How Nature makes O₂: an electronic level mechanism for water oxidation in photosynthesis.

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Abstract: In this report we combine broken symmetry density functional calculations and electron paramagnetic resonance analysis to obtain the electronic structure of the penultimate S₃ state of Nature’s water oxidising complex and determine the electronic pathway of O–O bond formation. Analysis of the electronic structure changes along the reaction path shows that two spin crossovers, facilitated by the geometry and magnetism of the water oxidising complex are used to provide a unique low energy pathway. The pathway is facilitated via formation and stabilisation of the [O₂]³⁻ ion. This ion is formed between ligated deprotonated substrate waters, O₅ and O₆, and is stabilised by antiferromagnetic interaction with the Mn ions of the complex. Combining computational, crystallographic and spectroscopic data we show that an equilibrium exists between an O₅ oxo and O₆ hydroxo form with an S=3 spin state and a deprotonated O₆ form containing a two-centre one electron bond in [O₅O₆]³⁻ which we identify as the form detected using crystallography. This form corresponds to an S=6 spin state which we demonstrate gives rise to a low intensity EPR spectrum compared with the accompanying S=3 state, making its detection via EPR difficult and overshadowed by the S=3 form. Simulations using 70% of the S=6 component give rise to a superior fit to the experimental W-band EPR spectral envelope compared with an S=3 only form. The computational, crystallographic and spectroscopic data are shown to coalesce to the same picture of a predominant S=6 species containing the first one-electron oxidation product of
two water molecules i.e. [O5O6]\(^3\). Progression of this form to the two-electron oxidised peroxo and three-electron oxidised superoxo forms, leading eventually to the evolution of triplet \(O_2\), is shown to be the pathway Nature adopts to oxidise water under ambient conditions. The study reveals the key electronic, magnetic and structural design features of Nature’s catalyst which facilitates water oxidation to \(O_2\) under ambient conditions.

**Introduction:** The water oxidising complex of photosystem 2 oxidises two water molecules to molecular oxygen at a rate approaching 1000 s\(^{-1}\) at ambient temperatures and pressure.\(^1\)–\(^3\) It is one of the most important reactions in biology, and is also of intense interest from a green energy perspective, where it is recognized to be the main barrier to the development of commercial solar devices for the generation of hydrogen from water.\(^4\) Water oxidation to dioxygen is a challenging due to the high endergonicity (\(E^\circ = 0.82\) V (vs NHE) at pH 7) for the reaction,

\[
2H_2O \rightarrow O_2 + 4H^+ + 4e^-
\]

and the associated need to remove four protons and four electrons with the formation of an oxygen-oxygen, O-O, bond. Two broad mechanistic proposals, either water nucleophilic attack of metal oxo or direct metal oxo radical coupling, have been proposed for artificial water oxidation catalysts.\(^5\) Somewhat similar proposals have been put forward for the WOC namely water nucleophilic attack\(^6\) or oxyl radical-oxo coupling\(^7\) These require the generation of a reactive oxo species in the final Kok-cycle S\(_4\) state. Artificial catalysts generally use very high strength oxidising agents to generate reactive oxo species, either radical oxygen species or highly charged metal electrophilic species. The WOC on the other hand is limited to the approximately 1 V oxidising power of the nearby tyrosyl radical, \(Y_Z^{\text{OX}}.\)\(^8\) The current mechanisms for the WOC which propose generation of a reactive “hot” oxo species in the \(S_4\) state fail to explain how such a species can be generated when the
oxidising capability available from the visible light energy available via the $S_3Y_Z^{ox}$ oxidant is around 1V. It is also unclear how triplet O$_2$ can be produced from peroxo with such a mechanism given that the last oxidising equivalent has been used.

An alternative mechanistic scenario is a dynamic equilibrium model of $S_3$ consisting of concerted reduction of Mn coupled to O-O bond formation. Such a mechanism would aesthetically require the WOC catalytic cluster design, Figure 1, to facilitate a lowering of the O-O bond formation barrier, permitting it to be readily transversed at room temperature without the generation of a reactive oxo form.

![Figure 1. WOC catalyst core structure and numbering scheme used throughout.](image)

For the four electron oxidation of two water molecules in the aqueous phase, Figure 2, it is the first one-electron oxidation of water to form an oxyl radical that represents the major energy barrier with a reduction potential $\geq 2$ V, well in excess of the 1 V available in $S_3Y_Z^{ox}$. 

