STRATEGIES OF REGIOSELECTIVE RADIOLABELING OF NANOFITIN BINDER FOR IMAGING

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Introduction

Recently, new strategies emerged in the field of monoclonal antibodies **radiolabeling** for PET imaging with the use of positron emitters such as **zirconium-89** or **gallium-68**. Despite their important role in the therapeutic world, antibodies have many disadvantages related to their structure. Moreover, conjugation of chelating agent often occurs on lysines, which is non-regioselective and leads to a heterogeneous mixture of products. In addition, the slow clearance of antibodies can be a problem to obtain a good contrast when they are used in imaging.

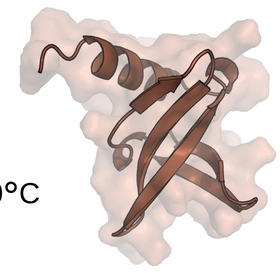
To address these different limitations, we developed a **chemistry-free chelating system** consisting of a **phosphorylatable peptide tag**. A specific phosphorylation step can generate a nanocluster of phosphate moieties that **can interact strongly with metal ions** like zirconium[1]. We used a peptide sequence which has been selected for its capacity to chelate lantanide ions such as terbium(III) to optimize this peptide tag and fuse it genetically to a Nanofitin, a protein scaffold developed as an alternative to antibodies, to ensure an efficient targeting of the radionuclide.

Objectives:

1) Adapt the labeling tag to the stereoselective chelation of rgallium-68 for PET imaging.
2) Validate the use of Nanofitin as a potent alternative tool for *in vivo* imaging.

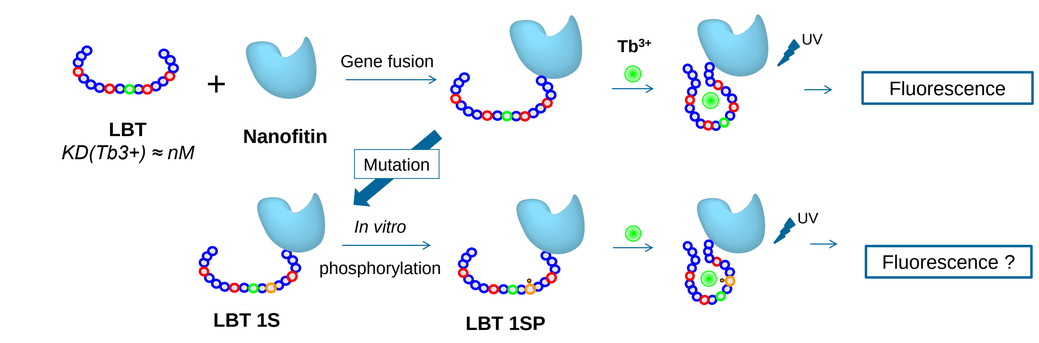
What are Nanofitins ?

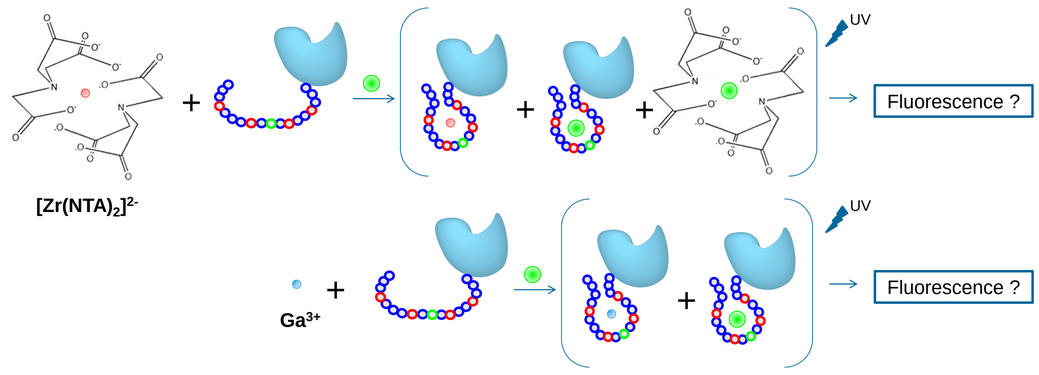
Small Protein:	10kDa
pH stability:	0-12
Temperature Stability:	Tm≈80°C
Production:	Generated in bacteria
Affinity:	nM



