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Comparison of Antibodies and Affimers as Affinity Agents for Quantitative Targeted Immuno-LC-MS/MS Approaches

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INTRODUCTION

An important part in the pharmaceutical development of new drugs is devoted to molecular biomarkers. A Molecular biomarker is an endogenous compound such as a peptide or a protein, that serves as an indicator of normal biological processes, pathogenic processes or pharmacologic responses to a therapeutic treatment. Objective quantification of biomarkers facilitates diagnosis of diseases, gives information about the potential of a drug, and shows how other exogenous compounds affect the metabolism. Therefore, the quantification of biomarkers is an essential tool in clinical studies [1].

However, the low concentration of most biomarkers combined with the very complex nature of biological matrices, make the quantification of a low-abundant peptide or protein a challenge and require advanced analytical methods. For that reason, it is necessary to reduce this complexity to a minimum, or isolate the target [2].

One possibility to achieve that is the combination of ligand binding assay principles and mass spectrometric detection, which offers selective enrichment with highly specific sequence based identification and quantification of the antigen – immuno-LC-MS/MS.

CONCEPT

In this work special attention is applied to the immunocapturing or also called immunoprecipitation (IP) step. In the immunoprecipitation step antibodies are presented to the sample and serve as a pole to capture the target protein or peptide. This target-antibody complex is isolated, the target released, eventually digested and subsequently injected into the LC-MS system. In doing so, the complexity of the biological sample is reduced immensely and the target is enriched. However, these methods highly depend on the quality of the used antibodies [6], [7]. To date the antibody is the undisputed first choice as affinity agent, but there are recently developed alternatives trying to compete, such as affimers.

