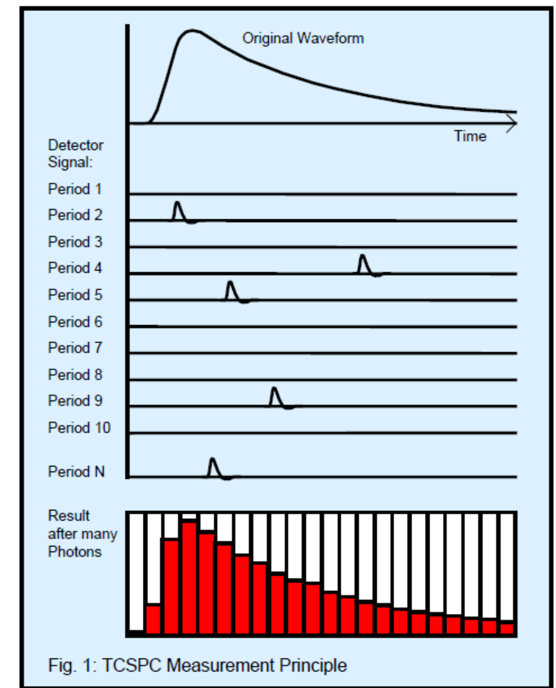
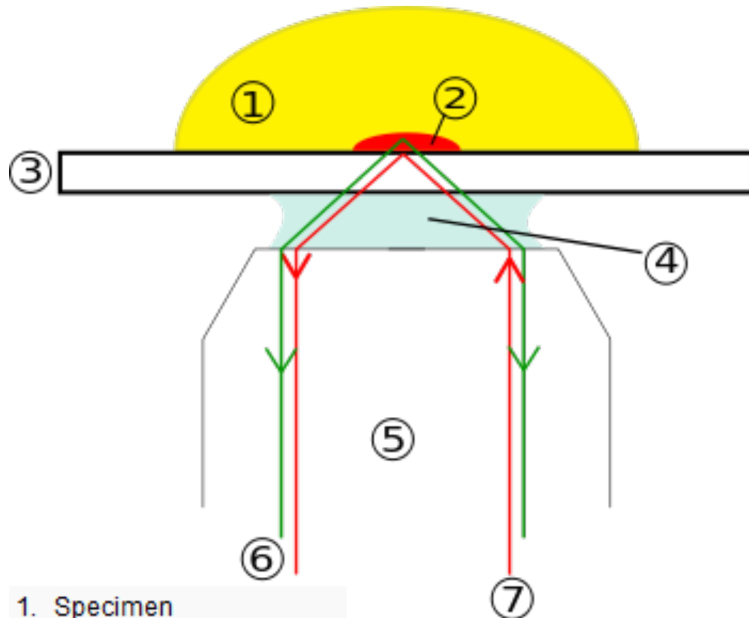


