Highly Sensitive Lanthanide Based Luminescent Particles for In Vivo Imaging of ROS Species in Deep Tissues

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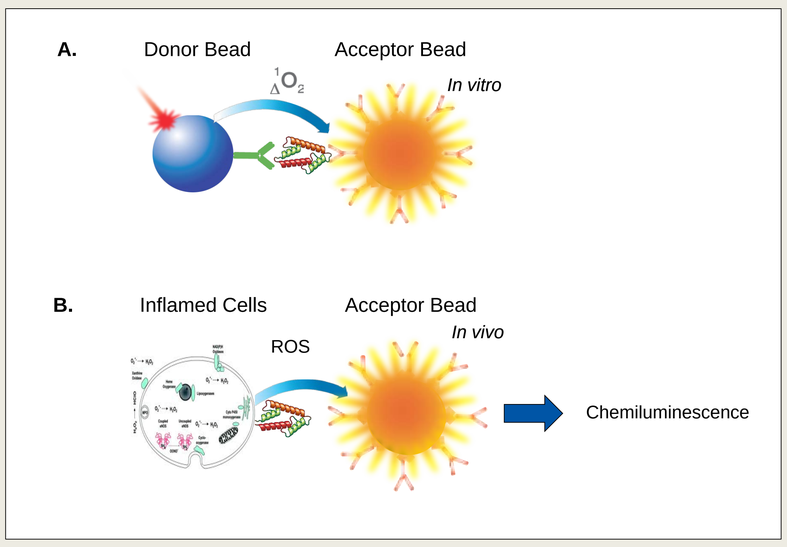
Abstract

Introduction: Reactive oxygen species (ROS) play a critical role in a wide variety of disease conditions like cancer, inflammation, neurodegenerative disorders and oxidative stress. Highly sensitive and specific optical probes (fluorescent, luminescent or chemiluminescent probes) are therefore required for detecting and studying the roles of different ROS in disease pathogenesis. However, very short life times of these species coupled with the presence of antioxidants in living systems make it extremely hard to detect these reactive species in vivo, especially in deep tissues. We employed the chemiluminescent properties of lanthanide acceptor beads to develop a highly sensitive probe for ROS detection by non-invasive optical imaging. In this approach when an acceptor bead comes in close proximity (200nm) to Singlet oxygen ($^1O_2$), energy is transferred from the singlet oxygen to thioxene derivatives within the acceptor bead, resulting in light production at 520-620 nm (EPRM®). The major advantages of this approach are: a. enabling detection of ROS by generating long-lived signal (half-life in seconds); b. Achieving high sensitivity due to lack of background signal and c. Generating long wavelength (620nm) signal thereby allowing deep tissue interrogations in living organisms.

Results: When incubated with ROS generating systems under light-proof conditions, the EPRM beads produced high amount of chemiluminescence (signal:background 5-15 fold) in presence of singlet oxygen. As a proof-of-concept, the beads were also tested in an animal model of LPS-induced lung inflammation. When delivered intravenously the beads produced 2-3 fold higher optical signal in inflamed versus normal lungs, further confirming their potential usage as an ROS probe for deep tissue imaging applications.

Summary: In conclusion, our results demonstrated the practical applicability of the probe for in vivo imaging of ROS. Future studies are aimed at targeting the beads with antibodies for investigating specific disease pathogenesis.

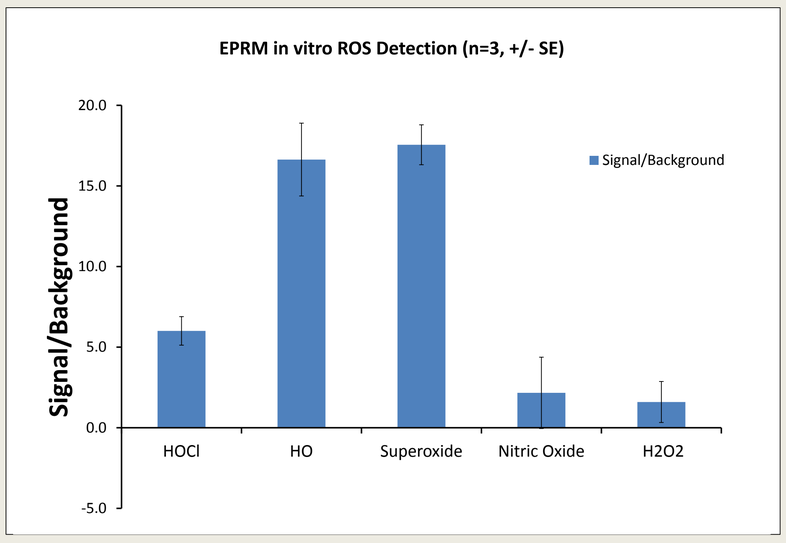
1 Principle of ROS Detection with AlphaLISA Acceptor Beads



A. Standard Detection with AlphaLISA System: Excitation of donor beads converts ground state $^3O_2$ to excited $^1O_2$ which initiate a cascade of reactions in the Acceptor beads, resulting in the emission of light .

