New perspectives for protein immunotherapy and vaccine development through antibody epitope identification using affinity-mass spectrometry

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Analysis of biomolecular interaction epitopes has recently become a key step in the development and molecular evaluation of therapeutic antibodies, biomedical peptide and protein biomarkers and molecular vaccines. Bioaffinity analysis using biosensors has been an established technique for detection and quantification of biomolecular interactions. However, a principal limitation of biosensors is their lack of providing chemical structure information of affinity-bound ligands. Proteolytic excision/extraction mass spectrometry (Protex-MS), hydrogen-deuterium exchange (HDX-MS) of peptide backbone hydrogens, and Fast- Photochemical Oxidation (FPOP) are major techniques for mass spectrometry based elucidation of protein- ligand epitopes, but these tools alone do not provide quantitative affinity data. Using a surface plasmon resonance (SPR) biosensor, we have developed a continuous proteolytic online biosensor-MS combination that enables the simultaneous affinity isolation, structural identification and affinity quantification of epitopes from a protein-ligand complex, immobilized on a microaffinity column or affinity chip. Key tool of the online biosensor-MS epitope analyser (DIGEST-PROTEX-MS) is an integrated, automated interface that provides sample concentration and in-situ desalting for the direct MS analysis of the ligand eluate [1, 2]. New biomedical application perspectives are opened by the online-biosensor-MS epitope analysis, which are illustrated by the elucidation of an unusual mixed-disulfide peptide antibody epitope of the rheumatoic target protein, HLA-B27; the interaction site identification of chaperone complexes of lysosomal enzymes [3, 4]; and the identification of M. Tuberculosis vaccine epitopes. Interaction epitopes as diverse as antigen-antibody and lectin- carbohydrate complexes [5], and affinity binding constants (K_D) from milli- to nanomolar ranges can be analysed. Most recently, epitope analysis of patient antibodies upon lysosomal enzyme replacement therapy enabled for the first time the development of hyposensitizing therapeutic peptide epitopes, that prevent or inhibit allergic and therapy-limiting immune reactions [6].

[1] Lupu L. *et al.*, *Anal. Bioanal. Chem.* **2017**, in press.
[2] Przybylski M. *et al.*, *Eur. Pat. Appl.* **2017**, in press.
[3] Iurascu M. *et al.*, *J. Am. Soc. Mass Spectrom.* **2016**, 27, 1105–1112.
[4] Moise A. *et al.*, *J. Am. Soc. Mass Spectrom.* **2016**, 27, 1071–1078.
[5] Moise A. *et al.*, *J. Am. Chem. Soc.* **2011**, *133*, 14844–14847.
[6] Kukacka Z. *et al.*, *Clin. Biochem.* **2017**, submitted.