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## Interaction of Au(III) with amino acids: a vade mecum for medicinal chemistry and nanotechnology†

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Au(III) is highly reactive. At odds with its reduced counterpart, Au(I), it is hardly present in structural databases. And yet, it is the starting reactant to form gold nanoclusters (AuNCs) and the constitutive component of a new class of drugs. Its reactivity is a world apart from that of the iso-electronic Pt(II) species. Rather than DNA, it targets proteins. Its interaction with amino acid residues is manifold. It can strongly interact with the residue backbones, amino acid side chains and protein ends, it can form appropriate complexes whose stabilization energy reaches up to more than 40 kcal mol<sup>-1</sup>, it can affect the pK<sub>a</sub> of amino acid residues, and it can promote charge transfer from the residues to the amount that it is reduced. Here, quantum chemical calculations provide quantitative information on all the processes where Au(III) can be involved. A myriad of structural arrangements are examined in order to determine the strongest interactions and quantify the amount of charge transfer between protonated and deprotonated residues and Au(III). The calculated interaction energies of the amino acid side chains with Au(III) quantitatively reproduce the experimental tendency of Au(III) to interact with selenocysteine, cysteine and histidine and negatively charged amino acids such as Glu and Asp. Also, aromatic residues such as tyrosine and tryptophan strongly interact with Au(III). In proteins, basic pH plays a role in the deprotonation of cysteine, lysine and tyrosine and strongly increases the binding affinity of Au(III) toward these amino acids. The amino acid residues in the protein can also trigger the reduction of Au(III) ions. Sulfur-containing amino acids (cysteine and methionine) and selenocysteine provide almost one electron to Au(III) upon binding. Tyrosine also shows a considerable tendency to act as a reductant. Other amino acids, commonly identified in Au–protein adducts, such as Ser, Trp, Thr, Gln, Glu, Asn, Asp, Lys, Arg and His, possess a notable reducing power toward Au(III). These results and their discussion form a vade mecum that can find application in medicinal chemistry and nanotech applications of Au(III).

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## 1. Introduction

Amino acids, the basic building blocks of proteins, interact with Au(III)<sup>1,2</sup> to determine the pharmacological profile of Au(III) drugs,<sup>3–9</sup> and trigger the protein-assisted synthesis of gold nanoclusters (AuNCs).<sup>10–14</sup>

Au(III) compounds are isoelectronic with Pt(II) complexes. In medicinal chemistry, it was initially suggested that the mechanism of pharmacological activity of Au(III) compounds would be similar to that of Pt(II)-compounds, *i.e.*, interaction with DNA. It then appeared that the modes of action of Au(III) compounds

are multifaceted and distinct from those of Pt species.<sup>3–9,15–22</sup> On the one hand, there is evidence suggesting that rather than nucleic acids some specific protein targets primarily mediate the biological effects of Au(III) compounds.<sup>3–9,15–24</sup> On the other hand, the cytotoxic activity of some Au(III) compounds exploit metalation and inactivation of a few key intracellular proteins that are effective cancer targets.<sup>3–9,15–23,25</sup>

Proteins further offer the opportunity to synthesize AuNCs directly in their pockets through a series of sequential steps of gold deposition, reduction, and aggregation.<sup>10–14</sup> A variety of functional groups, such as sulfhydryl, hydroxyl, carboxyl and amine groups, are suitable for accumulating and reducing Au(III) ions.<sup>10–14</sup> The bulky protein can also protect sterically the resulting AuNCs.<sup>10–14</sup> Many proteins (for instance, bovine and serum albumin, lysozyme, transferrin, trypsin, pepsin, insulin and others) were used to synthesize protein–AuNC hybrids.<sup>10–14</sup> External reducing agents (*i.e.*, NaBH<sub>4</sub>) or some of

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the protein amino acid residues trigger the reduction of the Au(III) ions leading to the synthesis of the AuNCs.<sup>10–14</sup> During the AuNC formation process, amino acid residues play a three-fold role of being anchoring sites for gold ions, reducing agents, and stabilizers.<sup>10–14</sup> AuNCs are among the most promising theranostic agents for applications in nanomedicine.<sup>10,26–33</sup>

Interaction of Au(III) with proteins may also be useful for (i) developing innovative photodynamic and photoactivated therapies;<sup>34,35</sup> (ii) radiotherapeutic purposes, since Au possesses two  $\beta$ -emitting isotopes, <sup>198</sup>Au and <sup>199</sup>Au,<sup>9</sup> and the proteins can be used as carriers;<sup>36</sup> (iii) catalysis and metal-mediated transformations in biological systems.<sup>37</sup>

The increasing interest in interactions between Au(III) and amino acids/peptides/proteins spurred a thorough investigation of the chemical mechanism governing their binding.<sup>38–46</sup> Comparison of the structural characteristics of known Au-protein adducts contained in the Protein Data Bank (PDB)<sup>2,47</sup> revealed that cysteine is the most preferred residue for Au binding, followed by histidine.<sup>2</sup> Side chains of other amino acids, such as Lys, Arg, Gln, Met, Glu, Asp, Asn, Thr, and Ser, as well as the terminal residues of proteins, interact with Au.<sup>2</sup> Sulfur, nitrogen, and oxygen lone pairs commonly bind Au ions.<sup>2</sup>

Au ions can interact with more than one side chains (*i.e.* His/Cys, His/Gln, Cys/Cys, Cys/Asp, or Cys/Asn dyads) to complete their coordination spheres or substitute ligands already present in the initial Au complex.<sup>2</sup> The binding of Au ions to proteins generally does not significantly affect the overall structure of the protein.<sup>2</sup>

