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Environmental effects on the structure of metal ion-DOTA complexes: An ab initio study of radiopharmaceutical metals.

Edmond Y. Lau[†], Felice C. Lightstone[†], and Michael E. Colvin[‡]

Biosciences Directorate, Lawrence Livermore National Laboratory, Livermore, CA 94550; School of Natural Sciences, University of California, Merced CA 95344

AUTHOR EMAIL ADDRESS: mcolvin@ucmerced.edu

RECEIVED DATE:

CORRESPONDING AUTHOR FOOTNOTE:

*Corresponding author email: mcolvin@ucmerced.edu

†Lawrence Livermore National Laboratory

‡University of California, Merced

ABSTRACT:

Quantum mechanical calculations were performed to study the differences between the important radiopharmaceutical metals yttrium (Y) and indium (In) bound by DOTA and modified DOTA molecules. Energies were calculated at the MP2/6-31+G(d)//HF/6-31G(d) levels, using effective core potentials on the Y and In ions. Although the minimum energy structures obtained are similar for both metal ion-DOTA complexes, changes in coordination and local environment significantly affect the geometries and energies of these complexes. Coordination by a single water molecule causes a change in the coordination number and a change in the position of the metal ion in In-DOTA; but, Y-DOTA is hardly affected by water coordination. When one of the DOTA carboxylates is replaced by an amide, the coordination energy for the amide arm shows a large variation between the Y and In ions. Optimizations including water and guandinium moieties to approximate the effects of antibody binding indicate a large energy cost for the DOTA-chelated In to adopt the ideal conformation for antibody binding.

INTRODUCTION:

DOTA (1,4,7,10-tetraazacyclododecane-1,4,7,10-tetraacetic acid) is a commonly used macrocycle to chelate metal ions in solution (Figure 1).[1] This metal chelator can octacoordinate a metal ion with its 4 ring nitrogens and 4 carboxylate sidechains to create a tightly bound complex. The structures of many metal ion-DOTA complexes have been solved using X-ray crystallography and NMR.[2-6] These structural studies showed that metal ion-DOTA complexes exist primarily in two conformations termed antiprism and inverted antiprism geometries.[6] The lowest energy geometry for a DOTA complex is dependent on the metal ion coordinated. These complexes are highly stable, and metal lose by DOTA is minimal in solution, making them useful as magnetic resonance imaging probes when containing lanthanides.

Because of the great stability of these metal complexes, yttrium-DOTA based molecules have been used as site specific targeted therapeutic radiopharmaceuticals in attempts to deliver radionuclides to cancerous tumors.[7] Yttrium-90 is a common radionuclide (β-emitter) used for cancer therapy but it is not easily imaged within the body because of its short-range emission. The biodistribution of yttrium-90 is commonly estimated in the body using the surrogate ion indium-111 (γ-emitter) because the ionic charge for these two metals is the same (+3), and the half-life of these radionuclides is almost identical.[8] Although these ions share similarities, there are indications that the physical properties of indium chelated DOTA may not be exactly the same as yttrium chelated DOTA. Studies of a DOTA binding antibody showed the uptake of ^{90}Y-DOTA was almost 3 orders of magnitude greater than ^{111}In-DOTA.[9] Recent HPLC measurements by Liu et al has shown the lipophilicity of these metal ions bound to a

modified DOTA differ along with the solution equilibrium as determined by NMR.[10] Because of the critical importance in accurately knowing the biodistribution of radionuclides within the body and their localization within tissue,[11] a computational study was performed to better understand the similarities and differences between Y-DOTA and In-DOTA and their modified complexes.

METHOD:

All calculations were performed using the programs Gaussian98 and Gaussian03.[12,13] Full geometry optimizations of the metal ion-DOTA complexes were performed at the Hartree-Fock level of theory. The Hay-Wadt LANL2DZ effective core potential (ECP) was used for the yttrium and the indium ions (calculated in their +3 state), and the 6-31G(d) basis set was used for all other atoms.[14-16] This combination of methods was found to give structures in good agreement with experiment.[17,18] The energies of the complexes were obtained from MP2/6-31+G(d) single point calculations using the optimized Hartree-Fock geometries (MP2/6-31+G(d)//HF/6-31G(d)). Harmonic frequency calculations were performed on all the optimized geometries. The zero-point vibrational energies (ZPE) from the frequency calculations were scaled by 0.893.[19]

RESULTS AND DISCUSSION:

