# Biological evaluation of $^{64}$Cu-radiolabeled gastrin-releasing peptide receptors antagonist conjugated to DOTHA$_2$ a new bifunctional chelator bearing hydroxamic acid arms

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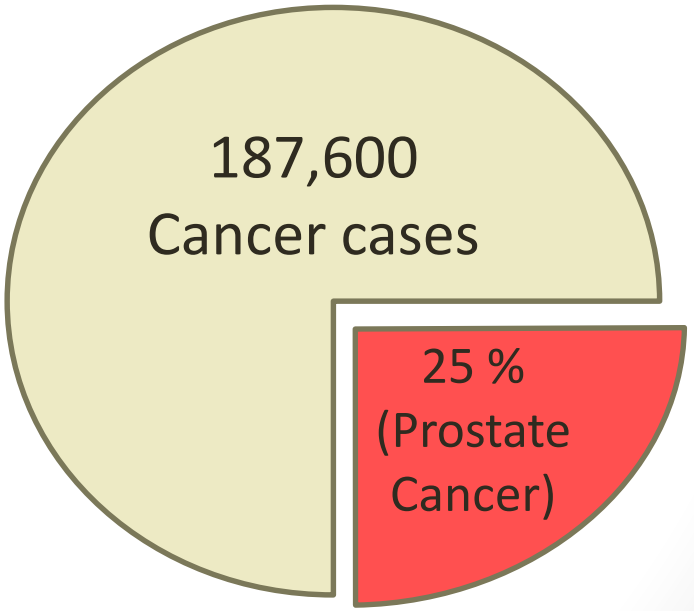
24th June 2015

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- Introduction
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# Introduction
# Prostate cancer statistics in 2013 (Canada)

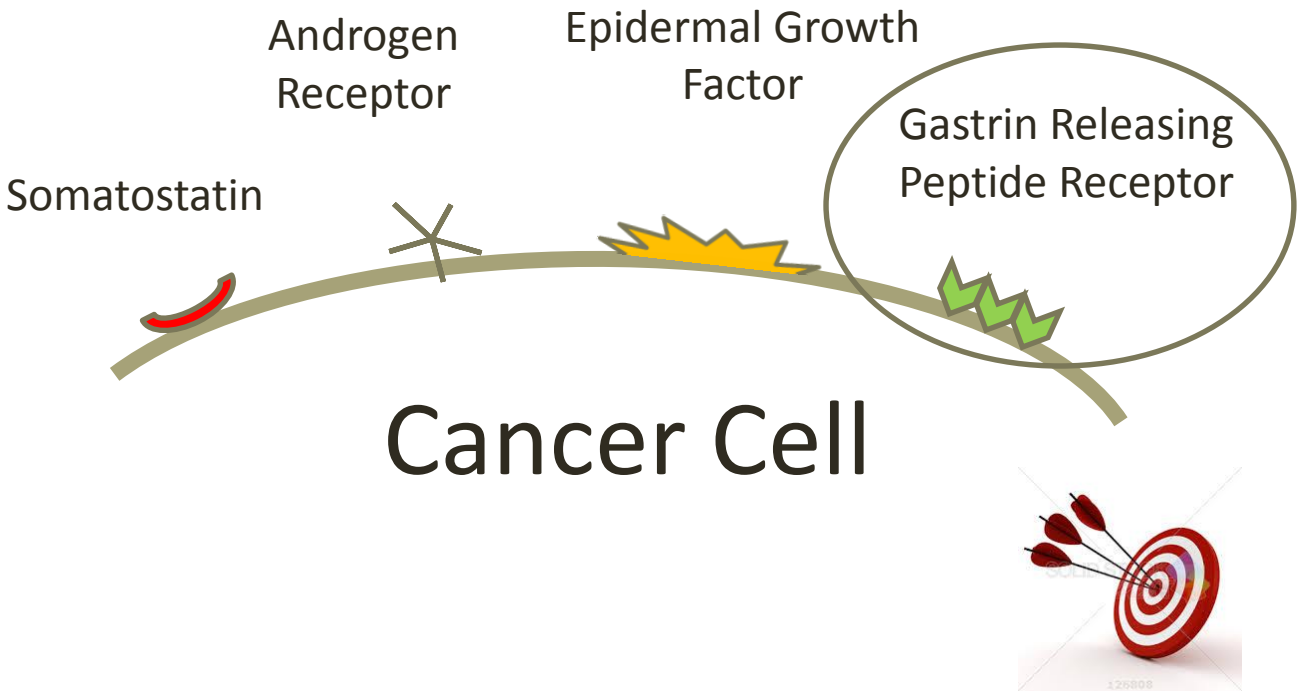
- Cancer is the leading cause of death in Canada.
- Prostate cancer is the 3rd cause of « cancer » death in men.
- On average (daily):
  - 65 Canadian will be diagnosed.
  - 11 Canadian men will die.






- Advisory Committee on Cancer Statistics (2013). Canadian Cancer Statistics 2013. Toronto, ON, Canadian Cancer Society

# Introduction
# Expression of Peptide Receptors



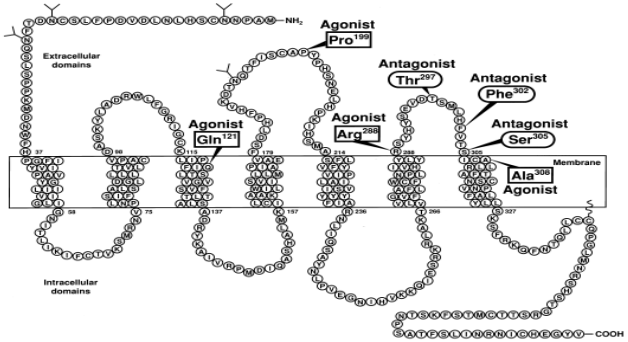

Targets for peptide-based radiopharmaceuticals

- Wenbin et al. *Molecular Imaging*. ISBN: 978-953-51-0359-2.
- Roesler R, et al. *Neuropsychol*. 2007;1(2):118-123

# Introduction
# Gastrin Releasing Peptide Receptor (GRPR)

- They are members of the G-protein coupled receptor superfamily (GPCR). They have 7 transmembrane domains.

- GRPR is expressed on pancreas.




- Expression on tumor:
  - Prostate (63 – 100%) [Marrone B, et al. *The Prostate.* 2012; 72:318 -325]
  - Bone metastases (50%) [Ananias H, et al. The Prostate. 2009; 69:1101 -1108]
  - Lungs, Breast, …




- Heppeler A, et al. *Current Meicinal Chemistry*. 2000;7:971-994
- Moody T, et al. *Drugs Fut*. 1998;23(12):1305

# Bombesin peptide (Clinical trial)

- Scopinaro et al and De Vincentis et al have shown the usefulness of ($^{99m}$Tc)- [$^{13}$leu]bombesin in several cancer patients.
  - (SPECT imaging, monoenergetic, low resolution)

- Hoffman et al used positron emission tomography with 68-Gallium ($^{68}$Ga) -BN to diagnose 13 prostate cancers.
  - (PET imaging, high resolution, whole body imaging, progress of the malignancy).

