Towards Single Metal Ion Sensing by Förster Resonance Energy Transfer

Jens U Sutter, Alexander MacMillan, David JS Birch and Olaf J Rolinski

Photophysics Group, Department of Physics, John Anderson Building,

University of Strathclyde, Glasgow G4 0NG, Scotland, UK

jens.sutter@strath.ac.uk

Biomolecular & Chemical Physics

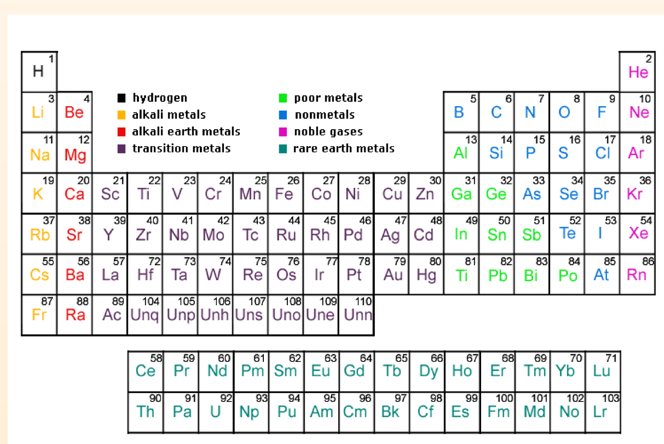
Abstract

Here we describe progress towards our objective of detecting single non-fluorescent hydrated metal ions

1. Transition metal ions

Transition metal ions like copper and nickel play an important role in biology as nutritional microelements as well as important ligands in proteins e.g. manganese in the photosystem II of plant cells or copper in the regulation of intracellular transport [1].

Sensing transition metal ions in biological systems by chemical methods proves to be difficult for concentrations are small and chemical sensing often interferes with the very process one desires to monitor

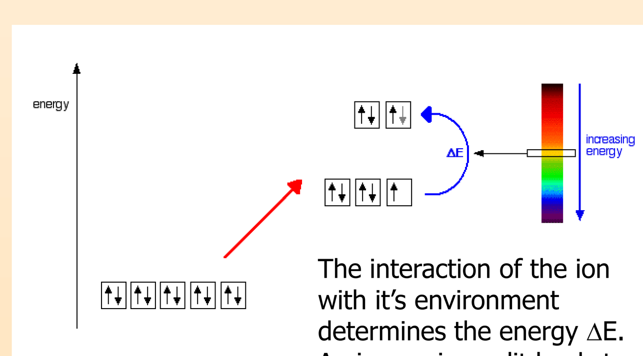


Periodic table of the elements showing important micronutrients within the first group and common contaminants throughout the block of transition metal ions.

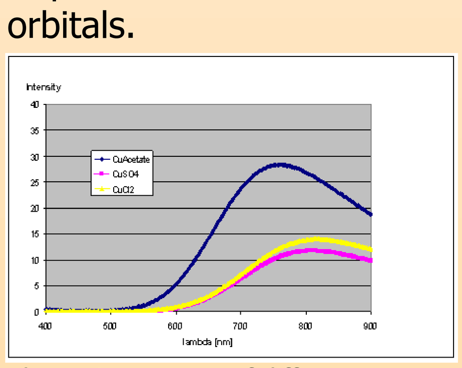
2. Light absorption by metal ions

When a transition metal ion interacts with one or more ligands the electrons of the ligand as well as the d-orbital of the ion repulse each other. This raises the energy level of the electrons in the d-orbital and leads to a split into two distinct energy bands.

With the d-orbitals incompletely filled, absorption of photons can lift an electron between the d-orbitals.



The interaction of the ion with it's environment determines the energy ΔE. An increasing split leads to a blue shift of the absorption spectrum



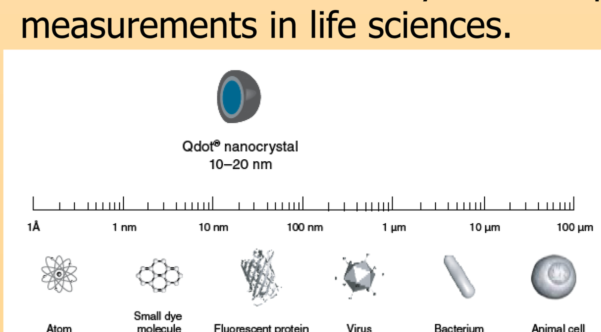
Absorption spectra of different copper salts

Thus the ligand of the ion has an influence on the extent of the split of the d-orbitals and the subsequent absorption spectrum of the ion.

