Electronic Supplementary Information

for

Bioinspired Polymer-Bound Mn-porphyrins with Artificial Active Center of Catalase

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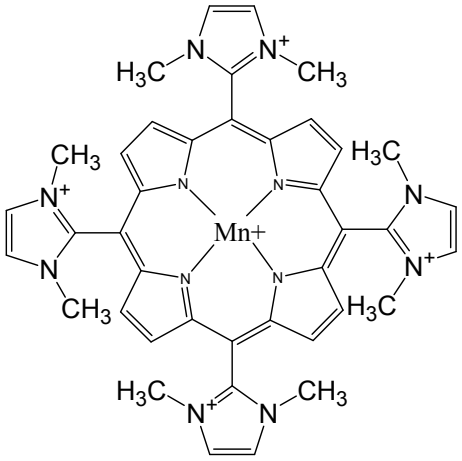
Experimental Section

Chemicals

Manganese(III)-5,10,15,20-tetrakis(N-methylpyridinum-4-yl) porphyrin ($MnM4Py_4P$), and Manganese(III)-5,10,15,20-tetrakis(1,3-dimethylimidazolium-2-yl) porphyrin ($MnMIm_4P$) were synthesized according to the previous report.[1,2,5] Both complexes were obtained as chloride salts. Carboxymethyl poly (1-vinylimidazole) (CM-PVIm) and poly (1-vinylimidazole) (PVIm) was synthesized and characterized according to our previous report.[3,4] Content of the carboxyl group in CM-PVIm is approximately 30%. Hydrogen peroxide (H_2O_2, 35~37%) and polyacrylic acid (PAA) with Mn (number average molecular weight) of 25,000 were purchased from Kanto Chemical Co., Inc.



Scheme S1. Chemical structure of $MnMIm_4P$.

Determination of Binding Constant

Binding constant between $MnM4Py_4P$ and CM-PVIm was determined by Scatchard plot. 1mM CM-PVIm and $MnM4Py_4P$ with various concentrations (0.25~1.0 mM) were mixed and stirred in 50 mM phosphate buffer (pH 7.4) for three days. After the mixing, the unbound $MnM4Py_4P$ was removed by ultra filtration (25℃, 10,000g, 60min) and quantified by UV/vis spectroscopy using molar absorption coefficient at 462 nm reported previously (ε=129000 cm^{-1} M^{-1}).[1] The bound $MnM4Py_4P$ was calculated from the difference between the concentration of initial $MnM4Py_4P$ and that of unbound $MnM4Py_4P$. Scatchard plot was prepared according to the following formula:

$$r/Cu=nK_b-K_br$$

where K_b:binding constant, n:number of binding sites, r: number of bound substrate, Cu: concentration of unbound substrate. K_b was determined from the slope of the plot of r/Cu versus r.

Measurement of Catalase Activity

Catalase activity (k_{CAT}) was measured by electrochemically monitoring the evolved molecular oxygen ($2H_2O_2 \rightarrow 2H_2O + O_2$) with Clark-type oxygen electrode. To 900μL solution of each concentration of Mn-porphyrin/CM-PVIm complex in 50 mM phosphate buffer (pH 7.4), was added

a 100μL of 10 mM H_2O_2 in Milli-Q water. The monitoring was started after the addition of the H_2O_2 solution. Observed-rate constant (k_{obs}) at each concentration of the complex was determined as a rate of O_2 production in initial ten seconds. From the slope of k_{obs} as a function of the complex concentration, k_{CAT} value was determined.

Stopped-flow kinetic analysis

Stopped-flow kinetic analysis was performed on SX.18MV stopped-flow reaction analyzer (Applied Photophysics) with 1 cm path length. Reaction temperature was maintained at 25±0.1℃ with thermostat water bath. MnM4Py$_4$P/CM-PVIm$_{10000}$ complex was rapidly mixed with aqueous solution of H_2O_2 (volume ratio was MnM4Py$_4$P/CM-PVIm$_{10000}$:H_2O_2aq=20/1(v/v)). Time-resolved UV/vis spectra were recorded from 400 nm to 500 nm with 400 scans in 1 second.

