

Complexes of nitric oxide with water and imidazole

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Abstract NO–Imi–H₂O complexes can be used as models to investigate the interactions of histidine with nitric oxide and water in biological systems like myoglobin. We discuss here the water–imidazole, water–nitric oxide dimers and the trimolecular complexes of nitric oxide with water and imidazole from the donor–acceptor point of view using the natural bond orbitals and localized molecular orbital energy decomposition analysis schemes. The comparison between trimolecular and bimolecular complexes shows that in general, the stabilization energies are more sensitive to changes in the interactions of imidazole with water than to changes in the interactions with nitric oxide. The effect of imidazole ring protonation on the geometry and stabilization of the complexes is also investigated. We found that cooperative effects are more relevant in charged complexes and planar structures than in neutral species and

nonplanar complexes. The driving forces governing the interactions between open and closed shell systems are also discussed with special emphasis on the role of lone pairs and unpaired electrons.

Keywords Nitric oxide · Imidazole · Water · NBO · DFT · Energy decomposition analysis · Heterodimers · Trimers

1 Introduction

Non-covalent interactions play an important role in many physical and chemical processes [1]. However, complexes between open and closed shell molecules are less understood than those only involving closed shell molecules. It is reported that the stabilization of hydrogen bonded systems is related to $n \rightarrow \sigma^*$ interactions between donor and acceptor [2]. Hernandez-Soto et al. investigated HO₂⋯X complexes (X = H₂O, H₂O₂, HONO, HONO₂, CH₃OH, HCOOH, CH₃COOH and H₂SO₄) [3] and found that the unpaired electron does not play an important role on their stabilization. To understand the competition between lone pairs and unpaired electrons, the complexes of ·CH₃, ·NH₂, ·OH and ·F radicals and water were previously investigated by us [4]. These radicals have an increasing number of lone pairs (from none to 3), and in most cases, the lone pairs showed the largest donor capacity compared to the unpaired electrons [4]. Most recently, the role of unpaired electrons in the interactions of aromatic radicals with water was also investigated by us, evidencing the effect of the aromatic ring in complex formation in radicals [5–7].

Nitric oxide (NO) is a radical involved in many biological processes [8–11]. Despite its small size, the theoretical study of NO associations with other molecules remains a

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challenge due to the radical character of the NO monomer and the van der Waals (vdW) character of its complexes. Thus, weak complexes between NO and inert gases are frequently used as models to understand vdW interactions [12–16]. The dimerization of NO [17] as well as its interactions with small molecules like N₂, CO, H₂O, CH₄, C₂H₆, C₆H₆, imidazole and amino acids was also investigated [18–27]. Weinhold et al. [2] examined the complexes between NO and HF using the donor–acceptor scheme based on the natural bond orbitals (NBO). In an unrestricted treatment, NBO of the NO molecule can be considered as hybrids of different spin [2]. Therefore, the unpaired electron can play an important role in the formation of complexes.

Histidine (His) is a polar amino acid that can be found in the active site of proteins like myoglobins. Histidine plays a major role in the regulation of nitric oxide diffusion near the myoglobin active site [28]. The polar molecule imidazole (Imi) forms the lateral chain of His, and it is therefore responsible for most of the properties of this amino acid. Since histidine is a suitable candidate for water substitution in proton transfer membranes, Imi–Imi proton transfer processes have been investigated [29, 30]. In deoxymyoglobin, the last coordinated position around Fe^{II} is occupied by a water molecule [28]. Thus, the NO–Imi–H₂O interactions could be important to regulate the access of nitric oxide to the active site of the enzyme.

Imi–H₂O complexes have been studied using both experimental and theoretical methods. Choi et al. [31] combined infrared laser spectroscopy and theoretical calculations and found two minima in which water could act either as proton acceptor or as donor. Similar complexes are reported by Carles et al. [32] using electron transfer spectroscopic techniques and semiempirical calculations. The interaction of protonated imidazole with water was theoretically investigated [33] as well as the interactions of imidazole dimers with water [34]. In both cases, it was found that water preferably interacts with the nitrogen atoms of the imidazole ring.

Cybulski et al. [35] studied the H₂O⋯NO interactions using RCCSD(T) calculations and symmetry-adapted perturbation theory. They found four complexes of which the most stable were those with water interacting with the nitrogen atom of nitric oxide. Similar results were reported by Myszkiewicz et al. [27] using UMP2, UMP4 y UCCSD(T) methods. Dozova et al. [36] studied the complexes between water and nitric oxide using matrix isolation and infrared spectroscopy techniques and identified experimentally one 1:1 NO⋯H₂O, three 1:1 NO⋯HDO and two 1:1 NO⋯D₂O complexes. In the same study, five NO⋯H₂O minima were calculated at the UB3LYP/6-311 ++G(2d,2p) and CCSD/6-311 ++G(2d,2p) levels of theory.

NO–Imi–H₂O complexes provide a simple model to investigate the interactions of histidine with nitric oxide

and water in myoglobin. Thus, we extend previous work on NO–Imi dimers [37] to assess the effect of one water molecule in nitric oxide–imidazole interactions. We discuss the water–imidazole, water–nitric oxide dimers and the trimolecular complexes of nitric oxide with water and imidazole from the donor–acceptor point of view using the NBO and the localized molecular orbital energy decomposition analysis (LMOEDA) scheme. The effect of the protonation of the imidazole ring on the geometry and stabilization of the complexes is also investigated. The driving forces governing the interactions between open and closed shell systems are discussed with special emphasis in the role of lone pairs and unpaired electrons.

2 Computational details

Intermolecular complexes of nitric oxide, imidazole and water were calculated at the UM05-2X/6-31 ++G(d,p) level of theory using Gaussian03 [38–40]. The reliability of the level of theory was tested comparing with calculations at higher levels of theory (Supporting Information). To analyze the effect of the protonation state of Imi, the molecule was considered in its neutral and protonated (ImiH⁺) forms. NO can act as Lewis acid and base. Both types of complexes were considered and analyzed in the broad context of interactions between radicals and closed shell molecules. Two interaction patterns are discussed here: (a) hydrogen bond interactions ((N–O)_{NO}⋯H–Z) and (b) interactions with NO acting as acid ((N–O)_{NO}⋯Z (Z represents non-hydrogen atoms)). Frequency calculations were performed to verify that the structures were minima on the potential energy surface.

Stabilization energies (ΔE) were calculated by subtracting the sum of the energies of the isolated monomers to the energy of the complex and using the LMOEDA method [41]. The basis set superposition error (BSSE) was estimated using the counterpoise method of Boys and Bernardi [42]. The electronic density analysis of all complexes was performed by means of the natural bonding orbital (NBO) scheme [2]. In this framework, the second-order perturbation theory allows to estimate the energy contribution associated with the departure from the Lewis structure as:

$$\Delta E_{a \rightarrow b}^{(2)} = -p \frac{\langle a | \hat{F} | b^* \rangle^2}{\varepsilon_{b^*} - \varepsilon_a} \quad (1)$$

$\langle a | \hat{F} | b^* \rangle$ are the matrix elements of the Fock operator between the full a and the empty b^* NBOs and p is the occupation of the NBOs (2 for restricted and 1 for unrestricted calculations). Then, the larger contributions are obtained for transitions with larger $\langle a | \hat{F} | b^* \rangle$ values and smaller energy gaps ($\Delta E_{a \rightarrow b} = \varepsilon_{b^*} - \varepsilon_a$) between a and b^* NBOs.

