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Exploring Indicator Displacement Assays for Phosphate Detection in Seawater

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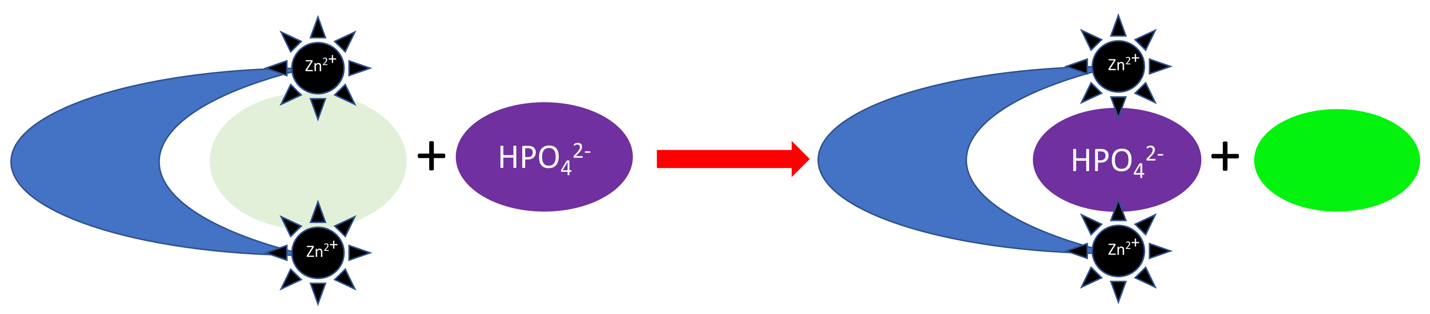
Introduction

According to the US EPA, phosphate in aquatic environments is the "limiting nutrient" for algae growth. Enhanced concentrations of phosphate can fuel harmful algal blooms, which have a variety of detrimental impacts on aquatic ecosystems.

We are working within the RI C-AIM Thrust III project portfolio to develop low-cost and easily deployable fluorescence-based sensors for phosphate and nitrate. The goal for the Thrust III project is limit-of-detection (LOD) of 1 ppb in saltwater for each anion.

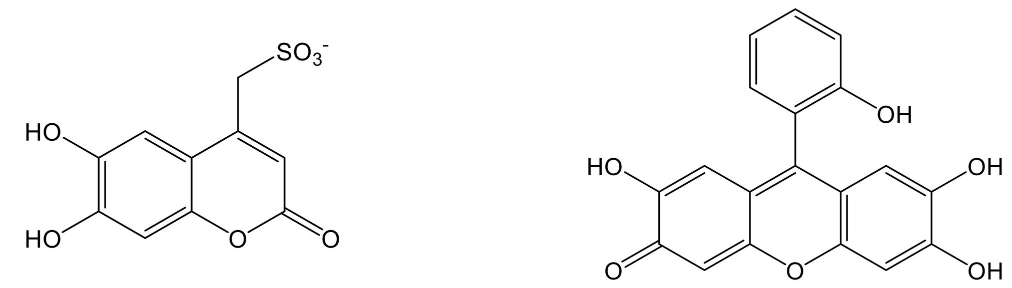
Indicator Displacement Assays

Indicator displacement assays (IDAs) are based on the optical signal modulation of a noncovalently bound indicator upon dissociation by an analyte species.[1] Many absorbance based IDAs have been developed and phosphate and pyrophosphate are common targets.[1-4] Our interest is in fluorescence based IDA's, particularly those involving indicator dyes that absorb and emit visible light.



Indicators

We have explored two fluorescent dyes for this work, 6,7-dihydroxy-coumarin-4-methylsulfonate (C) and salicyl fluorone (SF). C was used in one of the first fluorescent IDAs by B.D. Smith and co-workers[4] and SF has previously been used as a colorimetric indicator for Co^{2+}, Cu^{2+}, and Zn^{2+}.

