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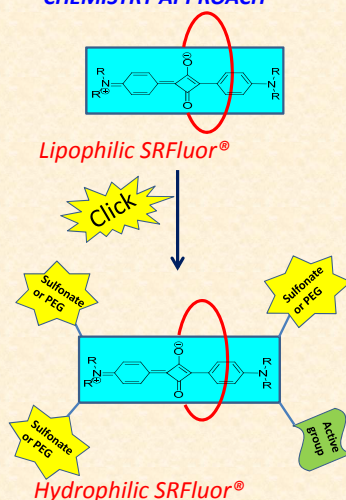


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INTRODUCTION

- ★ **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

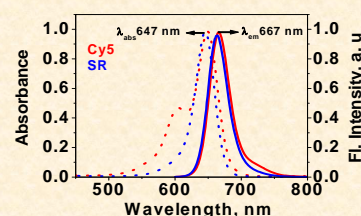


Figure 1: Absorption and emission (exc. 580 nm) spectra of SRFluor® and Cy5

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

| Dye | λ_{obs} (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

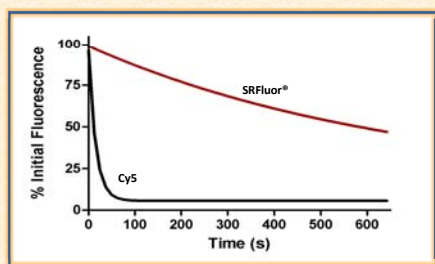
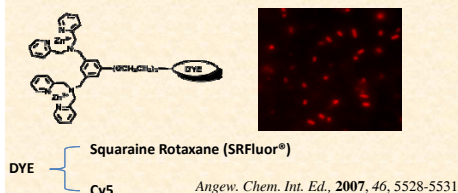


Figure 2: Photobleaching of bacterial cells stained with DPA probes undergoing continuous irradiation (620 nm \pm 30) with an X-cite 120 fluorescence illumination system through a Nikon 2000-TE epifluorescence microscope.

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

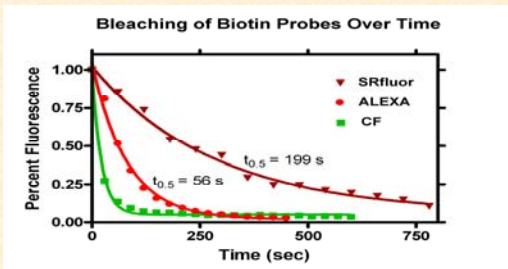
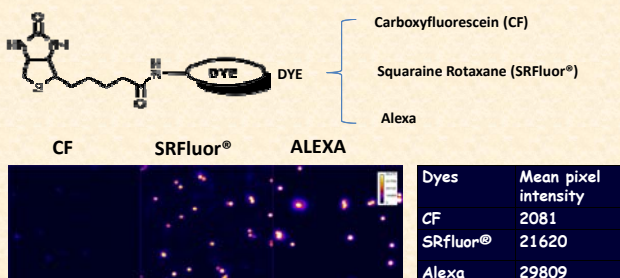


Figure 5: Photobleaching of biotinylated probes bonded to streptavidin coated nanoparticles undergoing continuous irradiation with an X-cite 120 fluorescence illumination system through a Nikon 2000-TE epifluorescence microscope.

RELATIVE STABILITY OF IgG PROBES

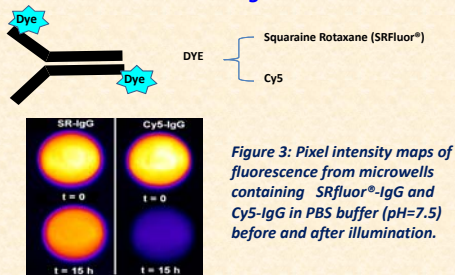


Figure 3: Pixel intensity maps of fluorescence from microwells containing SRFluor®-IgG and Cy5-IgG in PBS buffer (pH=7.5) before and after illumination.

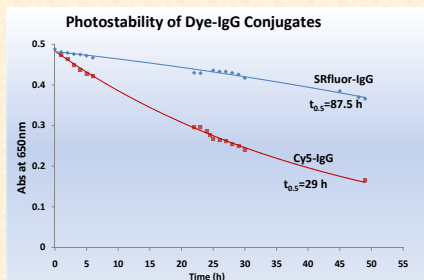


Figure 4: Solutions of SRFluor®-IgG (degree of labeling = 2.0) and Cy5-IgG (degree of labeling = 1.7) in PBS buffer (pH=7.5) were illuminated with a 100W bulb placed 12 inches away and the optical density of the solutions at 650 nm monitored over time.

RELATIVE STABILITY OF STREPTAVIDIN PROBES

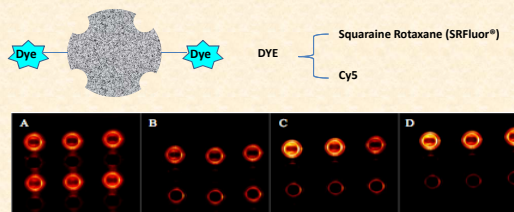


Figure 6: Microwells with biotinylated surfaces were treated with SRFluor®-streptavidin (top wells of each frame, degree of labeling = 1.8) or Cy5-streptavidin (bottom wells, degree of labeling = 2.0). A.) Emission prior to light exposure; B.) 10 minutes after light exposure; C.) 30 minutes after light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