To analyze the effect of an unpaired orbital, the corresponding interactions were deleted and the interaction energies recomputed. The interactions with values of $\Delta E_{a \rightarrow b}^{(2)}$ larger than 0.4 kJ/mol (a and b^* localized in different molecules) were considered as relevant. The NBO charges and spin densities are reported in the Supporting Information.

To analyze the nature of these interactions, the stabilization energies were decomposed into electrostatic, polarization, exchange, repulsion and dispersion components based on the LMOEDA method as implemented in the GAMESS code [43]. Single-point calculations were performed at the UM05-2X/6-31 ++G(d,p) level of theory, and the energy was decomposed using two and three molecular units to estimate the cooperative effects. The geometries, stabilization energies, NBO and LMOEDA analyses of all intermolecular complexes can be found in the Supporting Information.

3 Results and discussion

As mentioned above, we selected NO and H₂O as the open and closed shell molecules in this study because of their biological relevance, similar size and the fact that both feature oxygen as heteroatom. In addition, NO–Imi–H₂O complexes can be used as a model to investigate the interactions of histidine with nitric oxide and water in biological systems like myoglobin.

This way, we aim to answer the following questions:

- Is the unpaired orbital of NO involved in complex formation?

- In tri-molecular complexes of imidazole with an open shell molecule (NO) and a closed shell molecule (H₂O), which plays the most important role? How does the protonation of the Imi ring affect complexation?
- Why are these complexes stable?
- How important are cooperative effects in a open shell–water–closed shell system?

3.1 Is the unpaired orbital of NO involved in complex formation?

To investigate the role of the unpaired electron on the stabilization of the complexes, we considered the dimers of nitric oxide with water and imidazole. The geometries of these complexes have been previously reported [35–37, 44]. For consistency, we re-optimized the structures at the UM05-2X/6-31 ++G(d,p) level of theory. To discuss our main findings, we chose a set of representative complexes (Fig. 1). These complexes feature the most frequent interactions found in the trimers: ON_{NO}···O–Z (**A1**, **B1**), ON_{NO}···H–Z (**A2**, **B5**, **HB1**), NO_{NO}···H–Z (**A4**, **B7**, **HB2**). More information about other complexes can be found in the Supporting Information.

The donor–acceptor interactions with major energy contributions indicate how the complexes differ from the corresponding Lewis structures (Fig. 1; Table 1). To further verify the role of the interactions predicted as most important by the $\Delta E^{(2)}$ values, we removed these donor–acceptor interactions and performed new NBO calculations (Table 1). The resulting stabilization energies are reported as $\Delta E^{(a)}$.

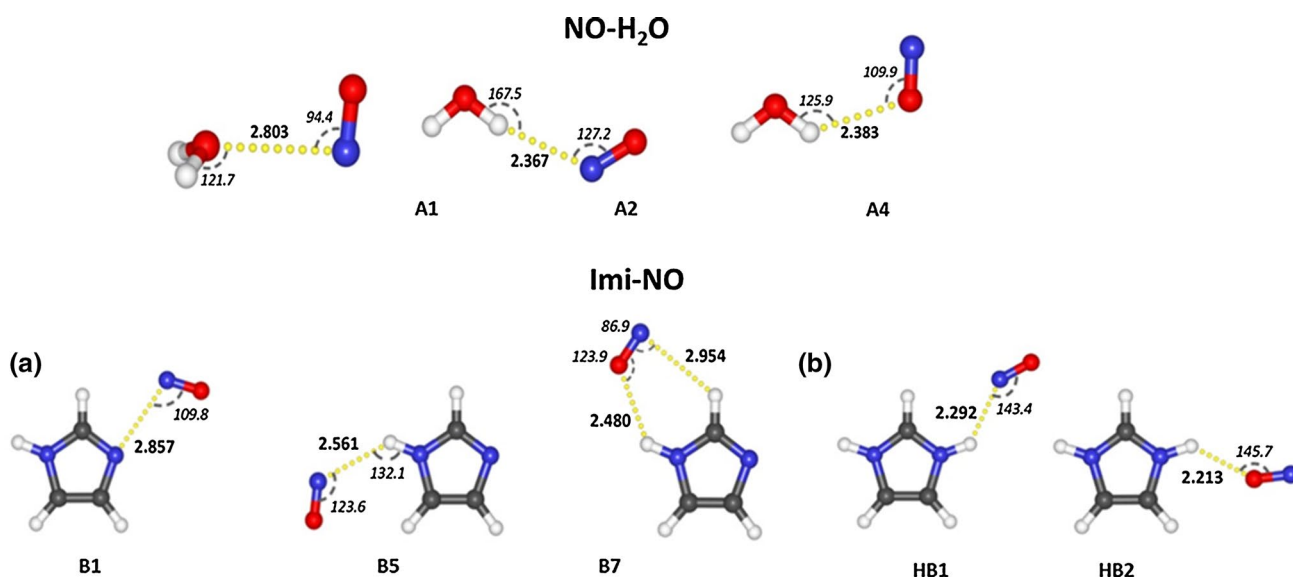


Fig. 1 Geometries of selected dimers (distances in Å and angles in degrees). Imidazole–NO dimers: **a** neutral planar, **b** protonated planar

Table 1 Major orbital interactions at the UM05-2X/6-31 ++G(d,p) level of theory for selected NO–H₂O, Imi–H₂O and ImiH⁺–H₂O complexes

	Interaction	Donor–acceptor <i>a</i> → <i>b</i>	$\Delta E_{a \rightarrow b}^{(2)}$		ΔE	$\Delta E^{(a)}$
			α Orbitals	β Orbitals		
Planar complexes						
A1	ON _{NO} ⋯OH _{water}	$n_{(O)} \rightarrow \pi_{(NO)}^*$	2.6	2.6	−7.9	0.6
A2	ON _{NO} ⋯HO _{water}	$n_{(N)} \rightarrow \sigma_{(OH)}^*$	7.5	–	−5.5	10.5
A4	NO _{NO} ⋯HO _{water}	$n_{(O)} \rightarrow \sigma_{(OH)}^*$	2.6	–	−5.0	1.2
B1	ON _{NO} ⋯N _{Imi}	$n_{(N)} \rightarrow \pi_{(NO)}^*$	1.8	2.3	−9.8	−0.9
B5	ON _{NO} ⋯HN _{Imi}	$n_{(N)} \rightarrow \sigma_{(NH)}^*$	4.5	–	−6.3	3.2
B7	NO _{NO} ⋯HN _{Imi}	$n_{(O)} \rightarrow \sigma_{(NH)}^*$	2.6	–	−5.7	0.3
HB1	ON _{NO} ⋯HN _{Imi}	$n_{(N)} \rightarrow \sigma_{(NH)}^*$	12.2	–	−15.4	8.0
HB2	NO _{NO} ⋯HN _{Imi}	$n_{(O)} \rightarrow \sigma_{(NH)}^*$	7.7	–	−13.5	2.8

