Electronic Supplementary Information (ESI)

Direct evidence for the catalase activity of $[Ru^{V}(edta)(O)]^{-}$

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Section S1 Experimental Procedures and Instrumentation

$K[Ru^{III}(Hedta)Cl]\cdot 2H_2O$ was prepared by following the published procedure and characterized accordingly.[1] $K_2[RuCl_5(H_2O)]$ (1 mmol) was reacted with Na_2H_2edta (1 mmol) in $HClO_4$ (1 mM). The reaction mixture was refluxed for 2 h and the volume of the pale-yellow solution was then reduced. Addition of cold ethanol precipitated the pale-yellow product, which was filtered off and washed several times with a water–acetone (1:9) mixture until free of chloride, and finally dried under vacuum. Anal. calculated for $K[Ru^{III}(Hedta)Cl].2H_2O$: C 24.0, H 3.42, N 5.59; Found. C 23.8, H 3.45, N 5.63. IR, ν/cm^{-1}: 1720 (free -COOH), 1650 (coordinated $-COO^{-}$). UV-Vis in H_2O: λ_{max}/nm ($\varepsilon_{max}/M^{-1}\,cm^{-1}$): 283 (2800 ± 50), 350 sh (680 ± 10).

IR spectra were recorded (using KBr pellets) on a Perkin Elmer Model Lambda 783. A Perkin-Elmer 240C elemental analyzer was used to obtain micro-analytical (C, H, N) data. UV-vis spectra and the kinetics of slow reactions were followed spectrophotometrically adopting a conventional mixing technique by using a Varian Model Cary 100 spectrophotometer. The instrument was thermostated at the desired temperature (± 0.1 °C) using a Cary Single Peltier accessory. A tandem cuvette was used for this purpose. Fast-kinetic experiments were performed in a reaction vessel using a Hellma 661.502-QX quartz Suprasil immersion probe attached *via* optical cables to a 150 W Xe lamp and a multi-wavelength J&M detector, which records complete absorption spectra at constant time intervals in the millisecond time range. Stopped-flow kinetics were carried out with a Hi-Tech SF-61 SX2 (TgK Scientific Ltd.) spectrophotometer using a 1 cm path length. Single-wavelength kinetic profiles were collected in photomultiplier mode, and data were processed using Hi-Tech KinetAsyst 3 software. The solution temperature was maintained to within ±0.1 °C using a circulating water bath (JEIO TECH RW-1025G).

All the instruments were thermostated at the desired temperature (± 0.1 °C). The pH of the solutions was measured with a Mettler Delta 350 pH meter. Acetate and

phosphate buffers were used to adjust the pH of the experimental solutions. Kinetic data are presented as an average of several kinetic runs (at least 5–8) and were reproducible within ±4 %.

Oxygen evolution studies were performed in a 25 ml closed glass vessel. A solution of Ru^{III}(edta) was purged with Ar prior to the addition of H_2O_2. The stock solution of H_2O_2 (2.0 M) was prepared in acetate buffer and also purged with Ar prior to the addition to the Ru^{III}(edta) solution. The solution temperature was maintained to within ±0.1 °C using a circulating water bath (JEIO TECH RW-1025G).The solution was pre-equilibrated at desired temperature by keeping the glass reactor in water bath for 30 mins prior to the addition of H_2O_2. An appropriate amount of H_2O_2 was added to the solution of Ru^{III}(edta) using a micro-syringe so that the desired concentration of H_2O_2 was attained in the final reaction mixture. The total volume of the aqueous phase was 10 ml. Oxygen evolved was identified and quantified by gas chromatographic studies. A Perkin Elmer gas chromatograph (Clarus-580) equipped with a 5A molecular sieve column (2 mm x 2mm) and Thermal Conductivity Detector (TCD), was used for this purpose. 500 μL of the gaseous phase was taken with a micro-syringe for injection. High purity Ar (99.999%) was used as carrier gas. Calibration of the GC parameters was accomplished with the use of self-prepared O_2/Ar mixtures of known concentration.

[1)] Diamantis, A.A.; .Dubrawski, J.V. *Inorg. Chem*., 1981, **20**, 1142.