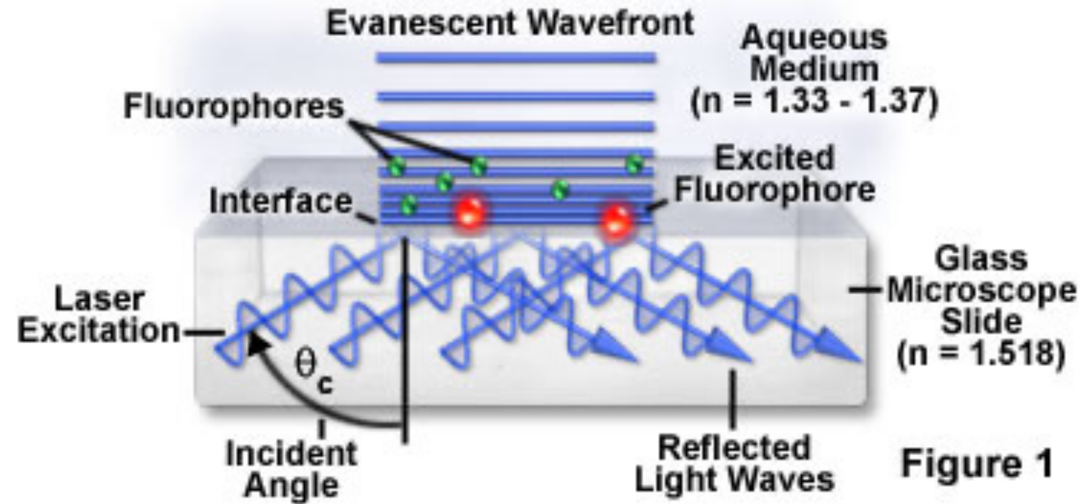
Fig. 1: TCSPC Measurement Principle

- **The experiment starts with the excitation pulse that excites the samples and sends a signal to the electronics.**
- **This signal is passed through a constant function discriminator (CFD), which accurately measures the arrival time of the pulse.**
- **This signal is passed to a time-to-amplitude converter (TAC), which generates a voltage ramp that is a voltage that increases linearly with time on the nanosecond timescale.**
- **A second channel detects the pulse from the single detected photon.**
- **The arrival time of the signal is accurately determined using a CFD, which sends a signal to stop the voltage ramp.**
- **The TAC now contains a voltage proportional to the time delay (delta t) between the excitation and emission signals.**
- **As needed the voltage is amplified by a programmable gain amplitude (PGA) and converted to a numerical value by the analog-to-digital converter (ADC).**
- **To minimize false readings the signal is restricted to given range of voltages.**
- **If the signal is not within this range the event is suppressed by a window discriminator (WD).**
- **The voltage is converted to a digital value that is stored as a single event with the measured time delay.**
- **A histogram of the decay is measured by repeating this process numerous times with a pulsed-light source.**

Total Internal Reflection Fluorescence (TIRF)



1. Specimen
2. Evanescent wave range
3. Cover slip
4. Immersion oil
5. Objective
6. Emission beam (signal)
7. Excitation beam



- **Fluorescent molecules are supported on a glass microscope slide.**
- **The refractive indices of the glass slide (1.518) and the aqueous medium (~ 1.35) are appropriate to support total internal reflection within the glass slide.**
- **With adjustment of the laser excitation incidence angle to a value greater than the critical angle, the illuminating beam is entirely reflected back into the microscope slide upon encountering the interface, and an evanescent field is generated in the medium immediately adjacent to the interface.**
- **The fluorphores nearest the glass surface are selectively excited by interaction with the evanescent field, and secondary fluorescence from these emitters can be collected by the microscope optics.**

[Demo: http:/// interactagram.com/physics/optics/refraction](http:///interactagram.com/physics/optics/refraction)

Other Fluorescence Detection techniques

- Flourescence imaging with one-nanometer accuracy (FIONA)
- Single-molecule high-resolution imaging with photobleaching (SHIRMP)
- Direct stochastic optical reconstruction microscopy (dSTORM)
- Blink Microscopy

Radioisotopic Labeling: Advantages

- Incorporation can be customized
  - Defined molecule
  - Defined level of radioactivity per molecule
- Easily detectable
- Flexible readout assays
- Quantitative

Radioisotopic Labeling: Disadvantages

- Special precautions required for working with radioactivity
- Emission can induce cellular damage and artifacts
- Isotope has a window of use
- Usually a trade-off between half-life and specific activity
- Further reading
  - http://stuff.mit.edu/people/ara/thesis08.pdf

Isotope	Half-Life	Specific Activity Max
^{3}H	12 years	28.8 Ci/mmol
^{35}S	88 days	1500 Ci/mmol
^{32}P	14 days	9000 Ci/mmol
^{33}P	25.4 days	5200 Ci/mmol

Rachel Graham, IGP Methodology, Metabolic Labeling 2005