NIR SQUARAINE DYES FOR CELL BILAYER BIOIMAGING: A STRUCTURE-ACTIVITY INVESTIGATION

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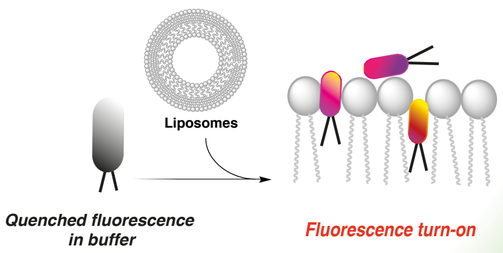
INTRODUCTION

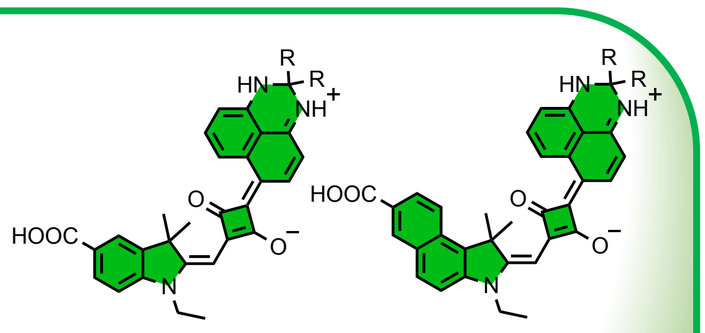
Cell bilayer membrane is a fascinating bio-supramolecolar structure playing key roles in many biochemical processes crucial for life. Membrane fluidity, fluid dynamics and polarization along with the specific interactions between phospholipids, proteins and channels, on and inside the bilayer, are fundamental to shed light on crucial biochemical pathways.

Fluorescence is a powerful and non-invasive technique which allows well-defined investigations in space and time of bio-chemical/physical events by using specific probes as Near InfraRed fluorophores and liposomal (DOPC and DPPC) lipid models .



PROBES DESIGN

Two series of novel unsymmetrical squaraine dyes were designed. A carboxyindolenine (**CI**) or a carboxybenzoindolenine (**CBI**) moiety have been placed on one side, while on the opposite one a perimidine derivative has been decorated with alkyl chains of different length (**C1**, **C2**, **C4** or **C8**). The COOH group improves the water solubility of the whole system, while the alkyl chains tune the interaction of the probe with the lipophilic components of the cell membrane.



