**An Organometallic Catalase Mimic with Exceptional Activity, \( \text{H}_2\text{O}_2 \) Stability, and Catalase/Peroxidase Selectivity**

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PAPER

An Organometallic Catalase Mimic with Exceptional Activity, H₂O₂ Stability, and Catalase/Peroxidase Selectivity

Zhuomin Lu,a Ian V. Lightcap,b and Andrew G. Tennyson*†a,c,d

Manganese–porphyrin and –salen redox therapeutics catalyze redox reactions involving O₂⁻, H₂O₂, and other reactive oxygen species, thereby modulating cellular redox states. Many of these complexes perform catalase reactions via high-valent Mn–oxo or –hydroxo intermediates that oxidize H₂O₂ to O₂, but these intermediates can also oxidize other molecules (e.g., thiols), which is peroxidase reactivity. Whether catalase or peroxidase reactivity predominates depends on the metal–ligand set and the local environment, complicating predictions of what therapeutic effects (e.g., promoting vs. suppressing apoptosis) a complex might produce in a given disease. We recently reported an organoruthenium complex (Ru) that catalyzes ABTS⁺– reduction to ABTS²⁻ with H₂O₂ as the terminal reductant. Given that H₂O₂ is thermodynamically a stronger oxidant than ABTS⁺–, we reasoned that the intermediate that reduced ABTS⁺– would also be able to reduce H₂O₂ to H₂O. Herein we demonstrate Ru₁-catalyzed H₂O₂ disproportionation into O₂ and H₂O, exhibiting an 8,580-fold faster catalase TOF vs. peroxidase TOF, which is 89.2-fold greater than the highest value reported for a Mn-porphyrin or –salen complex. Furthermore, Ru₁ was 120-fold more stable to H₂O₂ than the best MnSOD mimic (TON = 4000 vs. 33.4). Mechanistic studies provide evidence that the mechanism for Ru₁-catalyzed H₂O₂ disproportionation is conserved with the mechanism for ABTS⁺– reduction. Therapeutic effects of redox catalysts can be predicted with greater accuracy for catalysts that exhibit exclusively catalase activity, thereby facilitating the development of future redox therapeutic strategies for diseases.

Introduction

Catalases are metalloenzymes that catalyze the disproportionation of H₂O₂ into H₂O and ½ O₂.¹ where peroxidases catalyze the reduction of H₂O₂ into H₂O via oxidation of other substrates.² In both enzyme classes, H₂O₂ is reduced to H₂O and the Fe(III) enzyme resting state is oxidized to an O=Fe(IV)–porphyrin•+ intermediate (Scheme 1). In a catalase, this intermediate oxidizes a 2⁻equivalent of H₂O₂ to O₂, releasing a 2⁻equivalent of H₂O and regenerating the Fe(III) resting state. In a peroxidase, however, this intermediate oxidizes a substrate other than H₂O₂,³ proceeding via (a) 2e⁻ oxidation of one substrate molecule,⁴,⁵ (b) 1e⁻ oxidation of two substrate molecules,⁶,⁷ or (c) oxygen atom transfer to a substrate molecule.⁸–¹¹ Given the similarity between the O=Fe(IV)–porphyrin•+ intermediates in catalase and peroxidase, it is unsurprising that catalase can exhibit peroxidase reactivity at a sufficiently low [H₂O₂],¹² or that peroxidase can exhibit catalase reactivity at sufficiently high [H₂O₂].¹³

Scheme 1. Dual catalase / peroxidase activity from a common intermediate

Synthetic Mn complexes supported by porphyrin and salen ligands, designed as manganese superoxide dismutase (MnSOD) mimics, catalyze the disproportionation of O₂⁻ into O₂ and H₂O₂.¹⁴–¹⁹ However, these Mn-porphyrin and –salen complexes also catalyze the disproportionation of H₂O₂ into H₂O and ½ O₂,¹⁹,²⁰ which is unsurprising given that a Mn complex able to reduce O₂ to O₂⁺⁺ (E⁰⁺⁺ = −0.33 V vs. NHE)²¹ will likewise be able to reduce H₂O₂ to 2 H₂O (E⁰⁺⁺ = −1.35 V).²² Because the latter reduction is more thermodynamically favorable, many of the Mn-porphyrin and –salen complexes that are capable of MnSOD activity are also intrinsically capable of catalase activity.

