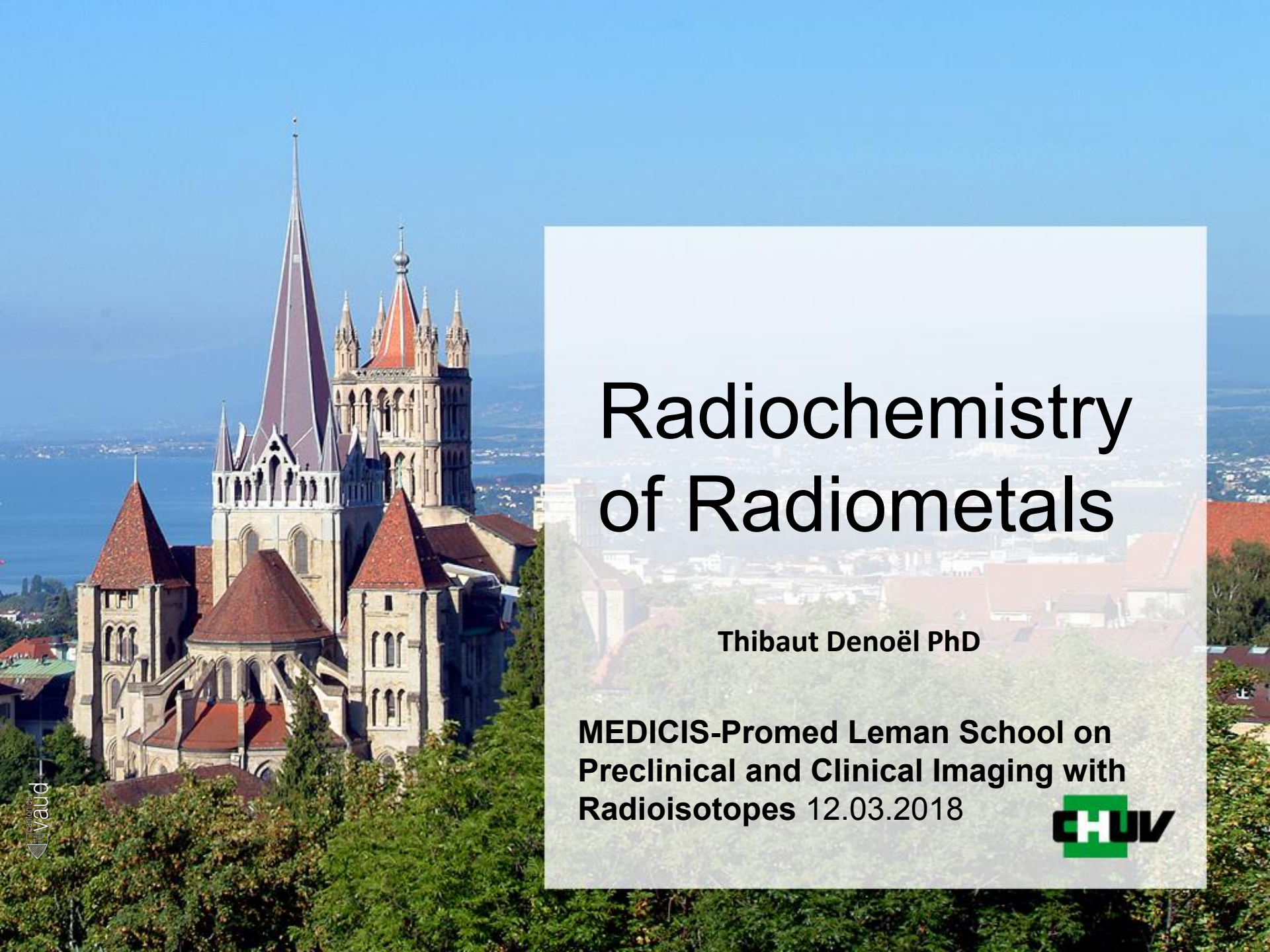


Radiochemistry of Radiometals

Thibaut Denoël PhD

MEDICIS-Promed Leman School on Preclinical and Clinical Imaging with Radioisotopes 12.03.2018

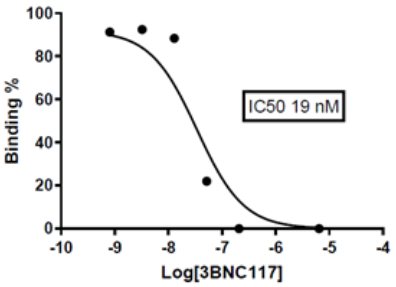
Radiochemistry

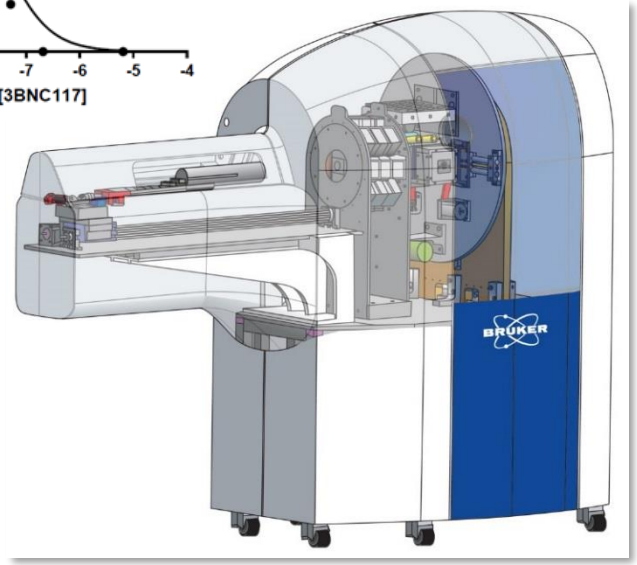
Radiochemistry is the chemistry of radioactive materials.

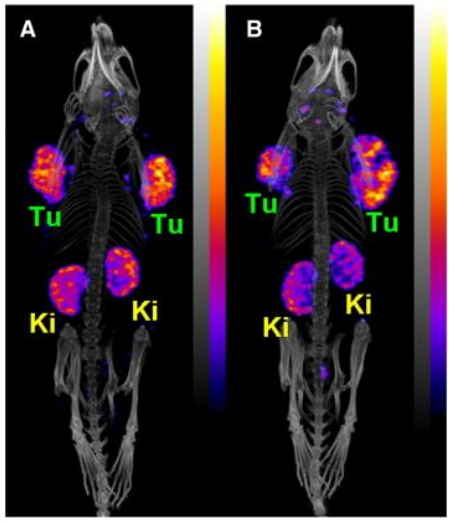
In this talk we try to answer this question:

How a radioactive isotope can be conjugated to a bioactive organic molecule to obtain a labeled compound, a radiopharmaceutical probe?

Radiotracers are tools that find ample application for *in vitro*, preclinical and clinical imaging.







In vivo SPECT/CT injection of ^{155}Tb-cm09 (~ 8.5 MBq).

C Müller, Nuclear Medicine and Biology 41, 2014, e58-e65

Overview of the presentation

- This presentation present main concepts, challenges and application in the **radiochemistry of radiopharmaceuticals**:

I) Labeling molecules with non-metal and metal radioisotopes

- Introduction
- Cases review
- Key concepts
- Nature of the biomolecule
- Choice of radioisotope (non-metal and metal)

II) Radiochemistry of metal radioisotopes

- Conjugation and chelation
- Choice of chelator
- Purification
- Analysis and quality control

Part I

Labeling molecules with non-metal and metal radioisotopes

Labeling molecules: introduction

Labeling a molecule leads, *unless for an isotopic substitution,* to **a new molecule**, an analog.

→ The analog is a different molecule and **cannot** strictly have the same pharmacology as the unlabeled molecule.

The aim of labeling is generally to **transform a known active biomolecule**, such as a receptor ligand, **to a new radioactive probe** displaying:

- A comparable binding to the receptor
- Good pharmacokinetic/pharmacodynamic (PK/PD)

The very low injected weight of radioactive probe (n.c.a.) does not induce a pharmacological effect due to sub microgram dosage.

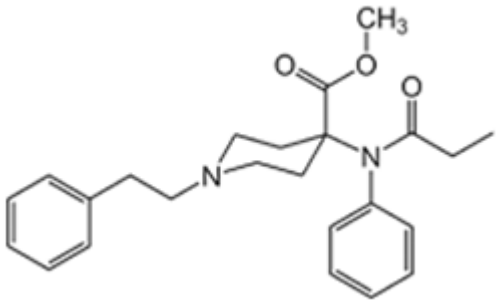
The only toxicity displayed by the probe will thus be from the radioelement.

A radiopharmaceutical hence can have **imaging** properties or **therapeutic** purpose.

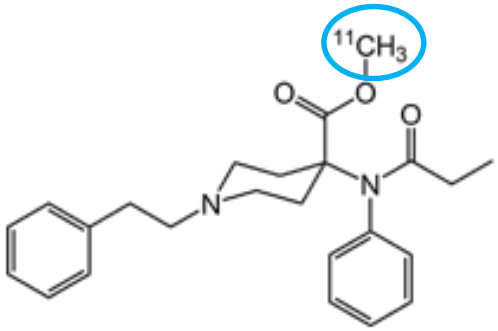
Labeling molecules: cases review

Exemples of radioanalogs of a known substance:

The best case:
a simple isotopic exchange: ^{11}C-Carfentanyl a radioanalog of Carfentanyl



Carfentanyl



^{11}C-Carfentanyl

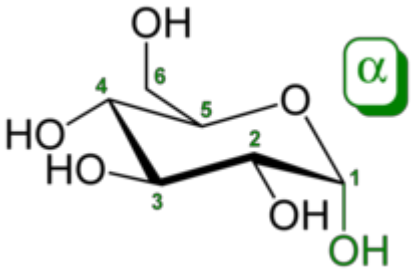
Isotopic exchange: $^{12}C \rightarrow {}^{11}C$
Radioisotopically labeled carfentanyl

^{11}C-Carfentanyl is **chemically and pharmacologically identical** to carfentanyl

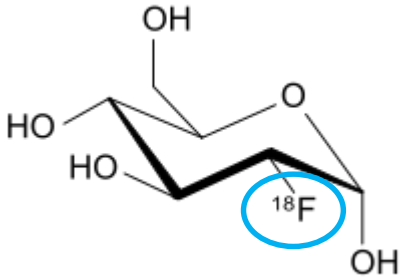
Labeling molecules: cases review

Exemples of radioanalogs of a known substance:

An atypical case:
an isosteric substitution: ^{18}F-FDG, a radioanalog of Glucose



Glucose



^{18}F-FDG

Isosteric substitution: ^{16}O → ^{18}F
Different PK/PD
Useful in sugar metabolism studies

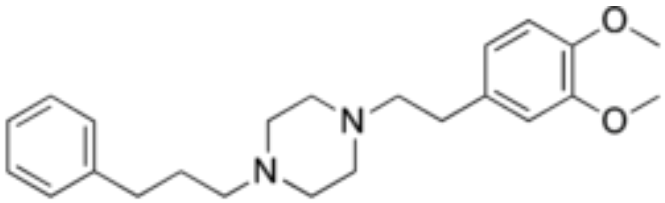
The different pharmacology (resistance to metabolism) leads in this case to a useful radiopharmaceutical!

