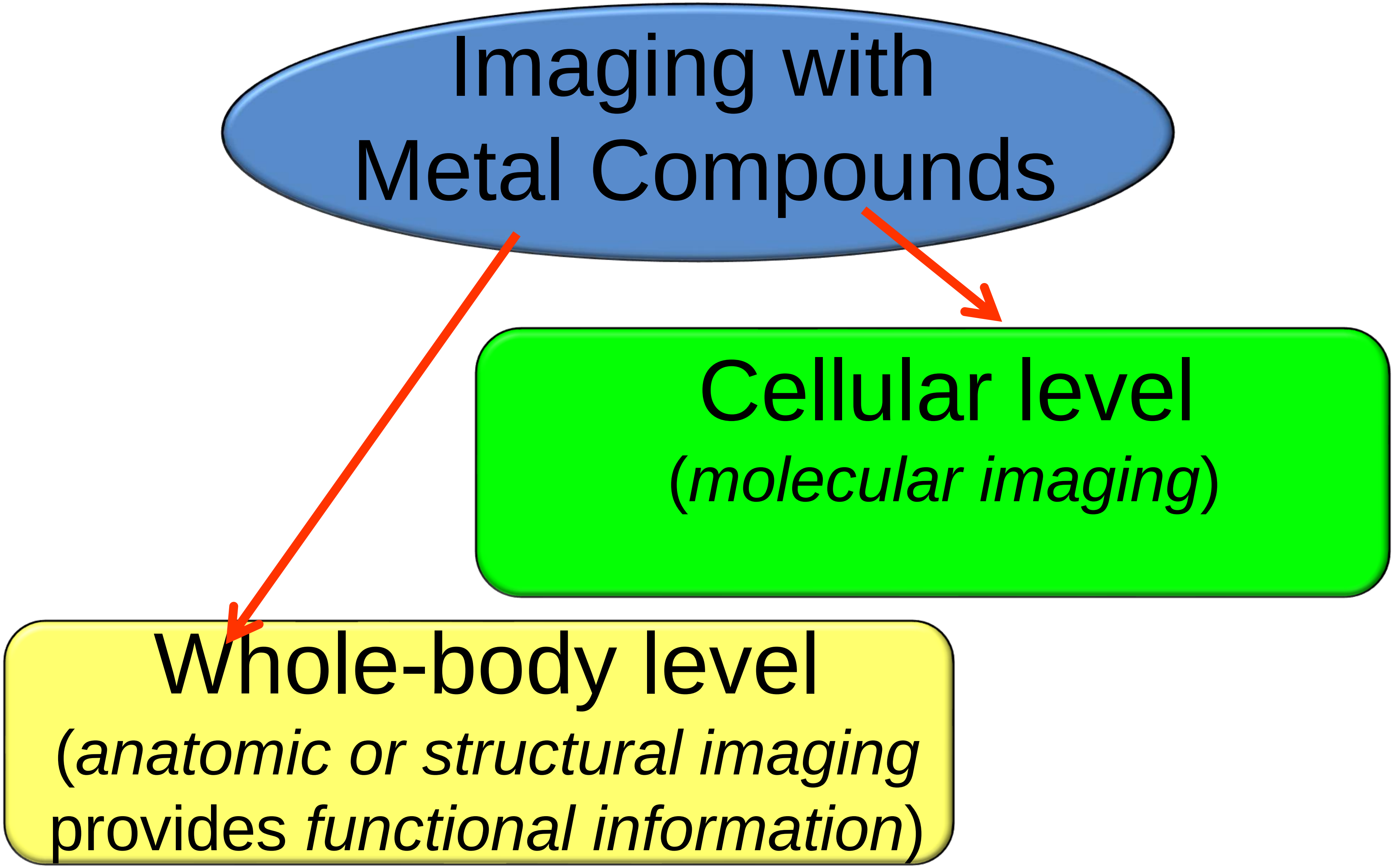
Imaging techniques

Imaging techniques comprise:

- X-ray (Computed Tomography, uses rotating X-rays, iodine-based contrast agents to enhance visibility of blood vessels and organs)
- Radio-imaging (SPECT, PET)
- Magnetic resonance (MRI)
- Optical imaging (e.g. fluorescence, mostly used in preclinical research)
- Ultrasound (ecography)



Imaging with Metal Compounds

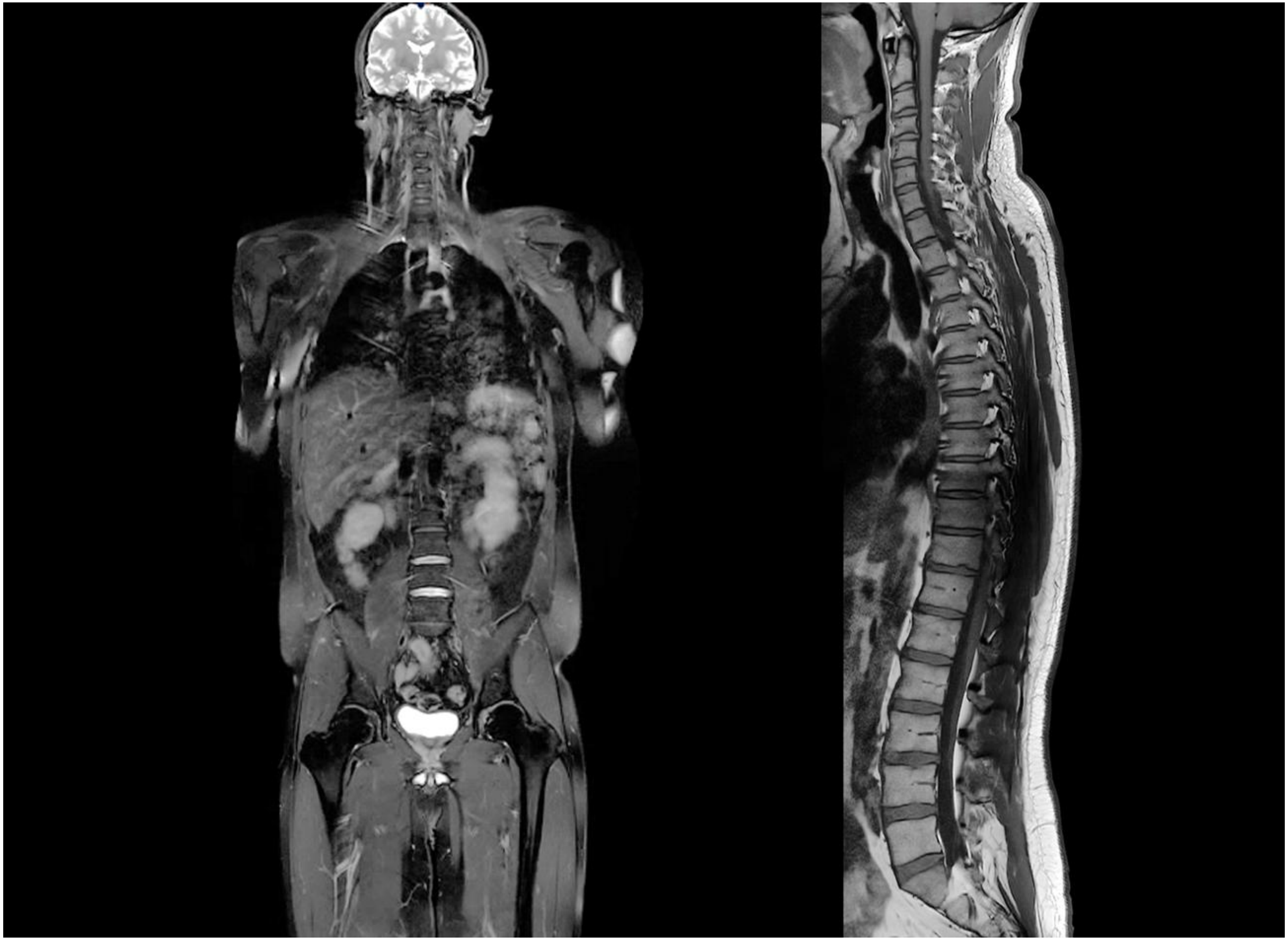
Cellular level

(*molecular imaging*)

Whole-body level

(*anatomic or structural imaging* provides *functional information*)

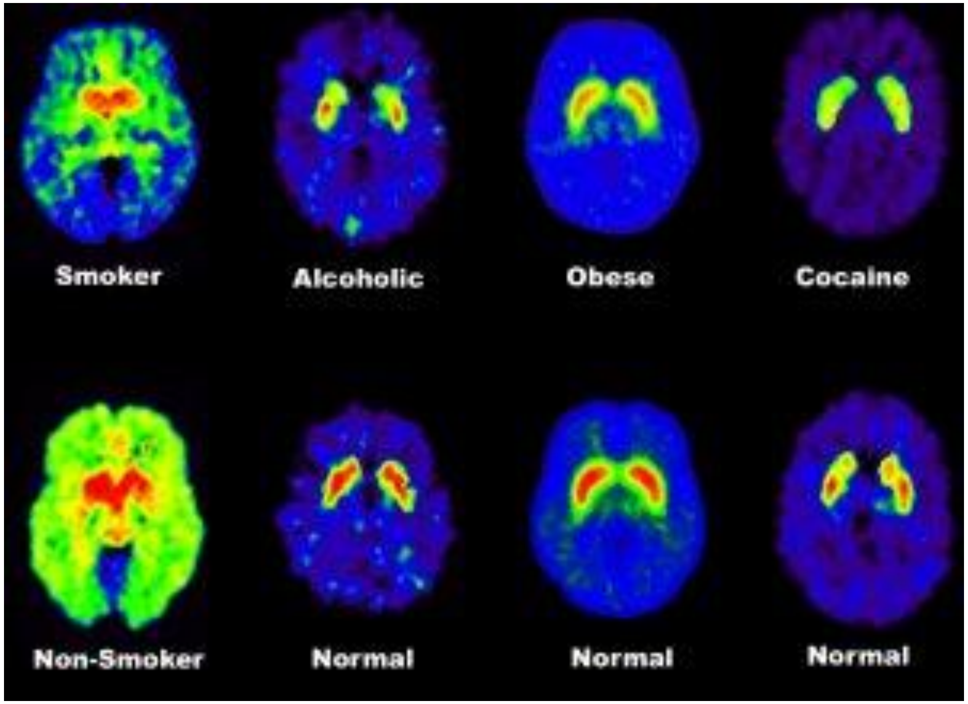
Example of full-body imaging

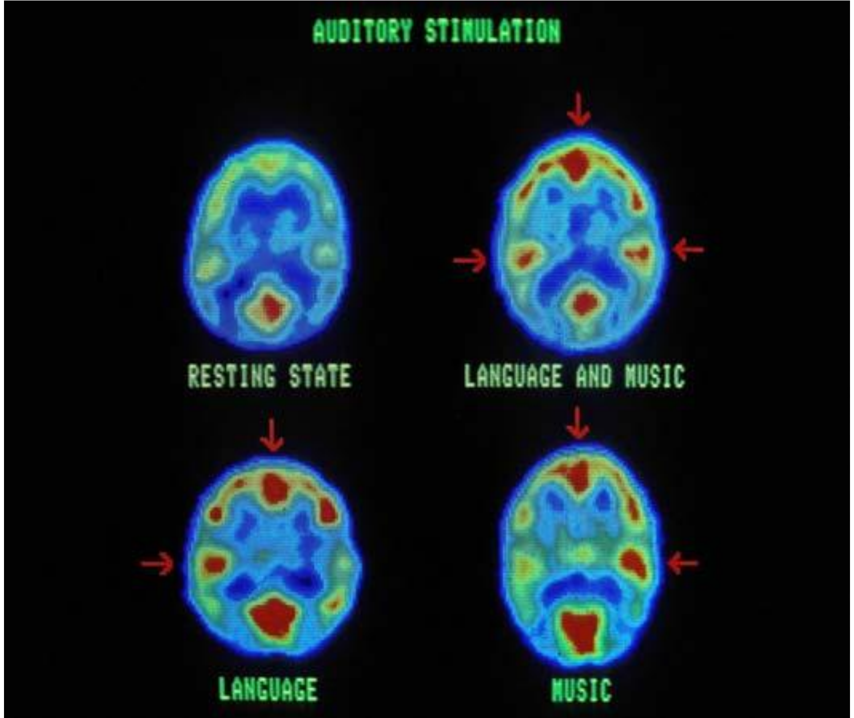


Definition of molecular imaging (2007):
molecular imaging concerns the visualization, characterization, and measurement of biological processes at the molecular or cellular level in humans or other living organisms.

Molecular Imaging

Molecular imaging depends on specialized probes (tagged with radioactivity, fluorescence, or magnetic properties) designed to bind to specific receptors, enzymes, or metabolic pathways.





As the concentration of the molecular targets is very low, besides specificity the probe must provide a very intense signal.

Spontaneous processes in radioactive nuclei

- Emission of particles (α, β^-, β^+)
- Electron capture (EC or ε)
- Emission of radiation (X, γ ray)

A β^- emission formally transforms a neutron into a proton. Both β^+ emission and EC formally transform a proton into a neutron.

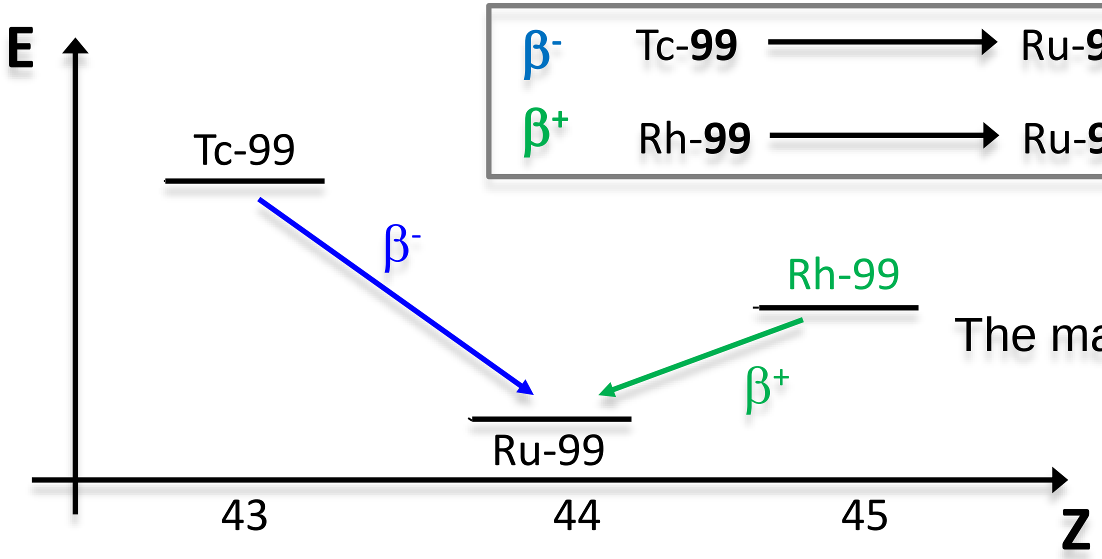
β^+ emission occurs when $\Delta E_{pf} > 1.022$ MeV
Electron capture occurs when $\Delta E_{pf} < 1.022$ MeV

Radioactivity: why are nuclei unstable?

a chemical energy scheme

β^- Tc-**99** $\longrightarrow$ Ru-**99** + e^- + antineutrino

β^+ Rh-**99** $\longrightarrow$ Ru-**99** + e^+ + neutrino



The mass number A does not change

- Isobars are atoms of different chemical elements that have the same total number of nucleons (i.e. mass number), and thus the **same number** of internucleon interactions

- One combination n-p ; p-p ; n-n must be **the most favorable one**, hence the most stable nucleus

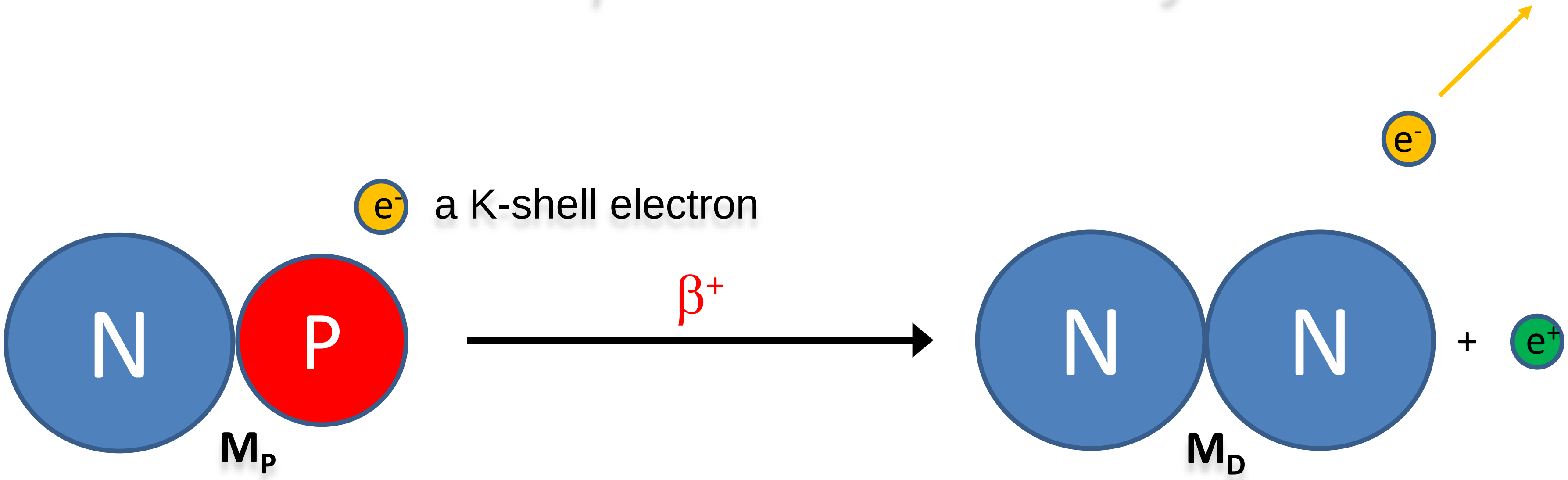
- All other combinations n/p are higher in energy, **hence unstable** stability on the atomic nucleus scale means MASS, hence, the lighter the more stable

Neutron: $m_n = 1.0086647$ a.u.

Proton: $m_p = 1.007276$ a.u.

(Electron): $m_e = 5.485799 \cdot 10^{-4}$ a.u.

