Synthesis of tiophene modified BODIPY based polarity sensors and their photophysical properties in lipid membranes

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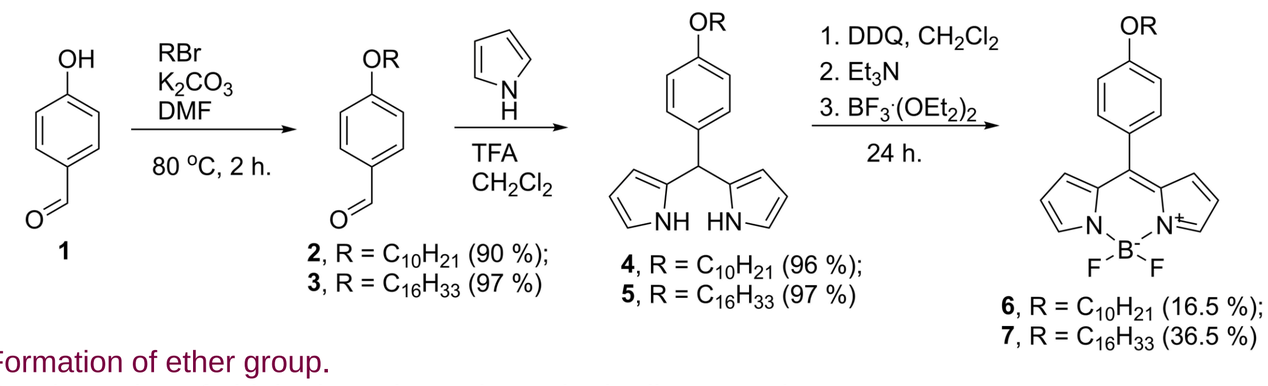
Studying lipid rafts with polarity sensors

- Lipid rafts are heterogeneous, dynamic cholesterol and sphingolipid enriched membrane nano-domains (10-200 nm) that are involved in crucial cellular processes.
- Polarity sensors are fluorophores capable of measuring polarity.
- Liquid-ordered (“rafts”) phase (Lo) of the membrane is more rigid than the liquid-disordered phase (Ld), thus it is less polar.
- Polarity sensors that stain lipid phases by different fluorescence intensity, color or lifetime can directly address the properties of separate lipid phases.

Synthesis of meso-phenyl BODIPY scaffold



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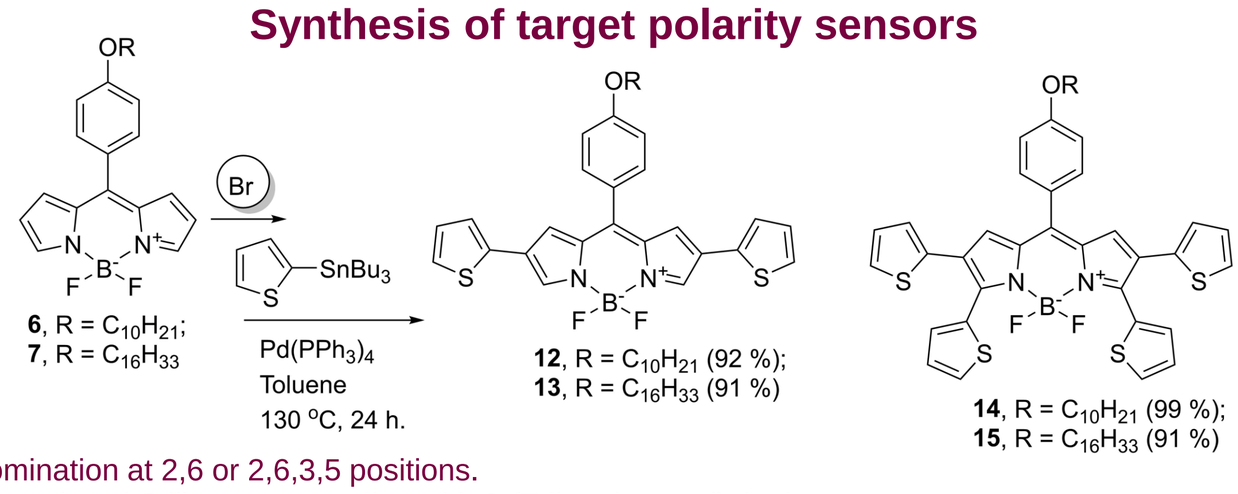
2, R = $C_{10}H_{21}$ (90 %);
3, R = $C_{16}H_{33}$ (97 %)

4, R = $C_{10}H_{21}$ (96 %);
5, R = $C_{16}H_{33}$ (97 %)

6, R = $C_{10}H_{21}$ (16.5 %);
7, R = $C_{16}H_{33}$ (36.5 %)

- Formation of ether group.
- Condensation of aldehyde and pyrrole to obtain dipyrromethane core.
- Oxidation by DDQ and complexation with $BF_3{\cdot}(OEt_2)_2$ to obtain BODIPY fluorophore.