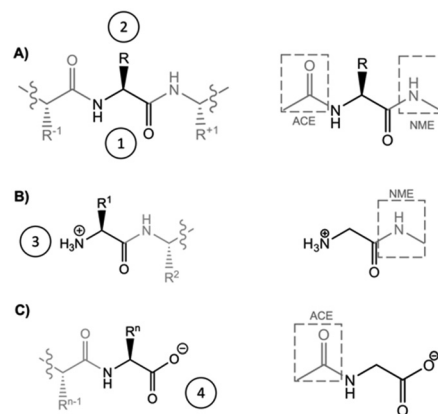
Amino acids can be the starting point to determine the mechanisms that control peptide/protein interactions with Au(III) ions.<sup>48</sup> Computational chemistry represents a tool to understand and design gold–bio interfaces.<sup>49–52</sup> Systematic studies of the interaction of amino acids with the Au(111) surface<sup>53,54</sup> or with the Au(I) ion<sup>55,56</sup> were already carried out, but surprisingly this analysis is lacking for Au(III). Here, using quantum chemical calculations, we investigated the interactions between twenty-one proteinogenic amino acids (including selenocysteine) and Au(III) ions.

## 2. Results and discussion

All the possible interaction sites between Au(III) and a protein/peptide were considered: (i) the protein/peptide backbone, (ii) the amino acid (AA) side chains, and (iii) N-terminal and C-terminal amino acids (Scheme 1). To mimic the usual interactions between Au(III) and an amino acid inserted into the peptide/protein sequence, the amino acids were capped at the N- and C-termini with acetyl (ACE) and *n*-methyl amide (NME) groups (ACE-AA-NME), while the N-terminal and C-terminal amino acids were capped only on one side.

### 2.1 Interactions of Au(III) with the peptide/protein backbone

Structural, spectroscopic and computational investigations of the interactions between Au(III) ions and model peptides indicated that Au(III) can



**Scheme 1** (A) Amino acids (in black) in a peptide/protein chain. (B) N-terminal and (C) C-terminal amino acids. On the left is the real system. On the right is the model system used in the calculations, capped with ACE/NME residues. Structures 1 to 4 represent the possible interaction sites of an Au(III) ion in a peptide/protein. 1. Peptide/protein backbone. 2. Amino acid side chains. 3. N-terminal and 4. C-terminal amino acids.

(a) deprotonate and bind the amide nitrogen of a peptide bond and/or the terminal amino nitrogen atom of these peptides,<sup>57,58</sup>

(b) be involved in the binding of the terminal carboxylate groups of the peptides,<sup>59–63</sup>

In the interaction of Au(III) with the  $\text{-NH}_3^+$  and  $\text{-COO}^-$  terminals, the binding of the Au(III) ion may induce deamination and decarboxylation of the amino acids.<sup>64,65</sup>

The interactions of Au(III) with the backbone and the N, C terminal tails usually take place cooperatively. To dissect and separate the individual contributions, we considered them singularly and calculated the interaction of the Au(III) ion with (i) the amino nitrogen at the N-terminal (Fig. 1A), (ii) the amide nitrogen (Fig. 1B) or the (iii) the carbonyl (Fig. 1C) of the peptide/protein backbone, and (iv) the carboxylate oxygen atoms at the C-terminal (Fig. 1D).

When the Au(III) ion interacts with the peptide bond, perhaps counterintuitively, the binding with the amide nitrogen ( $\Delta E_{\text{binding}} = -13.1 \text{ kcal mol}^{-1}$ ) is strongly favoured with respect to binding with the carbonyl group ( $\Delta E_{\text{binding}} = -5.1 \text{ kcal mol}^{-1}$ ).

The ability of Au(III) to induce amide deprotonation upon binding, already experimentally demonstrated,<sup>57,58</sup> explains this behaviour, making the lone pair of the amide available for the interaction with the Au(III) ion. Under physiological conditions, when the Au(III) ion interacts with the terminal amino acids, the binding with the carboxylate oxygen atoms of the C-terminal residue ( $\Delta E_{\text{binding}} = -17.1 \text{ kcal mol}^{-1}$ ) is preferred with respect to the interaction with the N-terminal amino nitrogen ( $\Delta E_{\text{binding}} = -11.1 \text{ kcal mol}^{-1}$ ), which becomes deprotonated upon Au(III) binding. When a neutral N-terminal group is taken into account, for example in solutions where the pH is above 10, the  $\Delta E_{\text{binding}}$  between Au(III) and the lone pair of the amine nitrogen becomes strongly favoured, with a  $\Delta E_{\text{binding}}$  value of  $-36.5 \text{ kcal mol}^{-1}$ .

However, since peptide bonds are usually engaged in the formation of secondary structures, the side chains of amino acids are important hot-spots to bind Au(III).



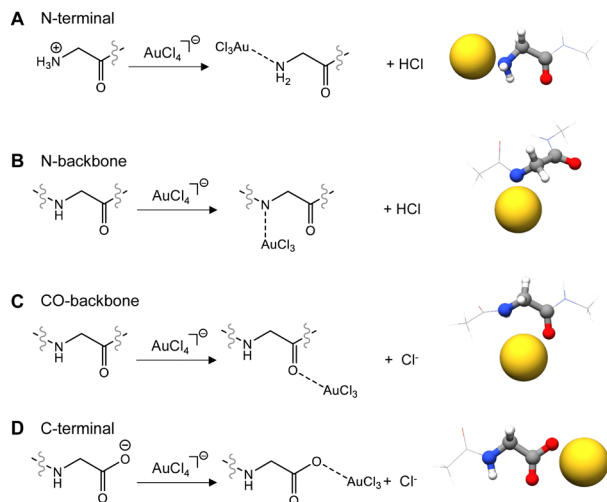


Fig. 1 Interaction of Au(III) with the (A) N-terminal amino acid, (B) N–H peptide/protein backbone, (C) C=O peptide/protein backbone, and (D) C-terminal amino acid. Chlorine atoms are removed for clarity.

