

*Eur. J. Inorg. Chem.* **2014** • © WILEY-VCH Verlag GmbH & Co. KGaA, 69451 Weinheim, 2014 • ISSN 1099–0682

**SUPPORTING INFORMATION**

**DOI:** 10.1002/ejic.201402263

**Title:** Mid- and Far-Infrared Marker Bands of the Metal Coordination Sites of the Histidine Side Chains in the Protein Cu,Zn-Superoxide Dismutase

**Author(s):** Bertrand Xerri, Hugo Petitjean, François Dupeyrat, Jean-Pierre Flament, Alain Lorphelin, Claude Vidaud, Catherine Berthomieu,\* Dorothée Berthomieu\*

Frequencies and modes of histidine side-chains.

*Imidazole and imidazolate ring vibrations*

Stretching modes  $\nu(\text{C}_4\text{-C}_5)$

Contributions from the  $\nu(\text{C}_4\text{-C}_5)$  vibrational modes are calculated in two spectral regions. The main  $\nu(\text{C}_4\text{-C}_5)$  histidine mode in oxidized and in reduced  $\text{Cu}^{\text{II}}$ ,Zn-SOD occurs at the highest frequency values (1623-1591  $\text{cm}^{-1}$ , Table S1) , while a second contribution is calculated, combined with major contributions of  $\delta(\text{C-N-C})$  bending modes at lower frequencies (1037-968  $\text{cm}^{-1}$ ). At high energies, the frequency values of the  $\nu(\text{C}_4\text{-C}_5)$  modes are expected to be larger for  $\text{N}\tau^-$  than for  $\text{N}\pi^-$  ligands. This is the case for the  $\nu(\text{C}_4\text{-C}_5)$  mode of His<sub>118</sub>  $\text{N}\tau$  ligand, predicted at 1618  $\text{cm}^{-1}$  in **9H(ox)**, as compared to  $\nu(\text{C}_4\text{-C}_5)$  mode frequencies of 1602-1598  $\text{cm}^{-1}$  for the His  $\text{N}\pi$  ligands (Table S1b). In contrast, the calculated  $\nu(\text{C}_4\text{-C}_5)$  signal from  $\text{N}\tau^-$  His<sub>46</sub> in  $\text{Cu}^{\text{II}}$ ,Zn-SOD is unexpectedly low, at 1598  $\text{cm}^{-1}$  ( $\nu_{59}$  in **9H(ox)**, Table S1b) in comparison with the other  $\text{N}\tau^-$  His<sub>118</sub> ligand. It is calculated in the range of the  $\text{N}\pi^-$  His ligands. The  $\nu(\text{C}_4\text{-C}_5)$  mode of the

bridging ligand His<sub>61</sub> is calculated at significantly lower frequency values of 1587 or 1591 cm<sup>-1</sup> for the **8H**(ox) or **9H**(ox) models, respectively.

In the reduced Cu<sup>I</sup>,Zn-SOD, the  $\nu(\text{C}_4\text{-C}_5)$  modes are calculated at slightly higher energies than in Cu<sup>II</sup> for the Cu ligands His<sub>44</sub> (+2 cm<sup>-1</sup>), His<sub>46</sub> (+6 cm<sup>-1</sup>) and His<sub>118</sub> (+15 cm<sup>-1</sup>) using the **9H** model (Table S1b), while the frequency of the  $\nu(\text{C}_4\text{-C}_5)$  mode of the Zn-ligand His<sub>78</sub> is slightly downshifted by 4 cm<sup>-1</sup>. The same trend is observed for His<sub>78</sub>, His<sub>44</sub> and His<sub>46</sub> in the **8H** model, with shifts by -4, +7 and +1 cm<sup>-1</sup>, respectively. However, the frequency of the  $\nu(\text{C}_4\text{-C}_5)$  mode of His<sub>118</sub> is calculated at lower energy using the **8H** model (and downshifted by 10 cm<sup>-1</sup> as compared to Cu<sup>II</sup>).

The largest change induced by the change in Cu redox state concerns the His<sub>61</sub>  $\nu(\text{C}_4\text{-C}_5)$  mode. Indeed, His<sub>61</sub> in Cu<sup>I</sup>,Zn-SOD is not an imidazolate bridging ligand as in Cu<sup>II</sup>,Zn-SOD but a N $\pi$  histidine ligand of Zn. In the reduced form, the  $\nu(\text{C}_4\text{-C}_5)$  frequency value of His<sub>61</sub> is calculated in the same range as for the others His N $\pi$ - ligands of Zn His<sub>78</sub> and His<sub>69</sub> (Table S1)

Upon H<sub>2</sub>O/<sup>2</sup>H<sub>2</sub>O exchange,  $\nu(\text{C}_4\text{-C}_5)$  frequencies downshift by 15-19 cm<sup>-1</sup> for the N $\tau$ - coordination *versus* 5-6 cm<sup>-1</sup> for the N $\pi$ - coordination (Table S1). According to these calculated downshifts, the  $\nu(\text{C}_4\text{-C}_5)$  peaks could overlap upon H<sub>2</sub>O/<sup>2</sup>H<sub>2</sub>O exchange and it could be difficult to discriminate N $\tau$ - and N $\pi$ - coordination for samples in <sup>2</sup>H<sub>2</sub>O. The different amplitude of the  $\nu(\text{C}_4\text{-C}_5)$  frequency downshift for N $\tau$ - coordination *versus* N $\pi$ - coordination upon H<sub>2</sub>O/<sup>2</sup>H<sub>2</sub>O exchange, may be however very useful, in addition to the  $\nu(\text{C}_4\text{-C}_5)$  mode frequency, to identify the type of coordination for histidine ligands. Upon <sup>15</sup>N/<sup>14</sup>N labelling,  $\nu(\text{C}_4\text{-C}_5)$  frequencies downshift by 5-9 cm<sup>-1</sup> for the N $\tau$ - coordination versus 3-5 cm<sup>-1</sup> for the N $\pi$ - coordination (Table S1) preventing from a full overlapping.

For Cu<sup>III</sup>,Zn-SOD histidines, combination of the  $\nu(\text{C}_4\text{-C}_5)$  with  $\delta(\text{C-N-C})$  bending modes for N $\tau$ - His and with  $\delta(\text{N}\pi\text{-C-N}\tau)$  bending modes for N $\pi$ - His is calculated in the domain 1006-1016 cm<sup>-1</sup> for all the His, except His<sub>61</sub> (Table S2). Similarly to high-frequency bands  $\nu(\text{C}_4\text{-C}_5)$ , at low frequency, the  $\nu(\text{C}_4\text{-C}_5)$  signals

are at higher values for  $N\tau^-$  than for  $N\pi^-$  ligands, and frequency values slightly change from  $Cu^{II}$  to  $Cu^I$ . However, in contrast with the first high-frequency band i)  $\nu(C_4-C_5)$  value in  $N\tau^-$  His<sub>46</sub> is calculated close to the  $N\tau^-$  His<sub>118</sub>; ii) in the reduced  $Cu^I$ ,Zn-SOD, the  $N\pi^-$ -His<sub>61</sub> ( $\nu_{148}$  Table S2) has a  $\nu(C_4-C_5)$  value in the range of the  $N\tau^-$  His. Indeed, the signal  $\nu(C_4-C_5)$  of the imidazolate ring of His<sub>61</sub>, that is combined with the  $\delta(C_4-N\pi-C_2)$  bending modes, is clearly calculated at higher energy than those of the five other histidines. While the shifts calculated upon  $^2H/^1H$  and  $^{15}N/^{14}N$  labelling are not always clearly assigned for the histidines, it clearly appears that upon  $^2H/^1H$  labelling no shift is expected for  $\nu_{145}$  of His<sub>61</sub> in the  $Cu^{II}$  form – in agreement with the imidazolate form- while a large downshift of  $11\text{ cm}^{-1}$  is expected upon  $^{15}N/^{14}N$  labelling.

Ring bending modes involving  $\delta(C_4-N\pi-C_2)$  and  $\delta(N\pi-C_2-N\tau)$  coordinates are predicted in the ranges 1007-1036 and 926-962  $\text{cm}^{-1}$  respectively (Table S2). The  $N\pi^-$ ,  $N\tau^-$  and imidazolate histidine characters are reflected in the modes, the His  $N\tau^-$  being predicted at higher frequencies than the His  $N\pi^-$ . The composition of the mode is different depending on the  $N\pi^-$ ,  $N\tau^-$  character of the His. Finally, the two signals from the imidazolate of His<sub>61</sub> in the  $Cu^{II}$  state are calculated at the highest frequency values ( $1036\text{ cm}^{-1}$ ,  $\nu_{145}$  and  $962\text{ cm}^{-1}$ ,  $\nu_{159}$ ).

As mentioned above, the main  $\nu(C_4-C_5)$  histidine mode in oxidized and in reduced  $Cu^{II/I}$ ,Zn-SOD appears at the highest frequency values ( $1623\text{-}1591\text{ cm}^{-1}$ , Table S1). According to the literature, frequency values are expected to be larger for  $N\tilde{\tau}$  than for  $N\tilde{\pi}$  ligands. But this is not the case for Cu, Zn-SOD because the  $\nu(C_4-C_5)$  for His<sub>46</sub>  $N\tau^-$  is calculated in the range of  $N\tilde{\pi}$  His. In order to clarify this unexpected behaviour, we considered three additional models, **0H**, **10H<sup>a</sup>** and **10H<sup>b</sup>** (see “theoretical calculation” section), and we calculated the frequency values of the  $\nu(C_4-C_5)$  modes. In Table S3, the calculated  $C_4-C_5$  bond lengths are

reported for each model, **0H**, **8H**, **9H**, **10H<sup>a</sup>** and **10H<sup>b</sup>**. It appears that the C<sub>4</sub>-C<sub>5</sub> bond is systematically longer in His<sub>46</sub> than in His<sub>118</sub> when atoms are fixed at their crystallographic positions while it is the opposite in the fully relaxed **0H** model (C<sub>4</sub>-C<sub>5</sub> bond shorter in His<sub>46</sub> than in His<sub>118</sub>). According to the calculated frequencies of  $\nu(\text{C}_4\text{-C}_5)$  mode, they are predicted at higher values for  $\text{N}\tilde{\tau}$  than for  $\text{N}\tilde{\pi}$  ligands for both His<sub>118</sub> and His<sub>46</sub> only in the fully relaxed structure **0H**.

### $\nu(\text{C-N})$ modes

$\nu(\text{C}_2\text{-N})$  contributes at different positions in the mid-IR domain.

Modes combining  $\nu(\text{C}_2\text{-N})$  and in plane  $\delta(\text{C}_2\text{-H})$  coordinates are predicted at 1509-1522 cm<sup>-1</sup> and 1503-1519 cm<sup>-1</sup> for Cu<sup>II</sup>,Zn-SOD **9H** (Table S1). The  $\nu(\text{C}_2\text{-N})$  mode involves the  $\nu(\text{C}_2\text{-N}\pi)$  and/or  $\nu(\text{C}_2\text{-N}\tau)$  modes, for the metal- $\text{N}\pi$  and metal- $\text{N}\tau$  histidine coordination respectively, and also a small contribution of in-plane  $\delta(\text{C}_2\text{-H})$  mode. For  $\text{N}\pi$ -His, the  $\nu(\text{C}_2\text{-N}\pi)$  mode is at higher frequency values than the  $\nu(\text{C}_2\text{-N}\tau)$  mode for  $\text{N}\tau$ -His. A unique  $\nu(\text{C-N})$  signature involving both asymmetric  $\nu(\text{C}_2\text{-N}\pi)$  and  $\nu(\text{C}_2\text{-N}\tau)$  and in plane  $\delta(\text{C}_2\text{-H})$  is calculated for the imidazolate ring of His<sub>61</sub> in Cu<sup>II</sup>,Zn-SOD. This mode leads to an imidazolate His<sub>61</sub> signal at 1493 cm<sup>-1</sup> clearly at lower frequency values in comparison with metal- $\text{N}\pi$  and metal- $\text{N}\tau$  imidazole ring modes at 1509-1522 cm<sup>-1</sup>. The large predicted frequency difference suggests a separation of the peaks in the experimental IR spectra. In the Cu<sup>I</sup> state, the  $\nu(\text{C}_2\text{-N}\pi)$  signal  $\nu_{63}$  for the Zn  $\text{N}\pi$ - His<sub>61</sub> ligand is calculated at 1516 cm<sup>-1</sup>, close to the two others  $\text{N}\pi$ - His connected to Zn  $\nu_{62}$  at 1519 and  $\nu_{64}$  at 1513 cm<sup>-1</sup> (Table S1). The  $\text{N}\pi$ - or  $\text{N}\tau$ - nature of the His is not reflected in the shifts induced upon <sup>2</sup>H/<sup>1</sup>H or <sup>15</sup>N/<sup>14</sup>N labelling (Table S1). Whatever the His,  $\text{N}\pi$ - or  $\text{N}\tau$ -, the <sup>2</sup>H/<sup>1</sup>H labelling induces downshifts of 4-5 cm<sup>-1</sup>. The downshifts induced by <sup>15</sup>N/labelling are in the range of 8-11 cm<sup>-1</sup>.

The  $\nu(\text{C}_2\text{-N})$  mode is predicted to contribute also as a combination with ring stretching modes at 1315-1348  $\text{cm}^{-1}$  for  $\text{Cu}^{\text{II}}$  and  $\text{Cu}^{\text{I}}$  in  $\text{Cu,Zn-SOD}$  (Table S1). These modes combine symmetric  $\nu(\text{C}_2\text{-N})$  and  $\nu(\text{C}_4\text{-N})$  motions. Whatever the valence state of copper, the highest energies correspond to ring  $\nu(\text{C}_2\text{-N}_\tau)$  modes of metal- $\text{N}\tau$  ligands (with contributions at 1348-1345  $\text{cm}^{-1}$ , Table S1b **9H(ox)**) while the bands at lower energies correspond to ring  $\nu(\text{C}_2\text{-N}_\pi)$  modes of metal- $\text{N}\pi$  ligands (Table S1,  $\nu_{102}, \nu_{103}$  and  $\nu_{105}$  bands at 1323-1315  $\text{cm}^{-1}$  for models **9H(ox)**). The  $\nu(\text{N}\tau\text{-C}_2)$  and  $\nu(\text{N}\pi\text{-C}_2)$  signature of  $\text{His}_{61}$  is calculated between these two groups (Table S1). Whatever the coordination type (His  $\text{N}\pi$ - and His  $\text{N}\tau$ - ligands), the downshifts in the labelled derivatives are comparable, being larger upon  $^{15}\text{N}$  labelling than upon  $^2\text{H}$  labelling. In the case of the imidazolate ring of the  $\text{His}_{61}$  bridging ligand (**9H(ox)**), a significant shift is only calculated upon  $^{15}\text{N}$  labelling.