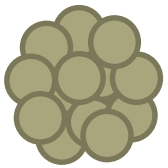



- Scopinaro F, Varvarigou AD, Ussof W, et al. Cancer Biother Radiopharm. 17:327-335, 2002.
- Hofmann M, Machtens S, Stief C, et al: Eur J Nucl Med Mol Imaging 31:S253, 2004 (abstr 207; suppl 2)

# Positron Emission Tomography (PET)

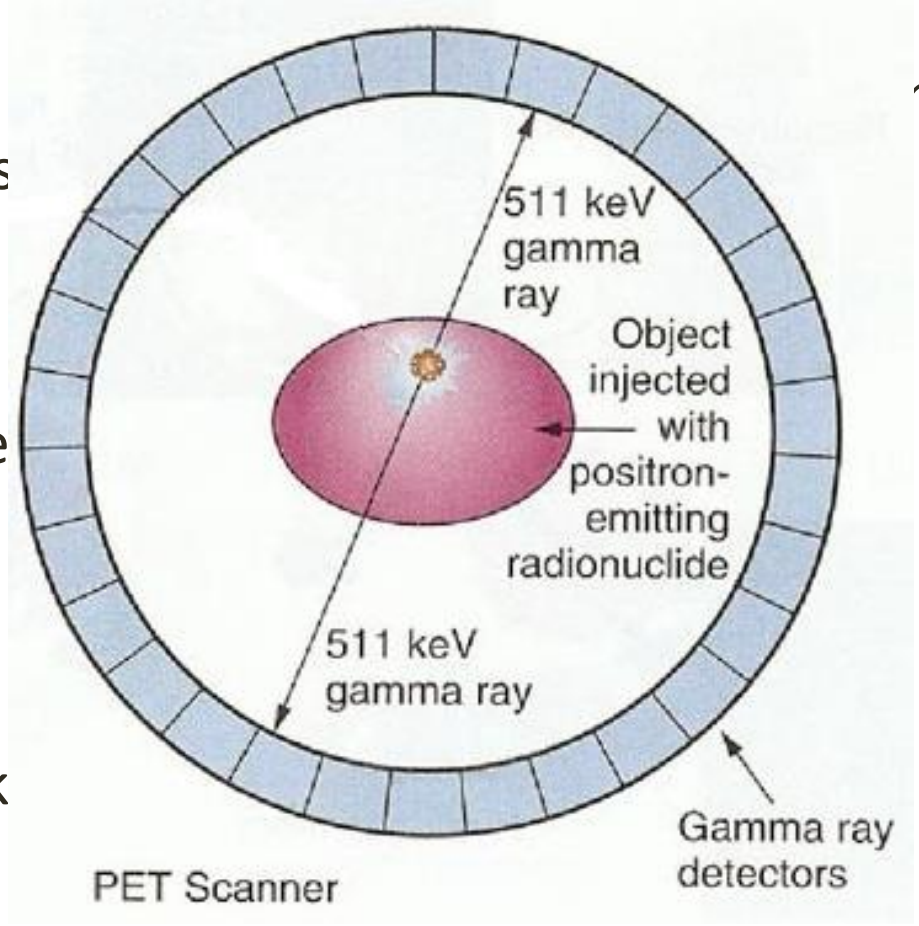
mma ray

Pos



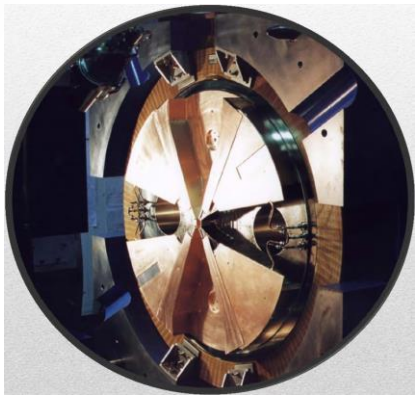
Radionuclide
Cu-64

511 k




- RBL 741 Radiation Science, 2014 Bentourkia; Guérin, Lecomte.
- http://www.cellsighttech.com/technology/pet.html

# Radionuclide Production ($^{64}Cu$)



Cyclotron TR-19 and TR24 (ACSI)

Production $^{64}Ni + p \longrightarrow {}^{64}Cu + n$

- Radionuclide: $^{64}Cu$, Half-life = 12.7 hr, $\beta^+$ = 18%  0.65 MeV, $\beta^-$ = 38.4%  0.573 MeV and Electron Capture = 43%



- RBL 741 Radiation Science, 2014 Bentourkia; Guérin, Lecomte.
- Carolyn J, et al.. C*ancer Biotherapy and Radiopharmaceuticals.* 2009; 24(4), 331-345.

# GRPR, tracers for PET imaging

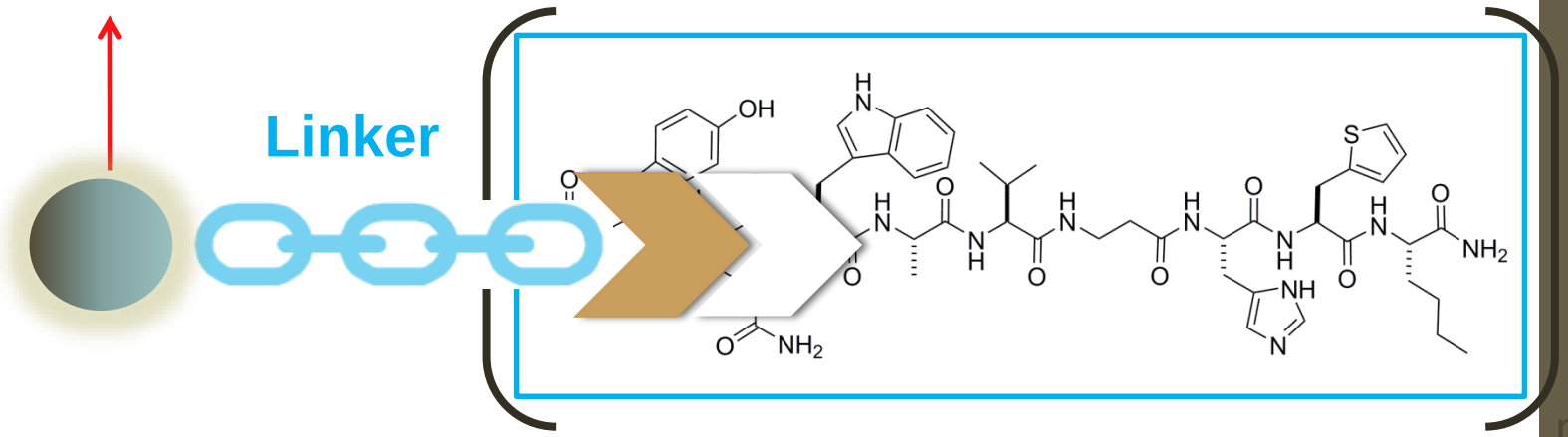
**Chelator** for metal radiolabelling

**Radiometal**

**Standard peptides**

**Demobesin 4 : GRPR agonist**

B.A. Nock *et al.* J. Med. Chem., 48 (2005), pp. 100–110.




