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Radiolabelled peptides and proteins for PET

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Prof. Anna Orlova

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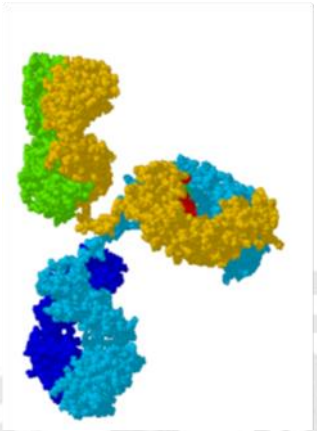
Peptide or protein?



Consists of ≤ 50 amino acids

Molecular weight of 1 aa is ca 100 Da

Molecular weight ca < 5000 Da

Consists of > 50 amino acids

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Positron Emission Tomography

\+ High sensitivity

High resolution

Quantification

\- Short life of positron emitting radioisotops

Not suitable for kinetics of large proteins (mAbs)

Cyclotron production

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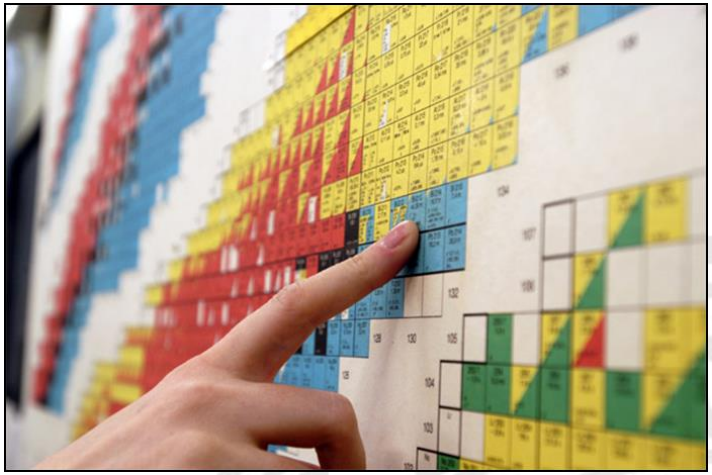
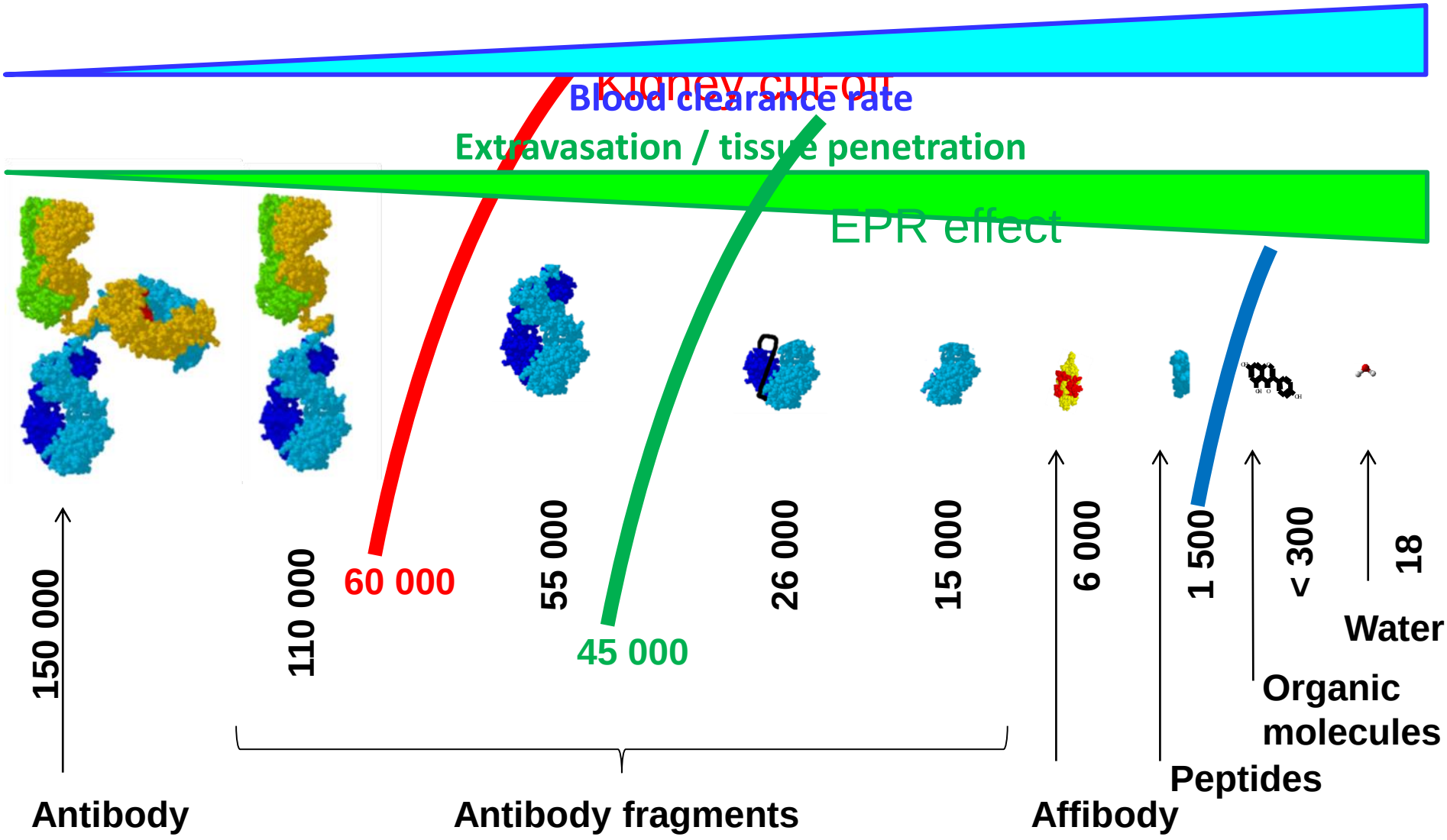
Choice of PET radioisotopes



- Half-life
- Availability
- PET image quality
- Labeling method
- What do you want to study?
- Influence of labeling method on studied protein

Proteins and peptides



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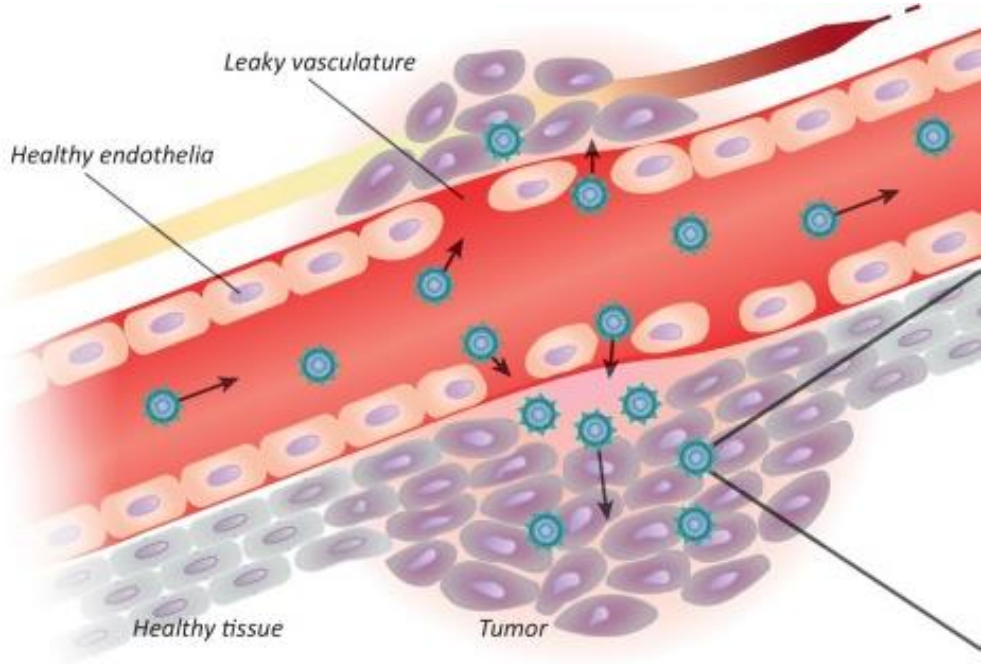
Enhanced permeability and retention (EPR) effect



Molecules > 45 kDa accumulate in tumors more than in healthy tissues due to ”leaky” vasculature in tumors

Nuclide	Half-life (hour)	Nuclear reaction for production	Nuclide	Half-life (hour)	Nuclear reaction for production
^{11}C	0.34	$^{14}N(p,a)^{11}C$	^{66}Ga	9.49	$^{66}Zn(p,n)^{66}Ga$
^{13}N	0.166	$^{13}C(p,n)^{13}N$	^{68}Ga	1.14	generator
^{15}O	0.0345	$^{15}N(p,n)^{15}O$	^{72}As	26.0	$^{72}Ge(p,n)^{72}As$
^{18}F	1.83	$^{18}O(p,n)^{18}F$	^{74}As	426.5	$^{74}Ge(p,n)^{74}As$
^{44}Sc	3.93	Generator/ $^{44}Ca\ (p,n)^{44}Sc$	^{75}Br	1.61	$^{74}Se(d,n)^{75}Br$
^{45}Ti	3.08	$^{45}Sc(p,n)^{45}Ti$	^{76}Br	16.2	$^{76}Se(p,n)^{76}Br$
^{48}V	383	$^{48}Ti(p,n)\ ^{48}V$	^{86}Y	14.7	$^{86}Sr(p,n)^{86}Y$
^{52}Mn	134.2	$^{52}Cr(p,n)\ ^{52}Mn$	^{89}Zr	78.4	$^{89}Y(p,n)^{89}Zr$
^{55}Co	17.53	$^{58}Ni(p,a)\ ^{55}Co$	^{94m}Tc	0.87	$^{94}Mo(p,n)^{94m}Tc$
^{61}Cu	3.4	$^{61}Ni(p,n)^{61}Cu$	^{110m}In	1.15	$^{110}Cd(p,n)^{110}In$
^{64}Cu	12.7	$^{64}Ni(p,n)^{64}Cu$	^{124}I	100.2	$^{124}Te(p,n)^{124}I$

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Availability

- Production using small cyclotron (expensive, logistic limitations, qualified staff)
- (p,n), (p,α), (d,n), (p,2n)-reactions
- most likely, the use of enriched targets
- necessity to regenerate target material

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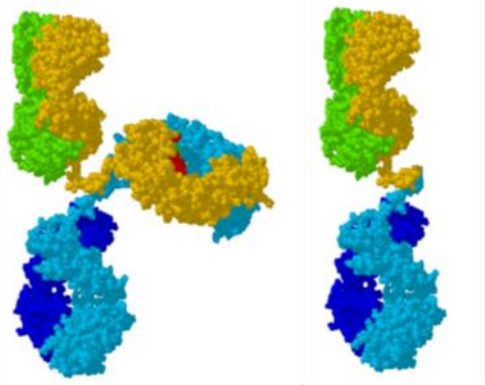
Does protein size matter?

Isotops with long half-life

Slow kinetic

Fast kinetic

Isotops with short half-life



150 000

110 000

55 000

26 000

15 000

6 000

1 500

< 300

18

Water

Organic molecules

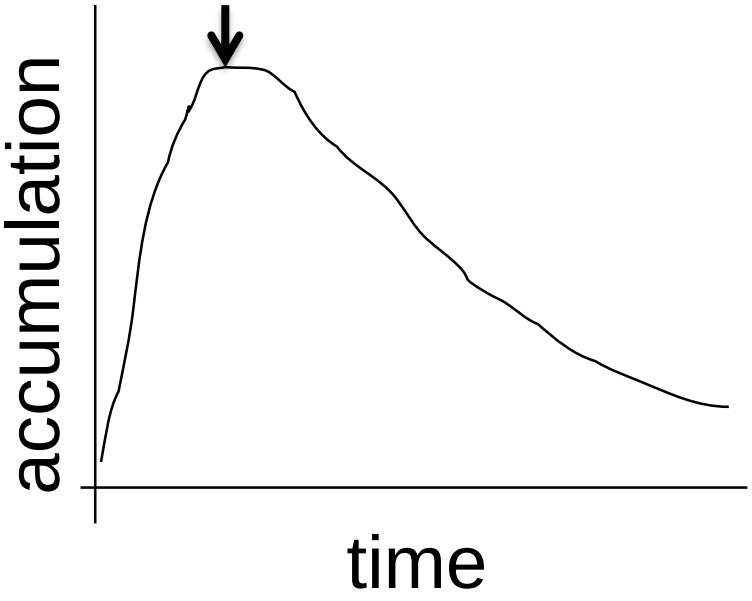
Peptides

Antibody

Antibody fragments

Affibody

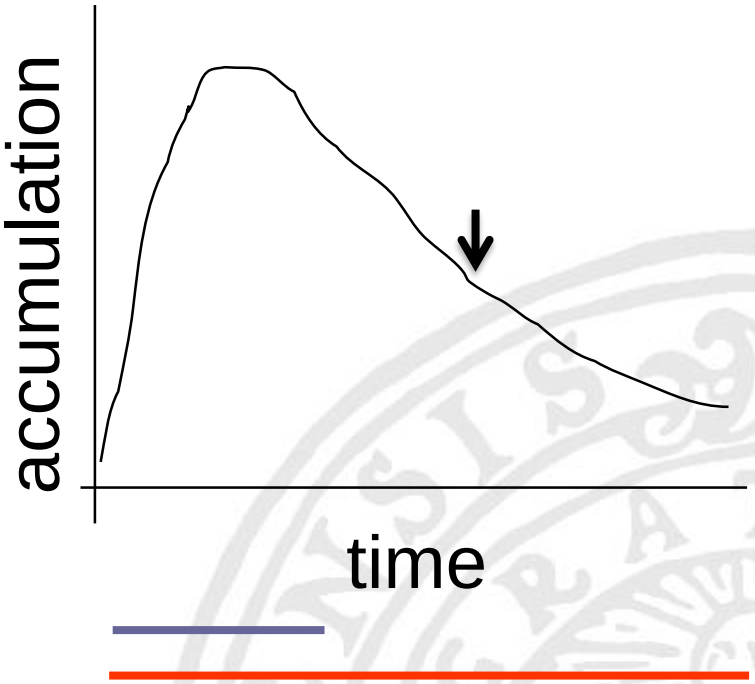
Half life of radioisotope



$T_{1/2}$

Observation

Case 1. Absolute accumulation



Case 2. Retention and wash out

We can observe signal in patients up to 3 x $T_{1/2}$ (for ^{68}Ga up to 4 h)

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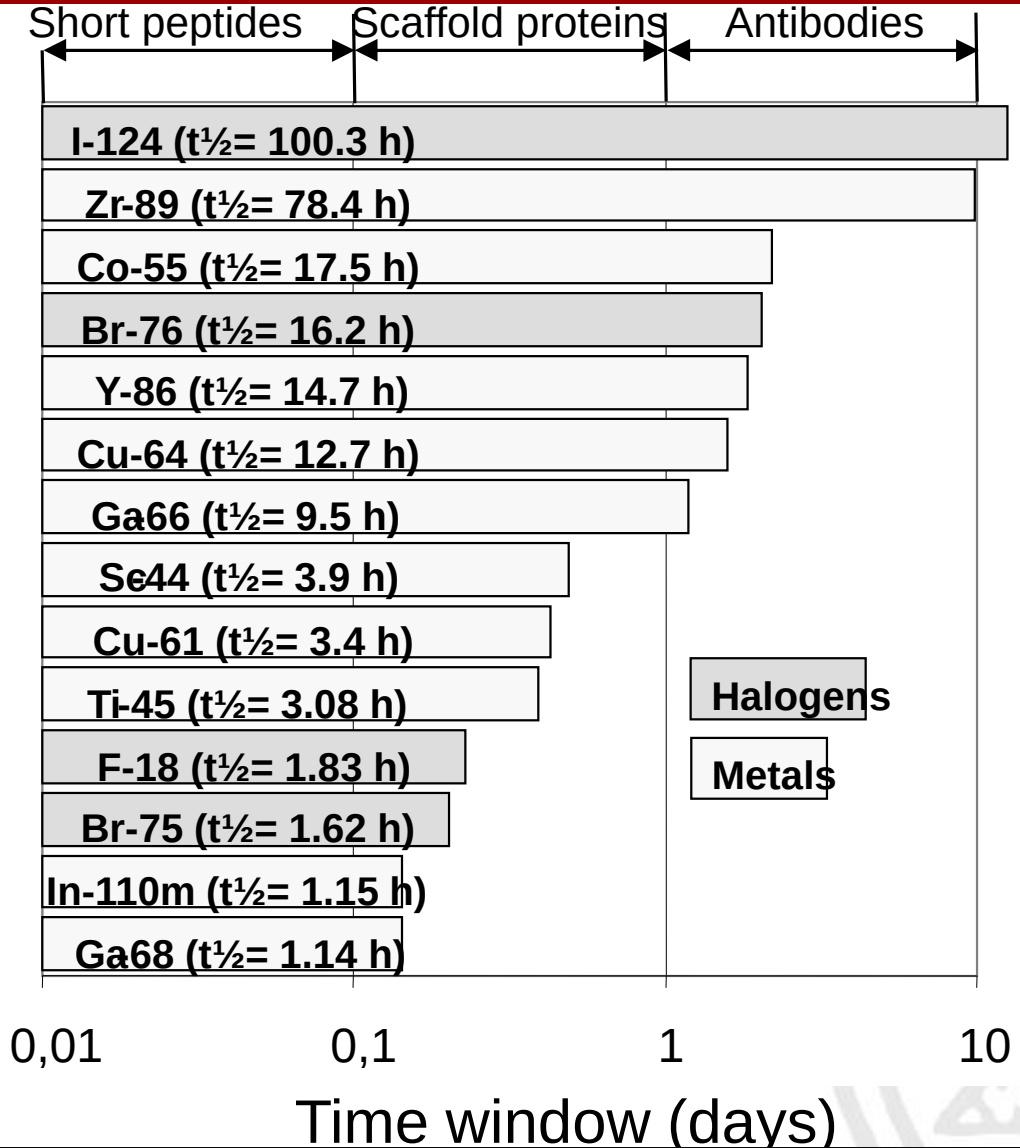
Does size matter?



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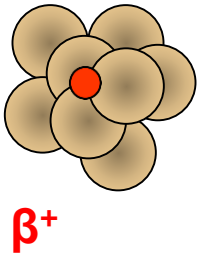
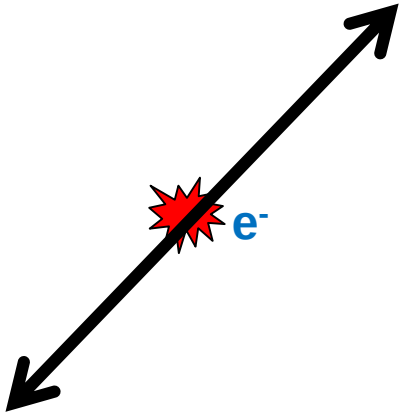
Availability

Optimal imaging time	Non-residualizing label	Residualizing label
Few hours pi	^{11}C (20 min) ^{18}F (110 min)	^{68}Ga (68 min) ^{61}Cu (3h) ^{44}Sc (3 h)
Next day pi	^{76}Br (16 h)	^{55}Co (17 h) ^{86}Y (14 h) ^{64}Cu (12.7 h)
3-5 days pi	^{124}I (100 h)	^{89}Zr (78 h)

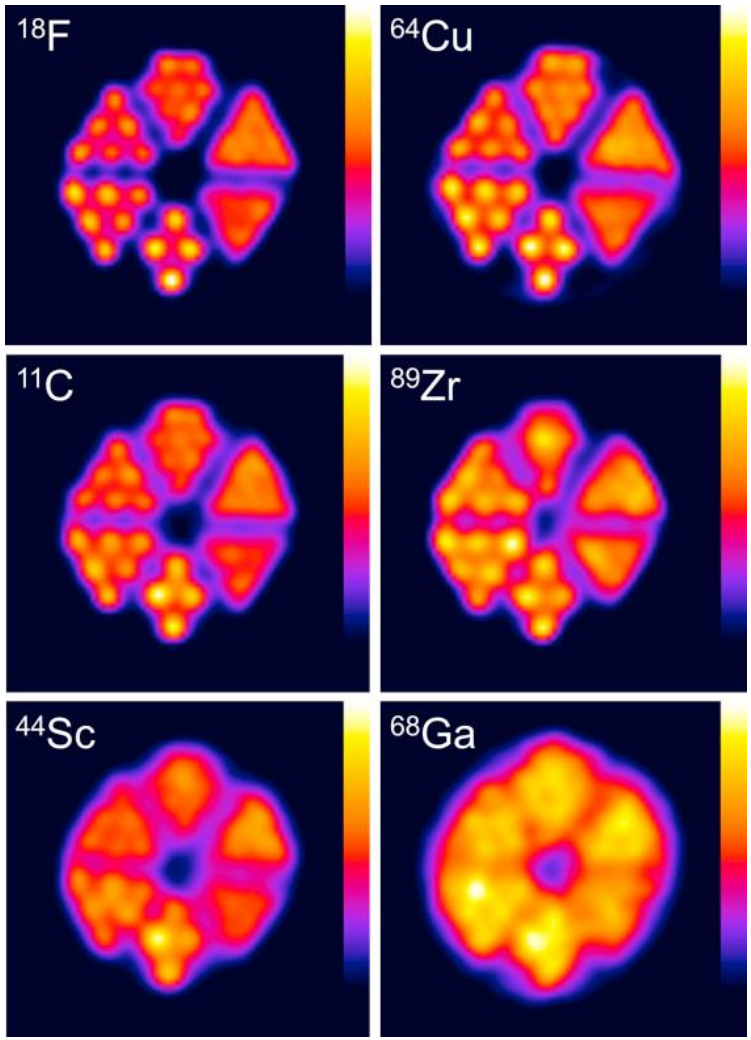
PET image quality





- **Abundance of positron emission**
  - ^{11}C – 100%
  - ^{18}F – 97%
  - ^{124}I – 23%
- **Positron energy**
  - distance from decay to annihilation

