Regioselective labeling of Nanofitin by using a phosphorylated peptide tag

Marine Goux[1,2,3,*], Mathieu Cinier [2] and Charles Tellier[1]

*[1]Unité de Fonctionnalité et Ingénierie des Protéines, University of Nantes, France ; [2]Affilogic SAS, Nantes, France and [3]Cyclotron Research Center, University of Liège, Belgium ; * contact: marine.goux@univ-nantes.fr*

Introduction

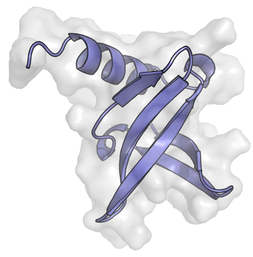
Recently, new strategies emerged in the field of monoclonal antibodies **radiolabeling** for PET imaging with the use of positron emitters such as **zirconium-89** or **gallium-68**. Despite their important role in the therapeutic world, antibodies have many disadvantages related to their structure. 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, with the alpha subunit of the human casein kinase II can generate a nanocluster of phosphate moieties that **can interact strongly with metal ions** like zirconium[1]. Two peptides sequences have been used as a starting material, one already described to promote the specific anchoring of protein on zirconium-phosphate surface, the other one selected for its capacity to chelate lantanide ions such as terbium(III).

Objectives:

1) Adapt the labeling tag to the stereoselective chelation of radionuclides for PET imaging.
2) Genetically fuse the tag to a Nanofitin, a protein scaffold developed as an alternative to antibodies, to ensure an efficient targeting of the radionuclide.

What are Nanofitins ?



Small Protein: 10kDa

pH stability: 0-12

Temperature Stability: Tm≈80°C

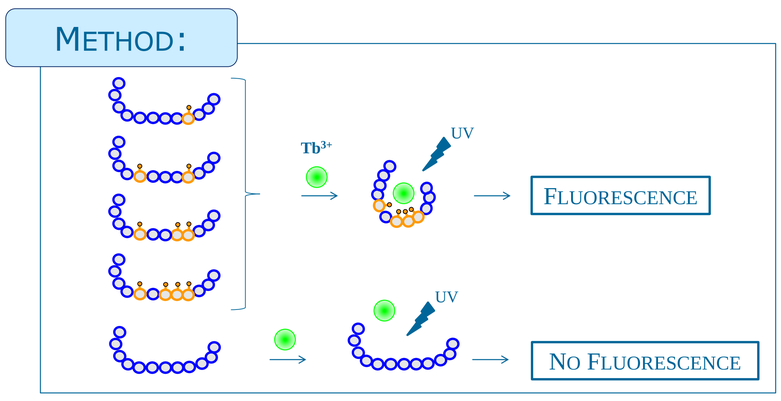
Production cost: Low (Generated *in vitro* and produced in bacteria)

Affinity: nM

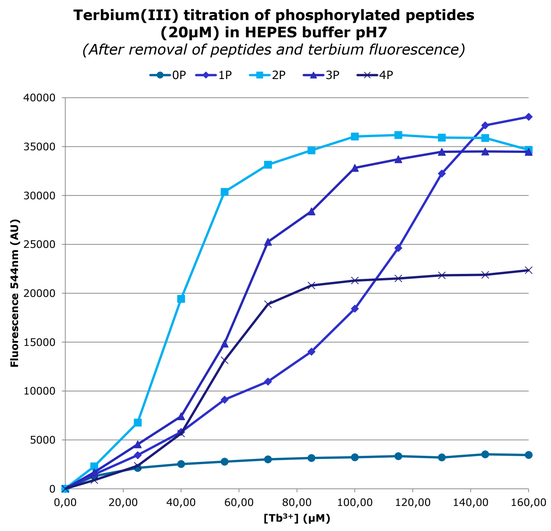
Results — Chelation and phosphorylatable tag

