

## Supplementary data

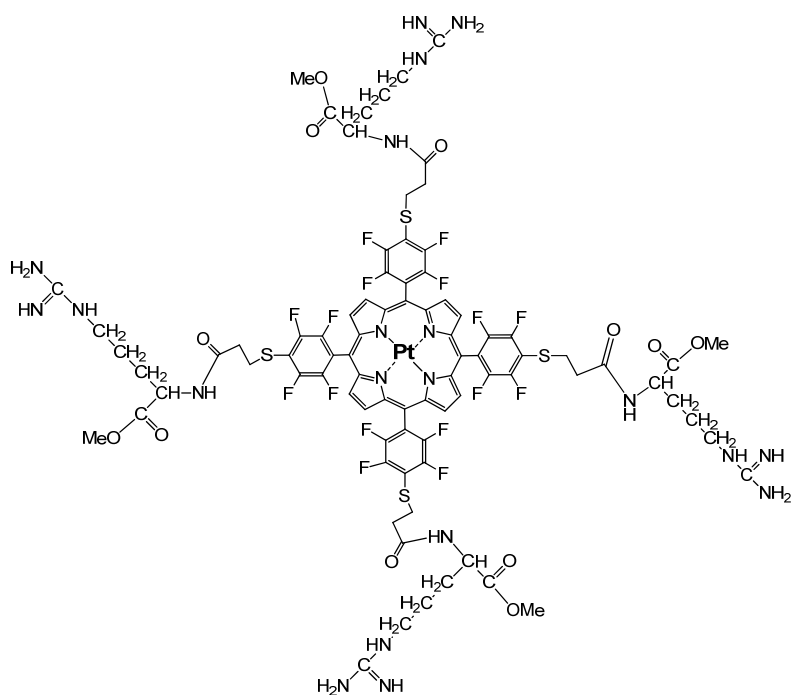
### SMALL MOLECULE PHOSPHORESCENT PROBES FOR O<sub>2</sub> IMAGING IN 3D TISSUE MODELS

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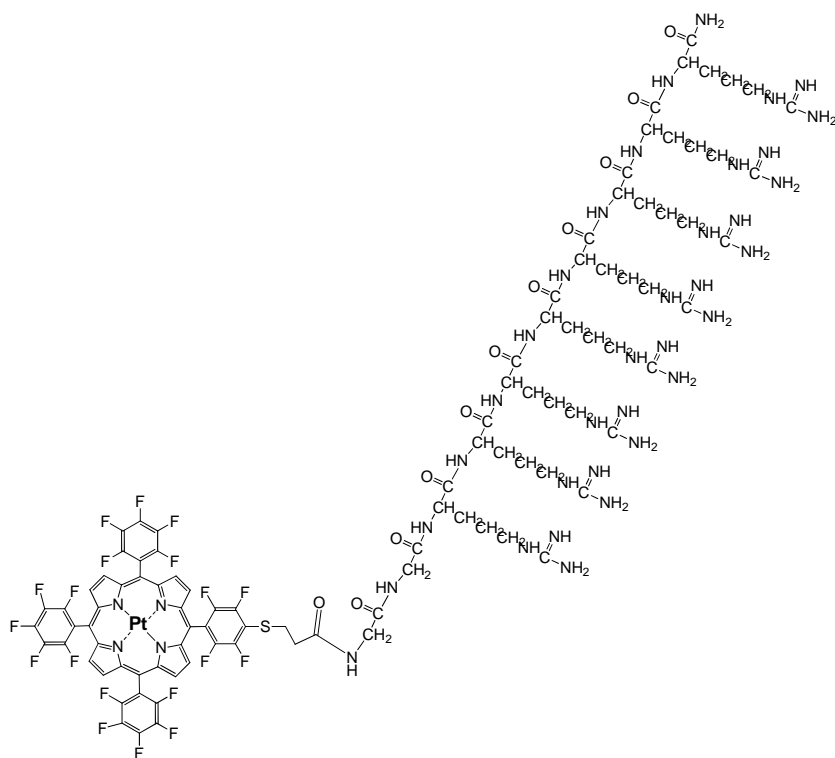
#### Contents.