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Electronic Supplementary Information (ESI) available: Details of experimental procedures, rate law equation derivations, and additional kinetics plots. See DOI: 10.1039/x0xx00000x

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As is observed with catalase itself, some Mn-salen and Mn-porphyrin complexes that (a) exhibit catalase activity can also (b) exhibit peroxidase activity,23, 24 such as oxidizing ABTS\(^{3-}\) to ABTS\(^{3+}\) (Scheme 2A).25-32 Even non-biomimetic Mn complexes will toggle between catalase activity33 vs. peroxidase activity34 upon simple ligand modification (e.g., \(R = Me\) vs. \(t-Bu\)). Thus, a single Mn complex is capable of producing either antioxidant or pro-oxidant effects in a given biological environment, with its behavior being determined by the metal–ligand set and the local conditions. For example, the Mn-porphyrin complexes that have entered clinical trials can (i) promote cancer cell death by shifting cellular redox states to more oxidized potentials,35, 36 or (ii) promote normal cell survival during radiotherapy by shifting cellular redox states to more reduced potentials.37, 38 Both outcomes are highly beneficial to patients and illustrate the enormous therapeutic potential for complexes capable of both catalase and peroxidase activity. However, it is difficult to predict in advance which reactivity mode will predominate in a given biological environment.

Our lab developed an organoruthenium complex (Ru1, Scheme 2B) that can catalytically reduce ABTS\(^{3-}\) to ABTS\(^{3+}\) by oxidizing biologically-relevant alcohols as the terminal reductant.39-41 We recently demonstrated that Ru1 can also harness H\(_2\)O\(_2\) as the terminal reductant, generating O\(_2\)(g) and a Ru–H intermediate, the latter then reducing 2 equiv. of ABTS\(^{3-}\) to ABTS\(^{3+}\) (Scheme 2C, top).42 Because the ABTS\(^{3+}\)/2-redox couple occurs at +0.68 V,43 and the standard reduction potential for H\(_2\)O\(_2\) to H\(_2\)O is much higher (+1.35 V),22 we reasoned that a Ru1-derived intermediate capable of reducing 2 ABTS\(^{3+}\) to 2 ABTS\(^{3-}\) will also be capable of reducing the more strongly-oxidizing H\(_2\)O\(_2\) to H\(_2\)O, thus exhibiting catalase activity (Scheme 2C, bottom). Moreover, given that Ru1 can use H\(_2\)O\(_2\) to reduce ABTS\(^{3+}\) to ABTS\(^{3-}\), we reasoned that Ru1 would show little to no oxidation of ABTS\(^{3-}\) to ABTS\(^{3+}\) with H\(_2\)O\(_2\), with this latter reaction being a spectroscopically-convenient probe for peroxidase reactivity.

Herein we report the catalytic disproportionation of H\(_2\)O\(_2\) into H\(_2\)O and \(\frac{1}{2}\) O\(_2\) by Ru1 with values for catalase TOF = 1.09 s\(^{-1}\), catalase TON ≥ 4000, and catalase/peroxidase selectivity = 8,580, which are individually 11.9-, 120-, and 89.2-fold greater, respectively, than the highest values reported\(^{44}\) for the Mn-porphyrin and Mn-salen MnSOD mimics.19, 20, 23-32 Using a combination of catalase TOF, catalase TON, and catalase/peroxidase selectivity values, the aggregated functionality of Ru1 as a catalase mimic is 14,200-fold higher than the best-performing synthetic MnSOD mimic.

### Results and Discussion

#### Catalase and peroxidase reactivity

Addition of 20 mM H\(_2\)O\(_2\) to a solution of 20 μM Ru1 in 3.00 mL PBS (pH 7.4, 25 °C) results in a gradual decrease in the absorbance at 240 nm (\(\varepsilon_{240} = 43.6\) M\(^{-1}\)cm\(^{-1}\) for H\(_2\)O\(_2\)) that is complete within 2 h (Figure 1, blue trace). The \(v_0\) measured is 17.1 ± 1.0 μM s\(^{-1}\),\(^{14, 44}\) corresponding to a catalase TOF = 0.868 ± 0.063 s\(^{-1}\).46, 47 When this experiment is repeated on a 100 mL scale, to facilitate quantification of O\(_2\)(g) evolution, 1.04 ± 0.02 mmol of O\(_2\)(g) is collected, corresponding to a TON = 1000 and in good agreement with the 25 mL theoretical volume if every 1.0 equivalent of H\(_2\)O\(_2\) that was consumed produced 0.5 equiv. of O\(_2\)(g). Four successive 20 mM aliquots of H\(_2\)O\(_2\) added every 2 h are all completely consumed by 20 μM Ru1 in PBS, yielding a minimum catalase TON = 4000 for Ru1. Headspace gas sampling to a thermocouple detector confirms that 100% of the evolved gas was O\(_2\)(g). In situ mass spectrometric analysis of these H\(_2\)O\(_2\) disproportionation reactions reveals the same intermediates as those in Ru1-catalyzed ABTS\(^{3-}\) reduction with H\(_2\)O\(_2\).\(^{42}\)

To assess potential peroxidase activity of Ru1, 100 μM ABTS\(^{3-}\) is introduced to a PBS solution containing 20 μM Ru1 followed by the addition of 5 μM H\(_2\)O\(_2\). The 4:1 Ru1/H\(_2\)O\(_2\) ratio is chosen to maximize coordination of H\(_2\)O\(_2\) to Ru1 and minimize the amount of free H\(_2\)O\(_2\) in solution available to undergo disproportionation by any Ru1/H\(_2\)O\(_2\) derived species. The 20:1 ABTS\(^{3-}\)/H\(_2\)O\(_2\) ratio was selected such that any Ru1/H\(_2\)O\(_2\) derived species would be significantly more likely to encounter ABTS\(^{3-}\) (peroxidase reactivity) over disproportionation of H\(_2\)O\(_2\) (catalase reactivity).