Labeling molecules: cases review

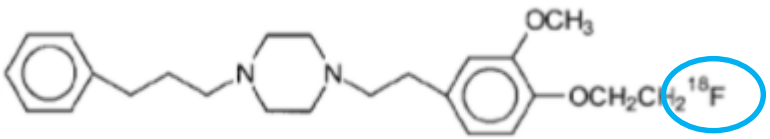
Exemple of radioanalogs of known substance:

A bad case:

^{18}F-FE-SA4503, a radioanalog of SA4503



SA4503



^{18}F-FE-SA4503

High selectivity $Sigma_1/Sigma_2$ (103)

Bad selectivity $Sigma_1/Sigma_2$ (0.33)

TABLE I. In vitro *data for sigma receptors and lipophilicity of FE-SA 4503 and SA4503*

Compound	Affinity for sigma receptors			Lipophilicity
	$Sigma_1$ (IC_{50}[a])	$Sigma_2$ (IC_{50}[a])	Selectivity $Sigma_1/Sigma_2$	log P
FE-SA4503	6.48	2.11	0.33	2.66
SA4503[b]	17.4	1,784.1	103	2.52

[a]Data are expressed in nM.
[b]Data from Kawamura et al. (2000a).

→ ^{18}F-FE-SA4503 display a reversal and a loss of selectivity!

"The in vitro **binding** characteristics for the sigma receptor subtypes was **dramatically altered** by replacement of the methoxy group for a fluoroethoxy group. The absolute affinity of [18F]FE-SA4503 has increased, whereas the **subtype-selectivity has disappeared**."

PH Elsinga, SYNAPSE 43:259–267(2002)

Labeling molecules: cases review

Exemple of radioanalogs of known substance:

The worse case:

… Analogs with a **complete loss of affinity**!

… there is **an infinity of examples**, but not worth a publication!

Labeling molecules: cases review

Conclusion:
There is the necessity to label a molecule in a way that the radiopharmaceutical:

- Preserves **the affinity** for the receptor of interest
- Preserves **the selectivity** toward other receptor subtypes or other receptor families
- Displays a good **stability** of the radionucleide on the organic molecule
- Displays a good **immunoreactivity** which is the % of radioactive substance that can effectively bind to a receptor
- Achieves a **biodistribution** that do not leads to unnecessary irradiation of organs at risk
- Presents an **improvement** over current radiopharmaceutical
- The labeling yield shall also be adequate as well as the synthesis time

→ The discipline ask for many quality control and most candidate radiopharmaceutical fails

Labeling molecules: key concepts

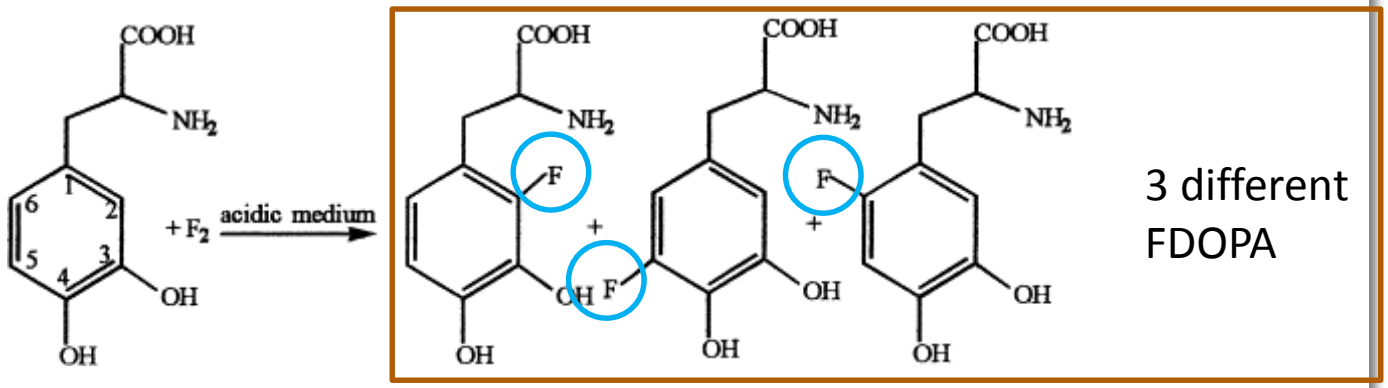
We just spoke of the most important attributes of a radiopharmaceutical

- affinity
- selectivity
- stability
- immunoreactivity
- biodistribution
- improvement

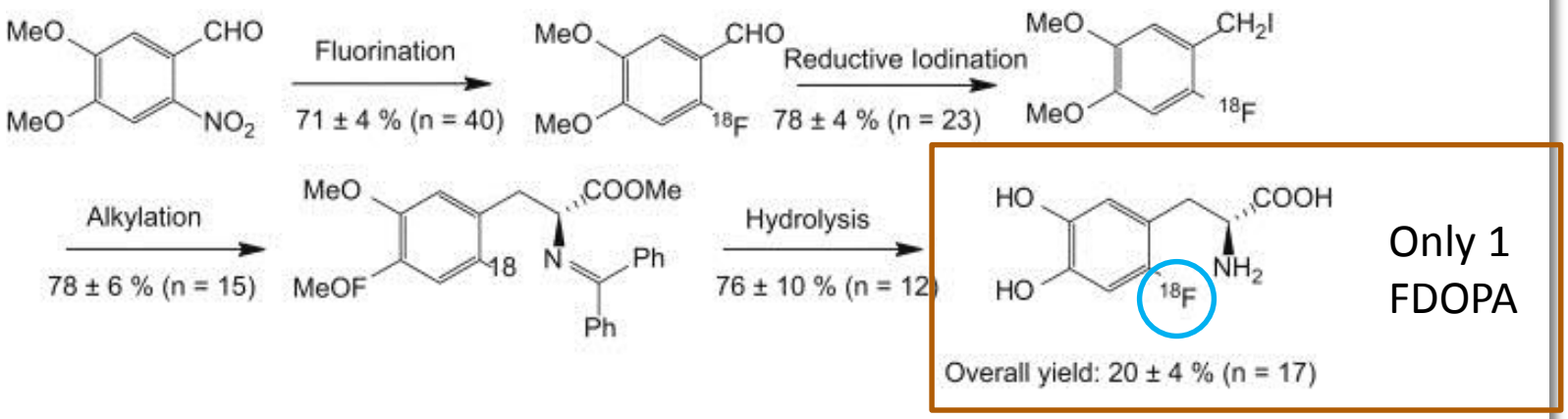
Keeping in mind those points, we will talk of the aspects of the radiolabeling reaction that helps to reach the ideal radiopharmaceutical

Labeling molecules: key concepts

The labeling reaction shall be **selective**



Electrophilic ^{18}F-FDOPA synthesis is **NOT selective**!



Nucléophilic ^{18}F-FDOPA synthesis is **selective**!

B Shen, Applied Radiation and Isotopes 67, 9, 2009, 1650-1653

Labeling molecules: key concepts

The labeling reaction shall use a radioisotope with a **high specific activity**

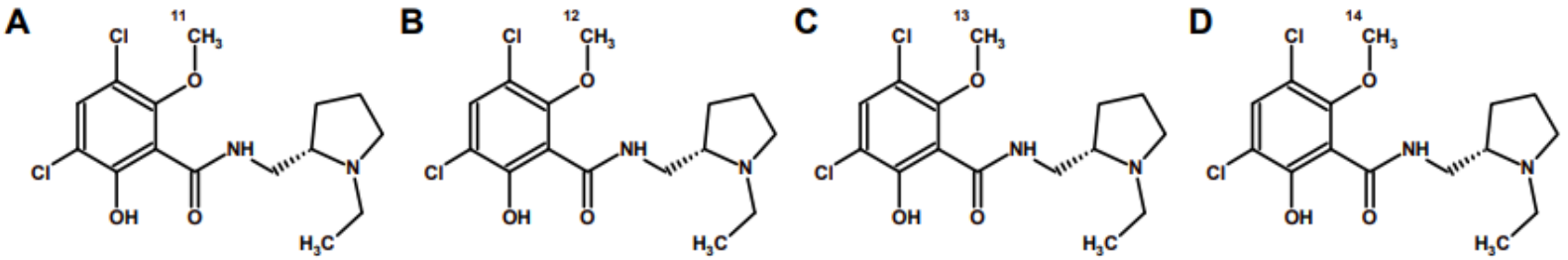


Fig. 1. Chemical structure of [^{11}C]Raclopride and chemically identical species which co-exists with the radioactive specie. All carbon atoms whose mass number is not specified are ^{12}C, ^{13}C or ^{14}C.

Specific activity is the ratio between the amount of radioactivity (in this case A containing ^{11}C-carbon) and the number of molecules (in mass or molar quantity) containing any of the four isotopes of carbon (A+B+C+D)

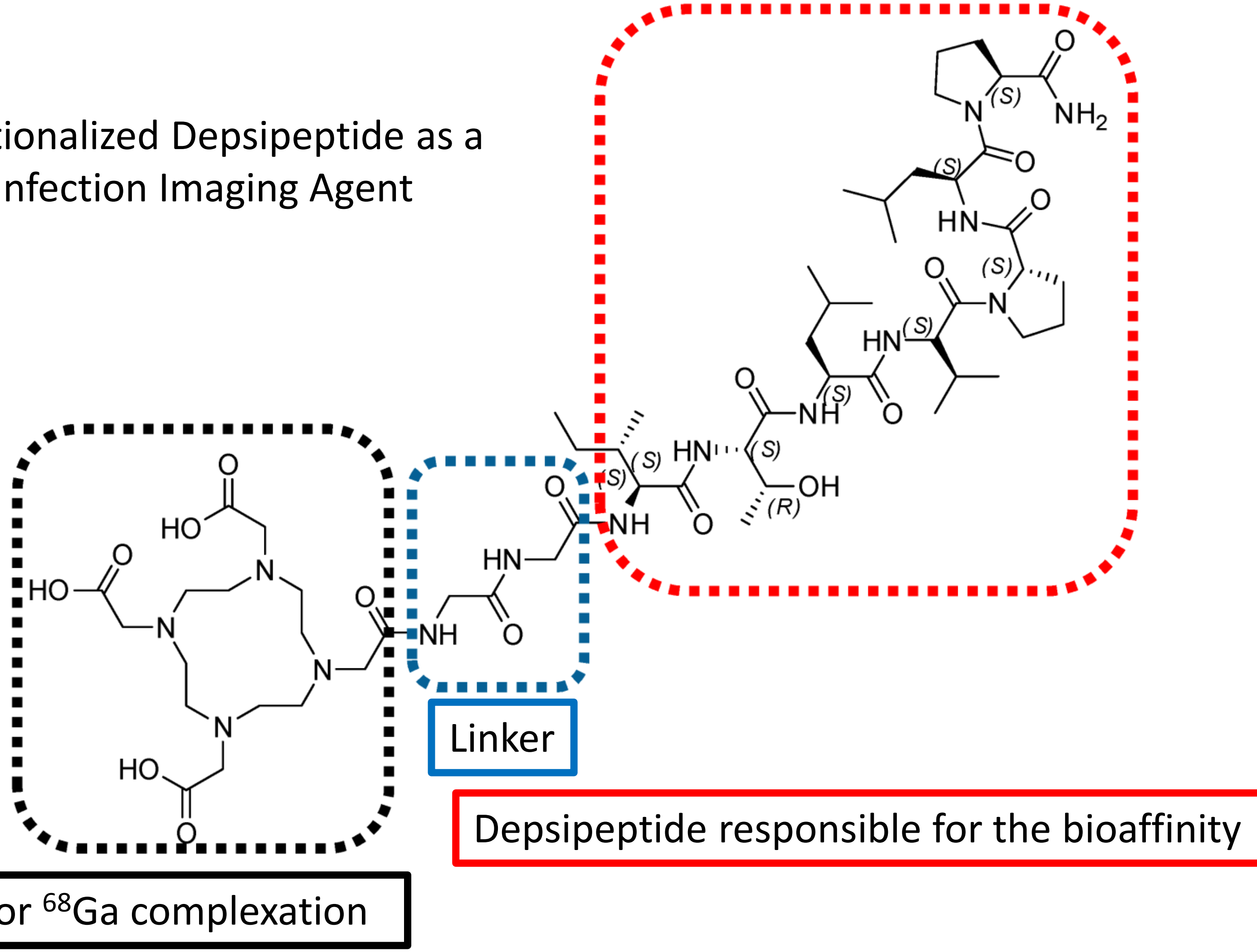
A **High specific activity** radiotracer is needed for imaging a **low abundance** of receptors *in vivo* (effect of binding competition)

