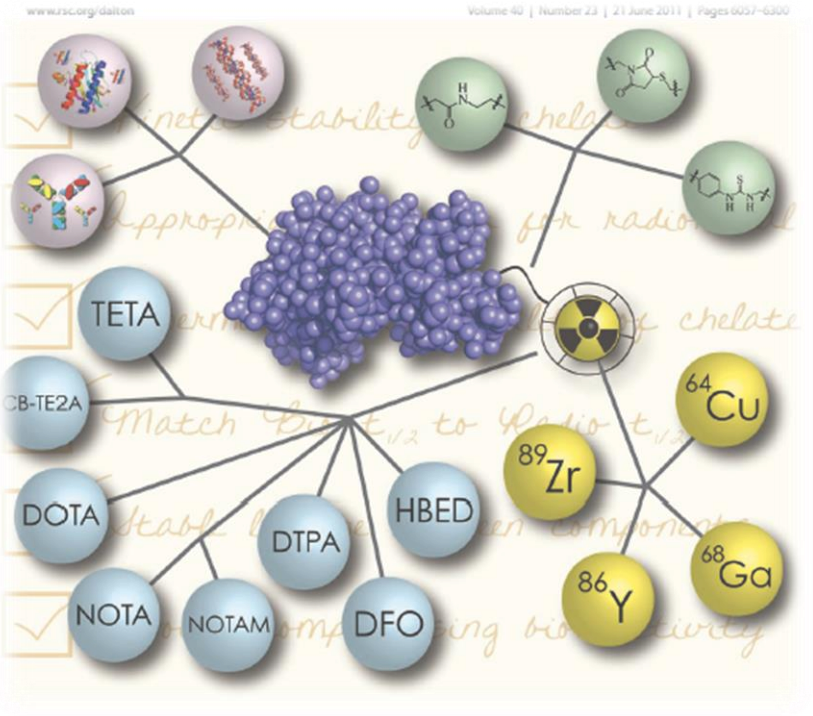
Le rôle du chimiste dans le développement de l'imagerie médicale



Franck DENAT

ICMUB - UMR CNRS 6302
9 avenue Alain Savary
21078 DIJON, FRANCE

Dijon
26 octobre 2016

Plan

1. Introduction sur l'imagerie moléculaire
2. Imagerie optique et fluorophores organiques
3. IRM et agents de contraste
4. Imagerie nucléaire (PET – SPECT)

   4.1 Marquage direct de petites molécules

   4.2 Marquage indirect : bioconjugués

   4.3 Nanoparticules
5. Vers la multimodalité et le théranostic

Imagerie moléculaire

L'imagerie moléculaire peut être définie comme «...la caractérisation et l'évaluation des processus biologiques chez l'animal et l'homme, au niveau cellulaire et moléculaire, en utilisant des procédés d'imagerie à distance. »

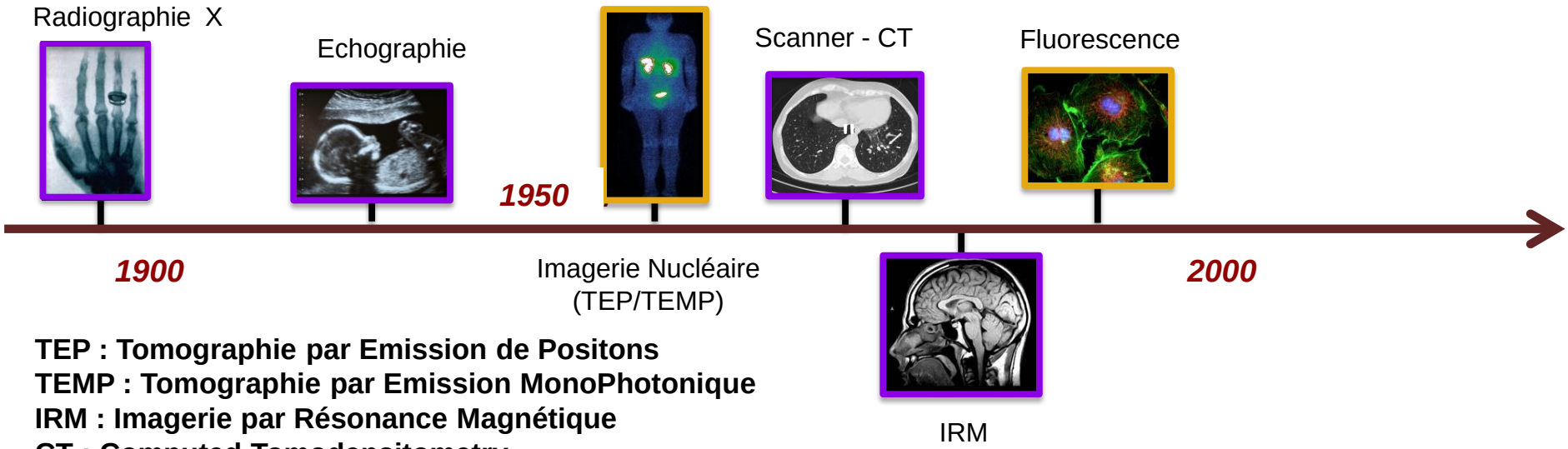
"Seeing is believing" est la force motrice de l'imagerie moléculaire, et la chimie joue un rôle central dans le développement de cette thématique interdisciplinaire. Les découvertes exceptionnelles dans divers domaines de la chimie permettent de fournir les outils pour la conception de sondes imageantes « intelligentes » nécessaires pour étudier des processus biologiques spécifiques, à la fois chez l'animal et chez l'homme.

Traduit de T.J. Meade, S. Aime

Un outil pour :

- **Diagnostic**
- **Suivi de l'évolution d'une maladie**
- **Développement de nouveaux médicaments "drug discovery"**

Imagerie moléculaire



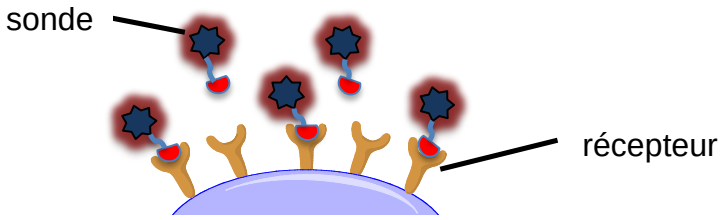
TEP : Tomographie par Emission de Positons
TEMP : Tomographie par Emission MonoPhotonique
IRM : Imagerie par Résonance Magnétique
CT : Computed Tomodensitometry
FMT : Fluorescence Molecular Tomography

Imagerie structurale : **informations anatomiques, morphologiques**
Imagerie fonctionnelle : **détection d'un évènement d'ordre cellulaire ou moléculaire**

Imagerie Fonctionnelle

Visualisation et quantification d'évènements à l'échelle cellulaire

Vectorisation spécifique d'un marqueur



Surexpression d'un récepteur

Weissleder, R.; Pittet, M. J. *Nature* 2008, *452* (7187), 580-589

Imagerie moléculaire

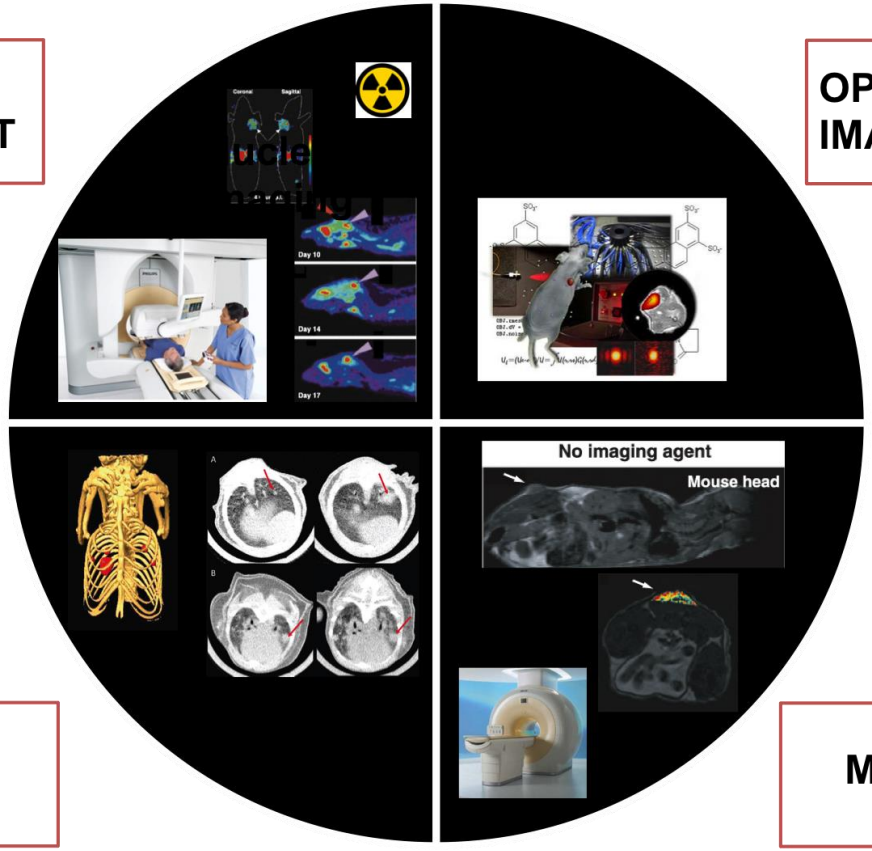
"A hallmark of modern medicine"

PET SPECT

- Pas de limite de pénétrabilité
- Excellente sensibilité
- Quantitatif (PET)
- Coût
- Résolution spatiale
- Radioactivité

OPTICAL IMAGING

- Coût
- Facilité de mise en oeuvre
- Sensibilité élevée
- Possibilité de multiplexage
- Pénétrabilité limitée
- Résolution spatiale
- Autofluorescence



CT

- Pas de limite de pénétrabilité
- Résolution spatiale élevée
- Résolution temporelle
- Faible sensibilité
- Information anatomique uniquement
- Applications en imagerie moléculaire limitées
- Radiations

MRI

- Pas de limite de pénétrabilité
- Résolution spatiale élevée
- Quantitatif
- Faible sensibilité
- Coût
- Faible résolution temporelle

James, M. L.; Gambhir, S. S. *Physiol. Rev.* **2012**, 92, 897-965.

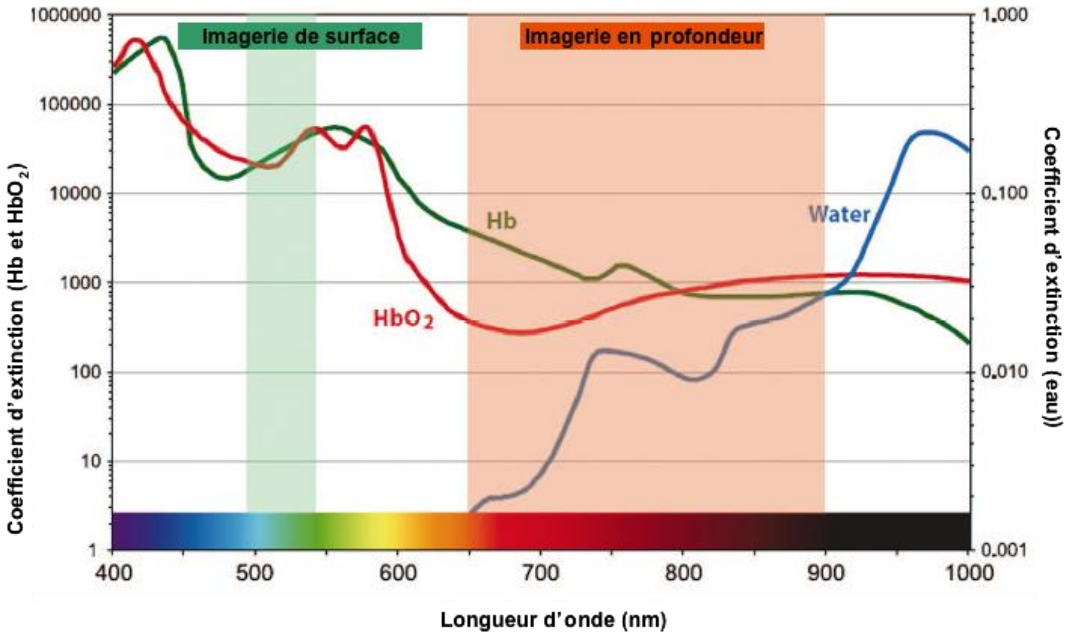
Combiner ≠ techniques d'imagerie : multimodalité

Imagerie optique

- Intérêt de l'imagerie optique

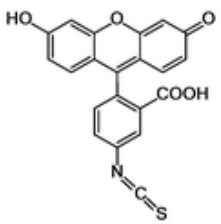
Faible coût, sensibilité élevée, pas de rayonnement : largement utilisé pour l'imagerie *in vitro*
Pb : pénétrabilité (cm) qui limite l'utilisation *in vivo*

- **vers des sondes émettant dans le proche infra rouge (fenêtre thérapeutique) où l'absorption des tissus est moindre**
- **limité cependant à une imagerie *in vivo* petit animal**
- **intérêt grandissant en clinique pour l'assistance à la chirurgie**

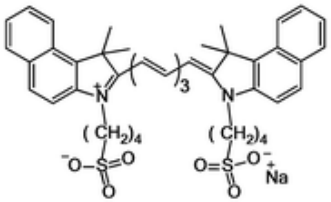


Fluorophores organiques

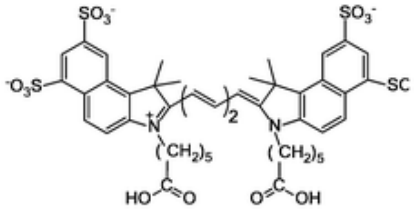
- Synthèse organique



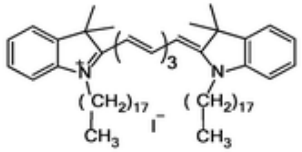
FITC[44]
λ_{em} = 520 nm



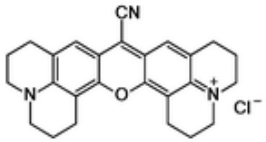
ICG[19]
λ_{em} = > 800 nm



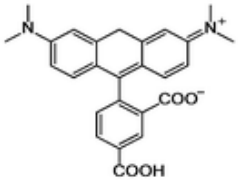
Cy-5[78]
λ_{em} = 660 nm



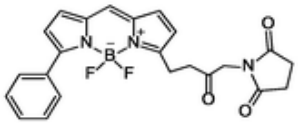
DiR[34]
λ_{em} = 780 nm



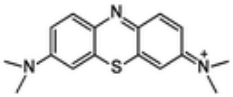
Rhodamine 800[79]
λ_{em} = 705 nm



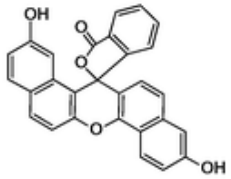
5-TAMRA[80]
λ_{em} = 570 nm



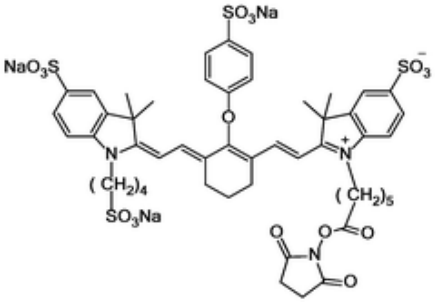
Bodipy 6RG[54]
λ_{em} = 550 nm



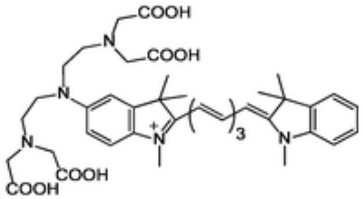
Methylene blue[58,81]
λ_{em} = 600–900 nm



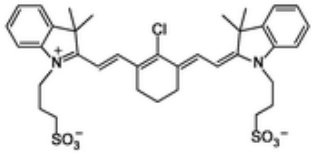
Naphtho-fluorescein[82]
λ_{em} = 665 nm



IRdye800CW[83]
λ_{em} = 790 nm



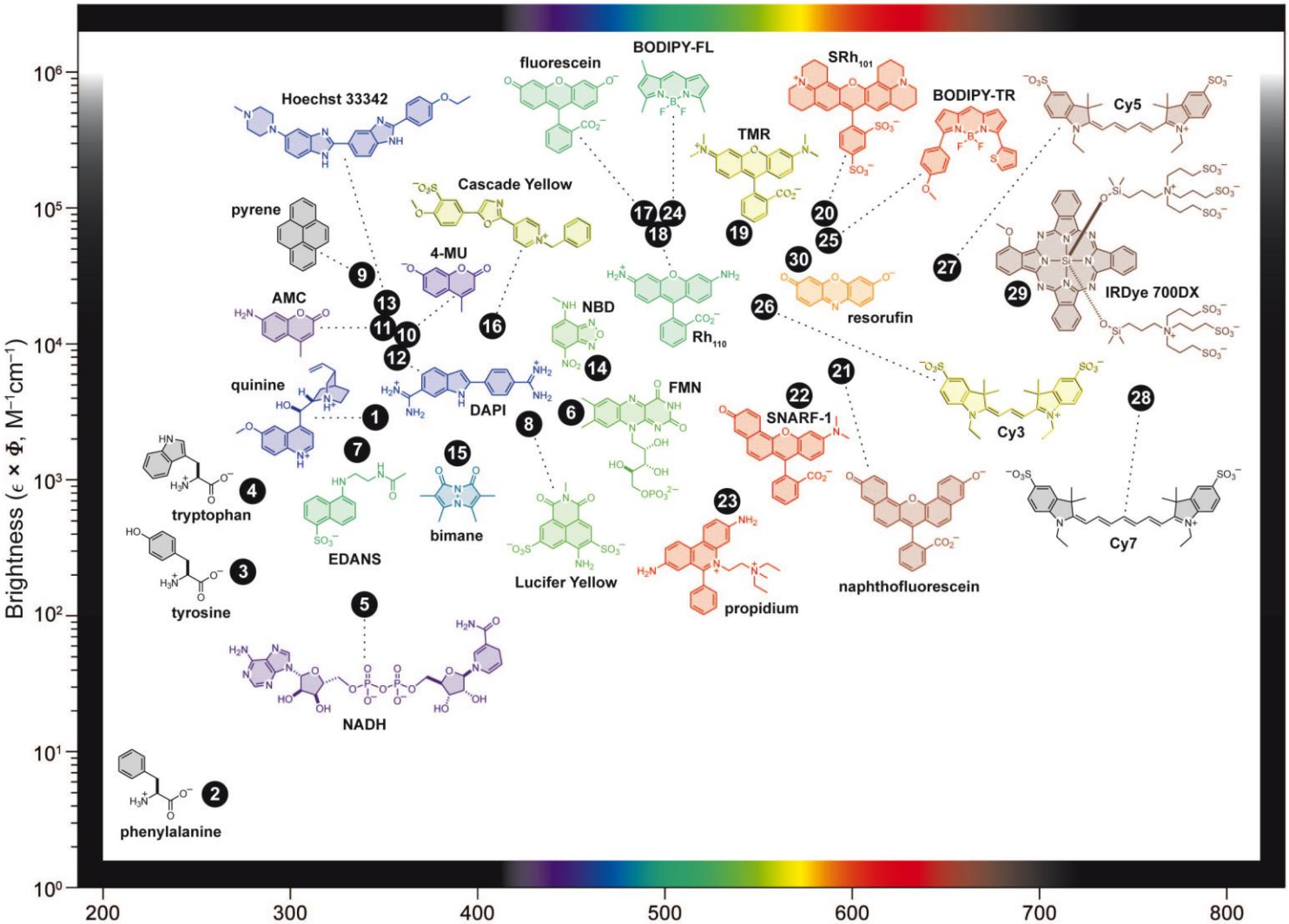
LS479[84]
λ_{em} = 690 nm



IR-783[69]
λ_{em} = 780 nm

A. Vollrath, S. Schubert, U. S. Schubert *J. Mater. Chem. B*, **2013**, 1, 1994

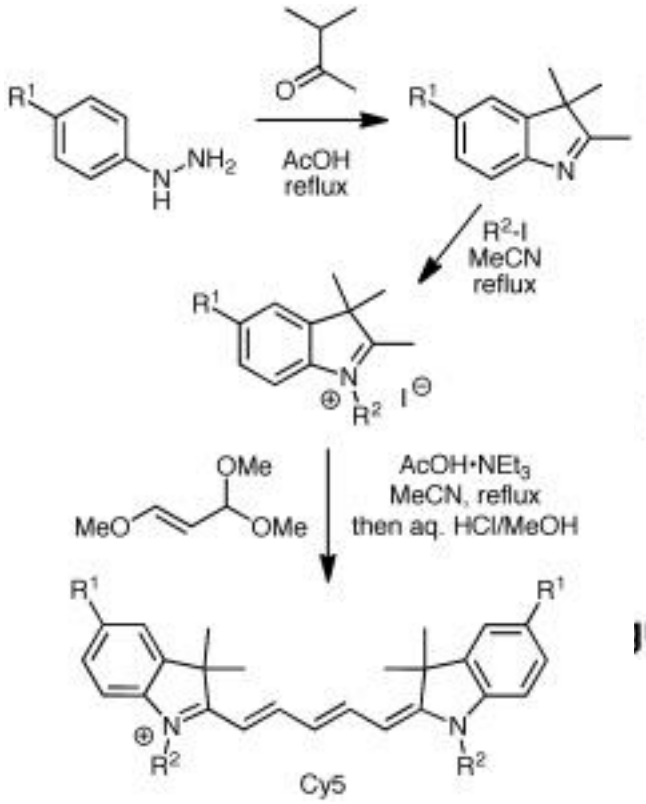
Fluorophores organiques

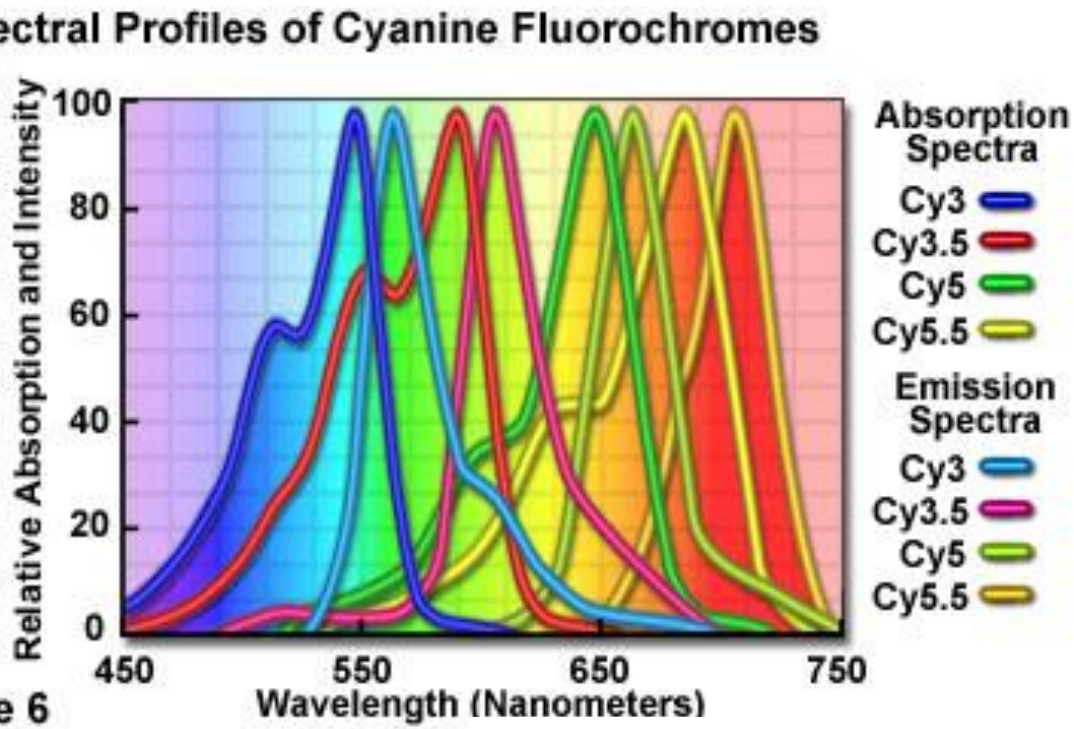


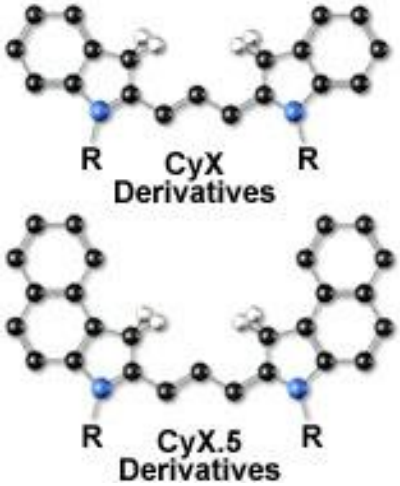
Lavis, L. D., Raines, R. T. *ACS Chem. Biol.* 2008, *3*, 142

Fluorophores organiques

- **Cyanines**



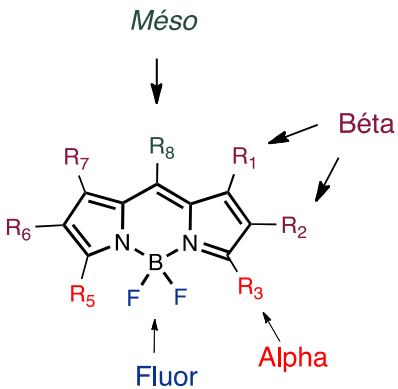




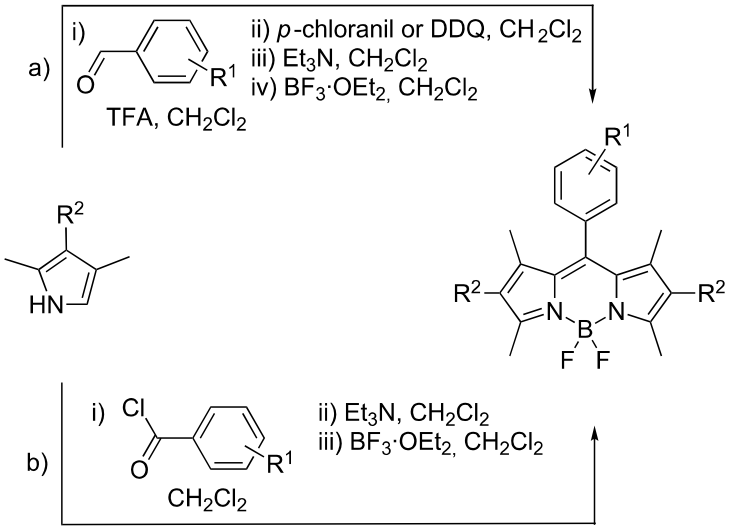
http://www.olympusmicro.com/primer/techniques/confocal/fluorophoresintro.html

