



Applications of Fluorescent Biosensors for Non-Invasive Glucose Monitoring

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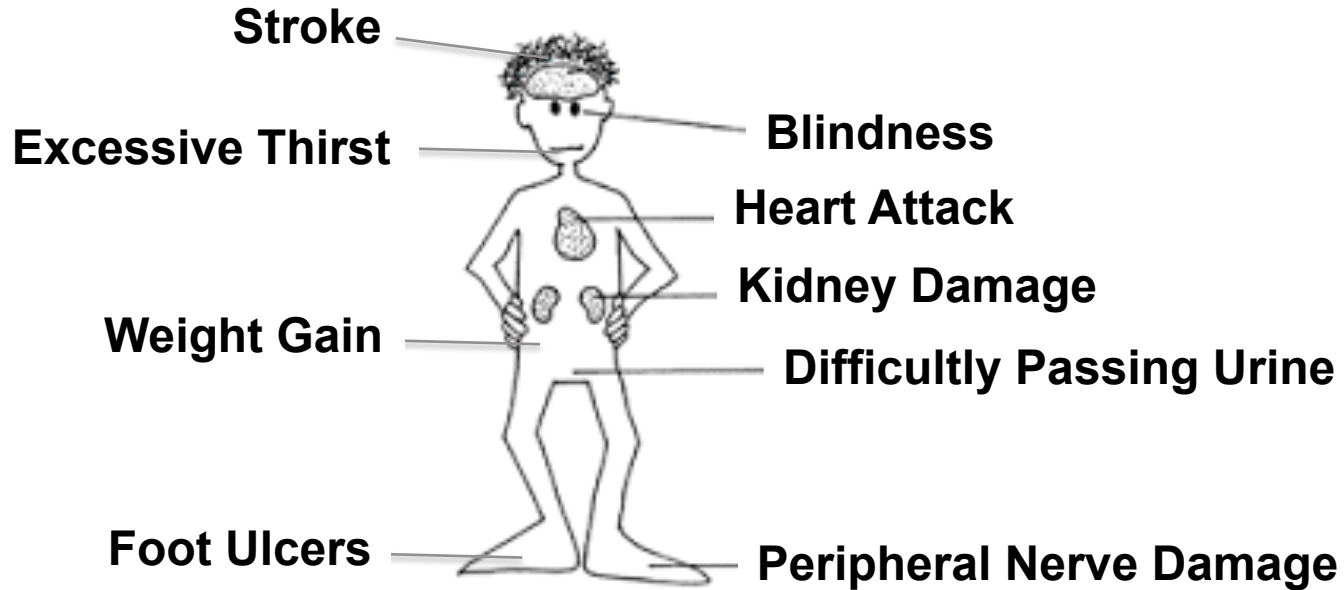
- Background
 - Diabetes – Side Effects
 - Monitoring Devices
- Project Goal
- Boronic Acids (BAs) for Sugar Recognition
- Direct Sensing
- Indirect Sensing
- Conclusions
- Future Work



Importance of Saccharide Sensing



- **Disease: Diabetes and the consequential side effects**



- **Monitoring glucose levels to prolong life expectancy**
- **Currently no noninvasive, continuous monitoring systems available**
- **Demonstrates a need for real-time, non-invasive monitoring**



Implanted Wearable Devices



Advantages:

- Real-time monitoring
- Continuous
- Coupled to insulin pump
- Eliminates injections *via* syringe

Disadvantages:

- Invasive

Finger Pricking Method



Advantages:

- Minimally Invasive

Disadvantages

- Not continuous
- Insulin injections required
- Miss episodes of hyper- and hypoglycaemia

<https://www.accu-chek.co.uk/gb/products/>



Electrochemical sensor in a wearable platform

Battery Powered



Interference from Electroactive Species in Ocular fluid



Use of Enzymes



NOVARTIS

H. Yao, et al, *Biosensors and Bioelectronics*, **2011**, 26, 3290-3296

B.E. Watt, et al, *Toxicol. Rev.*, **2004**, 23(1), 51-57



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Realistically....Not a Real Working Device



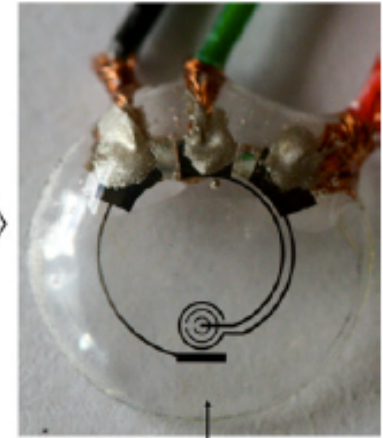
A 30 μL solution of glucose oxidase



A layer of GOD/titania sol-gel membrane



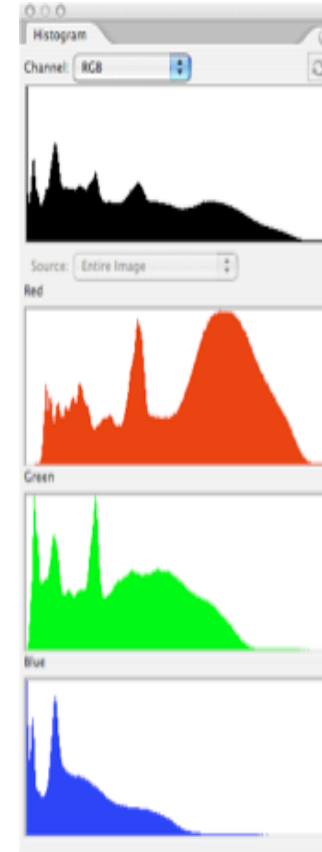
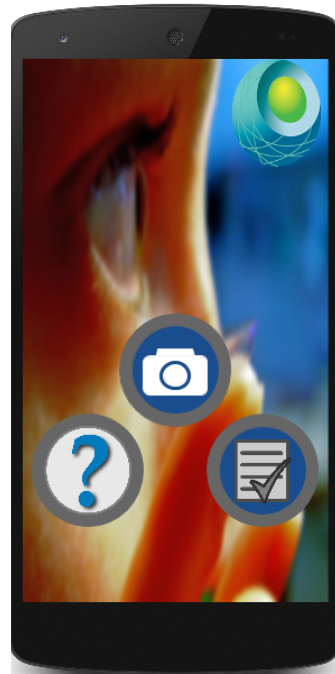
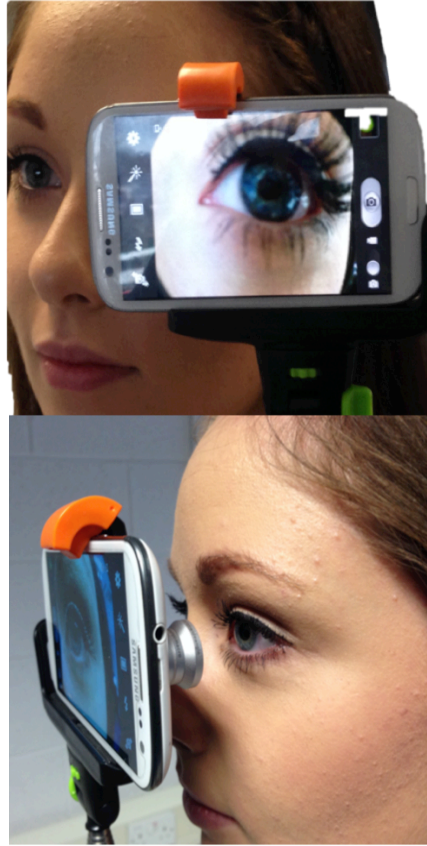
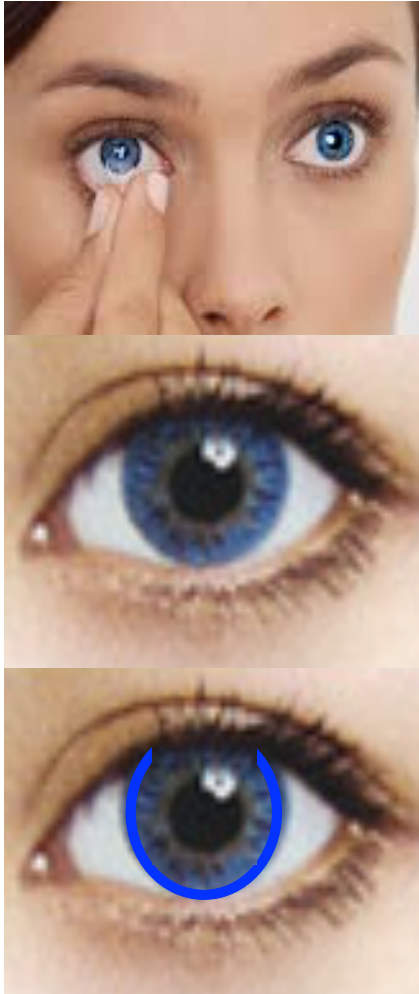
A spread of 30 μL Nafion® on sol-gel membrane



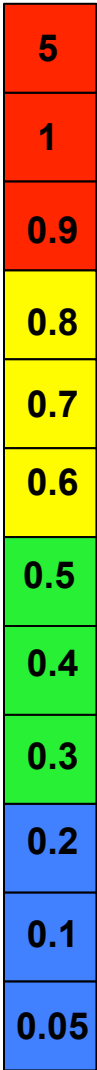
A transparent sensing area after rinsing with DI water

- Attached to a BASi Epsilon- EC Potentiostat +400 mV
- Sensing platform proposes glucose monitoring between 0.5-50 mM
- Ocular glucose range is 0.05-0.5 mM and up to 5 mM in diabetics
- Major shortcomings to meet immediate expectations

