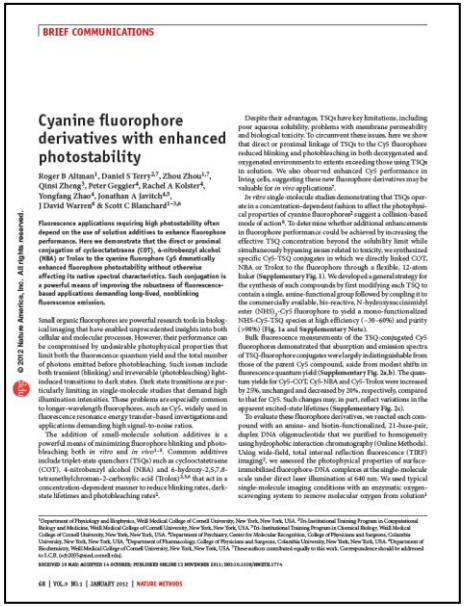
Cyanine fluorophore derivatives with enhanced photostability

Roger B Altman[1], Daniel S Terry[2,7], Zhou Zhou[1,7], Qinsi Zheng[3], Peter Geggier[4], Rachel A Kolster[4], Yongfang Zhao[4], Jonathan A Javitch[4,5], J David Warren[6] & Scott C Blanchard[1–3,6]

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.



BRIEF COMMUNICATIONS

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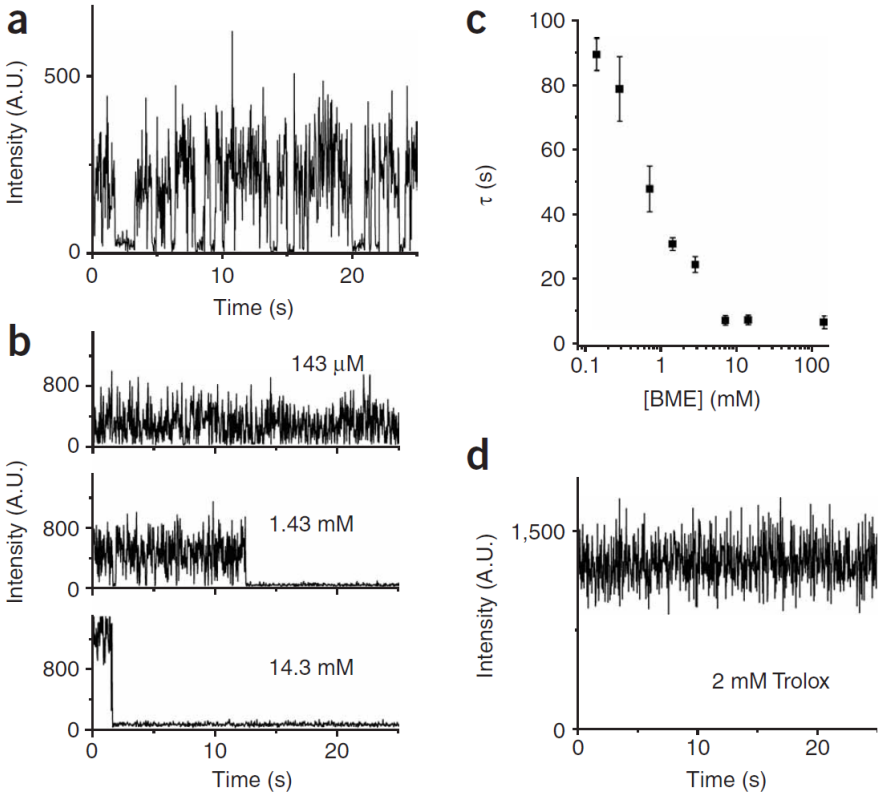
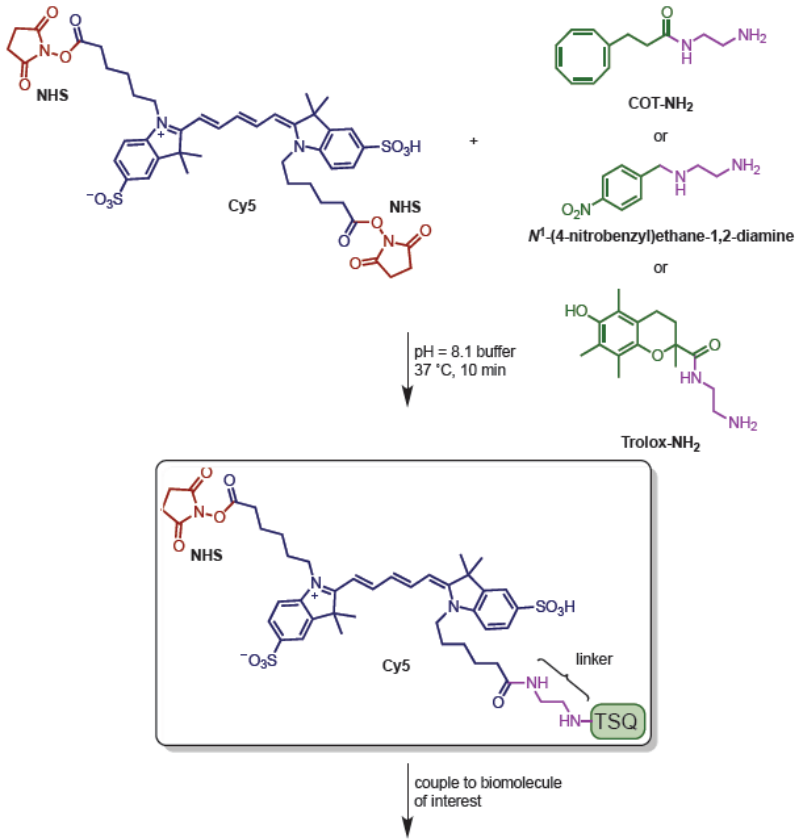
Photoblinking and photobleaching