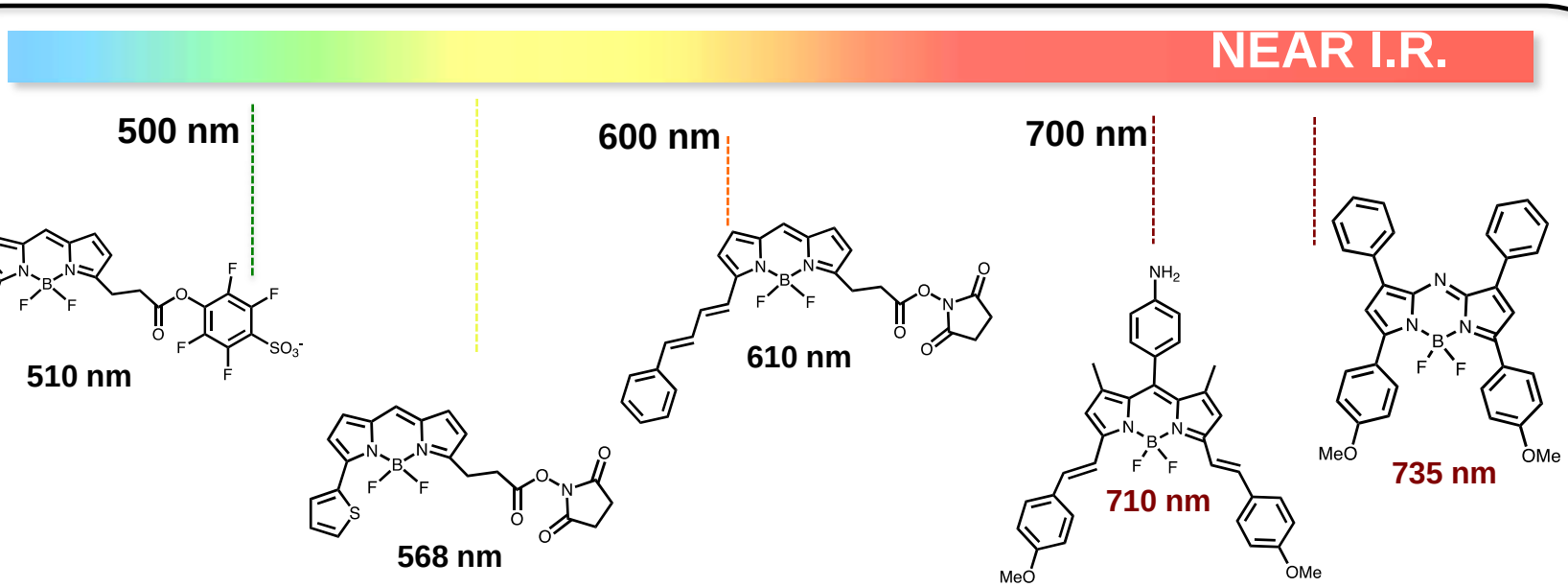
Fluorophores organiques

- BODIPY



- Bonne stabilité
- **Bande d'émission étroite**
- Rendement quantique élevé
- **Propriétés optiques facilement ajustables**





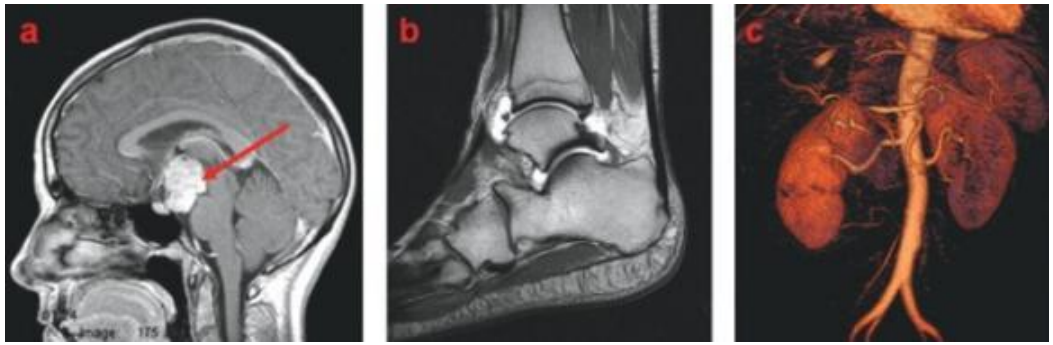
Handbook of Fluorescent Probes and Research Products, 9th ed, Molecular Probes, **2002.**

Akkaya, E. U. and coll. *Org. Lett*. 2005, *7*, 5187.

Agents de contraste pour l'IRM

- Intérêt de l'Imagerie par Résonance Magnétique

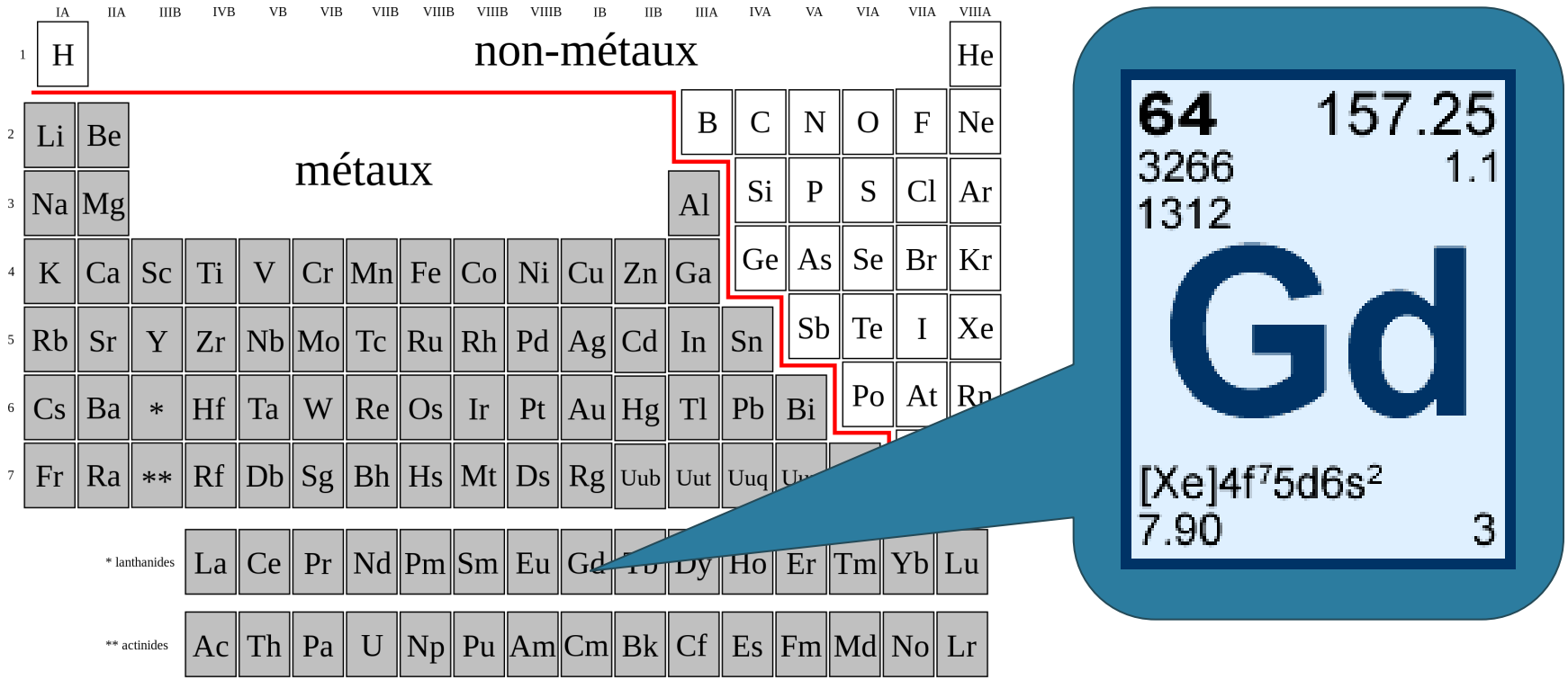
Imagerie non invasive permettant la visualisation de nombreux organes du corps humain (cerveau, cœur, poumon,…), tissus, vaisseaux…
➢Intérêt diagnostique : localisation et suivi de l'évolution de tumeurs, pathologies ostéo-articulaires, angiographie…



- Principe de l'Imagerie par Résonance Magnétique

Basée sur la résonance magnétique des noyaux d'hydrogène des molécules d'eau (70% du corps humain), c'est-à-dire leur réponse à l'application d'un champ magnétique (aimant) et d'une excitation électromagnétique.
Le contraste dépend de la concentration relative en eau et peut être augmenté en agissant sur les temps de relaxation des spins nucléaires :
➢Injection par voie IV d'un agent de contraste

Agents de contraste pour l'IRM

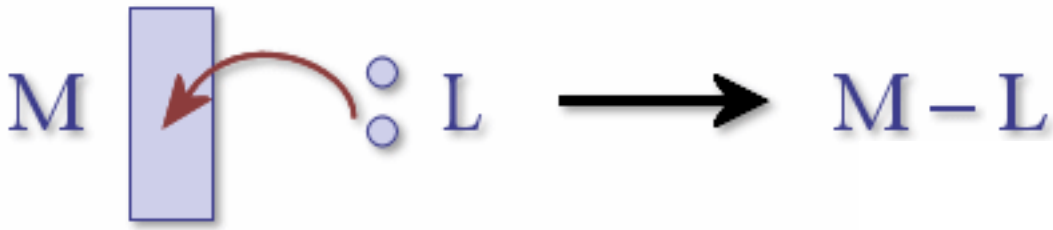


Ion Gd^{3+} : hautement paramagnétique (7 électrons célibataires)

☺ **-Elément "idéal" pour l'élaboration d'agents de contraste IRM**

☹ **-Gd^{3+} "ressemble" à Ca^{2+}, important dans l'équilibre de l'organisme : Gd^{3+} toxique, nécessité d'utiliser un complexe de gadolinium qui sera éliminé par voie rénale**

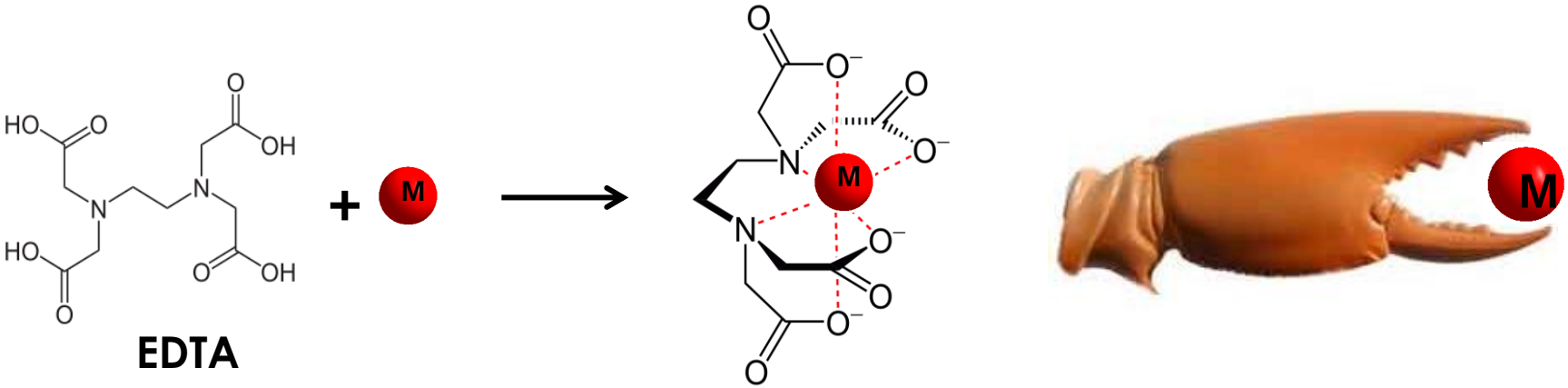
Chimie de coordination



Métal = acide de Lewis **Ligand = base de Lewis**

Métal + Ligand(s) = complexe

Ces ligands peuvent être monodentes ou polydentes suivant qu'ils coordinent le cation métallique par un ou plusieurs atomes
Les ligands polydentes sont également appelés « chélates »



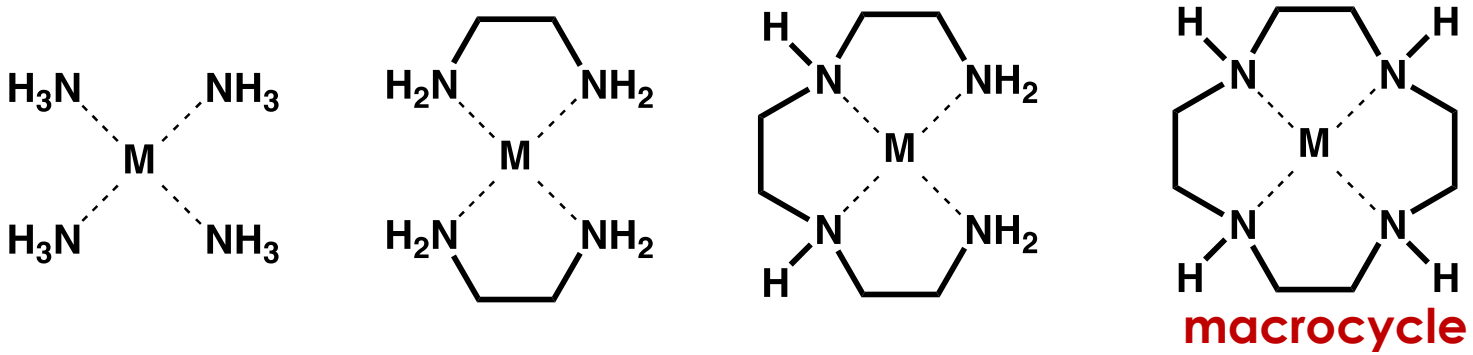
Chimie de coordination

La formation d'un complexe peut être schématisée par une succession d'équilibres

$$M + L \rightleftharpoons ML$$
$$ML + L \rightleftharpoons ML_2$$
$$ML_{n-1} + L \rightleftharpoons ML_n$$

auxquels sont associées des constantes d'équilibre qui permettent de définir une constante de stabilité

La stabilité d'un complexe métallique dépend donc de la nature du métal, de son degré d'oxydation, mais aussi de la nature du(des) ligands (neutre, anionique, nombre et nature des atomes donneurs, denticité,…)



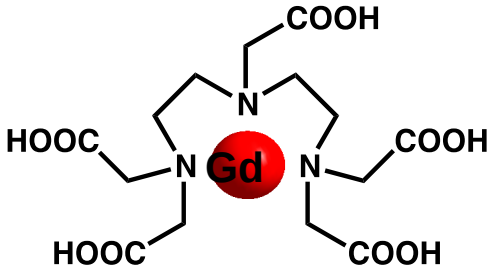
M = Pb	3,4	8,8	10,4	15,9
M = Cu	13,0	19,6	20,1	23,3

Agents de contraste pour l'IRM

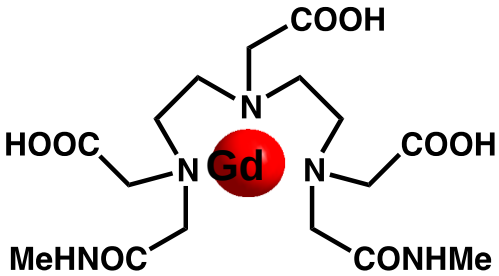
Nécessité d'injecter de grandes quantités (de l'ordre de 4 grammes d'agent de contraste / patient) et toxicité du Gd^{3+} ☹

➢ **Complexes de Gd^{3+} : élimination rapide par voie rénale, toxicité diminuée x100** ☺

Agents chélatants linéaires (dérivés du DTPA) :

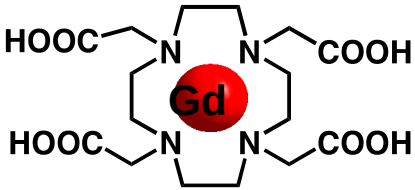


DTPAGd
Magnevist® (Bayer Shering)
AMM 1988

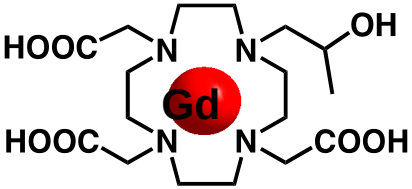


DTPA-BMAGd
Omniscan® (GE Healthcare)

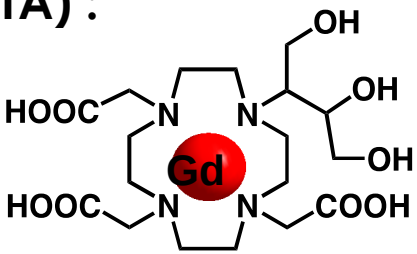
Agents chélatants macrocycliques (dérivés du DOTA) :



DOTAGd
Dotarem® (Guerbet)

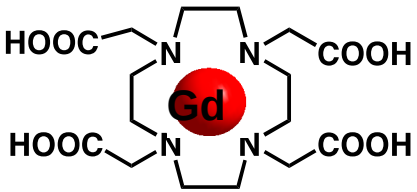


HPDO3AGd
ProHance® (Bracco)

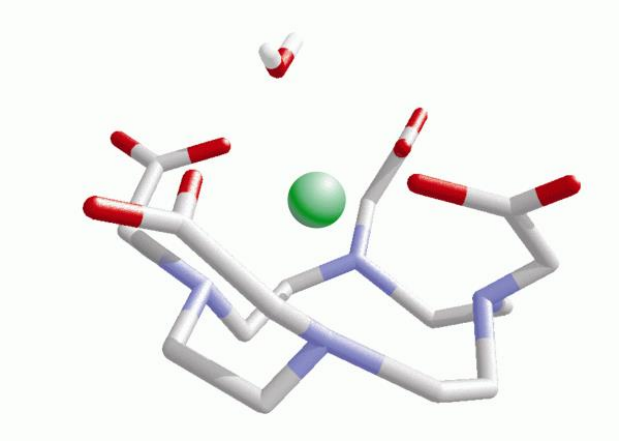


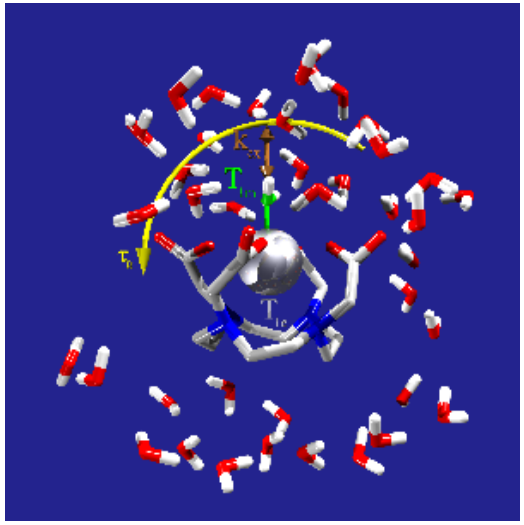
DO3ABGd
Gadovist® (Bayer Shering)

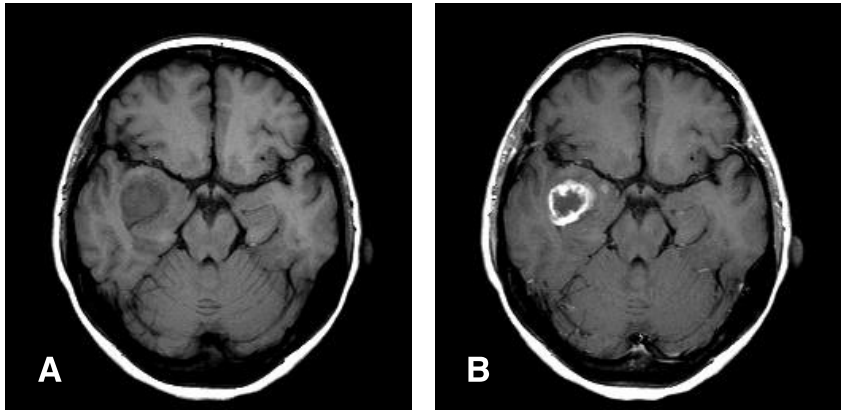
Agents de contraste pour l'IRM



DOTAGd
Dotarem® (Guerbet)







A.E. Merbach, E. Toth, Editors, *The Chemistry of Contrast Agents in Medical Magnetic Resonance Imaging,* John Wiley & Son, Ltd., Chichester, **2001**.

Agents de contraste pour l'IRM

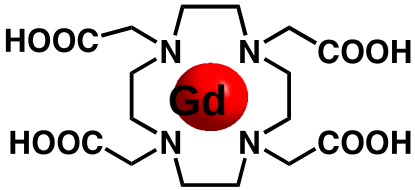
Pb : apparition (années 2000) d'une nouvelle pathologie, la fibrose systémique néphrogénique, chez des patients souffrant d'une insuffisance rénale sévère.

En 2006 : lien de cause à effet avec injection de complexes de Gd^{3+}

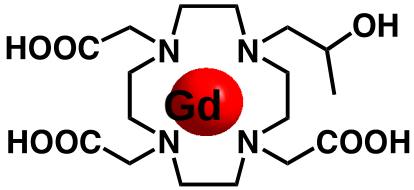
DTPAGd
Magnevist® (Bayer Shering)
AMM 1988

DTPA-BMAGd
Omniscan® (GE Healthcare)

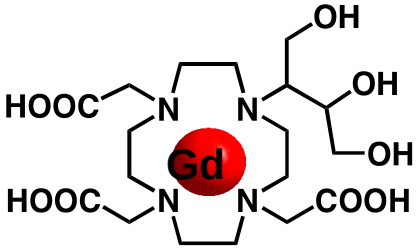
contre-indication en Europe



DOTAGd
Dotarem® (Guerbet)



HPDO3AGd
ProHance® (Bracco)



DO3ABGd
Gadovist® (Bayer Shering)

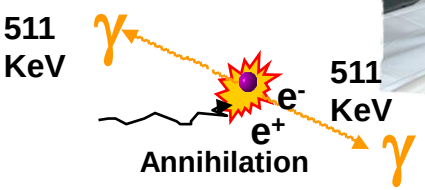
Médecine nucléaire

Diagnostic et/ou Thérapie

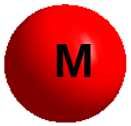
Imagerie
TEMP (SPECT)
émetteurs γ

^{111}In, ^{99m}Tc, ^{67}Ga, ^{123}I

Imagerie
TEP (PET)
émetteurs β^+



^{68}Ga, ^{64}Cu, ^{89}Zr, ^{18}F, ^{11}C



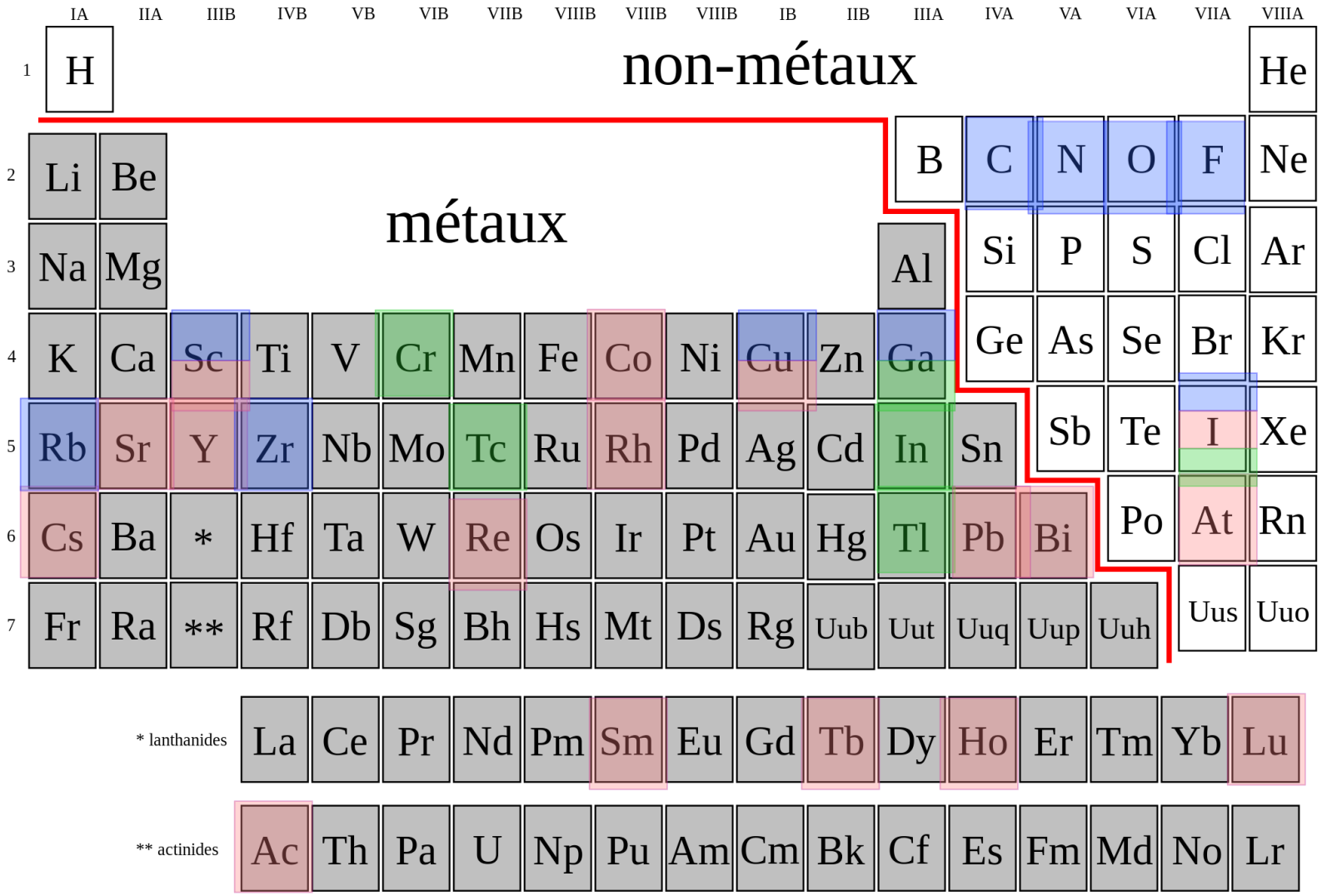
=

RadioImmunoThérapie (RIT) - the “magic bullet”

