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 spiropyrans with monoaza-15-crown-5, 15-crown-5 fragments or acyclic analogs in position 6' or the nitrogen atom of the indoline ring, as well as in position 8 of the benzopyrene part has a large influence on the photochromic properties of the molecule [2]. Previously we have reported the conjugation of spiropyran to a DO3A-macrocycle and further complexion of a gadolinium(III)-ion (Gd-ion) leading to the successful application as light responsive MRI CA [3, 4]. Due to the electrostatic interaction of the complexed metal ion with the indoline part of the photochromic molecule, the hydration state of gadolinium is altered dependent on the isomerization state of spiropyran, resulting in a modification of the contrast agent relaxivity. In this work, principle kinetic studies of the contrast agent's isomerization behavior in response to low level light illumination, establishing a basis for the application of bioluminescent enzymes for inducing the isomerization of the contrast agent and eventually leading to a change in relaxivity.

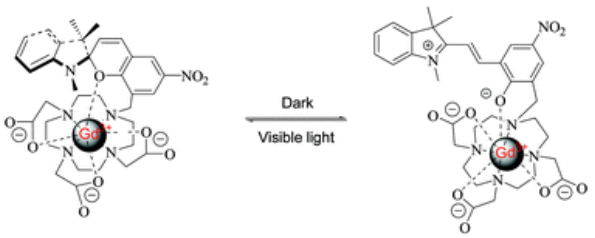

Figure 1: Proposed isomerization of spiropyran-DO3A-Gd (Figure from [4])

Experimental Synthesis of 1',3'-Dihydro-1',3',3'-trimethyl-6-nitrospiro[2H-1-benzopyran-2,2'-(2H)-indole] conjugated to the DO3A macrocycle (spiropyran-DO3A) was performed as previously reported [4]. For emission power modulation, resistors between 330 Ω and 1 MΩ were used in a serial circuit with the LEDs. For the illumination, a quartz cell was placed in front of the LED and irradiated for defined time periods (1s up to 10min), followed by UV-visible spectroscopy. The kinetic rate constants for the light-induced isomerization from the merocyanine to the spiropyran form were extrapolated from the slope of the plot using: $-k_t = \ln(\text{absorbance value}_{\text{timepoint}} / \text{absorbance value}_{\text{initial}})$.The total flux over the emission spectra was calculated based on measured power values. T_1 measurements were performed at constant temperature of 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.

Results Photon flux of a blue light emitting diode peaked at λ_{max}= 465nm and was compared with the spectral emission properties of recombinant *Gaussia princeps* luciferase, which also displayed a maximum emission at λ_{max}= 465nm (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 x10^{16} photons s^{-1} to 2.37 x10^{12} 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 x10^{12} 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, 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 x 10^{16} photons s^{-1} and 2.37 x 10^{12} photons s^{-1} induced a merocyanine to spiropyran conversion with calculated rate constants between k_t = 0.342 (± 0.013) s^{-1} and k_t= 1.28 x10^{-4} (± 1.89x10^{-5}) s^{-1} and a linear regression coefficient of R^2=0.999. To determine the MRI properties T_1 measurements were performed at 37ºC with a relaxometer. Spiropyran-DO3A-Gd kept under dark conditions possesses an r_1 of 2.93 mM^{-1} s^{-1} whereas the r_1 value decreased to 2.63 mM^{-1} s^{-1} after illumination for 60 seconds with the blue LED using a photonflux of 1.75 x10^{16} photons s^{-1}. This represents a light-induced change in relaxivity of 10.24 %. We also determined changes in relaxivity when illuminating with different photonfluxes clearly displaying a correlation between isomerization and change in T_1 values (Figure 3).

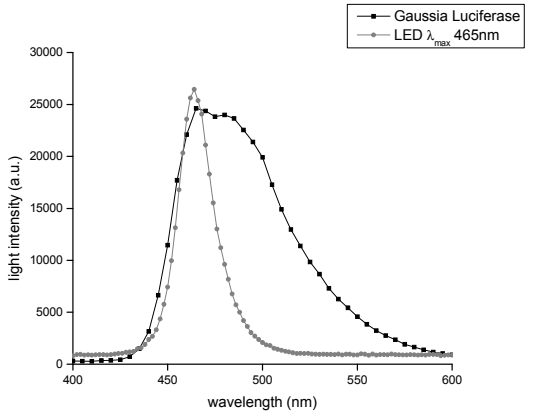

Figure 2: Comparison of LED and *Gaussia princeps* luciferase emission spectra

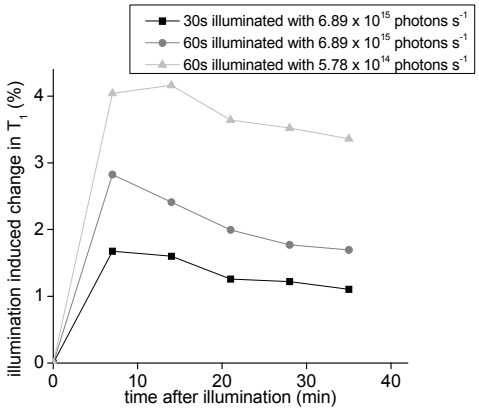

Figure 3: Light induced changes in relaxivity based on the isomerization of spiropyran-DO3A-Gd

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.

References 1.) Lukyanov, B.S. and M.B. Lukyanova, Chemistry of Heterocyclic Compounds, 2005. **41**(3): p. 281-311. 2.) Fedorova, O.A., S.P. Gromov, and M.V. Alfimov, Russian Chemical Bulletin, 2001. **50**(11): p. 1970-1983. 3.) Tu, C.Q., E.A. Osborne, and A.Y. Louie, Tetrahedron, 2009. **65**(7): p. 1241-1246. 4.) Tu, C.Q. and A.Y. Louie, Chemical Communications, 2007(13): p. 1331-1333. 5.)