A fourth  $\nu(\text{N-C})$  domain at 1081-1145  $\text{cm}^{-1}$  is dominated by  $\nu(\text{N}\tau\text{-C}_5)$  mode contributions combined with in-plane  $\delta(\text{C}_5\text{-H})$  modes (Table S2). These modes have been often identified experimentally for histidine metal-ligands in photochemically or electrochemically-triggered FTIR difference spectra. For  $\text{Cu,Zn-SOD}$ , these  $\nu(\text{N}\tau\text{-C}_5)$  modes are predicted at clearly larger frequency values for His  $\text{N}\tau$ - ligands than for  $\text{N}\pi$ - ligands. The calculated effects of  $\text{H}_2\text{O}/^2\text{H}_2\text{O}$  exchange and  $^{15}\text{N}/^{14}\text{N}$  labelling are not clearly identified because labelling induces mixing and overlaps between modes (Table S2). When calculated, in the labelled  $\text{Cu}^{\text{II/I}},\text{Zn-SOD}(^2\text{H}_{10})$ , the calculations show however that these combined  $\nu(\text{N}\tau\text{-C}_5)$  and  $\delta(\text{C}_5\text{-H})$  modes do not shift for His  $\text{N}\tau$ - but upshift by 10-20  $\text{cm}^{-1}$  for His  $\text{N}\pi$ -. In the labelled  $\text{Cu}^{\text{II/I}},\text{Zn-SOD}(^{15}\text{N}_{14})$  no significant difference between His  $\text{N}\pi$ - and  $\text{N}\tau$ - coordination type is calculated since these modes downshift by 4-9  $\text{cm}^{-1}$  for His  $\text{N}\pi$ - and by 4-5  $\text{cm}^{-1}$  for His  $\text{N}\tau$ -. Tentative assignments for the reduced  $\text{Cu}^{\text{I}},\text{Zn-SOD}$  suggest that these  $\nu(\text{N}\tau\text{-C}_5)$  modes calculated for  $\text{Cu}^{\text{II}},\text{Zn-SOD}$  remain at similar frequency value in  $\text{Cu}^{\text{I}},\text{Zn-SOD}$ .

### $\delta(\text{N-C-N})$ ring bending modes

Below  $1000\text{ cm}^{-1}$ , combinations of  $\delta(\text{N}\pi\text{-C}_2\text{-N}\tau)$  with  $\delta(\text{C-N}\tau\text{-C})$  and  $\delta(\text{C-C-N}\tau)$  or  $\nu(\text{C}_4\text{-N}\pi)$  are calculated in the region  $926\text{-}962\text{ cm}^{-1}$ .  $\delta(\text{N}\pi\text{-C}_2\text{-N}\tau)$  from His  $\text{N}\tau^-$  are at higher frequency values than from the His  $\text{N}\pi^-$  (Table S2). The mode involving  $\delta(\text{C}_4\text{-N}\pi\text{-C}_2)$  and  $\delta(\text{N}\pi\text{-C}_2\text{-N}\tau)$  coordinates from imidazolate His<sub>61</sub> exhibits the highest frequency values in the  $\text{Cu}^{\text{II}}$  state ( $\nu_{159}$  at  $962\text{ cm}^{-1}$  model **9H(ox)**) but it is only  $6\text{ cm}^{-1}$  above the peak from His<sub>118</sub> ( $\nu_{160}$  at  $956\text{ cm}^{-1}$ ). Calculated shifts from labelled  $\text{Cu}^{\text{II}},\text{Zn-SOD}(^2\text{H}_{10})$  and  $\text{Cu}^{\text{III}},\text{Zn-SOD}(^{15}\text{N}_{14})$  are quite similar whatever the His  $\text{N}\tau^-$  and His  $\text{N}\pi^-$ .

At lower frequencies, histidine ring torsions signal contributions are split in two regions.  $\tau(\text{C}_4\text{-N}\pi\text{-C}_2\text{-N}\tau)$  and  $\tau(\text{C}_4\text{-N}\tau\text{-C}_2\text{-N}\pi)$  modes at  $681\text{-}670$  (**9H(ox)**, Table S4b) and  $\tau(\text{C}_4\text{-C}_5\text{-N}\tau\text{-C}_2)$  and  $\tau(\text{C}_5\text{-C}_4\text{-N}\pi\text{-C}_2)$  modes at  $655\text{-}641\text{ cm}^{-1}$  (Table S4). In both domains, the ring torsion frequencies from the  $\text{N}\pi^-$  ligands and from the  $\text{N}\tau^-$  ligands are in the same range whatever the valence state of Cu,  $\text{Cu}^{\text{II}}$  or  $\text{Cu}^{\text{I}}$ . Band frequencies are affected by the change in redox state of the Cu, notably for the His<sub>118</sub> and His<sub>44</sub>. The bands are shifted upon  $\text{H}_2\text{O}/^2\text{H}_2\text{O}$  and  $^{15}\text{N}/^{14}\text{N}$  labelling but they could not be systematically clearly assigned.

### *N-H and C-H bending modes*

#### $\delta(\text{N-H})$ bending modes

$\delta(\text{N-H})$  bending modes are in plane  $\delta(\text{N}\tau\text{-H})$  bending modes for His  $\text{N}\pi^-$  ligands and in plane  $\delta(\text{N}\pi\text{-H})$  bending modes for His  $\text{N}\tau^-$  ligands. In plane  $\delta(\text{N-H})$  bending modes are split in two bands calculated at  $1421\text{-}1475\text{ cm}^{-1}$  and at  $1128\text{-}1186\text{ cm}^{-1}$  (Tables S1 and S2).

Modes at  $1421\text{-}1475\text{ cm}^{-1}$  are dominated by in-plane  $\delta(\text{N-H})$  motions. They largely downshift upon  $\text{H}_2\text{O}/^2\text{H}_2\text{O}$  exchange, by  $63\text{-}77\text{ cm}^{-1}$ , and upon  $^{15}\text{N}/^{14}\text{N}$  labelling, by  $18\text{-}23\text{ cm}^{-1}$ . Downshifts upon  $\text{H}_2\text{O}/^2\text{H}_2\text{O}$  exchange are not clearly identified for  $\text{Cu}^{\text{I}}$  in model **9H(red)** (Table S1b). These modes are predicted at

higher frequency values for His N $\pi$ - ligands than for N $\tau$ - ligands and are clearly different when they involve His N $\tau$ - ligands or N $\pi$ - ligands since  $\delta(\text{N}\pi\text{-H})$  are higher by at least 33 cm<sup>-1</sup> than  $\delta(\text{N}\tau\text{-H})$ . In the reduced Cu<sup>I</sup>,Zn-SOD these  $\delta(\text{N-H})$  modes have frequency values similar to those calculated for Cu<sup>II</sup>,Zn-SOD.

The His<sub>61</sub>  $\delta(\text{N}\tau\text{-H})$  mode ( $\nu_{68}$ ) calculated value is at higher energy by 16-22 cm<sup>-1</sup> than the two others N $\pi$ -His connected to Zn (1491 cm<sup>-1</sup> versus 1475 and 1469 cm<sup>-1</sup>, Table S1b). This may be due to the fact that the N $\tau$ -H from His<sub>61</sub> in the reduced Cu<sup>I</sup> exhibits a H-bond with the H<sub>2</sub>O molecule pseudo ligand of Cu<sup>I</sup>.

In plane  $\delta(\text{N-H})$  bending modes in the second region, at 1186-1128 cm<sup>-1</sup>, are combined with ring stretching modes. The in plane  $\delta(\text{N-H})$  bending modes are dominated by  $\nu(\text{C}_2\text{-N}\tau)$  stretching modes calculated at higher energies for N $\pi$ -His than the  $\delta(\text{N}\pi\text{-H})$  bending modes combined with  $\nu(\text{C}_2\text{-N}\pi)$  stretching modes calculated at lower energies for N $\tau$ -His (Table S2). As previously shown for the above in plane  $\delta(\text{N-H})$  bending modes at 1475-1421 cm<sup>-1</sup>, the  $\delta(\text{N-H})$  bending modes combined with  $\nu(\text{C}_2\text{-N})$  modes occur also at the same position for Cu<sup>II</sup> and Cu<sup>I</sup>. When the assignment was possible, calculations show that these modes are largely downshifted upon H<sub>2</sub>O/<sup>2</sup>H<sub>2</sub>O exchange by 54-62 cm<sup>-1</sup> and upon <sup>15</sup>N/<sup>14</sup>N labelling by 6-9 cm<sup>-1</sup>. Also, the N $\tau$ -H from His<sub>61</sub> in the oxidized Cu<sup>I</sup> ( $\nu_{124}$ ) is calculated at higher energy than the two N $\pi$ -His connected to Zn (1186 cm<sup>-1</sup> versus 1165 and 1158 cm<sup>-1</sup>, Table S2).

### $\delta(\text{C-H})$ bending modes

$\delta(\text{C-H})$  bending modes involve C<sub>2</sub> or C<sub>5</sub> atoms from the histidine rings. These calculated modes contribute in two different regions.

Only modes in the 1243-1227 cm<sup>-1</sup> region dominated by in-plane  $\delta(\text{C-H})$  motions are considered. In-plane  $\delta(\text{C-H})$  modes in the 1137-1081 cm<sup>-1</sup> region (Table S2) dominated by  $\nu(\text{N}\tau\text{-C}_5)$  mode contributions were detailed above. In the 1243-1227 cm<sup>-1</sup> region in-plane  $\delta(\text{C-H})$  modes are slightly downshifted upon



H<sub>2</sub>O/<sup>2</sup>H<sub>2</sub>O exchange and upon <sup>15</sup>N/<sup>14</sup>N labelling (Table S1). These modes are predicted at slightly larger frequency values for His Nπ<sup>-</sup> ligands than for Nτ<sup>-</sup> ligands. In the reduced Cu<sup>I</sup>,Zn-SOD these δ(C-H) modes have frequency values quite similar to those calculated for Cu<sup>II</sup>,Zn-SOD.

#### *N-H and C-H torsion modes*

Out-of-plane torsions are predicted in the region below 900 cm<sup>-1</sup>. Out-of-plane torsion φ(C-H) mode is more generally a combination of φ(C<sub>5</sub>-H) and φ(C<sub>2</sub>-H) from the histidine rings. They lead to two bands at 964-802 cm<sup>-1</sup> and 797-754 cm<sup>-1</sup> in **9H(ox)** while out-of-plane torsions φ(N-H) from the histidine rings are predicted below, at 607-568 cm<sup>-1</sup> in **9H(ox)**. φ(N-H) modes downshift by more than 100 cm<sup>-1</sup> upon <sup>2</sup>H labelling and by more than 7 cm<sup>-1</sup> upon <sup>15</sup>N labelling. These downshifts are in the range of 170 cm<sup>-1</sup> previously reported in N<sup>2</sup>H labelled Zn-methylimidazole complexes. More generally these out-of-plane torsion modes are predicted to be intense both in the Cu<sup>II</sup> and Cu<sup>I</sup> forms. More than one histidine ligand can participate to the mode.

Symmetric φ(C-H) modes are calculated at higher energies than the asymmetric φ(C-H) (Table S4). While frequencies of symmetric φ(C-H) modes are not ranked according to any obvious rule, frequencies of asymmetric modes are ranked following the His Nτ<sup>-</sup> and Nπ<sup>-</sup> types. Small or no shifts are predicted upon <sup>2</sup>H and <sup>15</sup>N labelling for these modes.

Finally, out-of-plane φ(NH) modes are predicted in the region 607-568 cm<sup>-1</sup> and occur at higher energy values for φ(NπH) than for φ(NτH). For these modes, the largest downshifts (> 120 cm<sup>-1</sup>) are predicted for labelled Cu<sup>II/I</sup>,Zn-SOD(<sup>2</sup>H<sub>10</sub>) and downshifts of ~7 cm<sup>-1</sup> are predicted for Cu<sup>II/I</sup>,Zn-SOD(<sup>15</sup>N<sub>14</sub>). These downshifts are similar for Nτ<sup>-</sup> ligands and Nπ<sup>-</sup> ligands. No φ(Nπ-H) specific position was calculated for protonated His<sub>61</sub>. This is most probably due to the fact that the H from NπH is very close to the water

molecule in the  $\text{Cu}^{\text{I}}$  coordination shell, thus affecting the low-frequency out-of-plane  $\phi(\text{NH})$  mode. This result is consistent with the significantly upshifted  $\delta(\text{NH})$  mode calculated in protonated  $\text{His}_{61}$  discussed above.



**Table S1a.** Calculated vibrational frequencies (all the frequencies are scaled by 0.98) and main normal mode description based on PED between 1655 and 1230  $\text{cm}^{-1}$  for models **8H(red)** and **8H(ox)** of  $\text{Cu}^{\text{II}}$ ,Zn-SOD and  $\text{Cu}^{\text{I}}$ ,Zn-SOD calculated within the B3LYP/6-31G(d,p) method (vib num = vibration numbers, main normal mode assignment are based on PED (potential energy distribution) and  $\Delta\nu$  shifts ( $\Delta\nu=\nu$  unlabelled – $\nu$  labelled) in the  $\text{N-}^2\text{H}$  and  $^2\text{H}_2\text{O}$  labelled models and in the  $^{15}\text{N}$  labelled models). Units are  $\text{cm}^{-1}$ .  $\nu(\text{XY})$  is the stretching vibration of the bond between atoms X and Y,  $\delta(\text{XYZ})$  is the bending vibration of the angles between atoms XYZ,  $\tau(\text{XYZW})$  is the torsion vibration,  $\delta(\text{X-H})$  is in-plane vibration of the X-H bond.

(a) ( $\pi$ ) or ( $\tau$ ) stand for  $\text{N}\pi$ - or  $\text{N}\tau$ - connexion type of the Histidine with the corresponding metal.

(b) - indicates that it was not possible from the calculations to determine the  $\Delta\nu$

		<b>8H(ox)</b>	$^2\text{H}$	$^{15}\text{N}$		<b>8H(red)</b>	$^2\text{H}$	$^{15}\text{N}$			
PED	vib num	$\nu \text{ cm}^{-1} * 0.98$	$\Delta\nu$	$\Delta\nu$	Involved Histidine residue <sup>a</sup>	vib num	$\nu \text{ cm}^{-1} * 0.98$	$\Delta\nu$	$\Delta\nu$	PED	Involved Histidine residue <sup>a</sup>
52% $\nu(\text{C}_4\text{-C}_5)$	$\nu_{52}$	1621	16	5	His118( $\tau$ )	$\nu_{52}$	1611	17	5	50% $\nu(\text{C}_4\text{-C}_5)$	His118( $\tau$ )
58% $\nu(\text{C}_4\text{-C}_5)$	$\nu_{53}$	1603	6	3	His69( $\pi$ )	$\nu_{56}$	1601	6	3	56% $\nu(\text{C}_4\text{-C}_5)$	His69( $\pi$ )
60% $\nu(\text{C}_4\text{-C}_5)$	$\nu_{55}$	1600	6	3	His78( $\pi$ )	$\nu_{58}$	1596	6	3	60% $\nu(\text{C}_4\text{-C}_5)$	His78( $\pi$ )
44% $\nu(\text{C}_4\text{-C}_5)$	$\nu_{56}$	1599	19	6	His46( $\tau$ )	$\nu_{57}$	1600	18	5	47% $\nu(\text{C}_4\text{-C}_5)$	His46( $\tau$ )
55% $\nu(\text{C}_4\text{-C}_5)$	$\nu_{57}$	1597	6	3	His44( $\pi$ )	$\nu_{54}$	1604	5	4	>47% $\nu(\text{C}_4\text{-C}_5)$	His44( $\pi$ )
55% $\nu(\text{C}_4\text{-C}_5)$	$\nu_{58}$	1587	0	1	His61	$\nu_{55}$ $\nu_{59}$	1603 1595	- <sup>b</sup> -	1 2	19% $\nu(\text{C}_4\text{-C}_5)$ 28% $\nu(\text{C}_4\text{-C}_5)$	His61
25% $\nu(\text{C}_2\text{-N}\pi)$ 22% $\delta(\text{C}_2\text{-H})$	$\nu_{60}$	1522	5	11	His78( $\pi$ )	$\nu_{62}$	1519	3	10	20% $\nu(\text{C}_2\text{-N}\pi)$ 18% $\delta(\text{C}_2\text{-H})$	His78( $\pi$ )

