Hydrocyanines: A New Class of Fluorescent Sensors That Can Image Reactive Oxygen Species in Cell Culture, Tissue and In Vivo

Niren Murthy
Georgia Institute of Technology, Department of Biomedical Engineering and the Petit Institute for Bioengineering and Biosciences
Atlanta GA 30332-0535

Statement of Purpose: The development of fluorescent probes for superoxide and the hydroxyl radical is a central problem in the field of chemical biology. Superoxide and the hydroxyl radical play a significant role in a variety of inflammatory diseases and probes that can detect these reactive oxygen species (ROS) have tremendous potential as medical diagnostics and research tools. Fluorescent sensors for superoxide and the hydroxyl radical, such as dihydroethidium (DHE), have been developed; however, they have had limited applicability due to their spontaneous auto-oxidation, rapid photobleaching, low emission wavelengths and multiple reaction products with ROS. New chemical probes for superoxide and the hydroxyl radical are therefore greatly needed. In this presentation, we present a new family of fluorescent ROS sensors, termed the hydrocyanines, which can be synthesized in one step from the commercially available cyanine dyes, and can detect superoxide and the hydroxyl radical in living cells, tissue samples and for the first time in vivo. We anticipate widespread interest in the hydrocyanines given their physical/chemical characteristics and ease of synthesis.

Methods: The synthesis and mechanism by which the hydrocyanines image ROS is shown in Scheme 1a. The hydrocyanines are synthesized by reducing the iminium cations of the cyanine dyes with $NaBH_4$. Hydrocyanines detect ROS through fluorescent imaging; they are weakly fluorescent because of their disrupted π conjugation, however oxidation with either superoxide or the hydroxyl radical, dramatically increases their fluorescence because it regenerates their extended π conjugation. Scheme 1b demonstrates the proof of principle of this methodology. Hydro-IR-676 has minimal fluorescence, however oxidation with superoxide causes a 100-fold

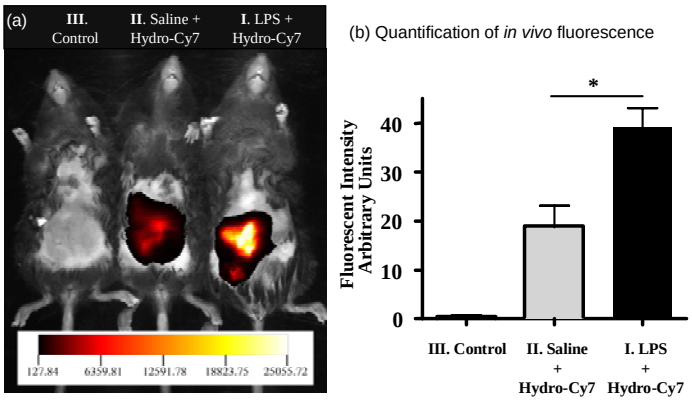
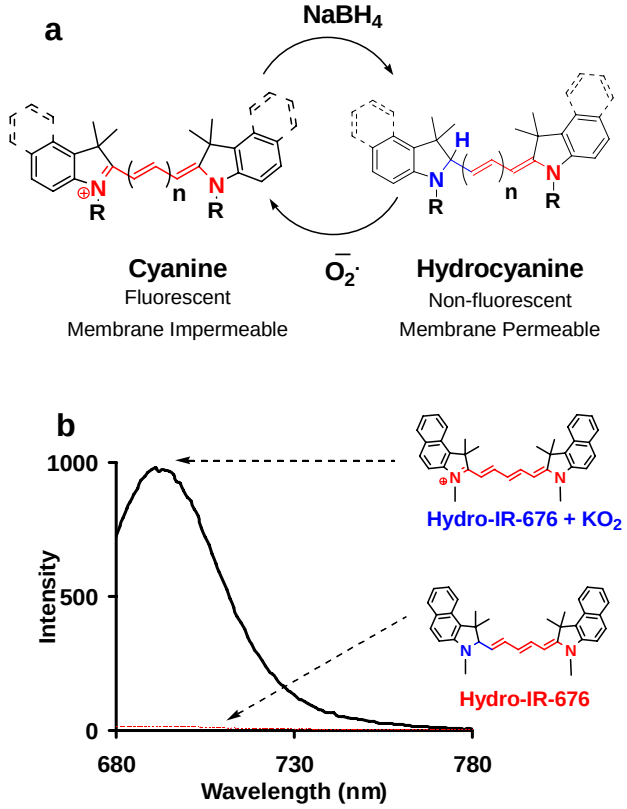


Figure 1a-b. *In vivo* imaging of ROS production from the peritoneal cavity of mice with hydro-Cy7, during an LPS mediated inflammatory response. [**a**] (I) LPS was injected into the peritoneal cavity of mice, followed by an IP injection of hydro-Cy7. (II) Saline was injected in the i.p. cavity of mice, followed by i.p injection of hydro-Cy7. (III) Negative control, neither LPS nor hydro-Cy7 was injected. Mice from each group were imaged at the same time after hydro-Cy7 injection. [**b**] Quantification of fluorescence intensities from LPS treated mice, saline treated mice and control (I, II and III from **a** respectively). Oxidized hydro-Cy7 fluorescence intensity is increased in LPS treated mice in comparison to saline treated mice (n = 3, *p<0.001).



Scheme 1. Hydrocyanines: a new family of ROS sensors synthesized from the cyanine dyes. [a] Hydrocyanines are synthesized via a one-step reduction with $NaBH_4$. Reaction with superoxide or the hydroxyl radical oxidizes the hydrocyanines into fluorescent cyanine dyes. [b] Hydro-IR-676 has negligible fluorescence emission, however, oxidation with superoxide causes a 100-fold increase in fluorescence (λ_{Ex} =675 nm, λ_{Em}=693 nm).

Figure 1a-b demonstrates that the hydrocyanine, hydro-Cy7 can image ROS in the peritoneal cavity of mice during an LPS induced inflammatory response.

Conclusions: In summary, the hydro-cyanines are a new family of ROS sensors, synthesized by $NaBH_4$ reduction of commercially available cyanine dyes, which have tremendous potential for the in vivo imaging of ROS.