Cyanine fluorophore derivatives with enhanced photostability

Roger B Altman¹, Daniel S Terry²,⁷, Zhou Zhou¹,⁷, Qinsi Zheng³, Peter Geggier⁴, Rachel A Kolster⁴, Yongfang Zhao⁴, Jonathan A Javitch⁴,⁵, J David Warren⁶ & Scott C Blanchard¹–³,⁶

Fluorescence applications requiring high photostability often depend on the use of solution additives to enhance fluorophore performance. Here we demonstrate that the direct or proximal conjugation of cyclooctatetraene (COT), 4-nitrobenzyl alcohol (NBA) or Trolox to the cyanine fluorophore Cy5 dramatically enhanced fluorophore photostability without otherwise affecting its native spectral characteristics. Such conjugation is a powerful means of improving the robustness of fluorescence-based applications demanding long-lived, nonblinking fluorescence emission.
Photoblinking and photobleaching

Rasnik et al., 2006
Cyanine fluorophore derivatives
Cyanine fluorophore derivatives

**COT-Cy5-NHS:**
LCMS: 25-65% B over 2.5 min, rt = 1.73 min
HPLC: 25-65% B over 25 min, rt = 13.01 min
ESI-MS: m/z calculated for $\text{C}_{54}\text{H}_{63}\text{N}_5\text{O}_{12}\text{S}_2$
\[ [\text{M+H}]^+ \text{ 1040.4, found: 1040.7} \]

**TX-Cy5-NHS:**
LCMS: 25-65% B over 2.5 min, rt = 1.80 min
HPLC: 25-65% B over 25 min, rt = 13.65 min
ESI-MS: m/z calculated for $\text{C}_{57}\text{H}_{71}\text{N}_5\text{O}_{14}\text{S}_2$
\[ [\text{M+H}]^+ \text{ 1114.5, found: 1114.8} \]

**NBA-Cy5-NHS:**
LCMS: 25-65% B over 2.5 min, rt = 1.28 min
HPLC: 25-65% B over 25 min, rt = 7.33 min
ESI-MS: m/z calculated for $\text{C}_{59}\text{H}_{60}\text{N}_6\text{O}_{13}\text{S}_2$
\[ [\text{M+H}]^+ \text{ 1017.4, found: 1017.7} \]
Cyanine fluorophore derivatives

![Absorbance vs. Wavelength](#)

- Cy5
- Cy5-COT
- Cy5-NBA
- Cy5-TX

![Relative Fluorescence Intensity vs. Wavelength](#)

- Cy5
- Cy5-COT
- Cy5-NBA
- Cy5-TX

![Relative Intensity vs. Time](#)

- Cy5
- Cy5-COT
- Cy5-NBA
- Cy5-TX
Single molecule traces

(a) Chemical structure of the Cy5 dye and its modifications. The figure shows the structure of the Cy5 dye conjugated with NHS (N-hydroxysuccinimide) and a linker. (b) Bar graph showing the lifetime ($\tau_{on}$) of Cy5 and its modifications in different solutions. The x-axis represents the solution type, and the y-axis represents the lifetime (s). (c) Graphs showing the intensity (photons) over time (s) for Cy5, Cy5-COT, Cy5-NBA, and Cy5-Trolox.
distance control
With Oxygen scavenging system

With +PCDTPCA

![](image1)

With -PCDTPCA

![](image2)

Legend:
- Cy5
- Cy5-COT
- Cy5-NBA
- Cy5-Trolox
on the living cell

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