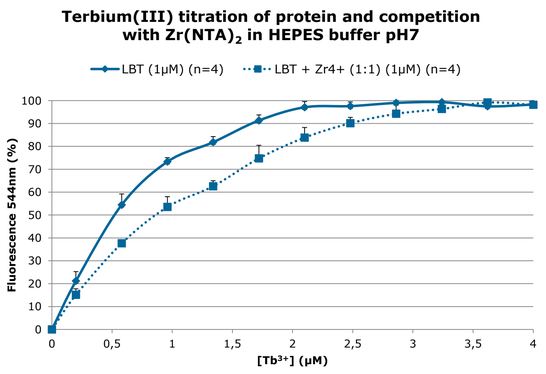
Chelation with gallium

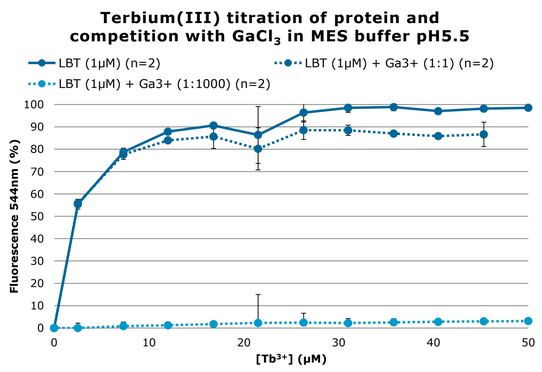
Method:

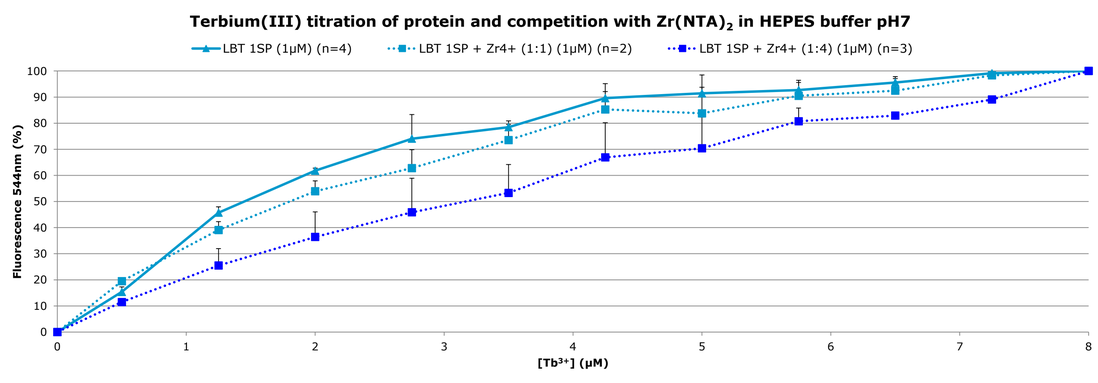




NB : Terbium emits fluorescence intrinsically. In aqueous solution, water molecules quench fluorescence and chelation prevents this quenching by expelling water molecules.







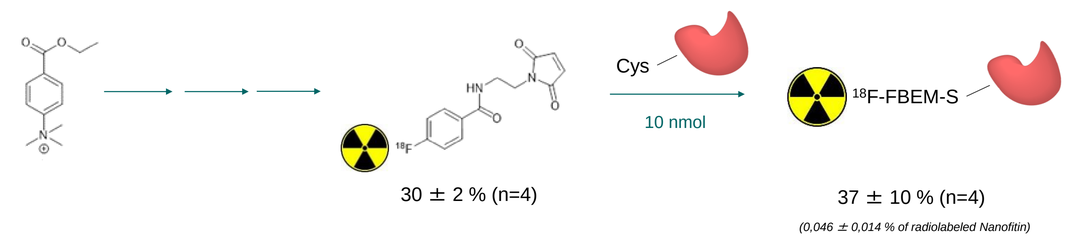
To increase the affinity for radionuclide, we worked on a sequence derived from calcium-binding proteins to chelate specifically lanthanides[2]. We optimized this sequence by incorporating a phosphate nanocluster to improve the chelation with radionuclides [3].

- Affinity for terbium(III) is in the sub-micromolar range for the lanthanide-binding tag fused to the Nanofitin and in the micromolar range for the mono-phosphorylated.
- Chelation of zirconium and gallium by the peptide tag was observed by a competition study.

