Electronic supplementary information

Ferrocifens labelled with an infrared rhenium tricarbonyl tag: synthesis, antiproliferative activity, quantification and nano IR mapping in cancer cells

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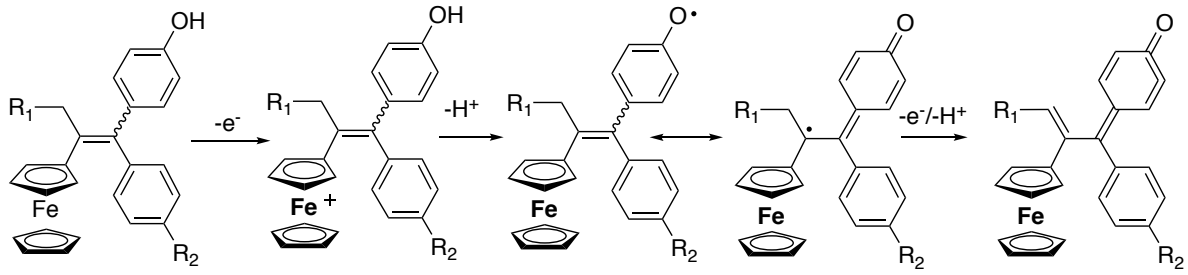
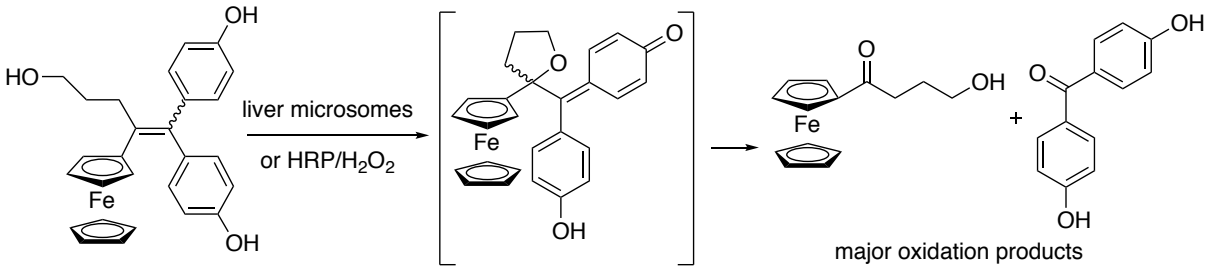


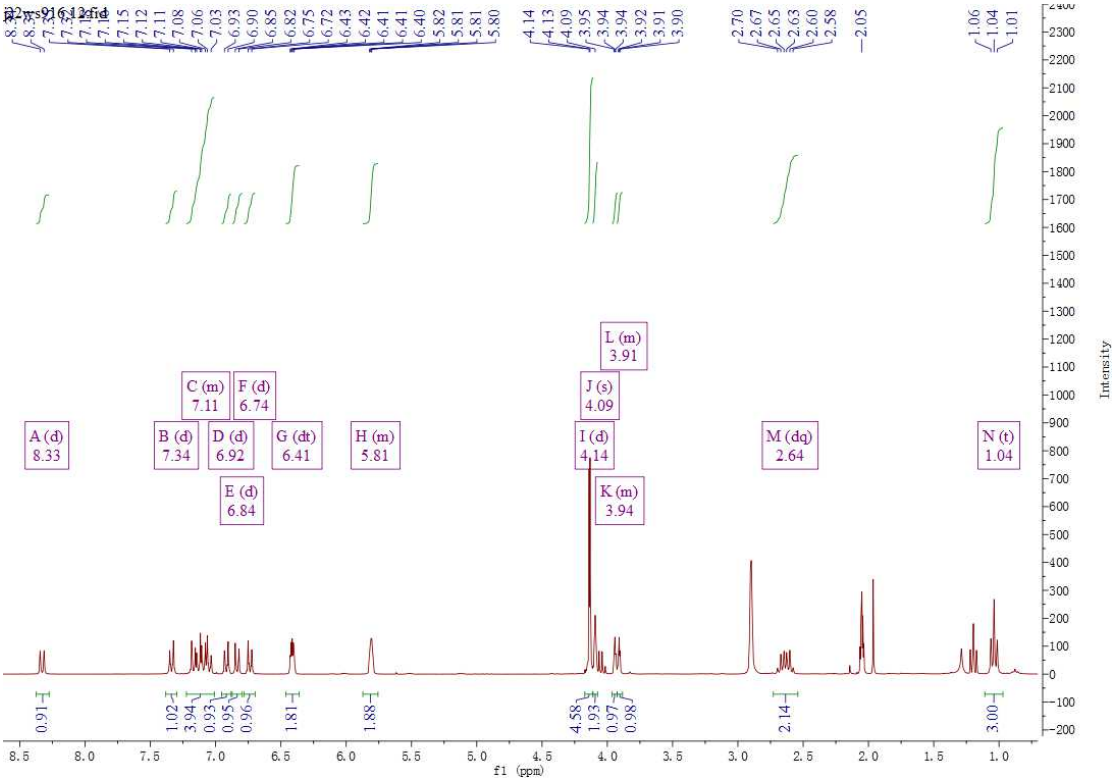
1a : $R_1 = CH_3$, $R_2 = OH$
1b : $R_1 = CH_3$, $R_2 = H$
4 : $R_1 = CH_3$, $R_2 = OCO\text{-}[\eta^5\text{-}C_5H_4)Re(CO)_3]$
6 : $R_1 = (CH_2)\text{-}OCO\text{-}[\eta^5\text{-}C_5H_4)Re(CO)_3]$, $R_2 = OH$

