Synthesis of A New Turn-On Fluorescent Dye – A Terephthalate Derivative for Staining Proteins

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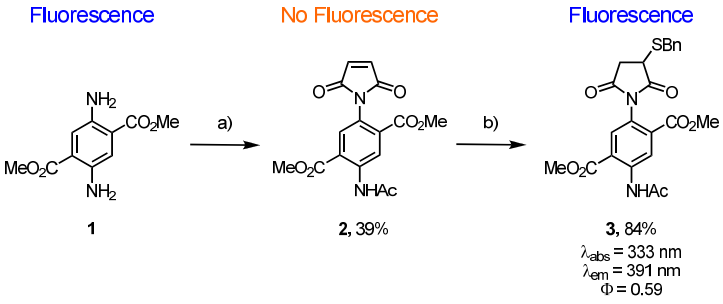
A diaminoterephthalate derivative with maleimide moiety was synthesized and used as fluorescence dye for sensing thiols. Whereas this "NiWa Blue" dye itself showed no emission, the conjugate addition of a thiol to the maleimide group turned on a fluorescence at about 390 nm when irradiating the dye at 330 nm. The fluorescent dye was used for labeling the protein recoverin. Conformational changes of recoverin in absence and presence of Ca^{2+} were followed by FRET studies.

Synthesis of the Maleimide Derivative

For the synthesis of maleimide **2**, the diaminoterephthalate **1** was converted with maleic anhydride to the ring opened maleic acid intermediate. The cyclization was performed with NaOAc and Ac_2O in 39% yield. No fluorescence emission was observed from the maleimide **2**. When treating a solution of compound **2** with mercaptans such as BnSH in CH_2Cl_2 and catalytic amount of NEt_3, a blue fluorescence turned on, therefore the compounds were titled "NiWa Blue".



Scheme 1. Reagents and conditions: a) 1. maleic anhydride, MTBE, 50°C, 2. NaOAc, Ac_2O, 140°C; b) BnSH, NEt_3, CH_2Cl_2.

Computational Chemistry

In order to shed light on the mechanistic details of the fluorescence properties of NiWa Blue, in particular on the process of fluorescence quenching of compound **2**, we preformed a theoretical analysis based on time-dependent density functional theory (TDDFT) using the program package Gaussian09.

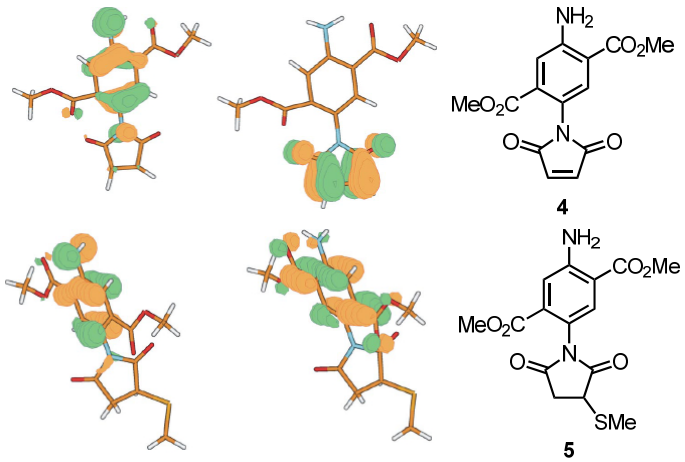


Figure 1. HOMO (left) and LUMO (right) of maleimide **4** and compound **5**. The lowest excited state of model compound **4** corresponds to an optically forbidden charge-transfer transition from the HOMO to the LUMO.

Biochemical Investigations

The neuronal sensor protein recoverin was labelled with "NiWa Blue" at Cys39. Recoverin undergoes significant conformational changes when calcium ions bind to its two functional calcium-binding sites (EF-hands). The emission spectra were recorded in the presence of either 500 µm $CaCl_2$ or 500 µm of Ca^{2+}-chelator EGTA and showed a maximum emission at 440 nm.
Furthermore, we excited tryptophan residues in the protein at 280 nm to detect a FRET-signal. Emission was carried out in the presence of either 163 nM or 1 mM Ca^{2+}_{free}, showing a strong dependence of the FRET-process on the Ca^{2+}-concentration.

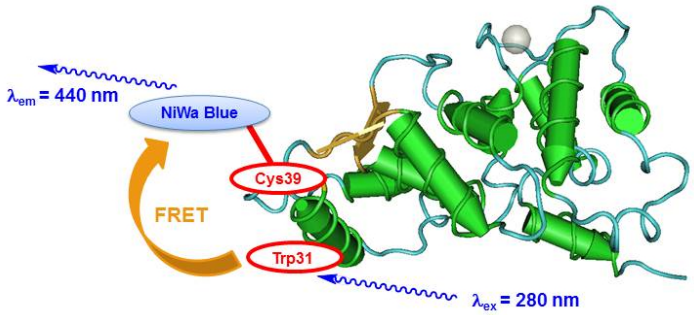


Figure 2. Recoverin labeled with NiWa Blue at Cys 39; FRET process from proximal Trp 31.

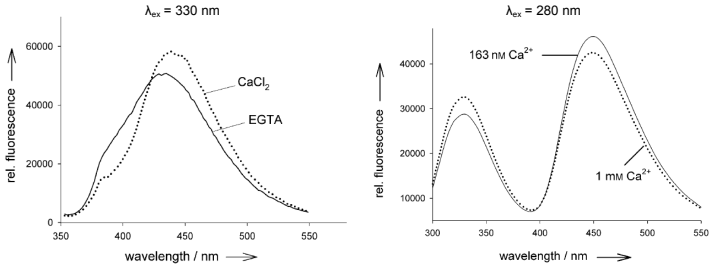


Figure 3. Fluorescence spectra of nonmyristoylated recoverin labeled with compound **2** in the presence of Ca^{2+} and without Ca^{2+} and FRET study by irritation at 280 nm.

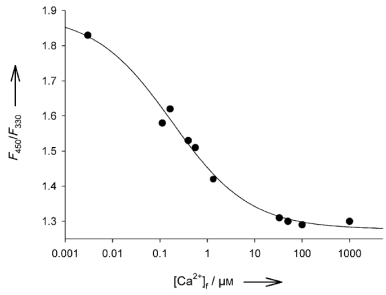


Figure 4. Ratios of fluorescence emission at 450 nm and 330 nm plotted as function of the free Ca^{2+}-concentration.

[1] N. Wache, C. Schröder, K.-W. Koch, J. Christoffers, *ChemBioChem* **2012**, *13*, 993−998.
[2] N. Wache, A. Scholten, T. Klüner, K.-W. Koch, J. Christoffers, *Eur. Org. J. Chem.* **2012**, in press.