Rasnik et al., 2006

Cyanine fluorophore derivatives



Cyanine fluorophore derivatives

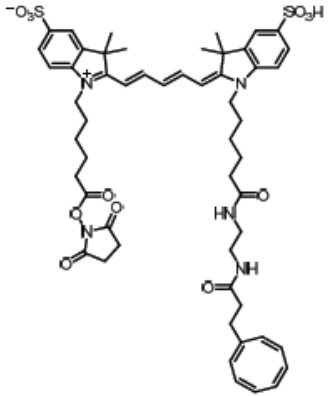
COT-Cy5-NHS:

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 $C_{54}H_{65}N_5O_{12}S_2$

$[M+H]^+$ 1040.4, found 1040.7



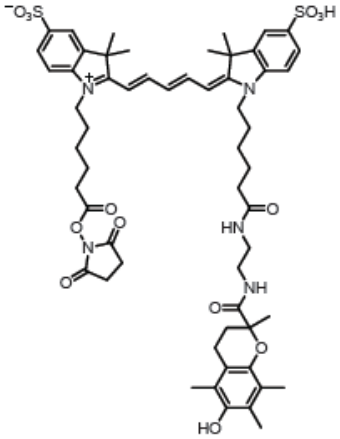
TX-Cy5-NHS:

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 $C_{57}H_{71}N_5O_{14}S_2$

$[M+H]^+$ 1114.5, found: 1114.8



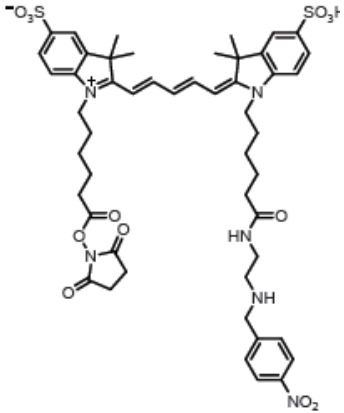
NBA-Cy5-NHS:

