Supplementary materials for:

Facile Synthesis of Monofunctional Pentamethine Carbocyanine Fluorophores

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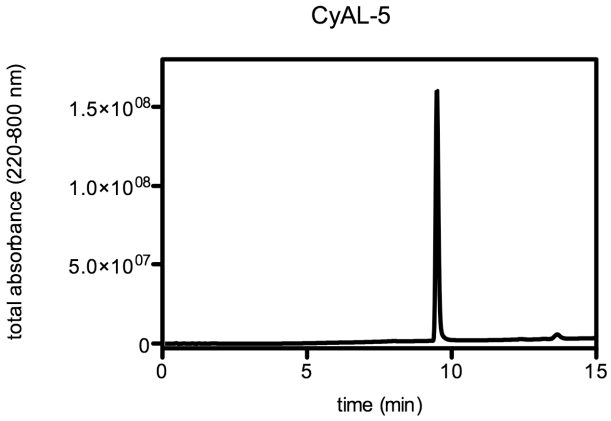
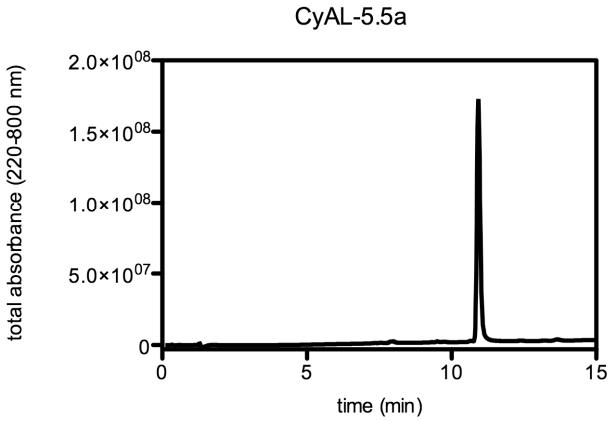




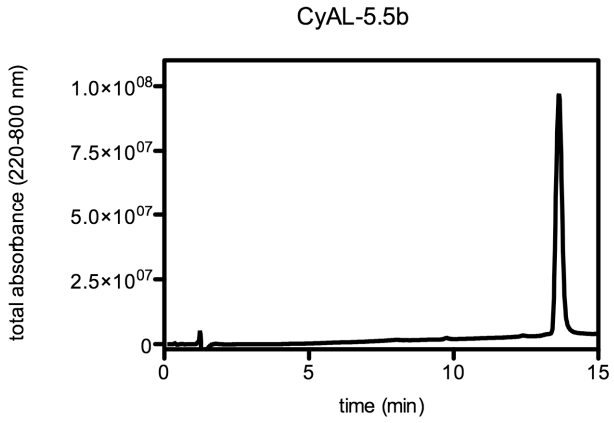
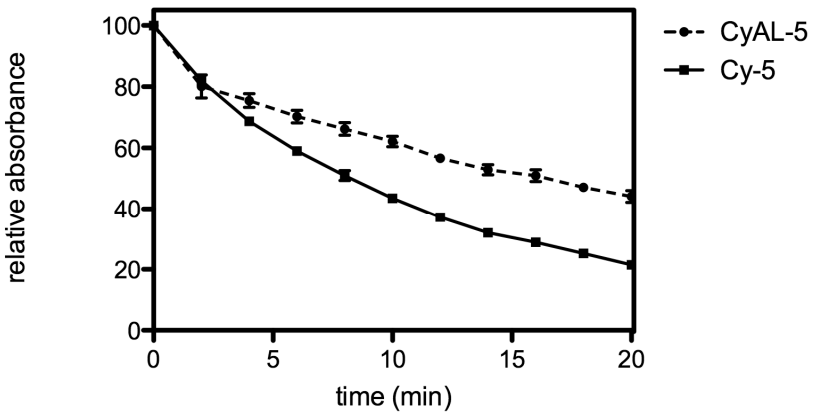


Figure S1. HPLC purity traces of CyAL-5 (*top*), CyAL-5.5$_a$ (*middle*), and CyAL-5.5$_b$ (*bottom*). The dyes were eluted using a gradient of 0-100% buffer B over 15 min and a flow rate of 0.3 mL/min. Buffer A is water with 0.1% trifluoroacetic acid, buffer B is acetonitrile with 10% water and 0.1% trifluoroacetic acid.

Photostability Studies.

The relative photostabilities of the new symmetric carboxylic acid modified fluorophores were assessed by comparison to analogous fluorophores: Cy-5 was used as a standard for CyAL-5 and Cy-5.5 was used as a standard for CyAL-5.5$_a$ and CyAL-5.5$_b$. Solutions of fluorophores (1-2 uM) in PBS (for Cy-5 and CyAL-5) or PBS with 10% DMSO (for Cy-5.5, CyAL-5.5$_a$, and CyAL-5.5$_b$) were irridiated with a 600 W high power sodium lamp and the photodecomposition was measured at 2 min intervals by UV/vis spectroscopy.



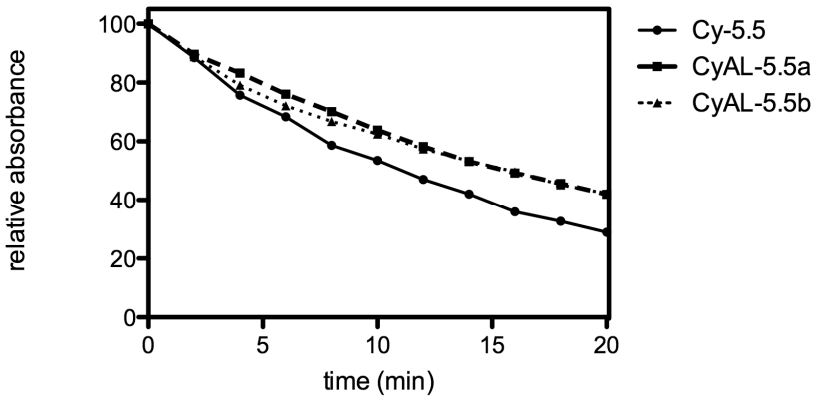


Figure S2. Traces (at the abs_{max} for each fluorophore) showing fluorophore photostability. Photostabilities of Cy-5 (solid line) and CyAL-5 (dashed line); top graph, and Cy-5.5 (solid line), CyAL-5.5_a (dashed line), and CyAL-5.5_b (dotted line); bottom graph, are shown. All data sets were performed in triplicate with error bars showing the standard deviation. All three dyes (CyAL-5, CyAL-5.5_a, and CyAL-5.5_b) show improved photostability in comparison to the standards.