1. Chemical structures of conjugates
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## 1. Chemical structures of PtPFPP conjugates

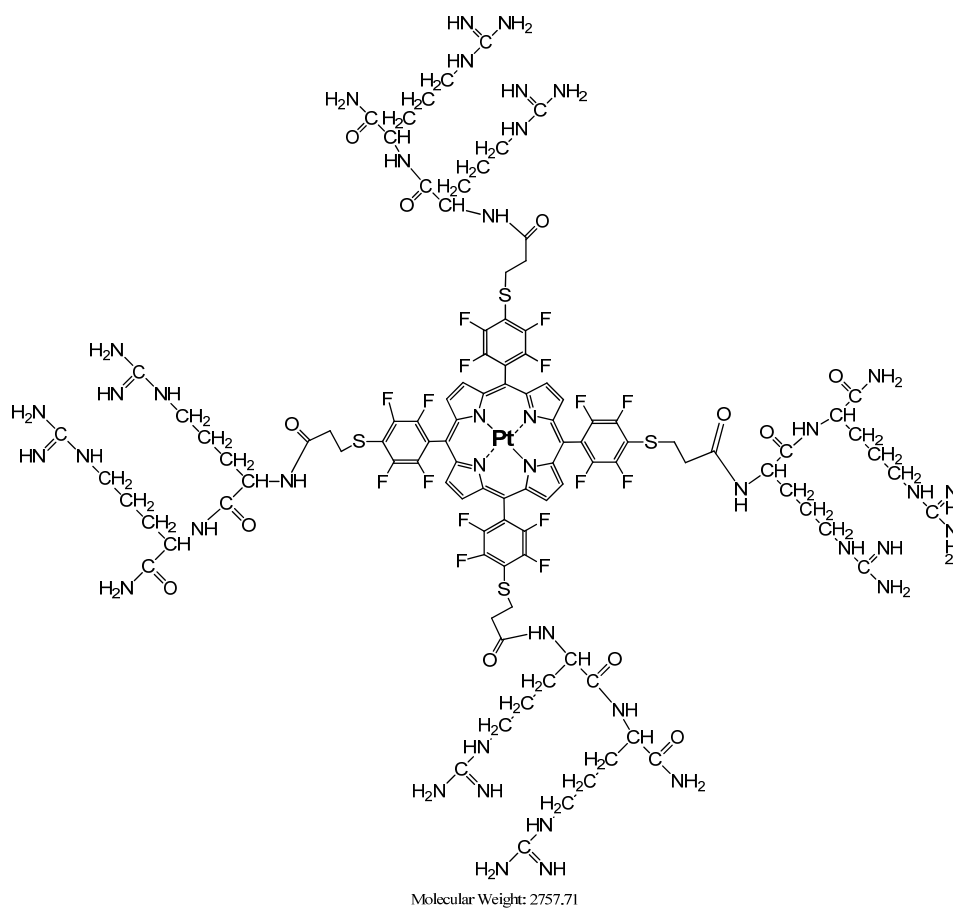


**Conjugate 1.** 4 x Arg (branched)

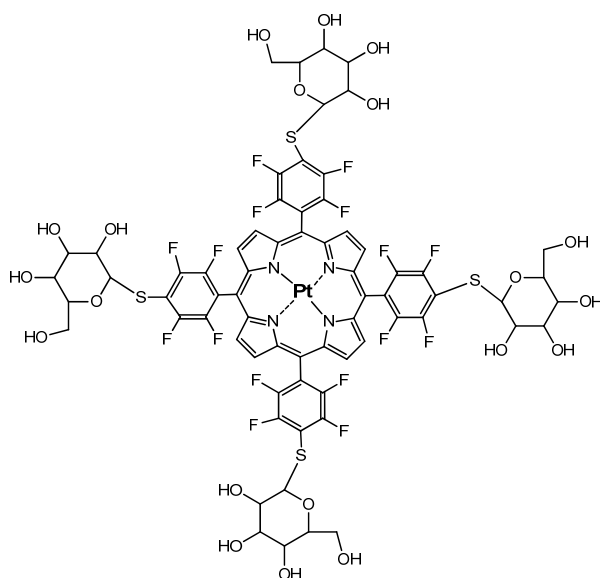


Molecular Weight: 2616.35

**Conjugate 2.** 8 x Arg (linear)



**Conjugate 3.** 8 x Arg (branched)

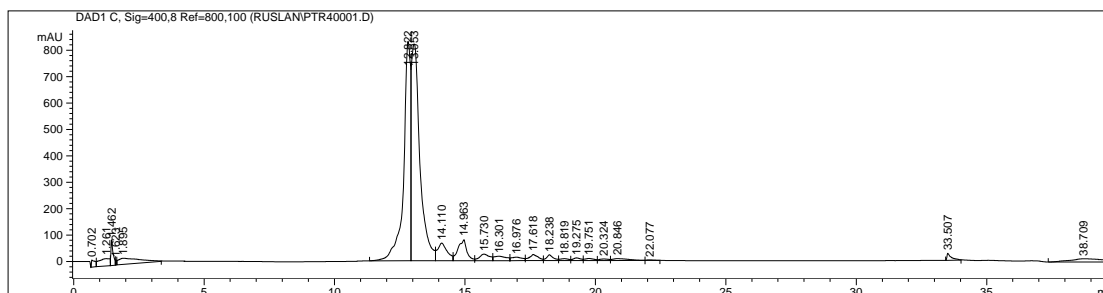


