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Relativistic effect behind the molybdenum *vs.* tungsten selectivity in enzymes†

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Molybdenum and tungsten, being congeners of the 6th group of d-block elements, are similar in many respects in terms of their properties. In fact, both participate in similar types of oxotransferase activity in their enzymes. Molybdenum is regarded as the heaviest essential trace metal in all forms of life; however, its next heavier congener, tungsten, as the heaviest metal, is found only in some prokaryotic organisms. Tungstoenzymes are generally selected by nature for carrying out low-potential redox activities under anaerobic conditions in prokaryotic organisms. This nature's molybdenum *vs.* tungsten selectivity for their biological functions under different working conditions (surrounding temperature and aerobic/anaerobic environment) is determined mainly by the relativistic effect, which is experienced to different extents by these two congeners. Understanding the mechanistic aspects of the relativistic effect-controlled enzymatic activities of tungstoenzymes is of immense biotechnological interest to develop eco-friendly and cost-effective methods for the commercial synthesis of acetaldehyde through the hydration of acetylene and commercial production of hydrogen (H_2, a green fuel) by producing tungsten-incorporated nitrogenase (W–N_2-ase) in CA6 (mutant strain) and to develop a biomimetic method to replace the hazardous Birch reduction in organic synthesis.

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1. Introduction

Molybdenum (Mo, atomic number 42, [Kr]$4d^5 5s^1$, a second-series transition metal) is ubiquitous as the heaviest essential trace metal in biological systems, while its next heavier congener, tungsten (W, atomic number 74, [Xe]$4f^{14}5d^4 6s^2$, a third-series transition metal), as the heaviest essential trace metal, is found only in some prokaryotes for performing similar oxotransferase biological reactions.[1–11] Tungsten is specifically selected in thermophilic and hyperthermophilic microorganisms by nature for carrying out low potential redox activities under anaerobic conditions.[6–8] In the periodic table, the congeners in a particular group show similar chemical properties; however, the heavier congeners experience the relativistic effect more.[12–27] Consequently, the congeners, tungsten and molybdenum, in the sixth group of the periodic table experience the relativistic effect to different extents, and it makes a difference in their many properties, including the redox potentials. It plays a crucial role in determining the Mo *vs.* W selectivity in their enzymes. The oxidative reactions catalyzed by Mo- and W-enzymes lead to the transfer of an oxygen atom from the M^{VI}=O group to the substrate (Y, say), causing the concomitant 2*e* reduction of the metal ($M^{VI} \rightarrow M^{IV}$), while in the reductive reactions catalyzed by these enzymes, one oxygen atom is transferred from the substrate (YO) to the metal, leading to a 2*e* oxidation of the metal centre ($M^{IV} \rightarrow M^{VI}$). In these catalysed oxygen atom transfer (OAT) reactions (also known as oxotransferase reactions), water is utilized as the ultimate source or sink of oxygen. The oxotransferase reactions catalysed by these enzymes are crucially important to run the global biogeochemical cycles of elements such as carbon, nitrogen, sulfur and arsenic.[3–9]

2. Molybdenum cofactor and tungsten cofactor in their enzymes

There are two scaffolds to produce two different types of molybdenum cofactors:[3–5,7–11] the iron–molybdenum cofactor [FeMo-co] in the Mo-based nitrogenase enzyme and the pterin (PT)-based molybdenum cofactor [MoPT-co] simply represented by [Mo-co] or molybdopterin (Mo-PT) in other Mo-based redox enzymes showing the oxotransferase activity (*e.g.* –CHO/–CO_2H, NO_3^-/NO_2^-, and SO_4^{2-}/SO_3^{2-}). There are different types of pterin-based [Mo-co] depending on the redox

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†This article is dedicated to Late Prof. D. Banerjea, Sir Rashbehari Ghose Professor, Department of Chemistry, University of Calcutta, India.

activity.[3,7–11] However, for the W-based enzymes, the pterin (PT)-based tungsten cofactor [WPT-co] simply represented by [W-co] or tungstopterin (W-PT) is known.[3,7–11] The pterin (PT)-based metal cofactor (M-PT) involves the non-innocent dithiolene chelating ligand ($R_2C_2S_2$) in the coordination sphere of Mo/W, and the remaining coordination sites are occupied by oxygen and/or sulfur, and/or selenium atoms depending on the nature of the enzymes.[3–5,7–11] The non-innocent dithiolene chelating ligand of M-PT can stabilize the mononuclear Mo/W enzymes at the physiological pH.[3,7–11] The non-innocent dithiolene ligand can exist in different redox states (Fig. 1) such as neutral dithiolene (*i.e.* dithioketone), anion radical ($1e^-$ reduced state, −1 charge) and dithiolate ($2e^-$ reduced state, −2 charge) to stabilize the variable oxidation states such as +4, +5 and +6 of Mo/W involved in the activity of their redox enzymes.[3,28–30] The molybdopterin (Mo-PT)-based redox enzymes are classified into three major families[2,3,31,32] (Fig. 2): xanthine oxidase (XO), dimethyl sulfoxide reductase (DMSOR) and sulfite oxidase (SO) and the tungstopterin (W-PT)-based enzymes are classified into two major families[2,3,31–33] (Fig. 3): DMSOR and W-AOR or WOR (tungsten oxidoreductase). The tungstopterin (W-PT)-based enzyme, acetylene hydratase (AH), belongs to the DMSOR family,[32] but this enzyme is involved in the hydration of acetylene (non-redox activity). In all these W-based enzymes, the W present in the W-cofactor is co-ordinated by the two pterin-based dithiolene groups. However, for the Mo-based enzymes, the two pterin-based dithiolene groups are present in the Mo-cofactor of only the DMSO reductase family, which also represents a family of W-based enzymes, while only one pterin-based dithiolene group is present for the remaining two other families (XO and SO). It may be noted that for both Mo- and W-based enzymes, the oxi-

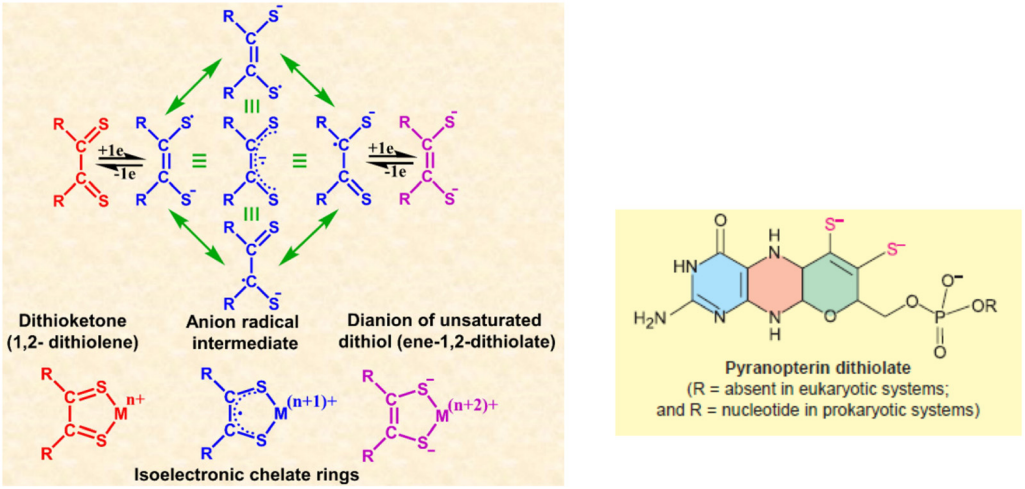


Fig. 1 Schematic of the different oxidation states (0, −1 and −2) and redox activity involving the intramolecular electron transfer of the non-innocent dithiolene ligand system ($R_2S_2C_2$) and its isoelectronic [$M(R_2S_2C_2)$] chelate rings to tune the different oxidation states of the metal centre.

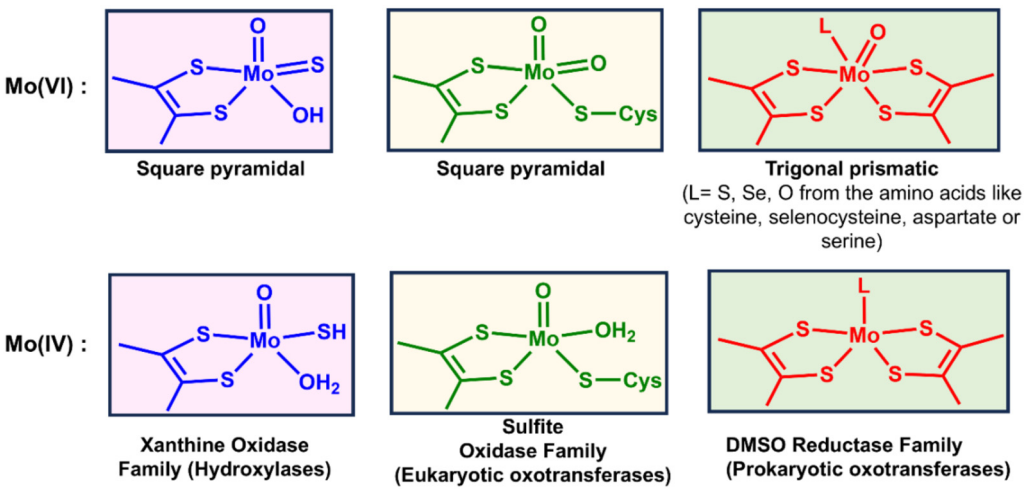


Fig. 2 Schematic of the coordination sphere of the three major families of Mo-enzymes catalyzing oxo-transfer (OAT) and hydrolase reactions. Pterin-based dithiolene chelating ligand (S, S donor sites).