V Gómez-Vallejo, http://cdn.intechweb.org/pdfs/27815.pdf

Labeling molecules: key concepts

The labeling reaction shall add a **minimal amount of stericity**

^{68}Ga-DOTA-Functionalized Depsipeptide as a Radiodiagnostic Infection Imaging Agent



T Ebenhan, Molecules 2017, 22, 1403

Labeling molecules: key concepts

The labeling shall be **stable** and not lead to a loss of the radionucleide due to:

- **Chemical reactivity** (for covalent bond)

Ex: ^{24}Na, ^{28}Mg, ^{45}Ca, ^{38}K does not form stable covalent bond with organic compounds

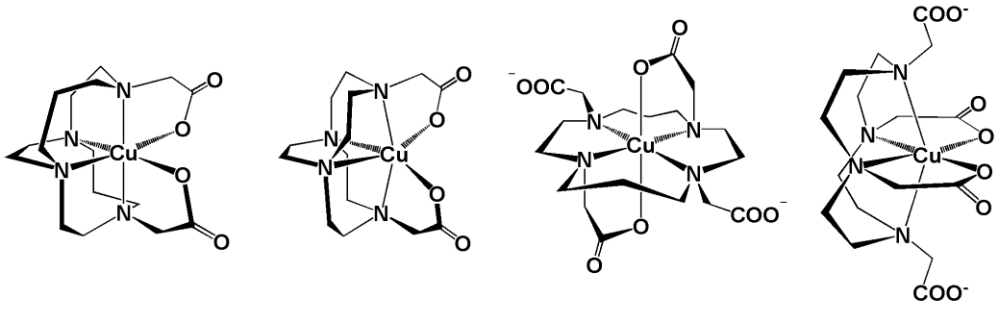
- **Radiolysis**

destruction of the chemical bonds by radioactive decay and free radicals formation

- **Enzymatic reactivity**

Ex: dehalogenase remove radioiodide bound to tyrosine..

- Insufficient **complex strength** (displacement by trace metals)



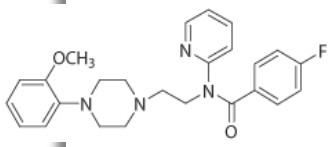
Coordination of metal cation by lone pairs of N and O

→ Different complex have different stability for a given element and for different elements

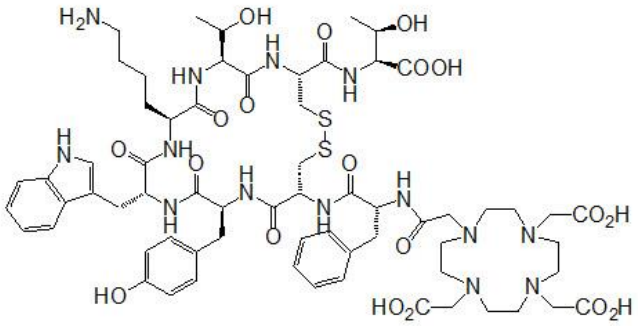
C Andrew Boswell, Mallinckrodt Institute of Radiology 2004

Labeling molecules: nature of the biomolecule

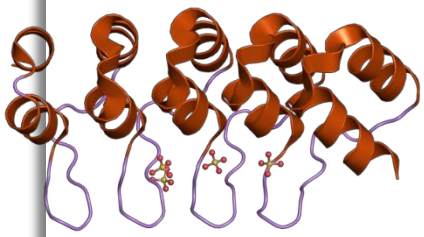
The molecules to be labeled are usually classified by molar weight (by size) and by structure



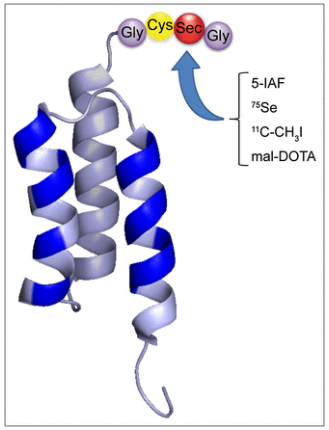
- Small organic molecules (< 1000 Da)

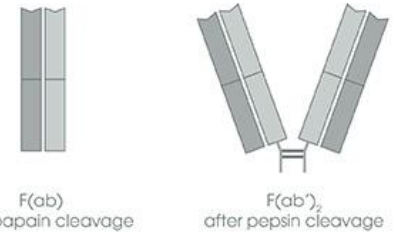


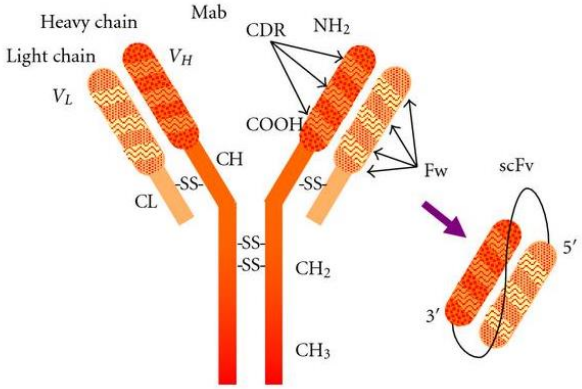
- Peptide derivatives (1-5 kDa)
- Proteins (5-170 kDa)



Affibodies
Single domain antibody
Aptamers
DARPin
Single chain Fv (scFv),
Fab,
F(ab')2,
diabodies
minibodies
Antibodies







> kDa

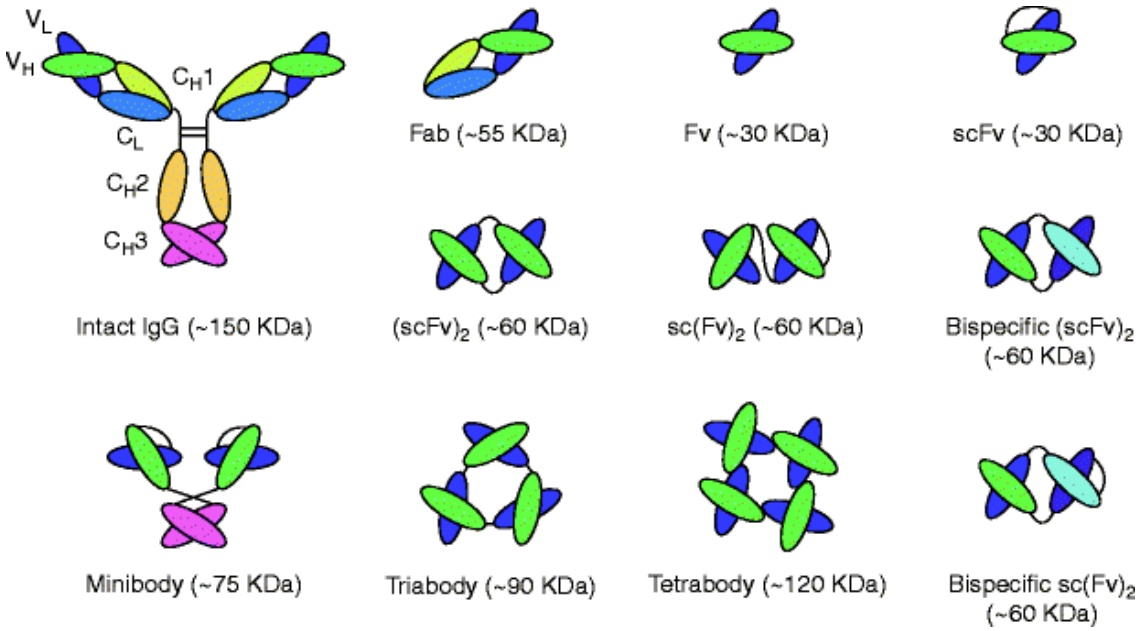
ZA Ahmad, Clinical and Developmental Immunology, 2012 ,ID 980250
H Wållberg, J Nucl Med September 1, 2012 vol. 53 no. 9 1446-1453

Labeling molecules: nature of the biomolecule

The molecules to be labeled are usually classified by molar weight (by size) and by structure

- Proteins (5-170 kDa)
  - Affibodies
  - Single domain antibody
  - Aptamers
  - DARPin
  - Single chain Fv (scFv),
  - Fab,
  - F(ab′)2,
  - diabodies
  - minibodies
  - Antibodies

> kDa



S Ozaki, Encyclopedia of Cancer 2011, https://doi.org/10.1007/978-3-642-16483-5_1603

Labeling molecules: choice of radioisotope

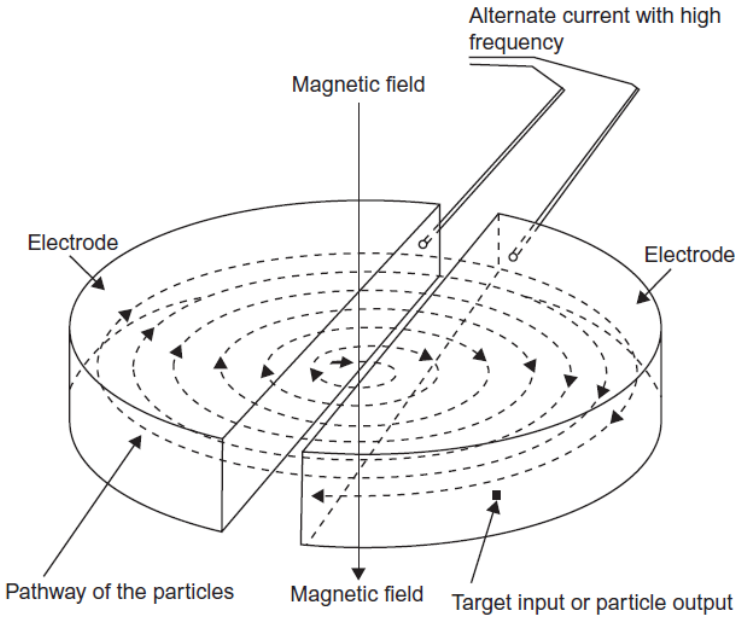
Most common non-metal radionucleides used in PET

^{11}C, ^{13}N, ^{15}O, ^{18}F

Radionuclide	Half-life (min)	Decay mode	Max. Energy (MeV)
Fluorine-18	109.8	97% β+ 3% EC*	0.69
Carbon-11	20.4	100 % β+	0.96
Nitrogen-13	9.98	100 % β+	1.19
Oxygen-15	2.05	100 % β+	1.70

Table 1. Physical characteristics of Fluorine-18, Carbon-11, Nitrogen-13 and Oxygen-15. *EC: Electron capture.