émetteurs β^- ou α

^{90}Y, ^{177}Lu, ^{67}Cu, ^{186}Re, ^{212}Pb, ^{131}I

Radioisotopes utilisés en médecine nucléaire

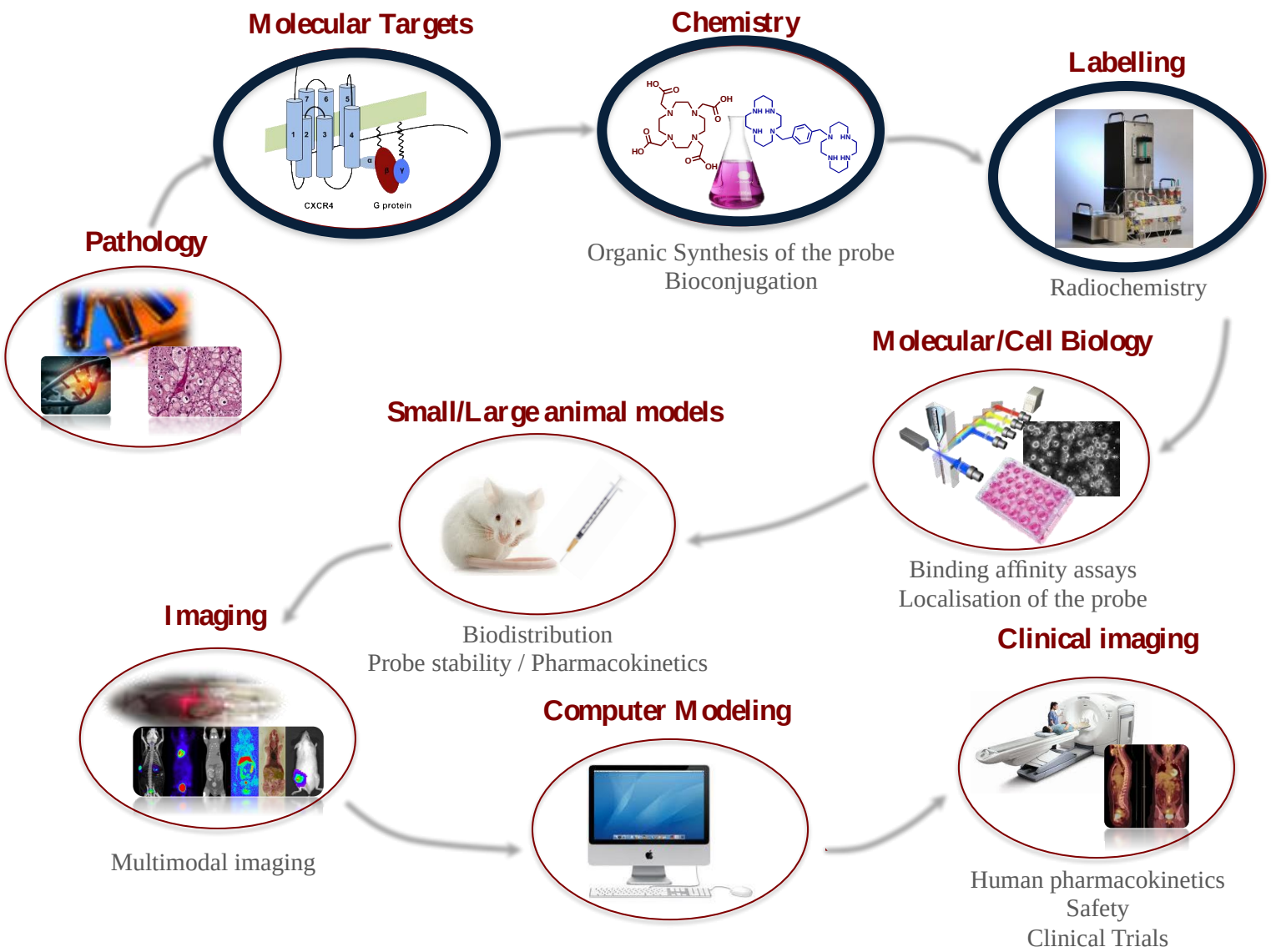


émetteurs γ
TEMP (SPECT)

émetteurs β^+
TEP (PET)

émetteurs β^- ou α
Thérapie

Imagerie moléculaire



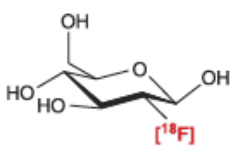
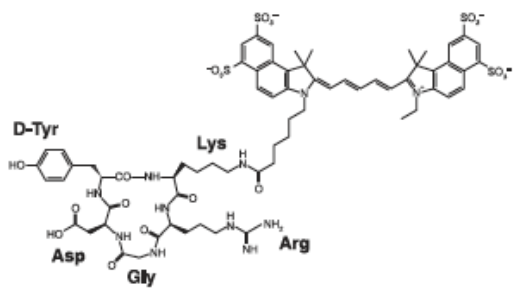
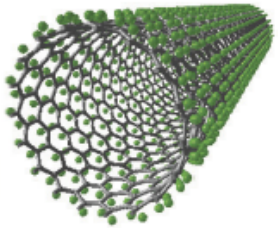
R. Weissleder, Molecular Imaging: Principles and Practice, *People's Medical Publishing House,* 2010

Imagerie moléculaire

Physiol Rev 92: 897–965, 2012
doi:10.1152/physrev.00049.2010

A MOLECULAR IMAGING PRIMER: MODALITIES, IMAGING AGENTS, AND APPLICATIONS

Michelle L. James and Sanjiv S. Gambhir

Type	Small molecule	Peptide	Affibody	Aptamer	Antibody	Nanoparticle
Size	<1 nm <0.5 kDa	~1–4 nm ~0.5–2 kDa	~5–10 kDa	~5–15 kDa	~150 kDa	~10–200 nm (no larger than 1,000 nm)
Example	 [18F]FDG	 RGD-Cy5.5	 [68Ga]-DOTA-MUT-DS	 Molecular Beacon	 ICG-Trastuzumab	 RGD-SWNT

Quencher Donor Signaling component

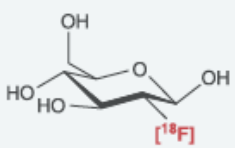
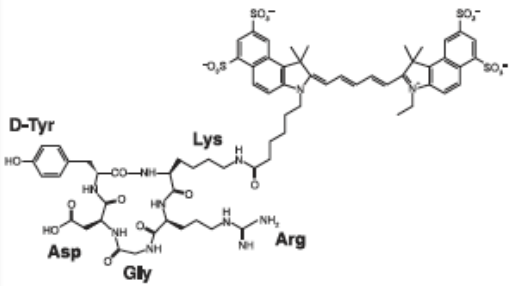
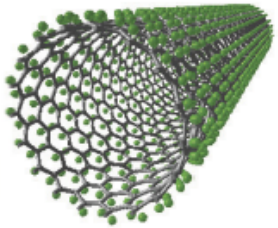
Imagerie moléculaire

Physiol Rev 92: 897–965, 2012
doi:10.1152/physrev.00049.2010

A MOLECULAR IMAGING PRIMER: MODALITIES, IMAGING AGENTS, AND APPLICATIONS

Michelle L. James and Sanjiv S. Gambhir

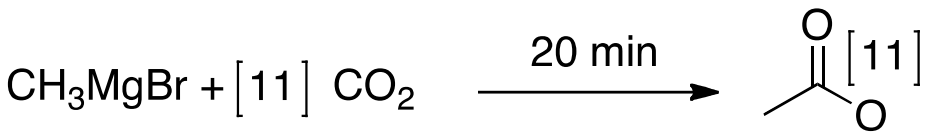
MARQUAGE DIRECT

Type	Small molecule	Peptide	Affibody	Aptamer	Antibody	Nanoparticle
Size	<1 nm <0.5 kDa	~1–4 nm ~0.5–2 kDa	~5–10 kDa	~5–15 kDa	~150 kDa	~10–200 nm (no larger than 1,000 nm)
Example	 [18F]FDG	 RGD-Cy5.5	 [68Ga]-DOTA-MUT-DS	 Molecular Beacon	 ICG-Trastuzumab	 RGD-SWNT

Quencher · Donor · Signaling component

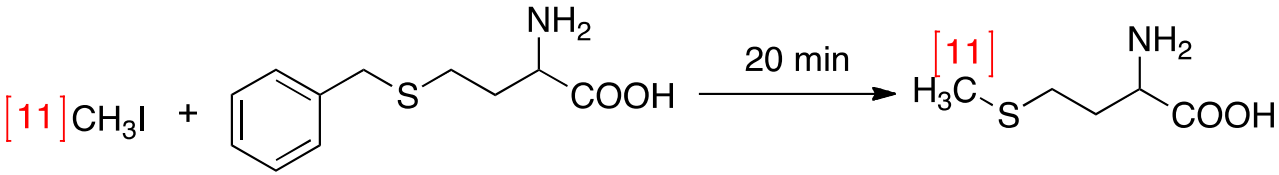
Petites molécules : marquage direct

[^{11}C] acétate



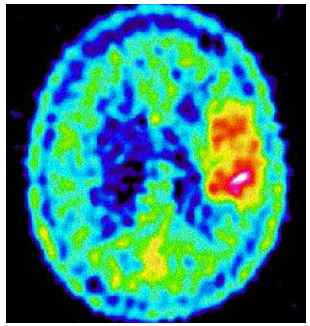
- ✓ **Oncologie : cancer prostate, reins, vessie**
- ✓ **Cardiologie : infarctus du myocarde**

[^{11}C] methionine



Tissu néoplasique cérébral

- ✓ **Gliomes**
- ✓ **Degré d' évolution des tumeurs**
- ✓ **Réponse aux traitements**

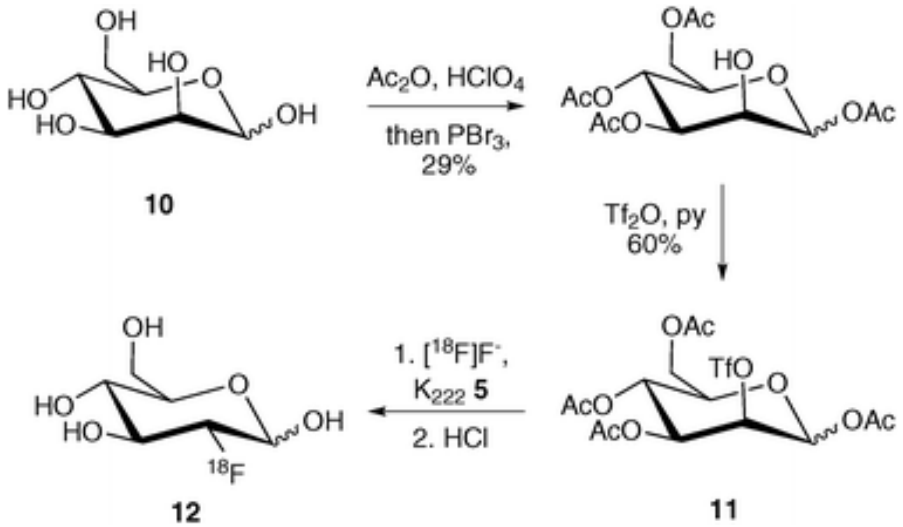


Petites molécules : marquage direct

[^{18}F] FDG

^{18}F
110 min
β^+

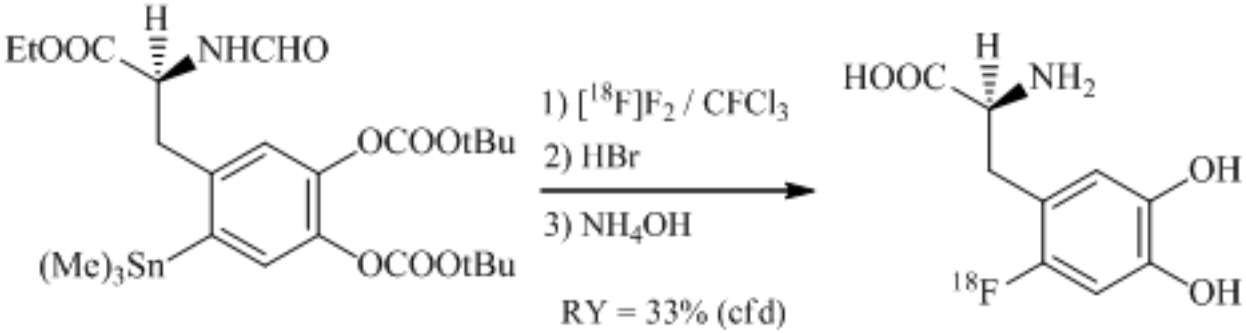
- ✓ **Onco/cardio/neuro**



K. Hamacher, H. H. Coenen and G. Stöcklin, *J. Nucl. Med., 1986, 27, 23*

[^{18}F] fluoro-L-dopa

^{18}F
110 min
β^+



- ✓ **Neuroimagerie : maladie d'Alzheimer**
- ✓ **Oncologie : mélanome, cancer du sein**

E. F. J. de Vries, G. Luurtsema, M. Brüssermann, P. H. Elsinga, W. Vaalburg *Appl. Rad. Isotopes*. 1999, *51,* 389

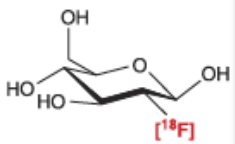
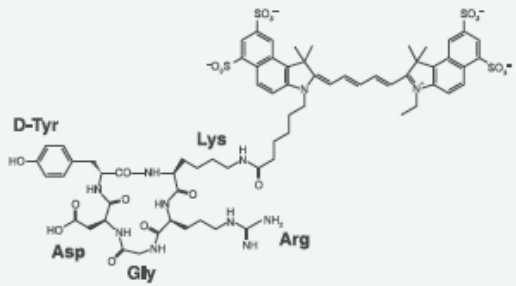
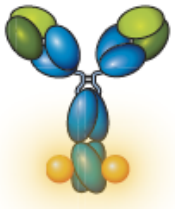
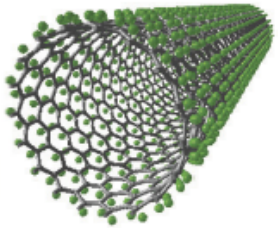
Imagerie moléculaire

Physiol Rev 92: 897–965, 2012
doi:10.1152/physrev.00049.2010

A MOLECULAR IMAGING PRIMER: MODALITIES, IMAGING AGENTS, AND APPLICATIONS

Michelle L. James and Sanjiv S. Gambhir

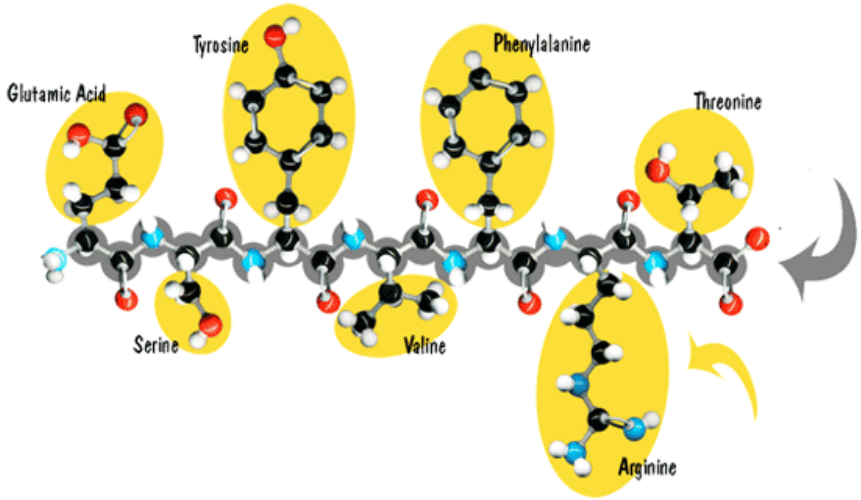
MARQUAGE DIRECT/INDIRECT

Type	Small molecule	Peptide	Affibody	Aptamer	Antibody	Nanoparticle
Size	<1 nm <0.5 kDa	~1–4 nm ~0.5–2 kDa	~5–10 kDa	~5–15 kDa	~150 kDa	~10–200 nm (no larger than 1,000 nm)
Example						
	[18F]FDG	RGD-Cy5.5	[68Ga]-DOTA-MUT-DS	Molecular Beacon	ICG-Trastuzumab	RGD-SWNT

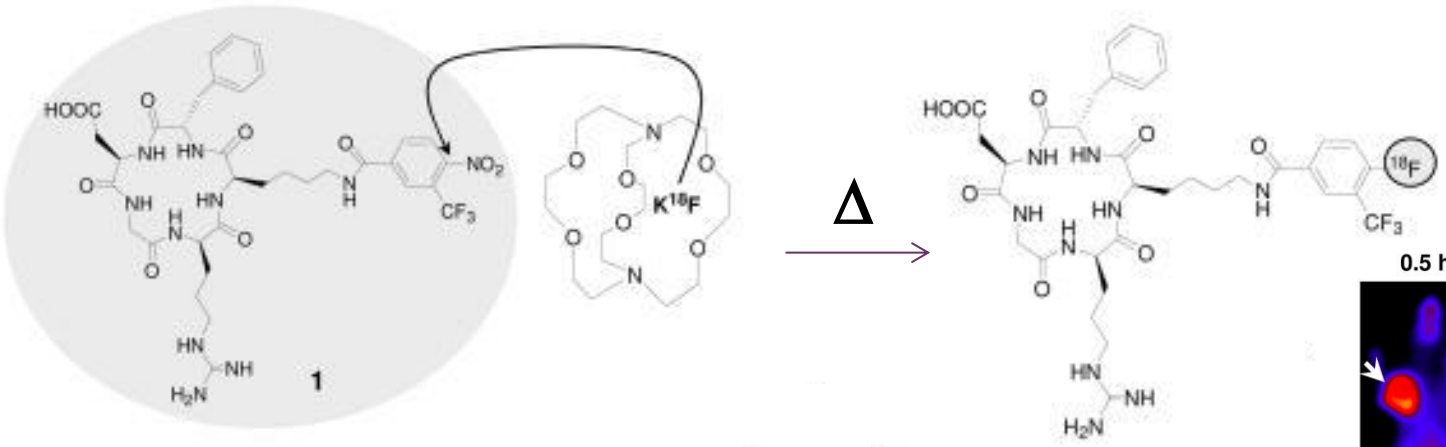
Quencher Donor Signaling component

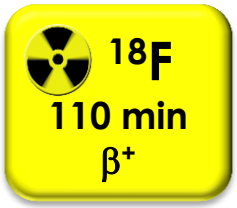
Marquage de peptides

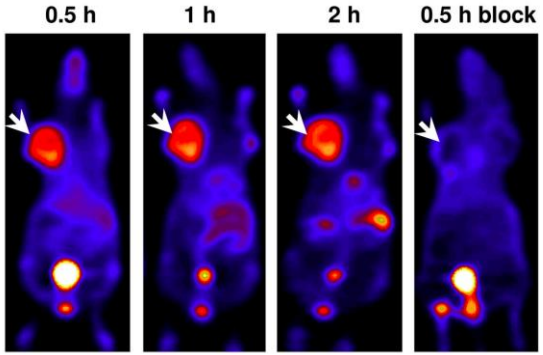
- Synthèse de peptides



Marquage direct au ^{18}F de 4-nitro-3-trifluoromethylbenzoyl-c(RGDfk)





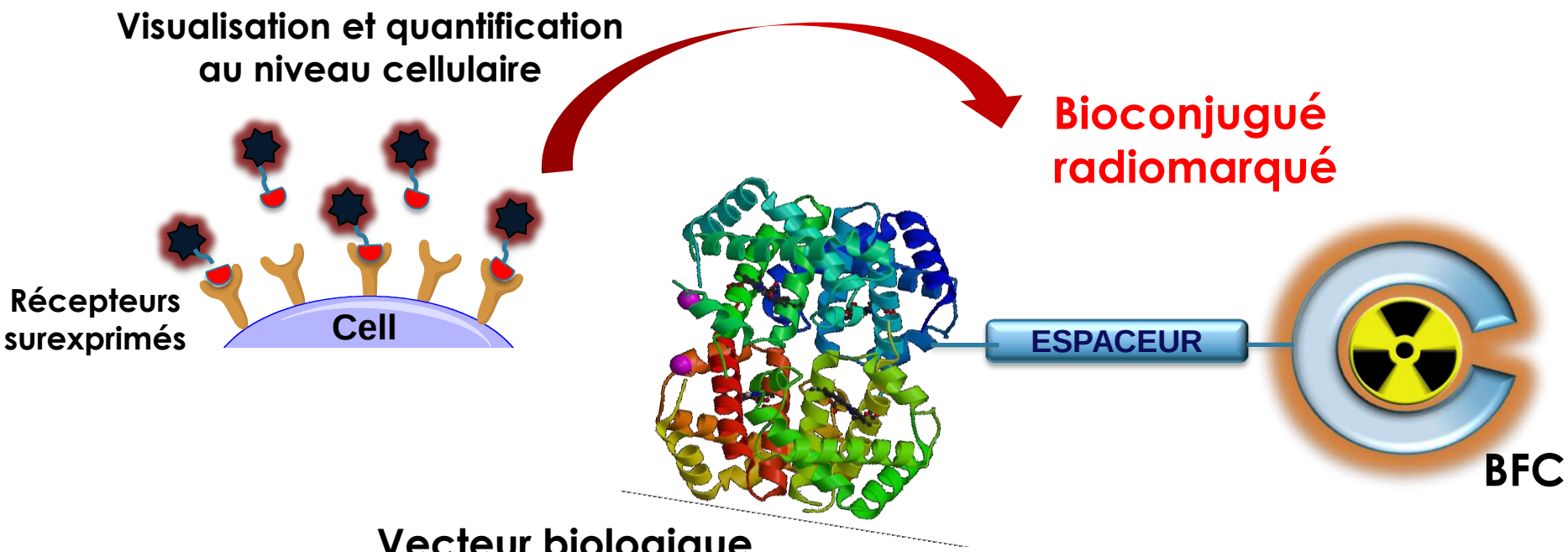


Imagerie de l'expression de l'integrine ⟨ vβ3

O. Jacobson et al., *Bioconjugate Chem.*, **2011**, 422-428

Marquage indirect

Marquage indirect par des radiométaux par le biais d'agents chélatants bifonctionnels (BFC)

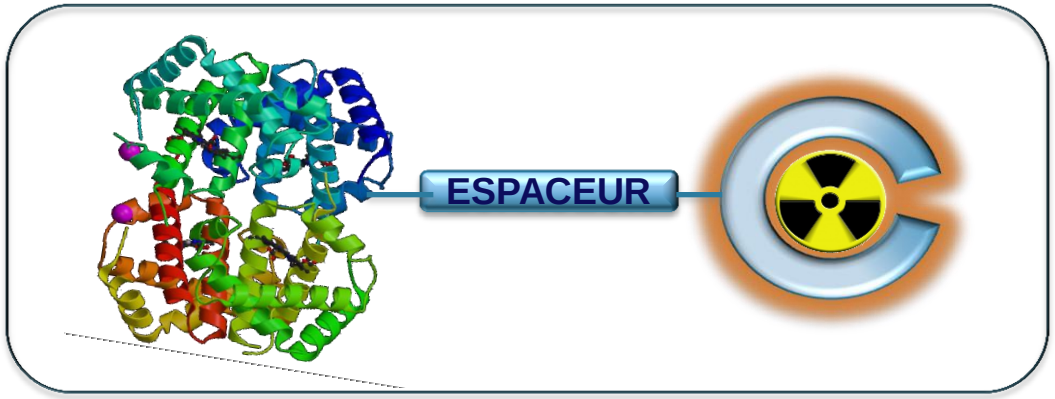


Vecteur biologique spécifique de la cible :

peptide, anticorps, fragment, ADC, affibody, minibody, nanobody…

- Synthèse organique
- Chimie de coordination
- Bioconjugaison

Design d'un bioconjugué radiomarqué



Cible :
Type de récepteur
Localisation
Degré d'expression

Biovecteur :
PK
Affinité
Spécificité
Clairance

Choix du radiométal :
Méthode d'imagerie
Demi-vie
Disponibilité

Chimie de coordination
Bioconjugaison

Choix du BFC :
Propriétés de coordination
Synthèse

ESPACEUR

Bio conjugaison:
Fonction de greffage
Propriétés de l'espaceur
Pre-targeting

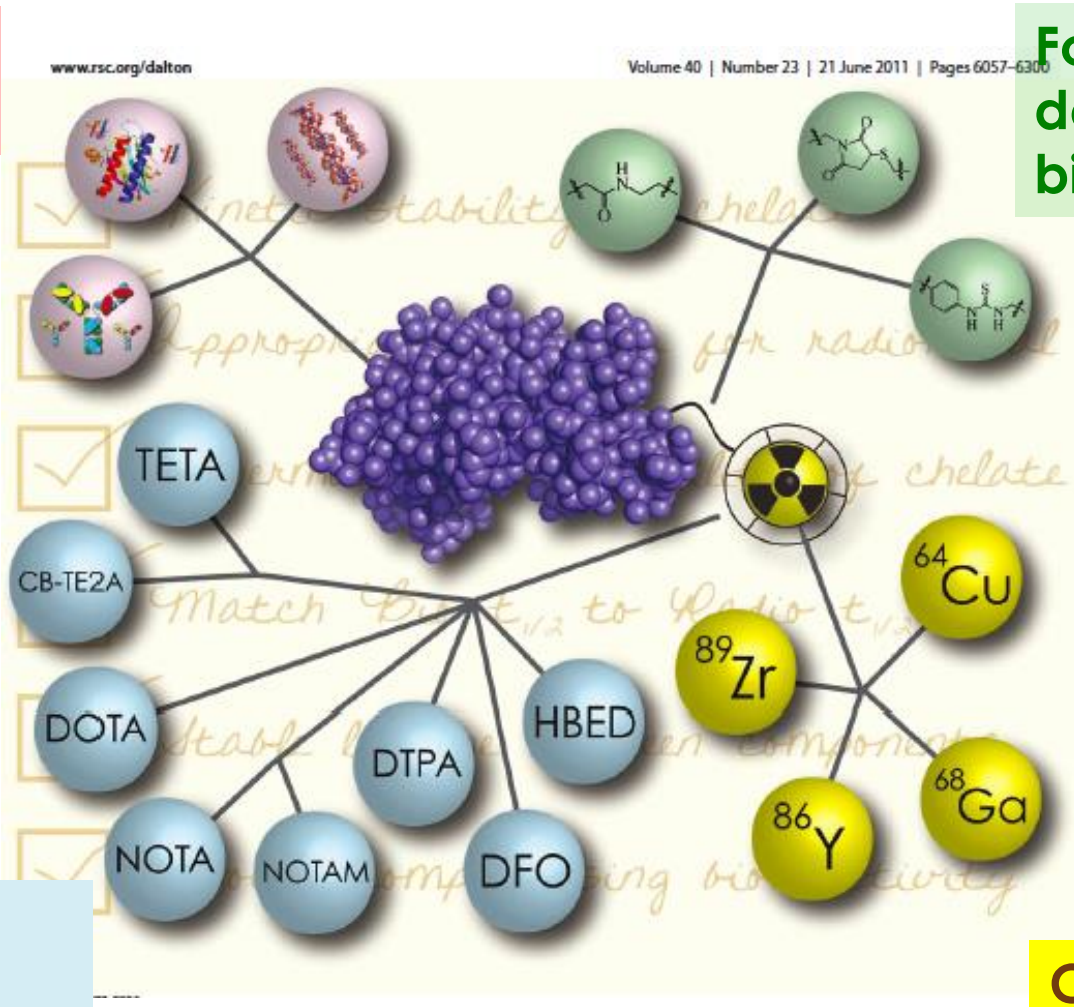
Radiomarquage
Imagerie

- Affinité – spécificité pour la cible
- Stabilité du bioconjugué en milieu physiologique
- Demi-vie dans le sang
- Perméabilité
- Vitesse et mode d'élimination