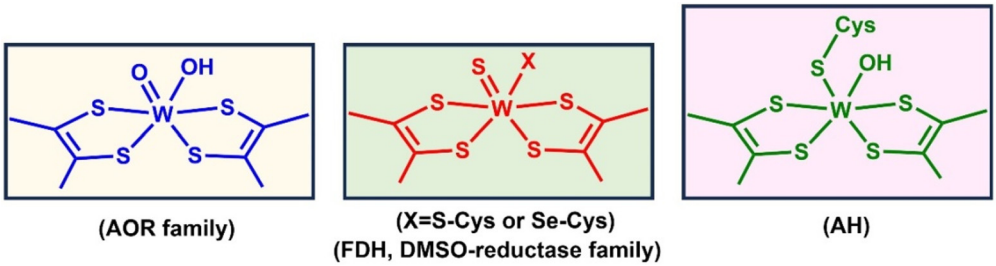


Fig. 3 Schematic of the coordination sphere of the major families of W-enzymes. Pterin-based dithiolene chelating ligand (S, S donor sites).

dation states of the metal centre range from +4 (d^2) to +6 (d^0). The +5 state (d^1, EPR active) exists as a transient intermediate species in the catalytic cycle M(VI)/M(IV). The presence of a bis (dithiolene) (two dithiolene ligands) moiety in all the W-based enzymes is probably required to stabilize the lower oxidation state, such as W(IV), which is relatively less stable compared to Mo(IV) in terms of the relativistic effect.

3. Selectivity of Mo *vs.* W in different enzymes

The pterin-based Mo-cofactor and W-cofactor are present at the active site of their enzymes to catalyze similar 2*e* transfer reactions, including the oxotransfer reactions, but their functioning environments (surrounding temperature and aerobic/anaerobic conditions) are different.[3,7–11,32,33] The Mo-based enzymes are also present in all forms of life, including humans, while the W-based enzymes are present only in some prokaryotic microorganisms (some bacteria and thermophilic archaea living at relatively high temperatures in the anaerobic regions surrounding the hydrothermal and volcanic vents in the sea bed).[3,7–11] However, some W-containing enzymes have also been found in organisms that can grow under milder environmental conditions.[32–34] This suggests a broader role of tungsten in biological systems. These W-based enzymes are generally stable in anaerobic conditions to catalyze low-potential redox processes. In general, Mo and W mutually antagonise in enzymatic activity; although in some cases, both Mo and W can be mutually exchanged to retain enzymatic activity.[3,32,33] Depending on the selectivity, the W-based enzymes are of two types:[3,32–34] obligate and facultative W-based enzymes. In obligate W-based enzymes, such as BCR (benzoyl–CoA reductase) and W–AOR (aldehyde oxidoreductase), catalyzing the redox reactions of very low redox potentials, and AH (acetylene hydratase), catalyzing an unusual nonredox hydration reaction to sustain enzymatic activity, the presence of the W-cofactor is essentially required and the corresponding Mo-substituted enzymes are inactive. The facultative W-based enzymes, such as FDH (formate dehydrogenase), may contain either Mo or W depending on the source.[3,32–35] In terms of the redox potentials of the catalyzed reactions, the tungsten enzymes are essentially required for the redox processes of lower potential, while the molybdoenzymes can catalyze the redox processes of a wider range of potential.[3,32–35] The relatively lower redox potentials of the W-couples compared to those of the corresponding Mo-couples play a crucial role in the selectivity of Mo *vs.* W in different redox enzymes, including nitrogenase. This review aims to rationalise this selectivity in terms of the relativistic effect.

4. Relativistic effects to control the properties of heavier elements

According to Einstein's relativity theory, the mass (m) of a moving particle is higher than its resting mass (m_0). The resting mass (m_0) and relativistic mass (m) are related in terms of the Lorentz factor (γ), which is also known as the gamma factor and is a dimensionless quantity, as follows:[12–17]

$$m = \frac{m_0}{\sqrt{(1 - v^2/c^2)}} = \gamma m_0,$$

where $\gamma = \frac{1}{\sqrt{(1-v^2/c^2)}} = \frac{1}{\sqrt{(1-v^2/137^2)}}$ and c (velocity of light) = 137 in atomic units (a.u.).

The atomic unit (a.u.) of velocity is the velocity of the bound electron in the first Bohr orbit (ground state) of the hydrogen atom in terms of the classical Bohr model = 2.188×10^6 m s^{-1}. This leads to $c = 2.998 \times 10^8$ m s^{-1} = 137 a.u. The increase in the mass of a moving particle is significant only if the velocity (v) of the particle is comparable to that of light ($c \approx 3.0 \times 10^8$ m s^{-1}). Consequently, this effect is not noticeable for common moving bodies with velocities far away from those of light. For the moving electrons in the atoms, depending on the condition, the relativistic increase in the mass of the electrons may be accountable. Without considering Dirac's relativistic quantum mechanics,[36,37] we can achieve this effect in terms of the Bohr–Sommerfeld theory of the atomic model.[38,39] In this atomic model (considering the electron as a charged particle), the velocity (v) of the revolving electron in a particular orbit of the principal quantum number (n) increases with the atomic number (Z), as shown below for H-like one-electron systems:[12–17]

$$v = \frac{Z}{n}\left(\frac{e^2}{2\varepsilon_0 h}\right) = \frac{Zc}{n}\left(\frac{e^2}{2\varepsilon_0 hc}\right) = \frac{Zc\alpha}{n} = \frac{Zc}{137n}$$
$$= \frac{Zc}{137} \text{ (for } n = 1).$$

The Sommerfeld fine structure constant (α) is a dimensionless quantity with a numerical value equal to (1/137) and is expressed as follows:

$$\alpha = \frac{e^2}{2\varepsilon_0 hc} = \frac{e^2}{4\pi\varepsilon_0 \hbar c} \approx \frac{1}{137}, \left(\hbar = \frac{h}{2\pi}\right).$$

The resting mass (m_0) and relativistic mass (m) are related in terms of the Lorentz factor (γ) and Sommerfeld fine structure constant (α) as follows:

$$m = \gamma m_0 = \frac{m_0}{\sqrt{(1 - v^2/c^2)}} = \frac{m_0}{\sqrt{(1 - \alpha^2 c^2 Z^2/c^2)}} = \frac{m_0}{\sqrt{(1 - \alpha^2 Z^2)}}, (\text{for } n = 1, \textit{i.e.}\ 1\text{s-electron}).$$

Thus, the velocity of the moving electrons of heavier elements is not negligible compared to that of light. Consequently, the relativistic effect of increasing the mass of the moving electrons is important only for the heavier elements and influences their properties. When the moving mass (m) of the revolving electron increases, it experiences a stronger attraction to the nucleus and the radius of the orbit shrinks or contracts; this phenomenon is described as the relativistic contraction of the orbit. It can be expressed as follows:[12–17]

$$r = \frac{Ze^2}{4\pi\varepsilon_0 mv^2} = \frac{Ze^2}{4\pi\varepsilon_0 \gamma mv^2} = \frac{r}{\gamma} = \frac{\text{nonrelativistic radius}}{\gamma}.$$

For hydrogen ($1s^1$), the speed of 1s-electron = $Zc/137 = c/137 \approx 0.0073c$ and $m/m_0 = \gamma = 1.00003$, *i.e.* the relativistic mass of the 1s-electron is only 1.00003 times the resting mass ($m/m_0 = \gamma = 1.00003$). For mercury ($Z = 80$), the speed of its 1s-electron is $80c/137 \approx 0.6c$, leading to $m/m_0 = \gamma = 1.23$, *i.e.* the relativistic mass (m) of the 1s-electron of mercury = $1.23m_0$, and the contraction of the radius of the 1s-orbital of mercury occurs by a factor of 1.23 (*i.e.* 23%).[12–17] With the increase in the velocity of the bound electron, its attractive force towards the nucleus increases; consequently, its binding energy increases.

The energy or velocity of an electron depends on the principal quantum number n, azimuthal or orbital angular momentum quantum number l, and atomic number Z (ignoring the spin–orbit coupling interaction). This dependence on n and Z can be understood even in terms of the nonrelativistic Bohrâ–Sommerfeld model.[38,39] However, understanding the dependence on l requires the rigorous mathematical treatment of relativistic wave mechanics.[17,36,37] The relativistic mass correction and relativistic correction to the kinetic energy of the electron lead to a relativistic correction energy term to the nonrelativistic kinetic energy. The mass-velocity correction energy term (ΔE_m) is given by the following equation (assuming m_0 = 1 a.u.) for H-like one-electron systems:[12,17]

$$\Delta E_{\mathrm{m}} = -\left[\frac{Z^4}{2n^3c^2}\right] \times \left[\frac{1}{(l+1/2)} - \frac{3}{4n}\right].$$

The above mass-velocity correction energy term indicates that ΔE_m is highly sensitive to Z and depends on Z^4. However, the non-relativistic energy depends on Z^2 and is given by $E_{\mathrm{n}} = -Z^2/2n^2$. The contribution of the relativistic mass-velocity correction energy term (ΔE_m) is of the order of $-Z^4/2n^3c^2$ (in a.u.). This relativistic correction energy term (ΔE_m) is a function of both n and l, while the non-relativistic electron binding energy is a function of n only. ΔE_m increases as n and l decrease. For a particular value of n, this relativistic correction is more important for smaller values of l. Obviously, this effect is most important for the s-orbital electron (l = 0), and the s-orbital electron is most tightly bound, *i.e.* the s-orbital is contracted to the maximum for a particular value of Z and n. This relativistic orbital contraction (described as the direct relativistic effect) occurs in the order s (l = 0) > p (l = 1) > d (l = 2) > f (l = 3) for the fixed values of Z and n.

