

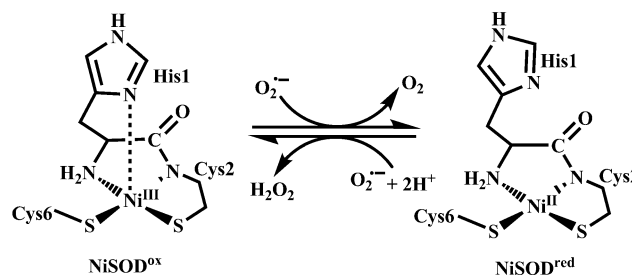
# Hexacoordinate Nickel(II)/(III) Complexes that Mimic the Catalytic Cycle of Nickel Superoxide Dismutase\*\*

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Dedicated to Professor Pradip K. Mascharak

**Abstract:** A functional model complex of nickel superoxide dismutase (NiSOD) with a non-peptide ligand which mimics the full catalytic cycle of NiSOD is unknown. Similarly, it has not been fully elucidated whether NiSOD activity is a result of an outer- or inner-sphere electron-transfer mechanism. With this in mind, two octahedral nickel(II)/(III) complexes of a bis-tridentate  $N_2S$  donor carboxamide ligand, *N*-2-phenylthio-phenyl-2'-pyridinecarboxamide ( $HL^{Ph}$ ), have been synthesized, structurally characterized, and their SOD activities examined. These complexes mimic the full catalytic cycle of NiSOD. Electrochemical experiments support an outer-sphere electron-transfer mechanism for their SOD activity.

The superoxide radical anions ( $O_2^{\cdot-}$ ) that inevitably form as a by-product of aerobic metabolism can cause severe cellular damage in biological systems.<sup>[1]</sup> To combat such oxidative damage, living organisms have developed defense metalloenzymes, called superoxide dismutases (SODs),<sup>[2]</sup> that catalytically convert  $O_2^{\cdot-}$  into  $O_2$  and  $H_2O_2$  by the reduction and oxidation of their redox-active metal centers, such as Fe, Mn, Ni, or Cu, in mononuclear FeSOD, MnSOD, NiSOD, and dinuclear Cu-ZnSOD, respectively, of which NiSOD was most recently identified. An analysis of the single-crystal X-ray structure of NiSOD revealed a square-planar  $N_2S_2Ni^{II}$  reduced form and a square-pyramidal  $N_3S_2Ni^{III}$  oxidized form as shown in Scheme 1.<sup>[3]</sup>



**Scheme 1.** Coordination geometry of oxidized (left) and reduced (right) forms of NiSOD active sites.

Efforts to synthesize nickel(II)/(III) complexes to structurally and/or functionally model NiSOD afforded several examples of such complexes with peptide-<sup>[4]</sup> and non-peptide-based<sup>[5]</sup> ligands. These reports account for important aspects of the structure–function relationship in NiSOD, such as the likely proton source for  $O_2^{\cdot-}$  reduction to  $H_2O_2$ ,<sup>[4b,k,5d–f]</sup> the role of “on” and “off” histidine-N (His-N) ligation axially to the nickel site,<sup>[4b,5b,h,i]</sup> and the rate of electron-transfer reactions.<sup>[4b,d,6]</sup> However, to understand the dismutation process, several key issues, as follows, require more intense study. 1) During dismutation, why does S-oxygenation of the cysteine-thiolato-sulfur residue not occur in an environment containing reactive oxygen species, such as  $O_2^{\cdot-}$  and  $H_2O_2$ ? Few models show S-oxygenation reactivity.<sup>[5b,c]</sup> 2) Does  $O_2^{\cdot-}$  or  $O_2$  interact with the Ni center either directly or indirectly through a secondary coordination sphere? Examples of Ni-dioxygen species are known.<sup>[7]</sup> 3) If no direct coordination to the Ni center occurs then is the Ni site required to have at least one vacant substrate binding site for its SOD activity? Biochemical evidence, both in favor<sup>[4e,f,h–k]</sup> and against<sup>[4b,5f,6]</sup> substrate binding to the nickel center, exists, in support of either an inner- or outer-sphere electron-transfer (ET) mechanism. Most of this evidence, including the low prevalence of S-oxygenation and very high rate (of an order of approximately  $10^9$ ) of catalytic  $O_2^{\cdot-}$  disproportion, suggest an outer-sphere ET mechanism, although this is still somewhat ambiguous. Recent investigations support the binding of a  $CN^-$  moiety to the Ni center in a peptide-ligand environment, giving impetus to the ongoing debate.<sup>[4b]</sup> Indeed, the native NiSOD active site itself and all existing model complexes have one or more empty binding sites around the nickel center. Therefore, during dismutation the interaction of  $O_2$ ,  $O_2^{\cdot-}$ , and  $H_2O_2$  with the Ni center cannot be excluded. To resolve these questions regarding the mechanistic pathway of SOD activity, more functional models are required. Apart

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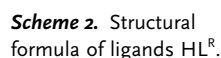
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from a few examples of nickel–peptide derivatives (containing peptide ligands in which a sequence of 6–12 amino acids provide the same coordination environment about the Ni center as in native NiSOD),<sup>[4]</sup> there is only one definite example of a nickel(III) complex containing a non-peptide-based ligand ( $\text{N}_3\text{O}_2$  donor).<sup>[5k]</sup> This complex can catalyze the oxidation of  $\text{O}_2^{\cdot-}$  to  $\text{O}_2$ , one of the two half reactions,  $\text{M}^{\text{ox}} + \text{O}_2^{\cdot-} \rightarrow \text{M}^{\text{red}} + \text{O}_2$ , of the NiSOD catalytic cycle. No model complex of NiSOD with a non-peptide ligand has yet been shown to mimic the full catalytic cycle nor has been

shown to demonstrate the outer-sphere ET mechanism for its SOD activity.