**Conjugate 4.** 4 x Glc (Gal) (branched)

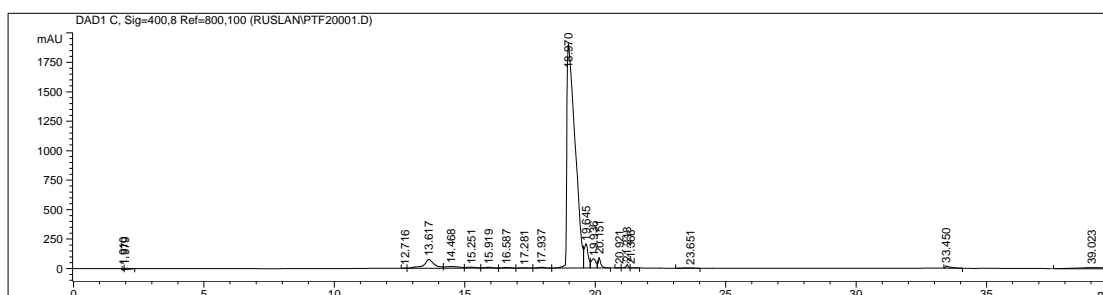
## 2. RP-HPLC analysis of PtPFPP conjugates

HPLC was performed on Zorbax Eclipse XDB-C18 column (5  $\mu$ m, 4.6 x 15mm, Agilent) using water - MeOH/0.1% TFA gradient. Signals represent absorbance at 400 nm.

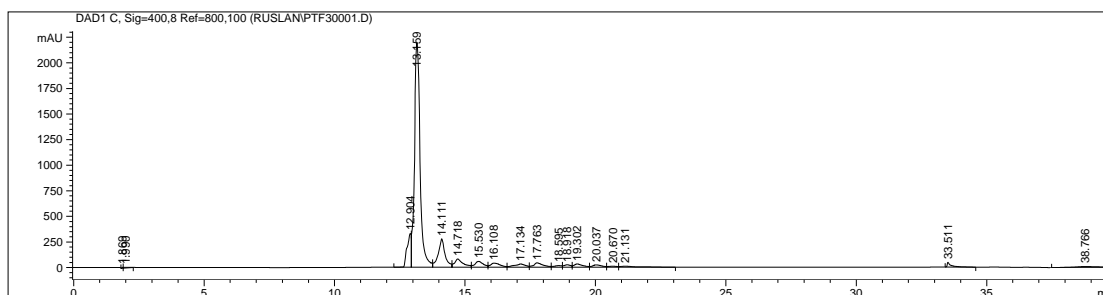
### Conjugate 1. 4 x Arg (branched)



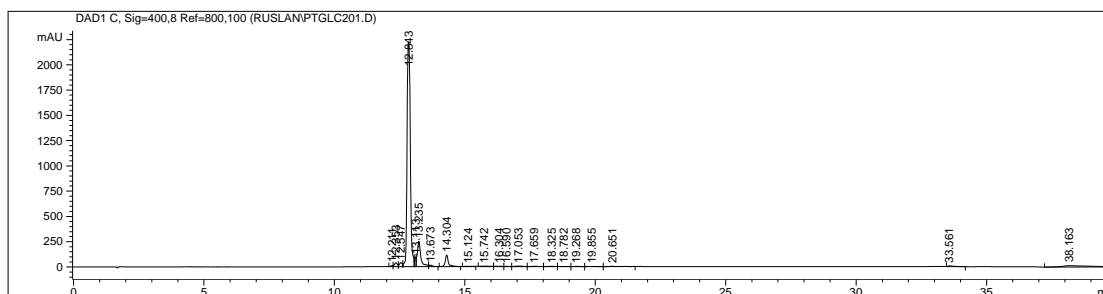
### Conjugate 2. 8 x Arg (linear)