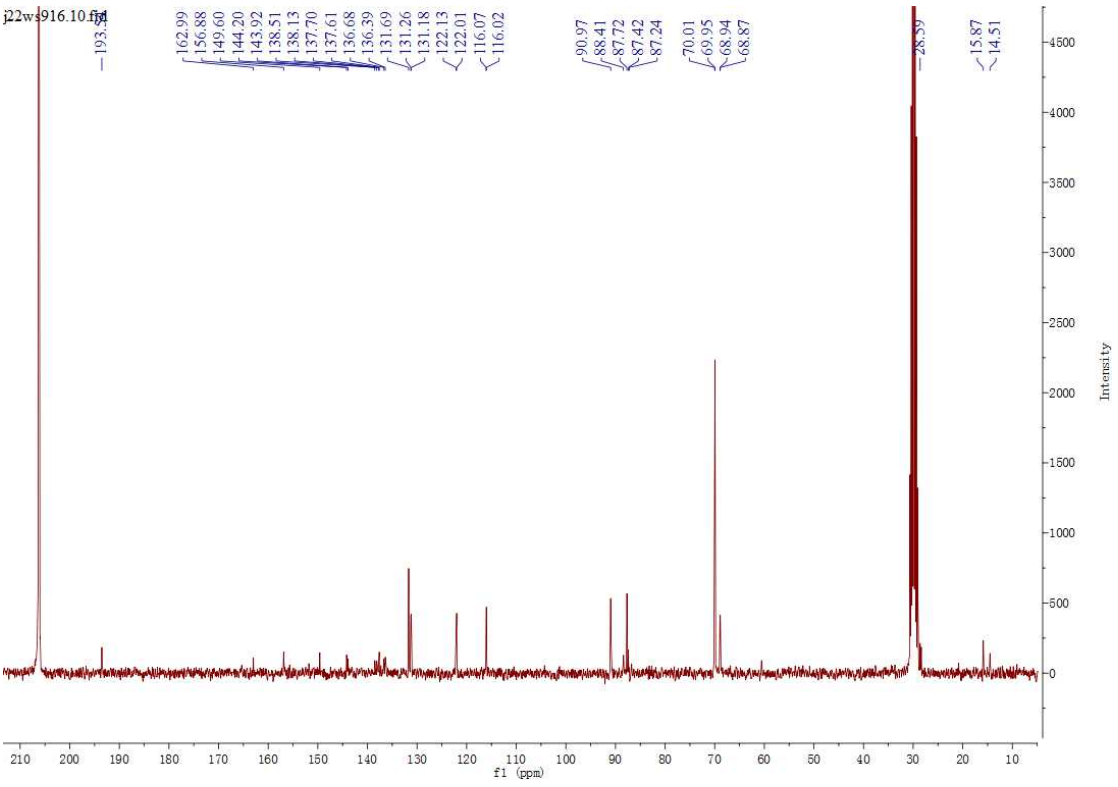
Scheme S1 : Proposed oxidation sequence of the complexes involving abstraction of 2 electrons and 2 protons and leading to the quinone methide QM (adapted from ref Hillard et al. Angew. Chem. Int. ed. 2006, 45, 285-290)



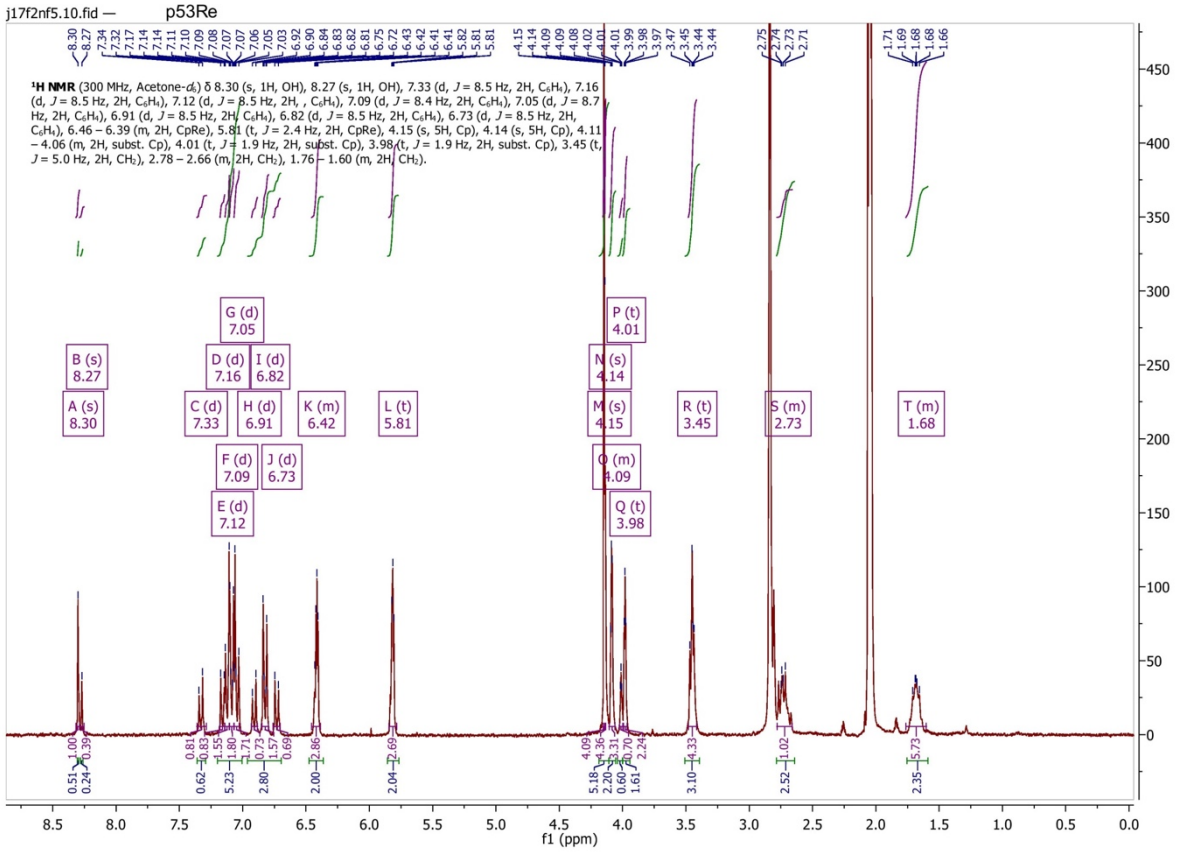
Scheme S2. Formation of the tetrahydrofuran substituted quinone methide by oxidation of **2** and its major metabolites. Adapted from ref Y. Wang et al., Chem. Sci. 2018, 9, 70 -78

