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Ratiometric detection of enzyme turnover and flavin reduction using rare-earth upconverting phosphors

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Supporting Information

General Experimental Details

All chemicals were purchased from Sigma-Aldrich and used as supplied. PETNR was prepared, purified and its activity tested according to a literature procedure[1] and the upconverting phosphors were kindly donated by Phosphor Technology Ltd.[2]

UV-visible spectra were recorded on a Cary 60 spectrometer (Agilent) using quartz cuvettes.

Steady state emission spectra were recorded in quartz cuvettes on an Edinburgh Instrument FP920 Phosphorescence Lifetime Spectrometer equipped with a 5 watt microsecond pulsed xenon flashlamp (with single 300 mm focal length excitation and emission monochromators in Czerny Turner configuration) and a red sensitive photomultiplier in peltier (air cooled) housing, (Hamamatsu R928P) and a custom built (Edinburgh Instruments) 980 nm diode laser operating in either continuous wave mode or pulsed mode, with variable repetition rate. Lifetime data were recorded following 980 nm excitation in pulsed mode using time correlated single photon counting (PCS900 plug-in PC card for fast photon counting). Lifetimes were obtained by tail fit on the data obtained, and the quality of fit judged by minimization of reduced chi-squared and residuals squared.

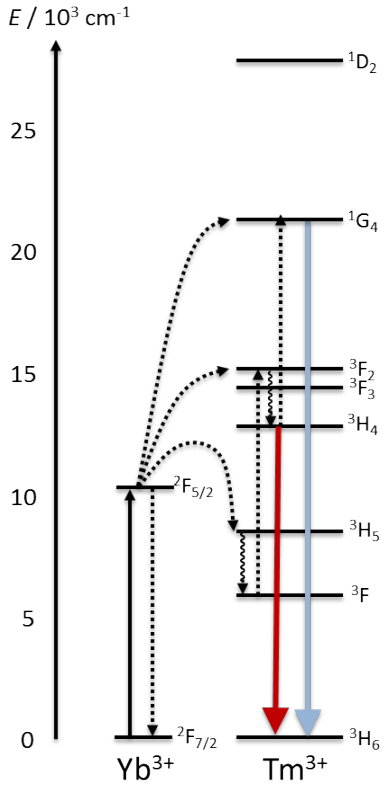

Fig. S1 Energy transfer upconversion scheme for PTIR 475 UCPs (λ_{exc} = 980 nm) to populate the $^1G_4$ and $^3H_4$ levels of Tm^{3+}.

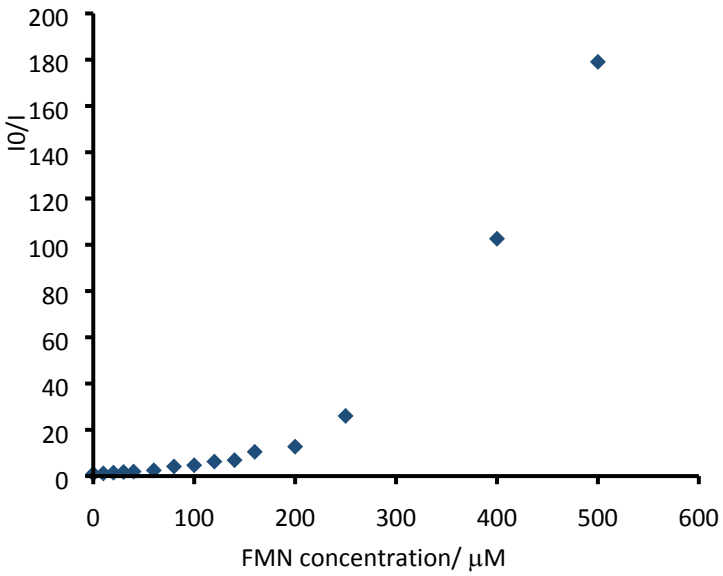

Fig. S2 Stern-Volmer analysis of the 475 to 800 nm emission intensity ratio upon FMN addition to an aqueous solution of UCPs (0.1 mg/mL), where I_0 is the emission intensity before enzyme addition and I is the intensity upon x µM addition of FMN.. Each value is the average of 3 runs. Estimated error 10%.

