

Fluorescent Oligomer Used in a Pathogen Biosensor

Presented By:

Danielle Okerblom of Cuesta College

UCSB Dept of Chemistry and Biochemistry

Mentor: Aidee Gonzalez

Advisor: Gui Bazan

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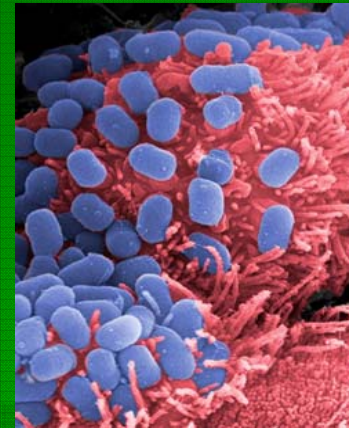


INSET



Recent News and Outbreaks

- **January 27, 2009- *More Salmonella Peanut Butter Recalls Coming* (5)**
- **June 2008- *Canada listeria outbreak 22 people died. Concluded that listeria is difficult to detect* (6)**
- **August 7, 2008- *Years After Anthrax Attacks, Bioterrorism Threat Still Looms* (3)**
- **June 18, 2008- *Investigation of Multistate Outbreak of E. coli* (6)**



Standard Methods of Detecting Bacteria

- Coliform Bacteria in Water Samples

- Lauryl tryptose broth or The Autoanalysis Colilert System.
- 24hrs to detect
- positive or negative results only does not identify of bacteria. Also marine bacteria have been shown to give false positive (1)

- Food Samples Salmonella-

- Grown in Trypticase soy broth
- To grow and detect CDC Biohazard level 2 guidelines must be met
- 30hrs to detect (2)

- Mail out for Identification to an Industry Lab

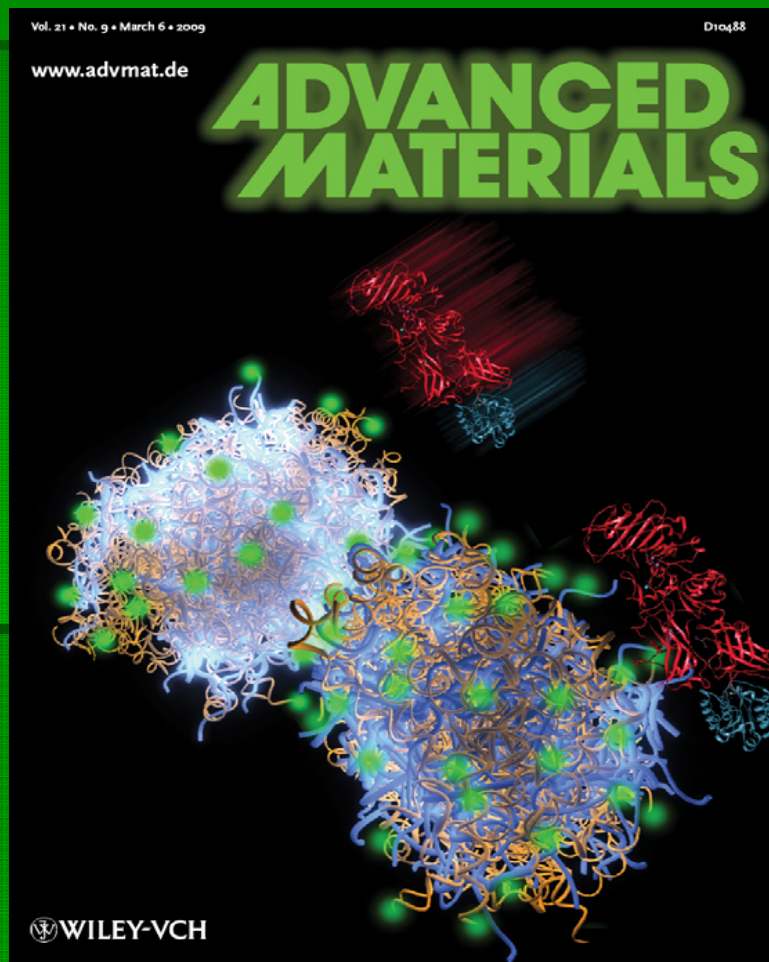
- \$50 per sample for test
- 10 business days
- Possible additional costs for meat samples. (4)