NMR spectra of complex **4**

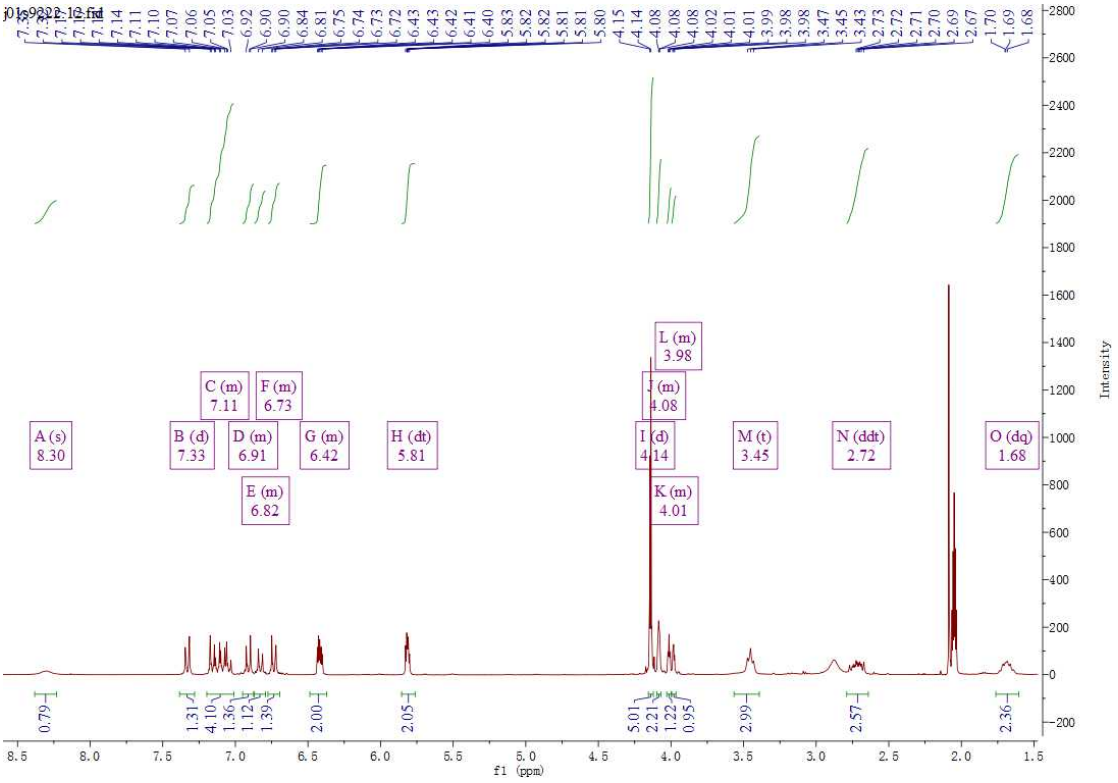


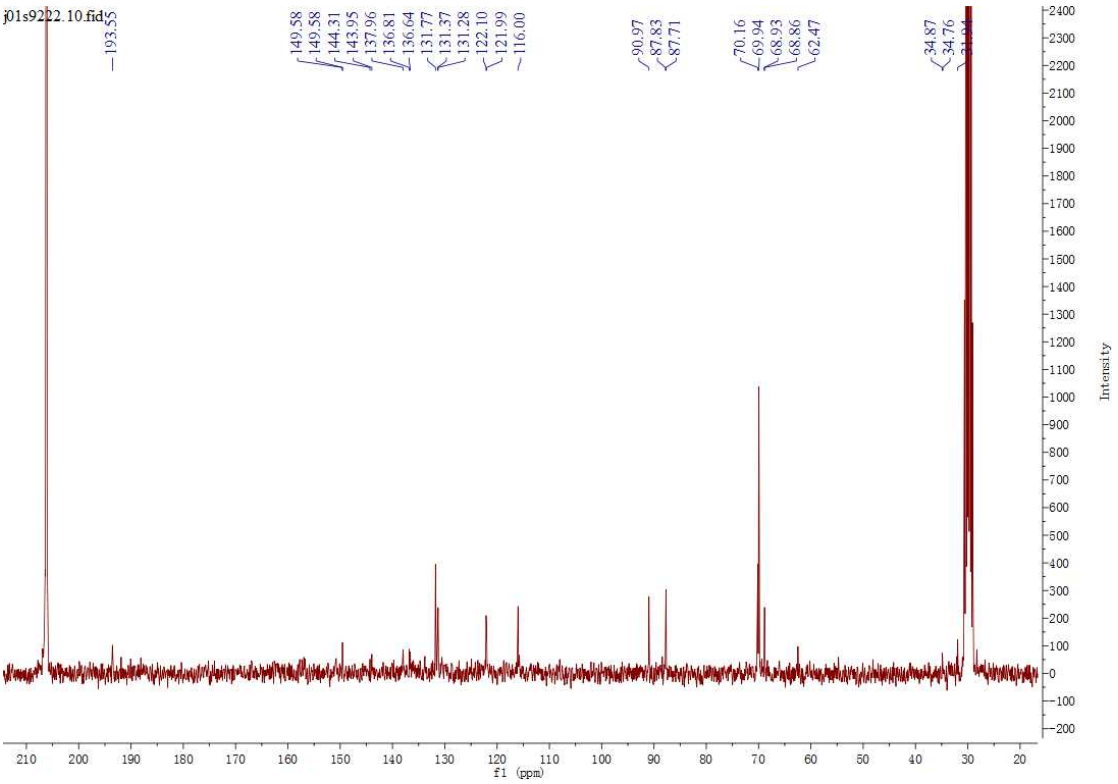


^{1}H NMR spectrum of complex **5**



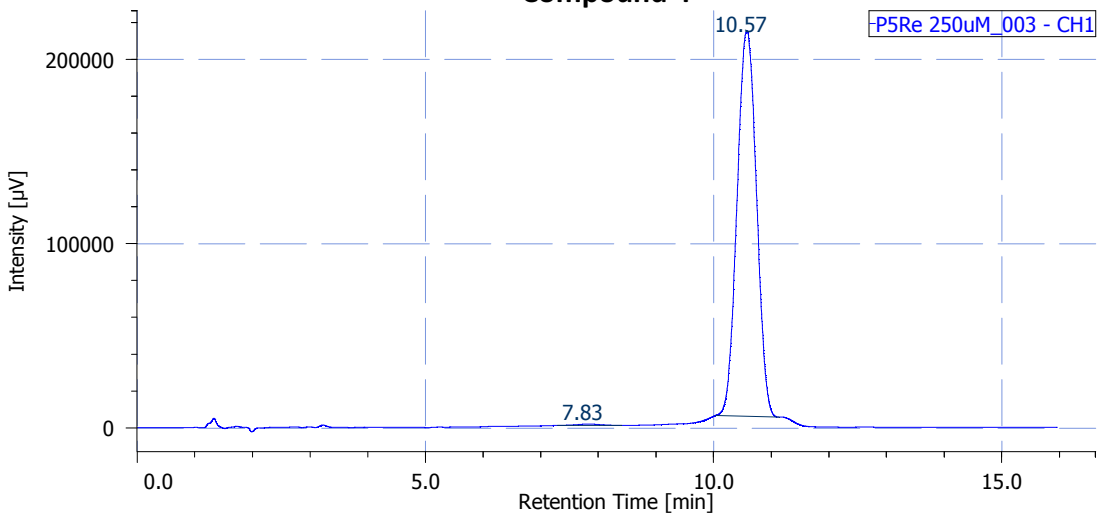
NMR spectra of complex **6**





Figures S1 – S3. RP-HPLC of complexes. Conditions: Nucleodur C18 Htec column (4.6 x 150 mm), MeOH/water (80/20) 1 ml/min, 254 nm