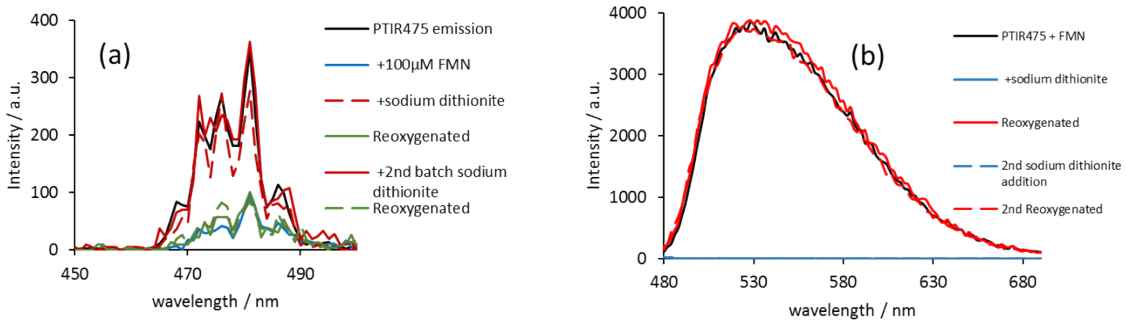

Fig. S3 Repetitive reduction and oxidation of FMN in the presence of PTIR 475 nanoparticles observed by UCP emission ((a) λ_{exc} = 980 nm) and flavin emission ((b) λ_{exc} = 475 nm).

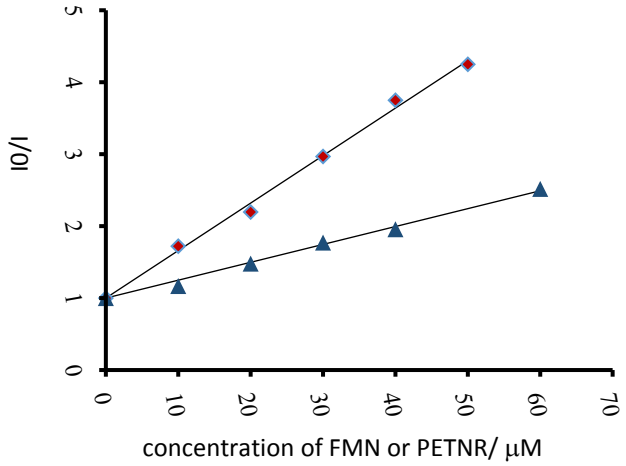

Fig. S4 Comparison of the Stern-Volmer plots for addition FMN (blue triangles) and PETNR (red diamonds) to an aqueous solution of UCPs (0.1 mg/mL) over the range 0-60 μM (PETNR: Ksv = 0.066 μM^{-1}, R^2 = 0.994; FMN: Ksv = 0.026 μM^{-1}, R^2 = 0.994). Analysis of the 475 to 800 nm emission intensity ratio as a function of added FMN/PETNR concentration, where I_0 is the emission intensity before enzyme addition and I is the intensity upon x μM addition. Each value is the average of 3 runs. Estimated error 10%.