H. Yao, et al, *Biosensors and Bioelectronics*, 2011, 26, 3290-3296

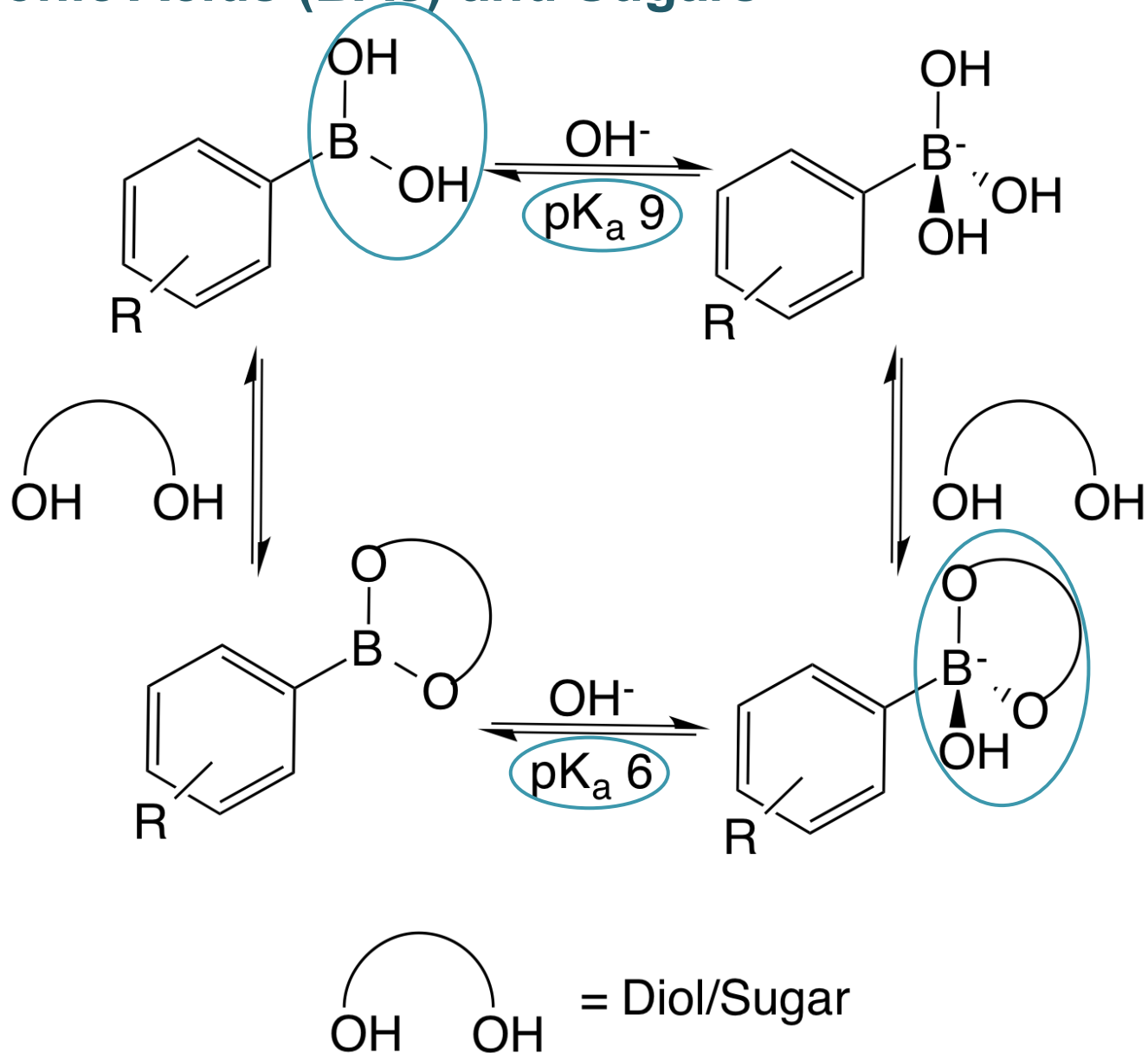


mM





Boronic Acids (BAs) and Sugars

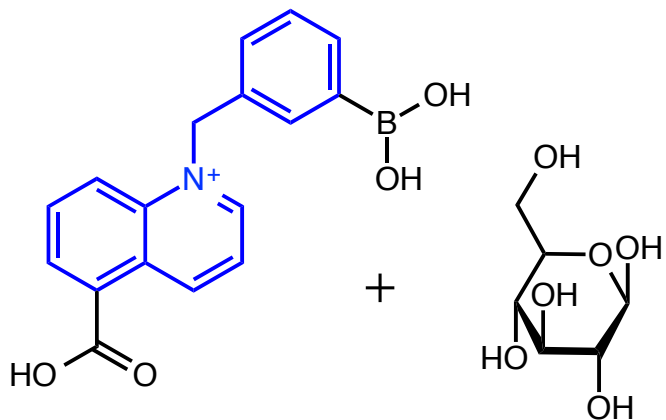




Direct vs. Indirect Sensing

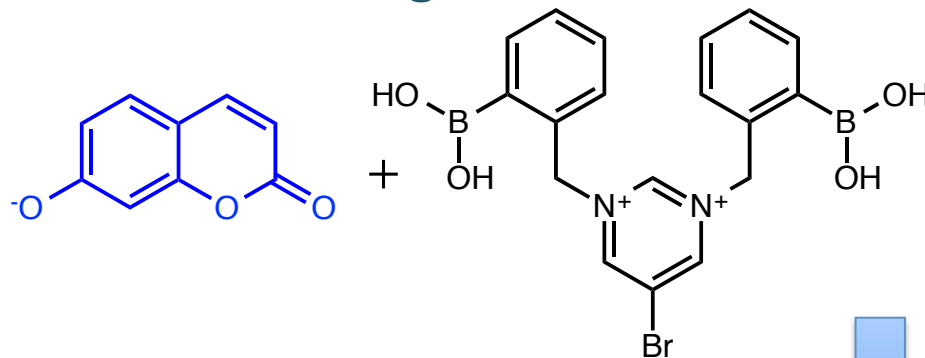


Direct Sensing

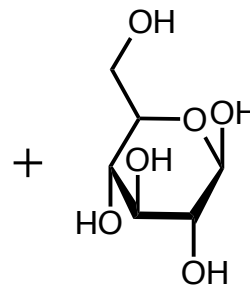


Fluorescence Decrease

Indirect Sensing



Fluorescence Decrease



Fluorescence Increase

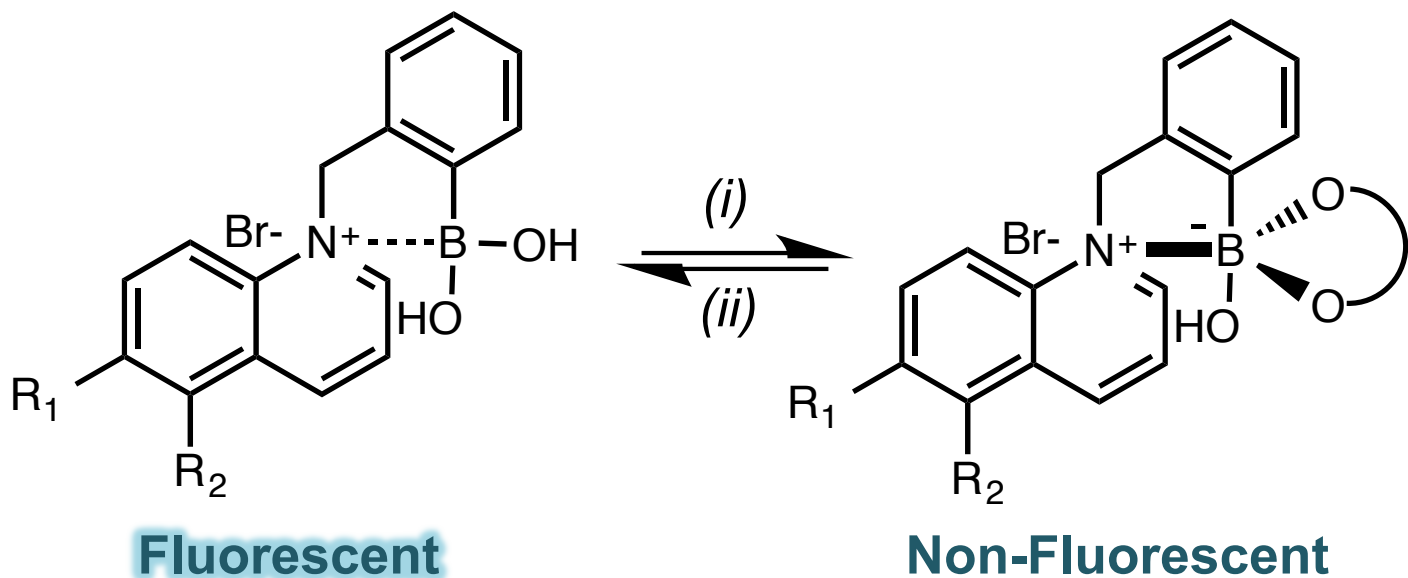
Fluorophore





Direct Sensing

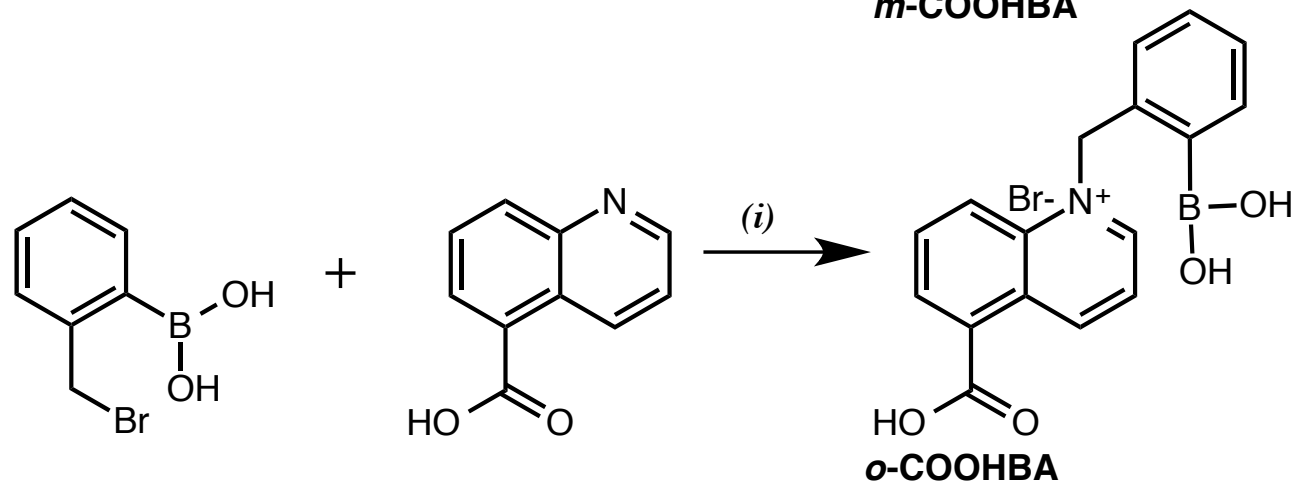
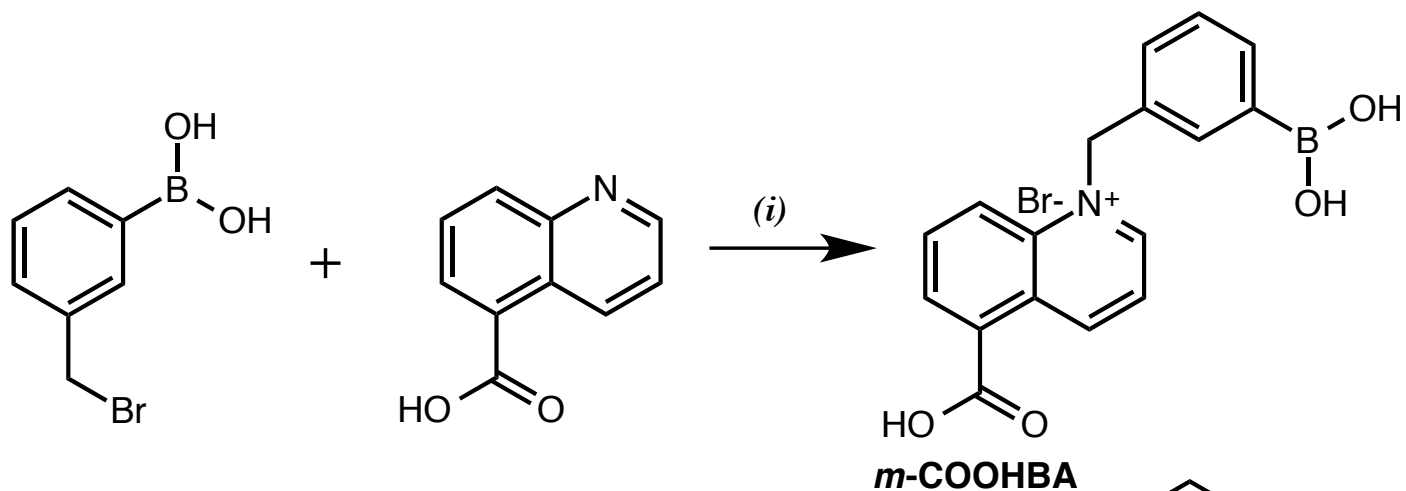




(i) Addition of OH^- ions/glucose

(ii) Addition of water/removal of glucose



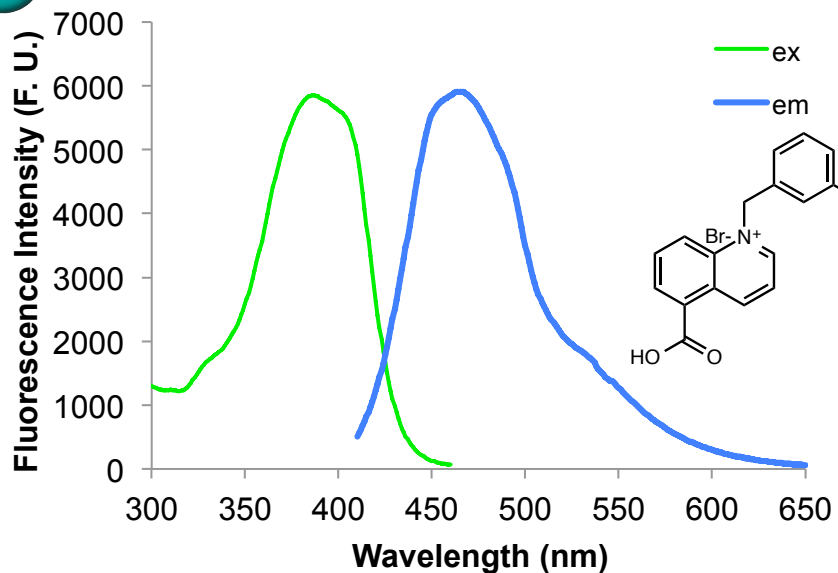


(i) Anhydrous dimethylformamide, N₂, 80 °C for 48h.

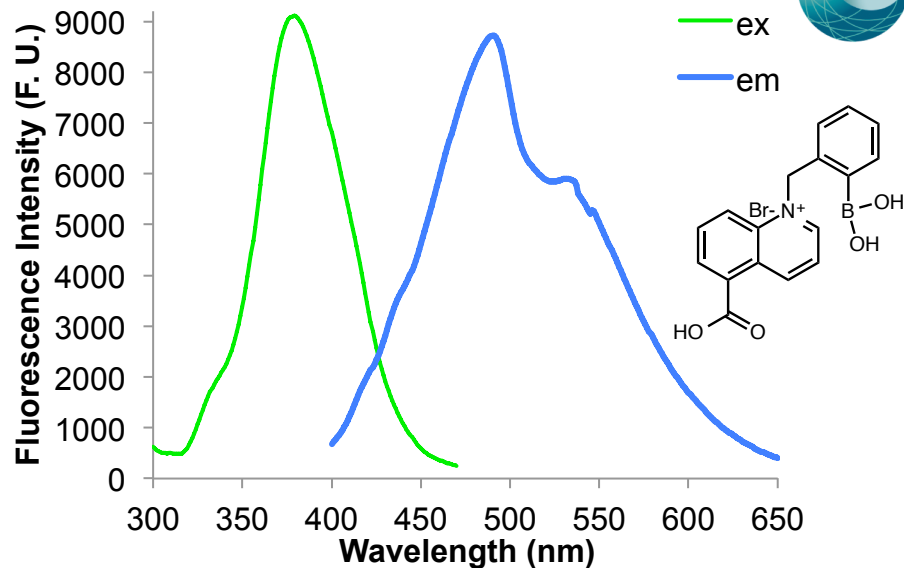
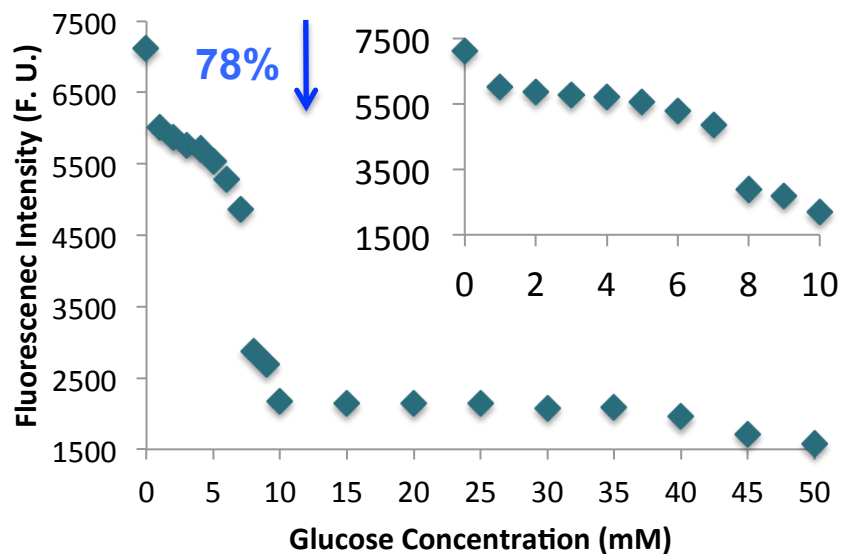
Successful synthesis of novel BA sensors were confirmed by NMR.



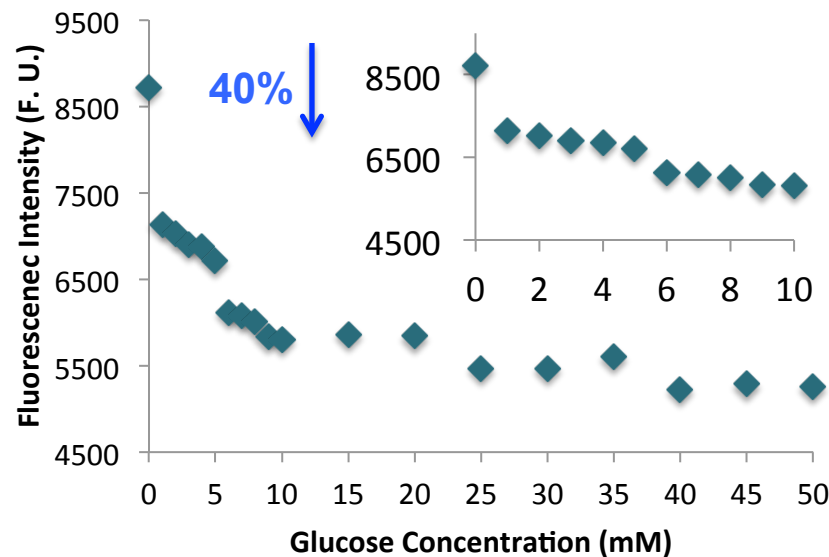
Fluorescence Results



m-COOHBA 0.5 mM in pH 7.4 phosphate buffer;
Excitation 390 nm; Emission 465 nm

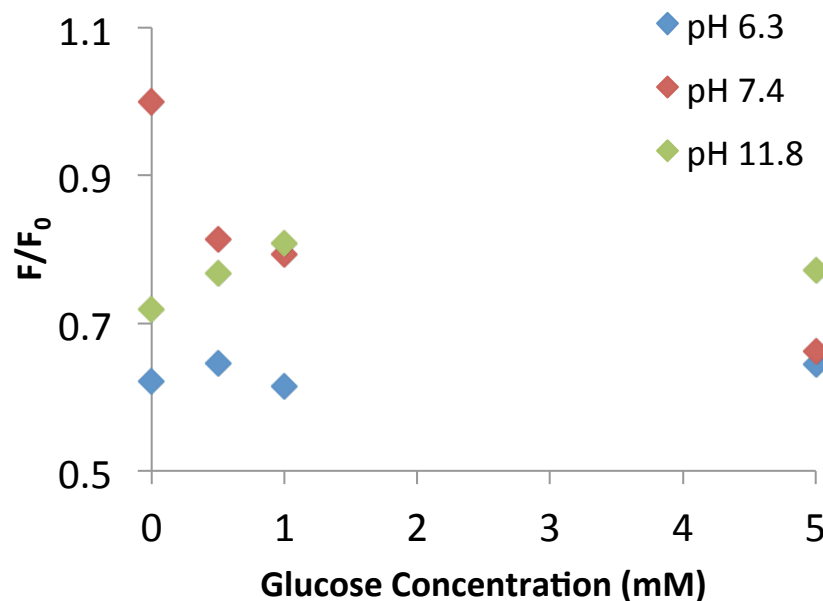
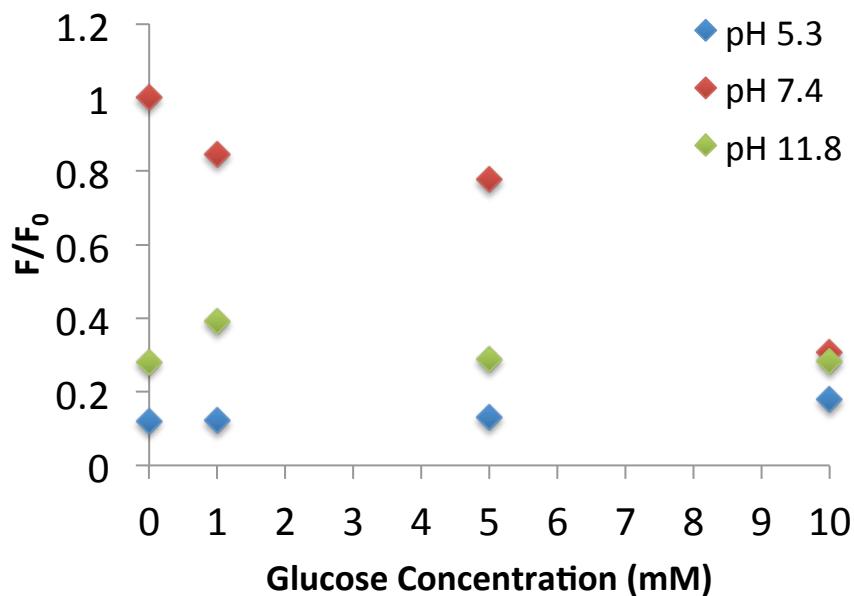


o-COOHBA 0.5 mM in pH 7.4 phosphate buffer;
Excitation 380 nm; Emission 485 nm

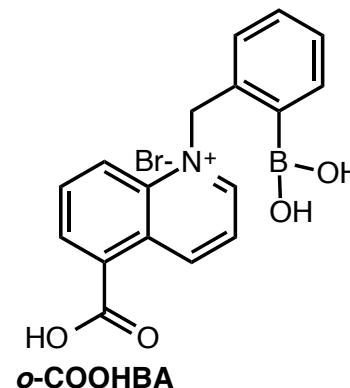
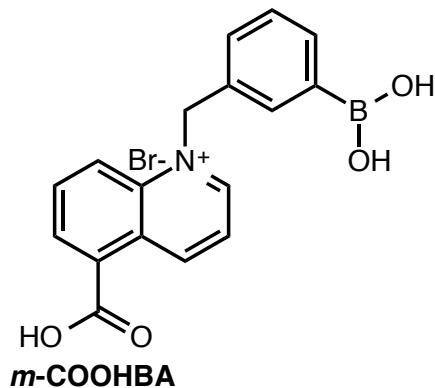




pK_a Investigation – Glucose Sensing pH Range



Glucose response for *m*-COOHBA and *o*-COOHBA (0.5 mM) in different pH buffer solutions ranging from pH 5-11.

