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# Labeling of (bio)molecules with radiometals

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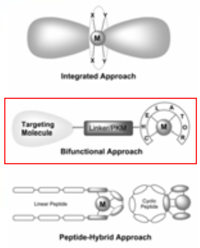
## Background radiometals

Different possibilities to complex a radiometal




Radiometal is vital part of receptor binding motive

Radiometal is kept away from receptor binding part

Complexation with radiometal results in good binding to receptor

S. Liu, *Chem. Soc. Rev*. 2004, 33, 445-461.

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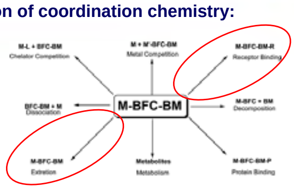
## Background radiometals

Contribution of coordination chemistry:




Distribution and elimination of a radiopharmaceutical following administration M–BFC–BM:

- M = radionuclide
- M' = metal ion in the blood stream
- BFC = bifunctional chelator
- BM = targeting (bio)molecule
- L = competing chelator

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## Background radiometals

Coordination chemistry ----- In vivo behaviour

- Redox properties
- Stability in vivo
- Stereochemistry
- Charge
- Lipophilicity

→The target determines the properties of the metal complex that you should use

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## Background radiometals

Modification of pharmacokinetics

- Chemical modification of:
  - * Biomolecule (*e.g.* introduction of hydrophilic groups)
  - * Metal chelate (*e.g.* use of different BFC with different charge and hydrophilicity)
- Use of different linker
- Choice of coligands

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## Overview radiometals

| Metal | SPECT | PET | Therapeutic |
|---|---|---|---|
| Technetium | $^{99m}Tc$ | | |
| Gallium | $^{67}Ga$ | $^{68}Ga$ | |
| Indium | $^{111}In$ | | |
| Copper | $^{67}Cu$ | $^{61/62/64}Cu$ | |
| Lutetium | $^{177}Lu$ | | $^{177}Lu$ |
| Yttrium | | $^{86}Y$ | $^{90}Y$ |
| Zirconium | | $^{89}Zr$ | |

Which radionuclide should I use???

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## Choice of radiometal

Which radionuclide should I use???

Dependant on time to reach target, target properties and the nuclear properties of radionuclide!!

Small molecule vs. Monoclonal antibody

- Half-life
- Type of radiation
- Energy

But also important: cost and availability!

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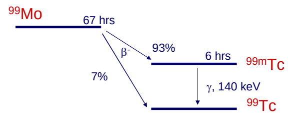
## $^{99m}$Technetium

- $^{99m}$Tc is produced from the parent radionuclide $^{99}$Mo, which is a fission product with a half-life of 2.78 days
- $^{99m}$Tc is a metastable form of $^{99}$Tc with a half-life of 6.02 hours
- Decay to the ground state of $^{99}$Tc: emission of 140 keV photons

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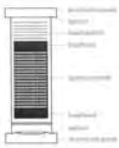
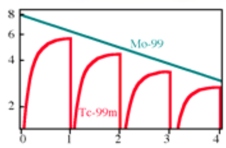
## $^{99m}$Technetium


- $^{99}MoO_4^{2-}$ absorbed to an alumina column
- $^{99m}$Tc is formed by decay of $^{99}$Mo
- $^{99m}TcO_4^-$ is eluted with saline

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## $^{99m}$Technetium: chemistry

- $^{99m}TcO_4^-$ can not be used as such
- Must be reduced, oxidation state depends upon:
  - * Reducing agent
  - * Chelator
  - * Reaction conditions

Rich chemistry, however difficult to control!!

