Monofunctional Carbocyanine Dyes for Bio- and Bioorthogonal Conjugation

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SUPPORTING INFORMATION

Table of Contents

Figure S1. HPLC traces present indicating the purity of the dye analogues.

Figure S2. Photostability of CyAM-5 acid in PBS buffer (pH 7.0).

Figure S3. Fluorescent imaging of CHO cells labeled with CyAM-5 alkyne and controls.



Figure S1. HPLC traces present indicating the purity of the Cy5 analogues. (a) CyAM-5 acid; (b) CyAM-5 azide; (c) CyAM-5 alkyne. HPLC was conducted on a Waters 2695 separations module equipped with a Waters 2996 photodiode array detector with gradient from 0 to 100% buffer B over 15 min.



Figure S2. Photostability of CyAM-5 acid and the standard Cy5 in PBS buffer (pH 7.0). After 60 minutes of irradiation at 620 nm, 42% of CyAM-5 acid (18 nM) is photobleached, whereas 54% of Cy5 at the comparable absorption is photobleached. Thus, CyAM-5 acid has 28% better photostability than Cy5.





Figure S3. Fluorescent imaging of CHO cells labeled with CyAM-5 alkyne and controls. Cells bearing azido-sugar (lane 1~4) and regular cells (lane 5~8) are labeled by CyAM-5 alkyne in the presence of both Cu(I) and ligand TBTA (lane 1,5), Cu(I) alone (lane 2,6), TBTA alone (lane 3,7), or neither of the two (lane 4,8). Cell nuclei stained with DAPI, fluorescence of labeled CyAM-5 alkyne and merge figures of the two above are shown in column *left, middle* and *right*, respectively. Details are listed in *experimental procedures*.