Design and Development of Nuclear Medicine

臺北榮民總醫院醫院 核醫部
張文議 藥師

Outline

- Nuclear medicine & Molecular imaging probe
- Molecular Imaging Probe
- Molecular Imaging Probe Design

Design of new radiopharmaceutical

1. What information do we intend to gather from the study?

Localization of the tracer (morphologic structure) or *physiological function of an organ*?

Ex. Liver imaging

a.Functional status: dyes, metabolic compounds primarily handled by hepatocytes.

b. Structural feature: ^{99m}Tc-sulfur colloid

Design of new radiopharmaceutical

2. How do we go about formulating the intended radiopharmaceutical?

a. reproducible method of preparation

b. simple and not to alter the desired property of labeled compound

c. optimum conditions (temp., pH, ionic strength, molar ratio…) should be maintained.

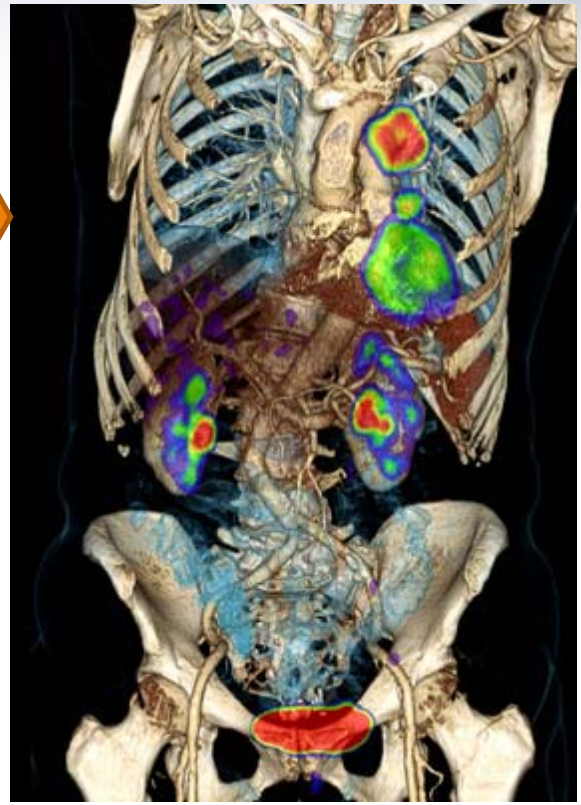
Radiopharmaceutical Research

1. Discovering new classes of RP--- finding major structure.
2. Selecting the most promising candidates within a class of compounds.
3. Clarifying the biological behavior of a radio compound.
4. Elucidating the relationship between chemical/structure properties of a radiotracer and its mechanism of biodistribution.

   --- Quantitative SAR

Isotopes in Medicine

Application		Isotopes
Diagnosis	In vitro	^{14}C, ^{3}H, ^{125}I, other
	In vivo	^{99m}Tc, ^{201}TI, ^{123}I, ^{67}Ga, ^{18}F, ^{11}C, ^{13}N, ^{15}O, ^{124}I, ^{68}Ga, ^{111}In
Therapy	Internal	^{131}I, ^{90}Y, ^{188}Re, ^{125}I, ^{177}Lu
	External	^{60}Co



Molecular Imaging

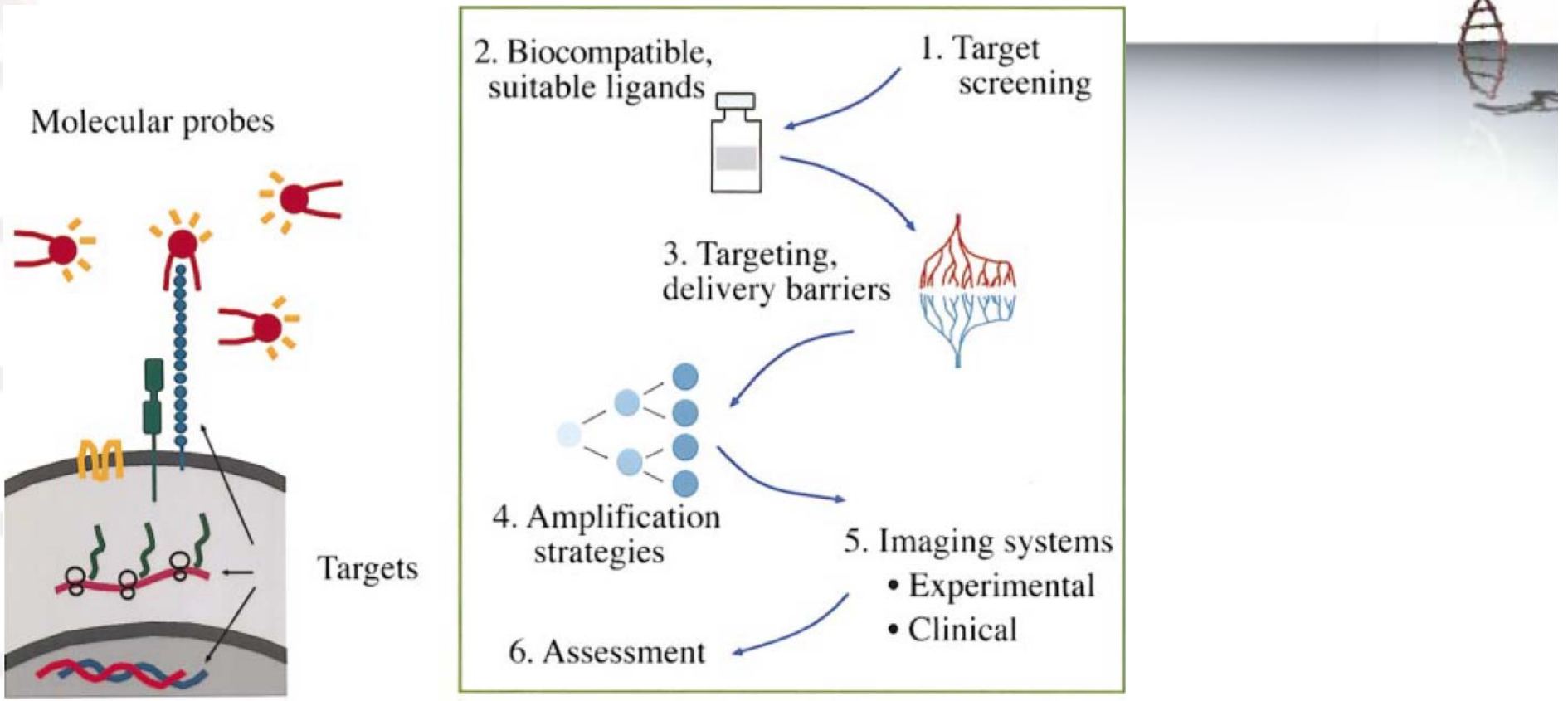
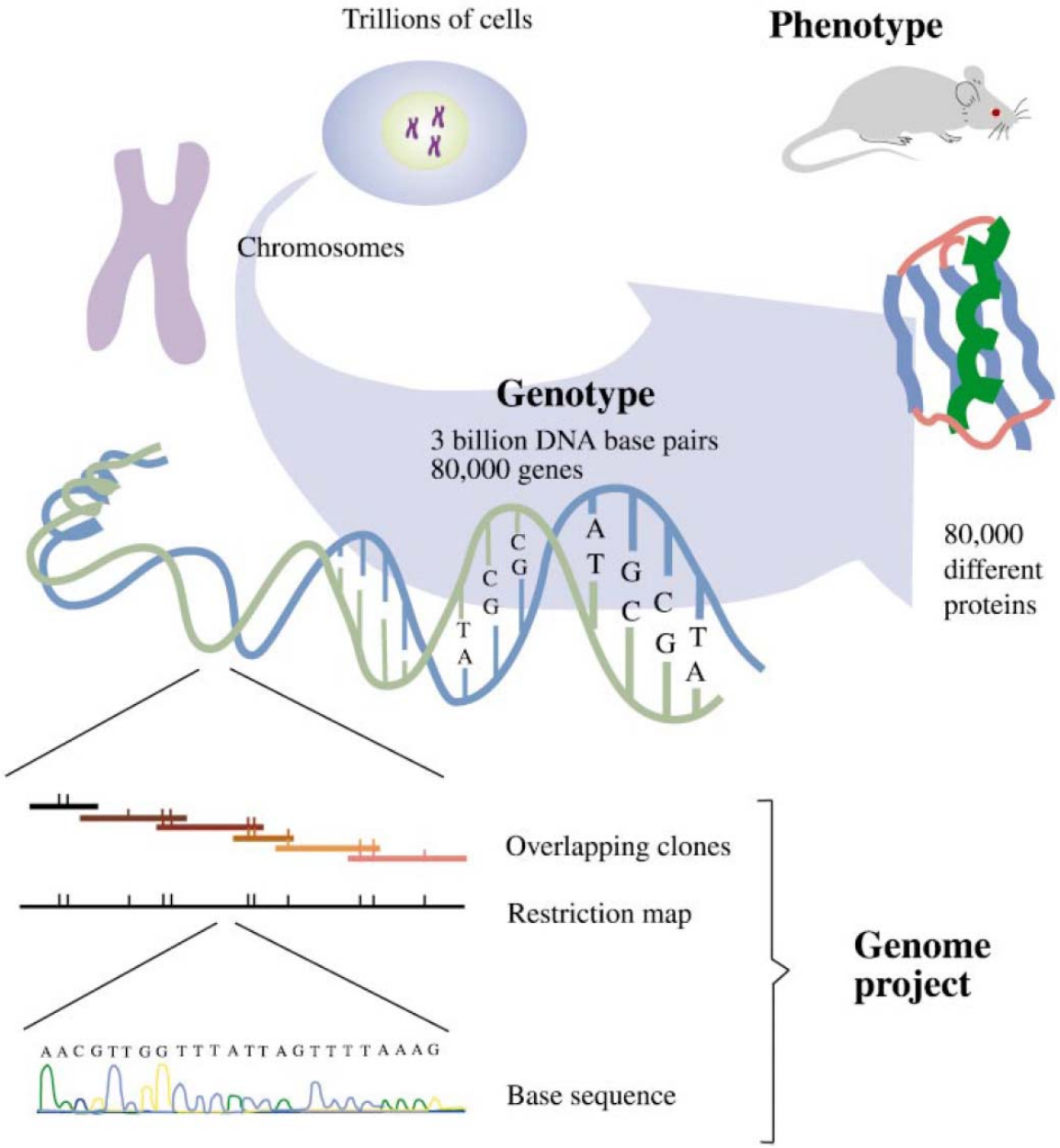
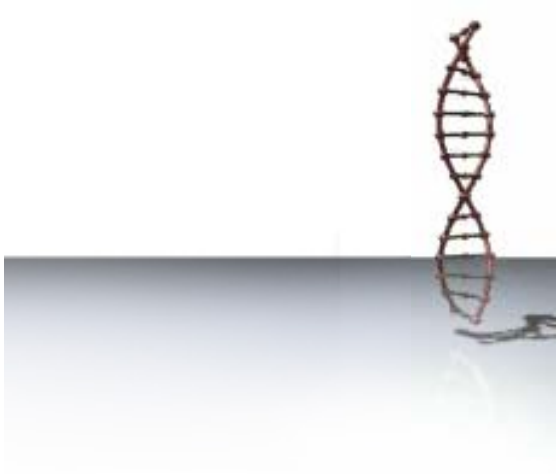


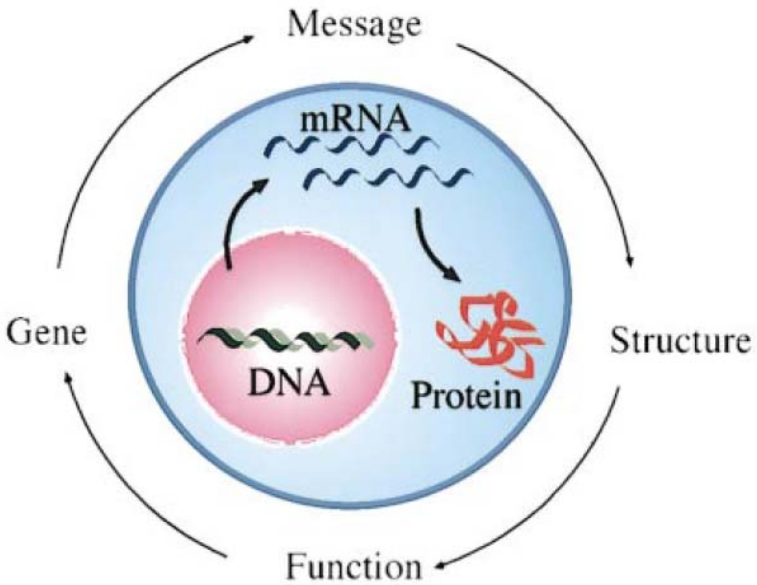
Figure 1. Schematics show prerequisites to in vivo molecular imaging. Potential targets can be at the DNA, RNA, or protein level (also see the text and Fig 2).


Trillions of cells
Phenotype
Chromosomes
Genotype
3 billion DNA base pairs
80,000 genes
80,000
different
proteins
Overlapping clones
Restriction map
AACGTTGGTTTATTAGTTTTAAAG
Base sequence
Genome
project




台北榮民總醫院 Taipei Veterans General Hospital
視病猶親 追求卓越 恪遵倫理 守法守信

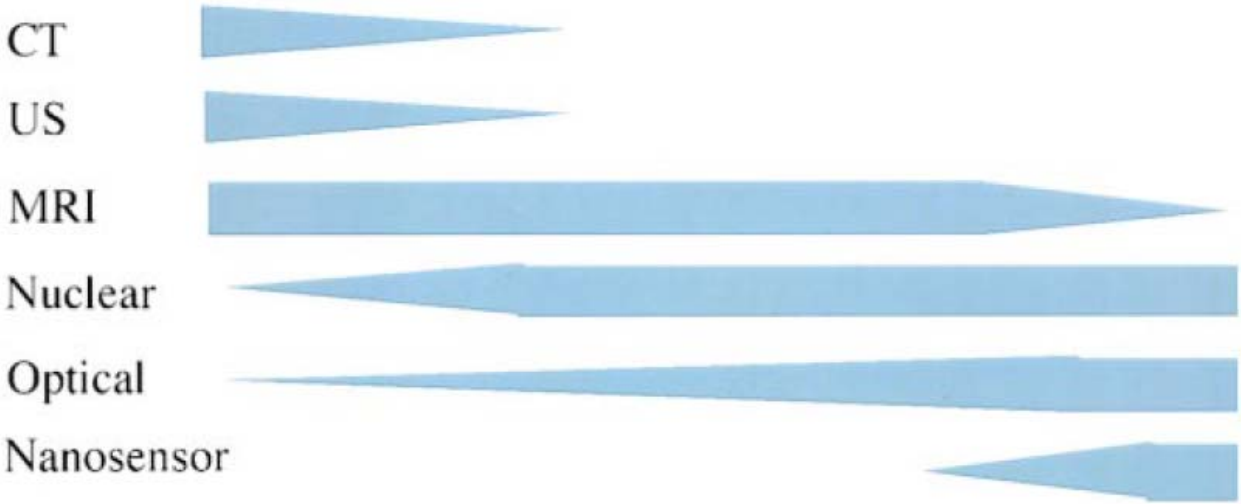
A.



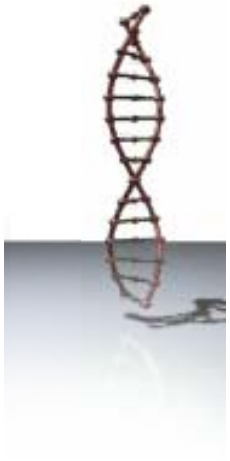
B.

Target	Number/cell
Gene (DNA)	2
Message	50-1,000
Protein	100 - 1,000,000
Function	massive

C.



Molecular Imaging Probe



Signal agent:
- PET: ^{18}F, ^{11}C, ^{64}Cu, etc.
- SPECT: ^{99m}Tc, ^{111}In, etc.
- Optical: near IR fluoresent dyes
- MR: magnetically active elements
  Gd (III) chelates; Iron oxide nanoparticles;
  Dynamic nuclear polarization; Para-Hydrogen
- Ultrasound: microbubbles; micelles; liposomes
- Multimodal agents
  MR/PET; MR/optical; CT/optical;
  MR/fluoresence/bioluminescence

Targeting moiety:
- Viruses
- Small molecules
- Peptides
- Proteins
- Antibodies
- Nanoparticles
- Dual recognition

Target:
- Enzyme
- Receptor
- DNA/RNA
- Physiological state

Fig. (1).
Schematic representation of molecular imaging probe.