Second-order energy contributions according the NBO donor–acceptor scheme ($\Delta E_{a \rightarrow b}^{(2)}$) and stabilization energies ($\Delta E^{(a)}$) computed removing the corresponding donor–acceptor interactions. All energies are reported in kJ/mol

For the **A1** dimer, the most relevant interaction is $n_{(O)} \rightarrow \pi_{(NO)}^*$, with the $n_{(O)}$ orbital located at the O atom of water as donor. For the **A2** and **A4** complexes, the main interactions involve in both cases density transfer from the NO molecule to water. Thus, in **A2** the $n_{(N)} \rightarrow \sigma_{(OH)}^*$ interaction contributes mostly to the stabilization energy. In **A4**, the main interaction is $n_{(O)} \rightarrow \sigma_{(OH)}^*$ involving the unpaired orbital $n_{(O)}$ centered in the O atom of NO; the larger $\Delta E^{(2)}$ corresponds to the interaction involving the orbital located in the N atom. The stabilization of **A2** and **A4** is related to donor–acceptor interactions between the unpaired electron of NO and the antibonding natural orbitals of the water molecule. The slightly higher stability of **A2** with respect to **A4** can be explained by the donor–acceptor model (Eq. 1) considering that the $n_{(N)}$ orbital is higher in energy than $n_{(O)}$. In addition, when optimizing anionic complexes analogous to **A2** and **A4**, the hydrogen bond structure becomes unstable and geometries similar to **A1** are obtained.

For NO–Imi, we found that the stabilization of the (N–O)_{NO}⋯H–N_{Imi} complexes is due to donor–acceptor $n_{(X)} \rightarrow \sigma_{(NH)}^*$ interactions with an important role of the unpaired electron (Table 1). These interactions are stronger for the protonated complexes (**HB1** and **HB2**), which have larger stabilization energies. In the planar complexes, $n_{(X)} \rightarrow \pi_{(NO)}^*$ donor–acceptor interactions are relevant showing little effect of the unpaired electron in the stabilization (like in **A1**).

An important factor in the stabilization of an interaction is the charge transfer between the molecules forming the complex. We found that, with the exception of **A1** and **B1**, complex formation is accompanied by a small spin density transfer from nitric oxide to water (about 0.01 e). This transfer, albeit small, is more effective in complexes in which NO is interacting via its nitrogen atom. The values of charge transfer and spin density transfer between the monomers do not change significantly when different schemes

to obtain the charges were considered. For the Imi–NO dimers, the charges of Imi and NO molecules within the complexes are similar to those obtained with atoms in molecules partition scheme at UMP2/6-311 ++G(2d,2p) level of theory [37].

In (N–O)_{NO}⋯H–Z complexes, the values of the charge and spin transfer are very similar, indicating that the *unpaired orbital plays a major role* in the stabilization. This is in contrast to the complexes stabilized by (N–O)_{NO}⋯Z interactions where NO acts as acid accepting electron density on their $\pi_{(NO)}^*$ orbitals. Consequently, the role of the unpaired electron in the stabilization of the dimers depends on the nature of the interaction. If NO acts as a Lewis base ((N–O)_{NO}⋯H–Z interactions), the unpaired electron is involved in the interaction. Conversely, when NO acts as Lewis acid, the unpaired electron does not have a significant impact on the stabilization of the complexes.

(N–O)_{NO}⋯H–Z dimers behave differently to open shell molecule–water complexes previously studied by us [4, 5], where the unpaired electron did not affect significantly the stabilization of the complexes. The distinct behavior of NO can be explained by the energy ordering of the NO orbitals. For instance, the energy gap between the unpaired orbital and the next occupied orbital with opposite spin is larger in NO (0.14 au) than in the hydroxyl radical (0.03 au). Consequently, the unpaired electron in nitric oxide is available to independently interact with closed shell molecules.

3.2 In tri-molecular complexes of imidazole with an open shell molecule and a closed shell molecule, which plays the most important role? How does the protonation of the Imi ring affect complexation?

Although the bimolecular complexes of nitric oxide with water [36] and imidazole [37] have been previously investigated, much less information is available concerning the

Fig. 2 Imidazole–water complexes (distances in Å and angles in degrees)

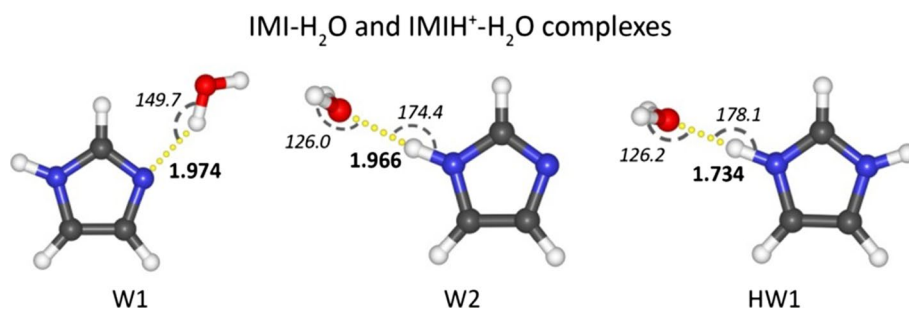
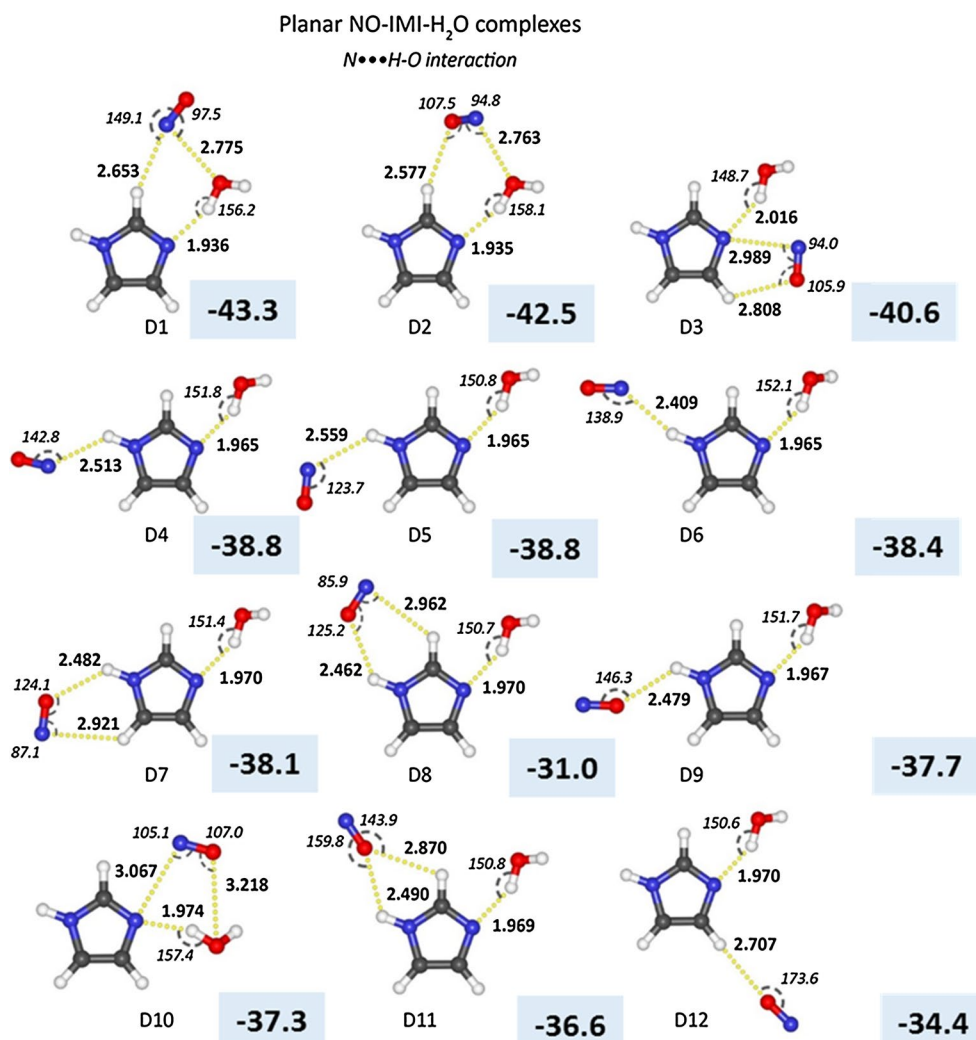