Herein, we report the first examples of nickel(II)/(III) complexes,  $[(\text{L}^{\text{Ph}})_2\text{Ni}]$  (**1**) and  $[(\text{L}^{\text{Ph}})_2\text{Ni}]-(\text{ClO}_4)_2 \cdot \text{H}_2\text{O}$  (**2**· $\text{H}_2\text{O}$ ), which are derived from a tridentate monocarboxamide  $\text{N}_2\text{S}$  donor ligand,  $\text{HL}^{\text{Ph}}$  (Scheme 2,  $\text{HL}^{\text{Ph}} = \text{N}$ -2-phenylthiophenyl-2'-pyridinecarboxamide) that functionally mimic the full catalytic cycle of NiSOD. As the Ni center is

**Scheme 2.** Structural formula of ligands  $\text{HL}^{\text{R}}$ .

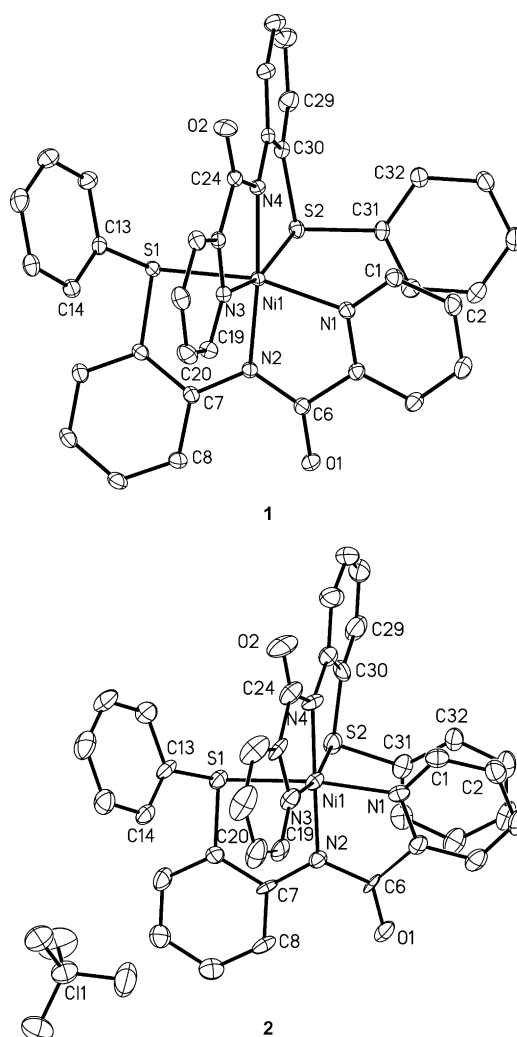


hexacoordinated in these complexes with no vacant substrate binding site, the outer-sphere ET mechanism for their SOD activity is more likely.

In NiSOD, all of the nitrogen and sulfur donor atoms coordinated to the nickel center are of different types (one nitrogen each of amide, amine, and imidazole, one thiolate-sulfur atom, and one hydrogen-bonded thiolate-sulfur atom, which acts like a thioether sulfur). These donor atoms appear to tune the electron density surrounding the Ni center with the result that the  $\text{Ni}^{\text{II/III}}$  redox couple is set to within the potential window of SOD activity. Recently, we reported that the amidato- $\text{N}^-$  donor atom of the deprotonated  $\text{HL}^{\text{Me}}$  ligand (Scheme 2), as a result of its strong  $\sigma$ -donor ability, can stabilize significantly the higher oxidation state(s) of metal ions, such as copper(II) and nickel(III).<sup>[8]</sup> The relative stabilities of various Ni oxidation states (0, +1, +2, +3) and their correlation to both the hard/soft nature and the  $\sigma$ -donor/ $\pi$ -acceptor properties of the ligand donor center(s) has also been demonstrated.<sup>[8b]</sup> Although the coulometrically generated  $\text{Ni}^{\text{III}}$  species,  $[(\text{L}^{\text{Me}})_2\text{Ni}]^+$ , was observed, attempts to isolate it in pure form by using the chemical oxidant,  $(\text{NH}_4)_2[\text{Ce}(\text{NO}_3)_6]$  (ammonium cerium(IV) nitrate, ACN) failed even at low temperature. As soon as  $[(\text{L}^{\text{Me}})_2\text{Ni}]^+$  formed, it reverts back to its precursor quite quickly. Similar examples of the successful synthesis of pure  $\text{Ni}^{\text{III}}$  species by electrochemical methods rather than using a chemical oxidant have been established.<sup>[9]</sup> Herein, however, using the  $\text{HL}^{\text{Ph}}$  ligand we have been able to isolate pure **2** in good yield from its precursor **1** by using ACN as an oxidant, possibly resulting from the fine tuning of the thioether-sulfur donor ability, imposed by a phenyl substituent which had a  $\pm \text{R}$  effect unlike the +I effect ( $\text{R} = \text{resonance or mesomeric}$ ,  $\text{I} = \text{inductive}$ ) of methyl group in the  $\text{HL}^{\text{Me}}$  ligand.

The  $\text{Ni}^{\text{II}}$  complex  $[(\text{L}^{\text{Ph}})_2\text{Ni}]$  (**1**) was synthesized in dimethylformamide (DMF) by reaction of  $\text{NaL}^{\text{Ph}}$  with  $[\text{Ni}(\text{H}_2\text{O})_6](\text{ClO}_4)_2$  in a 2:1 molar ratio. In the FTIR spectrum

of **1**, amide-nitrogen coordination to  $\text{Ni}^{\text{II}}$  is indicated by a change in the band of the carbonyl moiety, which undergoes a red shift ( $\nu_{\text{CO}} = 1620 \text{ cm}^{-1}$ ) compared to  $1680 \text{ cm}^{-1}$  for the free ligand. The magnetic moment of **1**,  $\mu = 2.85 \text{ B.M.}$ , measured by the Evans method in a solvent mixture of  $\text{CH}_2\text{Cl}_2$  and  $\text{C}_6\text{H}_6$ , indicates that **1** is paramagnetic with two unpaired spins on the nickel(II) center ( $S = 1$ ). The UV/Vis absorption spectrum of **1** measured in  $\text{CH}_3\text{CN}$  (with absorption bands  $\lambda_{\text{max}}/\text{nm}$  ( $\epsilon/\text{M}^{-1}\text{cm}^{-1}$ ) = 867 (66), 560 (25, shoulder), 340 (14880), 247 (28970) is comparable to those of other octahedral nickel(II) complexes.<sup>[8b]</sup> The single-crystal X-ray structure of **1**<sup>[10]</sup> revealed a distorted octahedral geometry about the  $\text{Ni}^{\text{II}}$  center. Two  $\text{L}^{\text{Ph}}$  ligands are bound to the  $\text{Ni}^{\text{II}}$  atom in a meridional fashion with two thioether-sulfur and two amidato-nitrogen donors of two ligands in *cis* and *trans* positions, respectively (Figure 1). The average  $\text{Ni}-\text{N}_{\text{py}}$ ,  $\text{Ni}-\text{N}_{\text{amide}}$ , and  $\text{Ni}-\text{S}$  bond lengths of 2.0566(12) Å, 2.0258(11) Å,