16% $\nu$ (C <sub>2</sub> -N <sub><math>\pi</math></sub> ) 21% $\delta$ (C <sub>2</sub> -H)	V <sub>62</sub>	1519	5	10	His69( $\pi$ )	V <sub>64</sub>	1515	5	10	28% $\nu$ (C <sub>2</sub> -N <sub><math>\pi</math></sub> ) 23% $\delta$ (C <sub>2</sub> -H)	His69( $\pi$ )
27% $\nu$ (C <sub>2</sub> -N <sub><math>\pi</math></sub> ) 18% $\delta$ (C <sub>2</sub> -H)	V <sub>63</sub>	1517	4	10	His44( $\pi$ )	V <sub>65</sub>	1513	5	10	31% $\nu$ (C <sub>2</sub> -N <sub><math>\pi</math></sub> ) 26% $\delta$ (C <sub>2</sub> -H)	His44( $\pi$ )
24% $\nu$ (C <sub>2</sub> -N <sub><math>\tau</math></sub> ) 15% $\delta$ (C <sub>2</sub> -H)	V <sub>64</sub>	1512	4	10	His118( $\tau$ )	V <sub>67</sub>	1505 1507	3	9	18% $\nu$ (C <sub>2</sub> -N <sub><math>\tau</math></sub> ) 13% $\delta$ (C <sub>2</sub> -H)	His118( $\tau$ )
24% $\nu$ (C <sub>2</sub> -N <sub><math>\tau</math></sub> ) 15% $\delta$ (C <sub>2</sub> -H)	V <sub>64</sub>	1512	4	10	His118( $\tau$ )	V <sub>66</sub>	1505 1507	4	9	12% $\nu$ (C <sub>2</sub> -N <sub><math>\tau</math></sub> )	His118( $\tau$ )
30% $\nu$ (C <sub>2</sub> -N <sub><math>\tau</math></sub> ) 20% $\delta$ (C <sub>2</sub> -H)	V <sub>65</sub>	1508	4	9	His46( $\tau$ )	V <sub>66</sub>	1507	4	9	19% $\nu$ (C <sub>2</sub> -N <sub><math>\tau</math></sub> ) 15% $\delta$ (C <sub>2</sub> -H)	His46( $\tau$ )
22% $\delta$ (C <sub>2</sub> -H) 10% $\nu$ (C <sub>2</sub> -N <sub><math>\tau</math></sub> ) 10% $\nu$ (C <sub>2</sub> -N <sub><math>\pi</math></sub> )	V <sub>66</sub>	1495		7	His61	V <sub>61</sub>	1521	6	10	10% $\nu$ (C <sub>2</sub> -N <sub><math>\tau</math></sub> ) 20% $\nu$ (C <sub>2</sub> -N <sub><math>\pi</math></sub> ) 20% $\delta$ (C <sub>2</sub> -H)	His61
						V <sub>68</sub>	1500	109	15	44% $\delta$ (N <sub><math>\tau</math></sub> -H)	His61
19% $\delta$ (N <sub><math>\tau</math></sub> -H)	V <sub>75</sub>	1469	75	18	His78( $\pi$ )	V <sub>78</sub>	1468	74	17	14% $\delta$ (N <sub><math>\tau</math></sub> -H)	His78( $\pi$ )
31% $\delta$ (N <sub><math>\tau</math></sub> -H)	V <sub>76</sub>	1468	74	19	His69( $\pi$ )	V <sub>77</sub>	1469	78	19	35% $\delta$ (N <sub><math>\tau</math></sub> -H)	His69( $\pi$ )
41% $\delta$ (N <sub><math>\tau</math></sub> -H)	V <sub>83</sub>	1458	76	18	His44( $\pi$ )	V <sub>82</sub>	1464	78	19	42% $\delta$ (N <sub><math>\tau</math></sub> -H)	His44( $\pi$ )
34% $\delta$ (N <sub><math>\pi</math></sub> -H)	V <sub>87</sub>	1424	59	20	His118( $\tau$ )	V <sub>90</sub>	1421	63	19	29% $\delta$ (N <sub><math>\pi</math></sub> -H)	His118( $\tau$ )
18% $\delta$ (N <sub><math>\pi</math></sub> -H)	V <sub>89</sub>	1421	66	23	His46( $\tau$ )	V <sub>92</sub>	1419	-	23	17% $\delta$ (N <sub><math>\pi</math></sub> -H)	His46( $\tau$ )

30%v(C <sub>2</sub> -N <sub>τ</sub> ) 10%v(C <sub>4</sub> -N <sub>π</sub> ) δ(N <sub>τ</sub> -C <sub>2</sub> - N <sub>π</sub> ) 17%δ(N <sub>π</sub> -C <sub>4</sub> -C <sub>5</sub> )	V <sub>99</sub>	1352	10	13	His118(τ)	V <sub>103</sub>	1341	10	103	33%v(C <sub>2</sub> -N <sub>τ</sub> ) 12%v(C <sub>4</sub> -N <sub>π</sub> ) 16%δ(N <sub>π</sub> -C <sub>4</sub> -C <sub>5</sub> )	His118(τ)
32%v(C <sub>2</sub> -N <sub>τ</sub> ) 13%v(C <sub>4</sub> -N <sub>π</sub> ) 16%δ(N <sub>π</sub> -C <sub>4</sub> -C <sub>5</sub> )	V <sub>100</sub>	1346	9	13	His46(τ)	V <sub>101</sub>	1345	19	12	32%v(C <sub>2</sub> -N <sub>τ</sub> ) 13%v(C <sub>4</sub> -N <sub>π</sub> ) 15%δ(N <sub>π</sub> -C <sub>4</sub> -C <sub>5</sub> )	His46(τ)
18%v(C <sub>2</sub> -N <sub>τ</sub> ) 23%v(C <sub>4</sub> -N <sub>π</sub> ) 24%δ(N <sub>π</sub> -C <sub>4</sub> -C <sub>5</sub> )	V <sub>101</sub>	1333	0	9	His61	V <sub>105</sub>	1321	3	14	41%v(C <sub>2</sub> -N <sub>π</sub> ) 10%δ(N <sub>τ</sub> -C <sub>5</sub> -C <sub>4</sub> ) 24%δ(N <sub>π</sub> -C <sub>4</sub> -C <sub>5</sub> ) 13%δ(C <sub>4</sub> -N <sub>π</sub> -C <sub>2</sub> )	His61
38%v(C <sub>2</sub> -N <sub>π</sub> ) 14%v(C <sub>4</sub> -N <sub>π</sub> ) 18%δ(N <sub>τ</sub> -C <sub>2</sub> - N <sub>π</sub> ) 20%δ(N <sub>τ</sub> -C <sub>4</sub> -C <sub>5</sub> ) 12%δ(C <sub>5</sub> -N <sub>τ</sub> -C <sub>2</sub> )	V <sub>103</sub>	1324	3	14	His69(π)	V <sub>104</sub>	1327	3	14	37%v(C <sub>2</sub> -N <sub>π</sub> ) 14%v(C <sub>4</sub> -C <sub>5</sub> ) 21%δ(N <sub>τ</sub> -C <sub>2</sub> - N <sub>π</sub> ) 24%δ(N <sub>τ</sub> -C <sub>4</sub> -C <sub>5</sub> ) 15%δ(C <sub>5</sub> -N <sub>τ</sub> -C <sub>2</sub> )	His69(π)
42%v(C <sub>2</sub> -N <sub>π</sub> ) 24%v(C <sub>4</sub> -N <sub>π</sub> ) 24%δ(N <sub>τ</sub> -C <sub>2</sub> - N <sub>π</sub> )	V <sub>104</sub>	1323	6	12	His44(π)	V <sub>107</sub>	1314	4	14	49%v(C <sub>2</sub> -N <sub>π</sub> ) 13%v(N <sub>τ</sub> -C <sub>5</sub> ) 22%δ(N <sub>τ</sub> -C <sub>2</sub> - N <sub>π</sub> ) 22%v(C <sub>4</sub> -N <sub>π</sub> )	His44(π)
40%v(C <sub>2</sub> -N <sub>π</sub> ) 12%v(C <sub>4</sub> -C <sub>5</sub> ) 12%δ(N <sub>τ</sub> -C <sub>2</sub> -N <sub>π</sub> ) 15%δ(N <sub>τ</sub> -C <sub>4</sub> -C <sub>5</sub> )	V <sub>106</sub>	1315	4	14	His78(π)	V <sub>106</sub>	1320	4	13	35%v(C <sub>2</sub> -N <sub>π</sub> ) 13%v(C <sub>4</sub> -C <sub>5</sub> ) 15%δ(N <sub>τ</sub> -C <sub>2</sub> -N <sub>π</sub> ) 17%δ(N <sub>τ</sub> -C <sub>4</sub> -C <sub>5</sub> )	His78(π)
33%v(C <sub>5</sub> -N <sub>τ</sub> )	V <sub>108</sub>	1278	7	9	His69(π)	V <sub>109</sub>	1278	8	10	28%v(C <sub>4</sub> -N <sub>τ</sub> )	His69(π)

36% $\nu(\text{C}_4\text{-N}_\pi)$	$\nu_{109}$	1273	8	9	His44( $\pi$ )	$\nu_{111}$	1269	5	7	38% $\nu(\text{C}_4\text{-N}_\pi)$	His44( $\pi$ )
42% $\delta(\text{N}_\tau\text{-C}_2\text{-N}_\pi)$ . 26% $\nu(\text{C}_2\text{-N}_\tau)$	$\nu_{113}$	1264	0	18	His61	$\nu_{110}$	1273	10	9	30% $\nu(\text{C}_4\text{-N}_\pi)$	His61 ( $\pi$ )
42% $\delta(\text{C}_5\text{-H})$ 21% $\nu(\text{C}_2\text{-N}_\pi)$	$\nu_{110}$	1266	8	5	His118( $\tau$ )	$\nu_{113}$	1263	9	5	50% $\delta(\text{C}_5\text{-H})$ 15% $\nu(\text{C}_2\text{-N}_\pi)$	His118( $\tau$ )
47% in plane $\delta(\text{C}_5\text{-H})$ 16% $\nu(\text{C}_2\text{-N}_\pi)$ 15% in plane $\delta(\text{C}_2\text{-H})$	$\nu_{114}$	1261	10	4	His46( $\tau$ )	$\nu_{114}$	1260	8	5	49% in plane $\delta(\text{C}_5\text{-H})$ 15% $\nu(\text{C}_2\text{-N}_\pi)$ 17% in plane $\delta(\text{C}_2\text{-H})$	His46( $\tau$ )
37% in plane $\delta(\text{C}_2\text{-H})$ 37% in plane $\delta(\text{C}_5\text{-H})$ 10% $\nu(\text{C}_2\text{-N}_\pi)$	$\nu_{115}$	1245	3	7	His44( $\pi$ )	$\nu_{117}$	1237	3	5	37% in plane $\delta(\text{C}_5\text{-H})$ 35% in plane $\delta(\text{C}_2\text{-H})$	His44( $\pi$ )
32% in plane $\delta(\text{C}_5\text{-H})$ 31% in plane $\delta(\text{C}_2\text{-H})$	$\nu_{116}$	1238	6	5	His69( $\pi$ )	$\nu_{118}$	1234	7	5	32% in plane $\delta(\text{C}_5\text{-H})$ 31% in plane $\delta(\text{C}_2\text{-H})$	His69( $\pi$ )
37% in plane $\delta(\text{C}_2\text{-H})$ 20% in plane $\delta(\text{C}_5\text{-H})$ 16% $\nu(\text{C}_2\text{-N}_\pi)$	$\nu_{117}$	1236	0	10	His61	$\nu_{116}$	1238	12	7	29% in plane $\delta(\text{C}_5\text{-H})$ 11% $\nu(\text{C}_2\text{-N}_\tau)$ 15% in plane $\delta(\text{C}_2\text{-H})$	His61
36% in plane $\delta(\text{C}_5\text{-H})$ 31% in plane $\delta(\text{C}_2\text{-H})$	$\nu_{118}$	1235	4	5	His78( $\pi$ )	$\nu_{119}$	1234	5	5	33% in plane $\delta(\text{C}_5\text{-H})$ 10% $\nu(\text{C}_2\text{-N}_\pi)$ 31% in plane $\delta(\text{C}_2\text{-H})$	His78( $\pi$ )

**Table S1b.** Calculated vibrational frequencies and main normal mode description based on PED between 1655 and 1230  $\text{cm}^{-1}$  for models **9H(ox)** and **9H(red)** of  $\text{Cu}^{\text{II}},\text{Zn-SOD}$  and  $\text{Cu}^{\text{I}},\text{Zn-SOD}$  respectively calculated within the B3LYP/6-31G(d,p) method (vib num = vibration numbers, main normal mode assignment are based on PED (potential energy distribution) and  $\Delta\nu$  shifts ( $\Delta\nu=v$  unlabelled  $-v$  labelled) in the  $^2\text{H}$  and  $^2\text{H}_2\text{O}$  labelled models and in the  $^{15}\text{N}$  labelled models). Units are  $\text{cm}^{-1}$ .  $\nu(\text{XY})$  is the stretching vibration of the bond between atoms X and Y,  $\delta(\text{XYZ})$  is the bending vibration of the angles between atoms XYZ,  $\tau(\text{XYZW})$  is the torsion vibration,  $\delta(\text{X-H})$  is in-plane vibration of X-H bond.

(a) ( $\pi$ ) or ( $\tau$ ) stand for  $\text{N}\pi$ - or  $\text{N}\tau$ - connexion type of the Histidine with the corresponding metal.