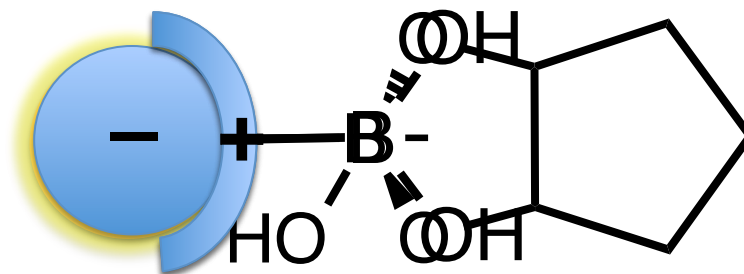


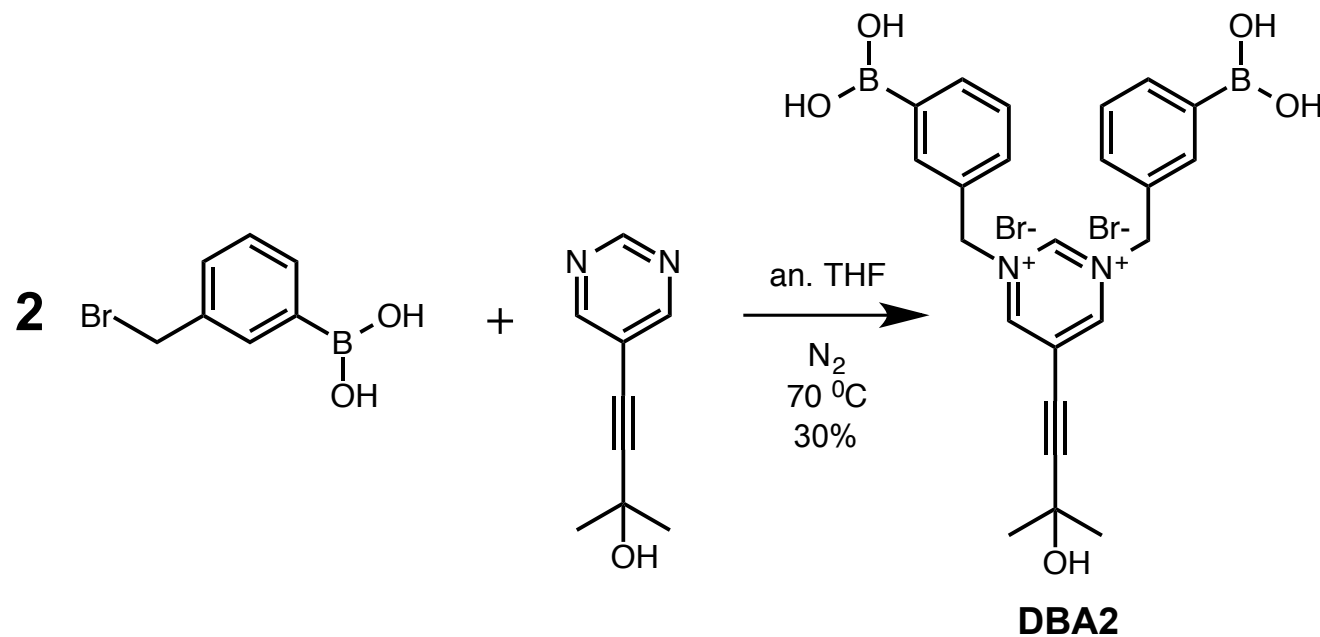
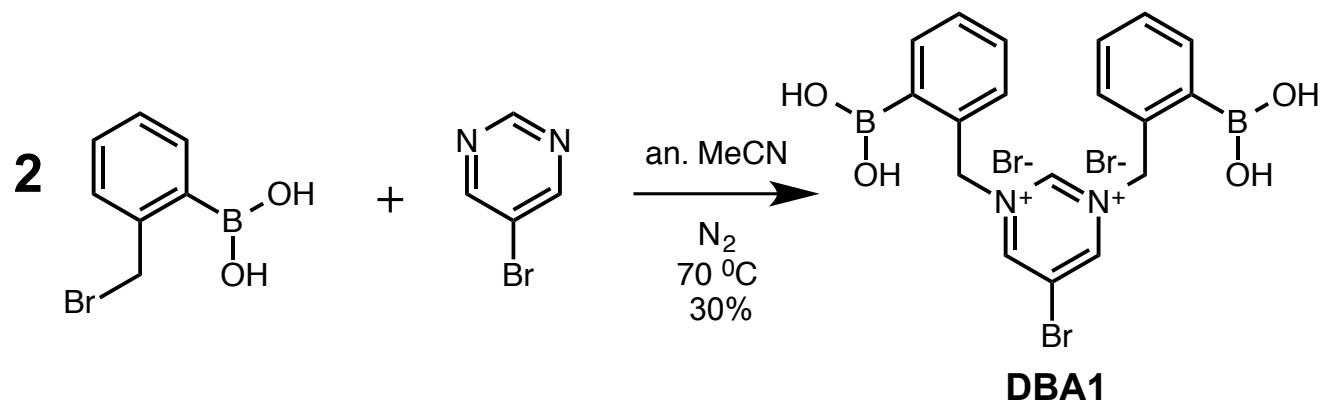


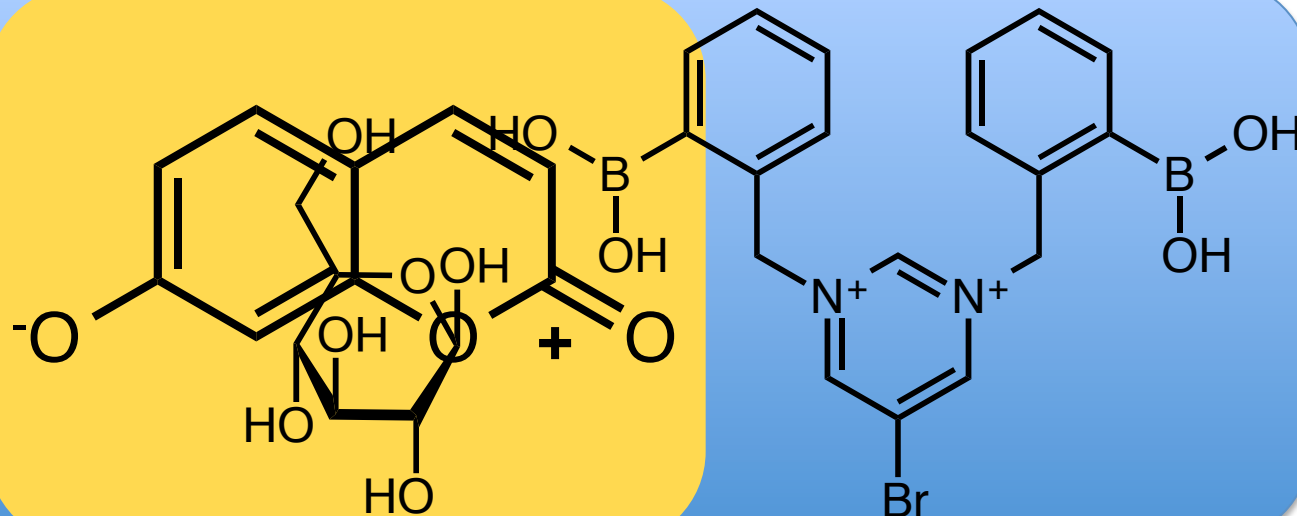
Indirect Sensing



Two-Component Sensing





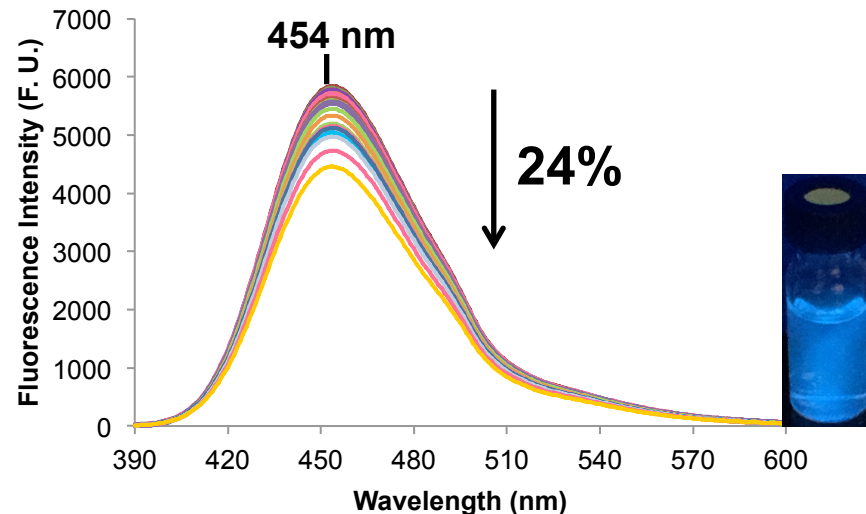
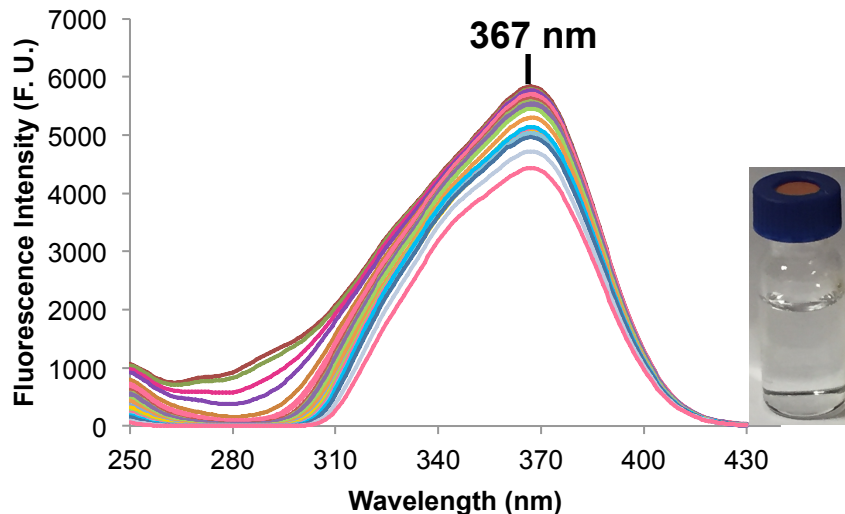


Fluorescent
Non-fluorescent

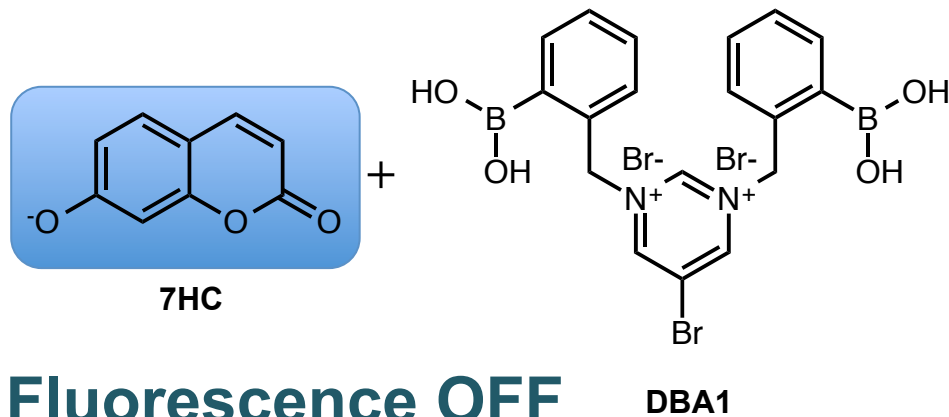
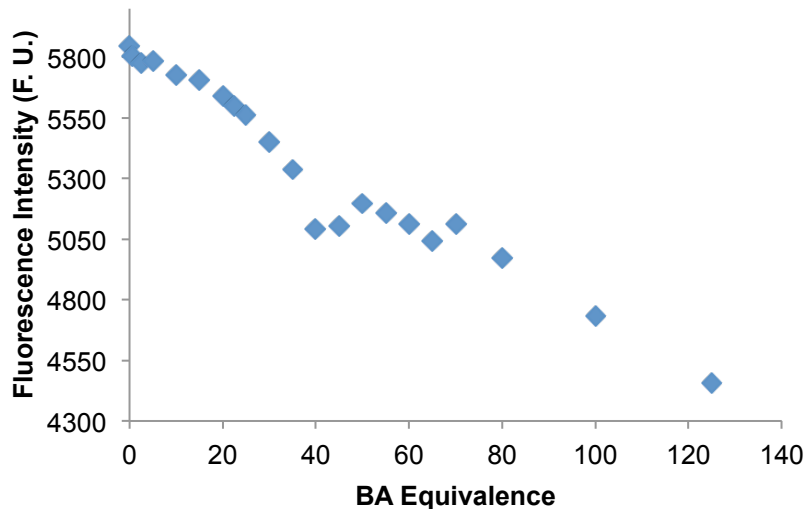




Two-Component Sensing – Fluorescence Quenching

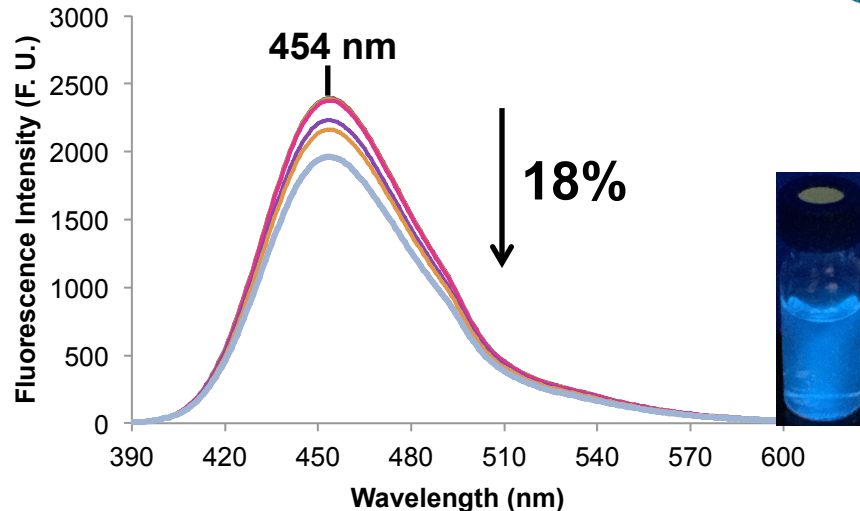
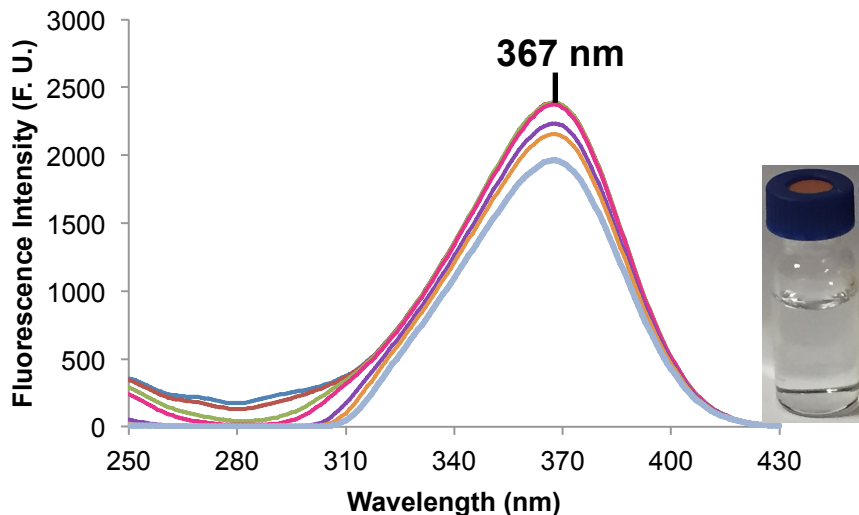