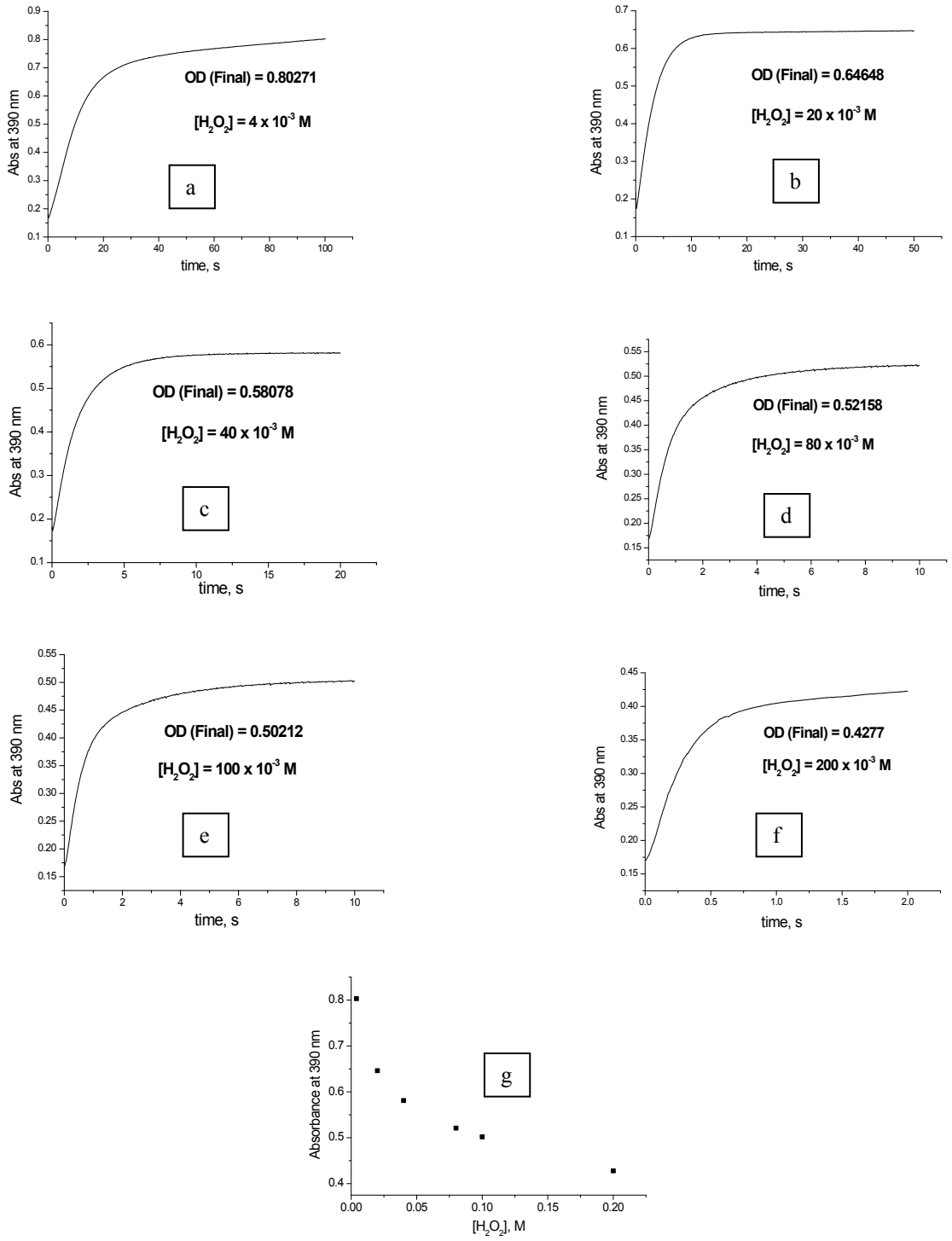


Fig. S1 Kinetic traces for the reaction of $[Ru^{III}(edta)(H_2O)]^-$ with H_2O_2 at 25 ^oC and pH 5.0 (10 mM acetate buffer). (a) $[H_2O_2]$ = 4.0 mM, (b) $[H_2O_2]$ = 20.0 mM, (c) $[H_2O_2]$ = 40.0 mM, (d) $[H_2O_2]$ = 80.0 mM, (e) $[H_2O_2]$ = 100.0 mM, (f) $[H_2O_2]$ = 200.0 mM and (g) plot of absorbance at 390 nm at different $[H_2O_2]$. $[Ru^{III}]$ = 0.2 mM.

Software Version : 6.3.2.0646
Sample Name :
Instrument Name : Clarus 580
Date : 9/4/2014

Peak #	Component Name	Time [min]	Area [uV*sec]	Height [uV]	Raw Amount
1		3.018	453839.50	27627.61	0.4538