Design d'un bioconjugué radiomarqué

Vecteur biologique

Anticorps
Fragment
Peptide
…



Fonction de bioconjugaison

NCS
Ester activé
Maleimide
Chimie Clic, …

Linéaire
Macrocyclique

Agent chélatant bifonctionnel

SPECT: ^{111}In, ^{99m}Tc, …
PET: ^{64}Cu, ^{89}Zr, ^{68}Ga, …
RIT: ^{90}Y, ^{177}Lu, ^{212}Pb, …

Choix du radiométal

B.M. Zeglis, J.S. Lewis, *Dalton Trans*, 40, **2011**, 6057-6300

Design d'un bioconjugué radiomarqué

Gamma emitters: SPECT imaging				
^{99m}Tc	6.0 h; 100% γ; γ141keV	Generator (^{99}Mo from reactor)	Worldwide GMP	Least expensive radionuclide The ^{99}Mo shortage will be solved in the near future and there is a real need for new ^{99m}Tc labeled tracers
^{123}I	13.2 h; 100% EC$^+$ β$^+$; γ 158 keV	Cyclotron	Limited supply	Too expensive, high energy, limited manufacturing capacity. Dedicated network of high energy cyclotrons is needed. Estimated remaining investment need: €100–120 M
^{111}In	2.80d; 100% EC; γ 245 keV, 171 keV	Cyclotron	Very limited supply	Very expensive as a diagnostic agent - could be reconsidered in the future as a therapeutic radionuclide
^{67}Ga	3.26d; 100% EC; γ 93.3 keV	Cyclotron	Worldwide GMP	Expensive radionuclide – use presently limited by the number of applications
^{186}Re	3.72d; 92.5%β$^-$; 7.5%EC β$^-$ 1069 keV, γ137KeV	Reactor	Worldwide GMP	Same chemistry as technetium, but much more expensive – number of applications presently limited
Positron emitters: PET imaging				
^{18}F	1.83 h; 100%EC$^+$ β$^+$; β$^+$ 633 keV	Cyclotron	Specific equipped locations	Still not available worldwide. Needs further investment in cyclotrons and GMP manufacturing sites
^{68}Ga	67.7 min; 100%EC$^+$ β$^+$; β$^+$ 1899 keV, γ 1077 keV	Generator (^{68}Ge from cyclotron)	GMP generator will be available	Highest potential – several companies are presently developing GMP grade generators. MA will come later
^{89}Zr	78.4 h; 100%EC$^+$ β$^+$; β$^+$ 902 keV, γ 909 keV	Cyclotron	Worldwide	Worldwide supply is easy to secure
^{124}I	4.18d; 100%EC$^+$ β$^+$; β$^+$ 1535 keV, γ 603 keV	Cyclotron	Worldwide	Worldwide supply is easy to secure. Issue: limited by high patient dosimetry
^{64}Cu	12.7 h; 61.5%EC$^+$ β$^+$, 38.5% β$^-$ β$^-$ 579 keV, β$^+$ 653 keV, γ 1346 keV	Cyclotron	Available only locally	Easy to produce, but large investment needed to supply at a worldwide level as existing cyclotrons are not adapted for this radionuclide production. Potential future in therapy (Auger electron)
^{86}Y	14.7 h; 100%EC$^+$ β$^+$; β$^+$ 1220 keV, γ 1077 keV	Cyclotron	Not available	Expensive, high energy
Beta and alpha emitters: therapeutic agents				
^{177}Lu	6.65d; 100%β$^-$; β$^-$ 498 keV, γ 208 keV	Reactor	Worldwide	Probably therapeutic radionuclide with the best profile. Care: two qualities of ^{177}Lu available and high specific activity will have preference
^{90}Y	2.67d; 100%β$^-$; β$^-$ 2280 keV	Generator (^{90}Sr from reactor)	Worldwide	Several sources available – no investment required, no risk of shortage
^{188}Re	17.0 h; 100%β$^-$; β$^-$ 2120 keV, γ155KeV	Generator (^{188}W from reactor)	Locally	Generator still needs to get its marketing approval – GMP generator under development
^{131}I	8.02d; 100%β$^-$; β$^-$ 606 keV, γ 363KeV	Reactor	Worldwide	In the future major applications will probably be limited to thyroid diseases. Long half-life, high energy, highly contaminating
^{166}Ho	26.8 h; 100%β$^-$; β$^-$ 1774 keV, γ 81KeV	Reactor	Locally	Presently only one reliable source. Not approved outside of South Korea
^{212}Pb	10.7 h; 100%β$^-$ ->^{212}Bi; β$^-$ 331 keV, γ 239KeV	Reactor	Not available	GMP manufacturing site under construction (^{212}Bi is also an alpha emitter)[c]
^{67}Cu	61.8 h; 100%β$^-$; β$^-$ 377 keV, γ184KeV	Cyclotron	Not available	Very expensive: Presently no technology available for large amounts production of high quality, highly pure ^{67}Cu
^{211}At	7.2 h; 58.2%EC, 41.8%β$^-$; α 5869 keV, γ 687KeV	Cyclotron	Only locally available	Will need a dedicated network of cyclotron. Same situation as ^{123}I, but more complicated chemistry.
^{117m}Sn	14.0d; IT γ 158KeV	Cyclotron	Not available	Expensive: network of manufacturing cyclotrons is missing.

Choix du radiométal :
Méthode d'imagerie
Demi-vie
Disponibilité

R. Zimmerman, *J. Nucl. Med. Bio.* **2012,**

Design d'un bioconjugué radiomarqué

Choix du BFC :
Propriétés de coordination
Synthèse

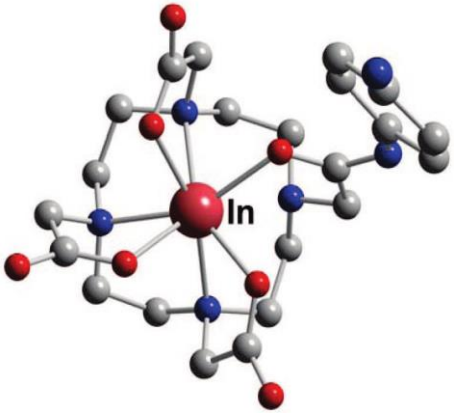
Fonctions coordinantes

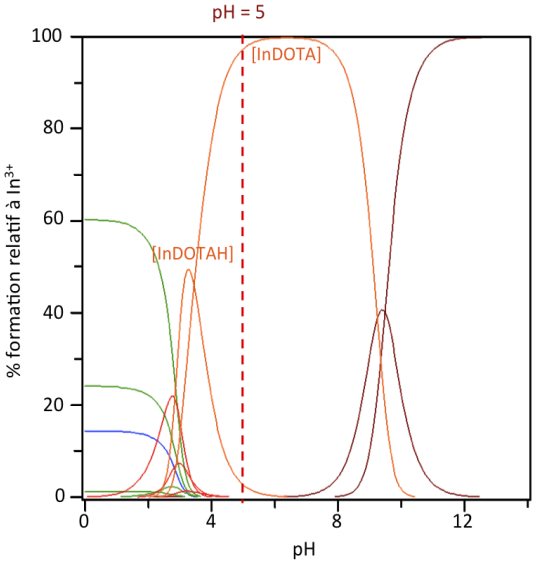
Atomes donneurs

Effet macrocyclique

Site d'attache de la fonction de bioconjugaison

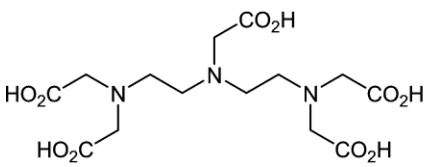
SYNTHESE DE LIGANDS ⟷ **ETUDES PHYSICO-CHIMIQUES**

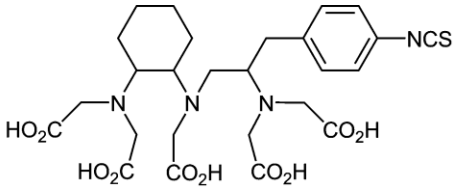


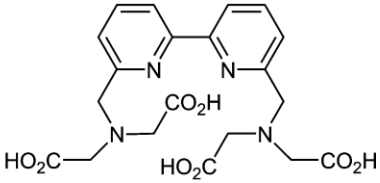


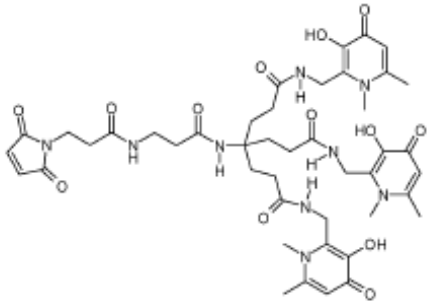
- **Affinité et sélectivité élevées**
- **Cinétique de complexation rapide / $T_{1/2}$**
- **Formation de complexes inertes pour éviter des réactions de transmétallation et transchélation *in vivo***
- **Activité spécifique élevée**
- **Formation de complexes non réductibles (Cu)**

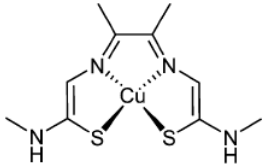
Quelques BFC cycliques et acycliques

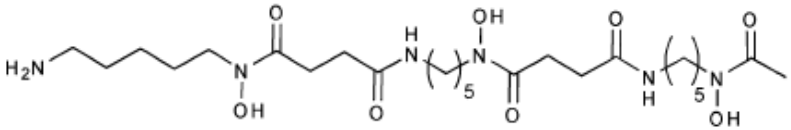




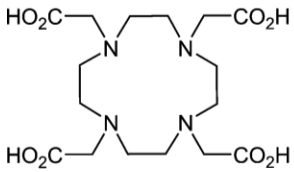


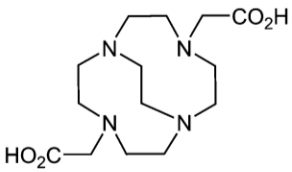


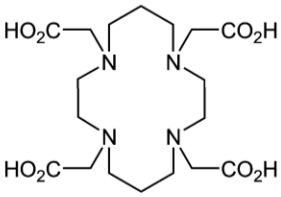


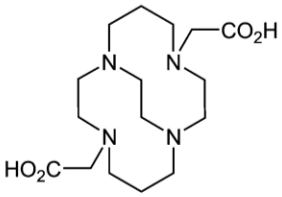


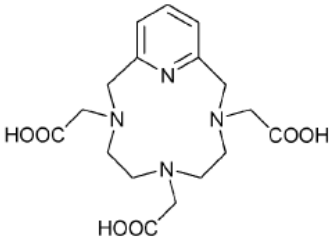
B.M. Zeglis, J.S. Lewis, *Dalton Trans*, 40, **2011**, 6057-6300 - I. Santos et al., *Dalton Trans.*, **2011**

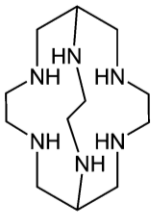


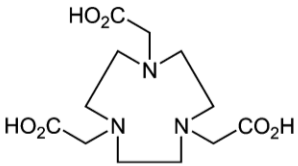


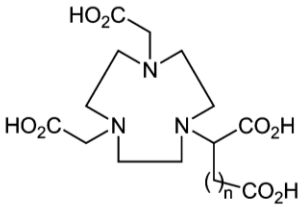


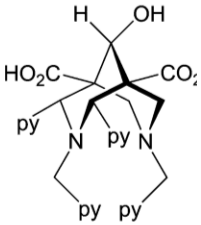


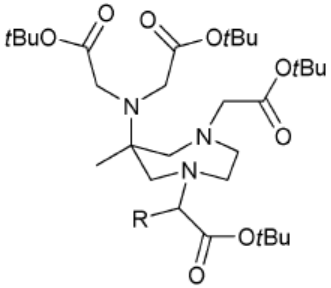




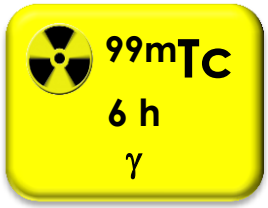








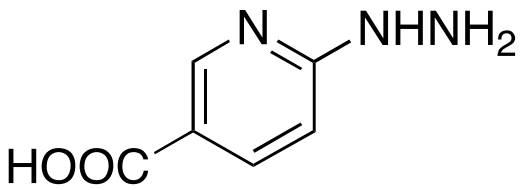
Marquage de peptides



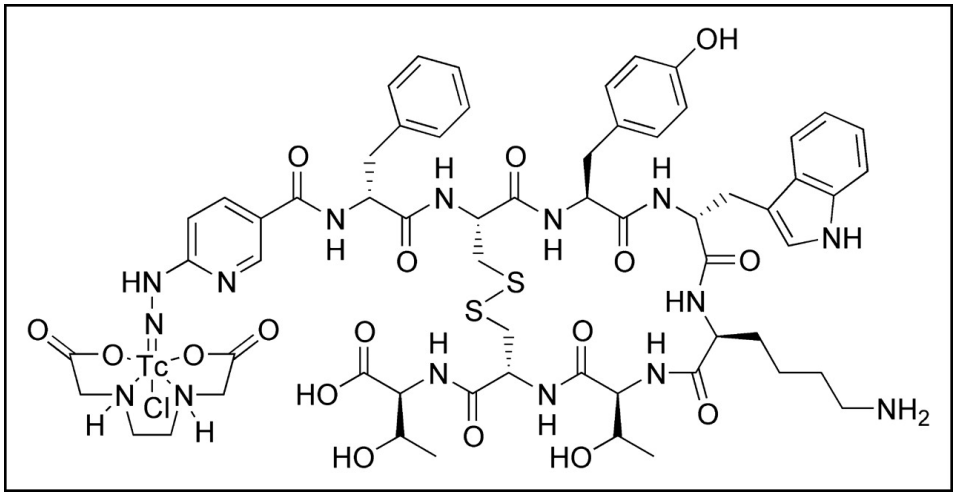
Radiométal le plus utilisé pour l'imagerie TEMP (SPECT) car facilement disponible (générateur)

Imagerie TEMP de tumeurs neuroendocrines par des peptides radiomarqués au ^{99m}Tc :

HYNIC: hydrazine-nicotamide

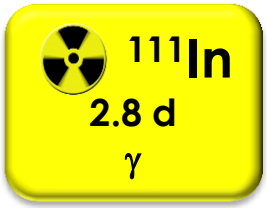


Agent bifonctionnel chélatant pour le ^{99m}Tc (souvent utilisé avec le co-ligand EDDA)
Très utilisé et validé en clinique pour le marquage de nombreuses protéines, peptides

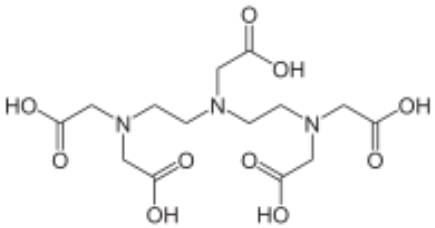


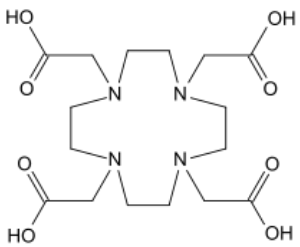
H. Maecke et al. *J. Nucl. Med*. 2011, 425-555.

Marquage de peptides

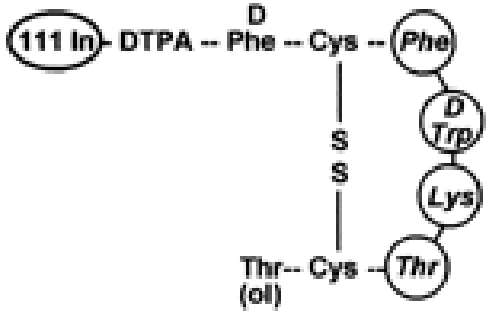


Production par cyclotron
Longue durée de demi-vie

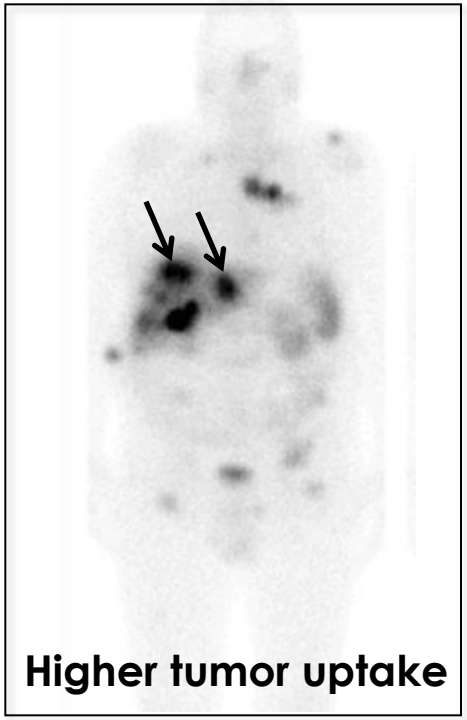




Imagerie TEMP (SPECT) de tumeurs neuroendocrines par des peptides radiomarqués à l'^{111}In :



^{111}In-DTPA (Octreoscan®) **vs** **^{111}In-DOTA (ant)**

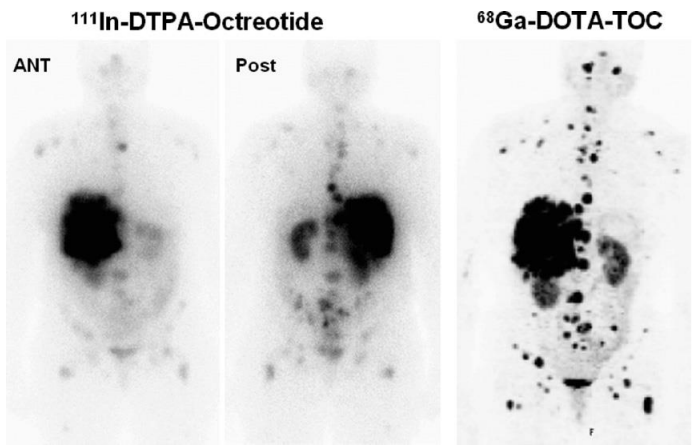


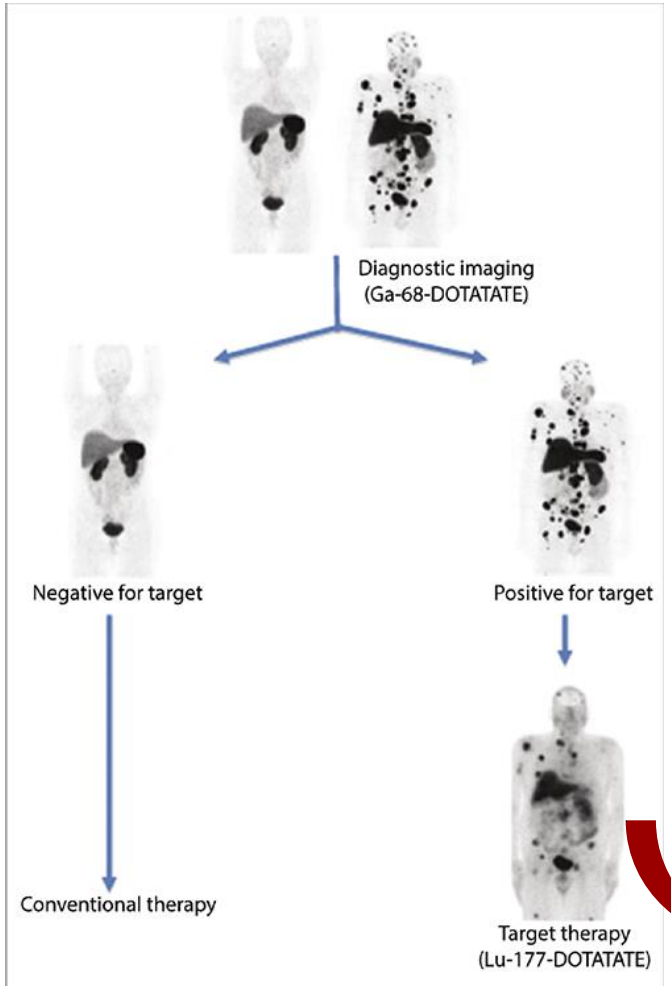
D. Wild, M. Fani, M. Behe, et al., *J Nucl Med* **2011**, *52*, 1412-17

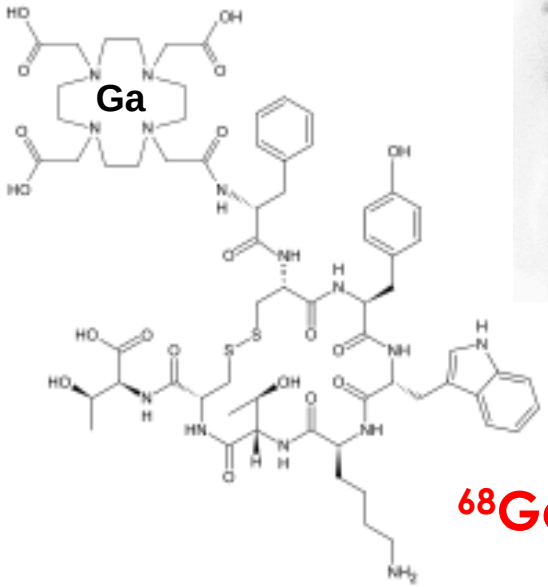
Marquage de peptides

^{68}Ga
67.7 min
β^+

Excellente disponibilité (générateur)
Durée de demi-vie assez courte, adaptée à l'imagerie TEP de peptides







^{68}Ga DOTATATE

Utilisation du même bioconjugué pour le diagnostic et la thérapie selon le radiométal utilisé : Théranostique

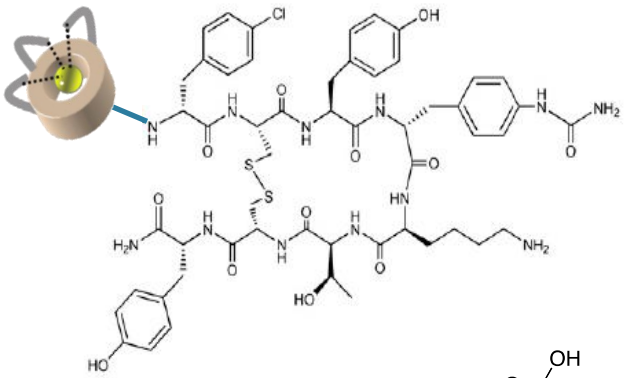
Suivi d'efficacité : compagnon diagnostic

“The chelate makes the difference”

PET of Somatostatin Receptor–Positive Tumors Using ^{64}Cu- and ^{68}Ga-Somatostatin Antagonists: The Chelate Makes the Difference

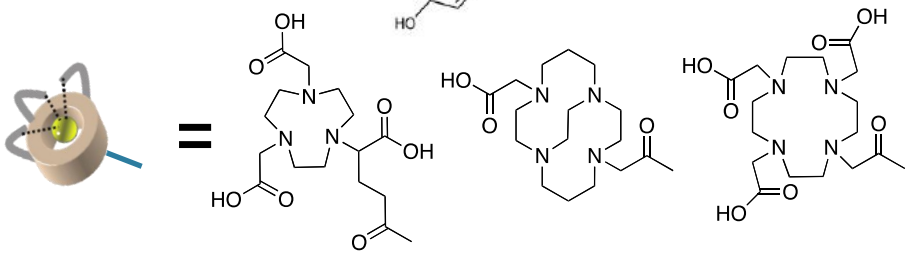
Melpomeni Fani[1,2], Luigi Del Pozzo[1,2], Keelara Abiraj[1], Rosalba Mansi[1,2], Maria Luisa Tamma[1], Renzo Cescato[3], Beatrice Waser[3], Wolfgang A. Weber[2], Jean Claude Reubi[3], and Helmut R. Maecke[1,2]