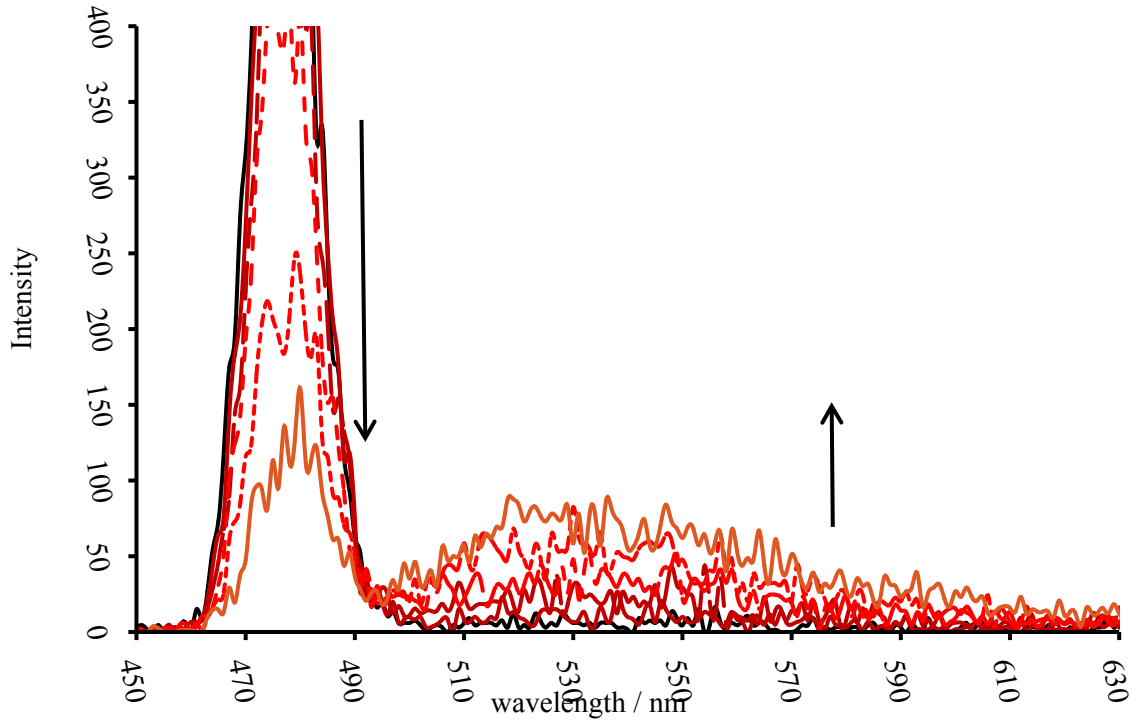

Fig. S5 Incremental addition of PETNR to a solution containing PTIR475 (0.01 wt%), highlighting the emergence of an emission band centred at 530 nm indicative of flavin emission as a result of FRET from the UCPs to FMN (λ_{exc} = 980 nm).

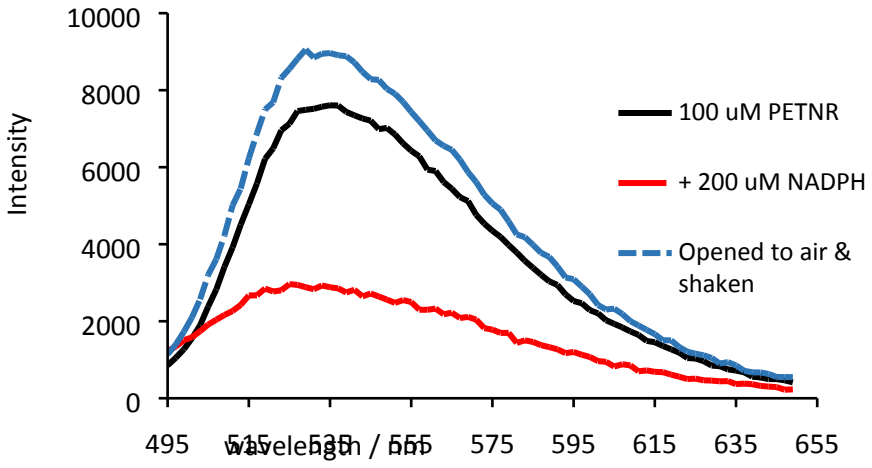

Fig. S6 PETNR emission (λ_{exc} = 475 nm) in anaerobic buffer solution in the presence of PTIR475 UCPs before (black line) and after (red dashed line) addition of NADPH, followed by reoxygenation in air (blue dashed line).