PET image quality



^{68}Ga<^{44}Sc<^{89}Zr<^{11}C<^{64}Cu<^{18}F

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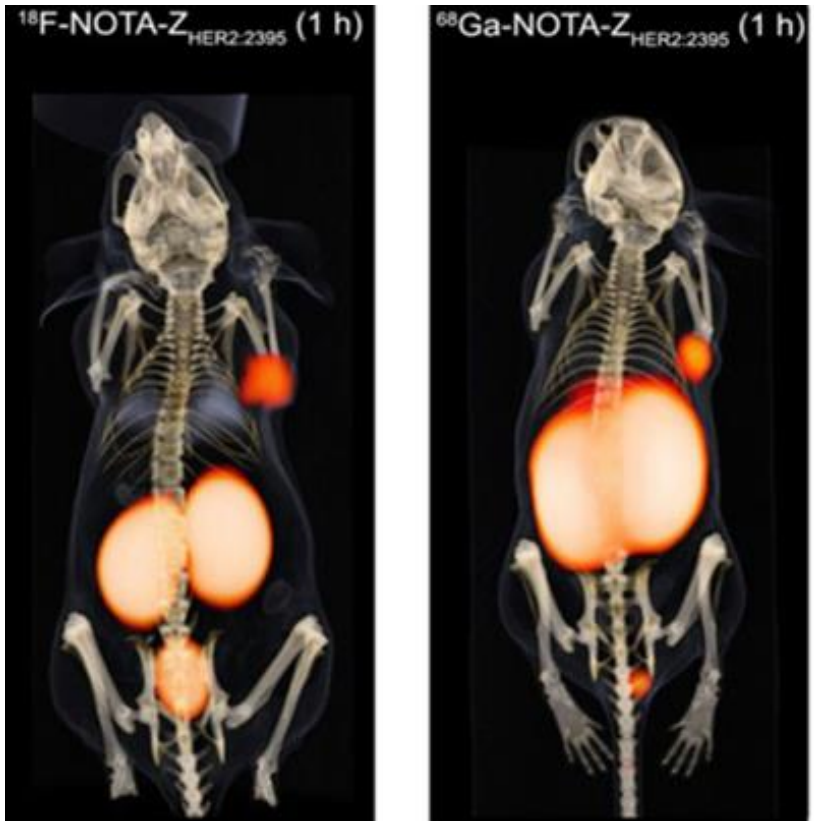
PET image quality

$^{68}Ga < ^{44}Sc < ^{89}Zr < ^{11}C < ^{64}Cu < ^{18}F$



Boerman, Tolmachev et al. J Nucl Med 2012

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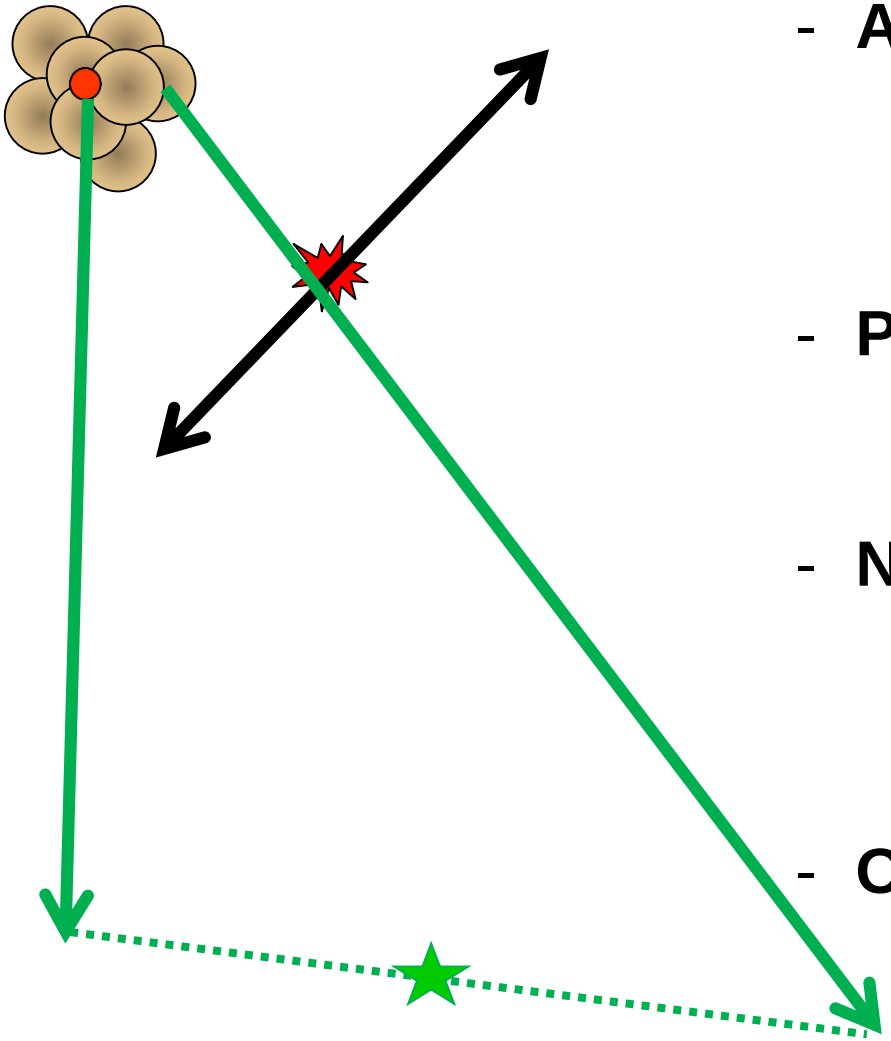
PET image quality



- **Abundance of positron emission**:
  - ^{11}C – 100%
  - ^{18}F – 97%
  - ^{124}I – 23%
- **Positron energy**
  - distance from decay to annihilation
- **Non-annihilation gamma**
  - 350-650 kEV
  - ^{86}Y – 443(17%), 628(32%), 646(9%)
- **Other radiation**
  - ^{64}Cu – β^- 37% (β^+ 18%)

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Applications of proteins

- **Basic research**
- **Preclinical development**: pharmacokinetic studies, targeting properties, therapeutic dose
- **Clinical diagnostic**: patient stratification, therapy responce
- **Treatment planning**: dosimetry estimation

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Labeling of proteins

- **Stability of tracer in blood**

  transchelation, free radionuclide, colloids

- **Chemical modifications**

  conformation, target recognition, molecular charge, lipophilicity

- **Cellular processing by malignant cells**

  residualising properties of radiocatabolites

- **Retention in healthy tissues**

  residualising properties of radiocatabolites

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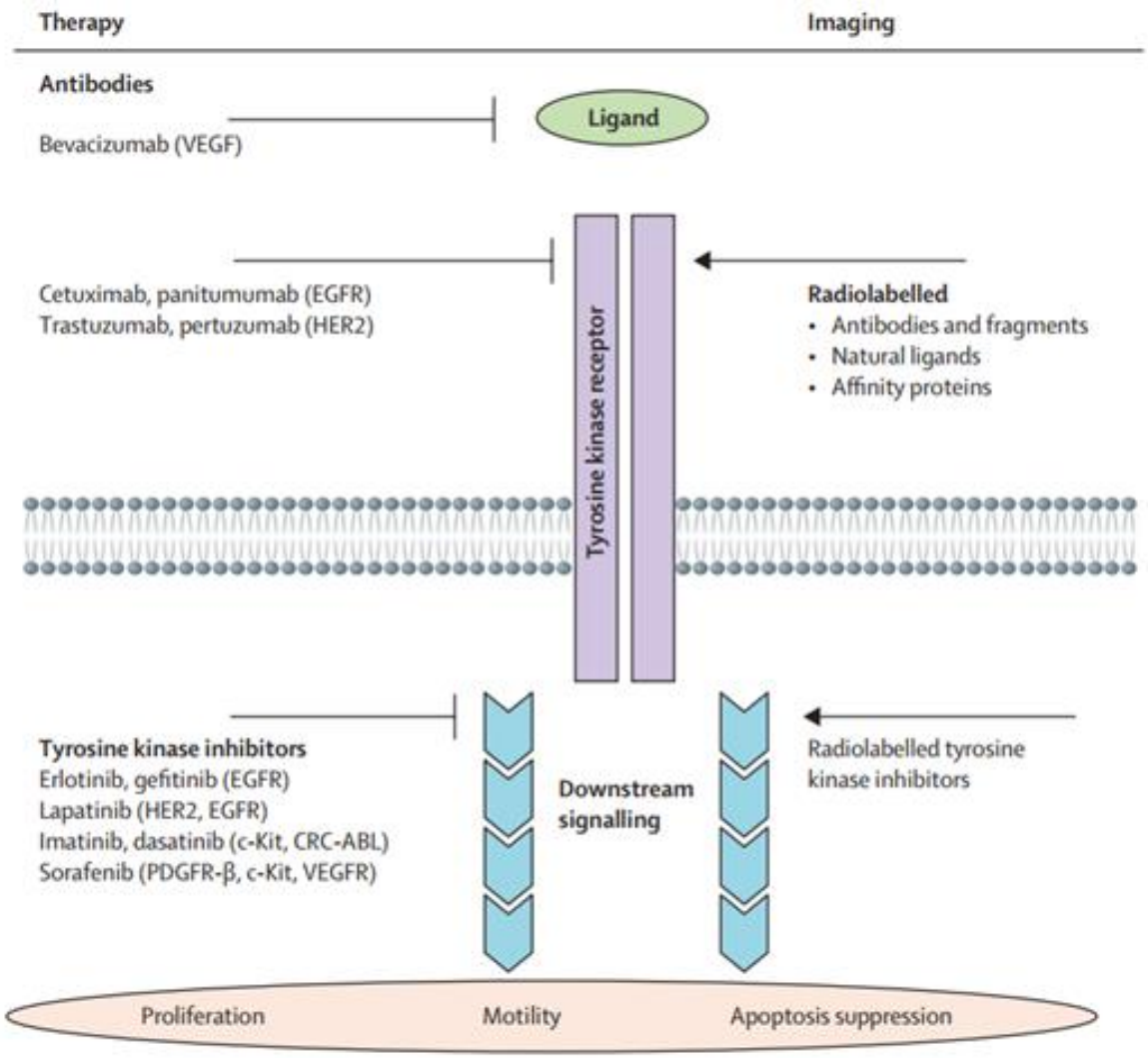
Receptor tyrosine kinases

Overexpressed in many tumors

Signalling

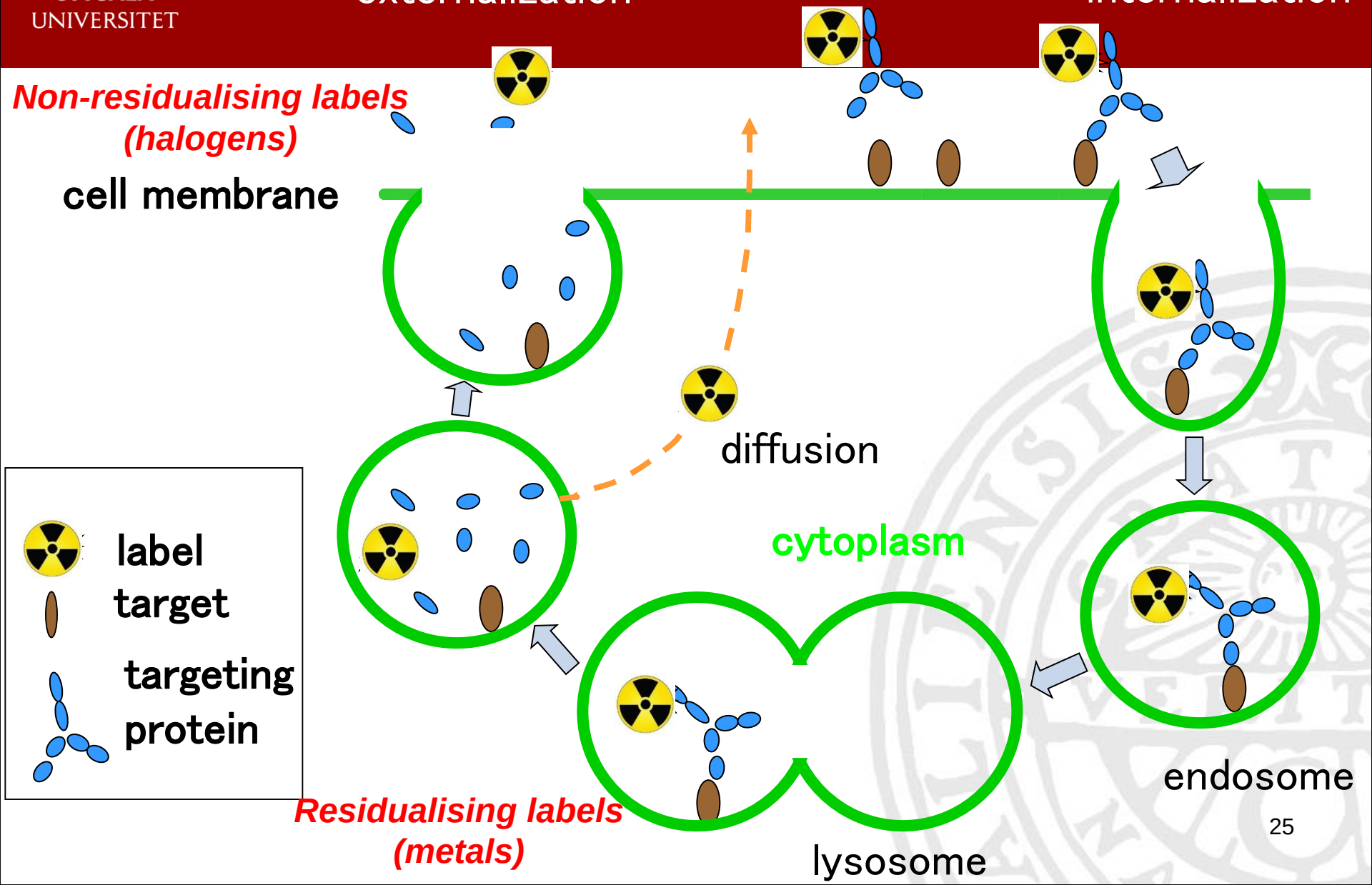
- **cell proliferation**
- **suppression of apoptosis**
- **increased motility**
- **recruitment of neovasculature**

RTKs are targets for anticancer drugs



Tolmachev et al. Lancet Oncology, 2010

Internalization of radiolabeled proteins



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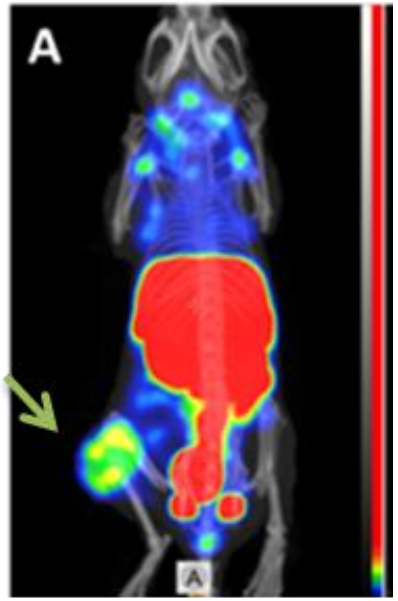
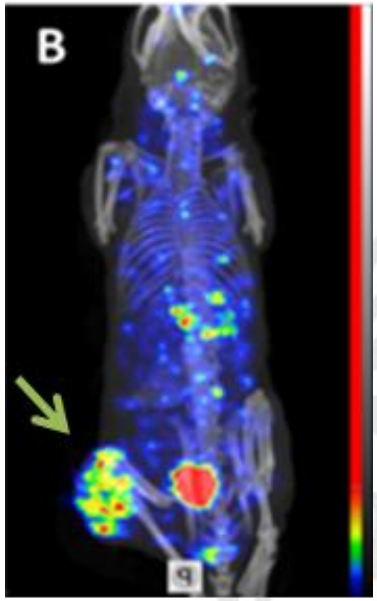
Same protein, different labels

Residualizing label (^{99m}Tc)



Retention in tumor, Retention in normal organs

Non-residualizing label (^{125}I halogen)



Retention in tumor, Wash-out from normal organs

Vorobyeva et al. Contrast Media & Molecular Imaging 2018

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Labeling of proteins

Optimal Imaging time	Non-residualizing label	Residualizing label
Few hours pi	^{11}C (20 min) ^{18}F (110 min)	^{68}Ga (68 min) ^{61}Cu (3h) ^{44}Sc (3h)
Next day pi	^{76}Br (16 h)	^{55}Co (17 h) ^{86}Y (14 h) ^{64}Cu (12.7 h)
3-5 days pi	^{124}I (100 h)	^{89}Zr (78 h)

Labeling chemistry

Amine chemistry: $^{11}CNBr$, $^{11}CH_2O$, $^{11}CH_3I$; **Sel-tag**: $^{11}CH_3I$

Prosthetic groups, AlF

Halogen chemistry

^{11}C (20 min)

^{18}F (110 min)

^{76}Br (16 h)

Chelation

^{124}I (100 h)

^{68}Ga (68 min)

^{61}Cu (3h)

^{44}Sc (3h)

^{55}Co (17 h)

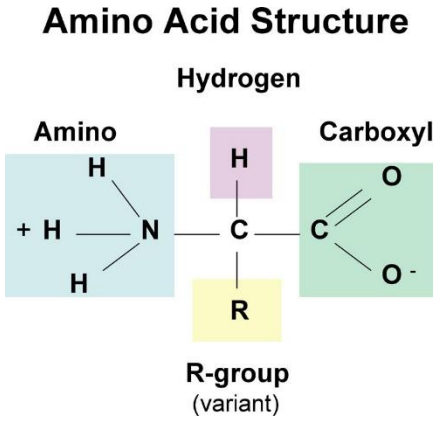
^{86}Y (14 h)

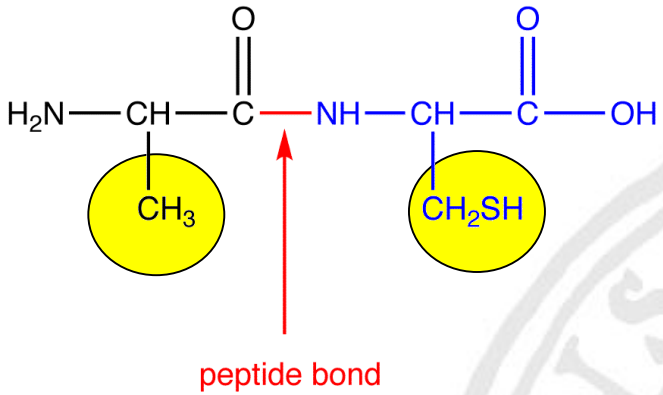
^{64}Cu (12.7 h)

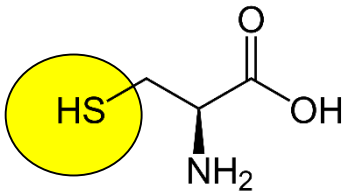
^{89}Zr (78 h)

Labeling chemistry

Labeling of proteins - Attachment to side chains of amino acids

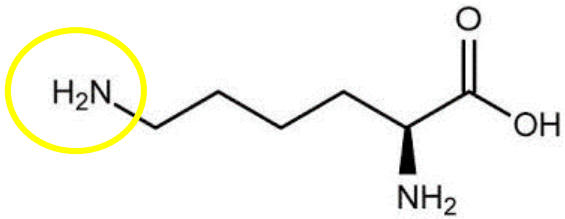






Cysteine- thiol group

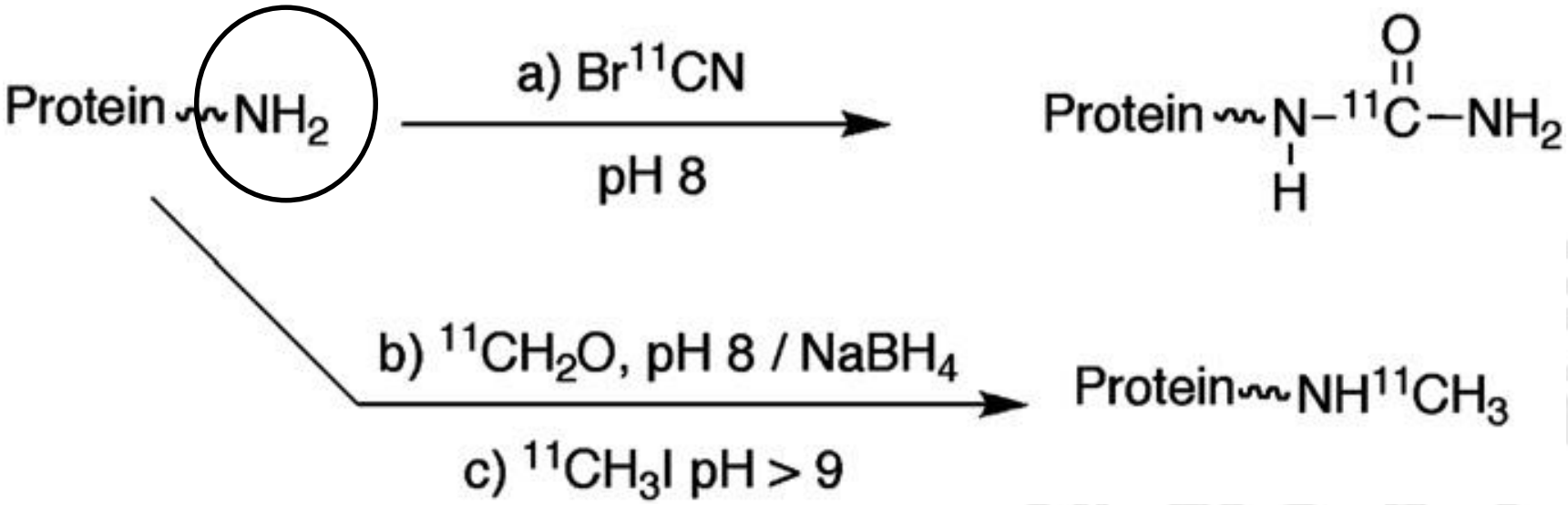
Site-specific labeling
Precise modification if single Cys
(1 label per molecule)



Lysine- amino group

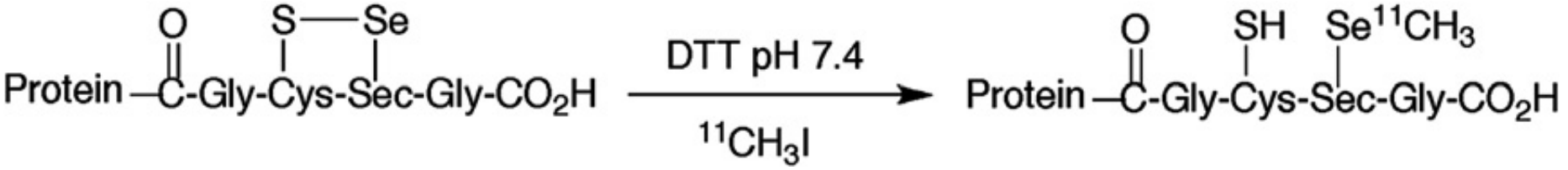
Non-specific (random) labeling
Less control over modification
If many -NH_2 (mAbs or proteins)

Labeling with ^{11}C (20 min)