www.water-research.net/images/coliformcolony.jpg

Expanding Uses of Organic Polymers and Oligomers

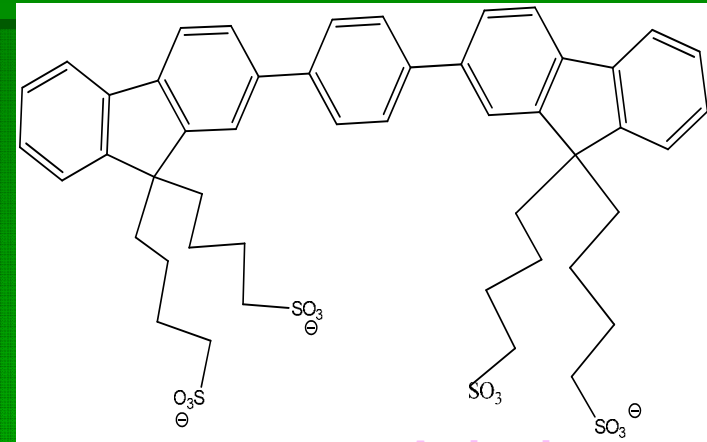
- Synthesizing Oligomers
- Prepare new applications for Oligomers
- Characterize oligomers
- Our lab has created aggregates which detect presence of proteins in solution
- Biospecies interacts with aggregates this modifies the fluorescence spectra



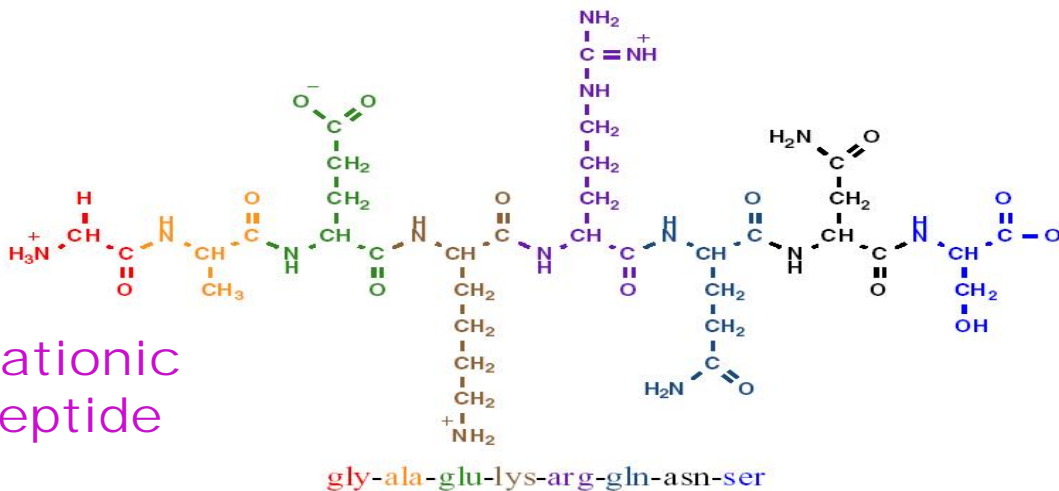
Huaping Li, Gui Bazan, Advance Material
Journal Volume 21 No 9 March 6th 2009

Synthesizing and Testing the Limitations of Aggregate Interactions

- Synthesize conjugated, water soluble oligomer
- Oligomer and peptides form aggregates by electrostatic interactions
- Test dependence of FRET signal on solvents