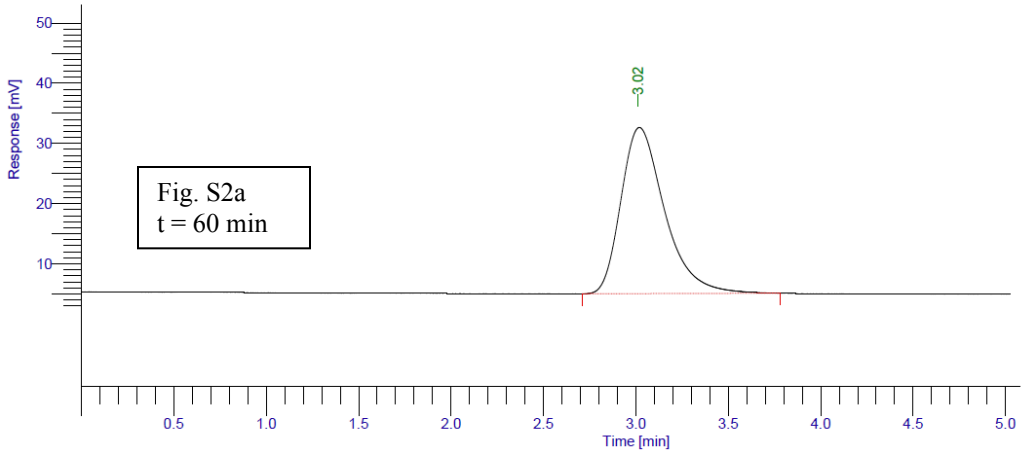


Fig. S2a
t = 60 min

Software Version : 6.3.2.0646
Sample Name :
Instrument Name : Clarus 580
Date : 9/5/2014

Peak #	Component Name	Time [min]	Area [uV*sec]	Height [uV]	Raw Amount
1		3.020	620201.18	39041.08	0.650

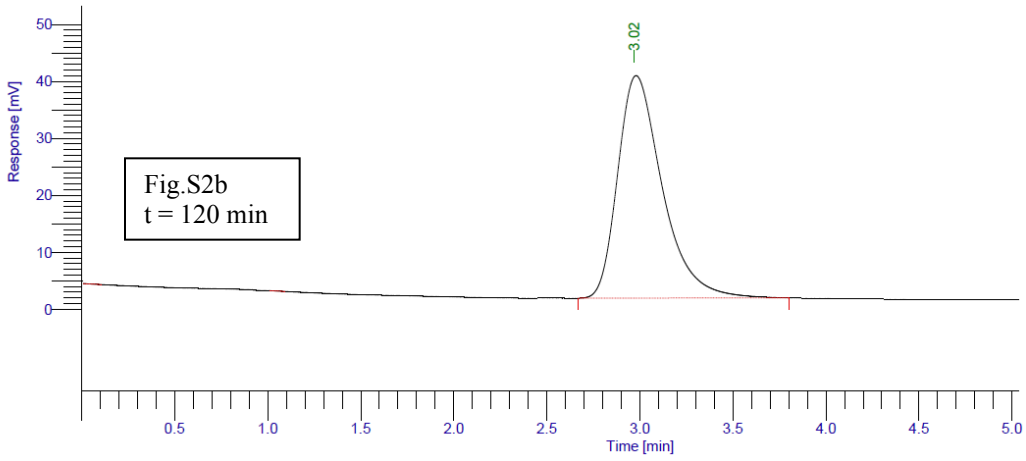
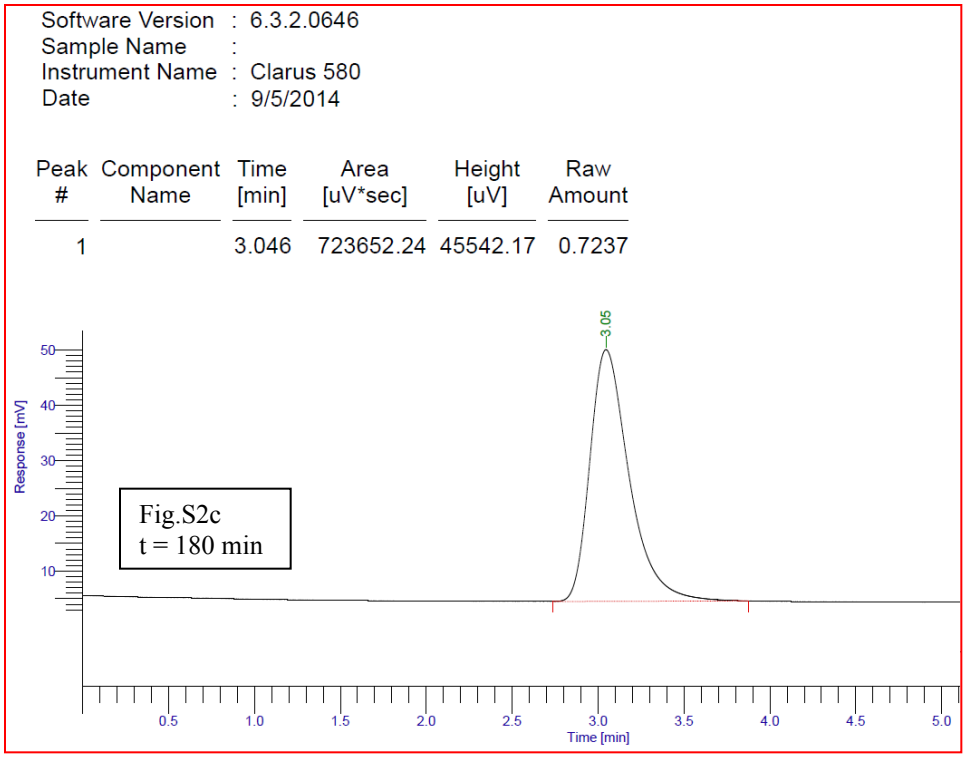