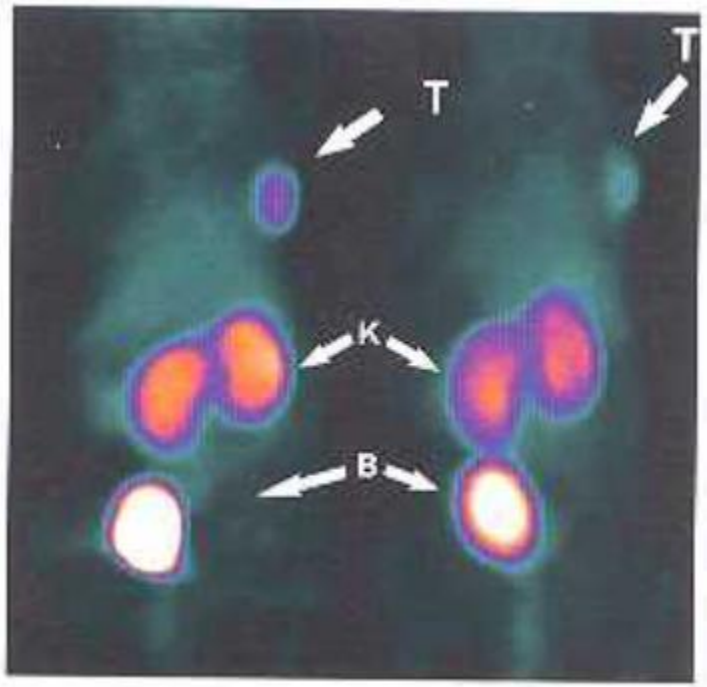
- \+ Available precursors
- − Random coupling
  Labeling conditions

Labeling with ^{11}C (20 min)



\+ Available precursor
Mild conditions
Site-specific labeling

− Absence of disulfide bonds
Bulky modification



^{11}C-Z_{HER2}
Wållberg et al. J Nucl Med. 2012

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Labeling with ^{18}F (2 h)

- Tough chemistry (low yields)

Several steps:
1) labeling of a prosthetic group
2) coupling of labeled prosthetic group to a protein

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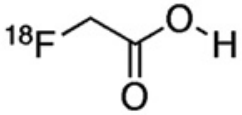
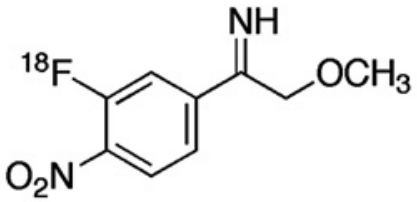
Labeling with ^{18}F

Amine-specific chemistry



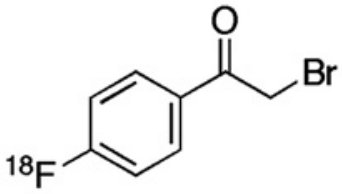
[^{18}F]FA

2 steps, 80 min, 50% [151]



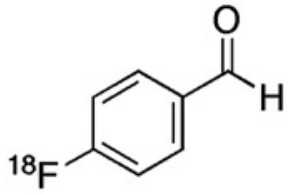
[^{18}F]FNB

2 steps, 50 min, 33% [152]



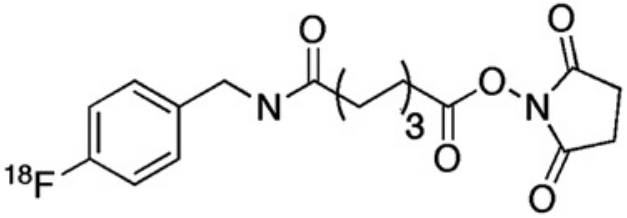
[^{18}F]FPB

3 steps, 75 min, 40% [152]



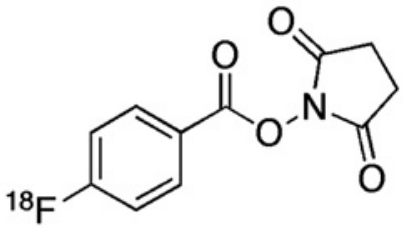
[^{18}F]FBA

1 step, 50 min, 70% [173]



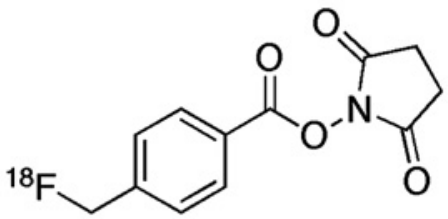
[^{18}F]SFBS

3 steps, 60 min, 40% [155]



[^{18}F]FSB

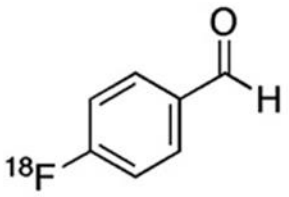
3 steps, 80 min, 35% [159]



[^{18}F]SFMB

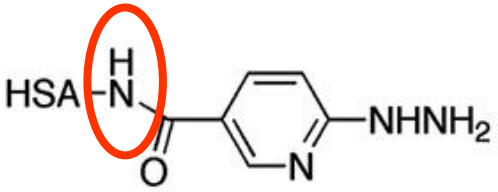
1 step, 50 min, 18% [168]

Labeling with ^{18}F

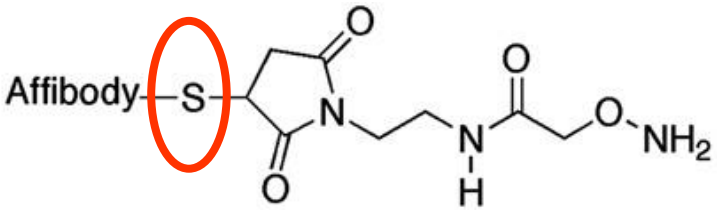


[^{18}F]FBA

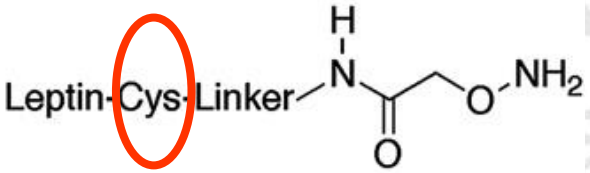
1 step, 50 min, 70% [173]



(a)



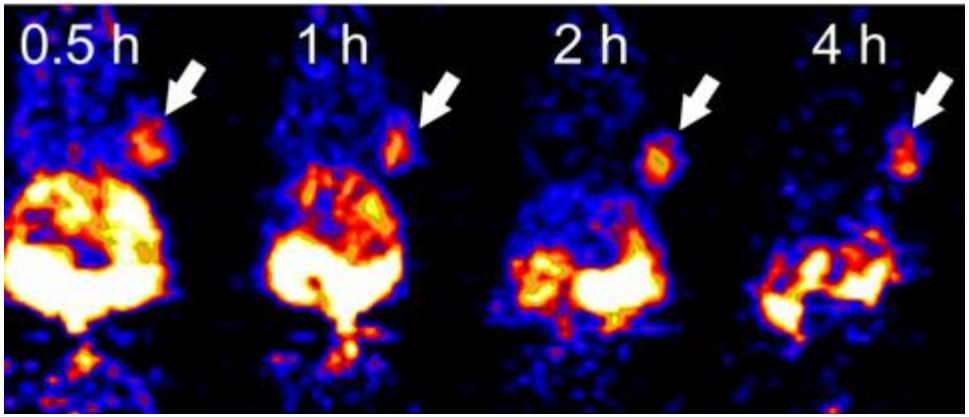
(b)



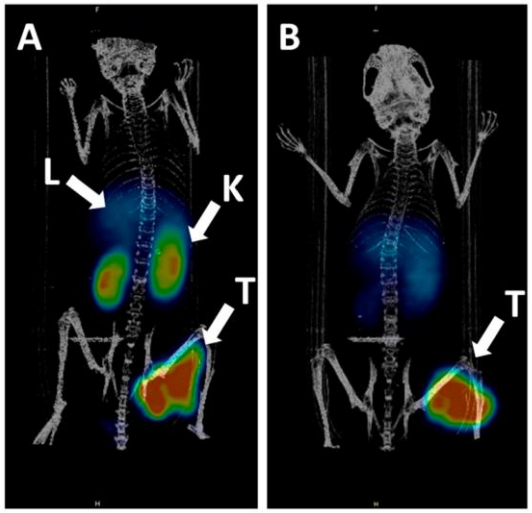
(c)

- Possible site-specific labeling

Labeling with ^{18}F

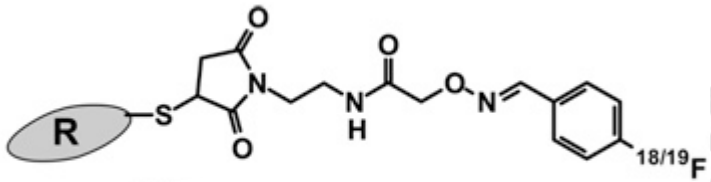


Cheng et al. J Nucl Med 2008; 49:804–813

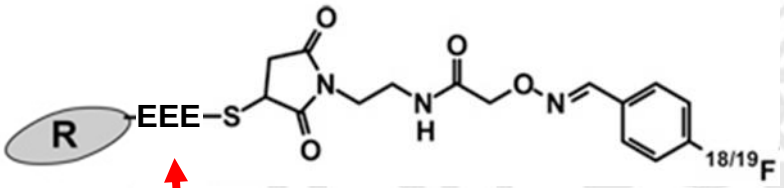


Rosik et al. Bioconjug Chem, 2014

^{18}F-Z_{HER2}-FBO



^{18}F-FBO-E_3-Z_{HER2}



Increased hydrophilicity

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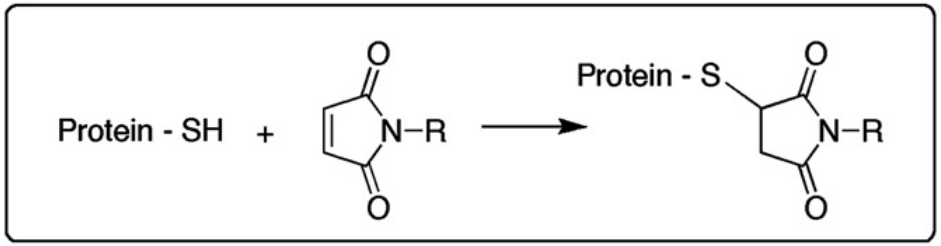
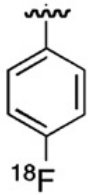
Labeling with ^{18}F

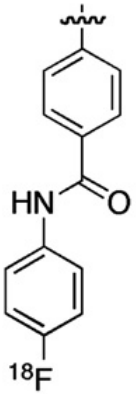


R =



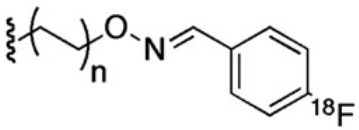
[^{18}F]FPPD

4 steps, 100 min, 15% [177]



[^{18}F]DDPFB

3 steps, 70 min, 10% [177]



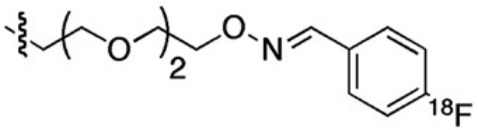
n=2 [^{18}F]FBABM

2 steps, 60 min, 35% [178]

2 steps, 92 min, 23% [179]

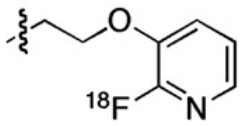
n=3 [^{18}F]FBAM

2 steps, 69 min, 29% [180]



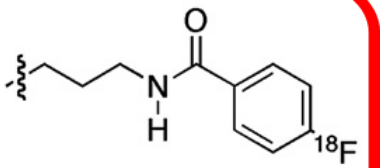
[^{18}F]FBOM

2 steps, 80 min, 19% [181]



[^{18}F]FPyME

3 steps, 110 min, 37% [182]

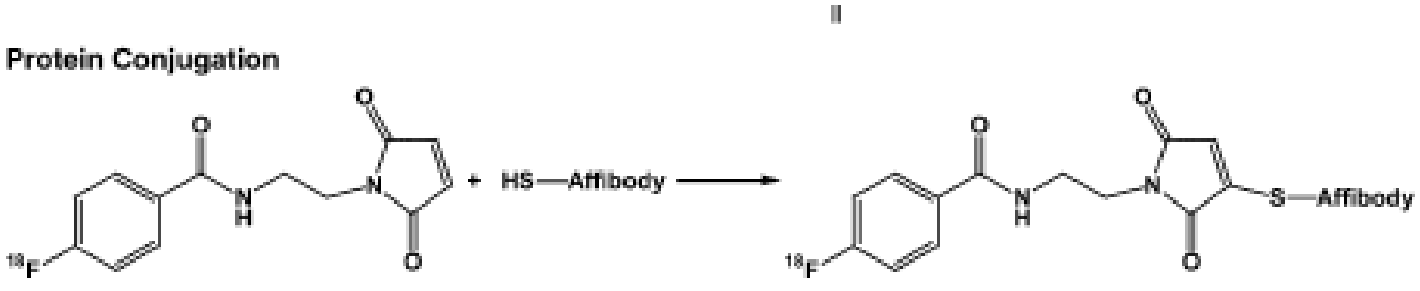


[^{18}F]FBEM

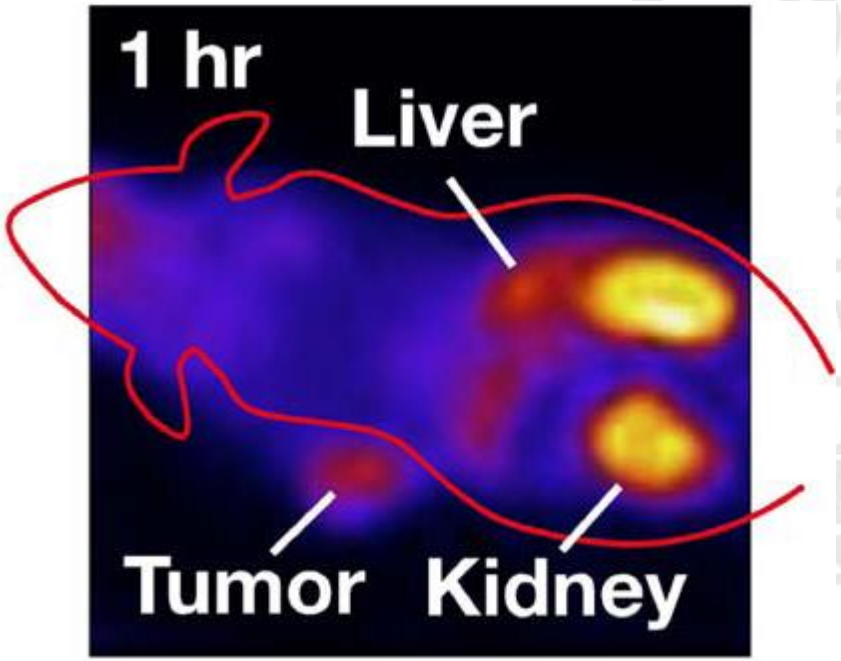
3 steps, 160 min, 5% [183]

3 steps, 75 min, 22% [184]

Labeling with ^{18}F



[^{18}F]FBEM



Kiesewetter et al. J Fluor Chem. 2008

Labeling with ^{18}F

Thiol-specific chemistry

\+ Site-specific labeling
Mild coupling

\− Low precursor yields
Lipophilic nature

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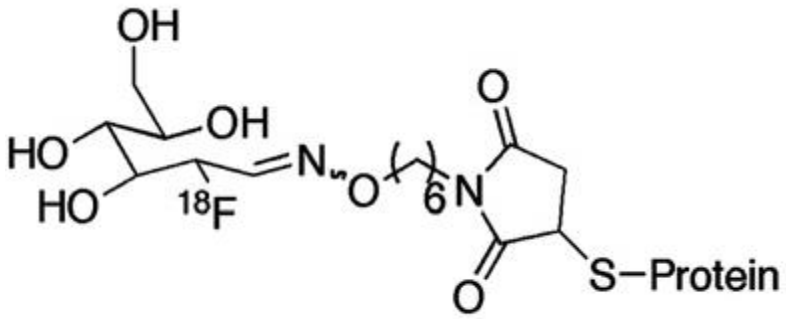
Labeling with ^{18}F

Thiol-specific chemistry



- FDG as precursor
  More site-specific labeling
  Mild coupling
  Hydrophilic group

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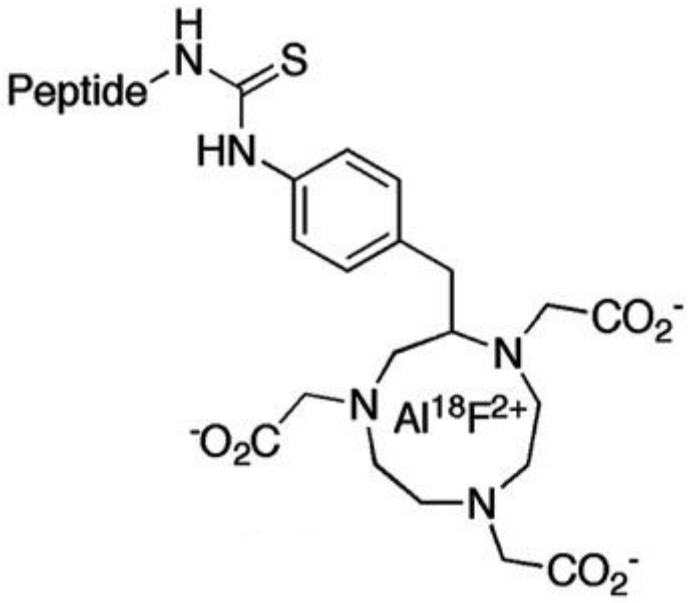
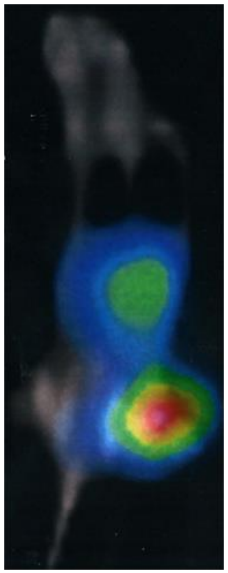
Labeling with ^{18}F

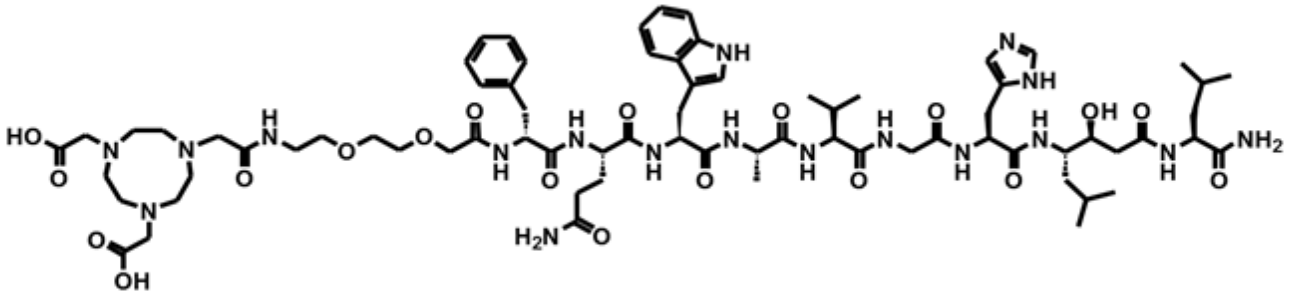


- 1-pot process
- Excellent stability
- Hydrophilic modification
- ”Metal” chemistry

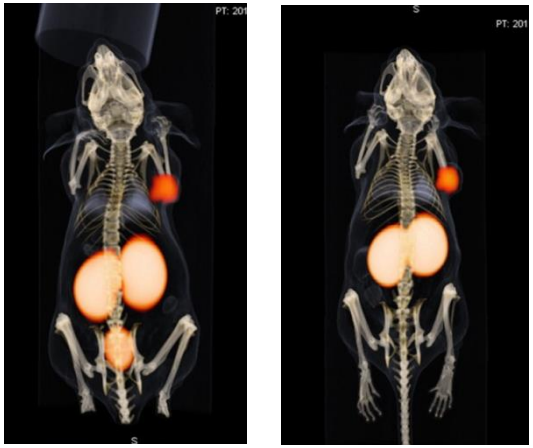
Labeling with ^{18}F



^{18}F-NOTA-BBN

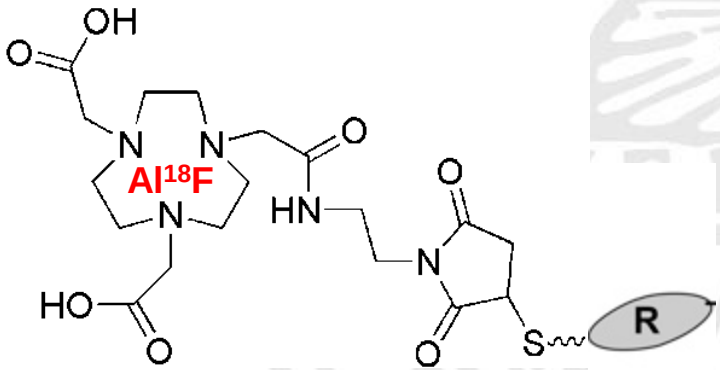


Varasteh et al. PLOS One 2013



Boerman, Tolmachev et al. J Nucl Med 2012

^{18}F-NOTA-Z_{HER2}



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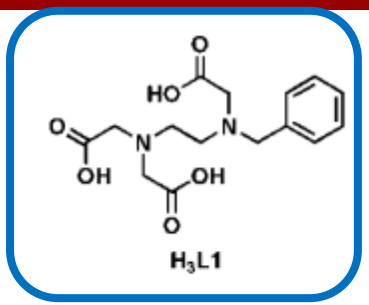
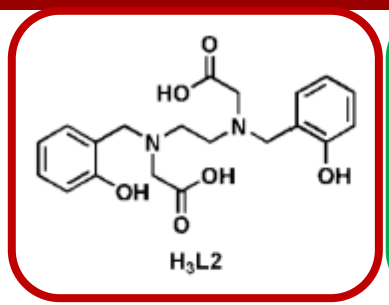
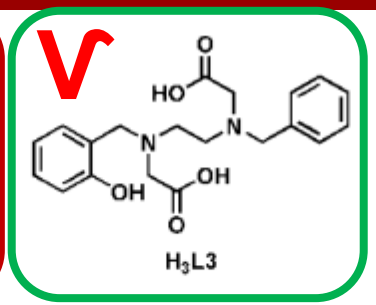
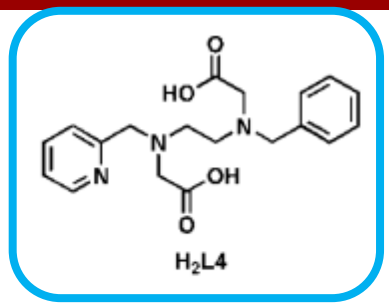
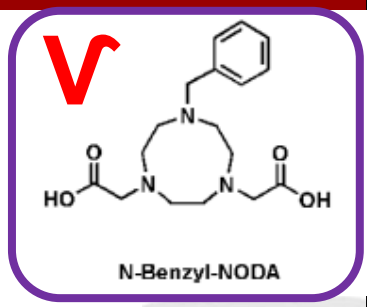
Labeling with ^{18}F

