

Nitrilotriacetate-Atto Dye Conjugates For The Fluorescence Detection Of Oligo-Histidine Tagged Proteins

Monika Bäuml¹, Emmanuel Guignet², Ruud Hovius², Roland Meier¹, Pierre Nording¹, Joachim Piguet², Alex Rück¹, Bernhard Schönenberger¹, Jean-Manuel Segura², Klaus Trummler¹, Horst Vogel², Michael Weber¹



¹ Sigma-Aldrich Switzerland, Industriestrasse 25, CH-9471 Buchs, ² Laboratory of Physical Chemistry of Polymers and Membranes, Ecole Polytechnique Fédérale de Lausanne, 1015 Lausanne, Switzerland

Summary

Chemical and biological labeling is fundamental for the elucidation of the functions of proteins within biochemical cellular networks. Fluorescent Ni-NTA conjugates (Fig. 1) have been used to detect oligo-histidine tagged proteins on blots, in gels or in solution. Recently, this approach has been extended to the detection and characterization of proteins in living cells¹. A major advantage of the labeling method is the flexibility to use the fluorophore best suited for the application. The binding characteristic of the NTA-probe to the His-tag is hardly affected by the nature of the fluorophore. Herein, we report the synthesis, characterization and application of Atto-dye labeled NTA conjugates. Due to their strong absorbance and high quantum yield, Atto dyes are optimal tools for modern fluorescent technologies even down to single molecule detection level.

Synthesis

After complexation of NTA-Lysine (N'-bis(carboxymethyl)-L-lysine) to Ni²⁺-ion, the compound (Figure 1, highlighted in yellow) was incubated with N-hydroxysuccinimidyl ester-activated Atto dye (e.g. Atto 488, Atto 550, Atto 647N) forming an amide-bond via the amino-group of the lysine-residue. Incubation was followed by a purification step using a silica column and recrystallization from methanol.

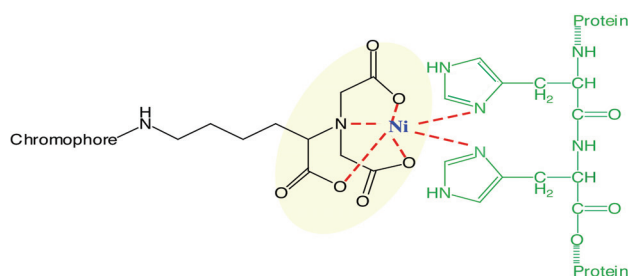


Figure 1: Interaction of a Ni-NTA-chromophore with an oligo-histidine tagged protein

Characterization of the Chromophore-NTA conjugate

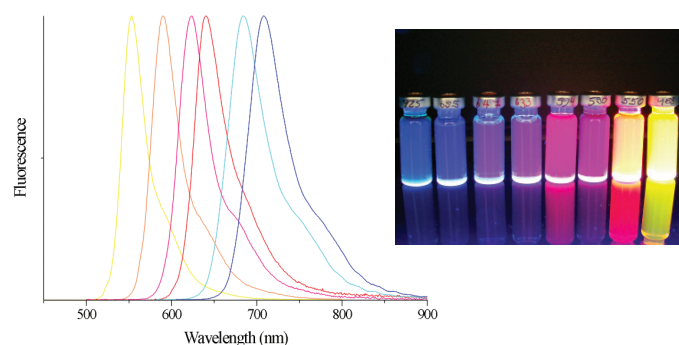


Figure 2: Normalized fluorescence spectra (left) of Atto dyes measured in ethanol. Series of Atto dyes (right) illuminated by UV-Light (312 nm). Atto dyes, characterized by their rigid molecular structures, are more photo-stable than some of the most widely used dyes which enable long-term measurements e.g. in biological systems.

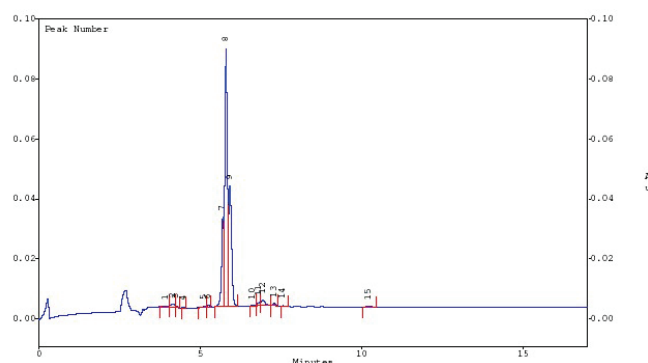


Figure 3: Capillary electropherogram of Atto 550-NTA. The graph exhibits a purity higher than 93%, where the three major peaks (peak numbers 7, 8 and 9) are attributed to the three existing isomers of Atto 550.