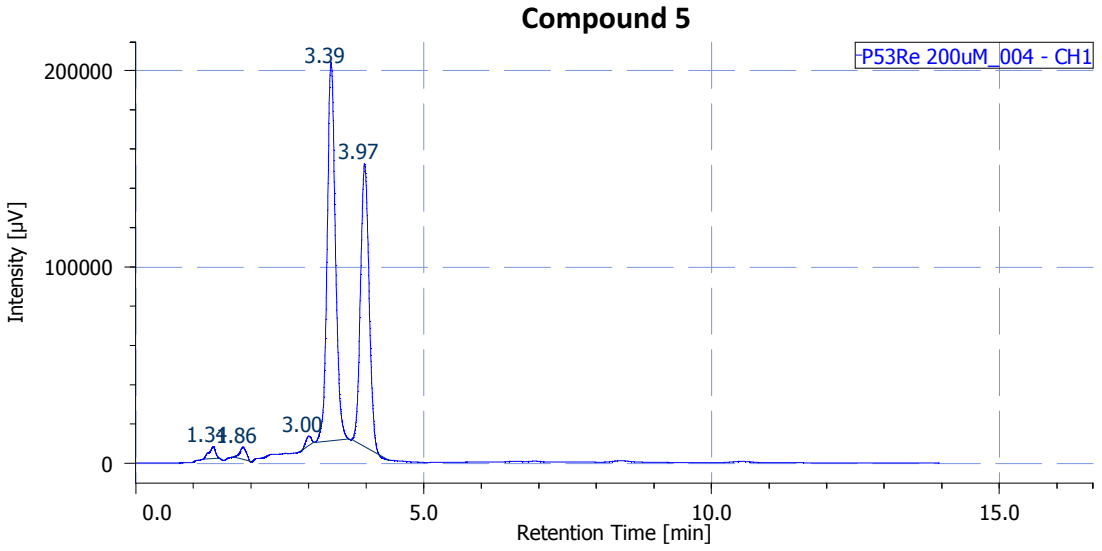
Compound 4



Chromatogram Name P5Re 250uM_003-CH1
Sample Name
Channel Name UV

#	Peak Name	CH	tR [min]	Area [µV·sec]	Height [µV]	Area%	Height%	Quantity	NTP	Resolution	Symmetry Factor	Warning
1	Unknown	1	7.83	19021	699	0.387	0.333	N/A	1930	4.073	0.951	
2	Unknown	1	10.57	4897891	209177	99.613	99.667	N/A	4426	N/A	1.017	

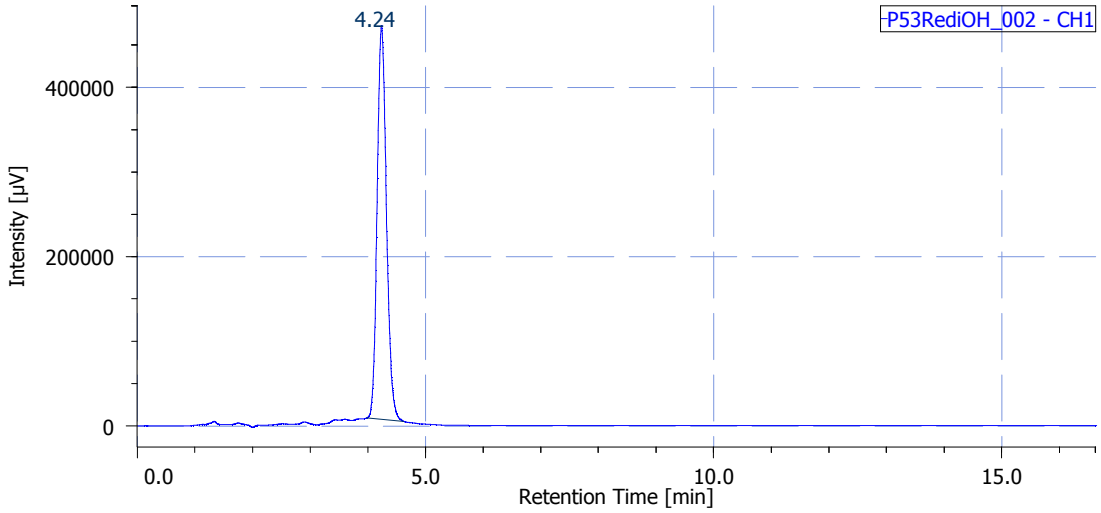
Compound 5



Chromatogram Name P53Re 200uM_004-CH1
Sample Name
Channel Name UV

#	Peak Name	CH	tR [min]	Area [µV·sec]	Height [µV]	Area%	Height%	Quantity	NTP	Resolution	Symmetry Factor	Warning
1	Unknown	1	1.34	48857	6127	1.381	1.731	N/A	538	2.333	0.803	
2	Unknown	1	1.86	49754	6205	1.406	1.754	N/A	1215	5.707	0.907	
3	Unknown	1	3.00	33577	4951	0.949	1.399	N/A	4004	1.749	0.858	
4	Unknown	1	3.39	1913586	192643	54.088	54.443	N/A	2861	2.214	1.081	
5	Unknown	1	3.97	1492157	143916	42.176	40.672	N/A	3345	N/A	1.073	

Compound 6



Channel & Peak Information Table

Chromatogram Name P53RediOH_002-CH1

Sample Name

Channel Name UV

#	Peak Name	CH	tR [min]	Area [µV·sec]	Height [µV]	Area%	Height%	Quantity	NTP	Resolution	Symmetry Factor
1	Unknown	1	4.24	5219940	464643	100.000	100.000	N/A	3342	N/A	1.149