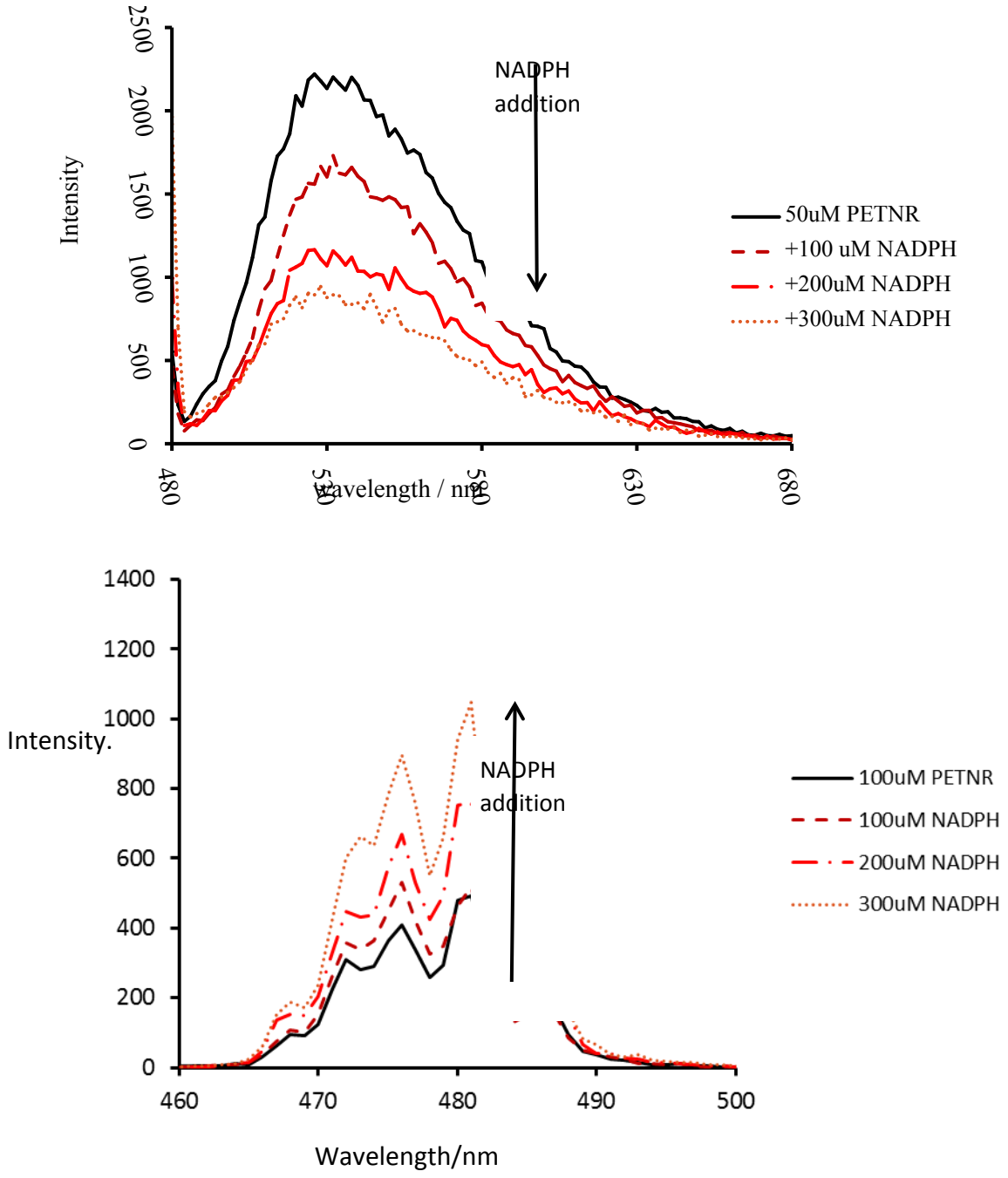

Fig. S7 Incremental addition of NADPH to an anaerobic solution containing PTIR475 (0.01 wt%) and PETNR (100 μM) showing the decrease in flavin emission as the enzyme is reduced; (top) λ_{exc} = 475 nm, and change in Tm(III) emission (bottom) λ_{exc} = 980 nm .