Fig. 3 Planar trimolecular complexes of imidazole with water and nitric oxide stabilized by the N⋯H–O interaction. Relevant intermolecular distances and angles are highlighted (distances are given in Å and angles in degrees). Stabilization energies BSSE corrected are provided in the blue boxes (in kJ/mol)

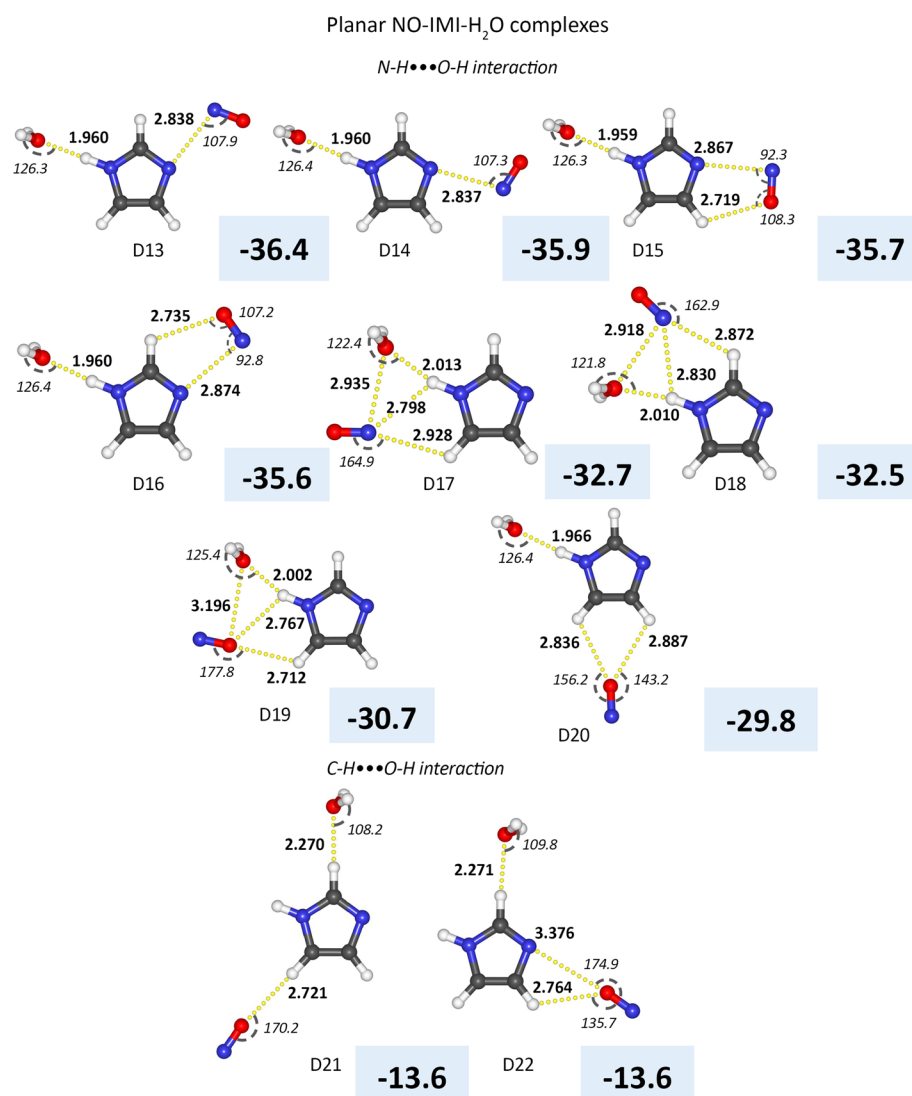


nitric oxide–imidazole–water trimers. Here we propose several geometrical arrangements for the NO–Imi–H₂O complexes (Figs. 3, 4, 5) which provide an excellent case study for the investigation of the competition between diverse interaction motifs, the role of specific molecular interactions, ring protonation and cooperative effects. For the NO–Imi–H₂O system, two main types of structures were considered: planar, in which the NO and H₂O molecules are in (or nearly in) the plane defined by the

imidazole ring, and nonplanar, in which the H₂O and/or the NO molecules lie over or under the imidazole ring plane.

To better understand the role of the dimer subunits in the stabilization of the trimers, the Imi–H₂O complexes were computed at the same level of theory. Two Imi–H₂O dimers have been previously reported [31, 45]: **W1** and **W2**, which are stabilized by the N_{Imi}⋯H–O_{water} and N–H_{Imi}⋯O–H_{water} interactions, respectively. In **W1**, the water molecule acts as Lewis acid and in **W2** as base, with

Fig. 4 Planar trimolecular complexes of imidazole with water and nitric oxide stabilized by the N–H...O–H and C–H...O–H interactions. Relevant intermolecular distances and angles are highlighted (distances are given in Å and angles in degrees). Stabilization energies BSSE corrected are provided in the blue boxes (in kJ/mol)



$N_{\text{Imi}}\cdots H-O_{\text{water}}$ and $N-H_{\text{Imi}}\cdots O-H_{\text{water}}$ distances of 1.97 Å in both cases (Fig. 2). The very similar interaction energies of both complexes (−33.3 kJ/mol for **W1** and −27.4 kJ/mol for **W2**) are in agreement with the results obtained by Choi et al. [31] that suggest the presence of an equimolar mixture of two complexes. For the protonated imidazole (ImiH⁺), only the $N-H_{\text{Imi}}\cdots O-H_{\text{water}}$ interaction (complex **HW1**) was found. The interaction energy (−69.3 kJ/mol) of **HW1** is almost two times larger than in **W2**. Thus, the protonation of the second nitrogen atom increases the hydrogen donor capacity of the amine group of imidazole. The Imi–H₂O dimers are significantly more stable than the NO–H₂O and Imi–NO dimers (Table 1 and Supplementary Information). Consequently, the planar trimers are classified according to the interaction between Imi and H₂O (Figs. 3, 4).