Fig.S2b
t = 120 min



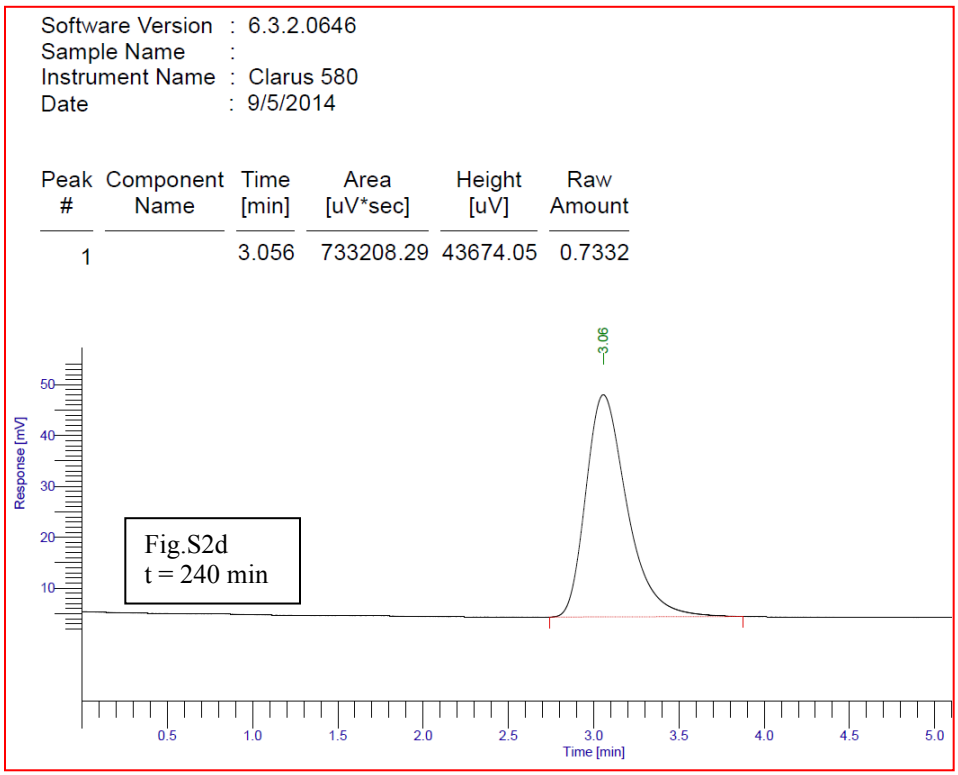


Fig. S2 Results of O_2 evolution studies as a function of time. [Ru^{III}] = 2.0 µmol, H_2O_2 = 0.4 mmol, Temp. 25 °C and pH = 5.0 (10.0 mM acetate buffer). GC recorded after (a) 60 min, (b) 120 min, (c) 180 min and (d) 240 min.

Software Version : 6.3.2.0646
Sample Name :
Instrument Name : Clarus 580
Date : 9/4/2014

Peak #	Component Name	Time [min]	Area [uV*sec]	Height [uV]	Raw Amount
1		3.124	137574.94	9636.21	0.1376

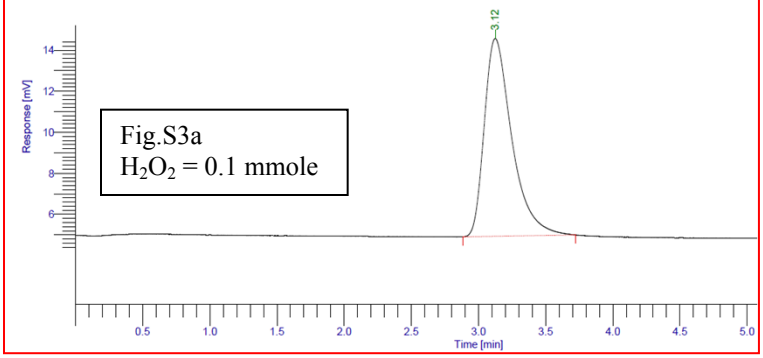


Fig.S3a
H_2O_2 = 0.1 mmole

Software Version : 6.3.2.0646
Sample Name :
Instrument Name : Clarus 580
Date : 9/4/2014

Peak #	Component Name	Time [min]	Area [uV*sec]	Height [uV]	Raw Amount
1		3.037	418717.13	24649.78	0.4187