**Figure 1.** ORTEP diagrams of  $[(\text{L}^{\text{Ph}})_2\text{Ni}]$  **1** (top) and  $[(\text{L}^{\text{Ph}})_2\text{Ni}]-(\text{ClO}_4)_2 \cdot \text{H}_2\text{O}$  (**2**· $\text{H}_2\text{O}$ ) (bottom). Thermal ellipsoids are set at 50% probability and the  $\text{H}_2\text{O}$  molecule of **2** and H atoms are omitted for clarity. Selected bond lengths [Å] in  $(\text{L}^{\text{Ph}})_2\text{Ni}$ , **1** and in  $[(\text{L}^{\text{Ph}})_2\text{Ni}]^+$  ion of **2**:  $\text{Ni1}-\text{N1}$  2.0557(12) [1.993(5)],  $\text{Ni1}-\text{N2}$  2.0182(11) [1.901(4)],  $\text{Ni1}-\text{N3}$  2.0575(12) [2.025(5)],  $\text{Ni1}-\text{N4}$  2.0334(11) [1.909(5)],  $\text{Ni1}-\text{S1}$  2.4615(4) [2.3222(17)],  $\text{Ni1}-\text{S2}$  2.4785(4) [2.3889(17)].

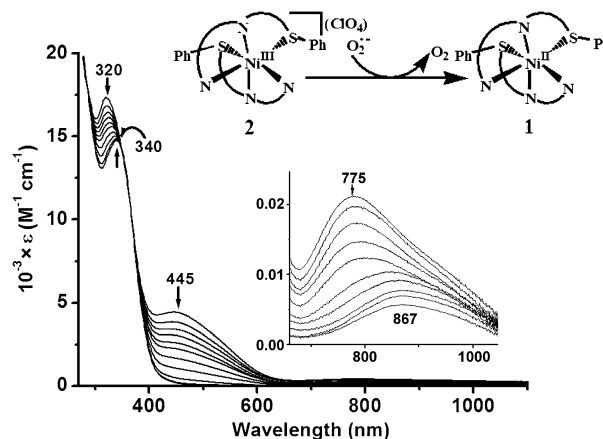
and 2.47(4) Å, respectively, are comparable to those of the complex  $[(L^{\text{Me}})_2\text{Ni}]$ , except for the Ni–S distance, which is approximately 0.026 Å longer in **1** (sulfur donor strength of -SPh is less than -SMe). The red-shifted UV/Vis absorption band at  $\lambda = 867$  nm in **1** compared to  $\lambda = 851$  nm for  $[(L^{\text{Me}})_2\text{Ni}]$  clearly follow the similar trends, as previously observed<sup>[8b]</sup> for a weaker donor ligand. The cyclic voltammogram (CV) of **1** displays a reversible redox wave (see Figure S4 in the Supporting Information) which corresponds to the  $\text{Ni}^{\text{II}}/\text{Ni}^{\text{III}}$  couple at  $E_{1/2} = +0.85$  V ( $\Delta E_p = 60$  mV) versus SCE (SCE = saturated calomel electrode), a slightly more anodic potential than that of  $[(L^{\text{Me}})_2\text{Ni}]$  as expected.

Addition of a  $\text{CH}_3\text{CN}$  solution of ACN to a yellow-colored solution of **1** in  $\text{CH}_2\text{Cl}_2$  immediately generates a dark, reddish-brown solution from which **2** has been isolated in good yield. In the FTIR spectrum of **2**, a  $\nu_{\text{OH}}$  band occurs at  $3520\text{ cm}^{-1}$  and a  $\nu_{\text{CO}}$  band at  $1635\text{ cm}^{-1}$ . The  $\nu_{\text{CO}}$  band is blue shifted compared to that of **1**, indicating coordination of the amidato- $\text{N}^-$  atom to a nickel ion with an oxidation state greater than +2, that is, to  $\text{Ni}^{\text{III}}$ . The EPR spectrum of **2** in the solid state at room temperature displays an isotropic signal at  $g = 2.09$  that in  $\text{CH}_2\text{Cl}_2$  solution splits to form a five-line signal<sup>[8b,11]</sup> (a  $\text{Ni}^{\text{III}}$  spin coupled to the nuclear spin of two *trans* amide-nitrogen atoms). This signal has a super-hyperfine coupling constant,  $A_{\parallel}^{\text{N}}$  (average), of 12 G (Figure S5) which confirms the presence of a  $\text{Ni}^{\text{III}}$  center and is further supported by its magnetic moment,  $\mu = 1.69$  B.M. ( $S = 1/2$ ), corresponding to one unpaired spin. The UV/Vis absorption spectrum of **2** in  $\text{CH}_3\text{CN}$  (with absorption bands at  $\lambda_{\text{max}}/\text{nm}$  ( $\epsilon/\text{M}^{-1}\text{cm}^{-1}$ ) = 930 (120, shoulder), 775 (200), 445 (4500), 320 (17 420), 250 (29 500, shoulder)) is comparable to the reported  $\text{Ni}^{\text{III}}$  complexes with a  $\text{N}_4\text{S}_2$  coordination sphere.<sup>[8b,9a,11c]</sup> Crystals suitable for X-ray diffraction were obtained by layering pentane on a  $\text{CH}_2\text{Cl}_2$  solution of **2**. The ORTEP plot of the crystal structure of **2** is shown in Figure 1 (bottom). In general, the structure shows similar structural features to the nickel coordination environment in **1**, but has, however, shorter Ni–N and Ni–S bond lengths. The average Ni– $\text{N}_{\text{py}}$ , Ni– $\text{N}_{\text{amide}}$ , and Ni–S bond lengths of 2.009(6) Å, 1.905(5) Å, and 2.3554(19) Å, respectively, are approximately 0.05 Å, 0.12 Å, and 0.11 Å shorter than those of **1**, clearly indicating that the nickel center is in a +3 oxidation state in **2**. DFT calculations on **2** were performed using the Gaussian 09 program and considering X-ray structural coordinates without symmetry restrictions.<sup>[12]</sup> Results showed that the unpaired electron spin mainly resides on the Ni center with a Mulliken atomic spin density of +0.79 (Figure S6). To our knowledge, this is the first structural example of a  $\text{Ni}^{\text{III}}$  complex with amide-nitrogen and thioether-sulfur donors.