![Figure 1. Plot of concentration vs. time for H\(_2\)O\(_2\) disproportionation (blue trace) and ABTS\(^{3-}\) oxidation (red trace) catalyzed by Ru1. Conditions: [H\(_2\)O\(_2\)]\(_0\) = 20 mM (blue trace) or 5 μM (red trace), [ABTS\(^{3-}\)]\(_0\) = 0 μM (blue trace) or 200 μM (red trace), [Ru1] = 20 μM, PBS (pH 7.4), 25 °C.](image)
When 5 μM H₂O₂ is added to a solution of 20 μM Ru1 and 100 μM ABTS⁺ in PBS, the absorbance at 734 nm (characteristic for ABTS⁺) gradually increases, reaching a maximum of 1.69 ± 0.13 μM after 28 min, and then gradually decays to zero over the course of 2 h (Figure 1, red trace). The ν₀ measured is 2.54 ± 0.03 nM s⁻¹, corresponding to a peroxidase TOF = 1.27 ± 0.01 × 10⁻⁴ s⁻¹. Dividing the catalase TOF by the peroxidase TOF yields the catalase/peroxidase selectivity ratio (C/P), which for Ru1 (at 20 μM) is 6,830. Notably, 5 μM H₂O₂ should have been sufficient to oxidize 10 μM ABTS⁺ to 10 μM ABTS⁺⁺, but a maximum of only 1.69 ± 0.13 μM ABTS⁺⁺ forms under these conditions, which demonstrates that only 17% of the total H₂O₂ is consumed by peroxidase reactivity, and the remaining 83% is consumed by catalase reactivity.

Catalase and peroxidase activities of Ru1 were measured again at 10 μM to match the concentrations employed in studies of Mn-based catalase mimics. At [Ru1]₀ = 10 μM, the catalase and peroxidase ν₀ values are 10.6 ± 0.5 μM s⁻¹ and 1.27 ± 0.12 nM s⁻¹, respectively (Figure S1), corresponding to a catalase TOF = 1.09 ± 0.13 s⁻¹, a peroxidase TOF = 1.27 ± 0.12 × 10⁻⁴ s⁻¹, and a C/P = 8,580 (Table 1). For comparison, MnP-1 (Figure 2) has the highest catalase activity (TOF = 9.14 × 10⁻² s⁻¹) of the porphyrin-based MnSOD mimics, which is 11.9-fold lower than Ru1. The highest catalase TON value achieved is 25.1 with MnP-2, 159-fold lower than Ru1. Using oxidation of ABTS⁺⁺ to ABTS⁺⁺⁺ as the measure of peroxidase activity, MnP-3 exhibited the highest catalase/peroxidase selectivity (C/P = 96.2), which is 89.2-fold lower than Ru1.

Among the salen-based MnSOD mimics, MnS-1 has the highest reported catalase TOF (5.87 × 10⁻² s⁻¹), which is 16.6-fold lower than Ru1. The highest catalase TON is 33.4 for MnS-2, 120-fold lower than Ru1. The best catalase selectivity among the Mn-salen MnSOD mimics is observed with MnS-3 (C/P = 9.16), which is 937-fold lower than Ru1. Porphyrin- and salen-based MnSOD mimics have been reported to exhibit perfect catalase selectivity (i.e., zero peroxidase activity with nonzero catalase activity), but they suffer from poor H₂O₂ stability (TON < 3), which could indicate that the observed catalase reactivity is non-catalytic.

![Figure 2](image-url)

**Figure 2.** Best-performing Mn·porphyrin (MnP) and Mn·salen (MnS) catalase mimics. The 5th ligands (Cl or OAc) for MnS have been omitted for clarity.