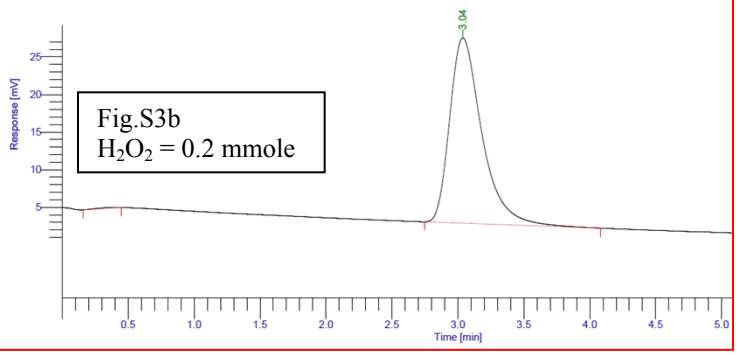


Fig.S3b
H_2O_2 = 0.2 mmole

Software Version : 6.3.2.0646
Sample Name :
Instrument Name : Clarus 580
Date : 9/4/2014

Peak #	Component Name	Time [min]	Area [uV*sec]	Height [uV]	Raw Amount
1		3.037	744531.69	48643.84	0.7445

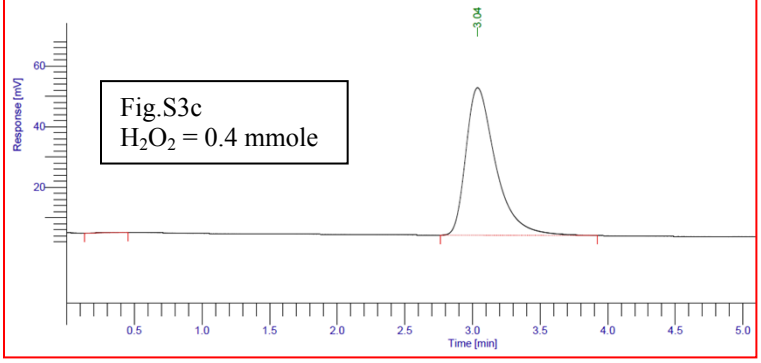


Fig.S3c
H_2O_2 = 0.4 mmole

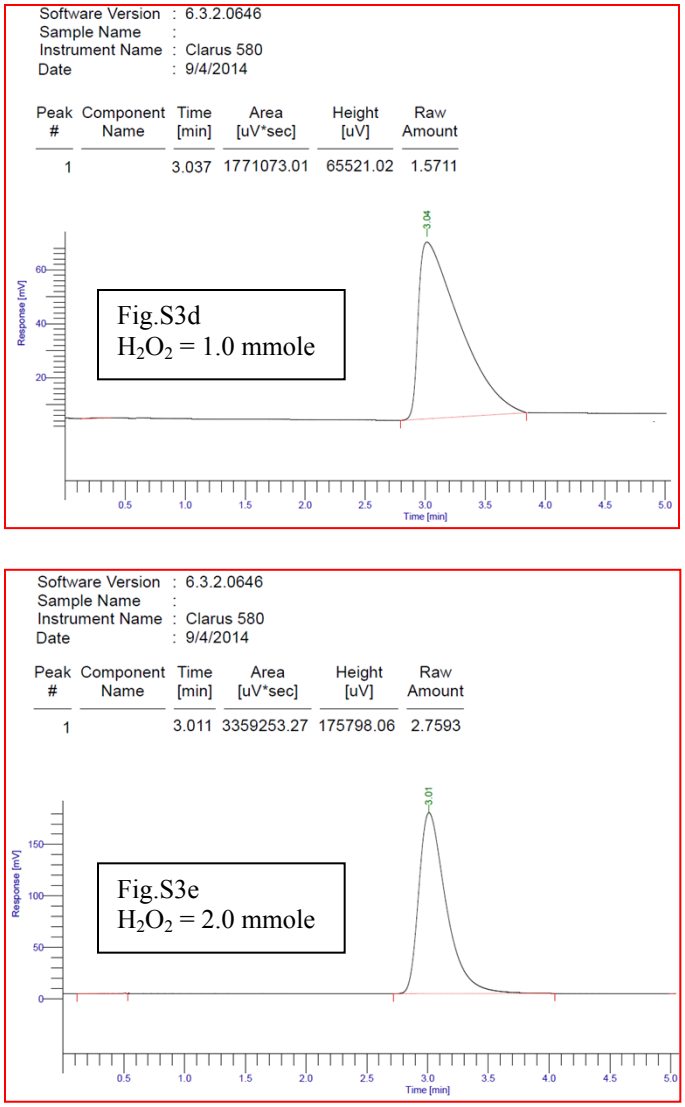


Fig. S3 Results of GC studies on O_2 evolution as a function of H_2O_2 concentration at 25 °C and pH = 5.0 (10.0 mM acetate buffer). $[Ru^{III}]$ = 2.0 µmole.

Table S1. Results of O_2 evolution studies as a function of H_2O_2 concentration at 25 °C and pH = 5.0 (10.0 mM acetate buffer). $[Ru^{III}]$ = 2.0 µmole.