To investigate the capability of complexes **1** and **2** towards the dismutation of  $\text{O}_2^{\cdot-}$  according to the following two half reactions of the NiSOD catalytic cycle [Eq. (1), (2)] and the concomitant shuttling between  $\text{Ni}^{\text{II}}$  and  $\text{Ni}^{\text{III}}$  oxidation states during the dismutation process, several experiments have been performed.



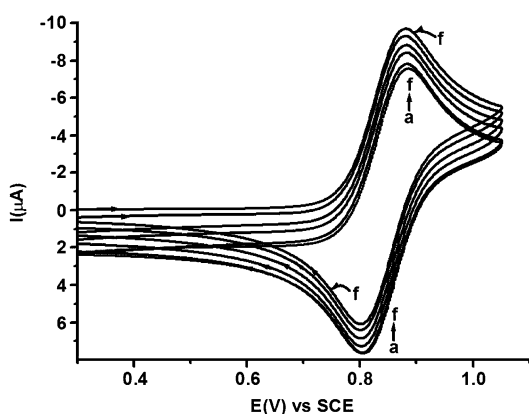
To investigate the SOD activity of **2**, a solution of **2** in  $\text{CH}_3\text{CN}$  ( $10^{-4}\text{ M}$ ) was titrated against a solution of  $\text{KO}_2$  in  $\text{CH}_3\text{OH}$  (1 equiv  $\text{KO}_2$  was dissolved in 100  $\mu\text{L}$   $\text{CH}_3\text{OH}$ , additions of this solution were then made in 10  $\mu\text{L}$  aliquots). The progress of the reaction was monitored using UV/Vis absorption spectroscopy (Figure 2). With increasing additions



**Figure 2.** UV/Vis absorption spectra showing the conversion of a  $\text{CH}_3\text{CN}$  solution of **2** into **1** after the addition of  $\text{CH}_3\text{OH}$  solution of  $\text{KO}_2$ . Inset: Expanded portion of absorption spectrum ( $\lambda = 650$ –1050 nm).

of  $\text{KO}_2$ , the absorption band of **2** at  $\lambda_{\text{max}} = 445$  nm vanishes and bands at  $\lambda = 775$  nm and  $\lambda = 320$  nm undergo red shifts to  $\lambda = 867$  nm and  $\lambda = 340$  nm, respectively. These bands correspond to the absorption bands evident in the spectrum of **1**. The isosbestic points at  $\lambda = 365$  nm and  $\lambda = 284$  nm indicate the clean transformation of **2** to form **1**. The molar extinction coefficients,  $\epsilon$ , of the absorption bands at  $\lambda = 340$  nm and  $\lambda = 320$  nm are comparable to those of the independently synthesized complexes **1** and **2** respectively, confirming that the conversion of **2** into **1** is stoichiometric. This conversion is very fast and we believe that the reaction proceeds according to Equation (1). The release of  $\text{O}_2$  gas is confirmed electrochemically (Figure S7, S8). In native NiSOD, the very high  $\text{O}_2^{\cdot-}$  dismutation rate of the order of  $10^9$  strongly supports an outer-sphere ET mechanism for its SOD activity. To gain insight herein into the nature of ET processes, cyclic voltammetry experiments on **2** in the presence of  $\text{KO}_2$  have been performed. Repeated scans of a solution of **2** in  $\text{CH}_3\text{CN}$  ( $10^{-3}\text{ M}$ ) after each addition of 20  $\mu\text{L}$  of a total of 100  $\mu\text{L}$  of a  $\text{CH}_3\text{OH}$  solution of stoichiometric  $\text{KO}_2$ , shown in Figure 3, revealed that the potential, current height, and  $\Delta E_p$  values of the  $\text{Ni}^{\text{II}}/\text{Ni}^{\text{III}}$  couple were retained. These results strongly support that no structural reorganization during this  $\text{Ni}^{\text{III}}$  to  $\text{Ni}^{\text{II}}$  reduction process occurs and consequently confirms the outer-sphere ET from  $\text{O}_2^{\cdot-}$  to a  $\text{Ni}^{\text{III}}$  metal center.

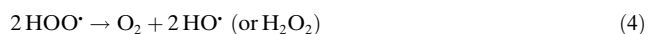
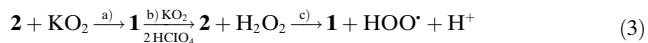
To examine the SOD activity of **1**, and to investigate whether the activity proceeds according to Equation (2), we have performed several experiments separately with  $\text{KO}_2$  and  $\text{HClO}_4$ . First, it was found that the yellow solution of **1** in  $\text{CH}_3\text{CN}$  in the presence of  $\text{KO}_2$  caused no change in the UV/Vis absorption spectrum with time. Second, when two equivalent of  $\text{HClO}_4$  were added to a solution of **1** in



**Figure 3.** Cyclic voltammogram of **2** in CH<sub>3</sub>CN (trace (a)) showing the conversion of **2** into **1** after the addition of a stoichiometric solution of KO<sub>2</sub> in CH<sub>3</sub>OH (traces (b)–(f) after each addition of 20 μL of the KO<sub>2</sub> solution).