## 2.2 Interactions of Au(III) with amino acid side chains at a glance

We determined the interaction energies of the amino acid side chains with Au(III), ranking their propensity to interact with the Au(III) ion (Fig. 2). A systematic search was carried out to analyse all possible binding sites of Au(III). We considered the amino acid side chains in their physiological protonation state.

The rank quantitatively reproduces the experimental tendency of Au(III) to interact with selenocysteine, cysteine and histidine and negatively charged amino acids such as Glu and Asp, which is observed by the analysis of the interactions of the known Au–protein adducts in the PDB.<sup>2</sup>

From the calculation, we can also classify the tendency of different amino acids to reduce Au(III), or alternatively to be oxidized by Au(III), considering the charge transfer from the amino acid to the Au(III) ion upon binding (Fig. 3).

The amount of charge transfer from the various moieties to the Au(III) provides a proxy for the tendency to reduce

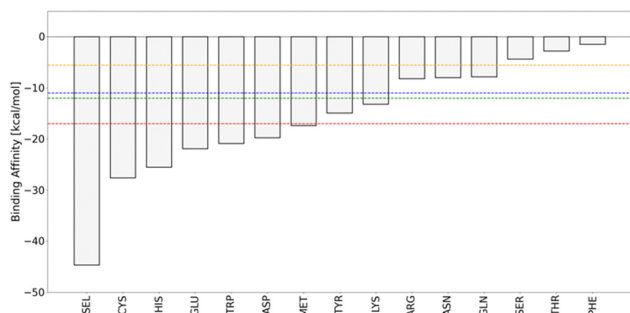


Fig. 2 Ranking of the interaction of Au(III) with amino acid side chains in their physiological protonation state. For comparison, the interaction energies of Au(III) with the N-terminal (blue line), N-backbone (green line), C-terminal (red line), and O-backbone (orange line) are shown. The side chains of SEL, CYS, HIS, GLU, TRP, ASP, MET, TYR, and LYS can successfully compete with the backbone to host Au(III).

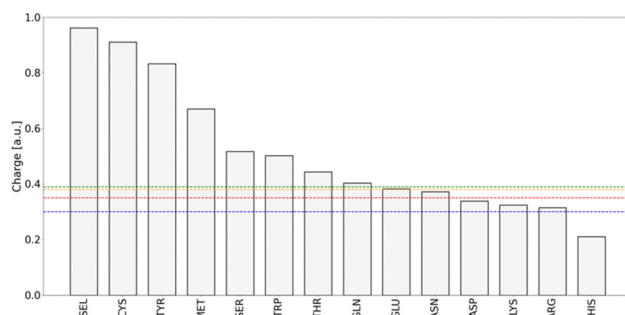


Fig. 3 Ranking of the reduction potential of amino acid side chains toward Au(III). For comparison, the reduction potential of the N-terminal (blue line), N-backbone (green line), C-terminal (red line), and O-backbone (orange line) toward Au(III) is shown. The side chains of SEL, CYS, TYR, MET, SER, TRP, THR, and GLN show a tendency to reduce Au(III) better than the backbone.

Au(III). Structural data of protein/gold complexes suggest that the reduction of Au(III) into Au(I) determines the mechanism of action of many biologically active Au(III) compounds, viz. the oxidation of catalytic residues such as selenocysteine or cysteine.<sup>2</sup> Following the binding of Au(III) ions to specific amino acid residues in a protein pocket, the reduction of Au(III) is also a crucial step for the formation of protein-protected AuNCs.<sup>10</sup>

Amino acids containing sulfur (cysteine and methionine) and selenium (selenocysteine) provide almost one electron to Au(III) upon binding. Tyrosine also shows a considerable tendency to act as a reductant. Other amino acids, commonly identified in Au–protein adducts, such as Ser, Trp, Thr, Gln, Glu, Asn, Asp, Lys, Arg and His, possess a notable reducing power toward Au(III).

These results indicate that, when Au(III) is bound in a protein by a dyad/triad of reducing amino acids, it can spontaneously be reduced to Au(I), without the need for an external reductant. Such a behaviour is observed in many drugs and AuNCs,<sup>2,10</sup> see for instance the interaction of the drug Auoxo6 with the model protein bovine pancreatic ribonuclease<sup>66</sup> or the binding of Au ions in Apo-ferritin (Fig. 4).<sup>67</sup>

Below, we analyse the results in detail, grouping the amino acids in families, following their chemical characteristics.