Figure 2. Energy level diagram for four sequential electron oxidations of water to O$_2$ in the aqueous phase.

If the WOC can reduce this barrier then sequential 4e oxidation of water is thermodynamically feasible with visible light energy. Here we demonstrate that the WOC is designed to achieve this task by stabilising the one-electron oxidation product of water as an [O$_2$]$^{3-}$ ion. Partial O-O bond formation and stabilisation of this species is brought about by the unique architecture and magnetism of the WOC which facilitates electron rearrangement between the O5 and O6 oxos engaged in O-O bond formation and the Mn1 and Mn4 ions of the WOC. This is combined with a stabilising antiferromagnetic alignment of the Mn1,3,4 ions with the unpaired electron of [O$_5$O$_6$]$^{3-}$. This stabilises nascent O-O bond formation in the S$_3$ state, permitting low barrier O-O bond formation and is supported by the XFEL structural crystallographic data and by the EPR spectra obtained on the 2-flash state of the WOC.
Results and Discussion

Electronic structure analysis

Our starting point on the pathway to O5-O6 bond formation is an O5 oxo O6 hydroxo form of the WOC complex, Figure 1, formed after initial formation of the $S_3$ state. This corresponds to the $S=3$ form detected by EPR with four Mn (IV) ions. O6 corresponds to the new oxygen atom detected by XFEL after the second flash. For this oxo-hydroxo model seven broken symmetry, Ms, states are possible at the optimised geometry. We have shown that two of these, both $M_S=3$, $[\text{Mn}_4(\downarrow\downarrow\downarrow)\text{Mn}_3(\uparrow\uparrow\uparrow)\text{Mn}_2(\uparrow\uparrow\uparrow)\text{Mn}_1(\uparrow\uparrow\uparrow)]$ and $[\text{Mn}_4(\uparrow\uparrow\uparrow)\text{Mn}_3(\downarrow\downarrow\downarrow)\text{Mn}_2(\uparrow\uparrow\uparrow)\text{Mn}_1(\uparrow\uparrow\uparrow)]$ are the lowest in energy and govern the spin density of the complex resulting in a spin distribution of close to 0.5, 0.5, 0.0 and 0.0 for Mn1-Mn4. This explains the set of two large (Mn1 and Mn2) and two small (Mn3 and Mn4) magnitude $^{55}\text{Mn}$ hfcs observed using EDNMR spectroscopy. Deprotonation of O6 leads to an O5 oxo - O6 oxo form. At the optimised geometry, two low energy BS states are found, an $M_S=3$ form $[\text{Mn}_4(\uparrow\uparrow\uparrow)\text{Mn}_3(\uparrow\uparrow\uparrow)\text{Mn}_2(\uparrow\uparrow\uparrow)\text{Mn}_1(\downarrow\downarrow\downarrow)]$ and $M_S=6 ([\text{Mn}_4(\uparrow\uparrow\uparrow)\text{Mn}_3(\uparrow\uparrow\uparrow)\text{Mn}_2(\uparrow\uparrow\uparrow)\text{Mn}_1(\uparrow\uparrow\uparrow)]$. The energies of these oxo-hydroxo and oxo-oxo states are plotted as a function of O5O6 distance in Figure 3. The oxo-hydroxo form is the lowest energy form for O5O6 distances 2.5 – 2.1 Å.
Figure 3 PES scans for oxo-hydroxo (blue), oxo-oxo Ms=3 (red) and oxo-oxo, Ms=6 (green). The spin alignments for the most stable species along the PES are illustrated.