Metal ion-DOTA complexes:

There are two minimum energy geometries apiece for the metal ion-DOTA complexes (Figure 2). The lowest energy conformer is the antiprism geometry (denoted

A) and the higher energy conformer is the inverted antiprism geometry (denoted IA) for both yttrium and indium DOTA complexes. Both conformations have been characterized by solution NMR for lanthanides bound by DOTA and the energy difference between the A and IA conformation can be reversed depending on the metal.[6,20] The metal ions are octacoordinated by one oxygen from each of the four carboxylate sidechains and the four nitrogens in the DOTA ring. The difference in the A and IA structures is due to the orientation of the carboxylate sidechains of DOTA. The change in sidechain orientation affects the coordination of the metals. Although the metal ion-O distances are relatively unchanged when going from the A to IA structure, the metal ion-N distances increases by 0.03 and 0.07 Å for yttrium and indium, respectively (Table 1). The energy difference between the A and IA structures are 3.66 and 5.60 kcal/mole for yttrium and indium bound DOTA, respectively. The overall structures of Y-DOTA and In-DOTA are similar, and the most significant difference is in the metal ion-oxygen distances. For both the A and IA conformations, the In-O bond (2.07 Å) is ~0.2 Å shorter than the Y-O bond (2.27 Å) due to the smaller ionic radii of indium relative to yttrium (0.92 and 1.02 Å, respectively).[21] The bond lengths obtained for Y-O and Y-N are in good agreement with the crystal structure. The In-O bond is shorter, and the In-N bond is longer than expected from the crystal structure.[2]

Metal ion-DOTA-H_2O complexes:

In the crystal structure of Y-DOTA and other lanthanides ions bound by DOTA, the metal ion also coordinates to a single water molecule.[3,22] NMR chemical shift and XAFS measurements of metal ion-DOTA complexes also show a single water molecule

coordinated to the metal ion in solution.[23,24] A recent crystal structure of the monoclonal antibody 2D12.5 bound with a modified Y-DOTA was shown to have the yttrium coordinated by a single water molecule. Since water coordination is a common feature of many metal ion-DOTA complexes, the calculated A and IA structures were optimized with a single water molecule coordinating the metal ion and placed above the carboxylate sidechains to determine if a change in coordination affects the structures.

The calculated distance of the oxygen of water to yttrium was almost identical for the A and IA conformers, 2.519 and 2.520 Å, respectively. Theses distances are in reasonable agreement with X-ray crystallography (2.424 Å).[5] For the Y-DOTA complexes, having the additional ligand has little effect on the geometry of the complex as compared to the complex without water. This result is consistent with the crystal structure. For both calculated conformers, there is an increase in the Y-O distances for the two carboxylates interacting with the hydrogens of the water but the two other Y-O distances are almost identical to the non-water coordinating distances. The most significant change occurs in the Y-N distances. The Y-N distances increase by ~0.07 and ~0.09 Å for A and IA conformers (Table 1), respectively, relative to the Y-DOTA structures without a water. The change in the Y-N distance is due to repositioning of the yttrium within the ring structure of DOTA. In the complexes without a water coordinating, yttrium sits 1.706 and 1.777 Å above the plane formed by the nitrogens in DOTA in the A and IA conformers, respectively. When water coordinates the metal ion, the yttrium raises to 1.807 and 1.906 Å above the plane for the A and IA conformers, respectively (Figure 3).

The crystal structures of indium complexed with modified DOTAs do not have a water molecule coordinating the indium.[2,25] The calculated oxygen of water to indium distances was 2.375 and 2.328 Å for the A and IA conformers, respectively. The In-DOTA geometries are more sensitive to coordination by a water molecule. When the In-DOTA complex is not coordinated with a water molecule the indium is 1.707 and 1.827 Å above the plane formed by the nitrogens in DOTA in the A and IA conformers, respectively. When a water molecule coordinates to the indium, the metal ion raises within DOTA significantly more than in the Y-DOTA. In the A conformer, coordination of a water molecule causes the metal ion to rise to 1.979 Å above the plane. An even larger change occurs in the IA conformer with water coordination. Water coordination causes the indium to rise by almost an additional 0.5 Å above the plane (2.270 Å) relative to the non-water coordinated structure. There is little change in the In-O distances (~0.04 Å variation, see Table 1) for either conformer if water is absent or present in the complex. The change in position of indium due to water coordination within DOTA significantly weakens the In-N coordination. The In-N distances increase by ~0.22 Å and ~0.33 Å in the A and IA conformers, respectively. This change in structure is consistent with structural studies of indium showing that the preferred coordination number for this ion is 6 or 7.[26,27] The calculated change in position and coordination of indium in DOTA relative to yttrium is also [5]in agreement with experimental observations that metal lose by a modified DOTA is more rapid for indium than yttrium.[28]

