Marie Curie project TRAMLANES (PI: Prof. M. Ward, Sheffield UK. Fellow: Dr. Atanu Jana) – FINAL REPORT

PART 1: transition-metal / lanthanide complexes for multi-modal cellular imaging

Phosphorescent metal complexes offer major advantages over conventional fluorescent organic molecules as the basis of luminescent probes for cell imaging. The long luminescence lifetimes associated with triplet emission from complexes of *e.g.* Pt(II), Ru(II), Ir(III), Re(I) and lanthanides allow simple rejection of short-lived background autofluorescence which might otherwise interfere. In addition, *variations* in luminescence lifetimes of such complexes in different cellular regions, caused by the presence of different analytes such as O_2, provide the basis of the recently-developed microsecond lifetime mapping techniques; phosphorescence lifetime imaging (PLIM) and time-resolved emission microscopy (TREM).

We developed a new rigid and conjugated ligand structure **Ir•Ln$_x$** (where, x = 1 and 2 respectively) connecting phenanthroline and poly(amino-carboxylate) binding sites (see Chart 1) to provide mixed d/f complexes (transition metal + lanthanide). These show high potential for use in dual modal (PLIM and magnetic resonance) imaging, and well as for luminescence imaging using either luminescence component independently. Several features of these carefully-drsigned compounds make them valuable for use in these ways. In these complexes, a strongly phosphorescent Ir(III) unit connected to a water-stable Ln(III) unit *via* a fully conjugated and rigid connector. This results in both (i) long-lived luminescence which can be used in PLIM imaging under one-photon or two-photon excitation, and (ii) unusually long relaxivity from a single Gd(III) centre as a consequence of the rigid design. The combination of Ir(III) and Gd(III) components for dual-imaging purposes has been very little explored and this report is the first demonstration of PLIM using a complex that also has high relaxivity for MRI (the relaxivity of **Ir•Gd** is 11.9 $mM^{-1}s^{-1}$ which is much more higher than the other reported values in current literature) purposes. The same ligand architecture also provides an effective through-bond coupling pathway for efficient Dexter Ir(III)→Eu(III) energy-transfer (EnT) in the isostructural **Ir•Eu** complex. Dual-luminescent d/f complexes are of interest for a range of applications from imaging to white-light emission and many of these applications hinge on the extent of d→f EnT which controls the balance of luminescence output from the two components.

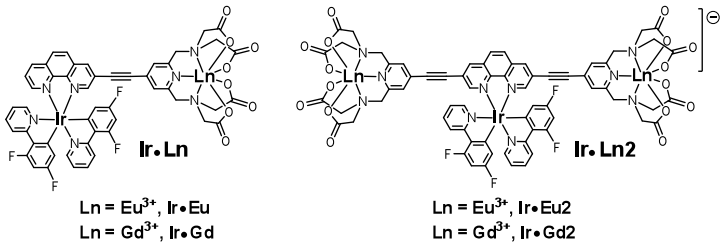

Chart 1. Structures of the luminescent probes.

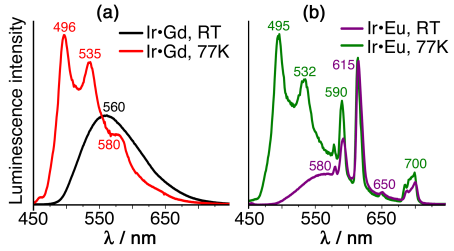

Figure 1: Luminescence spectra of DYADS.

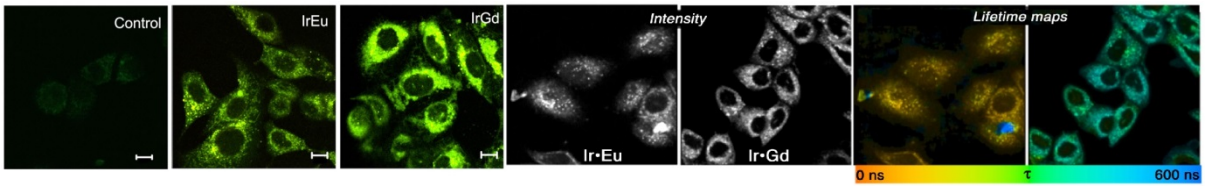

Figure 2: Luminescence images of MCF-7 cell lines incubated with DYADS and their lifetime maps.

Socio-economic impact: The value of these compounds is in healthcare: compounds of this nature that alow te bodym or tissue samples, to be imaged with high resolution can be used for diagnosis. MRI gives high anatomical resolution and deep tissue penetration, but lacks sensitivity. On the other hand, optical imaging gives high sensitivity but has limited tissue penetration. The combination of these two techniques will provide a more complete picture of the biological area of interest. Therefore, the new molecular architectures which demonstrates dual modal imaging approach could be promising probes for medicinal applications where the combination of two different imaging modes will provide two types of complementary imaging information from a single molecule.