The spin–orbit (SO) coupling effect (described as the 3rd relativistic effect) is a relativistic effect because the concept of electron spin exists in Dirac's relativistic wave mechanical model[36] but not in nonrelativistic wave mechanics. This relativistic effect considers the orbital angular momentum vector ($\boldsymbol{l}$) and electron spin momentum vector ($\boldsymbol{s}$) to couple to produce the resultant $\boldsymbol{j}$ vector. Thus, in relativistic wave mechanics, the total angular momentum is defined by the quantum number j (*i.e.* $j = l \pm s$) as a combination of l (orbital angular momentum quantum number) and s (spin quantum number). The corresponding total magnetic angular momentum quantum number (m_j) includes all the values ranging from $-j$ to $+j$ separated by one. The SO coupling interaction splits the orbitals (with the nonzero l value) into two sets defined by the j values: $j = l \pm s$. Obviously, the s-orbital (l = 0) with no orbital angular momentum does not experience SO coupling and does not split. Each set can accommodate a maximum of $2j + 1$ electrons. The SO coupling interaction energy (ΔE_{so}) for electrons with non-zero l values is given by the following equations for the H-like one-electron systems:[12,17]

$$\Delta E_{\mathrm{so}} = -\left\{\frac{Z^4}{2n^3}\right\} \times \left\{\frac{1}{[l(2l+1)]}\right\} \text{for} j = l - \frac{1}{2}$$

and

$$\Delta E_{\mathrm{so}} = \left\{\frac{Z^4}{2n^3c^2}\right\} \times \left\{\frac{1}{[(l+1)(2l+1)]}\right\} \text{for} j = l + \frac{1}{2}.$$

ΔE_{so} shows the same type of dependence on Z (as Z^4) and n (as n^{-3}) as noted for the mass-velocity correction energy term (ΔE_m). The above expression indicates that ΔE_{so} becomes more important for smaller values of n and l, as in the case of ΔE_m. It also indicates that for $j = l + \frac{1}{2}$, it destabilises, whereas for $j = l - \frac{1}{2}$, it stabilises. For the fixed l- and n (principal quantum number) values, the orbitals with lower j values are of lower energy. Thus, the energy order is $np_{3/2} > np_{1/2}$; $nd_{5/2} > nd_{3/2}$ and $nf_{7/2} > nf_{5/2}$.

The total relativistic energy correction term ($\Delta E_{\mathrm{n,j}}$) is approximately given by the following expression,[12,17] neglecting the contribution of higher-order correction terms beyond c^{-2}:

$$\Delta E_{\mathrm{n,j}} = -\left\{\frac{Z^4}{2n^3c^2}\right\} \times \left\{\left[\frac{1}{j+1/2}\right] - \frac{3}{4n}\right\}.$$

This total relativistic energy correction term ($\Delta E_{\mathrm{n,j}}$) is added to the non-relativistic energy – $Z^2/(2n^2)$ term to give the cor-

rected energy value of the orbital electron. Compared to the other correction terms, the mass-velocity correction energy term (ΔE_m) is more important. It is worth noting that in the correction terms, the Z^4 dependence for the hydrogen-like one-electron systems is approximately replaced by the Z^2 dependence for both the scalar (spin free) and SO relativistic effects on the valence electrons of many-electron systems.[12,16,17] This Z^2 dependence of the relativistic effects explains why the relativistic effects on the valence electrons are more important for heavier congeners in a particular group of the periodic table. In reality, these relativistic effects on the valence electrons become important only for the heavier elements (Z > 60–70), such as the 6th and 7th period elements[12,16,17–23]

The consideration of the relativistic effect reveals that for the valence shells, the electron binding energy increases with an increase in Z (Z^2 dependence in many-electron systems) and a decrease in n and l. Thus, for particular values of Z and n, the relativistic orbital contraction (direct relativistic effect) occurs in the following order: s (l = 0) > p (l = 1) > d (l = 2) > f (l = 3).[12,16,17,40] The electrons in these contracted inner s- and p-orbitals with smaller l values effectively screen the electrons of outer d- and f-orbitals with higher l values.[12,16,17] Consequently, the d- and f-electrons experience less electrostatic attraction towards the positively charged nucleus, causing the relativistic expansion (described as the indirect relativistic effect, a consequence of the direct relativistic effect) of the d- and f-orbitals. These two effects,[12,16,17,40] relativistic contraction (direct relativistic effect) of the s- and p-orbitals and relativistic expansion (indirect relativistic effect) of the d- and f-orbitals, are to be considered to understand the properties of the heavier elements because these effects are only important for the heavier elements. If we compare the two congeners Mo and W, for the heavier congener W, the 5d-valence orbital is relativistically more expanded (*i.e.* more destabilized) than the 4d-valence orbital of the lighter congener Mo. This is why the 5d-valence orbital electrons of the heavier congener (W) are more easily lost than the 4d-valence orbital electrons of the lighter congener (Mo) to attain a higher oxidation state. The stability order[12–17] W(VI) > Mo(VI) is obtained. The redox potential of the W(VI)/W(IV) couple is more negative than that of the Mo(VI)/Mo(IV) couple. This relativistic effect is quite important for understanding the selectivity of Mo *vs.* W in their redox enzymes. Besides, the relativistically expanded 4f- and 5d-orbitals of W make it more polarisable (*i.e.* softer in terms of HSAB theory, hard and soft acids and bases theory) than Mo.[12–17]

5. Relativistic effect and selectivity of Mo *vs.* W in the nitrogenase (N_2-ase) enzyme

Depending on the nature of metal (M) ions (iron and another metal, M) present at the active site of N_2-ase enzymes, in nature, there are three types of N_2-ase enzymes:[3,41–45] Mo-dependent (most common and most efficient), V-dependent (found only in the absence of Mo in soils) and only Fe-dependent (found only in the absence of both Mo and V in soils). Here, we consider only the most common and most efficient Mo-dependent N_2-ase enzymes bearing the Fe–Mo cofactor (denoted by [FeMo-co] or FeMoco) and compare the activity of Mo-dependent N_2-ase enzymes *vs.* W-incorporated N_2-ase enzyme produced under the W-enriched condition (*i.e.* [FeMo-co] *vs.* [FeW-co]).[46,47] The Fe_7MoS_9 heterometal cluster of [FeMo-co] consists of two void-cubane metal cluster units (Fe_3MoS_3 and Fe_4S_3), which are connected by three inorganic S^{2-} ions (known as belt-S sites) (Fig. 4). Besides these belt-S sites, a ligand C-atom (μ_6-C) is coordinated to the six central Fe-centres of the two void-cubane metal cluster units to connect them and provide the tetrahedral coordination geometry of the Fe-centres. This gives a trigonal prismatic structure of the Fe_7MoS_9 cluster in which the Mo centre is octahedrally coordinated by the ligand sites: 1 His-N site, 2 O donor sites of *R*-homocitrate and 3 belt-S^{2-} sites. There are two [FeMo-co] units at the active site of N_2-ase, and they act independently.

The overall ATP hydrolysis-driven N_2-ase-catalyzed reduction of N_2 is coupled with the reduction of H^+ to produce H_2:

$$N_2 + 8H^+ + 16\text{MgATP} + 8e^- \rightarrow 2NH_3 + H_2 + 16\text{MgADP} + 16P_i,$$
$$(P_i = \text{inorganic phosphate, such as } H_2PO_4^-)$$

Owing to the obligatory hydrogen evolution, the N_2-ase-catalyzed reduction of N_2 is an 8*e* transfer process (*cf.* nonenzymatic dinitrogen reduction is a 6*e* transfer process). The Thorneley–Lowe (T–L) kinetic model (Scheme 1) of Mo-nitrogenase activity suggests that proton-coupled electron transfer (PCET) from the reduced Fe-protein in 8 successive steps to the [FeMo-co] active site (E_0 resting state as isolated) produces the various reduced states of the enzyme (E_1–E_8) in the reduction of N_2 to produce NH_3 along with the obligatory evolution of H_2.[43–45] The various electronic states of the active site of the enzyme are denoted by E_n (n = 0–8), where the subscript n denotes the number of electrons transferred to the active site from the reduced Fe-protein. The E_0 state of the [FeMo-co] unit consisting of $Mo^{3+}4Fe^{3+}3Fe^{2+}$, where the metal centres are electronically coupled, cannot bind to the N_2 substrate. Hydrogen (H_2) evolution can occur through the hydride protonolysis (HP)[43,45] from the E_2, E_3 and E_4 states bearing the [Fe–H–Fe] bridging hydride(s) and protonated SH^+ group(s) of the FeMoco protein in the absence of N_2 substrate. The E_4 (EH_4) intermediate state possesses 2 hydridic-H as 2[Fe–H–Fe] bridging hydrides and 2 protonic-H as $2SH^+$ to balance the charge. Similarly, $E_2(EH_2)$ stores the two reducing equivalents as a hydride through the formation of a [Fe–H–Fe] hydride bridge along with a protonated sulfide group (SH^+). Thus, FeMoco can act as the 'hydride storage device' to store the reducing equivalents as the bridging hydrides.[43,45] FeMoco can store the 4 reducing equivalents as bridging hydrides in E_4 (EH_4), and the successive release of 2 molecules of H_2 from the E_4 (EH_4) state through hydride protonolysis (HP) leads to the initial E_0 state.[43,45]