Tc(V) complexes:
- Best controlled chemistry
- Simple σ donors (N, O, S)
- Stability: tetradentate > tridentate > bidentate > monodentate
- Thiol ligands are preferred and are more stable than alcohol ligands
- Most applied in nuclear medicine
- $SnCl_2$ is a good reducing agent to obtain Tc(IV) complexes

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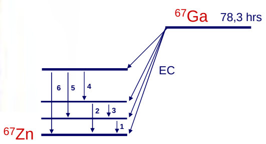
## $^{67}$Gallium

- $^{67}$Ga is a cyclotron produced isotope by the $^{68}$Zn(p,2n)-$^{67}$Ga reaction
- $^{67}$Ga has a half-life of 78,3 hours
- $^{67}$Ga decays by electron capture




| Transition | Energy (keV) | Abundance (%) |
|---|---|---|
| EC | | 100 |
| γ1 | 93 | 38.3 |
| γ2 | 185 | 20.9 |
| γ3 | 91 | 3.1 |
| γ4 | 209 | 2.4 |
| γ5 | 300 | 16.8 |
| γ6 | 393 | 4.7 |

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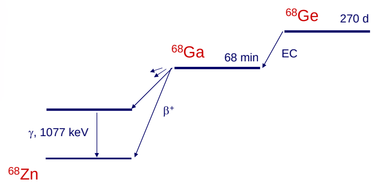
## $^{68}$Gallium

- $^{68}$Ga is produced from a $^{68}$Ge/$^{68}$Ga generator
- $^{68}$Ga has a half-life of 68 min
- $^{68}$Ga decays by 89% $β^+$-emission
- The long half-life of the parent isotope, $^{68}$Ge (280 d) gives the generator a shelf-life of 1-2 years

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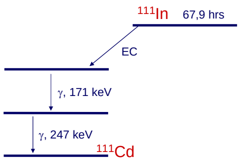
## $^{111}$Indium

- $^{111}In$ is a cyclotron produced isotope by the $^{111}Cd(p,n)$-$^{111}In$ reaction
- $^{111}In$ has a half-life of 67.9 hours
- $^{111}In$ decays by electron capture with two photon emissions at 173 and 247 keV






## Galium and Indium chemistry

- Gallium and Indium are in group IIIB of the periodic table
- Under physiological conditions, the only oxidation state in aqueous solution is +3.
- Due to their charge density, they both prefer hard donors, such as N and O.
- Ga(III) and In (III) have coordination number 3,4,5 and 6. (In(III) also 7 or 8)
- 6-coordinate complexes are most stable (also *in vivo*)
- Gallium and Indium are similar to Iron with respect to their coordination number and biological properties



## Galium and Indium chemistry

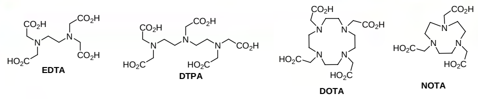
| | Ga | In | Fe |
|---|---|---|---|
| Ionic radius | 62 pm | 80 pm | 65 pm |
| Log $K_1$(OH) | 11.3 | 10.0 | 11.8 |
| Log $K_1(NH_3)$ | 4.1* | 4.0* | 3.8* |
| Log $K_1(RS^-)$ | 8.7 | 9.1 | 8.6* |

* Estimated value

- Because they are highly charged, hydrolysis of $Ga^{3+}$ and $In^{3+}$ at pH>4, remains a serious problem during radiolabeling in aq. media
- Ligand exchange with transferrin!
- Detached $^{67/68}Ga$ and $^{111}In$ accumulate in liver and lungs (due to strong binding capacity of transferrin)



## Galium and Indium chemistry




- Indium has a larger size than Ga
- EDTA, DTPA and DOTA bind In more securely than Ga
- Carboxylic groups of DOTA can be used for conjugation to a targeting molecule, while for NOTA this results in reduced stability