**Demobesin 1: GRPR antagonist**

Wang, LH *et al*. *J Biol Chem* 1990; 265:15695–15703

Valence

n


Guérin B et al. Organic letters, **2010**, 12(2)

Ait-Mohand S etal. Bioconjugate chemistry, **2011**, 22(8)

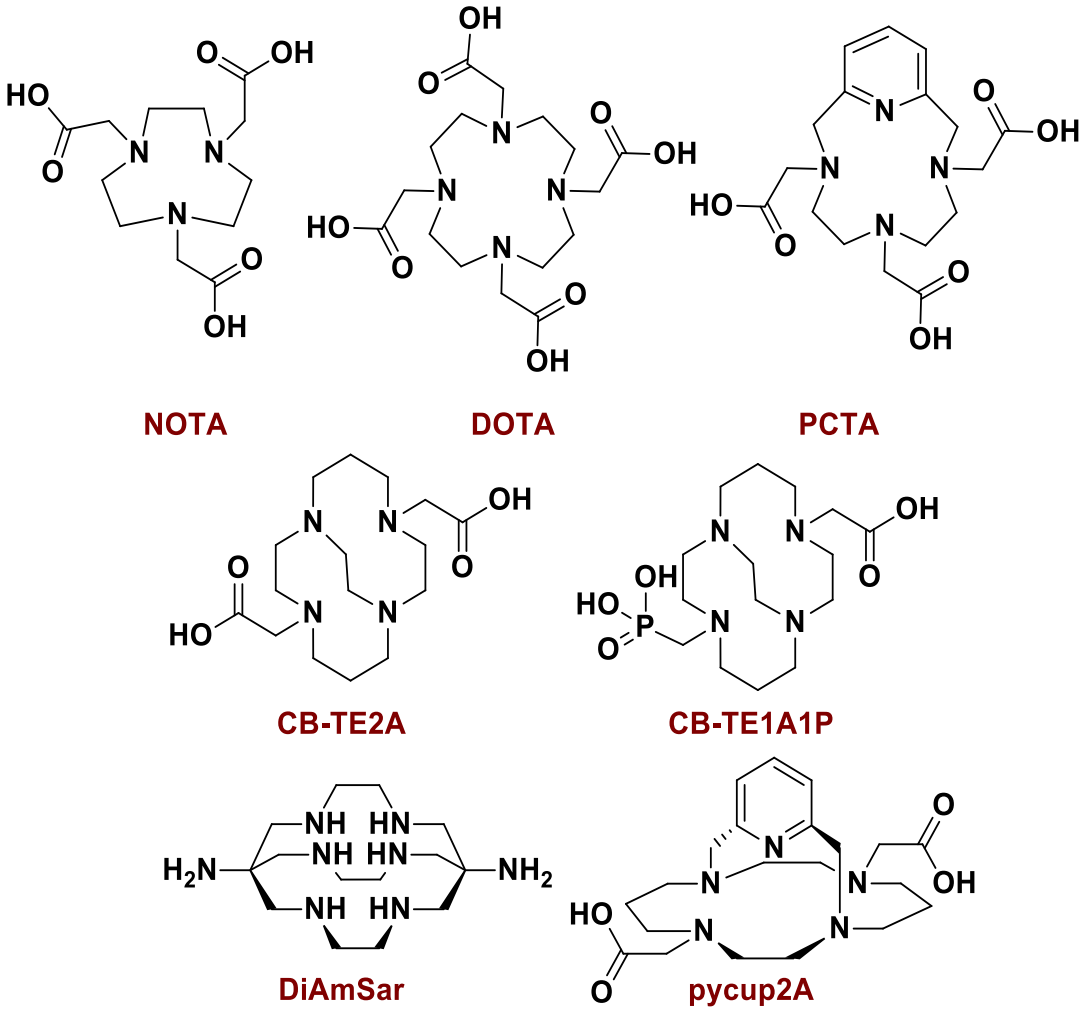
Fournier et al. *EJNMMI Research* **2012**, 2:8

Fournier P etal. Bioconjugate chemistry,, **2012** 23(8)

Inkster JA et al. Chemistry, **2012**, 18(35)

Inkster J et al, Bioorganic & medicinal chemistry letters, **2013**, 23(13)

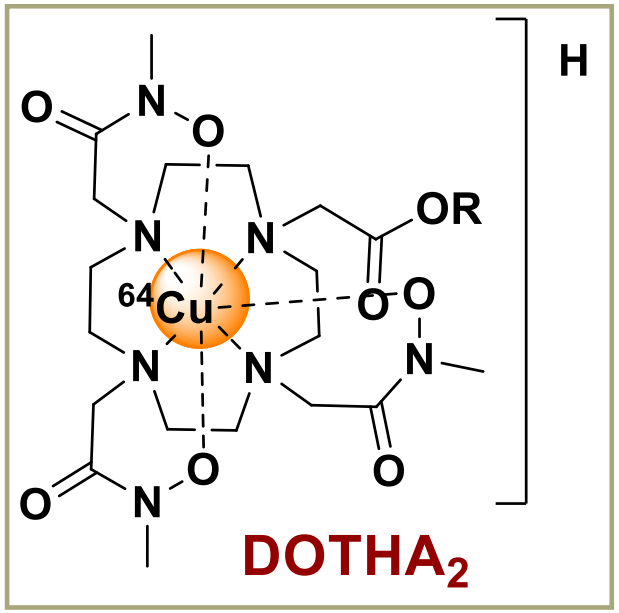
# Structures of various BFCs used for $^{64}Cu$ labelling



# Bifunctional Chelator (BFC) ideal Characteristics

- Small size.
- Simple chemical preparation
- Fast $^{64}Cu$ chelation
- Slow $^{64}Cu$ demetallation
- Resistance to transchelation
- Available conjugation

to a bioactive molecule.