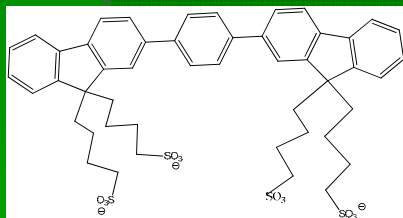
Anionic Oligomer



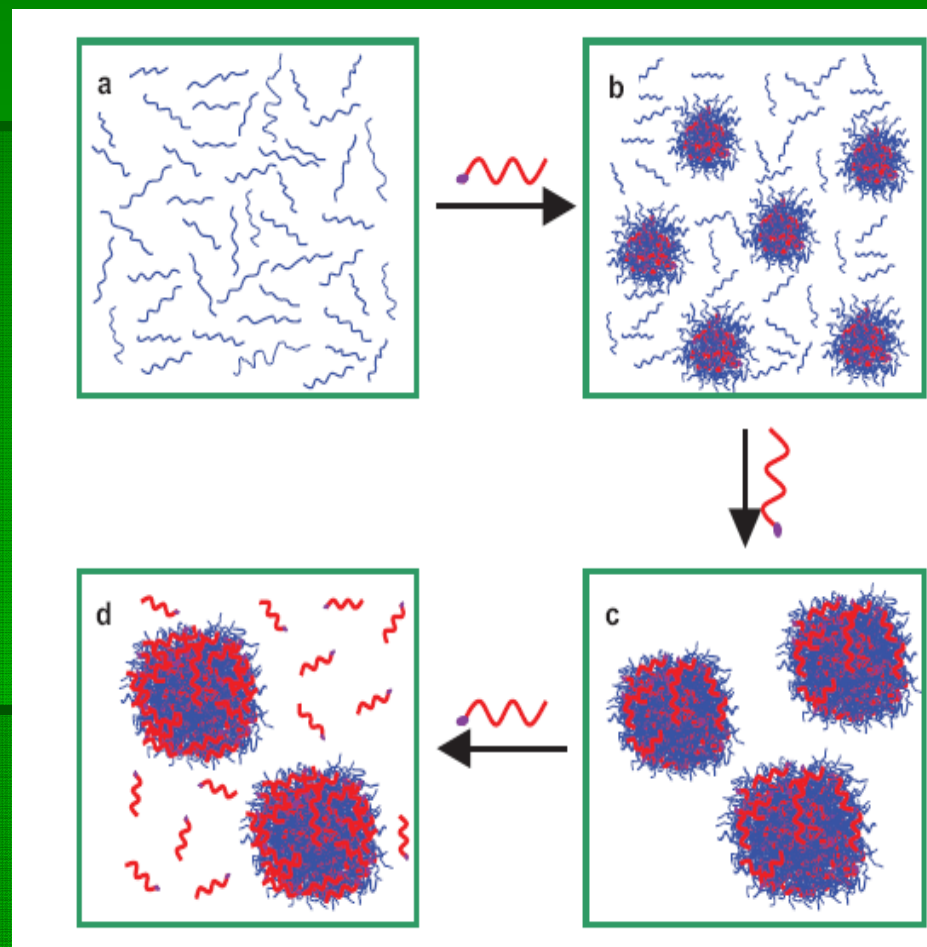
Cationic Peptide

<http://www.activorcorporation.net/sitebuildercontent/sitebuilderpictures/peptide10.jpg>