**2.2.1 Sulfur and selenium containing amino acids.** For centuries, it has been known that gold interacts with sulfur-containing molecules. Methionine and cysteine are the two sulfur-containing amino acids present in peptides and proteins. Antioxidant defence, protein folding, and redox sensing and control are well-known functions of these amino acids. Cysteine has also a prominent role in enzymatic catalysis. Cysteine is rapidly oxidized to cystine by Au(III) in aqueous solution, while Au(III) is reduced to Au(I).<sup>68</sup> The same results are obtained when cysteine is inserted into peptide sequences, such as in glutathione.<sup>68–70</sup> The exchange of an Au(III) ligand with Cys, in particular the binding of the sulfur atom of the side chain, is the first step of the process. Also, the redox reaction between [AuCl<sub>4</sub>]<sup>−</sup> and methionine involves initially a very fast substitution of one chloride ion by the sulfur atom of the Met



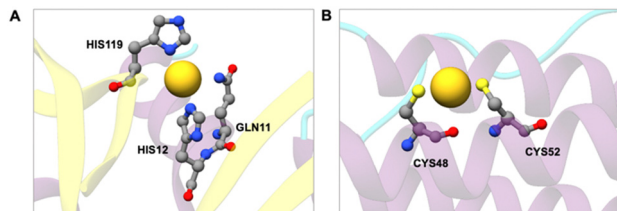


Fig. 4 (A) Interaction of the drug Auoxo6 with the model protein bovine pancreatic ribonuclease (PDB:4MXF).<sup>66</sup> The Au atom is simultaneously coordinated with imidazoles of His12 and His119. (B) Linear coordination structure of the Au atom with Cys 48 and Cys54 in apo-ferritin (PDB:5GU3) before the formation of AuNCs.<sup>67</sup> In both cases, the coordination geometries are nearly linear, strongly indicating that gold is in the +1 oxidation state.

side chain with the formation of a short lived Au(III)–methionine complex.<sup>71,72</sup> The second step of the reaction is the formation of methionine sulfoxide and Au(I).<sup>71,72</sup> The structure of the final product of Au(III)-induced oxidation of methionine-containing peptides was confirmed by X-ray crystallography.<sup>1</sup>

Selenocysteine (Sec) is considered to be the 21<sup>st</sup> proteinogenic amino acid. Sec is a cysteine analogue with selenium instead of sulfur. There are about 25 selenoproteins, *i.e.*, proteins that contain Sec, known in humans. They play biological functions essential for preserving redox equilibrium.

The biological activity of many Au(III) drugs are usually mediated *via* Au–S or Au–Se coordination *via* ligand replacement with protein thiols/selenols.<sup>2</sup> By directly inhibiting the active sites, cysteine (or selenocysteine) in proteins, Au(III) compounds can cause cytotoxicity.<sup>2</sup> For example, thioredoxin reductase (TrxR) enzymes possesses a Gly–Cys–Sec–Gly C-terminal active site motif, which is the target of several Au(III) drugs,<sup>73</sup> explaining their anti-tumor proliferative activities.

The most important difference between Cys and Sec is the basicity of selenolate, that is 3–4 pK<sub>a</sub> units lower than that of thiolate, due to the weaker bond to hydrogen and the larger size and increased polarizability of Se.<sup>74</sup> Thus, at neutral pH, Cys (pK<sub>a</sub> = 8.25) is mostly in the protonated state whereas selenocysteine is virtually entirely ionized to a selenolate (pK<sub>a</sub> = 5.43).<sup>74</sup> This behaviour determines the difference in the binding affinity of the Au(III) ion to Sec and Cys (Fig. 5). Even if the binding of Au(III) is able to deprotonate the Cys, the interaction energy of Au(III) with the negatively charged Se ( $\Delta E_{\text{binding}} = -44.7 \text{ kcal mol}^{-1}$ ) is obviously higher than that with Cys ( $\Delta E_{\text{binding}} = -27.6 \text{ kcal mol}^{-1}$ ).

However, even in a physiological environment, the thiol may exist as a thiolate.<sup>75</sup> Electropositive local environments tend to lower the pK<sub>a</sub> of Cys by stabilizing the thiolate.<sup>75</sup>  $\alpha$ -Helices exhibit a dipole moment with a more positive charge towards the N-terminal end that stabilizes the thiolate form of the Cys residues located in this region.<sup>75</sup> Also, the presence of specific residues near Cys can modify its pK<sub>a</sub>, for example proximity of Cys to His usually determine the deprotonation of the SH group to form a thiolate–imidazolium ion pair.<sup>76</sup> In this case, when Au(III) interacts with the thiolate, the interaction energy between Au(III) and Cys is comparable ( $\Delta E_{\text{binding}} = -45.4 \text{ kcal mol}^{-1}$ ) to that observed with Sec.

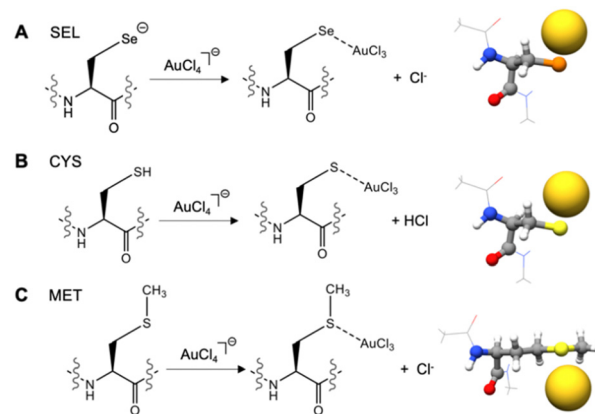


Fig. 5 Interaction of Au(III) with (A) selenocysteine (Sec), (B) cysteine (Cys), and (C) methionine (Met). Chlorine atoms are removed for clarity.

Also, Met strongly interacts with Au(III) ( $\Delta E_{\text{binding}} = -17.4 \text{ kcal mol}^{-1}$ ), even if it remains in its neutral form upon interaction with gold because of the lack of acid hydrogens on the sulfur atom, demonstrating the natural affinity of the sulfur atom toward gold.