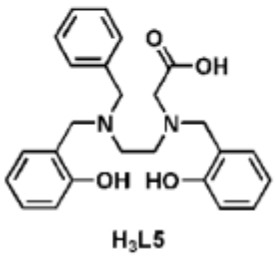


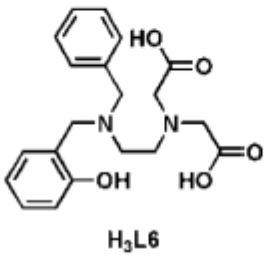


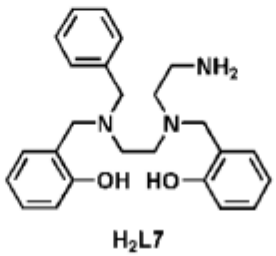


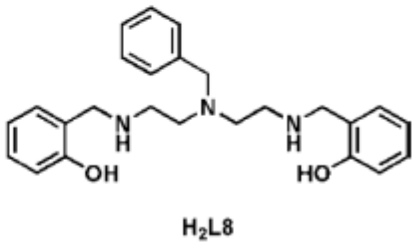


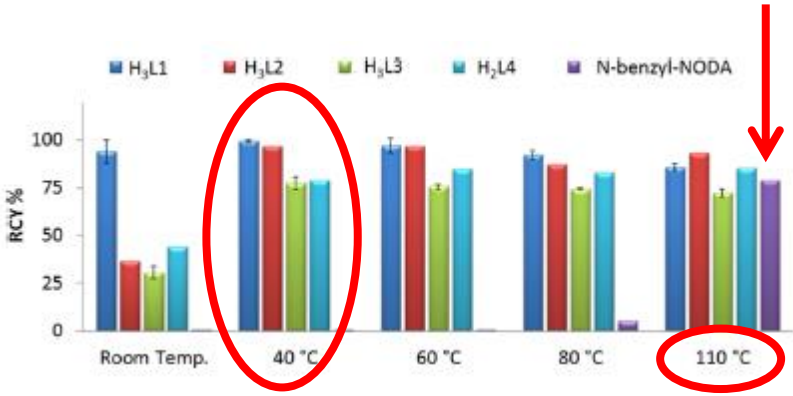


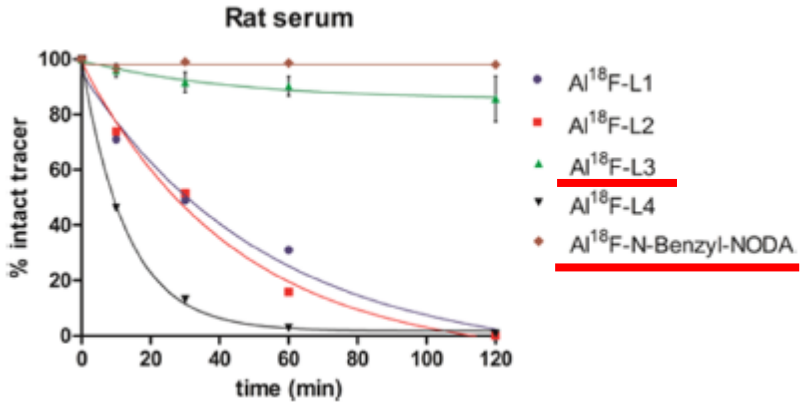






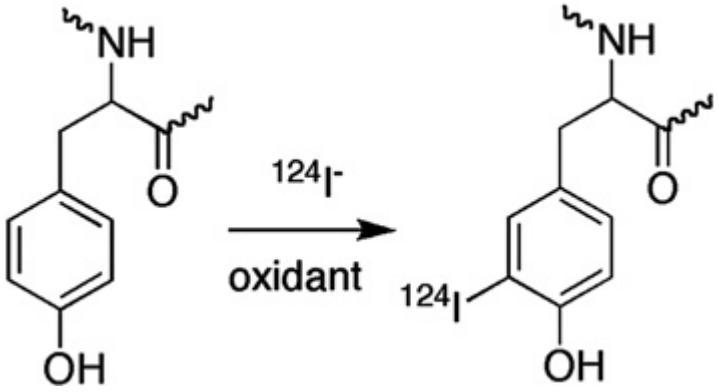






Cleeren et al. Bioconjug Chem, 2016

Radiohalogenation (^{124}I)



Direct radiohalogenation

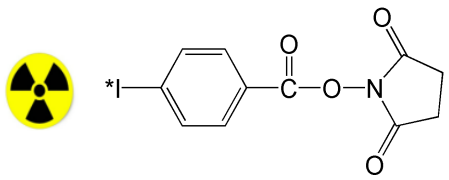
- simple and robust method
- forms stable covalent bond with protein
- good for proteins which are not internalised after binding to target
- high specific radioactivity can be obtained

- structure and properties can be damaged by ox-red processes;
- after internalization catabolites leak from target cells;
- tyrosines are overrepresented in binding sites of antibodies;
- difficult for bromine.

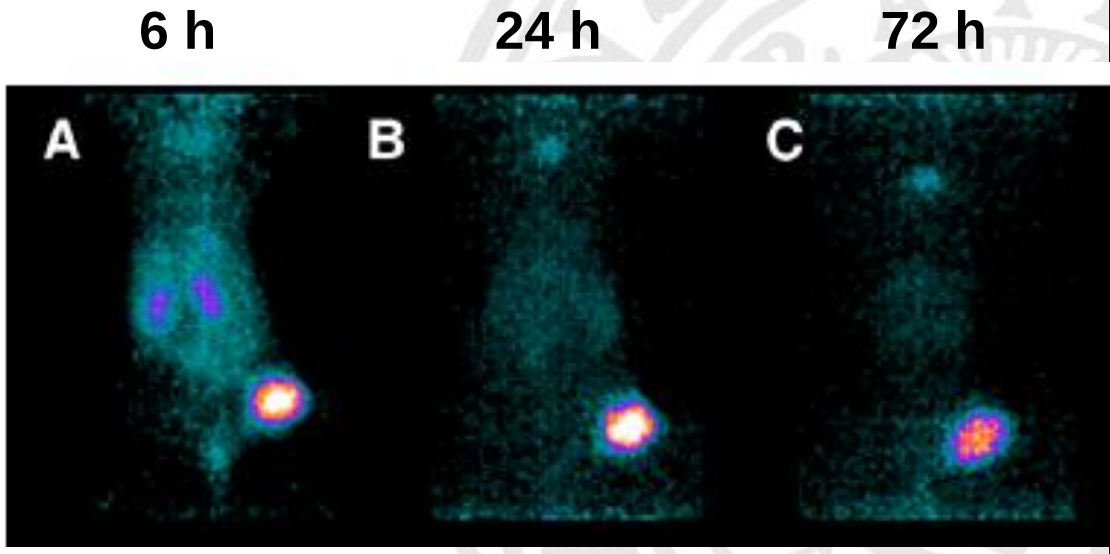
Radiohalogenation (^{124}I)

Indirect radiohalogenation

- Mild labelling conditions;
- Rapid excretion of radiocatabolites;
- Poor cellular retention of radiocatabolites.
- Yield and specific radioactivity are lower in comparison with direct labeling;

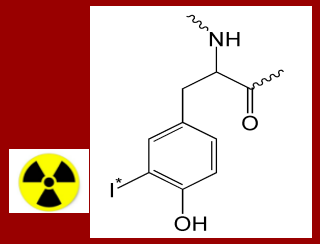


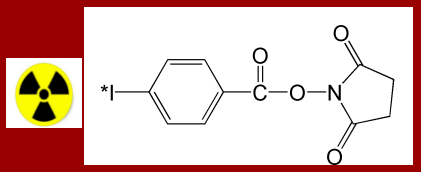
^{124}I-PIB-Z_{HER2}

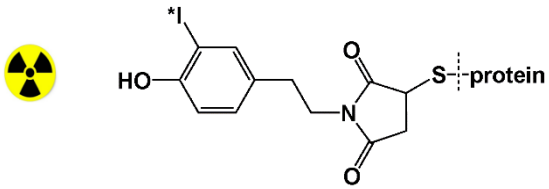


Orlova et al, JNM, 2009

Radioiodination of DARPin G3

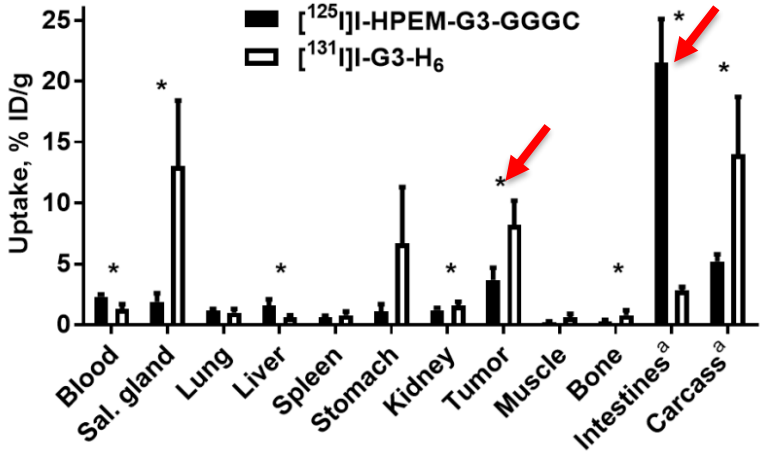






Indirect HPEM label

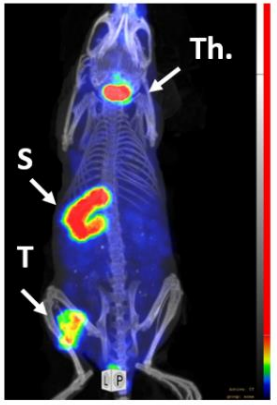
Site-specific labeling of G3-GGGC resulted in high hepatobiliary excretion

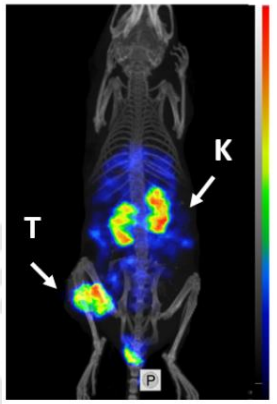


Vorobyeva et al. Int J Onc. 2019; 54(4), 1209-1220.

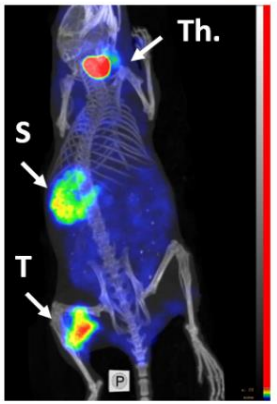
Direct label **Indirect PIB label**

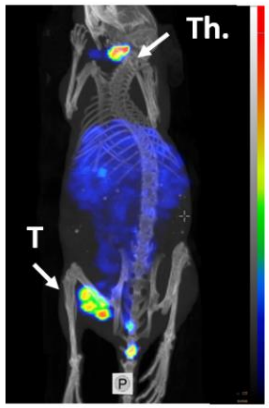
3 h





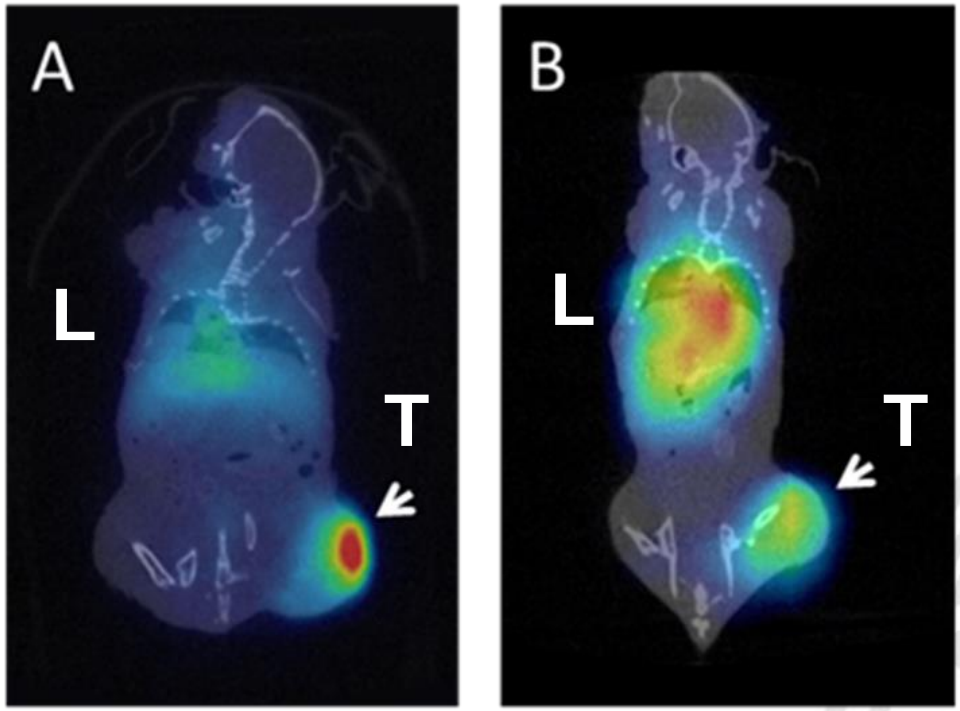
6 h





Vorobyeva *et al. Int J Mol Sci.* 2019, 20, 3047

Retention in excretory organs



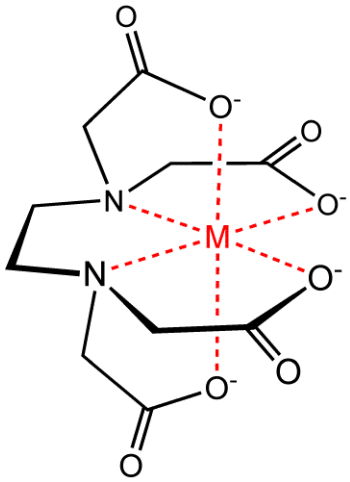
^{124}I: non-residualizing **^{111}In: residualizing**

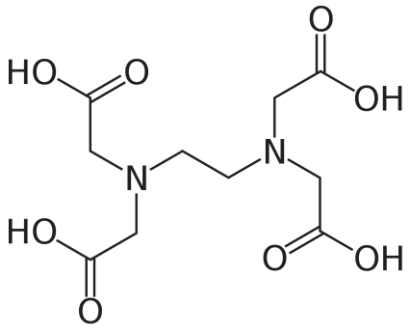
Internalization in excretory organs is much faster than in tumors

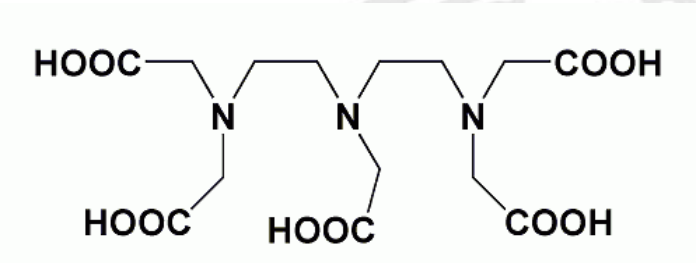
Tolmachev et al. Int J Oncol. 2014

Metal chelation

formation or presence of coordinate bonds between a polydentate (multiple bonded) ligand and a single central atom. These ligands are called chelants, chelators, chelating agents







EDTA

EthyleneDiamineTetraAcetic acid

Chelation therapy for heavy metal poisoning

Food industry, pharmacy, cosmetics etc.

DTPA

DiethyleneTriaminePentaAcetic acid

Chelation of radiometals

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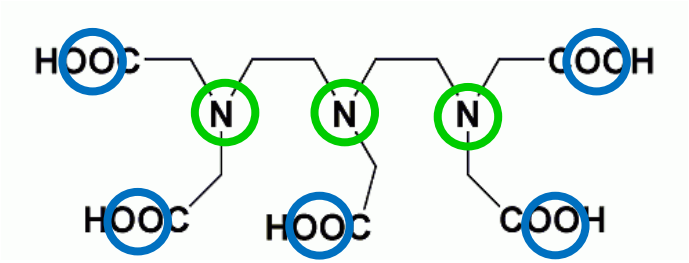
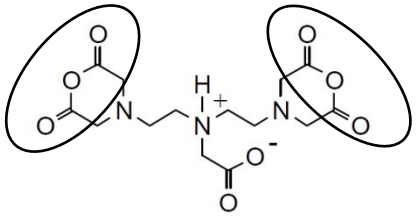
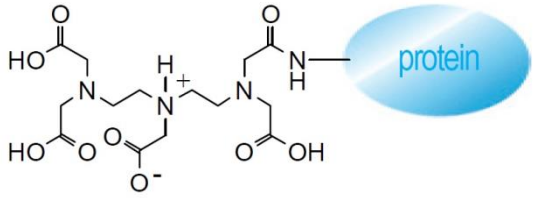
Acyclic chelators



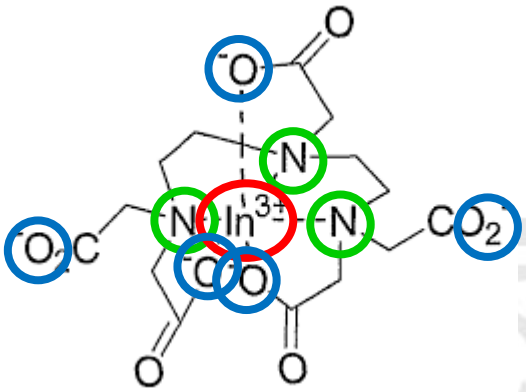
DTPA

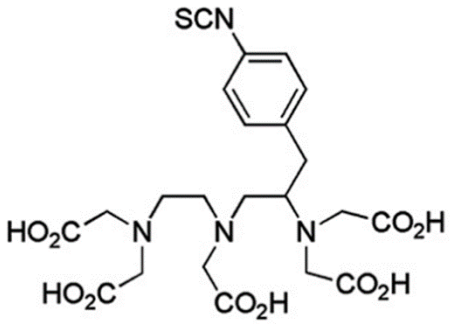


DTPA anhydride

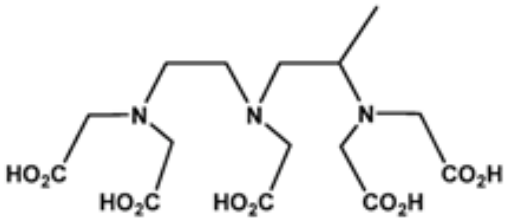


DTPA-conjugated protein

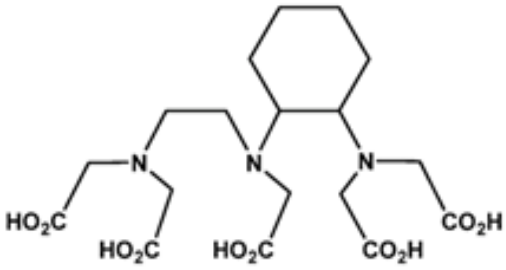




para-SCN-Bn-DTPA



MX-DTPA



CHX-DTPA

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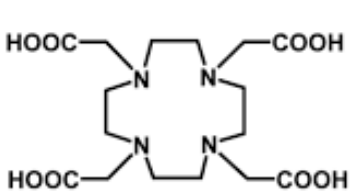
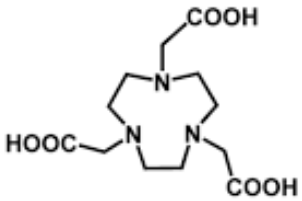
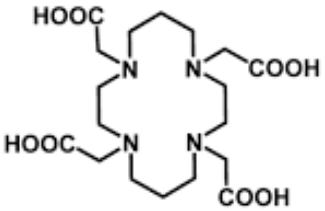
Cyclic chelators



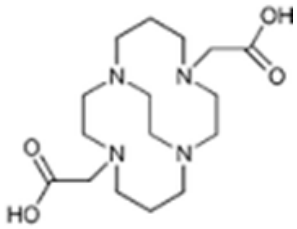
DOTA



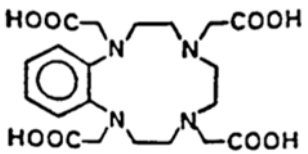
NOTA



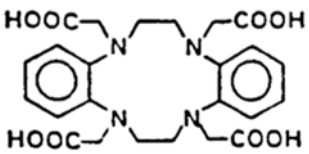
TETA



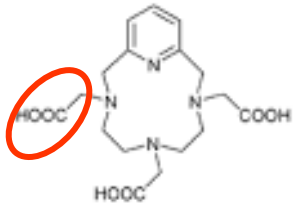
CB-TE2A



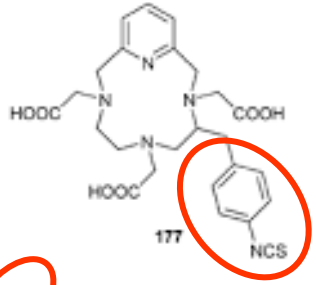
benzo-DOTA



dibenzo-DOTA



PCTA



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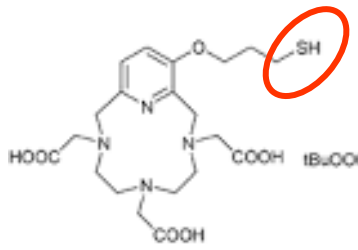

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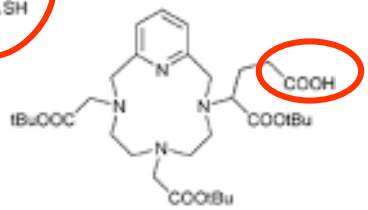

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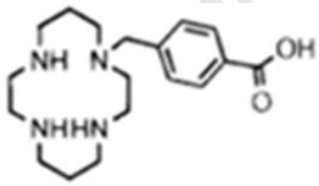
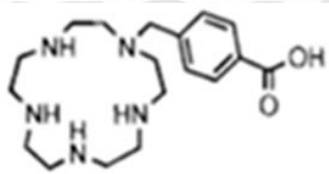


