Max Planck Institute for Biophysical Chemistry

Research News

April 12, 2010

Captivated: in the cage, in the cell

Max Planck scientists invent and apply novel class of masked fluorescent dyes

In life sciences, the term “caged” is often used for compounds whose function is temporally hampered by the chemical transformation of the initially active substances. However, the original properties can be restored by “uncaging”. UV or visible light is often used for the uncaging, as it may be applied non-invasively, i.e. without cellular damage, with high spatial and temporal precision.

Caged fluorescent dyes are of great interest for biological imaging because they may be used for protein tracking, multi color applications, optical nanoscopy and beyond. They are kept in a non-fluorescent state by incorporating a labile chemical group. This photosensitive masking group or ”molecular cage” may be destroyed to re-establish the fluorescent state. *o*-Nitrobenzyl residues are frequently utilized as the masking groups. However, the complex synthesis and unwanted by-products liberated during the photolysis limit the use of these and other bulky caging groups.

To overcome these drawbacks, Vladimir Belov and colleagues from the Department of NanoBiophotonics at the Max Planck Institute for Biophysical Chemistry in Göttingen invented and studied a novel class of caged compounds called Rhodamines NN, with an incorporated 2-diazoketone (COC**NN**) caging group (Figure 1a).[1,2]

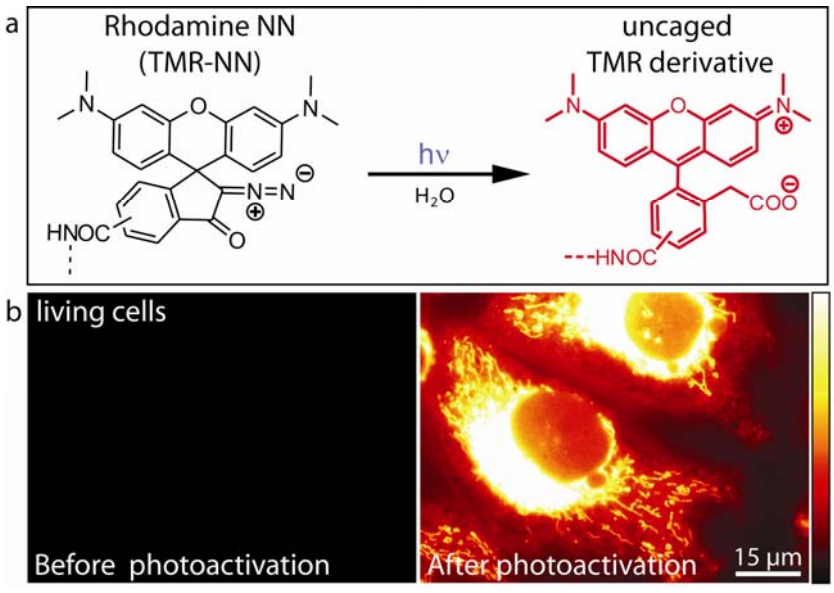

Figure 1. Structure and uncaging reaction of Rhodamine NN. (a) The nonfluorescent caged compound TMR-NN (left) is uncaged upon irradiation with UV light (≤ 420 nm), liberating a brightly fluorescent (uncaged) TMR derivative (right). (b) TMR-NN penetrates cellular membranes and can be used for imaging structures not only in fixed but also in living cells. Upon irradiation with low intensity UV light, the fluorescence signal increases by a factor of >40. *(Picture: Belov, Wurm / MPIbpc)*

This very simple and small chemical group is the core element of a new class of masked fluorescent dyes with remarkable properties. The rhodamines NN may be easily prepared and conjugated with biomolecules. Upon irradiation with UV light (at wavelengths ≤ 420 nm), they undergo rapid uncaging to form highly fluorescent derivatives and liberate only nontoxic nitrogen. Moreover, they may be photoactivated in aqueous buffers as well as in various embedding media normally used in imaging applications.

The novel caged rhodamines may be used in microscopy either as single labels, or in combination with conventional fluorescent dyes and photoswitchable rhodamine spiroamides. For example, a set consisting of Rhodamine NN, photochromic spiroamide of “Rhodamine S” and conventional (uncaged) *N,N,N′,N′*-Tetramethylrhodamine enabled the stepwise activation and detection of several fluorescent markers using only one excitation source and one detection channel. Based on these three fluorophores with very similar absorption and emission spectra, a monochromatic multi-label imaging scheme with low cross-talk was realized (Figure 2a). Moreover, the new caged dyes may be used in optical nanoscopy, e. g. for sub-diffraction imaging by single molecule switching (Figure 2b).