CI-squaraine series

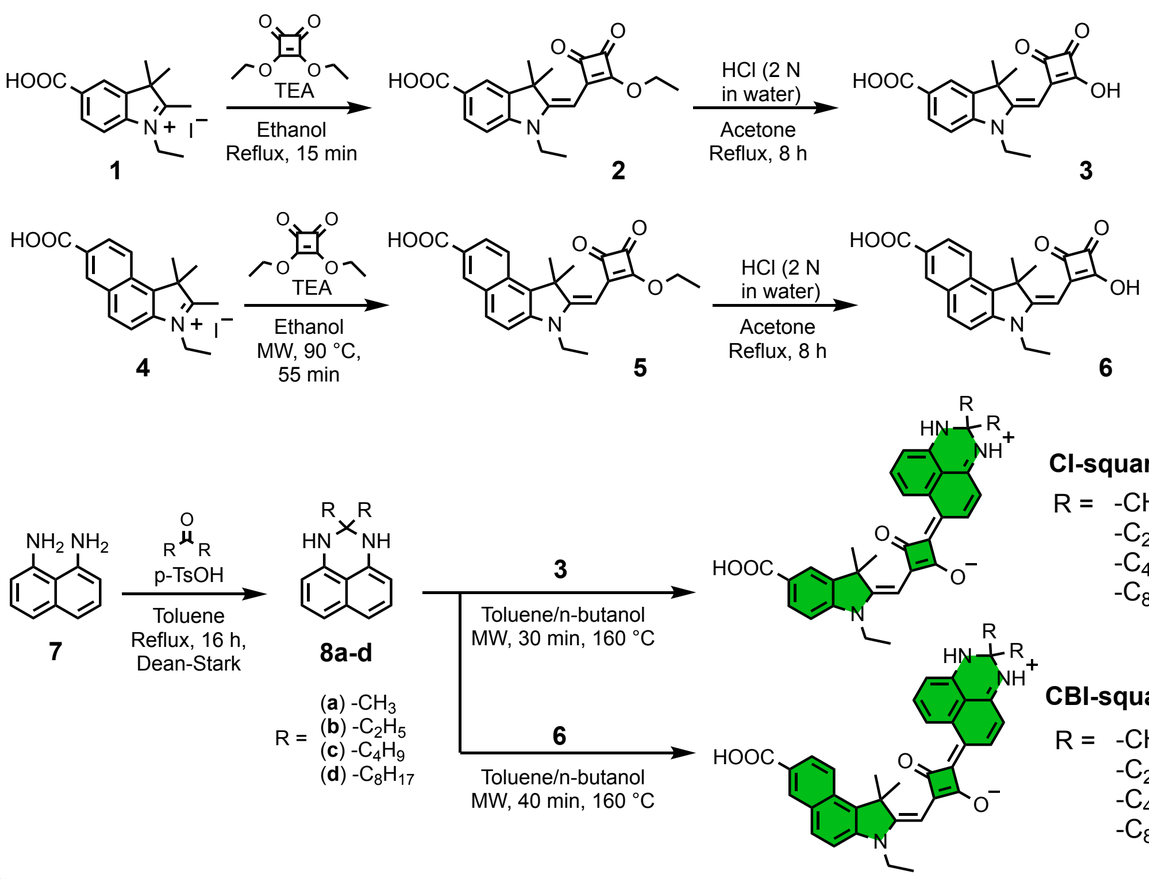
R = $-CH_3$ **CI-C1**
$-C_2H_5$ **CI-C2**
$-C_4H_9$ **CI-C4**
$-C_8H_{17}$ **CI-C8**

CBI-squaraine series

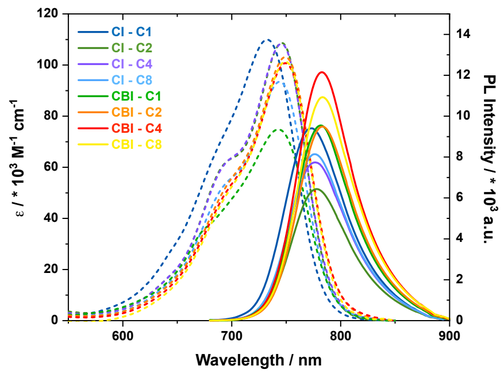
R = $-CH_3$ **CBI-C1**
$-C_2H_5$ **CBI-C2**
$-C_4H_9$ **CBI-C4**
$-C_8H_{17}$ **CBI-C8**

SYNTHESIS

Two series of novel unsymmetrical squaraine dyes were prepared as follows. A carboxyindolenine (**CI**) or a carboxybenzoindolenine (**CBI**) moiety has been placed on one side, while on the opposite one a perimidine derivative has been decorated with alkyl chains of different length (**C1**, **C2**, **C4** or **C8**). The squaraine dyes were synthetized by direct condensation between an emisquaraine (**3** or **6**) and a perimidine derivative (**8a-d**) in a microwave reactor. The emisquaraine partners were obtained following published procedures, while perimidines **8a-d** were prepared according to a novel acid catalyzed grams-scale method. The 1,8-diaminonaphthalene **7** was refluxed in toluene with the relative ketone in presence of few drops of *p*-toluenesulfonic acid.