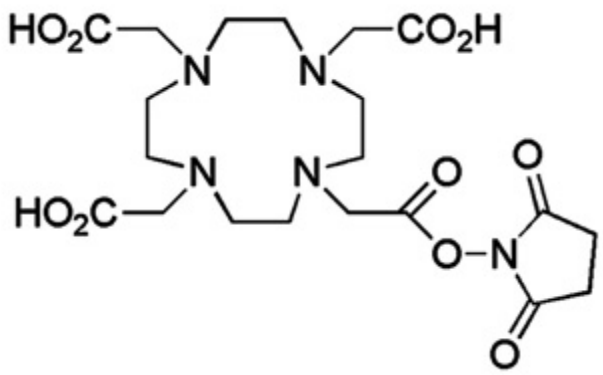
CPTA



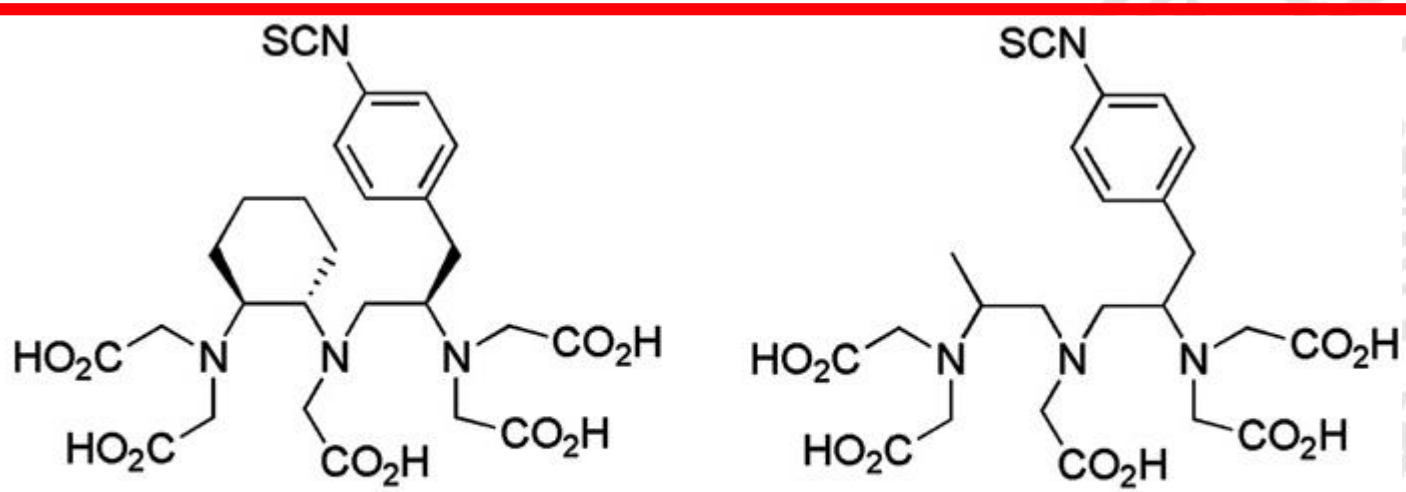
PCBA

Metal chelation

^{86}Y



- Stable comlexes
- Elevated temperatures and long incubation

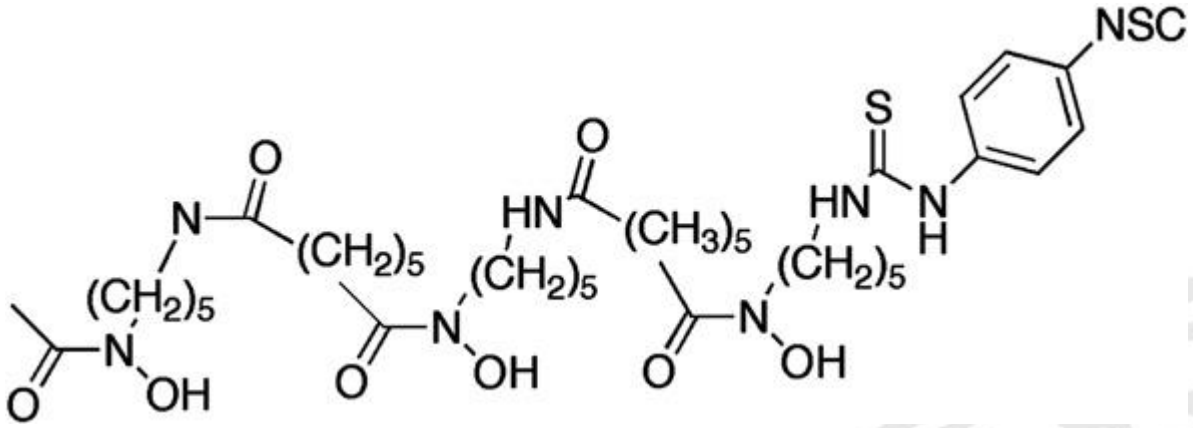


CHX-A"-DTPA

1B4M-DTPA

Metal chelation

^{89}Zr



Benzylisothiocyanate-derivative of desferrioxoamine (DFO)

Multi-step process
Mild conditions

Not very stable in blood circulation
Not optimal radionuclide
Popular for labeling of mAbs (long half-life)

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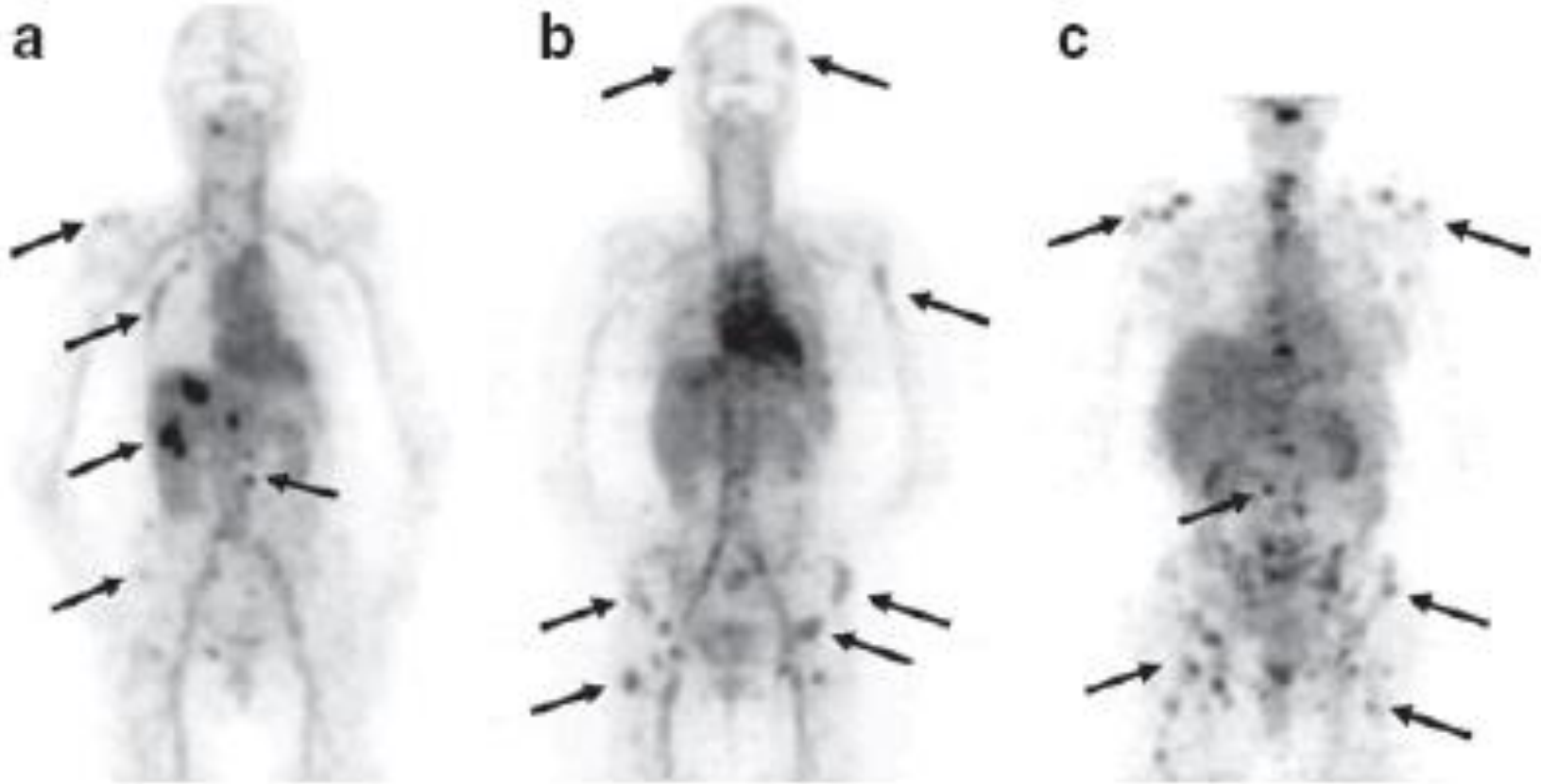
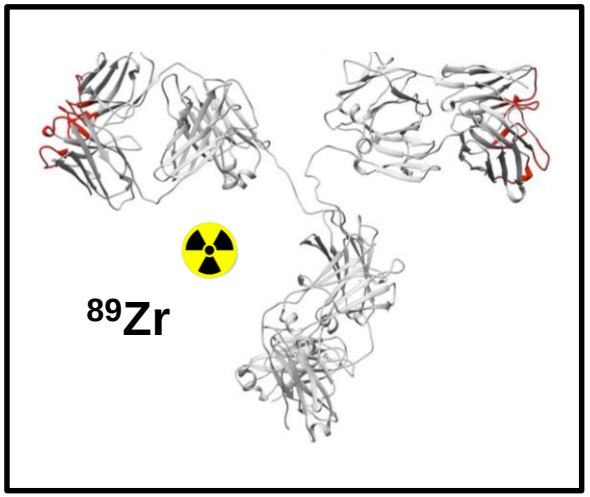
Clinical study: uptake of ^{89}Zr-trastuzumab 5 d pi

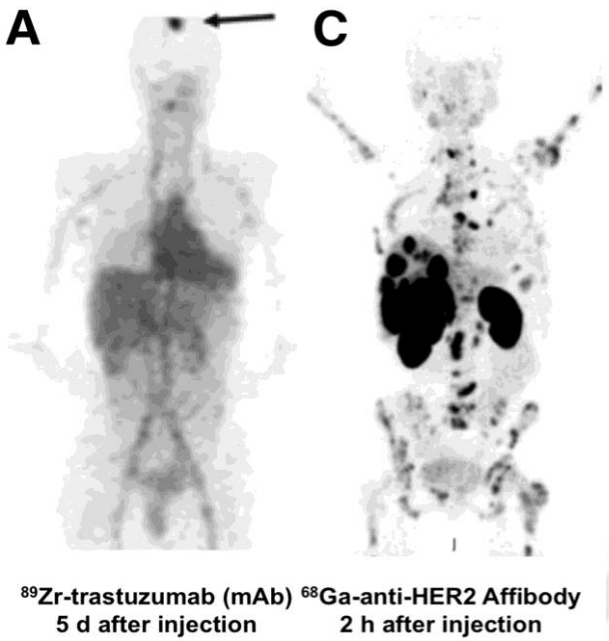


Dijrers et al, Clin Pharmacol Ther. 2010

Affibodies: small targeting probes



Antibody
150 kDa



89Zr-trastuzumab (mAb) 5 d after injection
68Ga-anti-HER2 Affibody 2 h after injection

Affibody
6 kDa

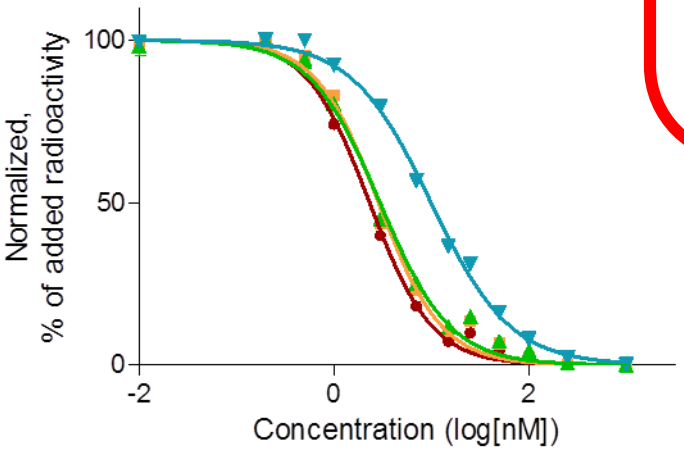
- Large size
- Long half-life in blood
- Imaging days after inj.
- EPR effect (false positive)
- Sensitive (T , pH)

- Small size
- Rapid pharmacokinetics
- Imaging hours after inj.
- High affinity to targets
- Robustness

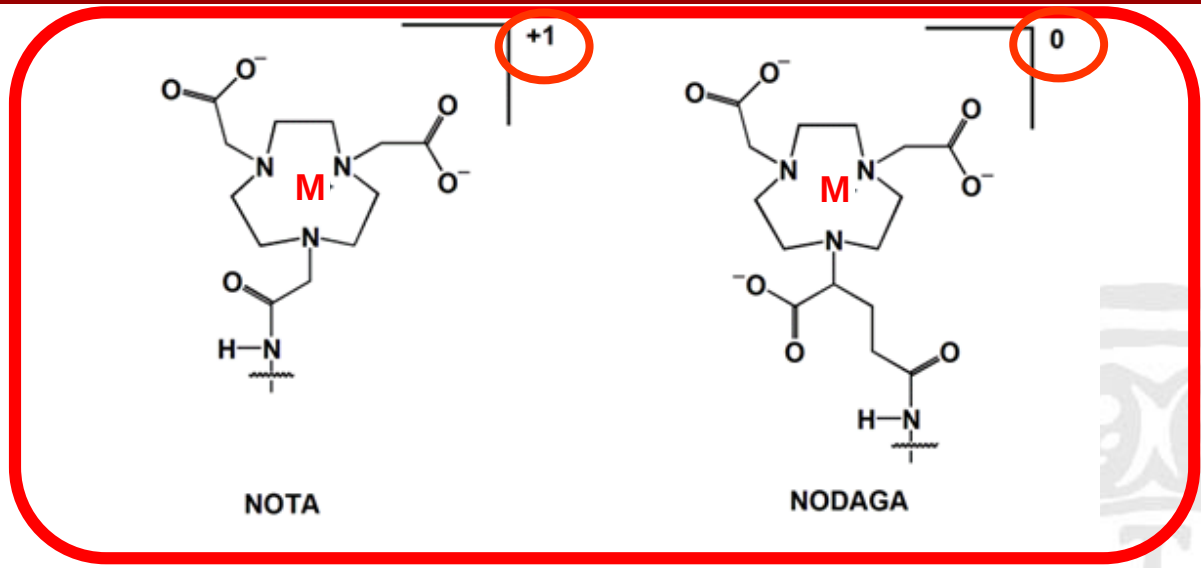
Krasniqi *et al*. *J Nucl Med* 2018;59:885-891

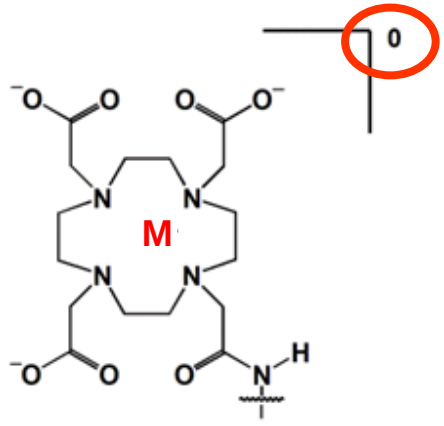
Metal chelation

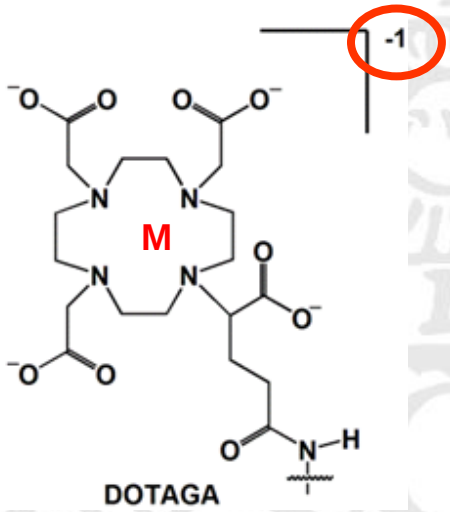
$^{68}Ga^{3+}$



Variant	IC_{50} (nM)
^{nat}Ga-NOTA-PEG_2-RM26	2.3±0.2
^{nat}Ga-NODAGA-PEG_2-RM26	3.0±0.3
^{nat}Ga-DOTA-PEG_2-RM26	2.9±0.3
^{nat}Ga-DOTAGA-PEG_2-RM26	10.0±0.6







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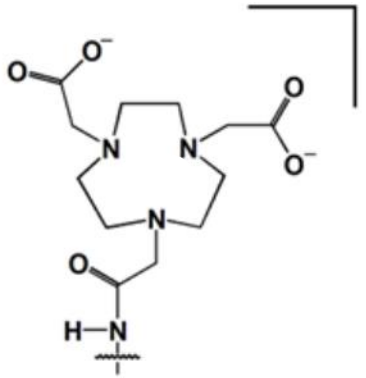
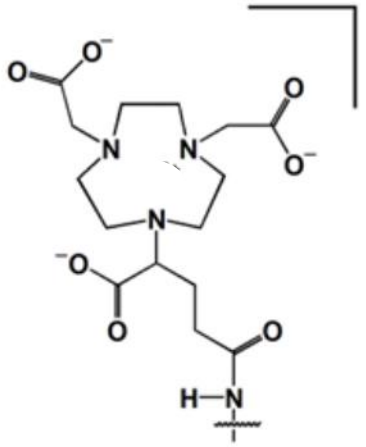
Metal chelation

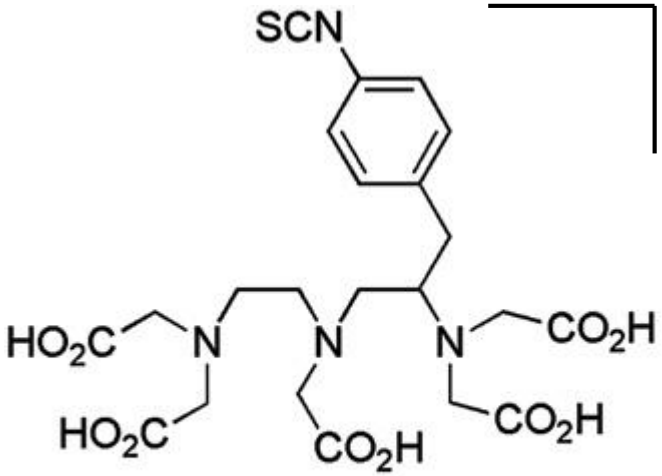
$^{55}Co^{2+}$



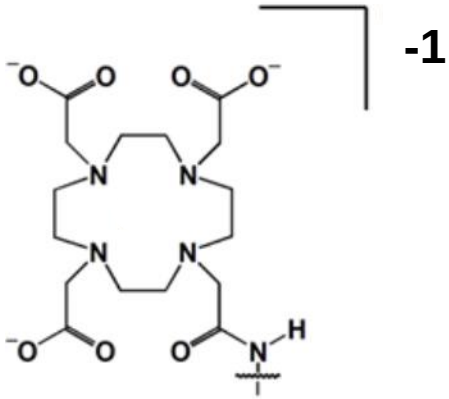
NOTA



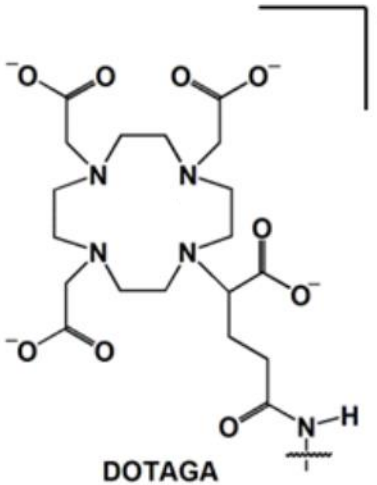
NODAGA



para-SCN-Bn-DTPA



DOTA



DOTAGA

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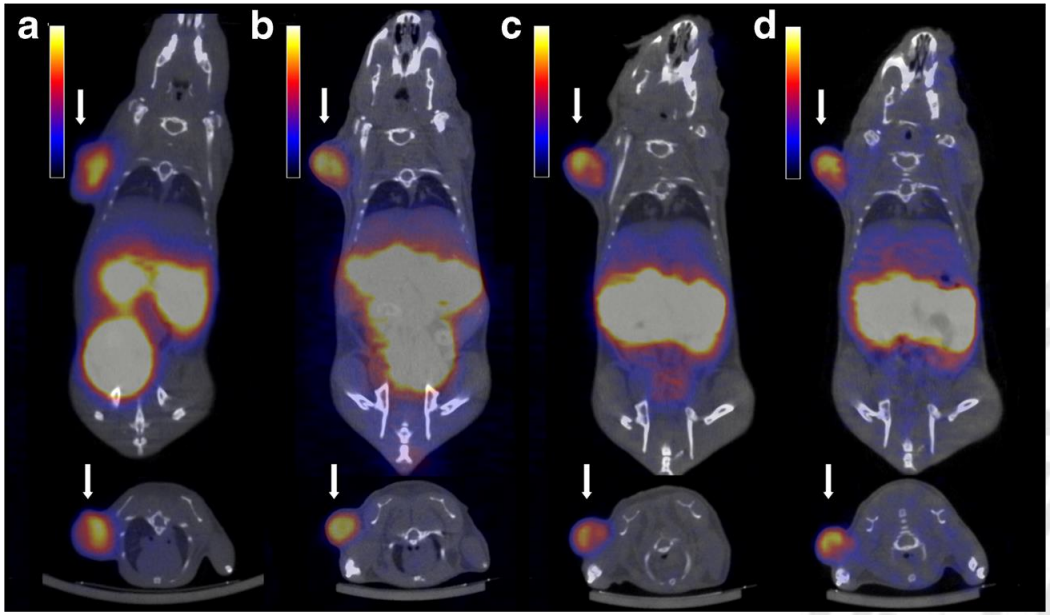
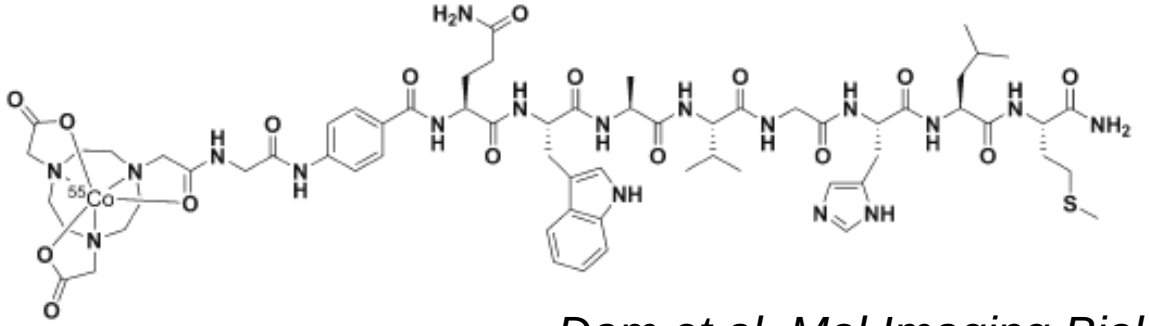
Same peptide, different nuclides