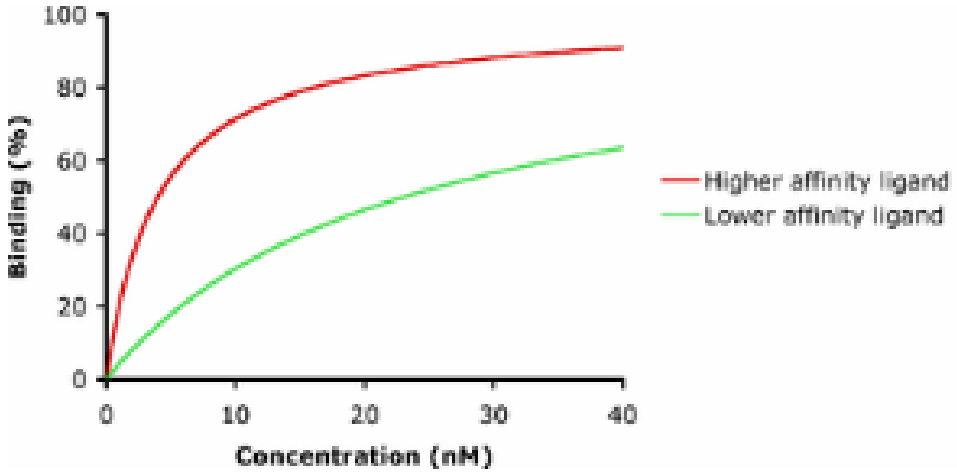
Molecular Imaging Probe

- **1.** High binding affinity to target.
- **2.** High specificity to target.
- **3.** High sensitivity.
- **4.** High contrast ratio.
- **5.** High stability in vivo.
- **6.** Low immunogenicity and toxicity.
- **7.** Production and economical feasibility.

Molecular Imaging Probe

- **1.** High binding affinity to target.

  Molecular imaging generally favors the acquisition of the images at early time after administration of a molecular probe. To obtain high uptake of the imaging probe to the target within limited circulation time frame requires that the imaging probe has binding property of fast on-rate (Kon) and slow off-rate (Koff).

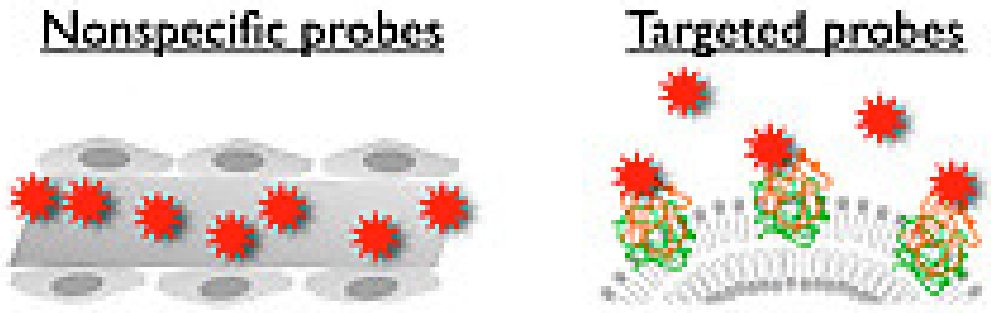


- **2.** High specificity to target.
- **3.** High sensitivity.
- **4.** High contrast ratio.
- **5.** High stability in vivo.
- **6.** Low immunogenicity and toxicity.
- **7.** Production and economical feasibility.

Molecular Imaging Probe

- **1.** High binding affinity to target.
- **2.** High specificity to target.

In contrast, target-specific molecular imaging probes can interact with particular biomarkers, such as enzyme, receptor, and transporters, which are involved in various biological processes associated with particular cell populations and subcellular compartments.



- **3.** High sensitivity.
- **4.** High contrast ratio.
- **5.** High stability in vivo.
- **6.** Low immunogenicity and toxicity.
- **7.** Production and economical feasibility.

Molecular Imaging Probe

- **1.** High binding affinity to target.
- **2.** High specificity to target.
- **3.** High sensitivity.

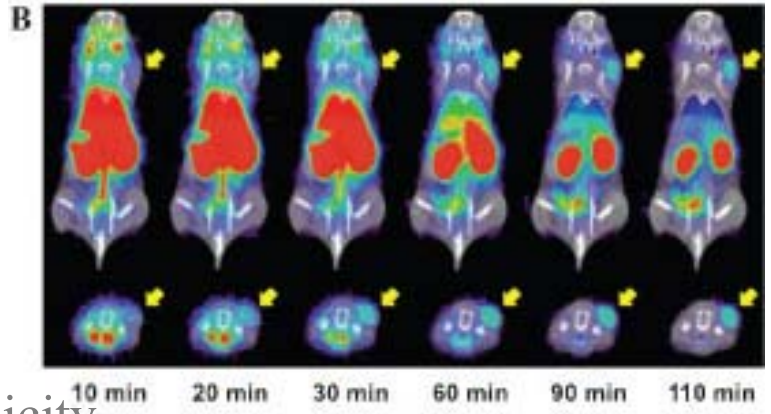
  To detect the biochemical process of the disease, especially at an early stage, frequently requires spying on the aberrant of a very small amount of targets.

- **4.** High contrast ratio.
- **5.** High stability in vivo.
- **6.** Low immunogenicity and toxicity.
- **7.** Production and economical feasibility.

Molecular Imaging Probe

- **1.** High binding affinity to target.
- **2.** High specificity to target.
- **3.** High sensitivity.
- **4.** High contrast ratio.

  High contrast images with high target-to-background or signal-to-noise ratio ensure appropriate interpretation of physiological and pathological conditions of the diseases.



- **5.** High stability in vivo.
- **6.** Low immunogenicity and toxicity.
- **7.** Production and economical feasibility.

Molecular Imaging Probe

- **1.** High binding affinity to target.
- **2.** High specificity to target.
- **3.** High sensitivity.
- **4.** High contrast ratio.
- **5.** High stability in vivo.

  Although only trace amount of imaging probe is normally given to the living subjects, maintenance of the intact structure of an imaging probe is a big challenge because numerous enzymes or proteases present in serum or targeted tissue may degrade the imaging probe. The image information given from the metabolites of the imaging probe undoubtedly complexifies the imaging readout and usually makes the understanding of disease highly vague.
- **6.** Low immunogenicity and toxicity.
- **7.** Production and economical feasibility.

Molecular Imaging Probe

- **1.** High binding affinity to target.
- **2.** High specificity to target.
- **3.** High sensitivity.
- **4.** High contrast ratio.
- **5.** High stability in vivo.
- **6.** Low immunogenicity and toxicity.

  A molecular imaging probe should have minimal or acceptable level of immunogenicity and toxicity before it can be safely employed in human.
- **7.** Production and economical feasibility.

  The low cost and excellent availability of molecular imaging probes are advantageous for their wide distribution and clinical routine use.

Molecular Imaging Imaging Probe Design

First of all, the molecular imaging probe must remain intact in the circulation to evade the reticuloendothelial system (RES). In addition, the molecular imaging probe must be able to reach and accumulate into the targeted tissues such as tumor. Moreover, if the molecular imaging probe is designed to image the intracellular target, the imaging probe has to penetrate the cell membrane and stay inside of the cells. One of major goals of molecular imaging probe design is to maximize probe's ability of crossing these biological barriers.

Molecular Imaging Imaging Probe Design

- **Lipinski's rule of five** (guideline for drug design)

1. No more than 5 hydrogen bond donors (the total number of nitrogen hydrogen and oxygen–hydrogen bonds)
2. Not more than 10 hydrogen bond acceptors (all nitrogen or oxygen atoms)
3. A molecular mass less than 500 daltons
4. An octanol-water partition coefficient log *P* not greater than 5
5. No more than 10 rotatable bonds

Molecular imaging probe design

1.Ionization constant (pKa value)
2.Lipophilicity (logP value)
3.Stability

Molecular imaging probe design

- Lipophilicity (logP value)

$$P = C_{organic} / C_{aqeous}$$

$$\log P = \log (C_{organic} / C_{aqeous})$$

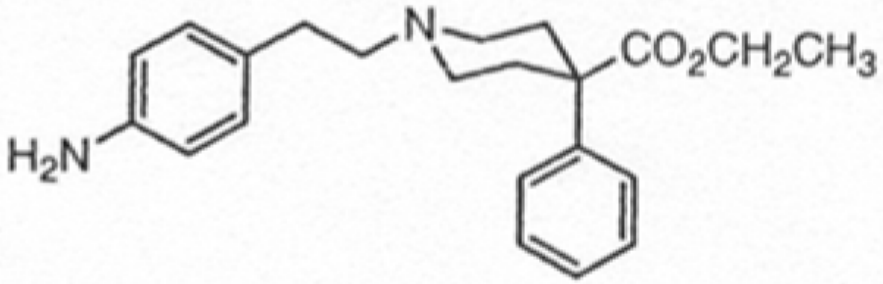
Lipophilicity: logP

Table 2.6. Hydrophilic-lipophilic Values (π V) for Organic Fragments (9)

Fragments	π Value
C (aliphatic)	+0.5
Phenyl	+2.0
Cl	+0.5
O_2NO	+0.2
IMHB	+0.65
S	0.0
O = C−O (carboxyl)	−0.7
O = C−N (amide, imide)	−0.7
O (hydroxyl, phenol, ether)	−1.0
N (amine)	−1.0
O_2N (aliphatic)	−0.85
O_2N (aromatic)	−0.28

Eq. 2.7 $$\text{LogP} = \Sigma\pi \text{ (fragments)}$$

Lipophilicity: logP



Fragments	π
2 amines	−2.0
9 aliphatic carbons	+4.5
2 phenyl rings	+4.0
1 ester	−0.7
logP	+5.8

Pharmacokinetics - ADME

intake

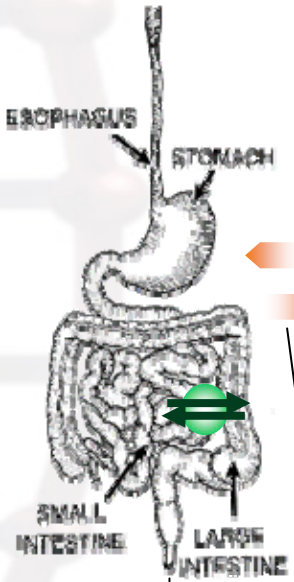
A: Absorption

D: Distribution

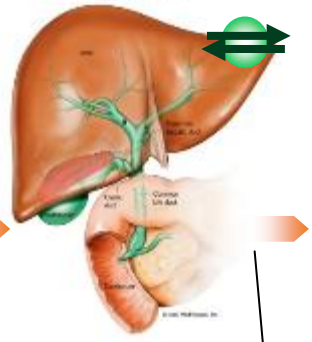
M: Metabolism

E: Excretion

Enterohepatic Circulation



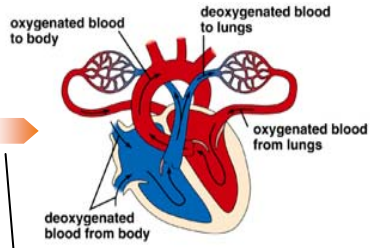
feces



liver

Portal V.

Mesenteric V.

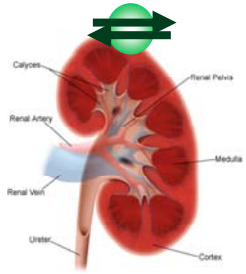


Heart/circulation system

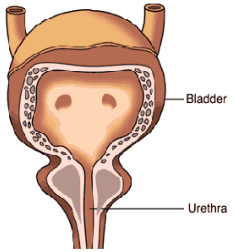
Inferior Vena Cava

Hepatic V.