### Conjugate 3. 8 x Arg (branched)

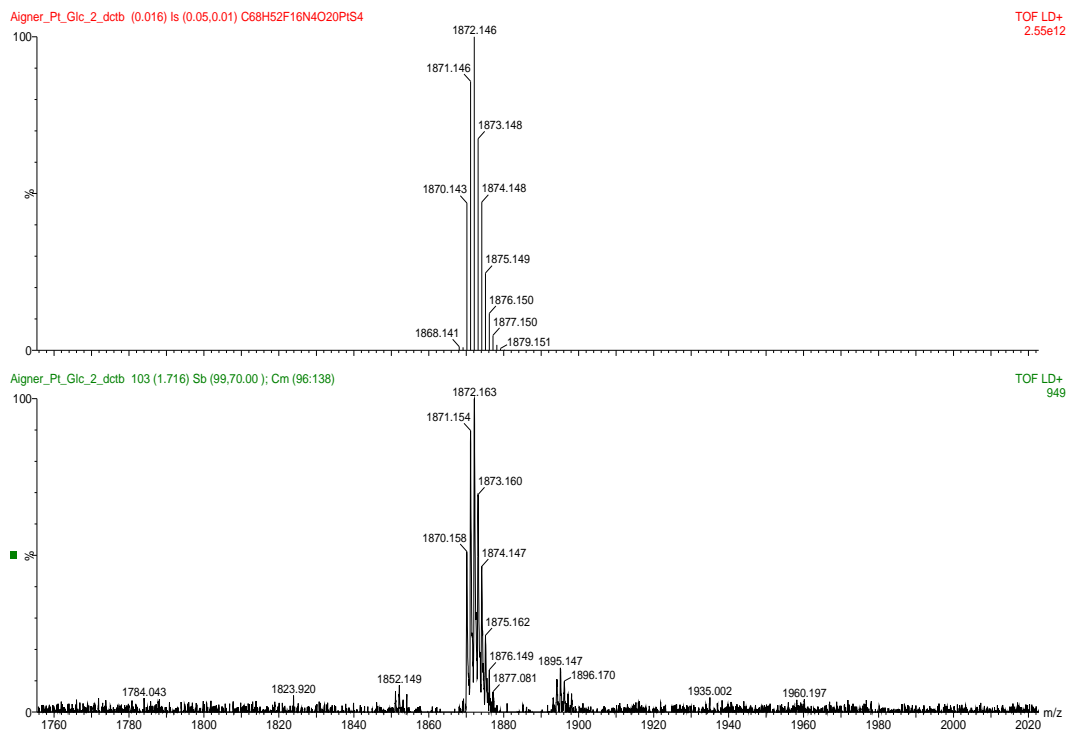


### Conjugate 4. 4 x Glc (branched)

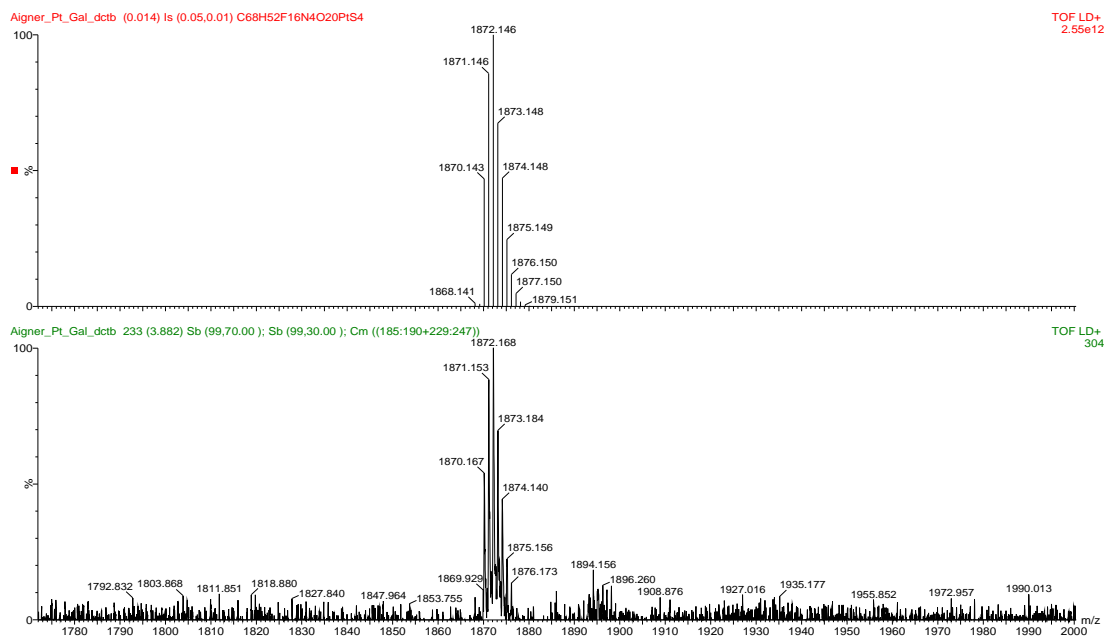