J. Nucl. Med., **2011**, 52, 7, 1110-1118

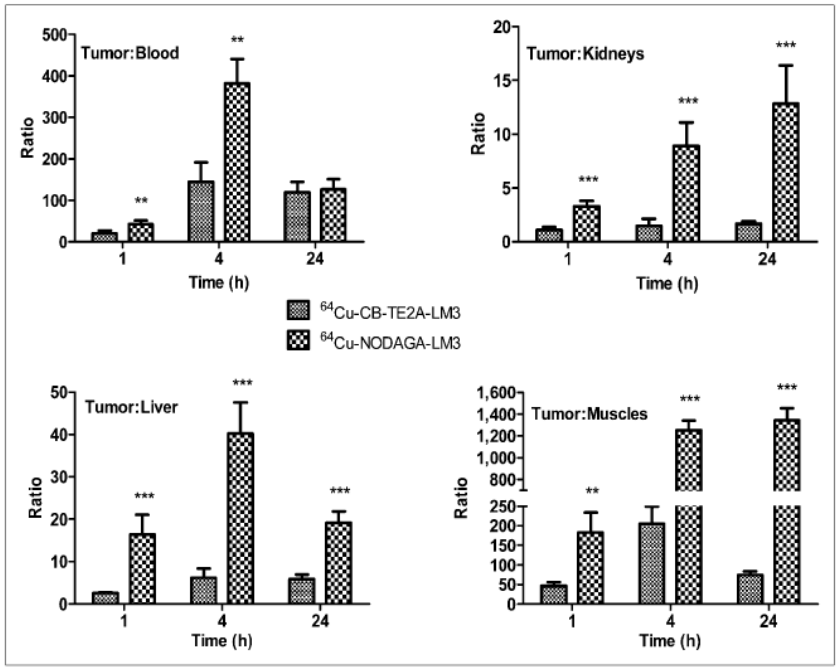


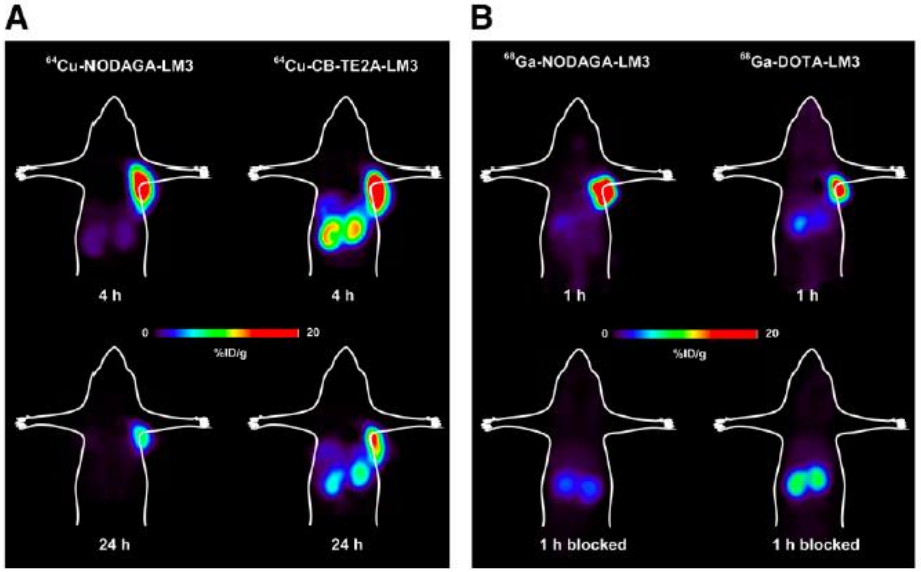
^{64}Cu
12.7 h
β^+ 653 keV

^{68}Ga
67.7 min
β^+ 1899 keV



NODAGA-LM3 CB-TE2A-LM3 DOTA-LM3





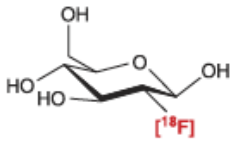
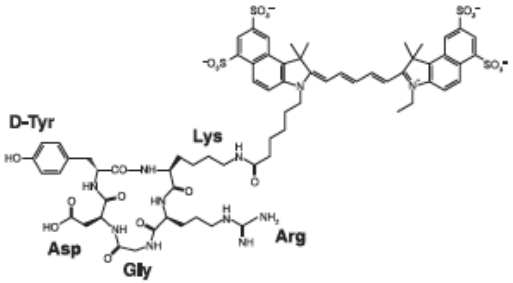
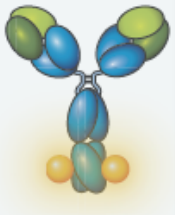
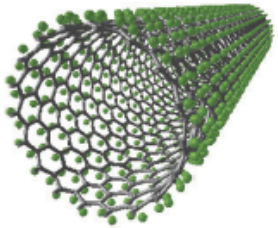
Imagerie moléculaire

Physiol Rev 92: 897–965, 2012
doi:10.1152/physrev.00049.2010

A MOLECULAR IMAGING PRIMER: MODALITIES, IMAGING AGENTS, AND APPLICATIONS

Michelle L. James and Sanjiv S. Gambhir

MARQUAGE INDIRECT

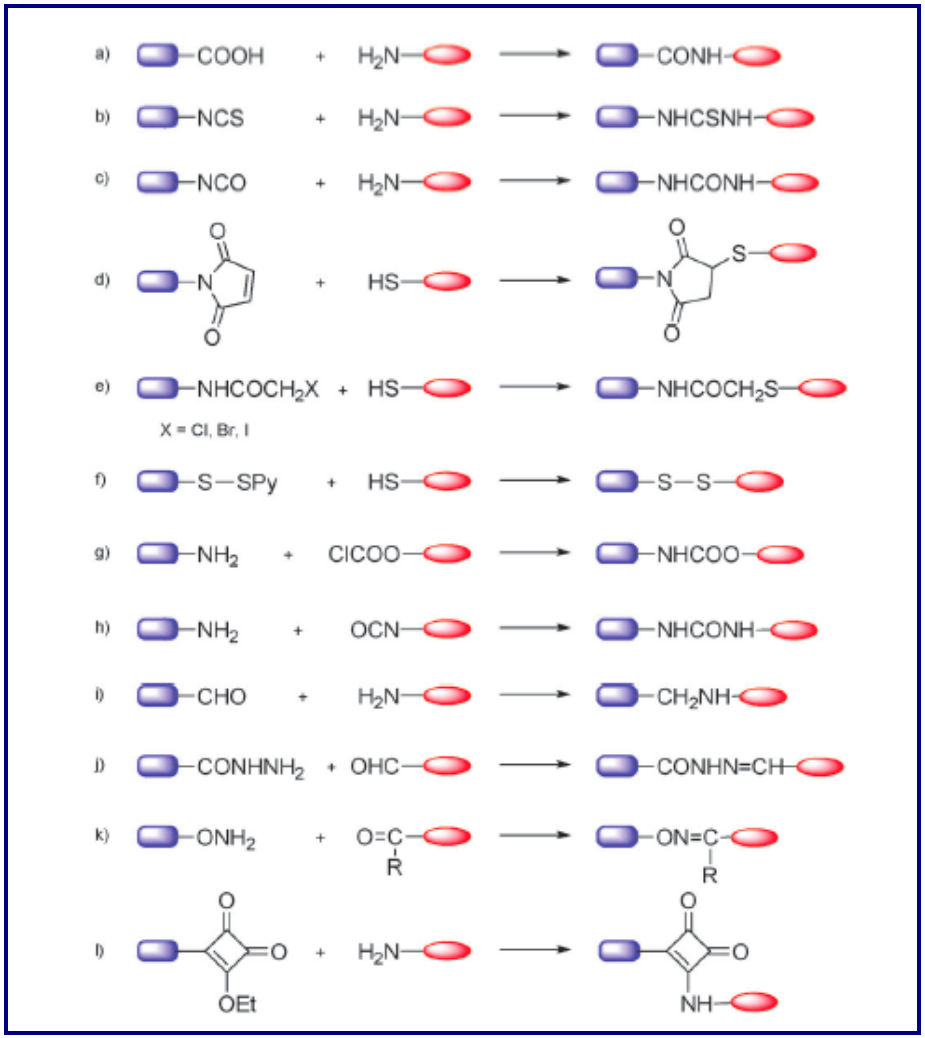
Type	Small molecule	Peptide	Affibody	Aptamer	Antibody	Nanoparticle
Size	<1 nm <0.5 kDa	~1–4 nm ~0.5–2 kDa	~5–10 kDa	~5–15 kDa	~150 kDa	~10–200 nm (no larger than 1,000 nm)
Example	 [18F]FDG	 RGD-Cy5.5	 [68Ga]-DOTA-MUT-DS	 Molecular Beacon	 ICG-Trastuzumab	 RGD-SWNT

Quencher · Donor · Signaling component

Stratégies de bioconjugaison

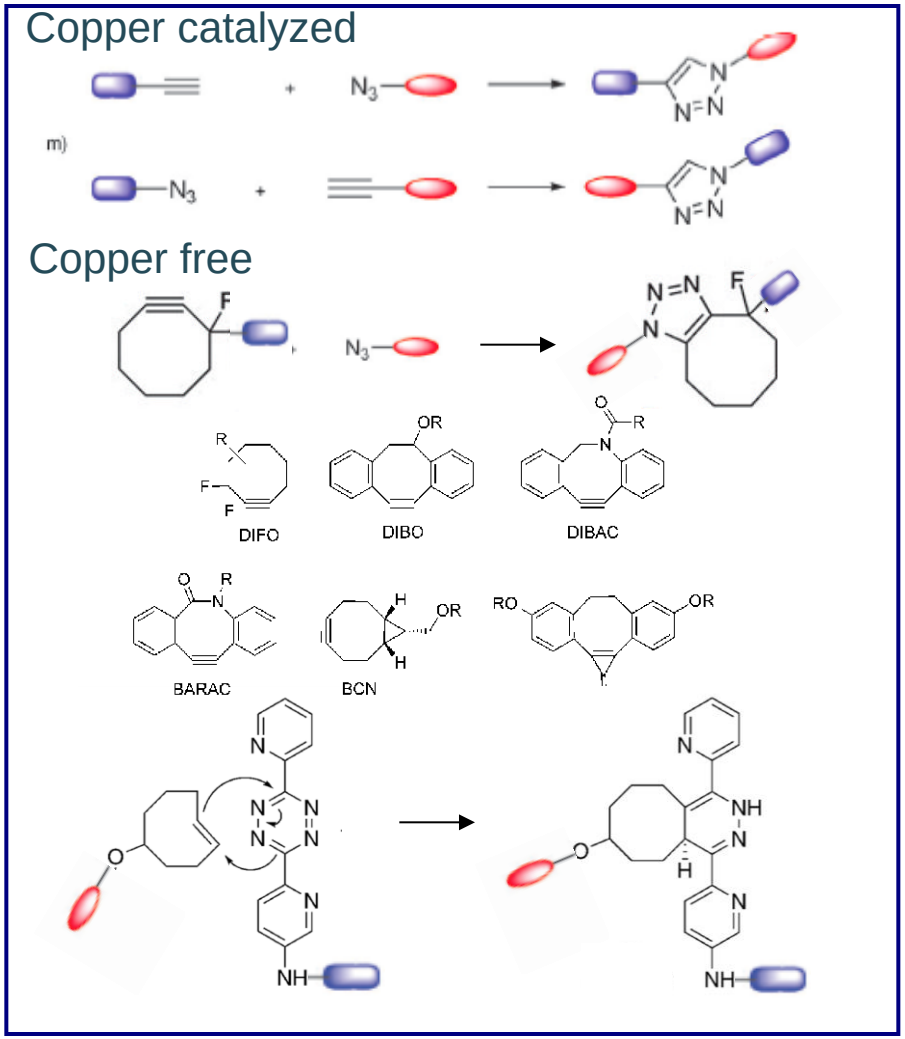
ESPACEUR
Bioconjugaison

Méthodes classiques



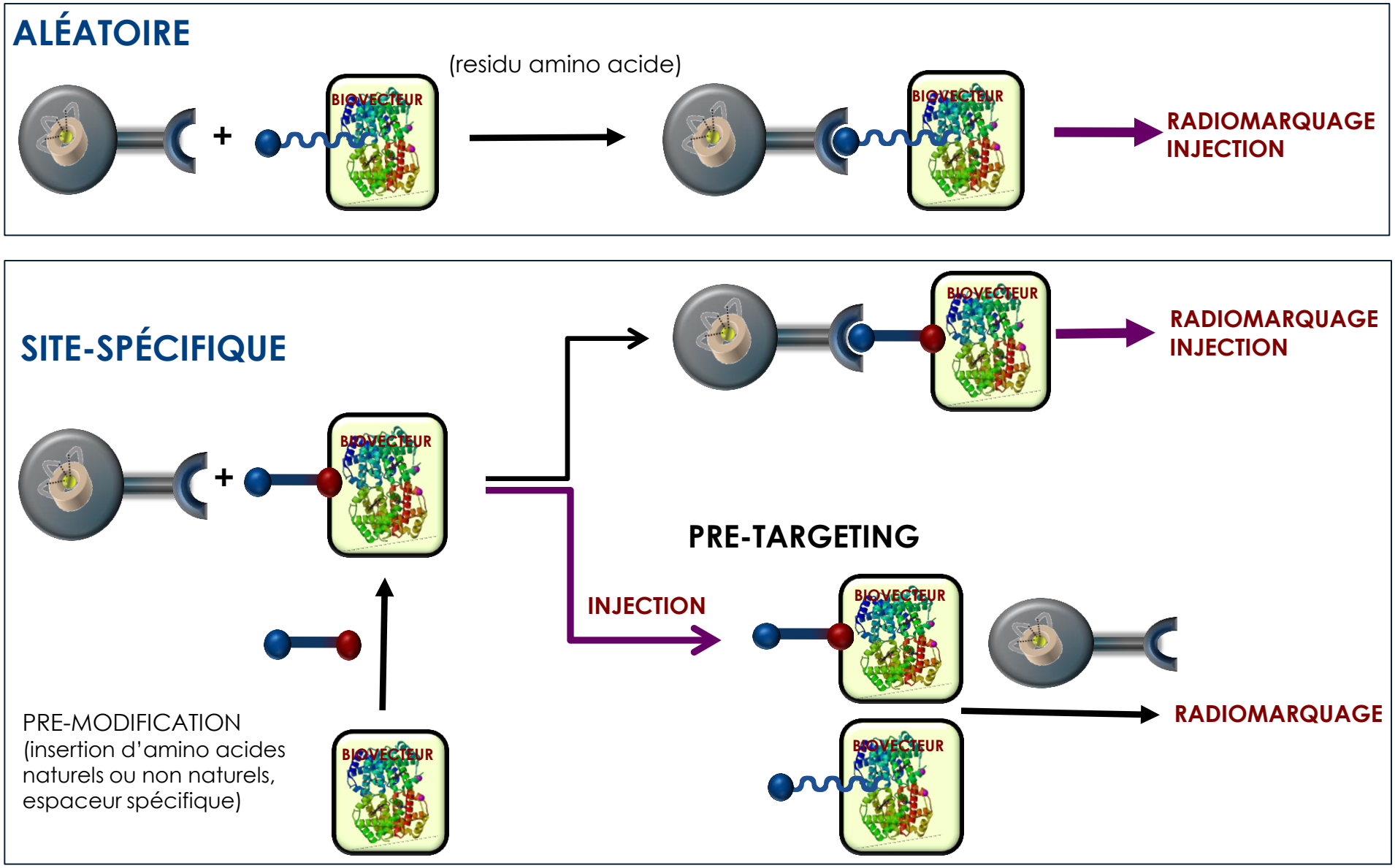
L. Lattuada et al., *Chem. Soc. Rev.*, **2012**

Chimie bioorthogonale
Chimie Click

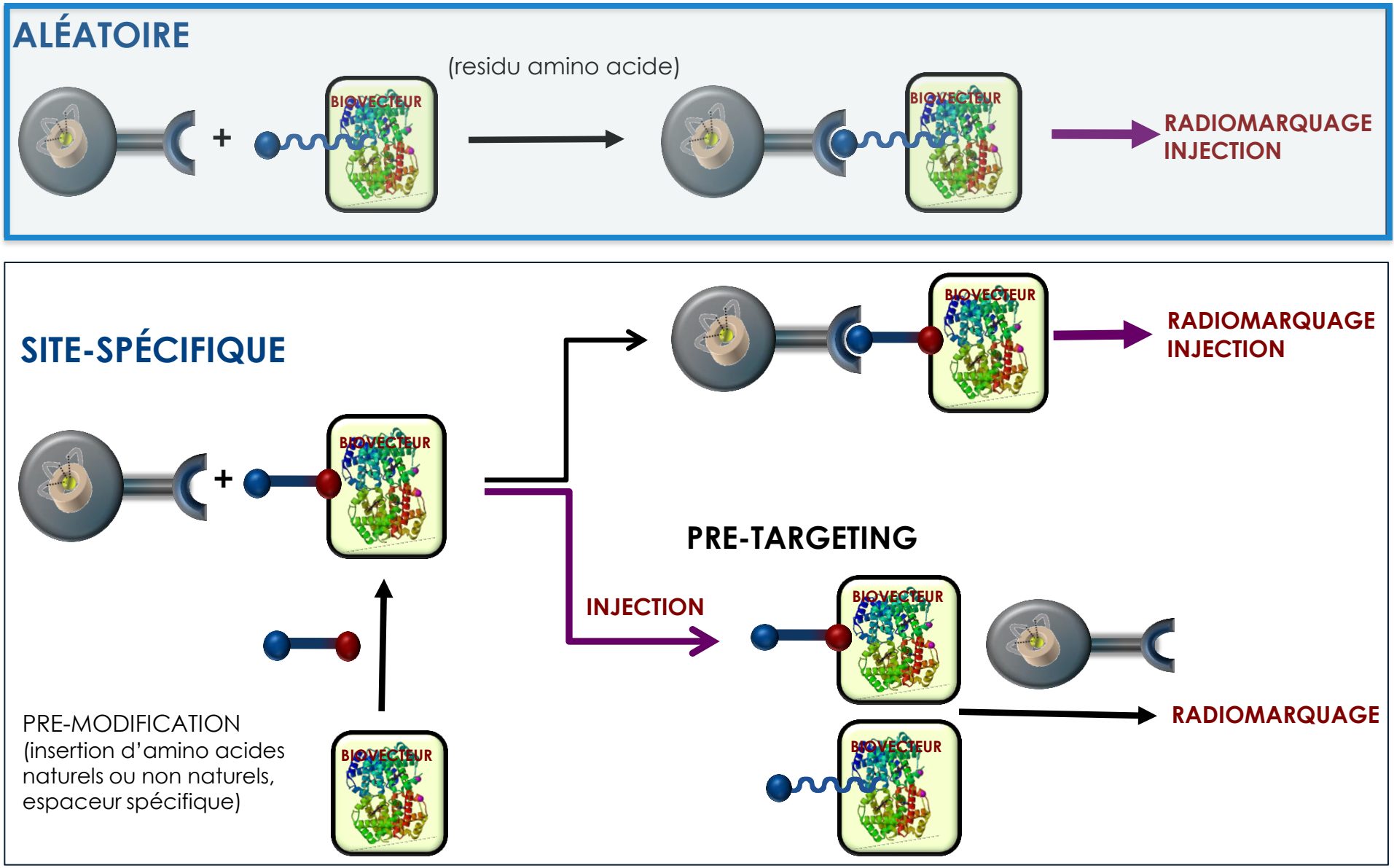


E. Fernandez-Megia et al., *Angew. Chem., Int. Ed.*, **2011**, 8794-8804
J.C. Jewett, C.R. Bertozzi, *Chem. Soc. Rev.*, **2010**, 1272-79

Stratégies de bioconjugaison

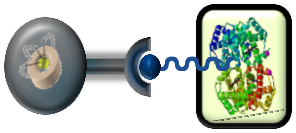


Stratégies de bioconjugaison

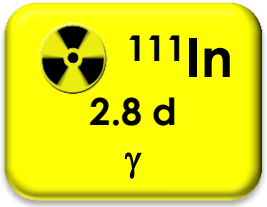


Marquage d'anticorps

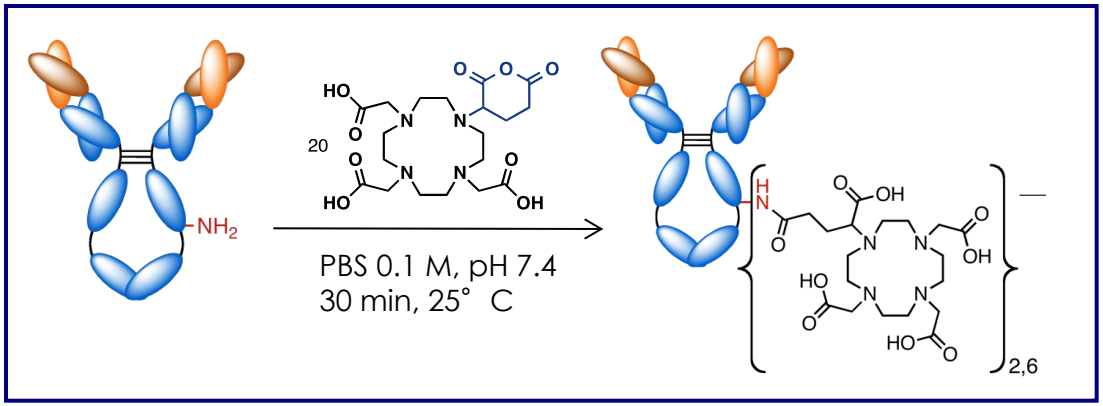
Marquage aléatoire



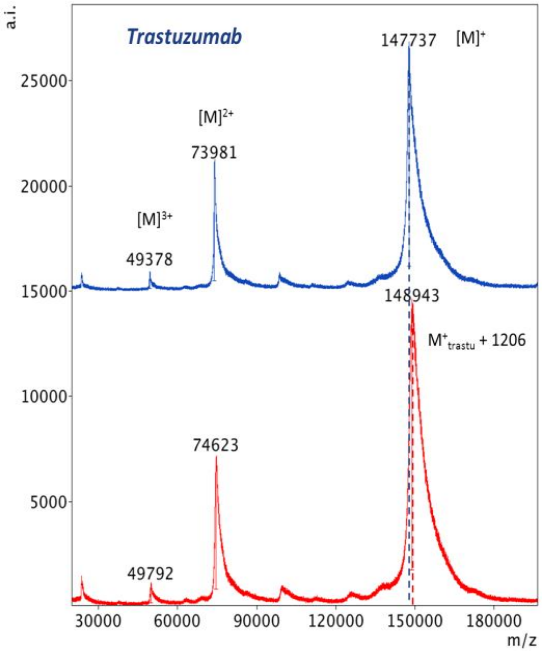
Marquage du trastuzumab par le DOTAGA-Anhydride



Le trastuzumab est un anticorps monoclonal ciblant les récepteurs HER2, surexprimés dans de nombreux cancers, en particulier le cancer du sein.



M. Moreau, O. Raguin, J.-M. Vrigneaud, B. Collin, C. Bernhard, X. Tizon, F. Boschetti, O. Duchamp, F. Brunotte, F. Denat *Bioconjugate Chem.* **2012**, *23*, 1181

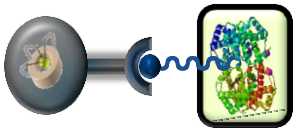


QC MALDI-TOF MS. Degree of conjugation of 2.6 macrocycles per antibody.

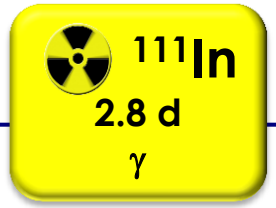
LCMuB · PHARM'IMAGE Platforms and competences for pharmacology · Onco design Biotechnology · CGFL Centre Georges François Leclerc Ensemble, dépassons le cancer · CheMatech macrocycle design technologies

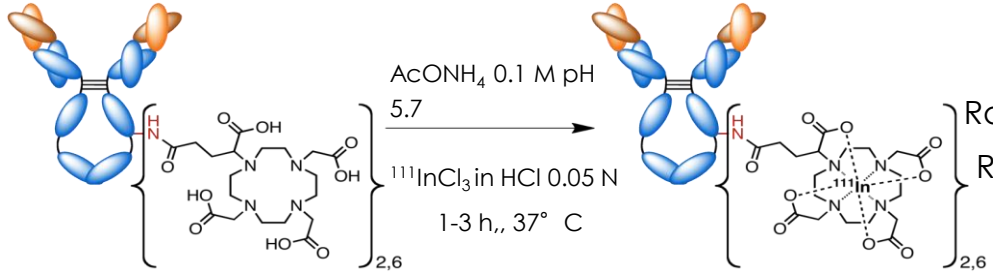
Marquage d'anticorps

Marquage aléatoire



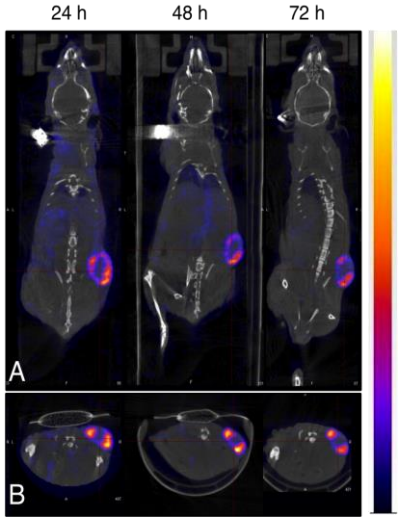
Marquage du trastuzumab par le DOTAGA-Anhydride



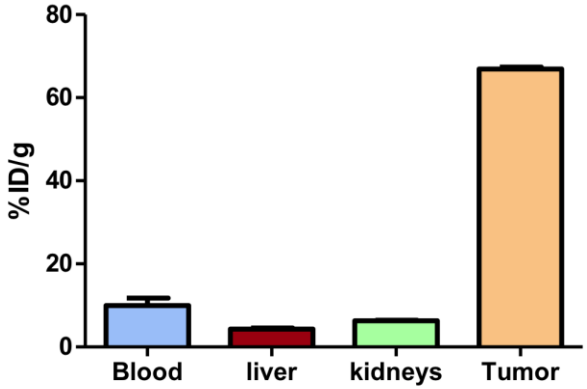


Radiolabeling yield : 75 %

Radiochemical purity : 99 %



Subcutaneous BT-474 human breast tumor imaging by SPECT/CT (24h/48h/72h post-injection of ^{111}In-radiolabeled compound).