Target



kidney



bladder

Urine

D Drug

Transporter

Molecular Imaging Imaging Probe Design

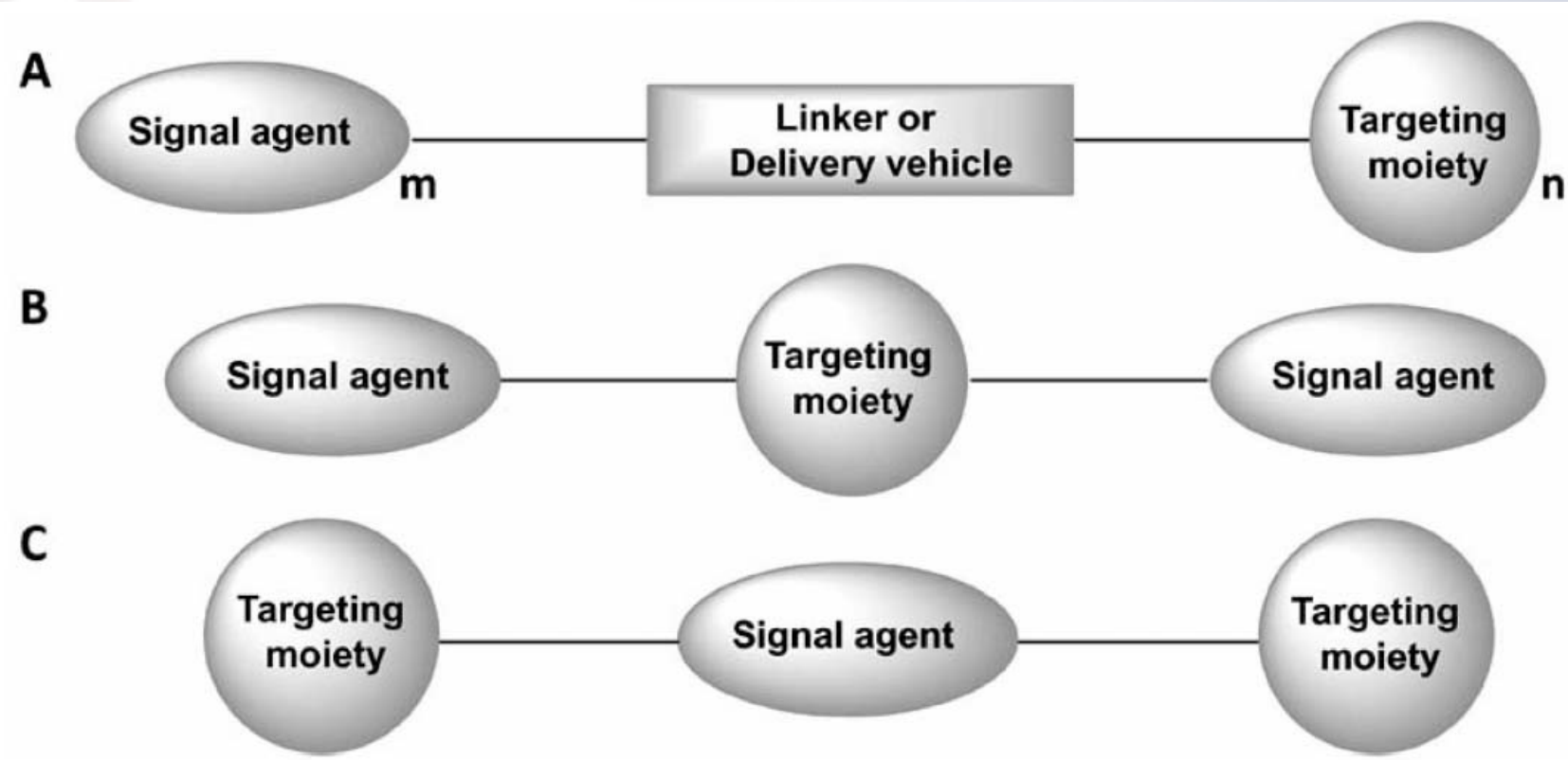
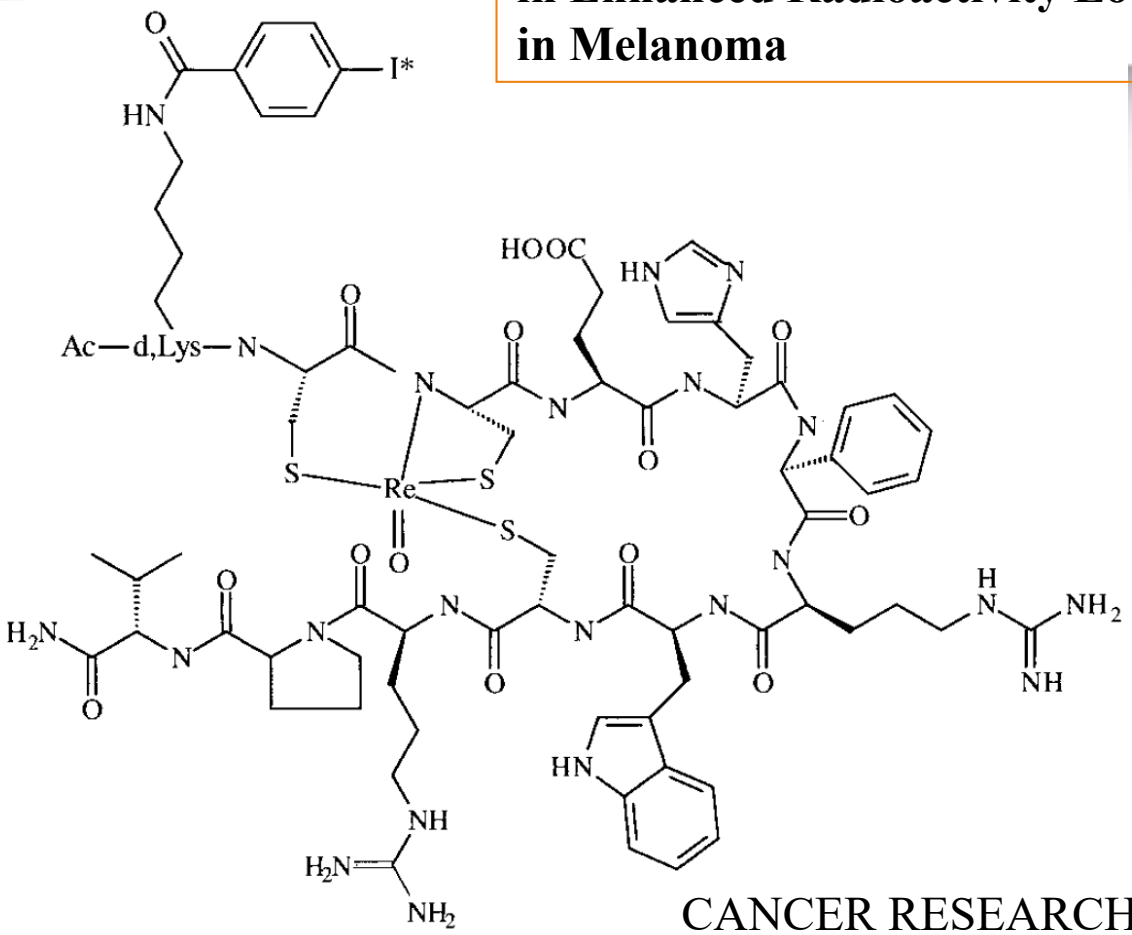
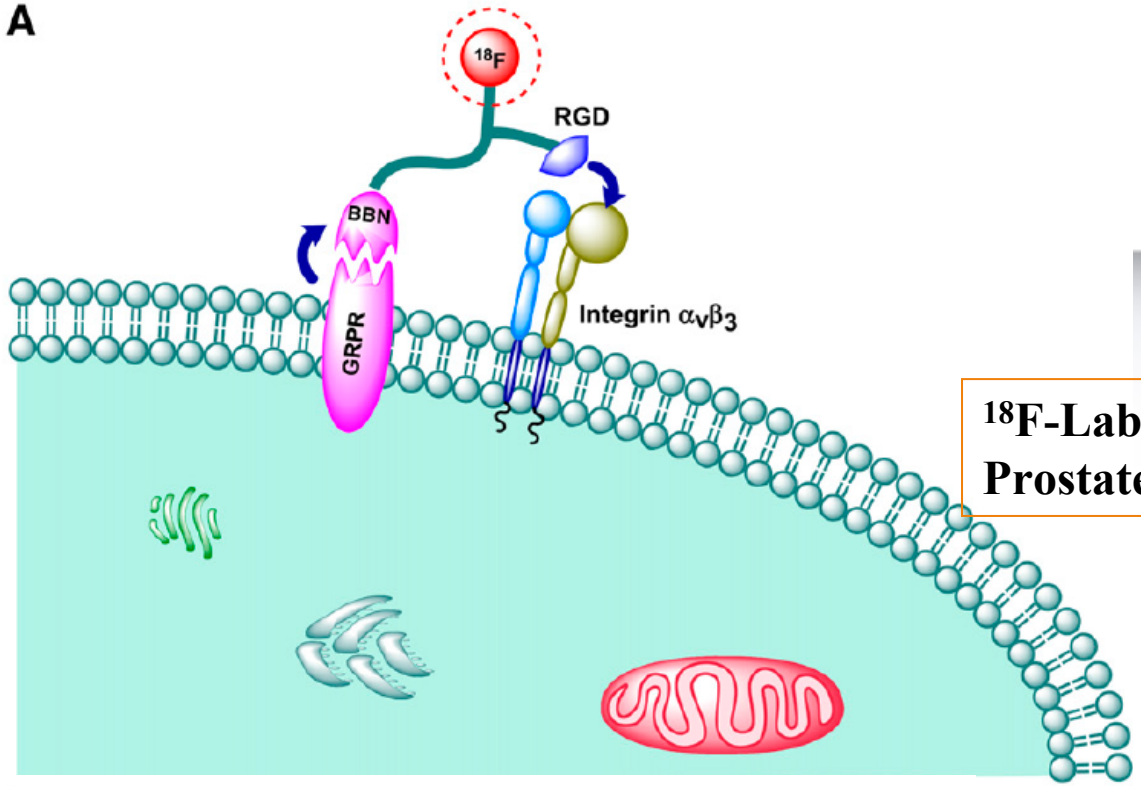


Fig. (2).
Schematic representation of general approaches of molecular imaging probe design.

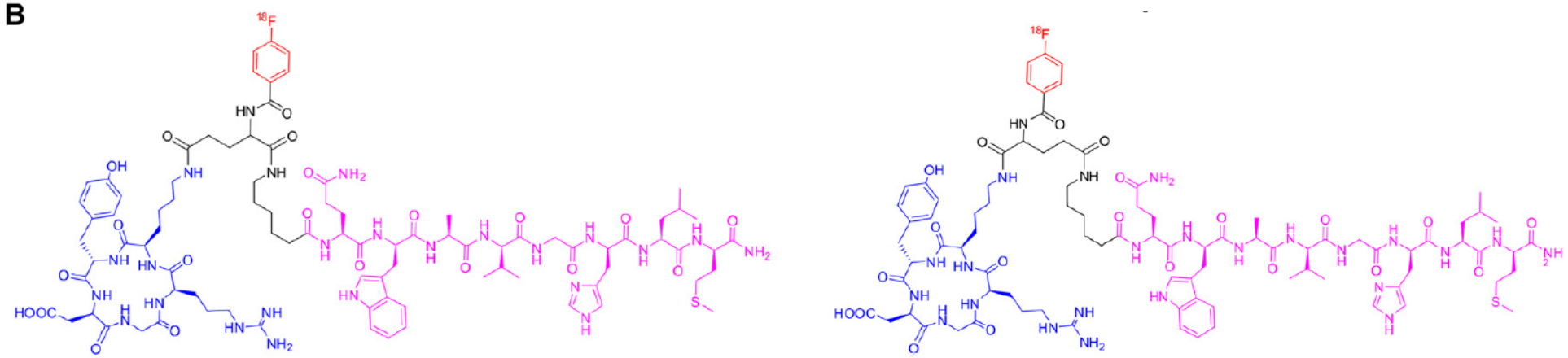
Radioiodination of Rhenium Cyclized α-Melanocyte-Stimulating Hormone (α-MSH) Resulting in Enhanced Radioactivity Localization and Retention in Melanoma



CANCER RESEARCH 64, 1411–1418, February 15, 2004



18F-Labeled BBN-RGD Heterodimer for Prostate Cancer Imaging



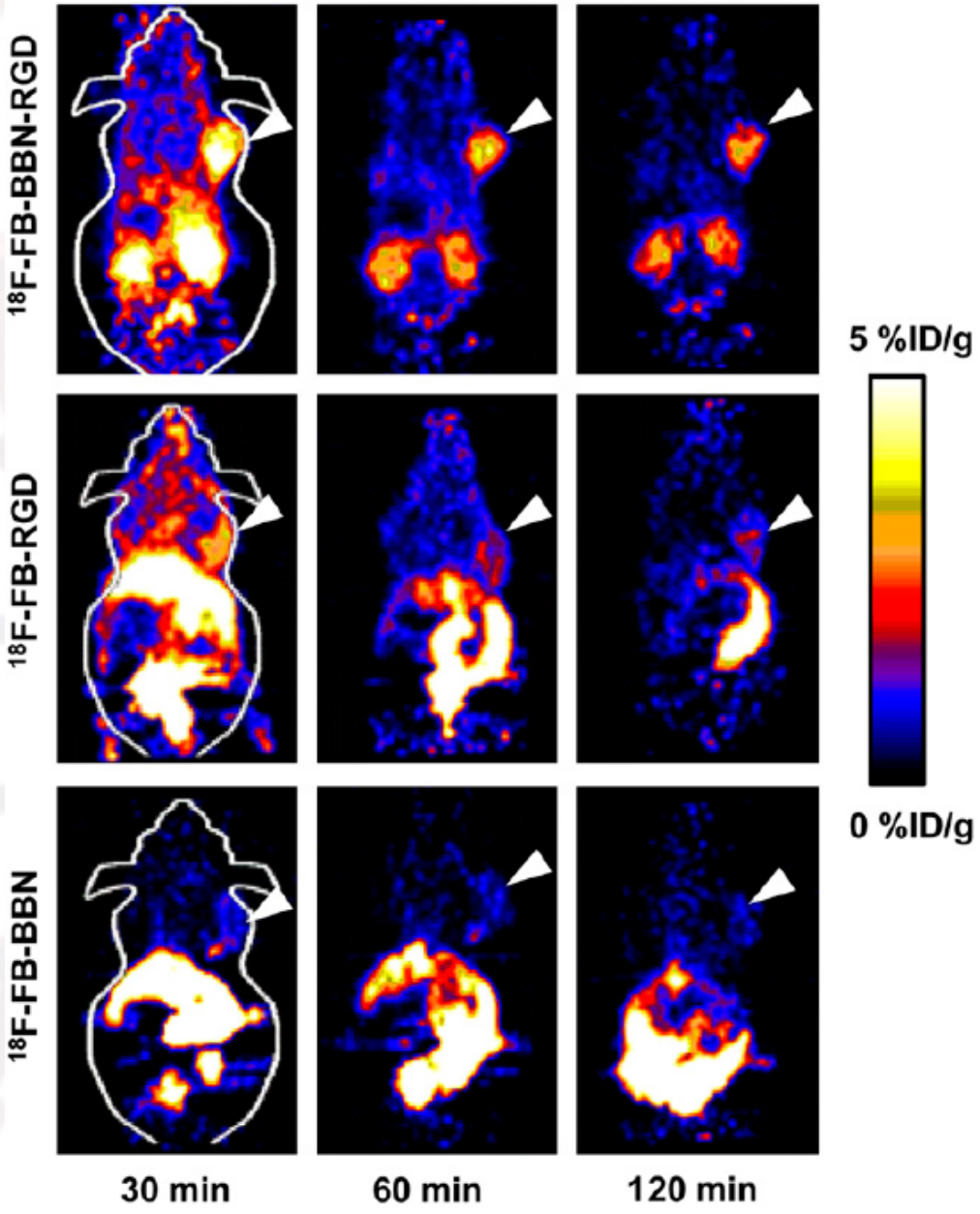
J Nucl Med 2008; 49:453–461

^{18}F-Labeled BBN-RGD Heterodimer for Prostate Cancer Imaging

Overexpression of gastrin-releasing peptide receptor (GRPR) has been discovered primarily in androgen-independent human prostate tissues and, thus, provides a potential target for prostate cancer diagnosis and therapy. Various approaches have been explored for the imaging of GRPR expression in vivo. Bombesin (BBN), which was originally isolated from the skin of a frog, is an analog of the gastrin-releasing peptide (GRP). The truncated sequence BBN was considered to be sufficient for the specific binding interaction with GRPR and metabolically stable for in vivo application.

It is well documented that most solid tumors are angiogenesis dependent and that integrin is a key player. In particular, integrin αvβ3 was found to be necessary for the formation, survival, and maturation of new blood vessels. Synthetic peptides containing the arginine-glycine-aspartate (RGD) sequence motif are active modulators of cell adhesion and can bind specifically to integrin αvβ3.

J Nucl Med 2008; 49:453–461



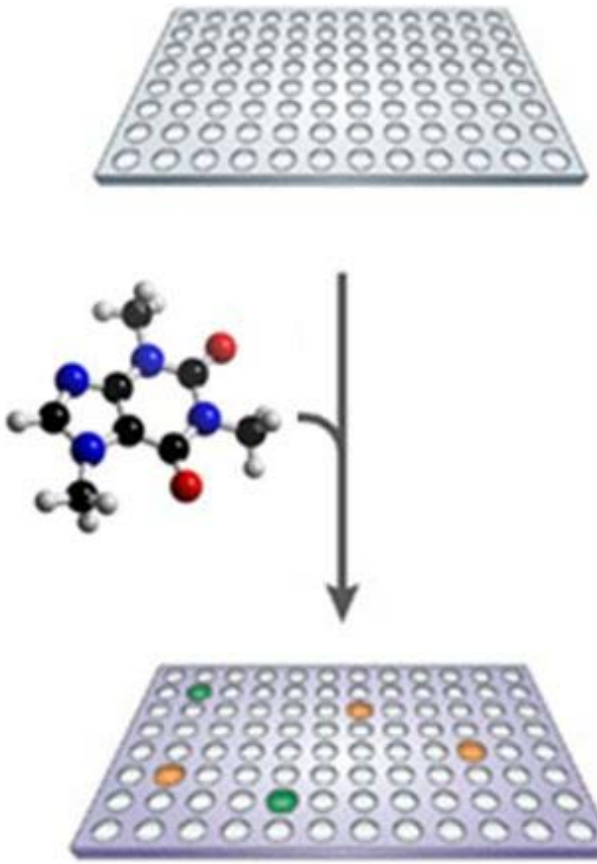
J Nucl Med 2008; 49:453–461

Molecular Imaging Imaging Probe Design

- Strategies for development of molecular imaging probes : In general, the strategies of molecular imaging probe development can be categorized into two major classes: random approach and rational approach. Under some circumstances, two approaches can be combined together to develop molecular imaging probes.

Molecular Imaging Imaging Probe Design

- Random approach



High-throughput screen
- Host-pathogen interactions
- Cell or organism level

Small molecule libraries
- FDA-approved drugs
- Diversity-oriented synthesis
- Natural products

Plate assay read-out
- Automated microscopy
- Host survival
- Pathogen invasion

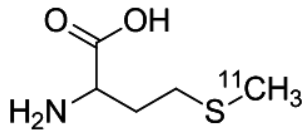
Molecular Imaging Imaging Probe Design

- **Generation from Naturally-Occurring Bioactive Molecules**
- **Generation from Drugs or Drug Candidates**
- **Generation from Established Molecular Imaging Probes**
- **Computer-Aided Molecular Imaging probe Development**

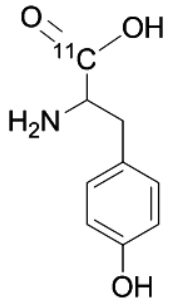
Molecular Imaging Imaging Probe Design

- **Generation from Naturally-Occurring Bioactive Molecules**

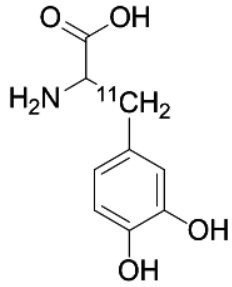
  Numerous naturally-occurring bioactive molecules have been discovered over the past decades. Many of these molecules are involved in both normal and disease biological process. These molecules can interact with enzymes, receptors, transporters, and proteins, and provided great sources and foundations for molecular imaging probe development.