Applications

Application I: NTA-Atto 647N on SDS-PAGE Gel and Western Blot Membrane

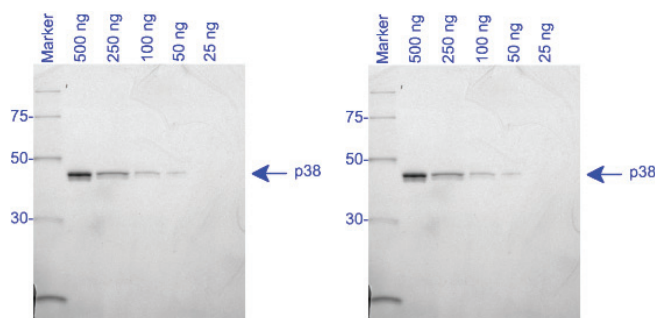


Figure 4: His-tagged p38-MAPK (500 ng – 25 ng) was separated on a 4-20% SDS-PAGE. The gel was fixed, washed and incubated with Ni-NTA-Atto 647N (1:1000) in the dark (left). The protein was transferred to a low-fluorescence PVDF-membrane by Western blot, blocked overnight, rinsed and incubated with Ni-NTA-Atto 647N (1:1000) in the dark (right). After washing, imaging was performed on both using a FLA-3000 Fuji® laser scanner with 633 nm excitation and a 675 nm emission filter.

Application II: Single molecule tracking using Atto 647N-NTA conjugates

An important application of the NTA-labeling method is the imaging of single fluorescently labeled molecules in living cells. In single molecule imaging the fluorescent labels are exposed to strong irradiation. Under these conditions most routine labels undergo photodestruction relatively rapidly, limiting the number of images that can be gathered.

Evaluation of the trajectories of single molecules

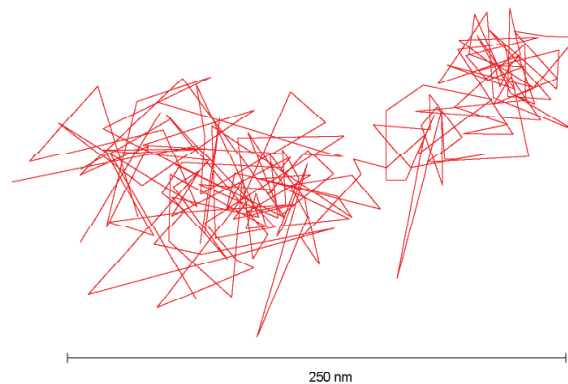


Figure 5: Trajectory of a single serotonin receptor, labeled with Atto 647N-NTA. Monitoring the trajectories of individual molecules over sufficiently long time periods allows the determination of individual molecule diffusion. The excellent properties of the Atto 647N enabled the acquisition of trajectory sequences up to about 100 frames.

Comparative experiment

The ionotropic serotonin receptor expressed in mammalian cells was labeled with either a Cy5 or an Atto 647N probe. Here, Atto 647N demonstrated its great potential for single molecule studies. Single molecule imaging revealed that the Atto 647N was more photostable than Cy5, having a two-fold lower bleaching rate (Figure 6).

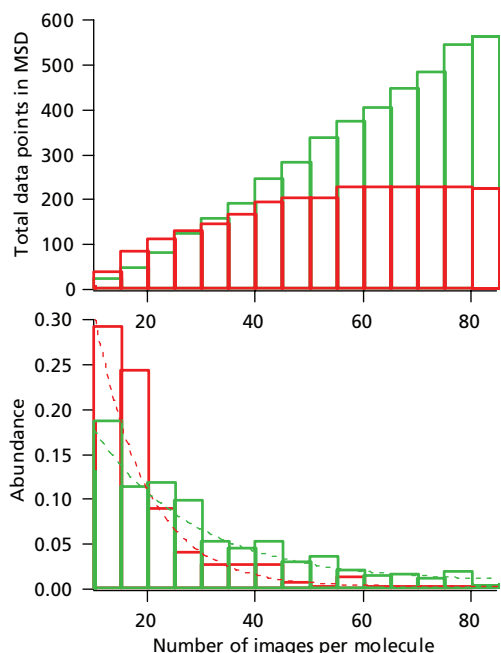


Figure 6: Atto 647N (green) shows higher photostability than Cy5 (red). The accumulative number of data points per molecule that is contributed by Cy5 labeled molecules levels at a value of about 200 for a maximal length of 55 frames (top). Trace length histograms of fluorescently labeled serotonin receptors show that Cy5 undergoes photobleaching twice as fast as Atto 647N (bottom).

Application III: Dot blot test proving activity of Atto 488-NTA conjugate



Atto 488-NTA
specifically bound
to polyhistidine

Blind-experiment:
Atto 488-NTA
has been removed
after washing

Figure 7: Dot blot experiments enable a fast detection of membrane-fixed proteins. Fluorescence image of Atto 488-NTA specifically bound to polyhistidine (top dot) on nitrocellulose membrane. Blind experiment (bottom dot) does not show fluorescence, which provides evidence of the absence of unspecific binding.

References

¹ Guignet et al. Nat. Biotech., Vol. 22, Nr. 4, 440 (2004)

Ordering Information

Cat. No.	Name	Pack Size
02175	NTA-Atto 647N	250 µg
94159	NTA-Atto 550	250 µg
39625	NTA-Atto 488	250 µg