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Synthesis and development of
Ca-ionselective fluorescent dyes for neuroscience

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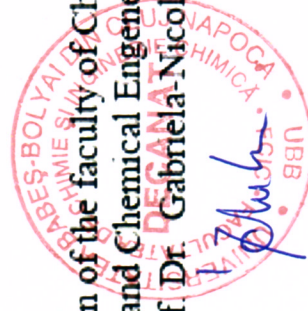
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Synthesis and development of Ca-ionselective fluorescent dyes for neuroscience

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The calcium ion has an essential role in numerous processes in the living organism, such as nervous system, cell signaling, muscle activity. In the central nervous system, the calcium ion transmits the neuronal activity as a secondary messenger. It was discovered earlier that the optical observation of the cellular Ca^{2+} ion concentration change in a neuron shows direct information about the neuronal activities. This led to the two-photon microscope technology, which is more and more spreading around the world in the field of neuron science, leading unbelievable breakthroughs in this field.

The calcium imaging fluorescent dye molecules are able to sense the free Ca^{2+} concentration in the intracellular space, following-up the time and space dependent changing of the activation potential.

The most frequently used ion chelating molecule is the 1,2-bis(*o*-aminophenoxy)ethane-*N,N,N',N'*-tetraacetic acid (BAPTA), which rapidly and selectively bound Ca^{2+} ions *e.g.* against Mg^{2+} ion. It was revealed that a covalent binding between BAPTA and a fluorescent dye controls significantly the emission intensity of the fluorescent part. Higher calcium concentration larger increase in intensity, so this concept proved to be an elegant and effective way to measure the relative concentration change in the cytoplasm. The emitted intensity is set by the so called photoinduced-electron-transfer (PET) phenomenon.

Our goal is to find a new and well tuned ion-sensitive fluorescent dye for human application with low and known toxicity profile, specifically developed for fix two-photon excitation and brightness detection.

We aimed:

- to increase the efficacy of PET effect;

- to fine tune the dissociation constant of the BAPTA part;
- to fine tune the fluorescent brightness under two-photon excitation;
- to lower the possible toxic effect.

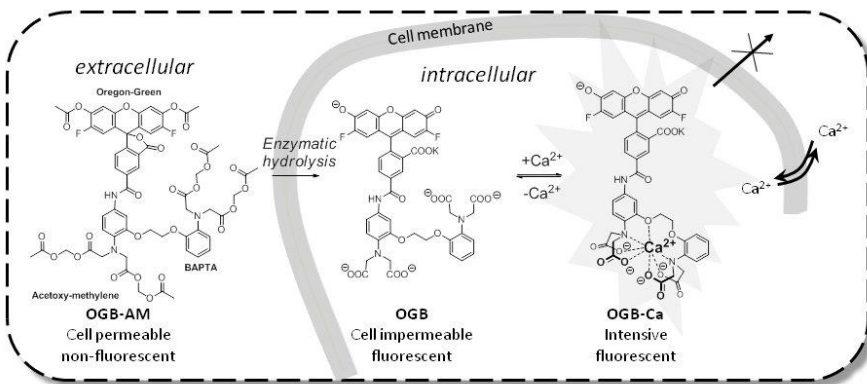


Fig. 1: A known example to describe the working mechanism of a Ca^{2+} ion sensitive fluorescent dye.

- [1] M. Collot, C. Loukou, A. J. Yakovlev, C. D. Wilms, *J. Am. Chem. Soc.* **2012**, 134, 14923–14931.
- [2] J. Yao, M. Yang, Y. Duan, *Chem. Rev.* **2014**, 114, 6130–6178.

SYNTHESIS AND DEVELOPMENT OF CALCIUM ION SELECTIVE FLUORESCENT DYES FOR NEUROSCIENCE

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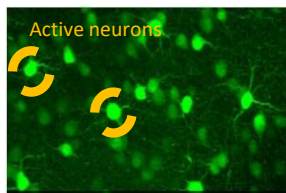
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Introduction

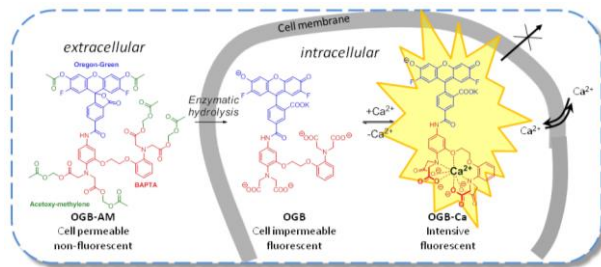


FEMTO 3D-AO microscope



In vivo fluorescence visualization of nervous system in a mouse

A second messenger calcium ion has a critical importance to neurons as it participates in the transmission of the depolarizing signal and contributes to synaptic activity. Optical in situ imaging of cellular calcium ion concentration provides direct information about the neuronal activities. Calcium sensitive fluorescent dyes composed of calcium ion chelating part binding to fluorescent indicator are able to sense the free calcium concentration in intracellular space, following-up the propagation of activation potential. This method led to the discovery two-photon microscopy technology spreading around the world in the field of neuroscience, leading unbelievable breakthroughs in this field.