A model β^+ emission decay



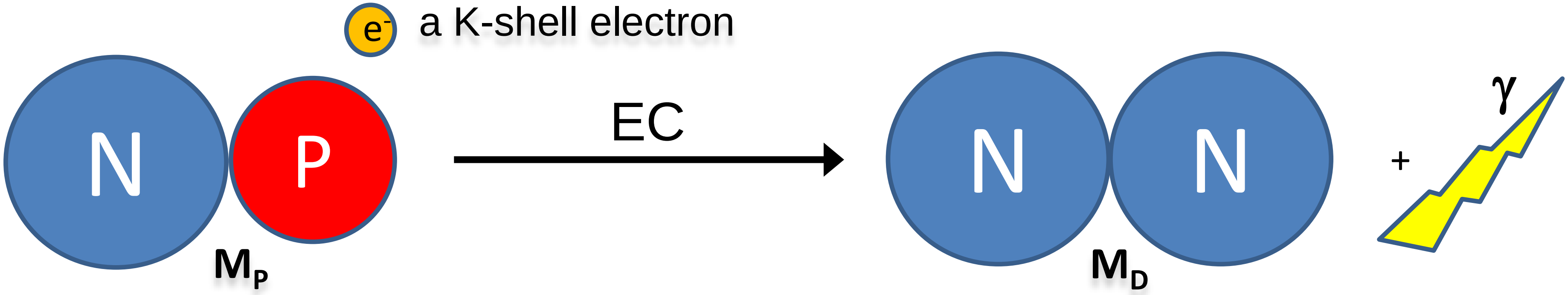
the new nucleus looses a shell electron with m_e = 0.0005485 amu

in addition, a β^+ particle with m = 0.0005485 amu is generated

in total, a mass = 0.001097 amu (= 1.022MeV) is generated

Thus, if the new nucleus has less than 1.022MeV additional binding energy, the β^+ decay is uphill and will not happen

A model EC decay



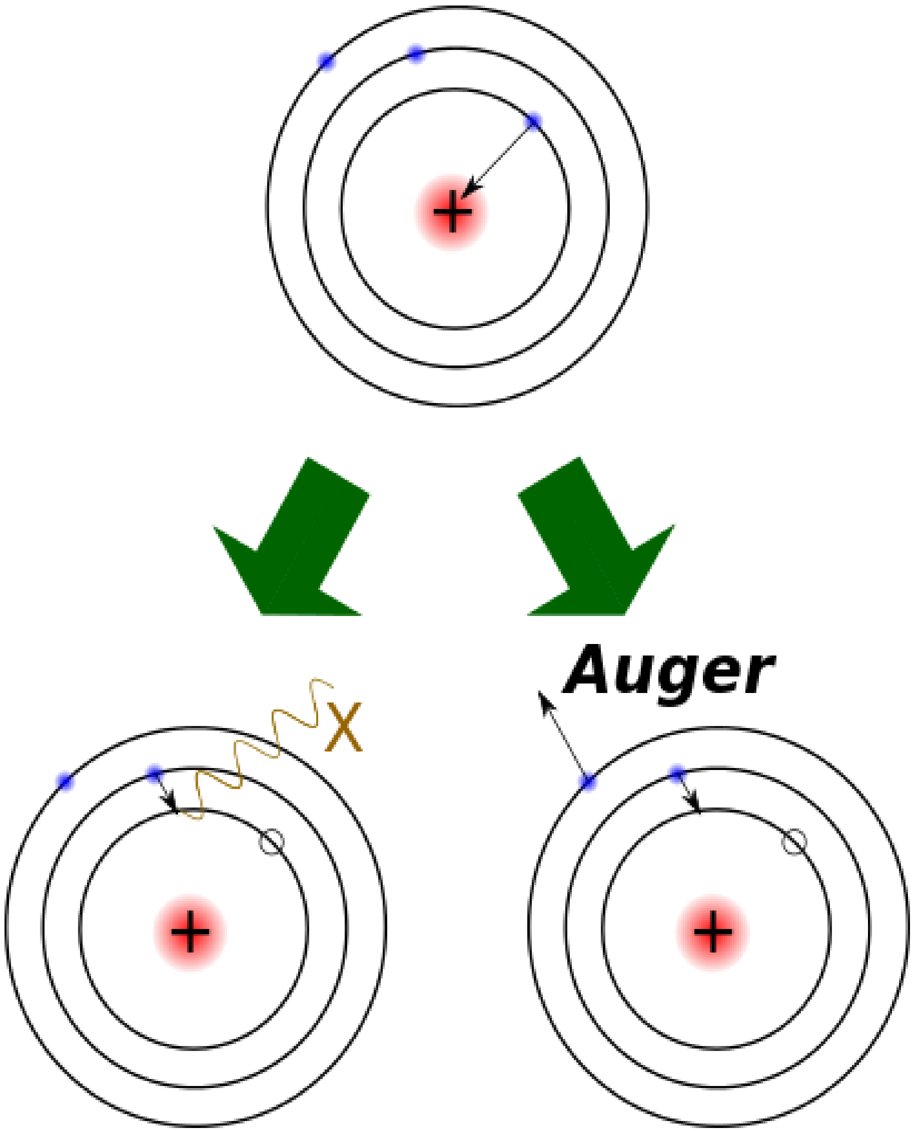
an electron from the K-shell is trapped by a proton inside the nucleus

Thus neither a positron nor an electron are lost

The decay energy Q is equivalent to M_P-M_D (0.0013887 amu) in form of a γ-quant

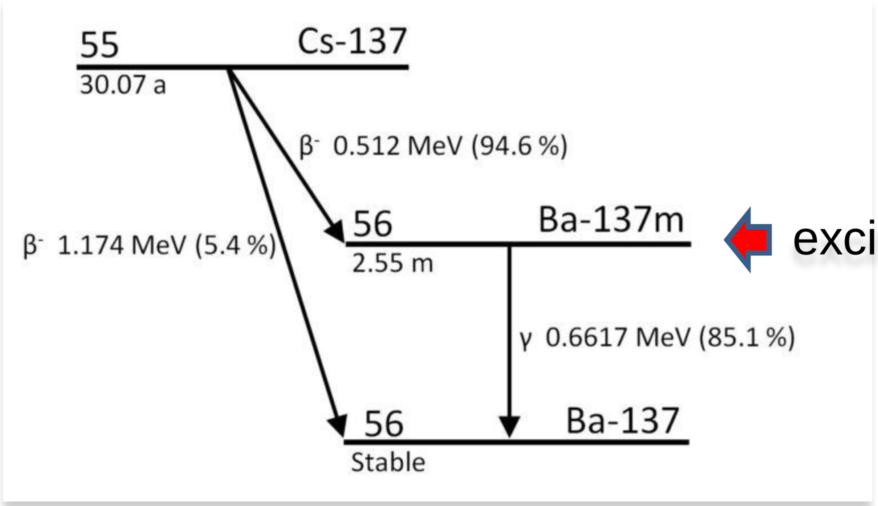
if Q < 1.022MeV, exclusively EC decay, if larger, both β^+ and EC decays may occur in parallel

Electron capture (EC) can produce Auger electrons



Phenomena following up the decay

After the β (and α) decay, the daughter nucleus is generally **in an excited state**



⇦ excited state

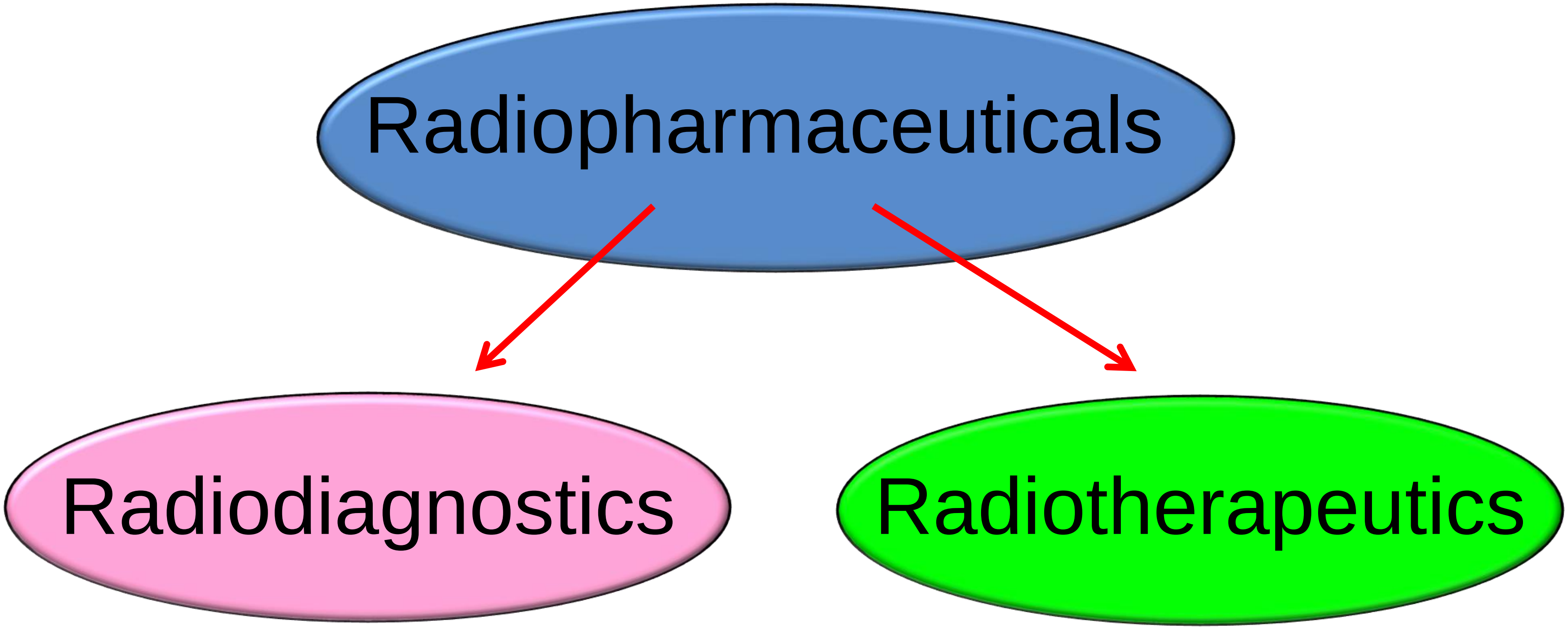
the excited state goes into the ground state by

emitting one (or more) γ-quants

or

by emitting electrons from the shell

⇨ Auger electrons



Radiopharmaceuticals

Radiodiagnostics

Radiotherapeutics

γ-emitters (SPECT)

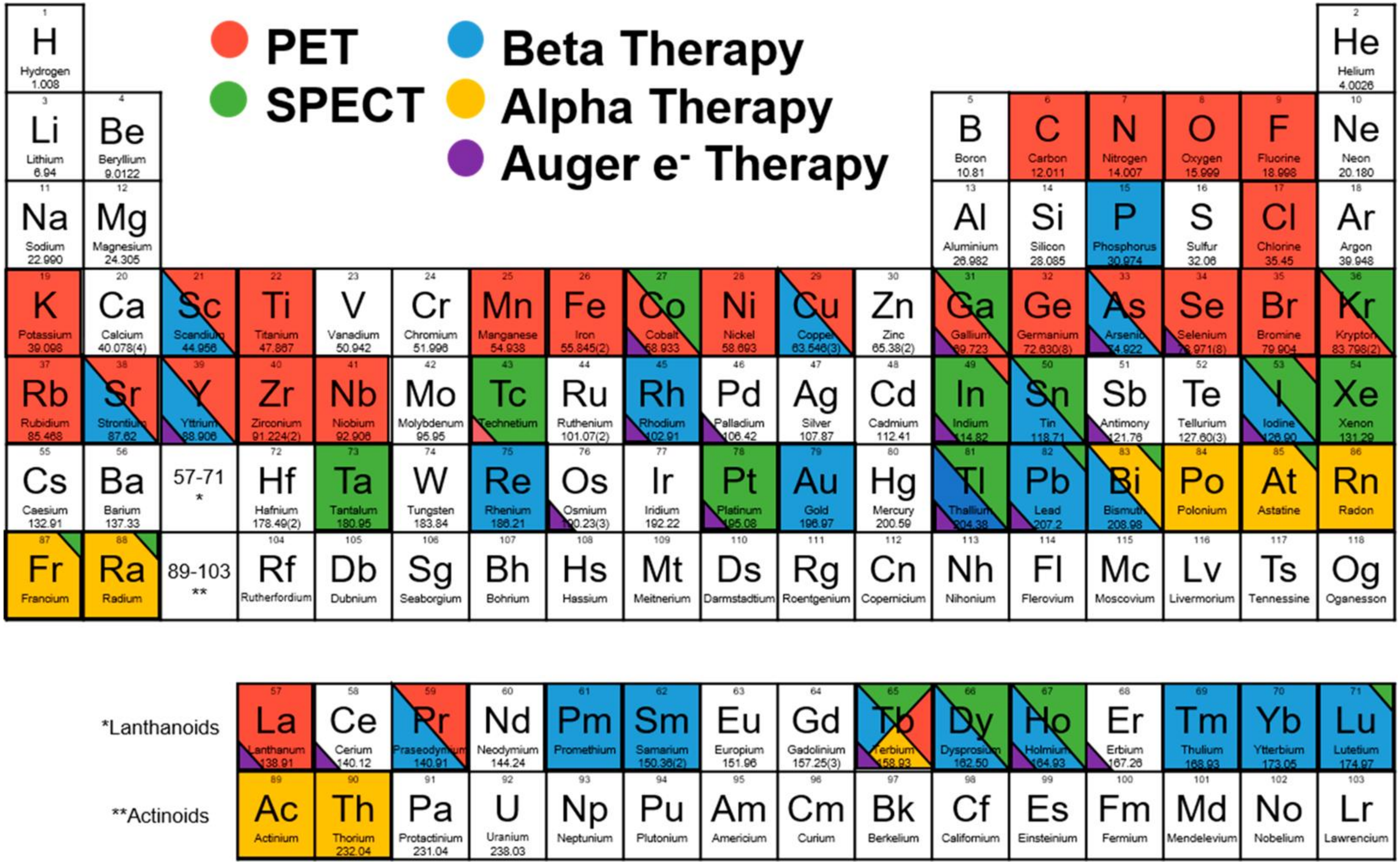
positron-emitters (β^+) (PET)

α or β^- emitters

SPECT = Single Photon Emission Computed Tomography

PET = Positron Emission Tomography

$10^{-6} – 10^{-8}$ M



🔴 **PET**

🟢 **SPECT**

🔵 **Beta Therapy**

🟡 **Alpha Therapy**

🟣 **Auger e^- Therapy**

1 H Hydrogen 1.008																	2 He Helium 4.0026
3 Li Lithium 6.94	4 Be Beryllium 9.0122											5 B Boron 10.81	6 C Carbon 12.011	7 N Nitrogen 14.007	8 O Oxygen 15.999	9 F Fluorine 18.998	10 Ne Neon 20.180
11 Na Sodium 22.990	12 Mg Magnesium 24.305											13 Al Aluminium 26.982	14 Si Silicon 28.085	15 P Phosphorus 30.974	16 S Sulfur 32.06	17 Cl Chlorine 35.45	18 Ar Argon 39.948
19 K Potassium 39.098	20 Ca Calcium 40.078(4)	21 Sc Scandium 44.956	22 Ti Titanium 47.867	23 V Vanadium 50.942	24 Cr Chromium 51.996	25 Mn Manganese 54.938	26 Fe Iron 55.845(2)	27 Co Cobalt 58.933	28 Ni Nickel 58.693	29 Cu Copper 63.546(3)	30 Zn Zinc 65.38(2)	31 Ga Gallium 69.723	32 Ge Germanium 72.630(8)	33 As Arsenic 74.922	34 Se Selenium 78.971(8)	35 Br Bromine 79.904	36 Kr Krypton 83.798(2)
37 Rb Rubidium 85.468	38 Sr Strontium 87.62	39 Y Yttrium 88.906	40 Zr Zirconium 91.224(2)	41 Nb Niobium 92.906	42 Mo Molybdenum 95.95	43 Tc Technetium	44 Ru Ruthenium 101.07(2)	45 Rh Rhodium 102.91	46 Pd Palladium 106.42	47 Ag Silver 107.87	48 Cd Cadmium 112.41	49 In Indium 114.82	50 Sn Tin 118.71	51 Sb Antimony 121.76	52 Te Tellurium 127.60(3)	53 I Iodine 126.90	54 Xe Xenon 131.29
55 Cs Caesium 132.91	56 Ba Barium 137.33	57-71 *	72 Hf Hafnium 178.49(2)	73 Ta Tantalum 180.95	74 W Tungsten 183.84	75 Re Rhenium 186.21	76 Os Osmium 190.23(3)	77 Ir Iridium 192.22	78 Pt Platinum 195.08	79 Au Gold 196.97	80 Hg Mercury 200.59	81 Tl Thallium 204.38	82 Pb Lead 207.2	83 Bi Bismuth 208.98	84 Po Polonium	85 At Astatine	86 Rn Radon
87 Fr Francium	88 Ra Radium	89-103 **	104 Rf Rutherfordium	105 Db Dubnium	106 Sg Seaborgium	107 Bh Bohrium	108 Hs Hassium	109 Mt Meitnerium	110 Ds Darmstadtium	111 Rg Roentgenium	112 Cn Copernicium	113 Nh Nihonium	114 Fl Flerovium	115 Mc Moscovium	116 Lv Livermorium	117 Ts Tennessine	118 Og Oganesson
*Lanthanoids			57 La Lanthanum 138.91	58 Ce Cerium 140.12	59 Pr Praseodymium 140.91	60 Nd Neodymium 144.24	61 Pm Promethium	62 Sm Samarium 150.36(2)	63 Eu Europium 151.96	64 Gd Gadolinium 157.25(3)	65 Tb Terbium 158.93	66 Dy Dysprosium 162.50	67 Ho Holmium 164.93	68 Er Erbium 167.26	69 Tm Thulium 168.93	70 Yb Ytterbium 173.05	71 Lu Lutetium 174.97
**Actinoids			89 Ac Actinium	90 Th Thorium 232.04	91 Pa Protactinium 231.04	92 U Uranium 238.03	93 Np Neptunium	94 Pu Plutonium	95 Am Americium	96 Cm Curium	97 Bk Berkelium	98 Cf Californium	99 Es Einsteinium	100 Fm Fermium	101 Md Mendelevium	102 No Nobelium	103 Lr Lawrencium

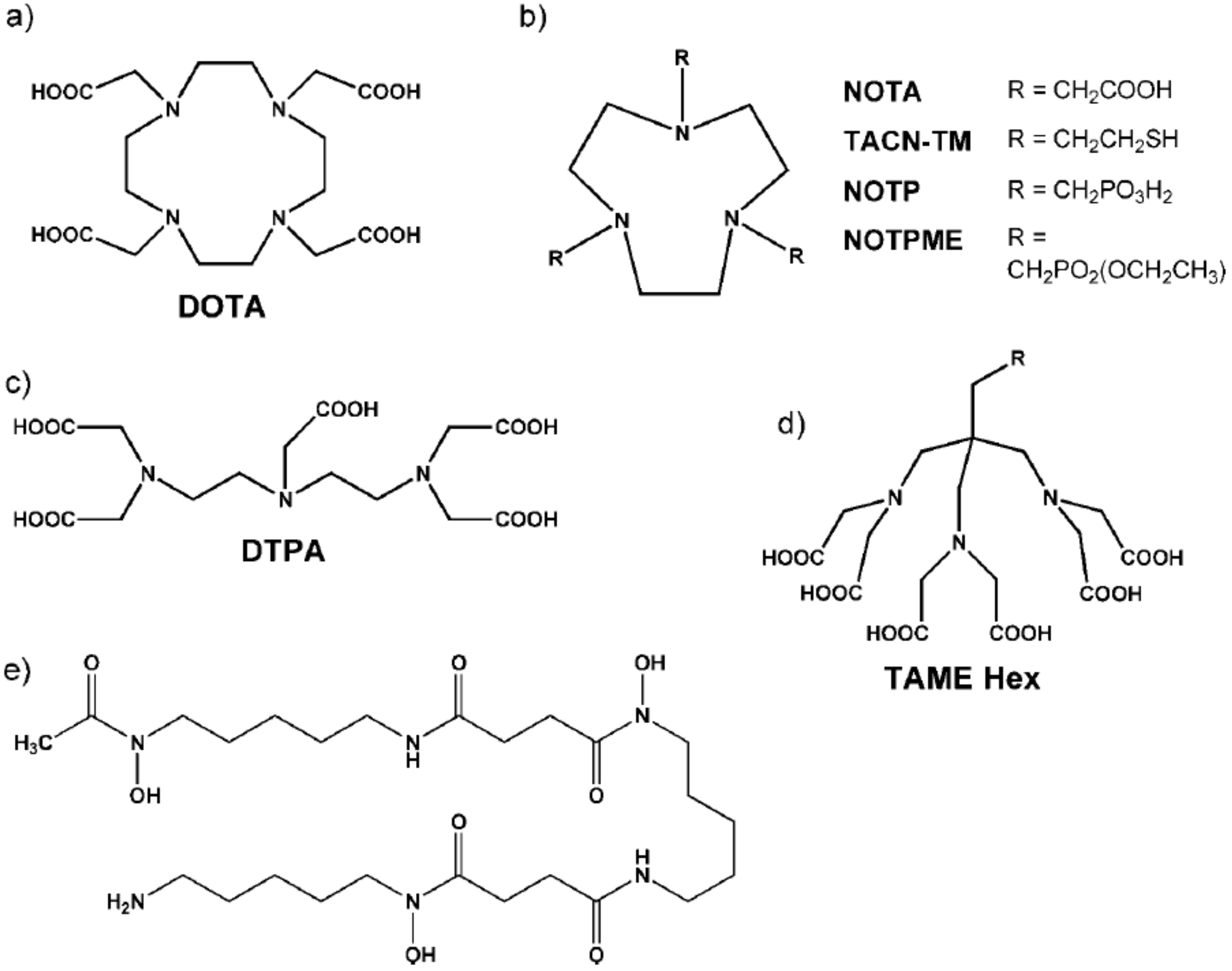
Preparation of a radiopharmaceutical, step 1: the radionuclide

Radio-isotopes can be obtained:

1. By decay of longer half-life radionuclides in a **generator**
2. Via a **cyclotron** by bombarding an appropriate element or its compound with accelerated charged particles, typically protons or deuterium nuclei
3. By nuclear bombardment with neutrons in a **nuclear reactor**

- Purification from parent isotope and by-products (also depends on isotopic purity of target nucleus)
- Incorporation into a compound, often via a polydentate chelator in case of metal radioisotopes