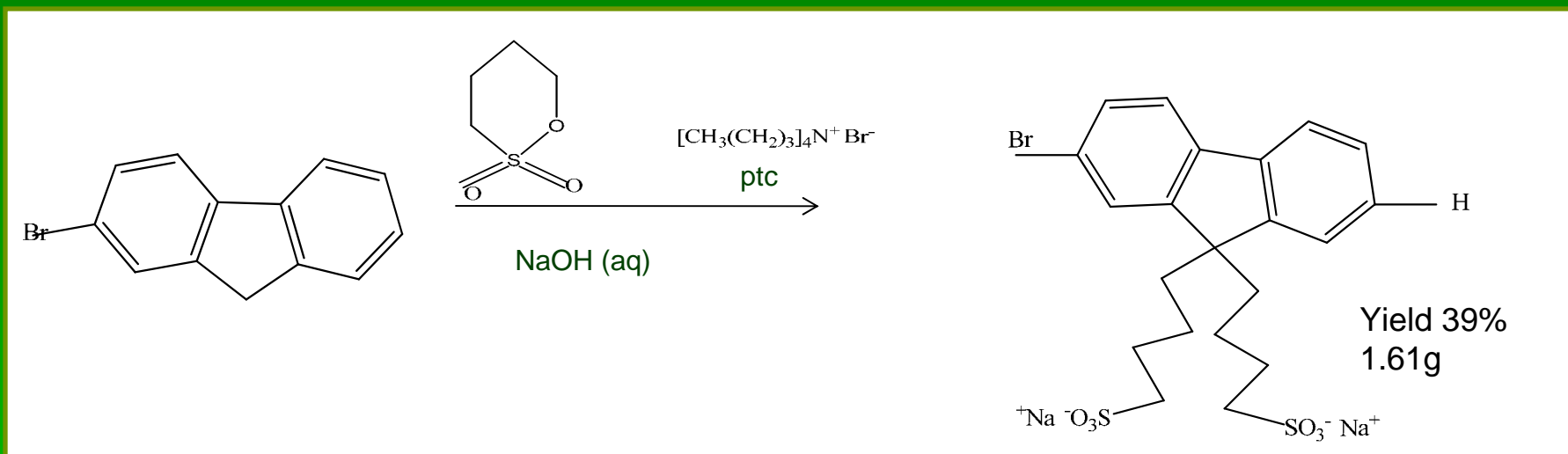
Electro-statically Formed Aggregates



- Oligomer is seen in blue
- Peptide tagged with Fluorescence is seen in red
- Negatively charged oligomer binds to positively charged peptide to form aggregate
- Energy transfer within aggregates measured with Forster Resonance Energy Transfer



Synthesis of Monomer:



Outline-

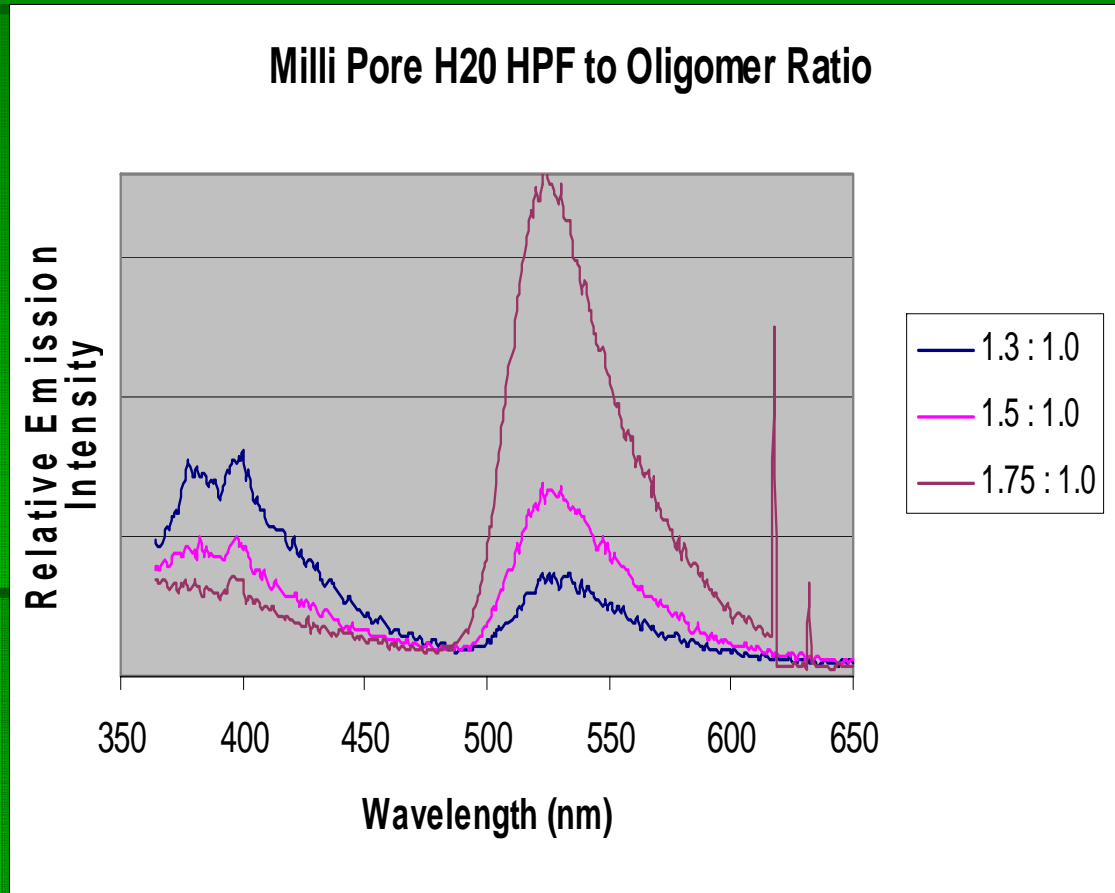
- 1) Reaction- Dissolve Fluorene, NaOH, ptc in DMSO. Add Sultone.
- 2) Transfer to acetone solution & precipitate
- 3) Purification
- 4) Crystallization
- 5) NMR Confirmation of product.
- 6) Used to create Oligomer