CH<sub>3</sub>CN, complete loss of the ligand from the Ni<sup>II</sup> center was observed (Figure S9). However, when the solution of **1** plus KO<sub>2</sub> is treated with HClO<sub>4</sub>, the reaction then proceeds according to Equation (2). Next, we studied the SOD activity of **1** which was prepared by two different methods: a) in situ generated **1** from the reaction of **2** with KO<sub>2</sub> [Eq. (3)], and b) isolated solid **1**, synthesized from the reaction of NaL<sup>Ph</sup> with [Ni(H<sub>2</sub>O)<sub>6</sub>](ClO<sub>4</sub>)<sub>2</sub>.

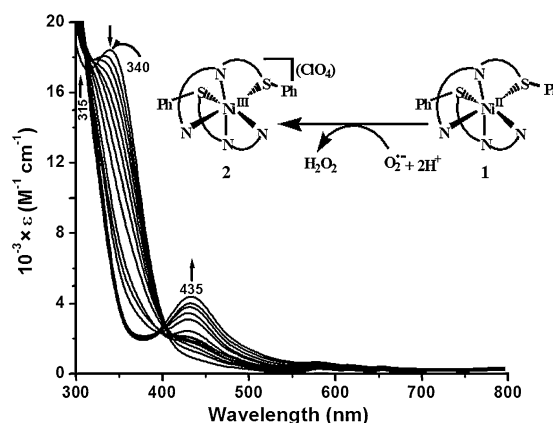
First, the SOD activity of in situ generated **1** was investigated. Stoichiometric production of **1** from a reaction of **2** with KO<sub>2</sub> [Eq. (3), step a] is evident from the UV/Vis absorption spectrum and the cyclic voltammogram as shown in Figures 2 and 3. **1** was treated with another equivalent of KO<sub>2</sub> followed by 2 equivalents of HClO<sub>4</sub> [Eq. (3), step b] after which the reddish brown color of **2** re-formed immediately. The formation of **2** was confirmed by the UV/Vis absorption spectrum and cyclic voltammetry (Figures S10, S11). An analysis of the UV/Vis absorption spectrum taken immediately after treatment with HClO<sub>4</sub> and focusing on the band at λ = 445 nm showed that approximately 75 % of **2** was re-formed. The remaining 25 % of **2** was converted back into **1** by reaction with produced H<sub>2</sub>O<sub>2</sub> [Eq. (3), step c] following a similar reaction mechanism (proton-coupled ET reaction), as proposed by Solomon et al.<sup>[13]</sup> In this case, half an equivalent of O<sub>2</sub> with respect to complex **2** was formed from HO<sub>2</sub><sup>•</sup> [Eq. (4)]. This formation of O<sub>2</sub> was confirmed electrochemically (Figure S12). In fact, following the proton-coupled ET reaction [Eq. (3), step c], the solution gradually faded within approximately five minutes from reddish-brown (resulting from generated **2**) to yellow (**1**).



The conversion of **2** into **1** using H<sub>2</sub>O<sub>2</sub> as a reagent [Eq. (3), step c] has been confirmed in a separate experiment by using a solution of the isolated solid **2** in CH<sub>3</sub>CN (Figure S13). Furthermore, the CV of the reaction mixture (composed of approximately 75 % of **2** and 25 % of **1**) taken

immediately after treatment of **1** with HClO<sub>4</sub> and KO<sub>2</sub> [Eq. (3), step b] displays the same current height of the Ni<sup>III</sup>/Ni<sup>II</sup> couple as that of **1** before the addition of acid (Figure S11), indicating the conversion of generated **2** into **1** [Eq. (3), step c] and not to any other species. As both **1** and **2** display the same cyclic voltammogram, the total current height thus remain same, independent of the ratio of **1** to **2** present in the reaction mixture. The reversible conversion between **1** and **2** was also qualitatively investigated (through the appearance of the reddish brown/yellow color) through several cycles after the sequential addition of KO<sub>2</sub> and 2 equiv HClO<sub>4</sub>. For this experiment, additional CH<sub>3</sub>OH/CH<sub>2</sub>Cl<sub>2</sub> was added to the reaction medium to prevent precipitation of **1** and KO<sub>2</sub> as they are highly soluble in this mixed solvent but not in pure CH<sub>3</sub>CN.

Second, the SOD activity of isolated **1** was investigated. As compounds **1**, **2**, and KO<sub>2</sub> were shown to be highly soluble in CH<sub>3</sub>OH/CH<sub>2</sub>Cl<sub>2</sub> (1:1 v/v), the SOD activity of isolated **1** was examined in this mixed solvent medium. Additionally, CH<sub>3</sub>OH helps to store protons by hydrogen bonding and supply it on demand in a controlled fashion to facilitate the transformation of **1** to **2** [Eq. (2)]. The solvent mixture was also suitable to monitor the formation of **2** by UV/Vis absorption spectroscopy (Figure 4). In fact, in this mixed

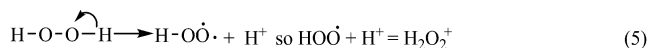


**Figure 4.** UV/Vis absorption spectra showing the conversion of a CH<sub>3</sub>OH/CH<sub>2</sub>Cl<sub>2</sub> (1:1 v/v) solution of **1** (10<sup>−4</sup> M) + KO<sub>2</sub> → **2** after the addition of 6 equiv HClO<sub>4</sub> (200 μL) in CH<sub>3</sub>OH solution. Each spectrum was recorded after the addition of 10 μL of the acid solution.