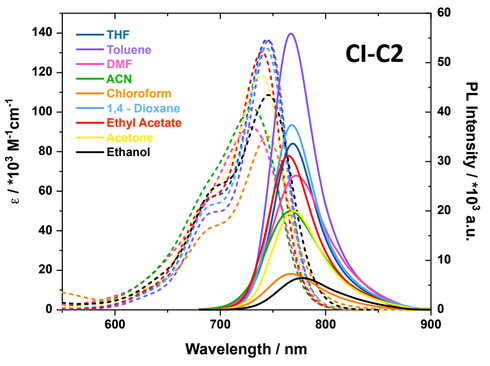
PHOTOPHYSICS



The molar extinction coefficient and quantum yield were estimated in ethanol for all the probes.

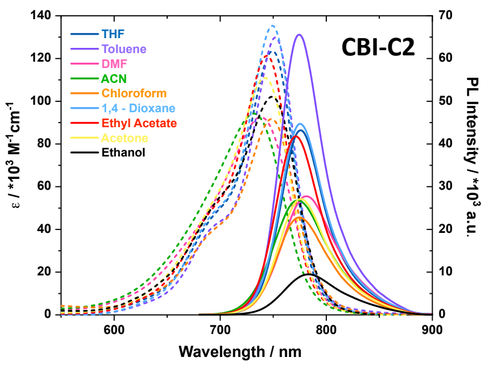
*Absorption (dashed) and emission (solid) spectra in **ethanol**. Emission spectra were normalized at 0.1 at excitation wavelength.*

Cpd	λ_{ABS} *nm*	λ_{EMISS} *nm*	Stokes Shift *cm⁻¹*	ε *M⁻¹ cm⁻¹*	QY *%*	Brightness *M⁻¹ cm⁻¹*
CI - C1	733	773	706	109423	1.46	1593
CI - C2	746	778	551	109635	0.97	1066
CI - C4	745	777	553	107712	1.14	1225
CI - C8	744	777	571	94504	1.26	1189
CBI - C1	744	782	653	75086	1.45	1090
CBI - C2	749	783	580	103029	1.43	1472
CBI - C4	749	783	580	103793	1.66	1724
CBI - C8	750	783	562	100755	1.65	1663



CI and **CBI** series solvatochromic properties were investigated by using **CI-C2** ad **CBI-C2** as examples. They exhibit sharp emission and absorption spectra, with high molar extinction coefficients ($4.94 \leq \log\varepsilon \leq 5.13$ in different solvents)

The N-H bonds may favor nonradiative pathways such as hydrogen bonding induced intersystem crossing. Protic solvents (*e.g.* ethanol) quenches QY, while fluorescence can be turned on by aprotic environment (*e.g.* toluene).