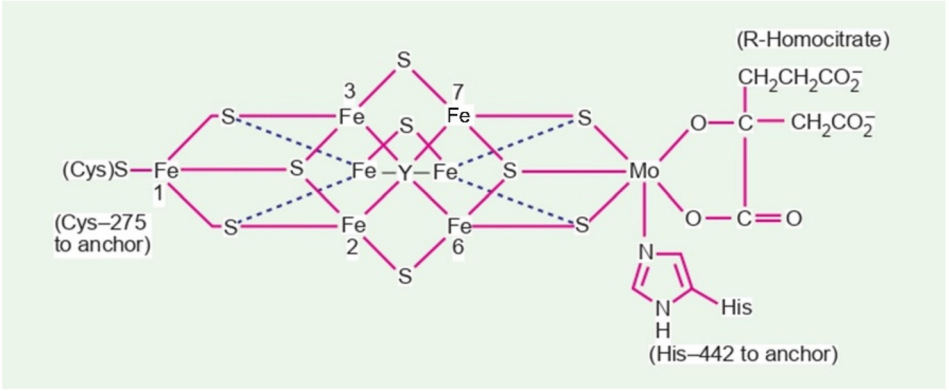
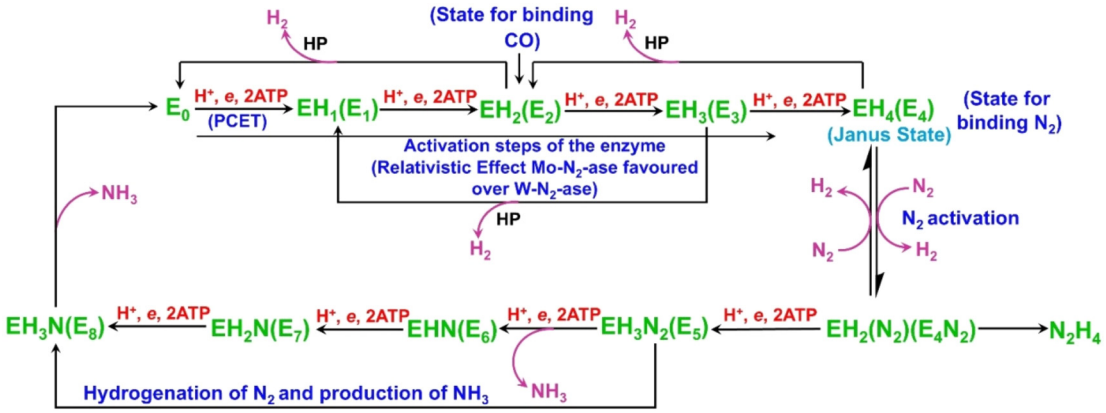


Fig. 4 Structural representation of heteronuclear $MoFe_7S_9$ metal cluster of [FeMoco] of Mo-nitrogenase enzyme. FeS face containing the Fe atoms 2, 3, 6, and 7 (numbering from the X-ray structure).[3,43,45]



Scheme 1 Schematic of the Thorneley–Lowe (TL) model of nitrogenase activity. The activation steps leading to the super-reduced state (E_3/E_4) are controlled by the relativistic effect to determine the Mo *vs.* W selectivity in nitrogenase. HP denotes hydride protonolysis.

The [FeMo-co] unit needs to be activated to bind N_2. This activation process requires the reduction of the resting state (E_0) to produce the reduced E_3/E_4 state (most probably the E_4 state, *i.e.* EH_4) of the metallocluster.[3,41–45] The 4 sequential proton-coupled electron transfer steps produce the super-reduced E_4 state that binds N_2 with the concomitant evolution of one molecule of H_2 through the reductive elimination (RE) and not hydride protonolysis (HP) (*i.e.* $EH_4 + N_2 \rightarrow (N_2)EH_2 + H_2$). The E_4 intermediate state containing two [Fe–H–Fe] bridging hydrides is described as the Janus state, which can act as a transition point in the catalytic cycle of N_2 reduction. From this state, by the successive release of two molecules of H_2 through the hydride protonolysis (HP), the initial E_0 state can be attained, and from this E_4 state, N_2 binding producing $(N_2)EH_2$, followed by the subsequent PCET steps, can also produce two molecules of NH_3 in the N_2 reduction stage. This is why the intermediate E_4 state is named after Janus, the Roman god of transitions.[43–45]

The π-acceptance property of the dinitrogen (N_2) ligand makes coordinated N_2 in $(N_2)EH_2$ electron-rich, thereby activating coordinated N_2 towards electrophilic attack by H^+ to experience the subsequent sequential PCET steps in the reduction to NH_3. The activation steps to produce the super-reduced E_4 state are required for N_2 binding because it is a very weak π-acceptor ligand with almost no σ-donor property (*i.e.* no synergistic interaction in the π-acceptance property).[3,48,49] In fact, the highly reduced state of the metal centre can act as a better π-donor site. It is worth noting that the stronger π-acceptor ligand CO, which is isoelectronic to N_2, does not require such a super-reduced E_4 state to bind. In fact, CO with the good σ-donor property (*cf.* a nonbonding MO as the HOMO localised on the less electronegative C-centre acting as a good σ-donor ligand while a σ-bonding MO as the HOMO localised between the two N-nuclei of N_2 acting as a poor σ-donor ligand) leading to the synergistic interaction in the π-acceptance property of CO can bind to the E_2 reduced state (a semi-reduced state). It is noteworthy that CO cannot bind to the resting state (E_0), for which the metallocluster of high oxidation states is not suitable for π-back donation, but the semi-reduced state E_2 is sufficiently electron-rich to bind CO.[43–45] This explains the evolution of H_2 in the presence of CO, which is a potential inhibitor to other nitrogenase substrates, including N_2 requiring the higher reduced states, such as like E_3 and E_4. Experimental findings indicate that carbon monoxide (CO)

becomes coordinated as a bridging ligand (μ_2-CO) between the Fe2 and Fe6 centres of FeMoco (E_2 state) by replacing the S2B belt sulfur (μ_2-bridging S2B).[43,45] From the other experimental findings, it is concluded that Fe2 and Fe6 in the trigonal prism of the FeMoco metallocluster are the potential sites for the exogenous ligands (Fe2 and Fe6 described as the privileged pair for ligand binding).[43] It is accepted that the Fe2, Fe3, Fe6 and Fe7 centres of the trigonal prism are probably involved in N_2 binding and its subsequent reduction.[45] In N_2 binding, μ_6-C remains intact, but it is not yet established whether the belt sulfur displacement occurs or not in N_2 binding. However, theoretical studies indicate that N_2 binds too weakly to FeMoco to force the displacement of a sulfide ligand, while CO binds more strongly to FeMoco to displace the sulfide ligand[43] (*cf.* CO is a potential pi-acid ligand, while N_2 is a very weak pi-acid ligand).

In the Mo-dependent nitrogenase enzyme, if Mo is substituted by its heavier congener W, then the enzyme becomes inactive for the reduction of dinitrogen (N_2).[46,47,50,51] In 2003, Siemann *et al.*[46] first characterised and isolated a tungsten-substituted nitrogenase enzyme (FeW-nitrogenase having the tungsten-substituted cofactor [FeW-co] in place of native [FeMo-co]) from a mutant of *R. capsulatus* developed under the tungsten-enriched environment. The W-substituted nitrogenase enzyme was confirmed from the recorded EPR spectrum at 4 K. The metal analysis of the isolated FeWco protein confirmed that it contains an average of 1 W-, 16 Fe-, and less than 0.01 Mo atoms per $\alpha_2\beta_2$-tetramer. From the redox titration experiments on the FeWco protein, the midpoint potential (E_m) value was found to be about −200 mV, which is significantly shifted to lower potentials with respect to that of the E_m value = −50 mV of the FeMoco protein present in the native enzyme. This more negative value of E_m of the FeWco protein is quite expected from the relativistic effect. In terms of the midpoint potential (E_m) value, the P clusters of both the W-substituted nitrogenase and the native Mo-nitrogenase enzymes were indistinguishable. From the recorded EPR spectra with the FeWco protein under turnover conditions, it was concluded from the extent of the decrease (only about 20%) in the intensity of the FeWco signal that the cofactor is enzymatically reduced under physiological conditions only to a smaller extent compared to that of the native FeMo cofactor.