Metal radionuclides need strong chelators



Diethylenetriamino-
pentaaceticacid

desferal

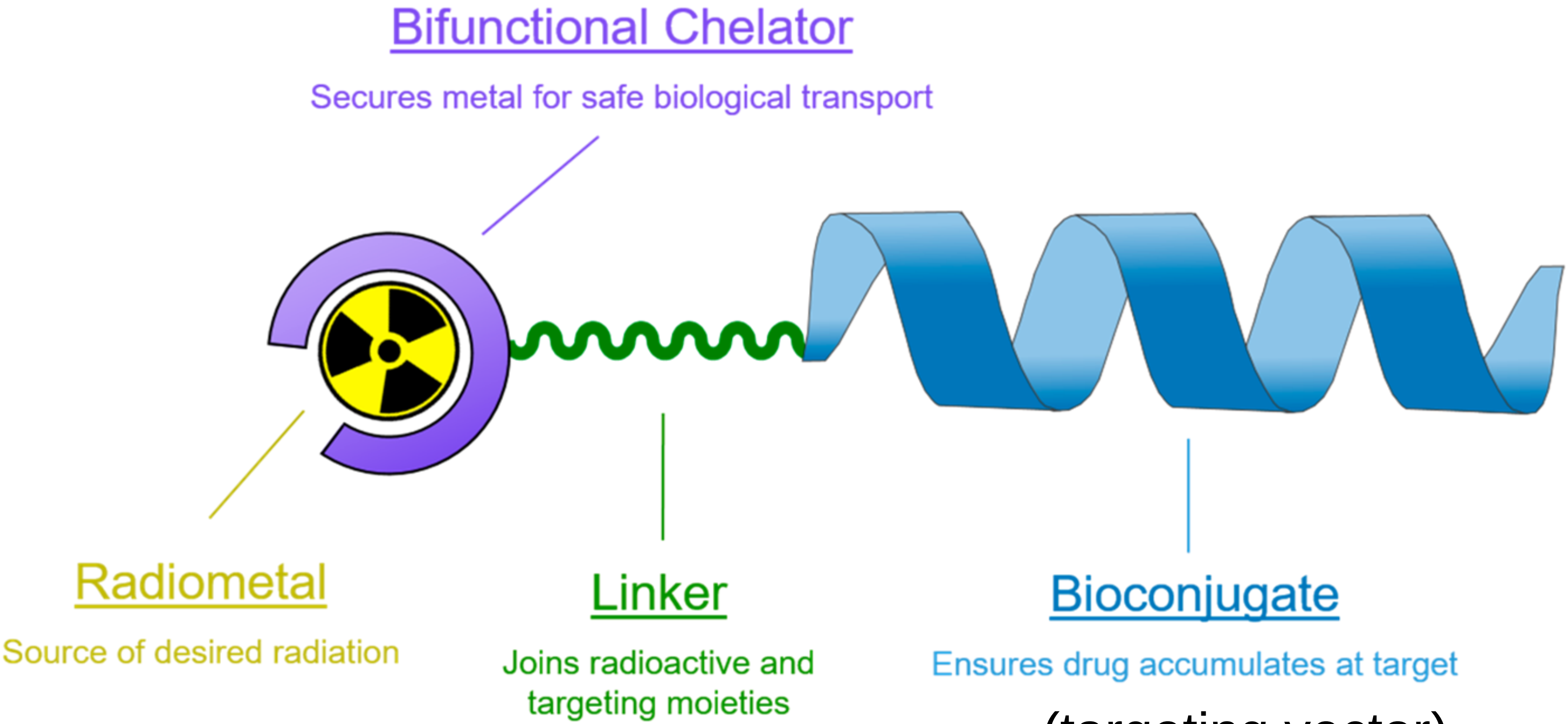
trans-metallation and *trans*-chelation reactions should be avoided

Metal compounds for radiopharmaceuticals

1st generation
Perfusion agents

2nd generation
Targeted agents

Targeted approach
(molecular *Trojan horse*)



Bifunctional Chelator

Secures metal for safe biological transport

Radiometal

Source of desired radiation

Linker

Joins radioactive and targeting moieties

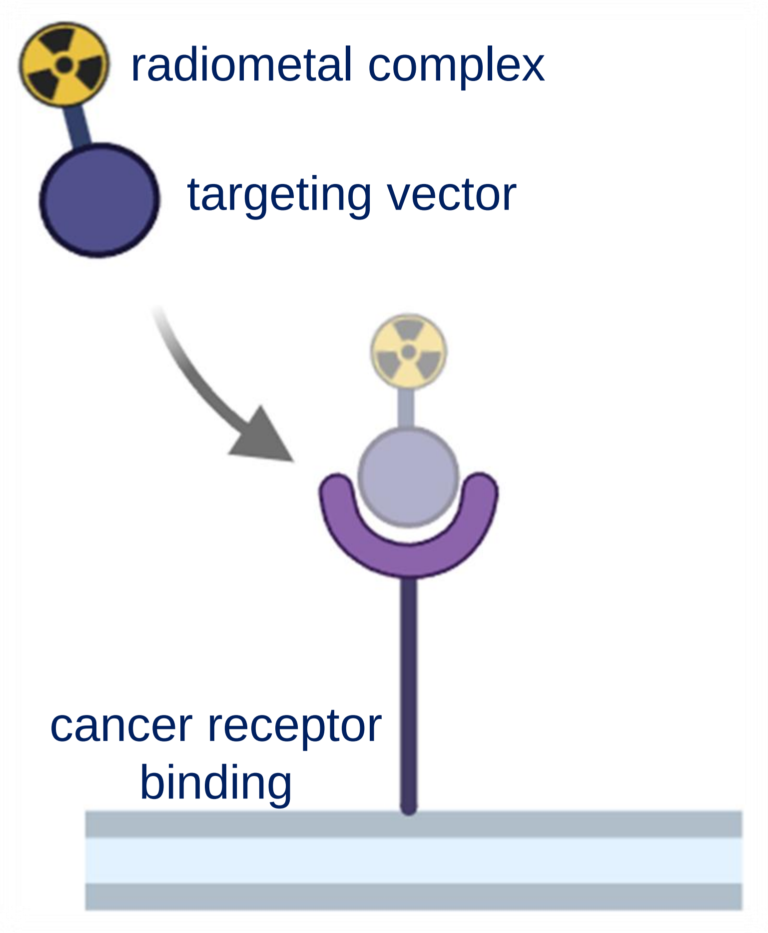
Bioconjugate

Ensures drug accumulates at target

(targeting vector)

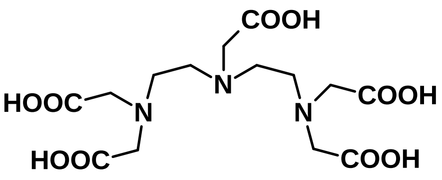
Targeting molecules: monoclonal antibodies, peptides, vitamins, carbohydrates,..

Targeted approach
(molecular *Trojan horse*)

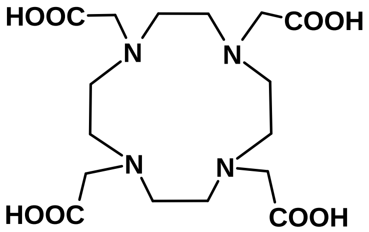


Match between physical and biological half-lifes

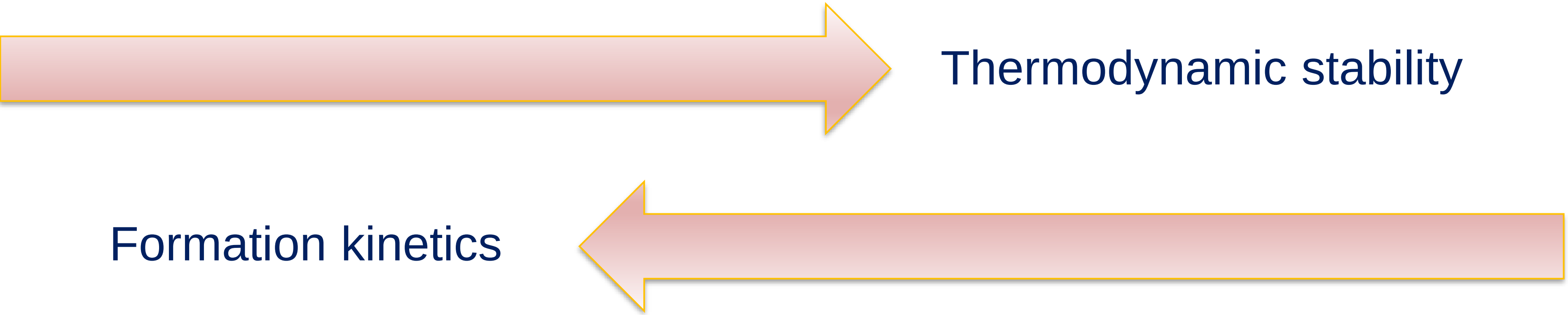
Thermodynamic vs kinetics



Linear (e.g. DTPA)
easy synthesis, rapid complexation, lower stability and kinetic inertness



Macrocyclic (e.g. DOTA)
slow formation kinetic, high stability and kinetic inertness



Thermodynamic stability

Formation kinetics

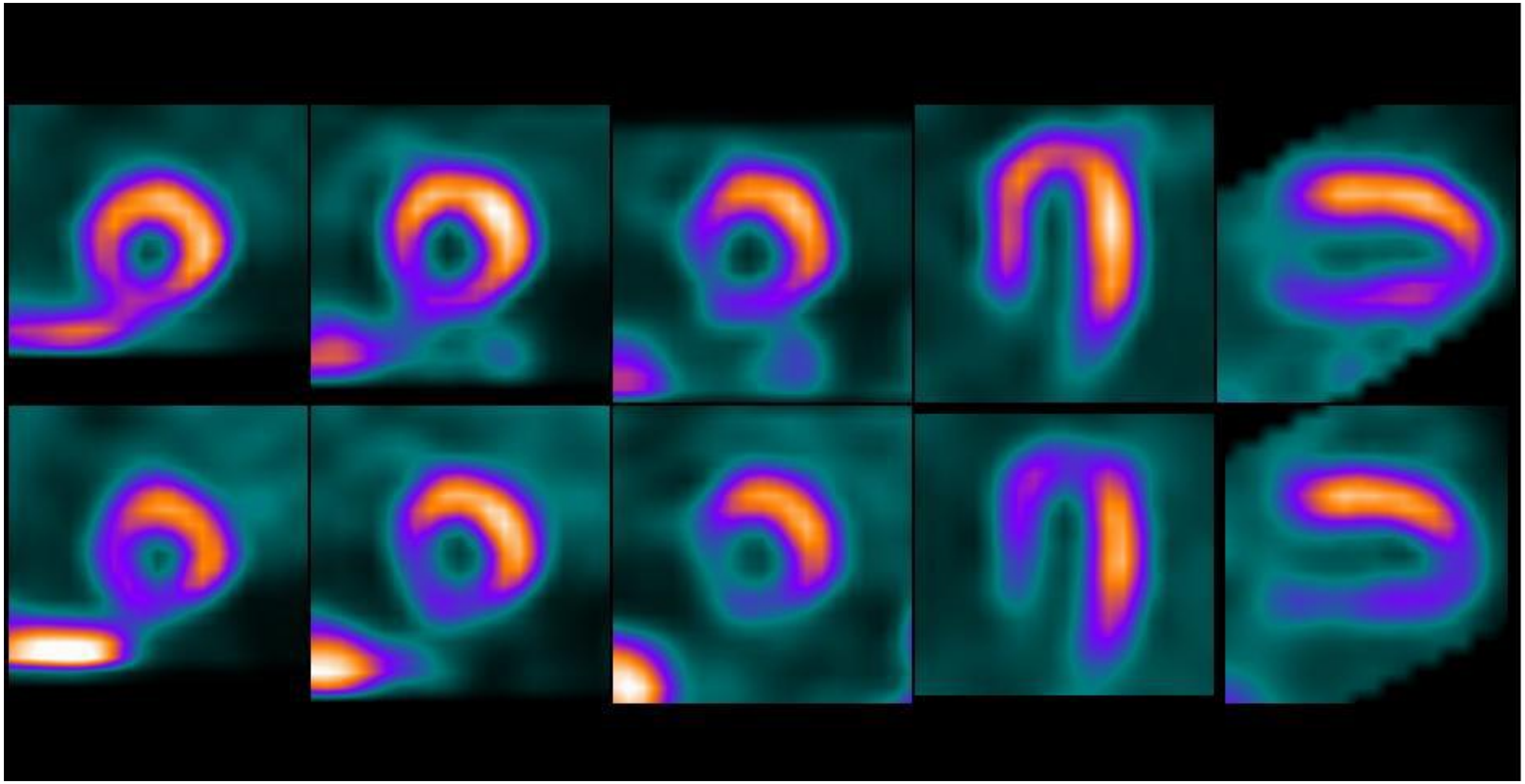
Preparation of radiopharmaceuticals: "*Shake and bake*" principle

- Preparation has to be done "on site" in clinics (kits).
- Yields must be > 98% (even at very low metal ion concentration).
- Compound must be ready for administration.
- No lenghty purification or separation.
- Saline aqueous solutions.
- Non-toxic reagents (e.g. Sn(II) componds as reducing agents) and byproducts.
- Characterization techniques (*cold* analogues).

SPECT: Single Photon Emission Computed Tomography

γ emitters, 100 – 250 keV

Myocardial perfusion



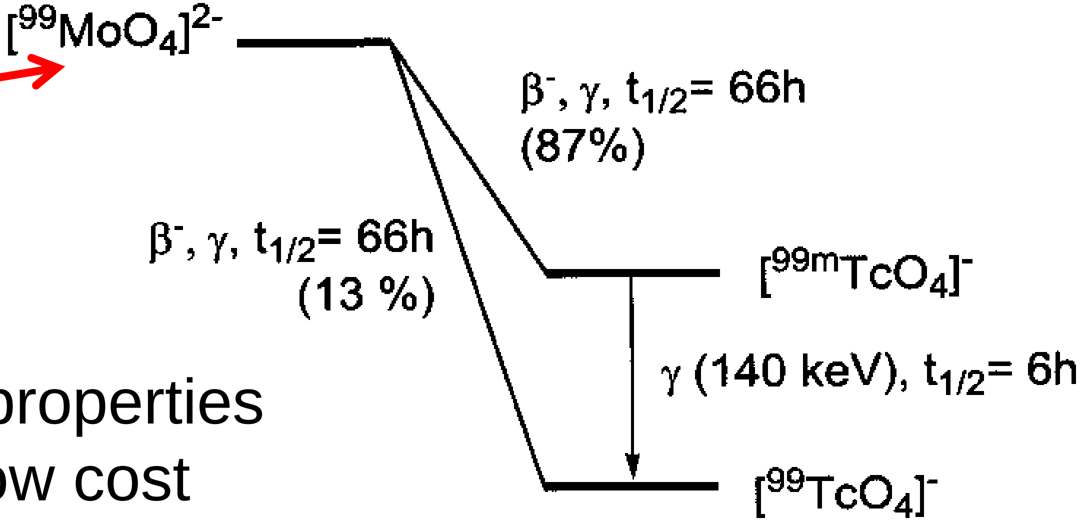
Main radionuclides for SPECT

Radionuclide	**Half life**	**Energy of main γ emission (keV)**
^{67}Ga (γ)	78 h	93, 185, 300
^{99m}Tc (γ)	6 h	140
^{111}In (γ)	67 h	171, 245
^{131}I (β, γ)	8 d	364

^{99m}Tc: the *workhorse* of radioimaging

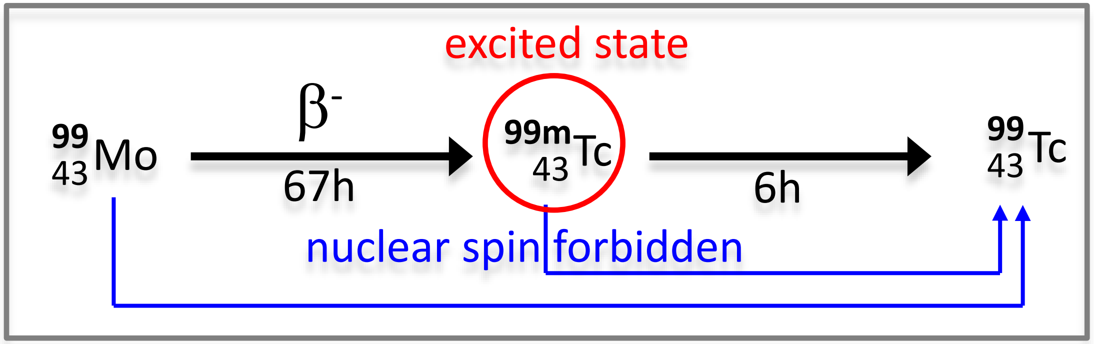
(used in >85% of radiodiagnostic scans, ca. 25 – 30 M per year)

Obtained from fission of ^{235}U or from ^{98}Mo upon (n, γ) reaction



Pure β-emitter, $t_{1/2}$= 21 ky

- Ideal physical decay properties
- Readily available at low cost
- Many oxidation states (+7 – -1)
- Various coordination geometries (4 – 9)
- *Cold* Re for characterization (matched-pair approach)



excited state

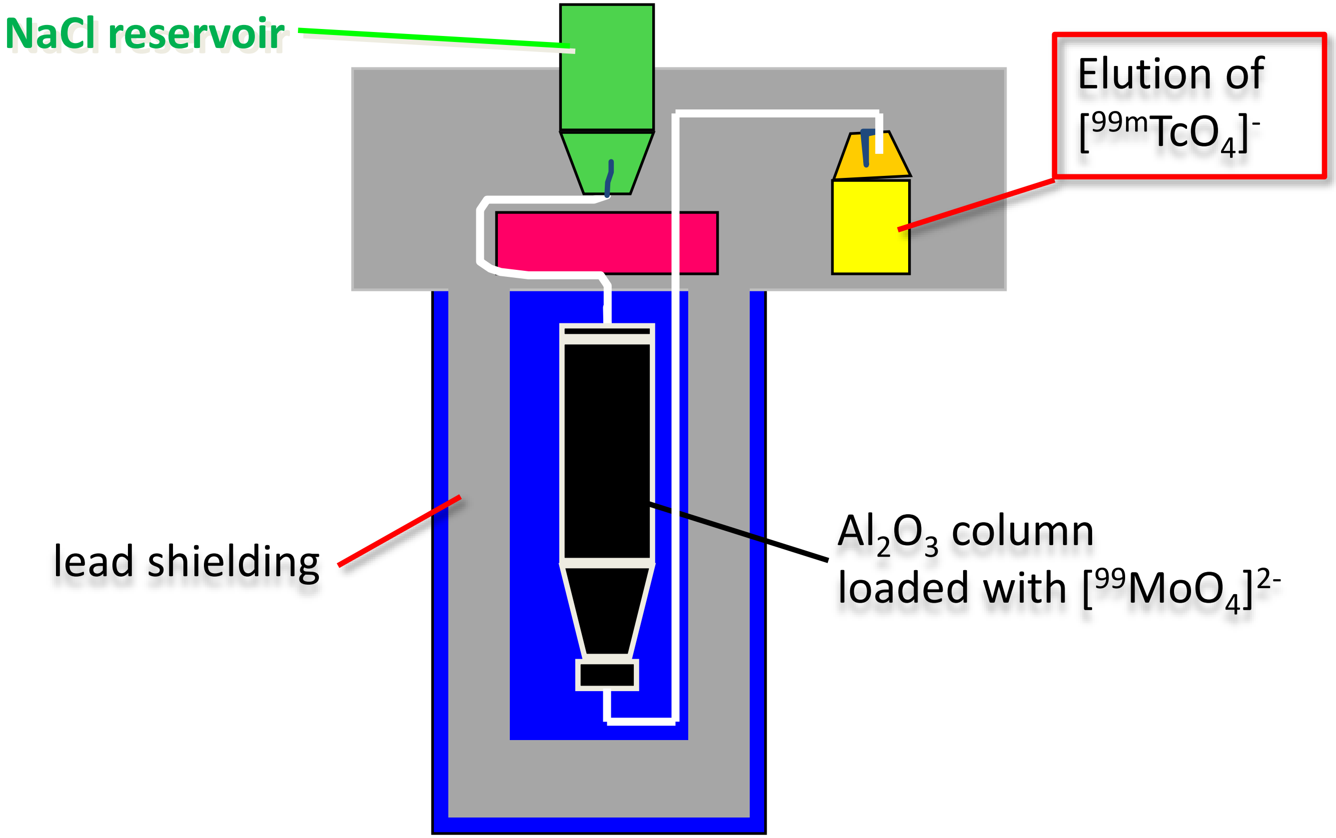
$$^{99}_{43}\mathrm{Mo} \xrightarrow[67h]{\beta^-} {}^{99m}_{43}\mathrm{Tc} \xrightarrow[6h]{} {}^{99}_{43}\mathrm{Tc}$$

nuclear spin forbidden

nuclear spin forbidden: compare to **spin forbidden transitions** in the electron shell ➡ **phosphorescence**

$^{99m}TcO_4^-$ generator

^{99}**Mo** (parent) $\xrightarrow[k_1]{}$ ^{99m}**Tc** (desired nuclide) $\xrightarrow[k_2]{}$ ^{99}**Tc** (final product)



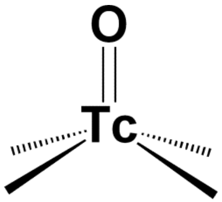
NaCl reservoir

Elution of $[^{99m}TcO_4]^-$

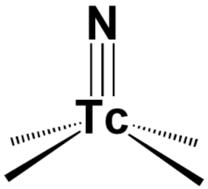
lead shielding

Al_2O_3 column loaded with $[^{99}MoO_4]^{2-}$

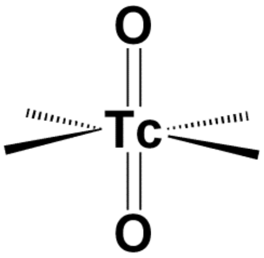
Most relevant oxidation states and core structures



