Development of Chemiluminescent Probes for Detecting Reactive Oxygen Species

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Introduction

Generated as the byproducts of respiration, reactive oxygen species (ROS) are reduced form of dioxygen that can act as signaling molecules in organisms, such as $O_2^{\cdot-}$, H_2O_2, $OH^{\cdot}$, HOCl etc. However, at high concentrations, ROS would damage cells and organs, eventually leading to cell death and a wide range of diseases, such as cancer and asthma. Hence, real-time monitoring of ROS with molecular probes can unravel the pathogenesis of diseases and aid the development of drug development.

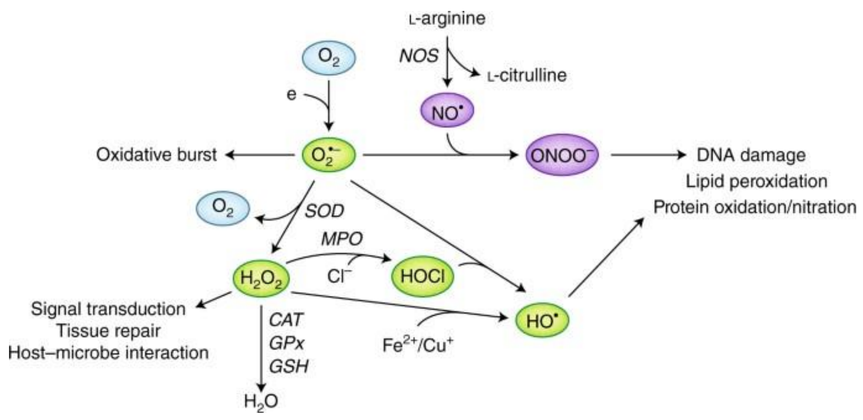
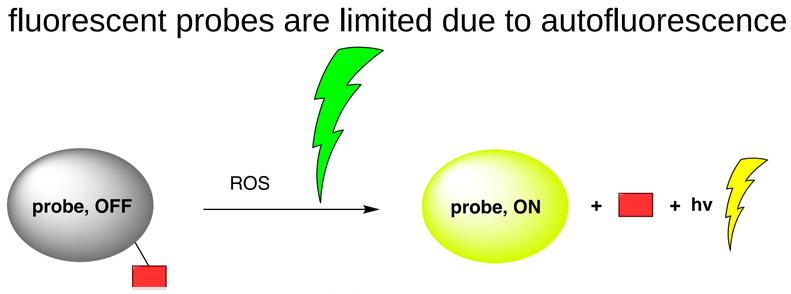


Figure 1: Production of ROS in cells

Fluorescent probes have traditionally been used for ROS imaging in living cells and organisms since it can be turned on easily. However, applications of fluorescent probes are limited due to autofluorescence and scattering issues.



Scheme 1: Working principle of fluorescent ROS probes

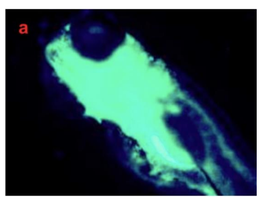


Figure 2: Fluorescent imaging of ROS in zebrafish embryo

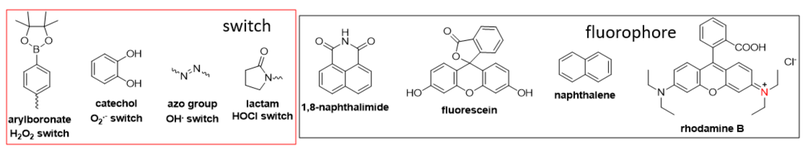


Figure 3: Switch and fluorophore adopted in design of ROS fluorescent probes

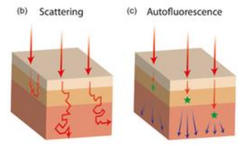
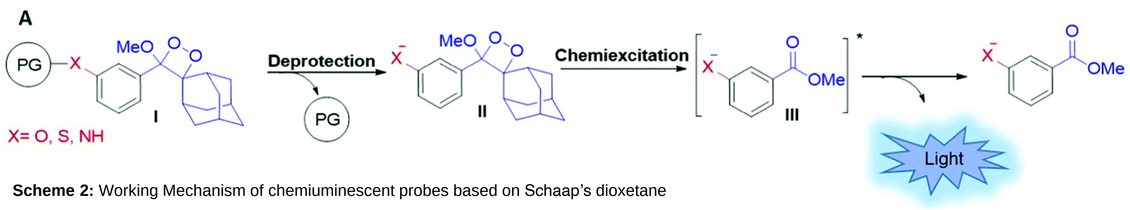


Figure 4: Illustration of scattering and autofluorescence.

Recently, turn-on chemiluminescent probes for ROS detection are synthesized based on Schaap's adamantiylidene-1,2-dioxetane for real-time *in vitro* and *in vivo* ROS detection. They do not require external excitation and can emit light once cleaved by the ROS substrate. They also exhibit high signal-to-noise ratio and improved tissue-penetration ability, which can serve as an alternative for traditional fluorescent probes in *in vivo* ROS detection.