**2.2.2 Histidine.** Histidine represents the most significant metal binding site in biological systems.<sup>77,78</sup> Histidine quickly coordinates Au(III) through its imidazole nitrogen atoms.<sup>79</sup> The binding is usually followed by its oxidation.<sup>80</sup> In peptides, both the N<sup>3</sup> and N<sup>1</sup> nitrogen atoms of the imidazole ring were found as potential anchoring sites for Au(III).<sup>1</sup>

Histidine was analysed individually because it is difficult to classify this amino acid residue in a single family because it is an aromatic amino acid, potentially charged in a physiological environment, it is polar and characterized by acid–base properties (a characteristic relevant to the catalytic mechanism of many enzymes). The unique chemical properties of His are due to its imidazole ring. At physiological values (the pK<sub>a</sub> of the imidazole group in proteins is 6.04), the two nitrogens of the imidazole ring can bind or release a proton to form the acid or the base form of His. In its neutral form, two tautomers of His exist (N<sup>1</sup>–H or N<sub>δ</sub> and N<sup>3</sup>–H or N<sub>ε</sub> tautomers), depending on which nitrogen the proton of the imidazole ring resides.

Interactions with the Au(III) indicate that histidine in its neutral form interacts using preferentially the lone pairs of the histidine imidazole nitrogens (Fig. 6), with the N<sub>δ</sub> tautomer slightly preferred ( $\Delta E_{\text{binding}} = -25.5 \text{ kcal mol}^{-1}$ ) to the N<sub>ε</sub> tautomer ( $\Delta E_{\text{binding}} = -23.1 \text{ kcal mol}^{-1}$ ). Due to the resonance effect, also the C<sup>4</sup> position of the His ring represents a potential Au(III) binding site, even if these interactions are considerably smaller ( $\Delta E_{\text{binding}} = -10.3 \text{ kcal mol}^{-1}$ ), when compared to the imidazole nitrogens.

The pK<sub>a</sub> value of histidine is very close to the physiological pH value, which makes it very common to find protonated histidines in proteins. However, also when the histidine is in its protonated form, deprotonation easily occurs upon Au(III) binding, releasing the lone pairs of the two nitrogens, that can bind Au(III) with  $\Delta E_{\text{binding}}$  values of  $-20.3 \text{ kcal mol}^{-1}$  for N<sub>ε</sub> and  $-16.3 \text{ kcal mol}^{-1}$  for N<sub>δ</sub>.



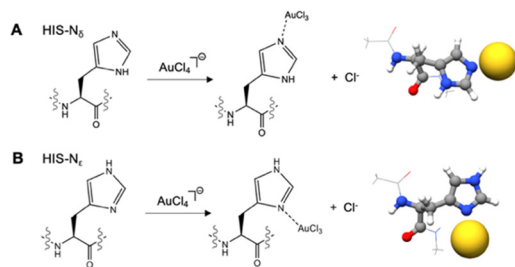


Fig. 6 Interaction of Au(III) with (A) histidine (His)-N $\delta$  tautomer and (B) histidine (His)-N $\epsilon$  tautomer. Chlorine atoms are removed for clarity.

**2.2.3 Aromatic amino acids.** Among the 20 standard amino acids, tryptophan (Trp), tyrosine (Tyr) and phenylalanine (Phe) are classified as aromatic. The folded structures of numerous proteins are stabilized by aromatic amino acid interactions<sup>81</sup> and for this reason, they are usually confined to the cores of globular proteins. When the aromatic residues appear on the protein surface, they are often involved in recognition processes,<sup>82</sup> and they are key components of the protein–protein<sup>82</sup> or protein–ligand interaction interfaces.<sup>82</sup> Aromatic amino acids are also involved in electron transfer processes.<sup>83</sup>

Aromatic amino acids such as tryptophan, tyrosine and phenylalanine may interact with gold ions with (i) their aromatic  $\pi$ -system,<sup>2</sup> (ii) with the lone pairs of the heteroatoms (when present),<sup>2</sup> or (iii) with the nucleophilic carbon atoms, composing the aromatic ring.<sup>2</sup> Analysis of the protein data bank and quantum chemical calculations evidenced Au $\cdots\pi$  interactions involving Au(I) and aromatic amino acids (Phe, Tyr, and Trp).<sup>84</sup> These Au $\cdots\pi$  interactions (alternatively defined as regium- $\pi$  bond) are not observed in the interactions between Au(III) ions and aromatic residues. The softer nature of the Au(I) ion, compared to Au(III), is at the basis of this different behaviour. The interaction of Au(III) with Trp and Tyr is structurally interesting, because even if free lone pairs are available due the presence of O/N heteroatoms in the amino acid side chains, Au(III) interacts preferentially with nucleophilic carbon atoms of the aromatic system. Trp and indole are  $\pi$ -excessive aromatic heterocycles that are able to strongly interact with electrophiles, such as Au(III).<sup>85</sup> In the case of Trp, Au(III) shows the strongest interaction with the C3 position of the indole ring ( $\Delta E_{\text{binding}} = -20.9 \text{ kcal mol}^{-1}$ ), similarly to the well-known gold-catalyzed functionalization reactions of C2-substituted indoles (Fig. 7).<sup>85</sup> Alternative interaction sites can also be identified in the Trp ring. Au(III) can deprotonate the N–H bond in Trp. In this case, two competitive Au(III) interaction sites can be identified, *i.e.*, C3 with a  $\Delta E_{\text{binding}}$  value of  $-19.9 \text{ kcal mol}^{-1}$  and N1 with a  $\Delta E_{\text{binding}}$  value of  $-16.4 \text{ kcal mol}^{-1}$ .

