Fluorophores for live cell imaging of AGT fusion proteins across the visible spectrum

Antje Keppler¹, Claudio Arrivoli², Lucia Sironi³, and Jan Ellenberg¹
¹European Molecular Biology Laboratory (EMBL), Heidelberg, Germany and ²Institute of Chemical Sciences and Engineering, École Polytechnique Fédérale de Lausanne (EPFL), Lausanne, Switzerland

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Absorption and Emission Spectra

For benzylguanine diethylaminomethyl coumarin (BGDEAC) and BGCy5, absorption and emission spectra were acquired with a SpectraMax® photometer (Molecular Devices Sunnyvale, CA, USA). For BG Rhodamine Green (BG505), CP tetramethylrhodamine (CP-TMR), BGDy547 (a derivative of Cy™3), and BGDy647S (a derivative of Cy5), absorption spectra were acquired with a SmartSpec™ Plus photometer (Bio-Rad Laboratories, Hercules, CA, USA). Fluorescence emission spectra were acquired with a Sapphire™ 1 plate reader (Tecan Group Ltd., Männedorf, Switzerland). The absorption and emission spectra of BG diacetyl fluorescein (BGAF), BG Oregon Green 488 (BGOG), and BGCy3 have been published before (1).

Relative Quantum Yields

The quantum yields of the underivatized fluorophores of BGDy547, BGDy647S, and BGD2 (a derivative of Cy5) were determined as described previously (2). Cy3 (for BGDy547) and Cy5 (for BGDy647S and BGD2) were chosen as standard fluorophores due to their high structural and spectral similarity to these dyes (3). As the quantum yields of Cy3 and Cy5 had been determined in phosphate-buffered saline (PBS), all dyes were solved in PBS (pH 7.4). Their absorbance was determined on a UV-VIS Cary 100 spectrometer (Varian, Palo Alto, CA, USA), and their fluorescence emission spectra was determined on a Cary Eclipse fluorescence spectrometer. The relative quantum yields for BG- and AGT-bound fluorophores with respect to the underivatized fluorophore (relative quantum yield set as 1) are listed in Table 1.

REFERENCES

Supplementary Figure S2. Spectra and structure of benzylguanine Rhodamine Green (BG505). Normalized absorption (Abs.) and emission (Em.) spectra of BG505 coupled to O\textsuperscript{6} alkylguanine-DNA alkyltransferase (AGT) at pH 7.5. The emission spectrum was acquired at an absorption wavelength of 450 nm.

Supplementary Figure S3. Spectra and structure of CP tetramethylrhodamine (CP-TMR). Normalized absorption (Abs.) and emission (Em.) spectrum of CP-TMR coupled to O\textsuperscript{6} alkylguanine-DNA alkyltransferase (AGT) at pH 7.5. The emission spectrum was acquired at an absorption wavelength of 450 nm.
Supplementary Figure S4. Spectra and structure of BGDy547 (a derivative of Cy3). Normalized absorption (Abs.) and emission (Em.) spectrum of BGDy547 coupled to O6-alkylguanine-DNA alkyltransferase (AGT) at pH 7.5. The emission spectrum was acquired at an absorption wavelength of 520 nm.

Supplementary Figure S5. Spectra and structure of BGDy647S (a derivative of Cy5). Normalized absorption (Abs.) and emission (Em.) spectrum of BGDy647S coupled to O6-alkylguanine-DNA alkyltransferase (AGT) at pH 7.5. The emission spectrum was acquired at an absorption wavelength of 600 nm.
Supplementary Figure S7. (A) Labeling of endogenous O\(^6\)-alkylguanine-DNA alkyltransferase (AGT) in PtK\(_2\) cells with BGCy3 and (B) diffusion of microinjected excess dye (BGCy3) out of the cell over time.

(A) A PtK\(_2\) wild-type cell was microinjected with 30 \(\mu\)M BGCy3 and imaged as described, demonstrating that labeling of endogenous AGT in this cell-type is below the detection limit. (B) A PtK\(_2\) cell stably expressing AGT-histone 2B (H2B) was microinjected with 30 \(\mu\)M BGCy3. Already 10 min after injection, only the Cy3 dye covalently linked to AGT-H2B was still detectable inside the cell.

Supplementary Figure S6. Spectra and structure of BGCy5. Normalized absorption (Abs.) and emission (Em.) spectrum of BGCy5 coupled to O\(^6\)-alkylguanine-DNA alkyltransferase (AGT) at pH 7.2. The emission spectrum was acquired at an absorption wavelength of 610 nm.