Scheme 2: Working Mechanism of chemiuminescent probes based on Schaap's dioxetane

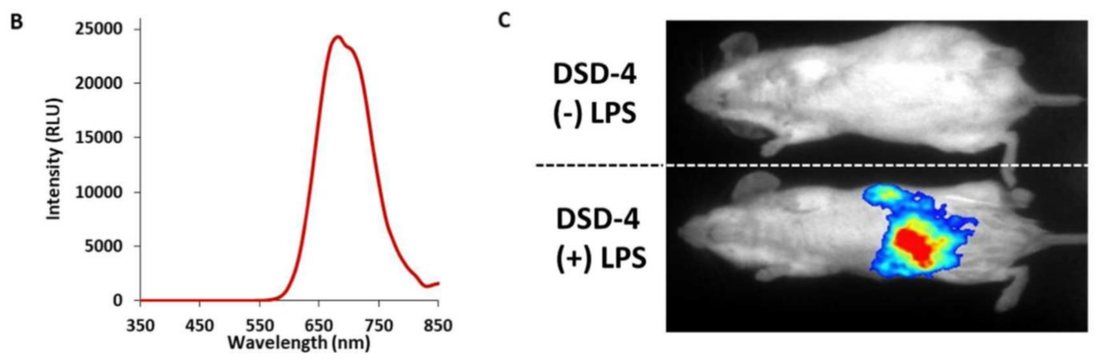


Figure 4: *In vivo* imaging and chemiluminescent measurements of DSD-4, a chemiluminescent probe.

In this work, the author reports two newly designed chemiluminescent probes for HOCl detection **(HOCl-DF-510, HOCl-DF-540)** and one probe for $O_2^{\cdot-}$ detection **($O_2^{\cdot-}$-540)**. Preliminary chemiluminescent tests are also carried out to evaluate the kinetics and performance of the probes.

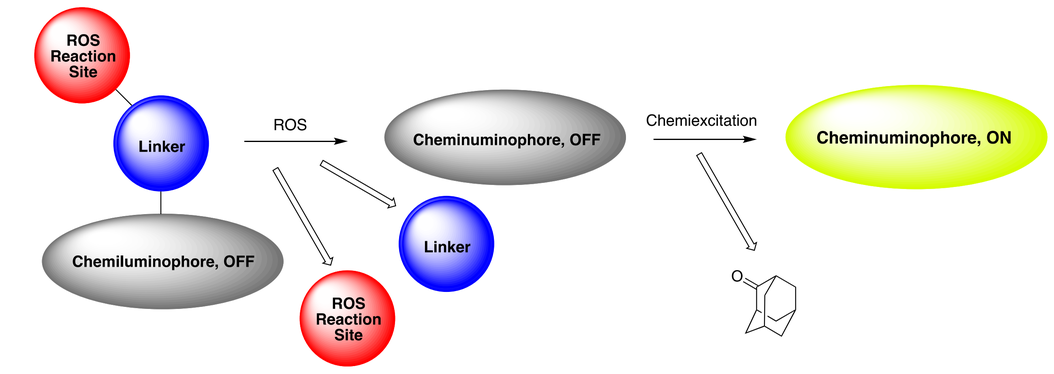
- **HOCl**: Elevated levels associated with rheumatoid arthritis & autoimmune diseases; promote drug chemotherapy when delivered precisely.

- **$O_2^{\cdot-}$**: One of upstream ROS. Secondary products induces inflammation, cancer etc. Elevated levels associated with damage of Fe-S proteins and cells.

Results

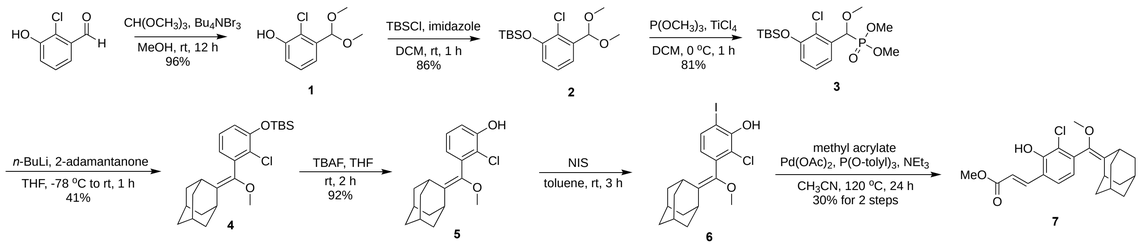
(i) HOCl Probe:

HOCl-DF-510 and **HOCl-DF-540** consist of three major components: HOCl reaction site, linker and the chemiluminophore. Upon reaction with HOCl, the reaction site and linker will self-immolate and activates the chemiluminophore.