(a) **Atanu Jana**, Elizabeth Baggaley, Angelo Amoroso, Michael D. Ward, *Chemical Communications*, **2015**, *51*, 8833–8836; (b) **Atanu Jana**, Elizabeth Baggaley, Angelo Amoroso, Michael D. Ward, *Manuscript under preparation.*

Part 2: Mixed porphyrin / platinum(II) dinuclear compleses for multi-modal cellular imaging

Singlet oxygen ($^1O_2$) is a reactive species highly desired for many medicinal applications including photodynamic therapy (PDT) because of its high oxidative ability. A photosensitizer is necessary to produce $^1O_2$ because direct excitation from triplet molecular oxygen ($^3O_2$) to $^1O_2$ is energetically forbidden. The porphyrin derivatives are the most widely used photosensitizers because of their high singlet oxygen quantum yield (Φ_Δ) and large molar extinction coefficient values. However, it is still challenging to produce effective $^1O_2$ photosensitizers for PDT drug with much higher efficiency and light-capture capability in the therapeutic window. I developed a series of novel porphyrin Pt(II)C^N^N-acetylides (see Chart 2) which are efficient photosensitizers for $^1O_2$ generation which could be a promising candidate sfor biomedical research.

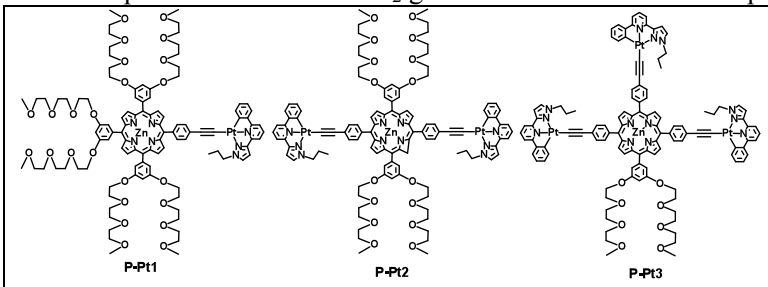

Chart 2: Structures of the porphyrin-Pt(II)-acetylide complexes.

All these compounds show good (Φ = 0.55 to 0.63) singlet oxygen generation efficiency. Moreover due to the presence of a strong fluorophoric unit (porphyrin) and heavy metal atoms (Pt^{2+}), these compounds were also used for dual modal imaging purposes (luminescence + transmission electron microscopy, TEM) to get a better idea on intracellular imaging and cellular localization.

As with the compounds produced in part 1, these will be of particular value as they combine several different imaging methods: they allow cells to be visualised by florescence microscopy (using the porphyrin unit) and by transmission electron microscopy (using the heavt Pt atoms). In addition their ability to generate cytotoxic singlet oxygen will allow them to have a therapeutic, as well as an imaging, functional behaviour which is very unusual.

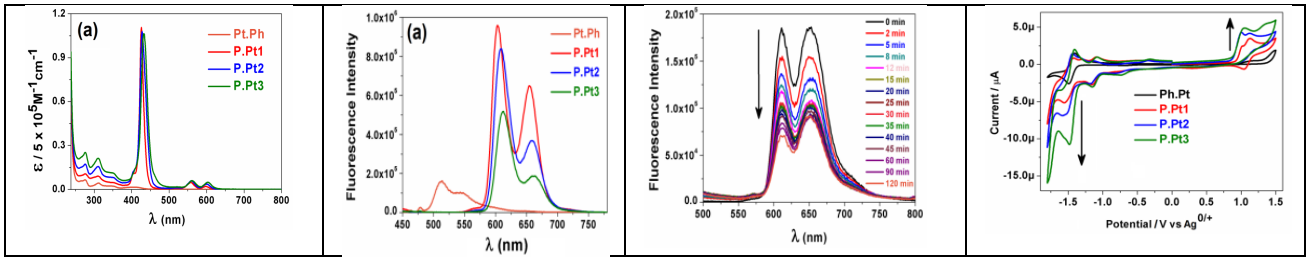

Figure 3: Photophysical and electrochemical data for the porphyrin-Pt(II)-acetylides.

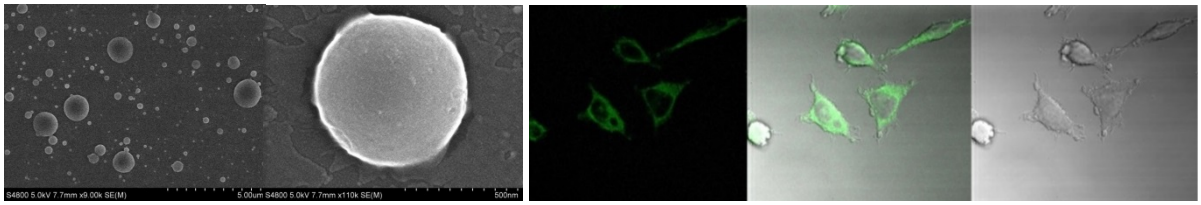

Figure 4: Electron-microscopy images of **P.Pt1** showing aggregation in aqueous medium (LHS); Two-photon fluorescence images of HeLa cell lines incubated with **P.Pt3**.

Socio-economic impact: It is still challenging for the chemists to produce efficient photosensitizer for biomedical research. Many of the compounds are not perfect for their poor aqueous solubility. Therefore, I demonstrate a new kind of porphyrin based supramolecular system containing heavy metal atoms which could be promising alternative for singlet oxygen production and imaging purposes. Still it is in very early stage and I am trying to make these systems much more water soluble by using different functional groups for an effective biological application.

Atanu Jana, Luke McKenzie, Jonathan Shewring, Masatoshi Ishida, Jonathan P. Hill, Julia A. Weinstein, Michael D. Ward, *Manuscript under preparation.*