Three types of interactions are possible between the neutral ring of imidazole and water: $N_{\text{Imi}}\cdots H-O_{\text{water}}$,

$N-H_{\text{Imi}}\cdots O-H_{\text{water}}$ and $C-H_{\text{Imi}}\cdots O-H_{\text{water}}$. In addition, the $C-H_{\text{Imi}}\cdots O-H_{\text{water}}$ interaction which is not present in the bimolecular complexes is identified in the trimer. Planar structures featuring the $N_{\text{Imi}}\cdots H-O_{\text{water}}$ interaction (**D1** to **D12**, Fig. 3) are the most stable of all NO–Imi–H₂O complexes (−43.3 to −34.4 kJ/mol). The NBO analysis shows that the main source of stabilization in these complexes is the individual contribution of Imi–H₂O donor–acceptor interactions. Similar tendencies are found (in terms of the effect of the unpaired electron) in the complexes stabilized by $(N-O)_{\text{NO}}\cdots H-Z$ or $(N-O)_{\text{NO}}\cdots Z$ interactions. The donor–acceptor interactions from the NO–H₂O and Imi–NO subunits of the trimers play a minor role in comparison with the $n_{(\text{N})} \rightarrow \sigma_{(\text{OH})}^*$ interactions in the $N\cdots O-H_{\text{water}}$ complexes or the $n_{(\text{O})} \rightarrow \sigma_{(\text{NH})}^*$ interactions in the $N-H_{\text{Imi}}\cdots O-H_{\text{water}}$ complexes.

Only the two less stable planar NO–Imi–H₂O complexes (**D21** and **D22**) feature the $C-H_{\text{Imi}}\cdots O-H_{\text{water}}$

Fig. 5 Planar trimolecular complexes of protonated imidazole with water and nitric oxide. Important intermolecular distances and angles are highlighted (distances are given in Å and angles in degrees). Stabilization energies (BSSE corrected) are provided in the blue boxes

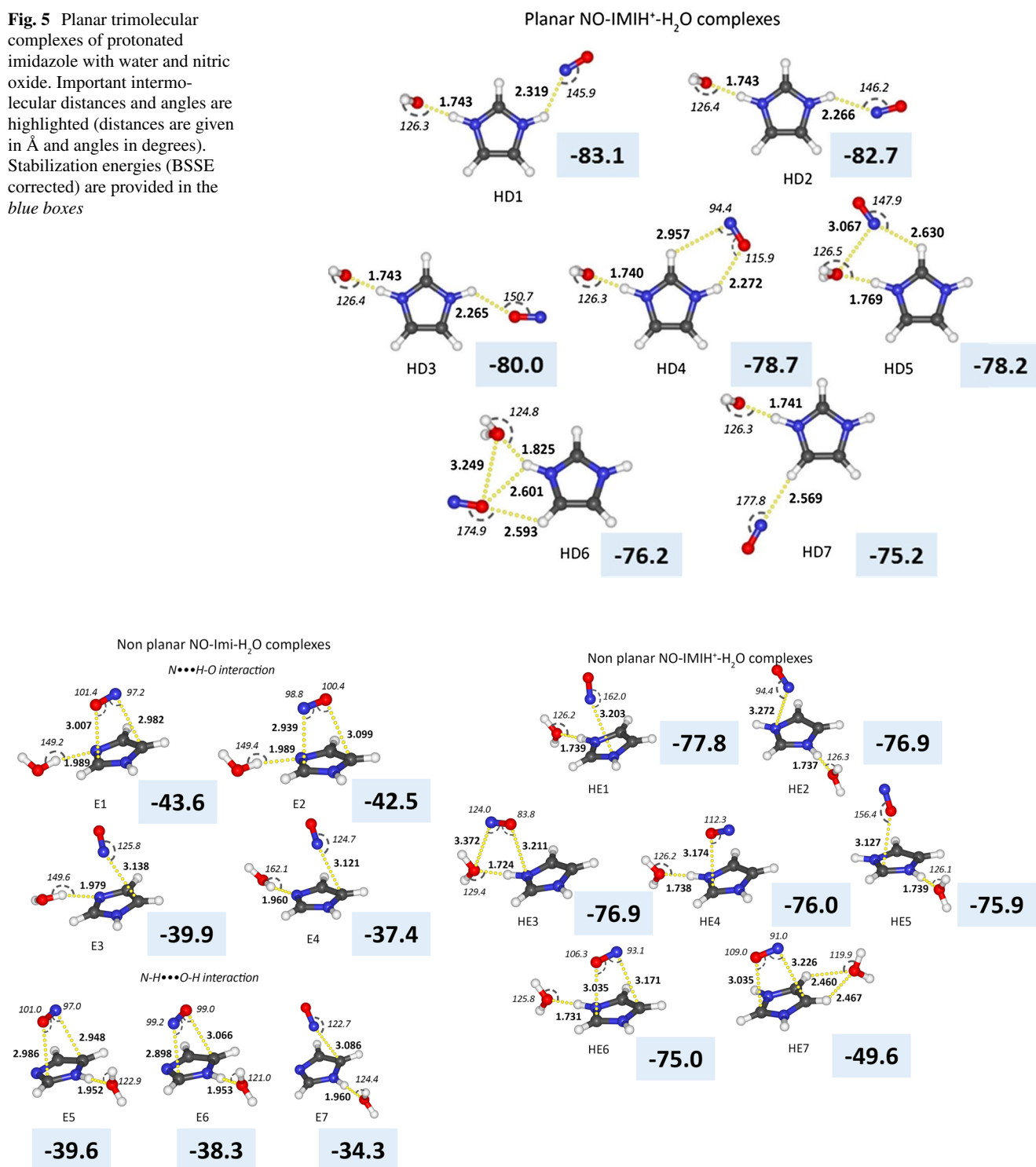


Fig. 6 Nonplanar trimolecular complexes of protonated imidazole with water and nitric oxide. Relevant intermolecular distances and angles are highlighted (distances are given in Å and angles in degrees). Stabilization energies (BSSE corrected) are provided in the blue boxes (in kJ/mol)

interaction (Fig. 4), which was not found in the Imi-H₂O dimers. This interaction is related to the $n_{(N)} \rightarrow \sigma_{(OH)}^*$ transition (Table S6), and the corresponding NBO total stabilization energies are the same in the two complexes.

In the last section, we evaluate the cooperative effect of these interactions.

When the imidazole ring is protonated, the resulting planar complexes feature the N-H_{Imi}...O-H_{water} interaction

between Imi and H₂O (Fig. 6). There, the imidazole–water and imidazole–nitric oxide distances are shorter, while nitric oxide and water monomers become more apart. The corresponding stabilization energies of the protonated complexes are also higher than those of the neutral complexes (Fig. 5). The NBO analysis shows that the most important Imi⋯H₂O interaction becomes stronger upon protonation of the imidazole ring (See Supplementary Information). For the interactions between NO and Imi, second-order perturbation energy values vary only slightly in the charged complexes. This indicates that Imi⋯H₂O interactions are more sensitive to ring protonation with respect to Imi⋯NO interactions.