- Produced in high yield in cyclotrons
- Direct introduction into biomolecules
- Decay almost 100% positron emission
- Amenable to isotopic substitution or isosteric in the case of Fluorine

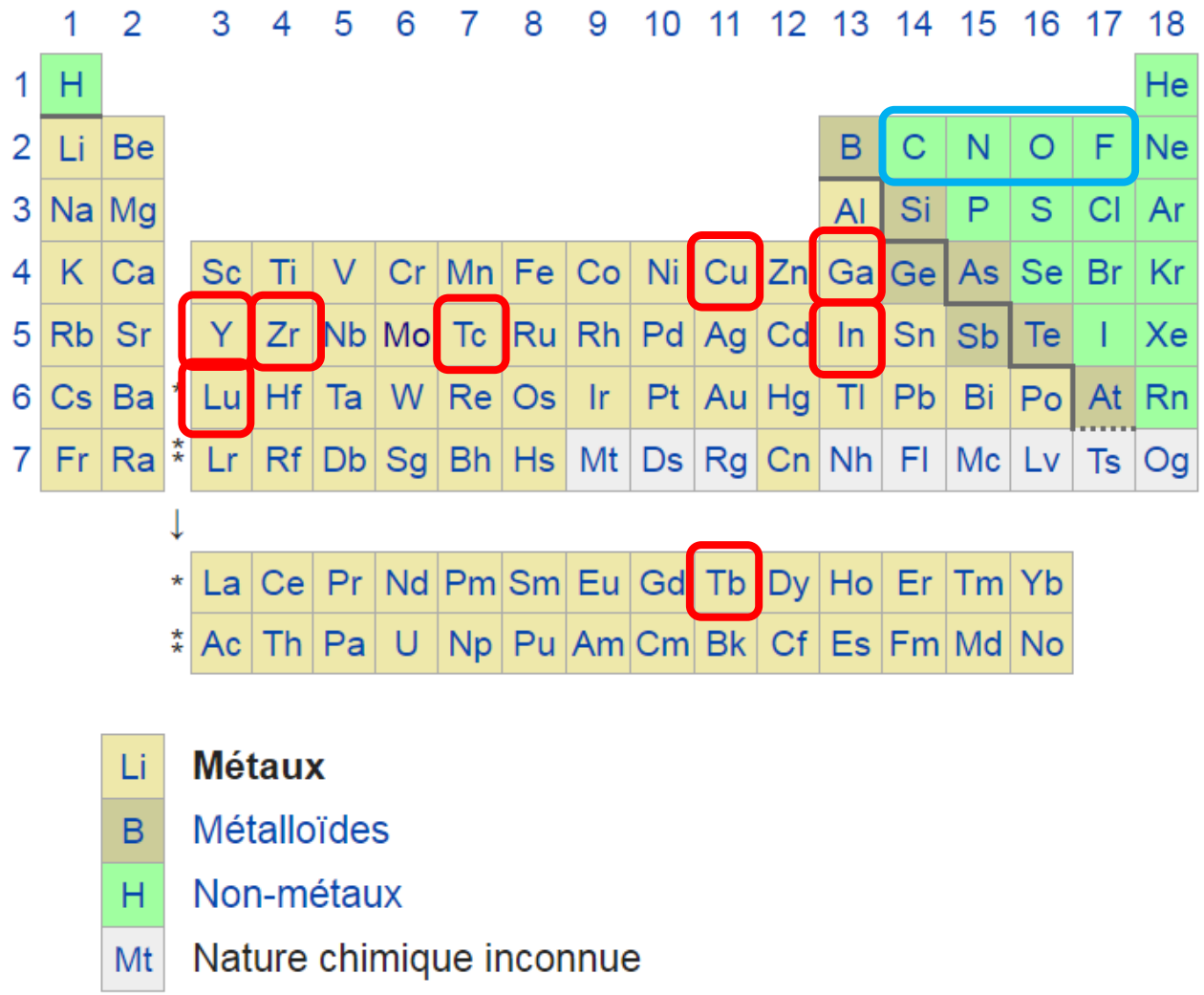


V Gómez-Vallejo, http://cdn.intechweb.org/pdfs/27815.pdf
J Konya, N Nagy, Nuclear and Radiochemistry 2012

Labeling molecules: choice of radioisotope

Most common metals for PET and SPECT

^{68}Ga (1.1 h, β+)
^{99m}Tc (6.0 h,γ)
^{111}In (67.2 h,γ)
^{177}Lu (159.4 h, β-)
^{90}Y (64.1 h, β-)
^{89}Zr (78.5 h, β+)
^{64}Cu (12.7 h, β+)
^{61}Cu (3.3 h, β+)
^{149}Tb (4.1 h, α)
^{152}Tb (17.5 h, β+)
^{155}Tb (127.7 h, γ)
^{161}Tb (165.4 h, β-)
^{44}Sc, ^{213}Bi, ^{153}Sm….



J Konya, N Nagy, Nuclear and Radiochemistry 2012
Wikipedia

Labeling molecules: choice of radioisotope

Table 1 Properties of some popular radiometal isotopes, EC = electron capture; some low abundance emissions have been omitted for brevity[1–4,15,16]

Isotope	$t_{1/2}$ (h)	Decay mode	E (keV)	Production method
^{60}Cu	0.4	β^+ (93%) EC (7%)	β^+, 3920, 3000, 2000	Cyclotron, ^{60}Ni(p,n)^{60}Cu
^{61}Cu	3.3	β^+ (62%) EC (38%)	β^+, 1220, 1150, 940, 560	Cyclotron, ^{61}Ni(p,n)^{61}Cu
^{62}Cu	0.16	β^+ (98%) EC (2%)	β^+, 2910	^{62}Zn/^{62}Cu generator
^{64}Cu	12.7	β^+ (19%) EC (41%) β^- (40%)	β^+, 656	Cyclotron, ^{64}Ni(p,n)^{64}Cu
^{66}Ga	9.5	β^+ (56%) EC (44%)	β^+, 4150, 935	Cyclotron, ^{63}Cu(α,nγ)^{66}Ga
^{67}Ga	78.2	EC (100%)	γ, 93, 184, 300	Cyclotron, ^{68}Zn(p,2n)^{67}Ga
^{68}Ga	1.1	β^+ (90%) EC (10%)	β^+, 1880	^{68}Ge/^{68}Ga generator
^{44}Sc	3.9	β^+ (94%) EC (6%)	γ, 1157 β^+, 1474	^{44}Ti/^{44}Sc generator
^{47}Sc	80.2	β^- (100%)	γ, 159 β^-, 441, 600	^{47}Ti(n,p)^{47}Sc
^{111}In	67.2	EC (100%)	γ, 245, 172	Cyclotron, ^{111}Cd(p,n)^{111m,g}In
^{114m}In	49.5 d	EC (100%)	γ, 190	Cyclotron, ^{114}Cd(p,n)^{114m}In or ^{116}(
^{114}In (daughter)	73 s	β^- (100%)	β^-, 1989	
^{177}Lu	159.4	β^- (100%)	γ, 112, 208 β^-, 177, 385, 498	^{176}Lu(n,γ)^{177}Lu
^{86}Y	14.7	β^+ (33%) EC (66%)	β^+, 1221	Cyclotron, ^{86}Sr(p,n)^{86}Y
^{90}Y	64.1	β^- (100%)	β^-, 2280	^{90}Zr(n,p)^{90}Y
^{89}Zr	78.5	β^+ (23%) EC (77%)	β^+, 897	Cyclotron, ^{89}Y(p,n)^{89}Zr
^{212}Bi	1.1	α (36%) β^- (64%)	α, 6050 β^-, 6089	^{228}Pb/^{212}Pb generator
^{213}Bi	0.76	α (2.2%) β^- (97.8%)	α, 5549 β^-, 5869	^{228}Th/^{212}Pb generator

Tb 149		Tb 152		Tb 155	Tb 161
4.2 m	4.1 h	4.2 m	17.5 h	5.32 d	6.90 d
ε β+ α 3.99 γ 796; 165…	ε α 3.97 β+ 1.8 γ 352; 165…	Iγ 283; 160… ε; β+… γ 344; 411…	ε β+ 2.8… γ 344; 586; 271…	ε γ 87; 105;… 180, 262	β- 0.5; 0.6… γ 26; 49; 75… e-

C Müller, 2012 J. Nucl. Med. 53 1951
EW Price, Chem. Soc. Rev., 2014, 43, 260

Labeling molecules: choice of radioisotope

Factors influencing the choice of the radionucleide

- Imaging or therapy?
- Quality of the image
- Intrinsic parameters such as half life
- Cost of production
- Specific activity
- Ease of chelation

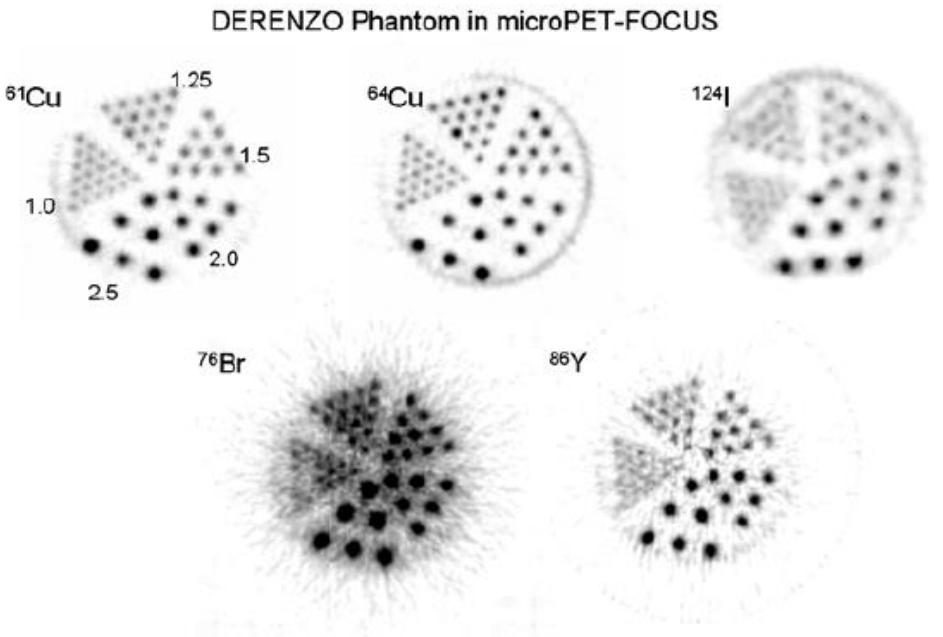


Fig. 3. A mini Derenzo phantom filled with various radionuclide imaged on the microPET Focus scanner. This phantom consists of radioactive rods of specified diameter separated by four times the diameter. These images were reconstructed utilizing the filtered back projection. It is seen that the nuclides with higher energy positrons and prompt gamma rays produce the images that are degraded compared to those with a single low energy positron (for example, ^{64}Cu)

