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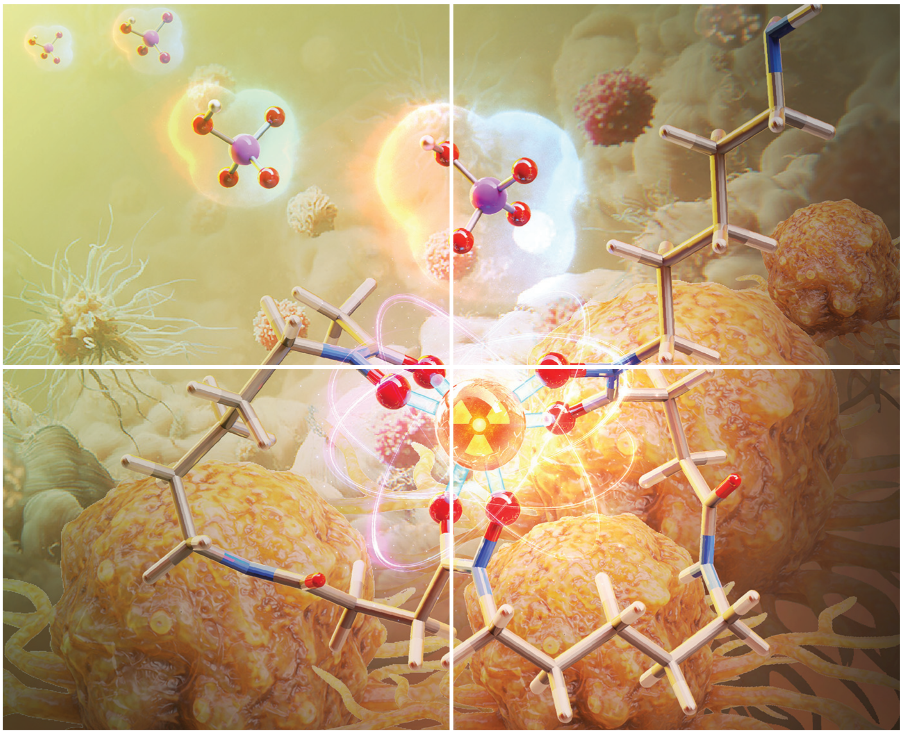

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RESEARCH ARTICLE

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Synergistic coordination in ^{89}Zr–DFO (deferoxamine) complexes: computational and experimental insights into auxiliary ligands†

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This study combines computational and experimental methods to investigate how auxiliary ligands enhance thermodynamic stability in hexacoordinate ^{89}Zr–DFO (deferoxamine) complexes. Strong electrostatic interactions favour HPO_4^{2-} over H_2O, Cl^-, CO_3^{2-} and $C_2O_4^{2-}$, indicating superior stability for advanced medical diagnostics and treatment applications.

The potential applications of the radioisotope ^{89}Zr ($t_{1/2}$ = 78.4 h, β^+ = 22.8%, $E_{\beta^+\text{max}}$ = 901 keV) in medical imaging and therapy have attracted significant attention.[1–11] Deferoxamine (DFO), known for its excellent biocompatibility, is widely used as a hexadentate chelator in clinical and preclinical trials for binding with ^{89}Zr.[1,10–23] However, due to the large ionic radius and octahedral coordination preference of ^{89}Zr,[12,24–37] the Zr^{IV}–DFO hexadentate chelate often experiences *in vivo* demetallation, potentially affecting bone uptake,[28,33,38] bone marrow radiation dose, and nuclear medicine diagnostics precision.[12,31,39–42] To address DFO's incomplete coordination, auxiliary ligands, including water molecules and other anions in the solution, fill vacant coordination sites. Additional research indicates that these auxiliary ligands directly affect the spectroscopic, magnetic, and electronic structure properties of the complex. Moreover, they can alter the oxidation state of the central atom.[43–51] Therefore, a systematic investigation of interactions between $^{89}Zr^{IV}$–DFO complexes and auxiliary ligands not only reveals the essence of these interactions but also emphasizes the crucial role of auxiliary ligands in competing for coordination positions.

