Chelate chemistry for molecular imaging

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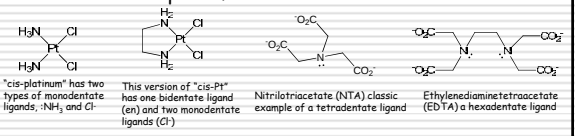
Take home lessons: What you should learn from todays lecture

- Choose the proper ligand for a particular metal ion. Why is this important?
- Macrocyclic ligands *versus* acylic ligands: pros & cons
- Which is more important for metal chelate applications *in vivo*? Thermodynamics or kinetics

Ligands *versus* Chelates

A ligand is defined as any anion or organic molecule that donates a pair of electrons to a metal ion.



"cis-platinum" has two types of monodentate ligands, $:NH_3$ and Cl^-

This version of "cis-Pt" has one bidentate ligand (en) and two monodentate ligands (Cl^-)

Nitrilotriacetate (NTA) classic example of a tetradentate ligand

Ethylenediaminetetraacetate (EDTA) a hexadentate ligand

Multidentate ligands bind to a metal ion by displacing solvent molecules (often water) to form a ML complex

- Multidentate ligands are called chelating agents (claw).
- The resulting ML complexes are called chelates.

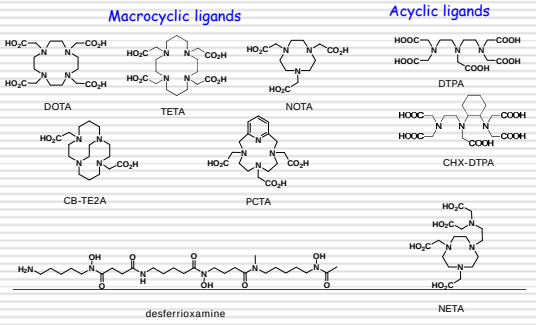
Typical chelates

$Gd(DTPA)^{2-}$

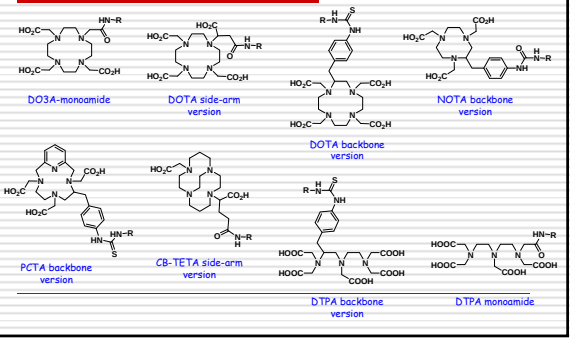
~~$^{68}Ga(NTA)$~~

$Fe(EDTA)^-$

Some common ligands used for chelating metal ions



Bifunctional ligands are widely used to attach a ligand to a targeting moiety ⇒ bifunctional chelates (BFC)



Bifunctional ligands are widely used to attach a ligand to a targeting moiety ⇒ bifunctional chelates (BFC)

Question: So, with all these choices, how does one choose the best ligand or bifunctional ligand for your particular molecular imaging application?

Answer: It depends!

- On size matching of metal ion and ligand cavity
- Thermodynamics (how stable does the chelate need to be?)
- Kinetics of ML complex formation
- Charge on resulting chelate; perhaps it needs to be charged or neutral

Some common radionuclides and ligands used in molecular imaging

Imaging Modality	Most common metal ion	Most common ligand(s)	Ligand characteristics (coord #, donor types)
MRI	Gd^{3+}	DOTA-like, DTPA-like	8-coordinate, needs one water site, prefers "hard" donor atoms
PET	$^{64}Cu^{2+}$	cyclen, DOTA, TETA, bridged TETA, NOTA	Prefers N donors, only 4 coordinate necessary
	$^{68}Ga^{3+}$	DOTA, NOTA	Prefers octahedral and "hard" donor atoms
	$^{86}Y^{3+}$	DOTA-like	8-9 coordinate like Gd^{3+}
	$^{89}Zr^{2+}$	Desferrioxamine-like	"hard" oxygen donors
SPECT	$^{99m}Tc^{2+}$	Methoxyisobutylisonitrile (Sestamibi)	N donor atoms
	$^{111}In^{3+}$	DTPA-like	8-coordinate, somewhat "softer" ion