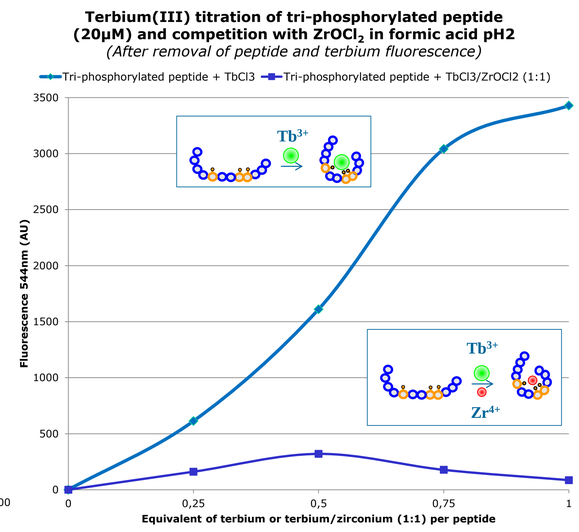
To optimize the sequence of the phosphorylatable tag, we studied the chelation of different mimetic peptides (from 0 to 4 phospho-serine) with a lanthanide (terbium).

Method:



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



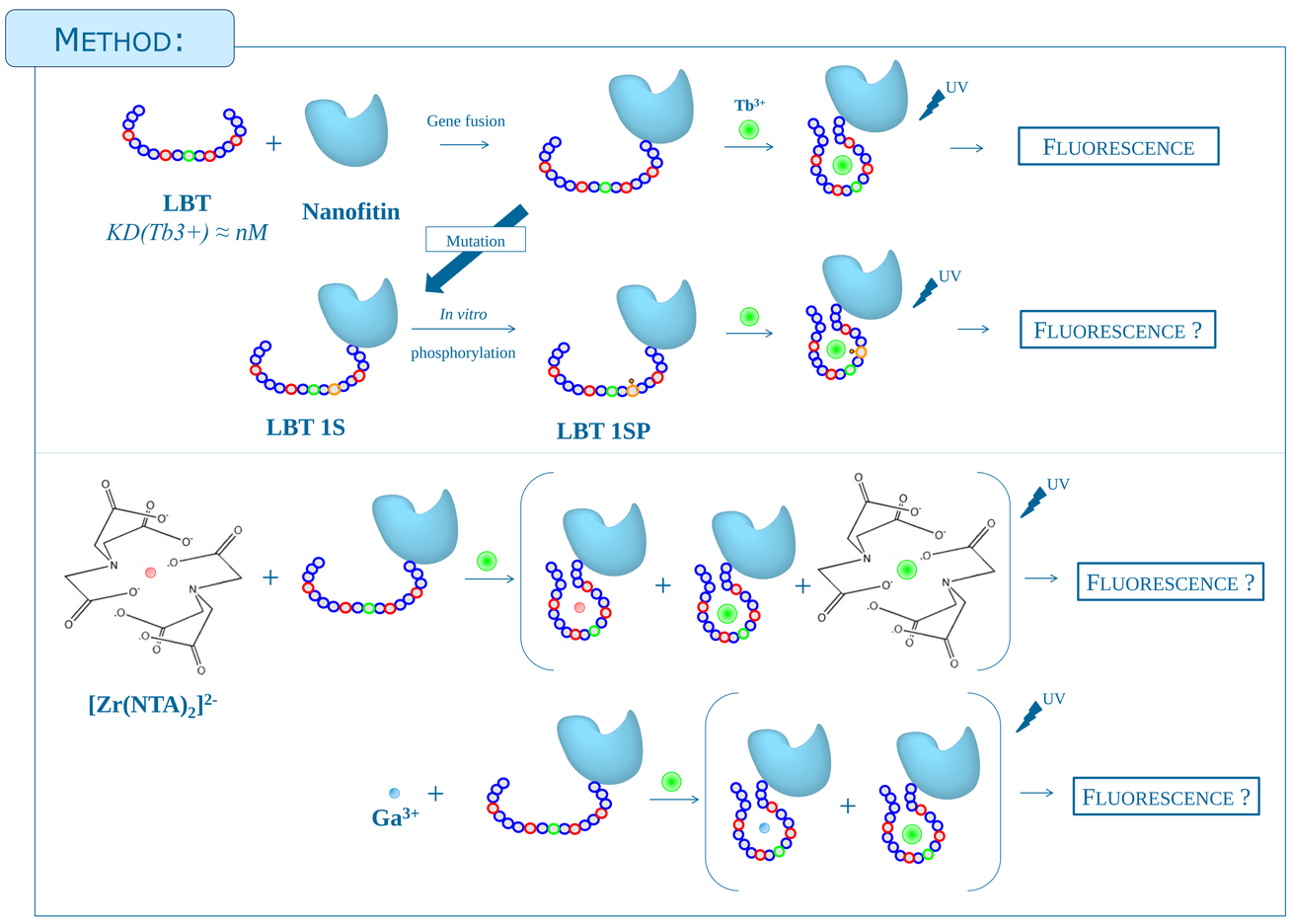


- Affinity for Tb^{3+} in the micromolar range : 2P>3P≈4P>1P>0P
- Chelation of the Zr^{4+} by the peptide tag was confirmed by competition.