Small molecules or ions, with minimal susceptibility to steric repulsion effects, efficiently bind into the first coordination sphere of metal complexes, demonstrating remarkable specificity in these interactions.[45,46,52] As a result, this work systematically characterizes a range of DFO aqueous complexes that may form during the synthesis of ^{89}Zr-labeled chelators,[40,53,54,69] involving tetravalent Zr^{IV} coordinated with both monodentate and bidentate ligands. These auxiliary ligands that form such complexes are commonly present under typical experimental conditions, such as H_2O (solvent) Cl^- (for ion exchange or elution), $C_2O_4^{2-}$ (for complexation), and CO_3^{2-} and HPO_4^{2-} (as buffer components).[55–59,69]

All calculations were performed using density functional theory at the PBE-D3/TZ2P level; solvent effects were incorporated into all calculations using the conductor-like screening solvation model (COSMO), as detailed in Part 1 of ESI.† The study comprehensively assesses thermodynamic stability and intrinsic coordination mechanism through simulations of interactions between auxiliary ligands and Zr^{IV}–DFO complexes. Experimentally, thermodynamic stability sequences were analyzed using radio-thin layer chromatography (radio-TLC) combined with high-performance liquid chromatography (HPLC), as detailed in Part 1 of ESI.† This dual approach elucidates the roles of different auxiliary ligands, providing insights into the coordination dynamics and stability of Zr^{IV}–DFO complexes.

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† Electronic supplementary information (ESI) available: Method details, eight possible geometrical isomer structures, optimized geometries, geometric parameters, comparison of structural parameters, discussion of the optimized geometry of the aquo complex, complex formation reactions, EDA-NOCV deformation densities of complexes, and Cartesian coordinates. See DOI: **https://doi.org/10.1039/d5qi00879d**

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We studied the eight possible geometric isomers of DFO,[60] where the most stable structure was used for the subsequent studies (Λ-*N-cis*, *cis*; see Part 2 of the ESI†). Initially, the Zr^{IV}–DFO complex, atomic plane 1 (green in Fig. 1a), consisting of O atoms from three N–O bonds, and atomic plane 2 (grey in Fig. 1a), consisting of O atoms from three C–O bonds, exhibits a shape reminiscent of a trigonal antiprism (TAP). This arrangement creates additional space for the complementary coordination of auxiliary ligands. Concurrently, Fig. 1b illustrates the octacoordinated structure of Zr^{IV} (see Part 3 of ESI† for details), resembling a square antiprism (SAP). Upon introducing auxiliary ligands (Fig. 1c) into Zr^{IV}–DFO, anionic ligands with high electronegativity (compared to the H_2O ligand) selectively occupy the synergic coordination (SC) 1 and 2 positions within the octahedral coordination sphere (depicted in Fig. 1b). This not only induces an expansion of the first coordination sphere but also leads to an increase in the twist angles ω_1 and ω_2 (see Part 4 of ESI†). These findings imply that the integration of auxiliary ligands may improve the thermodynamic stability of the chelate complex, which has been reported in previous studies.[12,61] Furthermore, the computed bond lengths of the Zr^{IV}–DFO complex closely correspond to previously reported values,[7,12,35,61–63] providing robust validation for the reliability of our methodology (see Part 5 of the ESI†).

Intriguingly, the introduction of water molecules unfolds in a unique way. Occupying the first coordination site, one water molecule impedes subsequent entry due to repulsion from the electron density shield formed by its oxygen atoms and those in DFO.[5,12,24,27,61] Consequently, access to the first coordination layer becomes challenging, and it has also been mentioned in previous literature that there is at least a very tight bound intrabulbar water in the complex of Zr^{IV}–DFO and H_2O,[27,61,64] which is consistent with the formation of a dynamical fluctuating $[Zr^{IV}(DFO)(^{in}H_2O)(^{out}H_2O)]^+$ complex with a relatively 'loose' 7-/8-coordination (see Part 6 of ESI† for details). This inclination facilitates easy exchange between the complex and other solvent-coordinated molecules.