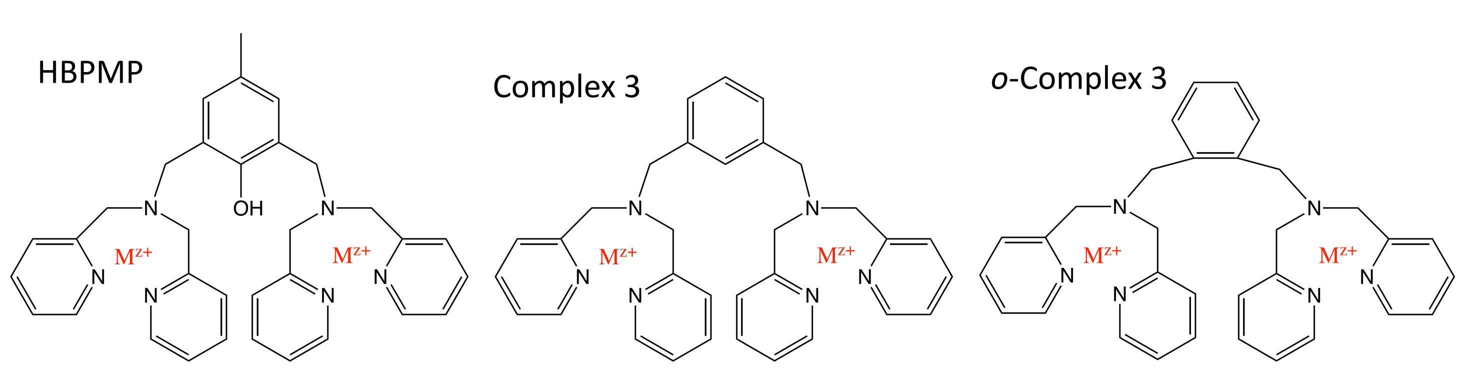


6,7-dihydroxy-coumarin-4-methylsulfonate

Salicyl Fluorone

Ligand:Metal Ion Complexes

We have prepared three ligands, each with two di(2-picolyl)amine (also called DPA or *bis*(2-pyridylmethyl)amine) groups starting from 2,6-*bis*(chloromethyl)-4-methylphenol, 2,6-*bis*(chloromethyl) benzene, and 1,2-phenylenedimethylamine. The first two ligands, HBPMP and Complex 3 have shown good selectivity for phosphate and pyrophosphate.



While we have briefly and unsuccessfully investigated other metal ions such as Cu^{2+}, Eu^{3+}, and Fe^{3+}, all experiments in this poster are with Zn^{2+}.

References

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2. M.S. Han and D. H. Kim. *Angew. Chem., Int. Ed.* **2002**, 41, 3809 – 3811.
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Fluorescence Assays: 6,7-dihydroxy-coumarin-4-methylsulfonate (C) with HBPMP/Zn^{2+}

The fluorescence indicator displacement assays follow and expand on the work of B. D. Smith and coworkers (Tetrahedron Letters 45 (2004) 8721 – 8724).

Solutions of the dye alone are colorless; however, additions of HBPMP/Zn^{2+} leads to the solution changing color to a yellow color. This observation can also be seen in the absorption where the λ_{max} shifts towards longer wavelengths upon the addition of HBPMP/Zn^{2+}.

Following addition of phosphate, the yellow color diminishes.