Excitation and emission spectra of 4 μM 7HC in pH 8.12 buffer solution with increasing DBA1 concentrations up to 0.5 mM (125 eq.); Medium sensitivity; 2.5 nm bandwidth

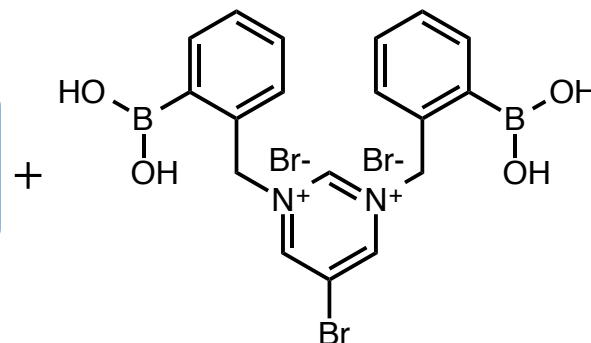
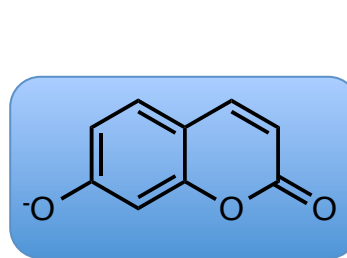
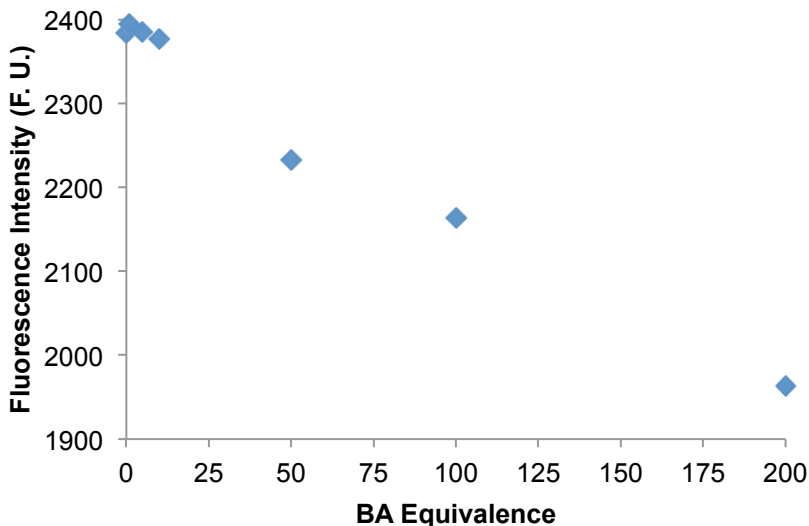




Two-Component Sensing – Fluorescence Quenching



Excitation and emission spectra of 4 μM 7HC in pH 8.88 buffer solution with increasing DBA1 concentrations up to 0.8 mM (200 eq.); Medium sensitivity; 2.5 nm bandwidth

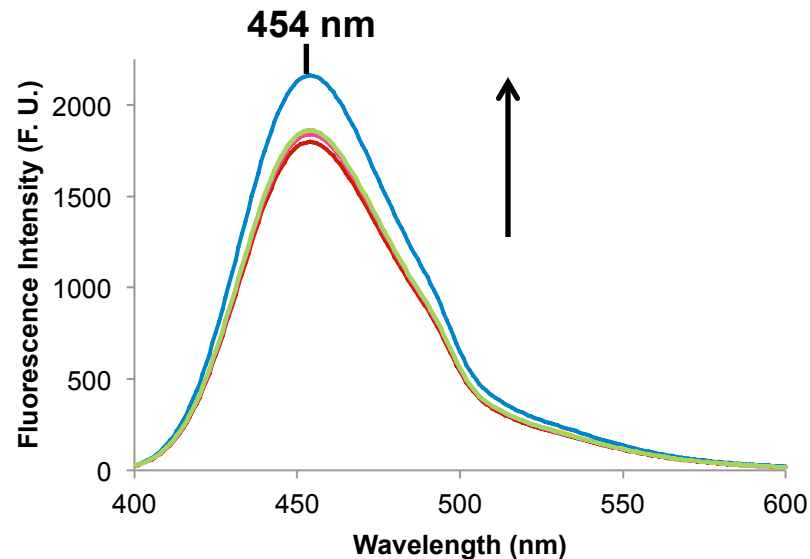
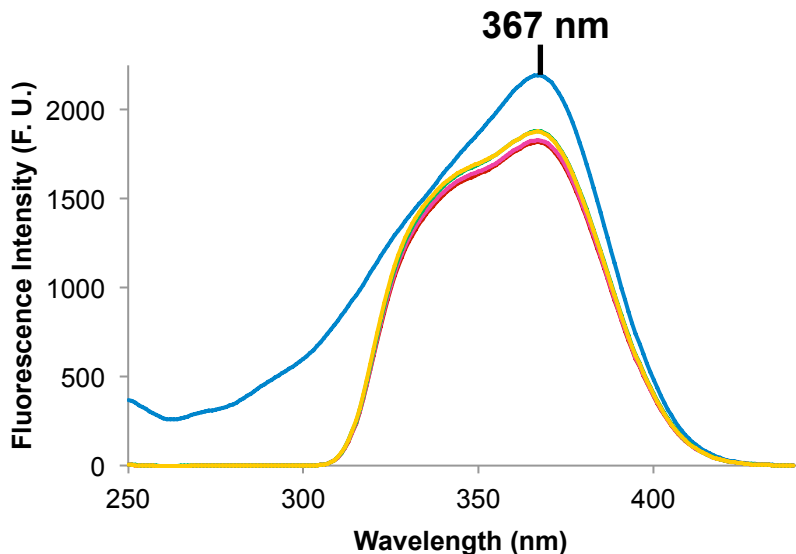


Fluorescence OFF

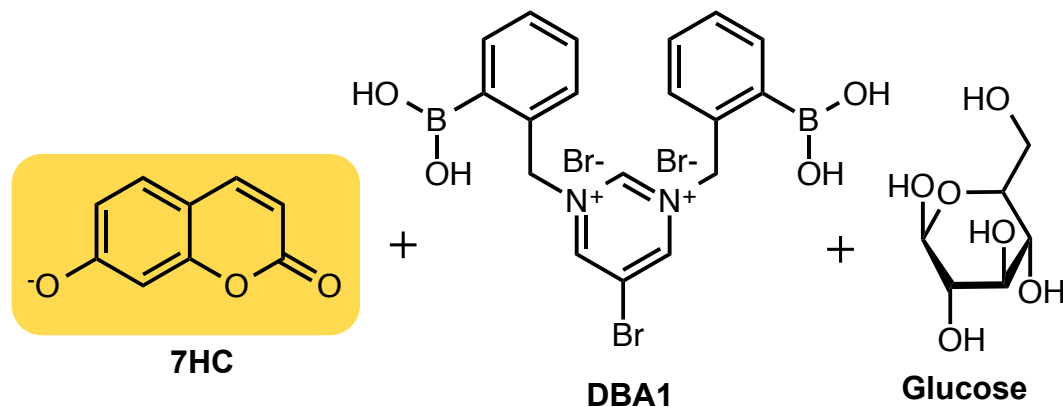
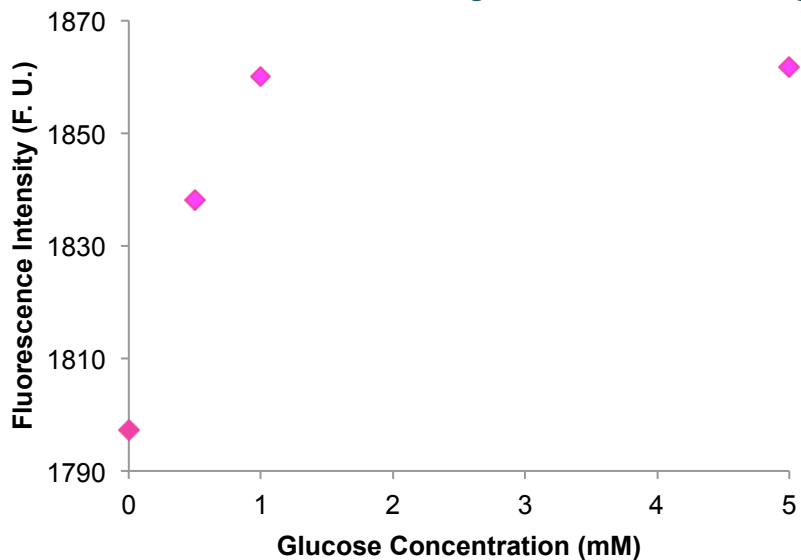
DBA1



Two-Component Sensing – Fluorescence Recovery



Excitation and emission spectra of 7HC (4 μM) and DBA2 (700 μM) (1:175 eq.) in pH 8.12 buffer solution with increasing concentrations of glucose up to 5 mM; Medium sensitivity; 2.5 nm bandwidth

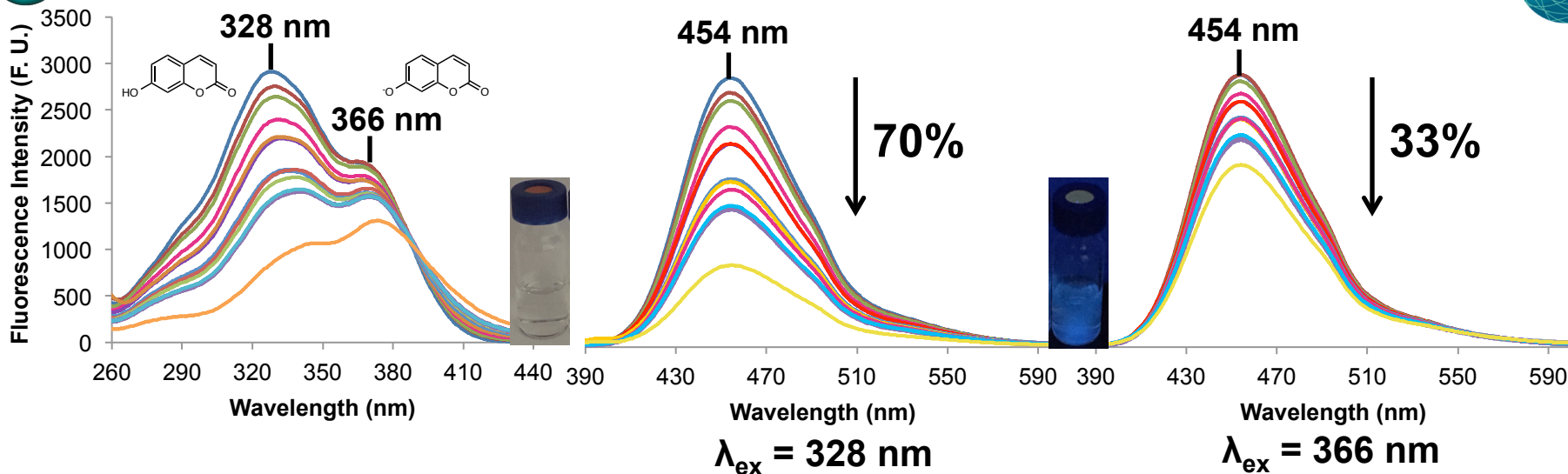


Fluorescence ON

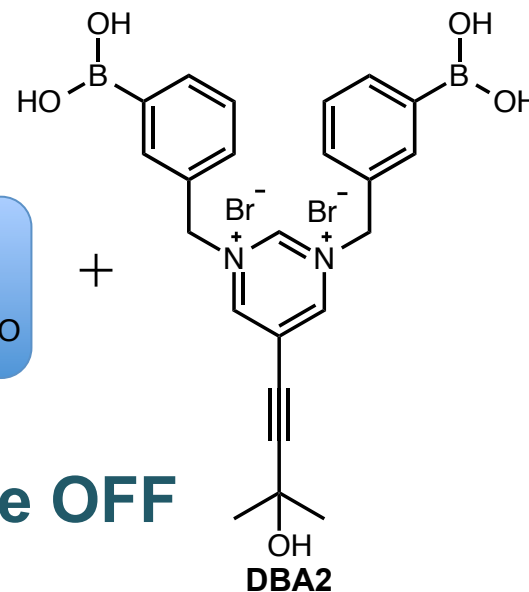
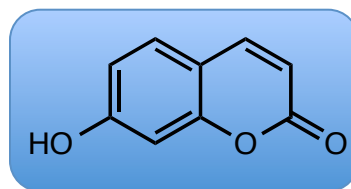
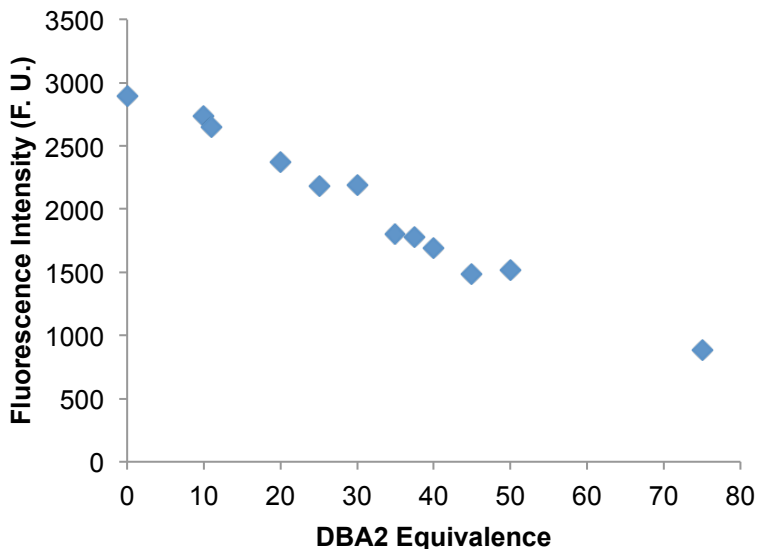




Two-Component Sensing – Fluorescence Quenching



Excitation and emission spectra of 4 μM 7HC in pH 7.4 with minimal MeOH (40 μL) with increasing DBA2 concentrations up to 0.3 mM (75 eq.); Medium sensitivity; 2.5 nm bandwidth

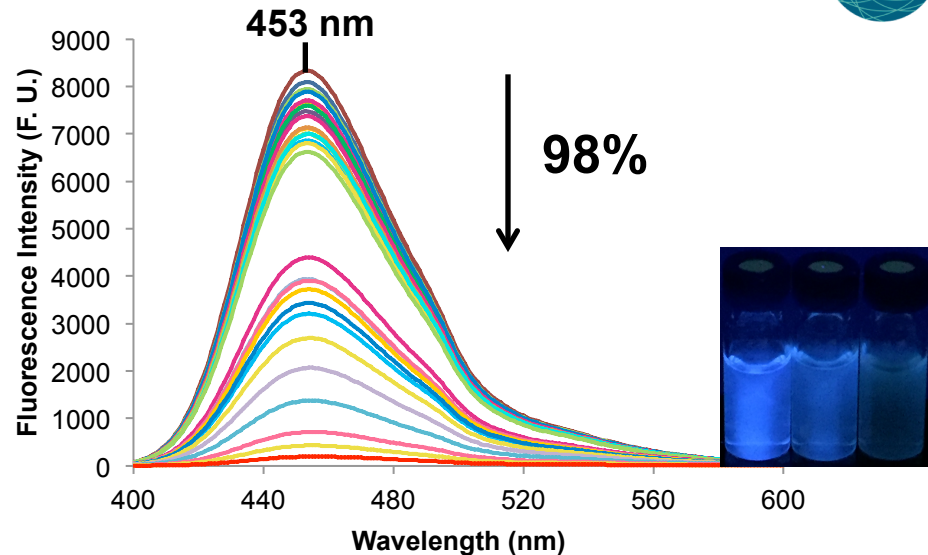
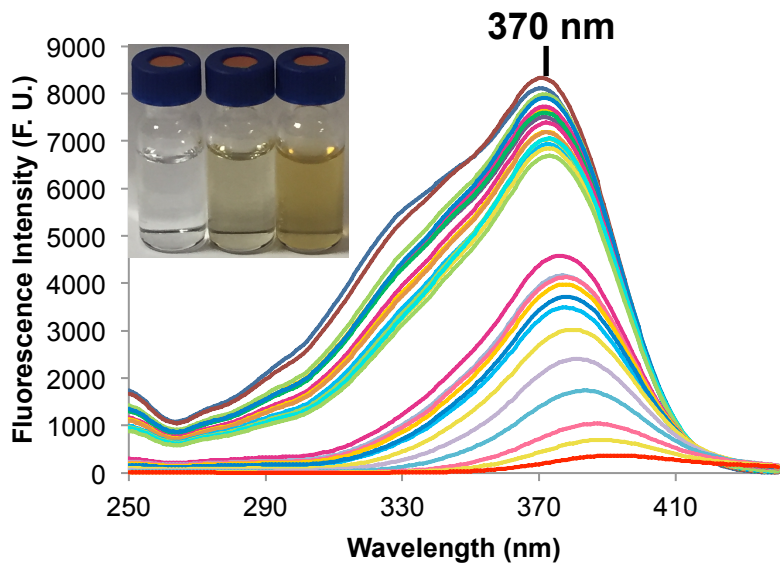