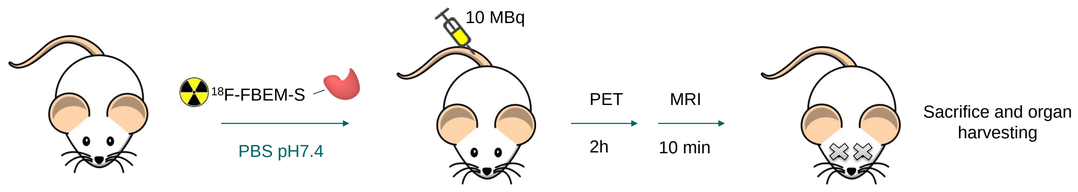
Labelling with fluorine-18

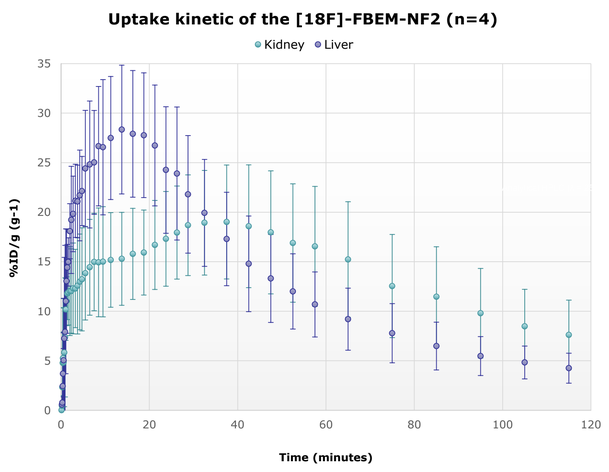
Method:

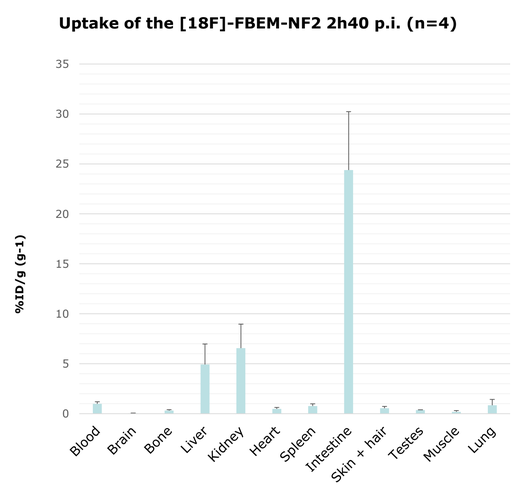
1) Automatic synthesis of [18F]-FBEM and radiolabeling of Nanofitin NF2 (Dammicco S. *et al.*)

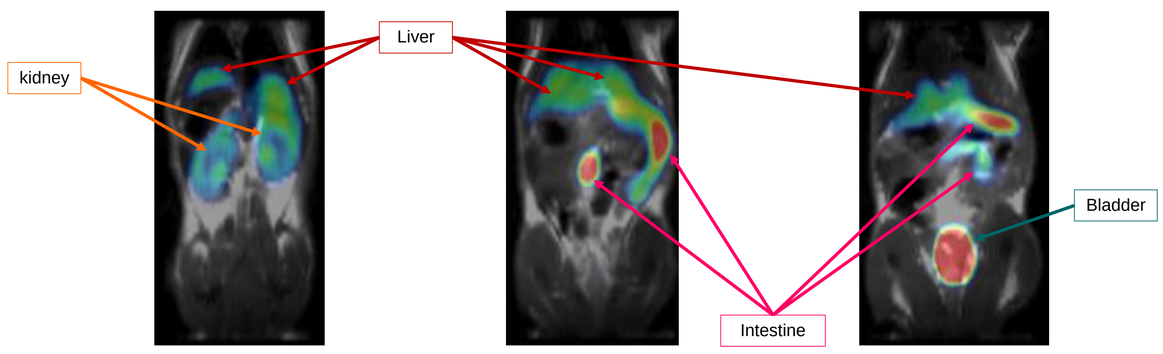


2) Injection of the Nanofitin radiolabeled in balb/c mice and PET/MRI imaging









Coregistred coronal sections of MRI and two-hours duration PET after injection of the NF2 radiolabeled

After radiolabeling of the Cys-tagged Nanofitin NF2 with [18F]-FBEM, the protein is injected in mice to evaluate its biokinetic. It seems that the Nanofitin is metabolized by the liver and reabsorpted in the cortical area of the kidneys. The metabolites obtained are excreted by kidneys through urine and by the liver via biliary excretion to the gut with feces.

References : [1] Cinier M. *et al.* (2012), *Journal of Biological Inorganic Chemistry*, 17, pp. 399–407 ; [2] Martin L. J. *et al.* (2007), *Journal of American Chemistry Society*, *129*(22), 7106–7113 ; [3] Pardoux R. *et al.* (2012), *PLoS ONE*, *7*(8).

Conclusions and perspectives

We succeeded to generate a phosphorylatable tag able to chelate terbium(III). Through competition studies, we have shown evidence for a capacity of chelation of zirconium(IV) and gallium(III). Radiolabeling studies with gallium-68 are on going to evaluate the powerfulness of such a strategy for the chelation of radionuclides. We have also obtained an hypothetic ADME profile of the Nanofitin NF2 and we are currently making use of its specific binding to a cell-surface receptor to target a very precise cell population by using a new animal model. Once the phosphorylatable tag optimized for regioselective radiolabelling and the Nanofitin targeting validated in an animal model, the next steps will be to combine these two approaches: we will fuse genetically the tag to the specific Nanofitin, radiolabel it with gallium-68 and perform the biokinetic study of this new radiopharmaceutical product.

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