Although the tungsten-substituted nitrogenase enzyme was found to be inactive for the reduction of N_2, it was found to be sufficiently active in the reduction of H^+ to produce H_2. The inability of a tungsten-substituted cofactor [FeW-co] to reduce dinitrogen arises from the thermodynamic barrier to reduce the metallocluster of [FeW-co] to the super-reduced state (E_4), which is required to bind and activate N_2 for its reduction through the PCET steps (*cf.* Scheme 1, TL model). It has already been stated that [FeW-co] cannot be reduced beyond its semi-reduced state under biological conditions. It is also supported by the more negative midpoint potential (E_m) value (redox titrational analysis experiment) of the FeWco protein compared to that of the native FeMoco protein (<$\hat{a}'$ 200 mV *vs.* $\hat{a}'$ 50 mV).[46] This thermodynamic barrier to reduce the metallocluster of [FeW-co] to the super-reduced state can be rationalised in terms of the relativistic effect, which is more important in the heavier congener, tungsten. The more pronounced relativistic effect causing the more relativistic expansion of the 5d-valence orbitals of tungsten favours the ionization of the 5d-valence electrons to stabilize the higher oxidation state of tungsten compared to that of the lighter congener molybdenum, for which the relativistic expansion of the 4d-valence orbital is less. This makes the redox potential of the couples of tungsten more negative. Consequently, the reduction of tungsten to its lower oxidation states experiences a higher thermodynamic barrier compared to that of molybdenum.[12–17] This is why the activation steps involving the proton-coupled 4*e*-reduction of the E_0 state of the metallocluster of [FeW-co] to the super-reduced E_4 state for N_2 binding and its activation towards its reduction are not thermodynamically feasible under biological conditions. The tungsten centre is relativistically more resistant to reduction; consequently, to produce the super-reduced state (E_4) of the metallocluster of [FeW-co], it requires more powerful reducing agents that are not biologically available. However, the native metallocluster of [FeMo-co] involving the Mo-centres with more positive redox potentials can be reduced to the super-reduced E_4 state required for N_2 reduction. It is noteworthy that for the reduction of H^+, according to the TL model, the required semi-reduced state (E_2) can be attained through the proton-coupled 2*e*-reduction of the [FeW-co] core by the available reducing agents under physiological conditions. Hydride protonolysis (HP) in the semi-reduced state (E_2) produces H_2. Thus, the relativistic effect can explain why W-substituted nitrogenase cannot reduce N_2 to NH_3 but can reduce H^+ to produce H_2.[46,51] In fact, it is noted by Noar *et al.* in 2015 that in the presence of tungsten, *A. vinelandii* CA6 can produce hydrogen efficiently under ambient conditions.[51] This aspect can be explored for developing a novel technique for the commercial production of hydrogen as a green fuel.

6. Relativistic effect and selectivity of W *vs.* Mo in the enzymatic activity of benzoyl–CoA reductase (W-BCR *vs.* Mo-BCR)

The tungsten-dependent native enzyme (W-BCR) catalyzes the biological Birch reduction of the aromatic ring of benzoyl–CoA to a cyclic diene (cyclohexa-1,5-diene-1-carboxyl–CoA, dienoyl–CoA) (Fig. 5). It involves the energetically unfavourable dearomatization of the aromatic ring.[3,33,52,53] The redox potential at the physiological pH ($E^{o\prime}$) of the benzoyl–CoA/dienoyl–CoA couple is −622 mV.[33] This highly negative value of $E^{o\prime}$ indicates that the reduction of the aromatic π-electron system in the substrate (benzoyl–CoA) is highly thermodynamically unfavourable (*i.e.* endergonic process, $\Delta G^{o\prime} > 0$). In fact, it needs a thermodynamically very strong reducing agent, and such a strong reducing agent is not available under physiological conditions to carry out the reduction of benzoyl–CoA. Therefore, benzoyl–CoA is thermodynamically highly resistant to its

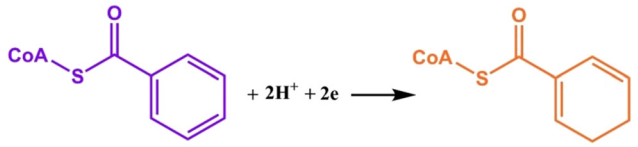
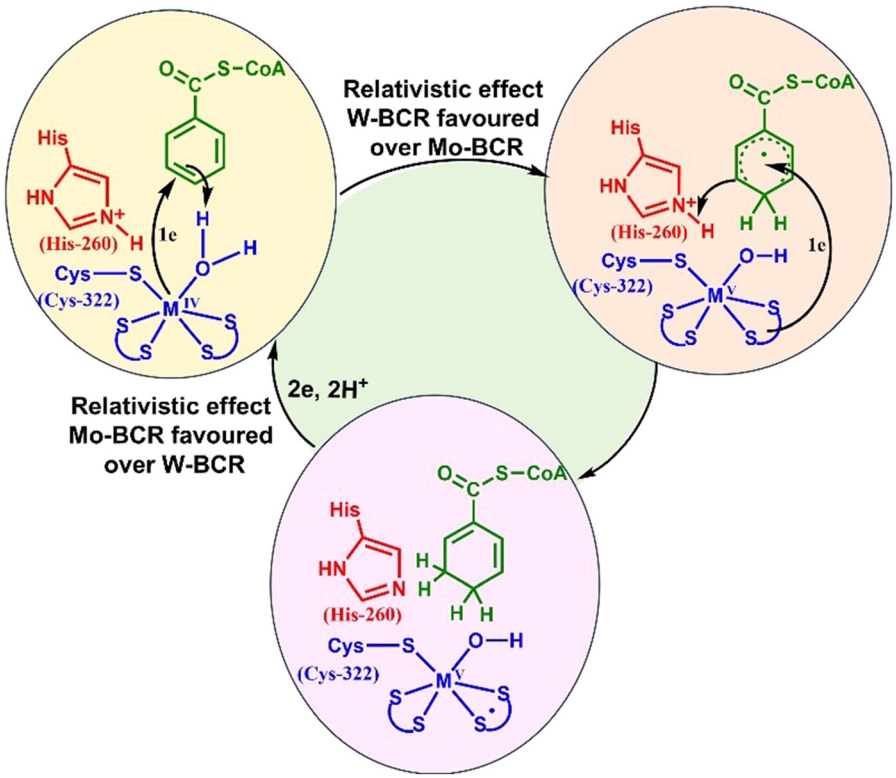


Fig. 5 W-BCR catalyzed biological Birch reduction of the aromatic ring of benzoyl–CoA to a cyclic diene.

reduction. Its reduction requires a highly exergonic reaction ($\Delta G^{\circ\prime} < 0$) to be coupled under physiological conditions so that the overall process becomes thermodynamically possible ($\Delta G^{\circ\prime} < 0$). This is attained in two ways by two different classes of BCRs (Class I and II).[3,33,53] Exergonic hydrolysis of ATP is coupled with the enzymatic activity of Class I BCRs present in the facultatively anaerobic bacteria in the reduction of benzoyl–CoA by a reduced ferredoxin acting as the electron source in the PCET steps, while the exergonic reduction of NAD^+ and/or menaquinone (MQ) is coupled in the enzymatic activity of Class II BCRs present strictly in the anaerobic bacteria.[33,52] The ATP-independent Class II BCRs occurring strictly in anaerobic sulfate-, metal–oxide-reducing or syntrophic bacteria earn far less energy in the oxidation of aromatics to CO_2 or acetate.[52] BCR catalysis leading to the benzene ring reduction is of much importance in terms of organic synthesis to develop a biomimetic method and replace the conventional hazardous Birch reduction.

The tungsten cofactor at the active site of W-BCR is developed by the coordination of four dithiolene sulfurs, a cysteinate-S (conserved Cys-322) and a water molecule to the W-centre. In the active form of the enzyme, tungsten exists as W(IV). The conserved His-260 residue is present close to the aromatic ring of the docked substrate at the active site and is suggested to deliver a proton during the reduction of the substrate in the proton-coupled electron transfer (PCET) process involved in the enzymatic activity.[3,54–56] Based on the theoretical studies,[55,56] the first step of the proposed mechanism (Scheme 2) of the reduction of benzoyl–CoA is an electron transfer from the W(IV) centre to the substrate (benzoyl–CoA) coupled with the proton transfer from the water molecule of the W(IV)—OH_2 linkage to the C_4 site of the substrate. This PCET step generates a W(V) state ligated by an OH^- ion and a resonance-stabilized substrate radical. The first step involving the 1*e*-oxidation of W(IV) to W(V) is the rate-determining step (rds). In the second step, the second electron is extracted from the pyranopterin cofactor with the dithiolene moiety (a non-innocent ligand capable of existing in variable oxidation states). This second step is also a PCET step involving proton transfer from the protonated histidine residue (His-260) to C_3 of the substrate radical intermediate generated in the first PCET step. This second step generates a cyclic diene product. The stepwise PCET steps are similar to those of the organic Birch reduction.

Theoretical studies on the corresponding Mo-BCR (*i.e.* molybdenum substituted enzyme) indicate that the activation



Scheme 2 Illustration of the probable mechanism of enzymatic activity of W-BCR/Mo-BCR. The first PCET step involves the extraction of an electron from M(IV) to reduce the substrate controlled by the relativistic effect to determine the selectivity of W *vs.* Mo in W-BCR/Mo-BCR.[3,55,56]

energy for the rate-determining step (rds), *i.e.* the first PCET step requiring the 1*e*-oxidation of Mo(IV) to Mo(V) intermediate, is larger (activation energy: 23.2 kcal mol^{-1} for W-BCR *vs.* 31.4 kcal mol^{-1} for Mo-BCR).[56] This larger activation energy barrier for the Mo-BCR catalyzed reduction of the substrate (benzoyl–CoA) can be rationalised from the relativistic effect that makes the redox potential of the Mo(V)/Mo(IV) couple more positive compared to that of the W(V)/W(IV) couple because of the lower stability of the higher oxidation states of the lighter congener, molybdenum, experiencing the less relativistic expansion of the 4d-valence orbital.[12–17] This relativistic effect makes the oxidation of Mo(IV) at the rds more difficult energetically compared to that of the native enzyme, W-BCR. In fact, for Mo-BCR, the first step of the proposed reaction mechanism is endothermic by about 16.7 kcal mol^{-1}.[56] These computational results justify the inactivity of Mo-BCR. It explains the natural selectivity of W *vs.* Mo in benzoyl–coenzyme A reductase.