B. ROS Detection with AlphaLISA Acceptor Beads: Induction of inflammation in animal cells produces ROS, which react with the Acceptor beads, resulting in the emission of Chemiluminescent light.

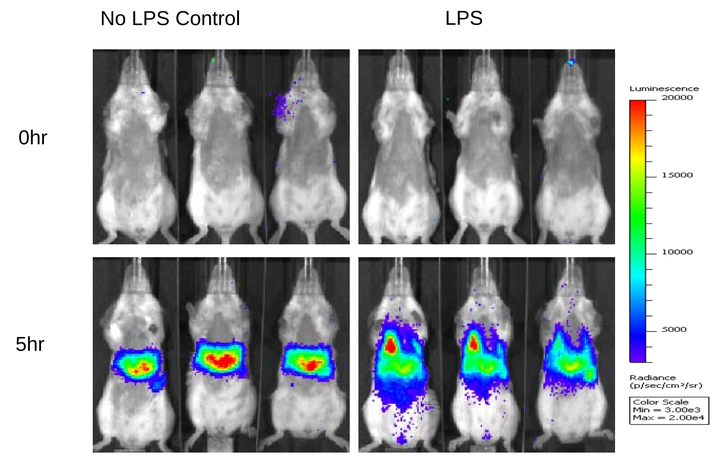
2 *In vitro* ROS Detection with AlphaLISA EPRM Acceptor Beads



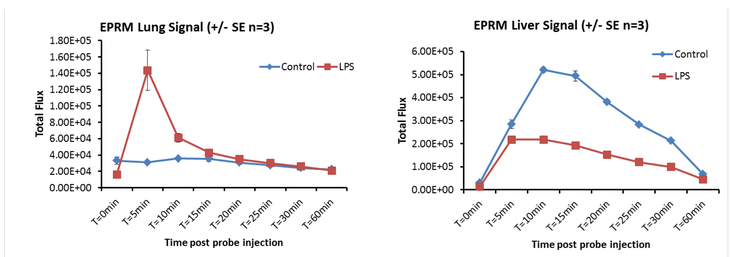
Using a series of chemical reactions, in vitro detection of the following ROS species: Hypochlorus Acid (HOCl), Hydroxl Radical (·HO), Superoxide, Nitric Oxide (NO^-), and Hydrogen Peroxide (H_2O_2), was evaluated with AlphaLISA EPRM Acceptor Beads. Hydroxl Radical and Superoxide have the highest signal above background with EPRM ROS detection.

3 *In vivo* ROS Detection with AlphaLISA EPRM Acceptor Beads

A. LPS Induction of ROS Activity



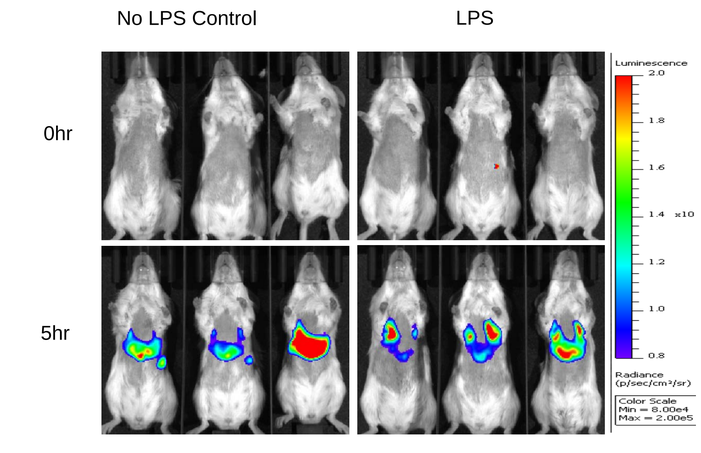
B. Quantification of Luminescent Signals



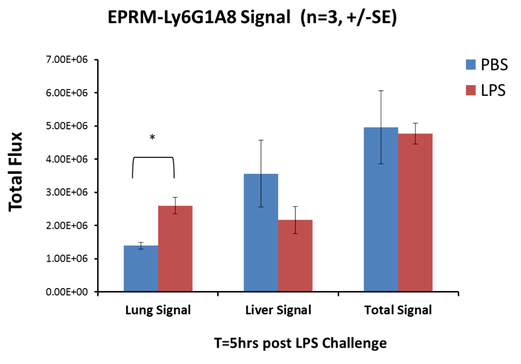
A. BALB/cJ mice were divided into two groups and were challenged with LPS (1 mg/kg i.n.) or PBS (negative controls). Induction of ROS activity was monitored at five hours post LPS challenge with i.v. injection of 10ug EPRM Acceptor beads. **B.** Quantitation of the luminescent signals from the lungs and liver is shown. Data presented as mean ± SEM (n=3).

