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Indium Phosphide Quantum Dot-Based FRET Probe for the Analysis of Phosphatase Activity

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BACKGROUND

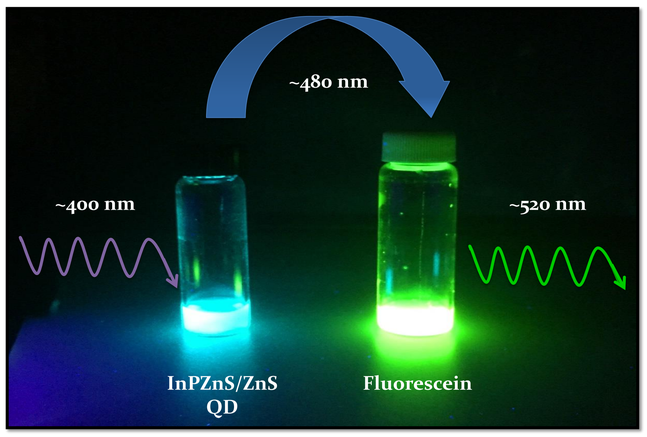
Dephosphorylation is a tightly regulated process that occurs regularly in cells. It is the removal of a phosphate group from an organic molecule. In biological processes, it is carried out by the enzyme phosphatase. If the phosphatase enzyme becomes damaged or defective, dephosphorylation can become uncontrollable, resulting in an excesses of phosphorylated proteins. The *tau hypothesis* suggests that it is this buildup of the phosphorylated tau protein that results in neurofibrillary tangles, neuron death, and Alzheimer's Disease. Alzheimer's is one of the most terrible and expensive diseases in the modern world. Very little is understood about it, therefore there are no cures or preventative measures. In order to monitor dephosphorylation in biological systems, a probe would have to be accurate, water-soluble, non-toxic, and sensitive.

MISSION STATEMENT

Design a probe that is sensitive enough to determine phosphatase specific activity and safe enough to use in cellular environments.

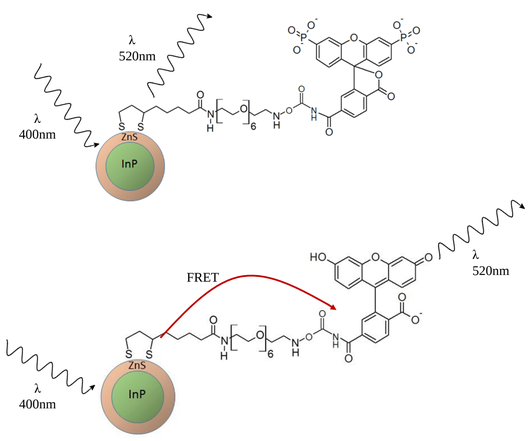
EXPERIMENTAL DESIGN

FIGURE 1: THE CONCEPT OF FLUORESCNECE RESONANCE ENERGY TRANSFER



If the donor species (quantum dot) and acceptor species (organic fluorophore) come within 1-10nm of each other, the donor species, instead of fluorescing, will donate its energy to the acceptor, who will become excited and then release its energy as photons lower in energy than the incident light[2].

FIGURE 2: COMPONENTS OF THE QUANTUM DOT BASED-FRET PROBE



This sensitive and water-soluble probe will borrow the best properties from three separate species. The quantum dot provides sensitivity, the PEG derivative provides water solubility, and the fluorescein dye provides a quenched fluorescence that is revealed with the cleavage of the phosphate groups.

FIGURE 2: SYNTHESIS OF InPZnS/ZnS CORE/SHELL QUANTUM DOTS[3]