## Copper isotopes

| Isotope | $T_{1/2}$ | $\beta^-$ MeV (%) | $\beta^+$ MeV (%) | EC (%) | γ MeV (%) |
|---|---|---|---|---|---|
| $^{60}Cu$ | 23.4 min | - | 3.92 (6%)<br>3.00 (18%)<br>2.00 (69%) | 7.4% | 0.85 (15%)<br>1.33 (80%)<br>1.76 (52%)<br>2.13 (6%) |
| $^{61}Cu$ | 3.32 h | - | 1.22 (60%) | 40% | 0.284 (12%)<br>0.38 (3%)<br>0.511(120%) |
| $^{62}Cu$ | 9.76 min | - | 2.91 (97%) | 2% | 0.511 (194%) |
| $^{64}Cu$ | 12.8 h | 0.573 (39.6%) | 0.655 (19.3%) | 41% | 1.35 (0.6%)<br>0.511(38.6) |
| $^{67}Cu$ | 62.0 h | 0.577 (20%)<br>0.484 (35%)<br>0.395 (45%) | - | - | 0.184 (40%)<br>0.092 (23%) |

This summary shows:
- Main decay of characteristics of the Cu-isotopes
- Half-life

Production via nuclear reaction, generator or spallation



## Copper chemistry

- Chemistry in aqueous solution restricted to two principal oxidation states: (I and II)

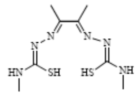
2 Cu (I) → Cu + Cu (II)

- Cu(I): * Tetrahedral configuration
  * Colorless
  * Soft donors like thioether, phosphine, isonitrile

- Cu(II): * Square planar configuration
  * Colored
  * Nitrogen (amine, pyridine) and sulfur (thiosemicarbazone) ligands

## Copper chemistry

- Design of copper chelators dependant on desired characteristics of the targeting molecule
- Short lived copper nuclides:
  - * Lipophilic neutral copper complexes → blood flow agents
  - * Stable enough to clear the blood and diffuse passively and efficiently into tissues like heart, brain, kidney or tumor upon 1st pass through of the blood to these tissues
  - * Cu(II) thiosemicarbazones: imaging hypoxia in the heart and in tumors



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## Copper chemistry

- Long lived copper nuclides:
  - * Biomolecules (peptides-mAb) for tumor imaging
  - * Copper nuclide must be firmly bound to the biomolecule → Cu-chelate with high *in vivo* stability!!
  - * Preferably a macrocyclic chelator!
  - * However, the choice of macrocyclic is very important!

↓

pharmacokinetics, distribution, metabolism

↓

clinical usefulness of the drug

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## Copper chemistry

Choice of macrocyclic is very important!

↓

Tetraazamacrocyclics superior to acyclic chelates (e.g. EDTA)

Most important DOTA and TETA

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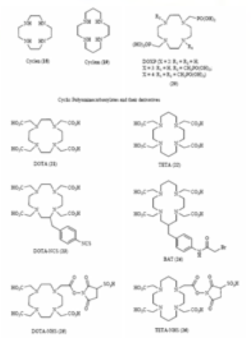
However, still instability observed *in vivo*



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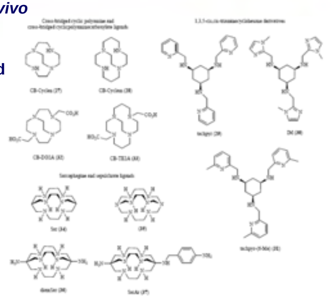
## Copper chemistry

Instability observed *in vivo*

↓

Complexes like depicted here show good in vivo stability!



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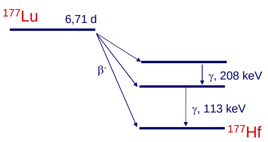
## $^{177}$Lutetium

- $^{177}$Lu has a half-life of 6.7 days
- $^{177}$Lu can be produced neutron bombardment of enriched $^{176}$Lu in a reactor
- It has three short range β-emissions: 0.176 MeV (12%) 0.384 MeV (9%) 0.497 MeV (79%)

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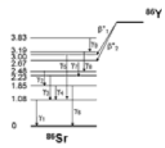
## $^{86}$Yttrium