solvent, species **2** formed from the reaction of **1** plus KO<sub>2</sub> with HClO<sub>4</sub> is more stable even in the presence of generated H<sub>2</sub>O<sub>2</sub>, provided excess HClO<sub>4</sub> (6 equivalents to 1 equivalent of **1**) is used. It is likely that when excess acid is used, the formation of HO<sub>2</sub><sup>•</sup> and H<sup>+</sup> from H<sub>2</sub>O<sub>2</sub> is prevented [Eq. 3, step c], and therefore the generated **2** is more stable and is not converted back into **1** [Eq. (3), step c]. In contrast, in pure CH<sub>3</sub>CN the reagent KO<sub>2</sub> is less soluble and so the added acid triggered de-metalation of the ligand (Figure S9) before complete transformation of **1** to **2** could occur. The formed **2** must react with H<sub>2</sub>O<sub>2</sub> (if no excess acid is used) and re-form **1** by a proton-coupled ET reaction [Eq. (3), step c]. When a CH<sub>3</sub>OH/CH<sub>2</sub>Cl<sub>2</sub> solution of **1** plus KO<sub>2</sub> is treated with HClO<sub>4</sub> (6 equivalents), a reddish-brown color is generated

resulting from the formation of **2** (Figure 4). The new band at  $\lambda_{\text{max}} = 435$  nm and the blue shift of the band at  $\lambda = 340$  nm to  $\lambda = 315$  nm correspond to those measured for **2** (same bands observed for isolated **2** in  $\text{CH}_3\text{OH}/\text{CH}_2\text{Cl}_2$ ). Based on the extinction coefficient of the band at  $\lambda = 435$  nm, the formation of **2** is stoichiometric (98 %), although this reddish brown color gradually fades (2 h). The lack of clean isosbestic point(s) in Figure 4 unlike in Figure 2 is a result of the follow-up reaction of generated **2** with  $\text{H}_2\text{O}_2$  and not with  $\text{HClO}_4$  [Eq. (3), step c]. This step depends on the concentration of  $\text{H}^+$  ions added, where a lower  $[\text{H}^+]$  promotes the reaction of **2** with  $\text{H}_2\text{O}_2$  but a greater  $[\text{H}^+]$  suppresses it. Thus, the progressive addition of  $\text{H}^+$  ions shifted the isosbestic points during the course of the experiment (Figure 4). Note, the solution of isolated **2** in  $\text{CH}_3\text{OH}/\text{CH}_2\text{Cl}_2$  is stable in the presence of  $\text{HClO}_4$ , with no change evident in the UV/Vis absorption spectrum upon addition of up to 12 equivalents of  $\text{HClO}_4$ . The CV of the reddish brown solution generated displayed two redox couples, one at  $E_{1/2} = -0.26$  V ( $\Delta E_p = 90$  mV), attributable to excess  $\text{HClO}_4$  present in the reaction medium, and the other at  $E_{1/2} = +0.84$  V which corresponds to the  $\text{Ni}^{\text{II}}/\text{Ni}^{\text{III}}$  couple of **2** (Figure S14). This CV clearly states that the generated **2** is stable in the presence of  $\text{HClO}_4$  and  $\text{H}_2\text{O}_2$  in this mixed solvent medium.

To understand the reason for the measured SOD activity of **1** and **2**, further cyclic voltammetry experiments were carried out. The CV of  $\text{KO}_2$  in a solvent mixture of  $\text{CH}_3\text{OH}/\text{CH}_2\text{Cl}_2$  (1:1 v/v) shows an irreversible wave which has  $E_{\text{pa}} = +0.32$  V and  $E_{\text{pc}} = -0.42$  V ( $E_{\text{pa}} + E_{\text{pc}}/2 = -0.05$  V) for the  $\text{O}_2^{\cdot-}/\text{O}_2$  couple. After addition of  $\text{HClO}_4$  to this solution, the CV shows a new irreversible wave with  $E_{\text{pa}} = +0.99$  V versus SCE for the  $(\text{HOO}^{\cdot} + \text{H}^+)/\text{H}_2\text{O}_2$  couple (Figure S15). This wave corresponds to the proton-coupled reduction of  $\text{O}_2^{\cdot-}$  to form  $\text{H}_2\text{O}_2$  [ $\text{KO}_2 + 2\text{H}^+ \rightarrow \text{HO}_2 + \text{H}^+ + \text{K}^+ \rightarrow \text{H}_2\text{O}_2 + \text{K}^+$ ;  $\text{H}_2\text{O}_2 + \text{e}^- \rightarrow \text{H}_2\text{O}_2^{\cdot-}$ ]. These redox potentials of  $\text{O}_2^{\cdot-}$  are very close to reported values of  $+0.04$  V and  $+1.03$  V versus  $\text{Ag}/\text{AgCl}$  respectively.<sup>[4a]</sup> The potential of  $+0.99$  V for the  $(\text{HOO}^{\cdot} + \text{H}^+)/\text{H}_2\text{O}_2$  couple is 110 mV more anodic than the  $E_{\text{pa}} = +0.88$  V of the reversible  $\text{Ni}^{\text{II}}/\text{Ni}^{\text{III}}$  couple of **1**. Therefore, it should be possible to oxidize the  $\text{Ni}^{\text{II}} \rightarrow \text{Ni}^{\text{III}}$ , that is from **1** to **2**, and at the same time the species  $\text{HOO}^{\cdot}$  and  $\text{H}^+$ , or  $\text{H}_2\text{O}_2^+$  [Eq. (5)], will be reduced to  $\text{H}_2\text{O}_2$  to complete the catalytic cycle.



The CV of the yellow solution of **1** [Eq. (3), formed in step a] plus  $\text{KO}_2$  displays the redox waves which correspond to the  $\text{O}_2^{\cdot-}/\text{O}_2$  and  $\text{Ni}^{\text{II}}/\text{Ni}^{\text{III}}$  redox couples. However, when  $\text{HClO}_4$  is added, inducing a color change from yellow to reddish brown, the  $\text{O}_2^{\cdot-}/\text{O}_2$  couple vanishes and the response of the  $\text{Ni}^{\text{II}}/\text{Ni}^{\text{III}}$  couple remains unchanged with almost same current height and  $\Delta E_p$  values (Figure S11). These results firmly establish the outer-sphere ET mechanism for this **1** to **2** conversion process [Eq. (2)]. Similarly, as  $E_{1/2} = +0.85$  V of the  $\text{Ni}^{\text{II}}/\text{Ni}^{\text{III}}$  couple is more anodic than the  $E_{\text{pa}} = +0.32$  V of the  $\text{O}_2^{\cdot-}/\text{O}_2$  couple, the  $\text{Ni}^{\text{III}}$  complex, **2**, can easily oxidize  $\text{O}_2^{\cdot-}$  to  $\text{O}_2$  and will itself be reduced to form  $\text{Ni}^{\text{II}}$  complex

**1** [Eq. (1)]. Interestingly, in the presence of weak acids, such as  $\text{CH}_3\text{CO}_2\text{H}$  and  $\text{CF}_3\text{CO}_2\text{H}$ , no formation of **2** is observed. Such weak acids are possibly unable to protonate  $\text{HO}_2^{\cdot}$  to form the oxidant  $\text{H}_2\text{O}_2^+$  and therefore are unable to oxidize  $\text{Ni}^{\text{II}}$  to  $\text{Ni}^{\text{III}}$ .