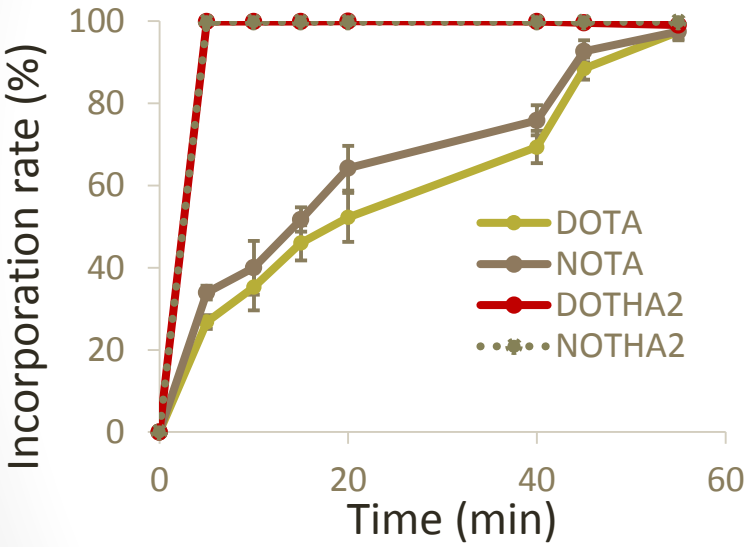




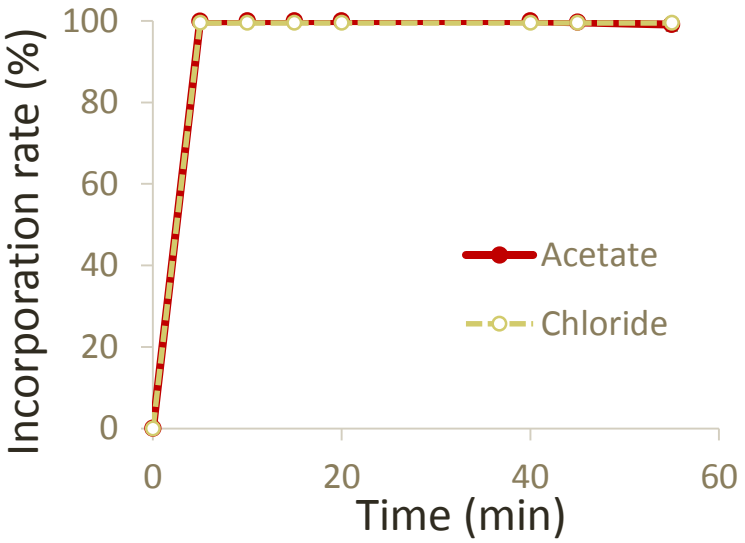

- S. Ait-Mohand, et al. *Organic Letters.* 2014; 16, 4512-4515

# $^{64}$Cu Radiolabelling Efficiency at room Temperature and pH 5.5

BFC in ammonium acetate buffer




$DOTHA_2$/$NOTHA_2$ with different $^{64}$Cu counterions




Complete complexation in 5 minutes at room temperature.

pH (4.5 – 10 )

- S. Ait-Mohand, et al. *Organic Letters.* 2014; 16, 4512-4515

# Stability under physiological conditions

| Condition | Time (h) | $^{64}$Cu/BFC stability (%) | | |
|---|---|---|---|---|
| | | NOTA | $DOTHA_2$ | $NOTHA_2$ |
| Plasma | 24 | >99 | >99 | >99 |
| In vivo | 4 | >99 | >99 | >99 |

- No decomposition in physiological conditions



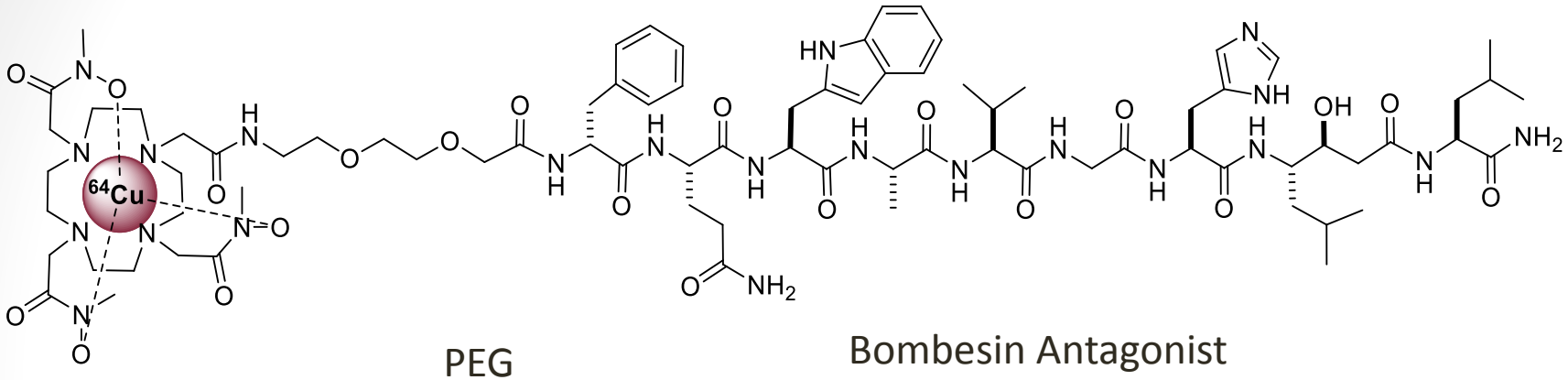
- S. Ait-Mohand, et al. *Organic Letters.* 2014; 16, 4512-4515

# Hypothesis of the Project

- Radiolabelled peptide antagonist targeting GRPR overexpressed on prostate cancer cells can be used as tools to improve cancer diagnosis by PET (Positron Emission Tomography ) imaging.

- The new class of chelator ($DOTHA_2$) would provide us with fast $^{64}Cu$ radiolabelling, high resistance for transmetalation and improve in vivo stability.