Energy decomposition analysis (EDA) combined with the natural orbitals for chemical valence (NOCV) method was

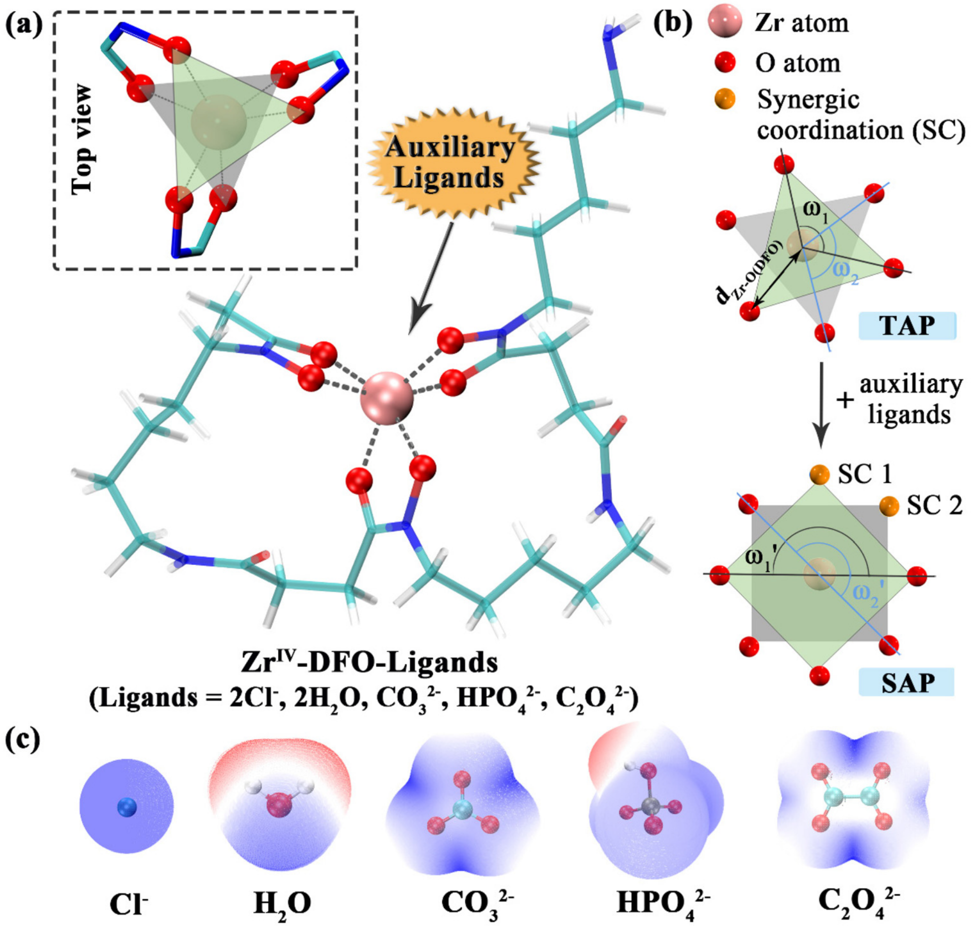

Fig. 1 (a) Optimized geometry of Zr^{IV}–DFO complex and top view of its hexacoordinate sites. (b) Schematic diagram of the transition of Zr^{IV} from hexa-coordinate to octa-coordinate configuration upon the addition of auxiliary ligands. The torsion angles ω_1, ω_2, ω'_1 and ω'_2 define the angles between the corresponding atoms in atomic plane 1 (P1) and atomic plane 2 (P2), respectively. Pink: Zr^{IV}; red: O; orange: synergic coordination (SC) atoms. (c) Electrostatic potential maps of mono- and bidentate ligands. Blue surface: electronegative regions; red surface: electropositive regions.

employed to delve into the chemical bonding properties. The EDA results indicate that the ΔE_{int} values for the anionic (auxiliary ligands) complexes (−232.17 to −288.72 kcal mol^{-1}) are significantly more negative than that of the water molecular complex (−28.79 kcal mol^{-1}), suggesting much stronger interactions (see Part 7 of ESI†). EDA analysis further reveals that electrostatic interaction (ionic bonds) are consistently dominant, with orbital interactions (covalent bonding) as secondary.