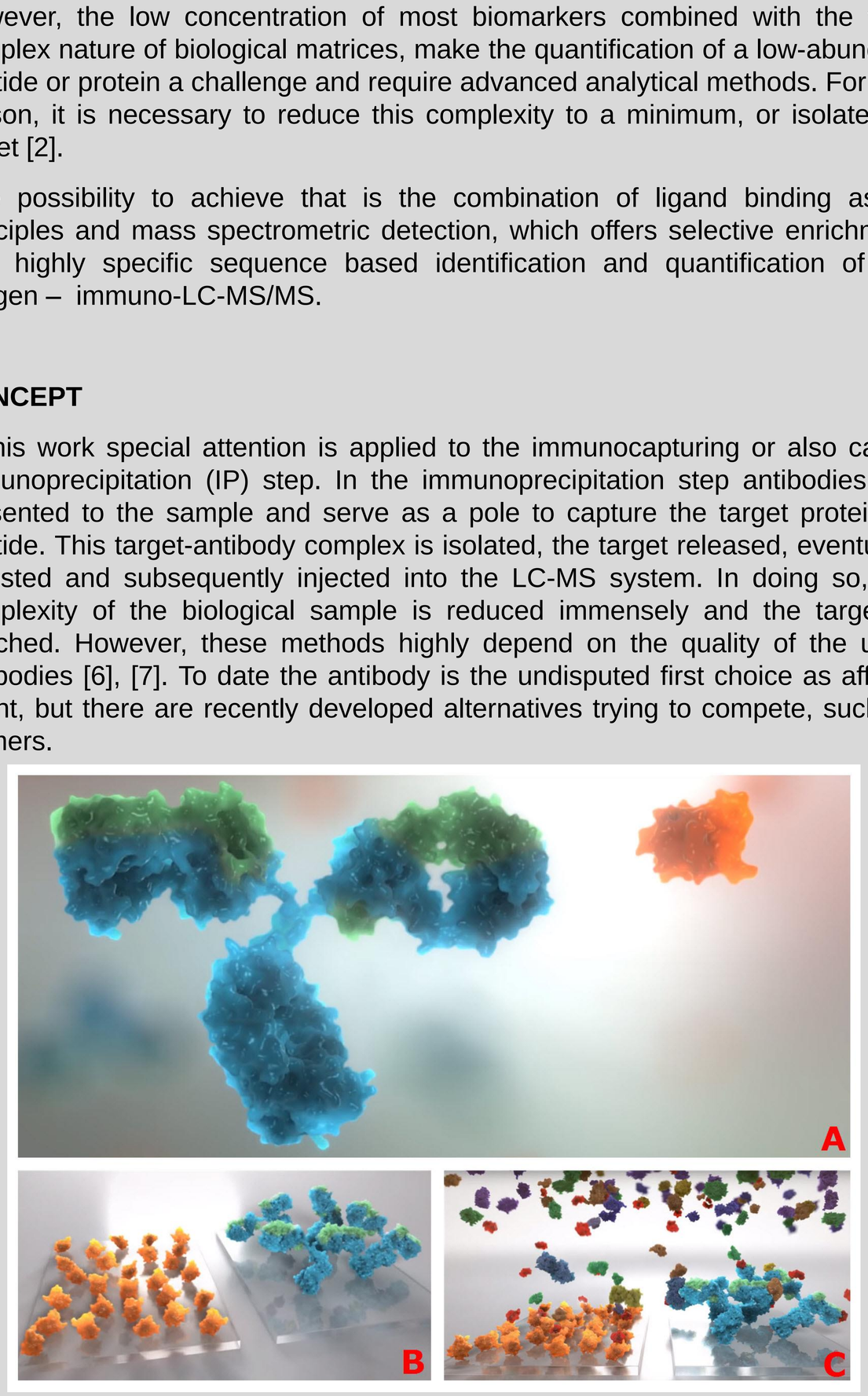

Fig. 1: (A) visualization of an antibody (left) and an affimer (right)). (B) immobilization on support. (C) immunoprecipitation process , capturing out of a complex solution/sample.

Theoretical advantages of Affimers are: expressed in *Escherichia coli,* therefore no need for animals, production time only 7 weeks; monoclonal nature, easy to modify; 10 x smaller than antibodies results in denser immobilization, more target is capturable, which increases assay sensitivity; cheaper in development than antibodies and no batch-to-batch variability [9].

To evaluate and proof this advantages, the overall task was the comparison of antibodies and affimers as affinity agents. As a second part, different supports were investigated to show potential improvements in the IP step.

RESULTS

Two different assay formats for the comparison of antibodies and affimers were available. One assay was for the targeted quantification of Apelin in human plasma, the other for the quantification of Ghrelin. A usual workup was in general as follows: 1) immobilization of the affinity agent on a support; 2) addition of different spiked concentrations of the target in buffer and in plasma; 3) discard of the supernatant and elution (Apelin assay) or digestion (Ghrelin assay) of the target; 4) Injection into LC-MS/MS system.

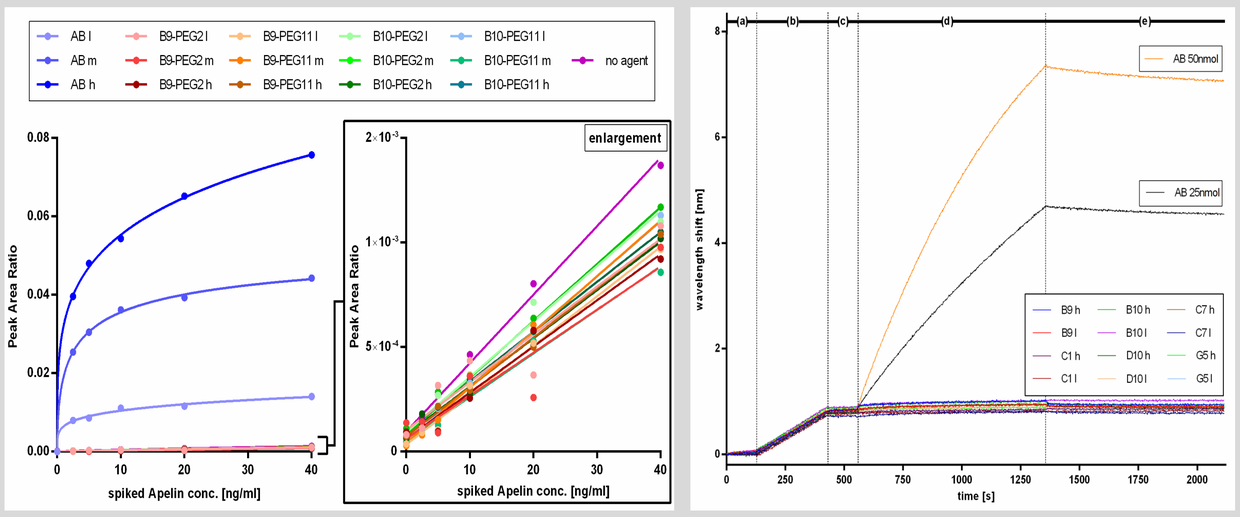

Fig. 2 and 3: (left) peak-area-ratio vs. concentration of spiked Apelin in buffer, different AB loadings show different saturations. Affimers are below negative control (enlargement). (right) results of affinity measurements of AB and a selection of affimers for Apelin.

The evaluation showed, that the affinity of the received affimers for Apelin and Ghrelin had a non-measurable low affinity.