# Compound Structure




DOTHA2 (chelator)

PEG (linker)

Bombesin Antagonist
[D-Phe$^{6}$-Sta$^{13}$-Leu$^{14}$-$NH_2$]BBN(6-14)

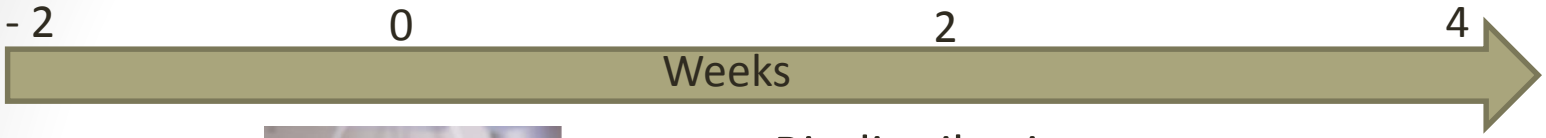
$^{64}Cu$-$DOTHA_2$-PEG-Bombesin (Antagonist)


- Linares M, et al. *J Pept Res*. 1999; 53(3):275-83
- S. Ait-Mohand, et al. *Organic Letters.* 2014; 16, 4512-4515

# Experimental Methods

- *In vitro* studies
  - Competition binding study (PC3 prostate cell line)
  - Cellular uptake and Efflux studies (PC3 and LNCaP prostate cell lines)
  - Plasma and *in vivo* stability studies (UPLC, Radio-TLC)
- *In vivo* studies
  - Biodistribution studies (normal and tumor bearing mice)
  - µPET imaging (tumor bearing mice)

# µPET imaging




Cells Preparation PC3 & LNCaP



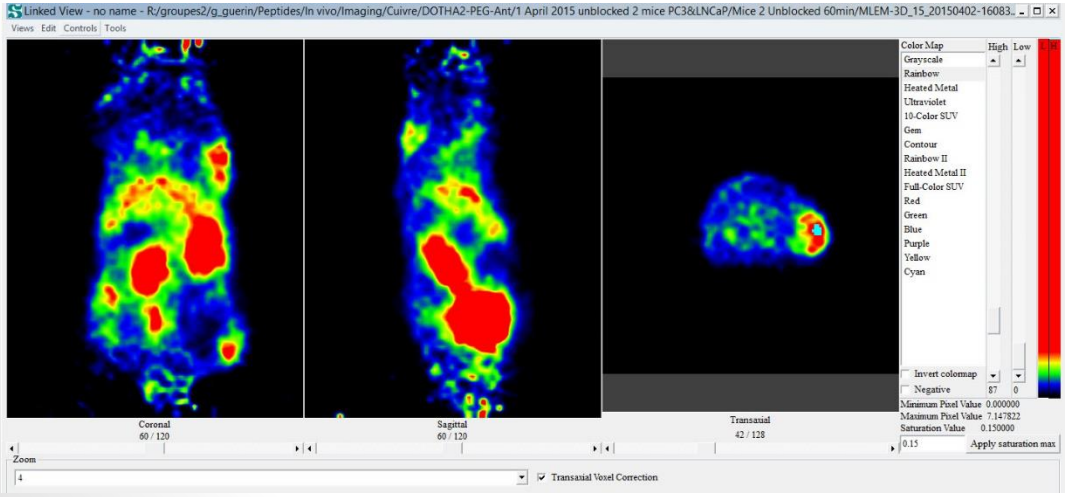
Cells inoculated on male athymic nude mice

Biodistribution or



1st PET dynamic scan

2nd PET dynamic scan




- Fournier P, et al. *Bioconjugate Chemistry.* 2012; 23, 1687-1693

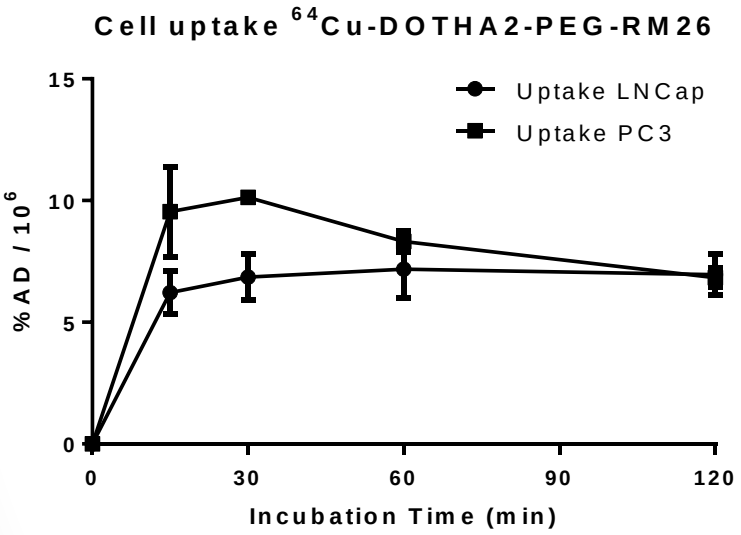
Quantification of the accumulation of the tracer in tumor, kidneys, liver and muscle

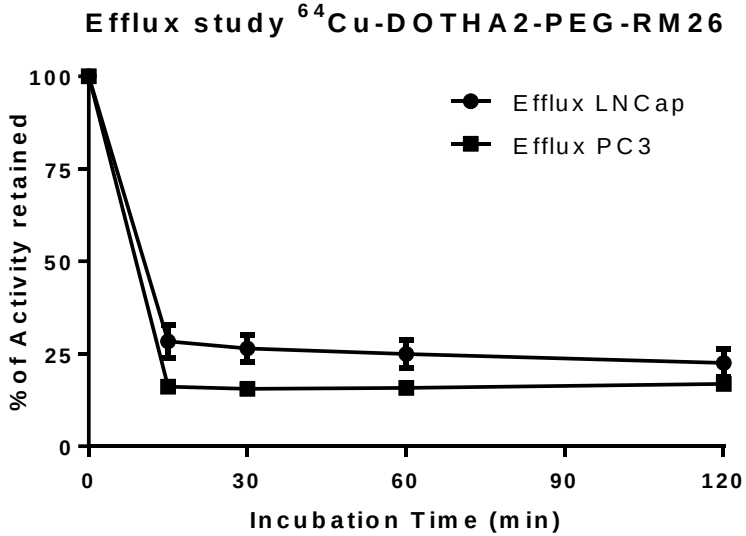
Data presented as the percentage of injected dose per gram (%ID/g).

# Results
# Radiolabelling and i*n vitro* studies

- Efficient labelling >95% in 5 min.
- Specific activity > 55 TBq/mmol.
- Competition binding study [$^{125}$I]-[Tyr$^{4}$]-BBN
  - Ki for GRPR on PC3 ($^{125}$I-BBN): 0.15 nM
  - Ki for GRPR on PC3 (Cu/DOTHA2-PEG-RM26): 0.68 ± 0.19 nM

# Results
# Cellular uptake and efflux studies



# Results
# Plasma and *in vivo* stability studies

- Radiolabelled compound injected in fresh mouse plasma (Female balb/c) (Reversed phase-HPLC) to detect $^{64}Cu$ metabolites.