Synthesis of target polarity sensors



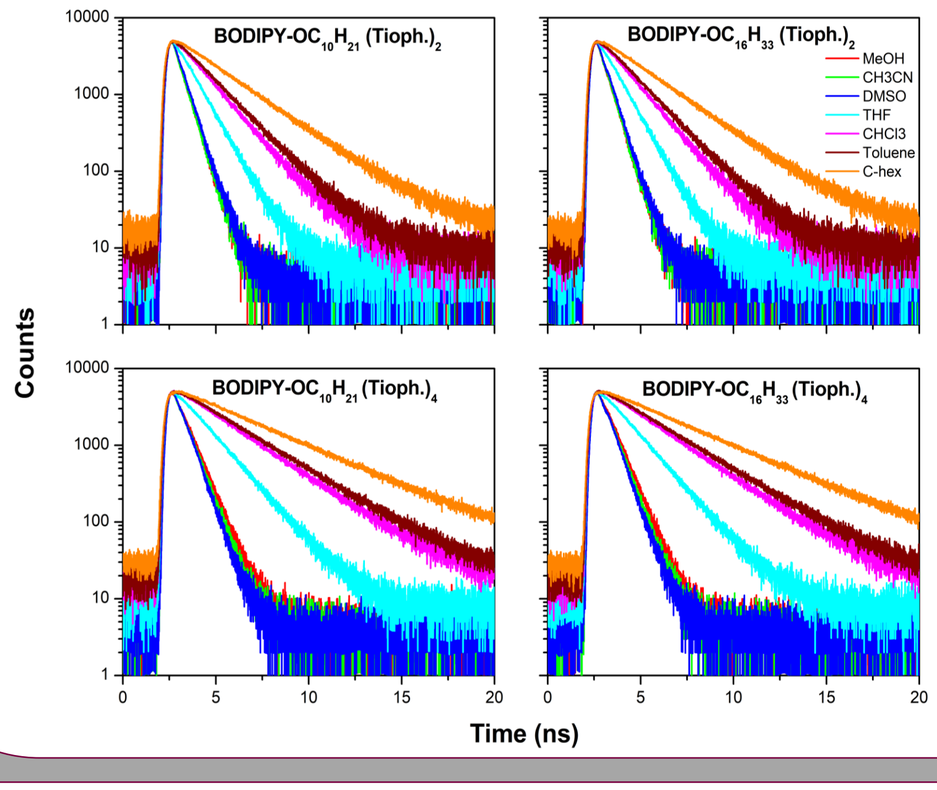
6, R = $C_{10}H_{21}$;
7, R = $C_{16}H_{33}$

12, R = $C_{10}H_{21}$ (92 %);
13, R = $C_{16}H_{33}$ (91 %)

14, R = $C_{10}H_{21}$ (99 %);
15, R = $C_{16}H_{33}$ (91 %)

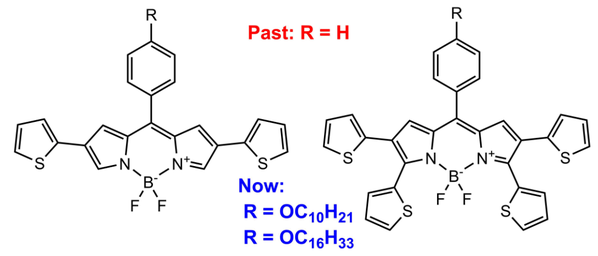
- Bromination at 2,6 or 2,6,3,5 positions.
- Pd catalyzed Stille cross-coupling with 2-(Tributylstannyl) tiophene.

Fluorescence lifetime decay in solvents

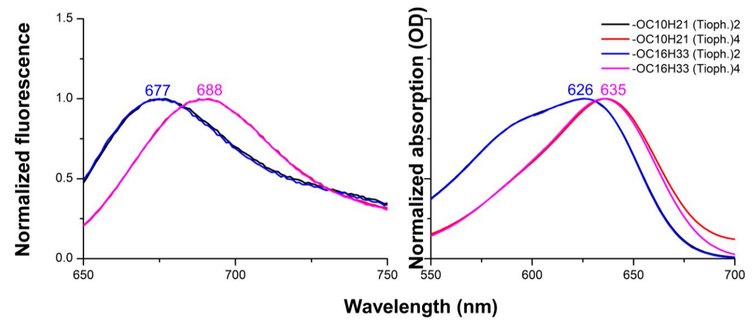


Previous work

- Previously, we investigated tiophene modified BODIPY fluorophores that were sensitive to polarity and emitted in red region, but could not discern between liquid-ordered and liquid-disordered lipid phases.
- We hypothesised that our past probes would aggregate or inefficiently attach into the lipid bilayer, thus failing to be usable in lipid phase studies.
- Thus, we sought to introduce alkoxy chains (-$OC_{10}H_{21}$; -$OC_{16}H_{33}$) into the meso position of the probe that would ensure an effective insertion into the lipid bilayer by increasing van der Waals interactions with lipid tails.