The EDA–NOCV calculations allow for a further breakdown of ΔE_{orb} into pairwise orbital interactions (see Fig. 2).[65,66] The results show that the pairwise orbital interactions in the $[\text{Zr}^{\text{IV}}(\text{DFO})(\text{HPO}_4)]^-$ and $[\text{Zr}^{\text{IV}}(\text{DFO})(\text{CO}_3)]^-$ complexes (−45.74 and −46.02 kcal mol^{-1}) are more negative than those in the other complexes, indicating more intense charge transfer. The $\Delta\rho_1$ of $[\text{Zr}^{\text{IV}}(\text{DFO})(\text{HPO}_4)]^-$ and $[\text{Zr}^{\text{IV}}(\text{DFO})(\text{CO}_3)]^-$ complexes primarily involves the donation of electrons from the nonbonding molecular orbitals (NBMO) to the 4d/5s shell orbitals of Zr^{IV}. Similar to actinyl(VI) complexes, small synergistic ligands with partial π bonding significantly influence the coordination, supporting our conclusions.[44] Conversely, the pairwise orbital interactions in $[\text{Zr}^{\text{IV}}(\text{DFO})(^{\text{in}}\text{H}_2\text{O})(^{\text{out}}\text{H}_2\text{O})]^+$ (−15.64 kcal mol^{-1}) are the weakest. Information on other contributions $\Delta\rho$ are given in Part 7 of ESI.†

Further, the relative thermodynamic stabilities of the auxiliary ligands binding to Zr^{IV}–DFO were evaluated through quantum chemical calculations (see Table 1 and Parts 1 and 8 of ESI†). The results from complex formation and ligand substitution reactions indicate that when an anion undergoes synergistic coordination, the thermodynamic stability of the complex exceeds that of the corresponding aqueous complex. Specifically, the sequence is as follows: HPO_4^{2-} (−107.98 kcal mol^{-1}) > CO_3^{2-} (−25.35 kcal mol^{-1}) > $\text{C}_2\text{O}_4^{2-}$ (−20.65 kcal mol^{-1}) > Cl^- (−13.95 kcal mol^{-1}) > H_2O (≈0 kcal mol^{-1}, due to water acting as both the solute and solvent molecules). Notably, the stability of HPO_4^{2-} binding to Zr^{IV}–DFO is significantly superior. Further theoretical simulations of the complex stability constant (logβ) (see Part 1 of ESI† for the calculation details), yielded results consistent with the thermodynamic stability analysis (see Part 8 of ESI†). To investigate the source of the enhanced stability of the $[\text{Zr}^{\text{IV}}(\text{DFO})(\text{HPO}_4^{2-})]^-$ complex, we analyzed the bonding interactions between the auxiliary ligand and Zr^{IV}–DFO using bond critical points (BCPs) from the quantum theory of atoms in molecules (QTAIM). Our analysis revealed that the HPO_4^{2-} ligand forms additional hydrogen bonds with H atoms on the DFO chain (C–H⋯O$_{86}$: 1.96 Å; C–H⋯O$_{87}$: 1.98 Å, further details are provided in Part 3 of the ESI†). These additional hydrogen bonds may contribute to the higher thermodynamic stability of the $[\text{Zr}^{\text{IV}}(\text{DFO})(\text{HPO}_4^{2-})]^-$ complex compared to the other complexes. In contrast, for CO_3^{2-}, $\text{C}_2\text{O}_4^{2-}$, and Cl^-, the interactions with Zr^{IV} are predominantly driven by direct coordination, with little to no hydrogen bonding.