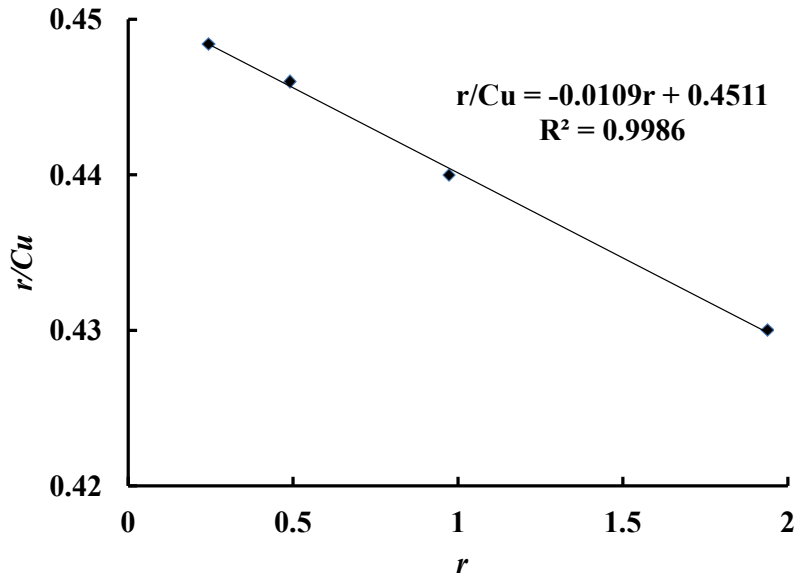


Fig. S1 Scatchard plot by MnM4Py$_4$P and CM-PVIm$_{10000}$.

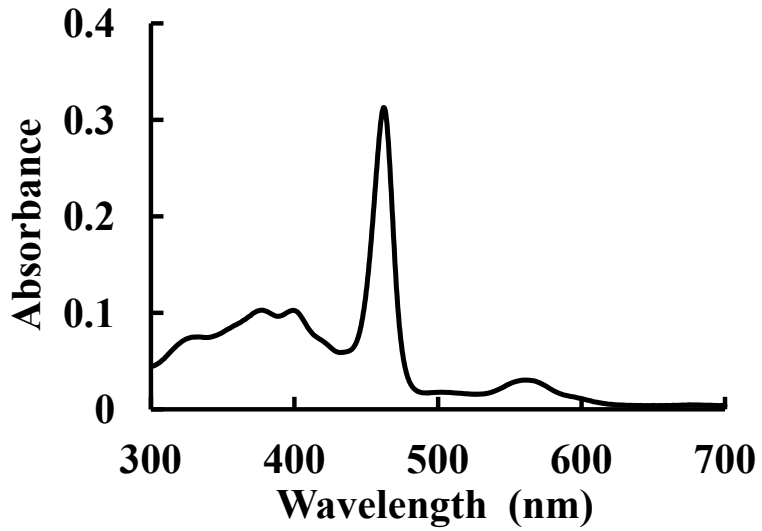


Fig. S2 UV/vis spectrum of 2.5 μM $MnM4Py_4P$ in 50 mM phosphate buffer (pH 7.4) before mixing with $CM\text{-}PVIm_{10000}$. Soret band was observed at 462 nm (ε=129000 $M^{-1}cm^{-1}$).

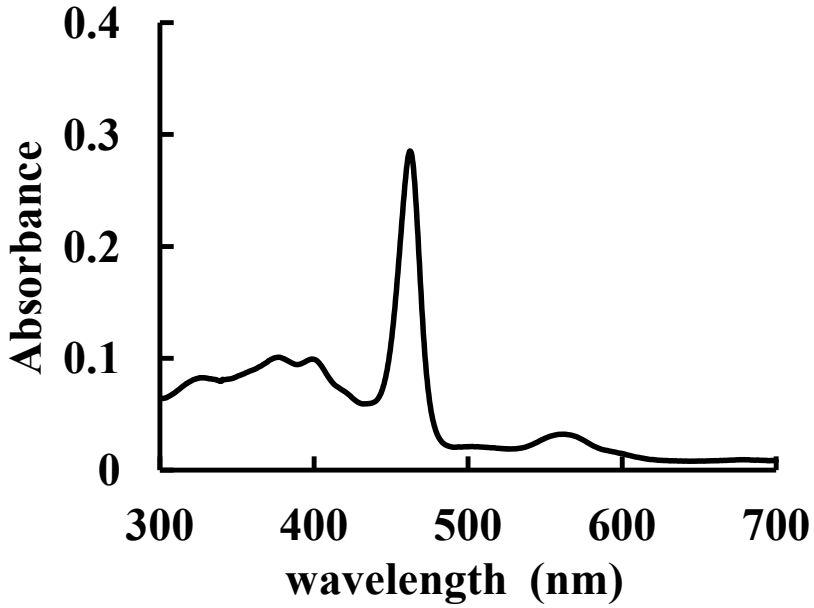


Fig. S3 UV/vis spectrum of 2.5 μM $MnM4Py_4P$ after three days mixing with $CM\text{-}PVIm_{10000}$ in 50

mM phosphate buffer (pH 7.4). The spectrum was measured under conditions where MnM4Py$_4$P was stoichiometrically bound to CM-PVIm. Soret band was observed at 462 nm.