^{68}Ga, 1 h ^{55}Co, 1h 4 h 24 h



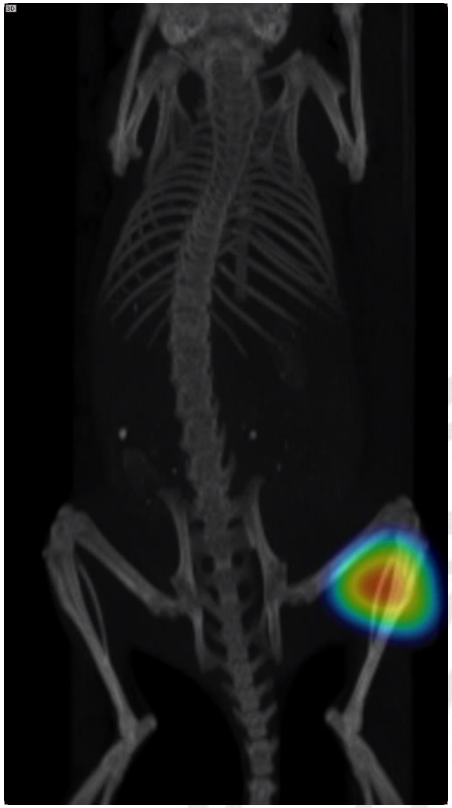
BBN agonist



Dam et al. Mol Imaging Biol, 2015

Same peptide, different nuclides

$^{68}Ga^{3+}$

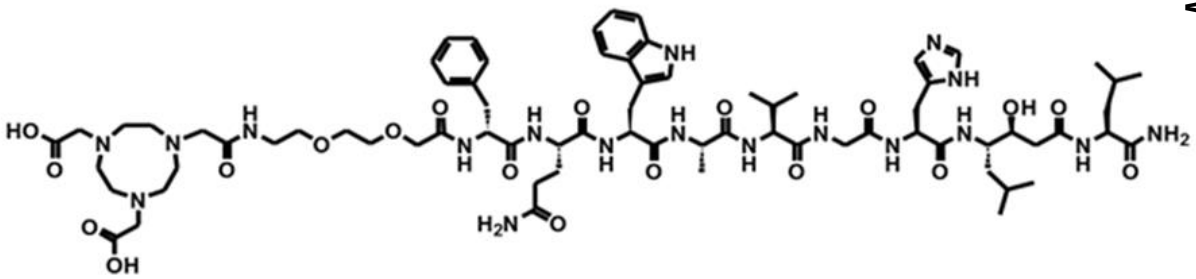


$^{55}Co^{2+}$

Varasteh et al, Bioconjug Chem, 2013

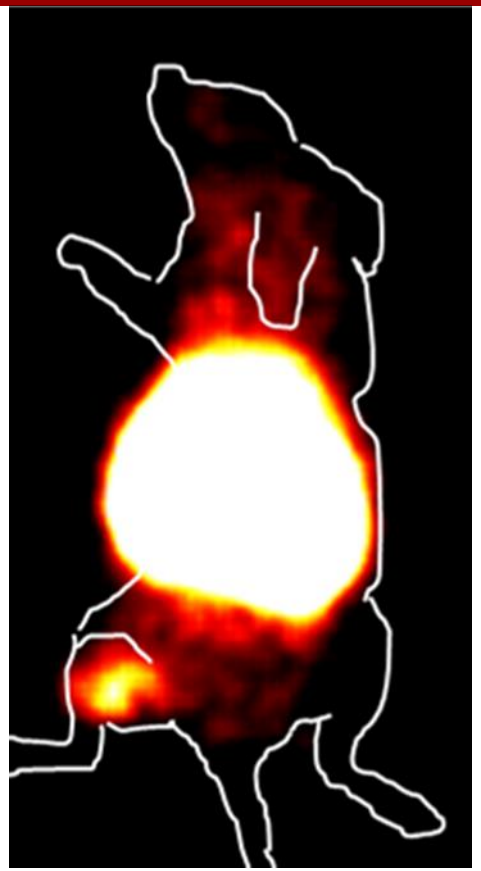
Mitran, unpublished results

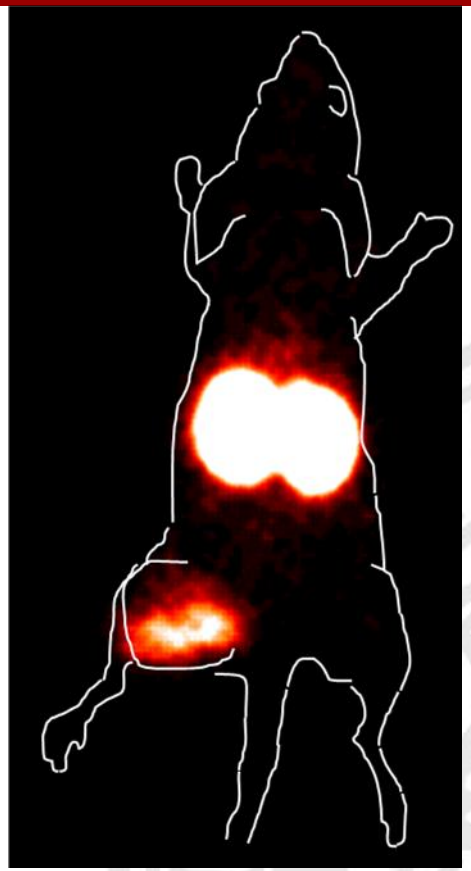
BBN antagonist



Same peptide, different nuclides

$^{111}In^{3+}$





$^{55}Co^{2+}$

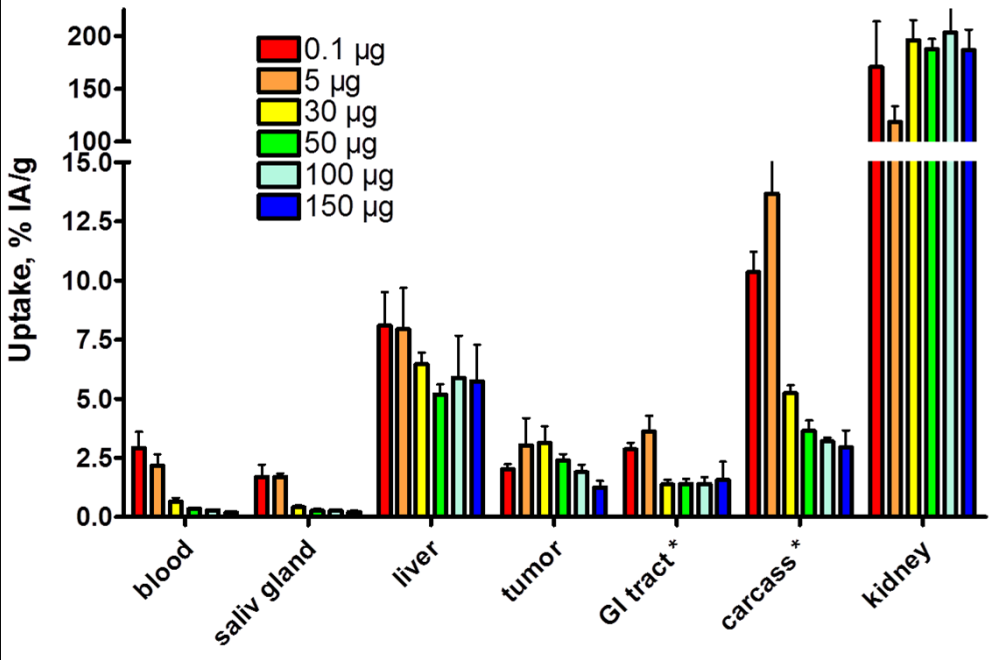
Tolmachev et, Eur J Nucl Med Mol Imaging, 2010

Garousi, unpublished results

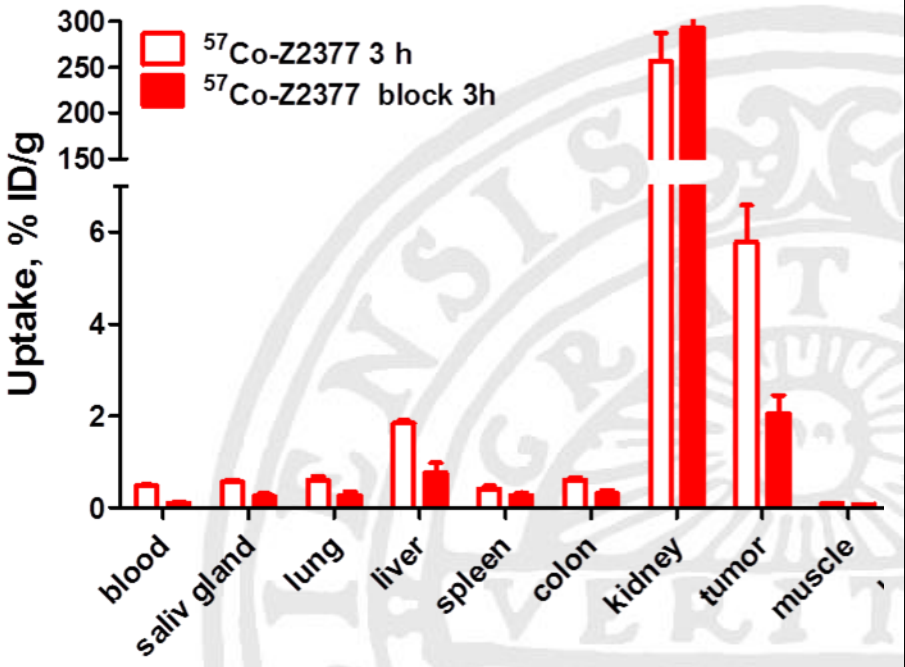
Anti-HER1 affibody

HER1 targeting with affibody

^{111}In-DOTA-ZHER1



^{57}Co-DOTA-ZHER1

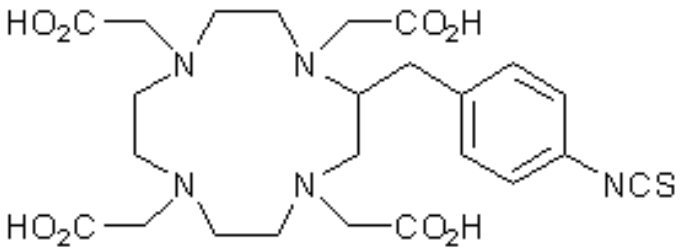


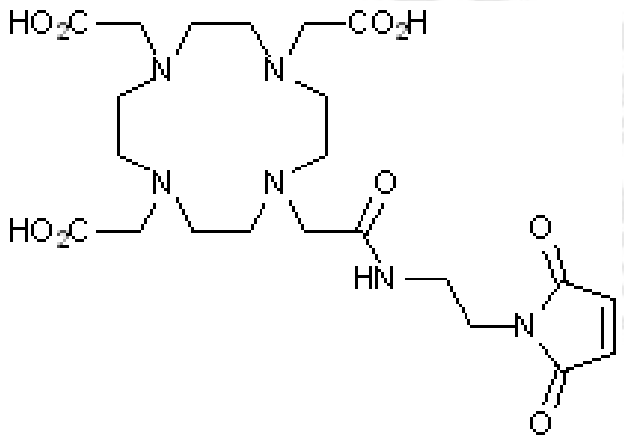
Tolmachev et, Eur J Nucl Med Mol Imaging, 2010
Garousi, unpublished results

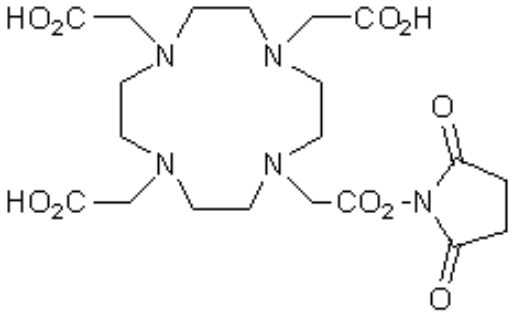
Metal chelation

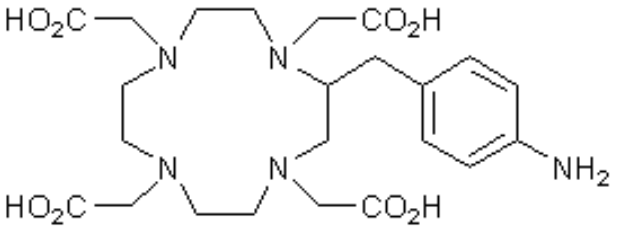
^{44}Sc

T1/2 = 3.97 h
(β+ branching of 94.3%)





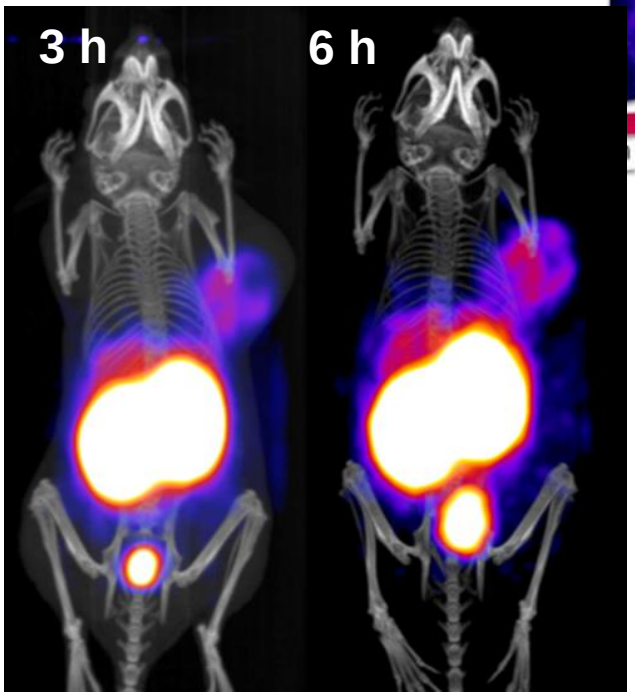




Metal chelation

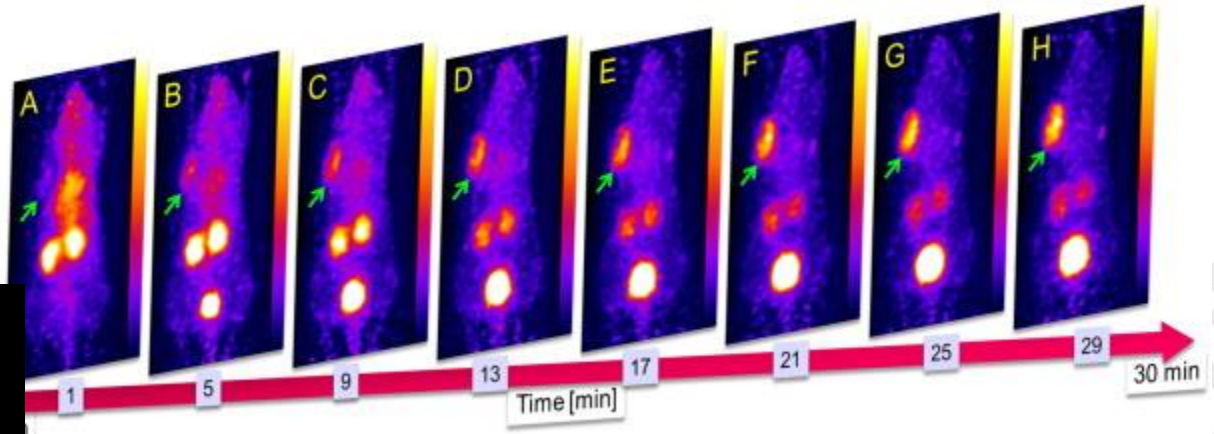
^{44}Sc

T1/2 = 3.97 h



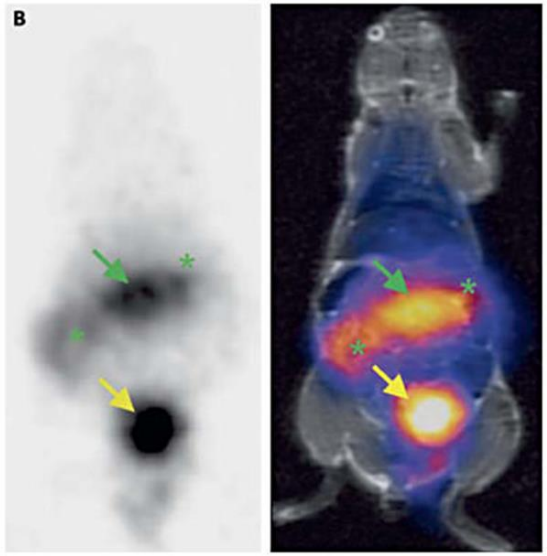
^{44}Sc-Z_{HER2}

Honarvar et al. Nucl Med Biol. 2017



van der Meulen et al. Nucl Med Biol. 2015

^{68}Ga



Stelter et al. Neuroendocrinol. 2008

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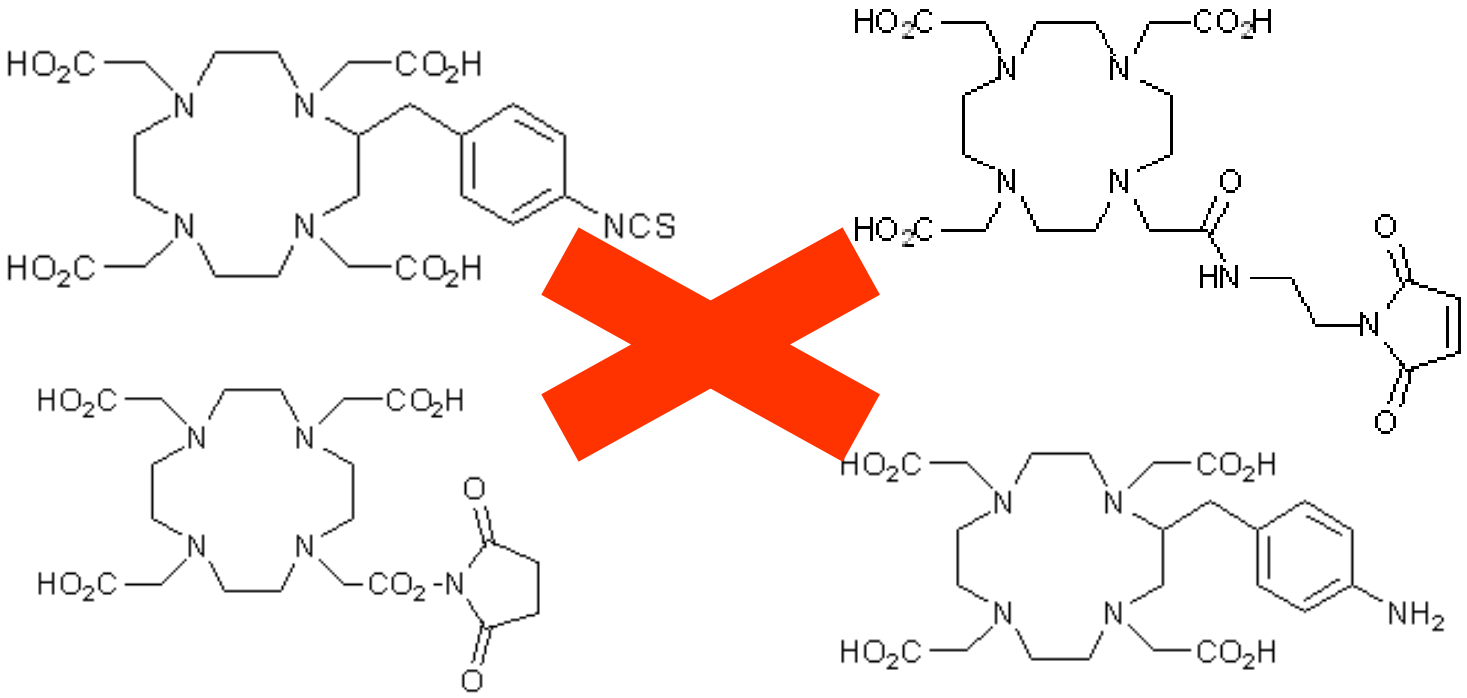
Metal chelation

^{61}Cu/ ^{64}Cu



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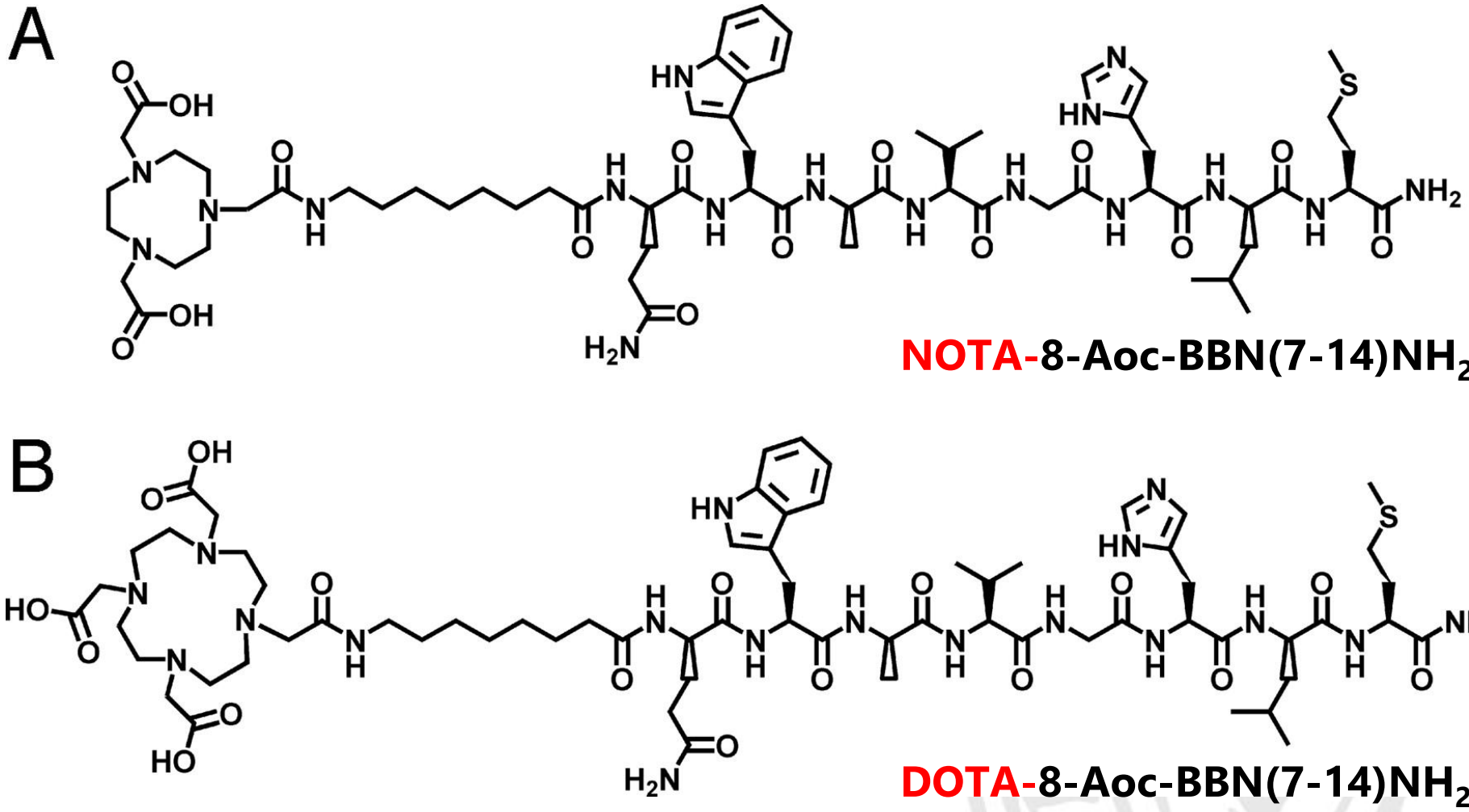
Metal chelation