Fluorescence OFF

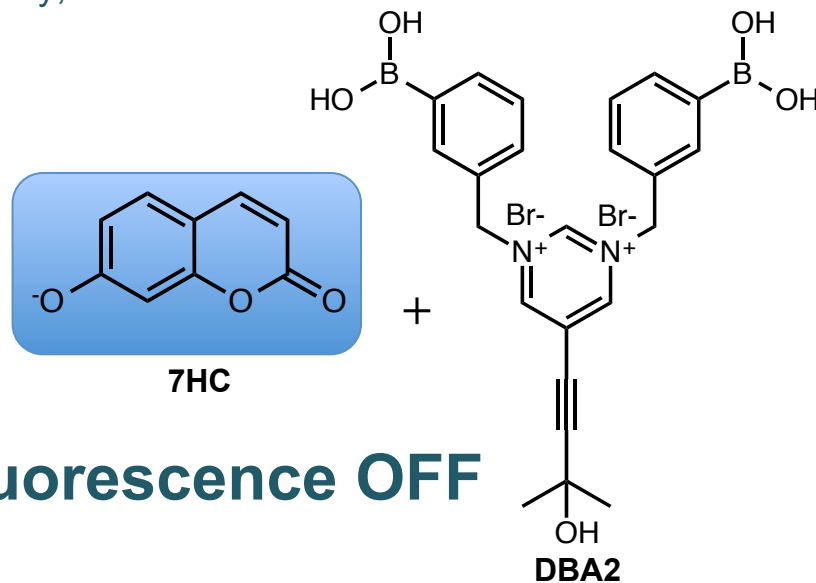
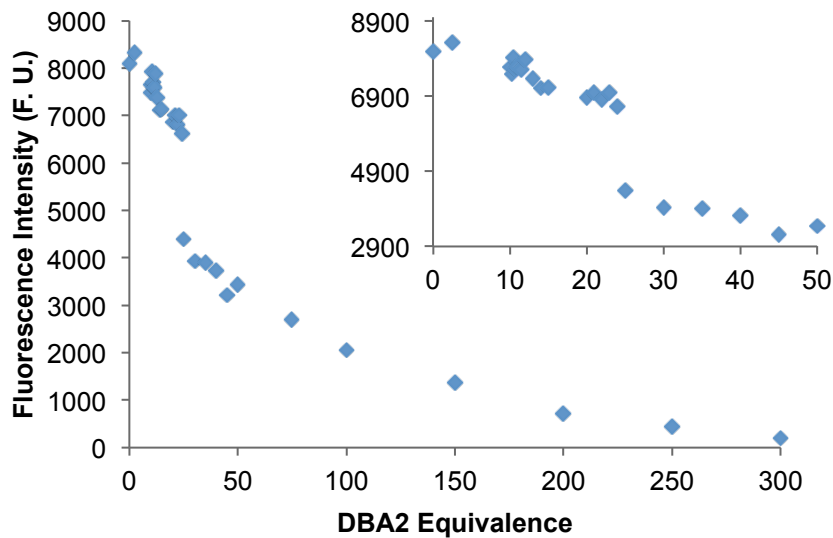




Two-Component Sensing – Fluorescence Quenching

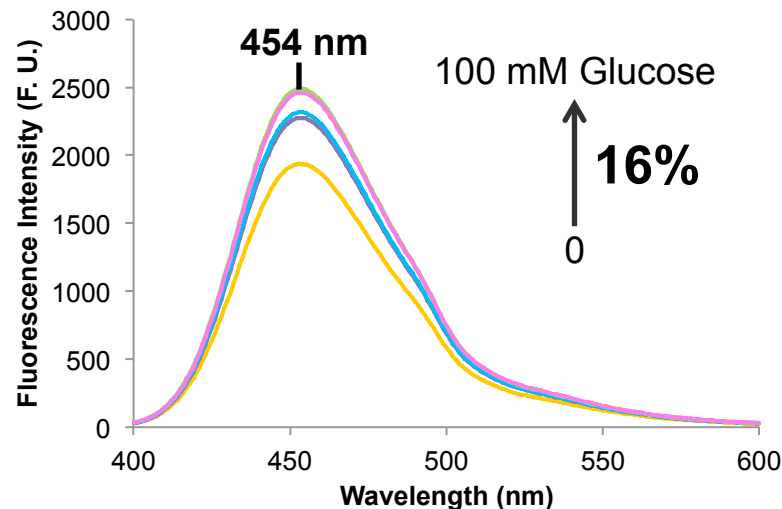
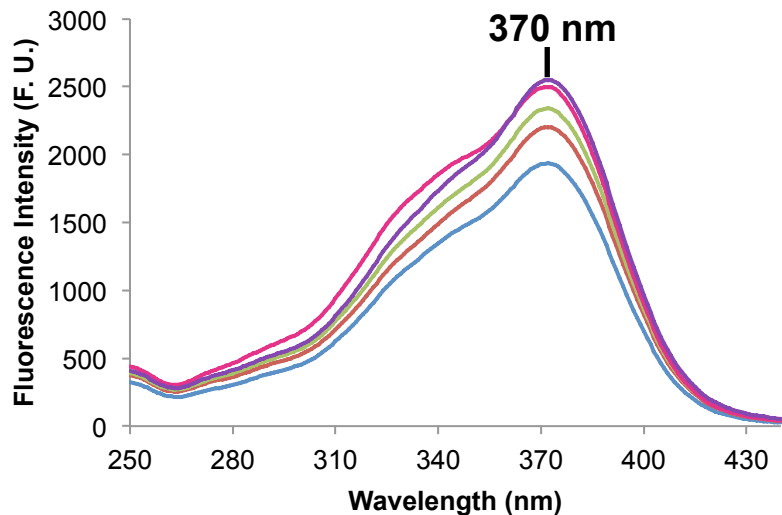


Excitation and emission spectra of 4 μM 7HC in pH 7.4:MeOH (1:1) (pH 8.6) with increasing DBA2 concentrations up to 1.2 mM (300 eq.); Medium sensitivity; 2.5 nm bandwidth

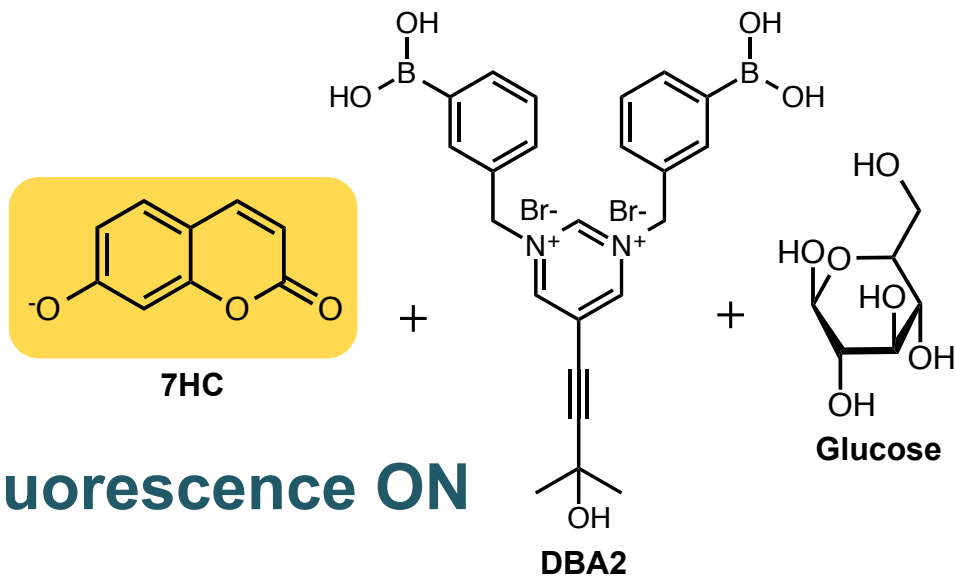
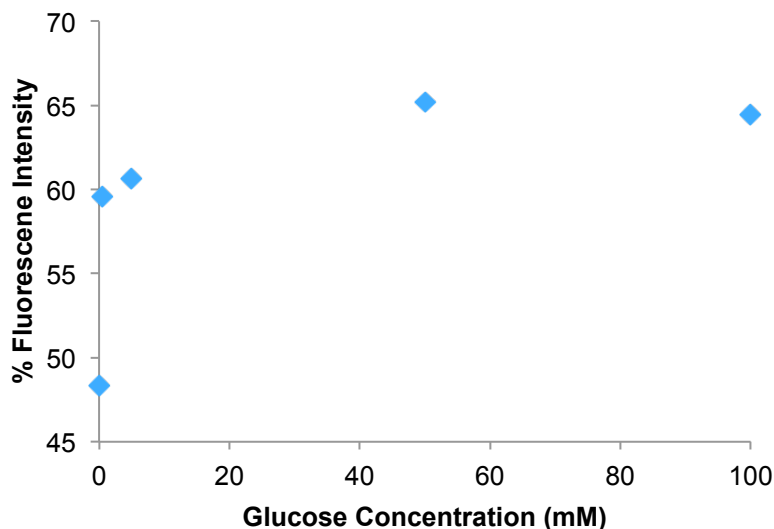




Two-Component Sensing – Fluorescence Recovery



Excitation and emission spectra of 7HC (4 μM) and DBA2 (80 μM) (1:20 eq.) in pH 7.4:MeOH (1:1) (pH 8.6) with increasing concentrations of glucose up to 100 mM; Medium sensitivity; 2.5 nm bandwidth



Fluorescence ON



Conclusions and Future Work

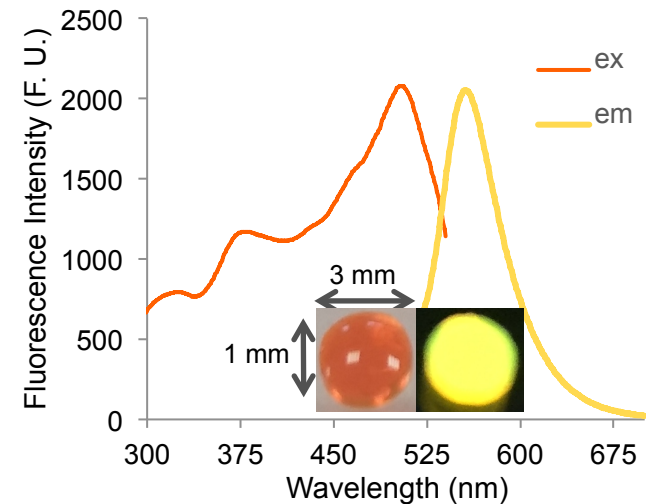
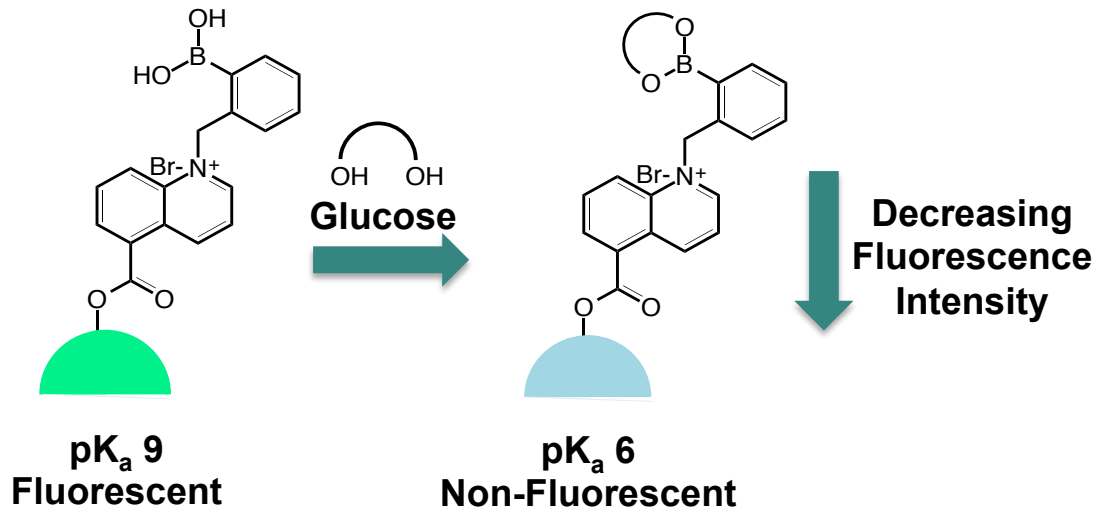


Conclusions

- Novel BAs are capable of direct and indirect glucose sensing
- -COOH substituent for desired anchoring possibilities
- Two-Component Sensing depends on the pK_a of the fluorophore and hence, the pH of the buffer solution

Future Work

- Immobilisation of the sensors on to a lens-like platform
- The incorporation of the two component sensing system in to ionogels

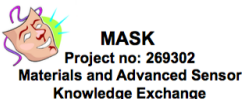
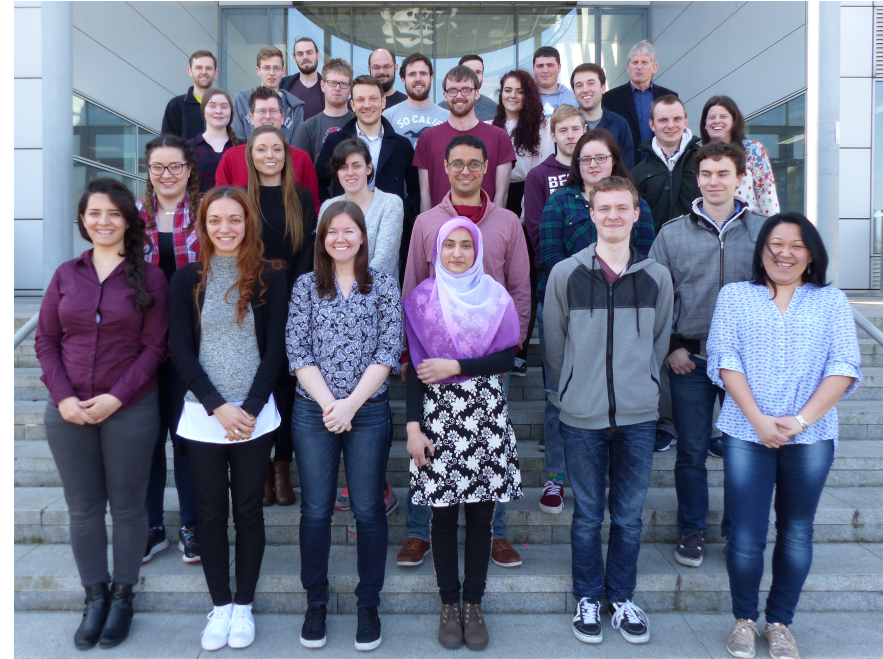