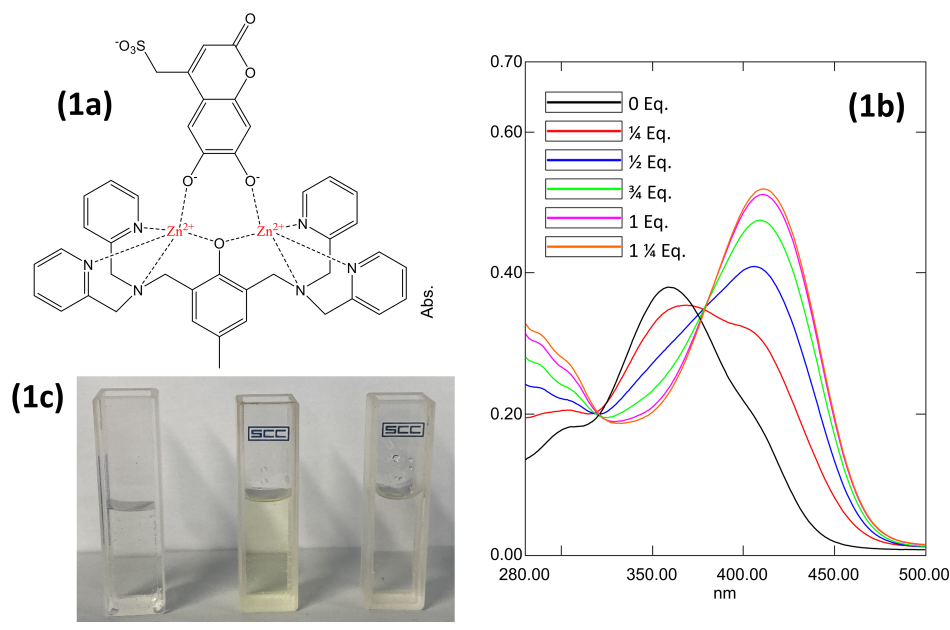


Figure C1: (a) A schematic of the C•HBPMP/Zn^{2+} complex, (b) the absorbance spectra of a 10 μM solution of C with additions of HBPMP/Zn^{2+} in pH 7.2 0.010 M HEPES, and **(c)** solutions of C, C•HBPMP/Zn^{2+} and C•HBPMP/Zn^{2+} with > 1 equivalent phosphate.

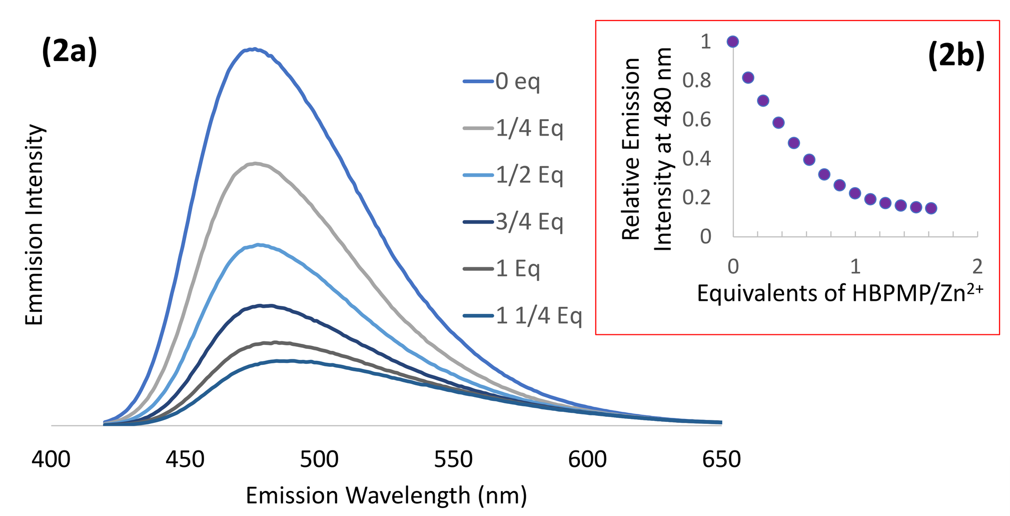


Figure C2: (a) The emission spectra of a 50 μM solution of C in pH 7.2, 0.010 M HEPES with additions of HBPMP/Zn^{2+} (λ_{ex} = 347 nm) and **(b)** the relative emission intensity at 480 nm with increasing HBPMP/Zn^{2+} indicating a 1:1 complex.

