Exploiting the nitrilotriacetic acid moiety for biolabeling with ultrastable perylene dyes

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Chemical and biological labeling is essential for the exploration of protein function, as fluorescent probes allow detection of molecular interactions, mobilities, and conformational changes. Especially fluorescence resonance energy transfer (FRET) has become an important tool to study conformational distributions and dynamics of biomacromolecules. This, however, requires the ability to introduce fluorescent reporters at specific sites. One of the most frequently used approaches for selective labeling of proteins is to introduce a non-native cysteine at a desired location, while the chromophore possesses a thiol reactive group. This strategy suffers from the disadvantage that the number and position of native cysteines are practically important for the structure and biological function of various proteins such as cysteine-based oxidoreductases, phosphatases, and proteases.

The most widely used genetically encoded tag is the polyhistidine tag, which usually consists of 2–6 consecutive histidine residues. It was originally developed for the purification of recombinant proteins by immobilized metal-affinity chromatography. Different modifications of this molecular recognition technique have been exploited, including target protein detection and protein structure studies. This strategy has been also utilized to introduce a chromophore containing a nitrilotriacetic acid (NTA) moiety into His-tagged proteins. All of the reported chromophores, however, suffered from the same drawback—a severe loss of the fluorescence upon binding of the paramagnetic nickel ion. When Ni\(^{2+}\) was complexed with the NTA moiety that was attached to commercial Cy3 and Cy5 dyes, the fluorescence quantum yield dropped by 75%. Although the quenching of the fluorescence is distance dependent, an 80% loss of the fluorescence of Atto-565 was measured, even when a longer spacer between the NTA moiety and the fluorophore was introduced.

Herein, we report a different NTA-modified fluorophore—water-soluble perylene(dicarboximide) dye, connected to a NTA unit, which in contrast to all the other examples reported before contains a much shorter spacer between the dye and the NTA (only two methylene groups). Remarkably, the photophysical properties of the chromophore remain unchanged upon Ni\(^{2+}\) binding. The new fluorescent reporter has relatively small molecular weight (1555 g/mol), which makes it a good candidate for labeling proteins at the same time avoiding limitations due to the large size of autofluorescent proteins or quantum dots.
bleaching and increased population of the nonfluorescent triplet state reduced the brightness per molecule. Similar saturation behavior was observed for Atto-565, which was bound to bovine serum albumin (BSA). In contrast, the molecular brightness of the PDI-NTA steadily increased up to an excitation power of 1.2 mW, reaching 120 kHz and supporting the high photostability of the perylene chromophore. At this high laser power, the tripped yield was only 11% compared to about 45% for Atto-565 bound to BSA. In the presence of Ni²⁺, the relative brightness of the perylene derivative was reduced to about 77%. Binding of the PDI-NTA-Ni²⁺ to the His tags of F₁-ATPase did not reduce the molecular brightness further but rather increased slightly.

In summary, we have presented the synthesis of a new water-soluble perylene(dicarboximide) dye functionalized with a nitrolotriacetic acid moiety. This chromophore combines the exceptional photophysical properties of the rylene(dicarboximide) dyes and a recognition unit for site-specific labeling of proteins. An important feature of the label is the unchanged emission of the dye upon complexion with nickel ions. Moreover, the suitability of the fluorophore for labeling His-tagged proteins was demonstrated by FCS. Due to its small size, its proven exceptional photostability and the possibility for site-specific attachment, the PDI-NTA offers great potential for the characterization of protein functions and interactions.

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Supporting Information Available: Detailed description of the synthesis, purification, and spectroscopic characterization of the compounds, and a description of the FCS experiments. This material is available free of charge via the Internet at http://pubs.acs.org.

References


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