PA Schubiger, PET Chemistry: The Driving Force in Molecular Imaging 2002

Part II

Radiochemistry of metal radioisotopes

Radiochemistry of metals: Conjugation and chelation

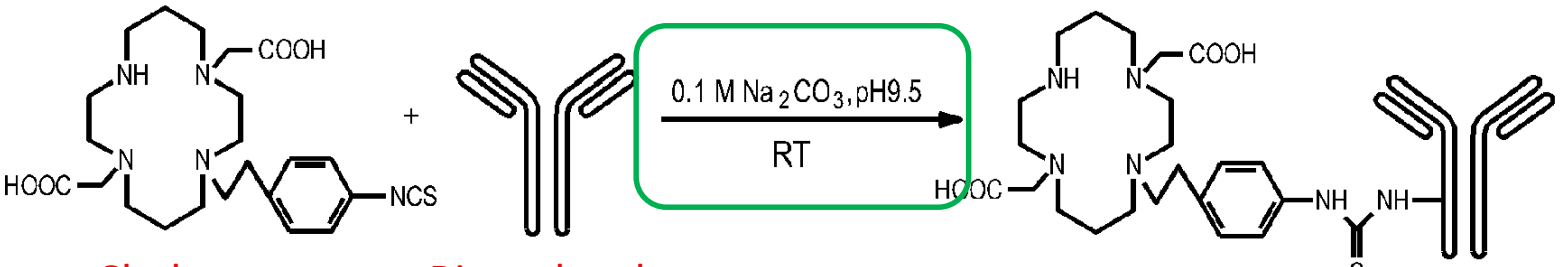
Small organic molecules are usually directly labelled by a covalent bond with a non-metal radioisotope in an organic solvent

The bigger molecules (peptides, proteins) are usually conjugated with a chelator for radiolabeling with metals in a two steps process in water

2 steps:

1) **Conjugation**
2) **Chelation**

Conjugation

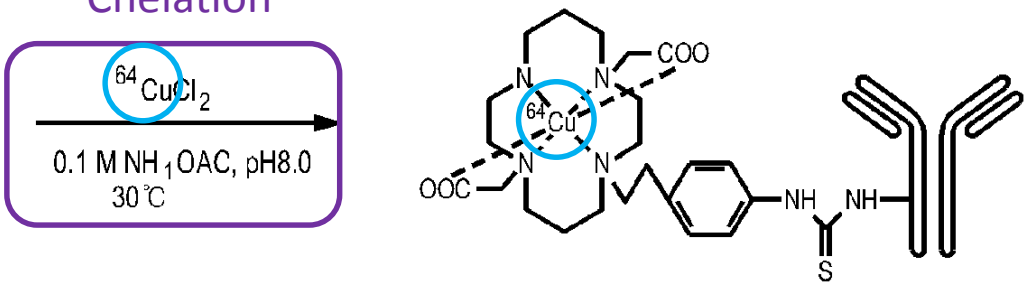


Chelator
14

Biomolecule
trastuzumab

Conjugated Biomolecule
39

Chelation



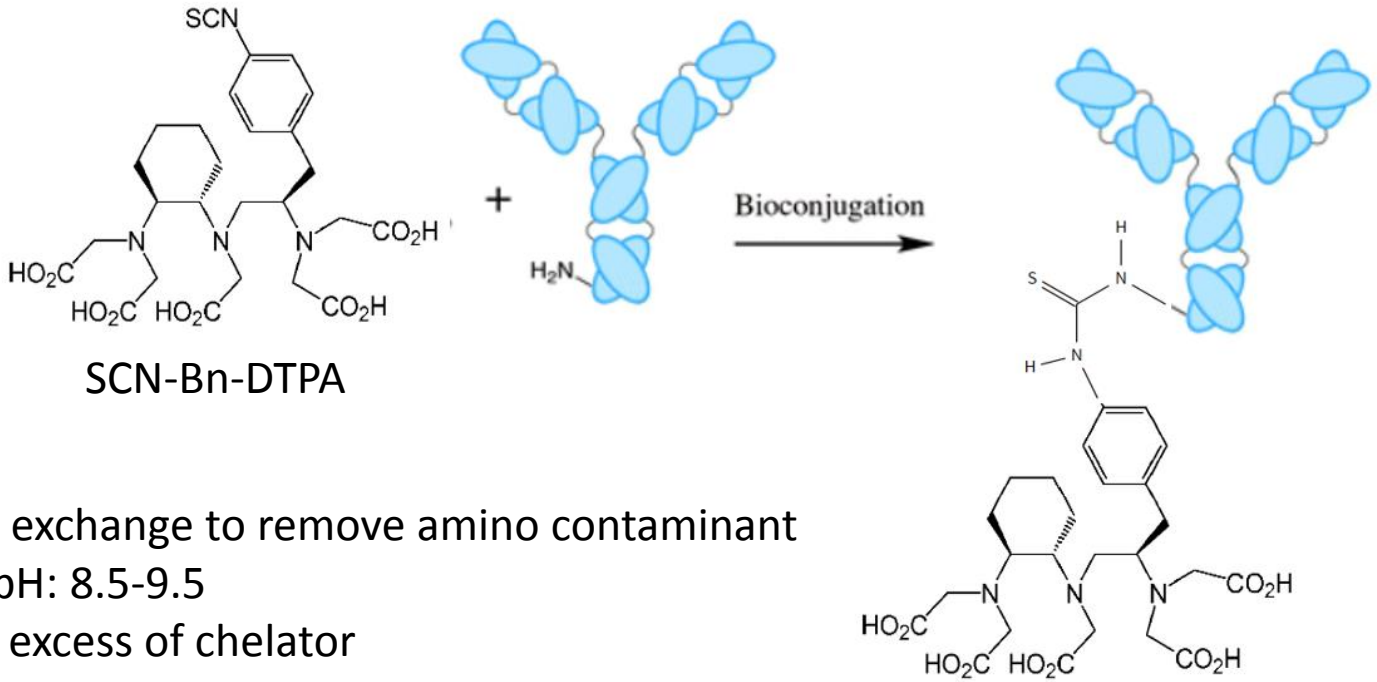
Radiolabeled Biomolecule
40

Radiochemistry of metals: Conjugation and chelation

The conjugation step

Peptides and proteins are a succession of amino acids.

A chelator can be conjugated with the **lysine** amino groups or with **cysteine** thiols of the biomolecule.



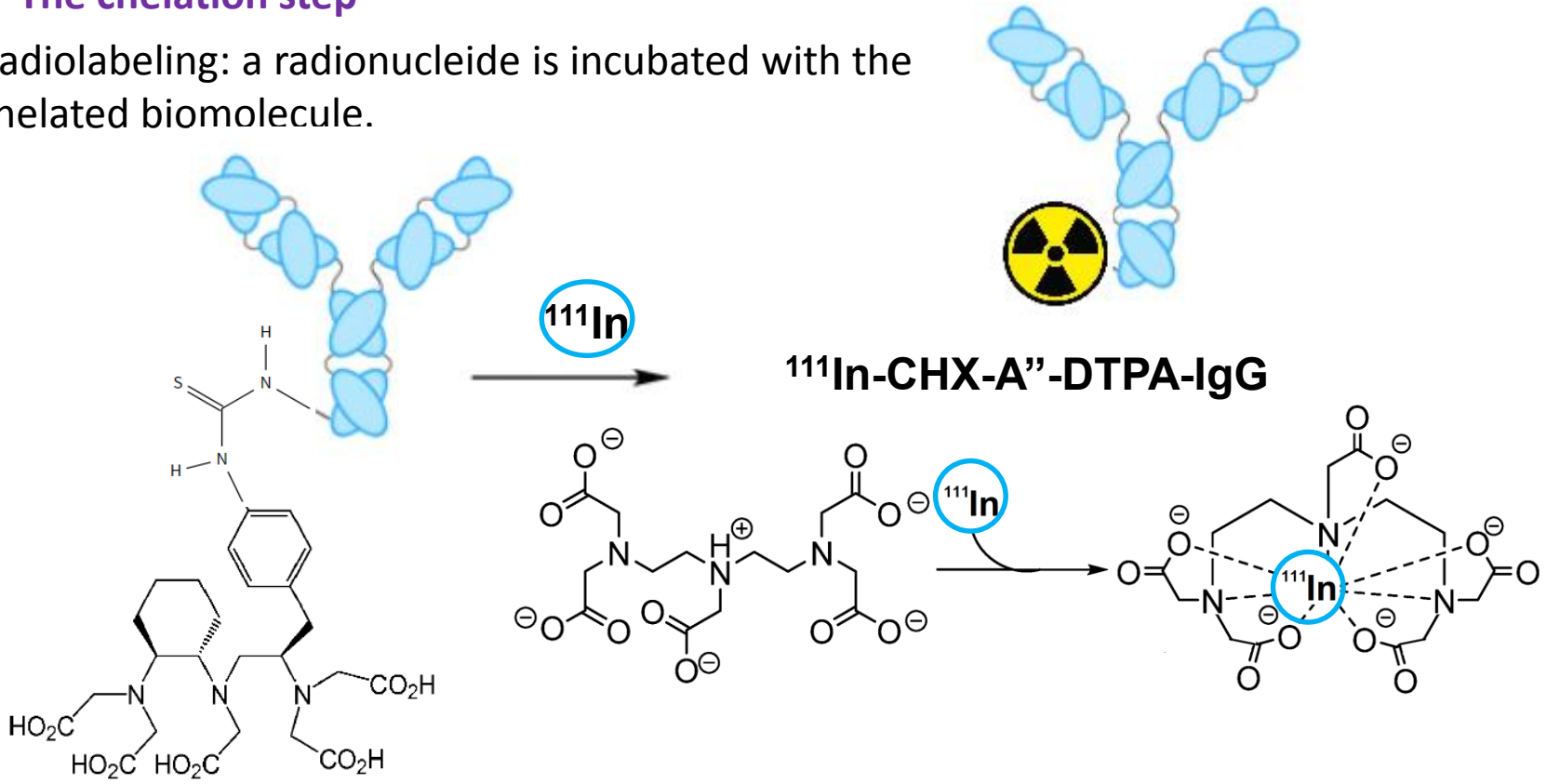
SCN-Bn-DTPA

- Buffer exchange to remove amino contaminant
- Basic pH: 8.5-9.5
- Molar excess of chelator
- Incubate
- Remove excess of unconjugated chelator
  (dialysis, ultracentrifugation, Size-exclusion chromatography)