<table>
<thead>
<tr>
<th>Mimic</th>
<th>catalyst TOF (s⁻¹)</th>
<th>catalase TON</th>
<th>peroxidase TOF (s⁻¹)</th>
<th>C/P selectivity</th>
<th>CCN</th>
<th>CPCN</th>
<th>Reference</th>
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<tr>
<td>Ru1</td>
<td>1.09</td>
<td>≥ 4000</td>
<td>1.27 × 10⁻⁴</td>
<td>8.58 × 10⁻⁵</td>
<td>4.36 × 10⁻¹</td>
<td>3.43 × 10⁻²</td>
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<tr>
<td>MnP-1</td>
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<td>—</td>
<td>1.65</td>
<td>—</td>
<td>1.73 × 10⁻⁵</td>
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<td>25.1</td>
<td>—</td>
<td>—</td>
<td>0.73</td>
<td>—</td>
<td>2.41 × 10⁻⁵</td>
</tr>
<tr>
<td>MnP-3</td>
<td>2.00 × 10⁻²</td>
<td>19.4</td>
<td>2.08 × 10⁻⁴</td>
<td>96.2</td>
<td>0.388</td>
<td>—</td>
<td>1.87 × 10⁻⁵</td>
</tr>
<tr>
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<td>28.8</td>
<td>1.23 × 10⁻²</td>
<td>4.77</td>
<td>1.69</td>
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</tr>
<tr>
<td>MnS-2</td>
<td>5.69 × 10⁻²</td>
<td>33.4</td>
<td>1.49 × 10⁻²</td>
<td>3.82</td>
<td>1.90</td>
<td>128</td>
<td>—</td>
</tr>
<tr>
<td>MnS-3</td>
<td>6.11 × 10⁻³</td>
<td>2.4</td>
<td>6.67 × 10⁻⁴</td>
<td>9.16</td>
<td>1.47 × 10⁻²</td>
<td>22.0</td>
<td>—</td>
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(a) Calculated by assigning to MnP-1 and MnP-2 a C/P selectivity value equal to that for MnP-3.
The catalase activity, H₂O₂ stability, and catalase/peroxidase selectivity values for Ru1 are each greater than the highest individual selectivity value among the MnSOD mimics (11.9-, 120-, and 89.2-fold greater, respectively). However, this understates the performance of Ru1 as a whole relative to each individual Mn complex in its totality. If we define a “catalase comparison number” (CCN) as catalase TOF × catalase TON, the CCN for Ru1 (4,360) is 2,290-fold higher than the best-performing Mn-based complex MnS-2 (CCN = 1.90). If we divide CCN by peroxidase TOF to account for any competing peroxidase activity, i.e., CPCN = catalase TOF × C/P, the CPCN for Ru1 (3.75 × 10⁵) is 14,200-fold higher than for MnP-2 (CPCN = 2.41 × 10³). Thus, not only are each individual catalase activity, H₂O₂ stability, and catalase/peroxidase selectivity of Ru1 among the MnSOD mimics by 1-2 orders of magnitude, the overall catalase performance of Ru1 as a whole is 3-4 orders of magnitude greater than the best-performing MnSOD mimics.

Proposed mechanism

We envision a mechanism for Ru1-catalyzed H₂O₂ disproportionation (Scheme 3A) that is conserved with the previously-established mechanism for Ru1-catalyzed ABTS•⁻ reduction with H₂O₂. Addition of Ru1 to an aqueous PBS solution will result in rapid exchange between Cl and H₂O₂, initiating the catalytic cycle by forming the aquo complex [L₆Ru-OH]⁺. Loss of H₂O and coordination of H₂O₂ will yield [L₆Ru(H₂O₂)]²⁺ (step 1) and subsequent H⁺ dissociation to the buffered aqueous solution will afford the hydroperoxo complex [L₆Ru-OOH] (step 2). We hypothesize that fragmentation of the hydroperoxo ligand will release O₂(g) and the resulting 2e⁻ will be transferred to the [L₆Ru] core, along with the hydrogen, to generate [L₆Ru-H] (step 3). Alternatively, the H-atom could transfer to the ligand CO₂ group as H⁺, leaving behind an O₂ ligand on a Ru center with an oxidation state of 0, 1+, 2+, depending on the O₂ binding mode. Independent of the structure of this intermediate, the key to the exceptional H₂O₂ stability and catalase/peroxidase selectivity of Ru1 is the fact that the 1st equivalent of H₂O₂ functions as a reductant. The Ru-H intermediate, or some other Ru1-derived species reduced by H₂O₂, will then be oxidized by the 2nd equivalent of H₂O₂, which affords H₂O and HO⁻, with HO⁻ remaining coordinated to the metal in [L₆Ru–OH] (step 4). This hydroxo complex will then acquire an H⁺ from solution to regenerate [L₆Ru·OH]³⁺ (step 5) and thereby restart the catalytic cycle.

If the proposed mechanism in Scheme 3A completely describes all aspects of Ru1-catalyzed H₂O₂ reduction, then the fact that all proposed Ru-containing intermediates are mononuclear will require the initial rate (v₀) of Ru1-catalyzed H₂O₂ reduction to be first order in [Ru1]₀. Although a plot of v₀ vs. [Ru1]₀ can be fitted by a linear equation (Figure S2A), the y-intercept corresponds to an uncatalyzed H₂O₂ degradation rate of 5 μM s⁻¹, which is too high by many orders of magnitude. Forcing the linear equation to have a y-intercept of zero results in a poor match between the fit and data (Figure S2B).