At 2.1 Å a spin crossover to the Ms=6 oxo-oxo form is indicated. For the Ms=3 oxo-oxo form the crossover with the oxo-hydroxo PES occurs at a higher energy at an O5O6 distance of 2.0 Å. The Ms=6 state remains the lowest energy form up to an O5O6 distance of 1.6 Å where a spin crossover to the Ms=3 occurs as peroxo is formed. At O5O6 distances less than 2.0 Å the oxo-hydroxo form becomes unstable and convergence is not achievable. The PES scan in Figure 3 suggests that two spin crossovers, facilitated by the unique geometry and magnetism of the WOC complex, provide a low energy pathway for O5-O6 peroxo bond formation. To monitor the changes in electronic configuration and rationalise the relative energies of the different BS states as we traverse the PES, we monitor the changes in the intrinsic localised bond orbitals involved. These changes are demonstrated for the oxo-oxo Ms=6 and the oxo-oxo Ms=3 states in Figures 4 and 5 respectively.
**Figure 4.** Ms=6 IBO analysis. Top, potential energy surface (PES) for O5-O6 bond formation (black) with accompanying IBO changes colour coded by orbitals shown beneath. Representative IBOs are given at the points labelled on the PES above showing $\alpha$ and $\beta$ spin evolution, see text for details.
Figure 5. Ms=3 IBO analysis. Top, potential energy surface (PES) for O5-O6 bond formation (black) with accompanying IBO changes colour coded by orbitals shown beneath. Representative IBOs are given at the points labelled on the PES above showing α and β spin evolution, see text for details.

For the oxo-hydroxo form no changes are found in the IBOs in the region of 2.5 – 2.0 Å and as mentioned above this model becomes unstable at bond distances less than 2.0 Å. By contrast significant changes are observed for both oxo-oxo forms. The IBOs which undergo
significant changes are located by monitoring the root-mean-square deviation of every IBO from the initial partial charge distribution along the PES.\textsuperscript{12,13} Figures 4 and 5 identify four main IBOs participating in bond making and breaking during the reaction. These are, the $\alpha$ and $\beta$ spin manifolds of the Mn$_4$O$_5$ $\sigma$-bond, the $\beta$ spin manifold of the Mn$_1$O$_6$ $\sigma$-bond and the $\alpha$ spin manifold of one of the $\pi$-bonding lone pair orbitals on O6. As the O5-O6 bond distance is decreased from the non-bonded oxo-oxo form, the $\alpha$ electron density of the Mn$_4$O$_5$ sigma bond evolves into a $dz^2$ orbital on Mn$_4$ at an O5-O6 bond distance of around 2.2 Å for Ms=6 and 2.0 Å for Ms=3. Concurrently with this electron density rearrangement, the $\alpha$ density of the $\pi$- lone pair on O6, left by deprotonation, evolves to a $\sigma$ bond between the O6 and O5 oxygens. A Mayer bond order analysis,\textsuperscript{14} Figure 6, also illustrates such a change with a decrease in the Mn$_4$-O5 bond order from near 1.0 to near 0.5 and an increase in the O5-O6 bond order from 0.0 to near 0.4. In a similar fashion, Mulliken spin population analysis, Figure 7 shows a change in spin population of Mn$_4$ from near 3.0 to 4.0 signalling a reduction from Mn$_4$(IV) to Mn$_4$(III). At an O5O6 distance of 1.6 Å, a spin crossover from the Ms=6 to Ms=3 state occurs. For the Ms=3 state, progression along the PES shows the $\beta$-electron of the Mn$_4$-O5 sigma bond evolves to become the $\beta$ component of the O5-O6 sigma bond and the Mn$_1$-O6 sigma bond $\beta$ electron density evolves into a $dz^2$ orbital on Mn$_1$. For Ms=6, the $\beta$-electron of the Mn$_4$-O5 sigma bond again evolves to become the $\beta$ component of the O5-O6 sigma bond while in this case a Mn$_1$-O6 $\pi$ bond $\beta$ electron density evolves into a $d\pi$ orbital on Mn$_1$. Mulliken spin populations, Figure 7, correspondingly show an increase in spin population from 3 to 4 for Mn1 illustrating reduction of Mn1 to high spin Mn(III) for Ms=3 whereas for Ms=6 the electron transfer of a $\beta$ electron to Mn1 results in occupation of a $d\pi$ orbital (see Figure 4) resulting in a spin population of 2 and corresponding to a low spin form of Mn(III). The Mayer bond order values for both Ms states of Figure 6 show an increase in the O5-O6 bond order to near 1.0 as the peroxo is formed.
Figure 6. Variation of Mayer bond orders for the Ms=6 and Ms=3 BS states along the O5O6 PES.
**Figure 7:** Variation of Mulliken spin populations along the O5O6 PES for Ms=3 (blue) and Ms=6 (red). A. Mn1 (x) and Mn4 (circle). B. O5 (x) and O6 (circle)
In summary for the Ms=6 state, as the O5O6 distance decreases the Mn4 spin population increases from a value of around 3 indicating a Mn(IV) oxidation state to a spin population of 4 at 2.2 Å. This state corresponds to a Mn4(↑↑↑↑)Mn3(↑↑↑)Mn2(↑↑↑)Mn1(↑↑)[O5O6]↓ state. The IBOs show that electron movement has occurred from O5 to Mn4 leading to a high spin Mn4 (III) and a nascent two centre one electron O5-O6 bond has formed. This state is favoured up to an O5O6 bond distance of 1.6 Å where a spin crossover to the Ms=3 [Mn4(↑↑↑↑)Mn3(↑↑↑)Mn2(↑↑↑)Mn1(↓↓↓↓)] state is favoured resulting in peroxo formation. This particular Ms=3 state is stabilised due to the favourable antiferromagnetic coupling between the Mn1 and Mn2 ions for the peroxo form. Corresponding orbitals demonstrating this phenomenon are described in the SI.