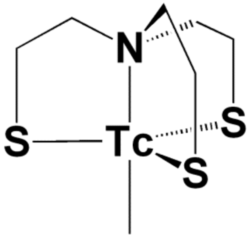
[Tc(V)]$^{3+}$



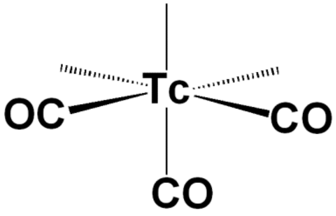
[Tc(V)]$^{2+}$



[Tc(V)]$^{+}$



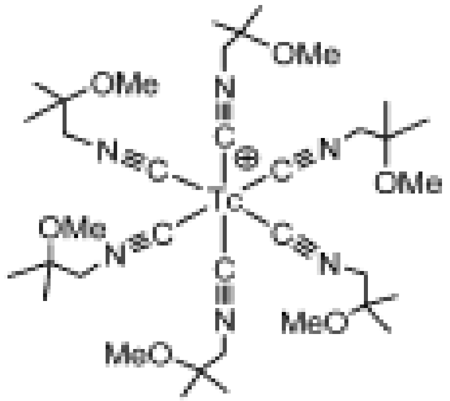
[Tc(III)]$^{+}$



[Tc(I)]$^{+}$

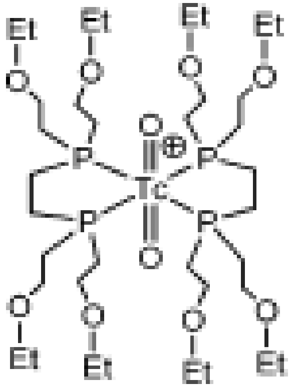
- "vacant sites must be occupied by stable/inert ligands
  - synthesis from [$^{99m}TcO_4$]$^-$ in 0.9% NaCl solution

First generations ^{99m}Tc agents



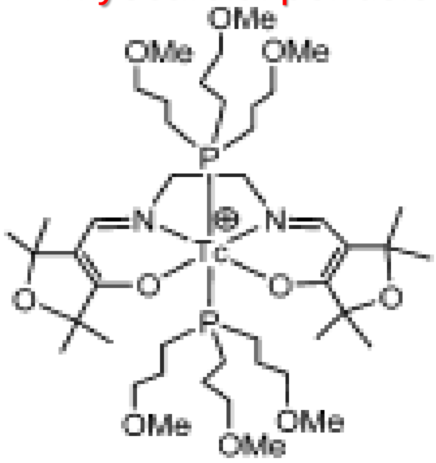
^{99m}Tc-Sestamibi

cardiac imaging



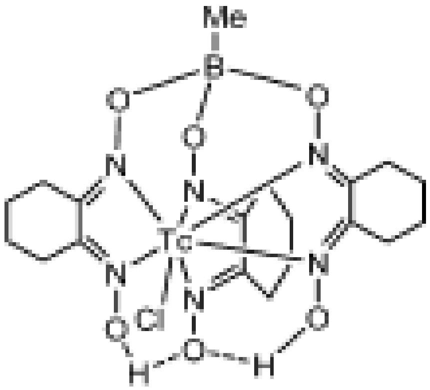
^{99m}Tc-Tetrofosmin

myocardial perfusion

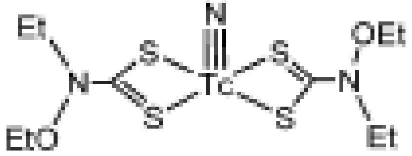


Q12

cardiac imaging

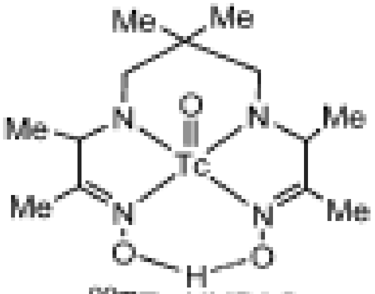


^{99m}Tc-Teboroxime



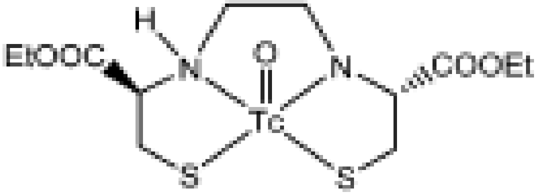
^{99m}TcN-NOET

myocardial perfusion



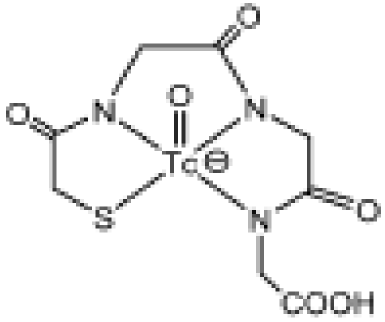
^{99m}Tc-HMPAO

cereberal perfusion



^{99m}Tc-Bicisate

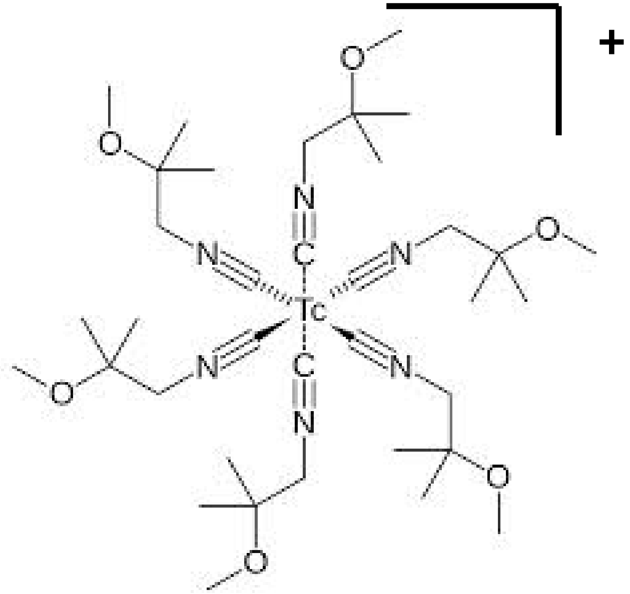
brain imaging



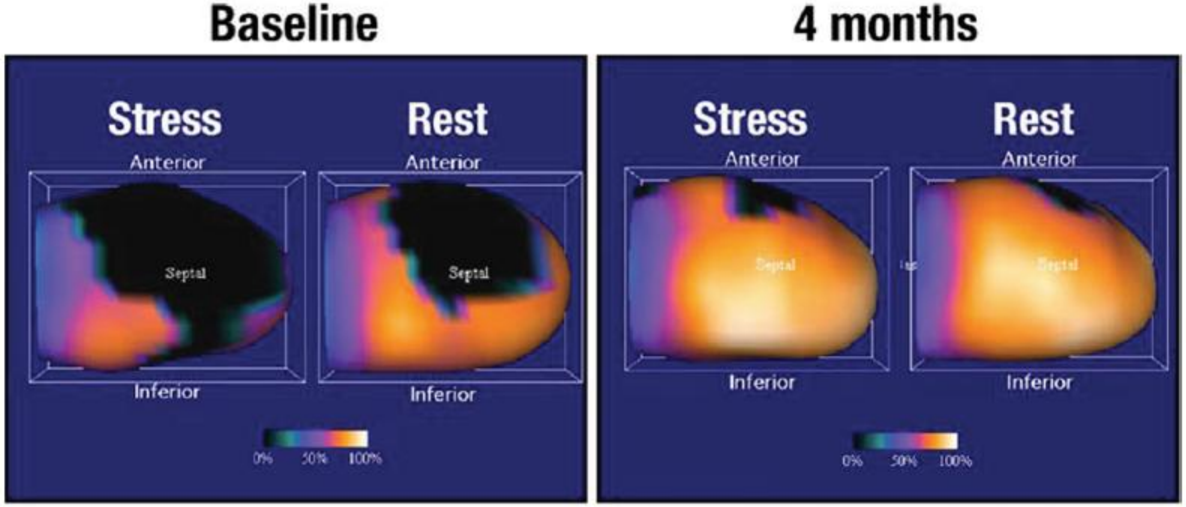
^{99m}Tc-MAG$_3$

renal imaging

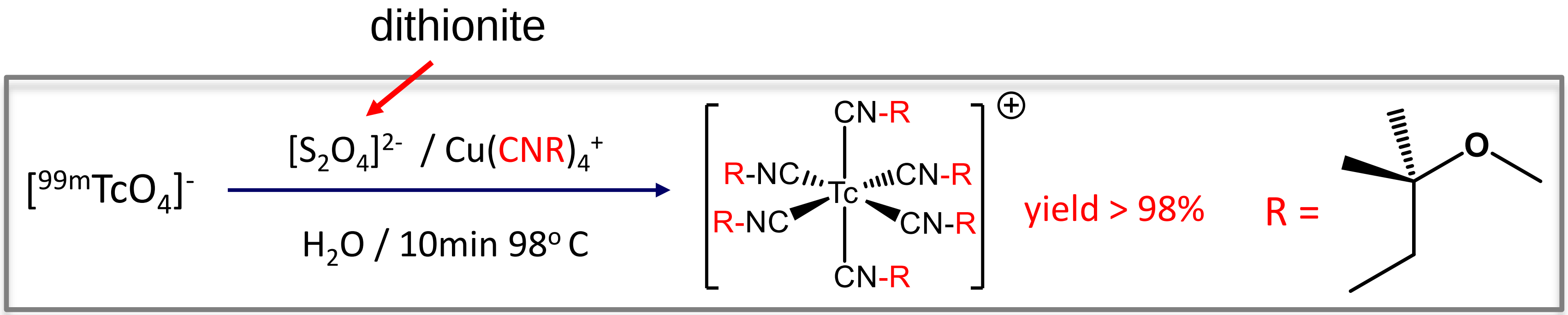
^{99m}Tc-sestamibi



Localizes in mithocondria



- Cardiac imaging (uptake proportional to blood flux)
- Cancer diagnosis (breast cancer)
- Thyroid imaging (adenoma)



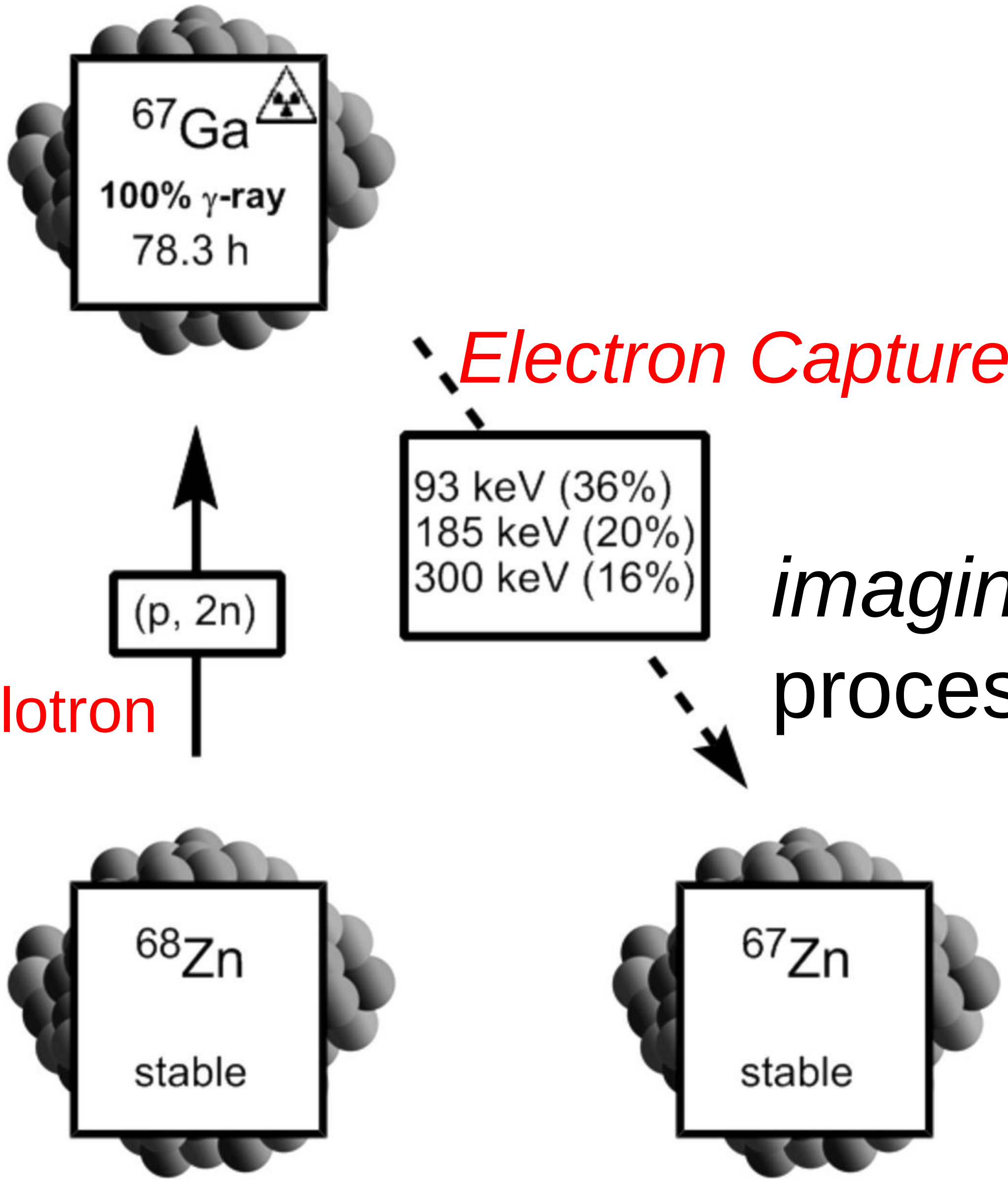
Chemistry is difficult, **redox chemistry** involved

$[^{99m}TcO_4]^-$ **Tc^{VII}** $\longrightarrow$ **Tc^{I}** $[^{99m}Tc(CNR)_6]^+$

6 electrons

- quantitative yield (**> 98%**)
- water as solvent, **boiling**
- high dilution, **kinetic** reaction **control**

>500 M$ per year



^{67}Ga

100% γ-ray

78.3 h

(p, 2n)

Cyclotron

Electron Capture

93 keV (36%)
185 keV (20%)
300 keV (16%)

^{68}Zn

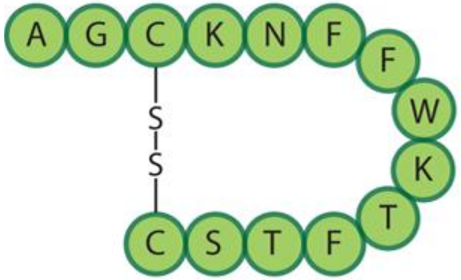
stable

^{67}Zn

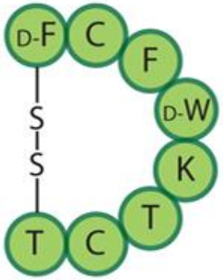
stable

imaging of inflammatory processes and tumors

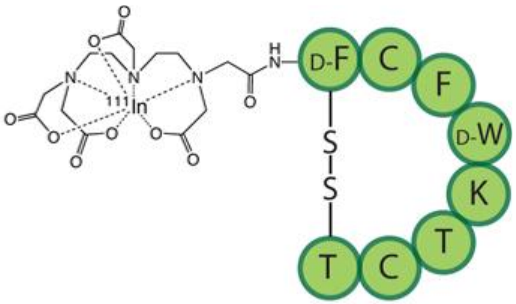
2nd generation SPECT agents: radio-immuno-scintigraphy



Somatostatin

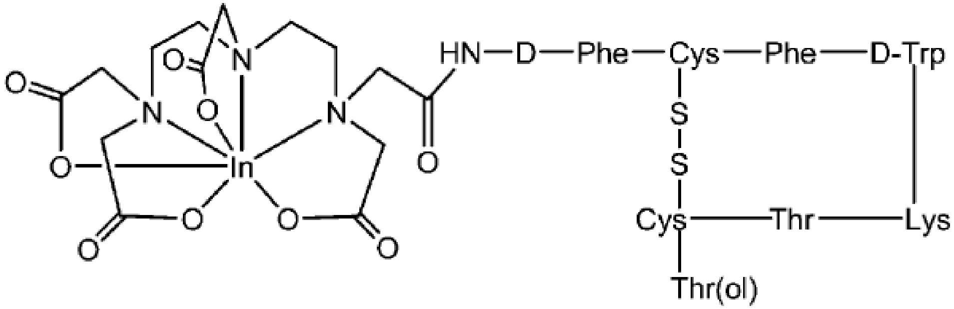


Octreotide



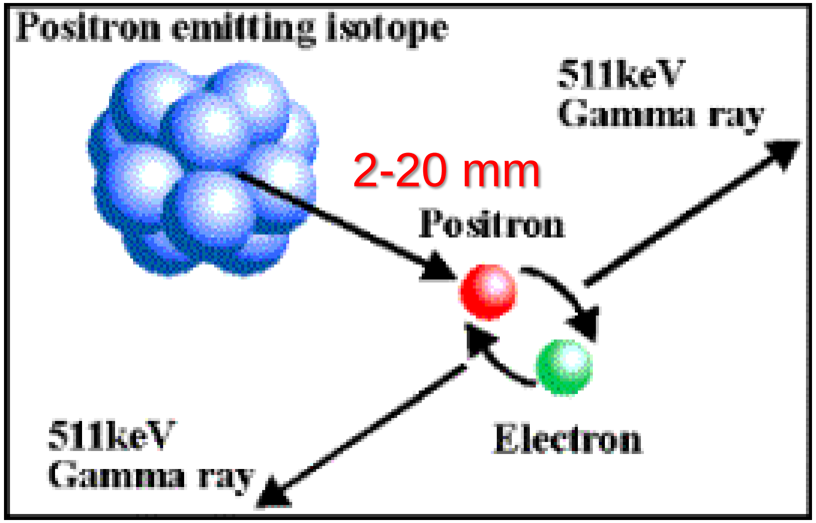
^{111}In-DTPA-Octreotide

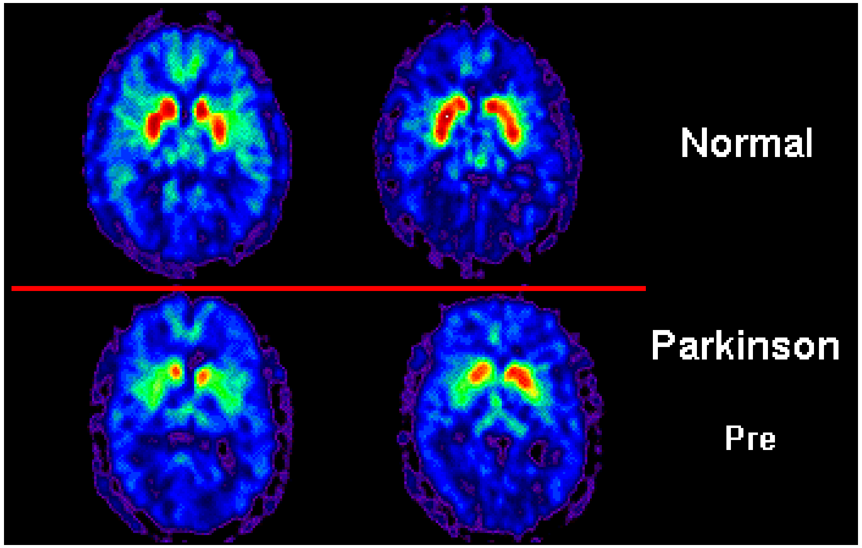
Somatostatin is a polypeptide hormone that regulates the endocrine system and cell growth and proliferation. Somatostatin receptors are trans-membrane proteins that are overexpressed in many types of **neuroendocrine tumors**. Octreotide is similar to somatostatin.

