Enhancing the Biocompatibility of Rhodamine Fluorescent Probes by Positional Isomerism Approach

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Fluorescence microscopy is an essential tool for understanding dynamic processes in living cells and organisms. However, many fluorescent probes for labeling cellular structures suffer from unspecific interactions and low cell permeability. We found that isomeric tuning could optimize rhodamine fluorescent probes' biocompatibility without affecting their photophysical properties. Later, we discovered that the neighboring group effect in novel isomer-4 rhodamines dramatically increases cell permeability of the rhodamine-based probes by stabilizing a fluorophore in a hydrophobic spiro-lactone state. However, 1st generation synthetic route to 4-carboxyrhodamines was based on Pd catalyzed fluorescein to rhodamine conversion, which limited structural diversity and had multiple protection and deprotection steps. Recently we have developed 2nd generation protecting group free synthesis route for 4-carboxyrhodamine class fluorescent dyes. This approach drastically reduces the number of synthesis steps, expands the achievable structural diversity, increases overall yields, and permits gram-scale synthesis of the dyes. By employing this synthetic route we synthesized and characterized a wide range of symmetrical and unsymmetrical 4-carboxyrhodamines covering the whole visible spectrum and targeted them to multiple structures in living cells – microtubules, DNA, actin, mitochondria, lysosomes, Halo-tagged and SNAP-tagged proteins.