Development of an in vivo phosphorylation system in Escherichia coli

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Recently, new strategies emerged in the field of monoclonal antibodies radiolabeling for PET imaging with the use of positron emitter such as zirconium-89 or gallium-68[1]. 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 highly phosphorylatable peptide tag. A specific phosphorylation step, with the alpha subunit of the casein kinase II (CKIIα), generates a nanocluster of four phosphates that can interact strongly with metal ions like zirconium. This strategy has already demonstrated its powerfulness for the stable and specific anchoring of protein on zirconium phosphate-based microarray[2]. We are now adapting the use of this labeling tag to the stereoselective chelation of radionuclides for PET imaging. To ensure an efficient targeting of the radionuclide, the tag was genetically fused to a Nanofitin®, a protein scaffold developed as an alternative to antibodies.

1. Chelation of the peptide tag with terbium(III) and zirconium(IV)

In order to optimize the sequence of the phosphorylatable tag, we studied the chelation of different mimetic peptides with terbium(III). The chelation of the zirconium by the peptide tag was confirmed by a competition study.

2. Co-expression system TOP10/pNF-pCKIIa

In early development, the phosphorylation was realized in vitro. In order to save phosphorylation steps and decrease production costs, we developed an in vivo phosphorylation system consisting in the co-expression of a Nanofitin® fused to the phosphorylatable tag (pNF) and the CKIIα.

3. Optimization of the phosphorylation

Extra addition of ATP after lysis improve significantly the rate of phosphorylation.

4. Evaluation of the phosphorylation rate by isoelectric focusing

The isoelectric focusing method is based on the fact that the pH of the Nanofitin® decreases as the protein is phosphorylated. Such a technique allowed us to isolate the different phosphorylation states of the phospho-Nanofitin® (from 0 to 4-phospho-serine).

5. Purification of the phosphorylated Nanofitin® by Fe-NTA and Ga-NTA

Using the specific interaction of phosphate moiety with gallium(III) or iron(III), we are currently developing a phosphoprotein purification protocol by immobilized metal affinity chromatography[3,4].