Metal ion-DO3AM complexes:

Although DOTA is commonly used to chelate metal ions in chemical studies, modified versions of DOTA are used in most biological studies. It is common to have a molecule such as somatostatin analogs linked to DOTA either at one of the carboxylate sidechains or to one of the ring carbons.[29,30] A recent experimental study investigated indium and yttrium coordinated by two DOTA analogs with a linker attached to one of the carboxylate arms, 1,4,7,10-tetraaza-4,7,10-tris(carboxymethyl)-1-cyclododecylacetylbenzylamine (DOTA-BA) and 1,4,7,10-tetraaza-4,7,10-tris(carboxymethyl)-1-cyclododecylacetyl-R-(+)- α-methylbenzylamine (DOTA-MBA) This study used NMR and HPLC measurements to show that the physico-chemical properties of the complexes differ in solution depending on whether they coordinate In or Y.[10] The HPLC retention times for the metals bound to the modified DOTA-BA and DOTA-MBA were significantly different between yttrium and indium even though the overall charge of the complexes were identical. The NMR studies showed the Y bound DOTA-BA and DOTA-MBA had only one major conformer in solution but the In-bound complexes showed significant line broadening implying multiple conformers. At elevated temperatures, the NMR spectrum of the Y-bound DOTA-BA and DOTA-MBA began to resemble the indium spectrum observed at lower temperatures.

The quantum mechanical calculations for metal ion coordination by a DOTA containing a single amide sidechain (denoted DO3AM) showed the lowest energy conformers (conformation A) are similar for indium and yttrium but are in equilibrium with different higher energy conformers (Figure 4). For Y-DO3AM, the lowest energy conformer is in equilibrium with conformer IA. Although the structure is similar to Y-

DOTA, the amide carbonyl oxygen is not bound as strongly as a carboxylate leading to a Y-O distance of 2.426 Å (conformer A) that is approximately 0.2 Å longer than the Y-O distance for a carboxylate oxygen (Table 2). The In-DO3AM lowest energy conformer (conformer A) is in equilibrium with a conformer (conformer B) that has the amide arm completely extended away from indium, and an adjacent carboxylate arms has rotated 71° degrees relative to conformer A. This conformer is only slightly higher in energy (0.7 kcal/mole) relative to conformer A, and the change in coordination results in a slight contraction in the In-O (carboxylates) distances. A second conformer with the amide sidechain extended away from indium was obtained and denoted conformer IB. The difference between the B and IB structures is the carboxylate sidechain opposite the amide is rotated (see Figure 4). Conformer IB for the In-DO3AM complex is also close in energy to conformer A. Interestingly, a recent crystal structure of In-DOTA-*p*-aminoanilide (DOTA-AA) is in the IA conformation.[25] The additional phenyl group in In-DOTA-AA may form interactions that make the IA the most stable conformation in the solid state. The four calculated low energy conformers for the Y- and In-DO3AM complexes are shown in Figure 4 with their relative energies to the A structure. The four structures for In-DO3AM only differ by 3.3 kcal/mole at most from one another, and the structures with the amide arm extended away from the indium differ by ~1.0 kcal/mole from the A structure. Conversely, the B and IB structures for Y-DO3AM are over 12 kcal/mole higher in energy than conformer A making them unlikely to be found in solution. These calculations are consistent with NMR results for Y- and In-DOTA-AA that shows Y-DOTA-AA is octacoordinated in solution but In-DOTA-AA has the amide sidechain dissociated from the metal ion.[25]

Coordination of a water molecule to the A and IA structures for these modified DOTA complexes had a similar effect on the structure as in the DOTA complexes. A water molecule was able to coordinate to the metal ion for conformer B of Y-DO3AM and did not significantly change the overall structure of this complex. The change in carboxylate coordination allows the water molecule to come in closer contact with the yttrium (2.492 Å, Y-O distance) relative to Y-DOTA. Interestingly, a water molecule was not able to coordinate to indium in conformer B of In-DO3AM. The inability of water to coordinate to the indium in conformer B may be due to the metal ion being positioned more deeply within DO3AM. The In-N distances for the B structure are significantly shorter than either the A or IA structures, and indium is heptacoordinated (a preferred number this ion).