LCMS: 25-65% B over 2.5 min, rt = 1.28 min

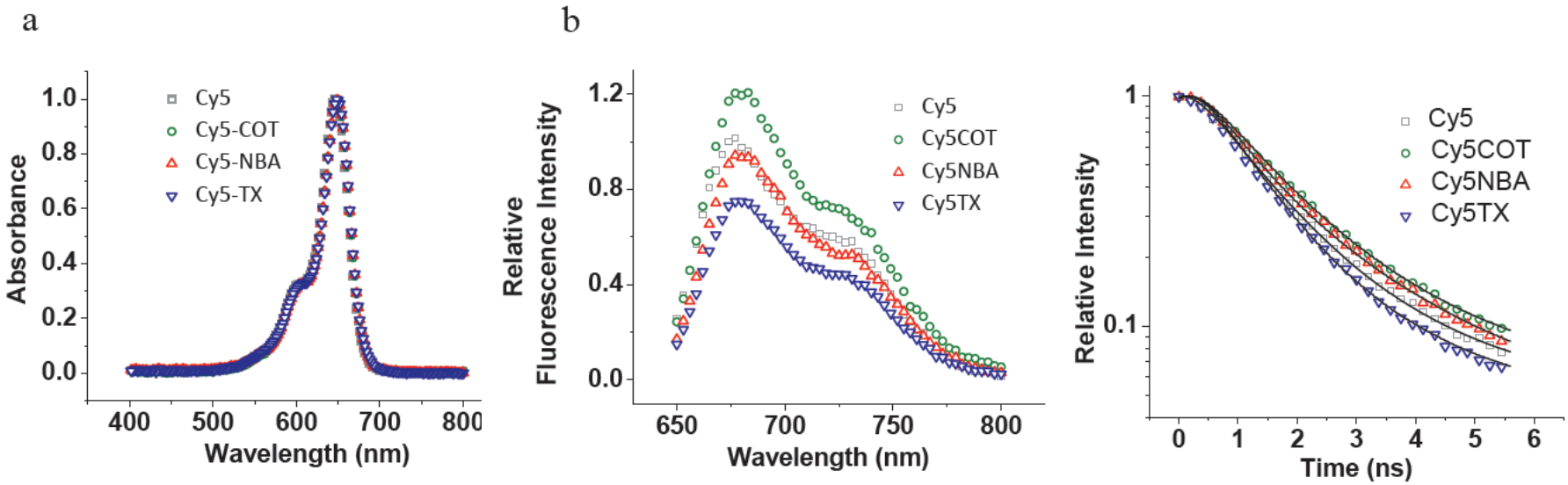
HPLC: 25-65% B over 25 min, rt = 7.33 min