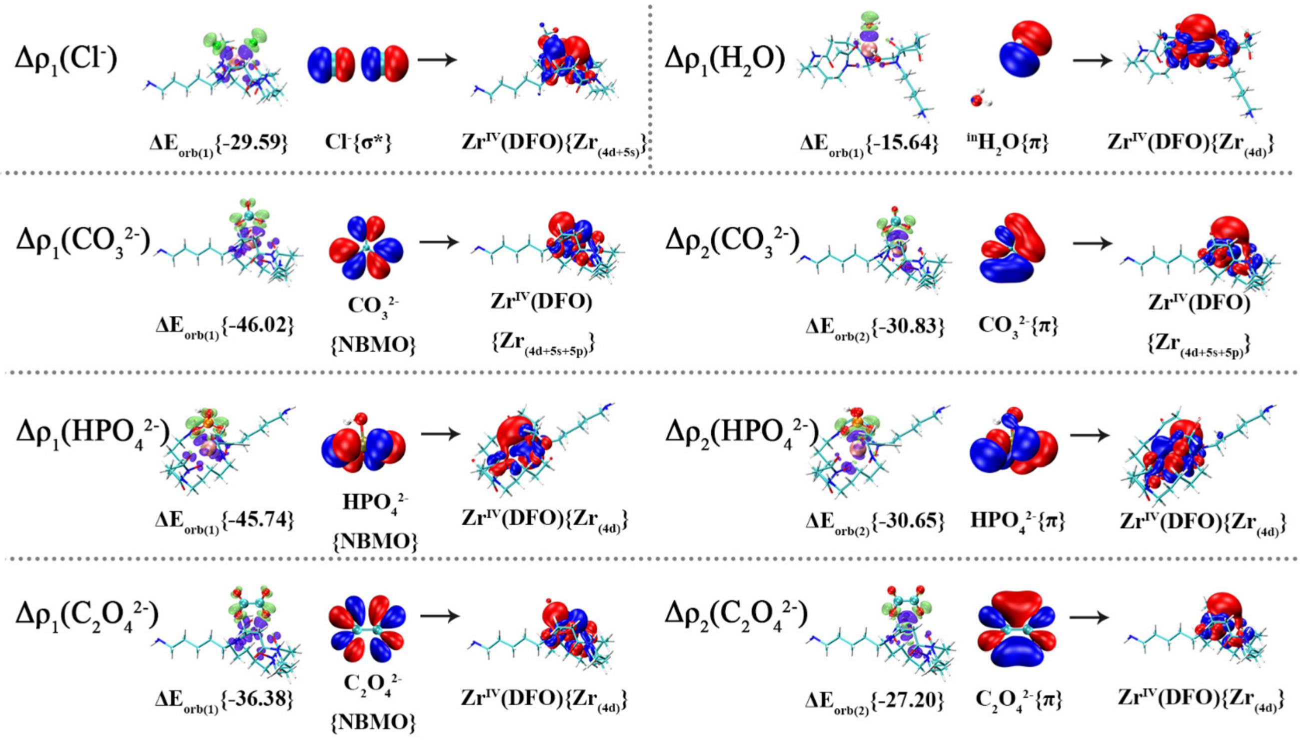

Fig. 2 Plots of EDA–NOCV deformation densities $\Delta\rho$ (isovalue = 0.0015) of the pairwise orbital interactions and the associated fragment molecular orbitals for the different forms of interacting fragments. Energy values for each interaction are enclosed in brackets (kcal mol^{-1}). The charge flow direction is depicted from green to purple. The labels π, NBMO and σ* represent π-bonding, non-bonding and σ-antibonding molecular orbitals, respectively.

Table 1 Gibbs free energy (kcal mol^{-1}) of complex formation reaction and ligand substitution reactions in aqueous solution obtained at PBE-D3/TZ2P levels