DOTA-antibody complexes:

Meares and coworkers have determined a crystal structure of the monoclonal antibody 2D12.5 binding a Y-DOTA analogue (Y-(S)HETD, linker is attached to the ring).[31] The DOTA analogue binds on the surface of the antibody in a depression and surprisingly there is a minimum of strong interactions between the antibody and DOTA. The binding site is made up mainly of tryptophans that are able to form hydrogen bonds with the carboxylates of DOTA and the methylene carbons of DOTA interact with the aromatic typtophans (Figure 5). A single salt bridge is formed between Arg98B and one of the carboxylate sidechains from DOTA. Interestingly, a single water molecule is coordinated with the yttrium and isolated from bulk solvent. This antibody is selective for Y-DOTA relative to In-DOTA. The measured binding constants showed Y-DOTA is

bound more than 100 times tighter than In-DOTA.[31,32] A better understanding of the differences of this system binding to Y-DOTA and In-DOTA could provide further insights to the differential behavior of these two molecules. Models for this system were constructed with metal ion-DOTA interacting with a single water molecule and methyl-guandinium (to represent the arginine side chain) and optimized.

Two minimum structures were found for each metal ion-DOTA model (Figure 6). The higher energy structure for the Y-DOTA model resembles the conformation found in the active site of the antibody (Figure 6A). The guandinium forms two interactions with the carboxylate (2.88 and 2.92 Å for the N-O distances) and the water oxygen is 2.56 Å from yttrium in the model system. The N-O distances are in agreement with the crystal structure although the Y-water oxygen distance is much shorter than observed in the crystal structure (2.81 Å). Although the higher energy In-DOTA model is similar to the Y-DOTA model, there is only one interaction between the guandinium and the carboxylate (2.92 Å, N-O distance). The second nitrogen of the guandinium interacts with the oxygen of water (2.90 Å, N-O distance) causing the water to be no longer coordinated with the indium (3.43 Å, In-O distance). Although these structures resemble the conformation found in the crystal structure a lower energy structure was found for these models. The low energy structure for both metal ion-DOTA complexes has the methyl-guandinium coordinated to two carboxylates and positioned flat against DOTA (Figure 6B,6D). This orientation also displaces the water molecule from the metal, leaving it no longer coordinated (4.11 and 4.22 Å, Y-O and In-O distances, respectively) but instead interacting with the methyl-guandinium. Although the structures obtained for Y-DOTA and In-DOTA from ab initio calculations have similar conformations, the

energy barrier separating the low energy and high energy structures differ significantly. For Y-DOTA, there is a 3.29 kcal/mole difference in energy between the structures. A larger barrier of 10.19 kcal/mole between the In-DOTA structures was obtained. One difference in the structures is that In-DOTA only forms one interaction with the methyl-guandinium and the other guandinium interaction is with the water molecule. Both oxygens of the carboxylate from Y-DOTA forms interactions with the methyl-guandinium and closely resemble the conformation found in the crystal structure. The ~3 kcal/mole difference in energy for the Y-DOTA structures likely makes binding of the Y-DOTA slow but at a reasonable level. The much larger 10 kcal/mole energy difference for the In-DOTA structures makes binding much less likely and is reflected in the measured binding difference between these DOTA complexes with this antibody.

CONCLUSIONS:

These ab initio calculations have provided insight into differences between yttrium and indium DOTA complexes. Y-DOTA and In-DOTA share similar structures, but as the coordination and ligands change In-DOTA is more affected than Y-DOTA. Calculations with even a minimal model for the active site of a DOTA binding antibody shows there are differences in both conformation and energetics between Y- and In-DOTA. Although it has been shown that in certain cases ^{90}Y- and ^{111}In-DOTA conjugated complexes are different but are biologically equilvalent in tumor uptake and tissue distribution.[33] But, recognition of In- and Y-DOTA complexes may differ as seen in the 2D15.2 antibody. This study has shown that In-DOTA may not always be

structurally similar to Y-DOTA and care should be taken when interpreting results from one metal ion to the other.

ACKNOWLEDGEMENTS:

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FIGURE LEGENDS:

Figure 1. Molecular structure of 1,4,7,10-tetraazacyclododecane-1,4,7,10-tetraacetic acid (DOTA).