(b) - indicates that it was not possible from the calculations to determine the  $\Delta\nu$

		<b>9H(ox)</b>	$^2\text{H}$	$^{15}\text{N}$		<b>9H(red)</b>	$^2\text{H}$	$^{15}\text{N}$		
PED	vib num	$\nu \text{ cm}^{-1}$ *0.98	$\Delta\nu$	$\Delta\nu$	Involved Histidine residue <sup>a</sup>	vib num	$\nu \text{ cm}^{-1}$ *0.98	$\Delta\nu$	$\Delta\nu$	Involved Histidine residue <sup>a</sup>
52% $\nu(\text{C}_4\text{-C}_5)$	$\nu_{52}$	1618	16	5	His118( $\tau$ )	$\nu_{52}$	1623	15	4	His118( $\tau$ )
56% $\nu(\text{C}_4\text{-C}_5)$	$\nu_{55}$	1602	6	3	His69( $\pi$ )	$\nu_{56}$	1603	6	3	His69( $\pi$ )
60% $\nu(\text{C}_4\text{-C}_5)$	$\nu_{56}$	1600	6	5	His78( $\pi$ )	$\nu_{59}$	1596	6	3	His78( $\pi$ )
38% $\nu(\text{C}_4\text{-C}_5)$	$\nu_{58}$	1598	18	9	His46( $\tau$ )	$\nu_{55}$	1604	17	5	His46( $\tau$ )
55% $\nu(\text{C}_4\text{-C}_5)$	$\nu_{57}$	1598	6	6	His44( $\pi$ )	$\nu_{57}$	1600	5	3	His44( $\pi$ )
53% $\nu(\text{C}_4\text{-C}_5)$	$\nu_{59}$	1591	-1	3	His61	$\nu_{54}$		- <sup>b</sup>	2	His61( $\pi$ )
53% $\nu(\text{C}_4\text{-C}_5)$	$\nu_{59}$	1591	-1	3	His61	$\nu_{58}$	1597	-	1	His61( $\pi$ )
27% $\nu(\text{C}_2\text{-N}\pi)$			5	11						
10% $\delta(\text{C}_2\text{-H})$	$\nu_{60}$	1522			His78( $\pi$ )	$\nu_{62}$	1519	5	11	His78( $\pi$ )
21% $\nu(\text{C}_2\text{-N}\pi)$	$\nu_{61}$		5	10						
11% $\delta(\text{C}_2\text{-H})$		1519			His69( $\pi$ )	$\nu_{64}$	1513	5	10	His69( $\pi$ )



30%v(C <sub>2</sub> -N <sub>π</sub> ) 11%δ(C <sub>2</sub> -H)	v <sub>64</sub>	1514	5	10	His44(π)	v <sub>65</sub>	1509	4	10	His44(π)
22%v(C <sub>2</sub> -N <sub>τ</sub> ) 15%δ(C <sub>2</sub> -H)	v <sub>63</sub>	1518	4	11	His118(τ)	v <sub>67</sub>	1503	4	8	His118(τ)
27%v(C <sub>2</sub> -N <sub>τ</sub> ) 10%δ(C <sub>2</sub> -H)	v <sub>65</sub>	1509	4	9	His46(τ)	v <sub>66</sub>	1506	4	9	His46(τ)
20%δ(C <sub>2</sub> -H) 11%v(C <sub>2</sub> -N <sub>τ</sub> ) 11%v(C <sub>2</sub> -N <sub>π</sub> )	v <sub>66</sub>	1493	-1	6	His61	v <sub>63</sub>	1516	5	10	His61(π)
						v <sub>68</sub>	1491	5	14	His61(π)
17%δ(N <sub>τ</sub> -H)	v <sub>73</sub>	1470	75	19	His78(π)	v <sub>72</sub>	1475	0	20	His78(π)
23%δ(N <sub>τ</sub> -H)	v <sub>75</sub>	1468	77	19	His69(π)	v <sub>77</sub>	1469	-	19	His69(π)
35%δ(N <sub>τ</sub> -H)	v <sub>83</sub>	1459	77	18	His44(π)	v <sub>81</sub>	1463	0	20	His44(π)
33%δ(N <sub>π</sub> -H)	v <sub>86</sub>	1426	63	19	His118(τ)	v <sub>90</sub>	1419	-	18	His118(τ)
17%δ(N <sub>π</sub> -H)	v <sub>87</sub>	1421	64	23	His46(τ)	v <sub>92</sub>	1418	-	20	His46(τ)
32%v(C <sub>2</sub> -N <sub>τ</sub> ) 10%v(C <sub>4</sub> -N <sub>π</sub> ) δ(N <sub>τ</sub> -C <sub>2</sub> -N <sub>π</sub> ) 16%δ(N <sub>π</sub> -C <sub>4</sub> -C <sub>5</sub> )	v <sub>98</sub>	1348	5	14	His118(τ)	v <sub>102</sub>	1348	15	12	His118(τ)
34%v(C <sub>2</sub> -N <sub>τ</sub> ) 12%v(C <sub>4</sub> -N <sub>π</sub> ) 14%δ(N <sub>π</sub> -C <sub>4</sub> -C <sub>5</sub> )	v <sub>99</sub>	1345	8	14	His46(τ)	v <sub>101</sub>	1349	20	12	His46(τ)

11%v(C <sub>2</sub> -N <sub>τ</sub> ) 21%v(C <sub>4</sub> -N <sub>π</sub> ) 25%δ (N <sub>π</sub> -C <sub>4</sub> -C <sub>5</sub> )	V <sub>101</sub>	1325		8	His61	V <sub>105</sub>	1321	4	13	His61 42%v(C <sub>2</sub> -N <sub>π</sub> ) 25%δ (N <sub>π</sub> -C <sub>4</sub> -C <sub>5</sub> )
38%v(C <sub>2</sub> -N <sub>π</sub> ) 14%v(C <sub>4</sub> -N <sub>π</sub> ) 17%δ(N <sub>τ</sub> -C <sub>2</sub> -N <sub>π</sub> ) 20%δ(N <sub>τ</sub> -C <sub>4</sub> -C <sub>5</sub> ) 12%δ(C <sub>5</sub> -N <sub>τ</sub> -C <sub>2</sub> )	V <sub>103</sub>	1323	3	14	His69(π)	V <sub>104</sub>	1329	3	14	His69(π)
40%v(C <sub>2</sub> -N <sub>π</sub> ) 30%v(C <sub>4</sub> -N <sub>π</sub> ) 24%δ (N <sub>τ</sub> -C <sub>2</sub> -N <sub>π</sub> )	V <sub>102</sub>	1325	4	13	His44(π)	V <sub>107</sub>	1317	5	14	His44(π)
40%v(C <sub>2</sub> -N <sub>π</sub> ) 12%v(C <sub>4</sub> -C <sub>5</sub> ) 12%δ (N <sub>τ</sub> -C <sub>2</sub> -N <sub>π</sub> ) 15%δ(N <sub>τ</sub> -C <sub>4</sub> -C <sub>5</sub> )	V <sub>105</sub>	1315	4	14	His78(π)	V <sub>106</sub>	1320	4	14	His78(π)
33%v(C <sub>5</sub> -N <sub>τ</sub> )	V <sub>107</sub>	1276	7	9	His69(π)	V <sub>108</sub>	1282	8	10	His69(π)
31%v(C <sub>4</sub> -N <sub>π</sub> )	V <sub>108</sub>	1273	8	8	His44(π)	V <sub>113</sub>	1267	5	8	His44(π)
26%δ(C <sub>5</sub> -H) 10%v(C <sub>2</sub> -N <sub>π</sub> )(His <sub>118</sub> ) 23% in plane δ(C <sub>5</sub> -H) (His <sub>46</sub> )	V <sub>110</sub>	1266	12	3	His118(τ)	V <sub>114</sub>	1265	7	4	His118(τ)
19%v(C <sub>2</sub> -N <sub>τ</sub> ) 15%v(C <sub>4</sub> -C) 17%δ (C <sub>5</sub> -N <sub>τ</sub> -C <sub>2</sub> ) 24%δ(N <sub>τ</sub> -C <sub>5</sub> -C <sub>4</sub> )	V <sub>111</sub>	1265	7	9	His78(π)	V <sub>111</sub>	1272	8	9	His78(π)

36% in plane $\delta(\text{C}_2\text{-H})$ 38% in plane $\delta(\text{C}_5\text{-H})$ 10% $\nu(\text{C}_2\text{-N}\pi)$	$\nu_{114}$	1243	4	6	His44( $\pi$ )	$\nu_{122}$	1227	-2	11	His44( $\pi$ )
33% in plane $\delta(\text{C}_5\text{-H})$ 32% in plane $\delta(\text{C}_2\text{-H})$	$\nu_{116}$	1238	6	5	His69( $\pi$ )					
12% in plane $\delta(\text{C}_5\text{-H})$ 28% $\delta(\text{HCC}_4)$ 16% $\phi(\text{HCC}_4\text{N}\pi)$	$\nu_{115}$	1240	0	3	His61	$\nu_{116}$	1239	-	7	His61( $\pi$ )
12% in plane $\delta(\text{C}_2\text{-H})$	$\nu_{119}$	1233	0	11	His61					
20% in plane $\delta(\text{C}_5\text{-H})$ 18% in plane $\delta(\text{C}_2\text{-H})$	$\nu_{117}$	1236	5	6	His78( $\pi$ )	$\nu_{118}$	1235	4	5	His78( $\pi$ )
15% in plane $\delta(\text{C}_2\text{-H})$ 13% $\nu(\text{C}_4\text{-N}\pi)$	$\nu_{118}$	1233	0	1	His118( $\tau$ )	$\nu_{117}$	1237	-	12	His118( $\tau$ )

**Table S2a.** Calculated vibrational frequencies and main normal mode description based on PED between 1190 and 945  $\text{cm}^{-1}$  for models **8H** of  $\text{Cu}^{\text{II}}$ ,Zn-SOD calculated within the B3LYP/6-31G(d,p) method (vib num = vibration numbers, main normal mode assignment are based on PED (potential energy distribution) and  $\Delta v$  shifts ( $\Delta v = v$  unlabelled  $-v$  labelled) in the N- $^2\text{H}$  and  $^2\text{H}_2\text{O}$  labelled models and in the  $^{15}\text{N}$  labelled models). Units are  $\text{cm}^{-1}$ .  $\nu(\text{XY})$  is the stretching vibration of the bond between atoms X and Y,  $\delta(\text{XYZ})$  is the bending vibration of the angles between atoms XYZ,  $\tau(\text{XYZW})$  is the torsion vibration,  $\delta(\text{X-H})$  is in-plane vibration of X-H bond.

(a) ( $\pi$ ) or ( $\tau$ ) stand for  $\text{N}\pi$ - or  $\text{N}\tau$ - connexion type of the Histidine with the corresponding metal.

(b) - indicates that it was not possible from the calculations to determine the  $\Delta v$

PED	vib num	$^8\text{H}(\text{ox})$ $\nu \text{ cm}^{-1} * 0.98$	$^2\text{H}$	$^{15}\text{N}$	Involved Histidine Residue <sup>a</sup>	vib num	$^8\text{H}(\text{red})$ $\nu \text{ cm}^{-1} * 0.98$	$^2\text{H}$	$^{15}\text{N}$	PED	Involved Histidine Residue <sup>a</sup>
58% $\nu(\text{C}_2\text{-N}_\pi)$ 12% $\nu(\text{C}_2\text{-N}_\tau)$ 15% $\delta(\text{C}_2\text{H})$	$\nu_{107}$	1296	0	15	His61	$\nu_{123}$	1191		9	32% $\nu(\text{C}_2\text{-N}_\tau)$ 15% $(\delta\text{N}\tau\text{H})$ 20% $(\delta\text{C}_2\text{H})$	His61( $\pi$ )
54% $\nu(\text{C}_2\text{-N}_\tau)$ 30% $(\delta\text{N}\tau\text{H})$	$\nu_{123}$	1165	63	9	His78( $\pi$ )	$\nu_{126}$	1163	62	9	54% $\nu(\text{C}_2\text{-N}_\tau)$ 30% $(\delta\text{N}\tau\text{H})$ 15% $(\delta\text{C}_2\text{H})$	His78( $\pi$ )
55% $\nu(\text{C}_2\text{-N}_\tau)$ 30% $(\delta\text{N}\tau\text{H})$	$\nu_{125}$	1159	59	9	His69( $\pi$ )	$\nu_{127}$	1157	60	9	52% $\nu(\text{C}_2\text{-N}_\tau)$ 29% $(\delta\text{N}\tau\text{H})$ 14% $(\delta\text{C}_2\text{H})$	His69( $\pi$ )
55% $\nu(\text{C}_2\text{-N}_\tau)$ 30% $(\delta\text{N}\tau\text{H})$	$\nu_{126}$	1150	56	9	His44( $\pi$ )	$\nu_{128}$	1153	56	8	53% $\nu(\text{C}_2\text{-N}_\tau)$ 31% $(\delta\text{N}\tau\text{H})$	His44( $\pi$ )
35% $\nu(\text{C}_2\text{-N}_\pi)$ 35% $(\delta\text{N}\pi\text{H})$	$\nu_{127}$	1135	-89	6	His118( $\tau$ )	$\nu_{129}$	1131	- <sup>b</sup>	6	36% $\nu(\text{C}_2\text{-N}_\tau)$ 37% $(\delta\text{N}\tau\text{H})$	His118( $\tau$ )
37% $\nu(\text{C}_2\text{-N}_\pi)$ 35% $(\delta\text{N}\pi\text{H})$	$\nu_{129}$	1130	-90	6	His46( $\tau$ )	$\nu_{131}$	1128	-	6	36% $\nu(\text{C}_2\text{-N}_\tau)$ 37% $(\delta\text{N}\tau\text{H})$	His46( $\tau$ )

29% $v(N_{\tau}-C_5)$ 25% $\delta(C_5-H)$	$v_{130}$	1 125	0	7	His61	$v_{135}$	1094	-11	4	35% $\delta(C_5-C_4-N_{\pi})$ 31% $\delta(C_5-H)$	His61( $\pi$ )
~50% $v(N_{\tau}-C_5)$ ~17% $\delta(C_5-H)$	$v_{131}$	1115	0	8	His118( $\tau$ ) mixed with His46( $\tau$ )	$v_{134}$	1104	0	9	53% $\delta(C_5-C_4-N_{\pi})$ . 11% $\delta(C_5-H)$	His118( $\tau$ )
~50% $v(N_{\tau}-C_5)$ ~17% $\delta(C_5-H)$	$v_{132}$	1109	0	8	His46( $\tau$ ) mixed with His118( $\tau$ )	$v_{132}$	1117	1	9	51% $\delta(C_5-C_4-N_{\pi})$ 13% $\delta(C_5-H)$	His46( $\tau$ )
50% $v(N_{\tau}-C_5)$ 23% $\delta(C_5-H)$	$v_{134}$	1088	-8	5	His44( $\pi$ )	$v_{138}$	1085	-14	5	45% $v(N_{\tau}-C_5)$ 28% $\delta(C_5-H)$	His44( $\pi$ )
50% $v(N_{\tau}-C_5)$ 29% $\delta(C_5-H)$	$v_{135}$	1087	-17	5	His78( $\pi$ )	$v_{136}$	1086	-17	4	49% $v(N_{\tau}-C_5)$ 29% $\delta(C_5-H)$	His78( $\pi$ )
48% $v(N_{\tau}-C_5)$ 30% $\delta(C_5-H)$	$v_{136}$	1087	-15	4	His69( $\pi$ )	$v_{139}$	1084	-	4	46% $v(N_{\tau}-C_5)$ 27% $\delta(C_5-H)$	His69( $\pi$ )
39% $\delta(C_4-N_{\pi}-C_2)$ 18% $v(C_4-C_5)$	$v_{146}$	1037	1	14	His61	$v_{149}$	1014	24	11	27% $\delta(C_4-N_{\pi}-C_2)$ 19% $v(C_4-C_5)$	His61( $\pi$ )
32% $\delta(C_4-N_{\pi}-C_2)$ 22% $v(C_4-C_5)$	$v_{147}$	1021	5	6	His118( $\tau$ )	$v_{148}$	1018	6	6	30% $\delta(C_4-N_{\pi}-C_2)$ 22% $v(C_4-C_5)$	His118( $\tau$ )
31% $\delta(C_4-N_{\pi}-C_2)$ 24% $v(C_4-C_5)$	$v_{148}$	1012	4	6	His46( $\tau$ )	$v_{150}$	1012	4	6	30% $\delta(C_4-N_{\pi}-C_2)$ 23% $v(N_{\tau}-C_2)$ .	His46( $\tau$ )
52% $\delta(N_{\pi}-C_2-N_{\tau})$	$v_{149}$	1009	8	24	His78( $\pi$ )	$v_{151}$	1009	33	22	48% $\delta(N_{\pi}-C_2-N_{\tau})$	His78( $\pi$ )
67% $\delta(N_{\pi}-C_2-N_{\tau})$	$v_{150}$	1008	15	24	His69( $\pi$ )	$v_{152}$	1005	30	23	67% $\delta(N_{\pi}-C_2-N_{\tau})$	His69( $\pi$ )
54% $\delta(N_{\pi}-C_2-N_{\tau})$	$v_{151}$	1003	3	0	His44( $\pi$ )	$v_{154}$	1003	32	10	80% $\delta(N_{\pi}-C_2-N_{\tau})$	His44( $\pi$ )
40% $\delta(N_{\pi}-C_2-N_{\tau})$ 30% $v(C_4-C_5)$	$v_{159}$	968	0	15	His61	$v_{165}$	937	32.8	13	51% $\delta(N_{\pi}-C_2-N_{\tau})$ 25% $v(C_4-N_{\pi})$	His61( $\pi$ )