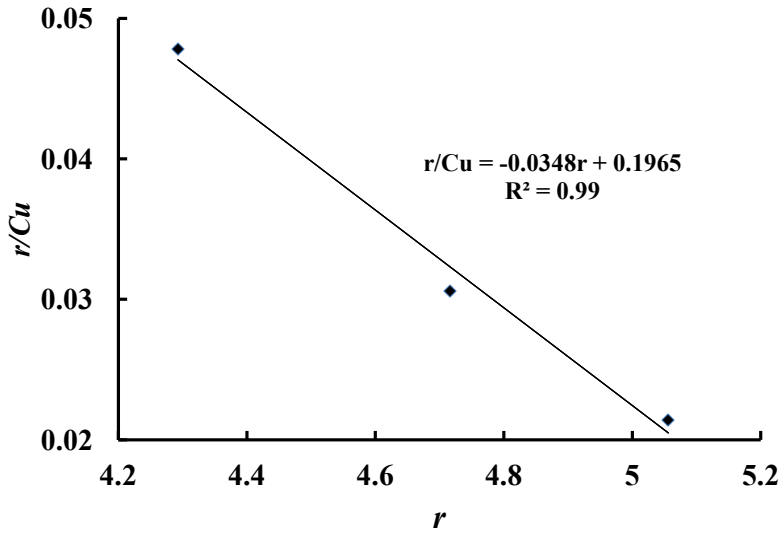


Fig. S4 Scatchard plot by MnM4Py$_4$P and PAA$_{25000}$. From the slope of the plot, K_b was determined to be 3.5×10^4 M^{-1}.

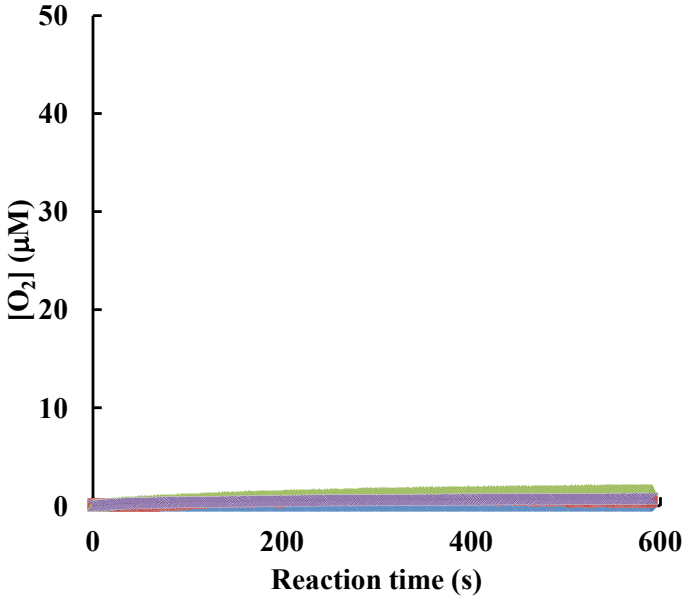


Fig. S5 Time-course of O_2 production from 1 mM H_2O_2 in the presence of MnM4Py$_4$P/PAA$_{25000}$ complex in 50 mM phosphate buffer (pH 7.4). The O_2 production was monitored at 1,3,6 μM per MnM4Py$_4$P.