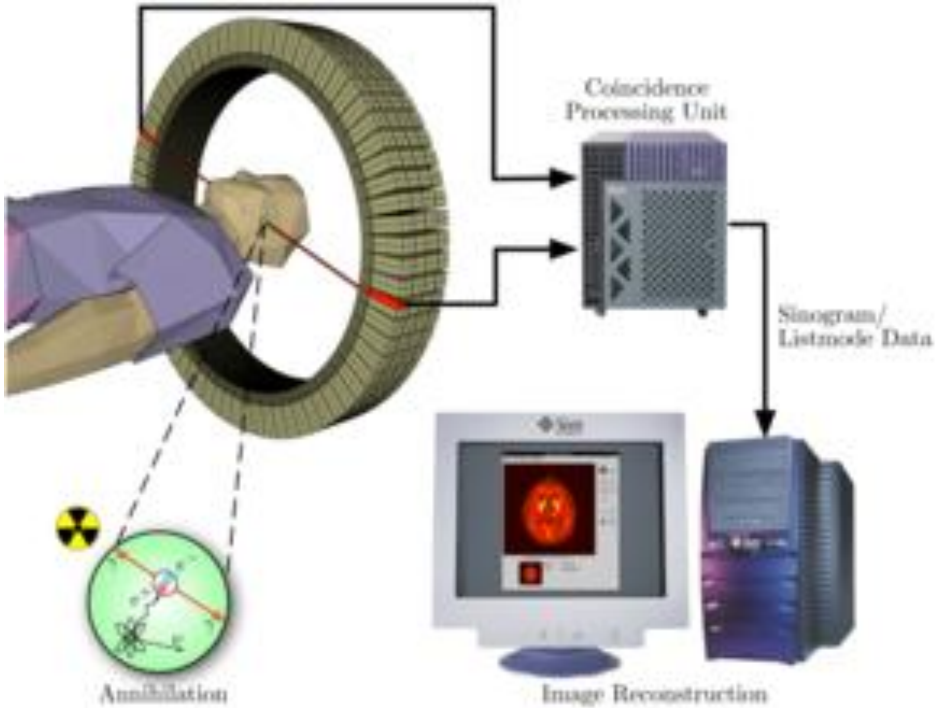


^{111}In-DTPA-Octreotide (OctreoScan®)

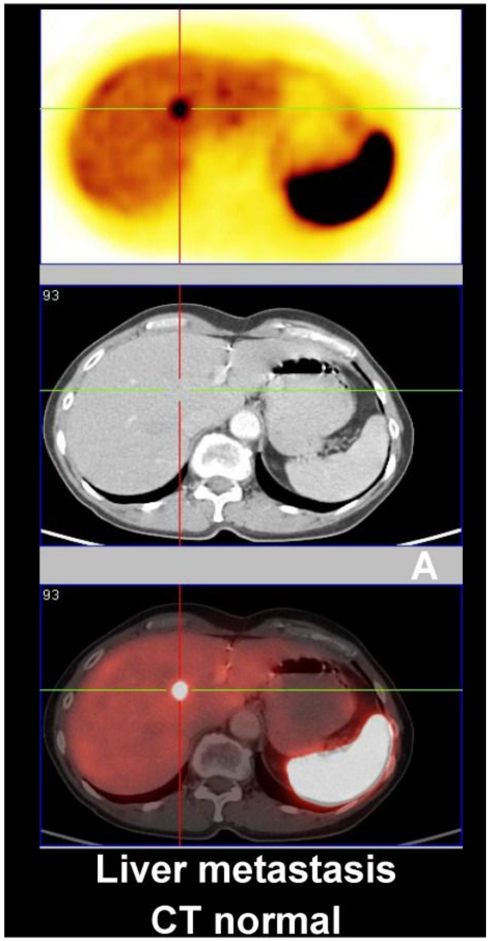
PET: Positron Emission Tomography







PET/CT: combination of imaging techniques (structural and functional)



Liver metastasis
CT normal

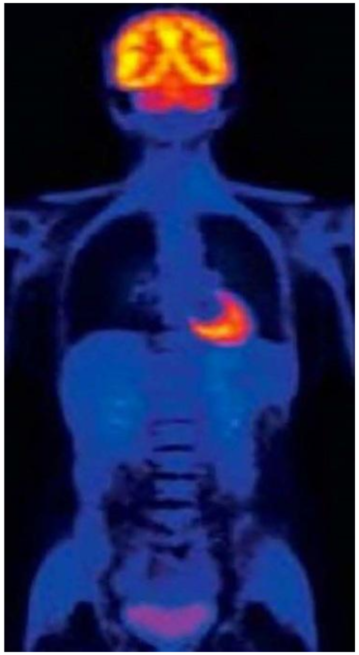
PET

CT

PET + CT

PET imaging and hybrid modalities

PET



Functional imaging

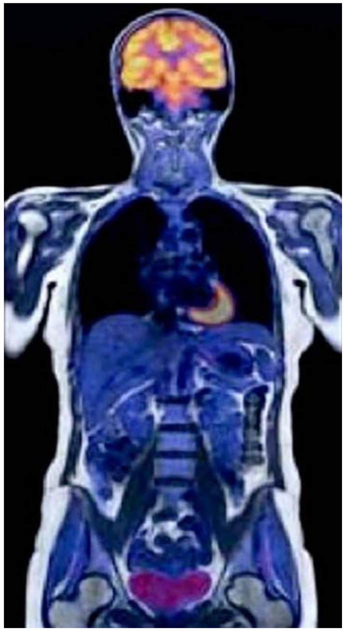
High sensitivity - Target specific

MRI

Anatomic / physiologic imaging

High resolution - Soft tissue contrast

PET/MRI



Improved clinical accuracy

Reduced radiation dose

Broader pathologic scope

Most relevant radionuclides for PET

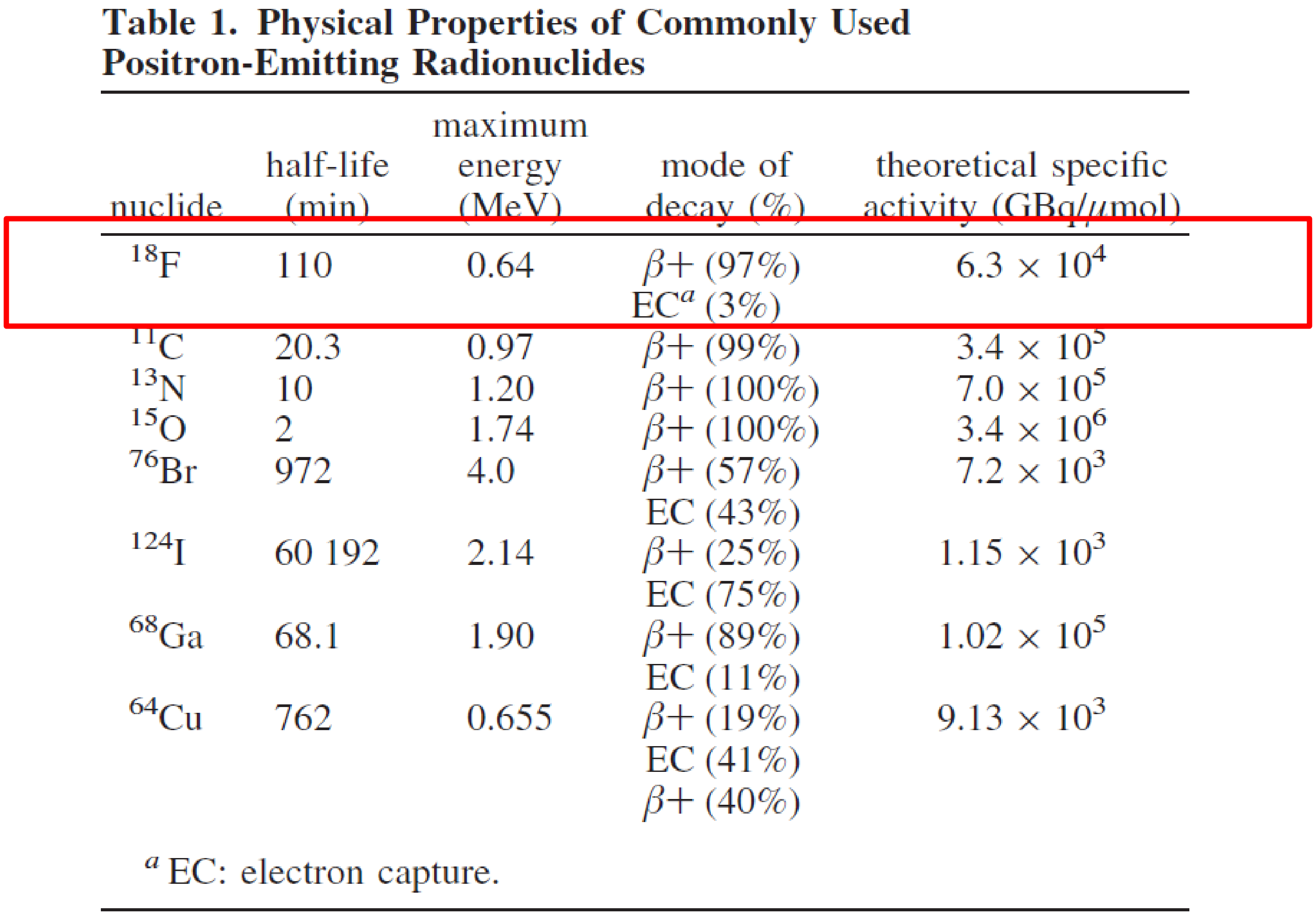


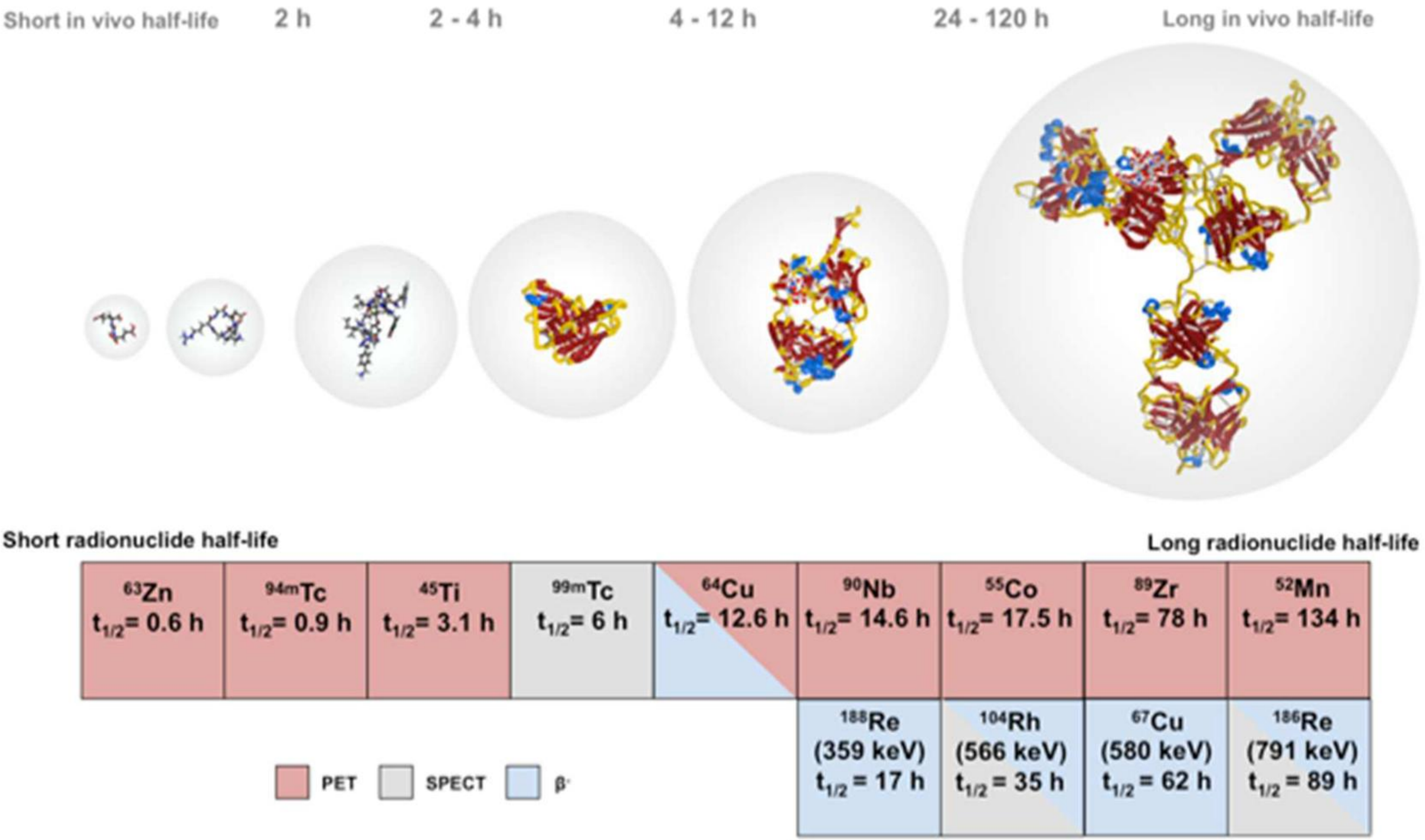
Table 1. Physical Properties of Commonly Used Positron-Emitting Radionuclides

nuclide	half-life (min)	maximum energy (MeV)	mode of decay (%)	theoretical specific activity (GBq/μmol)
^{18}F	110	0.64	$\beta+$ (97%) EC^{a} (3%)	6.3×10^{4}
^{11}C	20.3	0.97	$\beta+$ (99%)	3.4×10^{5}
^{13}N	10	1.20	$\beta+$ (100%)	7.0×10^{5}
^{15}O	2	1.74	$\beta+$ (100%)	3.4×10^{6}
^{76}Br	972	4.0	$\beta+$ (57%) EC (43%)	7.2×10^{3}
^{124}I	60 192	2.14	$\beta+$ (25%) EC (75%)	1.15×10^{3}
^{68}Ga	68.1	1.90	$\beta+$ (89%) EC (11%)	1.02×10^{5}
^{64}Cu	762	0.655	$\beta+$ (19%) EC (41%) $\beta+$ (40%)	9.13×10^{3}

[a] EC: electron capture.

An increase in the energy of the emitted positron leads to a decrease in resolution

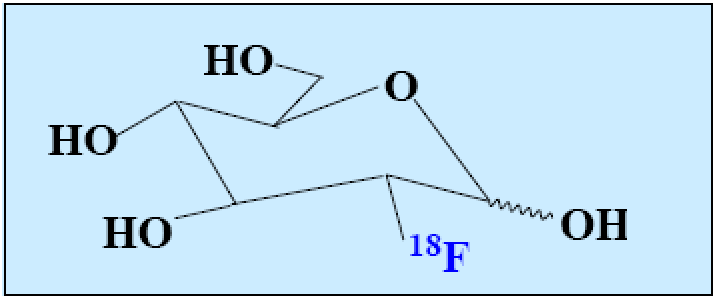
Radionuclide half-life and biodistribution half-life



^{63}Zn $t_{1/2}$= 0.6 h	^{94m}Tc $t_{1/2}$= 0.9 h	^{45}Ti $t_{1/2}$= 3.1 h	^{99m}Tc $t_{1/2}$= 6 h	^{64}Cu $t_{1/2}$= 12.6 h	^{90}Nb $t_{1/2}$= 14.6 h	^{55}Co $t_{1/2}$= 17.5 h	^{89}Zr $t_{1/2}$= 78 h	^{52}Mn $t_{1/2}$= 134 h
					^{188}Re (359 keV) $t_{1/2}$ = 17 h	^{104}Rh (566 keV) $t_{1/2}$ = 35 h	^{67}Cu (580 keV) $t_{1/2}$ = 62 h	^{186}Re (791 keV) $t_{1/2}$ = 89 h

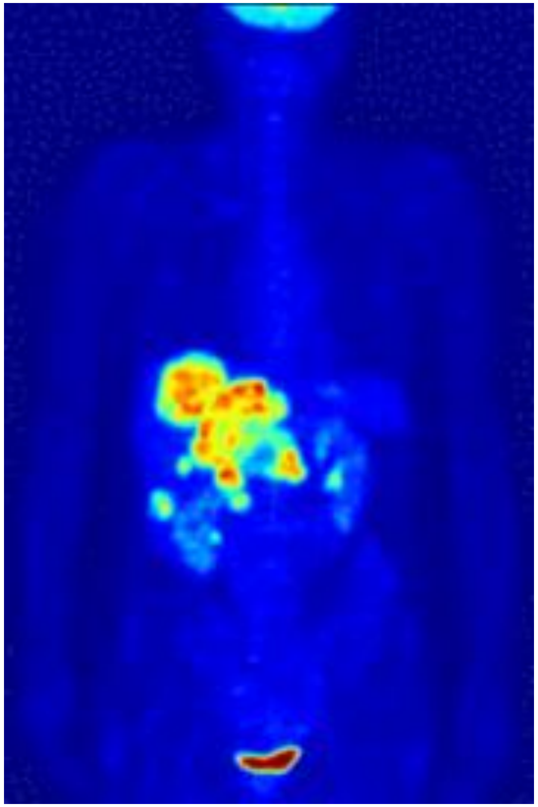
🟥 PET ⬜ SPECT 🟦 β^-

Bio-isosteric substitution with ^{18}F



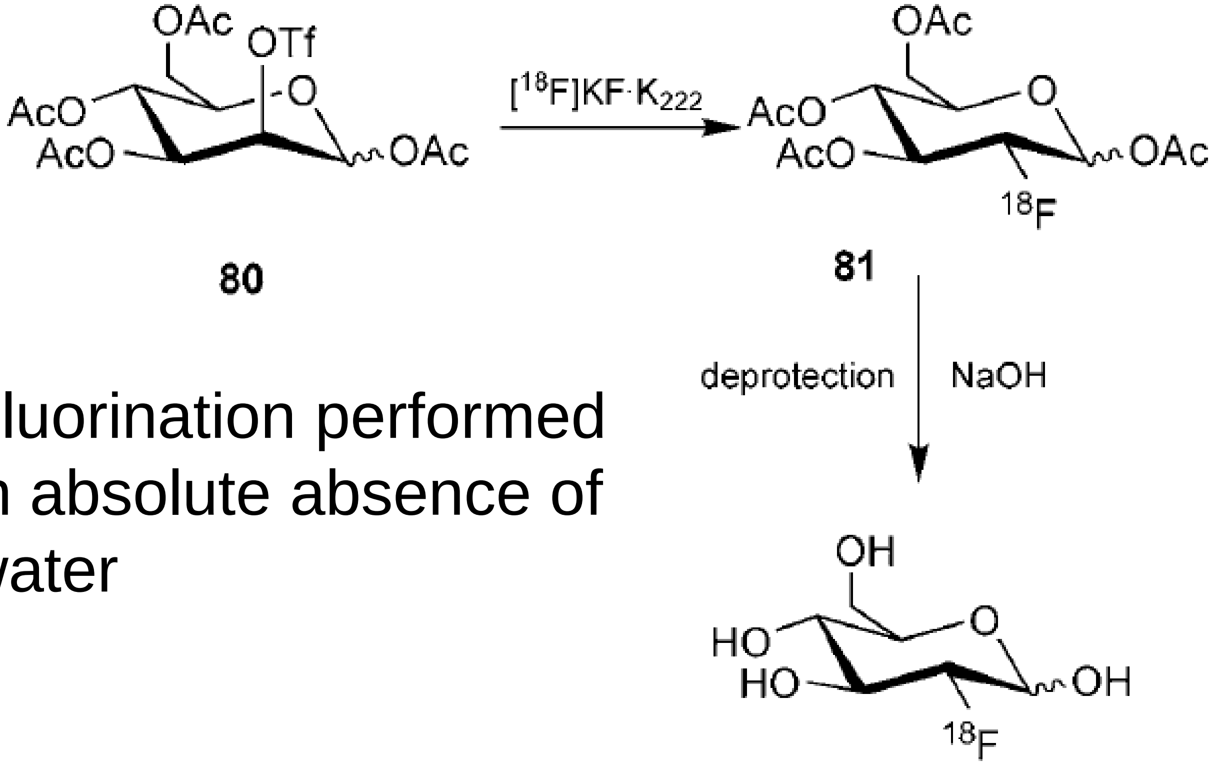
Fluorodeoxyglucose ([^{18}F] FDG)

[^{18}F] FDG follows glucose metabolism and thus allows tumor localization



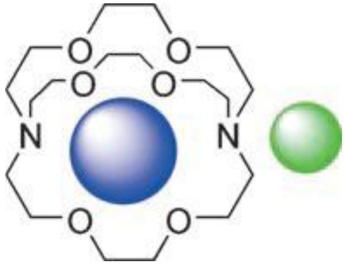
Example of nucleophilic direct fluorination ($^{18}F^{-}$)

$$^{18}O_{(\text{from } H_2O)}(p, n)^{18}F$$



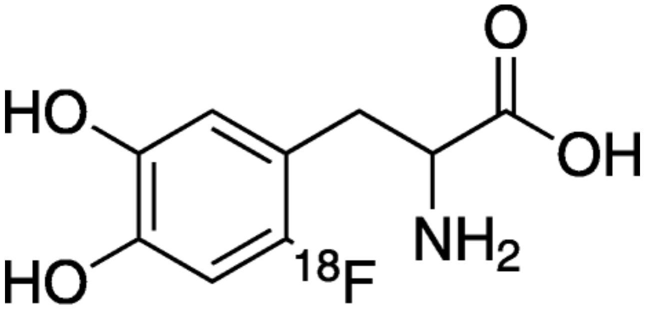
80

81



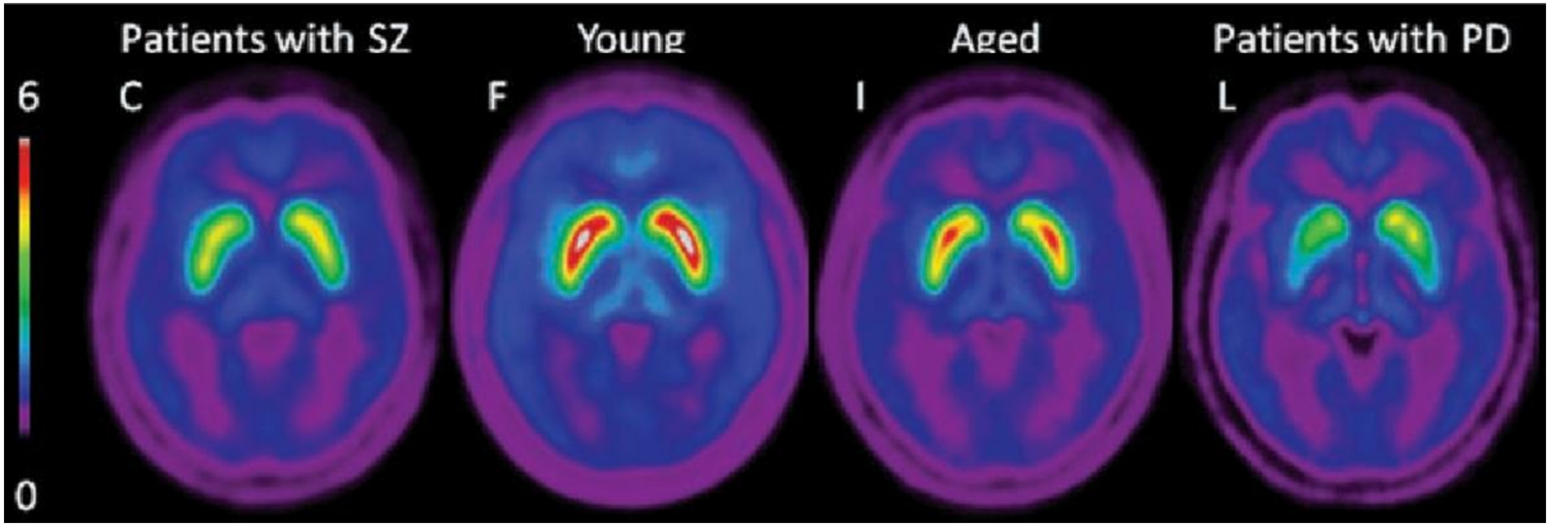
Fluorination performed in absolute absence of water