Factors to consider in the design of ligands for various metal ions

- Selection of ligand donor atoms
  - HSAB principles, matching coordination numbers
- Role of ligand architecture (cyclic versus acyclic)
  - Limited to size-match selectivity
- Kinetics of ML complex formation & dissociation
  - Ligands with flexible backbone structures form complexes more quickly but also dissociate faster
  - Macrocyclic ligands form complexes more slowly but also dissociate more slowly

Hard-Soft Acid-Base Principles (HSAB)

Ligand donor types (from softest to hardest): P < S < N < O < F

For 3d transition metal ions

21 Sc	22 Ti	23 V	24 Cr	25 Mn	26 Fe	27 Co	28 Ni	29 Cu	30 Zn

1) M^{2+} ions get smaller and harder from left to right (increasing nuclear charge)

Ti^{2+} (86 pm) > V^{2+} (79 pm) > Cr^{2+} (73 pm) > Mn^{2+} (67 pm) > Fe^{2+} (61 pm)

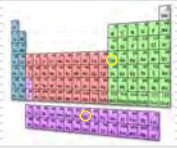
2) M^{+} (softer) < M^{2+} (intermediate) < M^{3+} (harder)

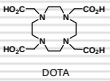
V^{2+} (79 pm) >> V^{3+} (64 pm) >> V^{4+} (53 pm)

As one progresses down a series (3d → 4d → 5d) for ions of the same charge

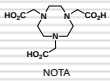
22 Ti	hardest	25 Mn	hardest	30 Zn	hardest
40 Zr	intermed.	43 Tc	intermed.	48 Cd	intermed.
72 Hf	softest	75 Re	softest	80 Hg	softest

Size does matter!





DOTA



NOTA

Gd^{3+} ($4f^7$) ionic radius ~1.0Å	log K_{st} = 24.7[a]	log K_{st} = 14.3[a]
Ga^{3+} ($3d^{10}$) ionic radius ~0.6Å	log K_{st} = 21[b], 26[c]	log K_{st} = 31[b]

[a]WP Cacheris, SK Nickle & AD Sherry, Inorg Chem, 26, 958-960 (1987)
[b]Clark & Martell, Inorg. Chimica Acta, 181, 273-280 (1991)
[c]Kubicek, et al., Inorg. Chem., 49, 10960-10969 (2010)

Some basic definitions: thermodynamic constants, kinetic constants, pKa's and basicity

$$Ln^{3+} + HL \underset{k_{d,HL}}{\overset{k_{f,HL}}{\rightleftharpoons}} LnL + H^{+}$$

K_{cond} = conditional stability constant (defined by conditions, i.e., pH)

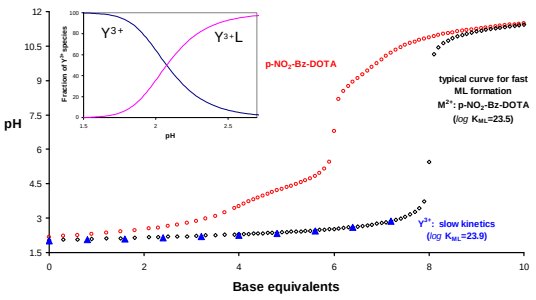
$$K_{ST} = \frac{[LnL]}{[Ln^{3+}]\cdot[L]} \qquad K_{HL} = \frac{[L]\cdot[H^{+}]}{[HL]}$$

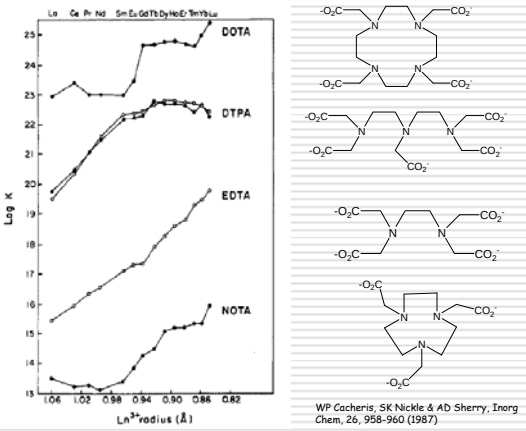
Thermodynamic ML stability constant

Highest pK_a of the ligand (most basic site)

the reverse rxn is referred to as the first protonation step or constant

Thermodynamic Stability Constant Determinations: Potentiometry is considered the "gold standard"





