

## Two-Photon Absorbing Nanocrystal Sensors for Ratiometric Detection of Oxygen

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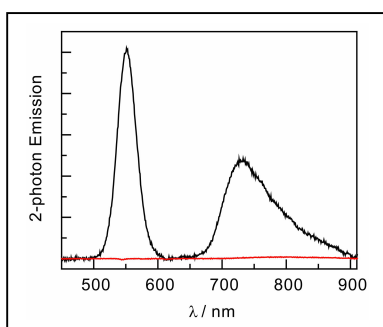
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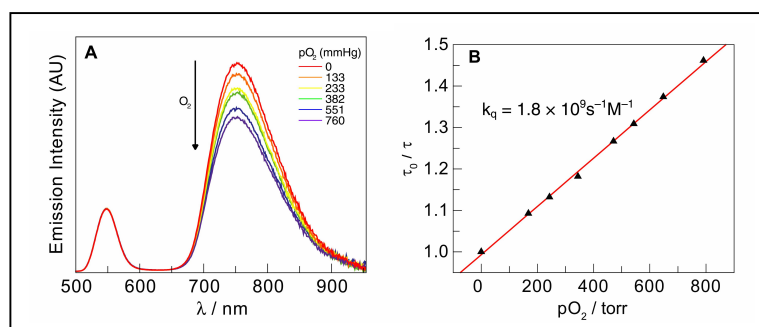
**Abstract** – Covalently tethering a phosphorescent complex to a semiconductor nanocrystal (NC) can engender oxygen sensitivity to the NC through fluorescence resonance energy transfer from the NC to the phosphor. The increased one- and two-photon absorbing properties of the NC are transferred to the phosphor, while the NC acts as a scaffold and an internal standard through its oxygen insensitive emission band. Sensors have been made employing Osmium(II) polypyridine phosphors and the design is being extended to group 10 metal porphyrins. The mechanism of oxygen sensing has been explored and the feasibility of the probe as an oxygen bioimaging agent is presented.

Previously, most imaging applications of semiconductor nanocrystals (NCs) have only involved the use of them as indicators of position; they have lacked a response to their local environment. Attaching an analyte-sensitive chromophore to a NC enables fluorescence resonance energy transfer (FRET) to be exploited as a signal transduction mechanism, sensitizing the NC. The mechanism for oxygen sensing involves kinetic quenching of the emission of the energy accepting dye in the presence of oxygen, while the emission of the energy donating NC remains stable. This mechanism was chosen owing to the unique ability of oxygen to quench emission from a phosphorescent compound, but not fluorescence from a NC. An additional benefit of FRET excitation includes inheritance of the very large one- and two-photon excitation cross-sections of NCs, as compared to small molecule phosphors. Together, these properties make the probes strong candidates for O<sub>2</sub> sensing applications in biological microenvironments, where probe concentrations may vary, and where the use of multiphoton excitation in microscopy presents significant advantages in imaging thick samples and in limiting extraneous tissue damage.

Construction of a ratiometric NC-based pO<sub>2</sub> sensor according to the design described above has been achieved using two specifically constructed Osmium(II) polypyridyl (Os(II)PP) complexes presenting a free amine enabling facile coupling to a carboxylic acid moiety on a NC. The Os(II)PP complexes also possess a red absorption that overlaps well with the NC emission, a requirement for FRET. Energy transfer from the NC to the Os(II)PP causes an enhanced Os(II)PP emission with a concomitant quenching of the NC emission. The photophysical properties of the sensor were further characterized to confirm the expected enhancement of the two-photon emission of the Os(II)PP by the NC. Figure 1 substantiated this claim with a very bright phosphor emission within the sensor and no detectable emission of Os(II)PP alone. Oxygen sensitivity of the complex was found to cover the entire range of possible pressures from 0 to 760 torr O<sub>2</sub> (Figure 2). The emission of the NC is insensitive to oxygen, even at 1 atm, and is well separated from the phosphor such that monitoring the baseline allows ratiometric measurements to be made in addition to the more typical lifetime detection mode, offering the advantage of simple signal detection. Such a sensor is attractive for highly scattering environments in biology, although the oxygen sensitivity range is much higher than that of biological systems (0-160 torr). Appendage of a longer-lifetime phosphor to NCs promises lower oxygen sensitivity and is currently underway.



**Figure 1:** Two-photon emission of the Os(II)PP complex (—) and the NC-Os(II)PP conjugate (—) with the same Os(II)PP concentration.



**Figure 2:** (a) Emission from the Os(II) polypyridine center of the NC conjugate with decreasing pO<sub>2</sub>. (b) Stern-Volmer plot of the excited state lifetime of the Os(II)PP center in the conjugate vs. pO<sub>2</sub>.