6-[^{18}F]-fluoro-3,4-dihydroxyphenylalanine (6-[^{18}F]FDOPA)

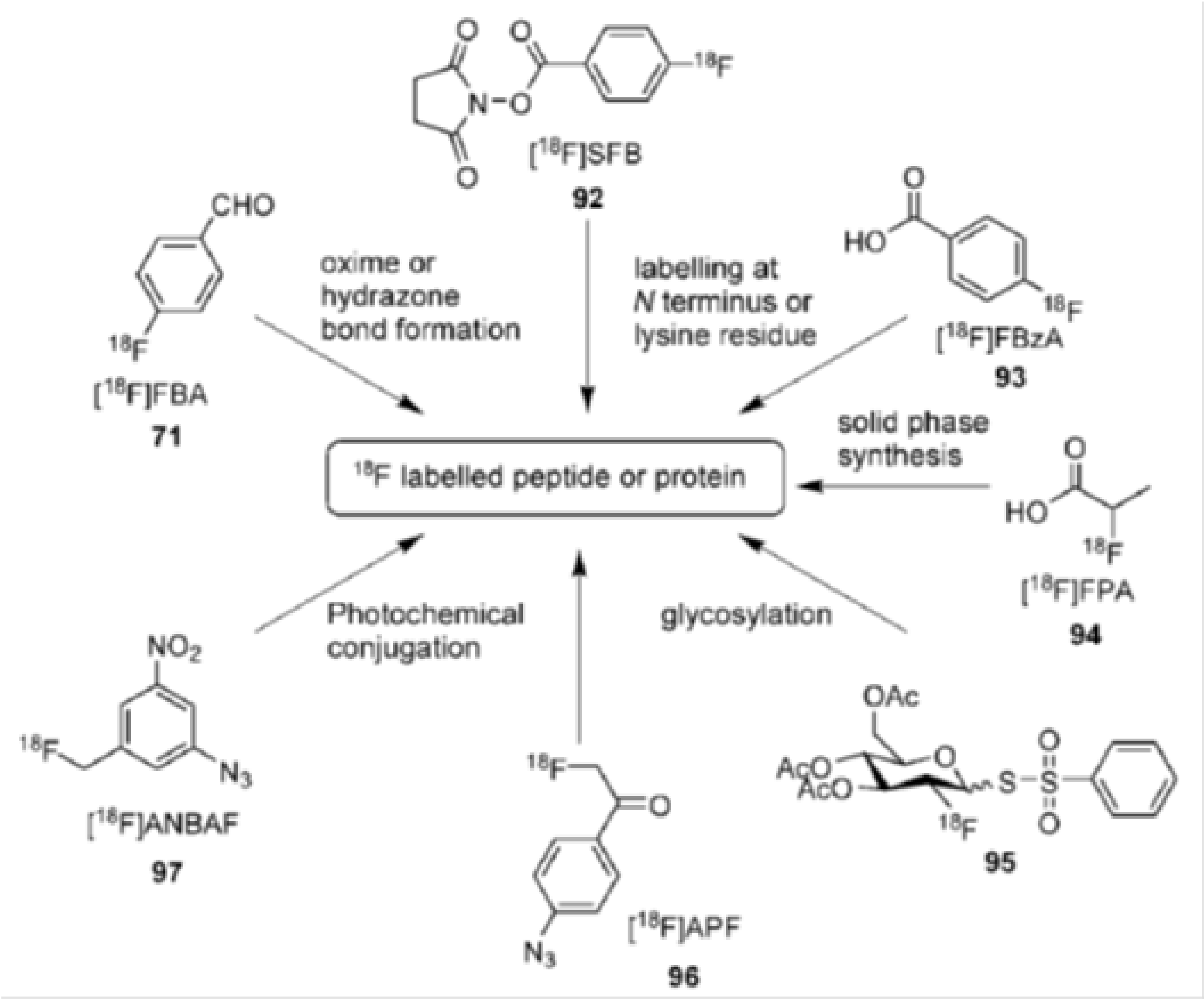


6-[^{18}F] FDOPA,

DOPA is the precursor of the neurotransmitter dopamine.
The neurotracer 6-[^{18}F]FDOPA is a powerful tool in PET imaging of neuropsychiatric diseases, movement disorders and brain malignancies. More recently, it also demonstrated good results in the diagnosis of other malignancies

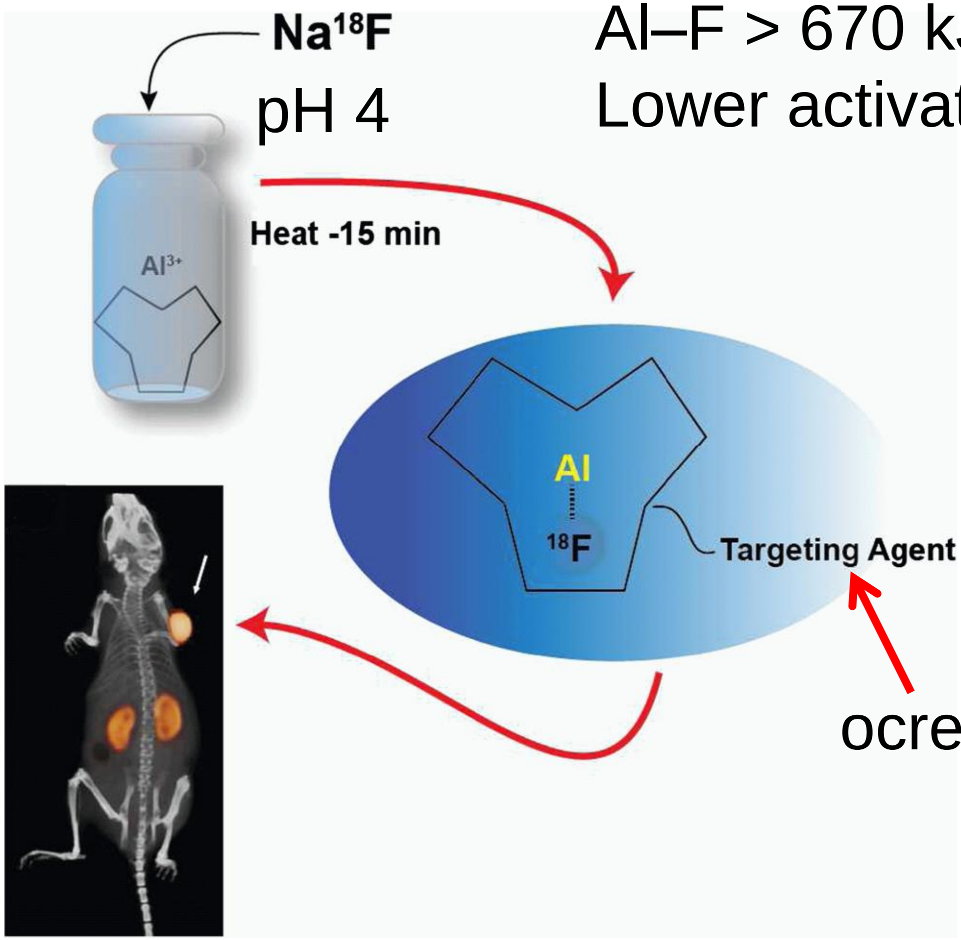


Prostetic groups for ^{18}F labeling of biomolecules



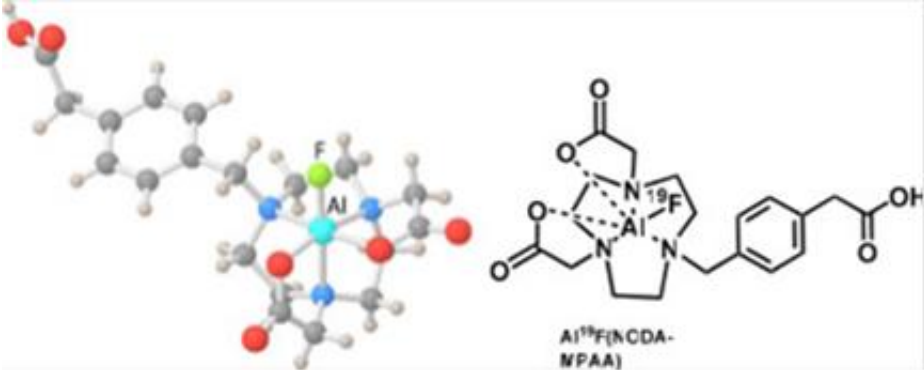
Inorganic fluoruration with $^{18}F^{-}$ (B, Si, Al…)

Al–F > 670 kJ mol^{-1} vs 480 kJ mol^{-1} for C–F
Lower activation energy



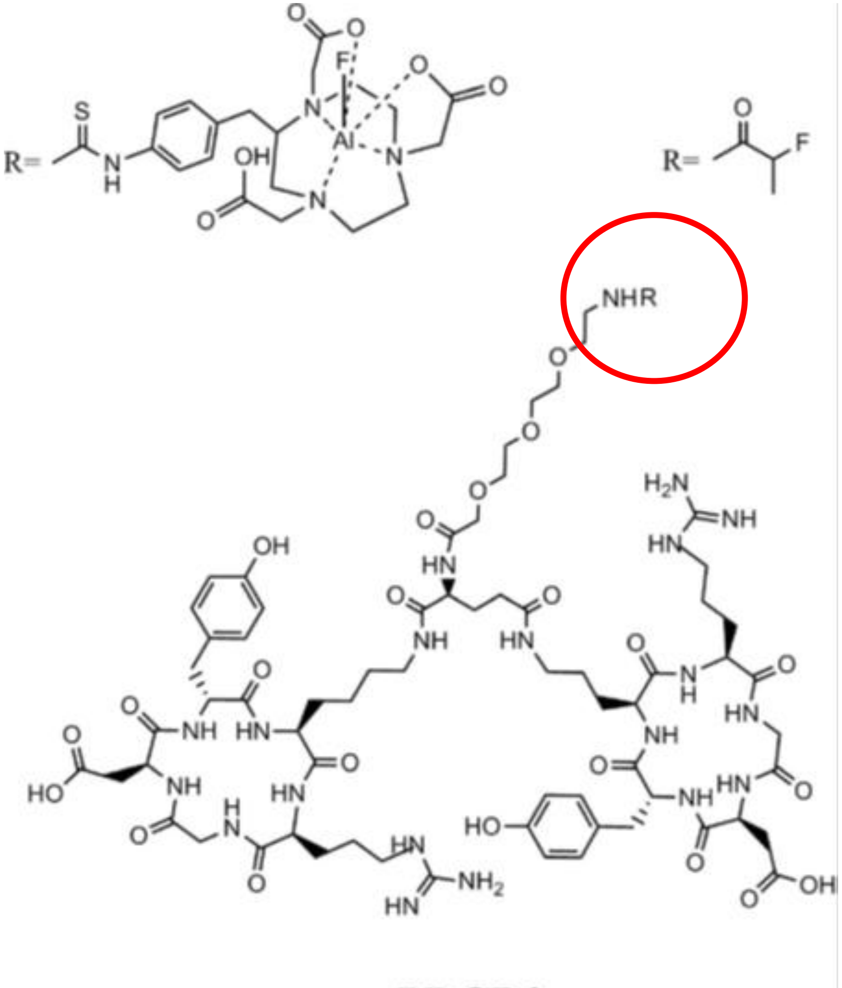
pH 4

ocreotide



$Al^{19}F$(NODA-MPAA)

Clinical study for PET imaging a lung cancer



PRGD2

Cyclic RGD peptides have high affinity and selectivity for the integrin receptor $\alpha_v\beta_3$

Visualizing and quantifying this integrin makes it possible to assess the neo-vascularization of a tumor and determine whether it is likely to respond to anti-angiogenic therapy

Metal radionuclides for PET

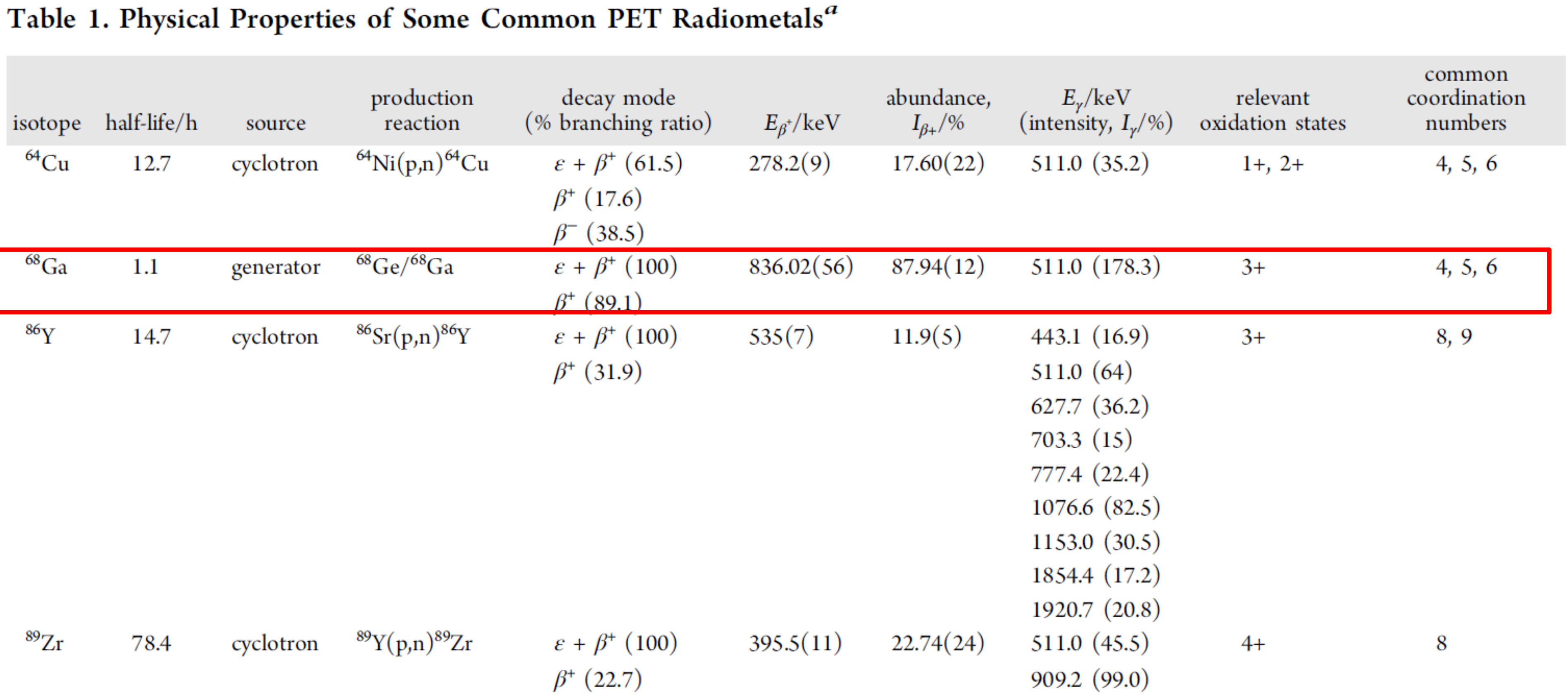


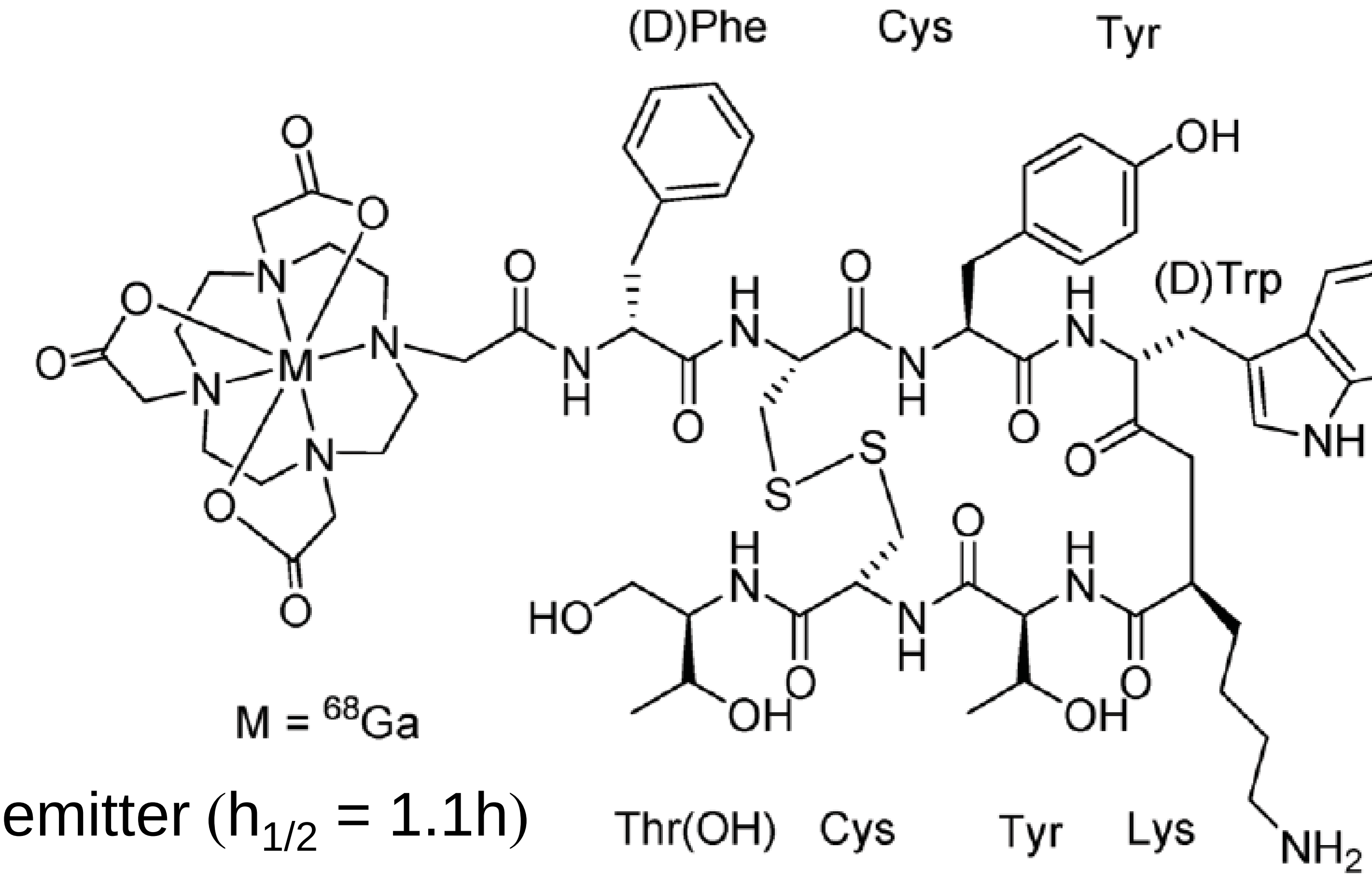
Table 1. Physical Properties of Some Common PET Radiometals[a]

isotope	half-life/h	source	production reaction	decay mode (% branching ratio)	E_{β^+}/keV	abundance, $I_{\beta+}$/%	E_γ/keV (intensity, I_γ/%)	relevant oxidation states	common coordination numbers
^{64}Cu	12.7	cyclotron	^{64}Ni(p,n)^{64}Cu	$\varepsilon + \beta^+$ (61.5) β^+ (17.6) β^- (38.5)	278.2(9)	17.60(22)	511.0 (35.2)	1+, 2+	4, 5, 6
^{68}Ga	1.1	generator	^{68}Ge/^{68}Ga	$\varepsilon + \beta^+$ (100) β^+ (89.1)	836.02(56)	87.94(12)	511.0 (178.3)	3+	4, 5, 6
^{86}Y	14.7	cyclotron	^{86}Sr(p,n)^{86}Y	$\varepsilon + \beta^+$ (100) β^+ (31.9)	535(7)	11.9(5)	443.1 (16.9) 511.0 (64) 627.7 (36.2) 703.3 (15) 777.4 (22.4) 1076.6 (82.5) 1153.0 (30.5) 1854.4 (17.2) 1920.7 (20.8)	3+	8, 9
^{89}Zr	78.4	cyclotron	^{89}Y(p,n)^{89}Zr	$\varepsilon + \beta^+$ (100) β^+ (22.7)	395.5(11)	22.74(24)	511.0 (45.5) 909.2 (99.0)	4+	8