In Tyr, the preferred interaction site is not the phenolic oxygen atom ( $\Delta E_{\text{binding}} = -3.6 \text{ kcal mol}^{-1}$ ), but the *ortho* ( $\Delta E_{\text{binding}} = -14.9 \text{ kcal mol}^{-1}$ ) and *para* ( $\Delta E_{\text{binding}} = -13.3 \text{ kcal mol}^{-1}$ ) positions of the phenyl ring. It is the well-known ability of cations to trigger the deprotonation of Tyr.<sup>86</sup> Au(III) induces deprotonation of the hydroxyl group of the Tyr residue, but delocalization of the lone pair makes the softer “*ortho*” and “*para*” carbons favourite over the hard oxygen-site.

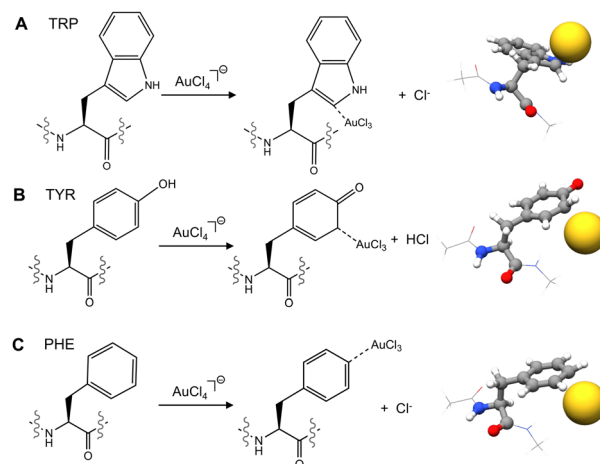


Fig. 7 Interaction of Au(III) with (A) tryptophan (Trp), (B) tyrosine (Tyr), and (C) phenylalanine (Phe). Chlorine atoms are removed for clarity.

Experimentally, the binding/reduction capability of Tyr can be greatly improved by adjusting the pH above the  $pK_a$  of Tyr ( $\sim 10$ ).<sup>10</sup> The calculations, repeated on a deprotonated Tyr, showed a huge increase in the binding energy ( $\Delta E_{\text{binding}} = -41.4 \text{ kcal mol}^{-1}$ ). This value is comparable to those of Sec and Cys, demonstrating the importance of this residue in the binding/reduction of Au(III), especially at  $\text{pH} > 10$ , when in deprotonated form.

Considering the aromatic system of Phe, Au(III) interacts more strongly with the backbone of the amino acid than with its side chain ( $\Delta E_{\text{binding}} = -1.5 \text{ kcal mol}^{-1}$ ), where the *para* position is favoured on the *ortho* ( $\Delta E_{\text{binding}} = -1.3 \text{ kcal mol}^{-1}$ ).

**2.2.4 Charged amino acids.** Among the 20 common amino acids, four amino acids have side chains that under physiological conditions are charged. Two amino acids are negatively charged, namely aspartate (Asp) and glutamate (Glu) (acidic side chains), and two are positively charged, namely lysine (Lys) and arginine (Arg) (basic side chains). The importance of charged groups in protein biochemistry has been studied extensively in terms of substrate binding and protein/protein (or protein/nucleic acid associations),<sup>87</sup> electron transfer<sup>88</sup> and selective transport of ions,<sup>89</sup> structural control of protein stability<sup>90</sup> and solubility,<sup>91</sup> to name a few processes. The formation of metal binding sites in proteins is probably one of the most important roles of charged amino acids.<sup>92</sup> Metal-binding sites with aspartate, glutamate, or both are found in many proteins that bind metal ions (“metalloproteins”) for structural or functional reasons.<sup>92</sup>

In this case, the strongly positive Au(III) clearly prefers the interaction with negatively charged Glu and Asp residues, than with the basic Lys and Arg (Fig. 8). Glu ( $\Delta E_{\text{binding}} = -21.9 \text{ kcal mol}^{-1}$ ) and Asp ( $\Delta E_{\text{binding}} = -18.9 \text{ kcal mol}^{-1}$ ) residues interact with the positive Au(III) ion giving electrostatic interactions with their negatively charged carboxylates. Lys ( $\Delta E_{\text{binding}} = -13.2 \text{ kcal mol}^{-1}$ ) and Arg ( $\Delta E_{\text{binding}} = -8.2 \text{ kcal mol}^{-1}$ ) instead use the lone pair of their basic nitrogens, after deprotonation induced by Au(III) binding, to coordinate the Au(III) ion.



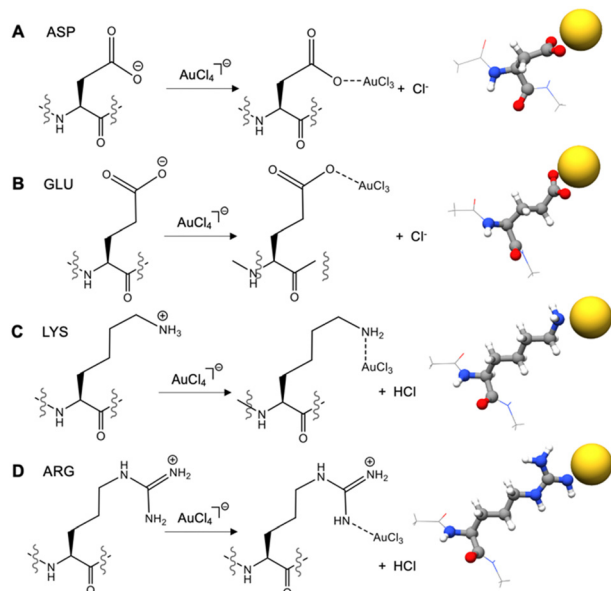


Fig. 8 Interaction of Au(III) with (A) aspartate (Asp), (B) glutamate (Glu), (C) lysine (Lys), and (D) arginine (Arg). Chlorine atoms are removed for clarity.