### 3. MALDI-TOF analysis of conjugate 4.

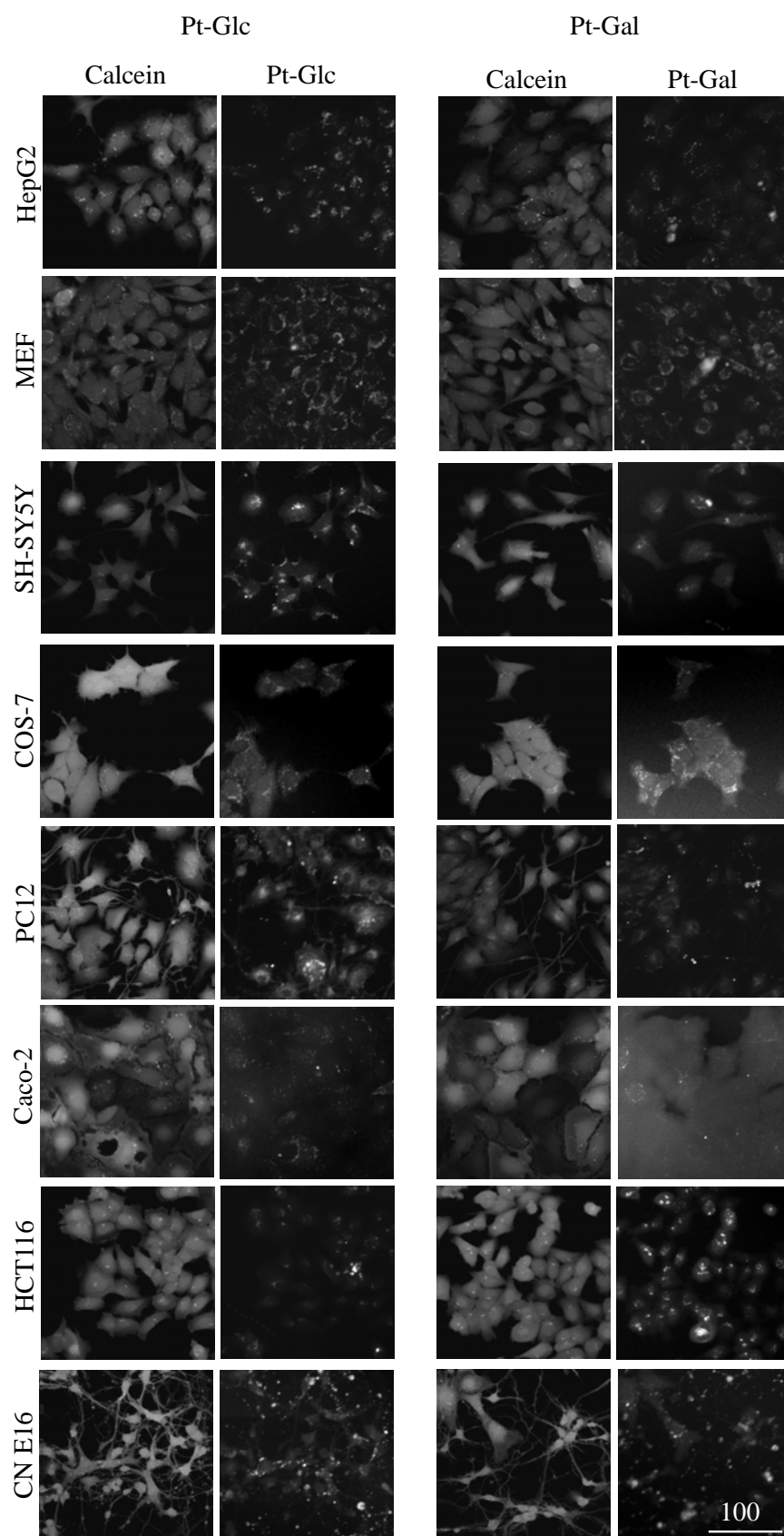
#### 4xGlc (branched). "Pt-Glc"