Prasanphanich et al., PNAS, 2007

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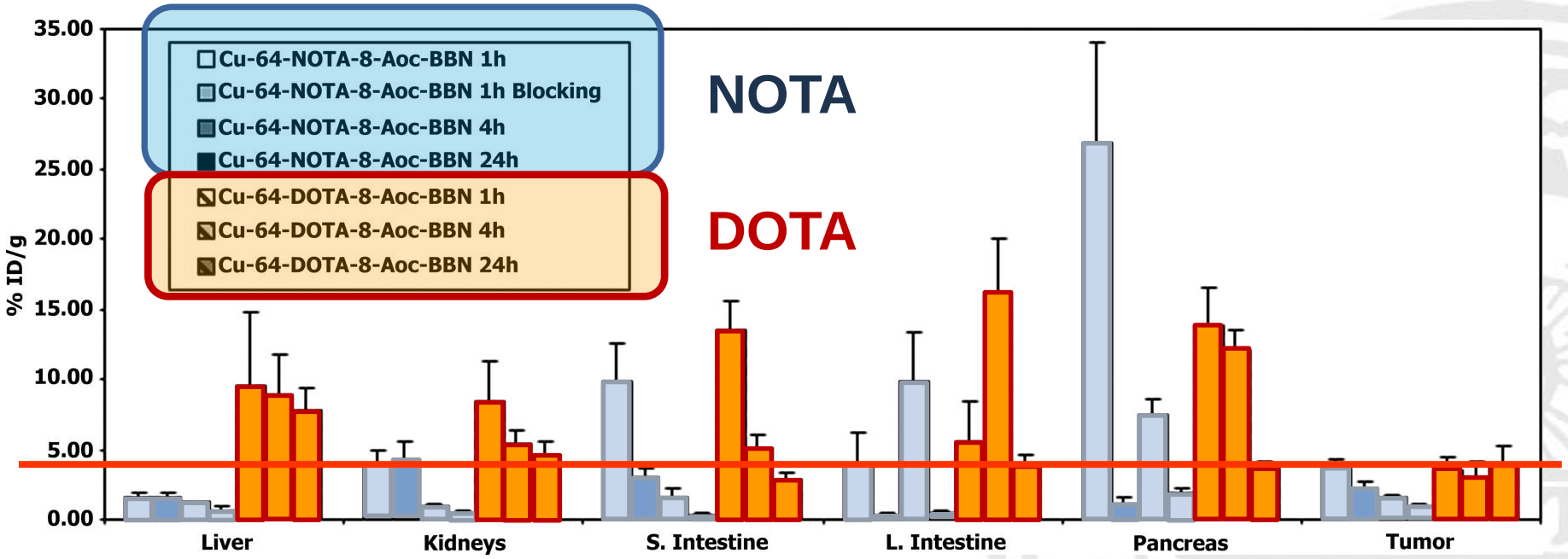
Metal chelation

^{61}Cu/ ^{64}Cu



Prasanphanich et al., PNAS, 2007

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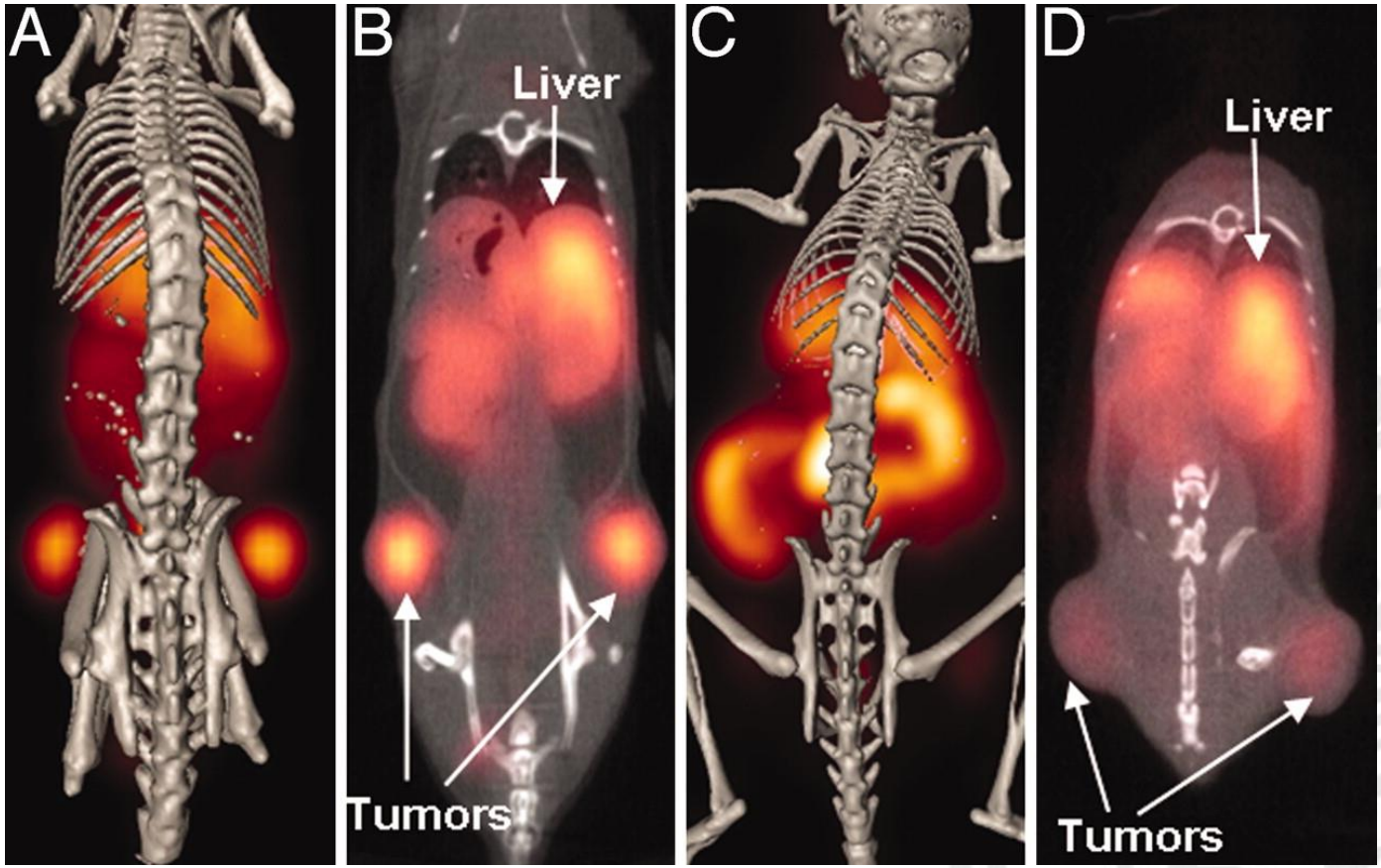
Metal chelation



^{64}Cu-NOTA-8-Aoc-BBN(7-14)NH_2

^{64}Cu-DOTA-8-Aoc-BBN(7-14)NH_2

Prasanphanich et al., PNAS, 2007

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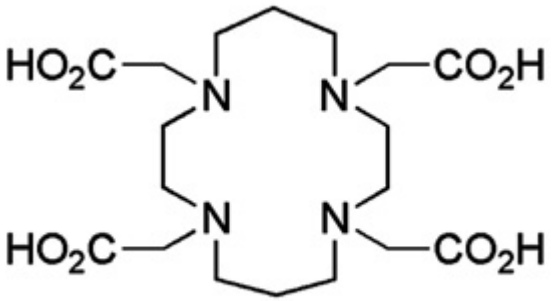
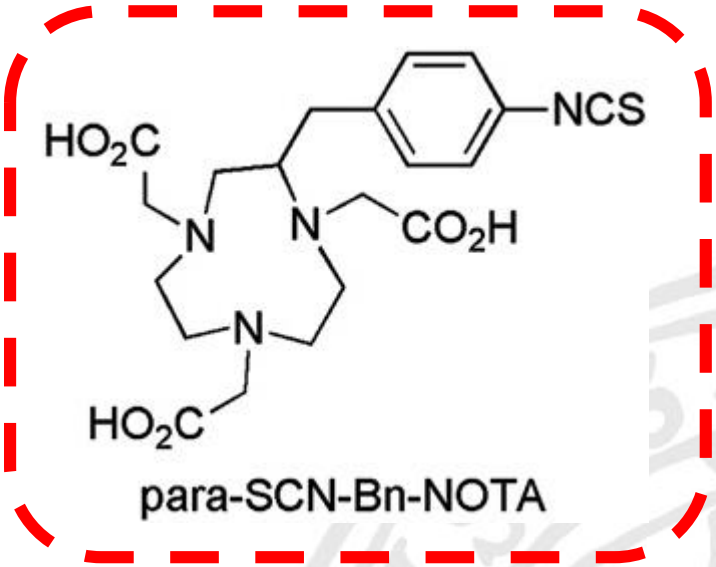
Metal chelation

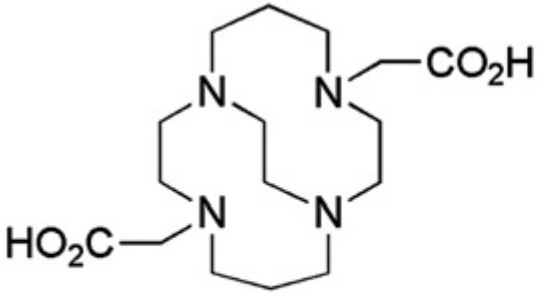
^{61}Cu/ ^{64}Cu



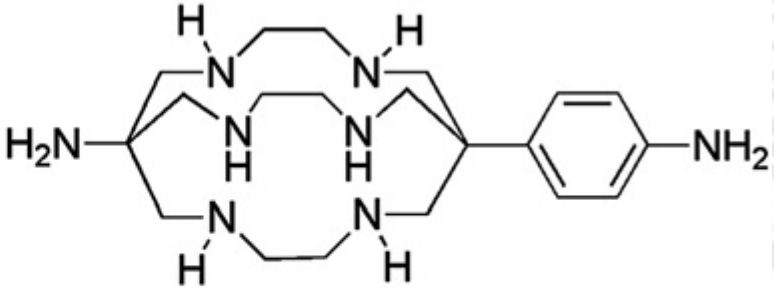
TETA



para-SCN-Bn-NOTA



CB-TE2A



SarAr

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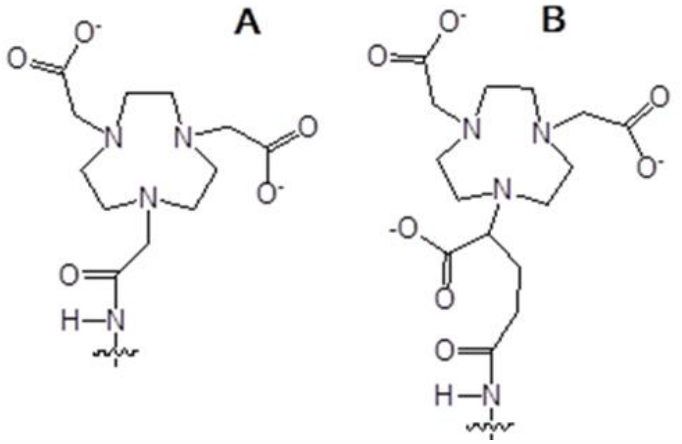
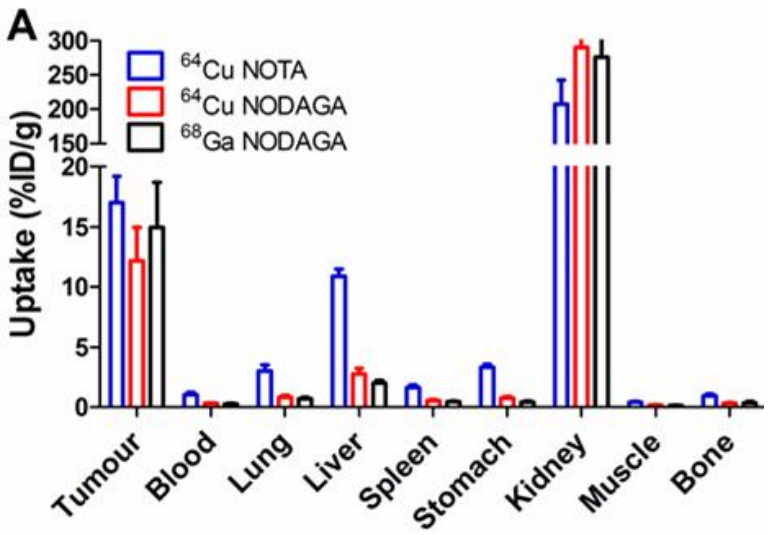
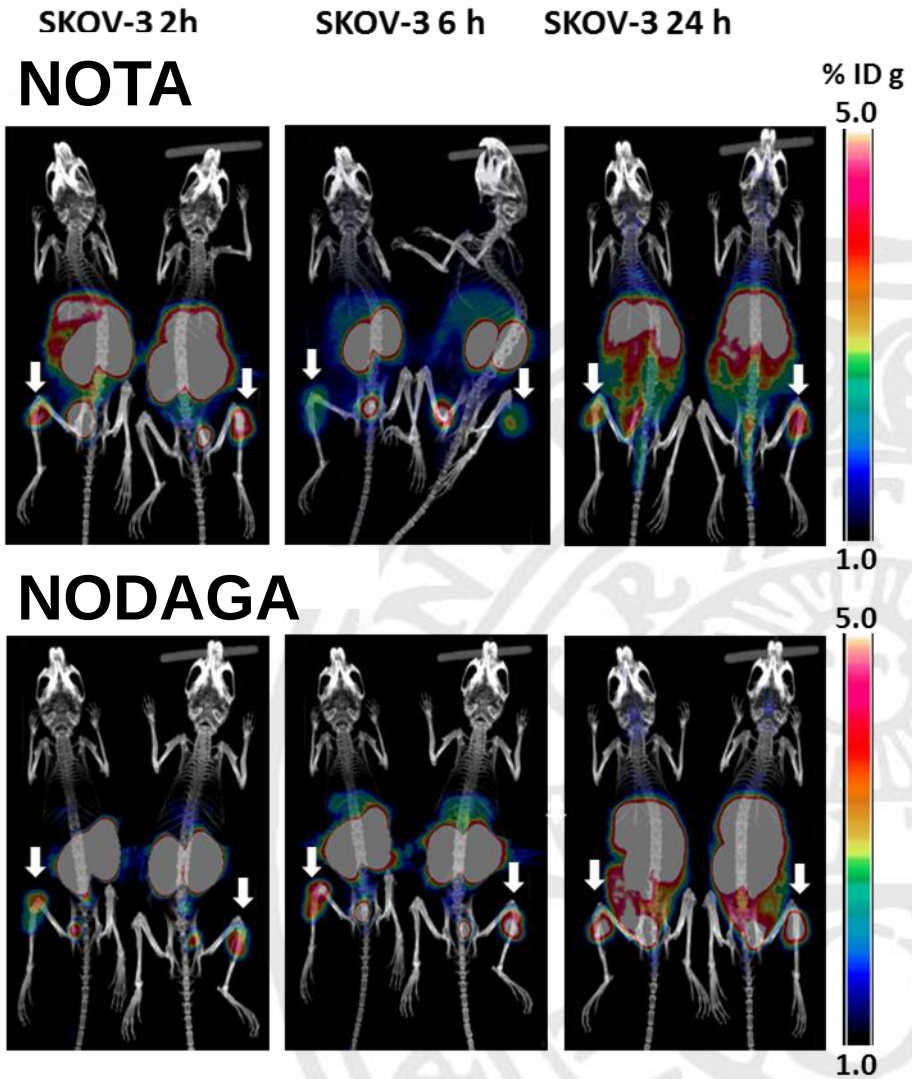
Metal chelation: Cu







Garousi et al. Contr Med Mol Imaging 2017

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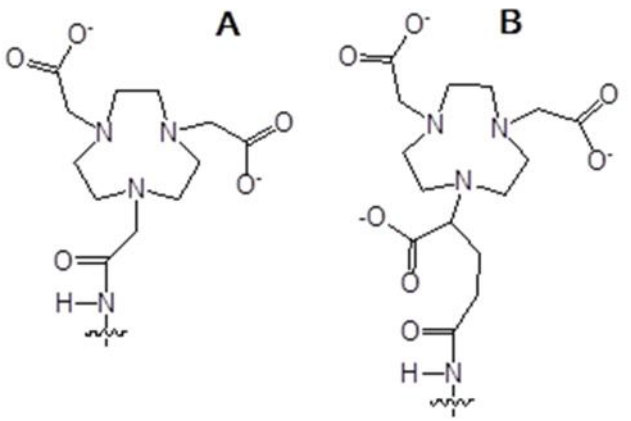
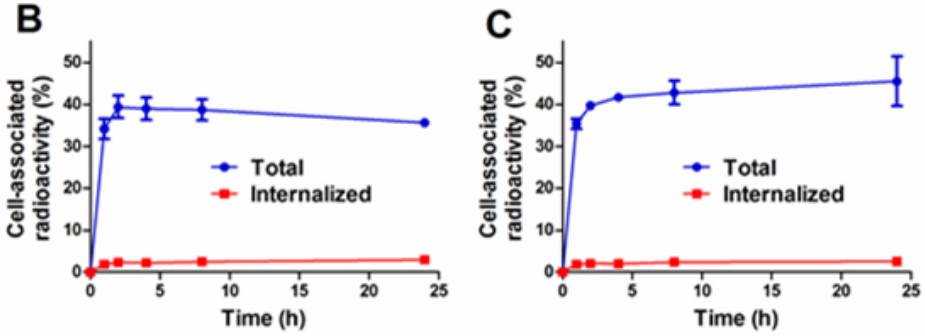
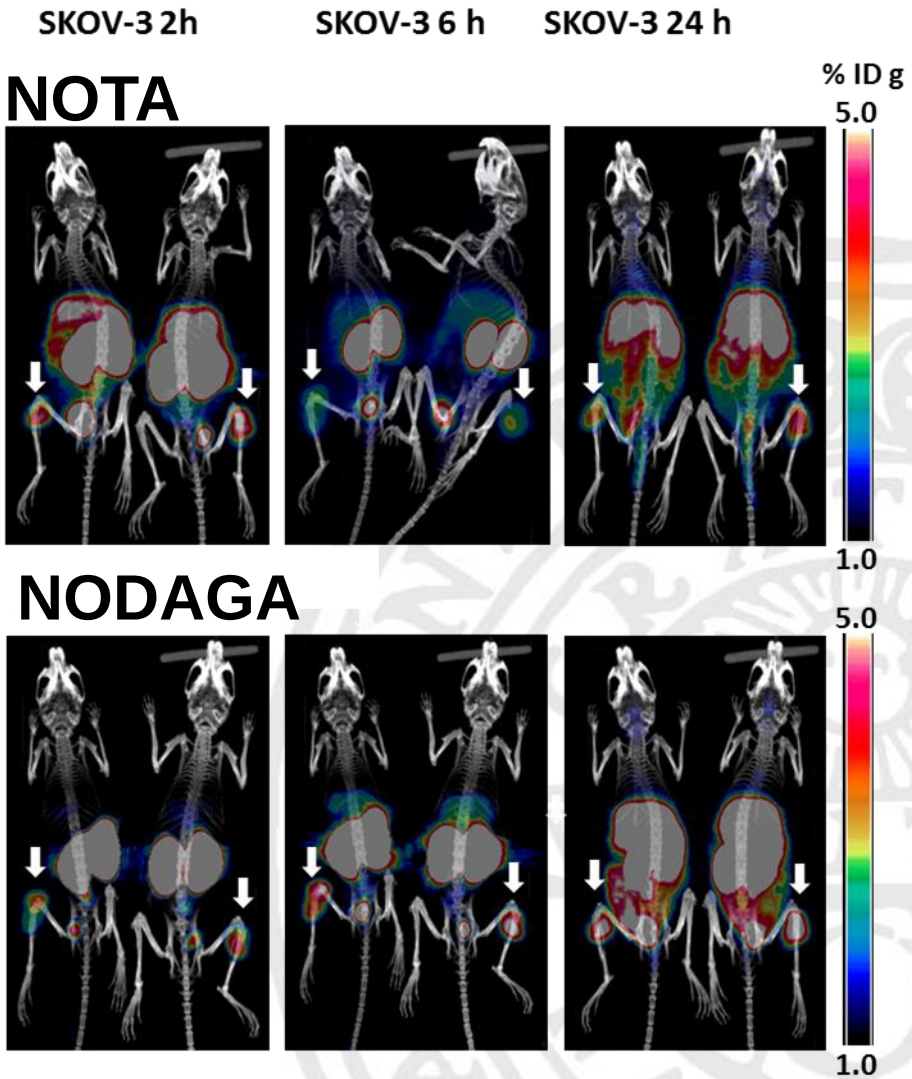
Metal chelation: Cu







Garousi et al. Contr Med Mol Imaging 2017

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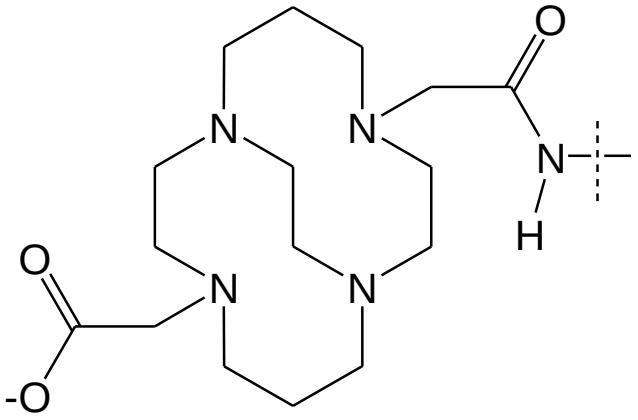
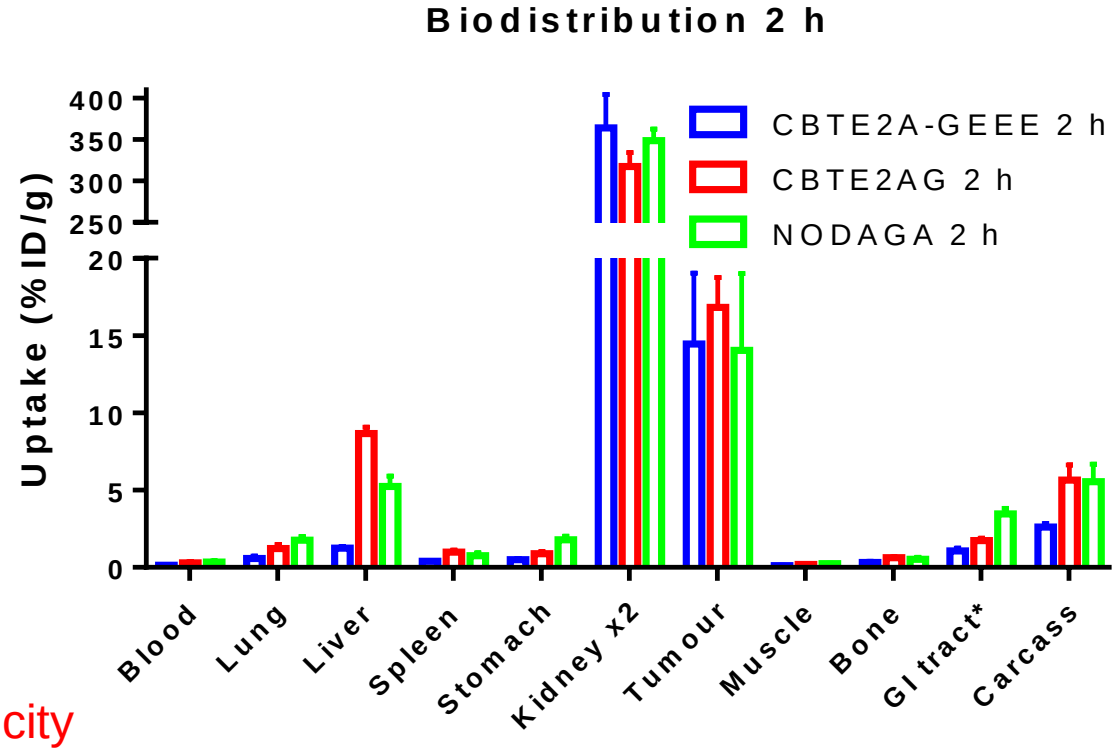
Metal chelation: Cu