^{11}C-Methionine



^{11}C-Tyrosine



^{11}C-L-DOPA

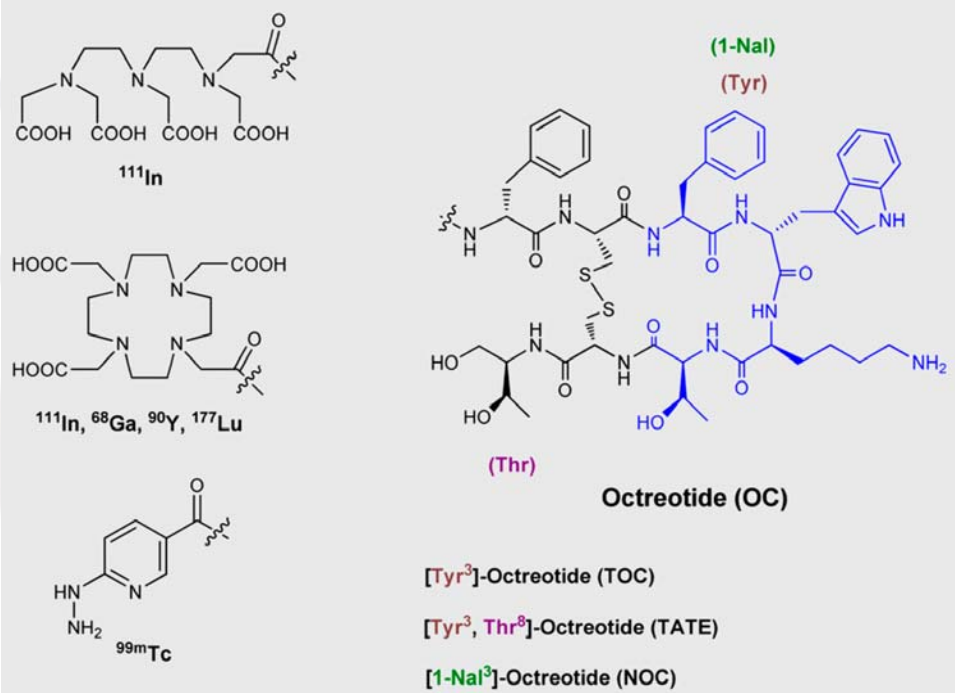
- Generation from Drugs or Drug Candidates
- Generation from Established Molecular Imaging Probes
- Computer-Aided Molecular Imaging probe Development

Molecular Imaging Imaging Probe Design

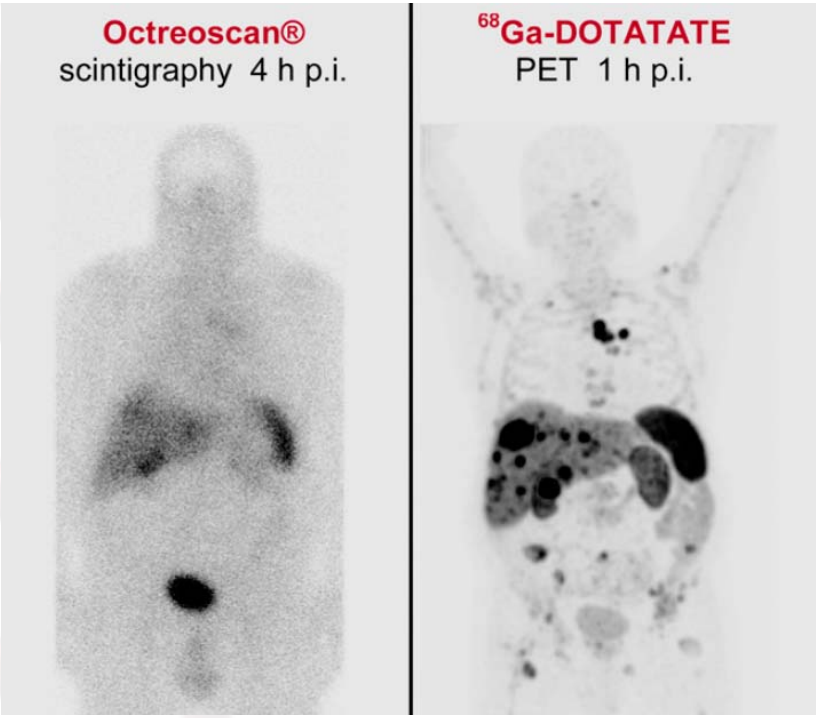
- **Generation from Naturally-Occurring Bioactive Molecules**
  In addition, many molecular imaging probes are developed based on the naturally-occurring peptides, including radiolabeled somatostatin and octreotide, bombesin analogs, neurotensin peptides, and α-MSH peptides.

- Generation from Drugs or Drug Candidates
- Generation from Established Molecular Imaging Probes
- Computer-Aided Molecular Imaging probe Development

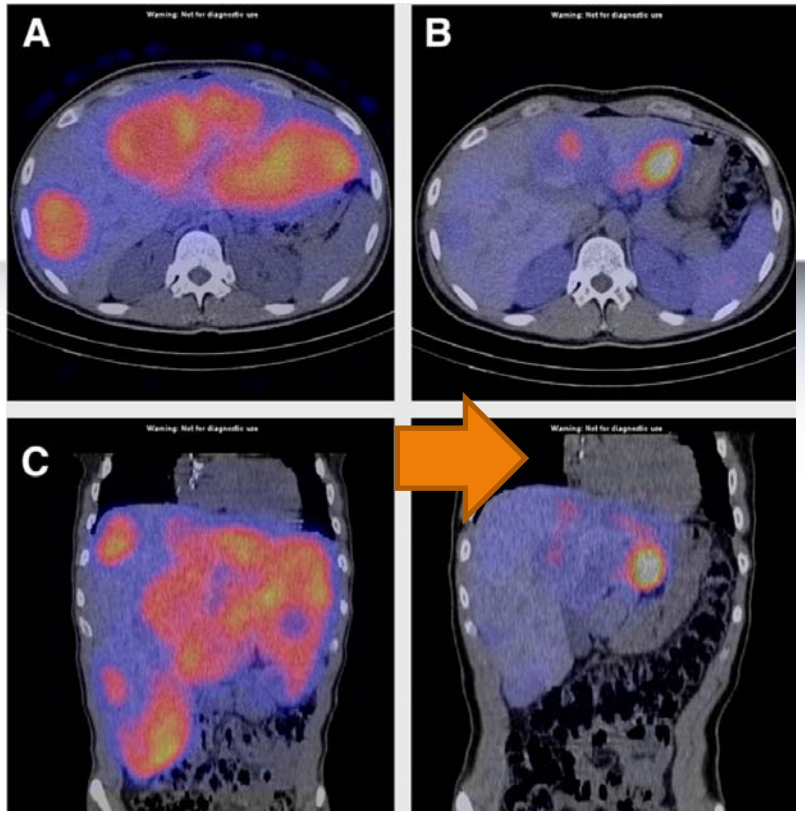
Somatostatin receptors have been identified at high density and incidence in most neuroendocrine tumors, including gastroenteropancreatic tumors, pheochromocytomas, pituitary adenomas, and paragangliomas but also in tumors of the nervous system such as neuroblastomas, medulloblastomas, and meningiomas.



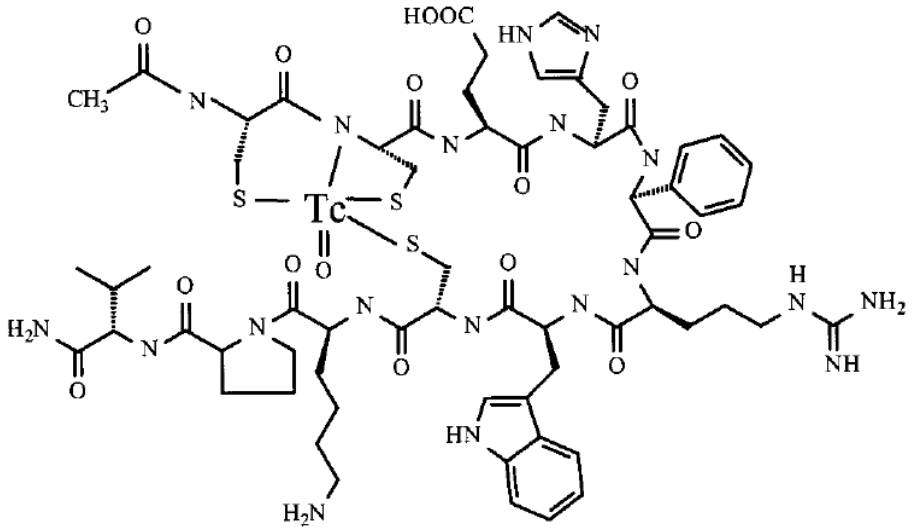
J Nucl Med 2011; 52:841–844



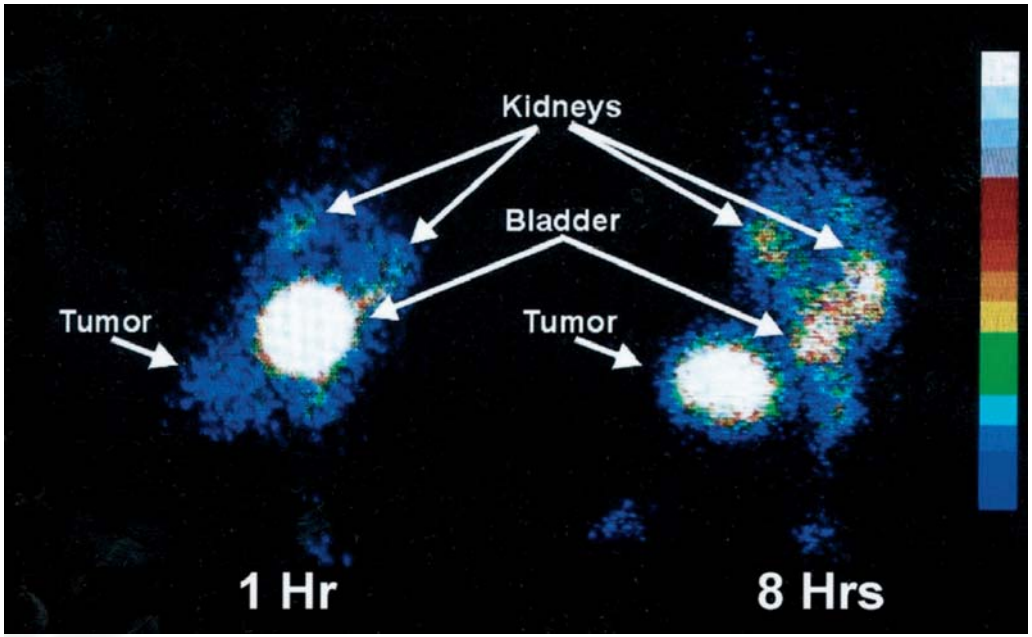
Patient with metastasized neuroendocrine tumor. Planar (left) and PET (right) images were obtained 3 wk apart. p.i. 5 after injection. (Courtesy of Damian Wild, University
Hospital Freiburg.)



Transaxial (A and B) and coronal (C and D) SPECT/CT images obtained after first (A and C) and second (B and D) therapy cycles with $^{90}Y/^{111}In$-DOTATOC (^{111}In is added for imaging). Impressive partial remission is seen after first therapy cycle. (Courtesy of Flavio Forrer and Guillaume Nicolas, University Hospital Basel, Switzerland.)



A structural model of ^{99m}Tc-CCMSH; Acetyl-Cys-Cys-Glu-His-dPhe-Arg-Trp-Cys-Lys-Pro-Val-NH_2.



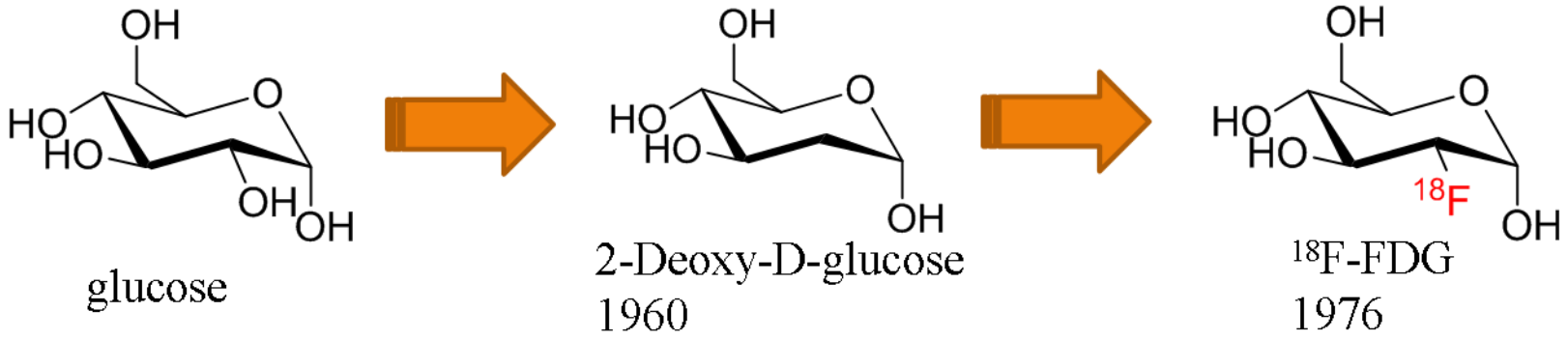
Imaging of B16/F1 murine melanoma bearing C57 mouse after 1 h (*left*) and 8 h (*right*) administration of 100 μCi of ^{99m}Tc-CCMSH

CANCER RESEARCH 60, 5649–5658, October 15, 2000

Molecular Imaging Imaging Probe Design

- Generation from Naturally-Occurring Bioactive Molecules
- **Generation from Drugs or Drug Candidates**

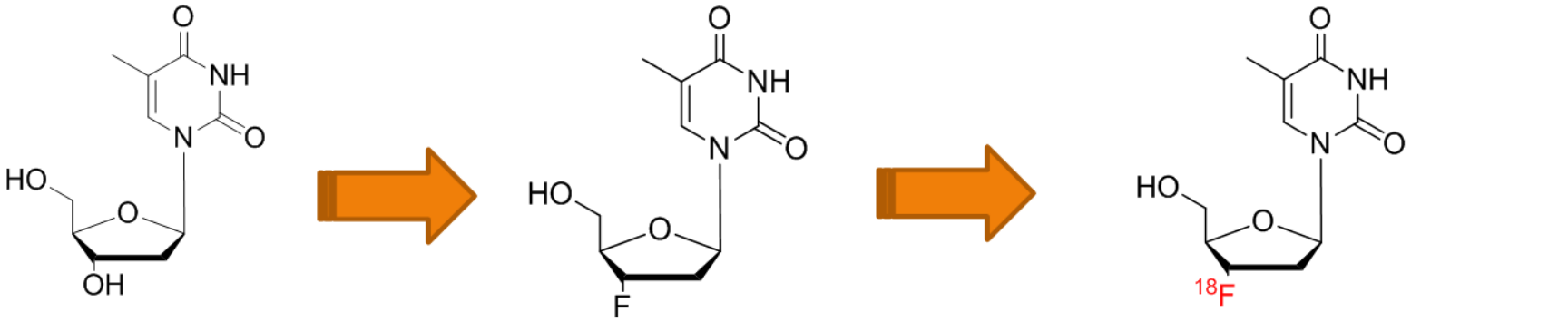
  Routinely used drugs as well as drug candidates in clinical trial provide valuable sources for molecular imaging probe development. The most successful PET probe, ^{18}F-fluoro-deoxy-D-glucose (^{18}F-FDG), is a good example.