#### 4 x Gal (branched). "Pt-Gal"

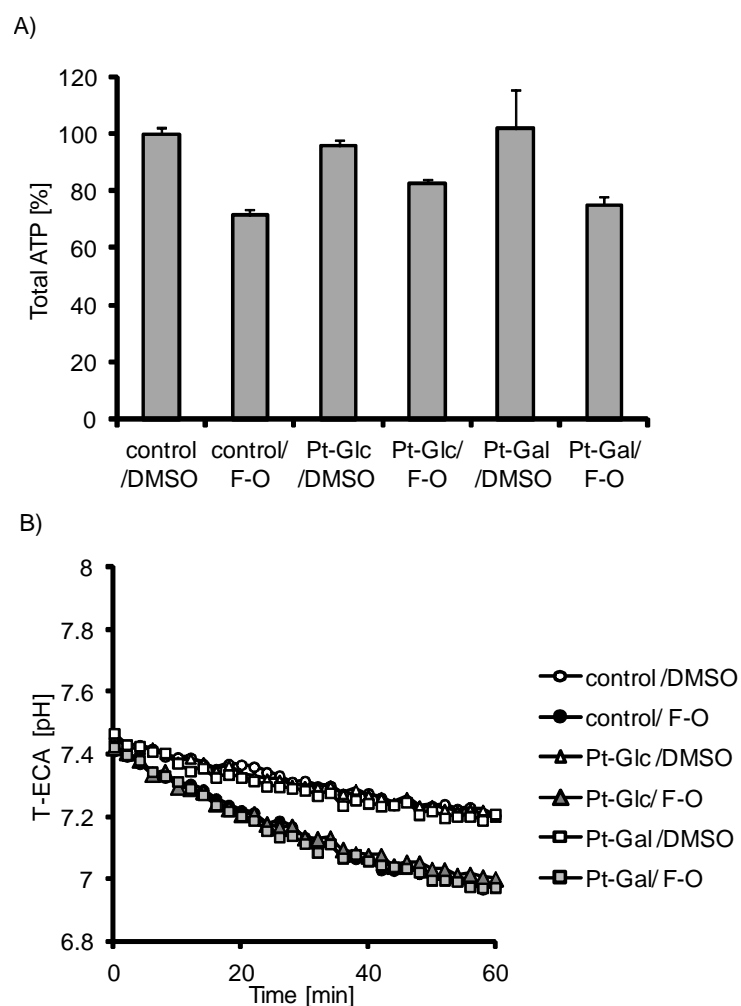


#### 4. Supplementary figures



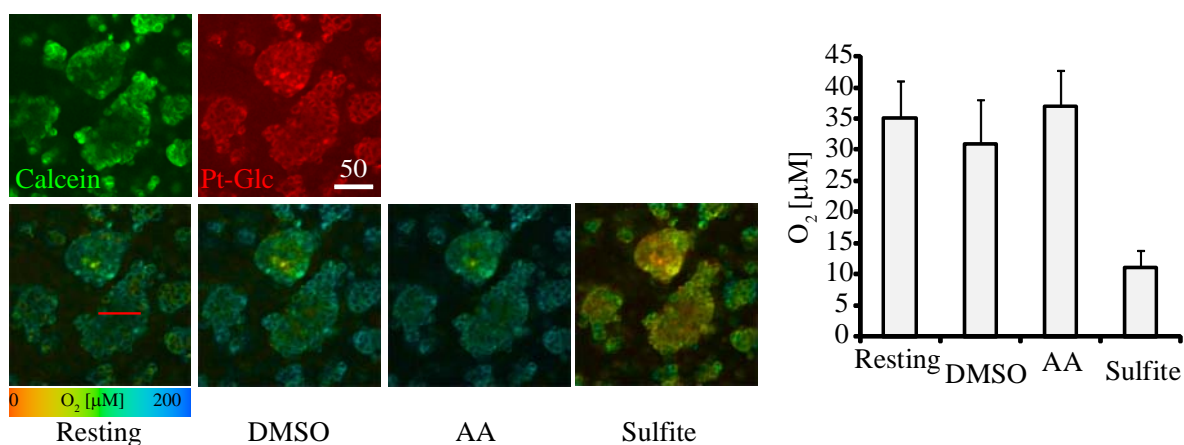
**Fig. S1. Staining of different cell types with Pt-Glc and Pt-Gal conjugates assessed by fluorescence microscopy.** Cells were stained with conjugates (2.5  $\mu$ M, 16 h), counter-stained with

Calcein Green (1  $\mu\text{M}$ , 0.5 h), treated with antimycin A (5  $\mu\text{M}$ ) and analysed by confocal TCSPC-PLIM. Single optical sections are shown. Scale bar unit is  $\mu\text{m}$ .