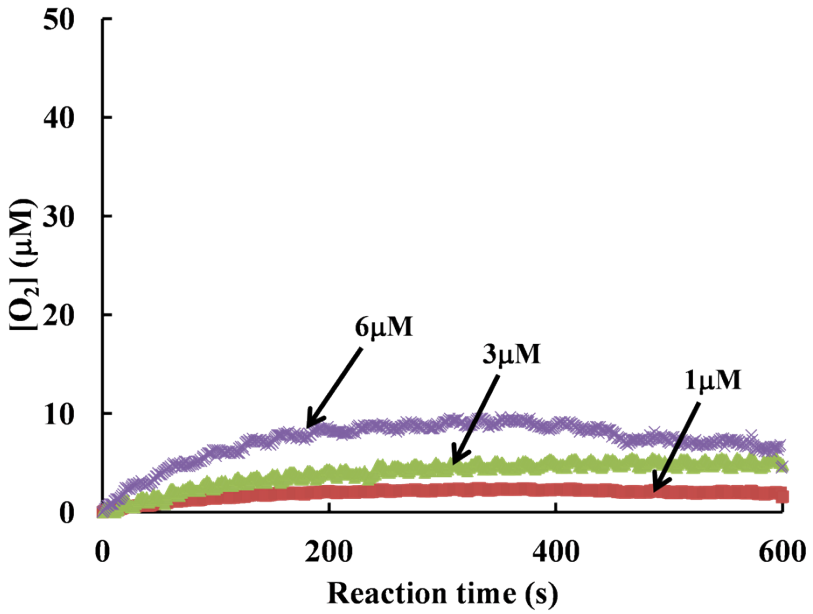


Fig. S6 Time-course of O_2 production from 1 mM H_2O_2 catalyzed by $MnM4Py_4P/PVIm_{10000}$ complex in 50 mM phosphate buffer (pH 7.4). Amount of O_2 production was increased with increasing concentration of the complex. k_{CAT} value was estimated to be 40~50 $M^{-1}s^{-1}$.

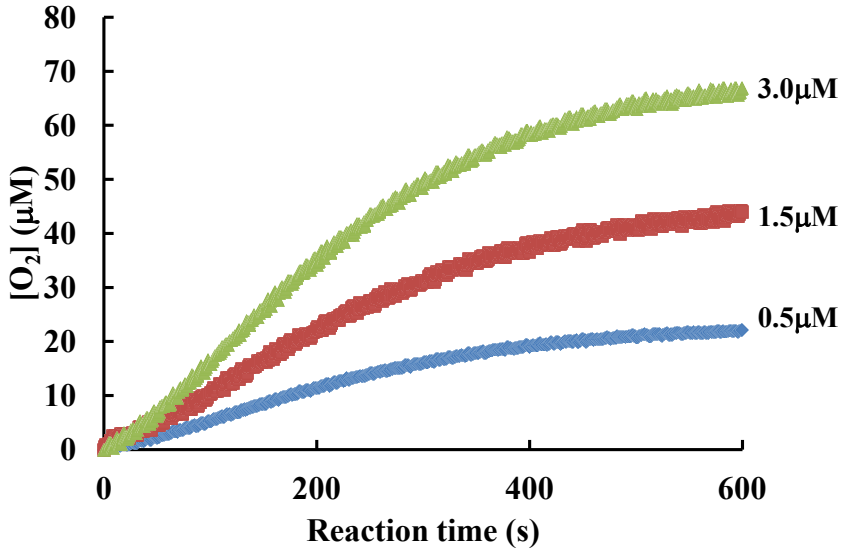


Fig. S7 Time-course of O_2 production from 1 mM H_2O_2 catalyzed by $MnMIm_4P/CM\text{-}PVIm_{10000}$ complex in 50 mM phosphate buffer (pH 7.4).

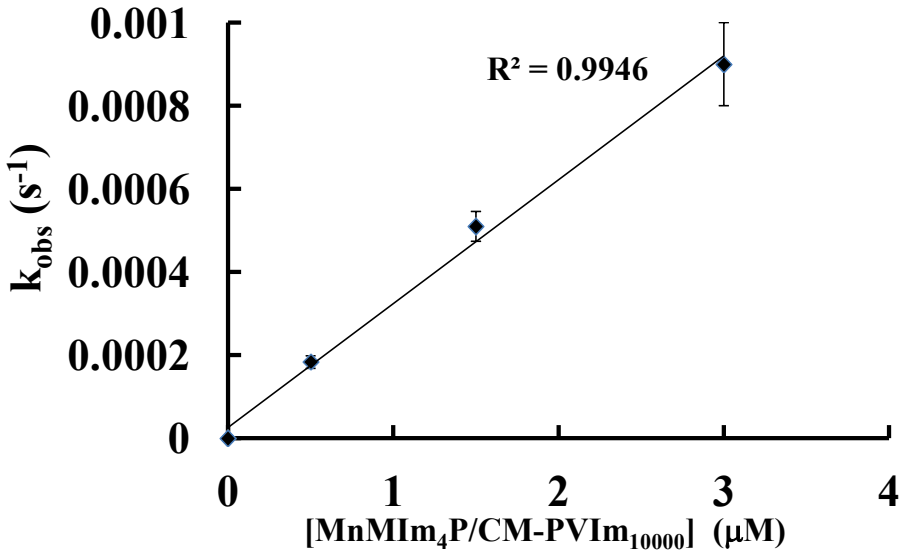


Fig. S8 Plot of k_{obs} as a function of $MnMIm_4P$/CM-$PVIm_{10000}$ complex.