Figure 2. Molecular structure of the two calculated low energy conformations of DOTA coordinating yttrium.

Figure 3. Picture showing the effect of water coordination on In-DOTA. Panel (A) shows the A conformation and panel (B) shows the same DOTA conformation with water coordinated to the indium. The box represents the plane formed by the nitrogens in the DOTA ring. The values are the indium to plane distances.

Figure 4. Optimized conformations for metal ion-DO3AM. The ΔE values are the energy difference relative to the A structure.

Figure 5. Picture of Y-(S)HETD (modified DOTA compound) in the active site of the 2D12.5 monoclonal antibody (PDB ID 1NC2).

Figure 6. Minimum energy structures of Y- and In-DOTA interacting with water and methyl-guandinium. Panels (A) and (C) show conformations similar to the crystal structure for Y- and In-DOTA, respectively. Panels (B) and (D) show the minimum energy structures for the model systems of Y- and In-DOTA, respectively.

Figure 1:

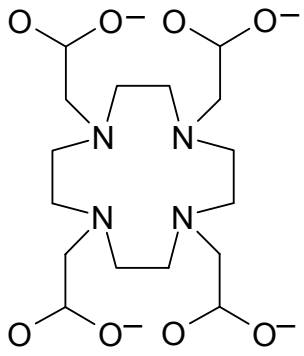
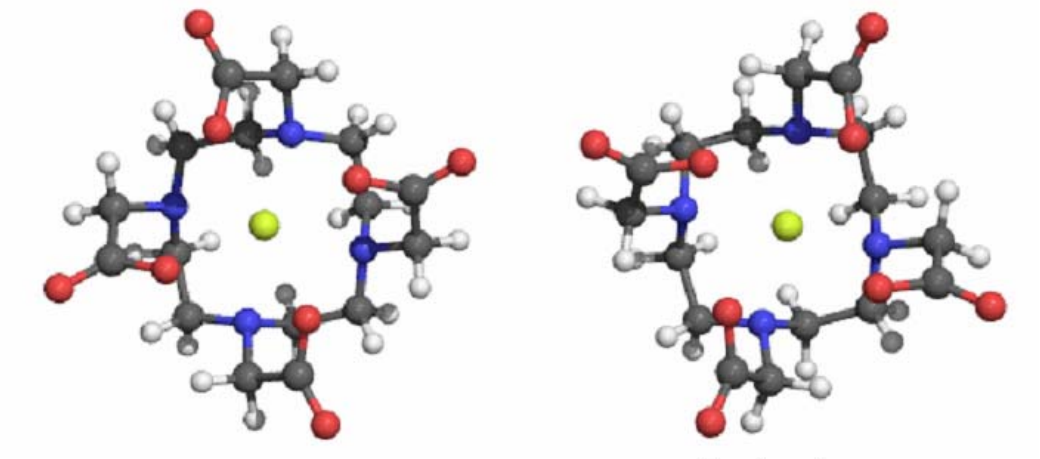


Figure 2:



A structure IA structure

Figure 3:

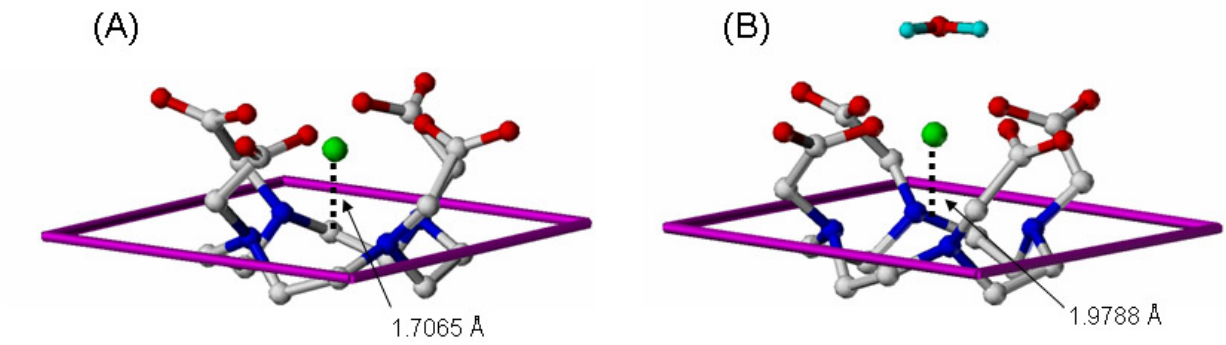


Figure 4:

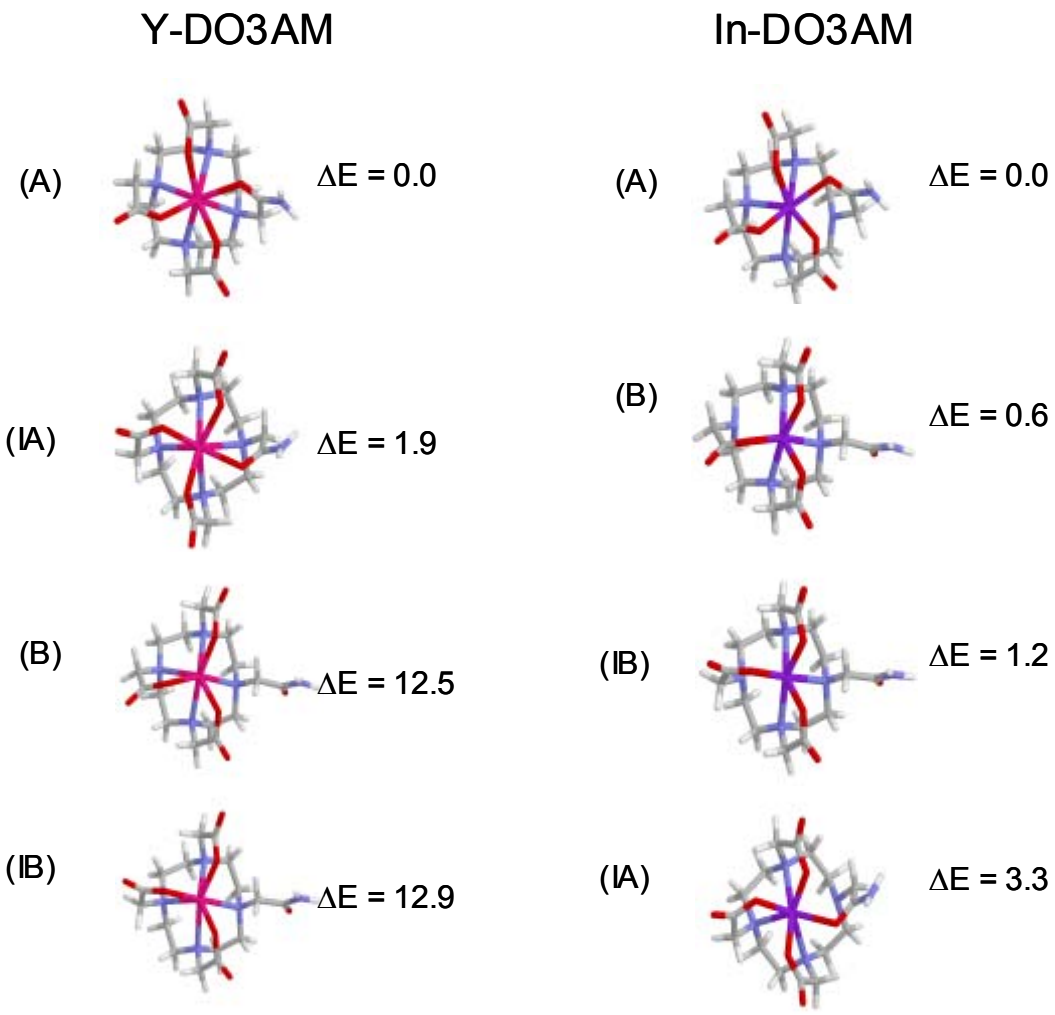

Y-DO3AM
In-DO3AM
(A)
ΔE = 0.0
(IA)
ΔE = 1.9
(B)
ΔE = 12.5
(IB)
ΔE = 12.9
(A)
ΔE = 0.0
(B)
ΔE = 0.6
(IB)
ΔE = 1.2
(IA)
ΔE = 3.3

Figure 5:

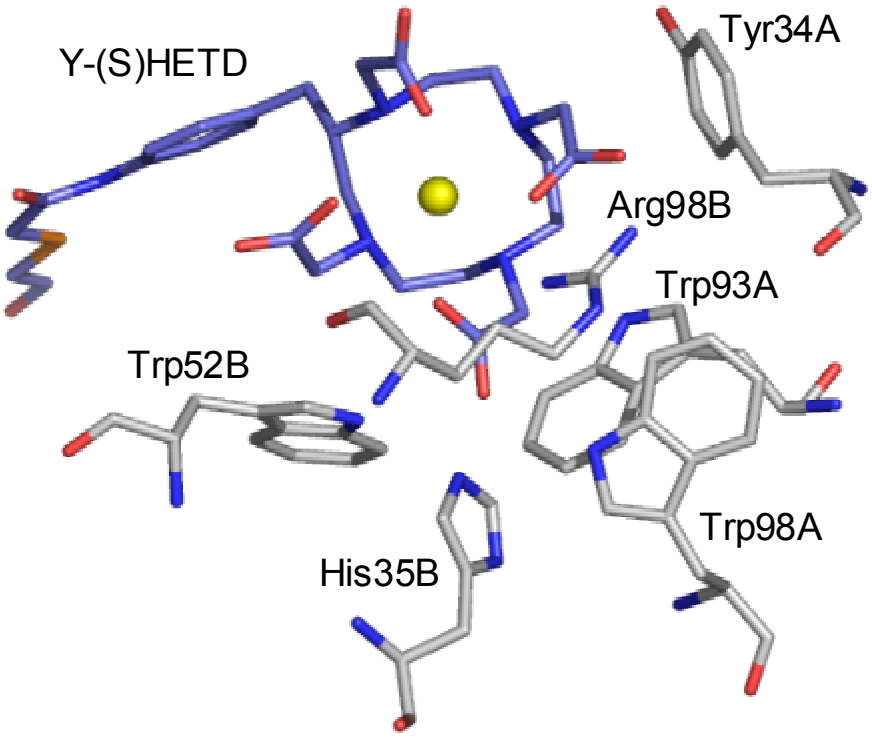


Figure 6:

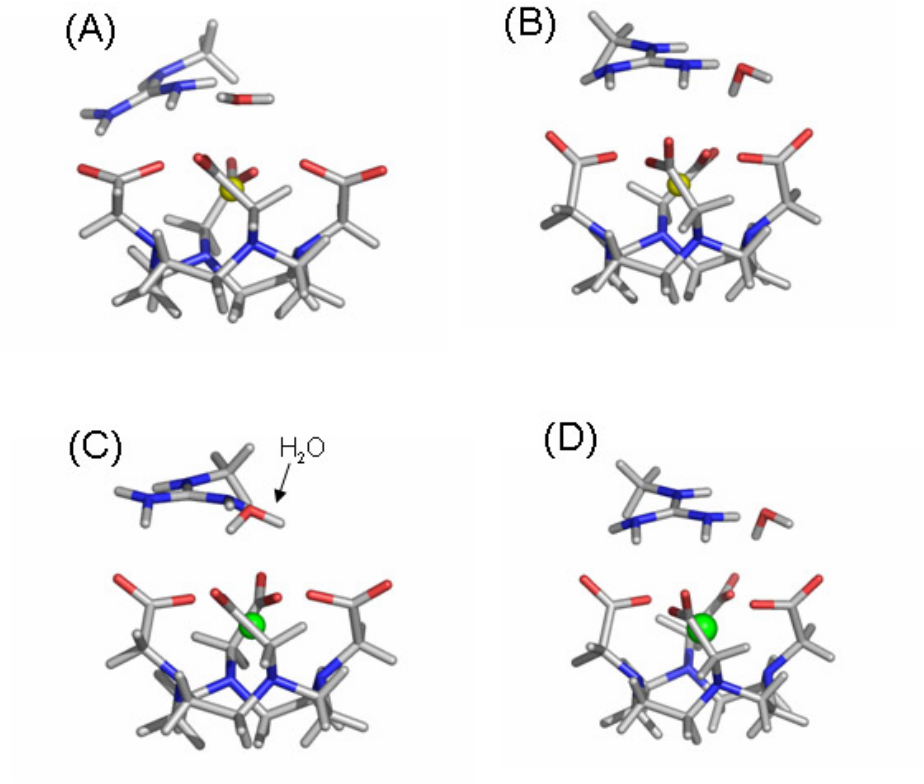


Table 1:		Y-DOTA			In-DOTA	
	A structure	IA structure	crystal structure (1)	A structure	IA structure	crystal structure (2)
	Y-O	Y-O		In-O	In-O	In-O
M-DOTA	2.272	2.272		2.067	2.061	2.157
	2.272	2.273		2.067	2.061	2.183
	2.272	2.272		2.067	2.061	2.202
	2.272	2.272		2.067	2.061	
	Y-N	Y-N		In-N	In-N	In-N
	2.725	2.756		2.703	2.771	2.386
	2.725	2.756		2.703	2.771	2.395
	2.725	2.756		2.703	2.771	2.327
	2.725	2.757		2.703	2.771	2.314
	Y-O	Y-O	Y-O	In-O	In-O	
M-DOTA+	2.326	2.320	2.330	2.079	2.058	
H2O	2.320	2.325	2.319	2.076	2.058	
	2.276	2.274	2.328	2.037	2.020	
	2.276	2.274	2.328	2.038	2.019	
	2.519 (H2O)	2.520 (H2O)	2.424 (H2O)	2.375 (H2O)	2.328 (H2O)	
	Y-N	Y-N	Y-N	In-N	In-N	
	2.821	2.847	2.628	2.976	3.124	
	2.796	2.874	2.654	2.928	3.167	
	2.765	2.843	2.633	2.865	3.078	
	2.795	2.822	2.666	2.922	3.045	

(1) 1,4,7,10-tetraazacyclododecane-1,4,7,10-tetraacetic acid (Ref. 5)
(2) 1,4,7,10-tetraazacyclododecane-1,4,7-triacetic acid (Ref. 2)

Table 2:	A structure	IA structure	B structure	IB structure	crystal structure
	Y-O	Y-O	Y-O	Y-O	Y-O (1)
Y-DO3AM	2.227	2.257	2.198	2.203	2.241
	2.213	2.216	2.188	2.187	2.254
	2.256	2.227	2.195	2.191	2.282
	2.426 (amide)	2.450 (amide)	5.464 (amide)	5.467 (amide)	2.318 (amide)
	Y-N	Y-N	Y-N	Y-N	Y-N
	2.693	2.711	2.689	2.616	2.388
	2.622	2.625	2.729	2.705	2.414
	2.644	2.704	2.589	2.693	2.434
	2.859 (amide)	2.820 (amide)	2.687 (amide)	2.689 (amide)	2.437 (amide)
	Y-O	Y-O	Y-O		Y-O (2)
Y-DO3AM+	2.242	2.267	2.211		2.345
H2O	2.245	2.251	2.200		2.274
	2.299	2.276	2.247		2.260
	2.443 (amide)	2.451 (amide)	5.501 (amide)		2.336 (hydroxyl)
	2.494 (H2O)	2.518 (H2O)	2.492 (H2O)		2.511 (H2O)
	Y-N	Y-N	Y-N		Y-N
	2.746	2.796	2.643		2.63
	2.687	2.695	2.866		2.66
	2.720	2.778	2.825		2.58
	2.928 (amide)	2.893 (amide)	2.733 (amide)		2.61 (hydroxyl)
	In-O	In-O	In-O	In-O	In-O (3)
In-DO3AM	2.007	2.033	1.994	2.024	2.219
	2.050	2.053	1.989	1.99	2.269
	2.038	2.010	2.020	2.002	2.275
	2.384 (amide)	2.399 (amide)	5.278 (amide)	5.282 (amide)	2.314 (amide)
	In-N	In-N	In-N	In-N	In-N
	2.601	2.636	2.550	2.434	2.372
	2.483	2.489	2.853	2.769	2.413
	2.529	2.615	2.386	2.536	2.417
	2.911 (amide)	2.875 (amide)	2.487 (amide)	2.486 (amide)	2.518 (amide)
	In-O	In-O	In-O		
In-DO3AM+	2.032	2.018	1.991		
H2O	2.043	2.039	1.997		
	2.045	2.020	2.039		
	2.253 (amide)	2.234 (amide)	5.318 (amide)		
	2.458 (H2O)	2.420 (H2O)	4.982 (H2O)		
	In-N	In-N	In-N		
	2.761	3.012	2.520		
	2.632	2.643	2.798		
	2.723	2.861	2.396		
	3.068 (amide)	3.169 (amide)	2.469 (amide)		

(1) DOTA-D-PheNH2 (Ref. 29)

(2) 10-(2-hydroxypropyl)-1,4,7,10-tetraazacyclodecane 1,4,7-triacetic acid (Ref. 4)

(3) 1,4,7,10-tetraazacyclododecane-1,4,7,10-tetraacetic acid mono(p-aminoanilide) (Ref. 25)