^{68}Ga-DOTA-tyr3-Octreotide

(^{68}Ga-DOTATOC, FDA-approved 2016)

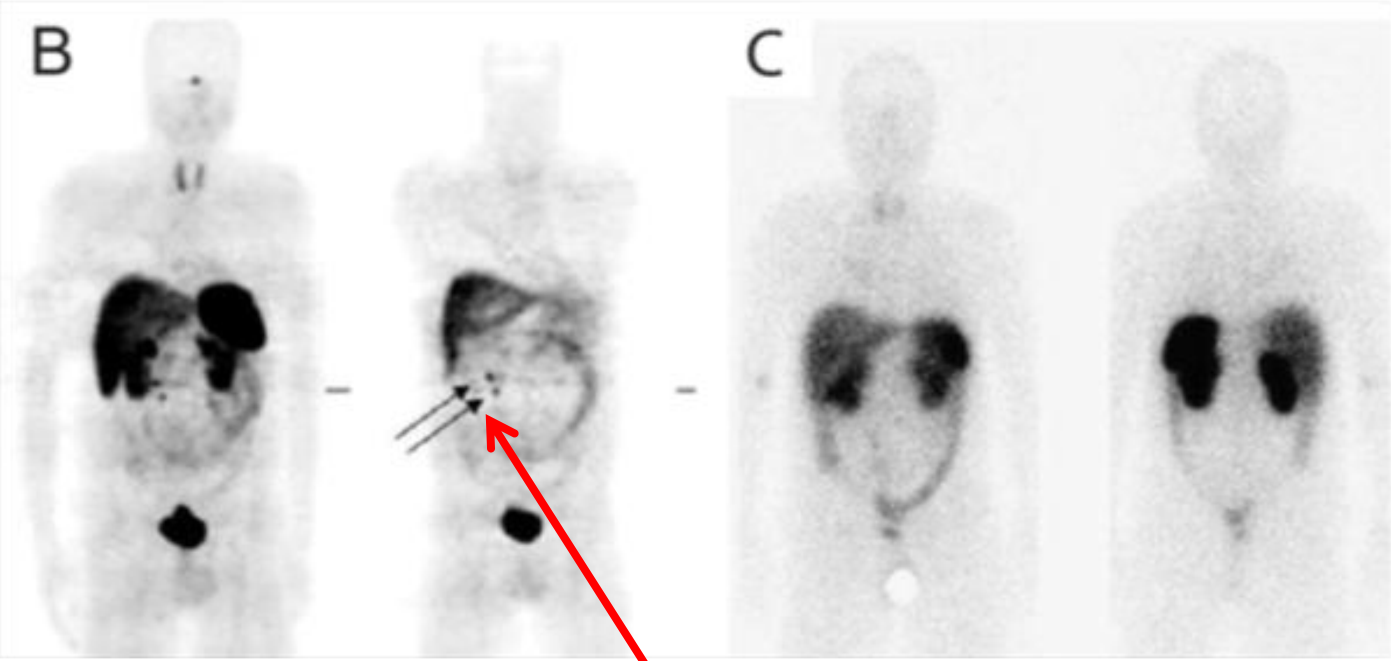
High resolution PET imaging of neuroendocrine tumors



β^+ emitter ($h_{1/2}$ = 1.1h)

^{68}Ga is obtained in a generator from ^{68}Ge through EC.

PET vs SPECT imaging of an endocrinous tumor



^{68}Ga-DOTATOC
(PET)

^{111}In-DPTA-ocreotide
(SPECT)

abdominal lymphnodes

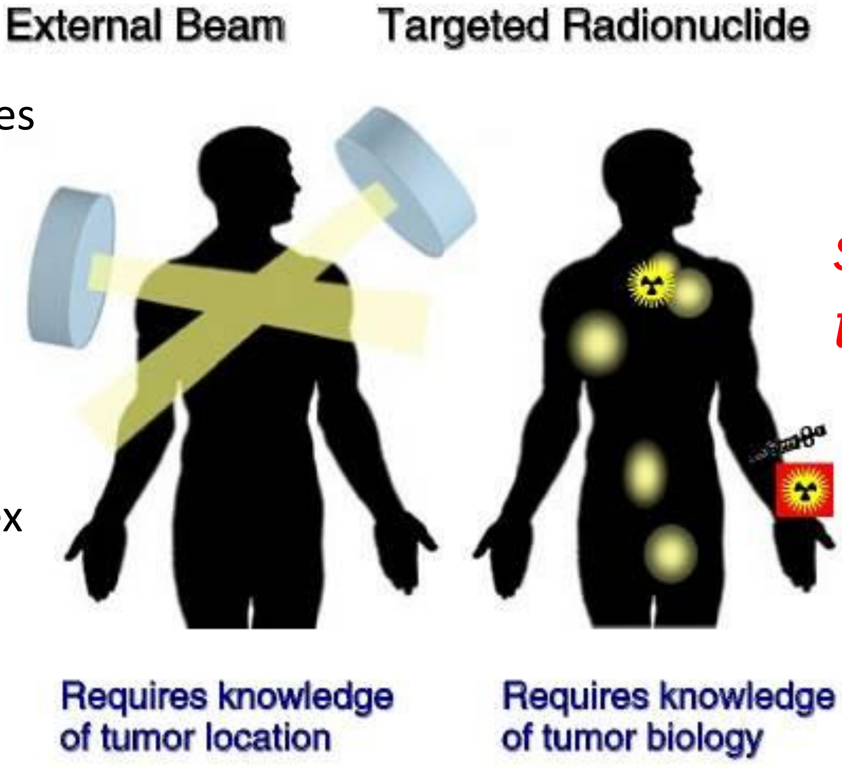
Targeted radiotherapy with α or β^- emitters

Required radiation dosages

- lymphomas: 1500–2000 cGy
- solid tumors: 3500–10000 cGy

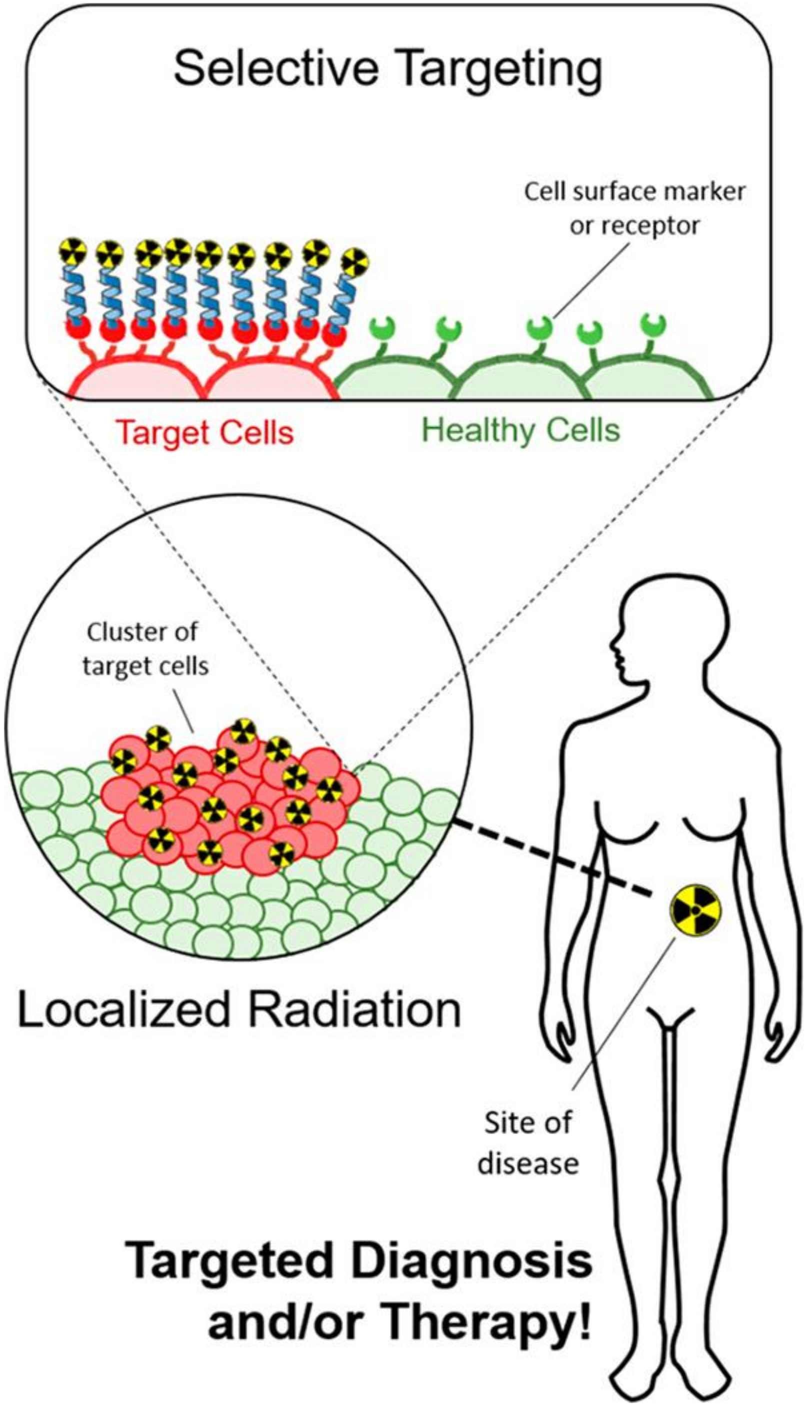
Required Therapeutic Index

- TI > 10 for kidney and lung
- TI > 50 for spinal cord



systemic treatment

TI = therapeutic index

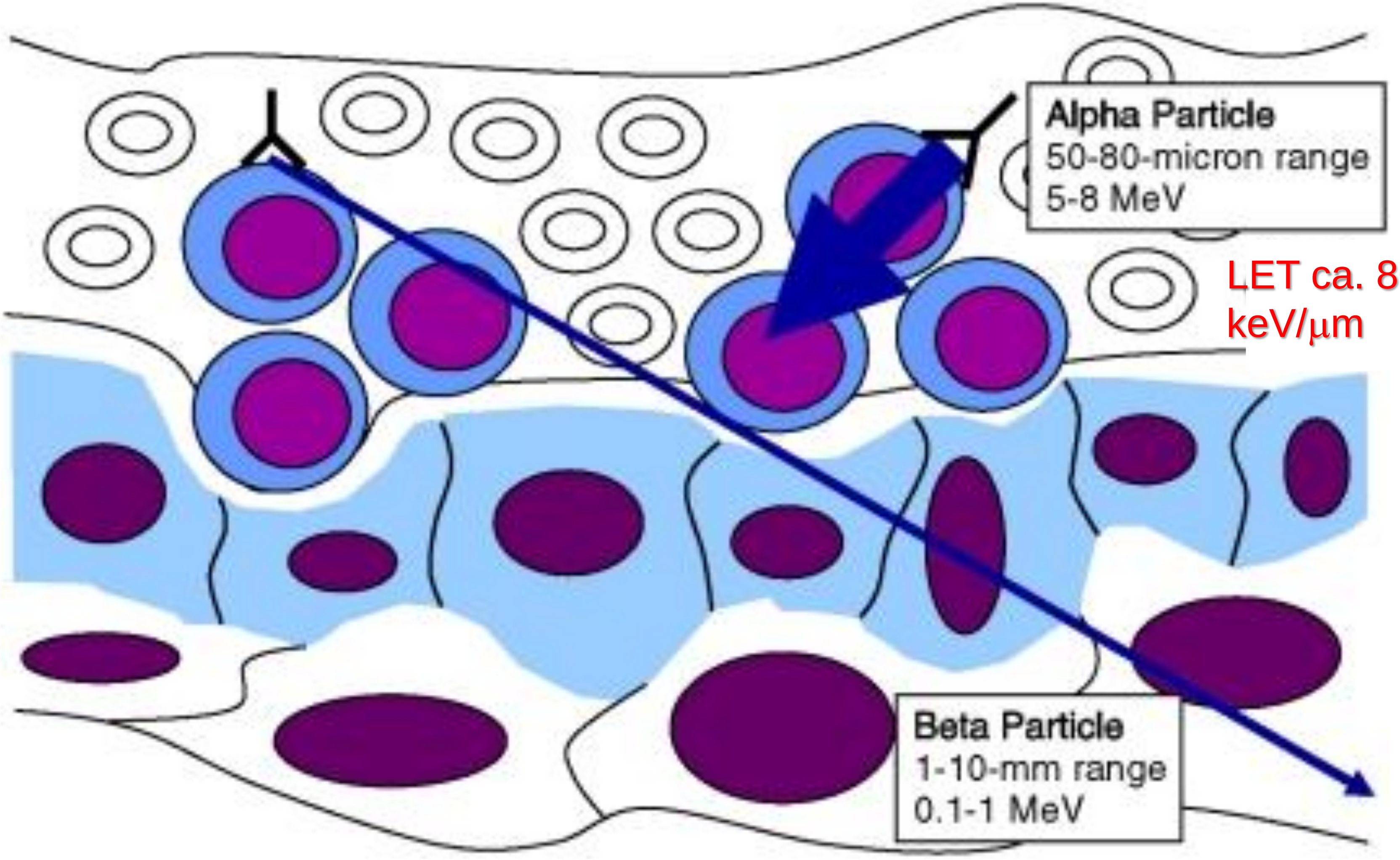


Targeted Diagnosis and/or Therapy!

Linear Energy Transfer (LET) refers to the amount of energy transferred by an ionizing particle to the material it passes through per unit distance traveled. It essentially quantifies the energy deposited by radiation into matter, with **higher LET generally leading to more damage in biological systems**

α-particles: helium nuclei, high linear energy transfer (LET, 100 keV/μm) → short pathlength in tissues (50–100 μm) → localized damage
β^- particles: electrons → indirect damage by ROS production → longer pathlength

Penetrating power of α and β particles



LET ca. 80-100 keV/μm

LET ca. 0.2 keV/μm

linear energy transfer (LET, keV/μm)

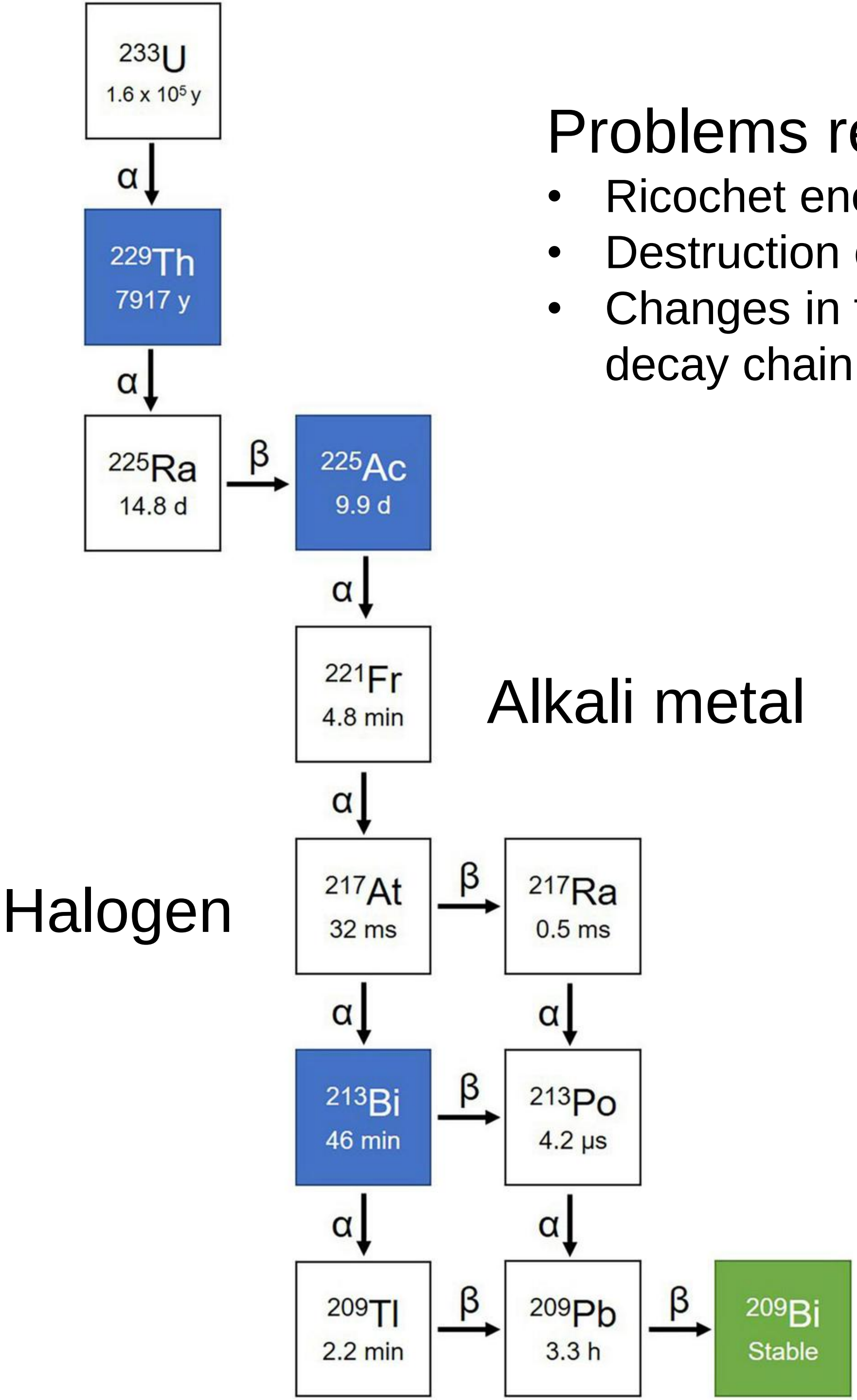
Main radionuclides for Targeted Radiotherapy

Radionuclide	**Half life**	**Average energy of decay particle (KeV)**
^{67}Cu (β, γ)	62 h	
^{90}Y (β)	64 h	934
^{153}Sm (β, γ)	46 h	
^{131}I (β, γ)	8 d	
^{177}Lu(β, γ)	6.6 d	134
^{188}Re (β, γ)	17 h	
^{213}Bi (α, β, γ)	1 h	
^{225}Ac ($5\alpha + 3\beta$)	10 d	5800 - 8400
^{223}Ra ($4\alpha + \beta$)	11.4 d	

Due to the different energy, the average tissue penetration of β particles is ca. 2 mm for ^{177}Lu *vs* 11 mm for ^{90}Y

Problems related to α-emission:

- Ricochet energy leading to metal release,
- Destruction of the ligand by the α particles,
- Changes in the nature of the nucleus along the decay chain.