Complex formation reactions	Gibbs free energies (ΔG)
$[Zr^{IV}(DFO)]^+ + 2Cl^- \rightleftharpoons [Zr^{IV}(DFO)(Cl)_2]^-$	−13.95
$[Zr^{IV}(DFO)]^+ + CO_3^{2-} \rightleftharpoons [Zr^{IV}(DFO)(CO_3)]^-$	−25.35
$[Zr^{IV}(DFO)]^+ + C_2O_4^{2-} \rightleftharpoons [Zr^{IV}(DFO)(C_2O_4)]^-$	−20.65
$[Zr^{IV}(DFO)]^+ + HPO_4^{2-} \rightleftharpoons [Zr^{IV}(DFO)(HPO_4)]^-$	−107.98
Ligand substitution reactions	**Gibbs free energies ($\Delta\Delta G$)**
$[Zr^{IV}(DFO)(^{in}H_2O)(^{out}H_2O)]^+ + 2Cl^- \rightleftharpoons [Zr^{IV}(DFO)(Cl)_2]^- + 2H_2O$	−13.65
$[Zr^{IV}(DFO)(^{in}H_2O)(^{out}H_2O)]^+ + CO_3^{2-} \rightleftharpoons [Zr^{IV}(DFO)(CO_3)]^- + 2H_2O$	−25.05
$[Zr^{IV}(DFO)(^{in}H_2O)(^{out}H_2O)]^+ + C_2O_4^{2-} \rightleftharpoons [Zr^{IV}(DFO)(C_2O_4)]^- + 2H_2O$	−20.35
$[Zr^{IV}(DFO)(^{in}H_2O)(^{out}H_2O)]^+ + HPO_4^{2-} \rightleftharpoons [Zr^{IV}(DFO)(HPO_4)]^- + 2H_2O$	−107.68
$[Zr^{IV}(DFO)(Cl)_2]^- + CO_3^{2-} \rightleftharpoons [Zr^{IV}(DFO)(CO_3)]^- + 2Cl^-$	−11.40
$[Zr^{IV}(DFO)(Cl)_2]^- + C_2O_4^{2-} \rightleftharpoons [Zr^{IV}(DFO)(C_2O_4)]^- + 2Cl^-$	−6.70
$[Zr^{IV}(DFO)(Cl)_2]^- + HPO_4^{2-} \rightleftharpoons [Zr^{IV}(DFO)(HPO_4)]^- + 2Cl^-$	−94.03
$[Zr^{IV}(DFO)(CO_3)]^- + C_2O_4^{2-} \rightleftharpoons [Zr^{IV}(DFO)(C_2O_4)]^- + CO_3^{2-}$	4.70
$[Zr^{IV}(DFO)(CO_3)]^- + HPO_4^{2-} \rightleftharpoons [Zr^{IV}(DFO)(HPO_4)]^- + CO_3^{2-}$	−82.63
$[Zr^{IV}(DFO)(C_2O_4)]^- + HPO_4^{2-} \rightleftharpoons [Zr^{IV}(DFO)(HPO_4)]^- + C_2O_4^{2-}$	−87.33

To date, numerous studies have shown that the radio-thin layer chromatography (radio-TLC) combined with high-performance liquid chromatography (HPLC) can effectively evaluate the purity, concentration, and *in vitro* stability of [^{89}Zr]Zr-labeled radiopharmaceuticals.[40,55–58,67,68] Based on this, we prepared fresh [^{89}Zr]Zr–DFO–CO_3^{2-} and [^{89}Zr]Zr–DFO–HPO_4^{2-}