51% $\delta$ (N $\pi$ -C <sub>2</sub> -N $\tau$ ) 23% $\nu$ (C <sub>4</sub> -N $\pi$ )	V <sub>160</sub>	955	-1	15	His118( $\tau$ )	V <sub>163</sub>	945	-3	17	53% $\delta$ (N $\pi$ -C <sub>2</sub> -N $\tau$ ) 15% $\nu$ (C <sub>4</sub> -N $\pi$ )	His118( $\tau$ )
58% $\delta$ (N $\pi$ -C <sub>2</sub> -N $\tau$ ) 20% $\nu$ (C <sub>4</sub> -N $\pi$ )	V <sub>161</sub>	943	-3	16	His46( $\tau$ )	V <sub>162</sub>	953	-2	16	46% $\delta$ (N $\pi$ -C <sub>2</sub> -N $\tau$ ) 17% $\nu$ (C <sub>4</sub> -N $\pi$ )	His46( $\tau$ )
50% $\delta$ (N $\pi$ -C <sub>2</sub> -N $\tau$ ) 33% $\delta$ (C <sub>5</sub> -N $\tau$ -C <sub>2</sub> ) 12% $\nu$ (C <sub>4</sub> -N $\pi$ )	V <sub>163</sub>	932	13	15	His44( $\pi$ )	V <sub>168</sub>	927	12	16	36% $\delta$ (N $\pi$ -C <sub>2</sub> -N $\tau$ ) 50% $\delta$ (C <sub>5</sub> -N $\tau$ -C <sub>2</sub> ) 12% $\nu$ (C <sub>4</sub> -N $\pi$ )	His44( $\pi$ )
~12% $\delta$ (N $\pi$ -C <sub>2</sub> -N $\tau$ ) ~72% $\delta$ (C <sub>5</sub> -N $\tau$ -C <sub>2</sub> ) ~16% $\delta$ (C <sub>4</sub> -C <sub>5</sub> -N $\tau$ )	V <sub>164</sub>	931	13	16	His69( $\pi$ )	V <sub>166</sub>	932	12	16	$\delta$ (C <sub>5</sub> -N $\tau$ -C <sub>2</sub> )	His69( $\pi$ )
~15% $\delta$ (N $\pi$ -C <sub>2</sub> -N $\tau$ ) ~69% $\delta$ (C <sub>5</sub> -N $\tau$ -C <sub>2</sub> ) ~16% $\delta$ (C <sub>4</sub> -C <sub>5</sub> -N $\tau$ )	V <sub>165</sub>	926	12	15	His78( $\pi$ )	V <sub>167</sub>	928	12	15	$\delta$ (C <sub>5</sub> -N $\tau$ -C <sub>2</sub> )	His78( $\pi$ )

**Table S2b.** Calculated vibrational frequencies and main normal mode description based on PED between 1190 and 945  $\text{cm}^{-1}$  for models **9H** of  $\text{Cu}^{\text{II}}$ ,Zn-SOD calculated within the B3LYP/6-31G(d,p) method (vib num = vibration numbers, main normal mode assignment are based on PED (potential energy distribution) and  $\Delta\nu$  shifts ( $\Delta\nu=\nu$  unlabelled  $-\nu$  labelled) in the  $\text{N-}^2\text{H}$  and  $^2\text{H}_2\text{O}$  labelled models and in the  $^{15}\text{N}$  labelled models). Units are  $\text{cm}^{-1}$ .  $\nu(\text{XY})$  is the stretching vibration of the bond between atoms X and Y,  $\delta(\text{XYZ})$  is the bending vibration of the angles between atoms XYZ,  $\tau(\text{XYZW})$  is the torsion vibration,  $\delta(\text{X-H})$  is in-plane vibration of X-H bond.

(a) ( $\pi$ ) or ( $\tau$ ) stand for  $\text{N}\pi-$  or  $\text{N}\tau-$  connexion type of the Histidine with the corresponding metal.

(b) - indicates that it was not possible from the calculations to determine the  $\Delta\nu$

PED	vib num	<b>9H(ox)</b> $\nu \text{ cm}^{-1} * 0.98$	$^2\text{H}$	$^{15}\text{N}$	Involved Histidine Residue <sup>a</sup>	vib num	<b>9H(red)</b> $\nu \text{ cm}^{-1} * 0.98$	$^2\text{H}$	$^{15}\text{N}$	Involved Histidine Residue <sup>a</sup>
51% $\nu(\text{C}_2\text{-N}_\pi)$ 13% $\nu(\text{C}_2\text{-N}_\tau)$ 16% $\delta(\text{C}_2\text{H})$	$\nu_{106}$	1299	0	14	His61	$\nu_{124}$	1186	- <sup>b</sup>	-	His61( $\pi$ )
54% $\nu(\text{C}_2\text{-N}_\tau)$ 30% $(\delta\text{N}\tau\text{H})$	$\nu_{123}$	1164	62	9	His78( $\pi$ )	$\nu_{125}$	1165	-	8	His78( $\pi$ )
55% $\nu(\text{C}_2\text{-N}_\tau)$ 30% $(\delta\text{N}\tau\text{H})$	$\nu_{125}$	1159	59	9	His69( $\pi$ )	$\nu_{127}$	1158	-	9	His69( $\pi$ )
54% $\nu(\text{C}_2\text{-N}_\tau)$ 30% $(\delta\text{N}\tau\text{H})$	$\nu_{126}$	1154	54	9	His44( $\pi$ )	$\nu_{128}$	1148	-	9	His44( $\pi$ )
30% $\nu(\text{C}_2\text{-N}_\pi)$ 33% $(\delta\text{N}\pi\text{H})$	$\nu_{127}$	1140	-	6	His118( $\tau$ )	$\nu_{131}$	1128	-	6	His118( $\tau$ )
31% $\nu(\text{C}_2\text{-N}_\pi)$ 32% $(\delta\text{N}\pi\text{H})$	$\nu_{128}$	1134	-	6	His46( $\tau$ )	$\nu_{130}$	1130	-	6	His46( $\tau$ )

14% $\nu$ (N $\tau$ -C <sub>5</sub> ) 11% $\delta$ (C <sub>5</sub> -H)	V <sub>130</sub>	1120	0	9	His61 mixed with His <sub>118</sub> and His <sub>46</sub>	V <sub>135</sub>	1094	-11	4	
~50% $\nu$ (N $\tau$ -C <sub>5</sub> ) 17% $\delta$ (C <sub>5</sub> -H)	V <sub>131</sub>	1113	1	9	His <sub>118</sub> ( $\tau$ ) mixed with His <sub>46</sub> ( $\tau$ )	V <sub>134</sub>	1114	0	8	
~50% $\nu$ (N $\tau$ -C <sub>5</sub> ) 17% $\delta$ (C <sub>5</sub> -H)	V <sub>132</sub>	1109	1	9	His <sub>46</sub> ( $\tau$ ) mixed with His <sub>118</sub> ( $\tau$ )	V <sub>132</sub>	1122	0	9	
41% $\nu$ (N $\tau$ -C <sub>5</sub> ) 20% $\delta$ (C <sub>5</sub> -H)	V <sub>137</sub>	1081	-	4	His <sub>44</sub> ( $\pi$ )	V <sub>139</sub>	1078		5	His <sub>44</sub> ( $\pi$ )
49% $\nu$ (N $\tau$ -C <sub>5</sub> ) 29% $\delta$ (C <sub>5</sub> -H)	V <sub>134</sub>	1088	-	5	His <sub>78</sub> ( $\pi$ )	V <sub>137</sub>	1085		5	His <sub>78</sub> ( $\pi$ )
48% $\nu$ (N $\tau$ -C <sub>5</sub> ) 30% $\delta$ (C <sub>5</sub> -H)	V <sub>135</sub>	1087	-	4	His <sub>69</sub> ( $\pi$ )	V <sub>138</sub>	1085		4	His <sub>69</sub> ( $\pi$ )
40% $\delta$ (C <sub>4</sub> -N $\pi$ -C <sub>2</sub> ) 22% $\nu$ (C <sub>4</sub> -C <sub>5</sub> )	V <sub>145</sub>	1036	1	11	His <sub>61</sub>	V <sub>148</sub>	1016	-	10	His <sub>61</sub> ( $\pi$ )
29% $\delta$ (C <sub>4</sub> -N $\pi$ -C <sub>2</sub> ) 23% $\nu$ (C <sub>4</sub> -C <sub>5</sub> )	V <sub>146</sub>	1018	4	5	His <sub>118</sub> ( $\tau$ )	V <sub>147</sub>	1018	9	9	His <sub>118</sub> ( $\tau$ )
28% $\delta$ (C <sub>4</sub> -N $\pi$ -C <sub>2</sub> ) 21% $\nu$ (C <sub>4</sub> -C <sub>5</sub> )	V <sub>147</sub>	1013	5	6	His <sub>46</sub> ( $\tau$ )	V <sub>149</sub>	1016	38	7	His <sub>46</sub> ( $\tau$ )
40% $\delta$ (N $\pi$ -C <sub>2</sub> -N $\tau$ )	V <sub>149</sub>	1008	9	24	His <sub>78</sub> ( $\pi$ )	V <sub>150</sub>	1011	-	0	His <sub>78</sub> ( $\pi$ )
56% $\delta$ (N $\pi$ -C <sub>2</sub> -N $\tau$ )	V <sub>151</sub>	1007	14	9	His <sub>69</sub> ( $\pi$ )	V <sub>153</sub>	1004	12	10	His <sub>69</sub> ( $\pi$ )
70% $\delta$ (N $\pi$ -C <sub>2</sub> -N $\tau$ )	V <sub>150</sub>	1007	10	8	His <sub>44</sub> ( $\pi$ )	V <sub>152</sub>	1006	18	12	His <sub>44</sub> ( $\pi$ )
28% $\delta$ (N $\pi$ -C <sub>2</sub> -N $\tau$ ) 27% $\nu$ (C <sub>4</sub> -C <sub>5</sub> )	V <sub>159</sub>	962	0	13	His <sub>61</sub>	V <sub>165</sub>	936	21	14	His <sub>61</sub> ( $\pi$ )
44% $\delta$ (N $\pi$ -C <sub>2</sub> -N $\tau$ ) 23% $\nu$ (C <sub>4</sub> -N $\pi$ )	V <sub>160</sub>	956	-1	15	His <sub>118</sub> ( $\tau$ )	V <sub>163</sub>	947	-1	17	His <sub>118</sub> ( $\tau$ )



51% $\delta(N\pi-C_2-N\tau)$ 18% $v(C_4-N\pi)$	$V_{161}$	946	-3	16	His46( $\tau$ )	$V_{162}$	956	-2	16	His46( $\tau$ )
50% $\delta(N\pi-C_2-N\tau)$ 33% $\delta(C_5-N_\tau-C_2)$ 12% $v(C_4-N\pi)$	$V_{163}$	931	13	15	His44( $\pi$ )	$V_{168}$	927	12	15	His44( $\pi$ )
$\sim 12\% \delta(N\pi-C_2-N\tau)$ $\sim 72\% \delta(C_5-N_\tau-C_2)$ $\sim 16\% \delta(C_4-C_5-N\tau)$	$V_{164}$	930	13	16	His69( $\pi$ )	$V_{166}$	933	13	16	His69( $\pi$ )
$\sim 15\% \delta(N\pi-C_2-N\tau)$ $\sim 69\% \delta(C_5-N_\tau-C_2)$ $\sim 16\% \delta(C_4-C_5-N\tau)$	$V_{165}$	926	12	15	His78( $\pi$ )	$V_{167}$	929	12	15	His78( $\pi$ )

**Table S3.** C<sub>4</sub>-C<sub>5</sub> (Å) bond length calculated geometrical parameters within the B3LYP/6-31G(d,p) method for models **0H**, **8H**, **9H**, **10H<sup>a</sup>** and **10H<sup>b</sup>** of Cu<sup>II</sup>, Zn-SOD. ( $\pi$ ) or ( $\tau$ ) stand for N $\pi$ - or N $\tau$ - connexion type of the Histidine with the corresponding metal. Scaled calculated  $\nu(\text{C}_4\text{-C}_5)$  frequencies within the B3LYP/6-31G(d,p) method in cm<sup>-1</sup> are calculated for models **0H**, **8H**, **9H**, **10H<sup>a</sup>** and refer to the B3LYP/6-31G(d,p) method.

Involved Histidine residue	d(C <sub>4</sub> -C <sub>5</sub> )					$\nu(\text{C}_4\text{-C}_5)$ cm <sup>-1</sup> *0.98			
	<b>0H</b>	<b>8H(ox)</b>	<b>9H(ox)</b>	<b>10H<sup>a</sup></b>	<b>10H<sup>b</sup></b>	<b>0H</b>	<b>8H(ox)</b>	<b>9H(ox)</b>	<b>10H<sup>a</sup></b>
<b>His<sub>118</sub> (<math>\tau</math>)</b>	1.37188	1.36841	1.36955	1.3695	1.37122	1614	1621	1618	1615
<b>His<sub>46</sub> (<math>\tau</math>)</b>	1.37076	1.37561	1.37561	1.37503	1.37346	1617	1599	1600	1600
<b>His<sub>44</sub> (<math>\pi</math>)</b>	1.37102	1.37101	1.37037	1.3721	1.37339	1596	1597	1598	1595
<b>His<sub>69</sub> (<math>\pi</math>)</b>	1.36887	1.36969	1.36974	1.36946	1.3695	1604	1603	1602	1603
<b>His<sub>78</sub> (<math>\pi</math>)</b>	1.36981	1.37026	1.37020	1.37014	1.37169	1604	1602	1600	1602
<b>His<sub>61</sub></b>	1.37504	1.36962	1.36789	1.36819	1.36984	1572	1587	1591	1588

**Table S4a.** Calculated vibrational frequencies and main normal mode description based on PED between 870 and 560  $\text{cm}^{-1}$  for models **8H** of  $\text{Cu}^{\text{II}}$ , Zn-SOD calculated within the B3LYP/6-31G(d,p) method ( $\nu$  wavenumbers, vibration numbers and main normal mode assignment based on PED (potential energy distribution) and  $\Delta\nu$  shifts in the  $\text{N}^{-2}\text{H}$  and  $^2\text{H}_2\text{O}$  labelled models). PED (in %) refers to the B3LYP/6-31G(d,p) method and model **8H**

(vib num = vibration numbers, main normal mode assignment are based on PED (potential energy distribution) and  $\Delta\nu$  shifts ( $\Delta\nu=\nu$  unlabelled – $\nu$  labelled) in the  $\text{N}^{-2}\text{H}$  and  $^2\text{H}_2\text{O}$  labelled models and in the  $^{15}\text{N}$  labelled models). Units are  $\text{cm}^{-1}$ .  $\nu(\text{XY})$  is the stretching vibration of the bond between atoms X and Y,  $\delta(\text{XYZ})$  is the bending vibration of the angles between atoms XYZ,  $\tau(\text{XYZW})$  is the torsion vibration,  $\delta(\text{X-H})$  and  $\phi(\text{X-H})$  are vibrations of X-H bond.