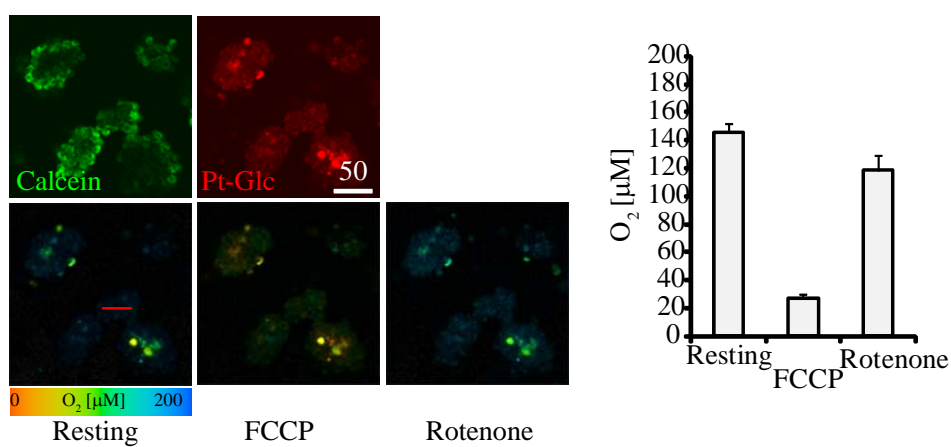


**Fig. S2.** Effects of conjugates (5  $\mu\text{M}$ , 16 h) on total cellular ATP (A) and total extracellular acidification (T-ECA, sealed system, B) analysed under resting (DMSO) and stimulated (maximal OxPhos activity, F-O) conditions.

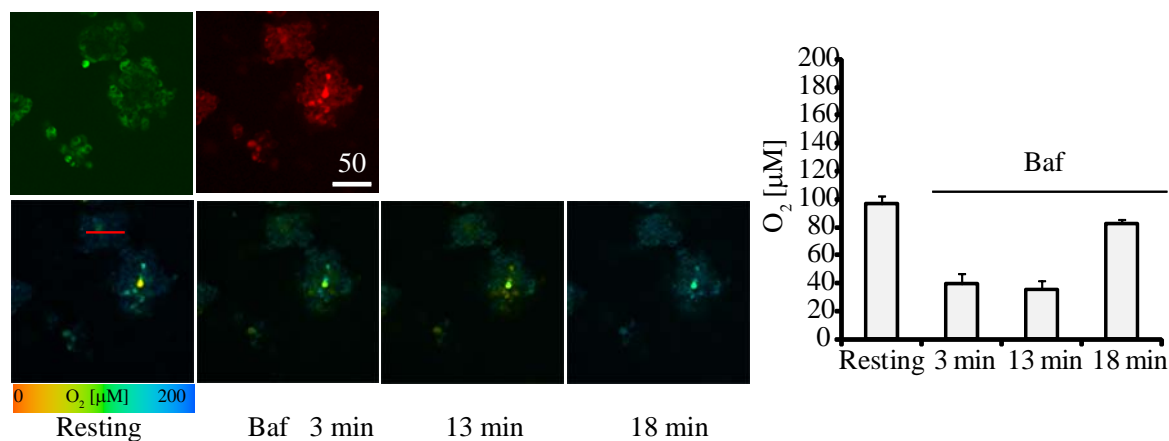
A)



B)

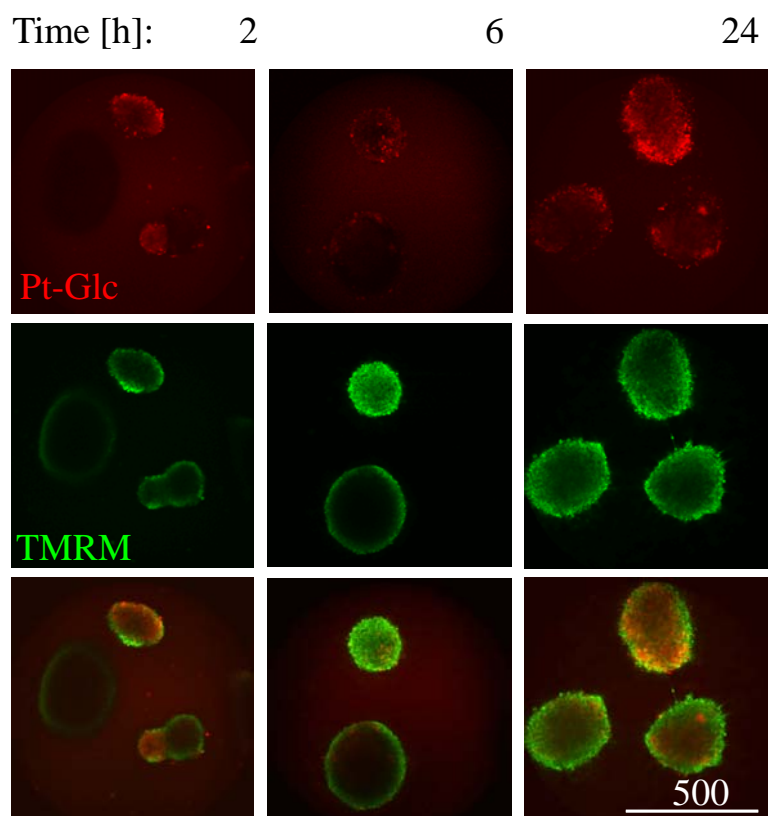


C)