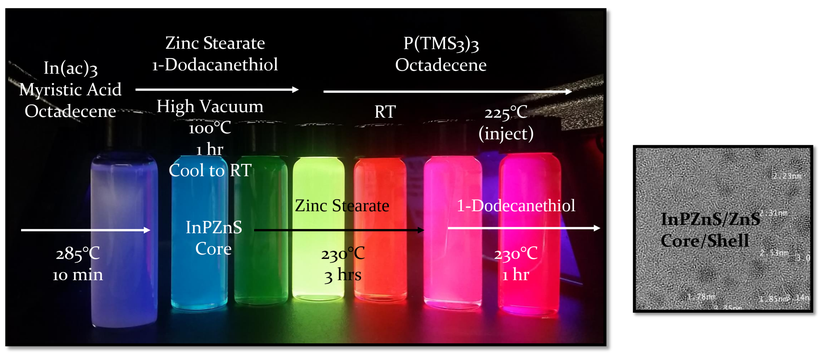
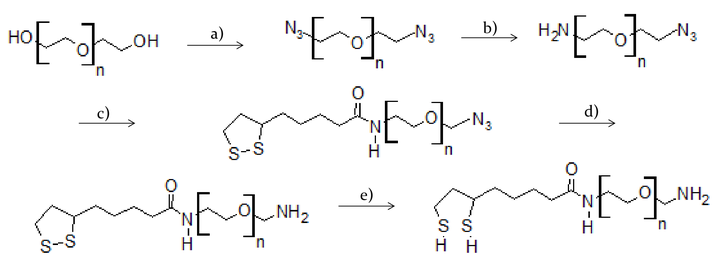
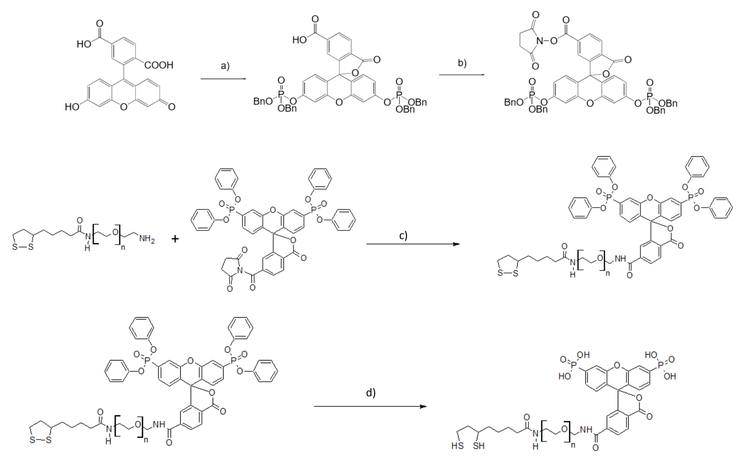


FIGURE 3: SYNTHESIZING DHLA-PEG$_{400}$-NH$_2$[4]



a) Mesyl-Cl, THF, NaN_3, H_2O; b) $(Ph)_3P$, EtOAc, 1M HCl ; c)Thioctic acid, DCC/DMAP, DCM d) $(Ph)_3P$, THF ; e) $NaBH_4$, MeOH/H_2O

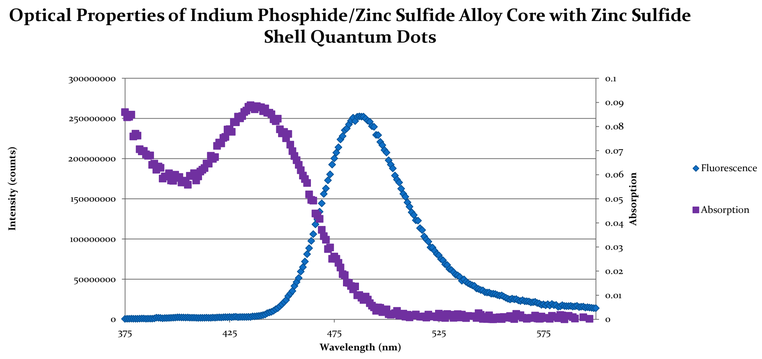
FIGURE 4: SYNTHESIS OF PHOSPHORYLATED FLUORESCEIN LIGAND[5]



a) $(BnO)_2PN(iPr)_2$, 1H-tetrazole, NEt_3 , mCPBA, $CHCl_3$; b) N-hydroxysuccinimide, EDC · HCl, $CHCl_3$; c) DMF ; d) Pd/C, H_2