We reasoned that dimerization of a Ru-containing species can potentially, and reversibly, generate a catalytically-incompetent intermediate, where (a) low [Ru1]₀ leads to negligible dimer formation and thus v₀ will increase with [Ru1]₀ in 1:1 fashion, but then (b) high [Ru1]₀ leads to significant dimer formation and v₀ will thus increase with [Ru1]₀ in less than 1:1 fashion. We propose ([L₆Ru]₂(μ₁,₂-O₂)) as the most likely bimetallic intermediate, which would be formed via reaction of [L₆Ru–OOH] with [L₆Ru-OH]²⁺ (Scheme 3B).

Although [L₆Ru-OH]²⁺ could itself dimerize to generate species such as ([L₆Ru]₂(μ-OH))¹⁺ or ([L₆Ru]₂(μ-O)), we believe the 1000-fold excess of H₂O₂ relative to Ru1 results in the concentration of [L₆Ru–OOH] being significantly higher than [L₆Ru·OH]³⁺, which will favor the formation of ([L₆Ru]₂(μ₁,₂-O₂)) (step 6, Scheme 3B) over ([L₆Ru]₂(μ-OH))¹⁺ or ([L₆Ru]₂(μ-O)). Furthermore, the greater Ru···Ru distance afforded by the μ₁,₂-O₂ bridging ligand will result in ([L₆Ru]₂(μ₁,₂-O₂)) being a lower energy species due to reduced steric clashing between L₆ sets compared to ([L₆Ru]₂(μ-OH))¹⁺ and ([L₆Ru]₂(μ-O)), which feature shorter Ru···Ru distances due to the shorter μ-OH and μ-O bridging ligands.

Additionally, the formation of ([L₆Ru]₂(μ₁,₂-O₂)) offers a pathway through which Ru1 can exhibit 1e⁻ peroxidase-like reactivity without the Ru-containing species being irreversibly shunted off the dominant catalase cycle. Specifically, the peroxo
bond in \([L_nRu_1]_2[μ_{1,2}-O_2]\) can be reduced by 2 equiv. of ABTS\(^2\)^- and acquire 2 \(H^+\) from the buffered solution to form 2 equiv. of \([L_nRu-OH]\) (Scheme 3C). As shown in Scheme 3A, \([L_nRu-OH]\) is an intermediate that participates in the catalytic cycle.

In other complexes, moieties such as Ru–H, Ru–OOH, and Ru–O–O–Ru can exhibit characteristic features observable by \(^1\)H NMR, IR, and Raman spectroscopy.\(^{50-55}\) However, these techniques require mM concentrations (or higher), but \([Ru_1]\) has limited solubility (ca 1 mM) in water-miscible organic solvents. Furthermore, the \([Ru_1]\)-catalyzed \(H_2O_2\) disproportionation reaction shuts down when the volume % of \(H_2O\) in the reaction solution drops below 70%. Thus, 300 \(μM\) is the highest \([Ru_1]\) concentration that can be achieved in aqueous solution that still affords catalytic \(H_2O_2\) disproportionation. We were unable to detect any Ru–H, Ru–OOH, or Ru–O–O–Ru intermediates (or any other \([Ru_1]\)-derived intermediates) in the \([Ru_1]\)-catalyzed \(H_2O_2\) disproportionation reaction using \(^1\)H NMR, IR, and Raman spectroscopy, due to the inability to access analyte concentrations above 300 \(μM\).

Rate law derivation

To test the validity of the proposed mechanism for \(H_2O_2\) disproportionation catalyzed by \([Ru_1]\), including the \([L_nRu-OOH]\) \(\Rightarrow [L_nRu_2]_2[μ_{1,2}-O_2]\) branch, we derived the general rate law and measured \(v_0\) as a function of \([Ru_1]_0\), \([H_2O_2]_0\), and \([H^+]_0\). Steps 1, 2, and 5 are relatively simple equilibria described by equilibrium constants \(K_1\), \(K_2\), and \(K_5\) respectively (Scheme 3A and 3B). From our prior mechanistic investigations of \([Ru_1]\)-catalyzed ABTS\(^-\) reduction with \(H_2O_2\), we reasoned that elimination of \(O_2\) from \([L_nRu-OOH]\) (step 3, Scheme 3A) would be turnover-limiting and effectively irreversible \((k_3 << k_4\text{ and }k_5 \gg k_3)\). The \(pK_a\) values reported for other \([L_nRu-OH]\)\(^{14}\) complexes range from 9–10, therefore we reasoned that protonation of \([L_nRu-OH]_n\) to generate \([L_nRu-OH_2]^+\) (step 5, Scheme 3A) would be faster than the formation of \([L_nRu-OH]_n\), which would render step 4 effectively irreversible as well \((k_3 \gg k_4\text{ and }k_5 \gg k_3)\).