WP Cacheris, SK Nickle & AD Sherry, Inorg Chem, 26, 958-960 (1987)

Ligand charge also plays a role

	DOTA	DO3A-N-propylamide	DO3A-SA	DOTP
log K_{st} (Gd^{3+})	24.7[a]	20.1[b]	27.2[c]	28.8[d]

[a]WP Cacheris, SK Nickle & AD Sherry, Inorg Chem, 26, 958-960 (1987)
[b]AD Sherry, RD Brown, CFGC Geraldes, SH Koenig, K-T Kuan & M Spiller, Inorg Chem., 28, 620-622 (1989)
[c]JP Andre, E Brucher, R Kiraly, RA Carvalho, H Maecke, CFGC Geraldes, *Helv. Chim. Acta, 88*, 633 (2005).
[d]AD Sherry, J Ren, J. Huskens, E Brucher, E. Toth, et al. *Inorg. Chem.* 1996, *35*, 4604 (1996)

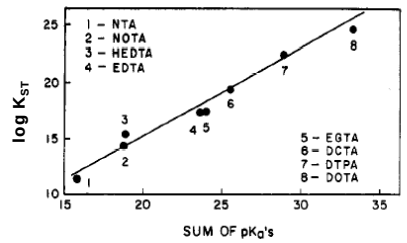
Correlation of $k_{f,HL}$, $k_{d,H}$, and *log* K_{ML} with the first protonation constant of the ligand (*log* K_{HL})

$$Ln^{3+} + HL \underset{k_{d,HL}}{\overset{k_{f,HL}}{\rightleftharpoons}} LnL + H^+$$

$$K_{eq} = \frac{[LnL]\cdot[H^+]}{[Ln^{3+}]\cdot[HL]} = \frac{k_{f,HL}}{k_{d,H}} = K_{ST} \times K_{HL}$$

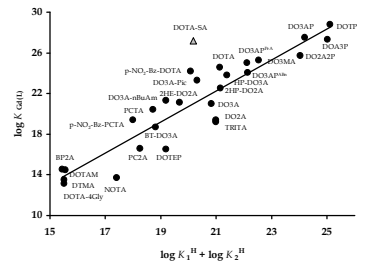
$$K_{ST} = \frac{k_{f,HL}}{k_{d,H} \cdot K_{HL}} \qquad \log K_{ST} = \frac{k_{f,HL}}{k_{d,H}} \; pK_a$$

log K_{ST} *vs* ΣpK_a's for a series of GdL complexes



From: WP Cacheris, SK Nickle & AD Sherry, Inorg Chem, 26, 958-960 (1987)

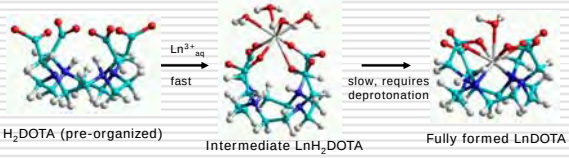
A more recent compilation: Brücher, et al., "Stability and Toxicity of Contast Agents", Chapter 5, in The Chemistry of Contrast Agents in Medical Magnetic Resonance Imaging, edited by Merbach, Toth & Helm, Wiley, 2012.



Importance of kinetics: rates of ML formation

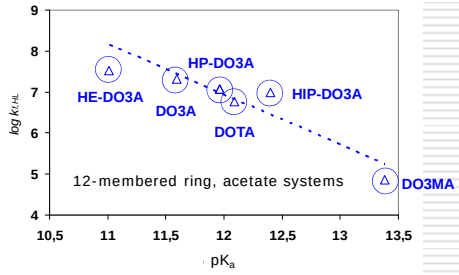
$$Ln^{3+} + HL \underset{k_{d,HL}}{\overset{k_{f,HL}}{\rightleftharpoons}} LnL + H^+$$

Why do macrocyclic ligands form complexes with metal ions so slowly?