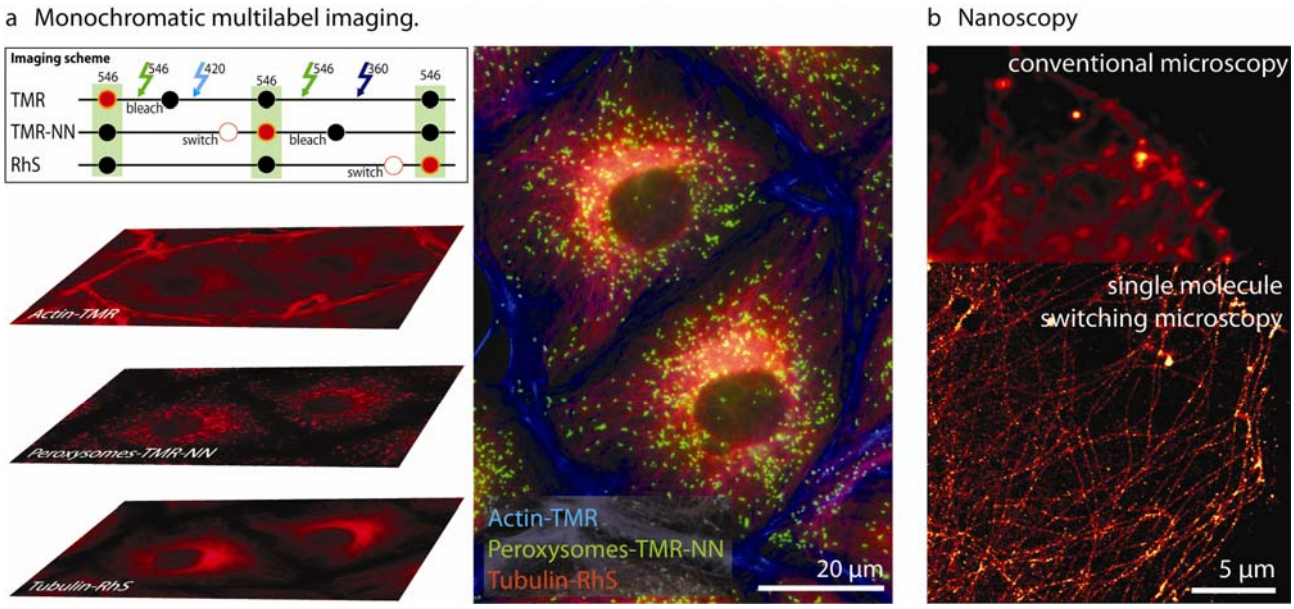

Figure 2. Application of the new caged compounds. (a) Three fluorophores with very similar absorption and emission spectra but different switching characteristics allow new multi-label and monochromatic imaging schemes. For this purpose, the actin cytoskeleton was labeled with conventional TMR fluorescent dye; the peroyxsomes were immunolabeled with caged TMR-NN and the tubulin cytoskeleton was immunostained with switchable Rhodamine S. The different structures can be distinguished and imaged by stepwise activation and bleaching of the individual fluorophores. (b) The new caged fluorescent dyes may be used in optical nanoscopy, e. g. in sub-diffraction imaging with single molecule switching. A conventional microscopy image of the tubulin cytoskeleton and its sub-diffraction representation are shown. *(Picture: Belov, Wurm / MPIbpc)*

In summary, the novel caging procedure can be applied to a wide variety of dyes (rhodamines, carbopyronines and probably other cationic dyes, e. g. triarylmethyl derivatives). Thus, the present approach can be extended to a larger set of (fluorescent) dyes and excitation sources.

Moreover, the compact structure and the very small size of the caging group facilitate the ability of Rhodamines NN to cross the membranes of living cells (see Figure 1b). In combination with the site specific labeling protocols, as realized for the Halo- and SNAP-tags, the availability of the new cell-permeable and caged Rhodamines NN may extend the set of live cell labelling strategies based on binding the genetically encoded protein tags with organic fluorophores. The spatially restricted photoactivation of Rhodamines NN followed by tracking of the uncaged molecules will enable the measurements of molecular dynamics (e. g. diffusion parameters or flow velocities). The combination of Rhodamine NN with nanoscopic techniques (e. g. STED or single molecule switching followed by localization) is expected to provide additional information on subcellular structures. Moreover, measurements of the fluorescence resonance energy transfer (FRET) signal of the newly uncaged fluorescent areas located in the close proximity to the “permanent” colored regions are likely to reveal additional structural details for the relatively short distances of 1-10 nm. (*vb/cw*)

References:

[1] V. N. Belov, C. Wurm, V. P. Boyarskiy, S. Jakobs, and S. W. Hell (2010): Rhodamines NN: a novel class of caged fluorescent dyes. *Angew. Chem. Int. Ed. 122* | DOI: 10.1002/ange.201000150

[2] S. W. Hell, V. N. Belov, V. P. Boyarskiy, C. A. Wurm, S. Jakobs, and C. Geisler (Max-Planck-Innovation GmbH), PCT/EP/2009/006578 (10.9.2009).

Further information:

Dr. Vladimir N. Belov, Department of NanoBiophotonics and Chemistry Facility
Max Planck Institute for Biophysical Chemistry, Göttingen
Phone: +49 551 201-2510/-2530
E-Mail: vbelov@gwdg.de

Prof. Dr. Stefan W. Hell, Department of NanoBiophotonics
Max Planck Institute for Biophysical Chemistry, Göttingen
Phone: +49 551 201-2500/-2503
E-Mail: shell@gwdg.de

Dr. Christian Wurm, Department of NanoBiophotonics
Max Planck Institute for Biophysical Chemistry, Göttingen
Phone: +49 551 201-2646
E-Mail: cwurm@gwdg.de