Radiochemistry of metals: Conjugation and chelation

The chelation step

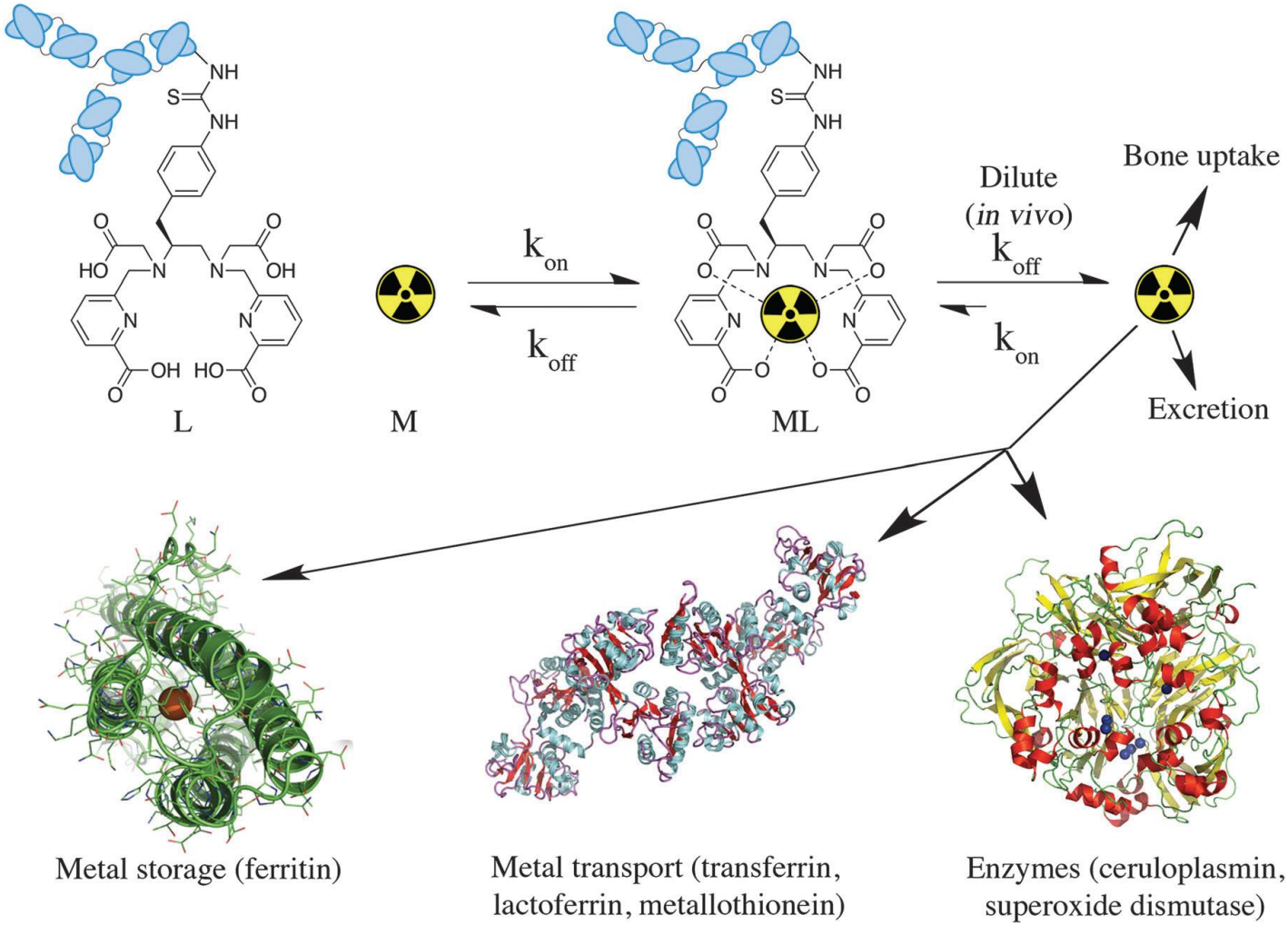
Radiolabeling: a radionucleide is incubated with the chelated biomolecule.



- Acidic pH: 4-6
- Molar default of radiometal
- Incubate
- Remove unchelated radiometal
  (dialysis, ultracentrifugation, Size-exclusion chromatography)

Radiochemistry of metals: choice of chelator

The chelate must have a good in vivo stability



A poor in vivo stability leads to a loss of the radionucleide!

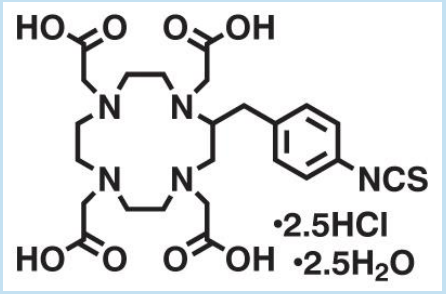
The radiopharmaceutical probe thus fail to achieve a good image or a selective therapeutic action at the target!

EW Price, Chem. Soc. Rev., 2014, 43, 260

Radiochemistry of metals: choice of chelator

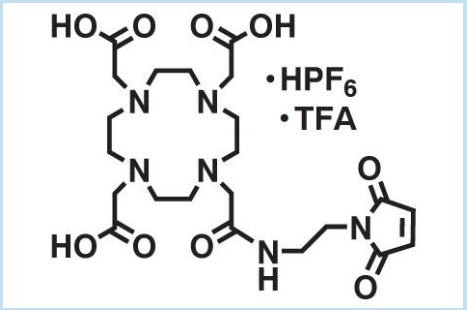
The reagent for the conjugation step is a bifunctional chelator

React with lysine:



p-SCN-Bn-DOTA

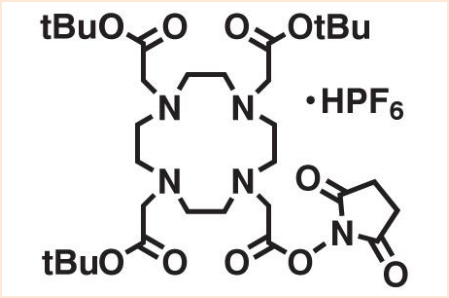
React with cysteine:



Maleimido-mono-amide-DOTA

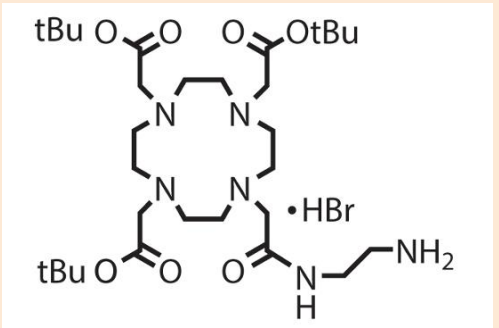
Aqueous reagents

React with amino group:



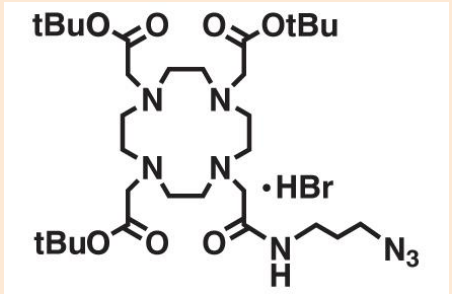
DOTA-mono-NHS-ester

React with carboxyl group:



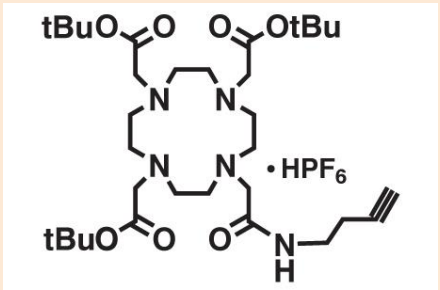
2-Aminoethyl-amide-DOTA

React with alkyne group:



Azido-amide-DOTA

React with azido group:

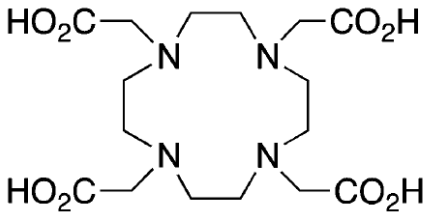


Butyne-DOTA

Organic reagents

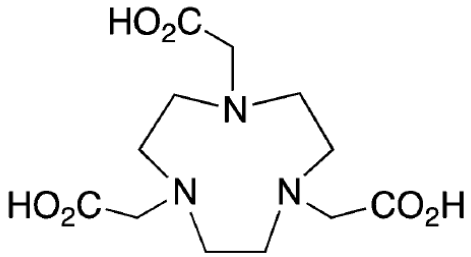
Radiochemistry of metals: choice of chelator

The reagent for the conjugation step is a bifunctional chelator



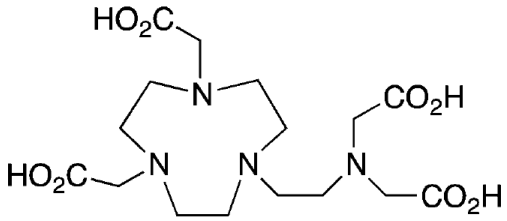
DOTA, 1,4,7,10-tetra-azacyclododecane-1,4,7,10-tetraacetic acid,

DOTA chelate well with:
$^{47}Sc^{3+}$, $^{111}In^{3+}$, $^{177}Lu^{3+}$, $^{90}Y^{3+}$, $^{225}Ac^{3+}$
Heating may be required!
Very stable in vivo



NOTA, 1,4,7-triazacyclononane-1,4,7-triacetic acid, CN = 6, N_3O_3

NOTA chelate well with:
$^{64}Cu^{2+}$, $^{68}Ga^{3+}$,
Room temperature



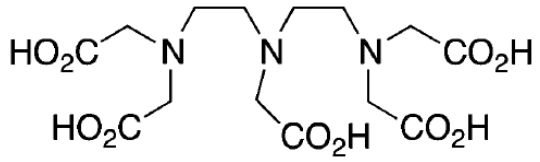
NETA, {4-[2-(bis-carboxymethylamino)-ethyl]-7-carboxymethyl-[1,4,7]triazonan-1-yl}-acetic acid, N_4O_4, CN = 8

NETA chelate well with:
$^{177}Lu^{3+}$, $^{90}Y^{3+}$, $^{213}Bi^{3+}$
Room temperature
Very stable in vivo

EW Price, Chem. Soc. Rev., 2014, 43, 260

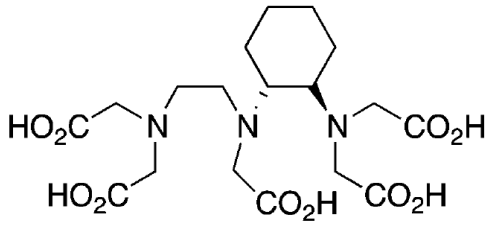
Radiochemistry of metals: choice of chelator

The reagent for the conjugation step is a bifunctional chelator



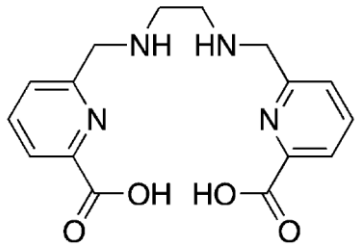
DTPA, diethylenetriaminepentaacetic acid, N_3O_5, CN = 8

DTPA chelate well with:
$^{111}In^{3+}$, $^{64}Cu^{2+}$, $^{177}Lu^{3+}$, $^{90}Y^{3+}$
Room temperature
Poor in vivo stability → obsolete



CHX-A″-DTPA, 2-(*p*-isothiocyanatobenzyl)-cyclohexyldiethylenetriaminepentaacetic acid, N_3O_5, CN = 8

CHX-A''-DTPA chelate well with:
$^{111}In^{3+}$, $^{177}Lu^{3+}$, $^{90}Y^{3+}$, $^{152}Tb^{3+}$
Heating may be required!