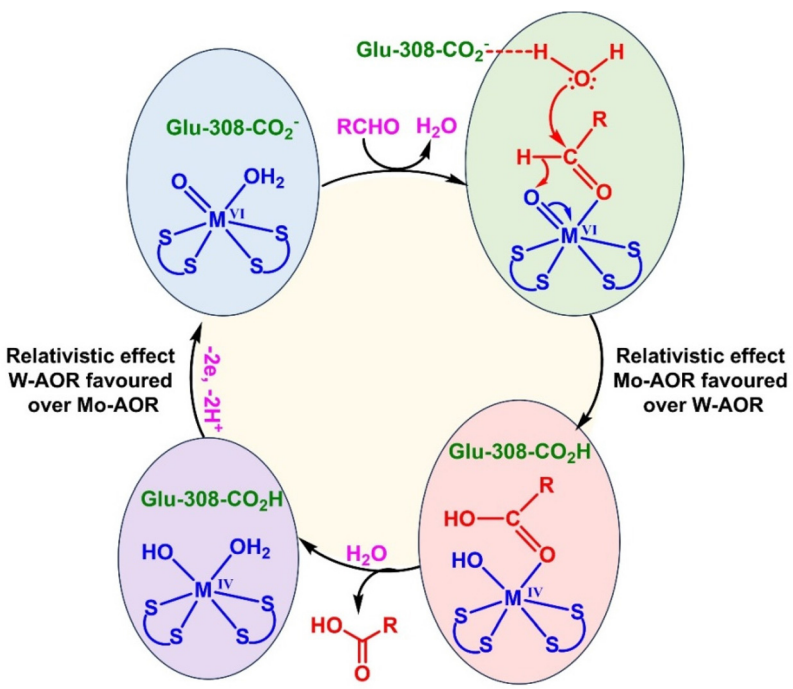
7. Relativistic effect and selectivity of W *vs.* Mo in the enzymatic activity of W-AOR (aldehyde oxidoreductase) *vs.* Mo-AOR

Tungsten oxidoreductase (WOR) (also known as the aldehyde oxidoreductase or W-AOR) is involved in the catalytic oxidation of different types of aldehydes.

$$RCHO + H_2O \rightleftharpoons RCO2H + 2H^+ + 2e^-$$

In nature, there is no corresponding Mo-based enzyme. At the active centre of the WOR, the pyranopterin-based W-cofactor is present. W-AOR (aldehyde ferredoxin oxidoreductase), FOR (formaldehyde ferredoxin oxidoreductase), GAPOR (glyceraldehyde-3 phosphate ferredoxin oxidoreductase), WOR4, and WOR5 are important members of the WOR family.[3,33,57] In the enzymatic activity of W-AOR (native metal W), the aldehyde is oxidised to carboxylic acid and W(VI) is reduced to W(IV). There are different possible mechanistic pathways for the reduction of W(VI) to W(IV).[34,58–60] Hydride transfer from the C—H bond of the substrate to the W^{VI}=O group causes the 2*e* reduction of W(VI) to W(IV) (Scheme 3). It is worth mentioning that in the Mo-based aldehyde oxidase activity, the hydride transfer from the C—H bond of aldehyde to the Mo^{VI}=S group also leads to the 2*e* reduction of Mo(VI). It may be noted that in W-AOR, there is no W^{VI}=S group. Besides, in Mo-based aldehyde oxidase, one dithiolene moiety coordinates to the Mo-cofactor, while in 'W-AOR', two dithiolene moieties coordinate to the W-cofactor.

To maintain the catalytic cycle, the active form of the W(VI)=O species must be regenerated from W(IV). This oxidation under biological conditions is carried out by the Fe_4S_4 ferredoxin protein (Fd). This regeneration of the catalytically active form of the W(VI)-enzyme by Fd is the crucial step in maintaining the catalytic cycle. In fact, because of the more negative redox potential of the W(VI)/W(IV) couple, this oxidation step, W(IV) to W(VI), by Fd is thermodynamically more



Scheme 3 Illustration of the probable mechanism of enzymatic activity of W-AOR/Mo-AOR. Regeneration of the active form of M(VI)-enzyme from the inactive form of M(IV)-enzyme by the physiologically available oxidant controlled by the relativistic effect to determine the selectivity of W *vs.* Mo in W-AOR/Mo-AOR.[3,58,60]

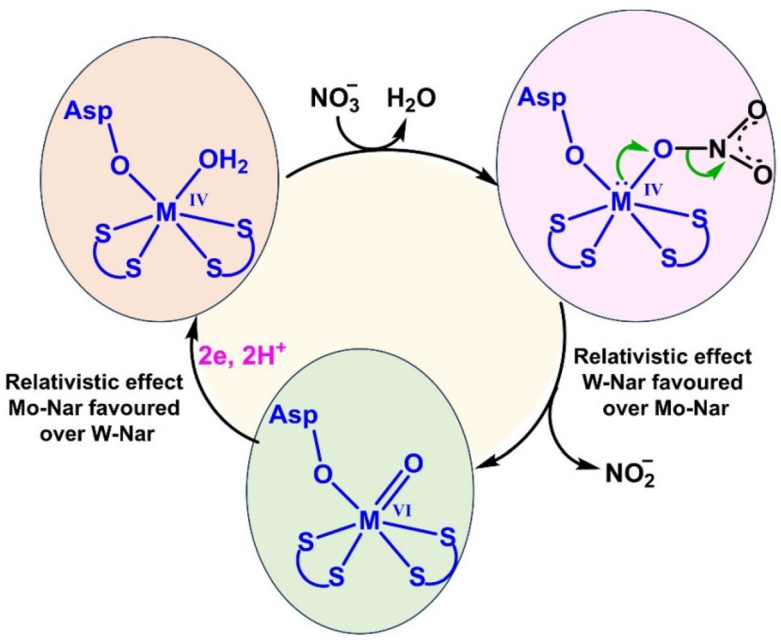
favourable than that of the corresponding Mo-substituted enzyme. Theoretically, the energetics of the enzymatic activity of Mo-FOR and W-FOR in the oxidation of formaldehyde have been studied and compared. It is found[60] that for Mo-FOR, the regeneration of the active Mo(VI)=O species from Mo(IV) by Fd as the available physiological oxidant of the enzyme is endothermic by about 14.0 kcal mol^{-1}. This rationalises the inactivity of Mo-FOR.

8. Relativistic effect and selectivity of W *vs.* Mo in the enzymatic activity of nitrate reductases

Nitrate reductases involved in the biogeochemical nitrogen cycle catalyze the two-electron ($2e^-$) reduction of nitrate to nitrite. There are four types of nitrate reductases: eukaryotic nitrate reductases belonging to the sulfite oxidase (SO) family and three distinct prokaryotic nitrate reductases belonging to the DMSOR family.[3,32,61–63] Prokaryotic nitrate reductases are classified by considering their cellular location, organization and active site structure:[3,32,61–63] periplasmic nitrate reductase (Nap), cytoplasmic nitrate reductase (Nas), and membrane-bound respiratory nitrate reductase (Nar). All the prokaryotic bacterial nitrate reductases and eukaryotic nitrate reductases possess a molybdenum cofactor at their active sites, and the corresponding tungsten-substituted nitrate reductases are inactive.[32,33,61–64] Herein, we illustrate a unique archeal Nar that can utilise both Mo and W-cofactor depending on the growth condition.

Bacterial Nars are inhibited in the presence of a high concentration of WO_4^{2-}.[64] *P. aerophilum* is the only hyperthermophilic denitrifying archaeon showing nitrate reductase activity even at a high concentration of WO_4^{2-}, which inhibits the bacterial Nars.[64,65] Nar purified from *P. aerophilum* grown in a tungstate enriched environment (4.5 μM WO_4^{2-}) is found to contain a W-cofactor similar to the Mo-cofactor of Mo-Nar purified from *P. aerophilum* grown at relatively low WO_4^{2-} concentrations.[64,66] Both Mo-Nar and W-Nar of *P. aerophilum* are active, but Mo-Nar shows a slightly higher activity.[64] From the redox titrational experiments, the midpoint potential (E_m) value was about 88 mV for Mo(V/IV) of the purified Mo-Nar, while for the purified W-Nar, the estimated E_m value for W(V/IV) was −8 mV.[64] This relatively small difference (by about 100 mV only) in the E_m values is consistent with the comparable enzymatic activities of W- and Mo-Nars of *P. aerophilum*. This indicates that the *P. aerophilum* Nar has evolved a special mechanism to efficiently utilize either the W-cofactor or Mo-cofactor depending on the growth conditions.