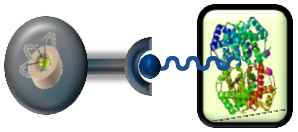


Ex vivo biodistribution of ^{111}In-DOTAGA-trastuzumab

M. Moreau, O. Raguin, J.-M. Vrigneaud, B. Collin, C. Bernhard, X. Tizon, F. Boschetti, O. Duchamp, F. Brunotte, F. Denat *Bioconjugate Chem.* **2012**, *23*, 1181

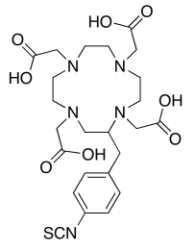
Marquage de fragments d'anticorps

Marquage aléatoire

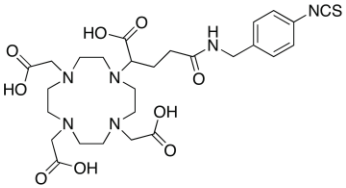


Comparaison de la stabilité *in vivo* de conjugués radiomarqués au ^{64}Cu

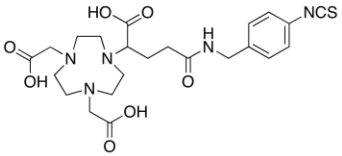
Comparaison de 4 BFC conjugués à des fragments de trastuzumab



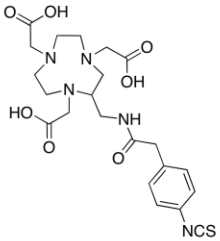
***p*-NCS-Bz-DOTA**



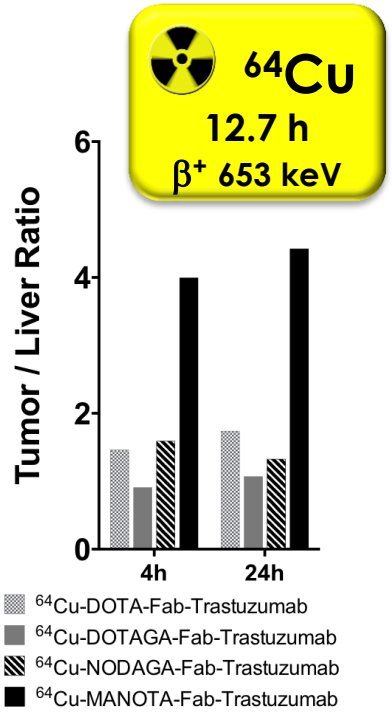
***p*-NCS-Bz-DOTAGA**

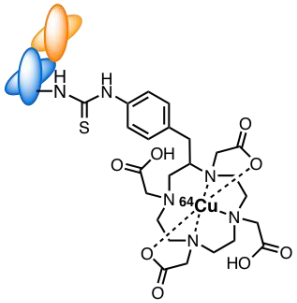


***p*-NCS-Bz-NODAGA**

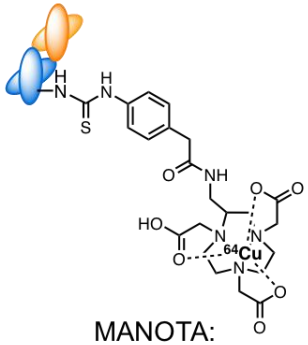


***p*-NCS-Bz-MANOTA**

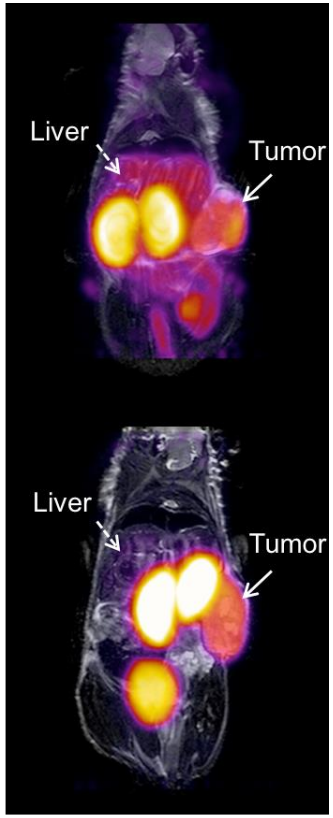




DOTA derivative:
high liver uptake



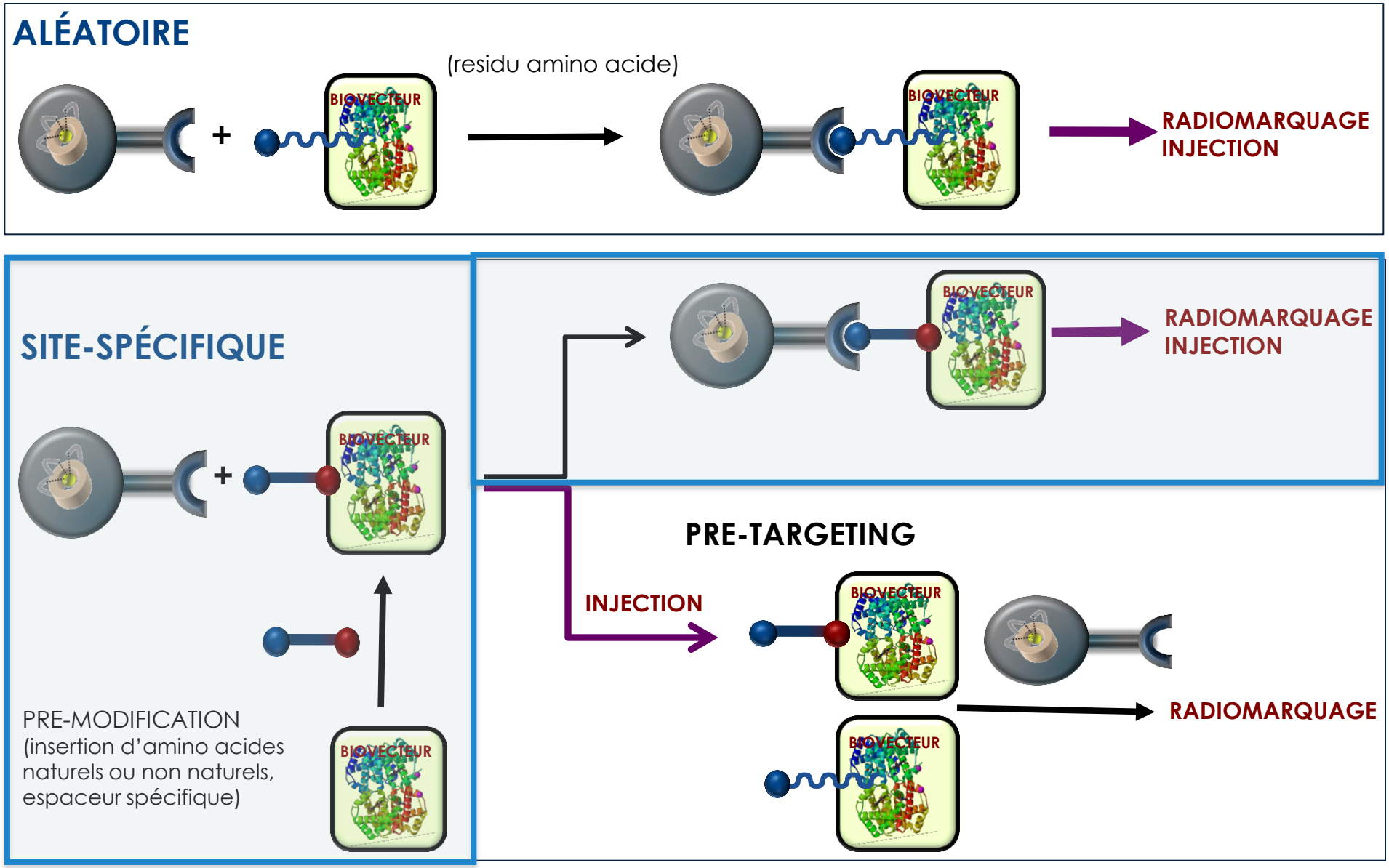
MANOTA:
low liver uptake



***p*-NCS-MANOTA est supérieur aux 3 autres BFC, incluant les "standards" DOTA et NODAGA**

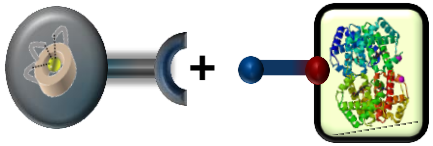
M. Moreau, S. Poty, J.-M. Vrigneaud, P. Walker, M. Guillemin, O. Raguin, A. Oudot, C. Bernhard, C. Goze, F. Boschetti, B. Collin, F. Brunotte, F. Denat *Submitted*

Stratégies de bioconjugaison

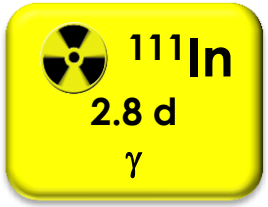


Marquage d'affibodies

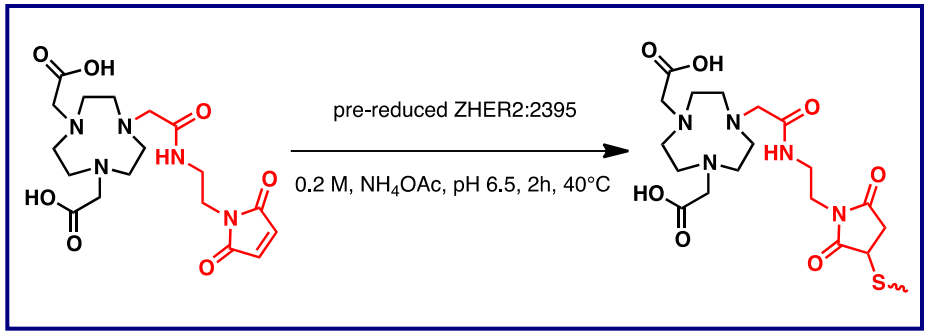
Marquage site spécifique

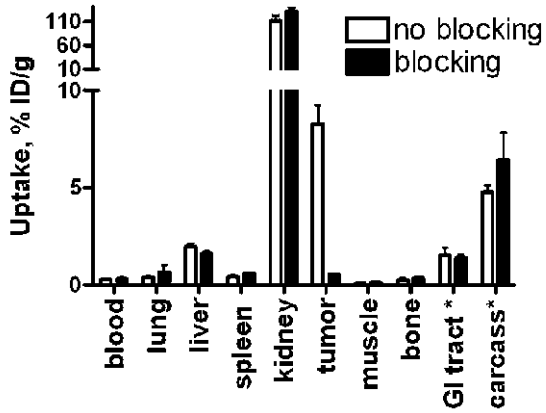


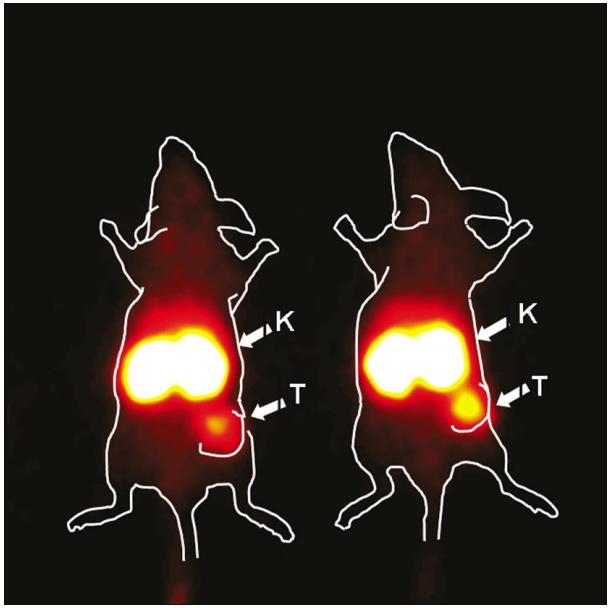
Affibody / stratégie « pre-réduction »



Marquage spécifique d'affibodies « pre-reduits » avec un BFC Maleimide-NOTA







SPECT images of nude mice bearing DU-145 xenografts 4h after injection of [^{111}In-MMA-NOTA-Cys^{61}]-$Z_{HER2:2395}$

V. Tolmachev, M. Altai, M. Sandström, A. Perols, A.E. Karlström, F. Boschetti, A. Orlova, *Bioconjugate Chem.* **2011**, *22*, 894

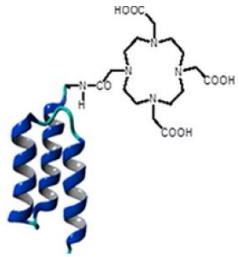
Marquage d'affibodies

Influence of DOTA Chelator Position on Biodistribution and Targeting Properties of ^{111}In-Labeled Synthetic Anti-HER2 Affibody Molecules

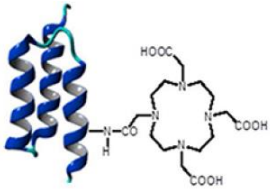
Anna Perols,[†] Hadis Honarvar,[‡] Joanna Strand,[‡] Ramkumar Selvaraju,[§] Anna Orlova,[§] Amelie Eriksson Karlström,[†] and Vladimir Tolmachev[‡,*]

Bioconjugate Chem., **2012**, 23, 1661-1670

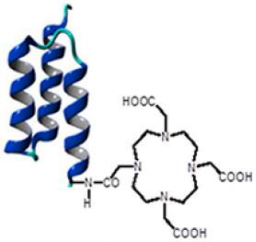
^{111}In
2.8 d
γ



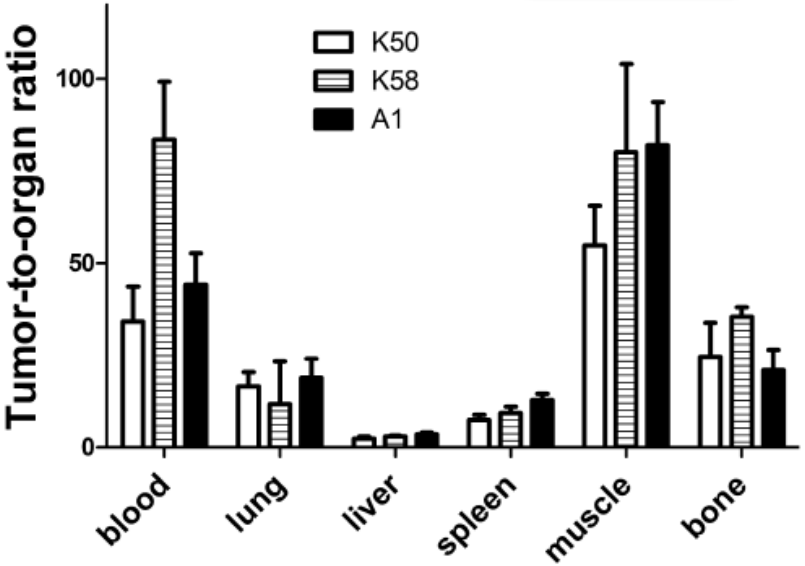
[DOTA-A1]–$Z_{HER2:S1}$



[DOTA-K50]–$Z_{HER2:S1}$



[DOTA-K58]–$Z_{HER2:S1}$



V. Tolmachev:

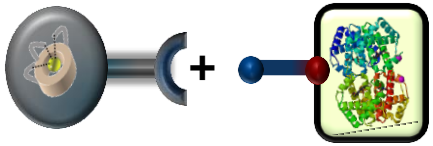
« Chemical properties and position of chelator/linker for labelling of Affibody molecules influence:

- **Predominant excretion pathway;**
- **Uptake and retention in excretory organs;**
- **Blood clearance rate »**

« Careful studies concerning influence of labeling chemistry on biodistribution and targeting may make a difference between success and failure ! »

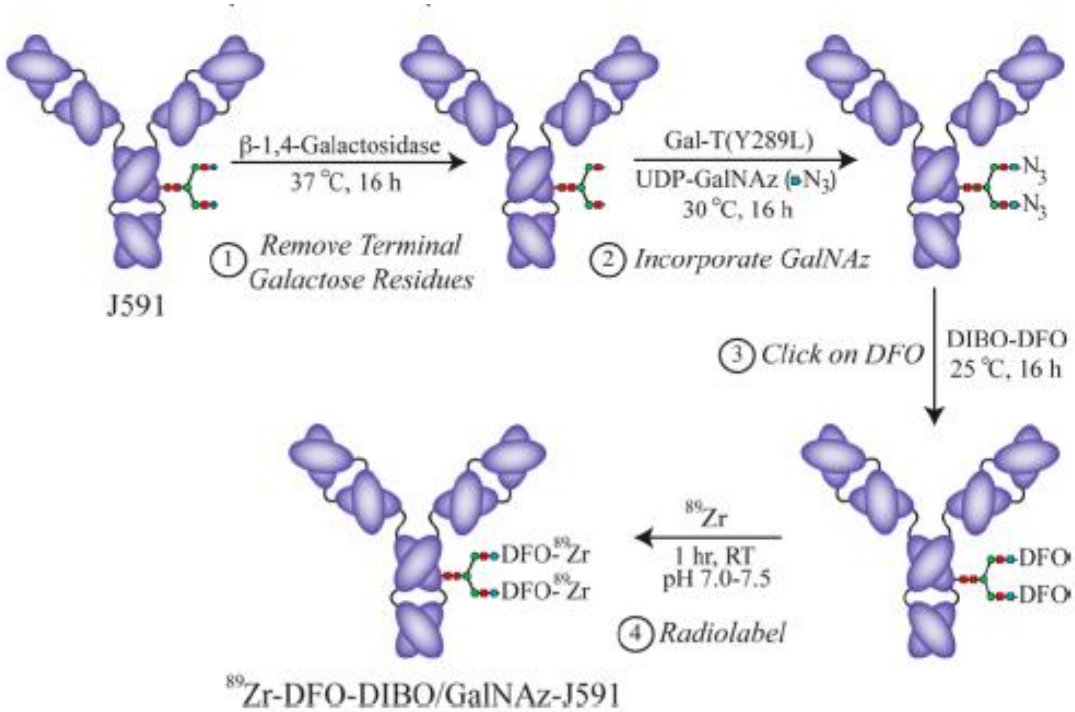
Marquage d'anticorps

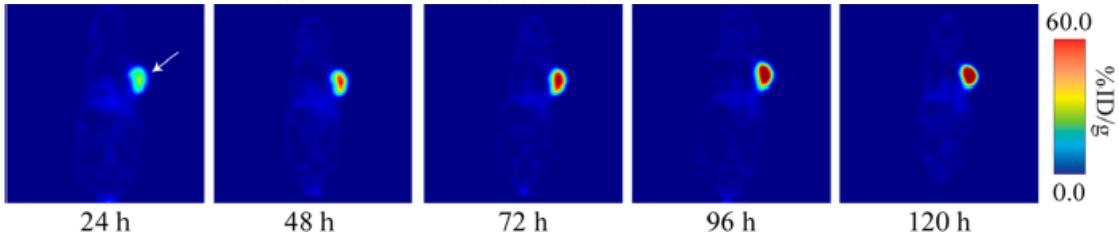
Marquage site spécifique

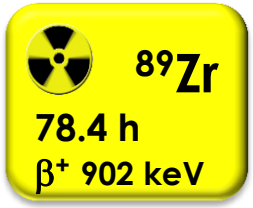


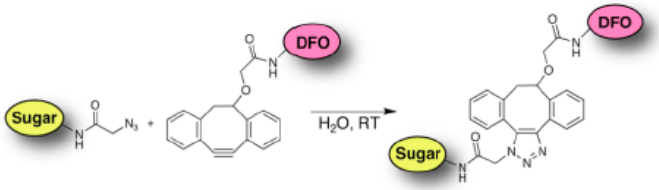
MAbs/ stratégie « chimie click »

Enzyme and click chemistry-mediated methodology for site-specific labeling of antibodies on the heavy chain by incorporation of azide-modified *N*-acetylgalactosamine monosacharides



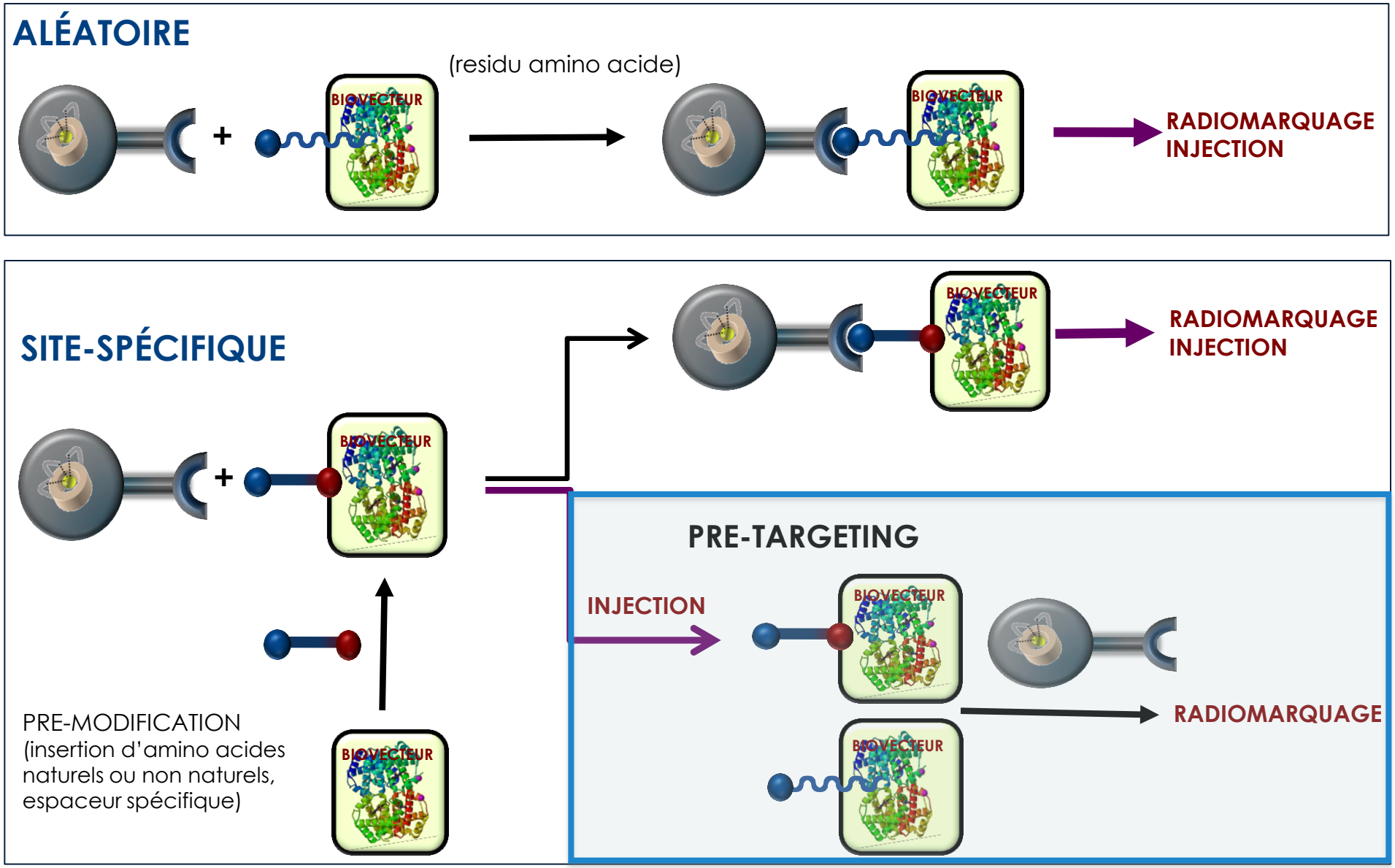






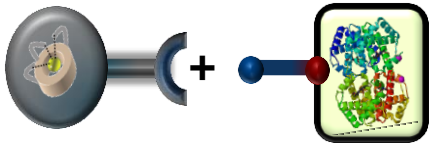
B. M. Zeglis et al. *Bioconjugate. Chem.*, **2013**, 24, 1057-1067

Stratégies de bioconjugaison



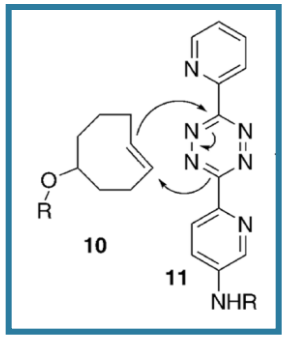
Marquage d'anticorps

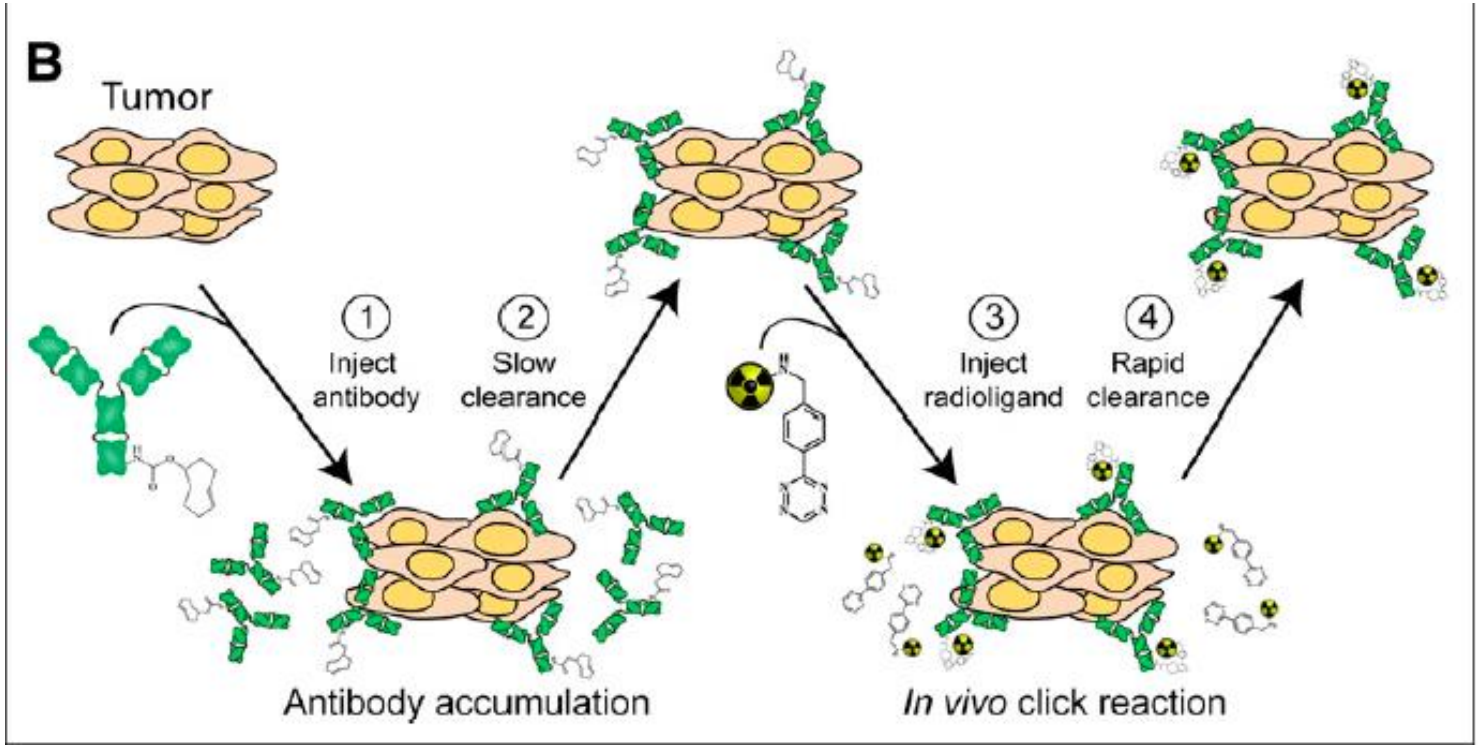
site spécifique / pre-targeting



Pre-targeting / stratégie « chimie click »

Selective *in vivo* binding of radioactive tetrazine probe to trans-cyclooctene tag of antibody via bioorthogonal reaction, **boosting target-to-nontarget ratios.**

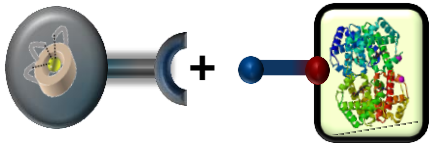




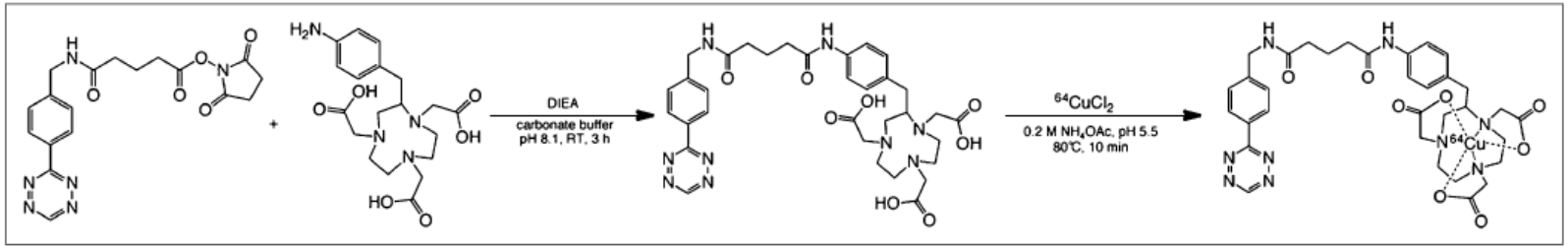
Weissleder, Lewis *J. Nucl. Med.* **2013**, *54*, 1389-1396

Marquage d'anticorps

site spécifique / pre-targeting



Pre-targeting / stratégie « chimie click »



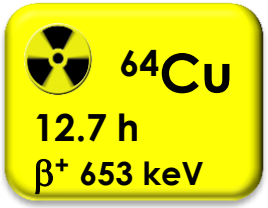


FIGURE 3. Synthesis of ^{64}Cu-Tz-Bn-NOTA.