The nonplanar neutral NO–Imi–H₂O complexes can be classified into two groups: the most stable complexes with the N_{Imi}⋯H–O_{water} interaction between the imidazole ring and water (**E1–E4**) and the N–H_{Imi}⋯O–H_{water} complexes (**E5–E7**) (Fig. 6). The stabilization energies vary in the same ranges that in the trimolecular planar complexes. For the first group of structures, the electron density transfer associated with the N_{Imi}⋯H–O_{water} interaction is a consequence of the $n_{(N)} \rightarrow \sigma_{(OH)}^*$ transition and involves transfer of electron density to an antibonding orbital of H₂O (Fig. 6 and Table S10, Supplementary Information). Conversely, for the N–H_{Imi}⋯O–H_{water} interaction, the charge transfer takes place from the water oxygen atom to the protonated nitrogen atom of imidazole $n_{(O)} \rightarrow \sigma_{(NH)}^*$.

When imidazole is protonated, no stable trimers with the water molecule out of the plane defined by the imidazole ring could be found. Two main interaction motifs are identified in the NO–ImiH⁺–H₂O complexes: the N–H_{Imi}⋯O–H_{water} interaction between the imidazole ring and water (**HE1** to **HE6**, Fig. 6) and the C–H_{Imi}⋯O–H_{water} interaction (**HE7**). Unlike the neutral structures and because ImiH⁺ lacks free electron pairs, water interacts only via its hydroxyl oxygen atom. The energies of the resulting complexes vary from –77.8 kJ/mol for **HE1** to –75.0 kJ/mol for **HE6**.

The comparison between the energies associated with the $n_{(O)} \rightarrow \sigma_{(NH)}^*$ interaction in protonated and unprotonated nonplanar trimolecular complexes shows that those values duplicate upon ring protonation. Since ImiH⁺ is an electron-deficient specie, the electron density transfer from water to the imidazole ring is favored in protonated complexes with respect to the neutral cases (Supplementary Information).

The natural charges of the protonated complexes show that the charge transfer between ImiH⁺ and H₂O takes values of about 0.05 e, while in neutral complexes with similar N–H_{Imi}⋯O–H_{water} interaction the charge transfer values are much lower (about 0.02). On the other hand, the charge transfer between ImiH⁺ and NO is much smaller than the

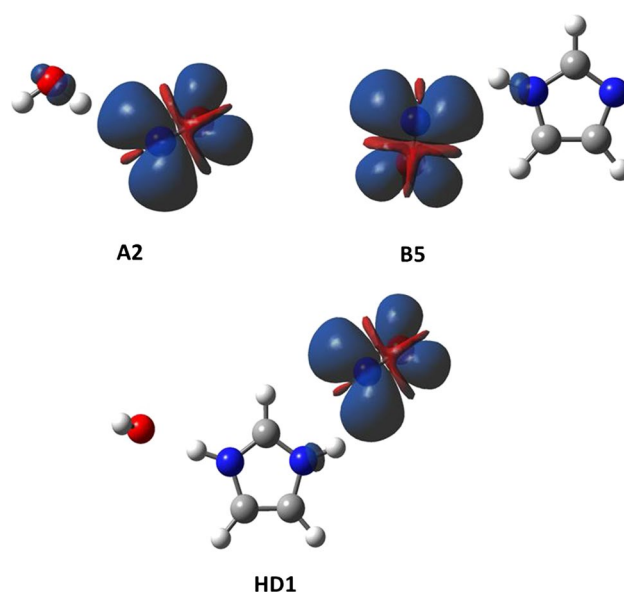


Fig. 7 Spin densities for **A2**, **B5** dimers and **HD1** trimer (contours: 0.005)

charge transfer between ImiH⁺ and H₂O. Since the spin density transfer is of the same order in nonplanar protonated and neutral complexes, it can be inferred that water plays a more relevant role than nitric oxide in the stabilization of the nonplanar NO–ImiH⁺–H₂O complexes. On the other hand, the spin density transfer is mainly from the NO molecule to the closest molecule. Figure 7 shows the spin density in three complexes featuring the Z–H⋯N–O interaction (trimer: **HD1** and dimers: **A2** and **B5**). A fraction of spin density is transferred from the NO molecule to the Z–H bonds, which is consistent with stabilization through ($n_{(N)} \rightarrow \sigma_{(ZH)}^*$) donor–acceptor interactions. Similar results are obtained for complexes stabilized by ($n_{(O)} \rightarrow \sigma_{(ZH)}^*$) interaction.

The comparison with bimolecular complexes evidences that in general, stabilization energies are more sensitive to changes in the interactions involving water than to changes in the interactions with nitric oxide. The NBO natural charges show that the Imi–H₂O charge transfer is in all cases (except **HE7**) much larger than the Imi–NO charge transfer. These results also indicate that the *stabilization of the charged complexes is primarily related to the interaction between ImiH⁺ and H₂O*.

We thus found that *the protonation of the ring stabilizes the system because it favors charge transfer interactions between Imi and H₂O*. The natural charge values show that only net charge transfer from NO and H₂O to Imi takes place with no evidence of charge transfer between H₂O and NO. This suggests that *the unpaired orbital of NO is not involved in the NO⋯H₂O interaction* in these complexes.

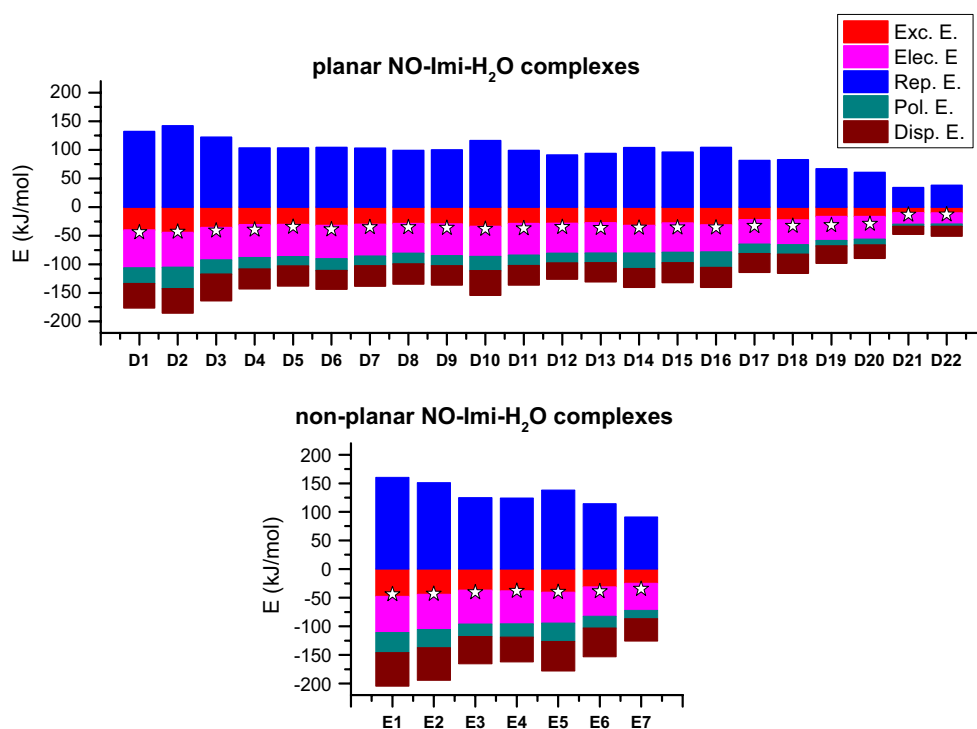


Fig. 8 Energy contributions to the stabilization energy computed using the LMOEDA scheme for NO–Imi–H₂O complexes, using three units in the analysis. The star represents the total stabilization energy

3.3 Why are these complexes stable?

LMOEDA analysis was carried out to understand the origin of the stabilization of the complexes and the interplay between different molecular units. We decomposed the stabilization energies of the dimers and trimers considering the individual units. The most important stabilizing energy components were dispersion, electrostatic and polarization. The competition between these components strongly depends on the involved molecules and the geometry.