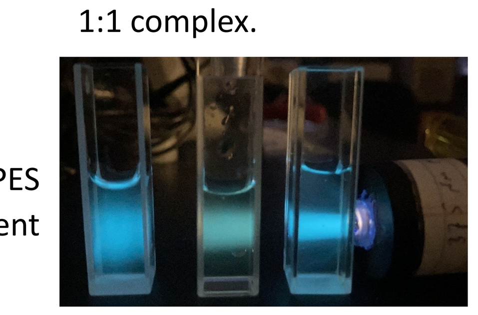
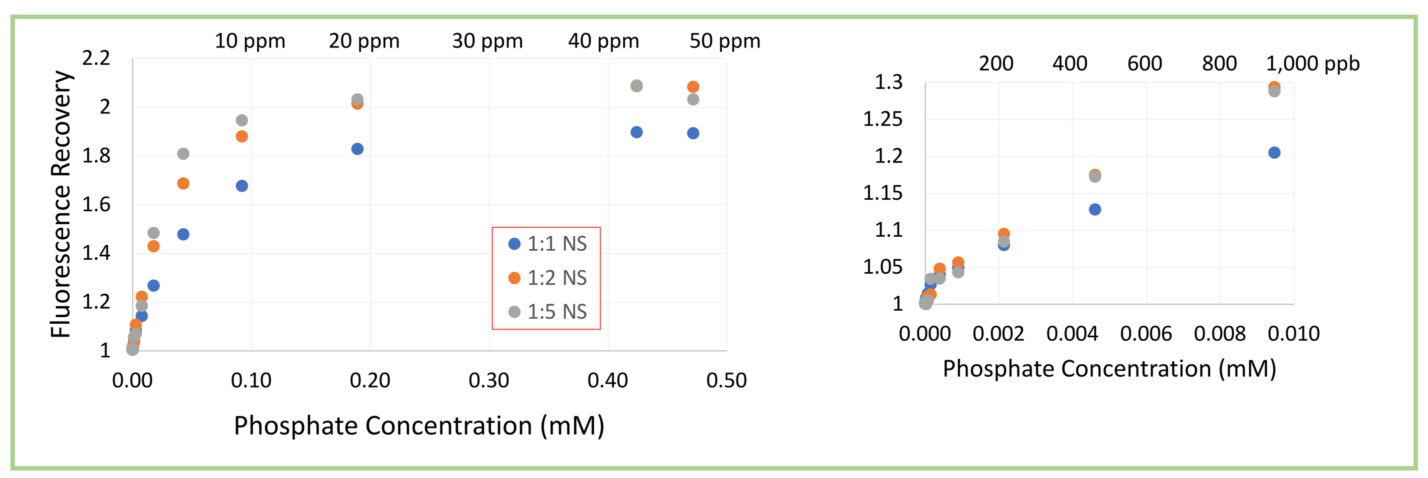
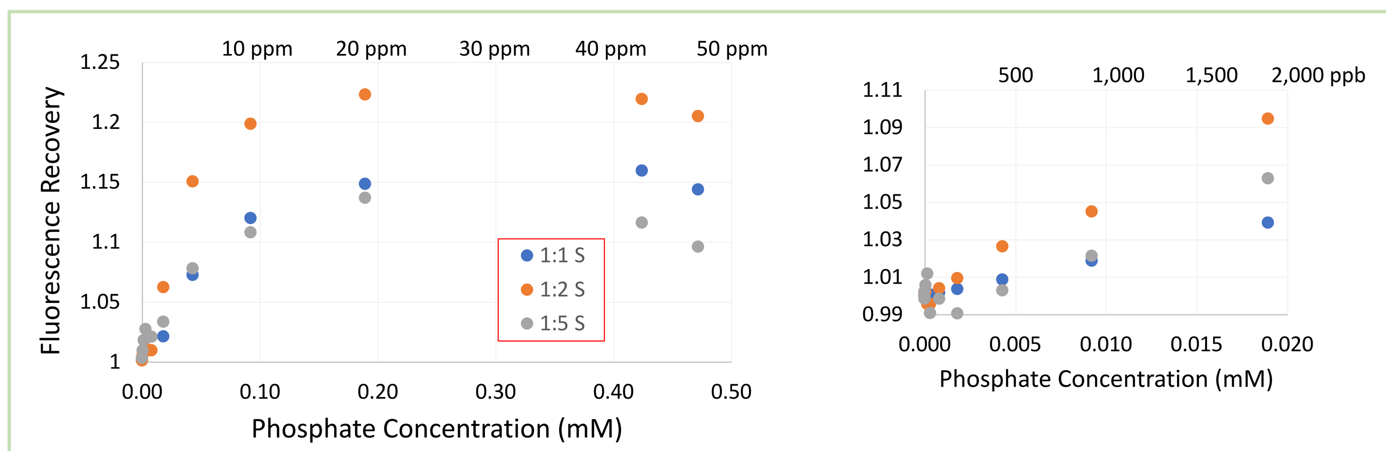


Figure C3: Pictured on the right is an image of pH 7.2, 0.010 M HEPES solutions of C, C•HBPMP/Zn^{2+} and C•HBPMP/Zn^{2+} with > 1 equivalent phosphate excited with a 370 nm LED.

Shown below are the results of six pairs of experiments (with and without salt) in which aliquots of phosphate is added to a solution of the C•HBPMP/Zn^{2+} complex and the change in the emission intensity is monitored at 480 nm (λ_{ex} = 347 nm). A 0.01 M phosphate solution was used for the experiments covering 0 – 0.5 mM while 0.0005 M and 0.001 M phosphate solutions were used for the experiments covering 0 – 0.01 M and 0 – 0.02 M. The ratio of C to HBPMP/Zn^{2+} was varied from 1:1 to 1:5 to investigate any significant improvement in the LOD.[5] In all experiments the displacement of the dye by phosphate leads to an increase in the fluorescence intensity.





