Kinetic Analysis of Model PET Redox Reporters Using Dynamic Bioluminescence Imaging with Biologically-Relevant Reactive Oxygen Species

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Introduction

Innate immunity is critical in physiological responses to pathogens, but can also exacerbate diseases through pro-inflammatory signaling. Innate immune cells rapidly generate high redox potential reactive oxygen and nitrogen species (RONS) as a host defense response.[1]

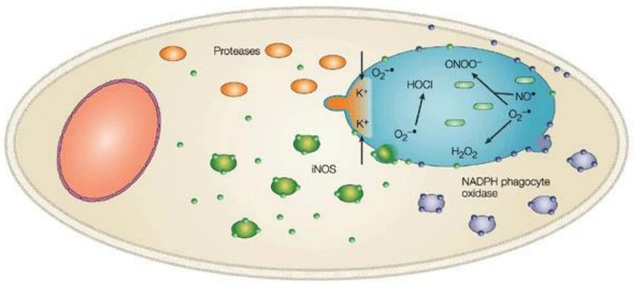


Fig. 1: Antimicrobial mechanisms of phagocytes[1]

These RONS are key effector molecules in inflammatory states, but excessive amounts lead to oxidative stress in cells and disease progression. Novel imaging approaches that efficiently localize inflammation-induced radical species would allow for precise imaging of immuno-activation sites *in vivo* that can be applied to study many inflammatory diseases and guide therapeutic choices.

Using imaging reporters to detect innate immune activity

- Luminol and L-012 are oxidized by RONS found in biological immune systems.[2,3]
- The bioluminescent emissions of these substrates when oxidized render them effective optical probes to localize superficial sites of inflammation.
- 4-[^{18}F]fluoro-1-naphthol ([^{18}F]4FN) has been tested in pre-clinical studies as a Positron Emission Tomography (PET) reporter for imaging innate immunity activation in deep tissues.
- L-012 bioluminescent activity was shown to strongly correlate with [^{18}F]4FN retention.

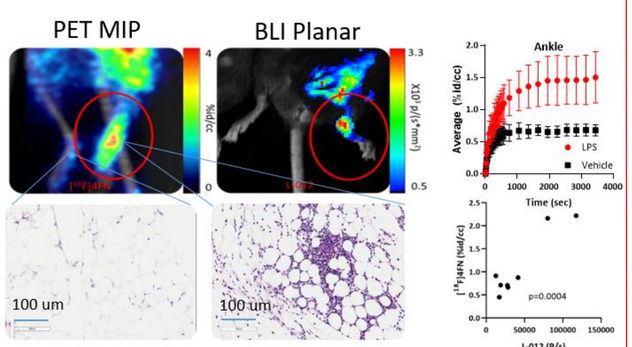
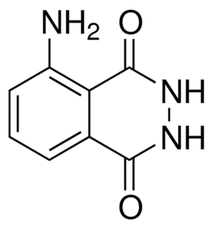


Fig. 2: *In vivo* arthritis model of inflammatory RONS production: correlation of L-012 BLI with the novel PET imaging reporter ([^{18}F]4FN.

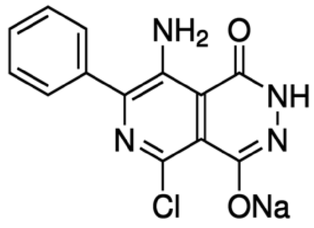
Methods

Structures of RONS imaging reporters:

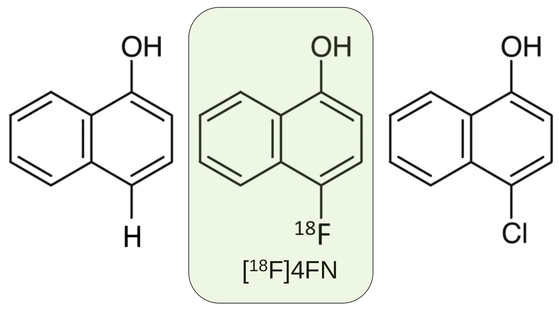
Luminol



L-012



Design of PET Tracer



Bioluminescence Imaging (BLI):

Luminol and L-012 were used as bioluminescence imaging substrates to be oxidized by various key biological RONS species found in living animals (Table 1).

Table 1. Reduction potentials of biologically relevant redox-coupled reactions

Redox Couple (# electrons)	Reduction Potential (V vs NHE)
•OH+H$^+$/H$_2$O (1e$^-$)	2.3
HOCl/H$_2$O,Cl$^-$ (2e$^-$)	1.48
Peroxynitrite/NO$_2$ (1e$^-$)	1.4 (pH=7)
MPO Compound I/Compound II (1e$^-$)	1.35 (pH=7)
MPO Compound I/Native MPO (2e$^-$)	1.16 (pH=7)
MPO Compound II/Native MPO (1e$^-$)	0.97 (pH=7)
O$_2$•$^-$/H$_2$O$_2$ (1e$^-$)	0.9 (pH=7)
OCl$^-$/Cl$_2$ (1e$^-$)	0.89
Singlet Oxygen/Superoxide anion (1e$^-$)	0.81
Naphthol radical/1-Naphthol (1e$^-$)	0.59
OCl$^-$ + H$_2$O/ 1/2Cl$_2$+2OH$^-$ (1e$^-$)	0.42
H$_2$O$_2$/H$_2$O+OH$^-$ (1e$^-$)	0.38
HOCl/HOCl$^-$ (1e$^-$)	0.25
O$_2$/O$_2^-$ (1e$^-$)	-0.16
•NO/^{3}NO$^-$ (1e$^-$)	-0.8

Photonic reactivities *in vitro* of L-012 or luminol (100 µM) with reactive oxygen and nitrogen species (100 µM) were detected through Bioluminescence Imaging (BLI). Reactions were monitored dynamically and quantified post-addition of various RONS for 40 additional minutes by BLI.