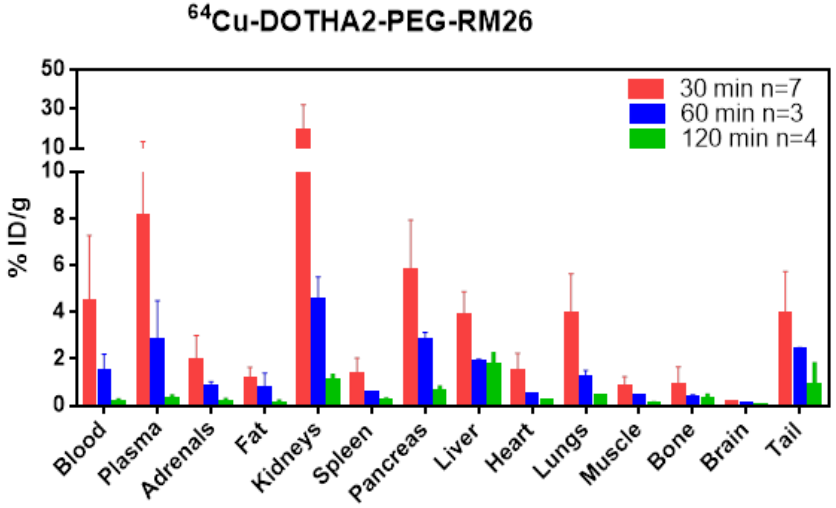
Compound stability in plasma > 95 %, 24 h.

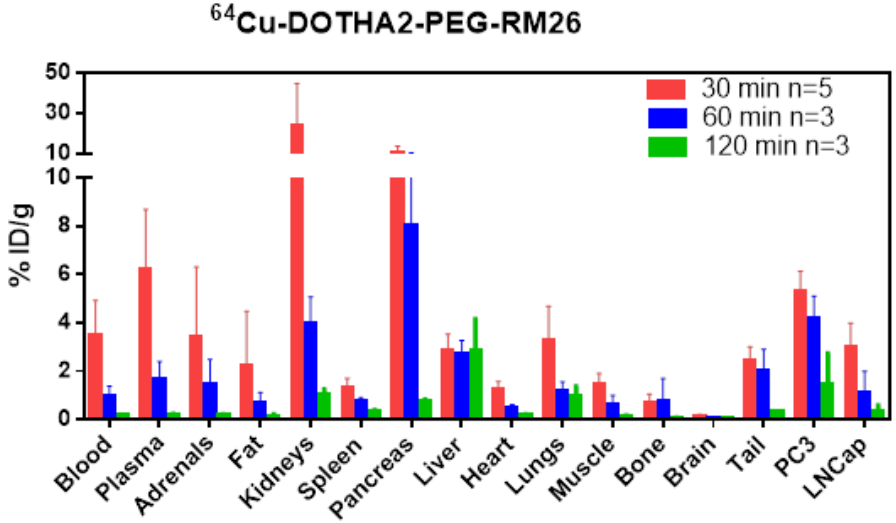


- Radiolabelled compound injected in female balb/c and blood collected at different time point (Radio-TLC) to presence of $^{64}Cu$.

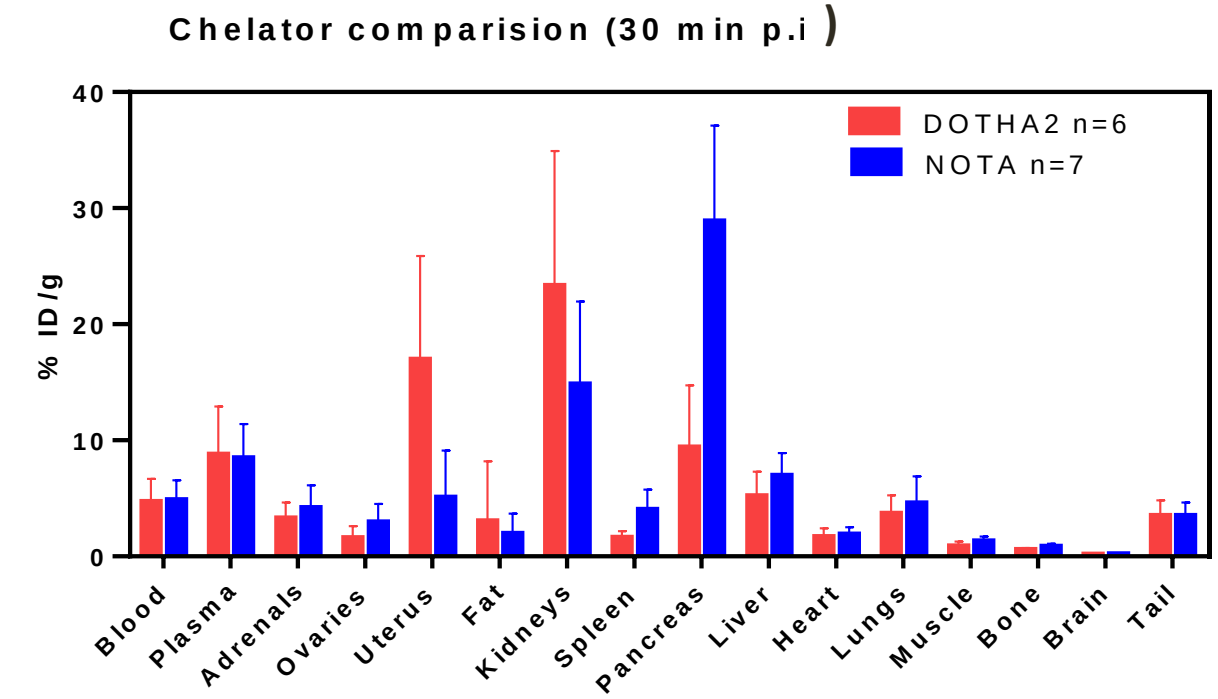
In vivo stability > 95%, 2h.