ESI-MS: *m/z* calculated for $C_{50}H_{60}N_6O_{13}S_2$

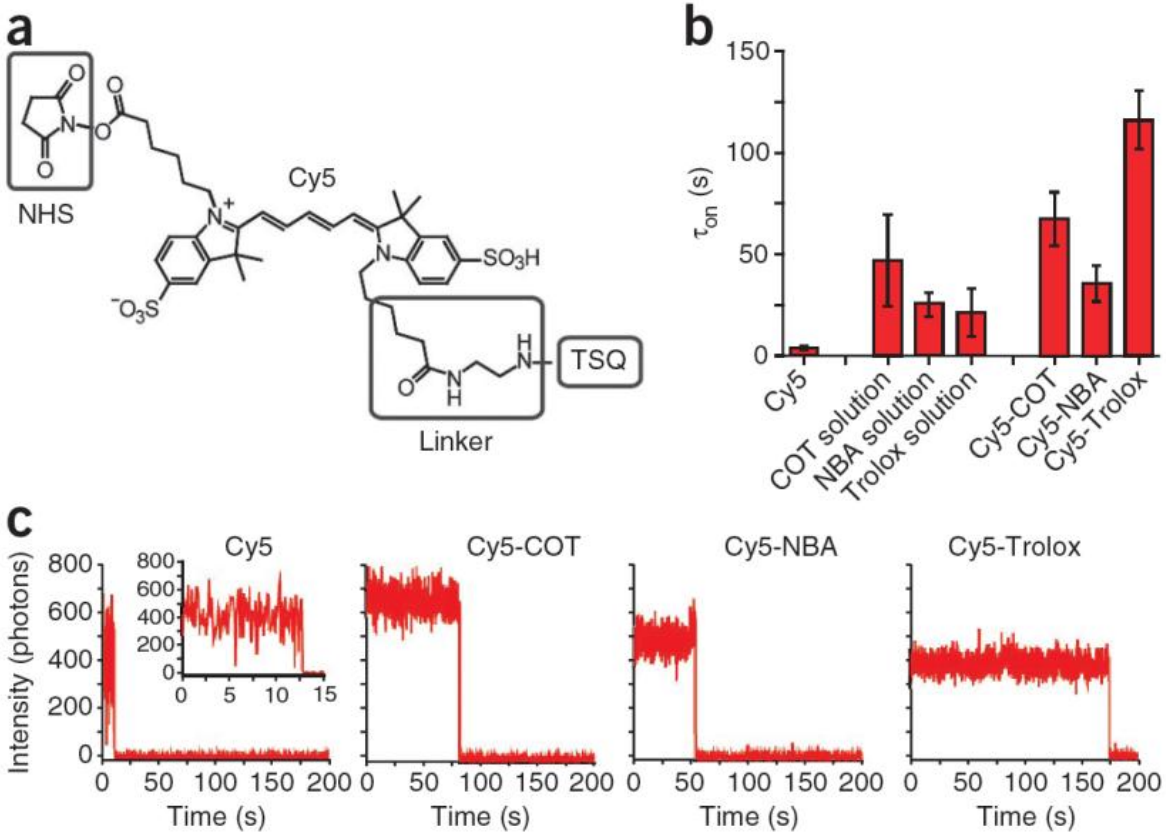
$[M+H]^+$ 1017.4, found: 1017.7



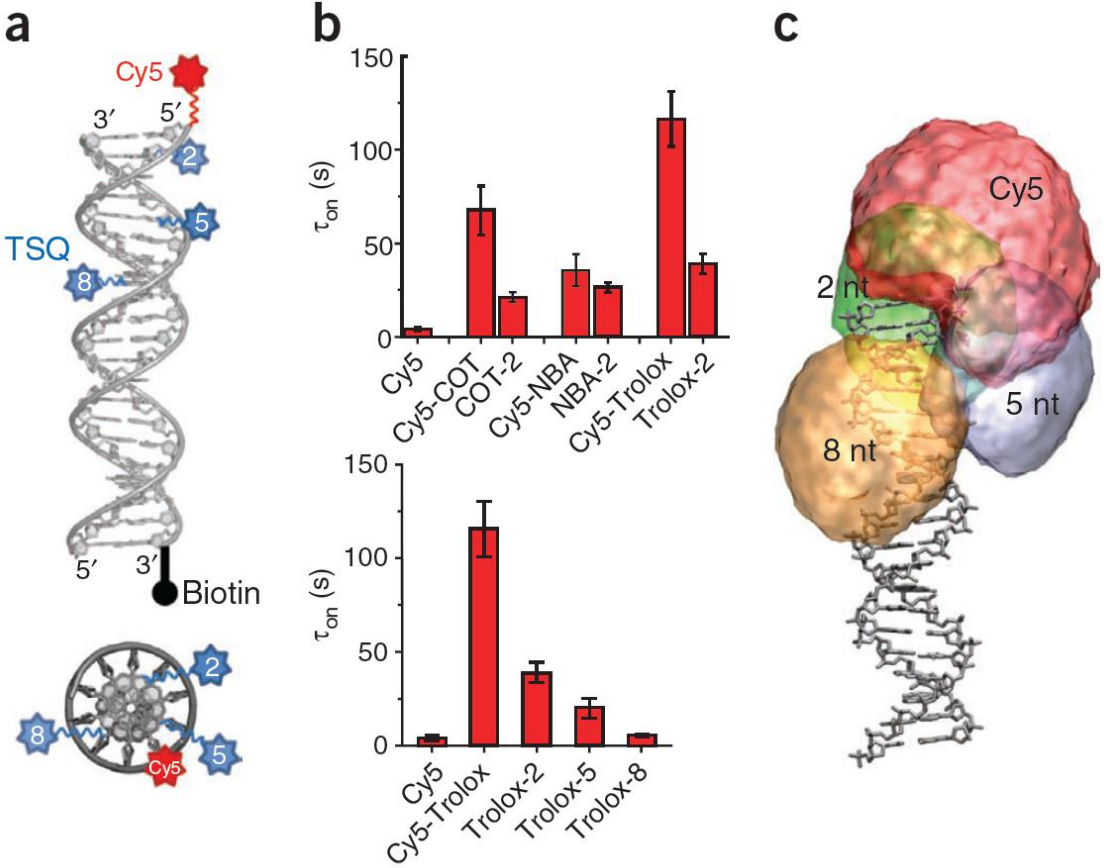