- Generation from Established Molecular Imaging Probes
- Computer-Aided Molecular Imaging probe Development

Molecular Imaging Imaging Probe Design

- Generation from Naturally-Occurring Bioactive Molecules
- **Generation from Drugs or Drug Candidates**



Thymidine

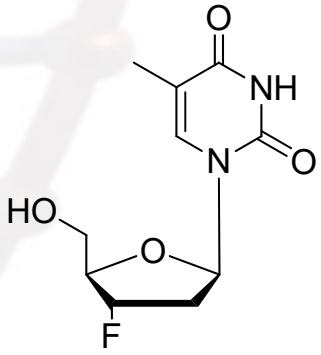
FLT Anti-HIV agent
JMC 1989, 32, 1743-1749

^{18}F-FLT
Tumor proliferation imaging

- Generation from Established Molecular Imaging Probes
- Computer-Aided Molecular Imaging probe Development

Table I. Comparative Potency and Selectivity of 2′,3′-Dideoxynucleoside Analogues as Inhibitors of HIV-1 Replication in MT-4 Cells

compd	ED_{50}[a], μM	CD_{50}[b], μM	SI[c]
4	484	500	1
5	>8	15	<2
6	>500	409	<1
7	484	500	1
10	11	240	22
12a	>500	>500	
14b	9.8	117	12
15a	>500	>500	
17a	0.38	535	1408
17b	0.41	24	59
17c	0.16	2.17	13.6
17d	46	348	7.6
19	1.7	7.7	4.5
21	>500	>500	
24	>20	40	<2
26	53	>500	>9.4
FddAdo[4]	50	557	11.1
AzddAdo[4]	5	10	2
ddAdo[8]	6.4	890	139
~~FddUrd[13]~~	~~0.04~~	~~16~~	~~400~~
FddThd[13]	0.001	0.197	197
AZT	0.003	4.8	1600



JMC 1989, 32, 1743-1749

Molecular Imaging Imaging Probe Design

- Generation from Naturally-Occurring Bioactive Molecules
- **Generation from Drugs or Drug Candidates**

Positron Emission Tomography (PET) Imaging with [^{11}C]-Labeled Erlotinib: A Micro-PET Study on Mice with Lung Tumor Xenografts

Cancer Res 2009; 69: (3). February 1, 2009

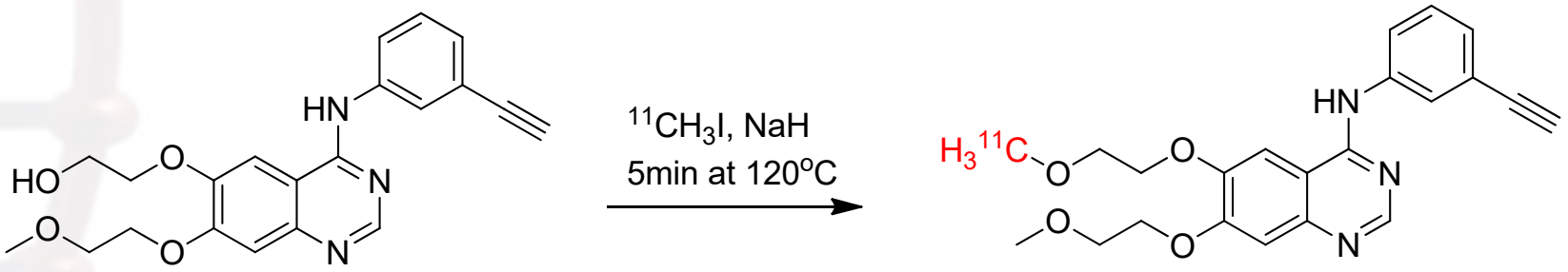
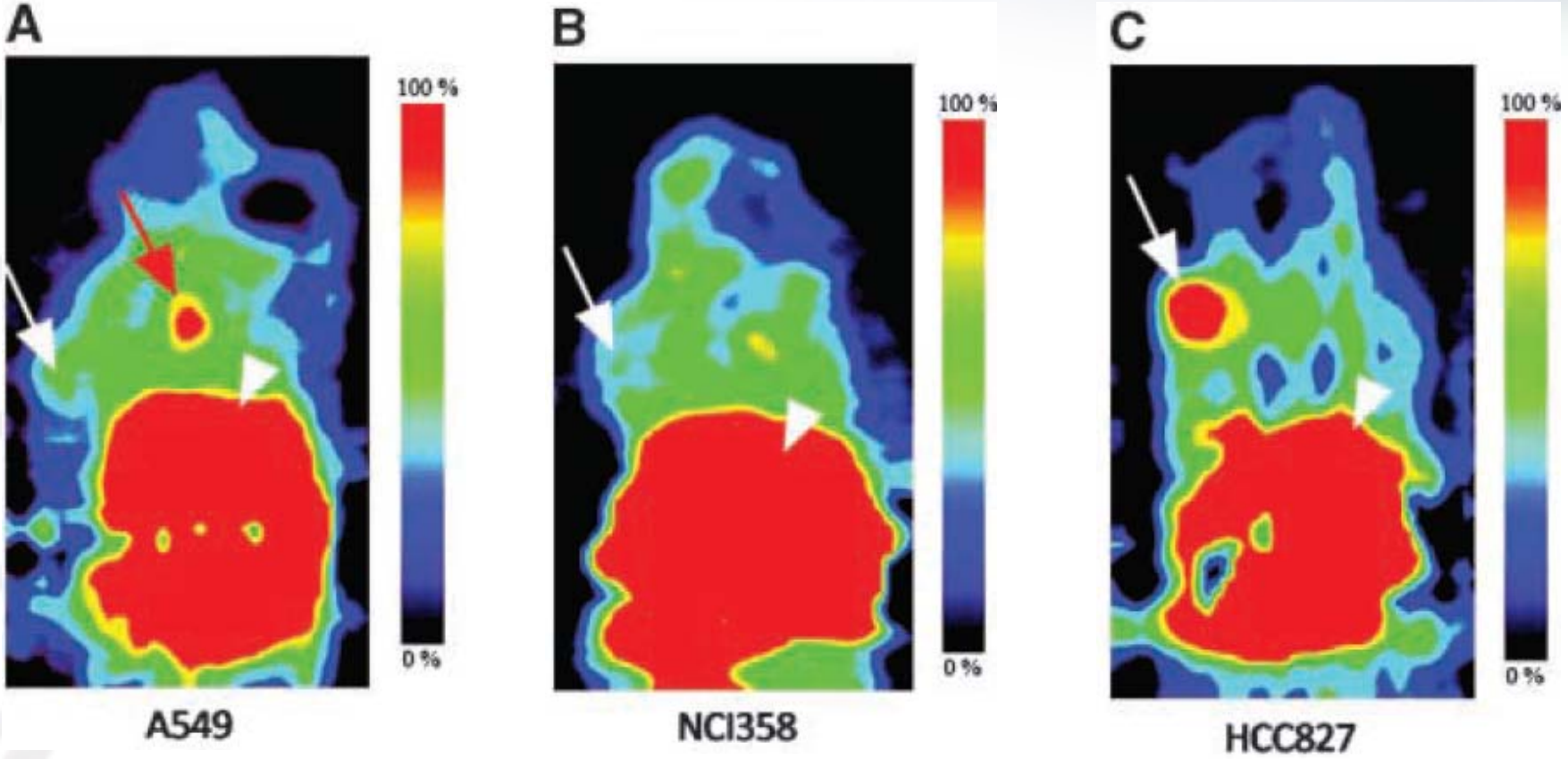


Diagram of [^{11}C]-erlotinib (Tarceva) radiosynthesis

- Generation from Established Molecular Imaging Probes
- Computer-Aided Molecular Imaging probe Development

Erlotinib (Tarceva) targets the epidermal growth factor receptor (EGFR), which is commonly overexpressed in human cancers, including lung cancer.

Cancer Res 2009; 69: (3). February 1, 2009

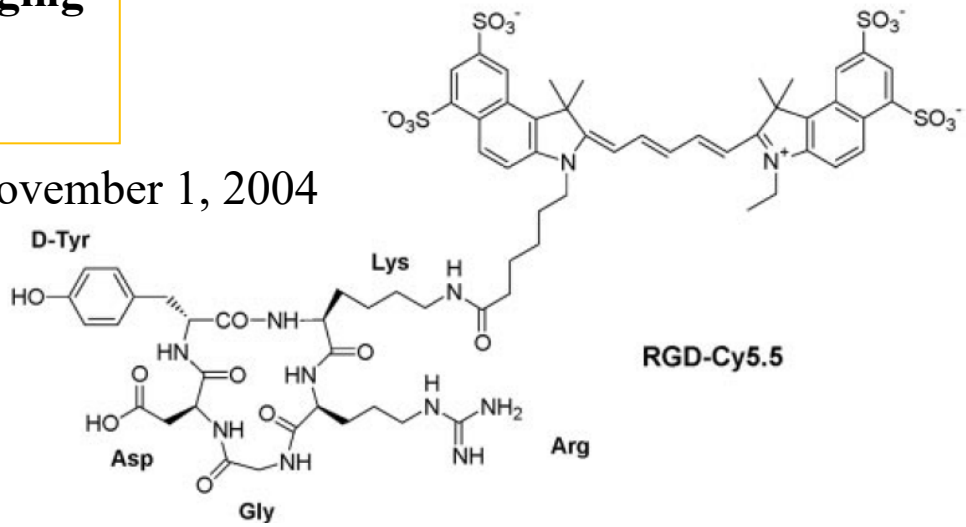


Molecular Imaging Imaging Probe Design

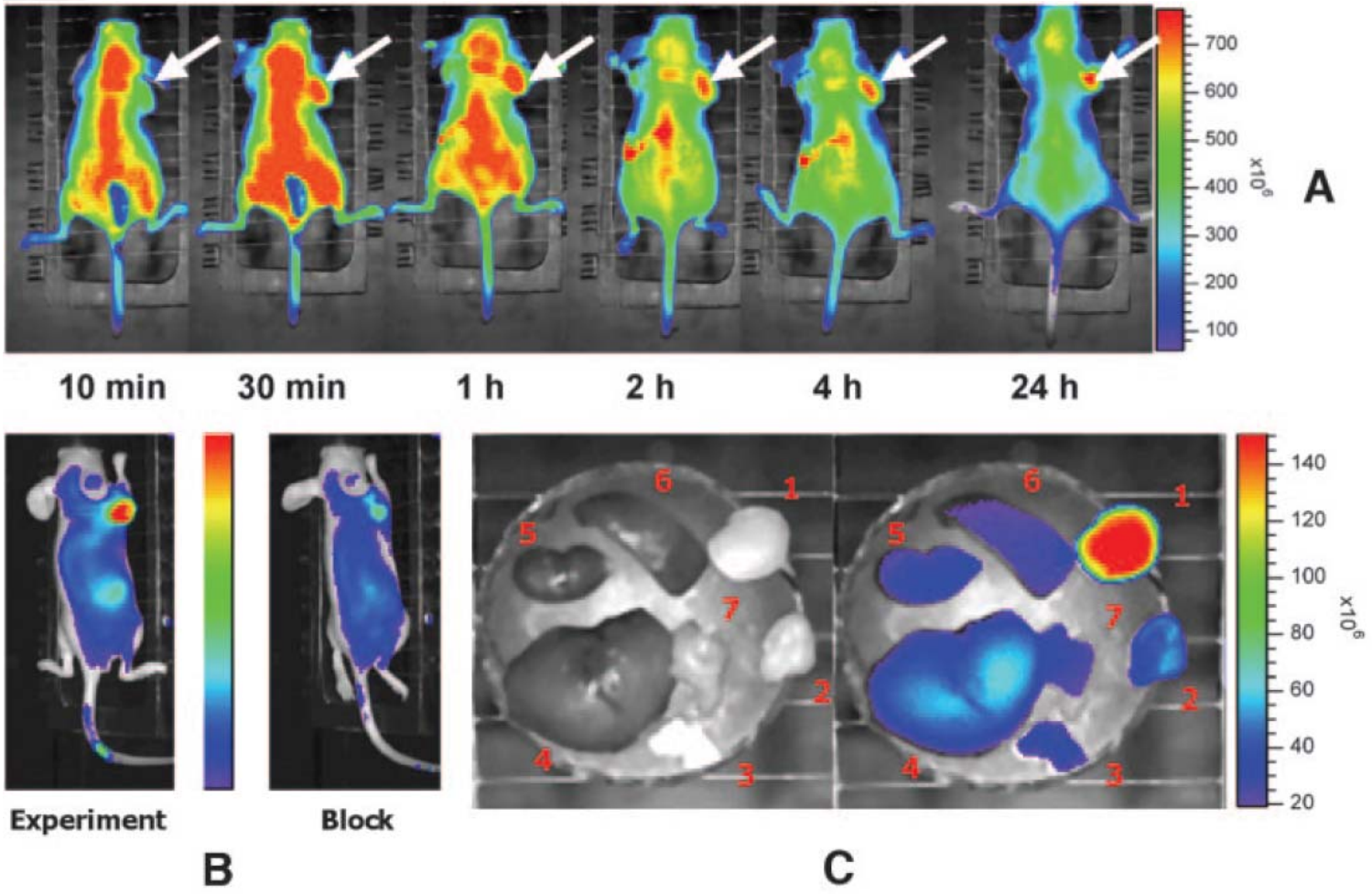
- Generation from Naturally-Occurring Bioactive Molecules
- Generation from Drugs or Drug Candidates
- **Generation from Established Molecular Imaging Probes**

***In vivo* Near-Infrared Fluorescence Imaging of Integrin $\alpha_v\beta_3$ in Brain Tumor Xenografts**

CANCER RESEARCH 64, 8009–8014, November 1, 2004



- Computer-Aided Molecular Imaging probe Development



CANCER RESEARCH 64, 8009–8014, November 1, 2004

Molecular Imaging Imaging Probe Design

- Generation from Naturally-Occurring Bioactive Molecules
- Generation from Drugs or Drug Candidates
- **Generation from Established Molecular Imaging Probes**

^{64}Cu-Labeled Alpha-Melanocyte-Stimulating Hormone Analog for MicroPET Imaging of Melanocortin 1 Receptor Expression

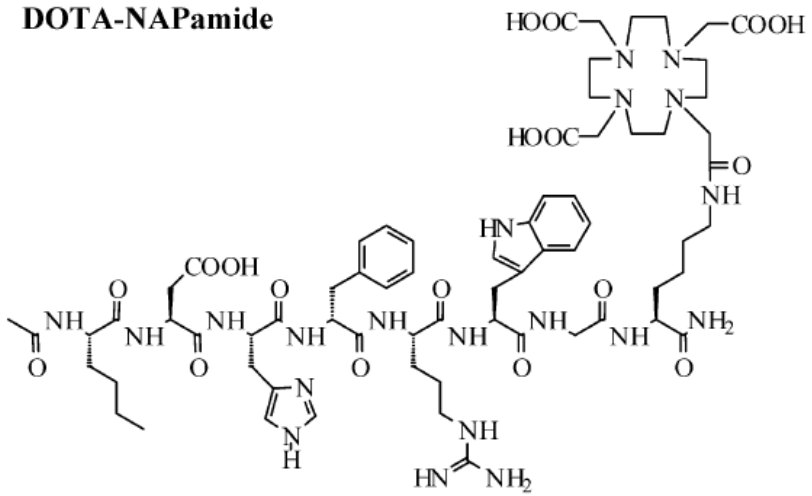
Bioconjugate Chem. **2007,** 18, 765-772

Small-Animal PET of Melanocortin 1 Receptor Expression Using a ^{18}F-Labeled α-Melanocyte-Stimulating Hormone Analog