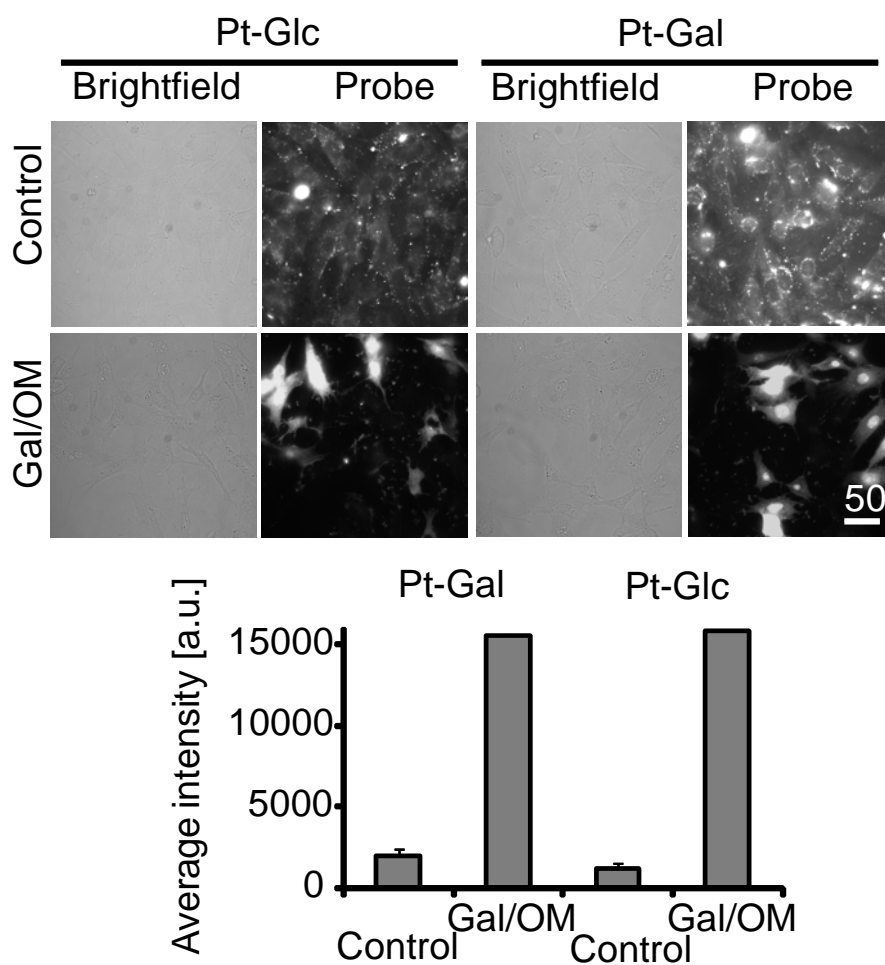


**Fig. S3. Monitoring of oxygenation of PC12 tumour spheroids under treatments and metabolic stimulations.** Cells were stained with Pt-Glc conjugate (2.5 μM, 16 h) and measured on confocal TCSPC PLIM microscope. Fluorescence images of calcein green and Pt-Glc (top rows) along with PLIM (bottom rows) are shown for each experiment. Averaged O<sub>2</sub> concentrations for selected line profiles (red arrows) are shown on right. A: consecutive treatment with mock (DMSO), antimycin A (10 μM, AA) and sulfite. B: consecutive treatment with FCCP (4 μM) and rotenone (2.5 μM). C: time-course of cells response to treatment with 0.25 μM bafilomycin A1 (Baf). Scale bar unit is μm. N=2.





**Fig. S4.** Kinetics of neurosphere staining with Pt-Glc conjugate (1  $\mu\text{M}$ ) in comparison with TMRM (20 nM). Scale bar unit is  $\mu\text{m}$ .



**Fig. S5. Effect of energy stress (glucose deprivation and inhibition of mitochondrial function) on the intracellular uptake of Pt-Glc and Pt-Gal.** MEF cells were pre-incubated in Glc-free medium, containing 10 mM Gal, then treated with oligomycin (10  $\mu$ M) to block energy production and then stained with Pt-Glc or Pt-Gal (2.5  $\mu$ M, 3 h), compared with control conditions (no energy depletion). Top: wide-field fluorescence microscopy images. Bottom: average staining efficiency calculated. Scale bar unit in  $\mu$ m.