Takeaways

1) The maximum recovery is ~ 1/3 of the dye alone signal.
2) The extent of quenching and dynamic range is reduced in the presence of 0.1 M NaCl.
3) The maximum recovery is reached with ~ 4 equivalents of phosphate. This is similar to our experiments with SF and reported colorimetric IDAs.
4) Our LODs are ~ 100 ppb in pH 7.2 0.01 M HEPES and ~ 500 ppb with 0.1 M NaCl.

Fluorescence Assays: Salicyl Fluorone with HBPMP/Zn^{2+}

The second dye we investigated was salicyl fluorone (SF).

Solutions of the dye alone are orange; however, additions of HBPMP/Zn^{2+} leads to the solution changing color to a pinkish color. This observation can also be seen in the absorption spectra on the right, where the λ_{max} shifts towards longer wavelengths upon the addition of HBMP/Zn^{2+}.

Following addition of phosphate, the pinkish color changes to a light purple.

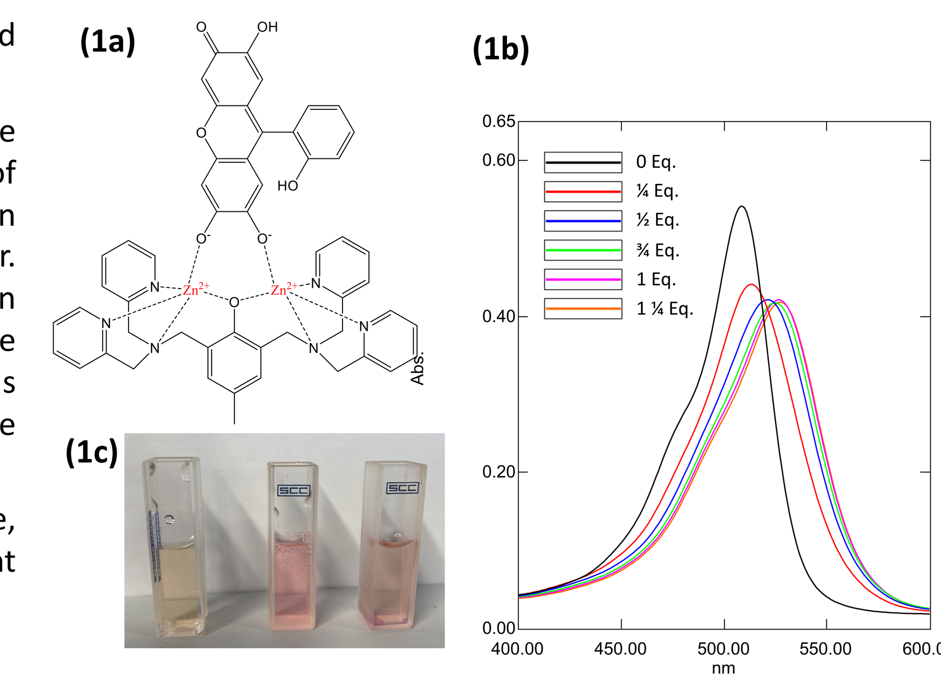


Figure SF1: (a) A schematic of the SF•HBPMP/Zn^{2+} complex, **(b)** the absorbance spectra of a 10 μM solution of SF with additions of HBPMP/Zn^{2+} in pH 7.2 0.010 M HEPES, and **(c)** solutions of SF, SF•HBPMP/Zn^{2+} and SF•HBPMP/Zn^{2+} with > 1 equivalent phosphate.