Cl^- small split
F^-
OH^-
H_2O
NH_3
CN^- large split

3. Quantum dots

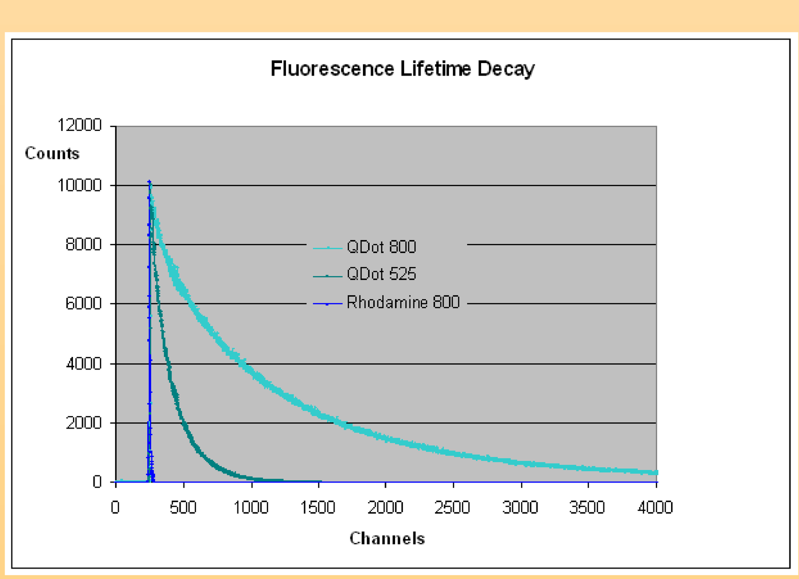
The relatively small size, the long fluorescence lifetimes and the photostability of semiconductor nanocrystals or "quantum dots" are of great advantage for measurements in life sciences.



Scale of quantum dots according to manufacturers (INVITROGEN QDot® range)

The main lifetimes on quantum dots exceed those of commonly used flourophors;

Rhodamine 800	0.7	ns
QDot525	25	ns
QDot800	220	ns



4. Förster Resonance Energy Transfer

In contrast to measurements that rely on direct binding of the ion the resonance energy transfer does not interfere with the process observed [2].

The fluorescence decay is measured then given by:

$$I(t) = I(0)\exp\left(-\frac{t}{\tau_0} - 2\gamma\left(\frac{t}{\tau_0}\right)^{\frac{1}{2}}\right)$$

With γ being the transfer coefficient between donor and acceptor, defined as:

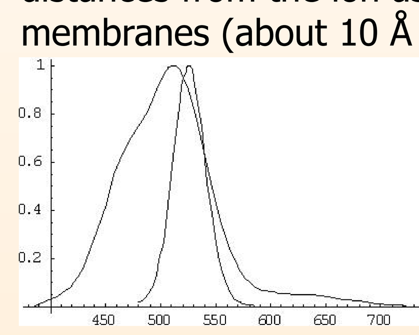
$$\gamma = \frac{[A]}{C_A}$$

$[A]$ is the concentration of the acceptor and C_A is the critical acceptor concentration as calculated from the overlap integral between donor emission and acceptor absorbance.

The value of the spectral overlap integral between acceptor and donor R_0 is

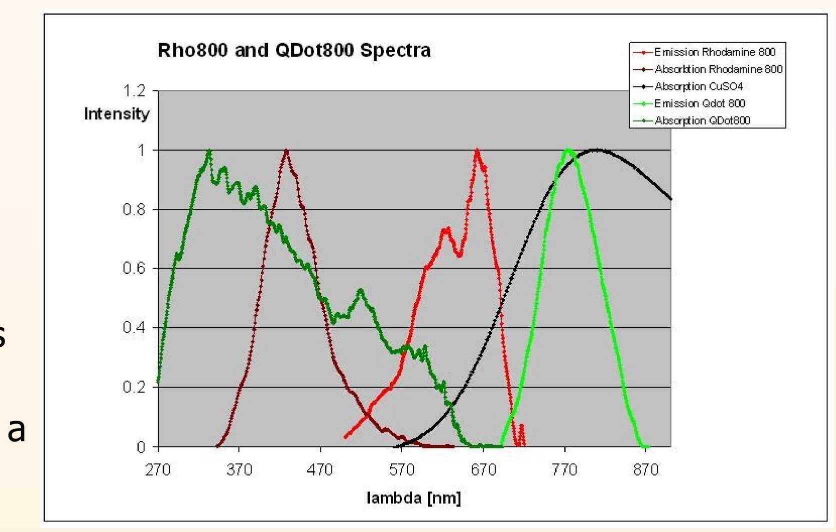
for Quantum Dot QDot525® with Co^{2+} 13.9 Å
and Quantum Dot QDot800® with Cu^{2+} 20.3 Å.