One turnover of the proposed catalytic cycle in Scheme 3A requires the consumption of 2 equiv. of \(H_2O_2\), with the 1\(^{st}\) equiv. being consumed during turnover-limiting formation of \([L_nRu-H]\) (step 3) and the 2\(^{nd}\) equiv. being consumed upon reaction with the \([L_nRu-H]\) (step 4, Scheme 3A). The time-dependent change in \([H_2O_2]\) can thus be described by Eqn. 1:

\[
-\frac{d[H_2O_2]}{dt} = k_4[H_2O_2][RuH] \quad (1)
\]

The time-dependent concentration of \([L_nRu-H]\), abbreviated as \([RuH]\), is unknown and cannot be measured directly. It is therefore necessary to express \([RuH]\) as a function of known variables and constants. This expression can be determined using (i) the equilibrium equations for steps 1, 2, 5, and 6, (ii) the steady-state first-derivative equation for \([RuH]\), and (iii) the constraint that the sum of the concentrations of all Ru-containing species must be equal to \([Ru_1]\). Using this approach, we can describe \([RuH]\) as a function of \([Ru_1]_0\), \([H_2O_2]_0\), and \([H^+]_0\). However, the formation of \([L_nRu_2]_2[μ_{1,2}-O_2]\) (step 6, Scheme 3B) introduces a non-linear term to the equation relating \([Ru_1]\) to \([RuH]\):

\[
[Ru_1]_0 = F_1 \cdot [RuH]^2 + F_2 \cdot [RuH] \quad (2)
\]

The derivation of Eqn. 2 and the full expressions for \(F_1\), a function of \([H_2O_2]_0\), and \(F_2\), a function of both \([H_2O_2]_0\) and \([H^+]_0\), can be found in the Supporting Information (Eqns. S1–S9). Because the expression for \([Ru_1]_0\) contains both \([RuH]\) and \([RuH]\) terms, solving for \([RuH]\) requires using the quadratic equation (Eqns. S10–S13), which yields a non-linear relationship between \([RuH]\) and \([Ru_1]\):

\[
[RuH] = \frac{F_2 + \sqrt{F_2^2 + 4F_1[Ru_1]_0}}{2F_1} \quad (3)
\]

The “\(^{\pm}\)” operator in the numerator of Eqn. S12 simplifies to “\(^+\)” in Eqn. 3 because equilibrium constants and rate constants cannot be negative, and thus having a “\(^-\)” operator would have resulted in a negative value for \([RuH]\), which is impossible. Plugging Eqn. 3 into Eqn. 1 (Eqns. S14–S17) affords an expression (Eqn. 4) for the rate of \(H_2O_2\) consumption as a function of \([Ru_1]\), \([H_2O_2]\), and \([H^+]\), where \(G_1\) is a constant:

\[
-\frac{d[H_2O_2]}{dt} = G_1 \cdot \left(-F_2 + \sqrt{F_2^2 + 4F_1[Ru_1]_0}\right) \quad (4)
\]

Measuring the rate of \(H_2O_2\) consumption at very short times \((v_0)\) allows the time-dependent \([H_2O_2]\) and \([H^+]\) terms in \(F_1\) and \(F_2\) to be replaced with the known constants \([H_2O_2]_0\) and \([H^+]_0\), respectively (Eqn. S18). By varying the initial concentration of only one of \([Ru_1]\), \([H_2O_2]\), or \([H^+]\), but holding the other two concentrations constant, Eqn. 3 simplifies to a single-variable function relating \(v_0\) (as the \(y\)-variable) to \([Ru_1]_0\), \([H_2O_2]_0\), or \([H^+]_0\) (as the \(x\)-variable). If \([Ru_1]_0\) is varied but \([H_2O_2]_0\) or \([H^+]_0\) are held constant (Eqns. S19–S22), the general rate law predicts the plot of \(v_0\) vs. \([Ru_1]_0\) will follow Eqn. 5. If \([H_2O_2]_0\) is varied but \([Ru_1]_0\) and \([H^+]_0\) are held constant (Eqns. S23–S26), the plot of \(v_0\) vs. \([H_2O_2]_0\) will follow Eqn. 6. If \([H^+]_0\) is varied but \([Ru_1]_0\) and \([H_2O_2]_0\) are held constant (Eqns. S27–S30), the plot of \(v_0\) vs. \([H_2O_2]_0\) will follow Eqn. 7. Note that \(a\), \(b\), and \(c\) are constants in Eqns. 5–7 and \([Ru_1]_0\) is a constant in Eqn. 6–7.

\[
y = (c)\left(-b + (b^2 + 4ac)^{1/2}\right) \quad (5)
\]

\[
y = (d)\left(-bx - c + (bx + c)^2 + 4a[Ru_1]_0x^{1/2}\right) \quad (6)
\]

\[
y = (d)\left(-bx - c + (bx + c)^2 + 4a[RU_1]_0b^{1/2}\right) \quad (7)
\]