www.bridgat.com/files/Caustoc_soda.jpg

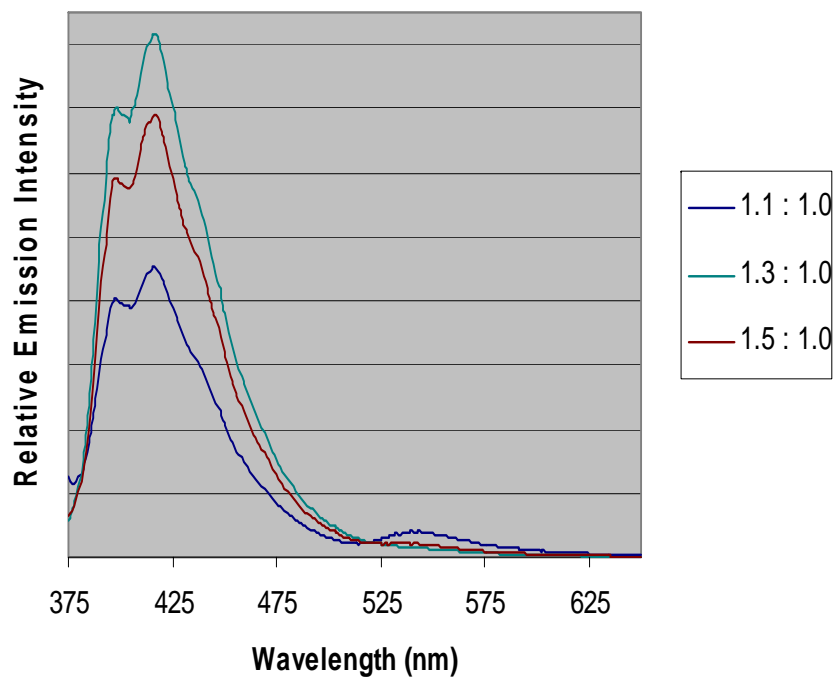
Solvent Investigations:

- The 400nm region is where the oligomer emissions is.
- The 550nm region is where the fluorescence emission is.
- Difference charge concentration which were tested.
- Stability of signals over 60 minutes time was measured.
- Relavance- 1st peak compared to 2nd peak intensity.