- $^{86}$Y has a half-life of 14.7 h
- $^{86}$Y can be produced in a cyclotron from $^{86}$Sr
- $^{86}$Y decays via emission of two $β^+$ particles and several gamma-rays




| | Energy (keV) | Abundance (%) |
|---|---|---|
| $β^+_1$ | 1221 | 11.9 |
| $β^+_2$ | 1545 | 5.6 |
| $γ_1$ | 1077 | 83.0 |
| $γ_2$ | 628 | 32.6 |
| $γ_3$ | 1153 | 30.5 |
| $γ_4$ | 777 | 22.4 |
| $γ_5$ | 1921 | 20.8 |
| $γ_6$ | 1854 | 17.2 |
| $γ_7$ | 443 | 16.9 |
| $γ_8$ | 703 | 15.4 |
| $γ_9$ | 646 | 9.2 |

M. Lubberink, H. Herzog *EJNMMI* 2011, 38, S1, S10-S18.

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## Lutetium and Yttrium chemistry

- Lu and Y favor 3+ oxidation state
- Similar charge, ionic radius and coordination chemistry
- Coordination number 7-10
- Bifunctional chelator: DOTA is preferred (but requires more strict reaction conditions) than DTPA

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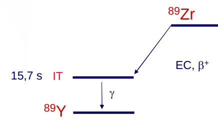
## $^{89}$Zirconium

- Production: nuclear reaction
  $^{89}$Y (p,n) $^{89}$Zr, obtained as $^{89}$Zr in Yttrium disk
  Proton energy 14 MeV optimal
- Work up: dissolve in 6M HCl, affinity chromatography
- Zr-89 has a half-life of 78.4 hr




| Transition | Energy (MeV) | Abundance (%) |
|---|---|---|
| β+ | 0.897 | 22.6 |
| EC | | 77.0 |
| γ | 0.909 | 99.0 |

IT= isomeric transition

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## Zirconium chemistry

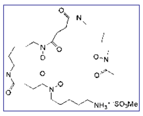
<u>Zr-complexation:</u>

- Zr(IV) is a hard Lewis acid and thus prefers hard Lewis bases as donor groups
- Zr(IV) forms very stable complexes with hydroxamate groups
- Zr(IV) prefers 8-coordination

<u>Desferrioxamine/desferal:</u>

- Consists of three hydroxamate groups and an amine group for linkage to protein
- Desferrioxamine has six metal-binding functionalities
- Used clinically for iron- and aluminum overload
- Can be used for complexation of $Fe^{3+}$,$Ga^{3+}$ and $Zr^{4+}$

Desferrioxamine is chelate of choice!



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## Targeting receptors

Does the metal ion or chelate matter? YES

Does the linker/spacer matter? YES

Multiple studies performed in which these effects have been evaluated

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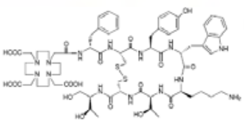
## Targeting receptors

Example 1: effect of different radiometal

Compound: DOTATOC

Radiometals: $^{67}$Ga, $^{111}$In and $^{90}$Y



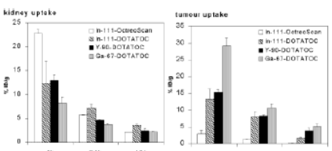



Figure 3. Biodistribution study of DOTATOC labelled with $^{67}$Ga, $^{111}$In and $^{90}$Y, in comparison to $^{111}$In-DTPA$^{0}$-D-Phe$^{1}$-octreotide, on nude mice bearing the AR4-2J tumour (% IDg$^{-1}$: % injected dose per gram).

Heppeler et al, *Chem. Eur. J.* 1999, 5, 1974-1981

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## Targeting receptors

Example 2: effect of different chelates and radiometals

Compounds: NODAGA-LM3, CB2-TE2A-LM3 and DOTA-LM3

Radiometals: $^{68}$Ga and $^{64}$Cu

| Metallopeptide | $IC_{50}$ (nmol/L) | Peptide charge |
|---|---|---|
| $^{64/nat}$Cu-NODAGA-LM3 | 6.7 ± 1.5 | 0 |
| $^{64/nat}$Cu-CB-TE2A-LM3 | 4.2 ± 1.6 | +2 |
| $^{68/nat}$Ga-NODAGA-LM3 | 1.3 ± 0.3 | +1 |
| $^{68/nat}$Ga-DOTA-LM3 | 12.5 ± 4.3 | +1 |

$IC_{50}$ values are mean ± SEM ($n$ = 2) for sst2, and log D values are mean ± SD ($n$ = 3).