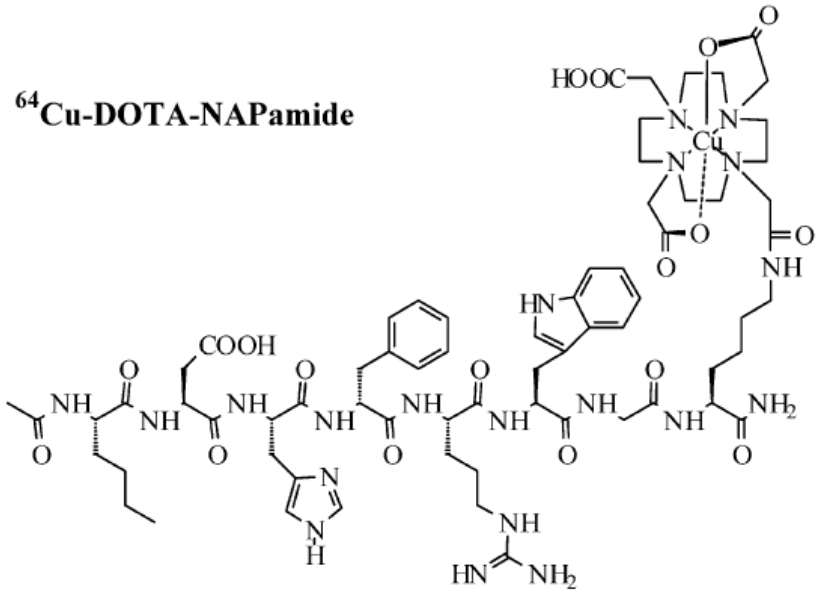
J Nucl Med 2007; 48:987–994

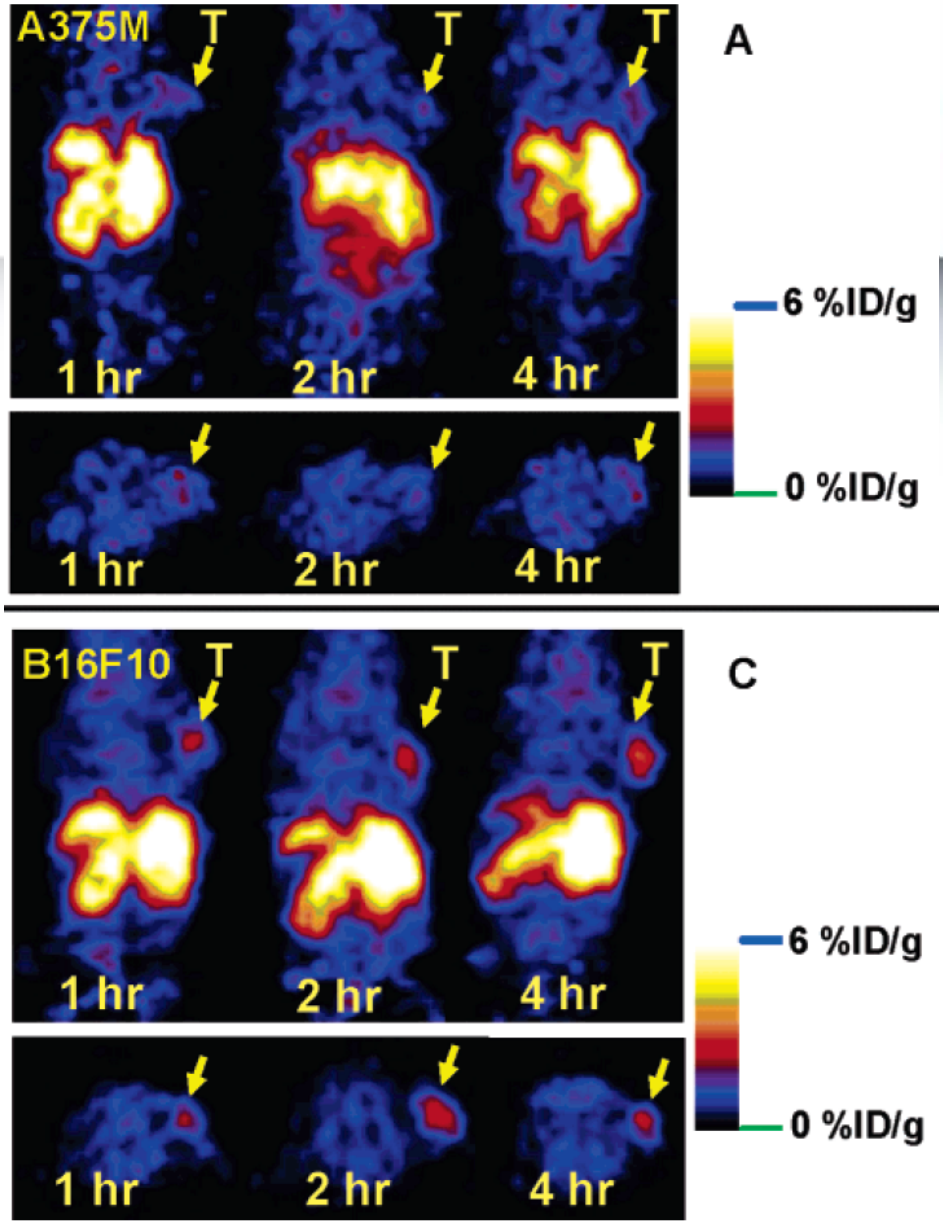
- Computer-Aided Molecular Imaging probe Development

DOTA-NAPamide



^{64}Cu-DOTA-NAPamide

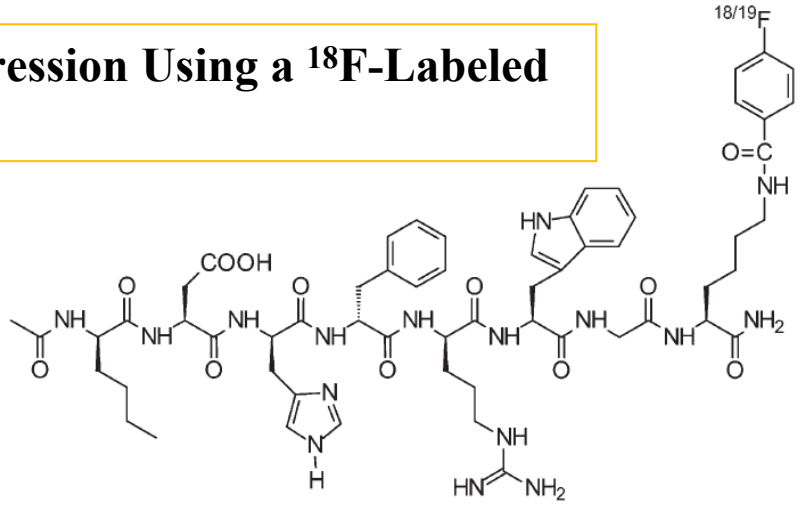


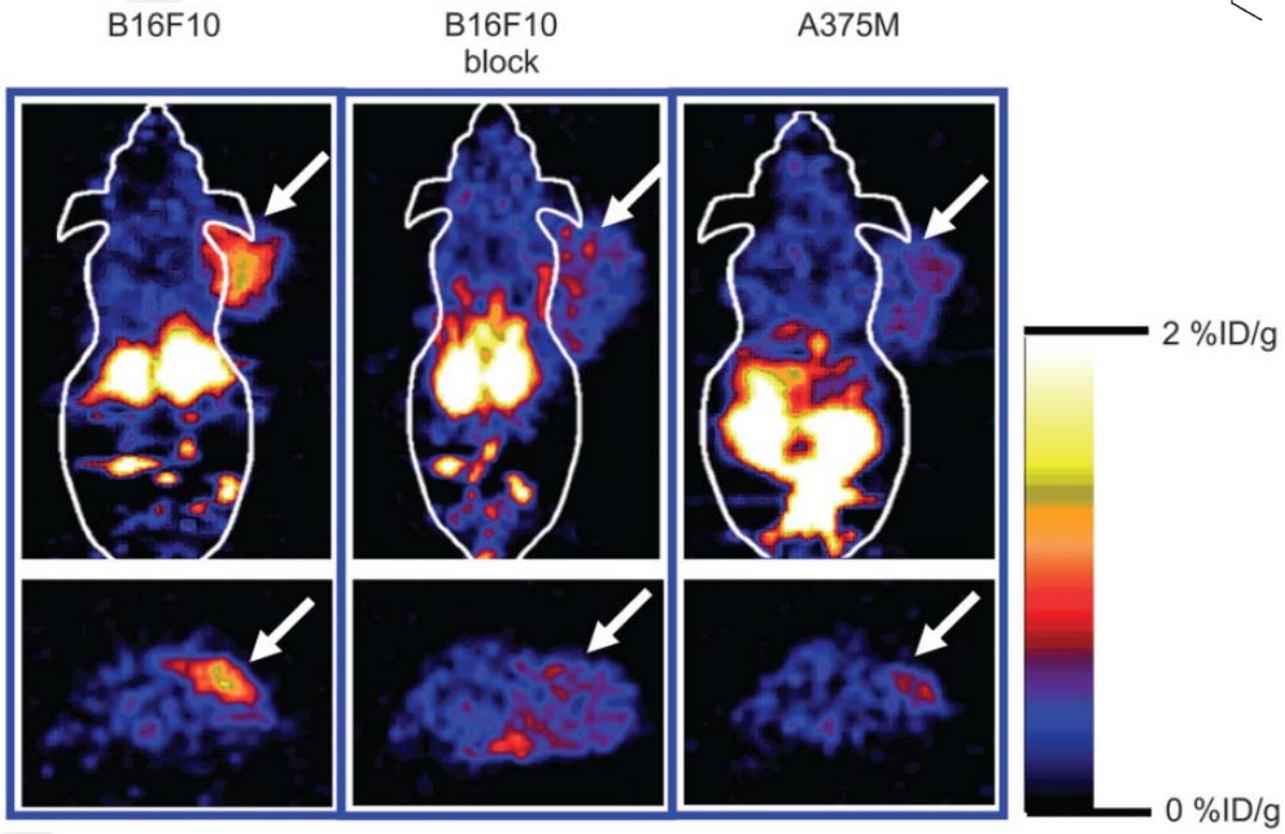


Bioconjugate Chem. **2007,** 18, 765-772

Small-Animal PET of Melanocortin 1 Receptor Expression Using a ^{18}F-Labeled α-Melanocyte-Stimulating Hormone Analog

J Nucl Med 2007; 48:987–994





Molecular Imaging Imaging Probe Design

- Generation from Naturally-Occurring Bioactive Molecules
- Generation from Drugs or Drug Candidates
- **Generation from Established Molecular Imaging Probes**

  Recently, molecular imaging and contrast agent database (MICAD) has been established by National Institutes of Health. This imaging agent database offers biomedical researchers easy access to a large number of molecular probes. It will help the researchers to quickly identify the probes for further optimization and modification, and thus facilitate the molecular imaging probe development.

- **http://www.ncbi.nlm.nih.gov/books/NBK5330/**

- Computer-Aided Molecular Imaging probe Development

Molecular Imaging Imaging Probe Design

- Generation from Naturally-Occurring Bioactive Molecules
- Generation from Drugs or Drug Candidates
- Generation from Established Molecular Imaging Probes
- **Computer-Aided Molecular Imaging probe Development**

  Computer-aided drug design (CADD) has been widely used in pharmaceutical development. It uses computational chemistry to discover, enhance, or study drugs and related biologically active molecules.

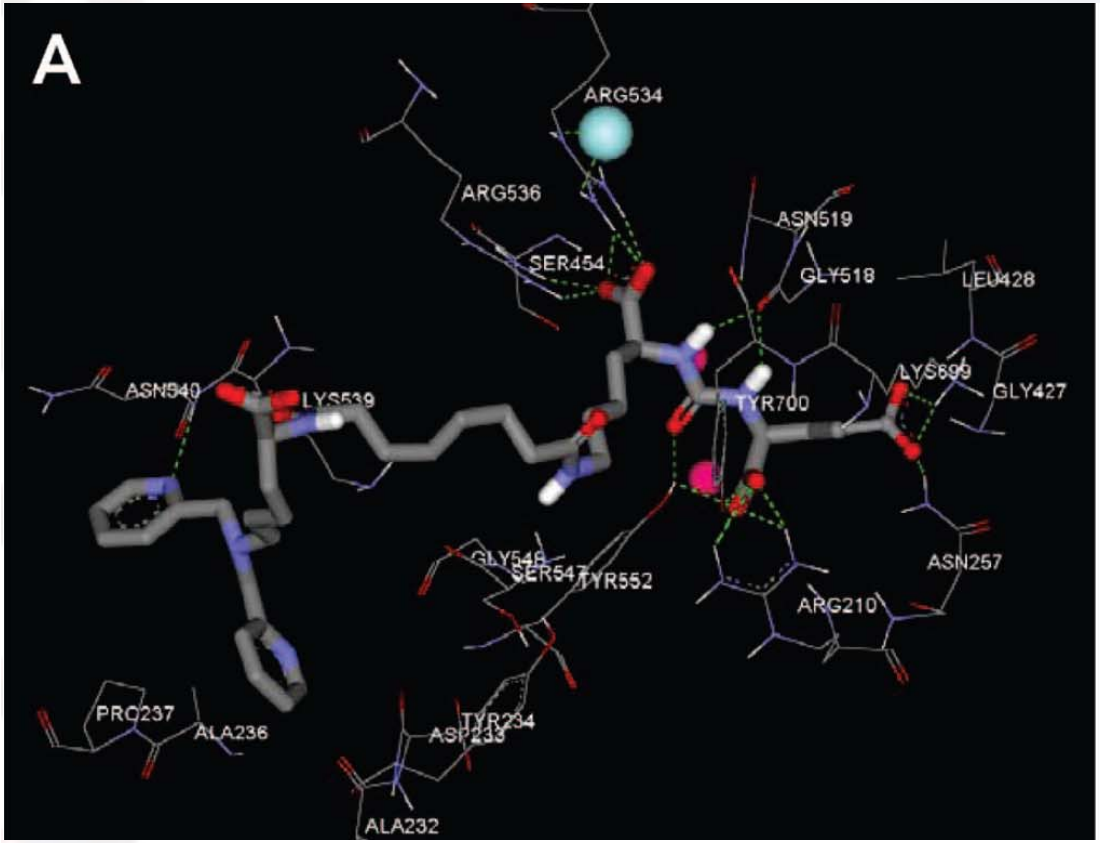
Synthesis and Evaluation of Technetium-99m- and Rhenium-Labeled Inhibitors of the Prostate-Specific Membrane Antigen (PSMA)

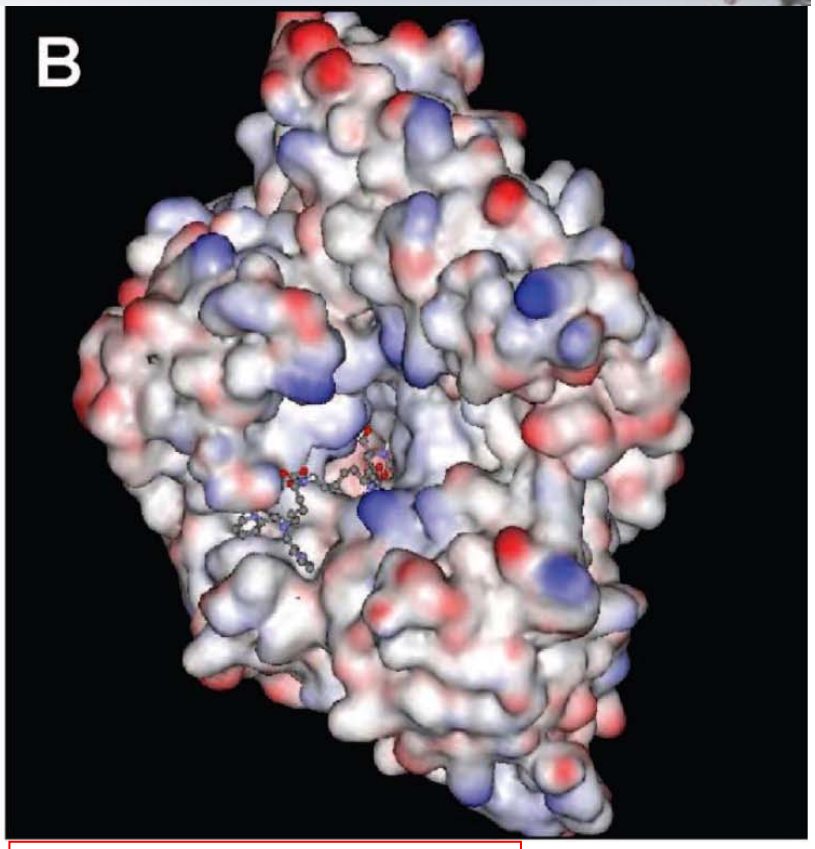
J. Med. Chem. **2008,** *51*, 4504–4517

Synthesis and Evaluation of a Near-Infrared Fluorescent Non-Peptidic Bivalent Integrin $\alpha_v\beta_3$ Antagonist for Cancer Imaging

Bioconjugate Chem. **2010,** *21*, 270–278

Synthesis and Evaluation of Technetium-99m- and Rhenium-Labeled Inhibitors of the Prostate-Specific Membrane Antigen (PSMA)

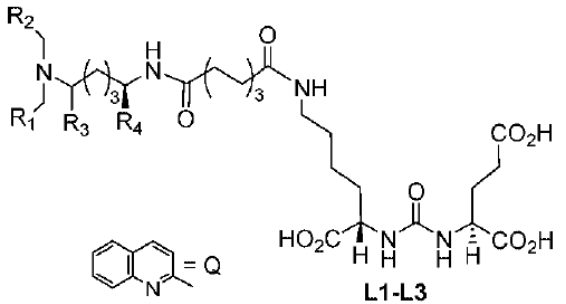




protein data bank (PDB)

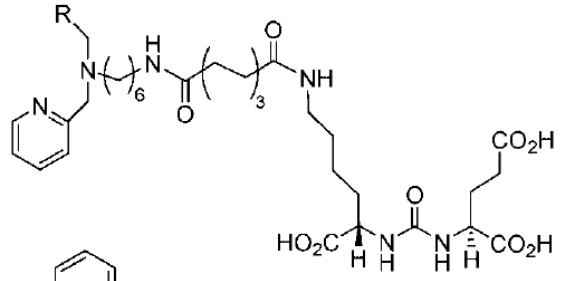
J. Med. Chem. **2008,** *51,* 4504–4517

Chart 2. Urea-Based PSMA Ligands **L1**−**L7**

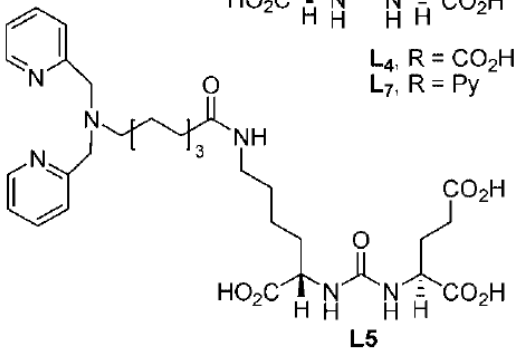


L1-L3

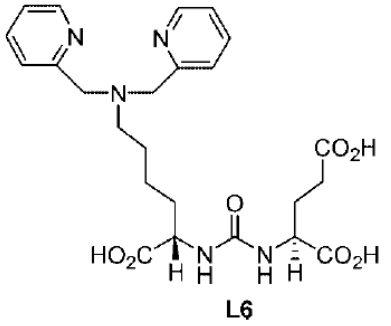
L1, $R_1 = R_2 = Py$, $R_3 = H$, $R_4 = CO_2H$
L2, $R_1 = R_2 = Q$, $R_3 = H$, $R_4 = CO_2H$
L3, $R_1 = R_2 = Py$, $R_3 = CO_2H$, $R_4 = H$