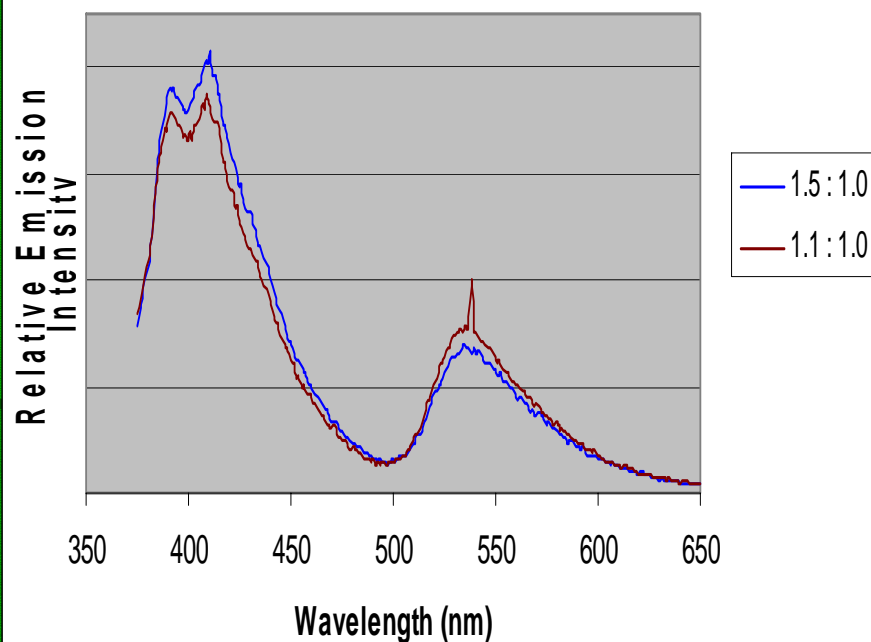


Further Solvent Investigations

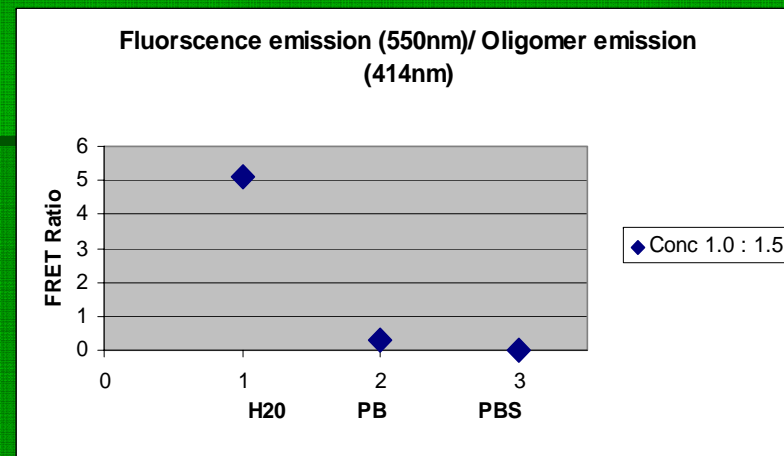
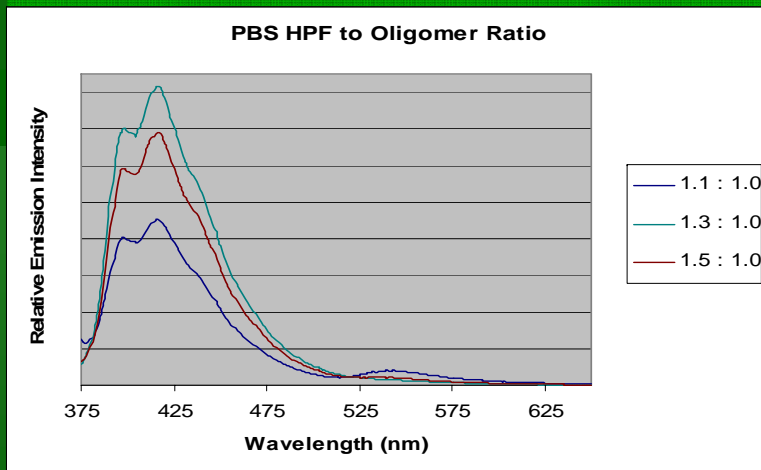
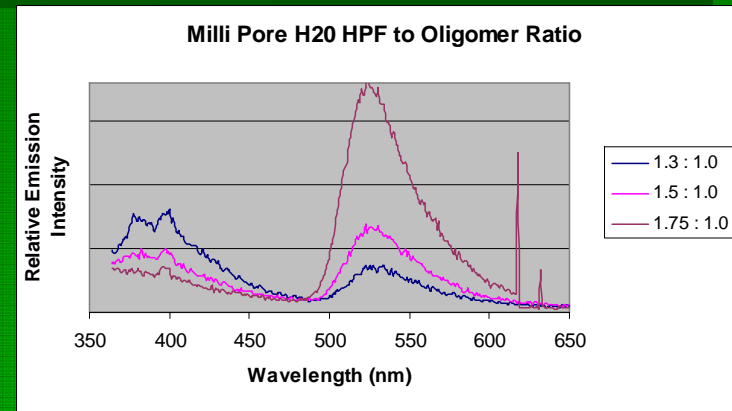
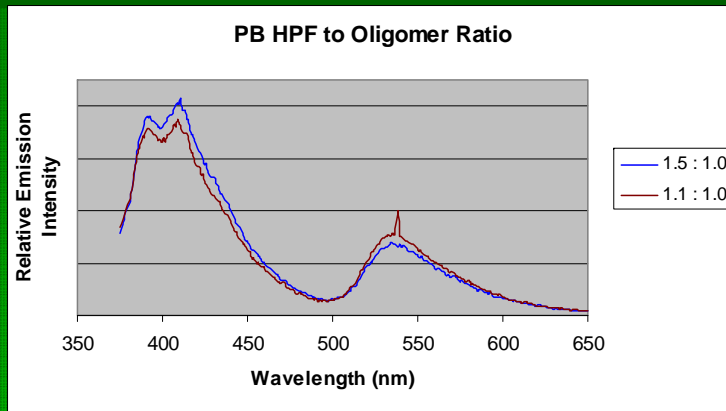
PBS HPF to Oligomer Ratio



