SRFluor®DYES: SUPERIOR NIR DYES FOR BIOIMAGING

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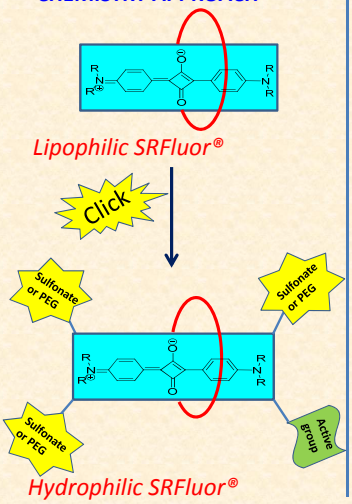
INTRODUCTION

- Molecular encapsulation of dyes as rotaxanes is a novel way to increase photostability.
- Squaraine rotaxane dyes exhibit excellent chemical and photostability that is superior to Cy5.
- Due to poor solubility under aqueous conditions their usefulness in biological applications remained a challenge.
- By introducing multiple sulfonate or PEG groups we have improved the solubility of this fluorophore and also reduced aggregation under lipophilic conditions.
- Preliminary bioconjugation studies showed excellent results.

SYNTHESIS USING CLICK CHEMISTRY APPROACH



ABSORPTION AND EMISSION COMPARISON OF SRFluor® WITH CY5

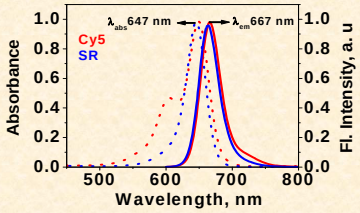
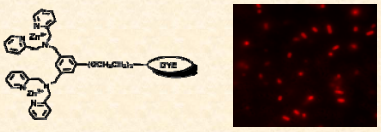


Figure 1: Absorption and emission (exc. 580 nm) spectra of SRfluor® and Cy5

Table 1: Absorption and emission characteristics of SRfluor® and Cy5

Dye	λ_{abs} (nm)	λ_{em} (nm)	ε ($M^{-1}cm^{-1}$)
SRfluor®	647	667	225,000
Cy5	647	667	250,000

RELATIVE STABILITY OF BACTERIA BINDING PROBES



DYE: Squaraine Rotaxane (SRFluor®); Cy5

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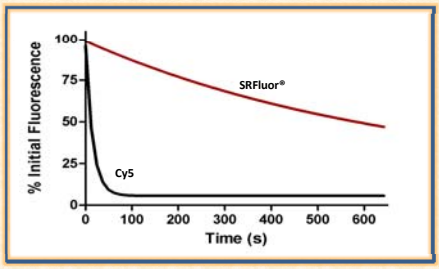
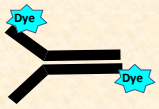


Figure 2: Photobleaching of bacterial cells stained with DPA probes undergoing continuous irradiation (620 nm ±30) with an X-cite 120 fluorescence illumination system through a Nikon 2000-TE epifluorescence microscope.

RELATIVE STABILITY OF IgG PROBES



DYE: Squaraine Rotaxane (SRFluor®); Cy5

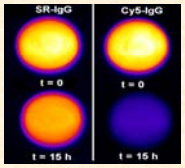


Figure 3: Pixel intensity maps of fluorescence from microwells containing SRfluor®-IgG and Cy5-IgG in PBS buffer (pH=7.5) before and after illumination.

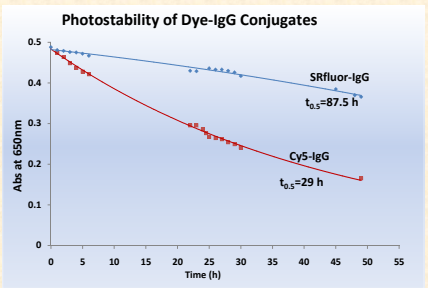
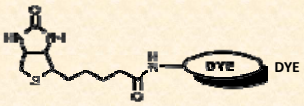


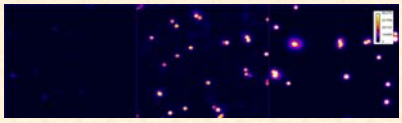
Figure 4: Solutions of SRfluor®-IgG (degree of labeling = 2.0) and Cy5-IgG (degree of labeling = 1.7) in PBS buffer (pH=7.5) were illuminated with a 100W bulb placed 12 inches away and the optical density of the solutions at 650 nm monitored over time.

RELATIVE BRIGHTNESS AND STABILITY OF BIOTIN PROBES ON THE SURFACE OF STREPTAVIDIN COATED NANOPARTICLES



DYE: Carboxyfluorescein (CF); Squaraine Rotaxane (SRFluor®); Alexa

CF SRFluor® ALEXA



Dyes	Mean pixel intensity
CF	2081
SRfluor®	21620
Alexa	29809

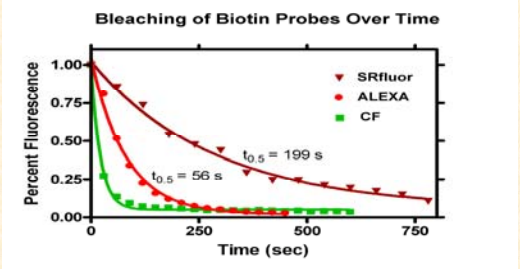
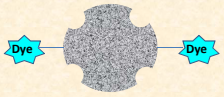


Figure 5: Photobleaching of biotinylated probes bonded to streptavidin coated nanoparticles undergoing continuous irradiation with an X-cite 120 fluorescence illumination system through a Nikon 2000-TE epifluorescence microscope.

RELATIVE STABILITY OF STREPTAVIDIN PROBES



DYE: Squaraine Rotaxane (SRFluor®); Cy5

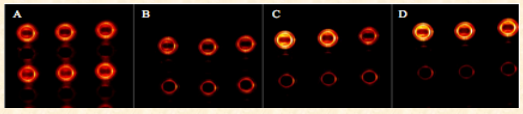


Figure 6: Microwells with biotinylated surfaces were treated with SRFluor®-streptavidin (top wells of each frame, degree of labeling = 1.8) or Cy5-streptavidin (bottom wells, degree of labeling = 2.0). A.) Emission prior to light exposure; B.) 10 minutes after light exposure; C.) 30 minutes after light exposure; D.) 60 minutes after light exposure

CONCLUSIONS

- SRfluor® dyes were successfully conjugated to DPA, IgG and streptavidin and compared with analogous Cy5 and Alexa probes.
- Measurements proved that SRfluor® probes are at least three times more photostable to Cy5 and Alexa dye based probes.
- New generation SRfluor® dyes are developed as a possible replacement of Cy5 dyes in optical imaging applications.

ACKNOWLEDGEMENT

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