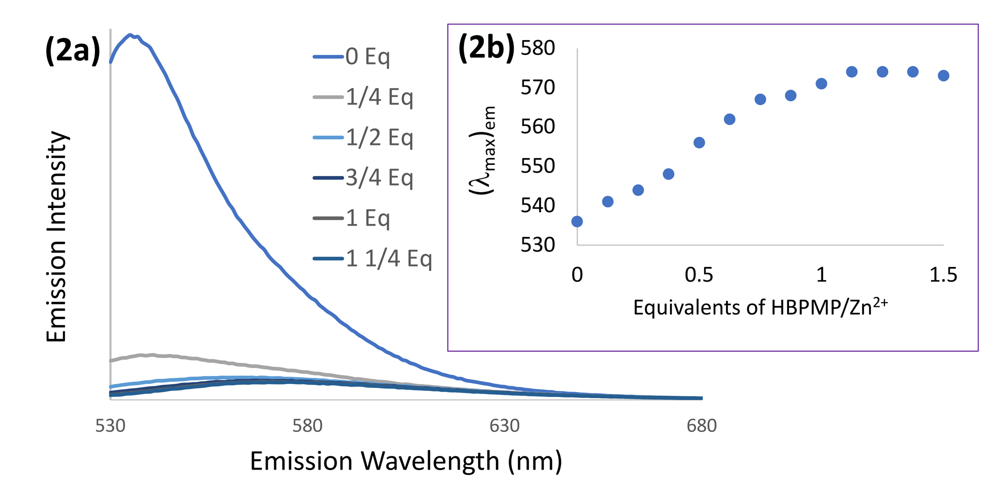


Figure SF2: (a) The emission spectra of a 25 μM solution of SF in pH 7.2 0.010 M HEPES with additions of HBPMP/Zn^{2+} (λ_{ex} = 508 nm) and **(b)** $(\lambda_{em})_{max}$ with increasing HBPMP/Zn^{2+} indicating a 1:1 complex.

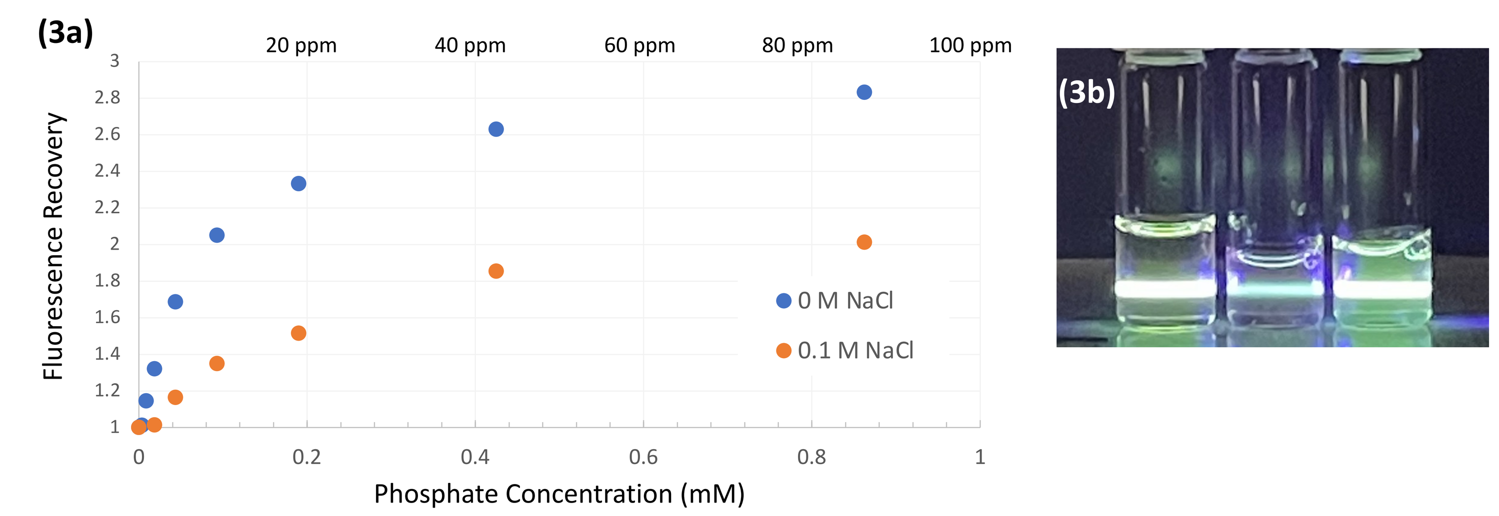


Figure SF3: (a) The fluorescence recovery of a 10 μM solution of SF: 10 μM HPBPMP/Zn^{2+} with increasing amounts of phosphate with and without 0.1 M NaCl (HEPES 0.010 M, pH 7.2, λ_{ex} = 508 nm, λ_{em} = 540 nm) and **(b)** an image of the fluorescence emanating from solutions of SF, SF•HBPMP/Zn^{2+} and SF•HBPMP/Zn^{2+} with > 1 equivalent phosphate excited with a 405 nm laser pointer.

Takeaways

1) SF•HBPMP/Zn^{2+} can be used in a fluorescent IDA with excitation and emission in the visible.
2) The maximum recovery is ~ 1/3 of the dye alone signal.
3) The extent of quenching and dynamic range is reduced in the presence of 0.1 M NaCl.
4) The maximum recovery is reached with ~ 4 equivalents of phosphate. This is similar to our experiments with C and reported colorimetric IDAs.
5) SF is not photostable, greatly hampering the LOD.

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