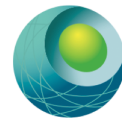
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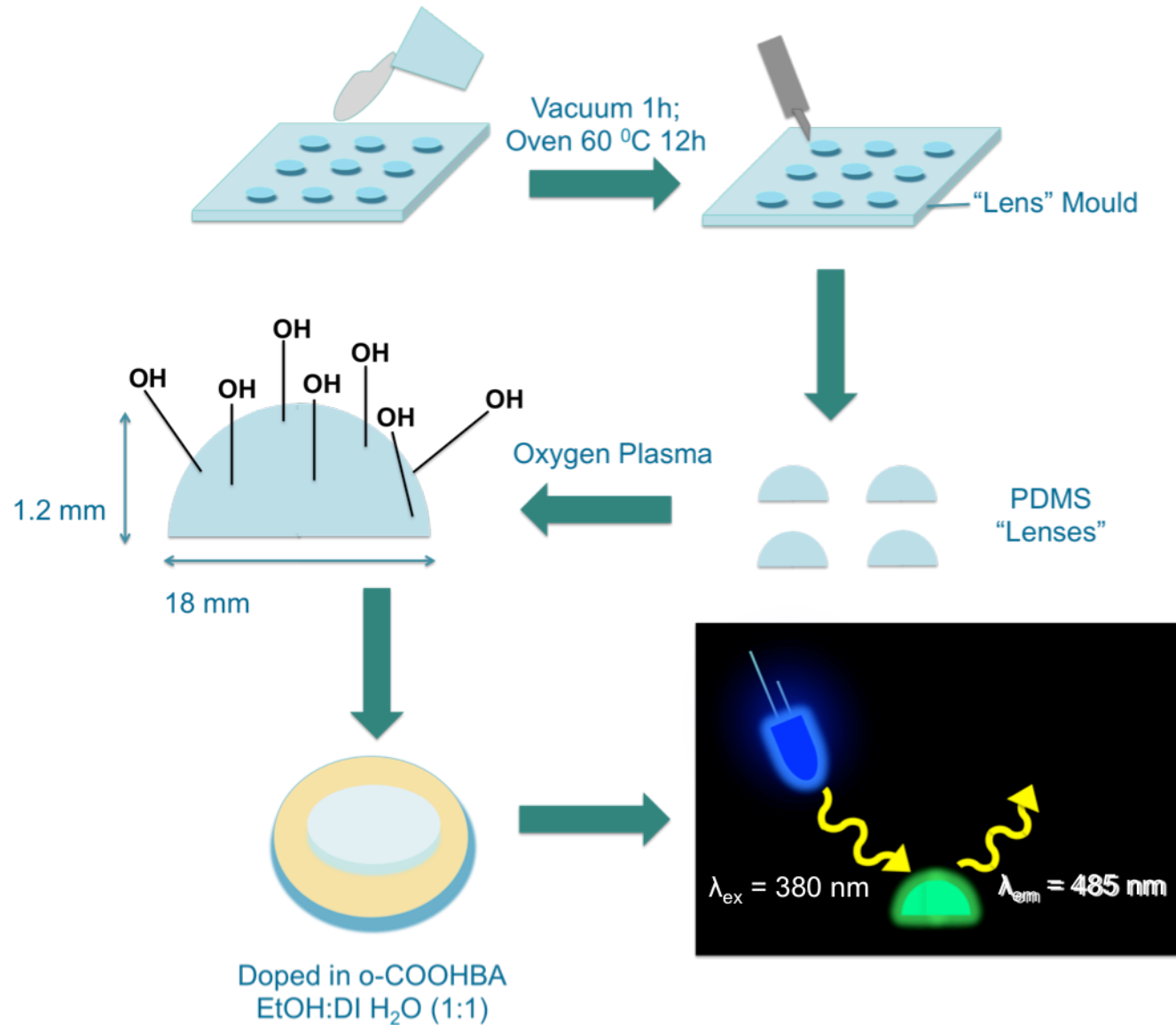


- Members of my research group in particular Dr. Colm Delaney, Dr. Larisa Florea and Prof. Dermot Diamond
- Adam McColgan, Cristiane Daikuzono, Jennifer Hynes, Jessica Cavalcante Abe and Eilana Schaefer
- Science Foundation Ireland & INSIGHT Centre (SFI/12/RC/2289)

Thank You for Your Attention!

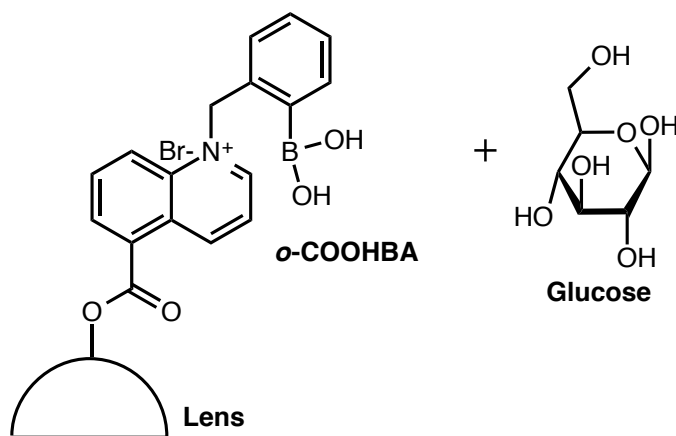
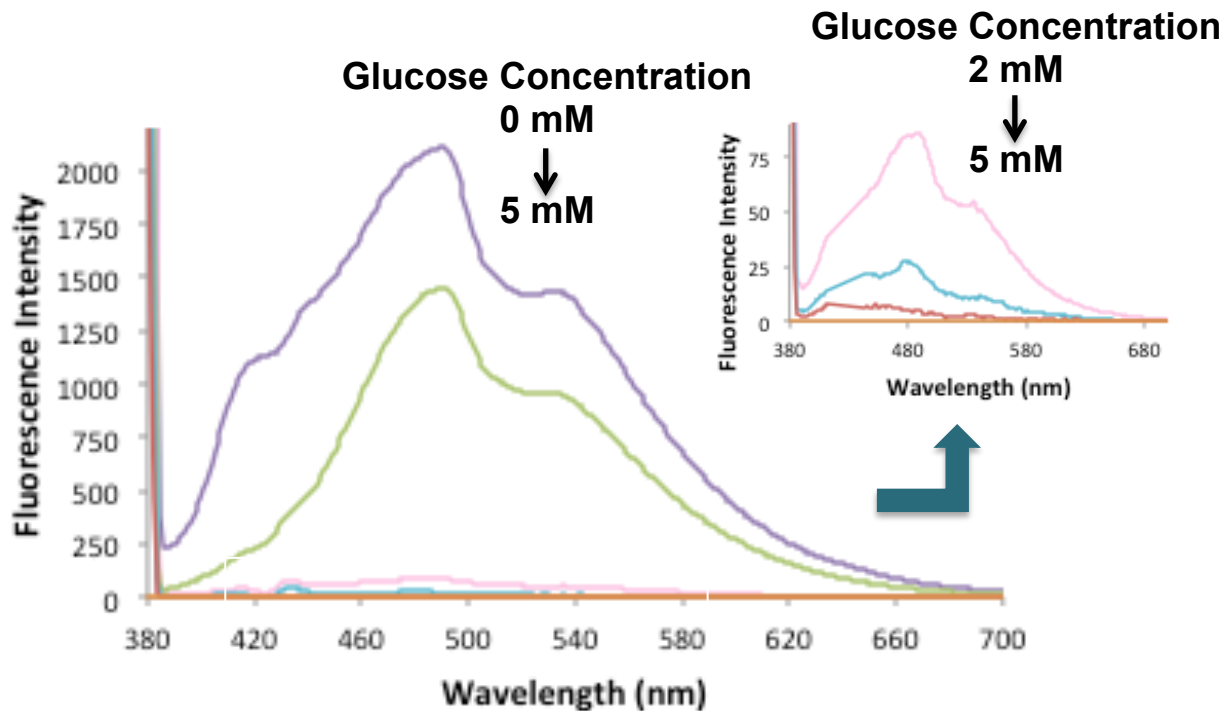






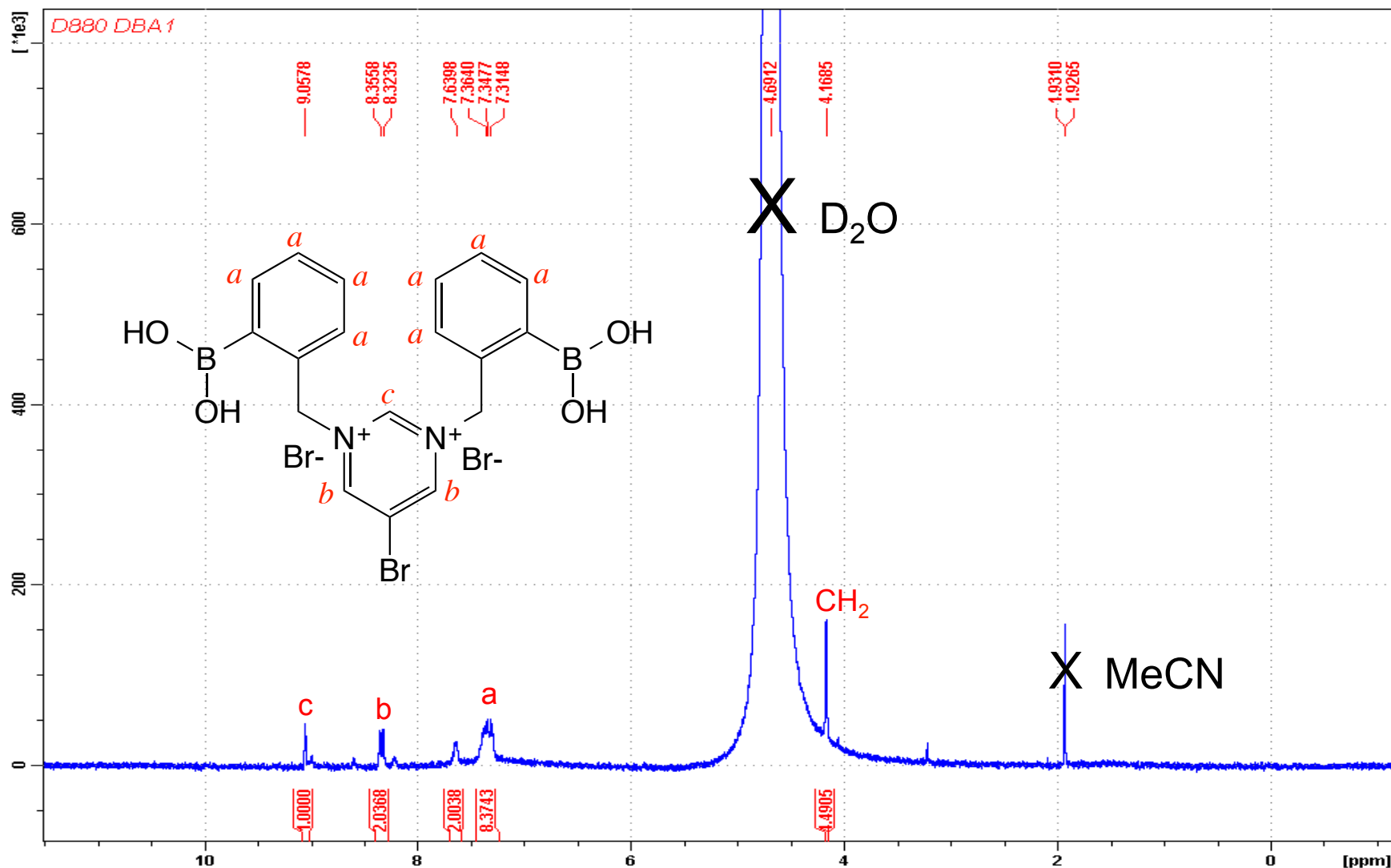


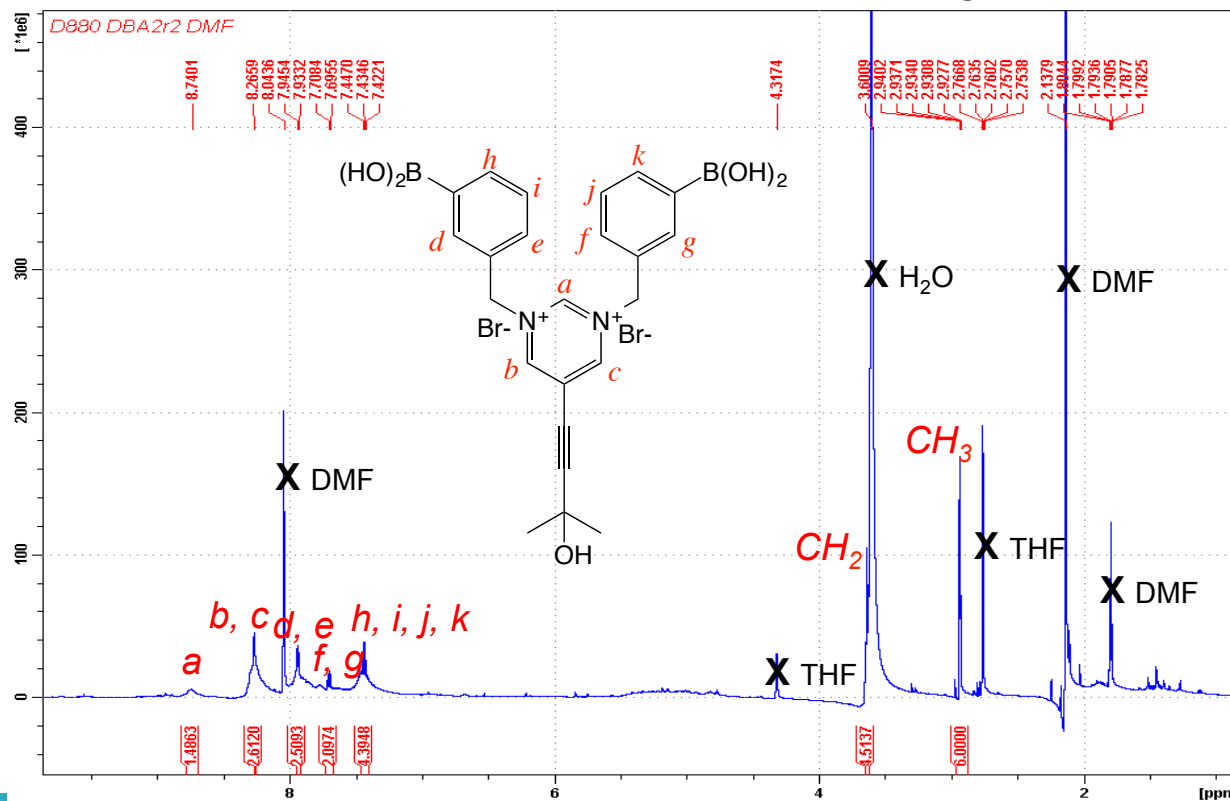
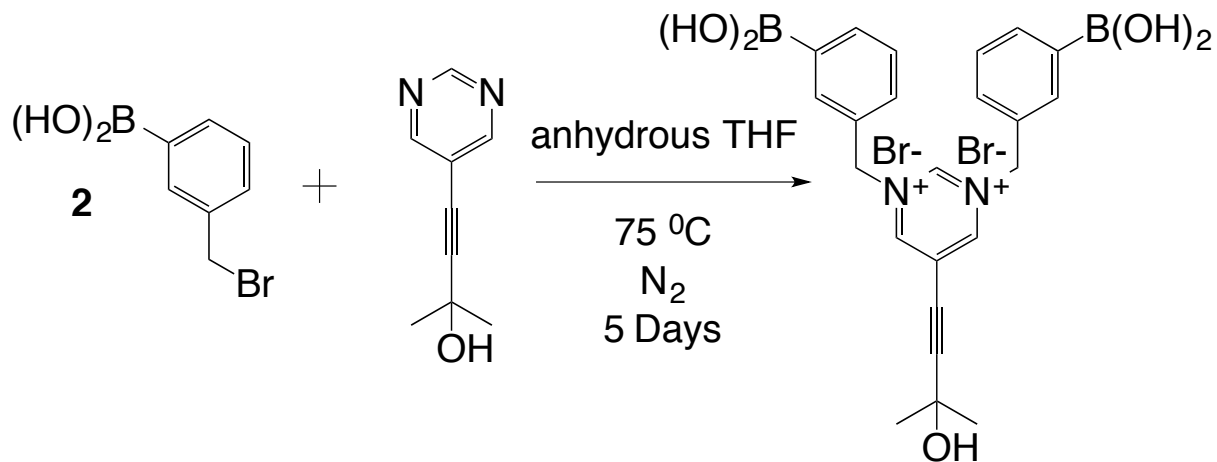
Functionalised PDMS Lens Fluorescence Studies