Cross-bridged TE-2A chelators





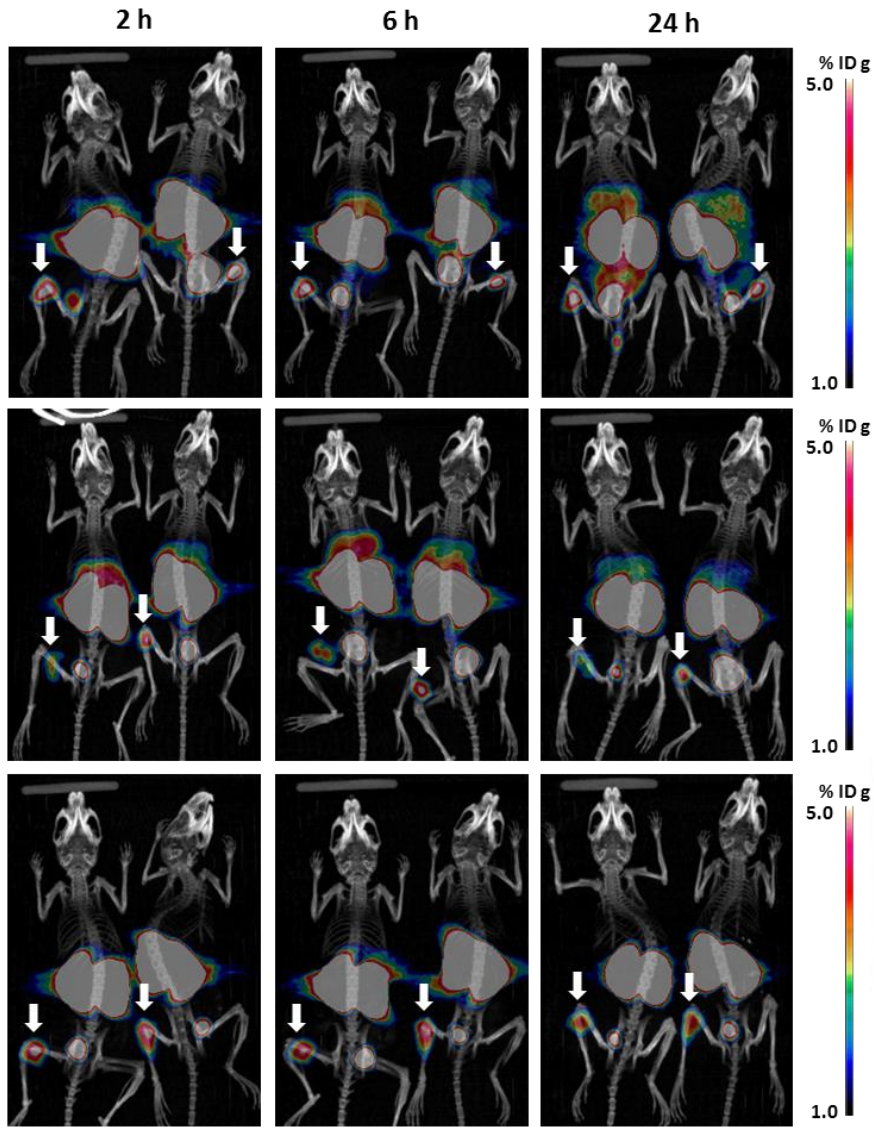
Linkers

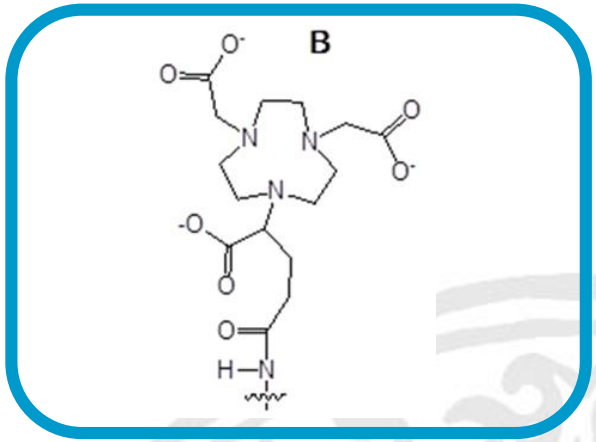
-G-

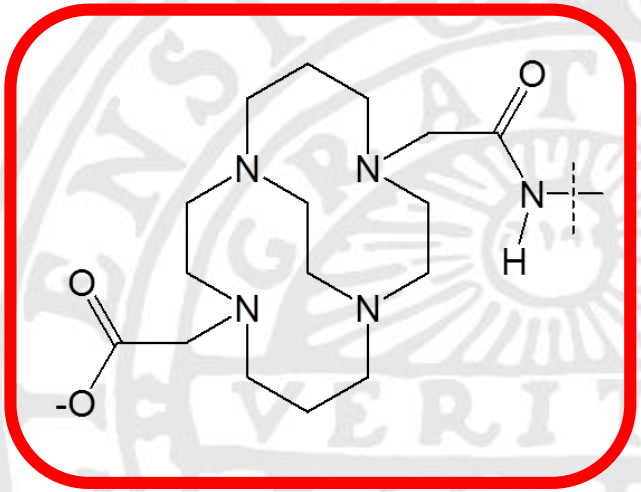
-GEEE- to increase hydrophilicity

Tolmachev Sci Rep 2018

Metal chelation: Cu



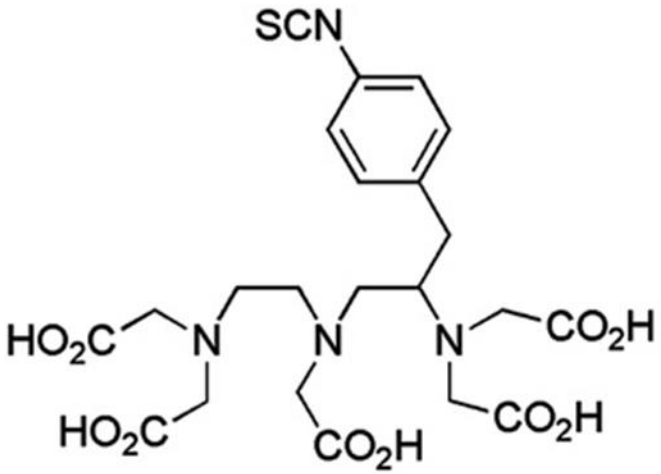




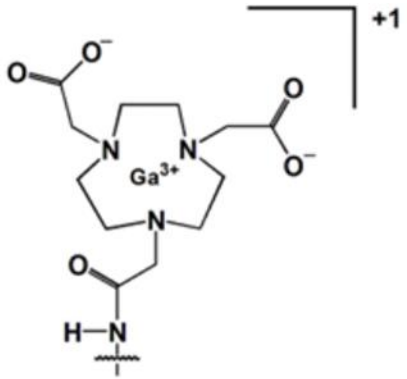
Tolmachev Sci Rep 2018

Metal chelation

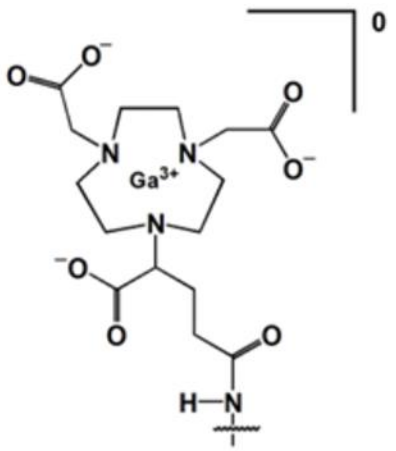
^{111}In



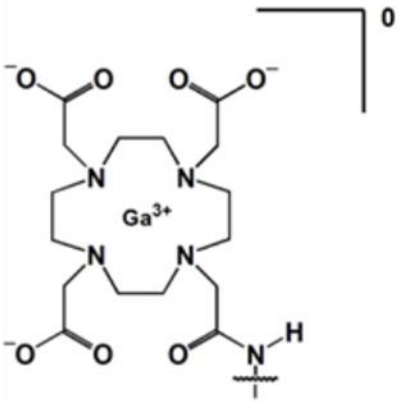
para-SCN-Bn-DTPA



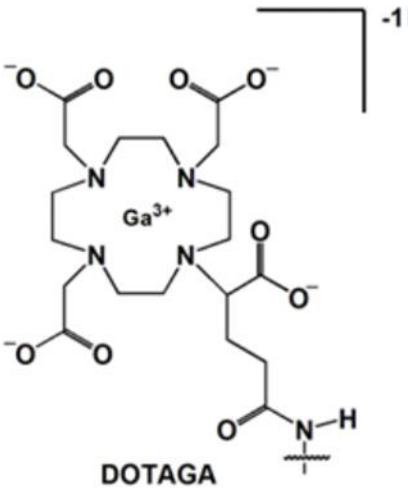
NOTA



NODAGA



DOTA



DOTAGA

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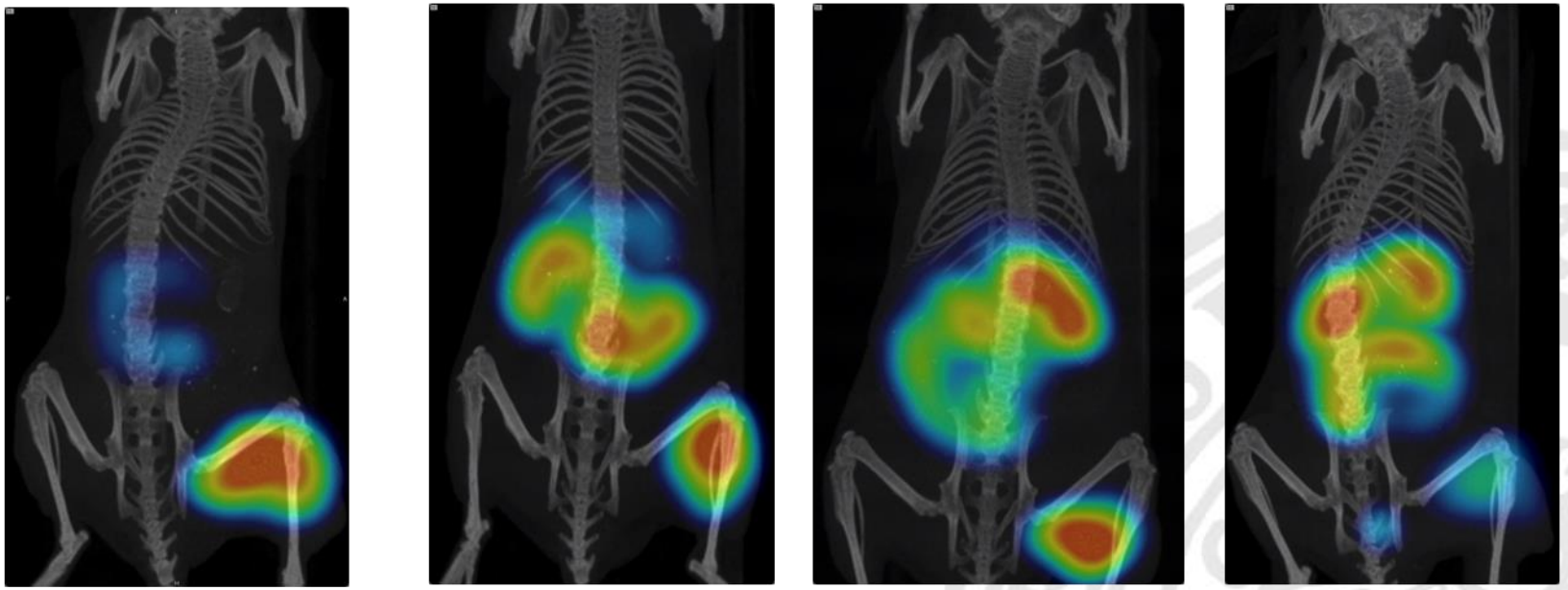
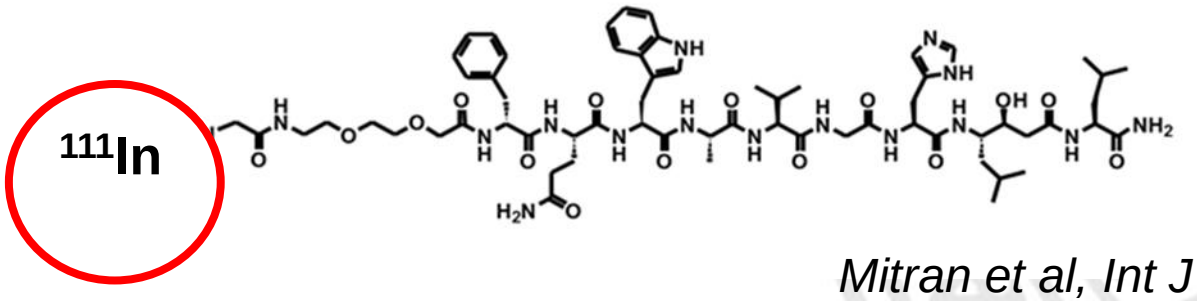
Influence of chelators on biodistribution of the peptide

DOTA | DOTAGA | NOTA | NODAGA

4 hrs p.i.





Mitran et al, Int J Oncol, 2016

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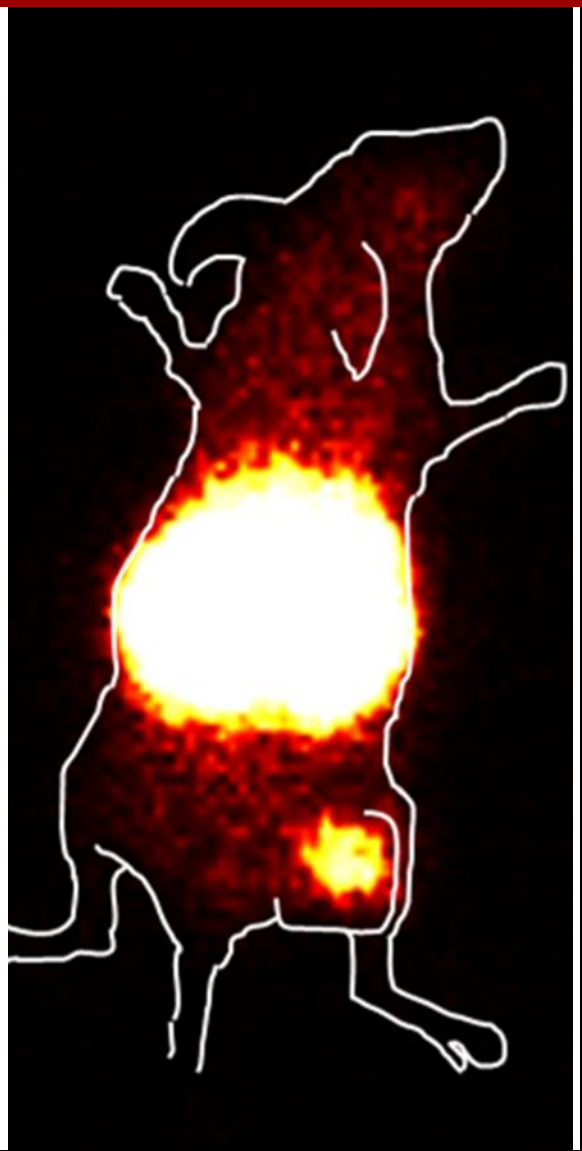
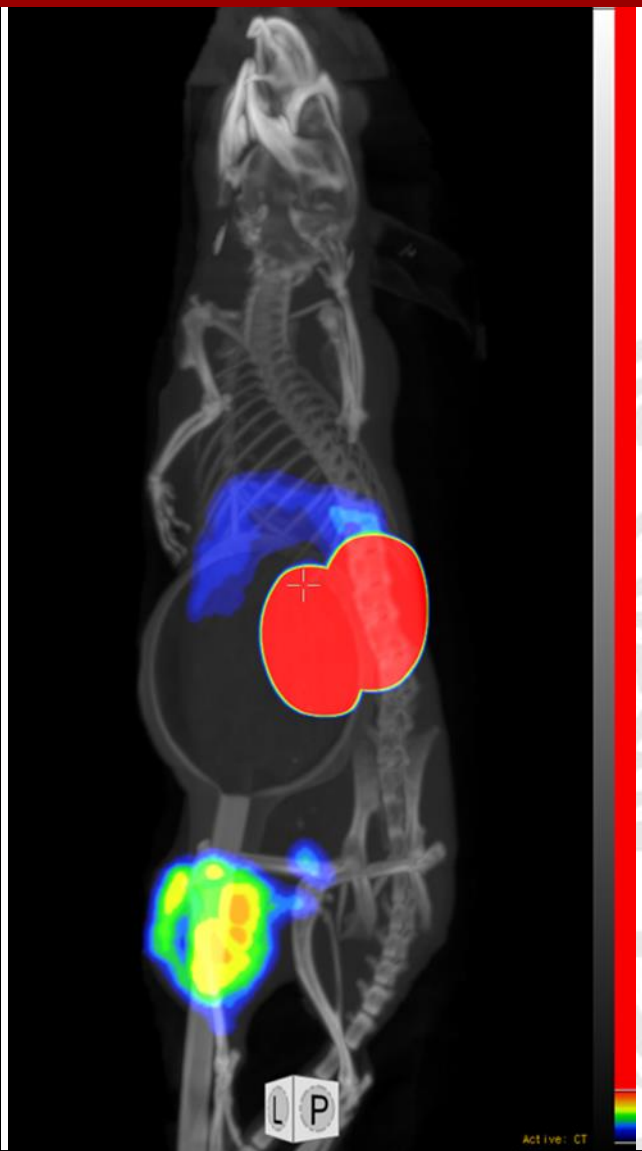
Optimal label (nuclide)!

Tolmachev, Eur J Nucl Med, 2010

^{111}In-DOTA-ZHER1





^{57}Co-DOTA-ZHER1

Garousi, unpublished data

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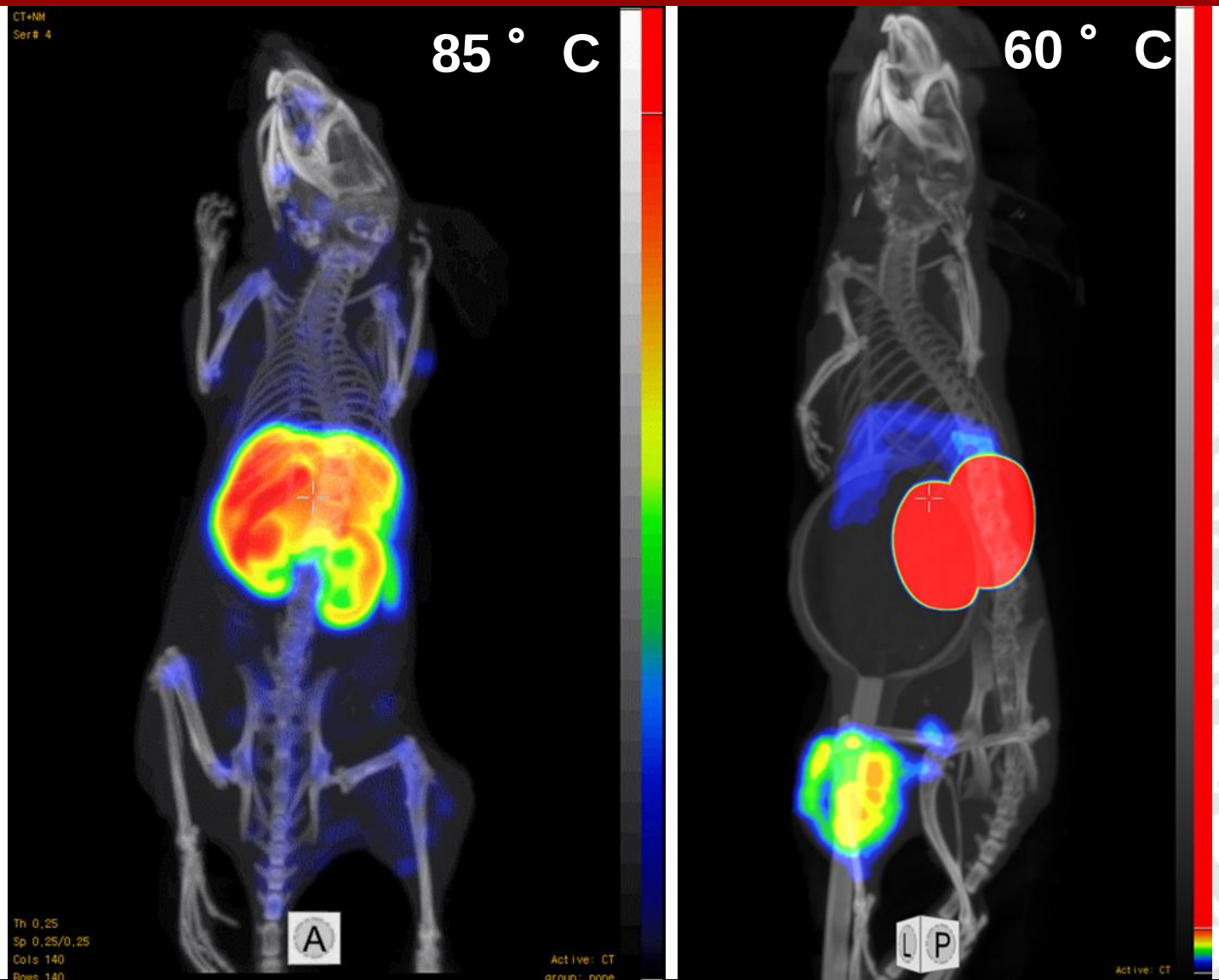
Optimal labeling conditions!



^{57}Co-DOTA-ZHER1

Garousi, unpublished data

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Take home message

Choice of labeling method (nuclide + chelator) depends on:

- biological half-life of targeting molecule
- cellular processing
- biological function
- binding site / amino acid composition
- lipophilicity