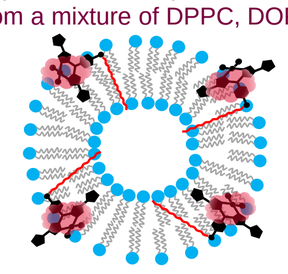


Fluorescence in solvents



Probes in lipid membranes

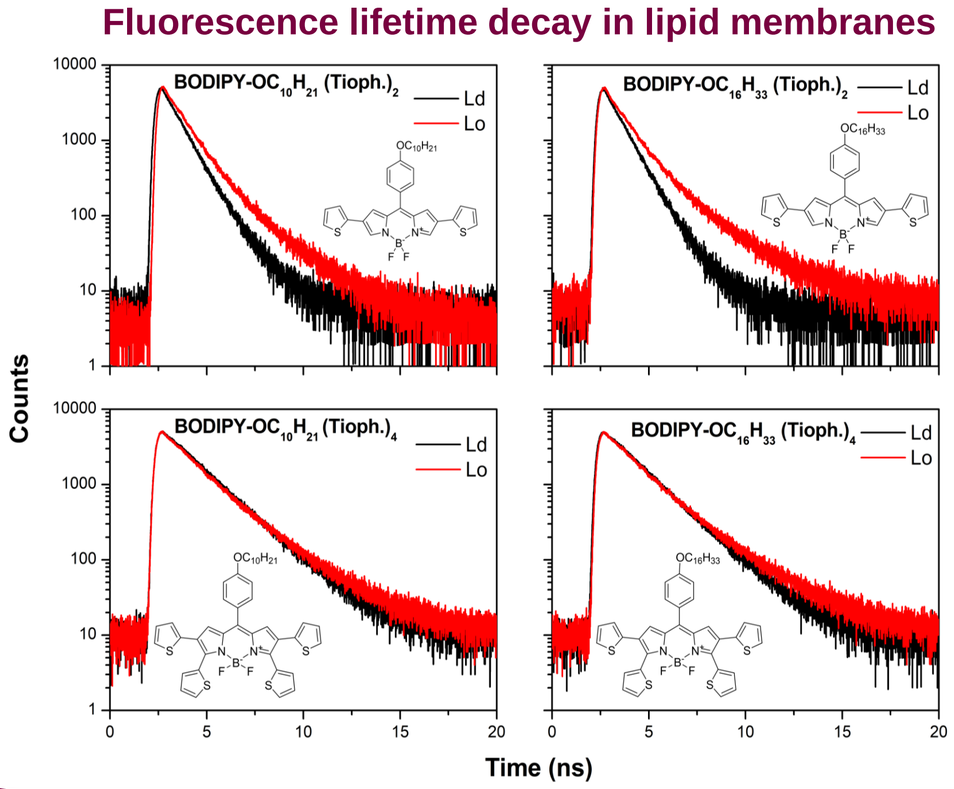
- Our probes were inserted into lipid membranes made by extrusion.
- Liquid-disordered phase was composed of DOPC, whereas liquid-ordered from a mixture of DPPC, DOPC and cholesterol.



- Illustration of the effect of alkoxy chains.
- Fluorescence lifetimes of our fluorophores in liquid-disordered and liquid-ordered phases.

Lifetimes in lipid membranes (ns)	Tiophene 2,6		Tiophene 2,6,3,5	
	Ld	Lo	Ld	Lo
BODIPY-$OC_{10}H_{21}$	0.86371	1.1585	1.7695	1.8112
BODIPY-$OC_{16}H_{33}$	0.84317	1.2668	1.7061	1.8258

Fluorescence lifetime decay in lipid membranes



Results

Tiophene modified BODIPY fluorophores bearing an alkoxy chain:

are polarity sensors
mono-exponential lifetimes in solvents
increase their fluorescence lifeti3mes as polarity decreases

In lipid membranes:

average lifetimes are longer in Lo phase
lifetimes become bi-exponential in Lo phase
disubstituted probes can discern between Ld and Lo phases

Conclusions

- Red-emitting BODIPY based polarity sensors were developed.
- Disubstituted dyes are more effective at discriminating between lipid phases than tetrasubstituted ones.
- We developed fluorophores that can discern between lipid phases by the difference of fluorescence lifetimes.

Future plans

- To insert probes into GUV’s.
- To analyse the influence of temperature on lifetimes of dyes in Lo phase.

Acknowledgment

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