Importantly the most stable state at an O5O6 distance around 2.0 Å, which corresponds closely to that measured by XFEL, corresponds to an effective [O5O6]3− ion. This species was identified previously by us15 as a shoulder on the Ms=3 state, see Figure 5. While a shoulder on the Ms=3 PES, it corresponds to a broad minimum energy structure on the Ms=6 surface due to the favourable antiferromagnetic coupling with all four Mn ions. We note that this species has been described by us15 and others16,17 previously as an O5 oxo O6 oxyl form but is correctly described as [O5O6]3− as the spin density is present on both O5 and O6 as clearly demonstrated by the spin density plot for this form in Figure 8.
Figure 8. Spin density contour plot for Ms=6 oxo oxo model at 2.0 Å on the PES surface demonstrating distribution of negative spin density on both O5 and O6 positions and signifying the presence of [O5O6]$^{3-}$.

This Ms=6 state is stabilised by the strong antiferromagnetic interaction occurring between the β electron density shared between O5 and O6 and the α electron spins on the Mn1, Mn3 and Mn4 ions. The strength of this antiferromagnetic coupling is quantitatively demonstrated by the large magnitude O5O6Mn1, O5O6/Mn3 and O5O6/Mn4 J values calculated for this electronic arrangement, see Table S1.
Scheme 1. Schematic electron flow pattern based on our PES and IBO analysis from Figures 3 and 4. O5O6 oxidation status indicated

Our PES and IBO analysis therefore shows that low barrier O-O bond formation is facilitated in the WOC by providing a concerted flow of electrons between the coupling oxos, O5 and O6, and the Mn1 and Mn4 ions providing low barrier spin crossovers to occur. Scheme 1 demonstrates key electron movements and spin flips involved. Figure 9 uses a simple molecular orbital scheme to illustrate the species involved with the concerted flow of electron from the $\sigma_{2p}^*$ orbital to Mn1 and Mn4 during O-O bond formation permitting formation of the O-O bond without double occupation of the high energy orbital.
Figure 9. Molecular orbital schematic of electronic rearrangements leading to O-O bond formation

The $\text{O}_2^{3-}$ species corresponds to a situation with doubly occupied $\sigma_{2p}$ and one-electron ($\beta$) occupied $\sigma_{2p}^*$ orbitals, resulting in an O-O bond order of 0.5. At the second spin crossover at a bond distance of 1.6 Å electron flow effectively occurs from the singly occupied $\sigma_{2p}^*$ orbital to Mn$^1$ forming a high spin Mn$^1$(III) ion and resulting in the formation of a O-O single bond in the peroxo form with a bond order close to 1. The key to low-barrier O-O formation in the WOC is the concerted removal of two electrons from the combining O6 and
O5 oxos by Mn$_1$ and Mn$_4$ respectively thereby resulting in an unoccupied $\sigma_{2p^*}$ orbital and low barrier O-O bond formation.