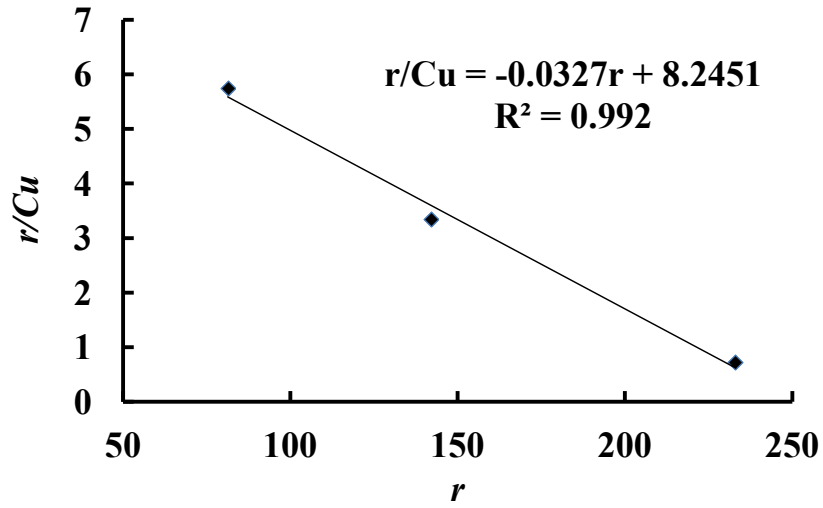


Fig. S9 Scatchard plot by $MnM4Py_4P$ and CM-$PVIm_{3000}$.

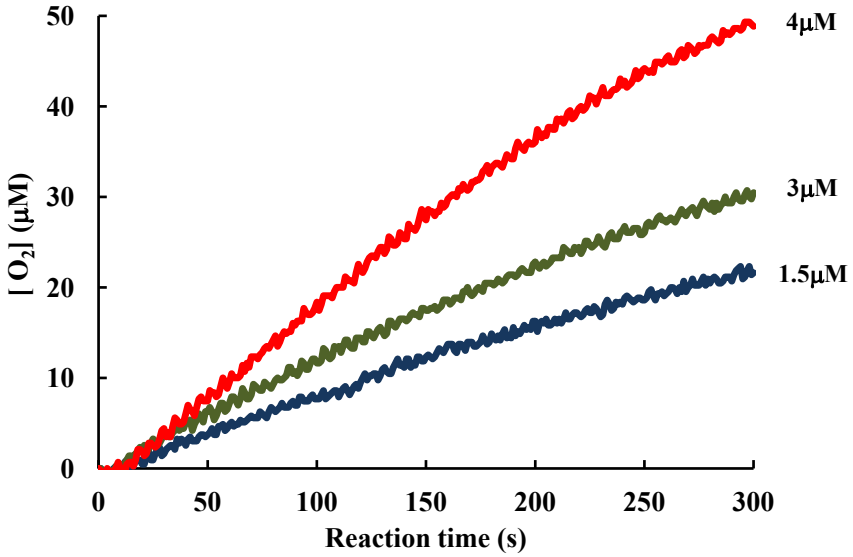


Fig. S10 Time-course of O_2 production from 1 mM H_2O_2 catalyzed by MnM4Py$_4$P/CM-PVIm$_{3000}$ complex in 50 mM phosphate buffer (pH 7.4).