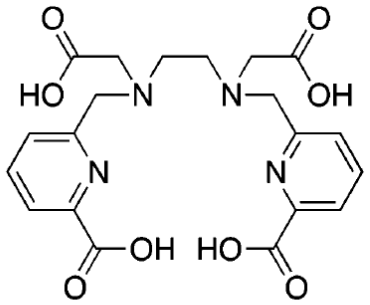
H_2dedpa, 1,2-[[6-(carboxy)-pyridin-2-yl]-methylamino]ethane, N_4O_2 CN = 6

H_2dedpa chelate well with:
$^{177}Cu^{2+}$, $^{68}Ga^{3+}$
Room temperature
Very stable in vivo

EW Price, Chem. Soc. Rev., 2014, 43, 260

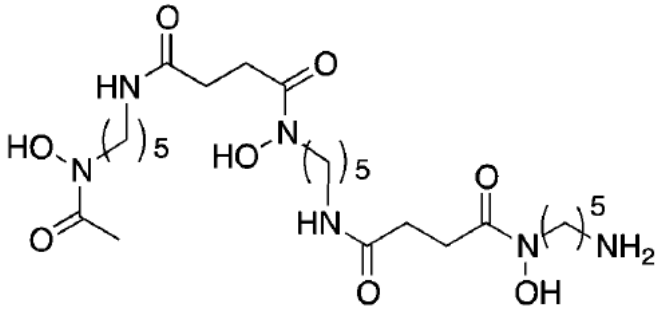
Radiochemistry of metals: choice of chelator

The reagent for the conjugation step is a bifunctional chelator



H_4octapa, *N,N′*-bis(6-carboxy-2-pyridylmethyl)-ethylenediamine-*N,N′*-diacetic acid, N_4O_4 CN = 8

H_2octapa chelate well with:
$^{111}In^{3+}$, $^{177}Lu^{3+}$
Room temperature
Very stable in vivo



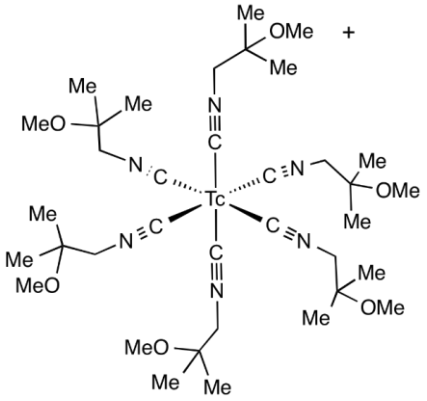
DFO, desferrioxamine B, O_6, CN = 6

DFO chelate well with:
$^{89}Zr^{4+}$
Room temperature
Slowly decompose in vivo
DFO is the only chelator of Zirconium

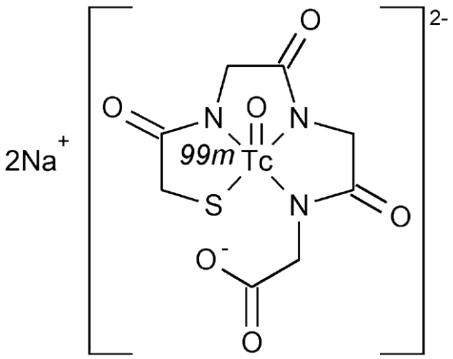
EW Price, Chem. Soc. Rev., 2014, 43, 260

Radiochemistry of metals: choice of chelator

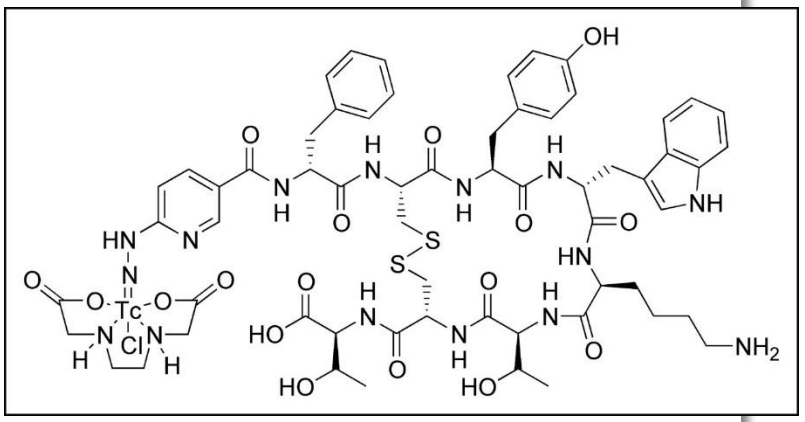
^{99m}Tc-Technetium is a particular case



Technetium ^{99m}Tc-sestamibi



^{99m}Tc-MAG3
(mercaptoacetyltriglycine)

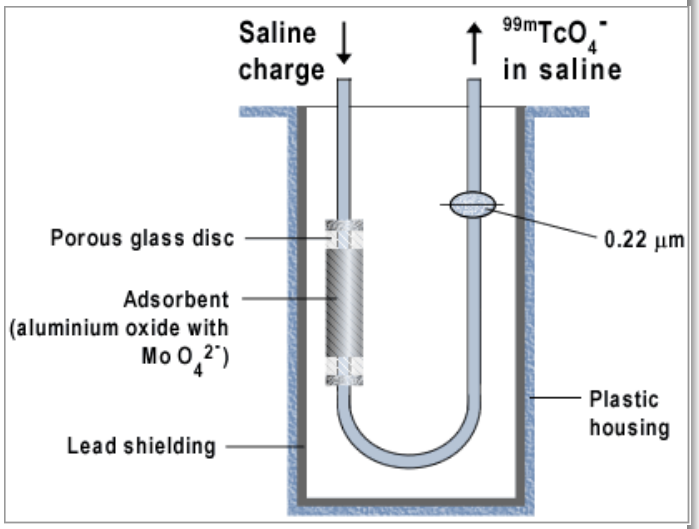


^{99m}Tc-octreotide

Several oxidation stage of ^{99m}Tc leads to multiple ligands and special synthetic techniques

Commercially available synthesis kit are in common use for ^{99m}Tc-radiopharmaceuticals

^{99m}Tc is very popular in nuclear medicine



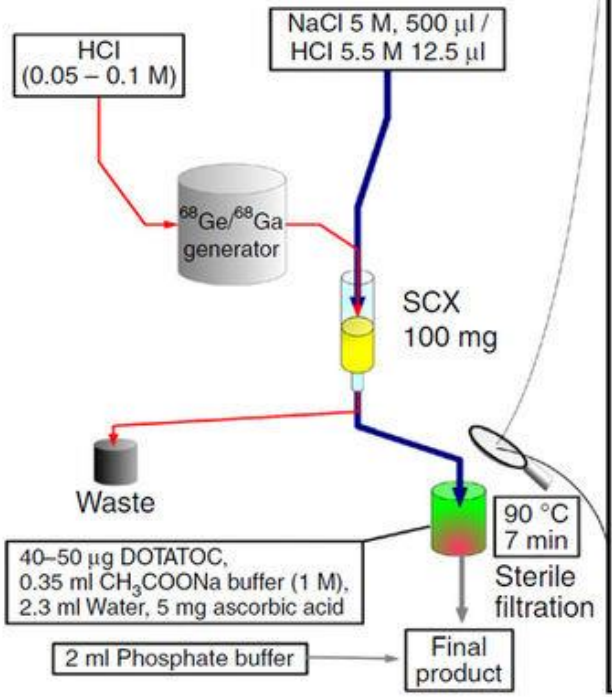
^{99m}Tc is prepared in the radiopharmacy using a generator (^{99}Mo → ^{99m}Tc)

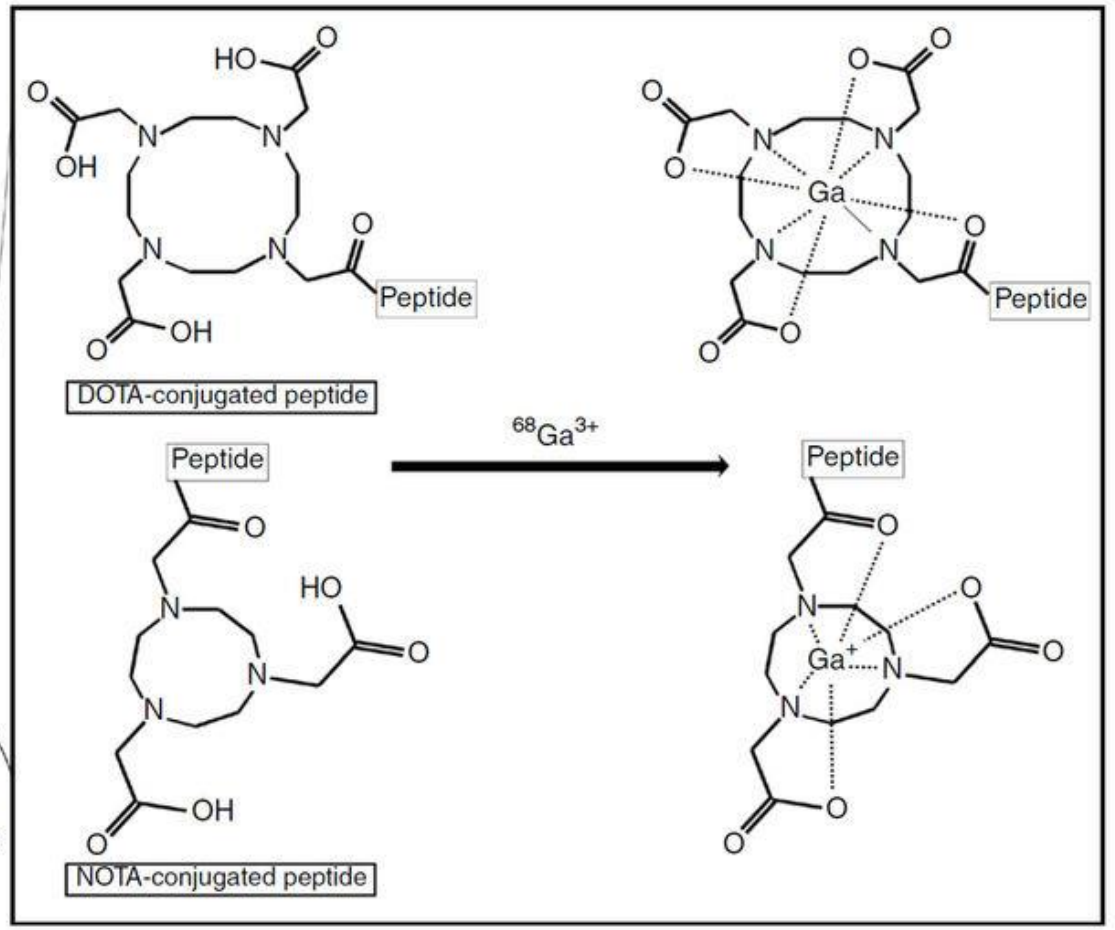
https://humanhealth.iaea.org/HHW/Radiopharmacy/VirRad/Eluting_the_Generator/Generator_Module/Design_principles