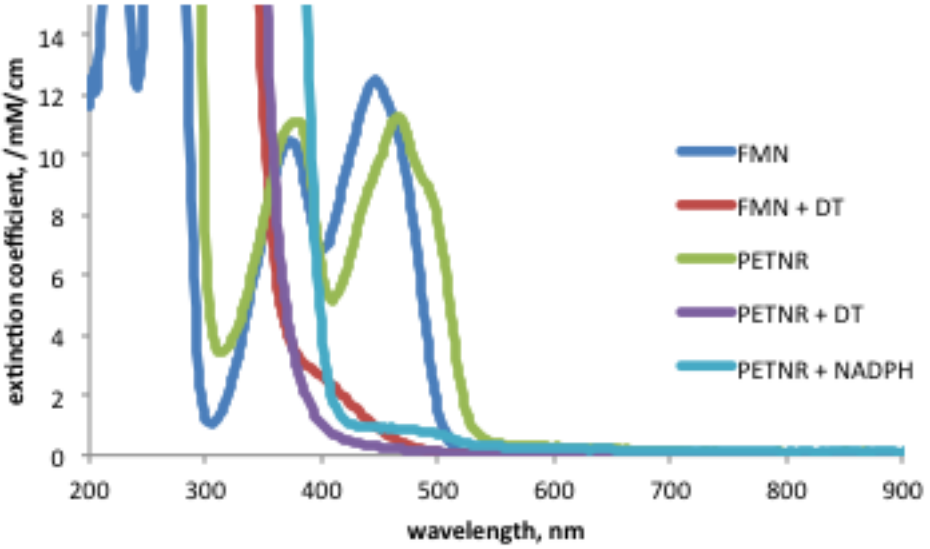

Fig. S8 Absorption spectra of oxidised FMN (dark blue trace), oxidised PETNR (green trace), dithionite reduced FMN (red trace), dithionite reduced PETNR (purple trace) and NADPH reduced PETNR (light blue trace) in TRIS buffer (pH 7); DT = dithionite.

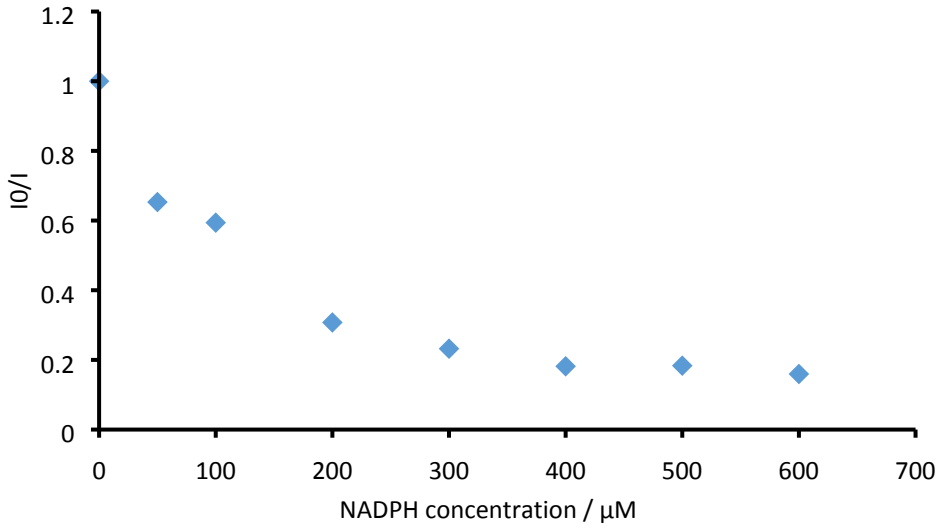

Fig. S9 I_0/I plot of the 475 to 800 nm emission intensity ratio upon the NADPH reduction of PETNR (100 µM) in an aqueous buffer solution of UCPs (0.01 wt%), where I_0 is the emission intensity before NADPH addition and I is the intensity upon x µM addition of NAPH. Each value is the average of 3 runs. Estimated error 10%.

References:

[1] H.S. Toogood, A. Fryskowska, M. Hulley, M. Sakuma, D. Mansell, G.M. Stephens, J.M. Gardiner and N.S. Scrutton, *Chem. Bio. Chem.*, 2011, **12**, 738.

[2] http://www.phosphor-technology.com