$[H_2O_2]$ (mM)	Total amount of H_2O_2 (mmol)	Peak Area (A)/mVs	O_2 produced in 15 ml of gaseous phase (mmol)	Total O_2 produced (mmol)
10.0	0.1	137.6	0.033	0.043
20.0	0.2	418.7	0.101	0.111
40.0	0.4	744.5	0.180	0.190
100.0	1.0	1771.1	0.430	0.440
200.0	2.0	3359.2	0.815	0.825

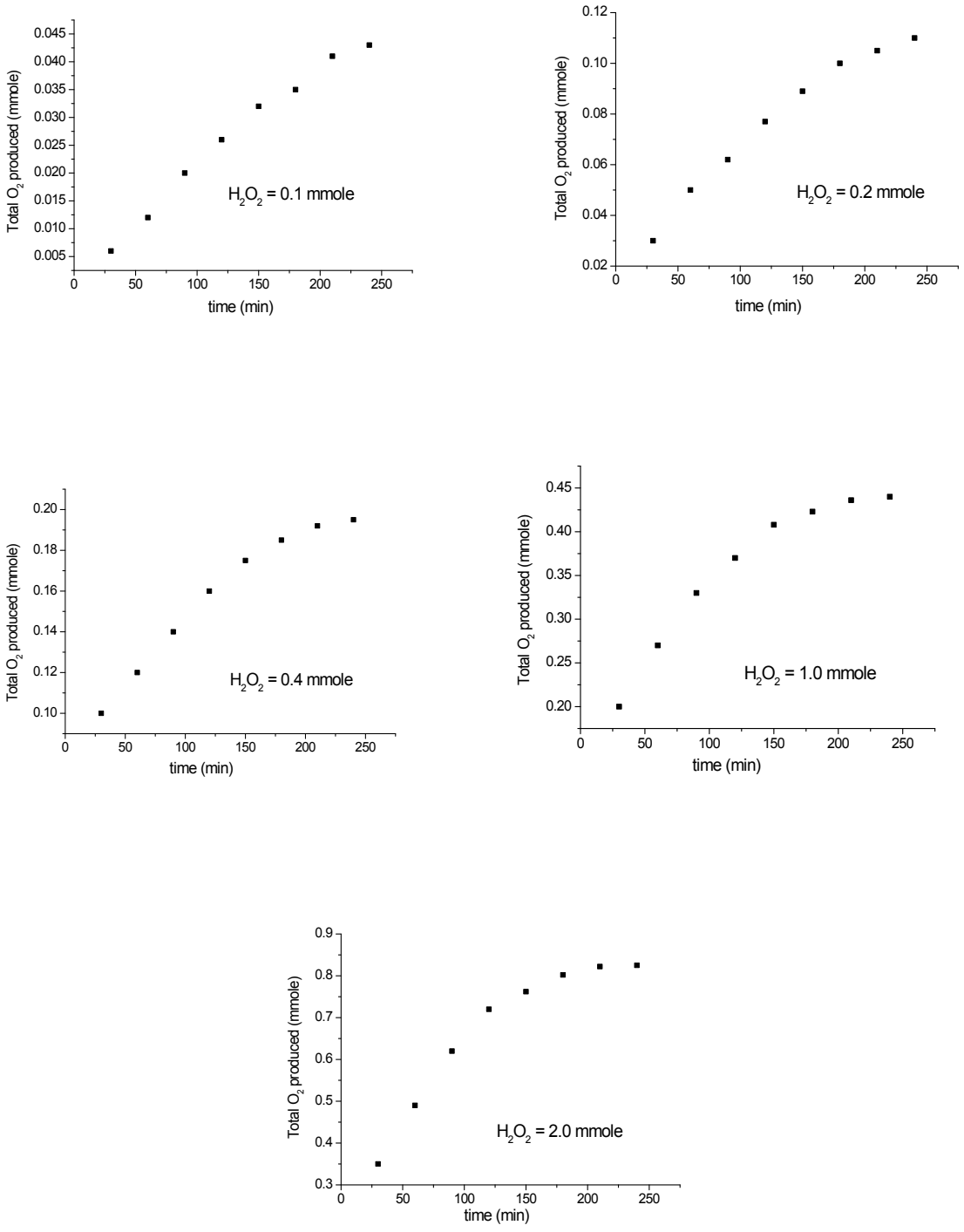


Fig. S4 Plots of O_2 evolution versus time as a function of H_2O_2 concentration at 25 °C and pH = 5.0 (10.0 mM acetate buffer). $[Ru^{III}]$ = 2.0 μmole.

