Editorial

HaloTag® Platform: From Proteomics to Cellular Analysis and Animal Imaging

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From *in vitro* proteomic protein analysis to in vivo animal models, various protein tagging systems are designed to provide useful functionalities. Commonly used affinity tags for protein purification and capture include GST, c-myc, metal ion affinity tag His6Tag, immuno-affinity FLAG tag, and maltose binding protein (MBP) tag [1-4]. For cellular imaging analysis, fluorescent proteins have served as markers for protein localization, translocation, interactions, and conformational change [5, 6]. However, in addition to the limited functionality, these protein fusion tags are further limited in that they cannot be used for *in vivo* animal models. Instead, bioluminescence, near-infrared (NIR) /IR800, Magnetic Resonance Imaging (MRI), positron emission tomography (PET), and single-photon emission tomography (SPECT) are used to detect protein markers *in vivo* [7, 8]. Due to limited capabilities for individual tags, studies involving multiple analytical methods often require the use of more than one tag and multiple genetic constructions.

HaloTag® technology provides an efficient way to use a single tag readily reconfigured to meet the needs of different applications, from *in vitro* protein purification to cellular protein complex analysis, and in animal models. SNAP-tag and CLIP-tag provide similar synthetic fluorophores with switchable spectroscopic properties to visualize biochemical activities in living cells [9, 10]. The HaloTag® technology possesses two distinct moieties: the HaloTag® reporter protein and the HaloTag ligand. The HaloTag reporter protein is fused to the N- or C-terminus of a protein of interest (POI). The reporter is a monomeric, 34 KDa engineered protein developed from a rare bacterial hydrolase. There is no endogenous equivalent in eukaryotic cells, and like most bacterial reporter/tag systems, it has extremely low background.

In this special issue, Dr. Encell *et al.* [11] describe the design and molecular evolution of the HaloTag® reporter system. Using directed mutagenesis, the binding kinetics was significantly improved for the initial version of the reporter HaloTag2 by opening up the chloroalkane binding pocket. Random mutagenesis was then used to further evolve HaloTag® together with its linker sequence into a more soluble and stable variant, HaloTag7, which is suitable for applications across mammalian and bacterial cells as well as *in vitro*. The chloroalkane ligand is the second component in the HaloTag® technology platform and it can covalently bind to HaloTag® protein. Chloroalkane ligands can carry many different functional groups including i) solid surface for protein immobilization and display that can be used for protein purification, interaction analysis and display of antigens; and ii) fluorescent dyes for protein quantification, gel labeling, and cellular imaging analysis. Also in this special edition, Drs. Urh and Rosenberg [12] provide a comprehensive review of the HaloTag® technology platform and its wide range of applications both *in vitro* and *in vivo*.

By comparing the performance of direct protein purification using different tags with 20 randomly selected ORFs encoded in the genome of *S. pneumonia,* Peterson and Kwon [13] demonstrated that HaloTag significantly enhances expression and solubility of target proteins. Furthermore, they presented a successful case study of using a HaloTag® fusion protein to identify protein-protein and protein-DNA interactions in *Y. pestis* Type 3 secretion system.

Unlike other fluorescence protein reporters where a separate construct needs to be made for each "color" of fluorescence, HaloTag® and HaloTag®-POI fusions are not fluorescent until the addition of a fluorescent HaloTag ligand. This important distinction allows for spatial and temporal control of labeling, and hence renders HaloTag an ideal technology for cellular imaging [14]. Ai *et al.* [15] exploited the advantages of the unique property of ligand interchangeability and demonstrated the localization and fate of human Proprotein Convertase Subtilisin-like Kexin type 9 (PCSK9), a protein important in regulating circulating low density lipoprotein-cholesterol (LDL-c) levels.

After imaging confirmation for proper localization and pharmacological behavior, the same construct can also be used for protein pull-down analysis for novel protein complex study using HaloLink resin. Indeed Ose *et al.* [16] demonstrated that galectin-1 and galectin-3 physically interact with ectopically expressed PCDH24- HaloTag® in HCT116 cells using a HaloTag® pull-down assay. The study supports a model that the galectin-anchoring activity of PCDH24 leads to the suppression of β-catenin signaling by

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the localization of β-catenin at the plasma membrane in PCDH24-expressing HCT116 colon cancer cells.

The irreversible, covalent bond formed between HaloTag ligand and reporter protein is in stoichiometric (1:1) ratio. Fluorescence intensities of protein bands following SDS-PAGE likely reflect the relative levels of HaloTag proteins after cell lysis. Oshima *et al.* [17] combined the advantages of HaloTag and the Dual Luciferase Assay to quantitatively examine splice variants (i.e., unspliced, aberrantly spliced, and correctly) of mRNA products to determine relative splicing efficiencies with and without mutations represented in a case of chronic granulomatous disease that was caused by a G→C mutation at position +5 in the 5'-splice donor site of intron 5 of the *CYBB* gene.

The advantages of the multifunctional nature of HaloTag® system do not stop at translating *in vitro* proteomics into cellular analysis. Recently, several papers have clearly demonstrated its utility in animal imaging using HaloTag MRI and PET ligands [18, 19]. Tseng *et al.* [20] spatially evaluated the in vivo application using a HaloTag® HCT116 tumor cell line overexpressing xenograft model and showed that flourophore-conjugated ligands are well tolerated after parenteral injection and exhibit enhanced tumor uptake for non-invasive *in vivo* imaging.

Recent studies from Dr. Crew's lab have provided an exciting new application of the HaloTag technology wherein labeling HaloTag® proteins with low-molecular-weight hydrophobic molecules (HyT) lead to targeted proteasomal degradation [21]. With tools for targeted genome engineering, we can i) integrate HaloTag® into endogenous genes of interest and study the localization of POI at endogenous levels using a variety of HaloTag® fluorescence ligands at cellular level; ii) pull down HaloTag®-POI using HaloTag® resin for protein complex analysis; and iii) apply HyT to knock down HaloTag®-POI at physiological level for functional analysis. The same concept can be applied to in vivo animal models by generating knock in HaloTag® mice. Development of HaloTag®-MRI and HaloTag®-PET ligands in concert with HyT will provide researchers integrated tools for studying drug targets in both cellular and animal models.

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