L4, R = CO_2H
L7, R = Py



L5

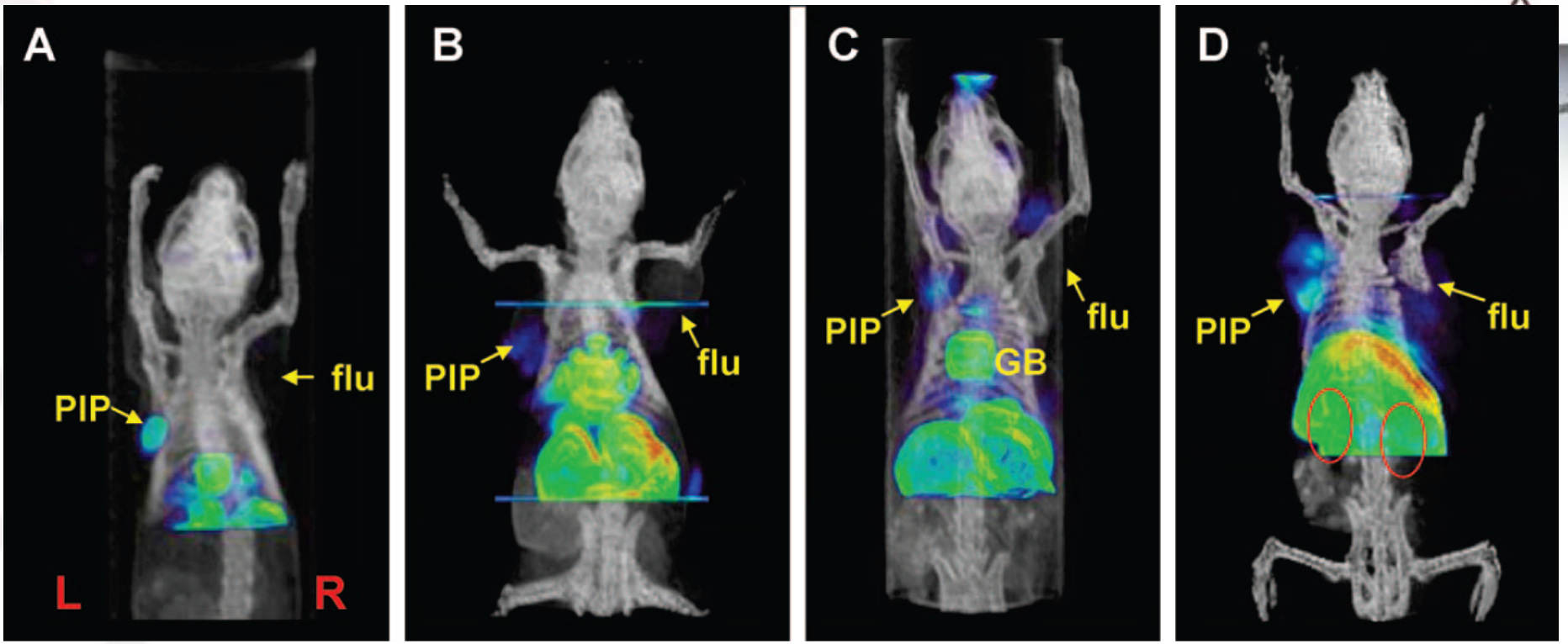


L6

Table 1. PSMA Inhibitory Activity and Calculated ClogD

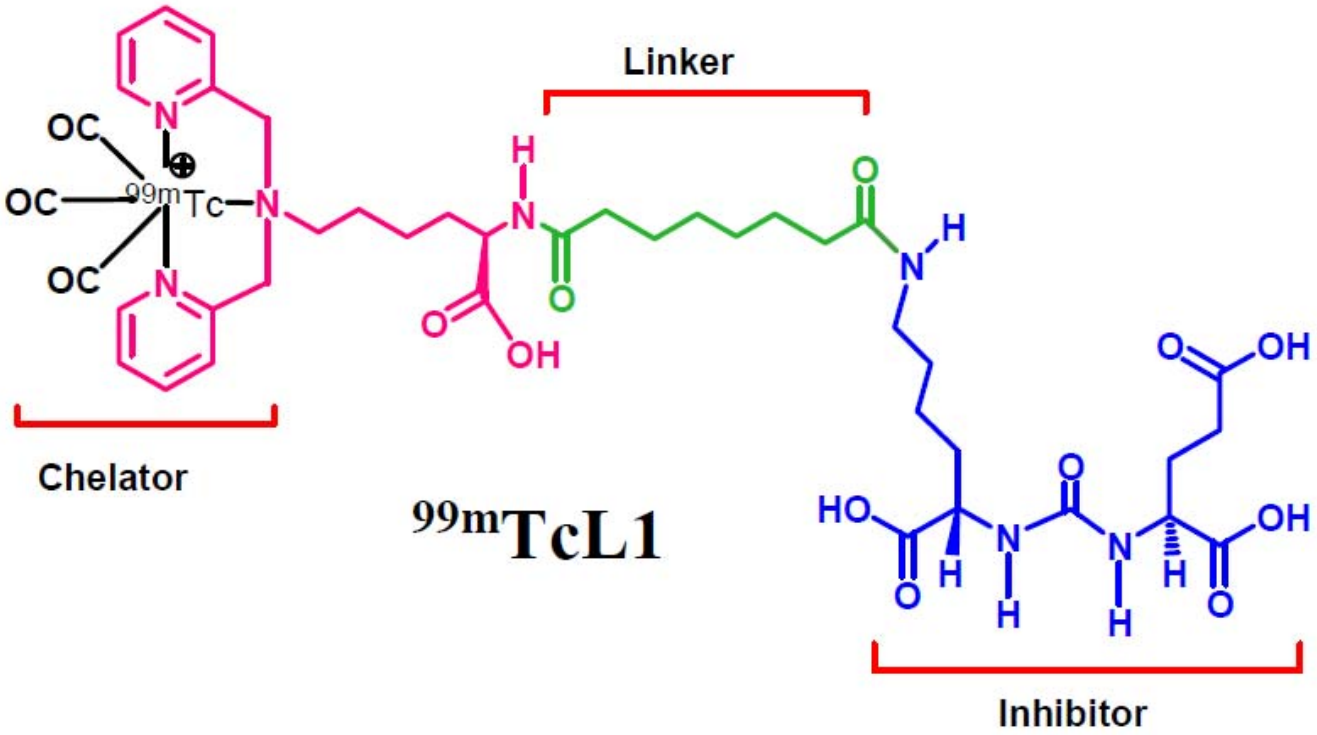
	K_i [nM]	95% CI[a]	ClogD
L1	15.25	7.93	−7.69
Re**L1**	10.75	3.81	
L2	0.17	0.05	−3.91
Re**L2**	0.50	0.07	
L3	1.08	0.14	−7.19
Re**L3**	10.34	3.76	
L4	2.54	0.60	−6.13
Re**L4**	0.17	0.08	
L5	1.86	0.21	−5.05
Re**L5**	0.91	0.44	
L6	7.53	5.65	−6.25
Re**L6**	199.56	135.26	
L7	0.45	0.25	−5.61
Re**L7**	2.06	0.25	
PMPA	0.20	0.06	−8.65

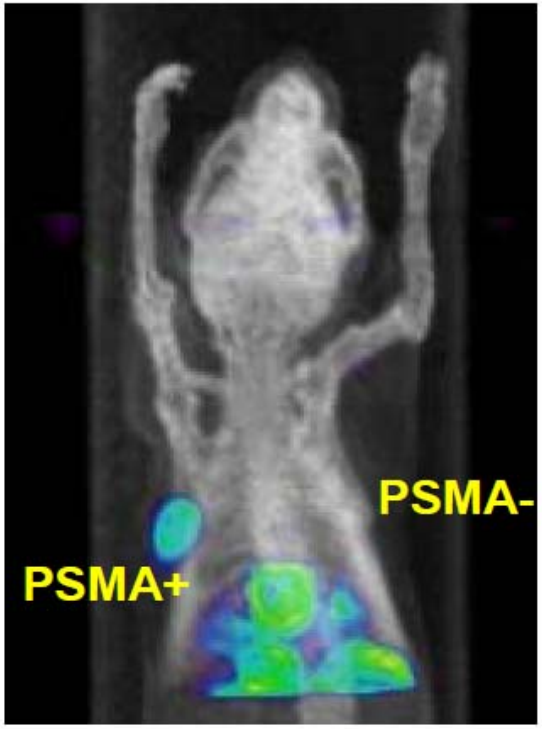
J. Med. Chem. **2008,** *51,* 4504–4517



SPECT-CT imaging of tumor bearing mice with [^{99m}Tc]**L1-L4**

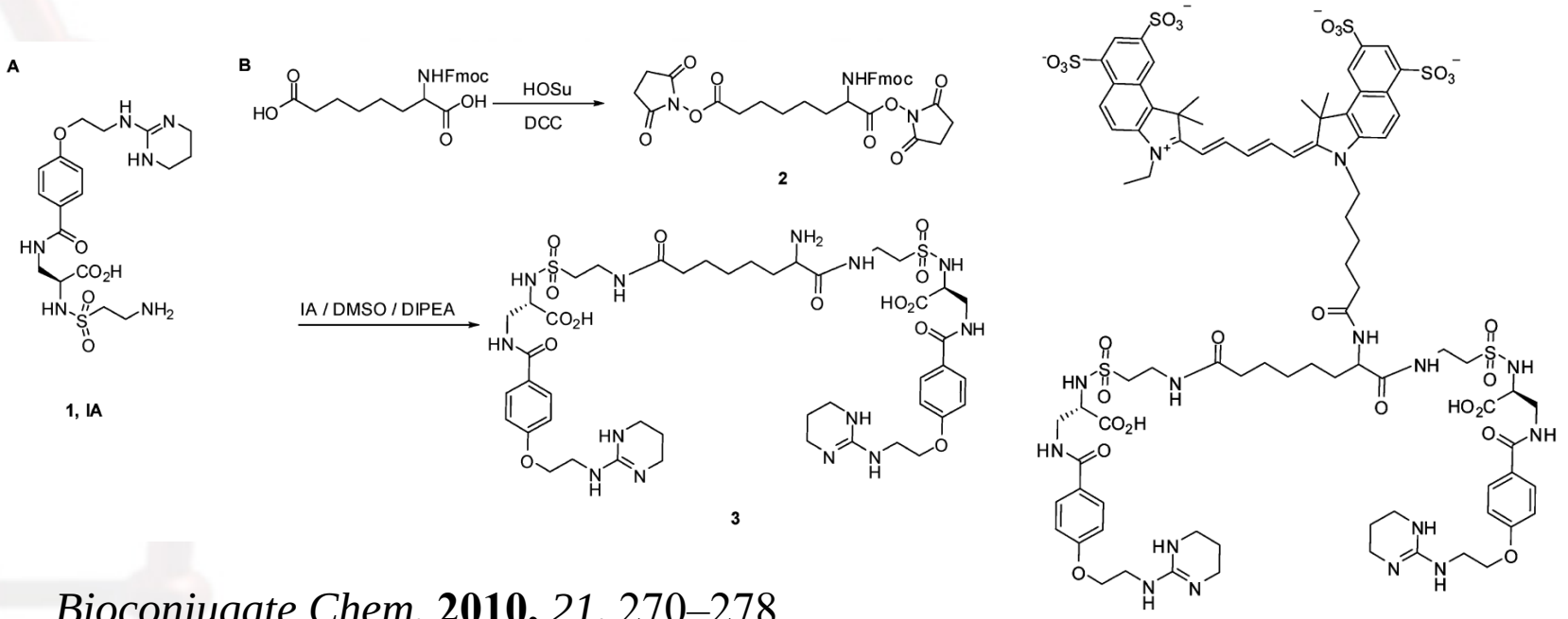
J. Med. Chem. **2008,** *51,* 4504–4517



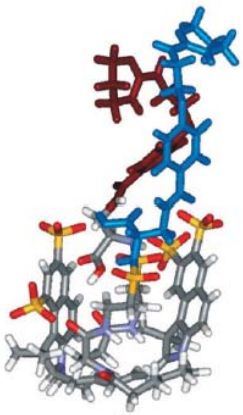


J. Med. Chem. **2008,** *51*, 4504–4517

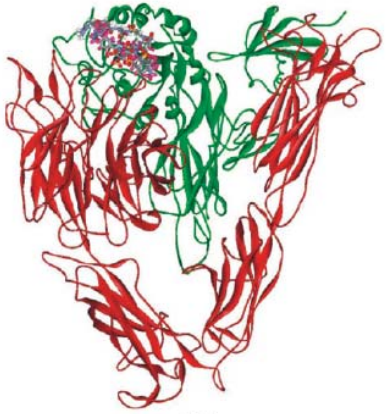
Synthesis and Evaluation of a Near-Infrared Fluorescent Non-Peptidic Bivalent Integrin $\alpha_v\beta_3$ Antagonist for Cancer Imaging



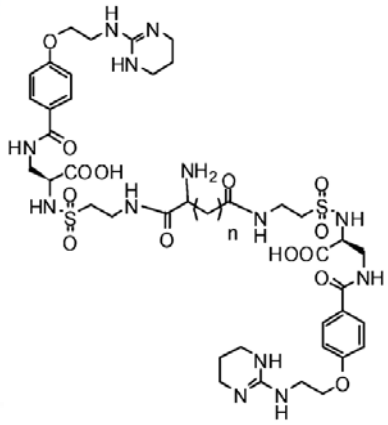
Bioconjugate Chem. **2010,** *21,* 270–278