QUANTUM DOT RESULTS

FIGURE 5: FLUORESCENCE AND ABSORPTION SPECTRA OF QUANTUM DOTS



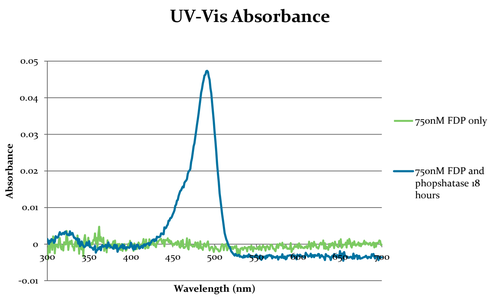
In order for the quantum dots to act as a successful donor species, they must emit light within the absorption spectrum of fluorescein (Figure 6a). The emission of these quantum dots has a peak at 483 nm, which is suitable to induce fluorescence in fluorescein. The absorption spectrum shows a strong absorption in the low 400nm range. This is important because in order to avoid direct excitation of the fluorescein, the wavelength of light that excites the quantum dot must be far from fluorescein's absorption peak (Figure 6a).

PHOSPHATASE ASSAY RESULTS

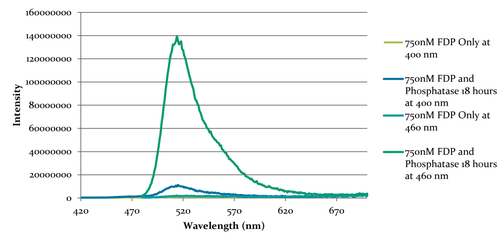
FIGURE 6 (a-d): CALCULATING SPECIFIC ACTIVITY OF ALKALINE PHOSPHATASE

6a: Effect of 18 hours Phosphatase Exposure on Fluorescein Diphosphate Absorbance

A distinct change in absorption around 480 nm is observable after the cleavage of the two phosphate groups from fluorescein diphosphate by alkaline phosphatase.



Fluorescence Excited from 400 nm and 460 nm



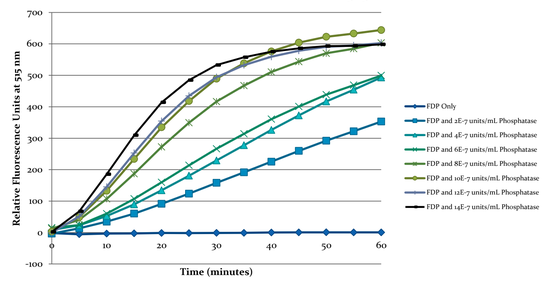
6b: Effect of 18 hours Phosphatase Exposure on Fluorescein Diphosphate Fluorescence at 400 nm and 460 nm

A distinct change in fluorescence is observable when excited at 460 nm, which is within the fluorescence spectrum of the quantum dots (Figure 4).

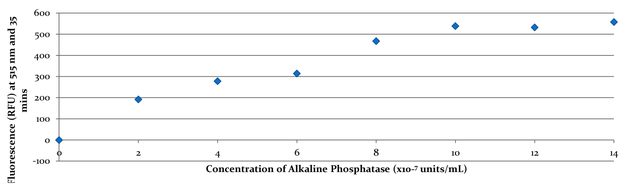
6c: Effect of Increasing Concentrations of Phosphatase in Fluorescein Diphosphate

Assuming one unit will hydrolyze 500 nmol in a total reaction volume of 1 mL in 1 minute at 37°C, the optimal concentration would be 10E-7 units/mL. Each data point is an average of three trials.

Fluorescence Emitted at 515 nm



Fluorescence Emitted at 515 nm after 35 Minutes Exposure



6d: Specific Activity of Phosphatase in Fluorescein Diphosphate

The specific activity of the enzyme is calculated using this graph. Signal change is related to the activity using enzyme kinetics equations.

CONCLUSIONS AND FUTURE WORK

The successful preparation of two of the three components of the probe and the assay suggests that this project should be continued. More research needs to be done on the synthesis of the phosphorylated fluorescein and the sequence in which the probe should be assembled. After testing the probe's sensitivity in the test tube environment, it should be tested in the cellular environment. From there, it can move onto animal and human subjects.

ACKNOWLEDGEMENTS

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