TABLE 1
Biodistribution Data for In Vivo ^{64}Cu Pretargeting Experiment

Organ	1 h	4 h	12 h	24 h
Blood	3.5 ± 0.6	2.6 ± 0.8	2.3 ± 0.4	2.1 ± 0.5
Tumor	4.1 ± 0.3	4.1 ± 0.6	4.2 ± 0.8	4.0 ± 0.9
Heart	1.1 ± 0.2	0.9 ± 0.3	0.9 ± 0.1	0.8 ± 0.2
Lung	1.6 ± 0.5	1.6 ± 0.4	1.1 ± 0.4	1.0 ± 0.3
Liver	2.2 ± 0.3	1.3 ± 0.3	0.9 ± 0.2	1.1 ± 0.2
Spleen	0.6 ± 0.1	0.5 ± 0.2	0.6 ± 0.3	0.4 ± 0.1
Stomach	0.5 ± 0.1	0.3 ± 0.1	0.5 ± 0.6	0.2 ± 0.0
Large intestine	0.5 ± 0.0	3.1 ± 1.1	2.3 ± 1.0	1.1 ± 0.4
Feces	11.9 ± 4.4	8.8 ± 3.4	2.5 ± 0.1	1.4 ± 0.5
Small intestine	0.0 ± 0.0	0.4 ± 0.1	0.8 ± 0.5	0.3 ± 0.0
Kidney	1.3 ± 0.1	0.9 ± 0.3	0.9 ± 0.3	0.7 ± 0.2
Muscle	0.2 ± 0.0	0.1 ± 0.0	0.2 ± 0.0	0.1 ± 0.0
Bone	0.3 ± 0.2	0.3 ± 0.2	0.4 ± 0.1	0.3 ± 0.1
Skin	0.3 ± 0.1	0.3 ± 0.1	0.4 ± 0.1	0.5 ± 0.2

Values are %ID/g ± SD. Mice (n = 4) bearing subcutaneous SW1222 xenografts were administered A33-TCO (100 μg) via tail vein injection. After 24 h, the same mice were administered ^{64}Cu-Tz-Bn-NOTA (0.55–0.75 MBq [15–20 μCi]) via tail vein injection (t = 0).

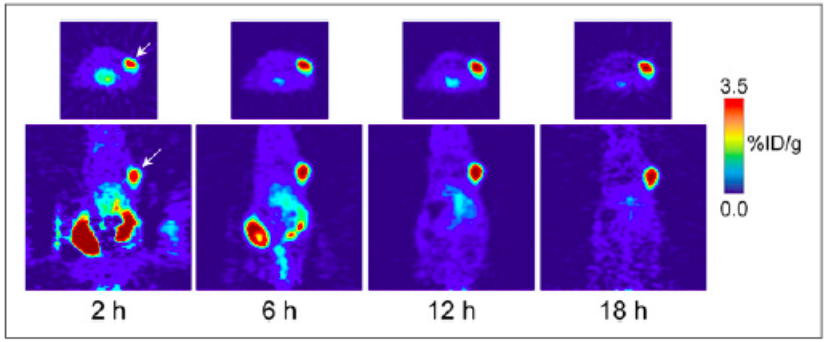
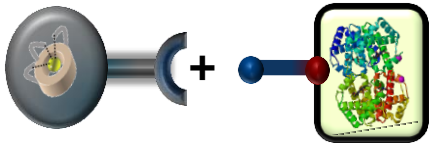


FIGURE 4. PET images of ^{64}Cu-Tz-Bn-NOTA/A33-TCO pretargeting strategy. Mice bearing subcutaneous SW1222 xenografts (100–150 mm^3, arrow) were administered A33-TCO (100 μg) via tail vein injection. After 24 h, the same mice were administered ^{64}Cu-Tz-Bn-NOTA (10.2–12.0 MBq [275–325 μCi], 1.2–1.4 μg, for 2.5–2.8 Tz-to-A33 ratio) via tail vein injection. Transverse (top) and coronal (bottom) planar images intersect the center of the tumors.

Weissleder, Lewis *J. Nucl. Med.* **2013**, *54*, 1389-1396

Marquage d'anticorps

site spécifique / pre-targeting



Pre-targeting / stratégie « chimie click »

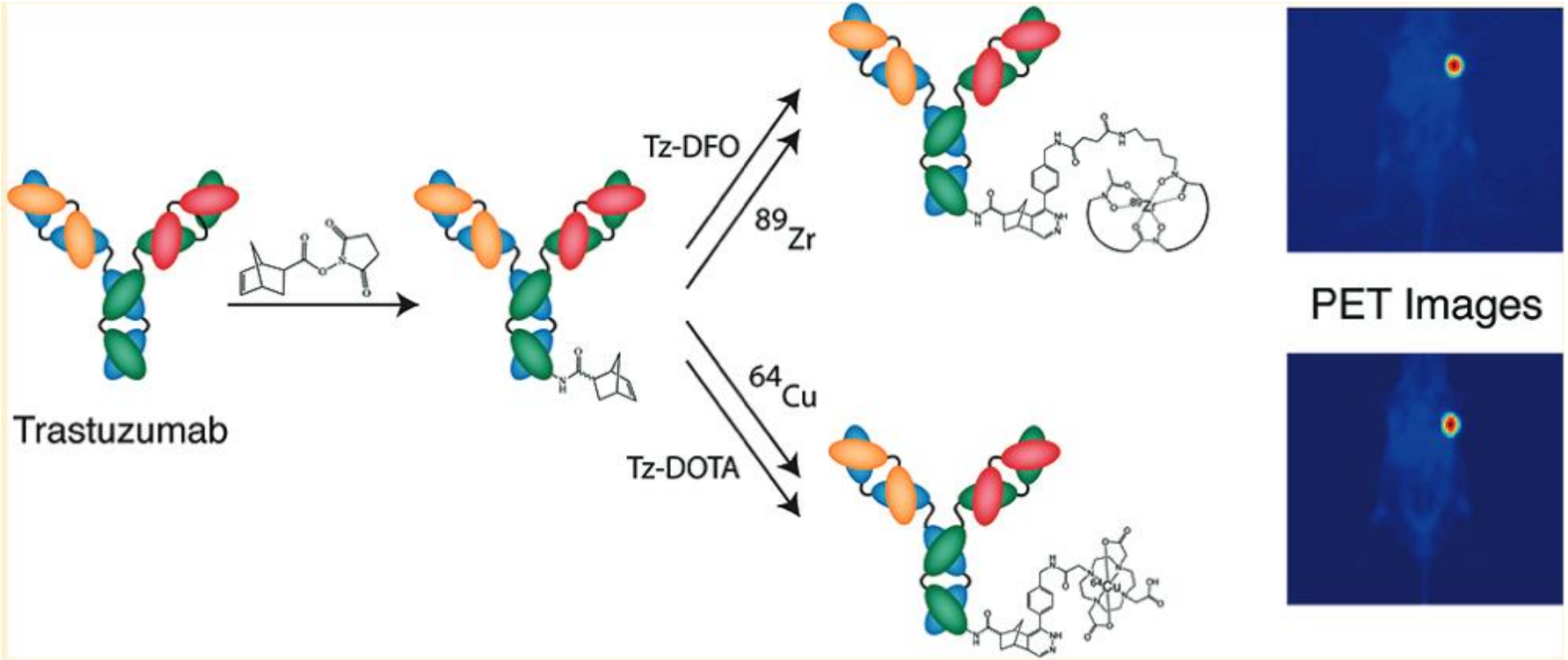
^{89}Zr
78.4 h
β^+ 902 keV

^{64}Cu
12.7 h
β^+ 653 keV

Bioconjugate Chemistry
ARTICLE
pubs.acs.org/bc

Modular Strategy for the Construction of Radiometalated Antibodies for Positron Emission Tomography Based on Inverse Electron Demand Diels–Alder Click Chemistry

Brian M. Zeglis,[†] Priya Mohindra,[†] Gabriel I. Weissmann,[†] Vadim Divilov,[†] Scott A. Hilderbrand,[‡] Ralph Weissleder,[‡] and Jason S. Lewis*[,†]



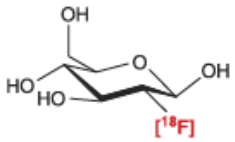
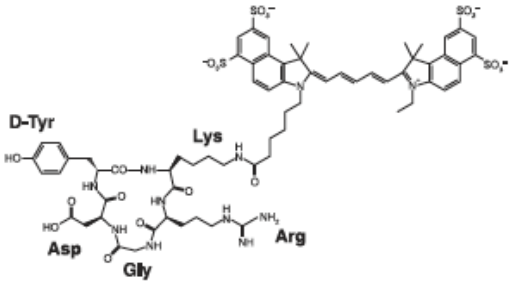
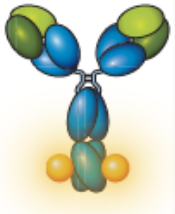
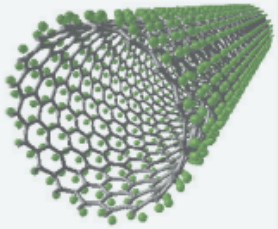
Imagerie moléculaire

Physiol Rev 92: 897–965, 2012
doi:10.1152/physrev.00049.2010

A MOLECULAR IMAGING PRIMER: MODALITIES, IMAGING AGENTS, AND APPLICATIONS

Michelle L. James and Sanjiv S. Gambhir

MODIFICATION DE NANOPARTICULES

Type	Small molecule	Peptide	Affibody	Aptamer	Antibody	Nanoparticle
Size	<1 nm <0.5 kDa	~1–4 nm ~0.5–2 kDa	~5–10 kDa	~5–15 kDa	~150 kDa	~10–200 nm (no larger than 1,000 nm)
Example	 [^{18}F]FDG	 RGD-Cy5.5	 [^{68}Ga]-DOTA-MUT-DS	 Molecular Beacon	 ICG-Trastuzumab	 RGD-SWNT

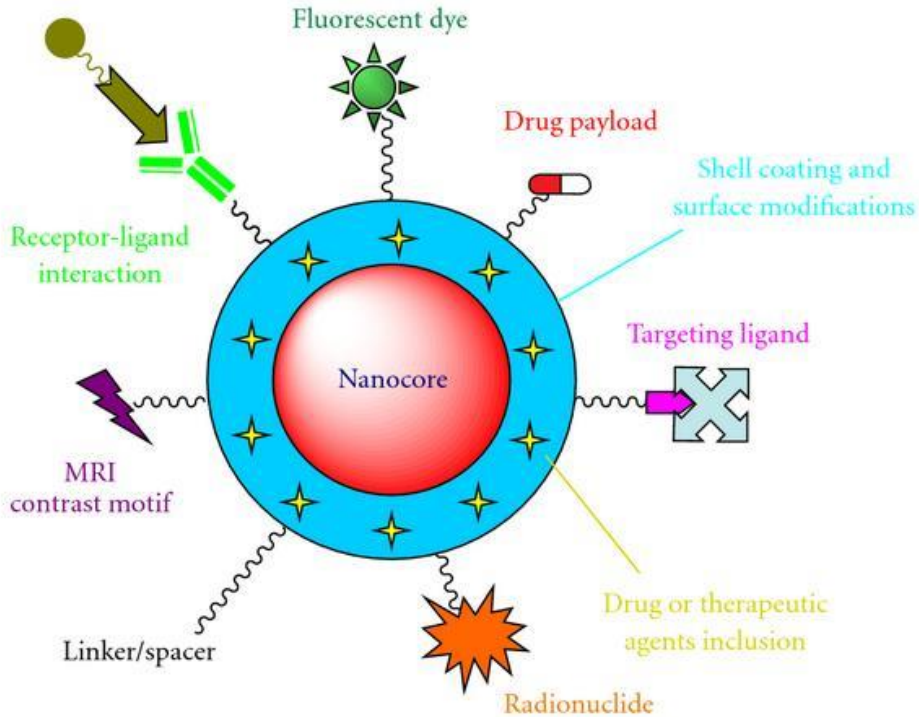
Quencher Donor Signaling component

Nanoparticules fonctionnalisées

Review Article

Advanced Nanomaterials in Multimodal Imaging: Design, Functionalization, and Biomedical Applications

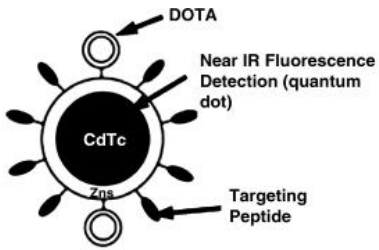
Zhe Liu, Fabian Kiessling, and Jessica Gätjens **J. Nanomater., 2010**



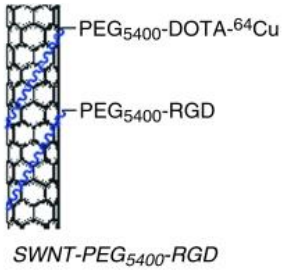
Copper-64 Radiopharmaceuticals for PET Imaging of Cancer: Advances in Preclinical and Clinical Research

Carolyn J. Anderson[1–3] and Riccardo Ferdani[1]

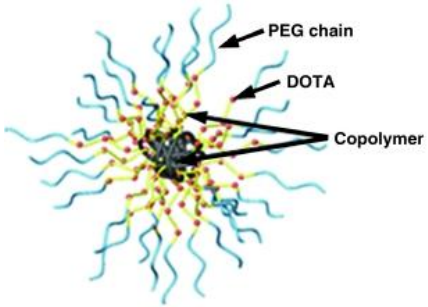
CANCER BIOTHERAPY AND RADIOPHARMACEUTICALS
Volume 24, Number 4, 2009



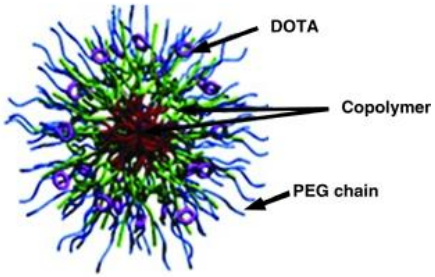
DOTA-Quantum Dot



SWNT-PEG_{5400}-RGD



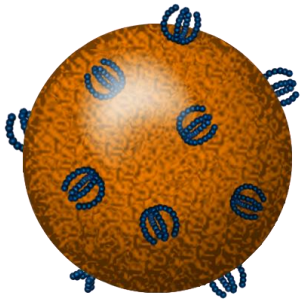
PEGylated DOTA-star copolymer

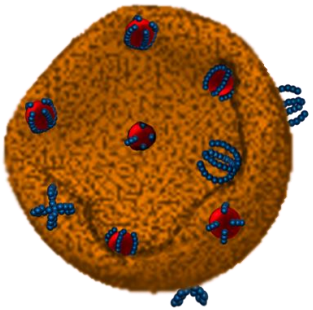


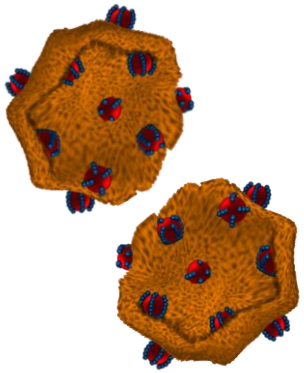
PEGylated DOTA-Shell Cross-Linked NPs

Nanoparticules fonctionnalisées

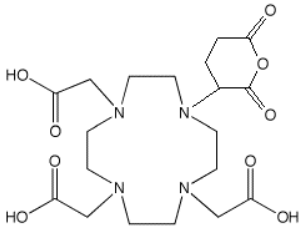
Nanoparticules AGUIX®





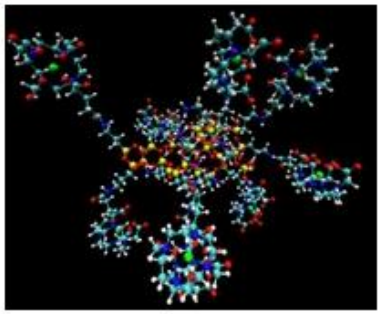


Gd_2O_3 polysiloxane APTES Np
+



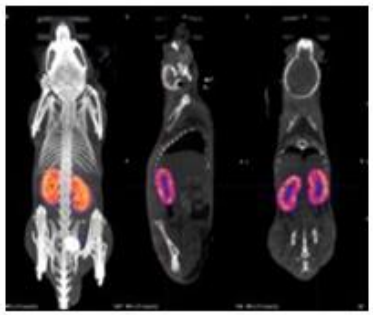
Dissolution du cœur oxyde de gadolinium,
Piégeage du gadolinium par le chélate

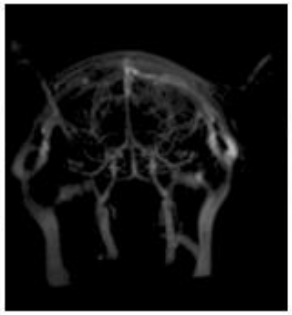
Ultrasmall size
4±1 nm, MW 8.5±2 kDa

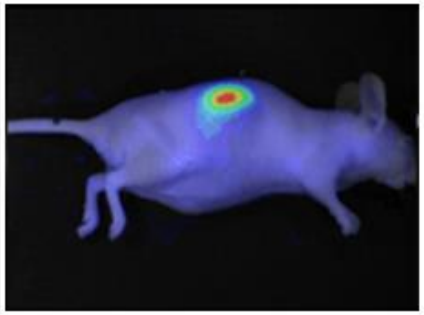


Multimodal imaging

Renal excretion

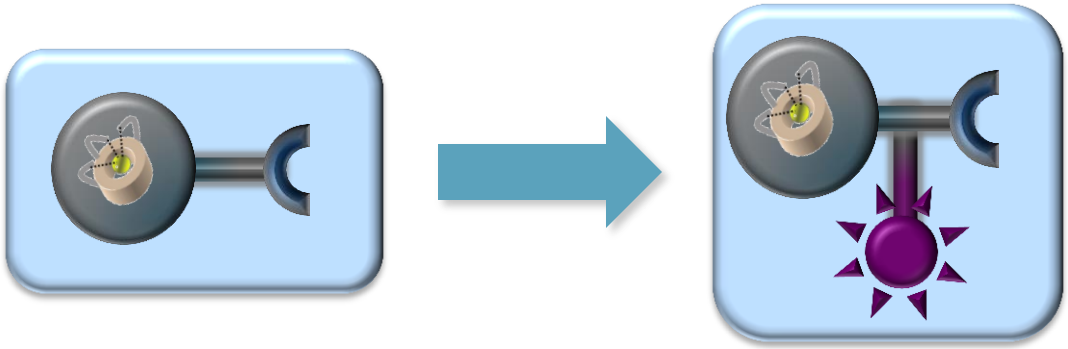






F. Lux et al. *Angew. Chem. Int. Ed.*, **2011**, *50*, 12299-12303

Vers la multimodalité et le theranostic



Approche multimodale

Combinaison de deux techniques d'imagerie de sensibilité comparable :
OI/SPECT-PET

Imagerie préclinique :

-Covalidation de la localisation de la tumeur

-Imagerie *in vivo* à des temps longs p.i.

-Microscopie *ex vivo*

Application clinique : chirurgie guidée par l' image

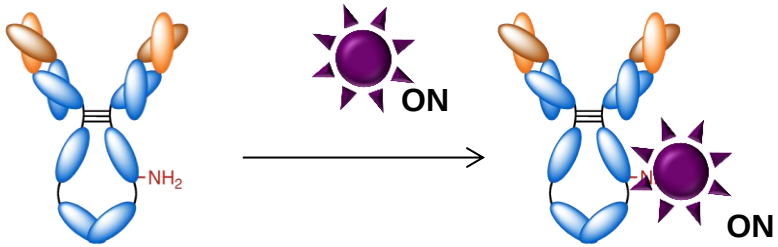
-Imagerie PET/SPECT pré-opérative pour identifier et localiser les tumeurs

-Imagerie OI (et SPECT) pour une meilleure résection de la tumeur

Culver et al. Combined Optical and PET/SPECT Methods *J. Nucl. Med.*, **2008**, 49, 169-172
C.A. Boswell, M.W. Brechbiel, *J. Med. Chem.*, **2007**, 50, 4759-4765
Z. Zhang, S. Achifelu., *Bioconjugate Chem.* **2005**, 16, 1232-1239

Bioconjugués pour l'imagerie optique

Chirurgie guidée par l' imagerie optique avec un anticorps fluorescent



translationnel

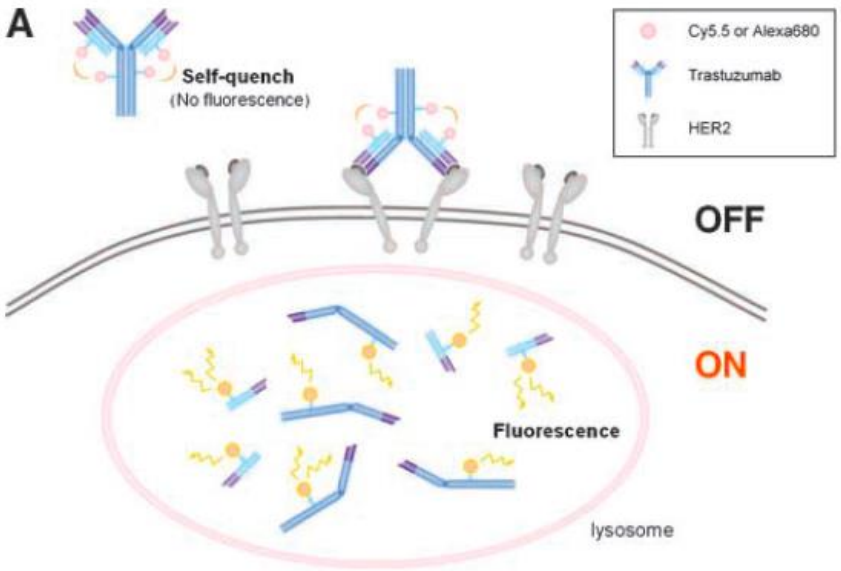
Etudes cliniques : chirurgie de cancers

Ann Surg Oncol (2014) 21:1405–1411
DOI 10.1245/s10434-014-3495-y

« SMART PROBES »
Ex : système OFF/ON

Fluorescence imaging *in vivo* Rao, Dragulescu-Andrasi and Yao

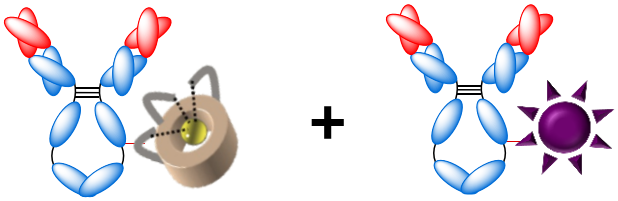
Ogawa et al. *Mol. Cancer Ther.* **2009**, 8,232-239



Vers la multimodalité et le theranostic

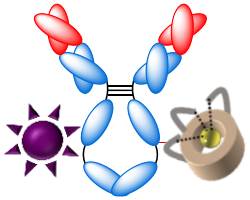
Plusieurs approches imagerie bimodale PET ou SPECT / OI

- **Approche cocktail : co-injection de deux bioconjugués ≠**



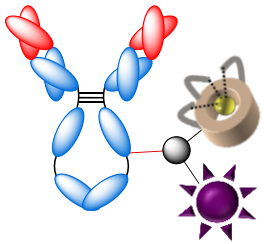
☹ Biodistribution ≠ ?