Results

In Vitro BLI assays:

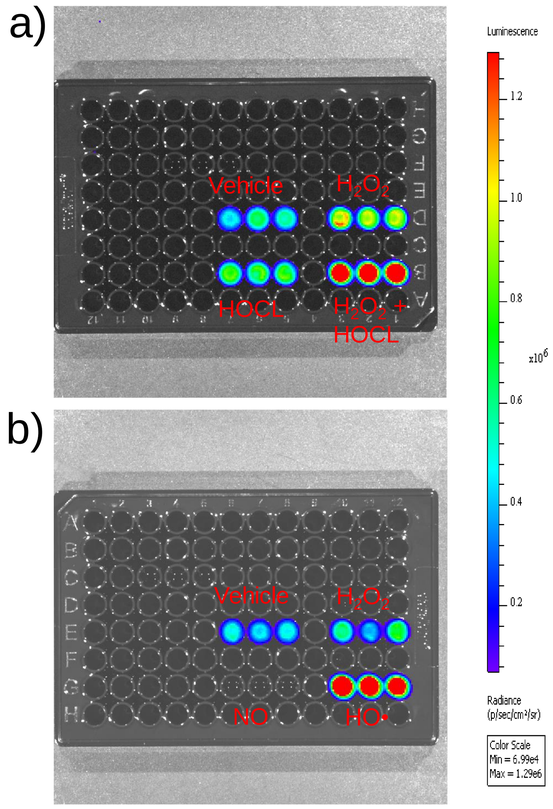


Fig. 3: Images of L-012 bioluminescence intensity of a) H_2O_2, HOCL, and singlet oxygen, and b) NO and hydroxyl radical *in vitro*.

Time series over 1 hour:

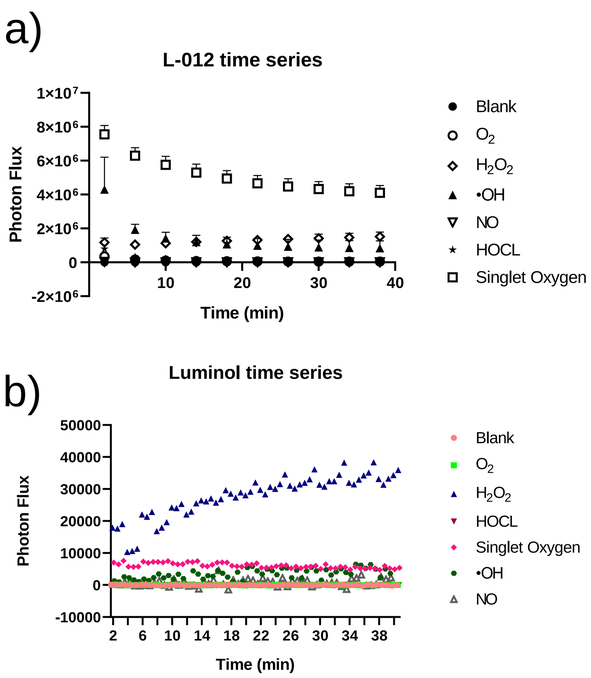
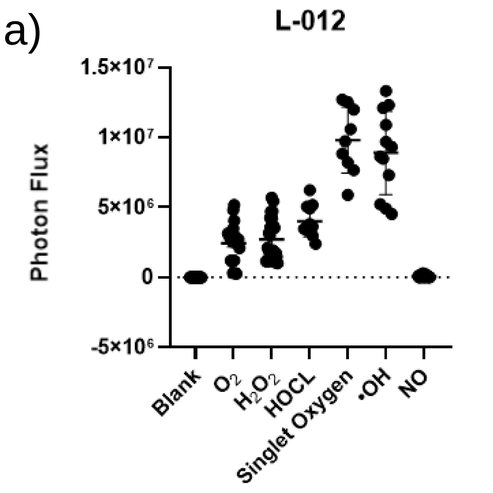


Fig. 4: Reactions with a) L-012 and b) Luminol rapidly reached a steady state with modest changes in photonic production rates for a few RONS over one hour.



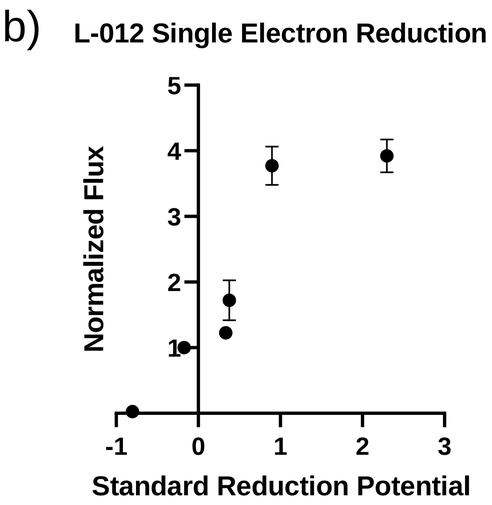


Fig. 5 The reduction potential of RONS species showed a positive correlation versus the bioluminescent flux of L-012 (r = 0.9429, p (two-tailed) = 0.0167).
a) Individual points
b) Normalized to control (O_2)

Conclusions

The radiance of model bioluminescent substrates positively correlated with the standard reduction potential of RONS. This property will help further quantitation of current probes and potentially aid development of new reporters for different regimes of reactive oxygen/nitrogen species.

Acknowledgements

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References

1) Fang et al. Nat Rev Microbiol 2004; 2: 820–832
2) Daiber et al. Free Radical Biology and Medicine 2004; 36: 101-111
3) Simons et al. Free Radical Biology and Medicine 1990; 8(3): 251-258