#	Warning
1	

Figure S4. ATR-IR spectrum of complex **4**

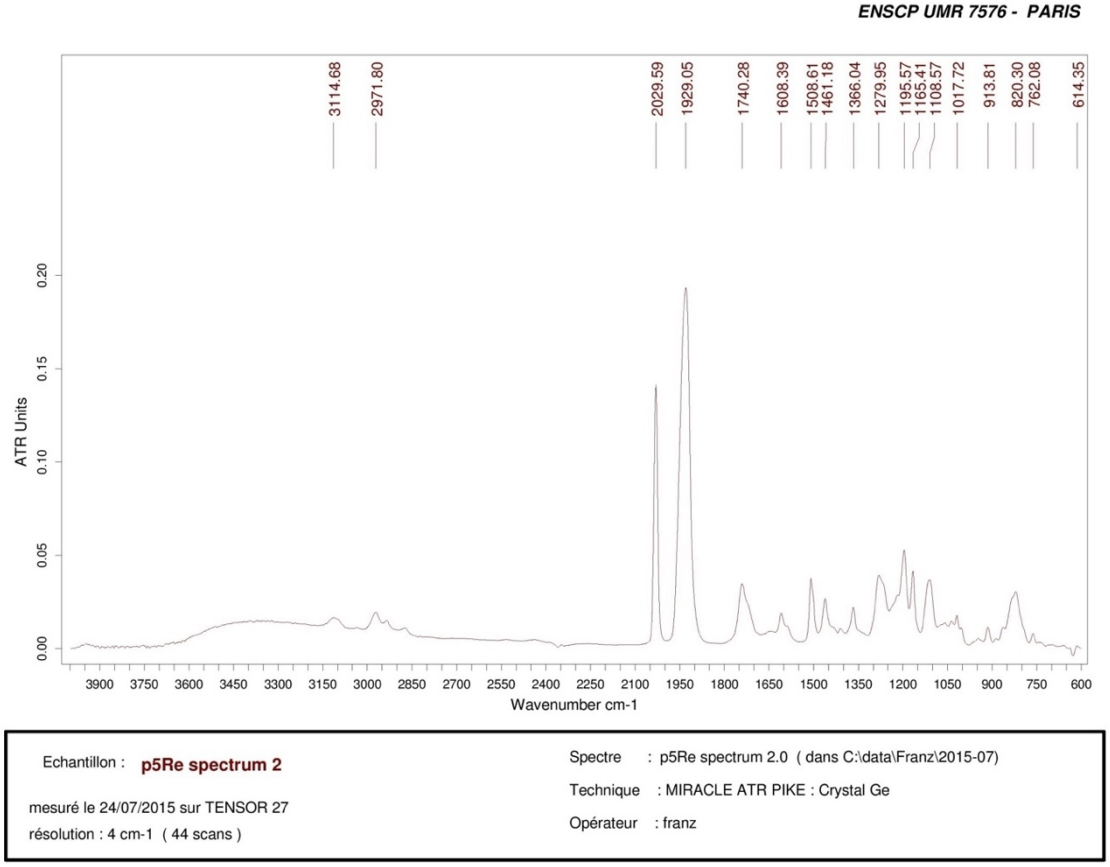


Figure S5. ATR-IR spectrum of complex **5**

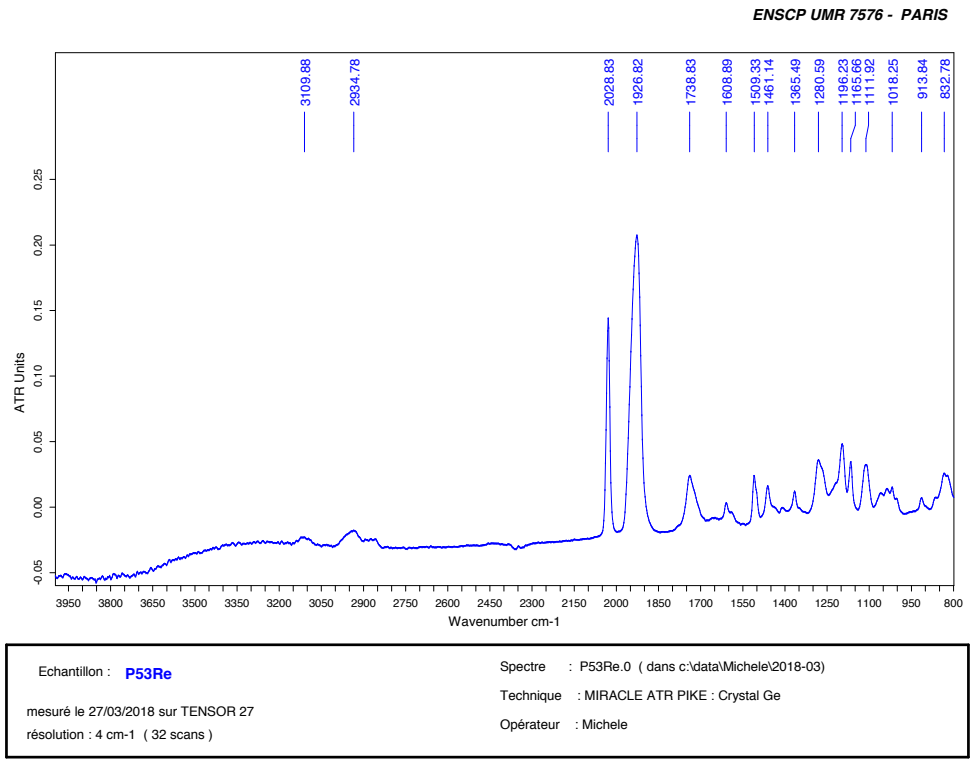


Figure S6. ATR-IR spectrum of complex **6**

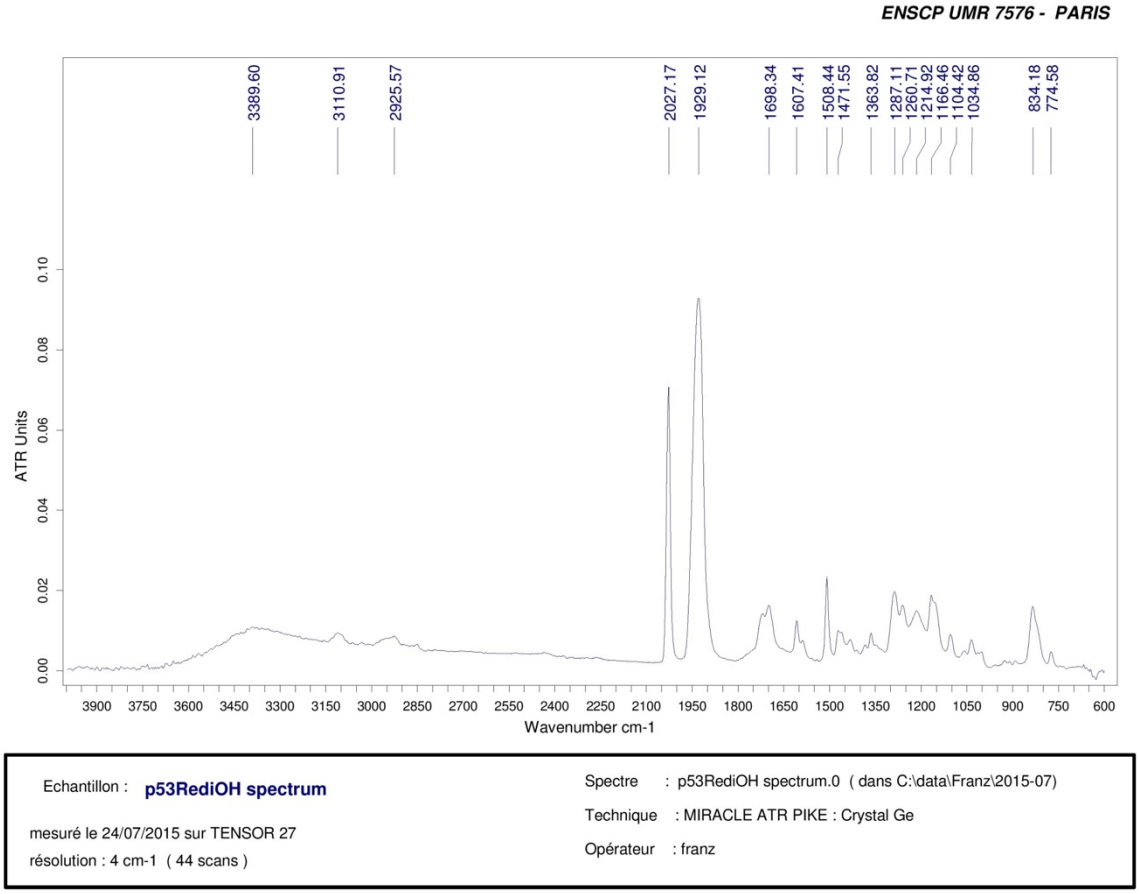
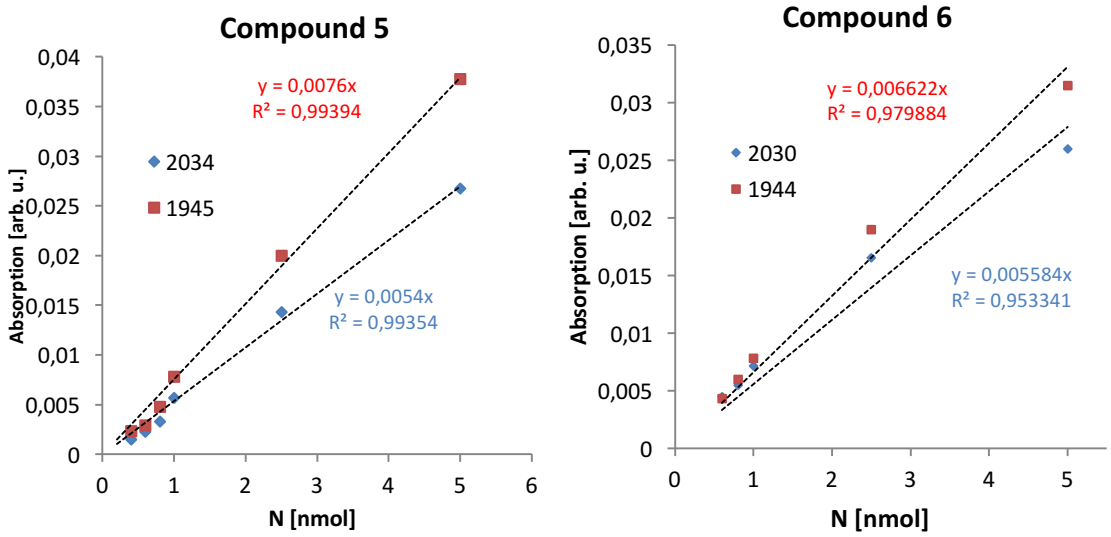