PB HPF to Oligomer Ratio



Solvent Investigation of FRET Signal : Phosphate Buffer vs Phosphate Buffer Saline vs Milli Pore Water



FRET ratio= Fluorescence emission / Oligomer emission

Future Work:

- Look at E. Coli isolated samples within the optimal peptide oligomer ratios
- Measuring aggregation formation with other peptides.
- Determine FRET signal using different bacteria and effect of media such soil and water.



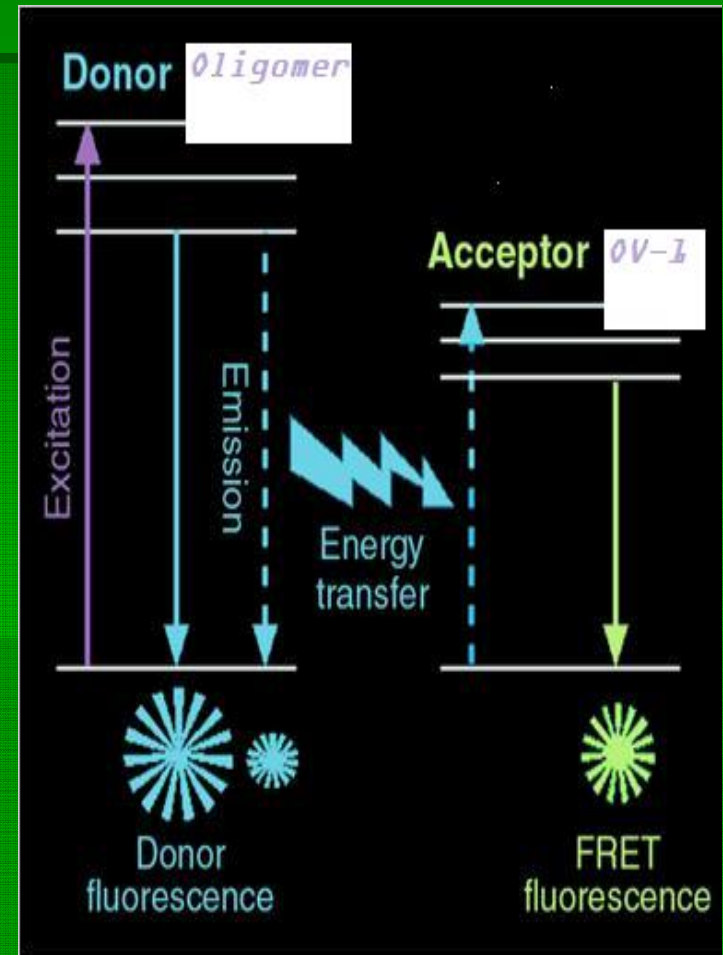
References:

- (1) EPA Analytic analysis drinking water
<http://www.epa.gov/nerlcwww/online.htm>
- (2) USDA Analysis of Meat, Poultry and Egg
http://www.fsis.usda.gov/PDF/MLG_4_04.pdf
- (3) http://www.pbs.org/newshour/bb/terrorism/july-dec08/bioterrorprep_08-07.html
- (4) MRPK Industry lab 1(866)888-6653
nbc33tv.com
- (5) <http://www.cdc.gov/salmonella/>

What is FRET

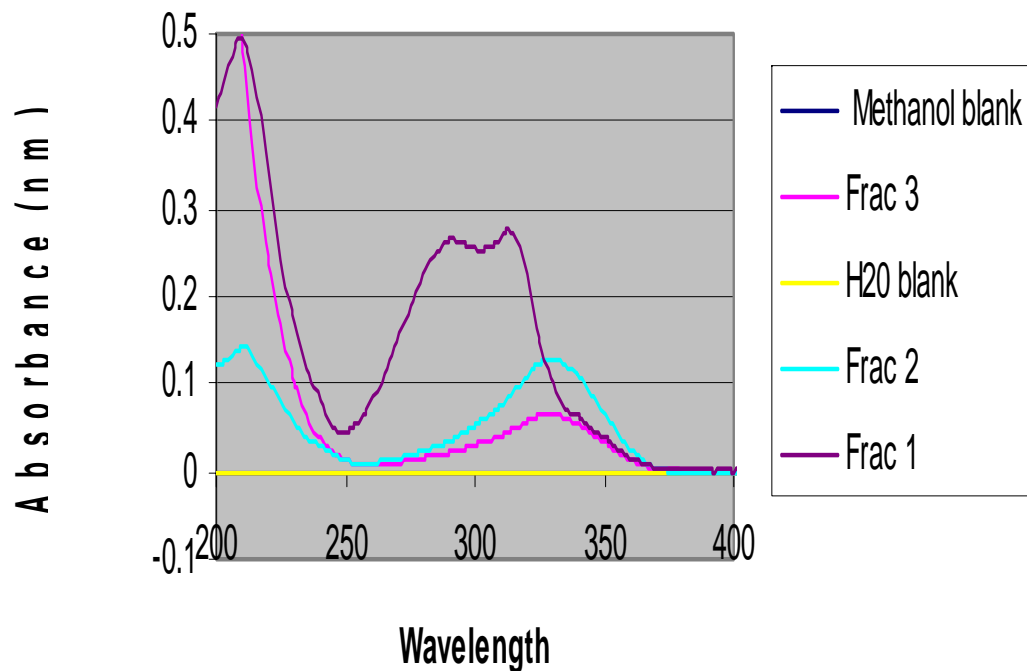
- FRET- Forester Resonance Energy Transfer
- describes energy transfer between two chromophores located less than 10nm from each other
- Excited electron from donor chromophore are transferred to lower levels of energy at acceptor chromophore.

*Chromophores almost always arise in one of two forms: conjugated pi systems.



Isolated Oligomer Fractions

Isolated FPFC4S03 Fract 1-3



*Fraction 2 is FPFC4S03.

*We use long UV waves lights to see the fluorescent glow