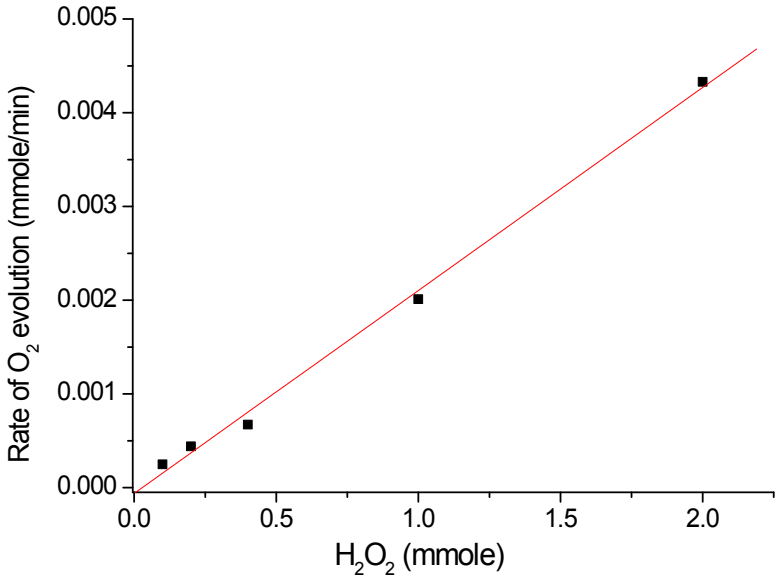


Fig.S5 Effect of H_2O_2 concentration on the rate of O_2 evolution at 25 ^oC and pH 5.0 (10 mM acetate buffer). $[Ru^{III}]$ = 0.2 mM.

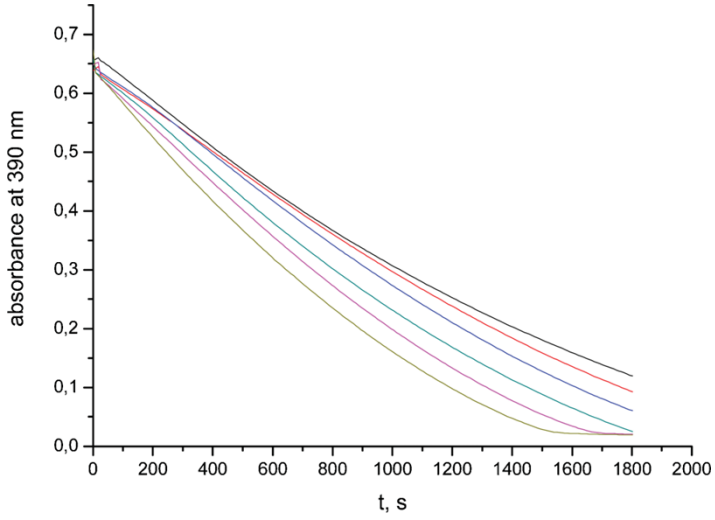


Fig.S6 Typical absorbance (at 390 nm) *versus* time traces for the reaction of $[Ru^V(edta)O]^-$ with different concentrations of cysteine. Experimental conditions: $[Ru^V{=}O]$ = 1 x 10^{-4} M, [cysteine] = (1–10) x 10^{-3} M) from top to bottom. (Taken from Ref.6a)

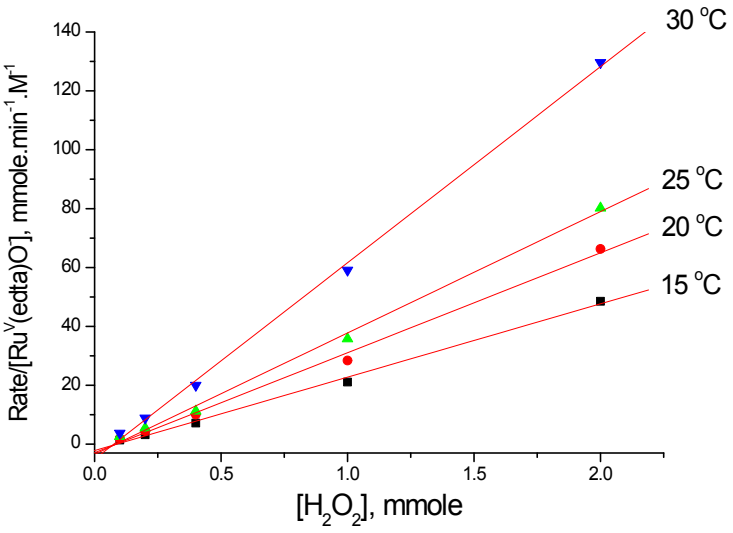


***Fig. S7** Plots of the rate/[Ru^{V}(edta)O^{-}] vesus H_2O_2 concentration at various temperature. at pH = 5.0 (10.0 mM acetate buffer).