Figure S7. Calibration curves of **5** and **6**. Known quantities of complex were deposited on 6 mm diameter nitrocellulose membranes by spotting 5 µl solutions in isopropanol. After air drying the membranes were analyzed by FT-IR spectroscopy in the transmission mode. The intensity of the two nCO bands is plotted as a function of quantity of complex and data are fitted according to a linear regression.



Monitoring of enzymatic oxidation of ferrocifens by HRP+ H_2O_2 mixture

Ferrocifens (50 µM) were oxidized by HRP (44 nM) and H2O2 (200 µM) in 0.2 M TRIS.HCl 1 mM EDTA pH 8.1 containing 10% DMSO (v/v). HRP (1.1 µM, 40 µL) and H_2O_2 (10 mM, 20 µL) were preincubated for 5 min and then added to the solution of complex (940 µL). The mixture was immediately transferred to a cuvette and the uv-vis spectrum was recorded between 250 and 700 nm every 30 s on a Cary 50 spectrometer (Varian). Rate constants k_{obs} were calculated using Kaleidagraph software by fitting OD_{371} (**4**), OD_{350} (**5**), OD_{566} (**6**), OD_{563} (**2**) or OD_{367} (**1b**) versus time according to the first order law equation: $OD = C_0 + C_1 \exp(-k_{obs} \times t)$

Figure S8. Uv-visible spectra of mixture of **4** (50 µM), HRP (44 nM) and H_2O_2 (200 µM) in 0.2 M TRIS.HCl, 1 mM EDTA pH 8.1; Inset: plot of OD_{371} and OD_{566} versus time.

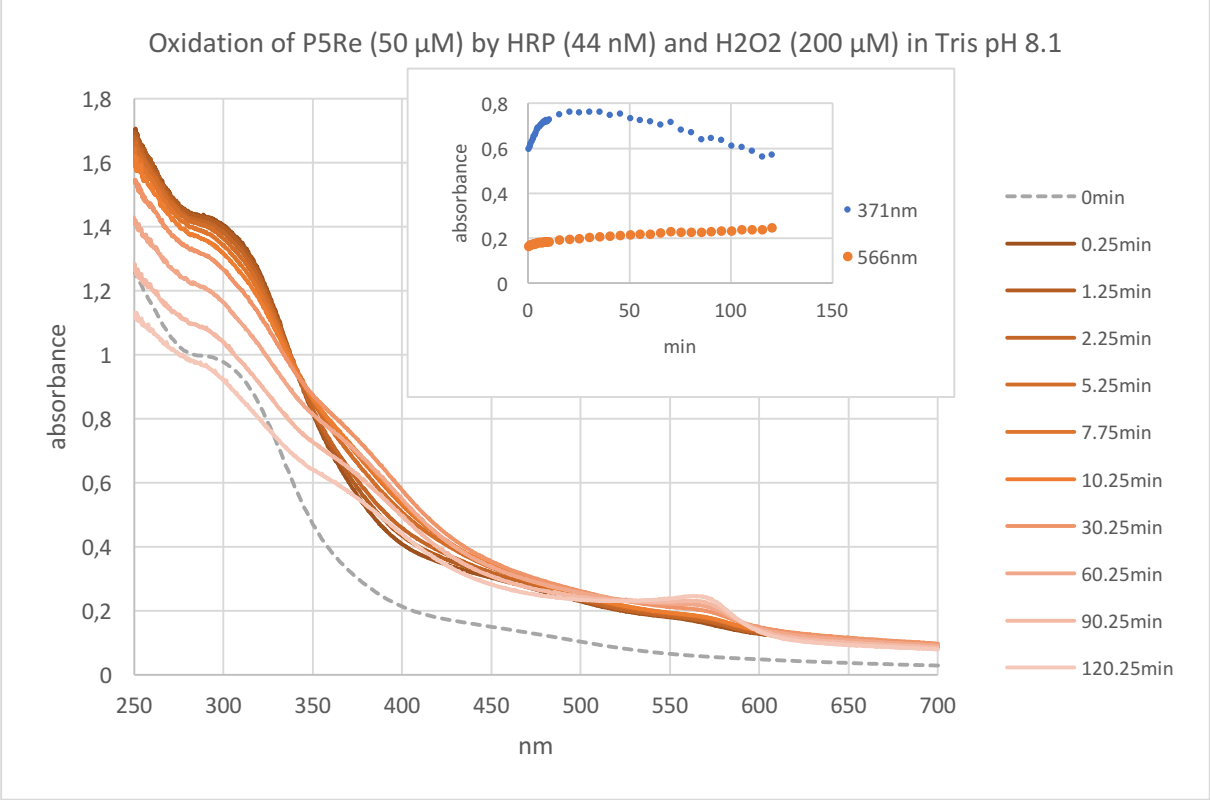


Figure S9. Uv-visible spectra of mixture of **5** (50 µM), HRP (44 nM) and H_2O_2 (200 µM) in 0.2 M TRIS.HCl, 1 mM EDTA pH 8.1; inset: plot of OD_{356} versus time.