These values of R_0 allow measurements over considerable distances from the ion as for example probing through cell membranes (about 10 Å in thickness).



Overlap QDot 525 with Co^{2+}

Calculations from measurements of the transfer coefficient γ and the acceptor concentration yield a R_0 value of 14 Å for QDot800 with Cu^{2+}.

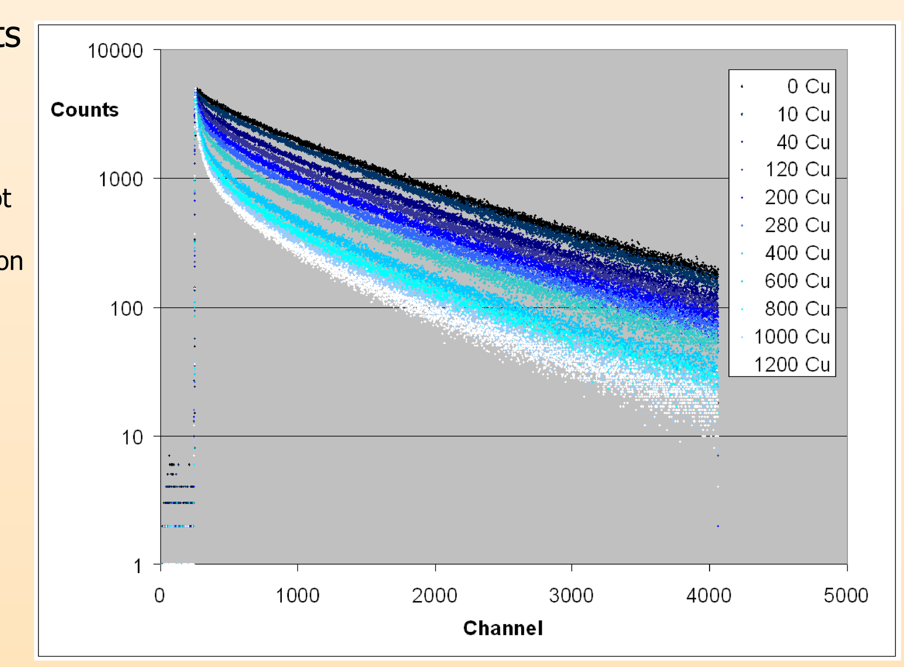


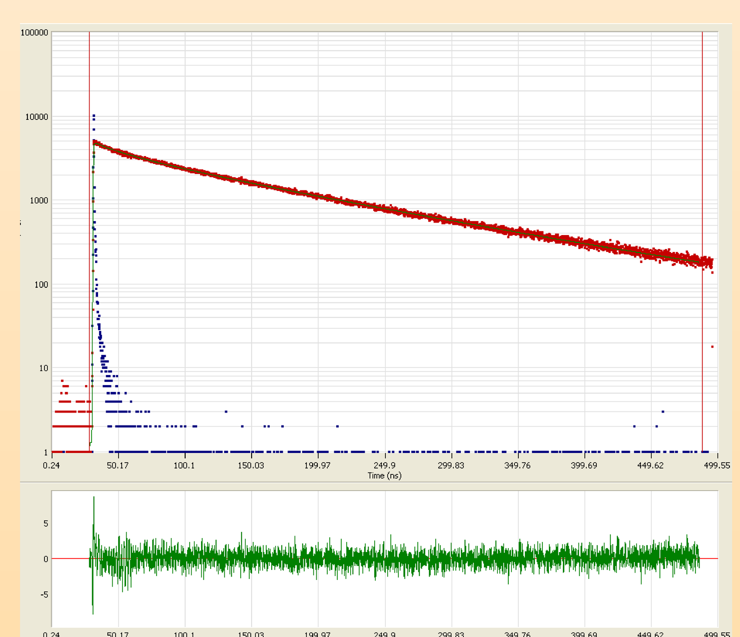
Emission and Absorption Spectra of Rhodamine 800 (red), QDot 800 (green) and $CuSO_4$ (black).