(a) ( $\pi$ ) or ( $\tau$ ) stand for  $\text{N}\pi$ - or  $\text{N}\tau$ - connexion type of the Histidine with the corresponding metal

(b) - indicates that it was not possible from the calculations to determine the  $\Delta\nu$

		<b>8H(ox)</b>	$^2\text{H}$	$^{15}\text{N}$		<b>8H(red)</b>	$^2\text{H}$	$^{15}\text{N}$			
PED	vib num	$\nu \text{ cm}^{-1}$ *0.98	$\Delta\nu$	$\Delta\nu$	Involved Histidine residue <sup>a</sup>	vib num *0.98	$\Delta\nu$	$\Delta\nu$	PED	Involved Histidine residue <sup>a</sup>	
80% $\phi(\text{C}_5\text{-H})$	$\nu_{168}$	868	-3	1	His44( $\pi$ )	$\nu_{172}$	824	2	0	80% $\phi(\text{C}_2\text{-H})$	His44( $\pi$ )
85% $\phi(\text{C}_2\text{-H})$ 14% $\phi(\text{C}_5\text{-H})$	$\nu_{170}$	833	0	1	His46( $\tau$ )	$\nu_{173}$	820	1	0	85% $\phi(\text{C}_5\text{-H})$	His46( $\tau$ )
31% $\phi(\text{C}_2\text{-H})$ 21% $\phi(\text{C}_5\text{-H})$	$\nu_{171}$	814	0	0	His61	$\nu_{175}$	809	-8	1	43% $\phi(\text{C}_2\text{-H})$ +25% $\phi(\text{N}\tau\text{-H})$ 20% $\phi(\text{C}_2\text{-H})$	His61( $\pi$ ) + $\epsilon$ His69( $\pi$ )
40% $\phi(\text{C}_2\text{-H})$	$\nu_{173}$	807	0	1	His69( $\pi$ )	$\nu_{174}$	815	4	1	58% $\phi(\text{C}_2\text{-H})$ 16% $\phi(\text{C}_2\text{-H})$	His69( $\pi$ ) + $\epsilon$ His61
50% $\phi(\text{C}_2\text{-H})$ 31% $\phi(\text{C}_5\text{-H})$	$\nu_{174}$	803	0	1	His118( $\tau$ )	$\nu_{176}$	807	1	1	48% $\phi(\text{C}_2\text{-H})$ +37% $\phi(\text{C}_5\text{-H})$	His118( $\tau$ )
62% $\phi(\text{C}_2\text{-H})$ 27% $\phi(\text{C}_5\text{-H})$	$\nu_{175}$	801	0	1	His78( $\pi$ )	$\nu_{177}$	802	0	1	64% $\phi(\text{C}_2\text{-H})$ +24% $\phi(\text{C}_5\text{-H})$	His78( $\pi$ )
25% $\phi(\text{C}_2\text{-H})$	$\nu_{171}$	814	- <sup>b</sup>	0	His46( $\tau$ )	$\nu_{180}$	768	0	1	91% $\phi(\text{C}_2\text{-H})$	His46( $\tau$ )

50% $\phi$ (C <sub>5</sub> -H) 34% $\phi$ (C <sub>2</sub> -H)	V <sub>177</sub>					V <sub>179</sub>						
		784	1	0	His118( $\tau$ )		792	15	0	38% $\phi$ (C <sub>5</sub> -H)+44% $\phi$ (C <sub>2</sub> -H)	His118( $\tau$ )	
58% $\phi$ (C <sub>5</sub> -H) 32% $\phi$ (C <sub>2</sub> -H)	V <sub>178</sub>					V <sub>184</sub>						
		774	0	0	His61		747	0	0	81% $\phi$ (C <sub>5</sub> -H)	His61	
94% $\phi$ (C <sub>2</sub> -H)	V <sub>179</sub>	763	1	1	His44( $\pi$ )	V <sub>182</sub>	757	0	0	74% $\phi$ (C <sub>5</sub> -H)+14% $\phi$ (C <sub>2</sub> -H)	His44( $\pi$ )	
43% $\phi$ (C <sub>5</sub> -H) 25% $\phi$ (C <sub>2</sub> -H)	V <sub>181</sub>					V <sub>183</sub>						
		755	0	0	His78( $\pi$ )		756	0	0	60% $\phi$ (C <sub>5</sub> -H)+30% $\phi$ (C <sub>2</sub> -H)	His78( $\pi$ )	
51% $\phi$ (C <sub>5</sub> -H) 16% $\phi$ (C <sub>2</sub> -H)	V <sub>182</sub>					V <sub>181</sub>						
		755		0	His69( $\pi$ )		760	0	0	71% $\phi$ (C <sub>5</sub> -H)+19% $\phi$ (C <sub>2</sub> -H)	His69( $\pi$ )	
$\tau$ (C <sub>4</sub> -N $\pi$ -C <sub>2</sub> -N $\tau$ )	V <sub>184</sub>	683	3	9	His44( $\pi$ )	V <sub>188</sub>	676	14	9		His44( $\pi$ )	
$\tau$ (C <sub>4</sub> -N $\pi$ -C <sub>2</sub> -N $\tau$ )	V <sub>186</sub>	676	9	8	His46( $\tau$ )	V <sub>190</sub>	668	5	9	66% $\tau$ (C <sub>4</sub> -N $\pi$ -C <sub>2</sub> -N $\tau$ )	His46( $\tau$ )	
$\tau$ (C <sub>5</sub> -N $\tau$ -C <sub>2</sub> -N $\pi$ )	V <sub>187</sub>	675	7	8	His78( $\pi$ )	V <sub>187</sub>	677	9	7	40% $\tau$ (C <sub>5</sub> -N $\tau$ -C <sub>2</sub> -N $\pi$ )	His78( $\pi$ )	
$\tau$ (C <sub>5</sub> -N $\tau$ -C <sub>2</sub> -N $\pi$ )	V <sub>189</sub>	674	5	8	His69( $\pi$ )	V <sub>186</sub>	678	8	9	50% $\tau$ (C <sub>5</sub> -N $\tau$ -C <sub>2</sub> -N $\pi$ )	His69( $\pi$ )	
$\tau$ (C <sub>4</sub> -N $\pi$ -C <sub>2</sub> -N $\tau$ )	V <sub>190</sub>	672	1	10	His61	V <sub>191</sub>	666	-3	8	22% $\tau$ (C <sub>4</sub> -N $\pi$ -C <sub>2</sub> -N $\tau$ )	His61( $\pi$ )	
$\tau$ (C <sub>4</sub> -N $\pi$ -C <sub>2</sub> -N $\tau$ )	V <sub>191</sub>	665	0	5	His118( $\tau$ )	V <sub>189</sub> V <sub>188</sub>	673 676	8 14	8 9	59% $\tau$ (C <sub>4</sub> -N $\pi$ -C <sub>2</sub> -N $\tau$ ) 16% $\tau$ (C <sub>4</sub> -N $\pi$ -C <sub>2</sub> -N $\tau$ )	His118( $\tau$ )	
$\tau$ (C <sub>5</sub> -C <sub>4</sub> -N $\pi$ -C <sub>2</sub> )	V <sub>195</sub>	656	0	4	His61							
$\tau$ (C <sub>5</sub> -C <sub>4</sub> -N $\pi$ -C <sub>2</sub> )	V <sub>196</sub>	654	28	3	His46( $\tau$ )	V <sub>197</sub>	654		14	42% $\tau$ (C <sub>5</sub> -C <sub>4</sub> -N $\pi$ -C <sub>2</sub> )	His46( $\tau$ )	
$\tau$ (C <sub>5</sub> -N $\tau$ -C <sub>2</sub> -N $\pi$ )	V <sub>197</sub>	654	31	2	His44( $\pi$ )	V <sub>204</sub>	640		2	70% $\tau$ (C <sub>5</sub> -N $\tau$ -C <sub>2</sub> -N $\pi$ )	His44( $\pi$ )	
$\tau$ (C <sub>4</sub> -C <sub>5</sub> -N $\tau$ -C <sub>2</sub> )	V <sub>200</sub>	649	32	1	His78( $\pi$ )	V <sub>199</sub>	649		2	68% $\tau$ (C <sub>4</sub> -N $\pi$ -C <sub>2</sub> -N $\tau$ )	His78( $\pi$ )	
$\tau$ (C <sub>5</sub> -C <sub>4</sub> -N $\pi$ -C <sub>2</sub> )	V <sub>201</sub>	648	27	4	His118( $\tau$ )	V <sub>198</sub>	649		2	74% $\tau$ (C <sub>5</sub> -C <sub>4</sub> -N $\pi$ -C <sub>2</sub> )	His118( $\tau$ )	
$\tau$ (C <sub>4</sub> -C <sub>5</sub> -N $\tau$ -C <sub>2</sub> )	V <sub>203</sub>	641	24	2	His69( $\pi$ )	V <sub>202</sub>	644		2	76% $\tau$ (C <sub>4</sub> -C <sub>5</sub> -N $\tau$ -C <sub>2</sub> )	His69( $\pi$ )	
						V <sub>206</sub>			-	77% $\tau$ (C <sub>4</sub> -N $\pi$ -C <sub>2</sub> -N $\tau$ ) +23% $\tau$ (C <sub>5</sub> -C <sub>4</sub> -N $\pi$ -C <sub>2</sub> )	His61( $\pi$ )	

36% $\phi(N_{\pi}\text{-H}) + \tau$ cycle	$v_{205}$	605	135	8	His46( $\tau$ )	$v_{209}$ $v_{210}$	594 589	133	5 6	15% $\phi(N_{\pi}\text{-H}) + \tau$ cycle + $\tau$ HOCuN 36% $\phi(N_{\pi}\text{-H}) + \tau$ cycle + $\tau$ HOCuN	His46( $\tau$ )
32% $\phi(N_{\pi}\text{-H}) + \tau$ cycle	$v_{206}$	599	131	8	His118( $\tau$ )	$v_{208}$ $v_{209}$	604 594	138	3 5	36% $\phi(N_{\pi}\text{-H}) + \tau$ cycle + $\tau$ HOCuN $\tau$ cycle + $\tau$ HOCuN	His118( $\tau$ )
45% $\phi(N_{\tau}\text{-H}) + \tau$ cycle	$v_{208}$	587	129	8	His44( $\pi$ )	$v_{214}$	546	124	6	67% $\phi(N_{\pi}\text{-H}) + \tau$ cycle	His44( $\pi$ )
42% $\phi(N_{\tau}\text{-H}) + \tau$ cycle	$v_{209}$	584	125	8	His78( $\pi$ )	$v_{211}$	585	125	8	38% $\phi(N_{\tau}\text{-H}) + \tau$ cycle	His78( $\pi$ )
57% $\phi(N_{\tau}\text{-H}) + \tau$ cycle	$v_{210}$	568	126	7	His69( $\pi$ )	$v_{212}$ $v_{213}$	584 580	125	2 6	22% $\phi(N_{\tau}\text{-H}) + \tau$ cycle 23% $\phi(N_{\tau}\text{-H}) + \tau$ cycle	His69( $\pi$ )

**Table S4b.** Calculated vibrational frequencies and main normal mode description based on PED between 870 and 560  $\text{cm}^{-1}$  for models **9H** of  $\text{Cu}^{\text{III}}/\text{Zn-SOD}$  calculated within the B3LYP/6-31G(d,p) method (vib num = vibration numbers, main normal mode assignment are based on PED (potential energy distribution) and  $\Delta\nu$  shifts ( $\Delta\nu=\nu$  unlabelled  $-\nu$  labelled) in the  $\text{N-}^2\text{H}$  and  $^2\text{H}_2\text{O}$  labelled models and in the  $^{15}\text{N}$  labelled models). Units are  $\text{cm}^{-1}$ .  $\nu(\text{XY})$  is the stretching vibration of the bond between atoms X and Y,  $\delta(\text{XYZ})$  is the bending vibration of the angles between atoms XYZ,  $\tau(\text{XYZW})$  is the torsion vibration,  $\delta(\text{X-H})$  and  $\phi(\text{X-H})$  are vibrations of X-H bond.

(a) ( $\pi$ ) or ( $\tau$ ) stand for  $\text{N}\pi-$  or  $\text{N}\tau-$  connexion type of the Histidine with the corresponding metal

(b) - indicates that it was not possible from the calculations to determine the  $\Delta\nu$

		<b>9H(ox)</b>	$^2\text{H}$	$^{15}\text{N}$			<b>9H(red)</b>	$^2\text{H}$	$^{15}\text{N}$	
PED	vib num	$\nu \text{ cm}^{-1}$ *0.98	$\Delta\nu$	$\Delta\nu$	Involved Histidine residue <sup>a</sup>	vib num	$\nu \text{ cm}^{-1}$ *0.98	$\Delta\nu$	$\Delta\nu$	Involved Histidine residue <sup>a</sup>
80% $\phi(\text{C}_5\text{-H})$	$\nu_{168}$	864	-4	1	His44( $\pi$ )	$\nu_{180}$	784	0	0	His44( $\pi$ )
97% $\phi(\text{C}_2\text{-H})$	$\nu_{169}$	855	3	1	His46( $\tau$ )	$\nu_{173}$	822	0	0	His46( $\tau$ )
67% $\phi(\text{C}_2\text{-H})$ +30% $\phi(\text{C}_5\text{-H})$	$\nu_{172}$	820	0	1	His61	$\nu_{175}$	809	-5	1	His61( $\pi$ )
53% $\phi(\text{C}_2\text{-H})$	$\nu_{175}$	802	1	1	His69( $\pi$ )	$\nu_{174}$	817	1	1	His69( $\pi$ )
10% $\phi(\text{C}_2\text{-H})$ +80% $\phi(\text{C}_5\text{-H})$	$\nu_{171}$	829	1	0	His118( $\tau$ )	$\nu_{172}$	829	1	0	His118( $\tau$ )
59% $\phi(\text{C}_2\text{-H})$ +26% $\phi(\text{C}_5\text{-H})$	$\nu_{176}$	802	0	1	His78( $\pi$ )	$\nu_{177}$	804	1	1	His78( $\pi$ )
72% $\phi(\text{C}_2\text{-H})$	$\nu_{173}$		1	0	His46( $\tau$ )	$\nu_{176}$	809	1	0	His46( $\tau$ )
83% $\phi(\text{C}_2\text{-H})$	$\nu_{177}$	797	1	1	His118( $\tau$ )	$\nu_{179}$	789	0	1	His118( $\tau$ )

64% $\phi$ (C <sub>5</sub> -H) +26% $\phi$ (C <sub>2</sub> -H)	V <sub>178</sub>	778	0	0	His61	V <sub>183</sub>	755	0	0	His61( $\pi$ )
92% $\phi$ (C <sub>2</sub> -H)	V <sub>179</sub>	767	0	1	His44( $\pi$ )	V <sub>184</sub>	739	0	1	His44( $\pi$ )
56% $\phi$ (C <sub>5</sub> -H) +34% $\phi$ (C <sub>2</sub> -H)	V <sub>181</sub>	756	0	0	His78( $\pi$ )	V <sub>182</sub>	758	0	0	His78( $\pi$ )
60% $\phi$ (C <sub>5</sub> -H) +28% $\phi$ (C <sub>2</sub> -H)	V <sub>182</sub>	754	0	0	His69( $\pi$ )	V <sub>181</sub>	760	0	0	His69( $\pi$ )
49% $\tau$ (C <sub>4</sub> -N $\pi$ -C <sub>2</sub> -N $\tau$ )	V <sub>184</sub>	681	13	9	His44( $\pi$ )	V <sub>189</sub>	676	8	9	His44( $\pi$ )
56% $\tau$ (C <sub>4</sub> -N $\pi$ -C <sub>2</sub> -N $\tau$ )	V <sub>186</sub>	676	9	9	His46( $\tau$ )	V <sub>190</sub>	668	6	8	His46( $\tau$ )
58% $\tau$ (C <sub>5</sub> -N $\tau$ -C <sub>2</sub> -N $\pi$ )	V <sub>187</sub>	676	9	8	His78( $\pi$ )	V <sub>186</sub>	680	8	8	His78( $\pi$ )
21% $\tau$ (C <sub>4</sub> -N $\pi$ -C <sub>2</sub> -N $\tau$ )	V <sub>188</sub>	673	<sup>b</sup>	-	His61					
58% $\tau$ (C <sub>4</sub> -N $\tau$ -C <sub>2</sub> -N $\pi$ )	V <sub>189</sub>	672	6	8	His69( $\pi$ )	V <sub>187</sub>	679	7	8	His69( $\pi$ )
63% $\tau$ (C <sub>4</sub> -N $\pi$ -C <sub>2</sub> -N $\tau$ )	V <sub>190</sub>	670	2	10	His61					
63% $\tau$ (C <sub>4</sub> -N $\pi$ -C <sub>2</sub> -N $\tau$ )	V <sub>191</sub>	668	7	6	His118( $\tau$ )	V <sub>188</sub>	676	6	8	His118( $\tau$ )
51% $\tau$ (C <sub>5</sub> -C <sub>4</sub> -N $\pi$ -C <sub>2</sub> )	V <sub>196</sub>				His61	V <sub>191</sub>	665	3	8	His61( $\pi$ )
						V <sub>206</sub>	625	0		75% $\tau$ (C <sub>4</sub> -N $\pi$ -C <sub>2</sub> -N $\tau$ ) 22% $\tau$ (C <sub>5</sub> -C <sub>4</sub> -N $\pi$ -C <sub>2</sub> ) His61( $\pi$ )
46% $\tau$ (C <sub>5</sub> -C <sub>4</sub> -N $\pi$ -C <sub>2</sub> )	V <sub>198</sub>	654	-	3	His46( $\tau$ )	V <sub>197</sub>	654	7	4	His46( $\tau$ )
14% $\tau$ (C <sub>5</sub> -N $\tau$ -C <sub>2</sub> -N $\pi$ )	V <sub>197</sub>	655	-	6	His44( $\pi$ )	V <sub>204</sub>	640	21	2	His44( $\pi$ ) mixed with His61 and His46