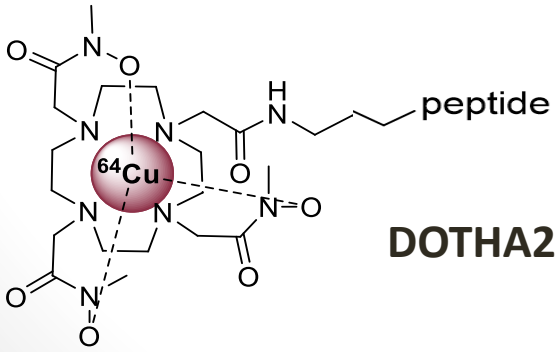
# Results
# Biodistribution studies

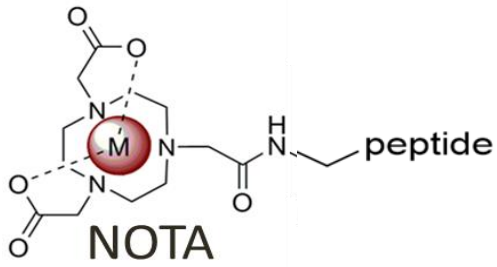


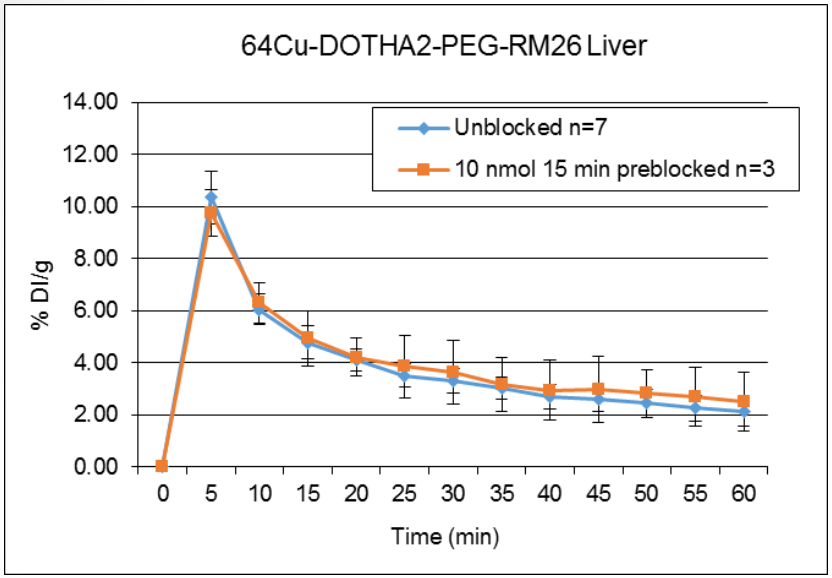
# Results
# Biodistribution studies

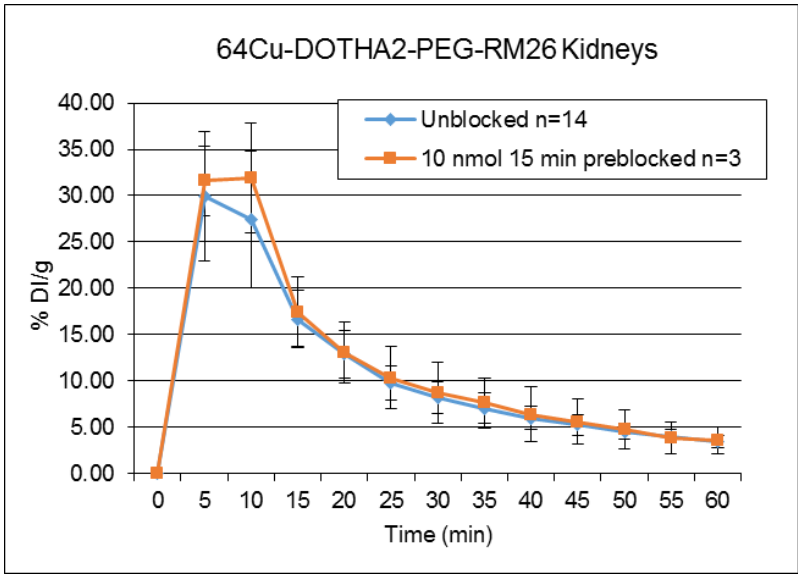


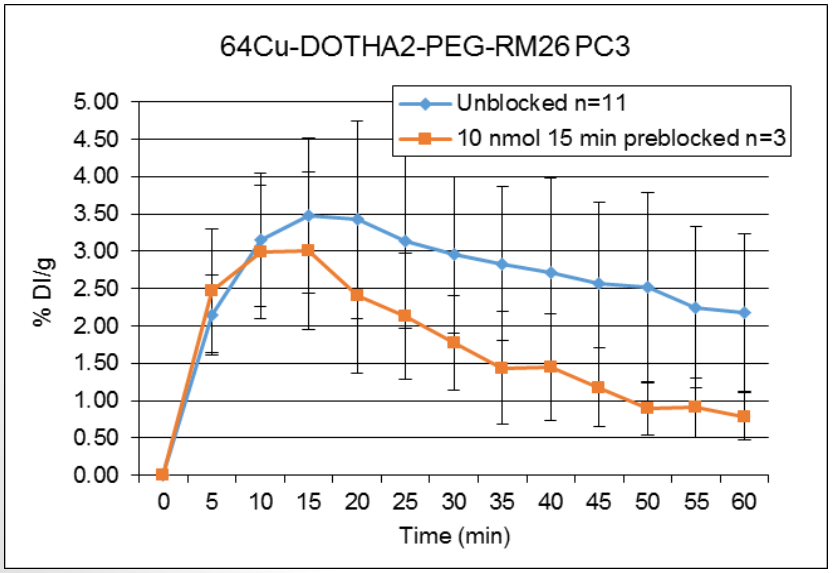



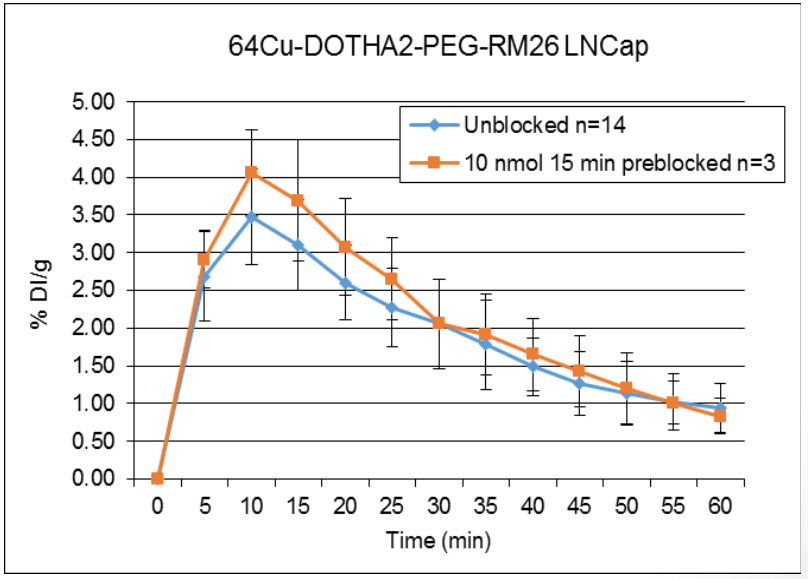



# Results
# µPET imaging



# Conclusions

- The compound showed high specific activity and high *in vitro* and *in vivo* stability.
- Biodistribution studies showed specific GRPR uptake (pancreas and tumor), excretions through the liver and mainly the kidneys and low non specific uptake.
- µPET imaging studies on tumor bearing mice showed high retention on tumor up to 60 min time point, normal excretion through the liver and fast kidney elimination.

