Available online at www.sciencedirect.com

SciVerse ScienceDirect

Current Opinion in Chemical Biology

Chemical tags: inspiration for advanced imaging techniques

Zhixing Chen, Virginia W Cornish and Wei Min

This review summarizes recent applications of chemical tags in conjunction with advanced bio-imaging techniques including single-molecule fluorescence, spatiotemporally resolved ensemble microscopy techniques, and imaging modalities beyond fluorescence. We aim to illustrate the unique advantages of chemical tags in facilitating contemporary microscopy to address biological problems that are difficult or near impossible to approach otherwise. We hope our review will inspire more innovative applications enabled by the mingling of these two growing fields.

Addresses
Department of Chemistry, Columbia University, New York, NY 10027, USA

Corresponding authors: Cornish, Virginia W (vc114@columbia.edu) and Min, Wei (wm2256@columbia.edu)

Current Opinion in Chemical Biology 2013, **17**:637–643

This review comes from a themed issue on **Molecular imaging**

Edited by **James Chen** and **Kazuya Kikuchi**

For a complete overview see the Issue and the Editorial

Available online 12th June 2013

1367-5931/$ – see front matter, Published by Elsevier Ltd.

http://dx.doi.org/10.1016/j.cbpa.2013.05.018

Introduction

Advances in microscopy have tremendously expanded our knowledge of biological processes at the microscopic level. The achievements therein are the result of close collaborations between physicists/engineers who build the imaging instruments and chemists/biochemists who design the corresponding probe molecules. One classic example representing this trend is the use of GFP to visualize specific proteins within living organisms by fluorescence microscopy [1]. Recent developments in more advanced imaging schemes (e.g. single-molecule fluorescence imaging, fluorescence lifetime imaging, triplet-state lifetime imaging, luminescence imaging, vibrational absorption imaging or magnetic resonance imaging) have emerged as next-generation tools to unravel complex biological processes in space and time from particular vantage points. In contrast to genetically encodable fluorescent proteins, the probes for these advanced imaging modalities, however, generally lack biocompatible targeting strategies to specific biomolecules. Since proteins are the most diversified functional biomolecules, protein-specific targeting capability, if achievable, will tremendously enrich the applications of corresponding imaging methods.

Chemical tags have emerged as a new generation protein labeling strategy compatible with live cells. Chemical tags are composed of a defined polypeptide sequence that is fused to a protein of interest, and which can be subsequently modified with a chemical reagent, such as an appropriately derivatized fluorescent dye. The first chemical tag, FlAsH, was invented in 1998 by the Tsien lab [2]. Since then, several commonly used chemical tags have been developed, including self-labeling FlAsH/ReAsH [3], SNAP/CLIP tag [4,5], TMP-tag [6], HaloTag [7], β-lactamase tag [8q] and enzyme-mediated labeling methods based on lipoic acid ligase [9]. Methodologically, chemists have used a variety of strategies to engineer and optimize chemical tags, including directed evolution [10], proximity-induced reactivity [11] and pro-drug loading [9,12]. With efforts from many research groups, chemical tags have reached a relatively mature stage, and the question has shifted from 'How to label' to 'What to label with', as discussed in several recent review articles [13••,14••,15]. In our opinion, the most powerful feature of chemical tags, compared to the classic fluorescent proteins, is the rendered chemical diversity in the label/reporter moiety. We discuss in this present review how this rendered chemical diversity perfectly matches this feature perfectly matches the demand of protein-specific imaging for a variety of advanced imaging methods.

In this review, we discuss the selected works that use chemical tags in combination with bio-imaging schemes beyond traditional fluorescence, such as wide-field or confocal microscopy. Reminiscent to the revolutionizing role of GFP to fluorescence microscopy, we highlight the bridging role of chemical tags that renders targeted protein specificity in modern advanced microscopies. And we also demonstrate the advantage of chemical tags in obtaining new and valuable information that would be difficult to collect otherwise (Table 1).

