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Cation- π lights up “Halo”

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TOC Graphic

Since its introduction by Dougherty in the 1990s, the significance of cation- π interaction(1) has been recognized in various aspects of chemistry and biology. For example, Dougherty's own work has clearly shown the importance of the cation- π interaction in neurotransmission, where cationic neurotransmitters (e.g., acetylcholine) are recognized by cell surface receptors with an aromatic box on the cognate receptor. Similar mechanisms have been described for readers of methylated histone proteins as well as bacterial phospholipases that selectively bind mammalian lipids. Furthermore, cation- π interaction has been reported to facilitate catalysis by stabilizing cationic transition states. Writing in a recent paper in Biochemistry, Zhang and coworkers revealed yet another exciting application of cation- π interaction, that is, to create fluorogenic fluorophores.(2)

Fluorescent labeling of a target molecule in a swamp of others is highly desirable for biological research. Even better, selective labeling reactions that induce strong fluorescence increases would allow direct visualization and potentially quantification of the target in live cells and organisms. Ideally, a labeling reaction should be fast and highly specific, with the labeling reagent being non-fluorescent and gaining fluorescence upon conjugation to the target molecule. Recent research in chemical biology has made great strides towards these goals.

Specificity toward a target protein can be achieved by introducing a recognition element, which could be an unnatural amino acid or a self-labeling protein tag.(3) Among these recognition elements, the self-labeling protein tags (SNAP, CLIP, HaloTag) enjoy the benefit of fast reaction, broad substrate scope and no need of unnatural amino acids. Excitingly, the HaloTag based labeling has been recently made to be fluorogenic. Since the initial report by Johnsson and coworkers in 2013,(4) several rhodamine-based substrates of HaloTag have

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been described,(5, 6) which elicit fluorescence emission via the spirolactone isomerization induced by conjugation to HaloTag (Figure 1a). The zwitterionic isomer displays much enhanced absorption (up to 21 fold), leading to much brighter fluorescence. Taking advantage of molecular rotors, Kool and coworkers described a different fluorogenic substrate of HaloTag, for which a 27 fold fluorescence increase was observed upon conjugation to the protein.(7) Specifically, a stilbene-based fluorophore (Figure 1b) was derivatized with a long chain chloride to enable facile conjugation to HaloTag. Once conjugated, these molecular rotors fit into the narrow channel of the protein, where bond rotations are restricted leading to strong fluorescence emission. In their recent Biochemistry paper, Zhang and coworkers reported yet another fluorogenic substrate of HaloTag, which is mechanistically distinct and gives an impressive 1,000 fold fluorescence increase upon protein conjugation. Interestingly, this dramatic fluorescence increase was found to originate from a cation- π interaction in the binding pocket of HaloTag (Figure 1c-e).

The Zhang lab sought to develop fluorogenic reporters of HaloTag with solvatochromic fluorophores. The team synthesized a number of HaloTag substrates based on the benzothiadiazole scaffold (e.g., P9 shown in Figure 1c). Fast conjugation to HaloTag relocates the fluorophores from bulk water to the binding pocket of the protein, a much less polar microenvironment where the solvatochromic fluorophores gains fluorescence emission. Although this mechanism has been well established for the design of turn-on fluorescence sensors, the 1000-fold fluorescence increase (Figure 1d) is rather unusual for solvatochromic fluorophores.(8) The secret of this impressive HaloTag-fluorophore pair was revealed by structural studies, which showed a Trp residue in close proximity of the chromophore. More specifically, the N, N-dimethylamino group of P9 is positioned right on top of the Trp ring (Figure 1e). Theoretical calculation shows a positive charge on the N, N-dimethylamino group of P9 in the excited state, which it is largely neutral in the ground state. This led the authors to hypothesize that the photoexcited state of the benzothiadiazole can be stabilized by the Trp through cation- π interaction. This stabilization is expected to better hold the excited chromophore in place, thereby minimizing the non-radiative decay through a twisted internal charge transfer mechanism. Indeed, time-resolved fluorescence measurement revealed the fluorescence lifetime of the P9-HaloTag conjugate is 100 times longer than that of P9 alone. The contribution of the cation- π interaction was further probed via incorporation of an unnatural amino acid: mutating the key Trp residue to 5-fluoro-L-Trp (5FW) significantly reduced the fluorescence intensity of the HaloTag-P9 conjugate. This result unequivocally confirms that the 1000 fold fluorescence increase is not purely due to the change of environment polarity of the solvatochromic chromophore P9. Rather, cation- π interaction contributes significantly to the fluorogenicity as 5FW is less electron rich and hence less favorable for forming cation- π interactions. The fluorogenicity of P9, together with the high specificity of HaloTag labeling, enabled live cell imaging of HaloTag labeled proteins under no-wash conditions. In addition, the authors show that the HaloTag-P9 pair allows quantification of HaloTag labeled proteins even at low nanomolar concentrations.

The newly discovered role of cation- π interaction in fluorogenicity is exciting. Although the discovery by Zhang and coworkers is not necessarily by design, it is intriguing to speculate how cation- π interactions can be rationally incorporated into the design of fluorescence reporters, which would certainly expand the toolbox for biological explorations.

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Figure 1.

Fluorogenic reporters of HaloTag. a) An example of rhodamine-based fluorescence reporter that acquires fluorescence via spirolactone isomerization; b) Fluorogenic reporters developed by Kool and coworkers based on molecular rotors; c) A solvatochromic dye-based reporter (P9) of HaloTag developed by Zhang and coworkers; d) Fluorescence emission of P9 in absence and presence of HaloTag showing 1,000 fold fluorescence increase; only

marginal fluorescence was observed with the non-reactive D106A mutant; e) Mechanistic views of the P9 fluorogenicity highlighting the importance of a cation- π interaction.

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