For the NO–H₂O complexes, the stabilizing electrostatic component is small with respect to dispersion and polarization (Supplementary Information). In the case of the ON_{NO}⋯OH_{water} interaction (**A1**), polarization is the dominant component. For the ON_{NO}⋯HO_{water} and NO_{NO}⋯HO_{water} interactions (**A2**, **A3** and **A4**, **A5** complexes), dispersion plays the most important role. In contrast to the NO–H₂O dimers, the Imi–H₂O complexes are largely stabilized by electrostatic terms with small contributions from dispersion, exchange and polarization terms. The protonation of the Imi ring increases the electrostatic component.

In the case of the planar Imi–NO dimers, we found that the geometry modulates the contributions of different energy terms (Supplementary Information). For planar complexes, the dispersive contribution is slightly larger than the electrostatic contribution. In the case of nonplanar

complexes, dispersion terms are more than twice the electrostatic stabilizing contribution. The ON_{NO}⋯N_{Imi} interactions in the complexes **B1** and **B2** have important dispersive contributions and some electrostatic character, while ON_{NO}⋯HN_{Imi} interactions (**B5** and **B6**) and NO_{NO}⋯HN_{Imi} interactions (**B7–B10**) are almost exclusively stabilized by dispersion, having a very small polarization component which is repulsive in some cases. The protonation of the Imidazole ring increases the role of the electrostatic contribution to the planar complexes stabilized by ON_{NO}⋯HN_{Imi} and NO_{NO}⋯HN_{Imi} interactions (**HB1** and **HB2**), but dispersion remains as the most important source of stabilization being the dominant contribution in nonplanar complexes.

The most important source of stabilization in planar trimers is the electrostatic component, followed by dispersion, exchange and polarization. The sum of the stabilizing components: electrostatic, polarization and exchange compensates the electrostatic repulsive interaction. Thus, the stabilization energies are very similar to the dispersive component. In the case of **D1**, the dispersion contribution is –42.2 kJ/mol and the stabilization energy is –44.2 kJ/mol (Fig. 8).

In the case of nonplanar complexes, dispersion contributions are in the same order of the electrostatic contributions, while polarization and exchange terms contribute similarly to the stabilization. The relevant role of dispersion is associated with the stacking between the Imi ring and the

Table 2 Association energies (kJ/mol) calculated at the UM05-2X/6-31 ++G(d,p) theory level for planar NO–Imi–H₂O complexes

	ΔE	Δ	$\Delta E - \Delta$
N...H–O interaction			
D1	–47.1	–44.3	–2.7
D2	–46.4	–43.9	–2.5
D3	–44.4	–47.4	3.0
D4	–41.6	–40.6	–1.0
D5	–41.7	–36.5	–5.2
D6	–41.3	–36.0	–5.2
D7	–41.1	–36.9	–4.2
D8	–41.0	–36.8	–4.2
D9	–41.0	–38.1	–2.9
D10	–41.5	–43.3	1.8
D11	–41.2	–39.2	–2.1
D12	–37.4	–36.8	–0.6
N–H...O–H interaction			
D13	–41.4	–40.2	–1.2
D14	–40.9	–39.9	–1.0
D15	–40.6	–39.4	–1.2
D16	–40.5	–39.6	–0.9
D17	–37.9	–39.1	1.2
D18	–37.7	–41.9	4.2
D19	–36.3	–38.1	1.9
D20	–34.8	–34.4	–0.4
C–H...O–H interaction			
D21	–17.2	–17.5	0.3
D22	–17.5	–16.7	–0.7

NO molecule. As it was found for the dimers, the nature of these interactions depends on the geometry (planar and nonplanar), which modulates the contributions of dispersion and electrostatic interactions.

In conclusion, LMOEDA analyses show that NO–Imi–H₂O trimers are stabilized by a combination of factors with an *important role of electrostatic, polarization and dispersion stabilizing terms*. The electrostatic contributions mainly come from the Imi–H₂O subunits of the trimers. The dispersion terms are related to the Imi–NO and NO–H₂O fragments. These calculations show that *NO–closed shell interactions are mainly dispersive*.

3.4 How important are cooperative effects in an open shell–water–closed shell system?

As mentioned above, the interaction energies of the complexes were calculated as:

$$\Delta E_{(\text{NO}\cdots\text{Imi}\cdots\text{H}_2\text{O})} = E_{(\text{NO}\cdots\text{Imi}\cdots\text{H}_2\text{O})} - (E_{(\text{Imi})} + E_{(\text{NO})} + E_{(\text{H}_2\text{O})}) \quad (2)$$

For a trimolecular complex, we define the value Δ as the sum of the energies of the dimer subunits forming the trimer (two-body contribution to the stabilization energy). Since we calculated the energies of the dimers subunits with the fixed geometries they adopt in the trimer, only electronic factors are considered and the effects of geometry relaxation are neglected.

$$\Delta = E_{(\text{Imi}\cdots\text{NO})} + E_{(\text{Imi}\cdots\text{H}_2\text{O})} + E_{(\text{NO}\cdots\text{H}_2\text{O})} - 2(E_{(\text{Imi})} + E_{(\text{NO})} + E_{(\text{H}_2\text{O})}) \quad (3)$$

Thus, two-body cooperative effects in the complex can be estimated as the difference between ΔE and Δ .

$$\begin{aligned} \Delta E - \Delta &= E_{(\text{NO}\cdots\text{Imi}\cdots\text{H}_2\text{O})} - E_{(\text{Imi})} - E_{(\text{NO})} - E_{(\text{H}_2\text{O})} \\ &\quad - (E_{(\text{Imi}\cdots\text{NO})} + E_{(\text{Imi}\cdots\text{H}_2\text{O})} + E_{(\text{NO}\cdots\text{H}_2\text{O})} \\ &\quad - 2E_{(\text{Imi})} - 2E_{(\text{NO})} - 2E_{(\text{H}_2\text{O})}) \Delta E \\ &\quad - \Delta = E_{(\text{NO}\cdots\text{Imi}\cdots\text{H}_2\text{O})} - E_{(\text{Imi}\cdots\text{NO})} \\ &\quad - E_{(\text{Imi}\cdots\text{H}_2\text{O})} - E_{(\text{NO}\cdots\text{H}_2\text{O})} + E_{(\text{Imi})} \\ &\quad + E_{(\text{NO})} + E_{(\text{H}_2\text{O})} \end{aligned} \quad (4)$$

This approximation allows us to roughly estimate the effect of introducing an additional molecule into a dimer to form the corresponding trimer. Here, we found that in almost all cases, the three-body cooperative effect is very low. As evidenced by frequency calculations, all the complexes are minima of the potential energy surface. Thus, positive $\Delta E - \Delta$ contributions could be related to the effect of geometry relaxation. This means that monomer geometry relaxation provides the extra energy required for trimer formation and that some dimers subunits can only be found within the trimer context.