Chemical tag-enabled imaging techniques based on single-molecule fluorescence

Single-molecule fluorescence imaging techniques have brought considerable excitement to biological research. These techniques enable characterization of biomolecules on the individual level, providing complementary data to that obtained from ensemble experiments. Because it only detects one molecule, the single-molecule fluorescence assay is technically demanding and requires high-photon-output fluorophores. A typical fluorescent protein molecule can emit roughly 4×10^5 photons before photobleaching [16], while the best organic dye molecules have a typical photon output on the order of 10^6 to 10^8 [17]. Therefore, dye molecules conjugated with chemical tags provide high

Table 1

Implementations of chemical tags in advanced microscopy discussed in this review

Imaging technique	Detected signal	Representative tag/probe	Significance	Ref.
PALM; STORM	Precise location of single-molecule fluorophores	Halo/azido DCDHF; TMP/Atto655	Reconstructed super-resolution images	[21,23, 26–28,29•]
Single-molecule tracking	Precise single-molecule trajectories	SNAP/Dy547; Halo/Alexa488	Dynamic information of target protein	[30–32]
CosMos	Colocalization of multi-color single-molecule fluorescence	TMP/Cy5; SNAP/DY549	Mechanistic analysis of macromolecular machines	[33••]
STED	Peripherally depleted fluorescence	Halo/Atto655; SNAP/SiR	Super-resolution imaging	[29•,37–40]
FLIM	Fluorescence lifetime	TMP/Cy3	Indicative of protein-specific micro-environment	[42•]
Triplet imaging	Triplet-state lifetime	SNAP/TMR	Indicative of cellular oxygen consumption	[43]
TR-FRET/LRET	Time-resolved luminescence resonance energy transfer	SNAP/K (europium cryptate); TMP/Lumi4 (terbium chelate)	Background-free detection of FRET for protein–protein interaction	[44,45]
OLID-FRET	Modulated donor fluorescence due to acceptor photoswitching	SNAP/NitroBIPS; SNAP/Cy3–NISO	Sensitive detection of FRET	[48,49]
EM	Electron beam	FlAsH–ReAsH	Protein-specific EM contrast	[3]
Infrared near-field microscopy	Infrared absorption	ACP/Alexa 488	Photobleaching-free	[51]
PET	Gamma ray from positron emission	Halo/^{64}Cu NOTA	*In Vivo* imaging of whole animal	[52]
MRI	Nuclear magnetic resonance	Halo/2CHTGd (gadolinium chelate)	Increased MRI sensitivity with protein-specificity	[53•]

photon budgets, more precise localization, longer observation time and a higher signal-to-noise ratio. These merits make chemical tags excellent tools for the the study of proteins by single-molecule fluorescence.

Single-molecule fluorescence detection enables the reconstruction of sub-wavelength resolution images by two fundamentally similar approaches: PALM (photoactivation localization microscopy) [18,19] and STORM (stochastic optical reconstruction microscopy) (Figure 1a) [20]. In PALM, target proteins are labeled with photoactivatable fluorophores, which are then photo-activated sparsely and repeatedly, allowing the record of a collection of single-molecule resolved images. The fluorophores are finally localized to a precise location using software and the super-resolution image is generated. Compared to the photoactivatable fluorescent proteins, chemical tags allow more accurate localization due to the larger number of detected photons. From a chemical point of view, chemical tag-based labeling methods provide diverse photo-chemical strategies toward dye photoactivation. In 2010, the Moerner group demonstrated the first example of live bacteria PALM imaging of a labeled target protein using the chemical tag, HaloTag/azido DCDHF conjugate [21]. Azido DCDHF has an extraordinary high quantum yield of photoactivation under UV exposure [22]. Therefore, a low-intensity UV light source can be used, reducing the UV-induced damage to living cells. More recently, the Johnsson group utilized a caged rhodamine derivative as an alternative probe for PALM imaging in conjunction with the SNAP-tag [23]. Similar to PALM, STORM takes advantage of the reversible photoswitching of fluorescent dyes. It has recently been shown that photoswitching is a rather universal process for a wide spectrum of organic dyes, especially rhodamines, cyanines and oxazines [24,25]. Live cell dSTORM (direct STORM) imaging of labeled intracellular protein H2B was demonstrated using a TMP–Atto655 conjugate, taking advantage of the photoswitching behavior observed in the presence of cellular oxygen and reductants [26]. Several alternative chemical tag/dye combinations have been successfully applied to live cell dSTORM [27,28], with a noteworthy example being the newly developed SNAP-tag/NIR fluorophore silicon–rhodamine [29•]. With the growing availability of PALM/STORM microscope and chemical tag-dye conjugates, we expect super-resolution imaging to become a routine protocol for live cell studies in the near future.

The high-photon output of synthetic fluorophores could further enable prolonged sub-resolution tracking of single proteins with high temporal-resolution inside live cells (Figure 1b). Appelhans *et al.* observed single-molecule diffusion behavior of mitochondrial proteins using HaloTag-rhodamine labels [30]. Benke *et al.* later reported dual-color single-molecule tracking of cellular proteins using multiple chemical tags [31]. In pursuit of brighter and more photostable material, Liu *et al.* reported a targeting strategy of quantum dots that combines both