**Crystallographic and Electron Paramagnetic Resonance analysis**

Studies using X-ray free electron laser (XFEL) atomic resolution structures of the 2-flash, predominantly S$_3$ state, generally support participation of O5 and O6 in O-O bond formation. While Young et al $^{18}$ found no evidence of an additional oxygen O6 insertion in S$_3$, Suga et al $^{19}$ reported an additional oxygen atom corresponding to O6 with an O5-O6 bond distance of 1.5 Å indicating peroxo formation.$^{9,20}$ Later studies proposed an additional oxygen O$_x$ similar to O6 of the Suga et al structure but with an extended O5-O6/O$_x$ bond length of 2.1 Å.$^{21,22,23}$ More recently Suga et al $^{16}$ propose a best fit O5-O6 bond length of 1.9 Å while not ruling out a peroxo structure. All structures of S$_3$ so far appear to rule out an oxo-hydroxo non-bonded form which requires an O5-O6 bond separation of at least 2.5 Å. Additional structural features are a relatively long Mn$_4$O5 bond length of 2.2 Å and a short Mn$_1$O6 bond distance of 1.7 Å. Comparison of our calculated minimum energy Ms = 6 structure with the experimental determinations is given in Table 1. This demonstrates excellent agreement with the minimum energy point of this state and the experimental XFEL values.

**Table 1:** Comparison of key calculated minimum energy structure bond distances (Å) and experimental XFEL determinations. XFEL bond distances reported are an average from both a and A chains of the deposited crystal structures.

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The interpretation of the $S=3$ signal EPR from the 2-flash state based on BS-DFT analysis of calculated hfcs is highly indicative of an oxo-hydroxo form for the $S_3$ state. An oxo-hydroxo model is not however compatible with the structure obtained by XFEL. As described above the $[O5O6]^3-$ model does agree with the XFEL structures. This corresponds to an $Ms=6$ spin state. This is not a true spin state, $S$. The true spin state energies can be obtained by diagonalization of the Heisenberg Dirac van Vleck Hamiltonian using $J$ values obtained by analysing all possible BS states. Table S1 shows the calculated $J$ values and energies of the ground spin states using this procedure. From this an $S=6$ spin state is calculated to be the ground state spin. This therefore cannot be attributed to the species observed by EPR/EDNMR which has an $S=3$ ground state spin. The PES shows that the two species are related by the protonation state of O6. Intriguingly an $S=6$ species was proposed to be formed in the 2-flash $S_3$ state of spinach samples and indeed was proposed to be the major component (80%) of native samples. The $S=6$ form was attributed to a so-called closed cubane form of the WOC cluster with a pentacoordinated Mn$_4$(IV) ion formed before the second substrate binds. So far no experimental support for such a closed cubane structure of the WOC has been obtained for any $S$-state. It is therefore more likely (see below) that this species corresponds to the $[O_2]^3-$ form alluded to in this manuscript, also with $S=6$.

Experimentally, no $S=6$ species has so far been reported in cyanobacteria samples where high resolution high field EPR spectra obtained are attributed to an $S=3$ form. Simulations of the $W$-band EPR spectra for the $S=3$ form are shown in Figure 10. Also shown are simulations for an $S=6$ form using the zero field splitting parameters reported for the spinach samples. From the simulations it is clear that the spectral intensity of the $S=6$ form is much less than that of the $S=3$ form. This suggests that the $S=6$ form would be difficult to detect in the $W$-band EPR experiment. More intriguingly as shown in Figure 10, at a percentage of $S=6$/$S=3$ of 70% the spectral features of the $S=3$ form still dominates the spectral envelope with the
S=6 form mainly contributing a distinctive shoulder at around 3500 – 4000 mT to the overall spectral shape.

Figure 10. Comparison 94 GHz EPR spectral simulations of S=6 (red), S=3 (blue) and a 0.7:0.3 mixture of S=6:S=3 (yellow). The experimental spectrum from Chrysina et al. is shown in black. Simulation parameters used are S=6, g=2, D = 1.523 cm$^{-1}$, E/D= 0.14; S=3, g=2, D = 0.179 cm$^{-1}$, E/D = 0.28.