6. Resolution of ion sensing

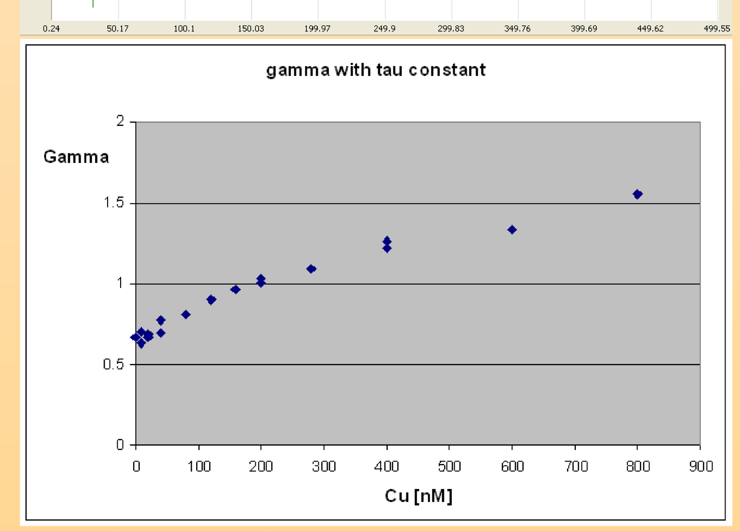
Recordings of the fluorescence lifetime of quantum dots emitting at 525 and 800 nm, show great sensitivity to addition of cobalt and copper ions respectively.

Fluorescence lifetime of 40 nM QDot 800 with addition of increasing amounts of copper sulphate. Addition of manganese does not change the fluorescence lifetime.

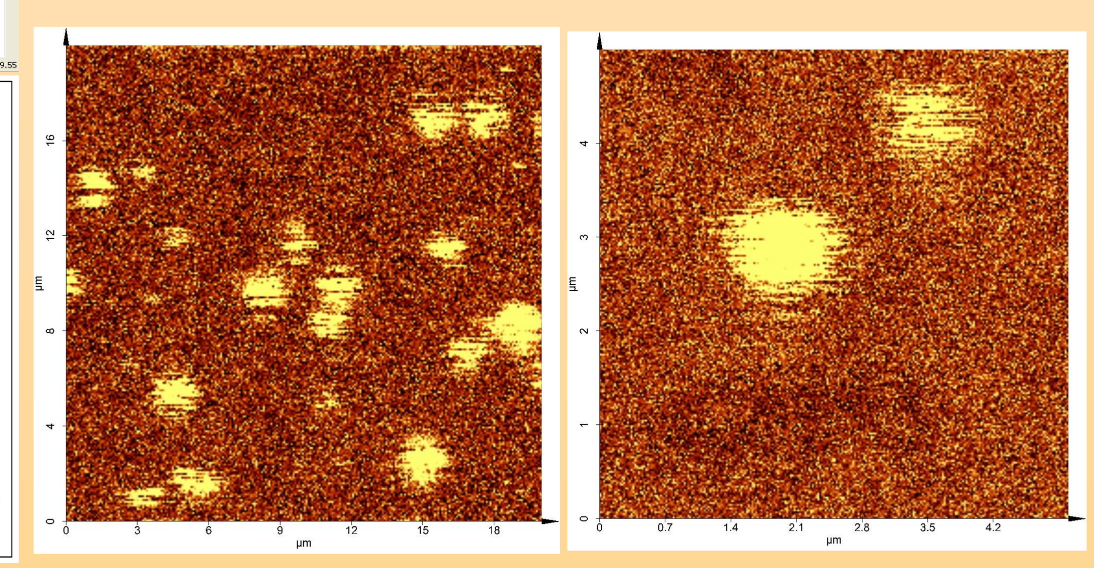




The recorded lifetimes can be fitted to a two exponential decay function. The set of recordings does well fit a Förster energy transfer Model.



Recordings of two datasets with 40 nM QDot800 in pH 9.4 Borate Buffer; τ = 220 ns. The donor to acceptor ratio ranges from 4:1 to 1:20



Quantum dots immobilized in sol gel and monitored using a confocal microscope.

7. Quantum dots are potent sensors for metal ions

Semiconductor nanocrystals provide excellent sensors for transition metal ions in biophysical systems.

Quantum dots can be generated with precise spectral properties, allowing to **target specific ions**. Furthermore the surface-properties of quantum dots allow relatively easy modification for binding of antibodies or even directly to target proteins.

Quantum dots provide very advantageous fluorescent characteristics; namely a high **photostability**, allowing for prolonged monitoring, wide excitation spectra, giving **flexibility in the excitation light source** and narrow emission granting good distinction of the target ion from other acceptors.

In bulk measurements a **single ion proves sufficient to yield a clear signal** from a quantum dot.

A **single quantum dot can be monitored** using confocal microscopy.

References:
[1] Liu J, Sitaram A, Burd CG. "Regulation of Copper-Dependent Endocytosis and Vacuolar Degradation of the Yeast Copper Transporter, Ctr1p, by the Rsp5 Ubiquitin Ligase." Traffic. 2007
[2] Birch DJS, Holmes AS & Darbyshire M. "Intelligent Sensor for Metal Ions based on Fluorescence Resonance Energy Transfer" Meas. Sci. Technol. 1995

The work presented here is kindly supported by the EPSRC Engineering and Physical Sciences Research Council

SUPA The Scottish Universities Physics Alliance