Analysis of reaction kinetics

To fit the experimental \(v_0\) vs. \([Ru_1]_0\) data and to not predict too high a rate of \(H_2O_2\) consumption in the absence of \([Ru_1]\), Eqn. 5 should produce a trace that \((i)\) has a more positive slope from \([Ru_1]_0 = 0\) to 10 \(μM\) than higher concentrations and \((i)\) increases
roughly linearly from \([\text{Ru}]_0 = 10\) to 40 \(\mu M\). An overlay of the Eqn. 5 trace (grey line, Figure 3A) on the plot of experimental \(v_0\) vs. \([\text{Ru}]_0\) data (black crosses, Figure 3A) shows that Eqn. 5 accurately describes the experimental data and satisfies the aforementioned constraints. If \([\text{Ru}]_0\) is very low, then \([(\text{L,Ru})_2(\mu_1,2-O_2)]\) formation is negligible, and \(v_0\) increases with \([\text{Ru}]_0\) in roughly 1:1 fashion (i.e., the mechanism follows Scheme 3A with negligible contribution by Scheme 3B). As \([\text{Ru}]_0\) increases, more \([(\text{L,Ru})_2(\mu_1,2-O_2)]\) forms, which decreases the relative amount of total Ru available to participate in the catalase cycle, and \(v_0\) increases with \([\text{Ru}]_0\) in less than 1:1 fashion (i.e., the mechanism follows a combination of the steps in Schemes 3A and 3B).

\[ \text{Scheme 3A} \]

The plot of \(v_0\) vs. \([H_2O_2]_0\) (Figure 3B) shows \(v_0\) increasing with increasing \([H_2O_2]_0\), but with a shallower slope at higher \([H_2O_2]_0\) values, behavior which can be accurately modeled by Eqn. 6. The shallower slope at higher \([H_2O_2]_0\) reflects the fact that \(H_2O_2\) coordinates to Ru before the turnover-limiting step (i.e., step 1, Scheme 3A). At sufficiently high \([H_2O_2]_0\), the equilibrium in step 1 saturates and \(v_0\) cannot increase any further.

Conversely, \(v_0\) decreases as \([H^+]_0\) increases, with the slope shallower at higher \([H^+]_0\) values (Figure 3C), whereby the variation in the \(v_0\) vs. \([H^+]_0\) experimental data can be accurately modeled by Eqn. 7. The general trend of greater \(v_0\) values at lower \([H^+]_0\) arises because \(H^+\) dissociation must occur (step 2, Scheme 3A) before the turnover-limiting step (step 3, Scheme 3A). The diminishing impact of increasing \([H^+]_0\) on \(v_0\) at higher values reflects the fact that step 2 is an equilibrium process.

Although the rate law equations that describe the \([H_2O_2]_0\) and \([H^+]_0\) dependencies of \(\text{Ru}\)-catalyzed \(H_2O_2\) disproportionation differ significantly from \(\text{Ru}\)-catalyzed ABTS** reduction with \(H_2O_2\), the general trends are similar, which suggests that the mechanism for the former reaction is partially conserved with the latter. The most noteworthy difference is that \(\text{Ru}\) does catalyze \(H_2O_2\) disproportionation in pure \(H_2O\), but \(\text{Ru}\) cannot catalyze ABTS** reduction with \(H_2O_2\). The origin of this is that \(\text{Ru}\)-catalyzed ABTS** reduction with \(H_2O_2\) becomes negligible. In contrast, \(\text{Ru}\)-catalyzed \(H_2O_2\) disproportionation releases 1 equiv. of \(H^+\) to solution in step 2 (Scheme 3A), but then acquires 1 equiv. of \(H^+\) from solution in step 5, with no net \(H^+\) generation or consumption during catalytic cycle turnover.

Eyring–Polanyi analysis of \(\text{Ru}\)-catalyzed \(H_2O_2\) disproportionation rate data collected at \(T = 20, 25, 30,\) and 35 °C (Figure 4) reveal that \(\Delta H^\ddagger = 13.6 \pm 0.7\) kcal mol\(^{-1}\) and \(\Delta S^\ddagger = -26.1 \pm 2.4\) cal mol\(^{-1}\) K\(^{-1}\). For comparison, \(\text{Ru}\)-catalyzed ABTS** reduction with \(H_2O_2\) exhibits \(\Delta H^\ddagger = 29.1 \pm 0.6\) kcal mol\(^{-1}\) and \(\Delta S^\ddagger = 25.5 \pm 1.9\) cal mol\(^{-1}\) K\(^{-1}\). Notably, the \(\Delta S^\ddagger\) value for \(\text{Ru}\)-catalyzed \(H_2O_2\) disproportionation is comparable in magnitude but opposite in sign to the value for \(\text{Ru}\)-catalyzed ABTS** reduction with \(H_2O_2\), which indicates significant differences in transition state structures and properties. Indeed, all our previous studies of \(\text{Ru}\)-catalyzed ABTS** reduction, with any terminal reductant, revealed positive \(\Delta S^\ddagger\) values.