Fani et al, *JNM* 2011, 52, 1110-1118

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## Targeting receptors

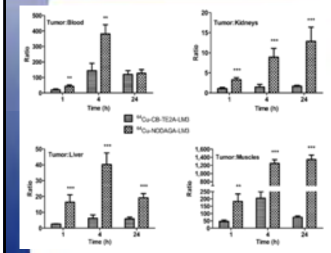


**FIGURE 4.** Tumor-to-normal-tissue ratios in HEK-sst2 tumor xenografts at 1, 4, and 24 h after injection are significantly higher for $^{64}$Cu-NODAGA-LM3 than for $^{64}$Cu-CB-TE2A-LM3. **$P < 0.01$. ***$P < 0.001$.

**TABLE 3**
Biodistribution Results of $^{68}$Ga-NODAGA-LM3 and $^{68}$Ga-DOTA-LM3 in Nude Mice Bearing HEK-sst2 Tumor Xenografts at 1 Hour After Injection

| Organ | $^{68}$Ga-NODAGA-LM3 | $^{68}$Ga-DOTA-LM3 |
|---|---|---|
| Blood | 0.91 ± 0.01 | 1.21 ± 0.38 |
| Heart | 0.50 ± 0.02 | 0.42 ± 0.12 |
| Liver | 1.08 ± 0.08 | 0.84 ± 0.20 |
| Spleen | 0.84 ± 0.03 | 0.58 ± 0.20 |
| Lung | 3.01 ± 0.54 | 1.73 ± 0.67 |
| Kidney | 19.68 ± 3.30* | 32.50 ± 0.66* |
| Stomach | 5.36 ± 1.45 | 1.08 ± 0.34 |
| Intestine | 0.87 ± 0.05 | 0.56 ± 0.16 |
| Adrenal | 2.55 ± 0.31 | 1.06 ± 0.64 |
| Pancreas | 9.15 ± 1.03 | 0.91 ± 0.22 |
| Muscle | 0.36 ± 0.02 | 0.32 ± 0.03 |
| Bone | 2.31 ± 0.66 | 1.63 ± 0.03 |
| Tumor | 37.27 ± 5.49 | 28.72 ± 5.56 |
| Tumor-to-normal-tissue ratio | | |
| Tumor to blood | 41.1 ± 6.2 | 24.2 ± 3.0 |
| Tumor to liver | 34.7 ± 7.5 | 34.3 ± 1.6 |
| Tumor to kidney | 1.9 ± 0.6 | 0.9 ± 0.2 |
| Tumor to muscle | 104.8 ± 22.1 | 90.6 ± 10.3 |

*$P < 0.05$, statistically significant.
Results are %ID/g ± SD ($n = 3$).

Fani et al, *JNM* 2011, 52, 1110-1118

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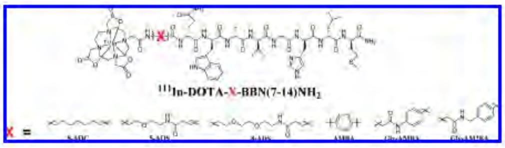
## Targeting receptors

**Example 3: effect of linker-spacer**

**Compounds: BBN(7-14)**
**Chelator: DOTA**
**Radiometals: $^{111}$In**




Garrison et al, *Bioconj Chem* 2008, 19, 1803-1812.

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## Targeting receptors


At 1 d p.i. radioconjugates containing aromatic groups showed the highest tumor retention

Garrison et al, *Bioconj Chem* 2008, 19, 1803-1812.

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