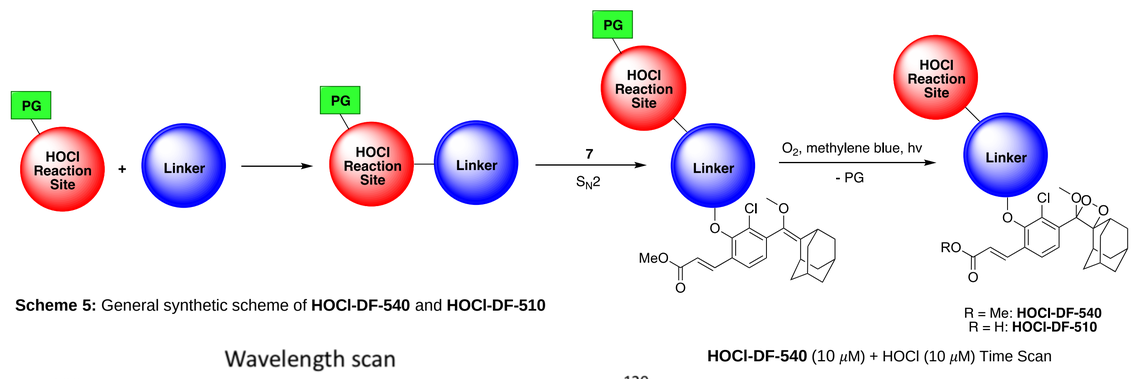


Scheme 3: General working principle of **HOCl-DF-510** and **HOCl-DF-540**

Parallel synthesis of the chemiluminophore precursor (**7**) and the linker plus reaction site is adopted. The HOCl reaction site (red) is first coupled with a newly designed linker (blue), and introduced to the chemiluminophore (black) via an S_N2 reaction. Singlet oxygen insertion and protecting group removal are conducted in the end.



Scheme 4: Synthesis of chemiluminophore precursor (**7**)



Scheme 5: General synthetic scheme of **HOCl-DF-540** and **HOCl-DF-510**

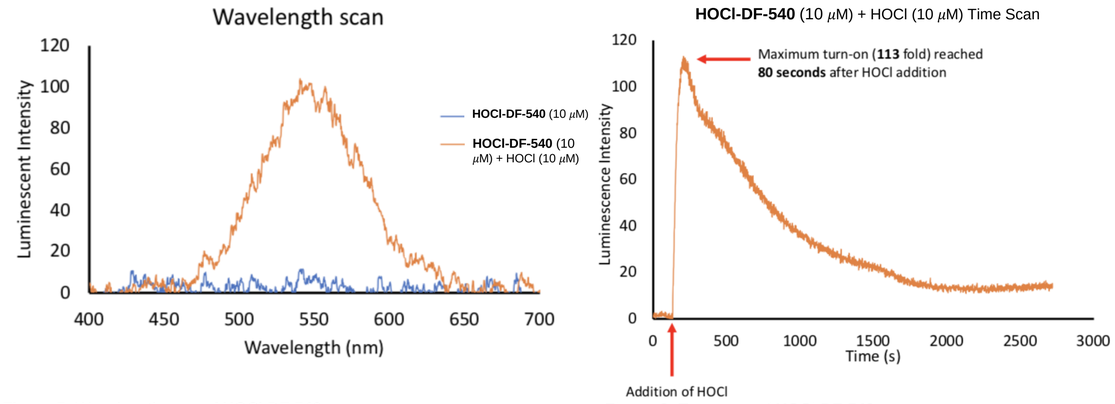
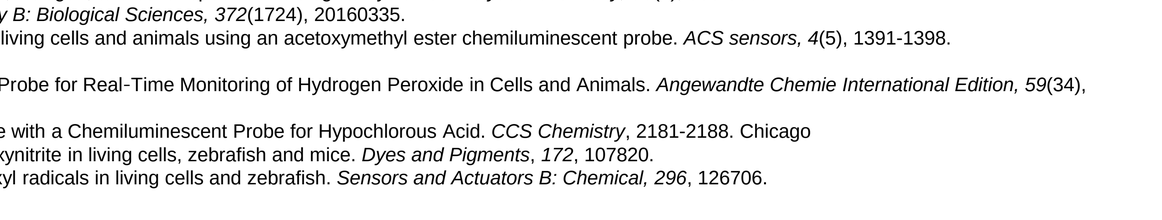


Figure 5: Wavelength scan of **HOCl-DF-540**

Figure 6: Time scan of **HOCl-DF-540**

(ii) $O_2^{\cdot-}$ probe:

$O_2^{\cdot-}$-540 consist of only the **$O_2^{\cdot-}$** reaction site and the chemiluminophore because the reaction is less affected by sterics. The same convergent approach is adopted as described in the HOCl probe. The **$O_2^{\cdot-}$** reaction site is first synthesized, and then linked to **7**. Finally, singlet oxygen insertion is conducted.



Scheme 6: General synthetic scheme of **$O_2^{\cdot-}$-540**

Summary

Two newly designed chemiluminescent probes for HOCl detection **(HOCl-DF-510, HOCl-DF-540)** and one probe for $O_2^{\cdot-}$ detection **($O_2^{\cdot-}$-540)** are designed and synthesized. Preliminary time scans have hinted that chemiluminescent probes with methyl ester moiety in the chemiluminophore only exhibit mediocre turn-on response. Further chemiluminescent tests shall be scheduled to fully explore the probe kinetics and sensitivity.

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