In summary,  $[(\text{L}^{\text{Ph}})_2\text{Ni}]$  (**1**) and  $[(\text{L}^{\text{Ph}})_2\text{Ni}](\text{ClO}_4) \cdot \text{H}_2\text{O}$  (**2**· $\text{H}_2\text{O}$ ) are the first examples of a set of NiSOD functional models with non-peptide ligands that mimic the full catalytic cycle of NiSOD.  $[(\text{L}^{\text{Ph}})_2\text{Ni}](\text{ClO}_4) \cdot \text{H}_2\text{O}$  (**2**· $\text{H}_2\text{O}$ ) is the first example of a structurally characterized  $\text{Ni}^{\text{III}}$  complex with an amide-nitrogen and thioether-sulfur donor environment. These complexes are also the first examples of NiSOD functional models where the nickel center is hexacoordinated. Thus for the first time, direct support for an outer-sphere electron-transfer mechanism for SOD activity is obtained. The present work will provide a new route in the assessment of the mechanistic pathway of NiSOD activity.

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- [1] a) J. S. Valentine, D. L. Wertz, T. J. Lyons, L. L. Liou, J. J. Goto, E. B. Gralla, *Curr. Opin. Chem. Biol.* **1998**, *2*, 253–262; b) D. M. Kurtz, Jr., *Acc. Chem. Res.* **2004**, *37*, 902–908; c) J. A. Imlay, *Annu. Rev. Microbiol.* **2003**, *57*, 395–418; d) A. C. Maritim, R. A. Sanders, J. B. Watkins III, *J. Biochem. Mol. Toxicol.* **2003**, *17*, 24–38.
- [2] a) A.-F. Miller, *Curr. Opin. Chem. Biol.* **2004**, *8*, 162–168; b) I. Fridovich, *Annu. Rev. Biochem.* **1995**, *64*, 97–112.
- [3] a) E. P. Broering, P. T. Truong, E. M. Gale, T. C. Harrop, *Biochemistry* **2013**, *52*, 4–18; b) D. P. Barondeau, C. J. Kassmann, C. K. Bruns, J. A. Tainer, E. D. Getzoff, *Biochemistry* **2004**, *43*, 8038–8047; c) J. L. Wuerger, Y.-I. Yim, H.-S. Yim, S.-O. Kang, K. D. Carugo, *Proc. Natl. Acad. Sci. USA* **2004**, *101*, 8569–8574.
- [4] a) J. Shearer, L. M. Long, *Inorg. Chem.* **2006**, *45*, 2358–2360; b) K. P. Neupane, K. Gearty, A. Francis, J. Shearer, *J. Am. Chem. Soc.* **2007**, *129*, 14605–14618; c) M. Schmidt, S. Zahn, M. Carella, O. Ohlenschläger, M. Görlach, E. Kothe, J. Weston, *ChemBioChem* **2008**, *9*, 2135–2146; d) J. Shearer, K. P. Neupane, P. E. Callan, *Inorg. Chem.* **2009**, *48*, 10560–10571; e) D. Tietze, H. Breitzke, D. Imhof, E. Kothe, J. Weston, G. Buntkowsky, *Chem. Eur. J.* **2009**, *15*, 517–523; f) D. Tietze, M. Tischler, S. Voigt, D. Imhof, O. Ohlenschläger, M. Görlach, G. Buntkowsky, *Chem. Eur. J.* **2010**, *16*, 7572–7578; g) M. E. Krause, A. M. Glass, T. A. Jackson, J. S. Laurence, *Inorg. Chem.* **2010**, *49*, 362–364; h) D. Tietze, S. Voigt, D. Mollenhauer, M. Tischler, D. Imhof, T. Gutmann, L. González, O. Ohlenschläger, H. Breitzke, M. Görlach, G. Buntkowsky, *Angew. Chem.* **2011**, *123*, 3002–3006; *Angew. Chem. Int. Ed.* **2011**, *50*, 2946–2950; i) M. E. Krause, A. M. Glass, T. A. Jackson, J. S. Laurence, *Inorg. Chem.* **2011**, *50*, 2479–2487; j) A. M. Glass, M. E. Krause, J. S. Laurence, T. A. Jackson, *Inorg. Chem.* **2012**, *51*, 10055–10063; k) J. Shearer, *Angew. Chem.* **2013**, *125*, 2629–2632; *Angew. Chem. Int. Ed.* **2013**, *52*, 2569–2572.
- [5] a) J. Shearer, N. Zhao, *Inorg. Chem.* **2006**, *45*, 9637–9639; b) H. Ma, S. Chattopadhyay, J. L. Petersen, M. P. Jensen, *Inorg. Chem.* **2008**, *47*, 7966–7968; c) R. M. Jenkins, M. L. Singleton, E. Almaraz, J. H. Reibenspies, M. Y. Darensbourg, *Inorg. Chem.*

