Reversible low-light induced photoswitching of a light sensitive magnetic resonance contrast agent

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Introduction The work performed here describes as a longterm goal a method to noninvasively map gene expression in deep tissues in vivo by developing magnetic resonance imaging contrast agents (MRI CA) that are responsive to commonly employed luminescent biomarker systems. Spiropyrans represent the most widely studied class of organic photochromes [1]. In general, spiropyran is stable in its closed-ring (spiropyran, SP) isomeric form, and is a colorless or pale yellow solution in non-polar solvents. After exposure to UV irradiation, this SP form is converted to a metastable open-ring isomer (merocyanine, MC), with an optical absorption peak at 550-600 nm. The original colorless closed-ring SP form, can be restored via visible light irradiation and/or thermal induction. Substitution of spiropyran with a bioluminescent enzyme (NanoLight, Pinetop, AZ, USA) was introduced into the expression vector pET28b (Addgene, Cambridge, MA, USA) and overexpressed in JM109 (DE3) bacterial cells. Bacteria were disrupted with sonification and the proteins were purified.

Results Photon flux of a blue light emitting diode peaked at \(\lambda_{max} = 465\text{nm}\) and was compared with the spectral emission properties of recombinant Gaussia princeps luciferase, which also displayed a maximum emission at \(\lambda_{max} = 465\text{nm}\) (Figure 2). The photon flux of the LED was changed, leading to a broad power range of 7.54 (±0.2) mW to 1.02 (±0.8) µW, corresponding to the emission of 1.75 \(\times 10^{16}\) photons s\(^{-1}\) to 2.37 \(\times 10^{15}\) photons s\(^{-1}\). We observed a consistent visible light-induced isomerization of the merocyanine to the spiropyran form with photon fluxes as low as 2.37 \(\times 10^{15}\) photons s\(^{-1}\). For the determination of rate constants for the light-induced MC to SP conversion of Gd(III), complexed and non-complexed spiropyran-DO3A was dissolved in water and/or ethanol, were illuminated with constant light for defined time periods and analyzed with UV-Vis spectroscopy. Illumination of spiropyran-DO3A-Gd dissolved in water with total light fluxes between 1.75 \(\times 10^{16}\) photons s\(^{-1}\) and 2.37 \(\times 10^{15}\) photons s\(^{-1}\) induced a merocyanine to spiropyran conversion with calculated rate constants between \(k_1 = 0.342\) (± 0.013) s\(^{-1}\) and \(k_2 = 1.28 \times 10^{4}\) (± 1.89 \(\times 10^{3}\)) s\(^{-1}\) and a linear regression coefficient of \(R^2=0.999\). To determine the MRI properties, \(T_1\) measurements were performed at 37°C with a relaxometer (Brukerminispec, mq60). For overexpression studies the 700 bp cDNA of humanized Gaussia princeps luciferase (NanoLight, Pinetop, AZ, USA) was introduced into the expression vector pET28b (Addgene, Cambridge, MA, USA) and overexpressed in JM109 (DE3) bacterial cells. Bacteria were disrupted with sonification and the proteins were purified.

Conclusion: The results demonstrate the potential for use of the described imaging probes in low light level applications such as sensing bioluminescence enzyme activity. The isomerization behavior of gadolinium(III)-ion complexed and non-complexed spiropyran-DO3A was analyzed in water and ethanol solution in response to low light illumination and compared to the emitted photon flux from over-expressed Gaussia princeps luciferase. We present here a simulation system to study experimentally the isomerization of a photoresponsive molecule to low light illumination and are currently optimizing the contrast agent properties and establishing an efficient over-expression system for the Gaussia princeps luciferase.