4 Improved Targeting of Lung ROS Signal with EPRM-Ly6G Conjugate

A. *In vivo* Evaluation of EPRM-Ly6G 1A8 Probe



B. Quantification of Luminescent Signals



C. Signal Distribution

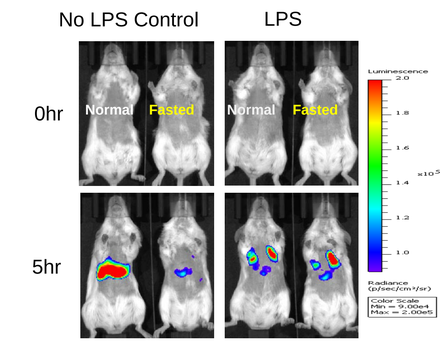
Percent of Total Signal

	Lung Signal	Liver Signal
PBS	28%	72%
LPS	55%	45%

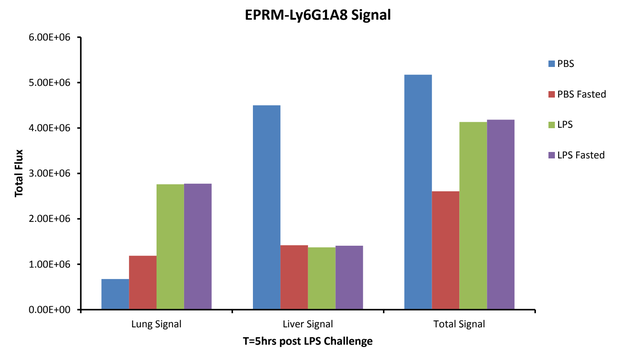
A. BALB/cJ mice were divided into three groups and were challenged with LPS (1 mg/kg i.n.) or PBS (negative controls). Induction of ROS activity was monitored at five hours post LPS challenge with i.v. injection of EPRM-Ly6G1A8 probe (50ug EPRM and 5ug Ly6G1A8) Ly6G1A8 is a neutrophil specific antibody. **B.** Quantitation of the luminescent signals from the lungs and liver is shown. Data presented as mean ± SEM (n=3). *p<0.05, by Mann-Whitney U test. **C.** Signal Distribution in lungs and liver.

5 Food Effect on in vivo ROS Detection

A. Evaluation of Fasting on *in vivo* ROS Detection



B. Quantification of Luminescent Signals



C. Signal Distribution

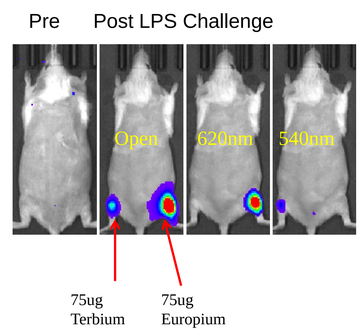
Percent of Total EPRM-Ly6G Signal

	Lung	Liver
PBS	13%	87%
PBS fasted o/n	46%	54%
LPS	67%	33%
LPS fasted o/n	66%	34%

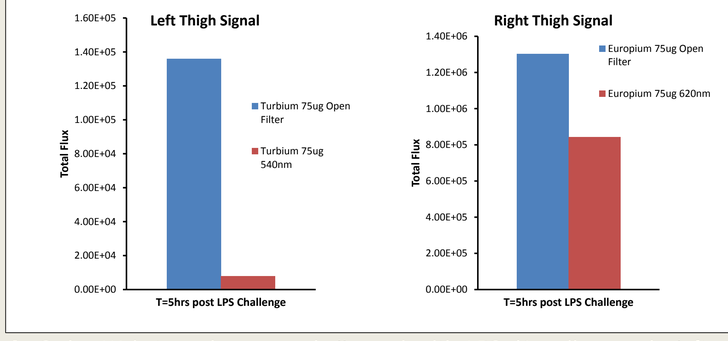
A. BALB/cJ mice were divided into two groups: LPS or PBS (negative controls), with one mouse in each group fasted o/n. Following o/n fast, mice were challenged with LPS (1 mg/kg i.n.) or PBS. Induction of ROS activity was monitored at five hours post LPS challenge with i.v. injection of EPRM-Ly6G1A8 probe (50ug EPRM and 5ug Ly6G1A8). **B.** Quantification of lung and liver signal. **C.** Signal distribution in lungs and liver.

6 *In vivo* ROS Detection with Terbium and Europium AlphaLISA Acceptor Beads

A. Spectral Imaging of ROS Activity



B. Quantification of Luminescent Signals



A. Swiss Webster mice were challenged with LPS (1mg/kg s.c. in left and right thigh). Induction of ROS activity was monitored at five hours post LPS challenge with s.c. delivery of 75ug Terbium beads in the left thigh and 75ug Europium beads in the right thigh. Mice imaged with Open filter, 620nm emission filter (Europium emission at 614nm), and 540nm emission filter (Terbium emission at 540nm). **B.** Quantification of Terbium and Europium thigh signals. At 540nm emission, Terbium signal is only 6% of total Terbium signal, while at 620nm emission, Europium signal is 65% of total Europium signal.

Summary

-Using AlphaLISA Acceptor Beads, we have developed a luminescent probe for detecting ROS *in vivo*.

-Conjugation of Ly6G1A8, a neutrophil specific antibody, to EPRM acceptor beads improves targeting of lung ROS activity.

-This in vivo ROS detection works with both Terbium (em 540nm) and Europium (em 614nm) AlphaLISA Acceptor Beads.

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