- 2009, 48, 7280–7293; d) C. S. Mullins, C. A. Grapperhaus, B. C. Frye, L. H. Wood, A. J. Hay, R. M. Buchanan, M. S. Mashuta, *Inorg. Chem.* **2009**, 48, 9974–9976; e) E. M. Gale, A. K. Patra, T. C. Harrop, *Inorg. Chem.* **2009**, 48, 5620–5622; f) V. Mathrubootham, J. Thomas, R. Staples, J. McCracken, J. Shearer, E. L. Hegg, *Inorg. Chem.* **2010**, 49, 5393–5406; g) E. M. Gale, B. S. Narendrapurapu, A. C. Simmonett, H. F. Schaefer III, T. C. Harrop, *Inorg. Chem.* **2010**, 49, 7080–7096; h) D. Nakane, Y. Funahashi, T. Ozawa, H. Masuda, *Chem. Lett.* **2010**, 39, 344–346; i) M. Gennari, M. Orio, J. Pécout, F. Neese, M.-N. Collomb, C. Duboc, *Inorg. Chem.* **2010**, 49, 6399–6401; j) E. M. Gale, A. C. Simmonett, J. Telser, H. F. Schaefer III, T. C. Harrop, *Inorg. Chem.* **2011**, 50, 9216–9218; k) E. M. Gale, D. M. Cowart, R. A. Scott, T. C. Harrop, *Inorg. Chem.* **2011**, 50, 10460–10471; l) W.-Z. Lee, C.-W. Chiang, T.-H. Lin, T.-S. Kuo, *Chem. Eur. J.* **2012**, 18, 50–53.
- [6] S. B. Choudhury, J.-W. Lee, G. Davidson, Y.-I. Yim, K. Bose, M. L. Sharma, S.-O. Kang, D. E. Cabeli, M. J. Maroney, *Biochemistry* **1999**, 38, 3744–3752.
- [7] a) K. Shiren, S. Ogo, S. Fujinami, H. Hayashi, M. Suzuki, A. Uehara, Y. Watanabe, Y. Moro-oka, *J. Am. Chem. Soc.* **2000**, 122, 254–262; b) D. Nakane, S. Kuwasako, M. Tsuge, M. Kubo, Y. Funahashi, T. Ozawa, T. Ogura, H. Masuda, *Chem. Commun.* **2010**, 46, 2142–2144; c) J. Cho, H. Y. Kang, L. V. Liu, R. Sarangi, E. I. Solomon, W. Nam, *Chem. Sci.* **2013**, 4, 1502–1508; d) M. T. Kieber-Emmons, C. G. Riordan, *Acc. Chem. Res.* **2007**, 40, 618–625.
- [8] a) R. C. Maji, S. K. Barman, S. Roy, S. K. Chatterjee, F. L. Bowles, M. M. Olmstead, A. K. Patra, *Inorg. Chem.* **2013**, 52, 11084–11095; b) S. K. Chatterjee, S. Roy, S. K. Barman, R. C. Maji, M. M. Olmstead, A. K. Patra, *Inorg. Chem.* **2012**, 51, 7625–7635.
- [9] a) K. Pramanik, S. Karmakar, S. B. Choudhury, A. Chakravorty, *Inorg. Chem.* **1997**, 36, 3562–3564; b) J. Hanss, H.-J. Krüger, *Angew. Chem.* **1998**, 110, 366–369; *Angew. Chem. Int. Ed.* **1998**, 37, 360–363.
- [10] Diffraction data were collected on a Bruker Smart APEX II CCD area detector diffractometer at 90(2) K for **1** and at 120(2) K for **2**. Structures were solved by direct methods (*SHELXS-97*, Sheldrick, **2008**) and refined by using full-matrix least squares on  $F^2$  (*SHELXL*, Sheldrick, **2013**) with all non-hydrogen atoms refined anisotropically. Crystal data for (**1**): Yellow block, monoclinic, space group  $P2_1/n$ ,  $a = 15.8112(13)$ ,  $b = 13.0936(11)$ ,  $c = 16.0002(13)$ ,  $\beta = 116.8880(10)$ ,  $V = 2954.3(4) \text{ \AA}^3$ ,  $Z = 4$ ,  $\rho_{\text{calcd}} = 1.505 \text{ Mg m}^{-3}$ ,  $2\theta_{\text{max}} = 62.7^\circ$ ,  $F(000) = 1384$ ,  $\lambda_{\text{Mo K}\alpha} = 0.71073 \text{ \AA}$ , total reflections 9686, unique reflections 7926, parameters 406,  $R1 = 0.0317$  and  $wR2 = 0.0741$  for 7926 data ( $I > 2\sigma(I)$ ). Crystal data for **2**: Purple block, monoclinic, space group  $P2_1/c$ ,  $a = 13.138(3)$ ,  $b = 25.212(6)$ ,  $c = 10.352(2)$ ,  $\beta = 101.146(4)$ ,  $V = 3364.3(13) \text{ \AA}^3$ ,  $Z = 4$ ,  $\rho_{\text{calcd}} = 1.554 \text{ Mg m}^{-3}$ ,  $2\theta_{\text{max}} = 51^\circ$ ,  $F(000) = 1620$ ,  $\lambda_{\text{Mo K}\alpha} = 0.71073 \text{ \AA}$ , total reflections 6206, unique reflections 3478, parameters 468,  $R1 = 0.063$  and  $wR2 = 0.1491$  for 3478 data ( $I > 2\sigma(I)$ ). CCDC-976750 (**1**), 977005 (**2**) contain the supplementary crystallographic data for this paper. These data can be obtained free of charge from The Cambridge Crystallographic Data Centre via [www.ccdc.cam.ac.uk/data\\_request/cif](http://www.ccdc.cam.ac.uk/data_request/cif).
- [11] a) T. Storr, E. C. Wasinger, R. C. Pratt, T. D. P. Stack, *Angew. Chem.* **2007**, 119, 5290–5293; *Angew. Chem. Int. Ed.* **2007**, 46, 5198–5201; b) H.-J. Krüger, R. H. Holm, *J. Am. Chem. Soc.* **1990**, 112, 2955–2963; c) A. K. Singh, R. Mukherjee, *Dalton Trans.* **2005**, 2886–2891.
- [12] Gaussian 09 (Revision B.01), Frisch, et al., Gaussian, Inc.: Wallingford, CT, **2010**.
- [13] R. K. Szilagy, P. A. Bryngelson, M. J. Maroney, B. Hedman, K. O. Hodgson, E. I. Solomon, *J. Am. Chem. Soc.* **2004**, 126, 3018–3019.