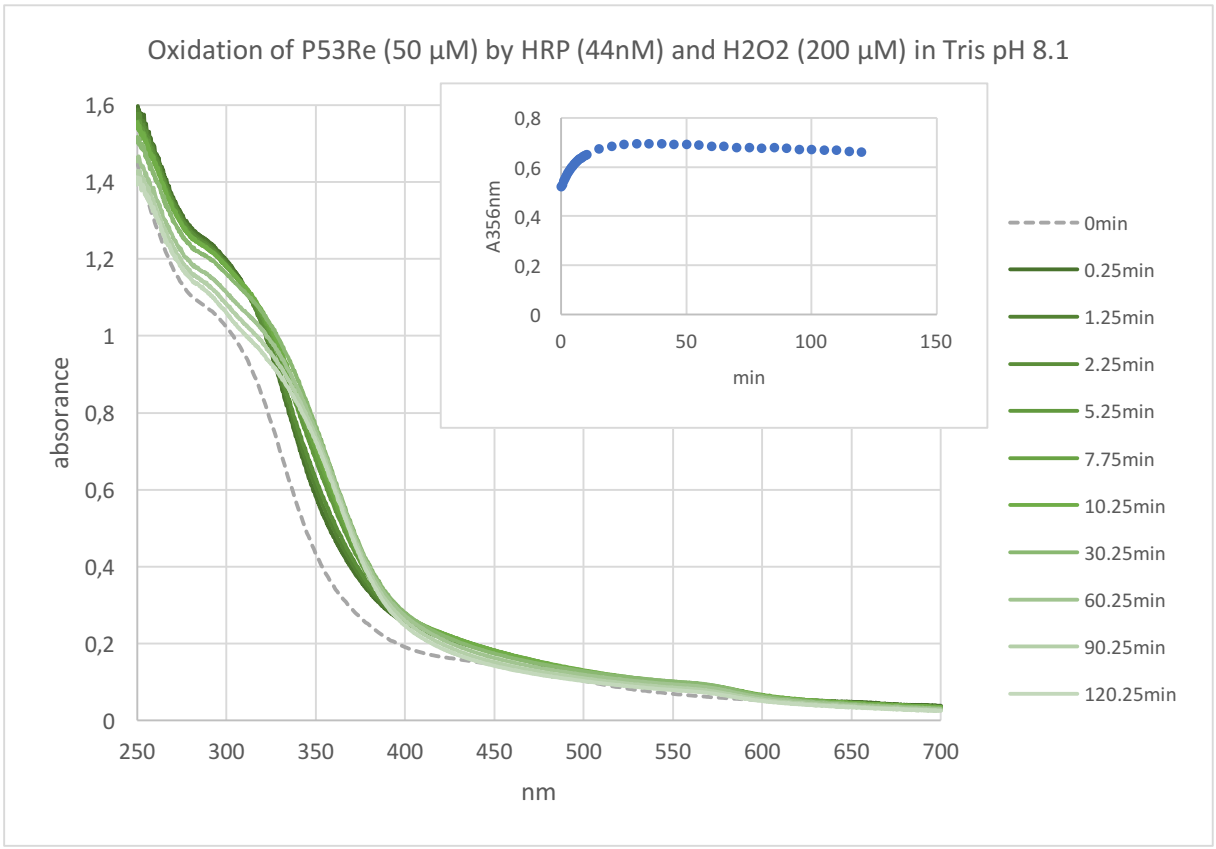


Figure S10. Uv-visible spectra of mixture of **6** (50 µM), HRP (44 nM) and H_2O_2 (200 µM) in 0.2 M TRIS.HCl, 1 mM EDTA pH 8.1; inset: plot of OD568 versus time.

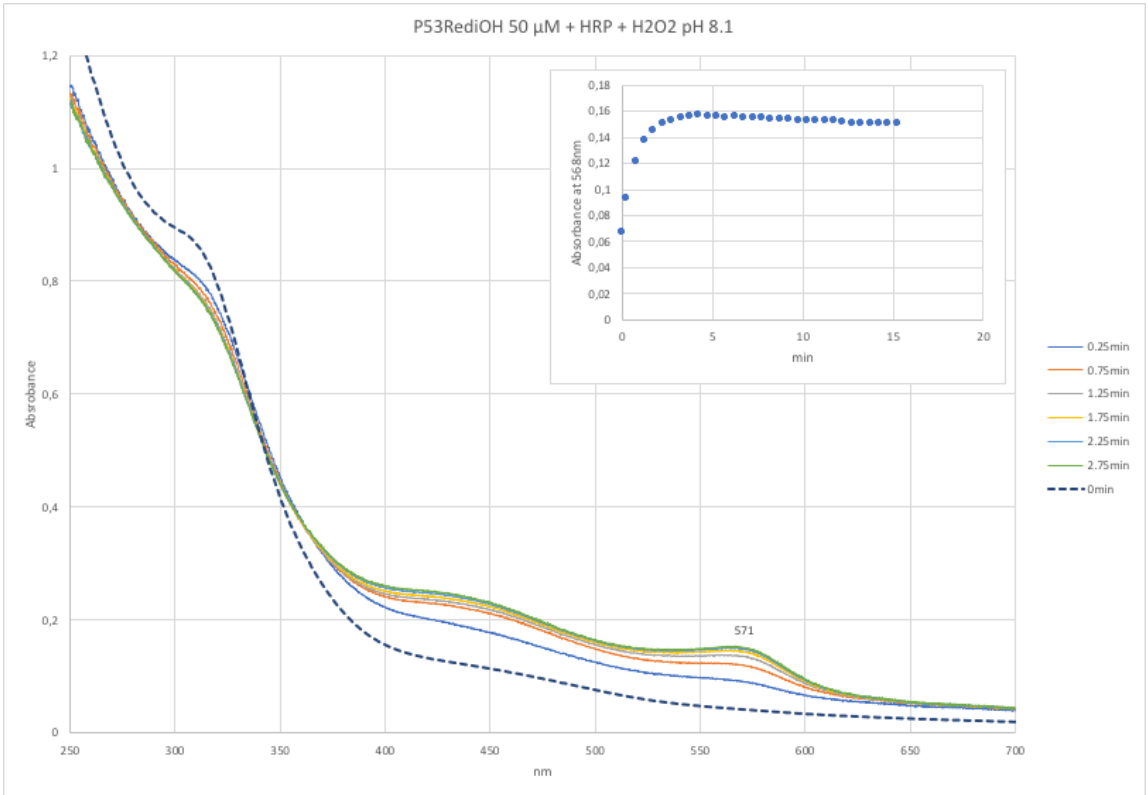


Figure S11. Uv-visible spectra of mixture of **2** (50 µM), HRP (44 nM) and H_2O_2 (200 µM) in 0.2 M TRIS.HCl, 1 mM EDTA pH 8.1; inset: plot of OD563 versus time.