55% $\tau$ (C <sub>4</sub> -C <sub>5</sub> -N <sub><math>\tau</math></sub> -C <sub>2</sub> )	V <sub>200</sub>	649	32	1	His78( $\pi$ )	V <sub>199</sub>	651	33	2	His78( $\pi$ )
37% $\tau$ (C <sub>5</sub> -C <sub>4</sub> -N <sub><math>\pi</math></sub> -C <sub>2</sub> )	V <sub>201</sub>	648	-	3	His118( $\tau$ )	V <sub>200</sub>	649	33	2	His118( $\tau$ )
59% $\tau$ (C <sub>4</sub> -C <sub>5</sub> -N <sub><math>\tau</math></sub> -C <sub>2</sub> )	V <sub>203</sub>	641	25	2	His69( $\pi$ )	V <sub>203</sub>	645	26	2	His69( $\pi$ )
$\tau$ (C <sub>5</sub> -C <sub>4</sub> -N <sub><math>\pi</math></sub> -C <sub>2</sub> )	V <sub>198</sub>	654	-	3	His46( $\tau$ )	V <sub>197</sub>	654	7	4	His46( $\tau$ )
$\tau$ (C <sub>5</sub> -N <sub><math>\tau</math></sub> -C <sub>2</sub> -N <sub><math>\pi</math></sub> )	V <sub>197</sub>	655	-	6	His44( $\pi$ )	V <sub>204</sub>	640	21	2	His44( $\pi$ )
$\tau$ (C <sub>5</sub> -C <sub>4</sub> -N <sub><math>\tau</math></sub> -C <sub>2</sub> )	V <sub>200</sub>	649	32	1	His78( $\pi$ )	V <sub>199</sub>	651	33	2	His78( $\pi$ )
37% $\tau$ (C <sub>5</sub> -C <sub>4</sub> -N <sub><math>\pi</math></sub> -C <sub>2</sub> )	V <sub>201</sub>	648	-	3	His118( $\tau$ )	V <sub>200</sub>	649	33	2	His118( $\tau$ )
59% $\tau$ (C <sub>5</sub> -C <sub>4</sub> -N <sub><math>\tau</math></sub> -C <sub>2</sub> )	V <sub>203</sub>	641	25	2	His69( $\pi$ )	V <sub>203</sub>	645	26	2	His69( $\pi$ )
37% $\phi$ (N <sub><math>\pi</math></sub> -H) + $\tau$ cycle	V <sub>205</sub>	607	136	8	His46( $\tau$ )	V <sub>209</sub>	594	139	6	His46( $\tau$ )
36% $\phi$ (N <sub><math>\pi</math></sub> -H) + $\tau$ cycle	V <sub>206</sub>	600	132	8	His118( $\tau$ )	V <sub>208</sub>	598	130	7	His118( $\tau$ )
38% $\phi$ (N <sub><math>\tau</math></sub> -H) + $\tau$ cycle	V <sub>207</sub>	591	126	8	His44( $\pi$ )	V <sub>214</sub>	540	121	6	His44( $\pi$ )
42% $\phi$ (N <sub><math>\tau</math></sub> -H) + $\tau$ cycle	V <sub>208</sub>	585	125	8	His78( $\pi$ )	V <sub>210</sub>	589	125	8	His78( $\pi$ )
55% $\phi$ (N <sub><math>\tau</math></sub> -H) + $\tau$ cycle	V <sub>210</sub>	568	125	7	His69( $\pi$ )	V <sub>213</sub> V <sub>211</sub>	582 588	126 131	6 2	His69( $\pi$ )



**Table S5.** Comparison of experimental data for Cu<sup>II</sup>,Zn-SOD and theoretical predictions<sup>a</sup> for Cu<sup>II</sup>,Zn-SOD and proposed assignment (see also

**Figure S3)**

Exp freq (cm <sup>-1</sup> ) Cu <sup>II</sup> $\Delta\nu^2\text{H}_2\text{O}$ $\Delta\nu^{15}\text{N}$	Exp freq (cm <sup>-1</sup> ) Cu <sup>I</sup> $\Delta\nu^2\text{H}_2\text{O}$ $\Delta\nu^{15}\text{N}$	Vib. Nb. <b>9H</b>	Calculated freq $\Delta\nu^2\text{H}_2\text{O}$ $\Delta\nu^{15}\text{N}$	Main calculated PED contributions	Proposed assignment
1618		$\nu_{52}$	1618, <b>-16</b> , -5	$\nu(\text{C}_4\text{-C}_5)$ His <sub>118</sub> (N $\tau$ )	$\nu(\text{C}_4\text{-C}_5)$ His <sub>118</sub> (N $\tau$ )
		$\nu_{55-57}$	1602-1598, <b>-6</b> , -3 to -6	$\nu(\text{C}_4\text{-C}_5)$ His <sub>69</sub> (N $\pi$ ), His <sub>78</sub> (N $\pi$ ), His <sub>44</sub> (N $\pi$ )	
1602, <b>-10</b> , -3		$\nu_{58}$	1598, <b>-18</b> , -9	$\nu(\text{C}_4\text{-C}_5)$ His <sub>46</sub> (N $\tau$ )	$\nu(\text{C}_4\text{-C}_5)$ His <sub>46</sub> (N $\tau$ ) Cu <sup>II</sup>
	1590, <b>-10</b> to <b>-16</b> , -3		1604, <b>-17</b> , -5	$\nu(\text{C}_4\text{-C}_5)$ His <sub>46</sub> (N $\tau$ )	$\nu(\text{C}_4\text{-C}_5)$ His <sub>46</sub> (N $\tau$ ) Cu <sup>I</sup>
1581, -3		$\nu_{59}$	1591, <b>1</b> , -3	$\nu(\text{C}_4\text{-C}_5)$ His <sub>61</sub>	$\nu(\text{C}_4\text{-C}_5)$ His <sub>61</sub> or $\nu(\text{C}_4\text{-C}_5)$ His(N $\pi$ )
	1233, -5	$\nu_{116-118}$ , $\nu_{122}$	1239-1227, <b>2 to -4</b> , -5 to -12	in plane $\delta(\text{C}_2\text{-H}) + \delta(\text{C}_5\text{-H})$	$\delta(\text{CH}) + \nu(\text{C}_2\text{-N}\pi)$ His (N $\pi$ ) and His (N $\tau$ )
1223-1225, -5		$\nu_{114}-\nu_{118}$	1243-1232, <b>0 to -4</b> , -1 to -6	in plane $\delta(\text{C}_2\text{-H}) + \delta(\text{C}_5\text{-H}) + \nu(\text{C}_2\text{-N}\pi)$	$\delta(\text{CH}) + \nu(\text{C}_2\text{-N}\pi)$ His (N $\pi$ ) and His (N $\tau$ )
	1112- 1111, <b>0</b> , -9	$\nu_{132}$ , $\nu_{134}$	1122-1114, <b>0</b> , -8 and -9	$\nu(\text{N}\tau\text{-C}_5) + \delta(\text{C}_5\text{-H})$ His <sub>118</sub> , His <sub>46</sub> (N $\tau$ )	$\nu(\text{C}_5\text{-N}\tau)$ His <sub>118</sub> (N $\tau$ ) and His <sub>46</sub> (N $\tau$ )
1097-1095, <b>0</b> , -9		$\nu_{13}$ , $\nu_{132}$	1113-1109, <b>-1</b> , -9	$\nu(\text{N}\tau\text{-C}_5) + \delta(\text{C}_5\text{-H})$ His <sub>118</sub> , His <sub>46</sub> (N $\tau$ )	$\nu(\text{C}_5\text{-N}\tau)$ His <sub>118</sub> (N $\tau$ ) and His <sub>46</sub> (N $\tau$ )
	844	$\nu_{172}-\nu_{180}$	829-784, <b>0 to -1</b>	$\phi(\text{C}_2\text{-H}) + \phi(\text{C}_5\text{-H})$	wagging mode of His side chains
826		$\nu_{168}-\nu_{176}$	864-802, <b>0 to -1</b>	$\phi(\text{C}_2\text{-H}) + \phi(\text{C}_5\text{-H})$	wagging mode of His side chains

	812	$\nu_{172}-\nu_{18}$	829-784, <i>0 to -1</i>	$\phi(\text{C}_2\text{-H}) + \phi(\text{C}_5\text{-H})$	wagging mode of His side chains
669-668, -7, -8		$\nu_{184}, \nu_{186}$ <i><math>-\nu_{191}</math></i>	681-668, <b>-2 to -13</b> , <i>-6 to -10</i>	$\tau(\text{C}_4\text{-N}\pi\text{-C}_2\text{-N}\tau) / \tau(\text{C}_5\text{-N}\tau\text{-C}_2\text{-N}\pi)$	His ( $\text{N}\pi$ ) and His ( $\text{N}\tau$ ) ring $\tau$
661, -11,		$\nu_{184}, \nu_{186}$ <i><math>-\nu_{191}</math></i>	681-668, <b>-2 to -13</b> , <i>-6 to -10</i>	$\tau(\text{C}_4\text{-N}\pi\text{-C}_2\text{-N}\tau) / \tau(\text{C}_5\text{-N}\tau\text{-C}_2\text{-N}\pi)$	His ( $\text{N}\pi$ ) and His ( $\text{N}\tau$ ) ring $\tau$
638-636, <b>0</b> , -6		$\nu_{197}-\nu_{204}$	640-651, <b>-7 to -33</b> , <i>-2 to -4</i>	$\tau(\text{C}_5\text{-C}_4\text{-N}\pi\text{-C}_2) / \tau(\text{C}_4\text{-C}_5\text{-N}\tau\text{-C}_2)$	His ( $\text{N}\pi$ ) and His ( $\text{N}\tau$ ) ring $\tau$
629-628, <b>0</b> , -5		$\nu_{196}-\nu_{203}$	656-641, <b>0 or -25 to -32 for Zn N<math>\pi</math> ligands</b> , <i>-1 to -6</i>	$\tau(\text{C}_5\text{-C}_4\text{-N}\pi\text{-C}_2) / \tau(\text{C}_5\text{-C}_4\text{-N}\tau\text{-C}_2)$	His ( $\text{N}\pi$ ) and His ( $\text{N}\tau$ ) ring $\tau$ modes of Cu ligands
622-620, <b>0</b> , -5		$\nu_{197}-\nu_{204}$	640-651, <b>-7 to -33</b> , <i>-2 to -4</i>	$\tau(\text{C}_5\text{-C}_4\text{-N}\pi\text{-C}_2) / \tau(\text{C}_4\text{-C}_5\text{-N}\tau\text{-C}_2)$	His ( $\text{N}\pi$ ) and His ( $\text{N}\tau$ ) ring $\tau$
338-335 <sup>1</sup> , <b>-2</b> , -4		$\nu_{226}, \nu_{227}$	344-347	$\delta(\text{C-C}_4\text{-N}\pi\text{His}_{118}) + \delta(\text{C-C}_4\text{-N}\pi\text{His}_{46})$	$\tau$ of His( $\text{Cu}^{\text{II}}$ ) and $\tau$ at $\text{Cu}^{\text{II}}$
323, <b>-2</b> , -4		$\nu_{226}, \nu_{228}$	310, <b>-6</b> , -3, 299, <b>-3</b> , -3	$\tau(\text{C-C}_4\text{-N}\pi\text{-C}_2\text{His}_{46}) / \delta(\text{N}\tau\text{His}_{46}\text{-Cu-N}\tau\text{His}_{118}) / \delta(\text{N}\pi\text{His}_{44}\text{-Cu-N}\tau\text{His}_{118})$	$\tau$ of His( $\text{Cu}^{\text{II}}$ ) and $\tau$ at $\text{Cu}^{\text{II}}$ $\text{Cu}^{\text{II}}$ -NHis61-Zn motif:
314-308, <b>0</b> , -2		$\nu_{227}$	301, -,	$\nu(\text{Cu-N}\tau\text{His}_{61}) + \nu(\text{Zn-N}\pi\text{His}_{61})$ .	$\nu(\text{Cu-NHis}_{61}) + \nu(\text{Zn-NHis}_{61})$

(a) model **9H(ox)** and **9H(red)** calculated within the B3LYP/6-31G(d,p) method. Values larger than  $600 \text{ cm}^{-1}$  are scaled by 0.98