The interactions with Asp, Glu, and Lys strongly resemble, energetically and structurally, the interactions with the C-terminal and N-terminal residues of a peptide/protein, but they are numerically more common and consequently more important. Even in this case, if basic pH solutions are considered, there is the possibility to deprotonate the side chains of Lys ( $pK_a = 10.79$ ) and Arg ( $pK_a = 12.48$ ), strongly improving their binding with Au(III) ( $\Delta E_{\text{binding}} = -23.2 \text{ kcal mol}^{-1}$  for Lys  $\Delta E_{\text{binding}} = -24.9 \text{ kcal mol}^{-1}$  for Arg).

The interactions with Asp, Glu, and Lys strongly resemble, energetically and structurally, the interactions with the C-terminal and N-terminal residues of a peptide/protein, but they are numerically more common and consequently more important.

**2.2.5 Polar amino acids.** Polar amino acids are characterized by the presence of hydrogen bond acceptors (heteroatoms present in the side chains, with their electron pairs) and hydrogen bond donors (N–H and O–H bonds). They are serine (Ser), threonine (Thr), asparagine (Asn), and glutamine (Gln). Ser and Thr contain aliphatic hydroxyl groups, while Asn and Gln contain an amide group in their side chains.

In analogy with the peptide bond, Au(III) interacts with Asn ( $\Delta E_{\text{binding}} = -8.0 \text{ kcal mol}^{-1}$ ) and Gln ( $\Delta E_{\text{binding}} = -7.8 \text{ kcal mol}^{-1}$ ) through their amide nitrogens, deprotonated upon binding (Fig. 9). The interaction energy of the Au(III) with the side chains of Asn and Gln is lower than the interaction with the peptide backbone because of the different nature of the amide group that is primary in the side chain of Asn and Gln and secondary, and thus more electron-rich, in the peptide backbone.

Also, the interaction of Au(III) with the hydroxyl groups of Ser ( $\Delta E_{\text{binding}} = -4.4 \text{ kcal mol}^{-1}$ ) and Thr ( $\Delta E_{\text{binding}} = -2.8 \text{ kcal mol}^{-1}$ ) may induce deprotonation, but small binding energies are observed in this case.

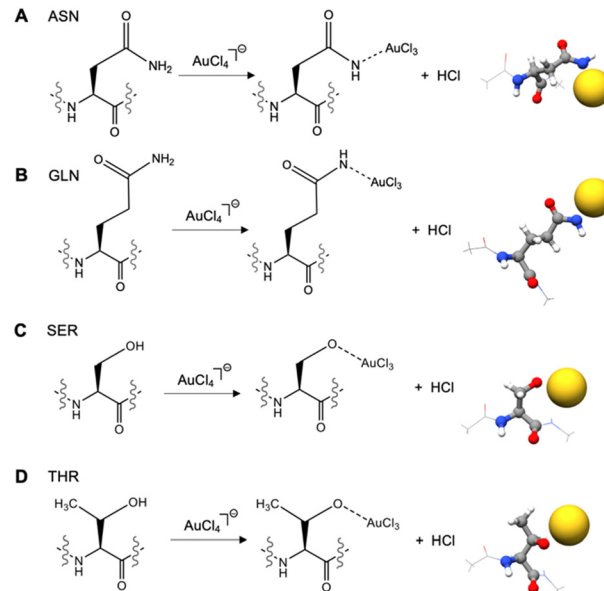


Fig. 9 Interaction of Au(III) with (A) asparagine (Asn), (B) glutamine (Gln), (C) serine (Ser), and (D) threonine (Thr). Chlorine atoms are removed for clarity.

Apart the direct interaction of the Au(III) ion with the side chain of polar amino acids, it was recently demonstrated that amino acids improve their properties as H-bond donors and become less efficient H-bond acceptors upon coordination with metal ions.<sup>93</sup>

**2.2.6 Aliphatic amino acids.** Aliphatic amino acids are characterized by an aliphatic side chain. These amino acids (glycine, alanine, valine, leucine, isoleucine, and proline) are hydrophobic and non-polar and do not contain heteroatoms or nucleophilic carbon atoms. As a consequence, Au(III) can interact only with the backbone of these residues.

### 3. Methodology

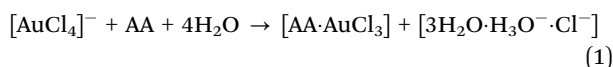
All structures were fully optimized by density functional theory (DFT) calculations employing the PBE functional<sup>94</sup> adding the atom-pair wise dispersion correction with Becke–Johnson damping (D3).<sup>95</sup> Frequency calculations were carried out at the same level of theory to check the nature of critical points of the potential energy surface, finding only real frequencies for the minima. In such structures, single point calculations were performed at the MP2<sup>96</sup> level of theory. In the manuscript, we provided the MP2 energies obtained using optimized DFT geometries. The atoms belonging to the amino acids (*i.e.*, N, C, O, H, S, and Se) and chlorine atoms were treated with the Karlsruhe basis set def2TZVP, and the Los Alamos electron core potential LanL2DZ was used to describe the metal center Au.<sup>97</sup> This computational procedure has been reported to accurately treat the interaction of amino acids with metals such as silver and gold,<sup>56</sup> reproducing the experimental findings.<sup>98</sup> To take into account the presence of the water media on the molecular structures and energetics, we used the integral equation



formalism polarizable continuum model (IEFPCM) approach,<sup>99</sup> employing water as a solvent. All calculations were carried out using the Gaussian16 software.<sup>100</sup> Cartesian coordinates of all the molecular structures can be found in the ESI.†