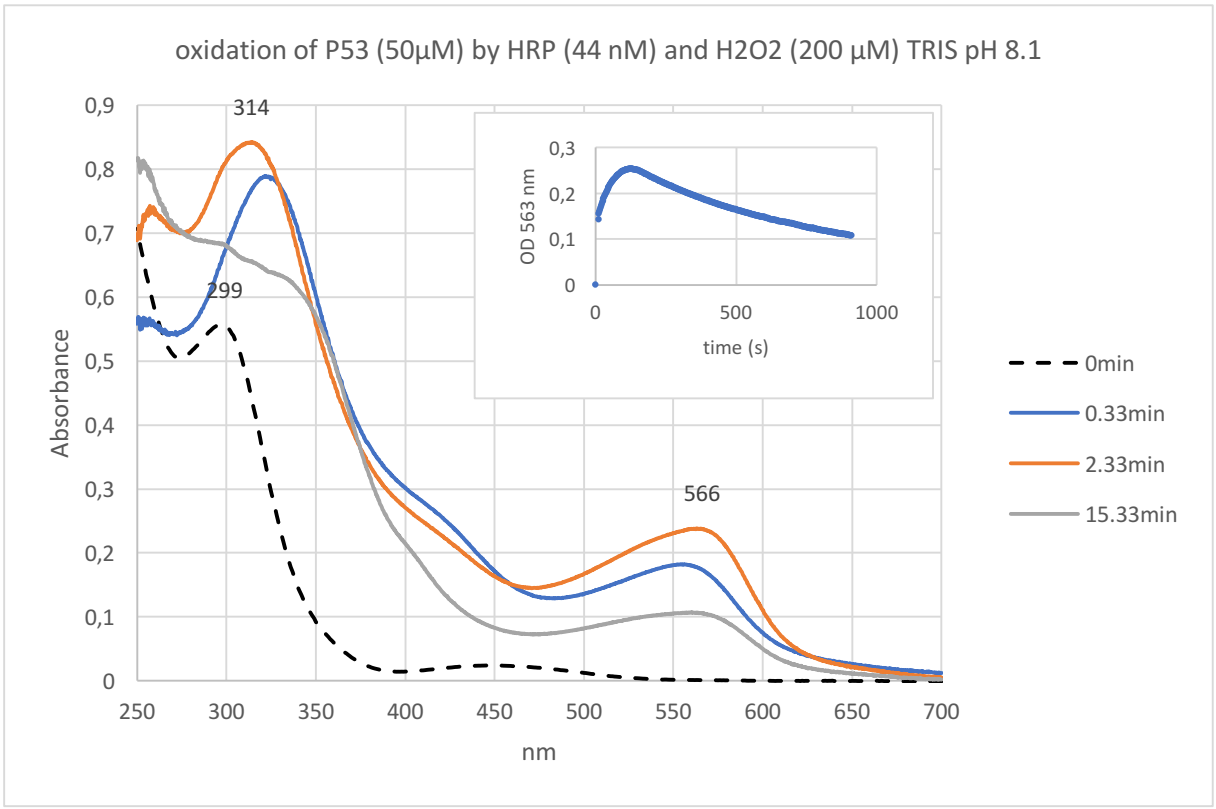


Figure S12. Uv-visible spectra of mixture of **1b** (50 µM), HRP (44 nM) and H_2O_2 (200 µM) in 0.2 M TRIS.HCl, 1 mM EDTA pH 8.1; inset: plot of OD367 versus time.

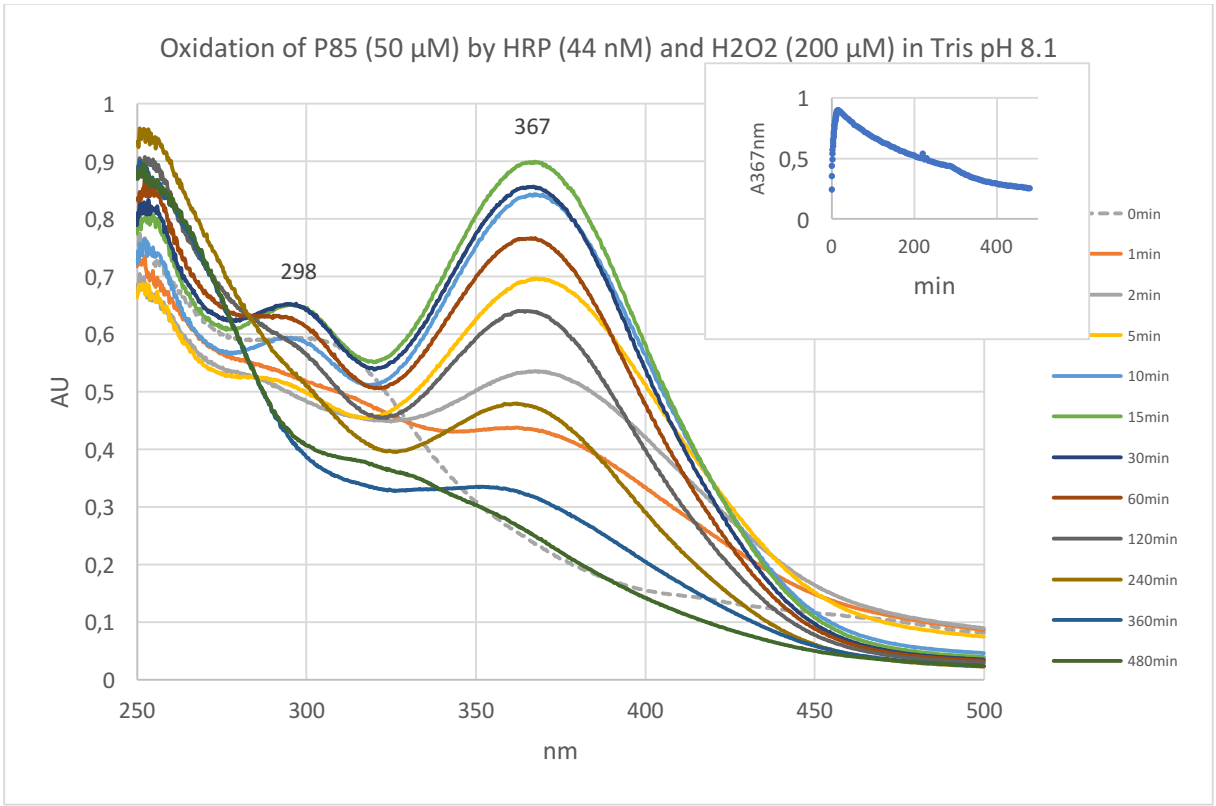


Figure S13. Uv-visible spectra of mixture of **1a** (50 µM), HRP (46 nM) and H_2O_2 (200 µM) in 0.2 M TRIS.HCl, 1 mM EDTA pH 8.1; inset: plot of OD560 versus time.