# Acknowledgments

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- **PET Imaging**
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- Maxime Paillé

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L'Institut de génétique / Institute of Genetics

CIMS
Centre d'imagerie moléculaire de Sherbrooke

CENTRE DE RECHERCHE CHUS

# Any Questions



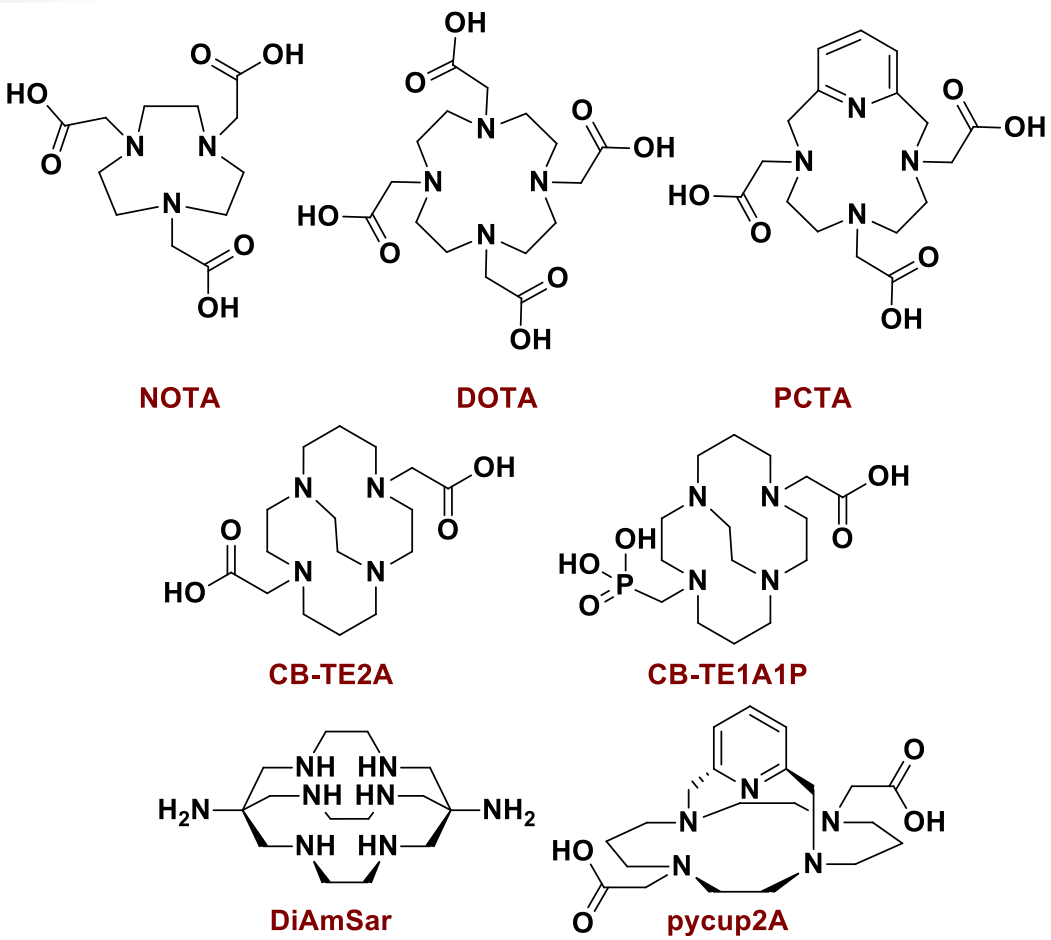
# Ideal radiotracers

- Easy to synthesize.
- High affinity for receptors targeting, rapid pharmacokinetics and long retention time.
- High ability in detection primary tumors and metastases.
- Low immunogenicity.
- Rapid chelation process.
- Obtain higher specific activity (low receptor density).
- Resistance to demetallation (radiometal leaves the tracer) and High *in vivo* stability.



- http://www.chem.ucla.edu/harding/IGOC/A/amino_acid.html
- Otto C, et al. *Seminar in Nuclear Medicine*. 2000;30(3):195-208
- Fani M, et al *Theranostics*. 2012;2(5):481-501

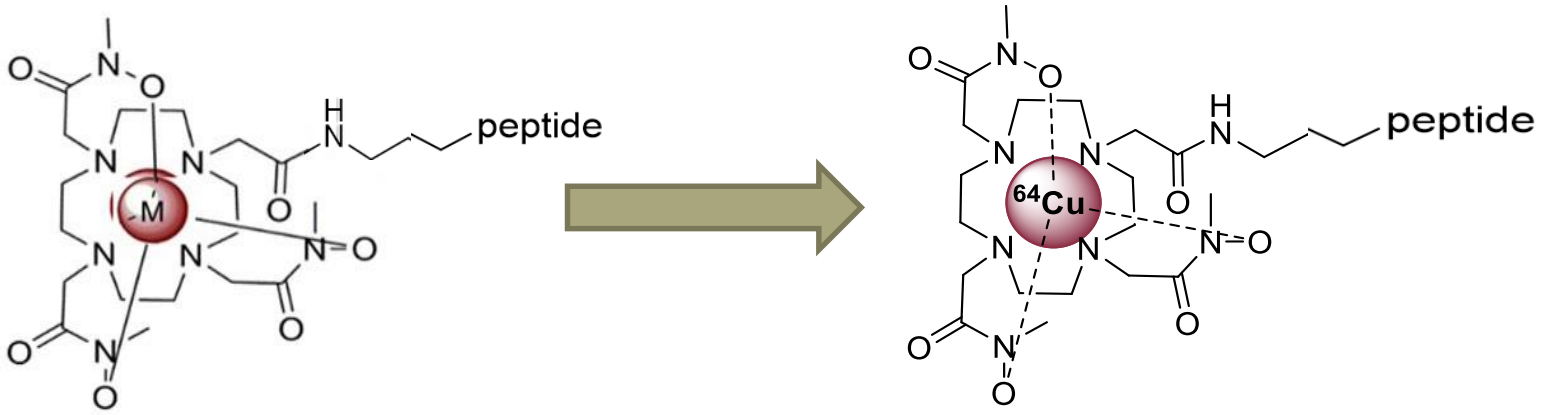
# Structures of Various BFCs Used for $^{64}$Cu Labeling




- Bifunctional chelators (BFCs)
- $^{64}$Cu $T_{½}$=12.7h
  - 39% $\beta^-$
  - 43% Electron Capture
  - 17.4% $\beta^+$
- $\beta^+$ maximal energy 0.656 MeV

# Radiolabelling with $^{64}Cu$

$$[^{64}Cu]CuCl_2 \xrightarrow[0.1M,\ pH\ 5.5]{\text{Ammonium acetate}} [^{64}Cu]Cu(OAc)_2)$$




5 min at pH 7 at room temperature.



- S. Ait-Mohand, et al. *Organic Letters.* 2014; 16, 4512-4515