^{233}U 1.6 x 10^{5} y

α

^{229}Th 7917 y

α

^{225}Ra 14.8 d

β

^{225}Ac 9.9 d

α

^{221}Fr 4.8 min

Alkali metal

α

Halogen

^{217}At 32 ms

β

^{217}Ra 0.5 ms

α

α

^{213}Bi 46 min

β

^{213}Po 4.2 μs

α

α

^{209}Tl 2.2 min

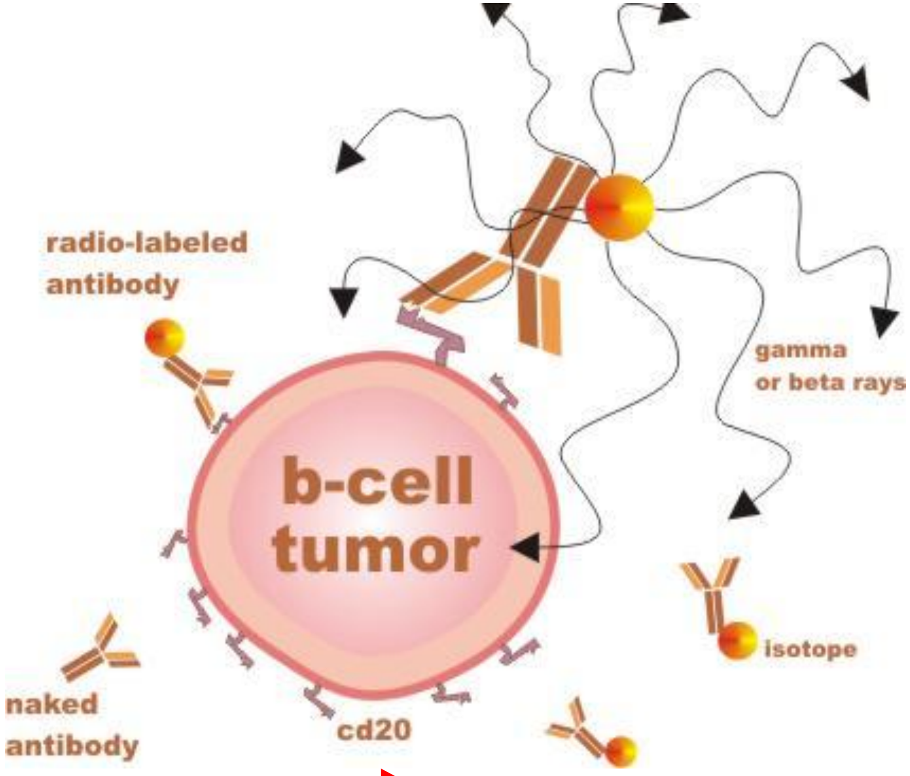
β

^{209}Pb 3.3 h

β

^{209}Bi Stable

Treatment of B-cell non-Hodgkin’s limphoma

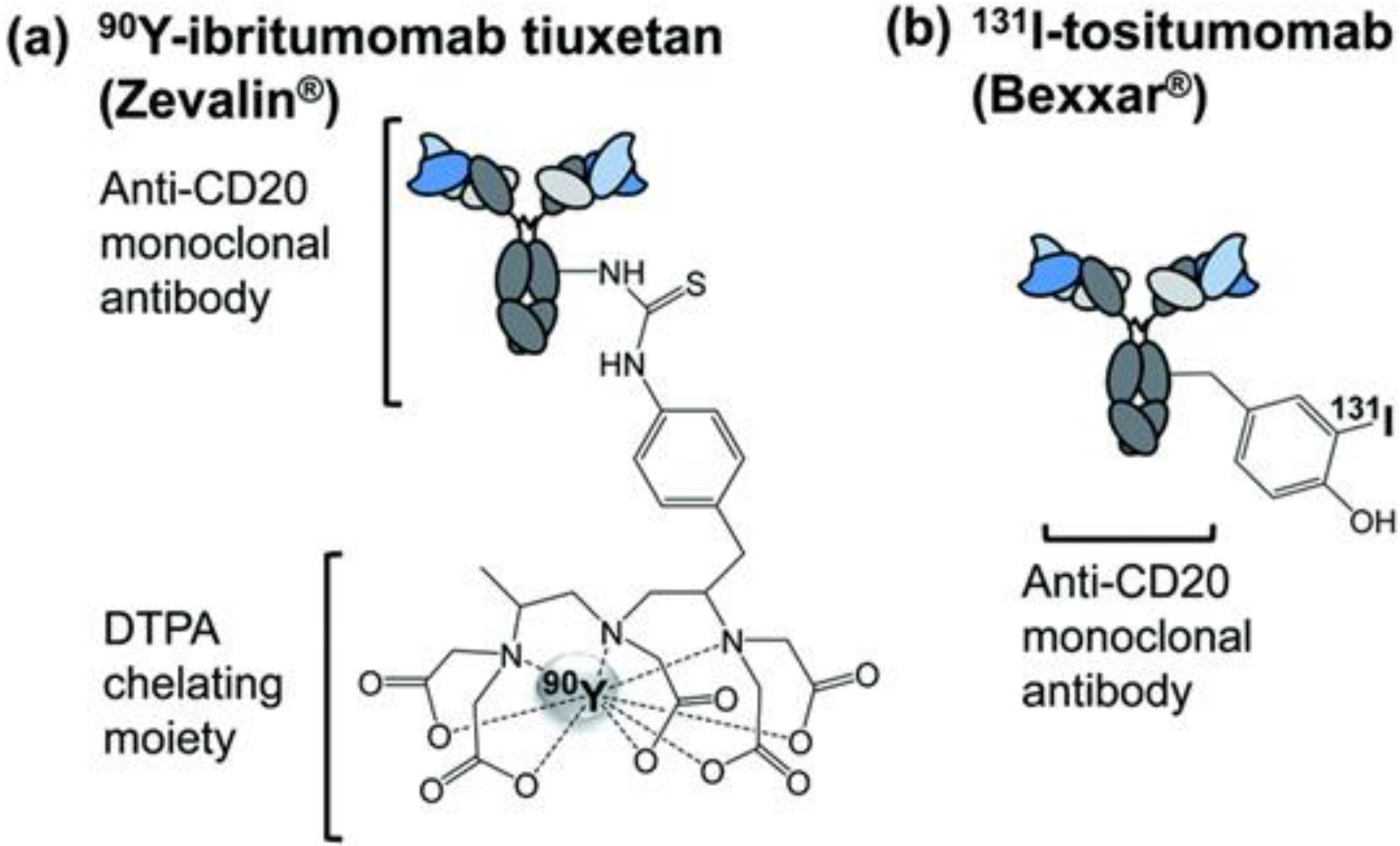


antigene

ideal antigen:

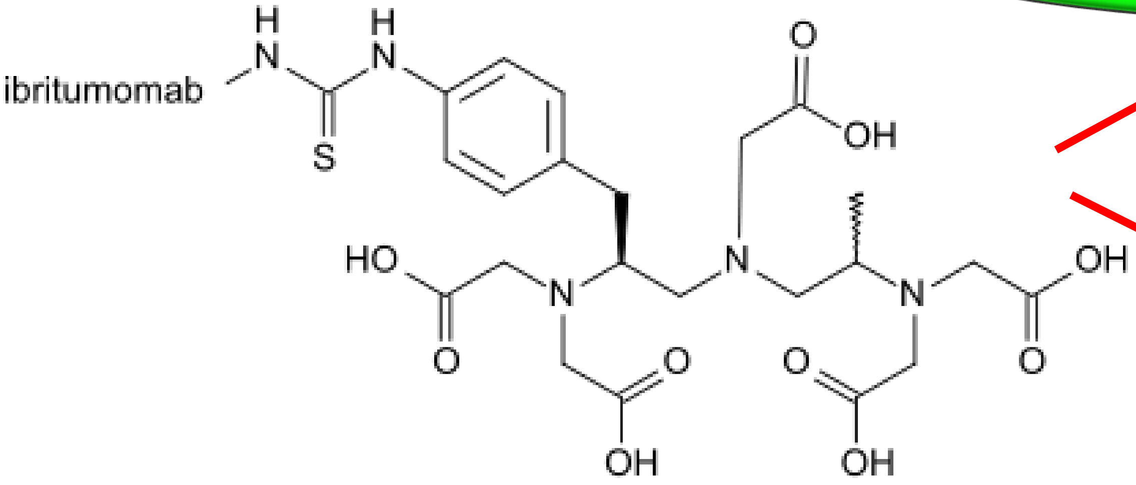
- highly expressed with uniform density on the surface of all tumor cells (> 10^5 sites per cell),
- not to be expressed (or much less) in healthy cells,
- antigen-antibody affinity in the nanomolar order
- internalization

FDA approved



Zevalin®

Ibritumomab (MC antibody) covalently conjugated to the ^{90}Y chelator tiuxetan



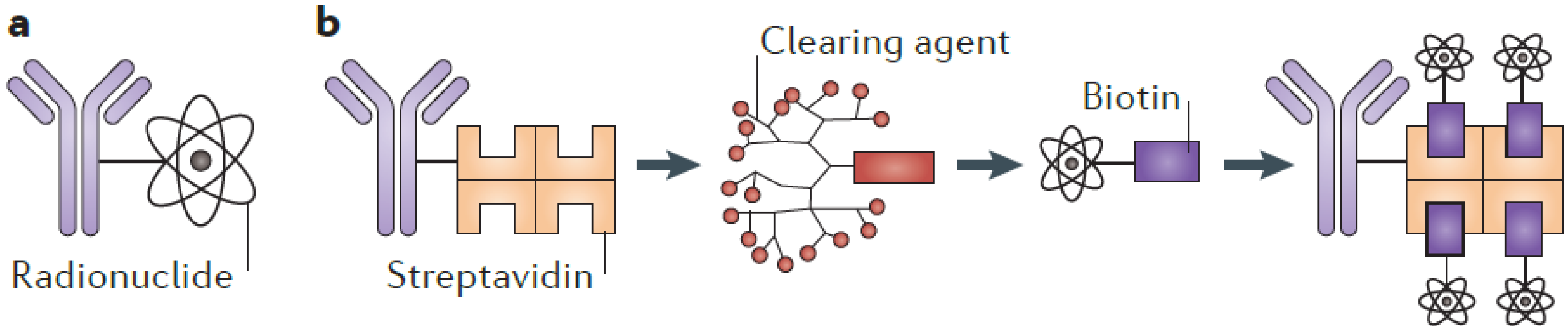
$^{111}\mathrm{In}^{3+}$ or – better – $^{86}\mathrm{Y}^{3+}$ for imaging

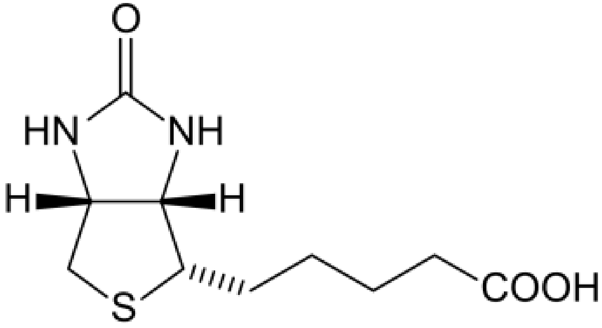
^{90}Y is a pure β^{-} emitter

$^{90}\mathrm{Y}^{3+}$ for therapy

Example of the matched-pair approach

multi-step pre-targeted radio-immunotherapy (PRIT)

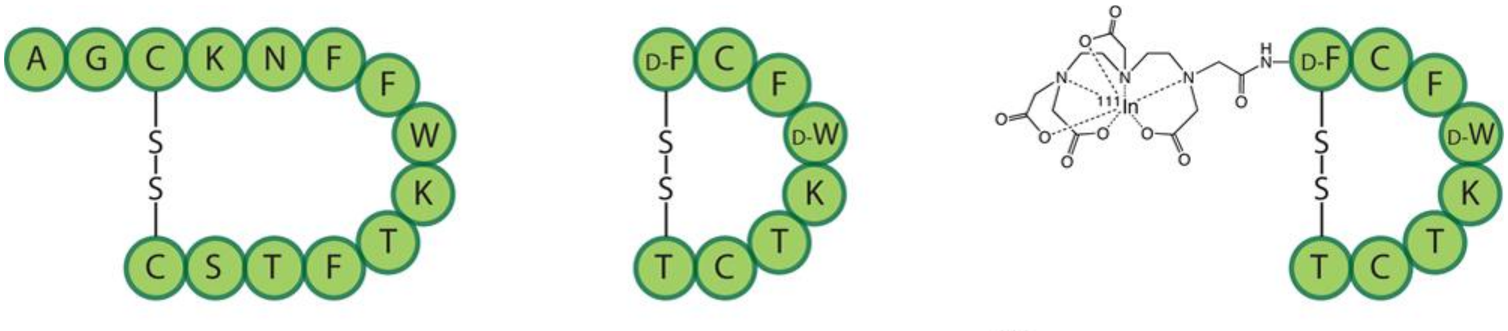




Biotin

The **streptavidin-biotin** binding constant is in the order of 10^{14} mol L^{-1}

Radio-immunotherapy of neuroendocrine tumors



Somatostatin

Octreotide

^{111}In-DTPA-Octreotide

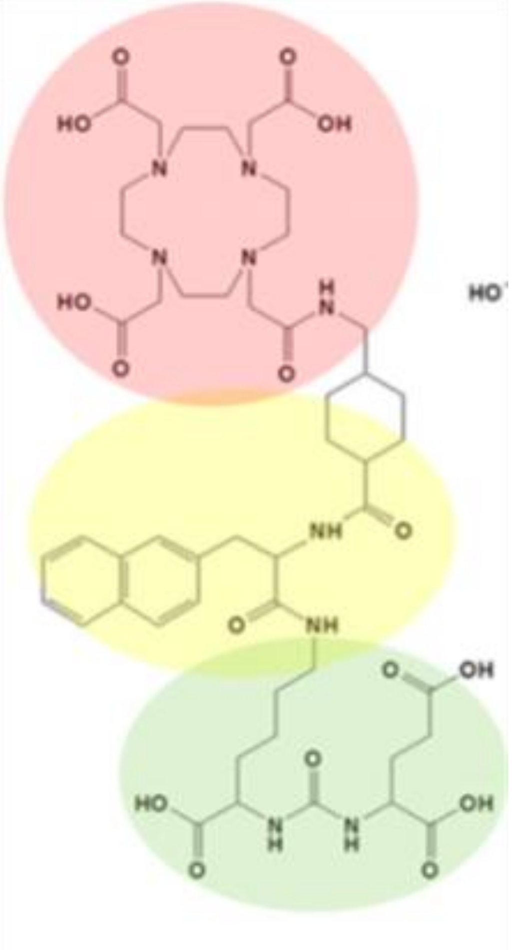
SPECT imaging
of neuroendocrine
tumors

^{68}Ga-DOTATOC for PET imaging

^{90}Y-DOTATOC or ^{177}Lu-DOTATATE for radiotherapy

^{177}Lu-DOTATATE (*Lutathera*) FDA approved in 2018
for treatment of neuroendocrine pancreatic tumors

^{177}Lu-PSMA-167



Linker

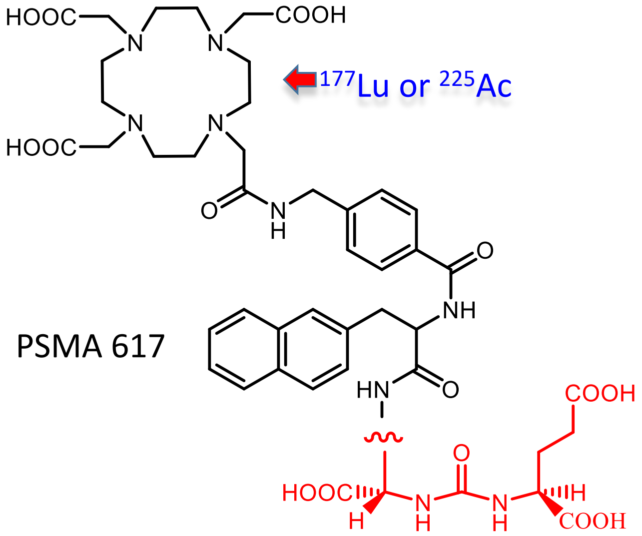
Peptidomimetic

PSMA-617

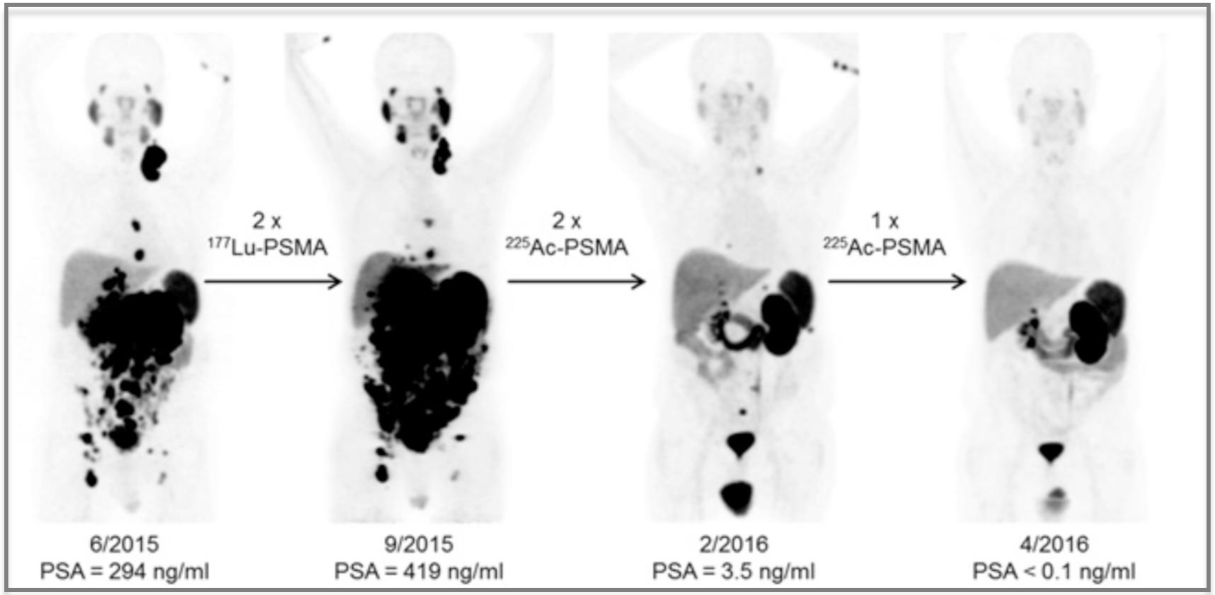
PSMA = *prostate-specific membrane antigen*

The Concept of Radiopharmaceutical Production: α-**emitters**

An example for ^{225}Ac PSMA617 α-emitters in radiotherapy

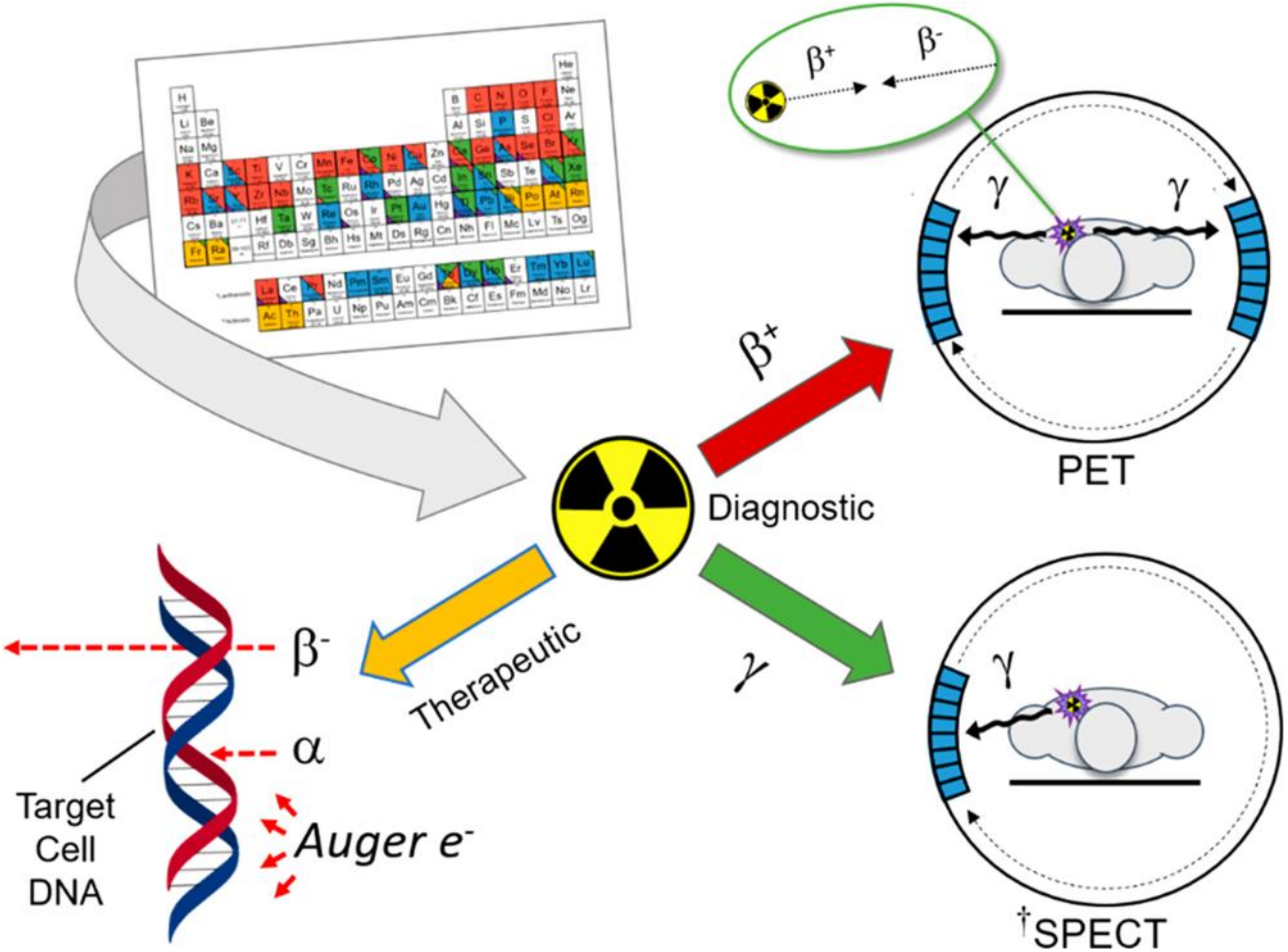


PSMA 617



- Patient **did not respond** to [^{177}Lu]LUPSMA-617 radionuclide treatment
- PCa showed continued **progression** after two rounds of ^{177}Lu
- Three cycles of [^{225}Ac]AcPSMA-617 treatment led to **complete response**

Kratochwil *et al.*, *J. Nucl. Med.*, **2016**, *57*, 1170


β+
β-
γ
γ
β+
PET
Diagnostic
γ
†SPECT
γ
Therapeutic
β-
α
Auger e-
Target Cell DNA