^1H NMR of 1,3-bis(2-boronobenzyl)-5-bromo-1,3-dinium bromide



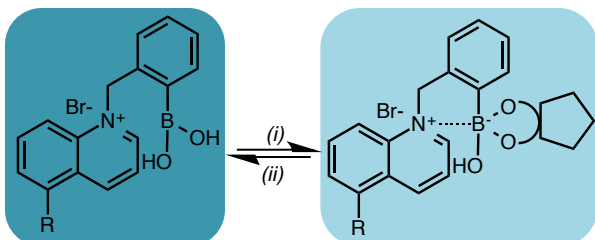




Direct vs. Indirect Sensing

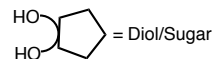


Direct Sensing



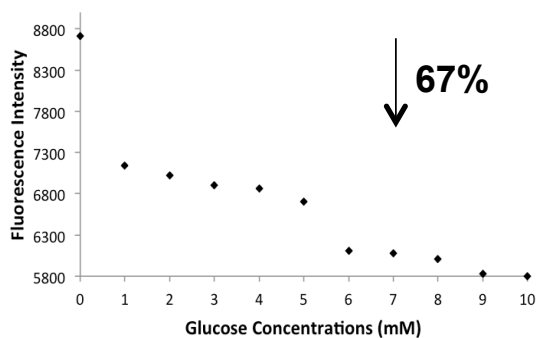
Fluorescence ON

Fluorescence OFF

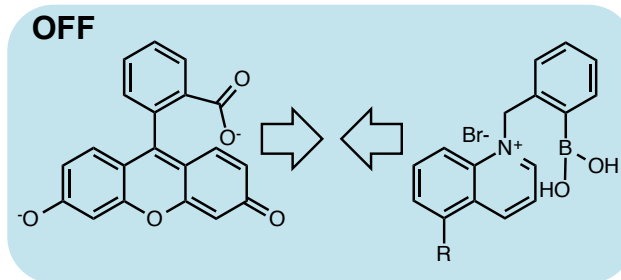
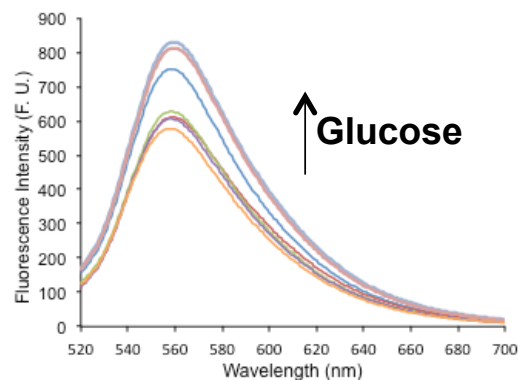
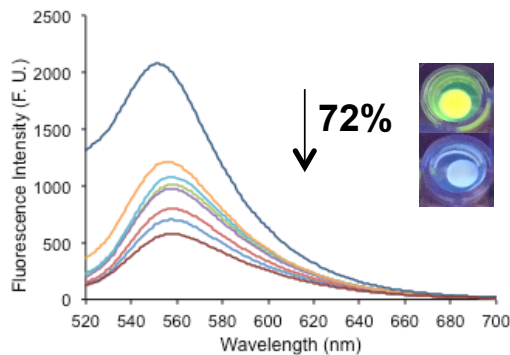


(i) Addition of OH^- ions or Diol/Sugar

(ii) Addition of Water

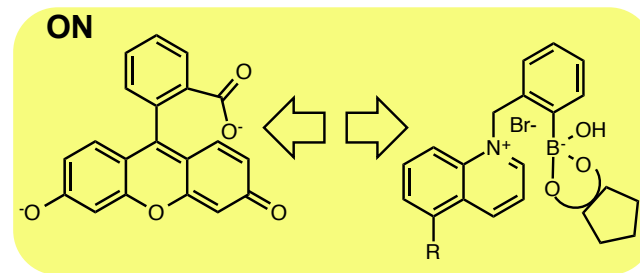
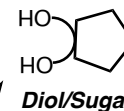


Indirect Sensing



Fluorescein

BA Derivative



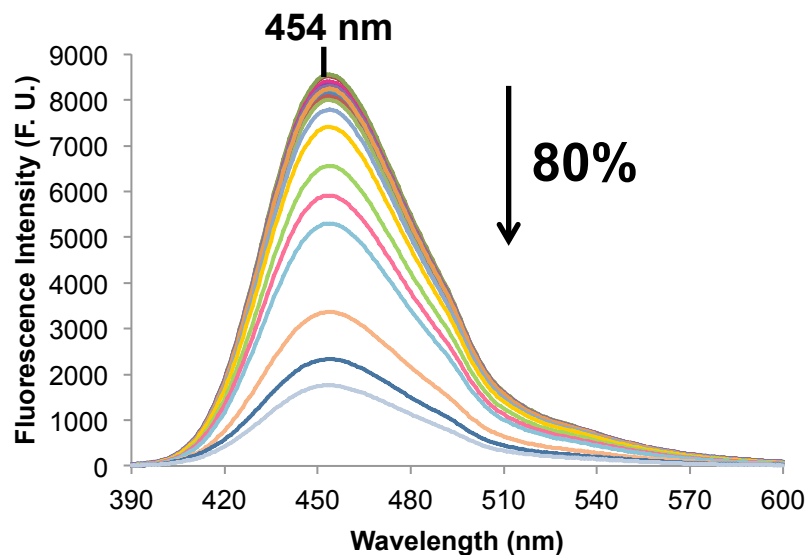
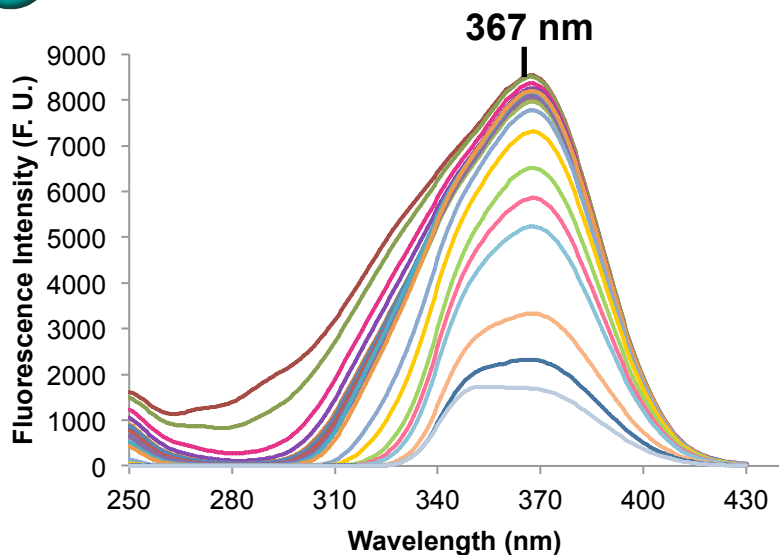
Fluorescein

BA-Sugar Bound Derivative

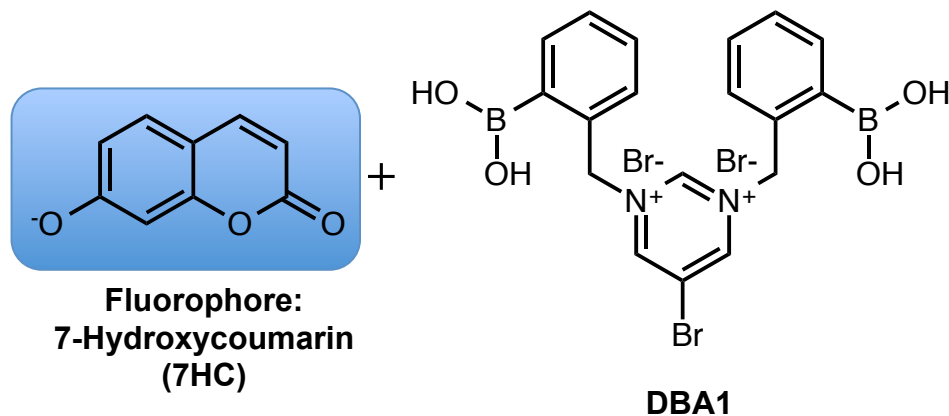
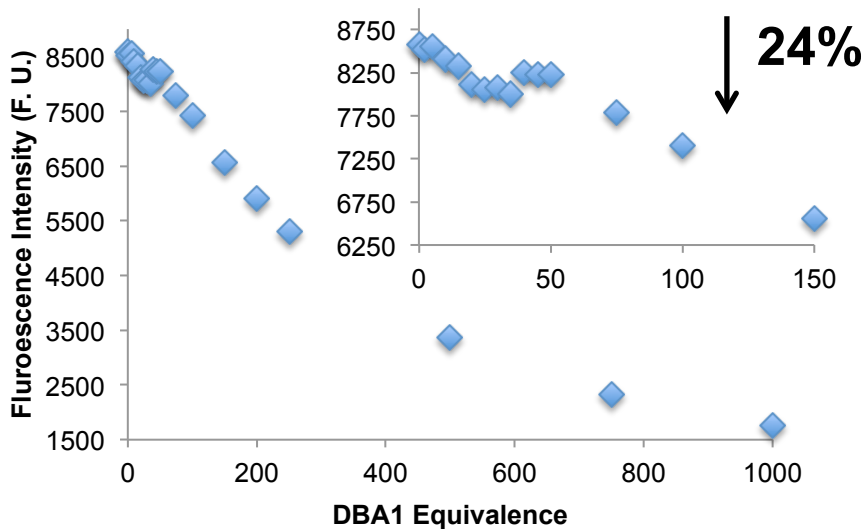




Two-Component Sensing – Fluorescence Quenching

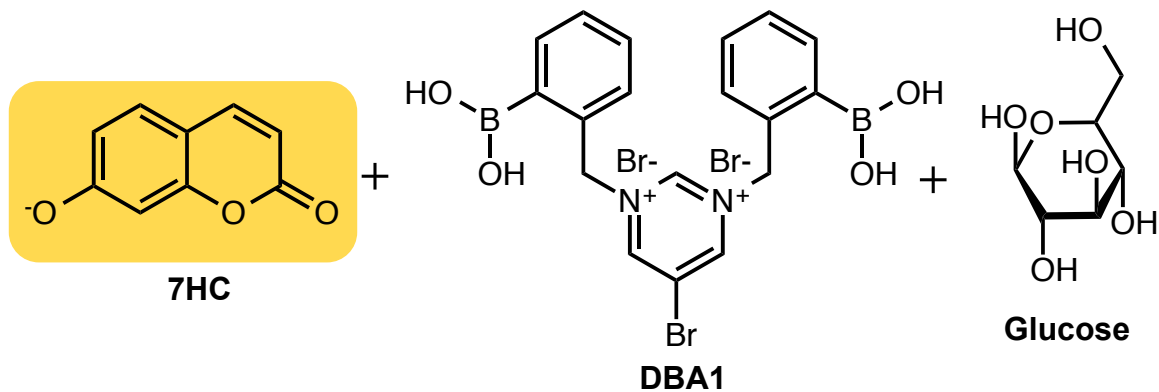
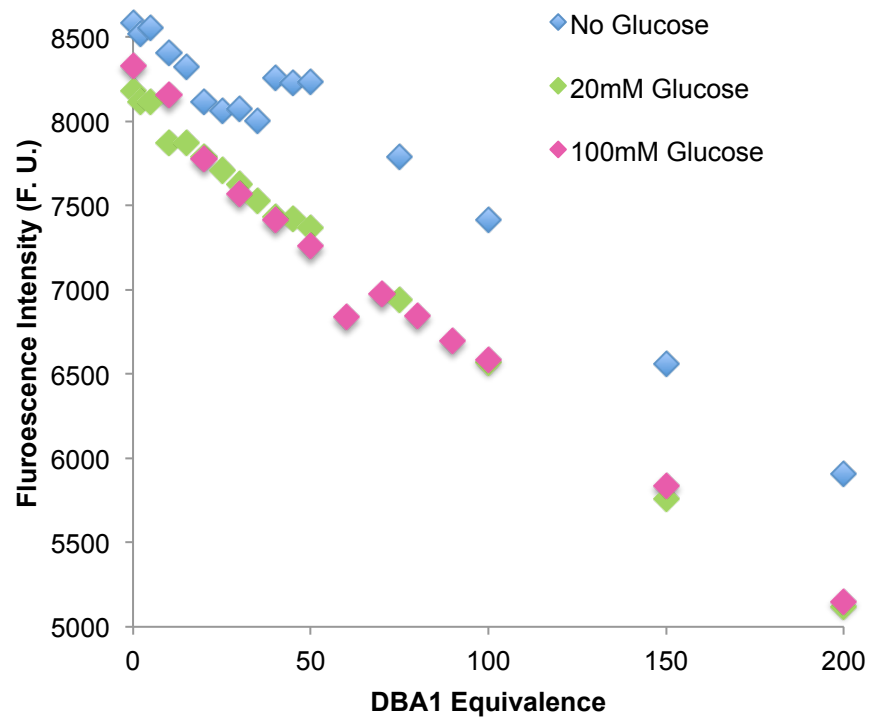


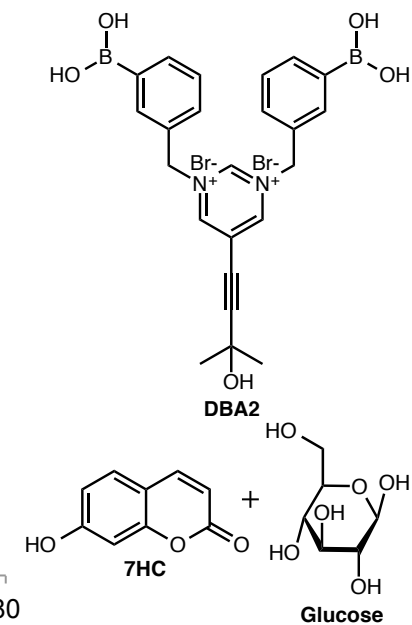
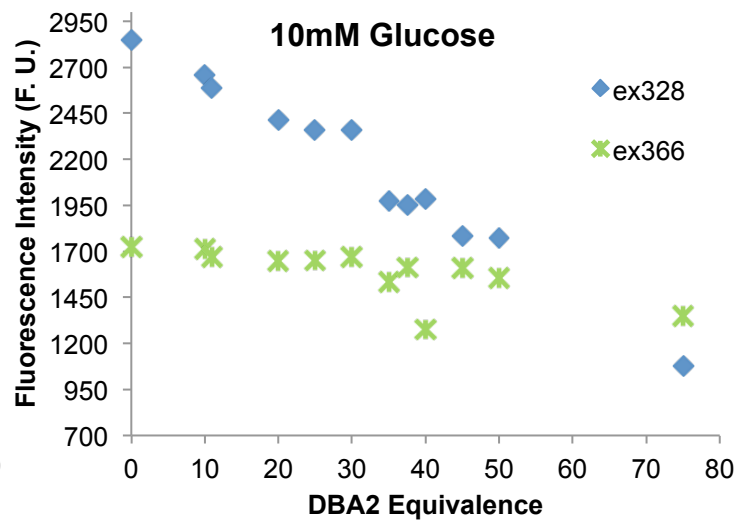
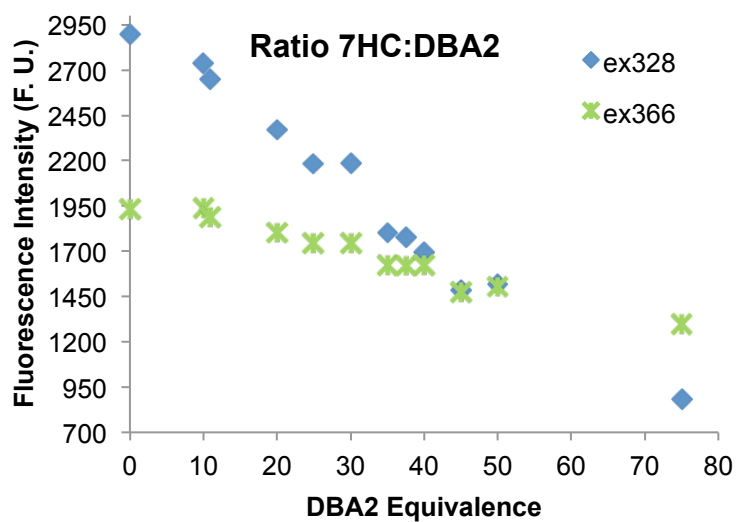
Excitation and emission spectra of 7 μ M 7HC in pH 8.12 buffer solution with increasing DBA1 concentrations up to 7 mM (1000 eq.); Medium 2.5 nm