It is clear from the spectra presented in Figure 3 of Chrysina et al. that a poorer fit between experimental and a simulated S=3 spectrum exists in this very region. Indeed inclusion of the S=6 form (70%) gives rise to a much improved fit to the experimental spectrum reproduced for comparison in Figure 10, see also additional simulations varying the ratio of the two spin systems, in the SI. We therefore suggest that the seeming incompatibility between the XFEL and EPR data for the S$_{3}$ state, lies in the fact that the oxo-hydroxo and $[O_{2}]^{3-}$ are in
equilibrium. The $[O_2]^{3-}$ form detected in the XFEL determined structure is not readily apparent in the EPR spectrum due to its S=6 nature and resultant low intensity compared with the S=3 form. It can be clearly identified however as a shoulder on the spectral envelope of the 2-flash sample, Figure 10. Further simulations presented in the SI suggest that the S=6 component is also likely a major component of the broadened W-band EPR spectrum caused by methanol and glycerol addition. It has been known for some time that the S=3 species does not correspond to all of the S=6 spin and that an EPR “undetectable” component observed only on NIR irradiation is also present in equilibrium with it. In our analysis, this undetectable component corresponds to the $[O_2]^{3-}$ form. This is a different assignment to that previously made for the S=6 form detected in spinach samples where the S=6 form was attributed to a closed cubane form of the WOC cluster with a pentacoordinated Mn$_4$ (IV) ion - an intermediate formed prior to binding of the second substrate water. This however, in striking contrast to the model proposed here, is not supported by the XFEL structural data. In addition it has been shown that Mn(III) is required for NIR excitation and the large D value of 1.523 cm$^{-1}$ for the S=6 form strongly suggests the presence of Mn(III) ion in the complex.

Our analysis indicates that O-O bond formation has begun between the O5 and O6 atoms in the S=3 state with the generation of the $[O5O6]^{3-}$ ion. This is the dominant species present in the S=3 state. Figure 1 shows that this provides a low barrier pathway to subsequent formation of the peroxo form and we suggest that this is formed after the fourth flash on generation of the S=3 $Y_Z^{OX}$ state and further removal of a proton from the WOC. Subsequent oxidation of the WOC by $Y_Z^{OX}$ leads to oxidation of peroxo leading to transient superoxo formation which will rapidly lead to triplet O$_2$ formation and release from the WOC. The initiation of O-O bond formation in the S=3 or S=3 $Y_Z^{OX}$ states is supported by kinetics findings which have shown that there is almost kinetic coincidence between the rate of O$_2$ evolution and $Y_Z^{OX}$
reduction.\textsuperscript{31} Time resolved X-ray emission studies\textsuperscript{32} have demonstrated that reduction as opposed to oxidation of the WOC occurs after the 3\textsuperscript{rd} flash fully supporting O-O bond formation in the S\textsubscript{3} and S\textsubscript{3}Y\textsubscript{Z}\textsuperscript{OX} states.

**Conclusions.**

Analysis of the electronic structure changes along the reaction path for O\textsubscript{5}O\textsubscript{6} bond formation in the S\textsubscript{3} state of the WOC shows that two spin crossovers, facilitated by the geometry and magnetism of the water oxidising complex are used to provide a unique low energy pathway. The pathway is facilitated via formation and stabilisation of the [O\textsubscript{5}O\textsubscript{6}]\textsuperscript{3-} ion. This [O\textsubscript{2}]\textsuperscript{3-} ion is stabilised by antiferromagnetic interaction with the Mn ions of the complex. Combined computational, crystallographic and spectroscopic data show that an equilibrium exists between an O\textsubscript{5} oxo and O\textsubscript{6} hydroxo form, S=3 spin state and a deprotonated O\textsubscript{6} form containing a two-centre one electron bond in [O\textsubscript{5}O\textsubscript{6}]\textsuperscript{3-} which we identify as the form detected using XFEL crystallography. This form gives rise to an S=6 spin state which we demonstrate gives rise to a low intensity EPR spectrum compared with the accompanying S=3 state, making its detection via EPR difficult and overshadowed by the S=3 form.

Simulations assuming a 70\% contribution of the S=6 form give rise to a superior fit to the experimental EPR spectrum compared with an S=3 only form. The study reveals the key electronic, magnetic and structural design features of Nature’s catalyst which allows water oxidation to O\textsubscript{2} to be uniquely performed under ambient conditions.

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**Supporting Information Available:** Details of calculations performed and models used, additional data tables and figures supporting the main text. XYZ coordinates of models used.
References