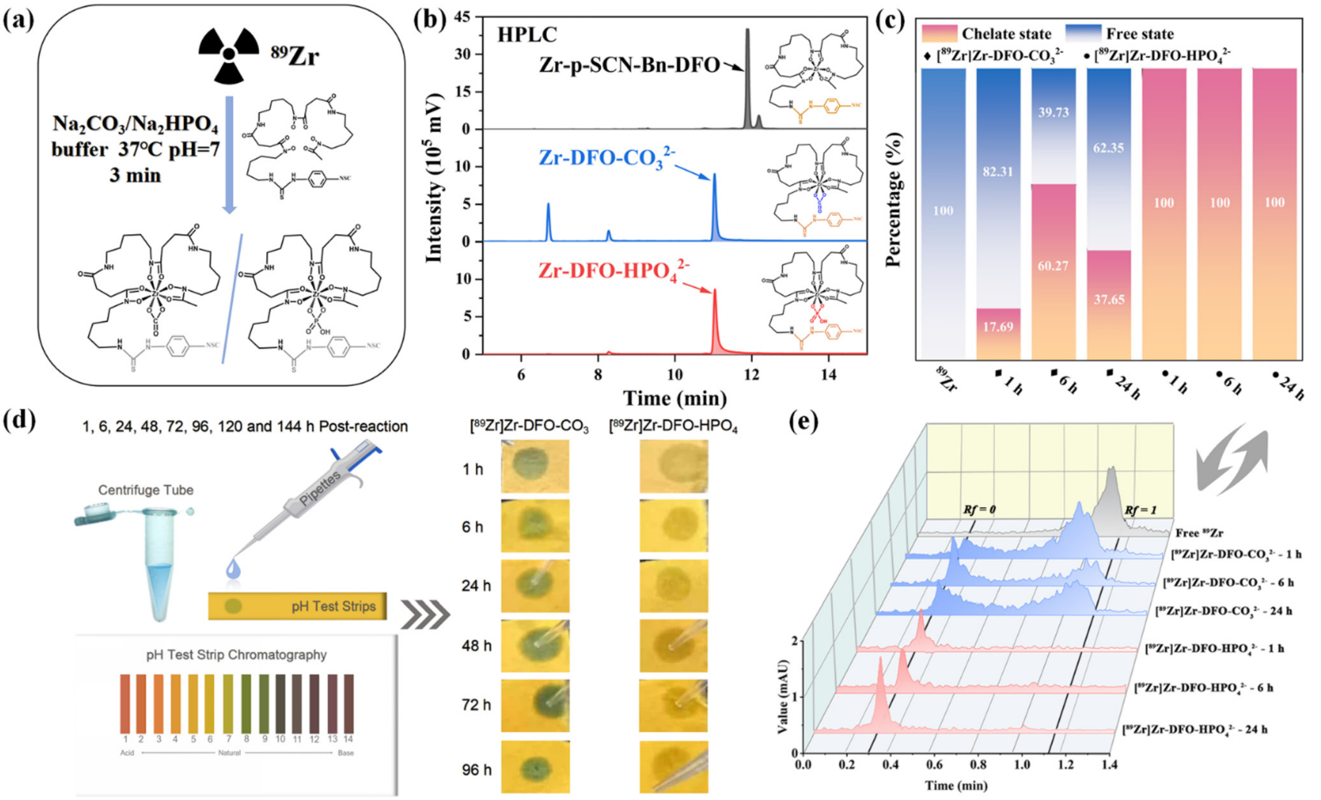

Fig. 3 Labeling and *in vitro* stability of [^{89}Zr]Zr–DFO–ligand complexes. (a) Labeling of DFO and auxiliary ligands (HPO_4^{2-} and CO_3^{2-}) with ^{89}Zr. (b) HPLC results for cold references Zr–DFO–ligand and Zr–DFO. (c) Percentage area plot from radio-TLC analysis of [^{89}Zr]Zr–DFO–ligand complexes. Blue and pink bars represent peak areas corresponding to free and chelated ^{89}Zr, respectively. The square and circle symbols represent [^{89}Zr]Zr–DFO–CO_3^{2-} and [^{89}Zr]Zr–DFO–HPO_4^{2-}. (d) pH changes for [^{89}Zr]Zr–DFO–ligand complexes over 144 hours. (e) Radio-TLC results of [^{89}Zr]Zr–DFO and [^{89}Zr]Zr–DFO–ligand complexes. Gray, blue, and red peaks correspond to free ^{89}Zr, [^{89}Zr]Zr–DFO–CO_3^{2-} and [^{89}Zr]Zr–DFO–HPO_4^{2-} complexes, respectively.

complexes to validate the stability sequence ($HPO_4^{2-} > CO_3^{2-}$). Initially, ^{89}Zr was produced using a cyclotron, followed by separation, purification, and elution. The radiochemical purity of purified ^{89}Zr exceeded 90% (see Part 9 in ESI†). Alkaline solutions of Na_2CO_3 and Na_2HPO_4, along with *p*-SCN–Bn–DFO, were then added to neutralize the mixture to pH 7. The [^{89}Zr]Zr–DFO–CO_3^{2-} and [^{89}Zr]Zr–DFO–HPO_4^{2-} complexes were successfully prepared by mixing at 37 °C for 60 minutes, as shown in Fig. 3a. A more detailed preparation process is provided in Part 1 of ESI.†