* The steady-state concentration of Ru(V) species at different [H_2O_2] was determined spectrophotometrically by using the molar-extinction coefficient value of [Ru^{V}(edta)O]$^{-}$ species at 390 nm is 8000 $M^{-1}cm^{-1}$ (Ref.14 in the text)

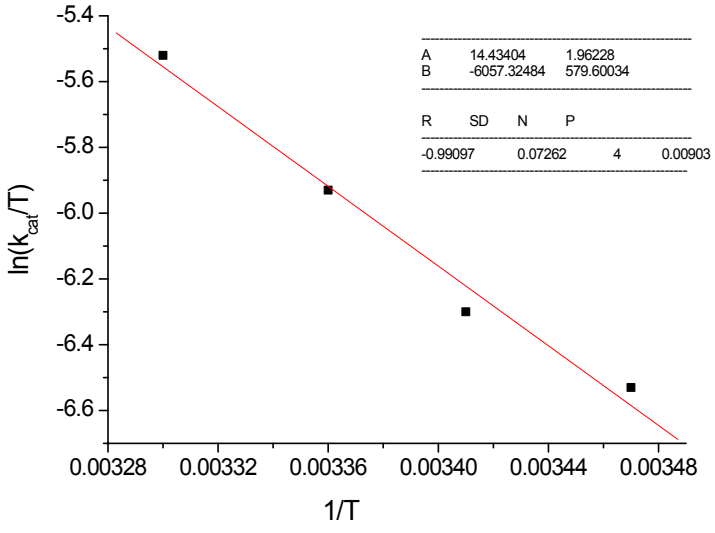


Fig.S8 Eyring plot for the reaction of [Ru^{V}(edta)O]$^{-}$ with H_2O_2 at pH = 5.0 (10.0 mM acetate buffer).

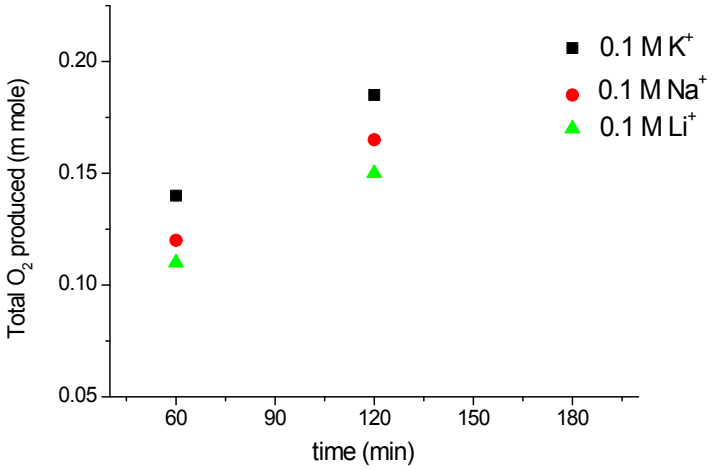


Fig. S9 Effect of alkali cations on O_2 evolution. [Ru^{III}] = 2.0 µmole, H_2O_2 = 0.4 mmol, Temp. 25 °C and pH = 5.0 (10.0 mM acetate buffer).

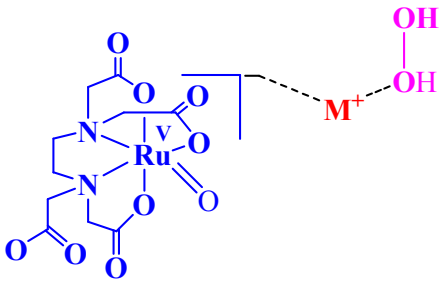


Fig. S10 Pictorial representation of intermediate **I**.

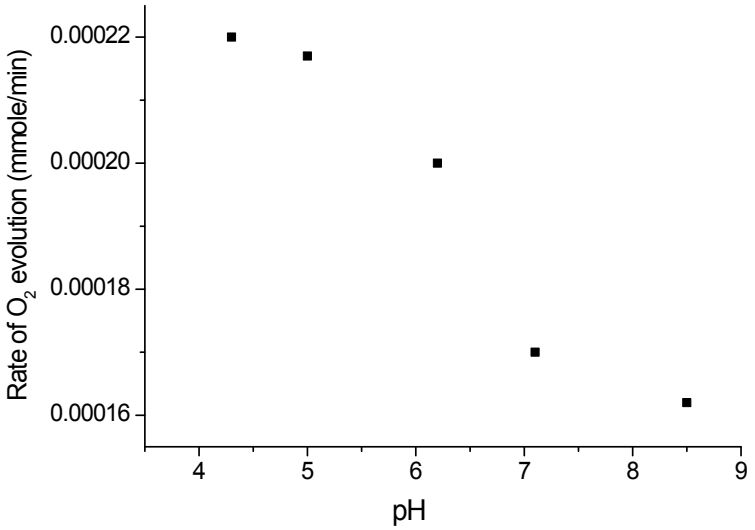


Fig.S11 Effect of pH on O_2 evolution. [Ru^{III}] = 2.0 μmole, H_2O_2 = 0.4 mmol, Temp. 25°C.