Recently, a density functional theory (DFT) study on their enzymatic activities using the model complexes derived from the protein X-ray crystal structure of W- and Mo-Nars of *P. aerophilum* is carried out to rationalise their relative activities.[67] Mo/W-Nar catalysed nitrate reduction is an oxo-transfer (OAT) reaction involving the reduction of nitrate (NO_3^-) to nitrite (NO_2^-) with the concomitant $2e^-$ oxidation of the metal centre (M^{IV} to M^{VI}) (Scheme 4). The mechanism considers the binding of the substrate (NO_3^-) with the metal centre (Mo/W)



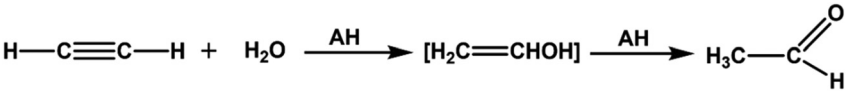
Scheme 4 Illustration of the probable mechanism of enzymatic activity of W-Nar/Mo-Nar in *P. aerophilum*. Regeneration of the active form of M(IV)-enzyme from the inactive form of M(VI)-enzyme by the physiologically available reductant controlled by the relativistic effect to determine the relative reactivities of Mo-Nar and W-Nar in *P. aerophilum*.[65–67]

of the reduced form of the enzyme (M^{IV}-enzyme), followed by an oxygen atom transfer (OAT) from the substrate to the metal centre. The computed results indicate that the energy barrier for the OAT from NO_3^- to the metal centre is 34.4 kcal mol^{-1} for the Mo active site model complex of Mo-Nar, while for the corresponding W active site model complex, the energy barrier is 12.0 kcal mol^{-1}. This higher energy barrier for the Mo-Nar model complex is expected because of the more positive redox potential of the Mo(VI)/Mo(IV) couple compared to that of the W(VI)/W(IV) couple. In terms of this OAT step, W-Nar is expected to be more active, but it contradicts the experimental findings.[64] In terms of the thermodynamic aspect of the oxidation of the educt complex, it has been estimated that the process is almost thermoneutral for the Mo active site model complex (−1.9 kcal mol^{-1}), but the process is strongly exothermic for the W-containing active site model complex (−34.7 kcal mol^{-1}).[67] This indicates that the regeneration of the active form of the enzyme (*i.e.* regeneration of the +IV oxidation state from the +VI oxidation state through 2*e* reduction) is more energetically difficult for the W active site model complex compared to that of the Mo active site model complex. This is due to the more positive redox potential of the Mo(VI)/Mo(IV) couple compared to that of the W(VI)/W(IV) couple. This is expected from the relativistic effect,[12–17] and it explains the higher activity (about twice) of the Mo-Nar compared to that of the W-Nar of *P. aerophilum.*

The reason the *P. aerophilum* Nar can utilise both Mo and W as a metal cofactor while the bacterial Nars can utilise only the Mo-cofactor and cannot utilise the W-cofactor is not clearly understood. The primary ligands present in the first coordination sphere of the metal cofactor (bis-dithiolene sulfur atoms and aspartate) are the same for both the *P. aerophilum* Nar and bacterial Nars.[64] Thus, the consideration of the ligand environment in the first coordination sphere cannot explain this observation. The nature of the second coordination sphere ligands in *P. aerophilum* Nar contributes to the accommodation of either the W-cofactor or Mo-cofactor at the active site.[64] This tuning probably makes a small difference (about 100 mV only) in the E_m values of Mo-Nar (+88 mV) and W-Nar (−8 mV) of *P. aerophilum*.[64] Probably, in the case of bacterial Nars, the E_m values of W-Nars are more negative to make the bacterial W-Nars inactive. Investigation is needed to understand this aspect clearly.

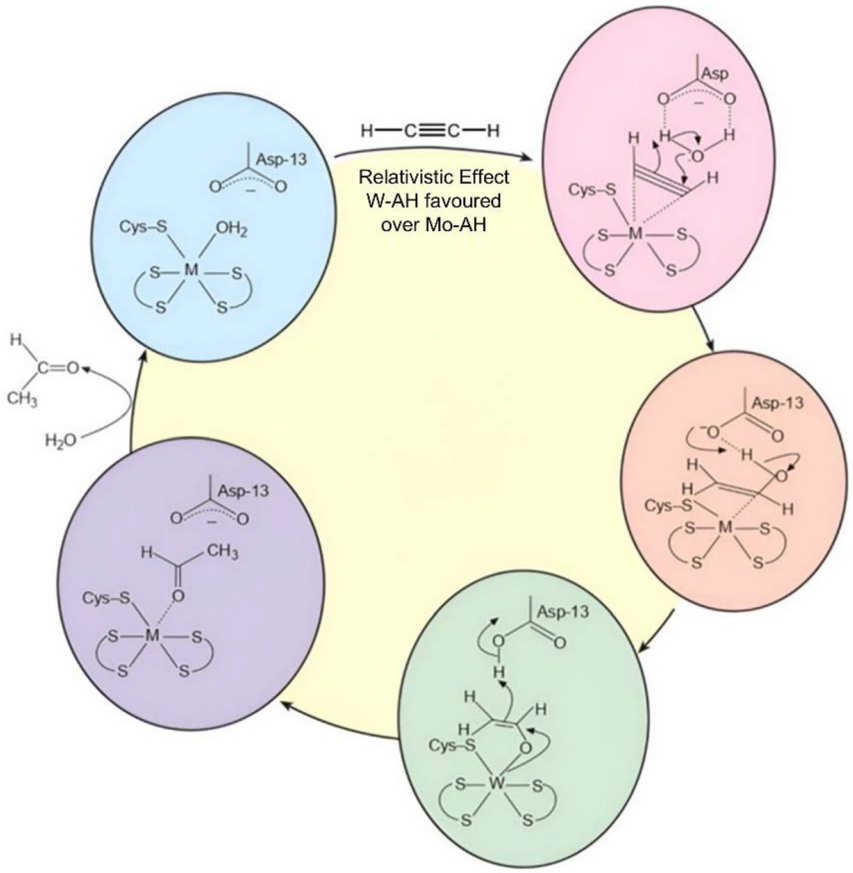
9. Relativistic effect and selectivity of W *vs.* Mo in the enzymatic activity of acetylene hydratase (AH)

In the W-cofactor of AH, W is coordinated by four dithiolene sulfur atoms: two pyranopterins, one cysteine sulfur, and one oxygen ligand. Compared to the enzymatic activities of other W-based enzymes showing oxidase-reductase activity, acetylene hydratase (W-AH) catalyzes a nonredox exothermic reaction ($\Delta G^{\circ\prime}$ = −111.9 kJ mol^{-1}),[3,68–71] hydration of acetylene, producing vinyl alcohol, which subsequently tautomerizes to the more stable product acetaldehyde that can be further oxidized to acetic acid to provide an important source of carbon and energy for certain prokaryotes.[54,68–71]



A theoretical study on the chemoselectivity of tungsten-dependent acetylene hydratase was carried out by Liao *et al.* using three representative substrates: propyne, ethylene, and acetonitrile.[72] It is found that all three substrates experience higher energy barriers for hydration compared with acetylene. Thus, AH is highly selective for acetylene.

In the enzymatic activity of W-AH, regarding the role and oxidation state of tungsten, there are different propositions.[68–70] However, the conserved Asp-13 residue present at the active site acts as an acid–base catalyst (proton shuttle) and plays a crucial role in enzymatic activity. Computational studies indicate that the 2nd coordination sphere mechanism for the W-AH-catalysed hydration of acetylene experiences unrealistically high energy barriers.[71] In fact, theoretical studies by DFT (density functional theory) suggest the energetically more viable 1st coordination sphere mechanism for the enzymatic activity of W-AH.[69,70] Let us consider the viable 1st coordination sphere mechanistic pathway of enzymatic activity (Scheme 5) that activates the substrate acetylene towards the nucleophilic attack by water to produce vinyl alcohol, followed by its prototropic tautomerization to acetaldehyde. This 1st coordination sphere mechanistic pathway mimics the mechanism of Hg(II)-catalyzed hydration of acetylene *in vitro*.[73,74] The 1st coordination sphere mechanism of enzymatic activity can explain the selectivity of W *vs.* Mo in acetylene hydratase An electron-rich substrate (4π-electrons) is typically soft and requires a soft metal centre to coordinate (HSAB matching condition). The 5d-valence orbitals of the heavier congener, tungsten, are radially more expanded owing to the higher relativistic expansion of the 5d-valence orbitals than the 4d-valence orbitals of the lighter congener, molybdenum. The relativistically more expanded 5d-valence orbitals make tungsten softer (*i.e.* more polarisable) than molybdenum for which the relativistic effect is less pronounced.[12–17] Besides, the presence of additional 10d-electrons and 14 4f-electrons in the relativistically expanded 4f-orbitals further softens the tungsten centre. The soft π-acceptor ligand acetylene as a 4*e*-donor ligand coordinates in an η^2-fashion to the metal centre. The two-filled orthogonal π-bonding MOs (*i.e.* π-BMOs) of acetylene are involved to account for its 4*e*-donor property through the σ- and π-type interactions with the metal d-orbitals.[75,76] The vacant two orthogonal π*-MOs of acetylene can participate in π- and δ-type interactions with the d-orbitals of the metal.[3,75,76] These bonding interactions (especially the π- and δ-type) of acetylene with the metal centre are favoured for the relativistically more expanded/diffused valence d-orbitals of the heavier congener, tungsten, compared to that for the lighter