Radiochemistry of metals: choice of chelator

^{68}Ga-Gallium is prepared in the radiopharmacy using a generator (^{68}Ge → ^{68}Ga)

^{68}Ge half-life 271 days
^{68}Ga half-life 68 minutes



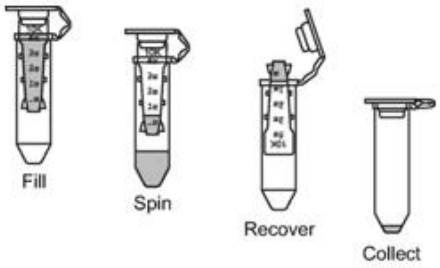


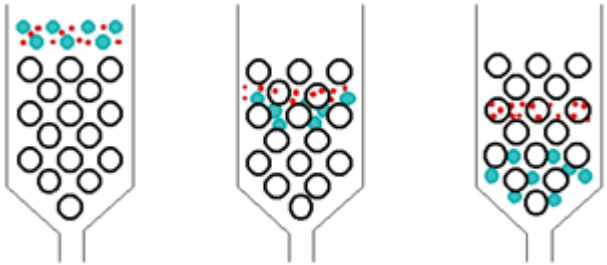
D Mueller, Nature Protocols 11, 1057–1066, 2016

Radiochemistry of metals: purification

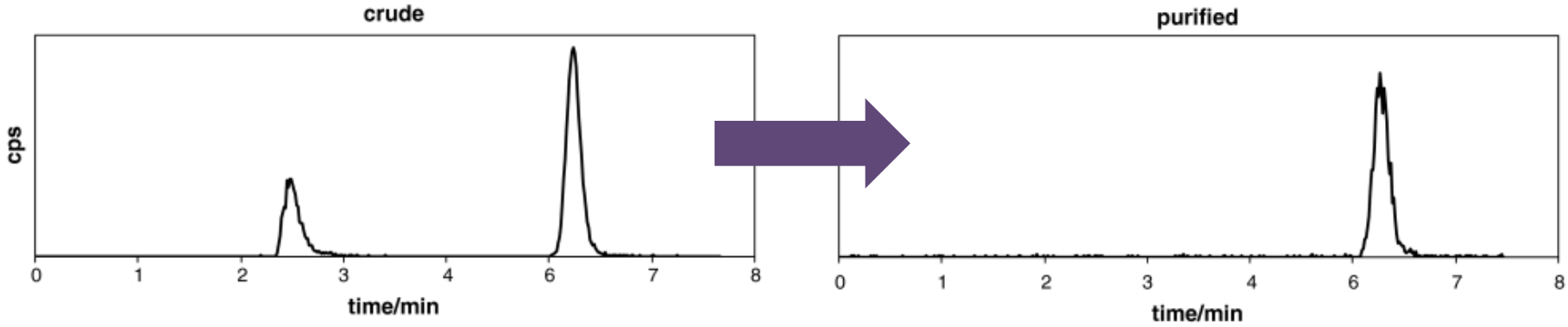
The purification of the final radiopharmaceutical can be done:

- No purification for synthesis kit
- Ultracentrifugation (MW > 10 kDa)
- Size Exclusion Chromatography (MW > 10 kDa)
- Cationic exchange column
- HPLC for small organic molecules





Purification is followed by a sterilisation step usually by sterile filtration (0.22 µm filter)



M Jauregui-Osoro, European Journal of Nuclear Medicine 2010 37(11):2108-16

Radiochemistry of metals: analysis and quality control

A Certificate of Analysis has to be completed

- The radiochemical purity is done using a radiochemical detector and:
  - Paper chromatography
  - Thin Layer Chromatography
  - High Pressure Liquid Chromatography (HPLC)
- The chemical purity is measured with an UV detector or others
- Residual solvents are measured by Gaz Chromatography

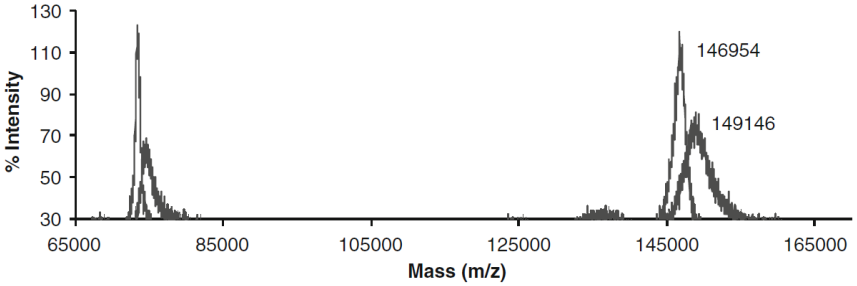
The analytical process has to be validated!

Radiochemistry of metals: analysis and quality control

The number of chelators on the biomolecule can be determined

- By MALDI-TOF Mass Spectrometry:

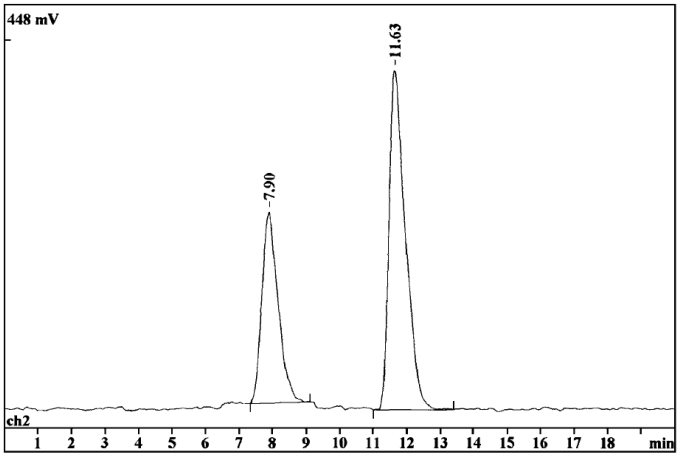
By knowing the average mass of the antibody and the center of the conjugated antibody average masses distribution an average number of conjugates linked to the antibody could be determined.



MS 149146 - 146954 = 2192 Da
DOTA MW 551 Da
2192 / 551 → 4 DOTA on Ab

- By Radio-HPLC:

Using 10 equivalents of cold isotope spiked with a trace amount of radioactive isotope. After incubation, the radioactive peak of the metal chelated with the biomolecule is compared with the total radioactive area.



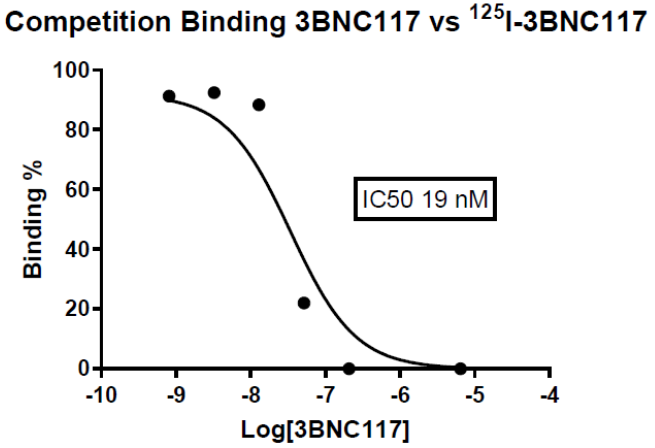
Area 7.90 ($^{177/nat}$Lu-DOTA-Ab) = 40%
Area 11.63 = ($^{177/nat}$Lu-DTPA) = 60%
Total = 10 eq vs Ab → 4 DOTA on Ab

F Forrer, Eur J Nucl Med Mol Imaging (2009) 36:1443–1452

Radiochemistry of metals: analysis and quality control

In vitro tests on the final molecule are also part of the validation

Competition Binding



36 – Competition binding of 3BNC117 vs ^{125}I-3BNC117 against gp120 heptamer 426c DMRS.

Immunoaffinity (*Scatchard*)

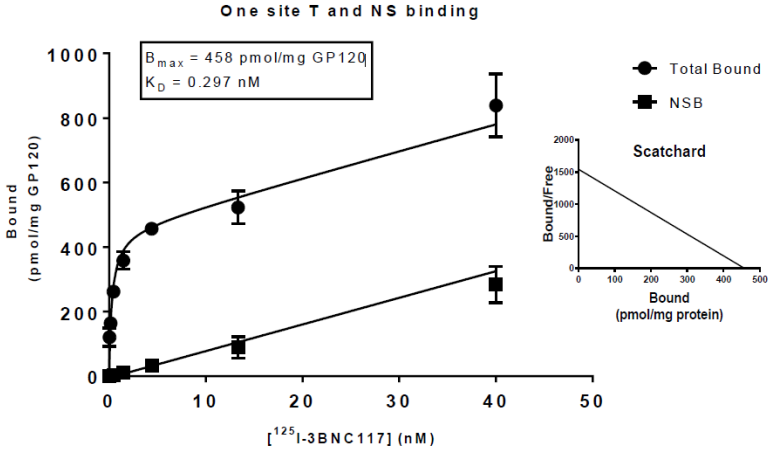


Figure 35 – Conventional saturation assay and Scatchard plot of ^{125}I-3BNC117 and gp120 heptamer 426c DMRS core C4b. Result of one of three experiments realized in triplicate.

Immunoreactivity (*Lindmo*)

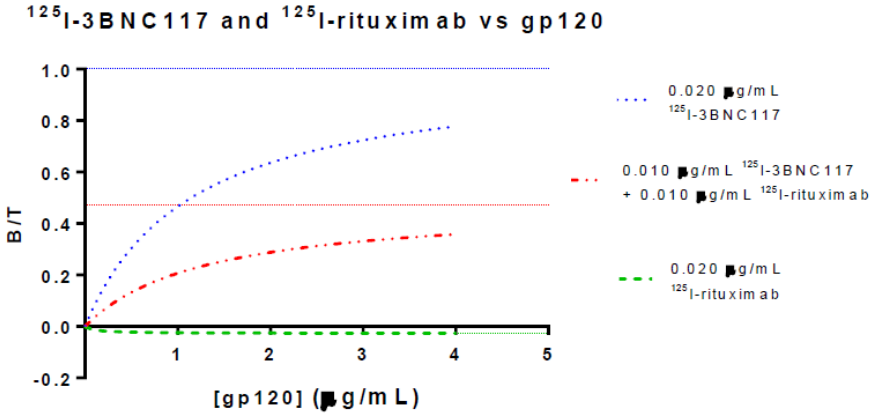


Figure 38 – Specificity test of the immunoreactivity assay with a specific and a non-specific radiolabeled antibody against gp120

canton de vaud

Thank You!