A large, negative \(\Delta S^\ddagger\) value for \(\text{Ru}\)-catalyzed \(H_2O_2\) disproportionation can be rationalized by the reaction of the Ru–H intermediate with \(H_2O_2\) (step 4, Scheme 3A). Because one OH group from \(H_2O_2\) is transferred to Ru to yield the Ru–OH intermediate, then the corresponding transition state most likely features an associative interaction between \(H_2O_2\) and Ru–H, potentially including H-bonding or direct coordination, which would give rise to the large, negative \(\Delta S^\ddagger\) value for \(\text{Ru}\)-catalyzed \(H_2O_2\) disproportionation. In contrast, the reaction of the Ru–H intermediate with ABTS** in \(\text{Ru}\)-catalyzed ABTS** reduction with \(H_2O_2\) involves 1\(e^-\) transfer from Ru–H to ABTS**. A large, positive \(\Delta S^\ddagger\) value for this process suggests outer-sphere electron transfer and coupling to the preceding step, fragmentation of Ru–OOH into Ru–H and O\(_2\). The larger, positive \(\Delta H^\ddagger\) value for \(\text{Ru}\)-catalyzed ABTS** reduction with \(H_2O_2\) likely reflects the fact that a negatively-charged electron must be removed from a neutral Ru–H intermediate and separated from the resulting cationic species, and then must be transferred to an already negatively-charged ABTS** molecule, both of which are energetically- unfavorable due to Coulombic effects. In contrast, both \(H_2O_2\) and the Ru–H intermediate are neutral, therefore unfavorable Coulombic effects are absent.
Conclusions

This report constitutes to the best of our knowledge the first instance of an organometallic complex that functions as a catalase mimic (i.e., catalyzes the reaction \( \text{H}_2\text{O}_2 \rightarrow \text{H}_2\text{O} + \frac{1}{2}\text{O}_2 \)), which is surprising given that catalytic \( \text{H}_2\text{O}_2 \) disproportionation has been reported for a multitude of non-organometallic complexes comprising a wide variety of metals.\(^{56-63}\) A similarly rich diversity of peroxidase model complexes also exists.\(^{64-72}\)

Compared to existing redox therapeutics, the Mn–porphyrin and –salen complexes initially developed to mimic MnSOD, Ru\(^1\) exhibits dramatically higher catalase activity, \( \text{H}_2\text{O}_2 \) stability, and catalase/peroxidase selectivity than the highest individual uncertainties when investigating the behavior of pro-oxidant reactivity. This selectivity will significantly decrease therapeutic will be almost exclusively antioxidant, with minimal faster than its peroxidase activity, its reactivity as a redox mimic is 4 orders of magnitude superior to any Mn–porphyrin or –salen biomimetic complex.

A major focus area of research involving Ru complexes in current Mn–porphyrin or Mn–salen complexes. The ability of Ru\(^1\) to prevent or alleviate biological oxidative stress will be detailed in a subsequent report.

Conflicts of interest

There are no conflicts to declare.

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Notes and references

64. Although catalytic H2O2 disproportionation has been reported for a wider variety of synthetic complexes than just Mn–porphyrin and Mn–salen complexes, detailed comparisons and discussion are only possible with MnSOD mimics for which both catalase and peroxidase reaction kinetic data are available.
65. Values of ν0 are calculated from the slopes of the [H2O2] vs. t plots at t0′, where t0′ was identified as the time at which the first-derivatives of the [H2O2] vs. t plots reached a minimum. Typically, t0′ occurred <4 min after H2O2 addition and when <10% of H21O12 had been consumed.
66. To be consistent with the reported kinetics for the Mn–porphyrin and Mn–salen complexes, catalase TOF values for Ru1 in units of s−1 were calculated by dividing the initial rate of H2O2 consumption in µM s−1 by the concentration of Ru1 in µM.
67. In this manuscript, catalase TOF and peroxidase TOF refer specifically to H2O2 consumption. Literature values for O2 production and ABTS+ production can be converted to H2O2 consumption via multiplication by 2 and 0.5, respectively.
68. R. Re, N. Pellegrini, A. Proteggente, A. Pannala, M. Yang and C. Rice-Evans, Free Radic. Biol. Med., 1999, 26, 1231–1237. There is dramatic variation in the catalase reaction kinetics reported for many Mn–salen complexes. There are even with instances of the same research group studying the same complex a second time and obtaining a second catalase TOF more than 10-fold lower than the TOF in the initial report. Fortunately, each report on Mn–salen complexes used for comparison to Ru1 included at least one Mn–salen complex that had been measured more than once. Dividing the corrected second value by the value in the initial report afforded a correction factor that was applied to each catalase TOF value.