Scheme 5 Illustration of the probable mechanism of the enzymatic activity of W-AH/Mo-AH. The first step in the activation of the substrate (acetylene) through complexation is controlled by the relativistic effect to determine the selectivity of W *vs.* Mo in W-AH/Mo-AH.[3,4,68–70]

congener, molybdenum, for which the corresponding 4d-valence orbitals are relativistically less expanded/diffused. Besides, the more effective nuclear charge of the heavier 5d-series congener due to the presence of low shielding d- and f-electrons (*cf.* lanthanide contraction, which is also partly a consequence of the relativistic effect) for a particular oxidation state makes the coordinated acetylene more electron deficient and activated towards the subsequent nucleophilic attack by water. All these factors, mainly the consequences of the relativistic effect experienced to different extents by the 4d- and 5d-series congeners, can justify nature's selection of tungsten in acetylene hydratase. It is noteworthy that this also explains the fact that the heavier congener Hg(II) is more efficient than the lighter congener Cd(II) to catalyze acetylene hydration *in vitro*.[73,74]

10. Relativistic effect and selectivity of W *vs.* Mo in the enzymatic activity of low potential redox processes, preferably under anaerobic conditions

W-based enzymes are mainly designed by nature to catalyze low-potential redox processes.[6–9,32,33] The more pronounced relativistic effect experienced by the heavier congeners of the d-block elements leads to a more relativistic expansion of their valence d-orbitals, causing a lowering of the ionisation potentials of the valence d-orbital electrons. It stabilises better the higher oxidation states for the heavier congeners of the d-block elements in the periodic table. For Group 6 elements, the stability order is as follows: W(VI) > Mo(VI) > Cr(VI).[12–17] This is why the redox potential of the Mo(VI)/Mo(IV) couple becomes more positive compared to that of the corresponding W(VI)/W(IV) couple. Thus, the relativistic effect suggests that the oxidation of W(IV) to W(VI) is relatively easier, while the reduction of Mo(VI) to Mo(IV) is relatively easier. Because of the relatively easier oxidation of tungsten(IV) to tungsten(VI), W-enzymes are more sensitive than Mo-enzymes to attack by atmospheric O_2, leading to aerial oxidation forming the W^{VI}═O bond, which is more stable than the corresponding Mo^{VI}═O bond.[77–80] The lower (about 300–400 mV more negative) redox potentials of the couples involving the +6, +5 and +4 oxidation states of tungsten compared to those of molybdenum can explain the nature's preference for tungsten enzymes to catalyze the low potential redox processes ($E^{o\prime}$ denotes the standard redox potential at the physiological pH 7.0, $E^{o\prime} < -420$ mV *vs.* NHE, *e.g.* $E^{o\prime} < -550$ mV for W-AOR, $E^{o\prime} = -620$ mV for BCR) in the biological systems.[32–34] In contrast

to the W-enzymes, the Mo-enzymes are selected for the enzymatic activity in a wider range of redox potential ($E^{\circ\prime}$ = −500 to +800 mV *vs.* NHE).[32–34] This rationalises the fact that for the inter-conversion process, CO_2/HCO_2^- ($E^{\circ\prime}$ = −430 mV, a fairly low potential process) catalyzed by formate dehydrogenase (FDH), both W- and Mo-based enzymes can participate.[32–35] However, in the development of nitrate reductase for the biological nitrate reduction to nitrite (*cf.* high redox potential, $E^{\circ\prime}$ = +420 mV for the NO_3^-/NO_2^- couple indicating the nitrate reduction to occur at +420 mV), nature has selected molybdenum, not tungsten. All the eukaryotic and prokaryotic bacterial nitrate reductases are molybdenum-dependent enzymes, and the corresponding tungsten-substituted nitrate reductases are inactive.[32,33,64] However, it has already been mentioned that one nitrate reductase, an archeal Nar present in the hyperthermophilic denitrifying archaeon, *P. aerophilum*, can utilise both Mo- and W-cofactor depending on the growth condition.[64–67] This is a unique example in which the midpoint potential (E_m) value (redox titrational experiment) of W-Nar (−8 mV) is more negative by only 100 mV compared to that of Mo-Nar (+88 mV).[64] This makes both Mo-Nar and W-Nar of *P. aerophilum* active.[64] This aspect is discussed to explain the observation by considering the effect of the second coordination sphere on the redox potential.

11. Relativistic effect and selectivity of W *vs.* Mo in the enzymatic activity at different working temperatures

Mo-based enzymes are selected to function under ambient conditions. However, the W-based enzymes are selected for the biological functions under anaerobic conditions at a relatively higher temperature (up to ∼100 °C) prevailing in the regions of volcanic and hydrothermal vents in the sea bed. In fact, at such a high temperature, the corresponding Mo-based enzymes are thermally unstable. The higher bond strength in the W-enzymes due to the more effective nuclear charge arising from the low screening power of the (n-1)d- and (n-2)f-orbital electrons imparts a higher thermal stability of the W-enzymes. The low screening power of the (n-1)d- and (n-2)f-orbital electrons of tungsten (n = 6, a 5d-series element) can be partly rationalised in terms of the relativistic expansion of the d- and f-orbitals. The lanthanide contraction, which is also partly a consequence of the relativistic effect,[12] is very often argued for the enhanced effective nuclear charge in the 5d-congeners compared to that of the corresponding 4d-congeners. The more relativistically expanded 5d-valence orbitals of tungsten can participate better in bonding interactions with the ligands to make the bond strength higher. This higher bond strength in the W-based enzymes makes the activation energy higher for the oxotransferase activity (*i.e.* the O-atom transfer reactions, OATs) requiring the $W^{VI}{=}O$ bond fission, and it can be attained at a relatively higher working temperature.[3,81] Because of this high activation energy, the W-based enzymes cannot function at normal working temperatures. This can be illustrated by the fact that W can be substituted by Mo in some enzymes of the organisms living at a relatively lower temperature but not in the hyperthermophilic organisms living in an environment of a higher temperature of about 100 °C.[3,81] Both W and Mo can function in FDH (formate dehydrogenase) present in mesophilic acetogen living at about 30 °C temperature.[33–35]

12. Conclusions and perspectives

Molybdenum and tungsten of Group 6 of the periodic table are similar in their chemical properties, and they are naturally used in similar biological redox activities, mainly oxotransferase activity using the M(VI)/M(IV) catalytic cycle, but under different working conditions. Tungstoenzymes mainly function under anaerobic conditions to catalyze low-potential redox processes at high temperatures. It may be noted that acetylene hydratase (W-AH) catalyzes a nonredox reaction, *i.e.* the hydration of acetylene. The natural selectivity of Mo *vs.* W in their enzymatic activities is mainly determined by the relativistic effect that modulates the properties of these two congeners, Mo and W. The relativistic effect experienced more by the heavier congener (W) destabilizes the valence 5d-orbital electrons through the more relativistic expansion of the 5d-valence orbital. It makes the W-centre more polarisable (softer in terms of HSAB theory) and stabilizes its higher oxidation states better than its lighter congener (Mo) for which the relativistic effect is less important. The more pronounced relativistic effect in tungsten makes the redox potentials of the tungsten couples involving the +4, +5 and +6 states more negative compared to those of molybdenum. This factor plays an important role in determining the selectivity of Mo *vs.* W in their redox enzymes. The enhanced softness of tungsten is responsible for the unique selection of tungsten in acetylene hydratase. All these aspects have been illustrated.

The structural insights and mechanistic pathways of W-BCR are of tremendous importance in developing biomimetic methods in biotechnology for the reduction of aromatic rings in organic green synthesis. The conventional Birch reduction of aromatic rings practised in organic synthesis is highly hazardous because it requires reducing agents such as highly reactive alkali metals (*e.g.* sodium) in liquid ammonia as a solvent kept at very low temperatures (*i.e.* under cryogenic conditions). However, the biological Birch reduction catalyzed by W-BCRs occurs under mild and ambient conditions, as desired in green synthesis to protect the environment. This biomimetic approach is a promising alternative to the conventional Birch reduction. More systematic theoretical and experimental investigations are required in this direction. Similarly, understanding the enzymatic activity of W-AH is important to develop a promising and green pathway for the hydration of acetylene in the industrial production of acetaldehyde to replace toxic Hg(II) as a catalyst in the hydration of acetylene. Understanding the mechanistic aspects of tungstoenzymes

controlled by the relativistic effect is quite important in biotechnology to mimic their enzymatic activities for the development of eco-friendly processes for the low-potential biocatalytic reduction of CO_2 (activity of W-FDH) and aromatic compounds. It has been rationalized in terms of the relativistic effect that the W-substituted nitrogenase enzyme fails to reduce dinitrogen (N_2) but can reduce H^+ to produce H_2 efficiently under ambient conditions. *Azotobacter vinelandii* CA6 (mutant strain) developed under the tungsten-enriched and molybdenum-depleted medium is quite efficient in producing H_2 under ambient conditions.[51] This possibility of industrial production of hydrogen, a green fuel, using the tungsten-incorporated nitrogenase in CA6 (mutant strain) may be explored to generate biocatalysts to meet the energy crisis in a greener way.

Data availability

No data were used for the research work described in this article.

Conflicts of interest

The authors declare that they have no known competing financial interests or personal relationships that could have appeared to influence the work reported in this paper.

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