Program Gama

Novel approach to labelling recombinant fragments of antibodies with copper radionuclides for PET imaging

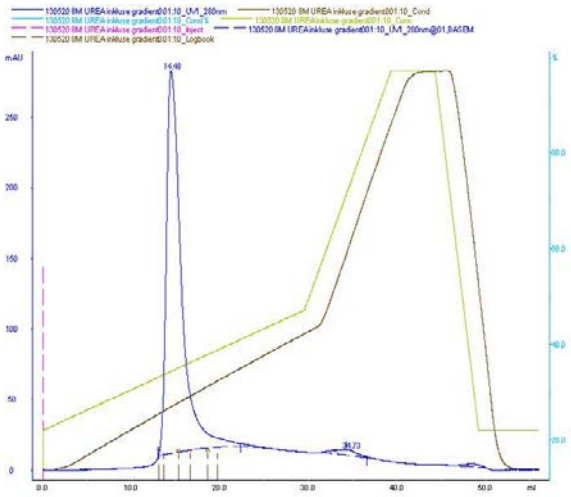
In an **overview**, the experiments have been focused onto „proof-of-concept“ for a new scheme of radioactive labelling of antibodies in their scFv format via a pair of complementary peptides of „coiled-coil“ structures capable to form strong non-covalent bonds. For such aim, supply of scFv tagged with one of the peptides has been established, while the counterpart peptide was chemically synthesized at Vidia a.s. company, in a manner allowing its modification with a preferable chelator of copper radionuclides. The initially considered pair of $(VAALKEK)_4$ and $(VAALEKE)_4$ peptides has been altered to peptides of the following sequences:

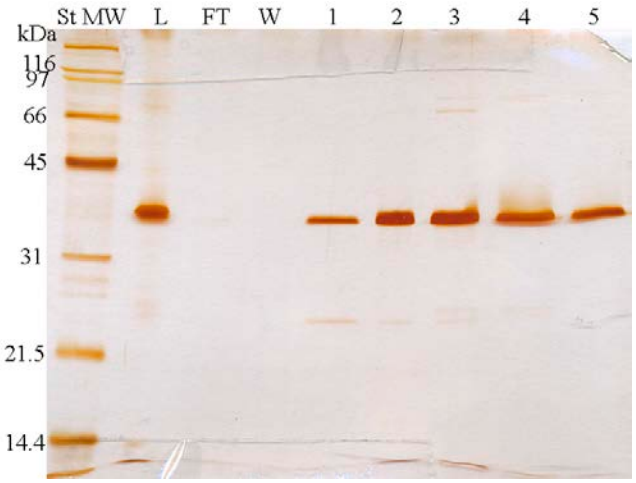
I A A L E S E I A A L E S E I A A L S K I A A L E S E (for scFv extension), and

I A A L K S K I A A L K S E I A A L K S K I A A L K S K (for the synthetic peptide),

in view of the progress achieved by others (Geoffrey M Lynn, … , Michal Pechar et al., Nature Biotechnology 33, 1201–1210, 2015).

Supply of the **recombinant fragment of M75 antibody tagged with 28-peptide** (as above) was established, involving construction of expression plasmid PelB-scFvM75(H-20aa-L-myc-IAALESE$_4$-His$_5$), accumulation of the product in *E. coli*, purification, and characterization. The final step of purification by MonoQ chromatography gave a homogenous and active product with a satisfactory yield of 0.6 mg/L of cultivation media:

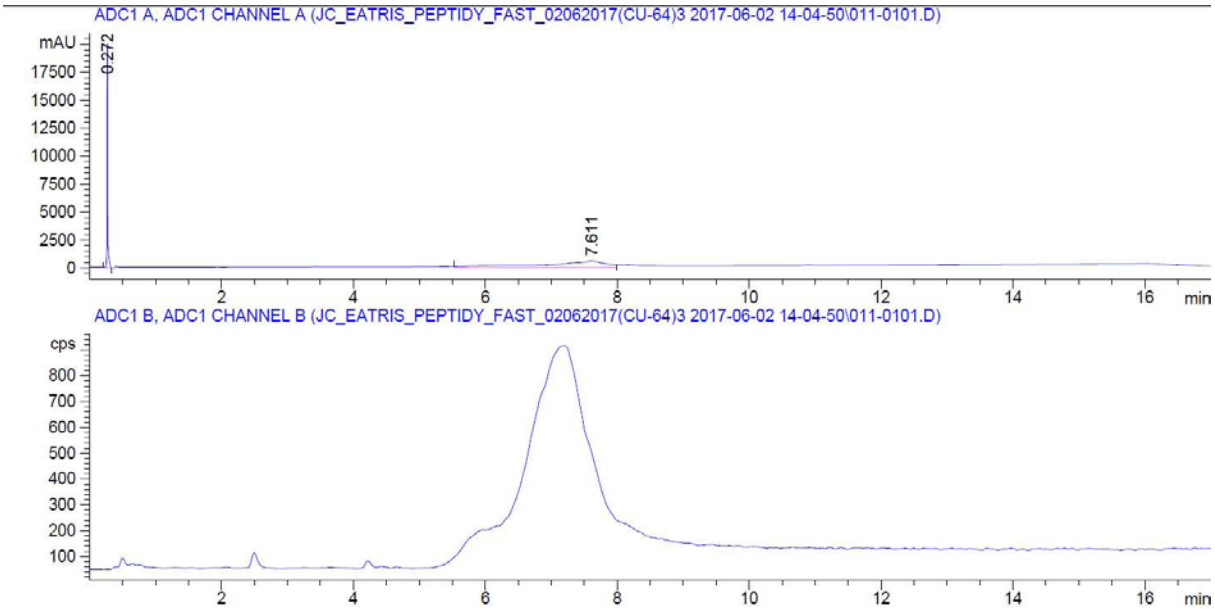




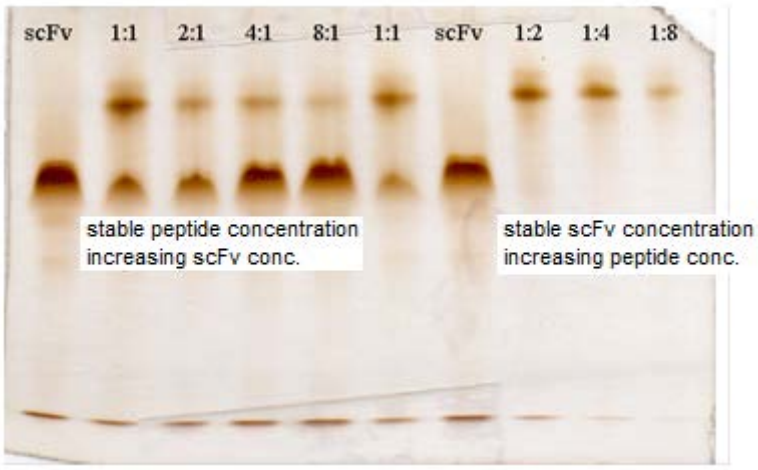
31-peptide conjugated with radionuclide-binding chelators. As the counterpart of the peptide extension of the modified scFv M75 (as described above), the following structure was designed

SarAr Y G S I A A L K S K I A A L K S E I A A L K S K I A A L K S K,

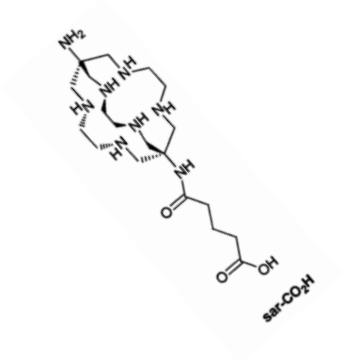
wherein SarAr represents a preferable chelator for copper radionuclides http://www.claritypharmaceuticals.com/ conjugated to the amino-terminal tyrosine residue of flexible linker YGS. The 31-peptide was supplied from the Vidia a.s. company; chemical reactions of the attachment of SarAr to it (i.e., diazotation and copulation) failed, possibly due to unfeasibility to reach sufficient concentrations of the reactants. As an alternative, the peptide was modified with a common chelating agent, NOTA, bound to the side-chain amino groups of the lysine residues using the thiocyanate chemistry. The product has five residues modified on average, as revealed by MS analyses. The radioactive labelling of this NOTA-peptide with Cu^{64} radionuclide was performed at the Institute of Nuclear Physics, Acad. Sci., in Řež; the yield was practically quantitative according to the chromatography analysis:



Interaction between 31-peptides and the peptide-tagged scFv was followed by native gel electrophoresis, PAGE: the complex displayed changed mobility, and its formation was found complete with a small excess of the peptide:



However, such desirable complex formation has not been attained with the NOTA-modified 31-peptide, possibly for detrimental effects of modifications of essential lysine residues. To tackle this problem, synthesis of otherwise modified peptide has been ordered at the Vidia a.s. company: the preferable Sar-COOH chelator will be attached to the amino-terminal tyrosine residue of the 31-peptide still bound to the synthesis carrier using the condensation chemistry:



Y G S I A A L K S K I A A L K S E I A A L K S K I A A L K S K

Since such modification will involve a flexible linker, not the coiled-coil structure, negative effects on the complex formation with the scFv tagged by the counterpart peptide are not to be expected. The Cu^{64} labeling experiments with such Sar-modified peptide have already been ordered at the Institute of Nuclear Physics, Acad. Sci., Řež.

Conclusions: Individual steps of a novel approach to radioactive labelling of recombinant fragments of antibodies have been confirmed. A complete proof-of-concept may be realistically expected in a short time after the end of this grant project.