- **Approche séquentielle : deux sondes ≠ sur le même vecteur**



☹ Contrôle du ratio des 2 sondes

- **Approche SAP « Single Attachment Point » : une seule molécule contenant les deux sondes : MOMIP « Monomolecular Multimodal Imaging Probe »**



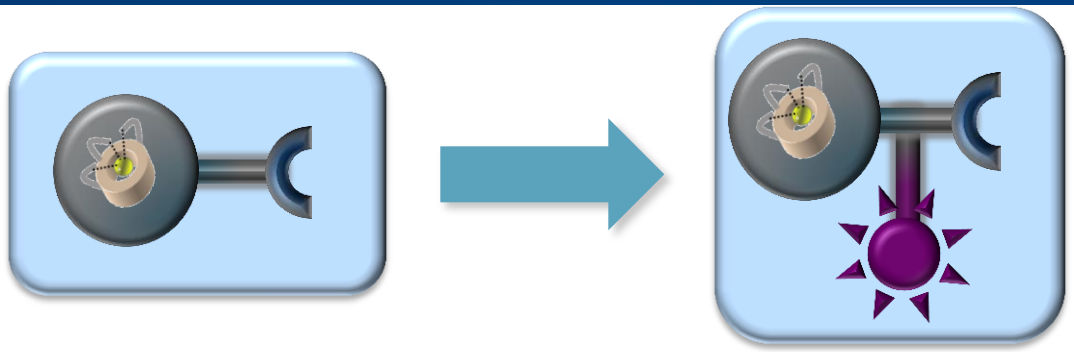
☺ Meilleur contrôle

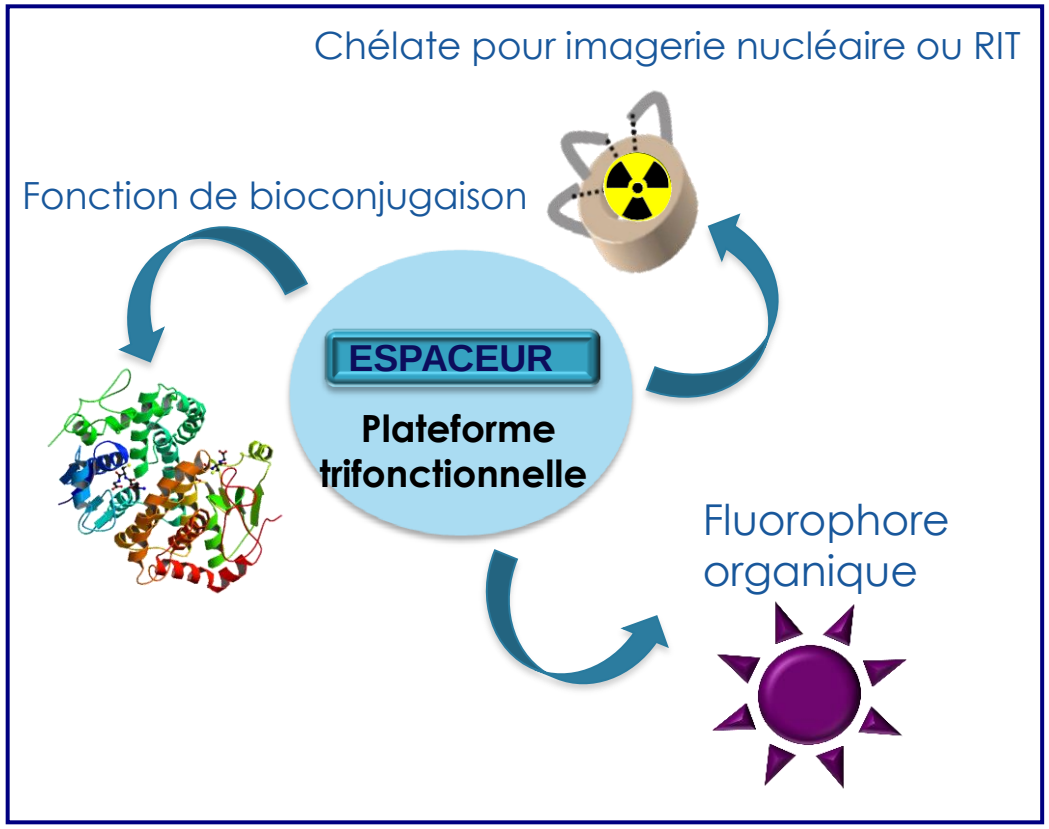
☺ Marquage site spécifique

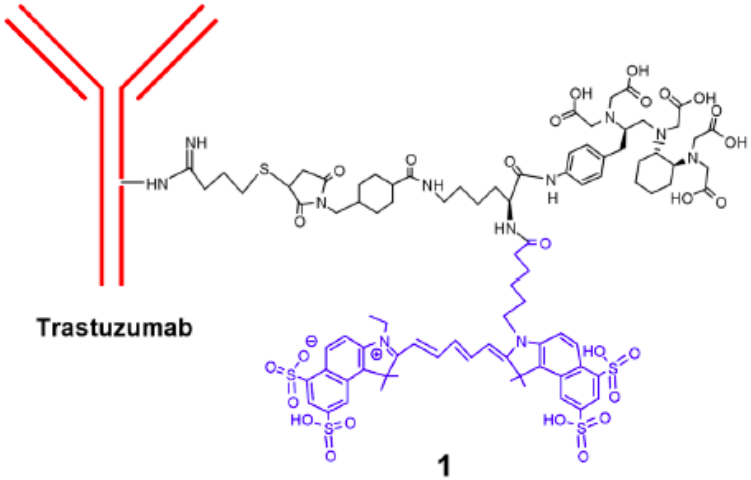
☹ Synthèse

Vers la multimodalité et le theranostic

MOMIP
Mon**O**molecular
Multimodality **I**maging
Probe



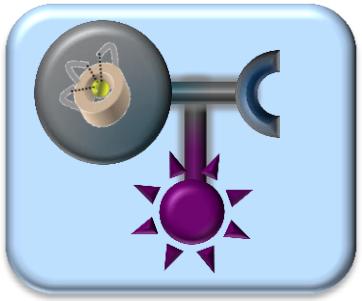




Brechbiel and coll. *Bioorg. Med. Chem.* **2009**

Culver et al. Combined Optical and PET/SPECT Methods *J. Nucl. Med.*, **2008**, 49, 169-172
C.A. Boswell, M.W. Brechbiel, *J. Med. Chem.*, **2007**, 50, 4759-4765
Z. Zhang, S. Achifelu., *Bioconjugate Chem.* **2005**, 16, 1232-1239

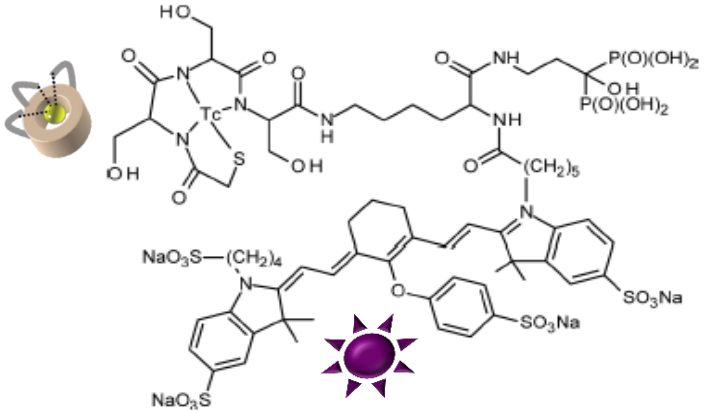
Vers la multimodalité et le theranostic



Multimodal radio- (PET/SPECT) and fluorescence imaging agents based on metallo-radioisotopes: current applications and prospects for development of new agents

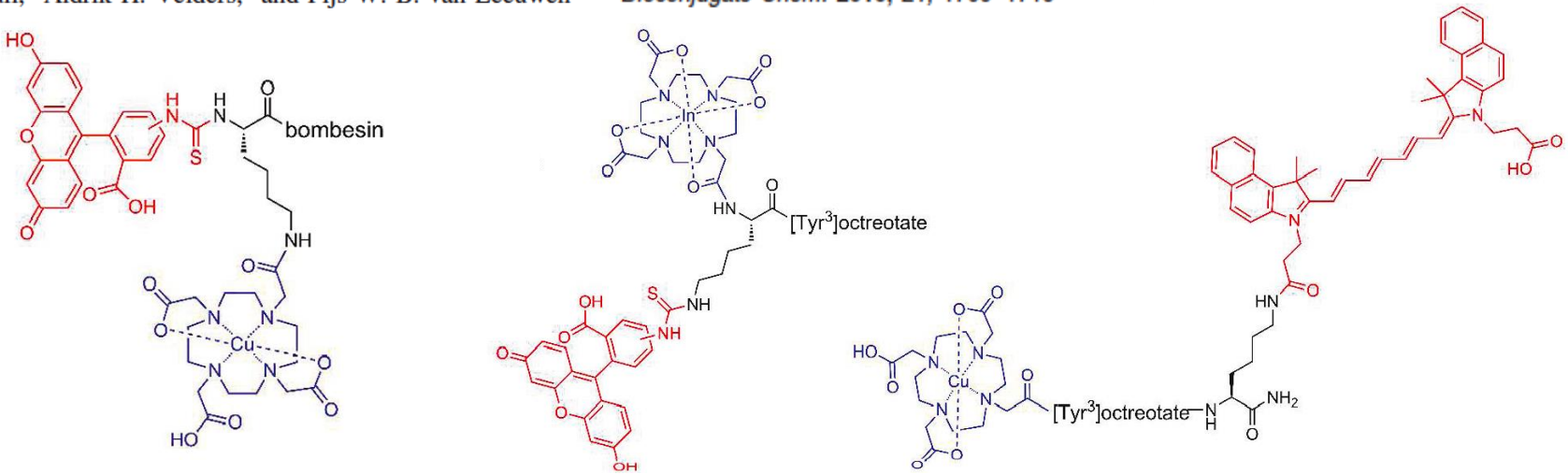
Flora L. Thorp-Greenwood and Michael P. Coogan*

Dalton Trans., **2011**

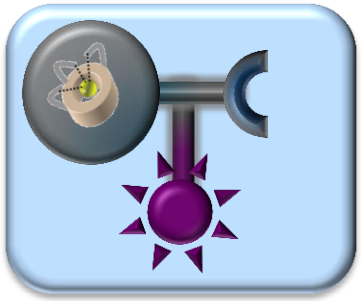


Multimodal Tumor-Targeting Peptides Functionalized with Both a Radio- and a Fluorescent Label

Joeri Kuil,† Aldrik H. Velders,‡ and Fijs W. B. van Leeuwen* *Bioconjugate Chem.* **2010**, *21*, 1709–1719



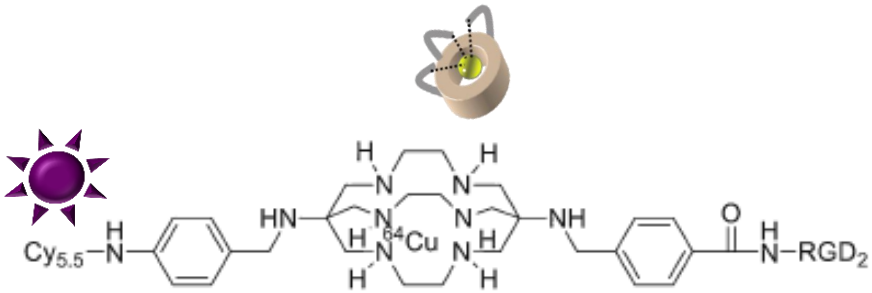
Vers la multimodalité et le theranostic



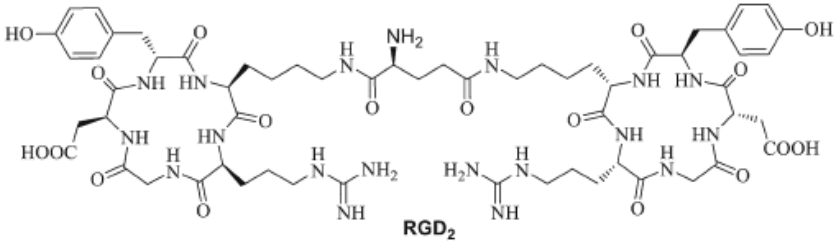
Efficient Construction of PET/Fluorescence Probe Based on Sarcophagine Cage: An Opportunity to Integrate Diagnosis with Treatment

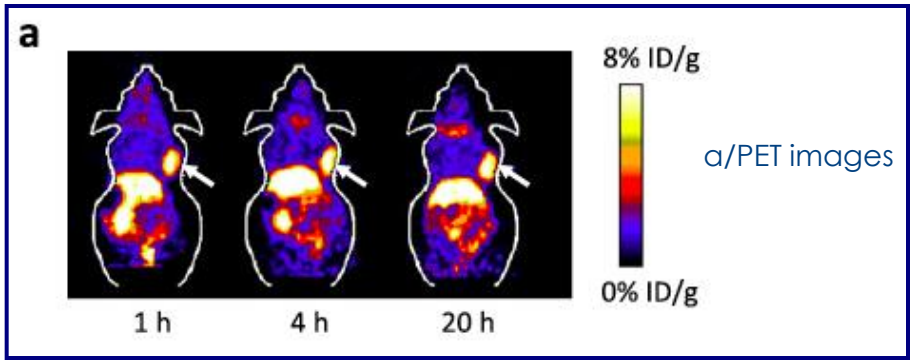
Mol Imaging Biol (2012) 14:718–724
DOI: 10.1007/s11307-012-0557-z

Shuanglong Liu,[1] Dan Li,[1,2] Chiun-Wei Huang,[1] Li-Peng Yap,[1] Ryan Park,[1] Hong Shan,[2] Zibo Li,[1] Peter S. Conti[1]

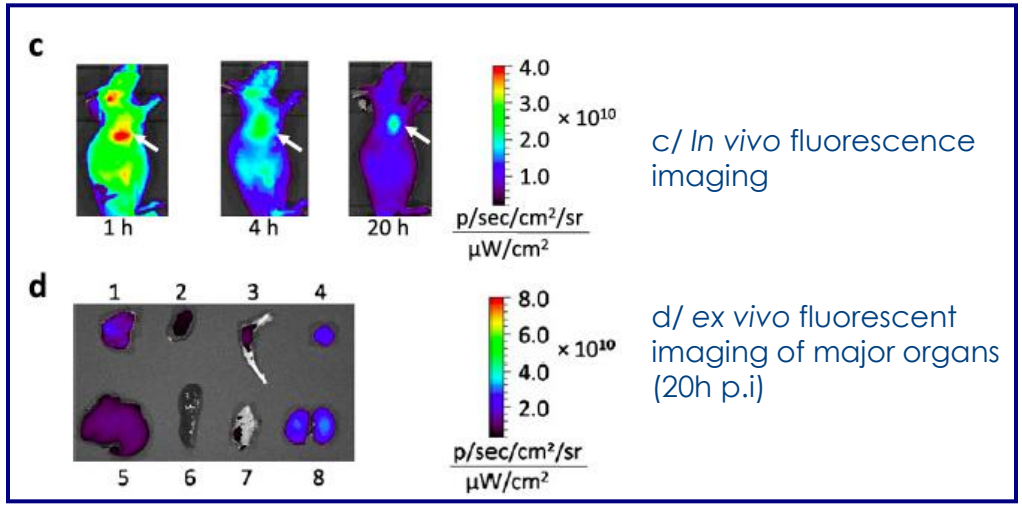


^{64}Cu-BaAnSar-RGD$_2$-Cy5.5





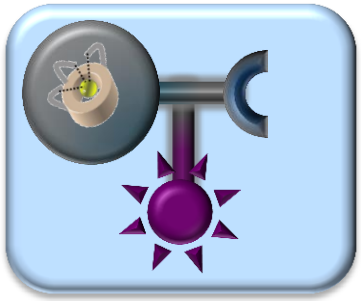
a/PET images



c/ *In vivo* fluorescence imaging

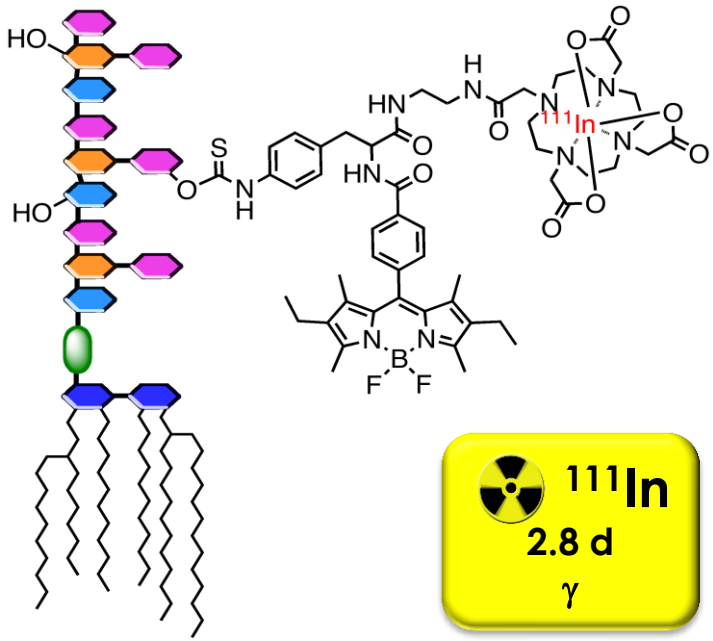
d/ *ex vivo* fluorescent imaging of major organs (20h p.i)

Vers la multimodalité et le theranostic

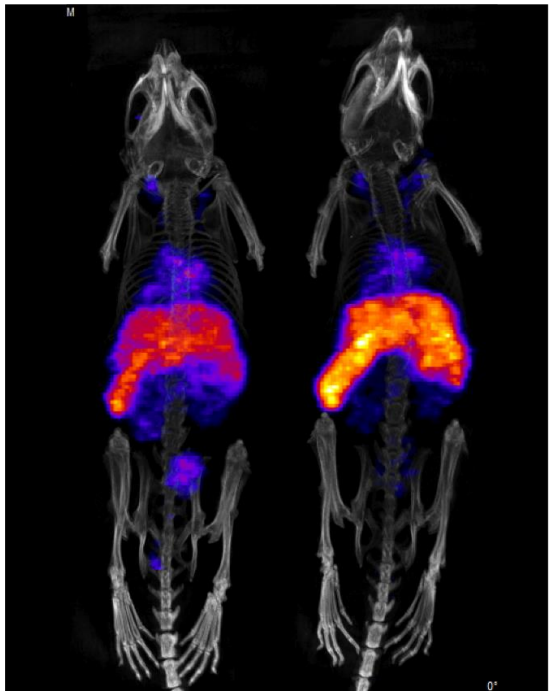


Double marquage de lipopolysaccharides (LPS) afin d'étudier le mode d'élimination de ces endotoxines

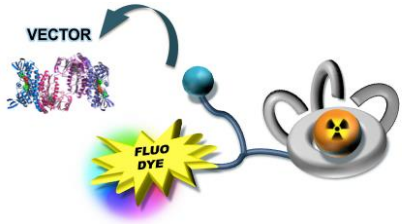
^{111}InDOTA-BOPIDY-LPS

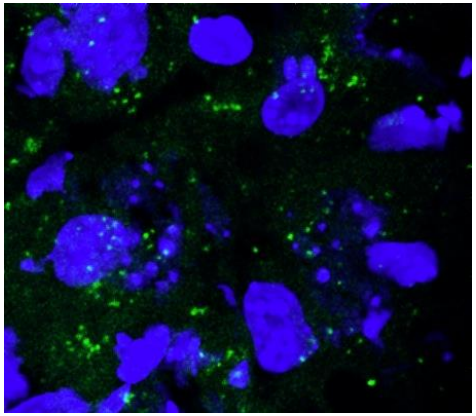


> Imagerie SPECT-CT + microscopie de fluorescence



Liver uptake (after 24h) visualized in the whole animal by SPECT/CT imaging

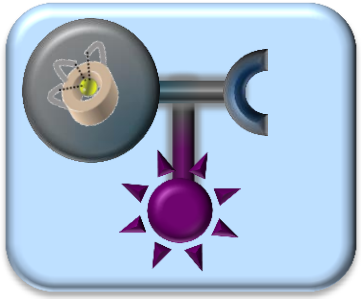




Histological analyses with fluorescence microscopy

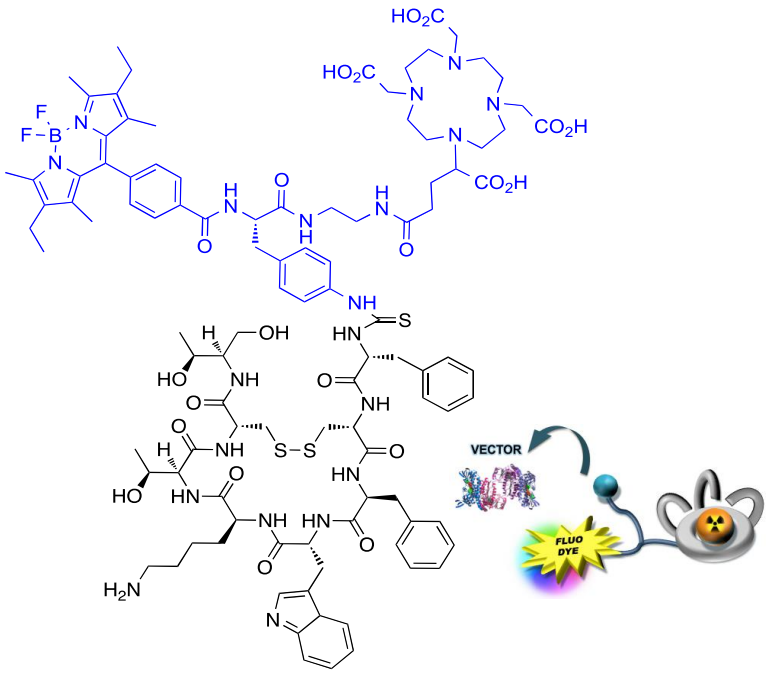
V. Duhéron, M. Moreau, B. Collin, W. Sali, C. Bernhard, C. Goze, T. Gautier, J.-P. Pais de Barros, V. Deckert, F. Brunotte, L. Lagrost, F. Denat, ACS *Chem. Biol Chem.* **2014**, 656-662

Vers la multimodalité et le theranostic



Double marquage de l'octréotide (récepteurs sst, tumeurs neuroendocrines)

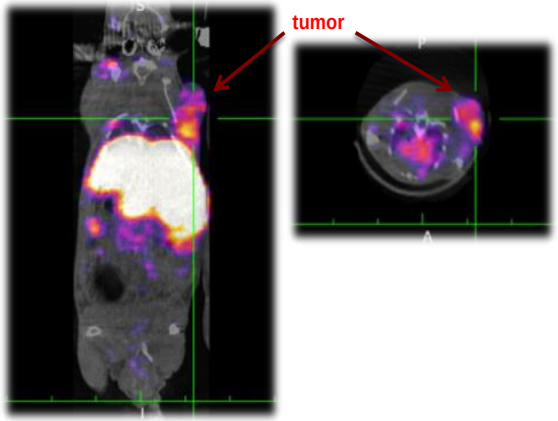
DOTAGA-BOPIDY-Octreotide



Radiomarquage avec Indium 111

Imagerie SPECT/CT

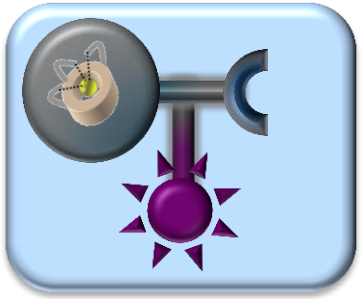
In vivo imaging of ^{111}In-DOTAGA-BOD-OC in male SWISS Nude mice bearing subcutaneous AR42J tumors was performed to assess the biological behavior of the conjugate.



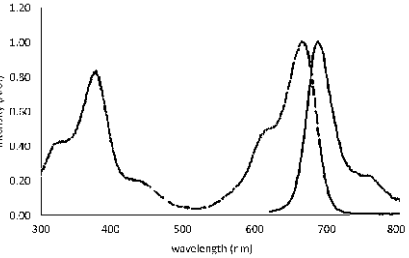
Subcutaneous AR42J tumors imaging by SPECT/CT 24h post injection (injected dose : 2.2-2.6 MBq/mouse (3.2-3.4 nmol)

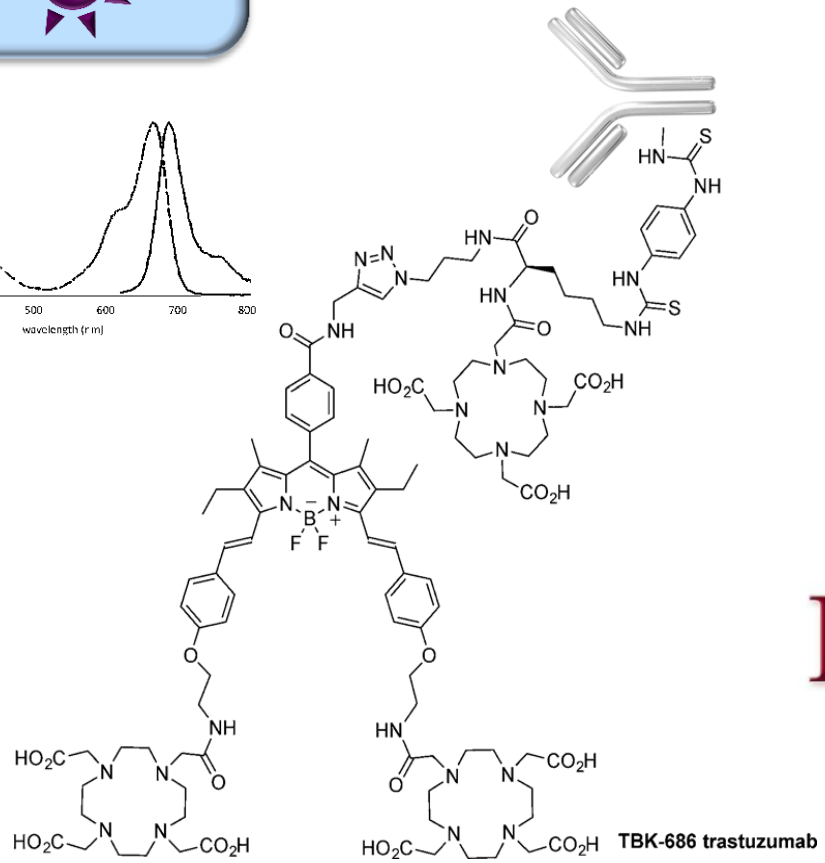
D. Lhenry, C. Bernhard, M. Larrouy, V. Goncalves, O. Raguin, P. Provent, M. Moreau, B. Collin, A. Oudot, J.-M. Vrigneaud, F. Brunotte, C. Goze, F. Denat, *Chem, Eur. J.*, **2015**, *21*, 13091-13099.

Vers la multimodalité et le theranostic

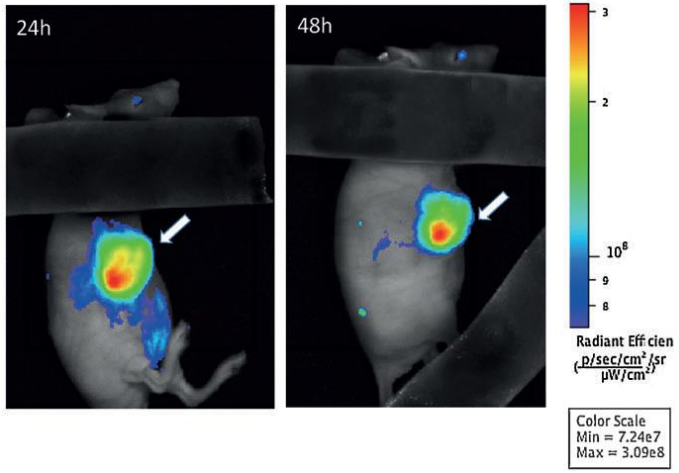


Double marquage du trastuzumab avec une nouvelle génération de MOMIP





- ✓ Le BODIPY émet dans le proche infrarouge
- ✓ La présence de 3 DOTA augmente le caractère hydrophile et l'efficacité de la radiométallation



N. Maindron, M. Ipuy, C. Bernhard, D. Lhenry, M. Moreau, S. Carme, A. Oudot, B. Collin, J.-M. Vrigneaud, P. Provent, F. Brunotte, F. Denat, C. Goze, *Chem, Eur. J.*, **2016**, 22, 12670-12674.

Conclusion

Le chimiste joue un rôle clef dans le futur de l'imagerie…

- ✓ Chimie organique pour la synthèse de :
  - ✓ fluorophores
  - ✓ petites molécules marquées (ex FDG)
  - ✓ chélates pour agents de contraste IRM
  - ✓ agents chélatants bifonctionnels pour l'imagerie PET / SPECT
  - ✓ sondes multimodales (MOMIP)
- ✓ Chimie de coordination
- ✓ Synthèse de peptides
- ✓ Bioconjugaison
  - ✓ développement de nouvelles techniques de bioconjugaison
  - ✓ chimie bioorthogonale (chimie click) pour marquage site-spécifique
- ✓ Synthèse et fonctionnalisation de nanoparticules
- ✓ Radiochimie
- ✓ …

… en travaillant au plus près des médecins, biologistes, pharmaciens, spécialistes de l'imagerie.