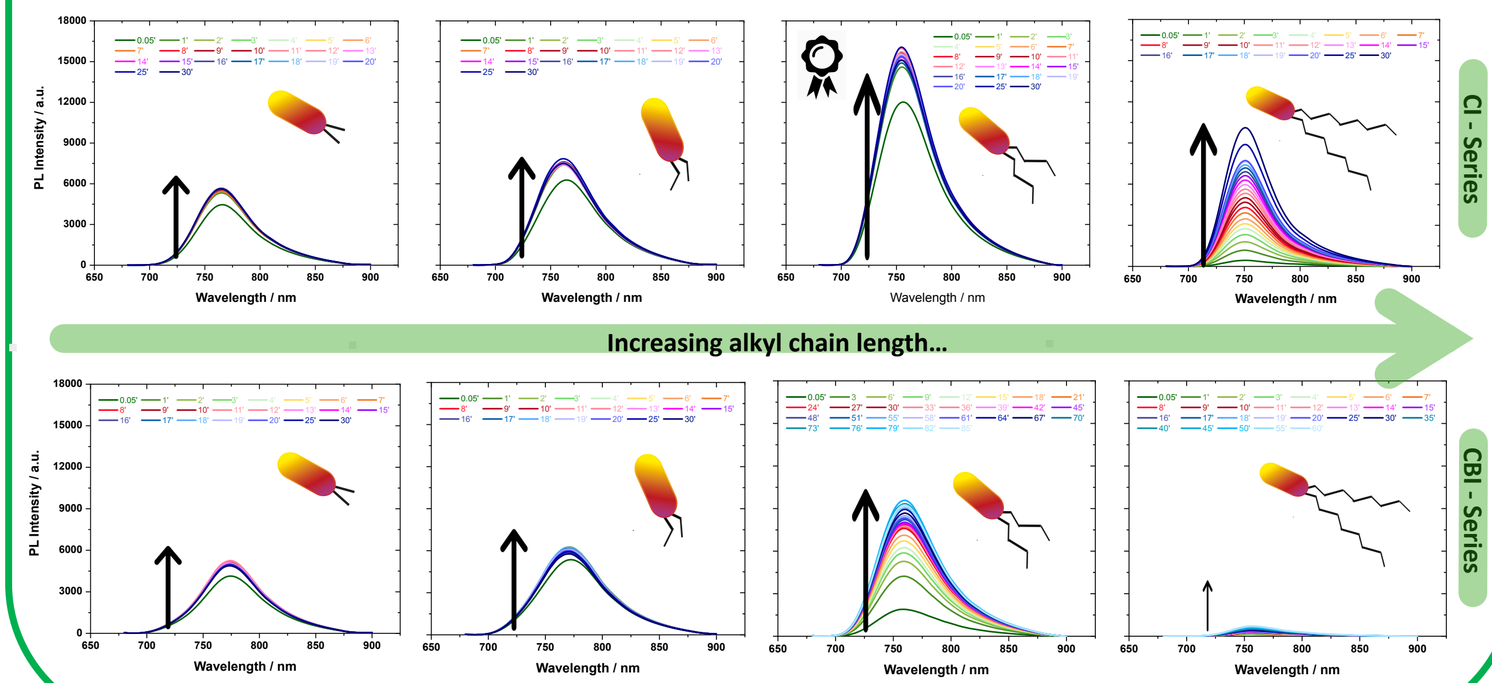


Absorption (dashed) and emission (solid) spectra in several solvents. Emission spectra were normalized at 0.1 at excitation wavelength.

Solvent	CI – C2 λ_{ABS} *nm*	CI – C2 λ_{EMISS} *nm*	CI – C2 ε *M⁻¹ cm⁻¹*	CI – C2 QY *%*	CBI – C2 λ_{ABS} *nm*	CBI – C2 λ_{EMISS} *nm*	CBI – C2 ε *M⁻¹ cm⁻¹*	CBI – C2 QY *%*
THF	745	769	136888	3.3	749	776	123482	4.2
Toluene	746	767	136348	5.8	752	774	129849	7.0
DMF	729	772	92441	3.3	739	781	92574	3.3
ACN	730	767	101904	2.2	732	773	94206	2.8
Chloroform	745	767	87634	0.9	749	775	91655	2.6
Dioxane	744	768	132502	2.8	749	775	135335	3.4
EtOAc	740	765	130459	2.9	745	771	121278	3.9
Acetone	739	770	118788	2.0	742	776	110907	2.7

PARTITIONING KINETICS INTO LIPOSOMES

The partitioning kinetic of the probes inside the membrane were investigated by monitoring the increase of the fluorescence intensity at defined times, upon addition of the fluorophores to the DPPC liposomes solution. The fluorescence in buffer is completely quenched, while in liposomes the environment change allows a fluorescence turn-onn.The alkyl chain length on the perimidine moiety showed to influence the probe intercalation.



CONCLUSIONS

Novel NIR squaraine dyes have been synthetized and optically characterized. Their partitioning kinetic into DPPC liposomes was evaluated, studying the influence of the alkyl chain length on the process. **CI-C4** and **CBI-C4** resulted to be the best candidate in the two series, allowing a complete intercalation within 60 minutes. The octyl chains slowed down the kinetics, slightly for **CI-C8** and more dramatically for **CBI-C8**. Finally, shorter chains (**C1** ad **C2**) probes partition into DPPC within few minutes after the addition.

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