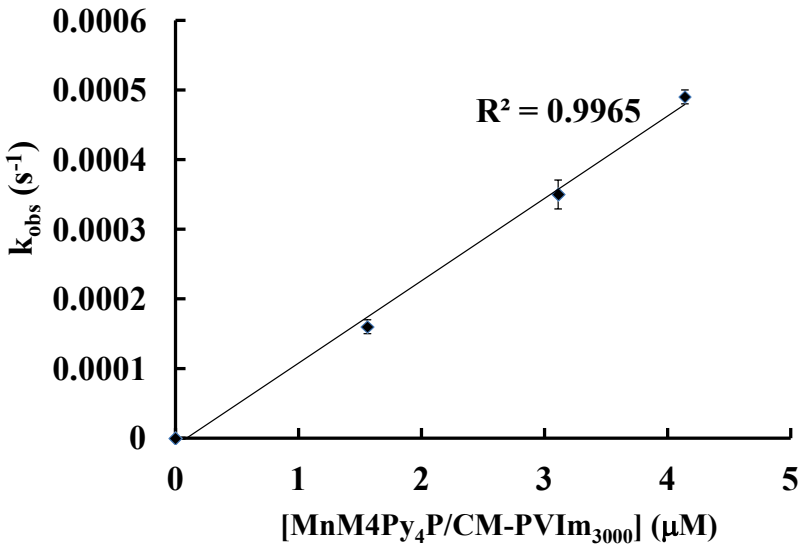


Fig. S11 Plot of k_{obs} as a function of MnM4Py$_4$P/CM-PVIm$_{3000}$ complex.

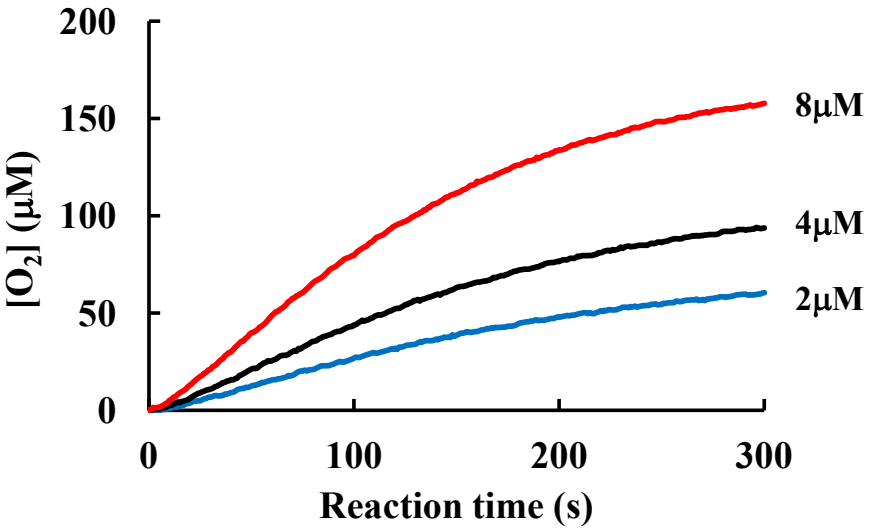


Fig. S12 Time-course of O_2 production from 1 mM H_2O_2 catalyzed by $MnMIm_4P/CM\text{-}PVIm_{3000}$ complex in 50 mM phosphate buffer (pH 7.4).

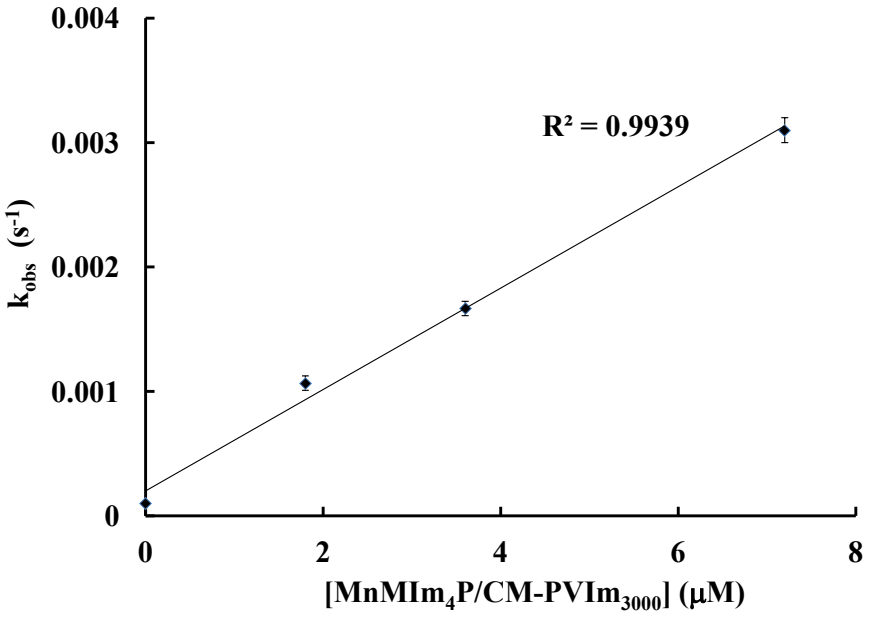


Fig. S13 Plot of k_{obs} as a function of $MnMIm_4P/CM\text{-}PVIm_{3000}$ complex.

References

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