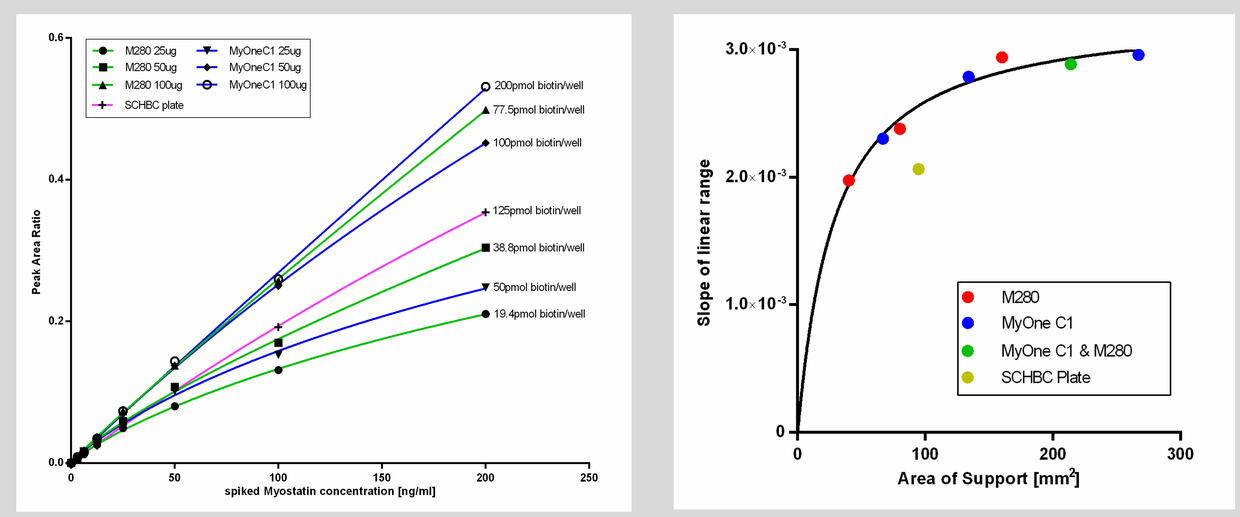

Fig. 4 and 5: (left) peak area ratio vs. spiked Myostatin concentration for different kinds of magnetic streptavidin coated beads and a SCHBC plate. The biotin binding capacity (bbc) is stated on every curve. (right) slopes of the linear range of the curves on the left, indicating that the surface area is the key factor and not the bbc of the manufacturer within one support type.

Different factors were evaluated to increase the sensitivity of an IP assay: the type of support, loading amount of affinity agent, sample volumes and presented surface area of different supports. With those improvements it was possible to increase the immuno-assay sensitivity up to 10 times at the same costs.

CONCLUSION

At this time point the developed affimers for Apelin and Ghrelin showed not enough affinity to their targets. It was the first time that such small peptides were used as targets for the affimer development. Nonetheless, their potential was shown in other works for bigger targets, which is a key reason to further investigate in this technology. The results of this work were a significantly influencing input for the manufacturer for the optimization of his process.

The IP support optimization yielded enough results to increase the immuno-LC-MS assay sensitivity. With those approaches the repertoire for the development of new immuno-LC-MS/MS assays for clinical studies of biomarkers could be enlarged.

REFERENCES

[1] J. Atkinson A.J., W. a. Colburn, V. G. DeGruttola, D. L. DeMets, G. J. Downing, D. F. Hoth, J. a. Oates, C. C. Peck, R. T. Schooley, B. a. Spilker, J. Woodcock, and S. L. Zeger, “Biomarkers and surrogate endpoints: Preferred definitions and conceptual framework,” *Clin. Pharmacol. Ther.*, vol. 69, no. 3, pp. 89–95, 2001.

[2] L. Dayon and M. Kussmann, “Proteomics of human plasma: A critical comparison of analytical workflows in terms of effort, throughput and outcome,” *EuPA Open Proteomics*, vol. 1, pp. 8–16, 2013.

[3] D. Lin, W. E. Alborn, R. J. C. Slebos, and D. C. Liebler, “Comparison of protein immunoprecipitation-multiple reaction monitoring with ELISA for assay of biomarker candidates in plasma.,” *J. Proteome Res.*, vol. 12, no. 12, pp. 5996–6003, Dec. 2013.

[4] M. a Gillette and S. a Carr, “Quantitative analysis of peptides and proteins in biomedicine by targeted mass spectrometry.,” *Nat. Methods*, vol. 10, no. 1, pp. 28–34, 2013.

[5] C. Kuan, “An Introduction To Affimers and Applications,” 2008. http://learning.avactalifesciences.com/affimers-and-applications