H_2DOTA (pre-organized) — Intermediate LnH_2DOTA — Fully formed LnDOTA

Correlation of $k_{f,HL}$ with the first protonation constant of the ligand (*log* K_{HL})



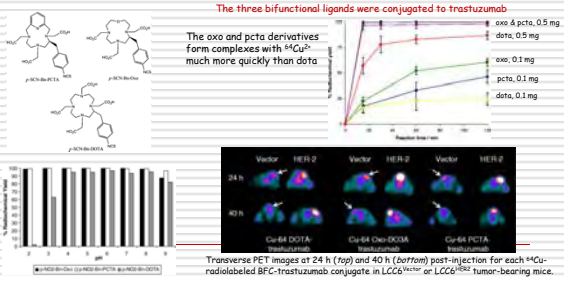
K. Kumar and M. F. Tweedle, *Inorg. Chem.*, **1993,** *32*, 4193-4199

Comparison of bifunctional chelates for $^{64}Cu^{2+}$-antibody imaging

CL Ferreira, DTTT Yapp, S Crisp, et al., Eur J Nucl Med Mol Imaging 37: 2117-2126 (2010)

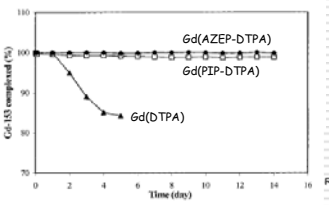
CuDOTA only moderately stable *in vivo*; CuNOTA and Cu-CB-TETA more stable but they also form complexes too slowly for efficient antibody labeling

The three bifunctional ligands were conjugated to trastuzumab



Transverse PET images at 24 h (*top*) and 40 h (*bottom*) post-injection for each ^{64}Cu-radiolabeled BFC-trastuzumab conjugate in LCC6^{Vector} or LCC6^{HER2} tumor-bearing mice.

Increased backbone rigidity improves the kinetic stability of complexes



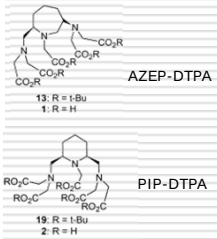
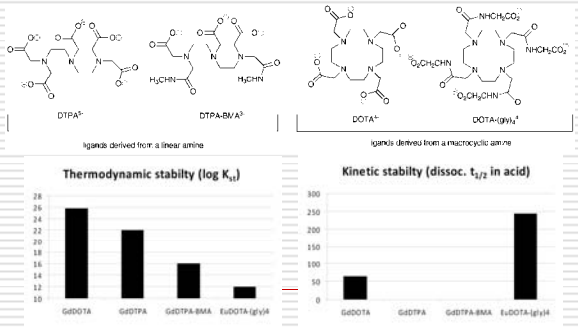


Figure 1. Serum stability of Gd(AZEP-DTPA) (□), Gd(PIP-DTPA) (♦), and Gd(DTPA) (▲)* at pH 7 and 37 °C. *Previous result in ref 8a.

H-S Chong, K Garmestani, LH Bryant, Jr., MW Brechbiel, *J Org Chem, 66,* 7745-7750 (2001)

Are LnDOTA-tetraamide complexes safe for *in vivo* use?



Take home lessons:

- Why it is important to choose the proper ligand for a particular metal ion for molecular imaging.
  - Ligand size and donor type should match the characteristics of the metal ion
- Macrocyclic ligands *versus* acylic ligands:
  - Macrocyclic ligands form more stable ML chelates but they also form more slowly (this kinetic barrier may be important in radiolabeling of antibodies which cannot be heated)
- Which is more important for metal chelate applications *in vivo*?
  - Thermodynamics is important but **kinetics** is likely even more important for *in vivo* applications!

Thanks

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  Prof. Zoltan Kovacs, Prof. Mark Woods, Bill Cacheris, Garry Kiefer, Prof. Gyula Tirsco, Won-dae Kim, Prof. Jimen Ren, Prof. Istvan Lazar, Shanrong Zhang, Prof. Carlos Geraldes, Prof. Jurriaan Huskens, Flavio Chavez, Prof. Navin Bansal, Yunkou Wu and, especially, Prof. Erno Brücher (Hungary) who taught me how to do potentiometry well.
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