The interaction of the Au(III) species, here investigated in the tetrachloroaurate ion  $[\text{AuCl}_4]^-$  form, with the amino acid (AA) leads to the formation of the  $[\text{AA}\cdot\text{AuCl}_3]$  complex. If the AA contains a deprotonable site, the formation of  $[\text{AA}\cdot\text{AuCl}_3]$  generates a hydrochloric acid molecule. Being in water media, HCl dissociates. As previously reported, the dissociation of HCl is observed computationally only considering a minimum of four water molecules.<sup>101</sup> Therefore, four molecules of  $\text{H}_2\text{O}$  were explicitly added to reliably reproduce the behavior of the acid in water solution.

Overall, the equilibrium considered throughout this work for a deprotonable AA is described in eqn (1):



And the corresponding binding affinity  $\Delta E_{\text{binding}}$  for the formation of  $[\text{AA}\cdot\text{AuCl}_3]$ , taking into account the energies at MP2 level of theory, is calculated by eqn (2):

$$\Delta E_{\text{binding}} = \{E[\text{AA}\cdot\text{AuCl}_3] + E[3\text{H}_2\text{O}\cdot\text{H}_3\text{O}^+\cdot\text{Cl}^-]\} - E[\text{AuCl}_4]^- + E(\text{AA}) + E(4\text{H}_2\text{O}) \quad (2)$$

In these cases, where the amino acid does not possess any deprotonable groups, eqn (3) is valid:



And the corresponding  $\Delta E_{\text{binding}}$  is obtained by eqn (4):

$$\Delta E_{\text{binding}} = \{E[\text{AA}\cdot\text{AuCl}_3] + E[4\text{H}_2\text{O}\cdot\text{Cl}^-]\} - E[\text{AuCl}_4]^- + E(\text{AA}) + E(4\text{H}_2\text{O}) \quad (4)$$

The reduction potential of the different amino acids toward Au(III) was estimated as their tendency to donate electrons to the gold complex, calculating the charge transfer (in terms of Mulliken charges) from the amino acids to the gold complex.

## 4. Conclusions

In order to comprehend and design protein–gold interactions, broad principles that govern the binding between amino acids and Au(III) can be obtained by analysing their binding energies and tendency to oxidation.

Experimentally, it was suggested that:

- (i) cysteine and, even more, selenocysteine residues are the primary targets of the interaction of Au(III);
- (ii) histidine and, in general, nitrogen-containing residues are key groups to complex/reduce Au (III) ions;
- (iii) oxygen-containing residues have a fundamental role in the complex formation/reduction of Au(III);
- (iv) aromatic residues are critical for the formation and stabilization of the Au nanoclusters in proteins;
- (v) a basic pH (10–12) is usually needed to favor the formation of Au nanoclusters in proteins;

(vi) the reduction of Au(III) can be activated by external reducing agents (*i.e.*,  $\text{NaBH}_4$ ) or directly by the protein amino acid residues.

The calculations provided in this paper address the different findings in a comprehensive manner:

(i) selenocysteine and cysteine are the two amino acids that undergo higher interaction with Au(III) due to the natural affinity of sulfur/selenium atoms to gold. In particular, selenocysteine is usually favored over cysteine because at physiological pH selenocysteine is virtually entirely ionized to a selenolate, while cysteine is mostly in the thiol state;

(ii) histidine possesses a free nitrogen lone pair, characterized by high coordination ability to bind Au(III). In cases when the lone pair of the nitrogen is unavailable because of protonation, N–H deprotonation easily occurs upon Au(III) binding, releasing the lone pair for the Au(III) coordination;

(iii) amino acid residues such as aspartate and glutamate hold a negative charge enabling them to electrostatically interact with the positive Au(III), while residues such as serine and threonine have an hydroxyl group in which the free lone pair of oxygen enables them to coordinate with the Au(III) ion;

(iv) aromatic residues such as tyrosine and tryptophan strongly interact with Au(III) with nucleophilic carbon atoms belonging to their aromatic system, resulting in soft–soft interactions;

(v) a basic pH (10–12) determines the deprotonation of cysteine, lysine and tyrosine in proteins, strongly increasing the binding affinity of Au(III) toward these amino acids;

(vi) many residues are able to donate, upon binding, an electron to Au(III), triggering its reduction without the need for external reducing agents.

The present data and discussion provide an essential platform to (i) fully comprehend the forces at play in the interaction between proteins and Au(III) and (ii) improve our capacity to engineer such complexes; and (iii) predict in advance the structure and strength of Au(III) adsorption on amino acids. They can be applied to (i) decipher the driving factors in the interaction of peptides/proteins with Au(III) drugs and understand their pharmacological mechanism and (ii) design protein pockets to synthesize AuNCs with desired properties. The complexation of Au(III) with amino acids is generally favourable thermodynamically; however, kinetic aspects should be clarified in future works, since processes such as ligand exchange, side chain deprotonation, and solvation and desolvation of the gold ion and the coordination sphere are crucial steps in the dynamics of the complexation process.

## Conflicts of interest

There are no conflicts to declare.

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