For planar complexes, $\Delta E - \Delta$ take modular values in the 5.2–0.4 kJ/mol range and the complexes featuring the N–H_{Imi}...O–H_{water} and C–H_{Imi}...O–H_{water} interactions have in general the lower cooperative effect (Table 2). In most cases, the stabilization energies can be estimated at chemical accuracy only considering two-body contributions. This effect is even less pronounced for nonplanar complexes ($\Delta E - \Delta$ values between 1.0 and 0 kJ/mol). Slightly higher cooperative effects are calculated for the N–H_{Imi}...O–H_{water} complexes. The same planar versus nonplanar tendency is found for the protonated complexes in which the cooperative effect is more pronounced than in the neutral structures ($\Delta E - \Delta$ values between 10.8 and 0 kJ/mol for planar complexes and of 1.0 kJ/mol and less for nonplanar complexes, Table 2 and Supporting Information). Thus, our results suggest that *three-body cooperative effects are more relevant in charged complexes and in planar structures than in neutral species and nonplanar structures*.

We computed the $\Delta E - \Delta$ contributions using the LMOEDA scheme defining the dimers as molecular units (Supplementary Information). In most of the cases, the

Table 3 NBO donor acceptor interactions in three selected trimers and the corresponding dimers

Complex	Interactions	Donor-acceptor pair	Stabilization energy		Dimer	Stabilization energy	
			α Orbitals	β Orbitals		α orbitals	β orbitals
D5	$\text{ON}_{\text{NO}} \cdots \text{H}-\text{N}_{\text{Imi}}$	$n_{(\text{N})} \rightarrow \sigma_{(\text{NH})}^*$	4.5	–	B5	4.5	–
	$\text{N}_{\text{Imi}} \cdots \text{H}-\text{O}_{\text{water}}$	$n_{(\text{N})} \rightarrow \sigma_{(\text{OH})}^*$	23.4	23.4	W1	45.7	–
D8	$\text{NO}_{\text{NO}} \cdots \text{H}-\text{N}_{\text{Imi}}$	$n_{(\text{O})} \rightarrow \sigma_{(\text{NH})}^*$	2.8	–	B7	2.6	–
	$\text{ON}_{\text{NO}} \cdots \text{H}-\text{C}_{\text{Imi}}$	$n_{(\text{N})} \rightarrow \sigma_{(\text{CH})}^*$	1.1	–		1.2	–
D13	$\text{N}_{\text{Imi}} \cdots \text{H}-\text{O}_{\text{water}}$	$n_{(\text{N})} \rightarrow \sigma_{(\text{OH})}^*$	23.4	23.4	W1	45.7	–
	$\text{ON}_{\text{NO}} \cdots \text{N}_{\text{Imi}}$	$n_{(\text{N})} \rightarrow \pi_{(\text{NO})}^*$	1.4	1.9	B1	1.8	2.3
	$\text{NH}_{\text{Imi}} \cdots \text{OH}_{\text{water}}$	$n_{(\text{O})} \rightarrow \sigma_{(\text{NH})}^*$	27.3	27.3	W2	53.6	–

electrostatic component of the interaction is additive. Dispersive and polarization contributions have the largest influence in the cooperative effects between the three monomers.

The $\Delta E-\Delta$ values also show that both complexes with favorable (the third molecule binds better to the dimer than to a single molecule, negative $\Delta E-\Delta$ values) as well as unfavorable cooperative effects can be found. In planar complexes, unfavorable cooperative effect took place when two molecules of the complex interacted with the same atom of the third molecule with noncomplementary charge transfers. **D3**, **D10**, **D17**, **D18**, **D19**, **E3**, **HD5** and **HE3** are stabilized by interactions between the three monomers that cannot be fully described as the sum of two-body components. The NBO analysis of **D3**, **D10**, **D17**, **D18** and **D19** complexes shows the unprotonated nitrogen atom of Imi acting as a density donor. In **D21**, the corresponding Imi-NO dimer is not stable in the trimer geometry, and there is an important geometry relaxation because of three-body interactions.

To investigate three-body effects on the NBO second-order energy contributions, the values of the most important interactions in **D5**, **D8** and **D13** trimers and their corresponding dimers were analyzed (Table 3, **D1**: **B5** + **W1**; **D8**: **B7** + **W1**, **D13**: **B1** + **W2**). These dimers are stable in nuclear configurations close to those of the corresponding trimers. The $\Delta E-\Delta$ values (with respect to the relaxed dimer structures) are -0.2 , -1.1 and -0.5 kJ/mol, respectively, showing the effect of geometry relaxation. The major orbital interactions change less than 1 kJ/mol with respect to the corresponding dimers.

4 Conclusions

Here, the bimolecular and trimolecular complexes of nitric oxide, imidazole and water are discussed. The NBO theory and the localized molecular orbital energy decomposition scheme are used to understand the nature of the intermolecular interactions. The prediction of the structures of NO-Imi-H₂O complexes not only allowed us to investigate

the interactions taking place in these systems, but also to elucidate the role of ring protonation and planarity in cooperative effects. These complexes provide a model for the investigation of the role of competing nonbonding interactions involving unpaired and paired electrons, which play a key role in enzymatic reactions. Our work indicates that the above-mentioned competition depends on the nature of each interaction and on the specific energy balance with respect to the closed shell molecule. This is related to the fact that orbitals with lone pairs and unpaired orbitals mostly show similar energies and are located in common regions of space. Accordingly, we expect this competition to play an important role in systems with many lone pairs.

We found that:

- In contrast to other radicals (i.e., HO, HO₂), when nitric oxide forms hydrogen bonds acting as a Lewis base ((N-O)_{NO}⋯H-Z interactions) the unpaired $n_{(\text{N})}$ or $n_{(\text{O})}$ orbitals play a significant role in the stabilization of the complexes.
- The dimers featuring the O-N_{NO}⋯H-Z interactions are more stable than the N-O_{NO}⋯H-Z complexes.
- When nitric oxide interacts with a heteroatom ((N-O)_{NO}⋯Z interactions), the $n_{(\text{Z})} \rightarrow \pi_{(\text{NO})}^*$ donor-acceptor has the most important contribution to complex stabilization.
- Protonation of the imidazole ring stabilizes the N-H antibonding orbitals in imidazole with the consequent stabilization of its complexes.
- The interplay between different stabilizing interactions in dimers and trimers strongly depends on the geometry (planar vs. nonplanar).
- Dispersion and polarization energy terms are the dominant contributions in the stabilization of NO-closed shell complexes.
- The stability of the trimers is more sensitive to changes in the interactions of imidazole with water than to changes in the interactions with nitric oxide.
- Our calculations suggest that the cooperative effect is more relevant in charged complexes and planar structures than in neutral species and nonplanar complexes.

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