The radiolabeled yield of the complex was measured using radio-TLC (Fig. 3c and e). For complex systems, specific labeled complexes are typically identified by measuring retention factor (R_f) values ranging from 0 to 1. Free ^{89}Zr (unbound $R_f \approx 1$) served as a control (grey area). The [^{89}Zr]Zr–DFO–CO_3^{2-} complex shows two peaks (bound $R_f \approx 0$ and unbound $R_f \approx 1$), while the [^{89}Zr]Zr–DFO–HPO_4^{2-} complex shows only one peak ($R_f \approx 0$). Further analysis revealed that the peak area ratios of the [^{89}Zr]Zr–DFO–CO_3^{2-} system did not increase linearly over 1, 6, and 24 h (Fig. 3c). The observed decrease in chelation content after 6 h suggests several important trends, as shown in our pH data (Fig. 3d): (1) significant pH changes, particularly in the [^{89}Zr]Zr–DFO–CO_3^{2-} system, may be due to the decomposition of carbonic acid. (2) After 6 hours, the pH of the complex solution showed an inverse correlation with the [^{89}Zr]Zr–DFO–CO_3^{2-} complex activity: higher pH values corresponded to lower complex activity. These observations suggest that two main complex forms exist in solution: [^{89}Zr]Zr–DFO and [^{89}Zr]Zr–DFO–CO_3^{2-}. The gradual loss of carbonate ions over time likely contributes to the observed decrease in [^{89}Zr]Zr–DFO–CO_3^{2-} complexation.

On the other hand, a small amount of $ZrCl_4$ and alkaline solutions (Na_2CO_3 and Na_2HPO_4) were reacted with excess DFO in water for 60 min under neutral conditions to prepare the nonradioactive Zr–DFO, Zr–DFO–CO_3^{2-}, and Zr–DFO–HPO_4^{2-} complexes at room temperature. The peaks in HPLC correspond to individual components, identified by their retention time. The results indicated that the retention times of Zr–DFO, Zr–DFO–CO_3^{2-}, and Zr–DFO–HPO_4^{2-} were 11.90, 11.03, and 11.04 minutes, respectively, confirming the identity of each complex (Fig. 3b). These finding highlight HPO_4^{2-}'s superior complexation capacity (thermodynamic stability) with ^{89}Zr–DFO over time. In addition, at 37 °C, [^{89}Zr]Zr–DFO–CO_3^{2-} and [^{89}Zr]Zr–DFO–HPO_4^{2-} were stable in human serum over 7 days. The results are shown in Part 10 of ESI.† In summary, the thermodynamic stability of ^{89}Zr–DFO complexes was rigorously evaluated *via* radio-TLC and HPLC, conclusively demonstrating that the [^{89}Zr(DFO)HPO_4]$^-$ complex exhibits superior stability compared to other complexes, consistent with theoretical predictions discussed above.

Conclusions

In summary, intermolecular electrostatic interactions, specifically ionic bonding, play a crucial role in determining the character, such as the structure and thermodynamic stability, of the complexes. Both theoretical and experimental evidence confirm that HPO_4^{2-} significantly improves the stability of complexes through its unique coordination pattern and hydrogen bonding interactions compared to alternative ligands such as CO_3^{2-}, showing good radiolabelling efficiency and *in vitro* stability (7 day integrity in serum). Considering the impact of different coordinating molecules on the octahedral complex of ^{89}Zr and practical factors such as the thermal stability of the complexes and subsequent modifications, we believe that this study will advance the development of highly stable chelating agents for ^{89}Zr, supporting precise quantitative applications of biomarkers such as immune positron emission tomography (PET) and prostate-specific membrane antigen (PSMA).

Author contributions

Yang Gao, Georg Schreckenbach and Xiaoan Li: conceptualization, funding acquisition, experimental support, review and editing; Jiarui Li and Chenghe Ding: investigation, methodology, writing – original draft, software, visualization, data curation and experimental test; Lili Wen, Pingping Zhao, Zhou Lu, Rui Luo, Mingsong Shi and Zhiming Wang: investigation, discussion and data curation.

Data availability

The ESI† includes method details of theory and experiments, possible geometrical isomer structures, optimized geometries, geometric parameters, bond critical point calculations, comparison with previous structural parameters, discussion of the optimized geometry of the aqueous complex, metal ligand complexation reactions, stability constant, EDA–NOCV deformation densities of complexes, characterization of radiochemical purity, and *in vitro* stability comparisons. The geometrical coordinates involved in this work are shown in the XYZ file.

Conflicts of interest

There are no conflicts to declare.

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