A



B

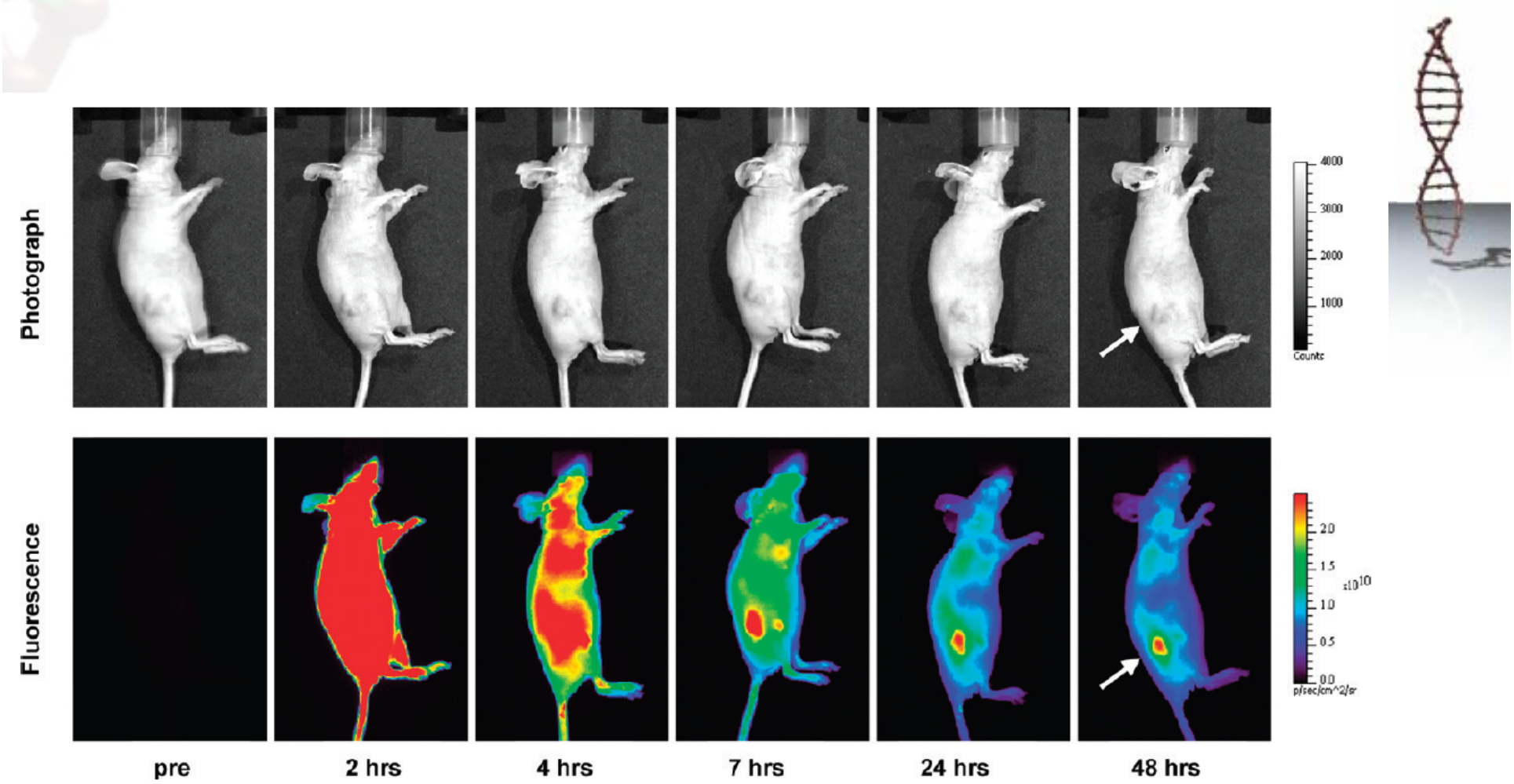


IA dimer

Conformational Energy of Bivalent IA and NIR Imaging Probe

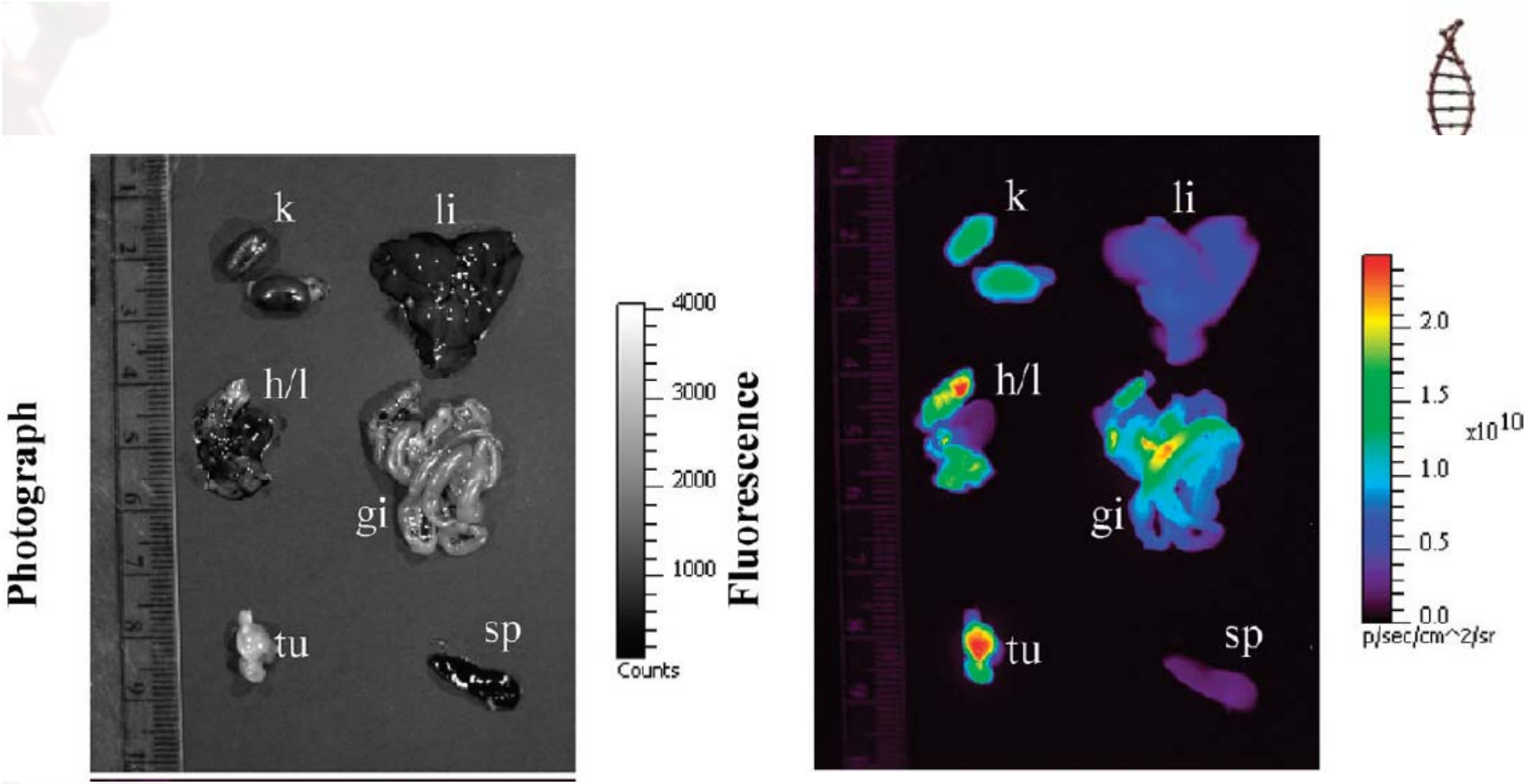
Form of IA	Linker composition	Linker Length	Conformational Energy (kcal/mol)
Monomer	N/A	N/A	-17.0 ± 2.0
Dimer (IA-X-IA) X = linkers	$(-C-)_n$	n = 1 n = 2 n = 3 n = 4 n = 5	-37.0 ± 2.0 -50.0 ± 2.0 -53.0 ± 2.0 -49.0 ± 2.0 -60.0 ± 2.0
Near Infrared (NIR) (IA-X-P-IA) X = linker; P =probe molecule	(-C-)n	n =5	-53.0 ± 2.0

Bioconjugate Chem. **2010,** *21*, 270–278



In vivo fluorescence imaging of live U-87 xenograft tumor-bearing mice. Photograph images (top panel) of representative mouse subject at selected time points.

Bioconjugate Chem. **2010,** *21,* 270–278



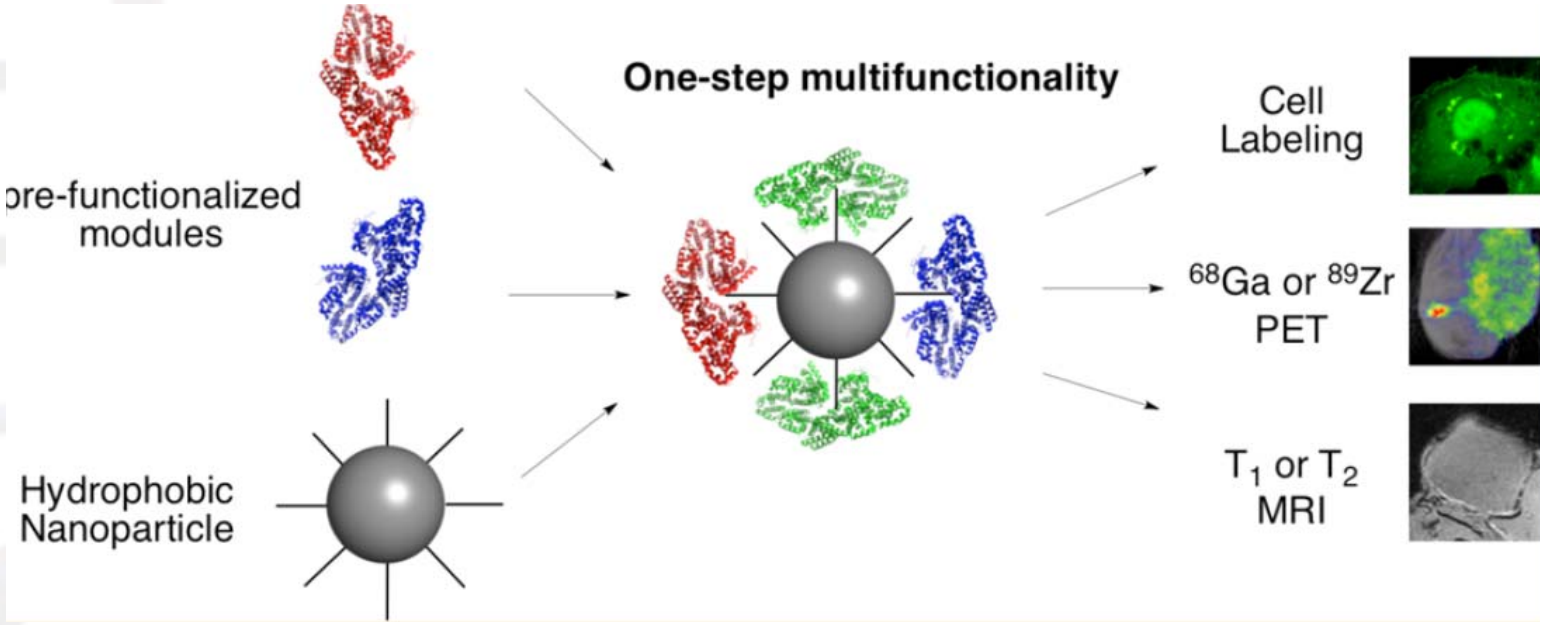
Representative *ex* V*i*V*o* NIR imaging of major internal organs harvested from a xenograft tumor-bearing mice at 24 h post i.v. injection of bivalent IA-Cy5.5.

Bioconjugate Chem. **2010,** *21*, 270–278

Molecular Imaging Imaging Probe Design

Parallel Multifunctionalization of Nanoparticles: A One-Step Modular Approach for in Vivo Imaging

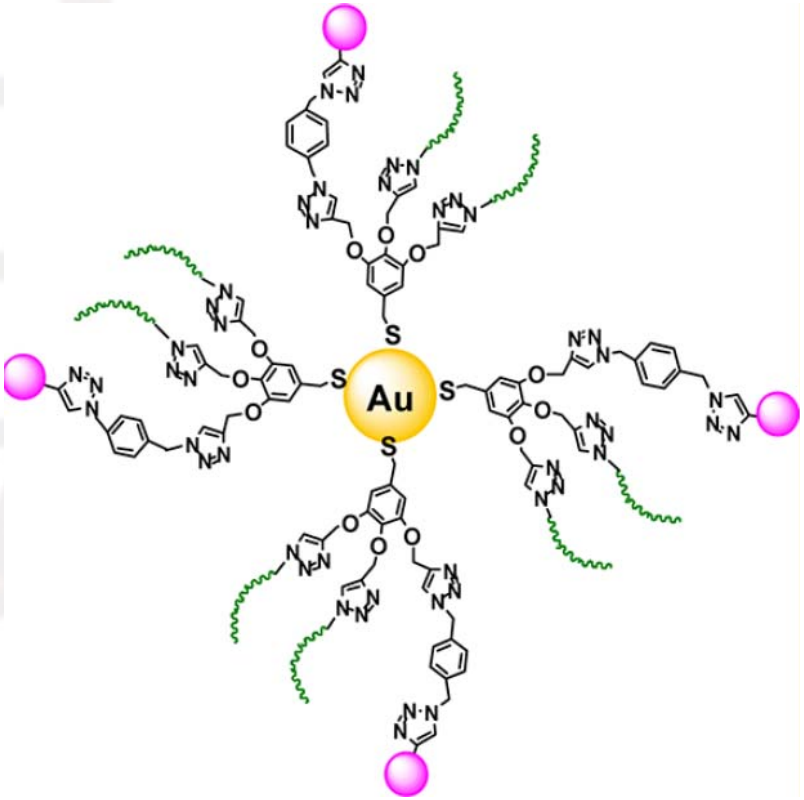
Bioconjugate Chem. 2015, 26, 153−160



Molecular Imaging Imaging Probe Design

"Click" Star-Shaped and Dendritic PEGylated Gold Nanoparticle-Carborane Assemblies

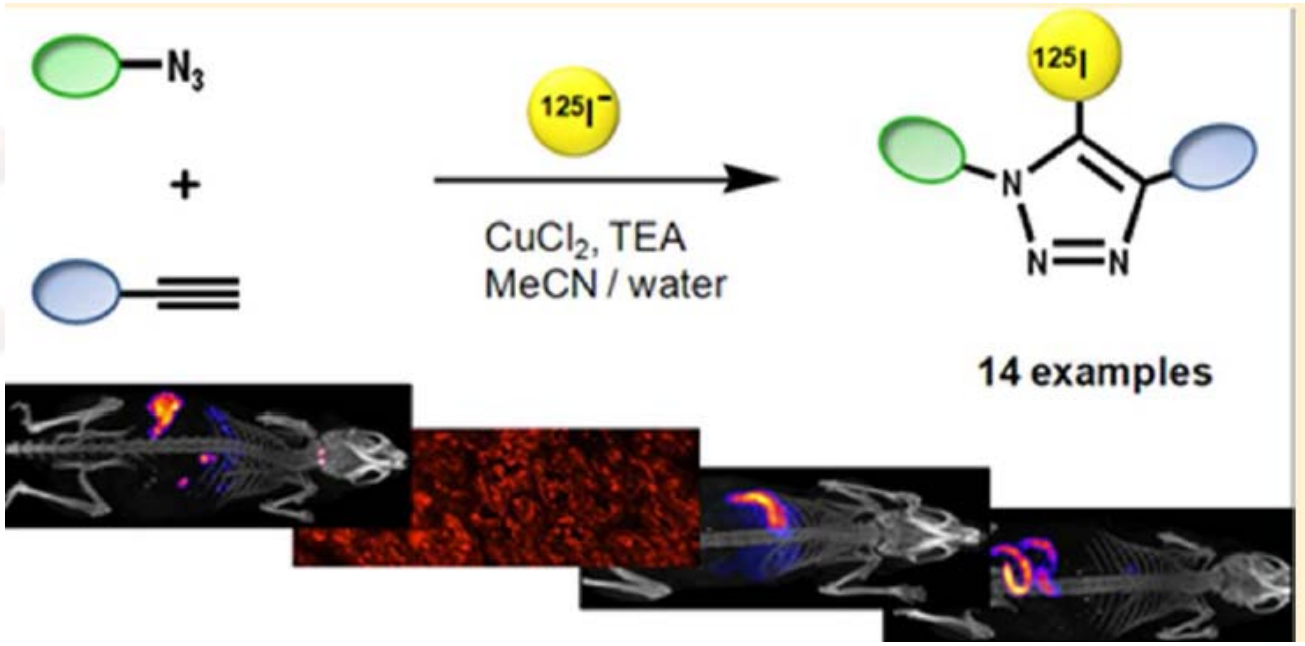
Inorg. Chem. 2013, 52, 11146−11155

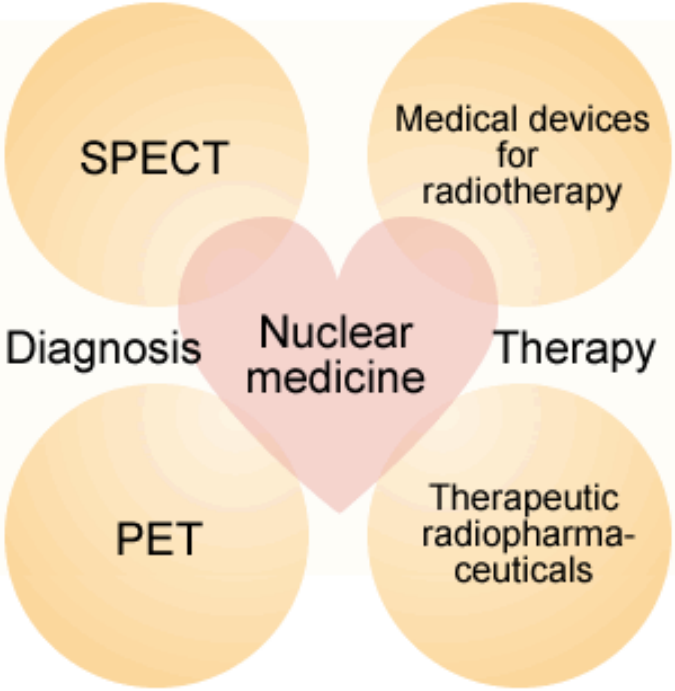


Molecular Imaging Imaging Probe Design

A One-Pot Three-Component Radiochemical Reaction for Rapid Assembly of ^{125}I-Labeled Molecular Probes

J. Am. Chem. Soc. 2013, 135, 703−709




SPECT
Medical devices for radiotherapy
Diagnosis
Nuclear medicine
Therapy
PET
Therapeutic radiopharma-ceuticals


Thank You