Results — Chelation and Lanthanide-Binding Tag

To increase the affinity for radionuclide from micromolar to nanomolar, 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].

Method:



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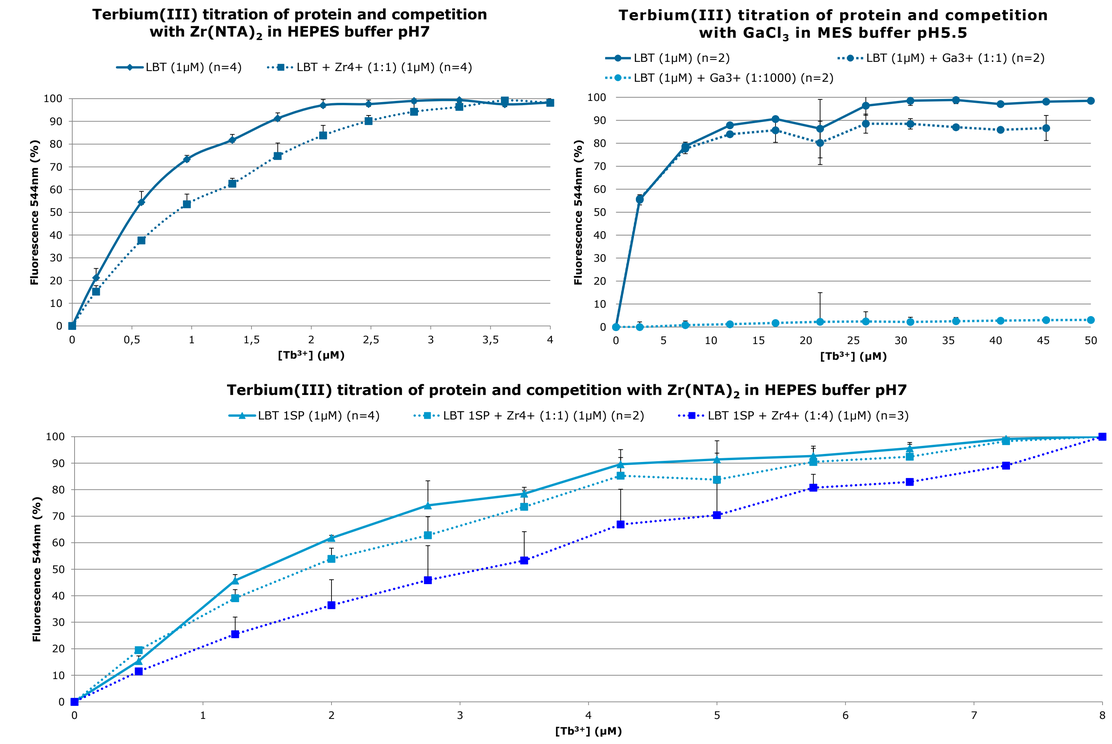

- Affinity for terbium(III) in the sub-micromolar range for the lanthanide-binding tag fused to the Nanofitin and in the micromolar range for the mono-phosphorylated.
- Chelation of zirconium and gallium by the peptide tag was observed by a competition study.

Conclusion

We succeeded to generate two types of 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.

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).

UFIP UMR CNRS 6286
2 rue de la Houssinière, 44322 Nantes, France

AFFILOGIC SAS
2 rue de la Houssinière, 44322 Nantes, France

Cyclotron Research Center
Allée du 6 Août, 4000 Liège, Belgium

Thesis project funded by "Région Pays de la Loire", into the Erasmus Mundus programme NanoFar

3rd NanoFar Autumn School
October 20-24, 2014
University of Louvain, Brussels, Belgium