Cyanine fluorophore derivatives



Single molecule traces



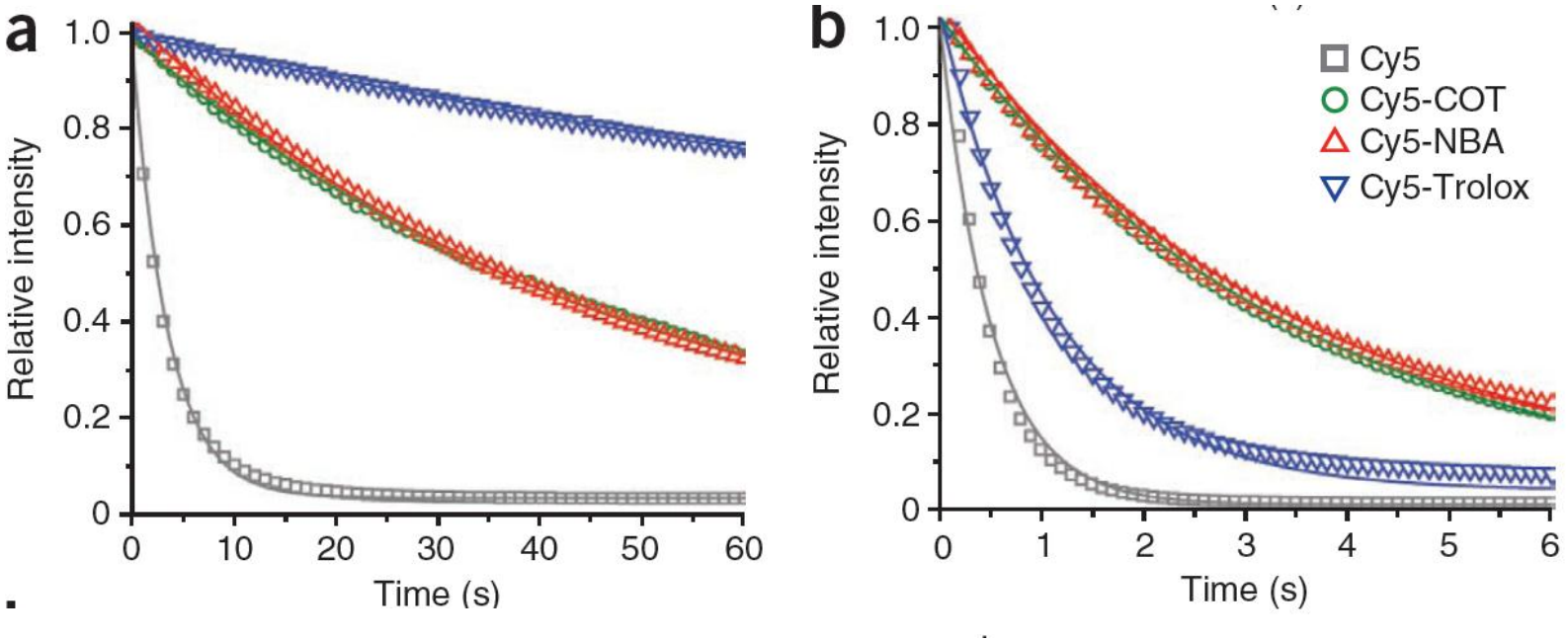
distance control



With Oxygen scavenging system

+PCDPCA

-PCDPCA



on the living cell