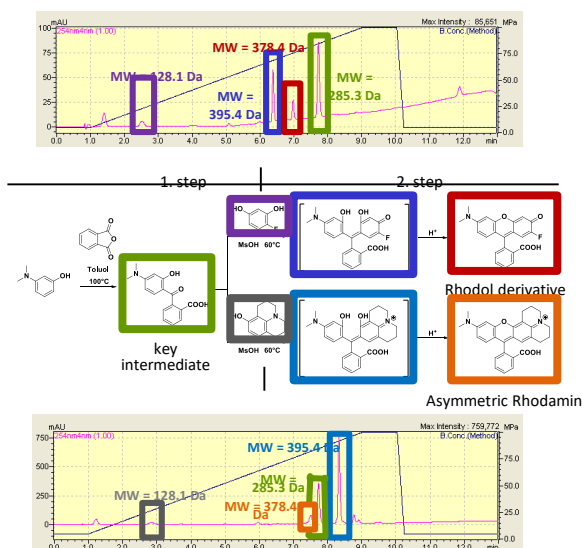
Calcium imaging by OGB-AM fluorescent dye

The most frequently used ion chelating structure is so called BAPTA, with high selectivity for calcium ion. It was revealed that a covalent bond between the BAPTA part and a fluorescent indicator controls significantly the overall intensity of the emission. Higher calcium concentration larger increase in intensity, which is regulated by the photoinduced electron transfer (PET) phenomenon, so this concept proved to be an elegant and effective method for measuring the relative concentration change in the cytoplasm.^{1,2}

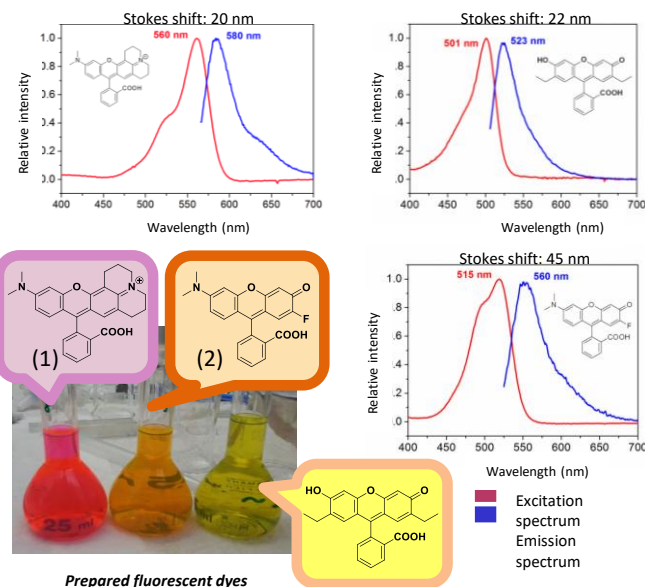
Our goal is to find a new and well tunable ion-sensitive fluorescent dye for human in vivo tests with low toxicity, developed for fix two-photon excitation and brightness detection.

Synthesis of fluorescent dyes

The reactions were monitored by HPLC-MS measurements which provides also the reaction mechanism.



Adsorption and fluorescent spectra of dyes synthesized



Summary

A known asymmetric rhodamine derivative (1) was synthesized successfully and an efficient synthetic route for a Rhodol compound (2) was worked out possessing excellent fluorescent properties.

Based on analytical results of compounds (1) and (2), it was proved that the UV absorption spectrum and the fluorescent properties (Stokes shift, quantum yield) of the new Rhodol-type compound can be valuable compounds for monitoring *in vivo* calcium ion concentration changes even in human brain tissues.

References

- [1] M. Collot; C. Loukou; A. J. Yakovlev; C. D. Wilms; *Journal of the American Chemical Society*, **2012**, (134), 14923-14931.
- [2] J. Yao; M. Yang; Y. Duan; *Chemical Reviews*, **2014**, (114), 6130-6178.
- [3] K. Huang; M. Liu; Z. Liu; D. Cao; J. Hou; W. Zeng; *Dyes and Pigments*, **2015**, (118), 88-94.

This project was supported by

NTP-SZKOLL-16-0047
KFI_16-1-2016-0177
NVKP_16-1-2016-0043
Hungarian tenders.