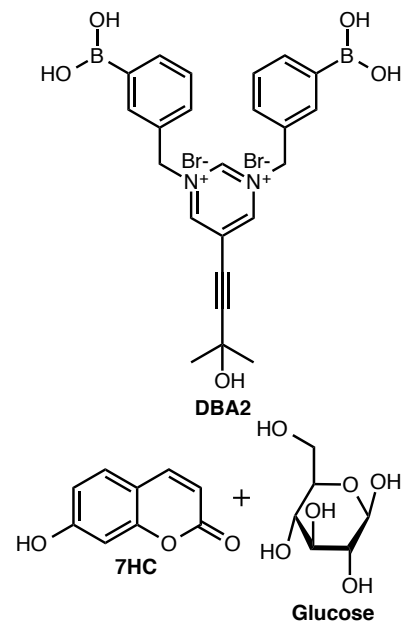
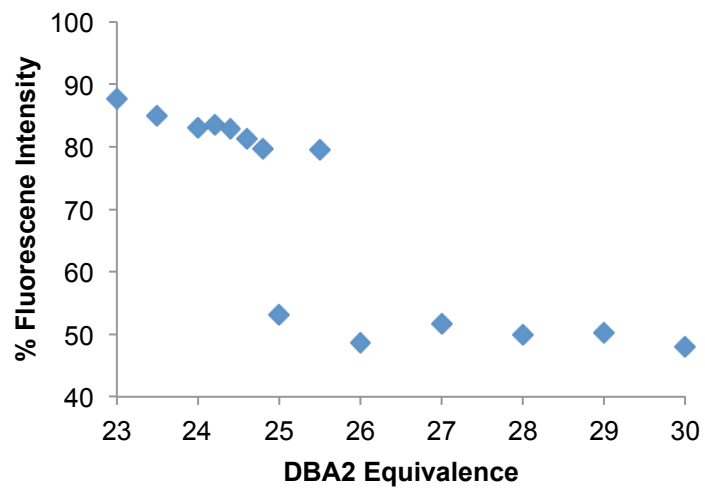


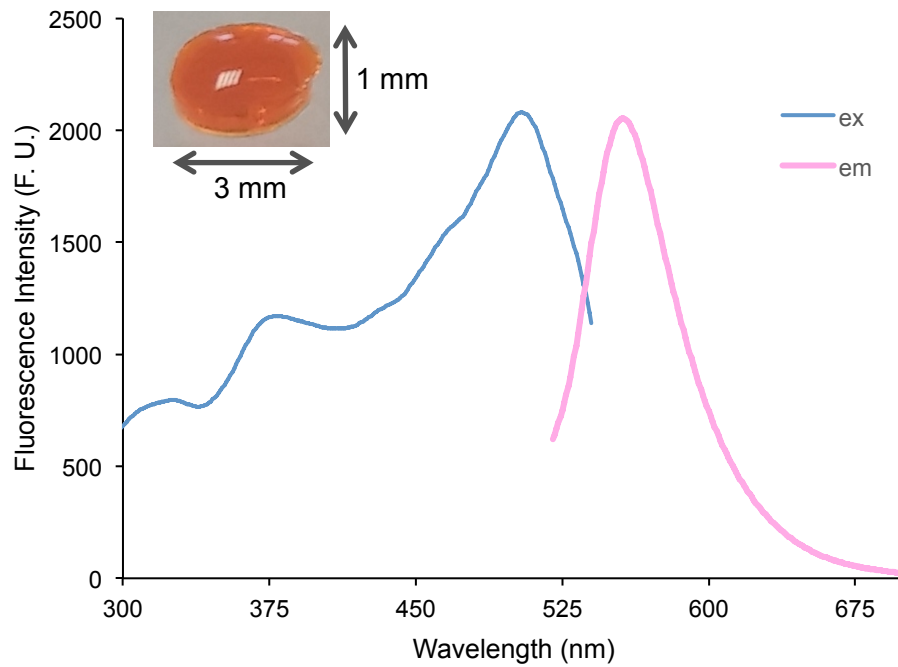
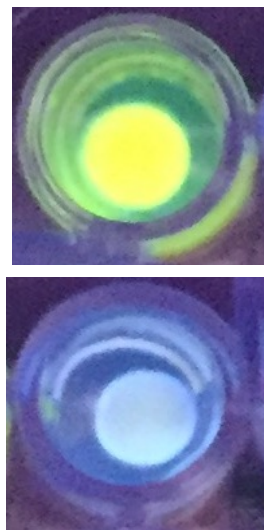
Fluorescence OFF

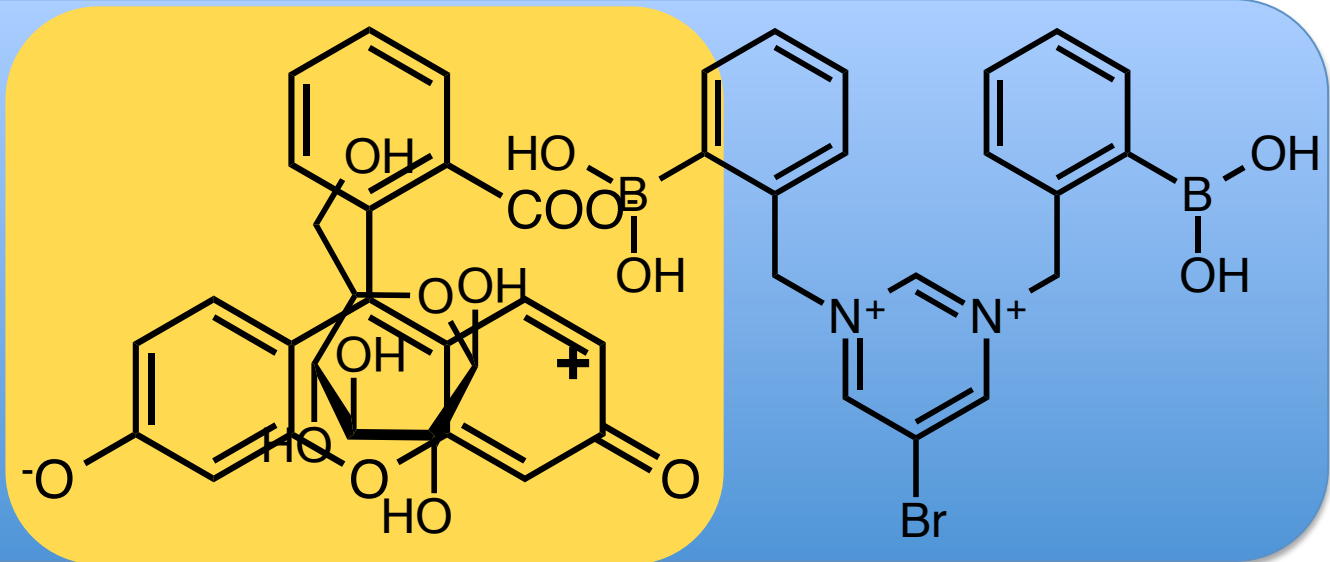






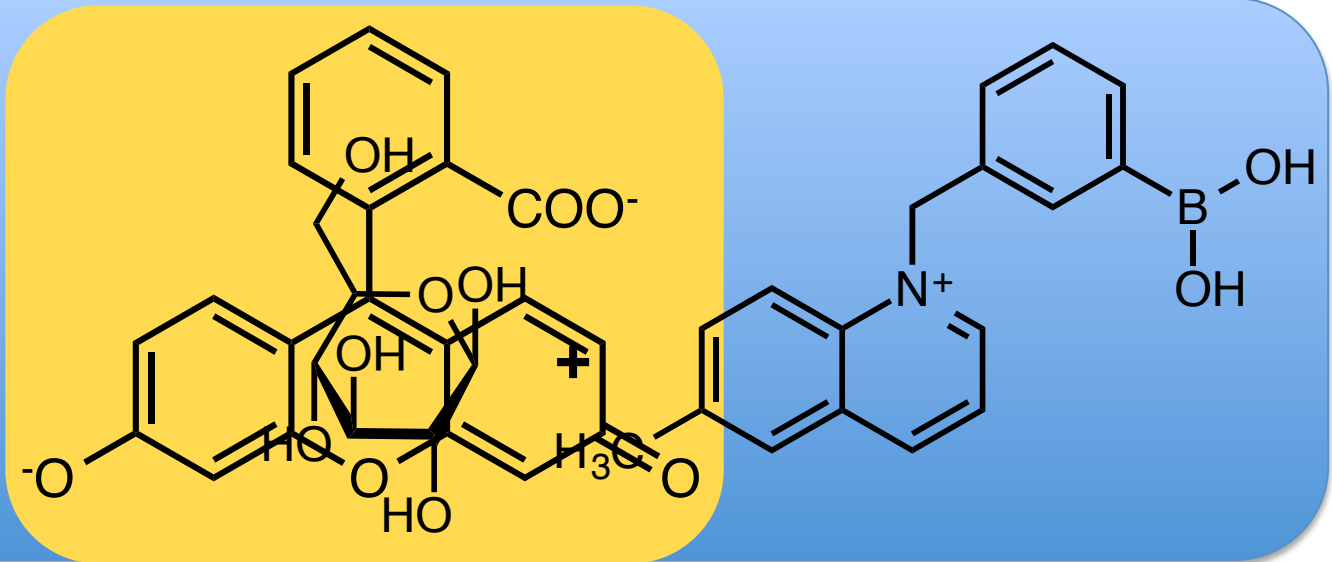




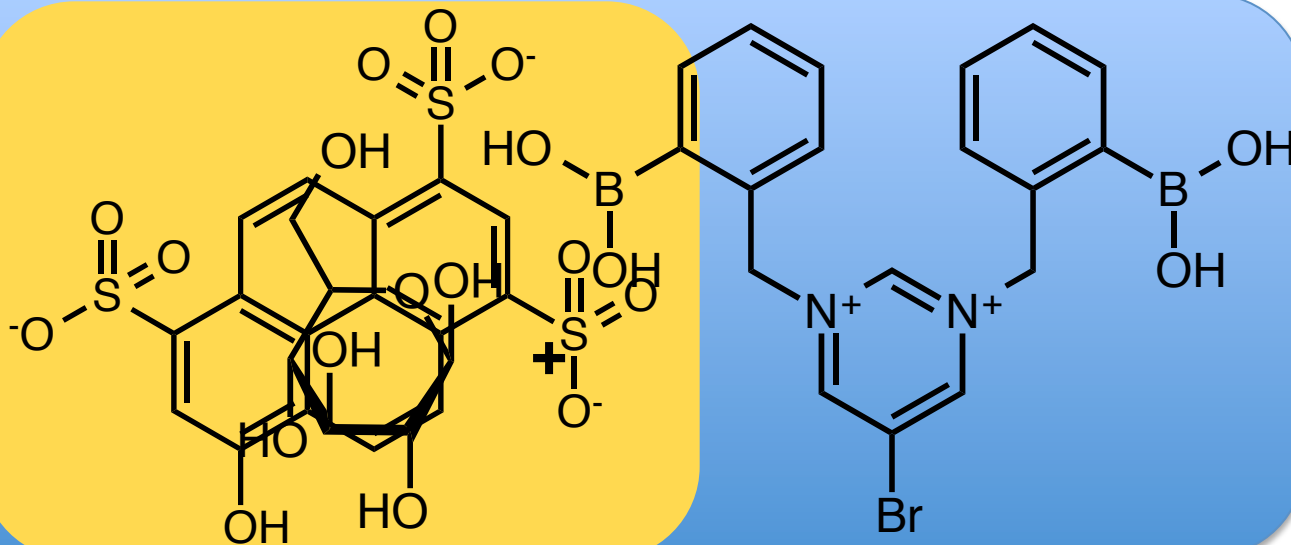


Fluorescent
Non-fluorescent

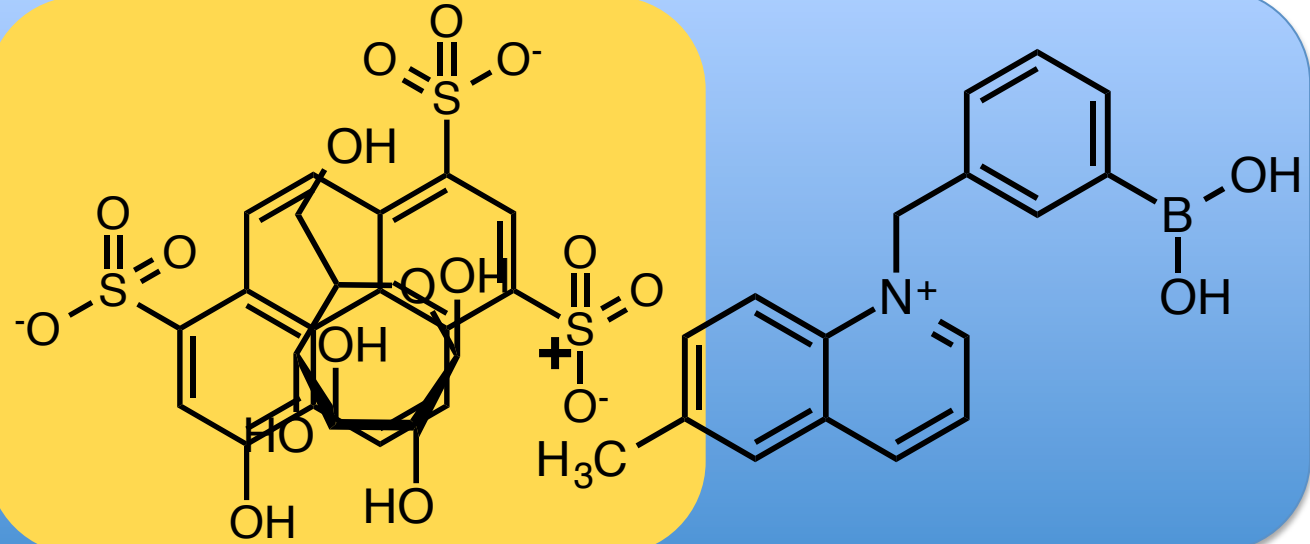




Fluorescent
Non-fluorescent

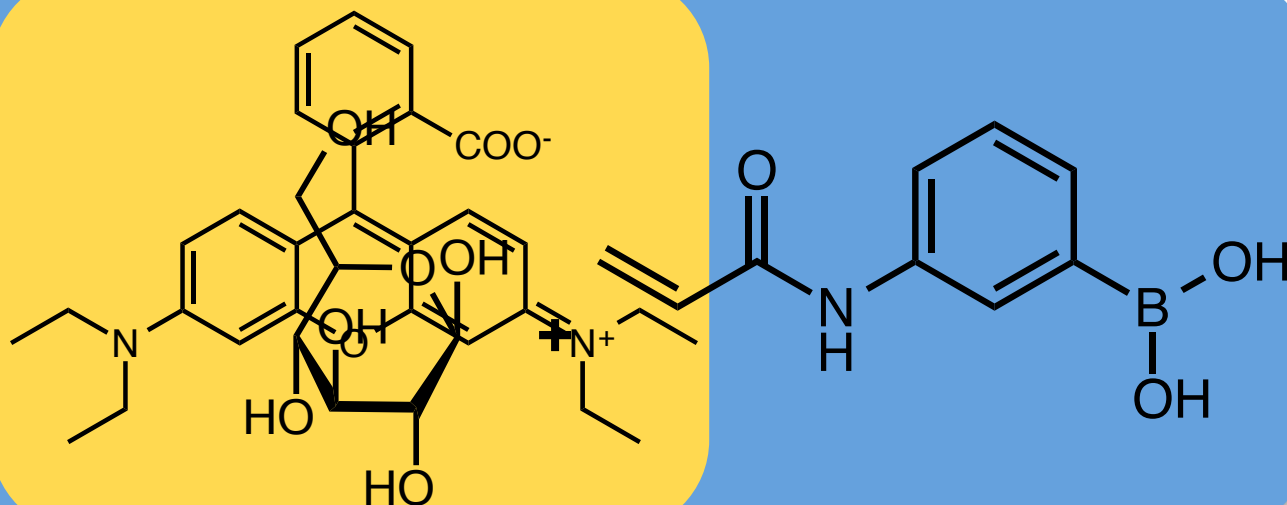


Fluorescent
Non-fluorescent



Fluorescent
Non-fluorescent





Fluorescent
Non-Fluorescent