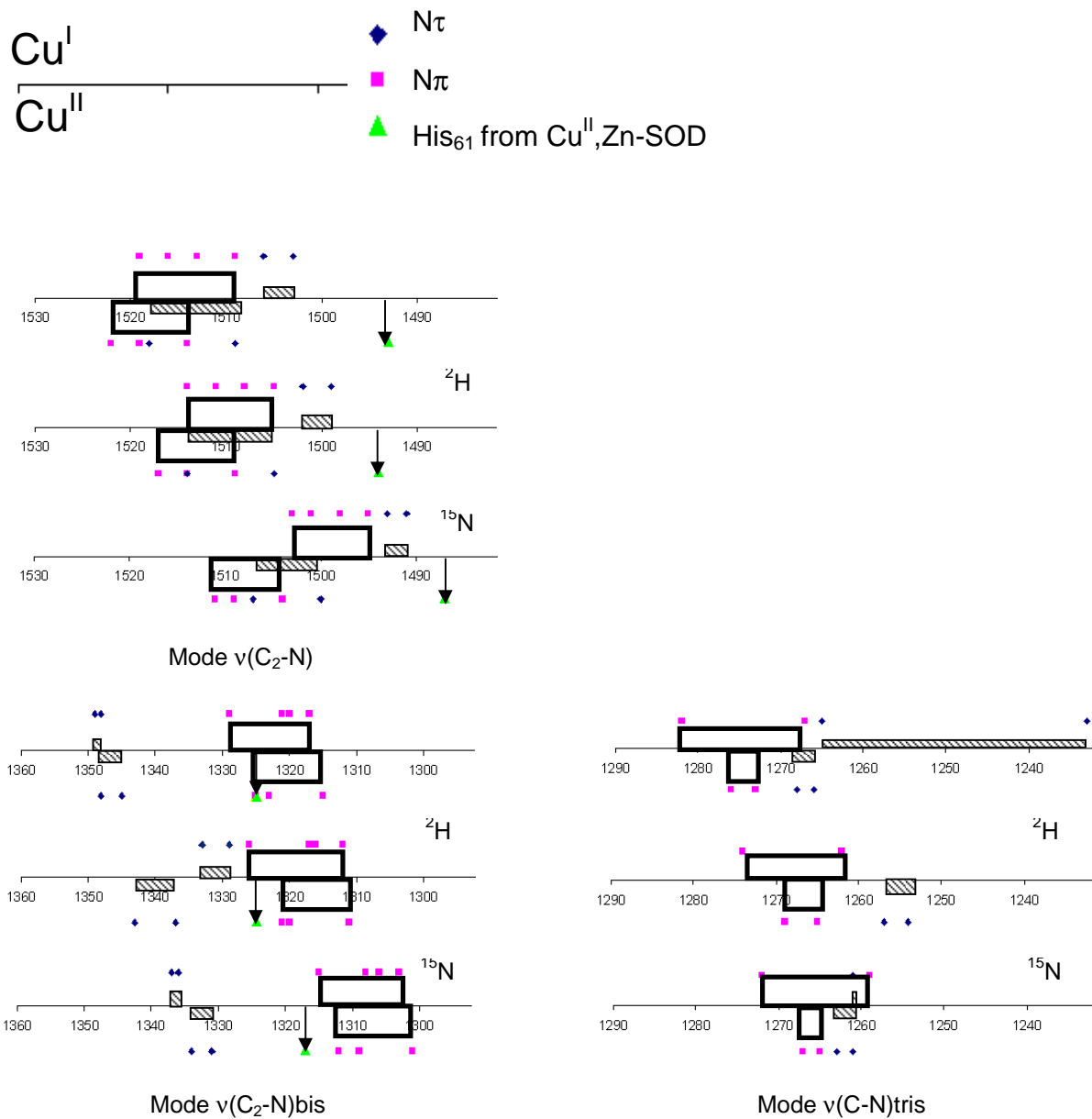
Vib.Nb are vibration number.  $\nu(\text{XY})$  is the stretching vibration of the bond between atoms X and Y,  $\delta(\text{XYZ})$  is the bending vibration of the angles between atoms XYZ,  $\tau(\text{XYZW})$  is the torsion vibration,  $\delta(\text{X-H})$  and  $\phi(\text{X-H})$  are vibrations of X-H bond  
Calculated frequency shift (frequency difference between labelled and unlabelled) upon  $\text{H}^2/\text{H}$  exchange of exchangeable protons are in bold, and upon  $^{14}\text{N}/^{15}\text{N}$  exchanged N are in italic.

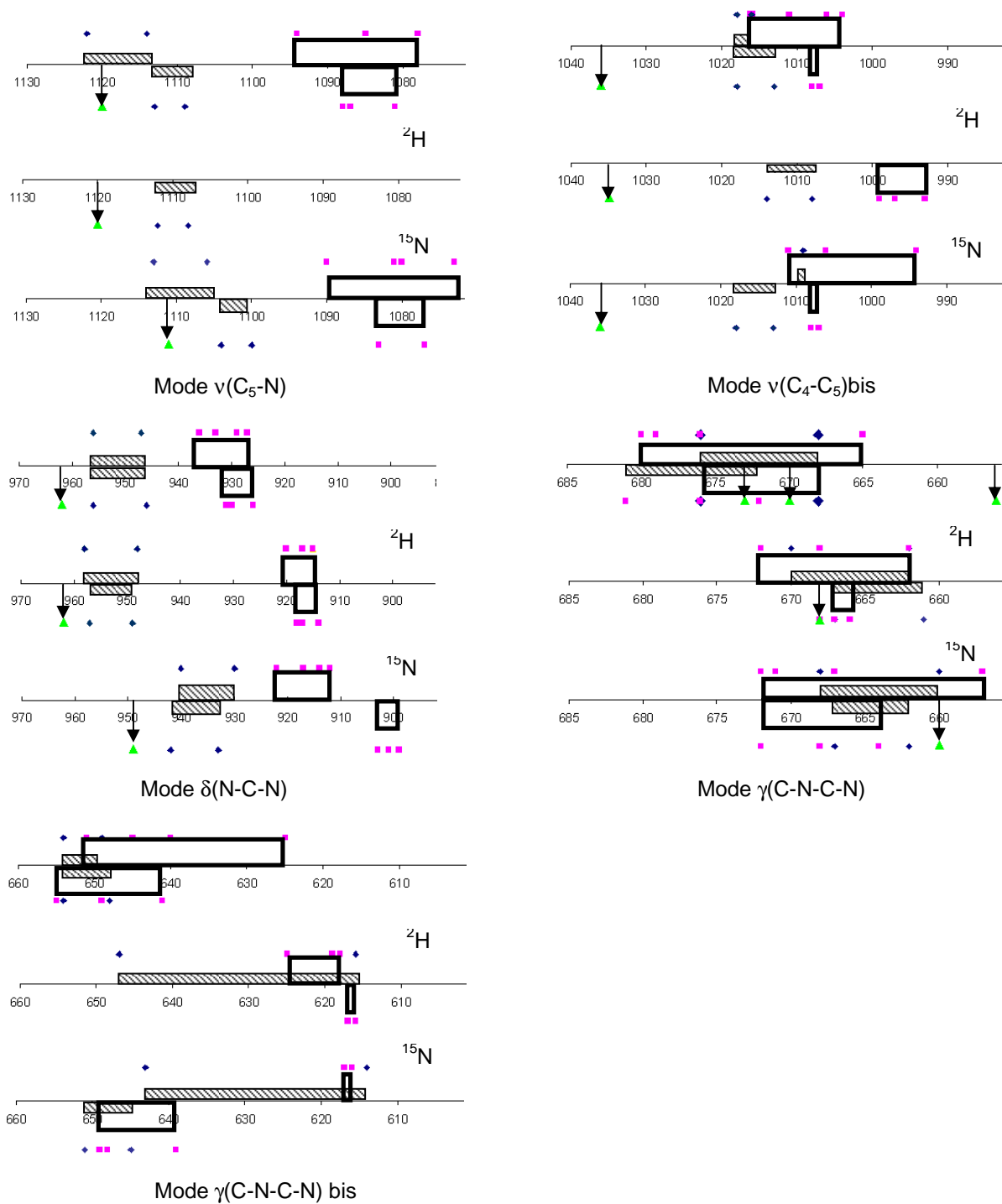
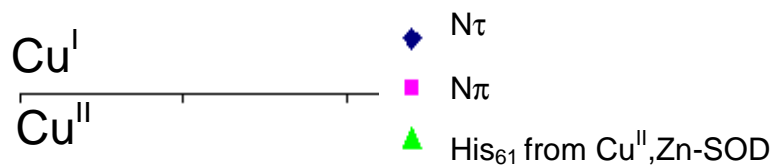
<sup>1</sup>See also Marboutin, L, Petitjean, H, Xerri, B, Vita, N, Dupeyrat, F, Flament, J-P, Berthomieu, D, Berthomieu C (2011)

Profiling the Active Site of a Copper Enzyme through Its Far-Infrared, *Angew. Chem. Int. Ed.* 50, 8062–8066



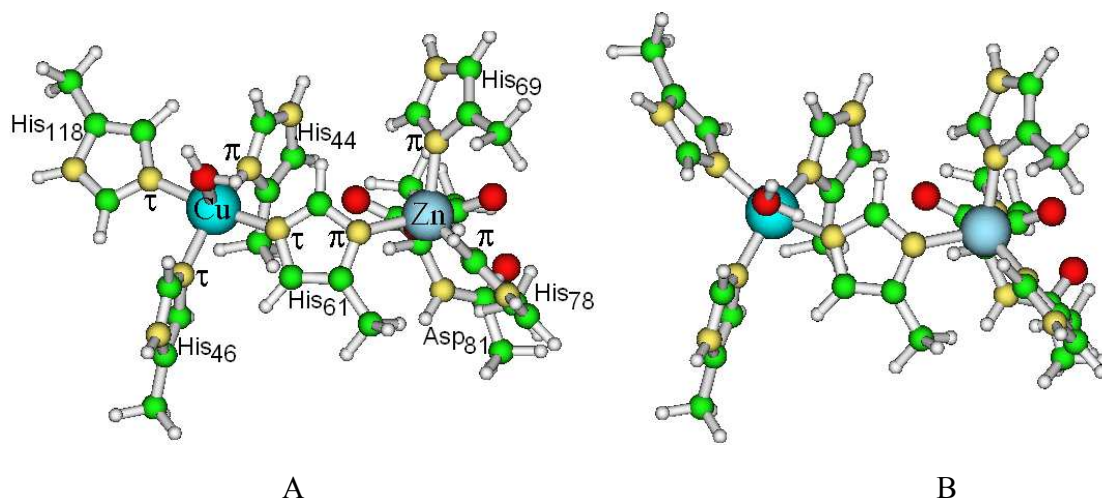
**Scheme S1.** Schematic presentation of calculated modes in  $\text{Cu}^{\text{II}}$ ,Zn-SOD **9H** for unlabelled and  $^2\text{H}$  and  $^{15}\text{N}$  labelled. The arrows are  $\text{His}_{61}$ , (and a green triangle for the imidazolate  $\text{His}_{61}$ ) the empty rectangles are  $\text{N}\pi$ -His (and small pink rectangles) and dashed rectangles are  $\text{N}\tau$ -His (and small blue diamonds). Numbers are wavenumbers in  $\text{cm}^{-1}$



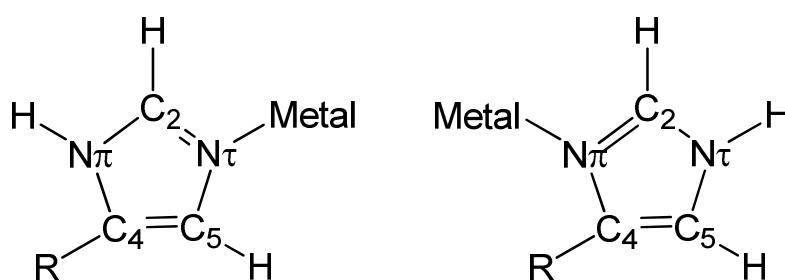


**Figure S1:** geometries of **8H** (A) and **9H** (B) models of Cu<sup>II</sup>,Zn-SOD active site. Grey balls are H atoms, green balls are C atoms, blue balls are N atoms, red balls are O atoms and sky blue balls are Cu and Zn atoms.

The histidines His<sub>46</sub> and His<sub>118</sub> are N $\tau$  ligands to copper and His<sub>44</sub> is N $\pi$  ligated to copper. The imidazole rings of His<sub>69</sub> and His<sub>78</sub> are N $\pi$  ligated to Zn site. The His<sub>61</sub> is N $\tau$  ligated to Cu and N $\pi$  ligated to Zn.

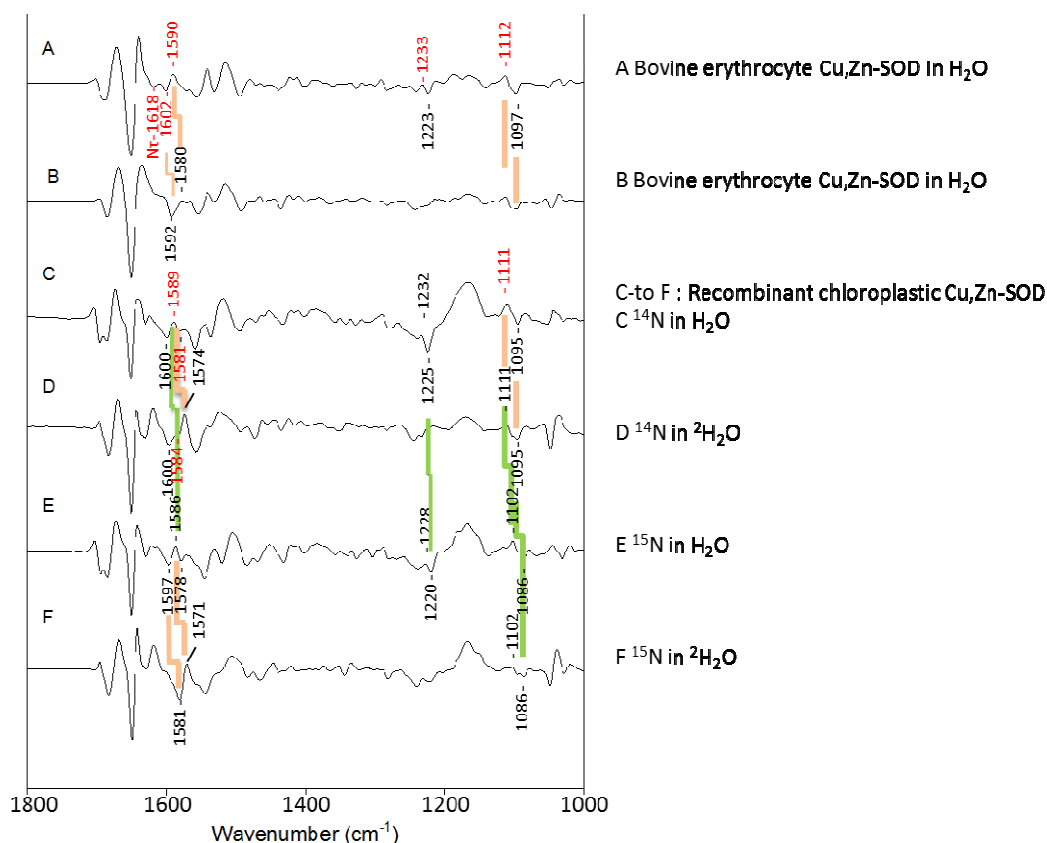


**Figure S2.** Histidine imidazole ring carbon atom labelings and nitrogen atom labelings. Metals can be either N $\tau$ - or N $\pi$ - connected to the histidine amino acid.

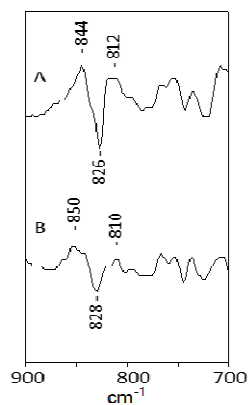


**Figure S3a** : Illustration of band assignments and shifts proposed in Table S5. The Cu(I)-minus-Cu(II) FTIR difference spectra are reported from F. Dupeyrat et al. (F. Dupeyrat, C. Vidaud, A. Lorphelin, C. Berthomieu, *J. Biol. Chem.* **2004**, 279, 48091). In the first spectrum (recorded with bovine erythrocyte Cu,Zn-SOD in H<sub>2</sub>O) the frequencies given in red are frequencies that correspond within a few cm<sup>-1</sup> with predicted IR modes of histidine side chains.

The lines in green correspond to shifts predicted for <sup>15</sup>N-labeling and the lines in red correspond to shifts predicted for samples in <sup>2</sup>H<sub>2</sub>O as compared to samples in H<sub>2</sub>O. The lines are broader when the correspondences between experimental and predicted shifts are close to each other.



**Figure S3b.** 900-700  $\text{cm}^{-1}$  zone with spectra recorded in  $\text{H}_2\text{O}$  and in  $^2\text{H}_2\text{O}$  with bovine erythrocyte Cu,Zn-SOD.



**Figure S3c.** 700-50  $\text{cm}^{-1}$  region using the same code as above. A bovine erythrocyte Cu,Zn-SOD in  $\text{H}_2\text{O}$ , C: chloroplastic  $^{14}\text{N}$ -labeled Cu,Zn-SOD in  $\text{H}_2\text{O}$ ; E: chloroplastic  $^{15}\text{N}$ -labeled Cu,Zn-SOD in  $\text{H}_2\text{O}$ .

