Regioselective labeling of Nanofitin by using a phosphorylated peptide tag

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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.

RESULTS
CHELATION AND PHOSPHORYLATABLE TAG

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

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[3]. We optimized this sequence by incorporating a phosphate nanocluster to improve the chelation with radionuclides[4].

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.


WHAT ARE NANOFITINS?
Small Protein: 10kDa
pH stability: 0-12
Temperature Tm=80°C
Stability: Low (Generated in vitro and produced in bacteria)
Affinity: nM

METHOD:

- Affinity for Tb³⁺ in the micromolar range: 2P > 3P = 4P = 1P > 0P
- Chelation of the Zr⁴⁺ by the peptide tag was confirmed by competition.

- 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.

METHOD:

- Chelating agent
- Peptide
- Nanofitin
- LBT (1µM) + Ga³⁺ (1:1) (n=2)
- LBT 1SP + Zr⁴⁺ (1µM) (n=3)
- LBT 1SP + Zr⁴⁺ (1µM) (n=3)
- LBT (1µM) (n=4)
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