TOF-SIMS examination of the dominant orientation of surface immobilized IgG antibodies: pH and surface density dependent studies

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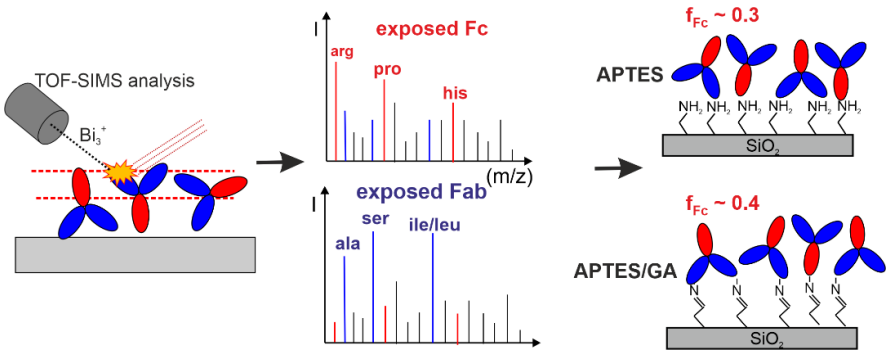
Surface immobilization of the IgG antibody requires a special attention. This molecule, which is used as a detection element in immunosensors, has an Y shape with an Fc trunk and two Fab arms ending with antigen binding sites. Various orientations of surface-immobilized IgG (flat-on, side-on, and vertical: tail-on and head-on) differ in the accessibility of antigen binding sites that drastically modifies the assay efficiency [1,2]. Therefore, it is of utmost interest to examine and control the dominant orientation of surface-immobilized antibodies. However, the IgG orientation is most commonly inferred from indirect methods, prone to high uncertainty. In contrast, antibody orientation can be resolved with TOF-SISM mass spectrometry, because of the technique sensitivity to the outermost nanometer region of adsorbed proteins and discrimination of the IgG domains (Fc and Fab) with different amino acid composition, enhanced by a multivariate Principal Component Analysis. This method is, however, limited to comparative analysis between samples that hindering an absolute determination of the dominant orientation on the particular surface. In this work, we present the novel approach of surface density dependent studies of antibody orientation with TOF-SIMS and PCA, which allows for direct tracking of orientation changes induced by the increasing molecules surface amount and for an accurate evaluation of the dominant orientation by estimation of share of molecules with head-on and tail-on alignment (f_{Fc} fraction) [3,4]. We examined the surface density dependent orientation of antibodies immobilized on silane-modified silicon by physical adsorption (APTES layer) and covalent coupling (APTES layer activated with glutaraldehyde APTES/GA, NHS-silane layer). In particular, differences in dominant vertical orientations, with the proportion of molecules with head-on to tail-on alignment: 3:1 for APTES/GA and 1:1 for APTES and NHS-silane, are revealed and discussed in terms of relevant molecule–molecule and molecule–surface interactions. Moreover, the impact of the pH of the IgG solution on the dominant vertical orientation of the antibodies immobilized on APTES and APTES/GA is determined and expressed by the f_{Fc} fraction. It is shown that under particular pH conditions the dominant antibody orientation can by significantly improved to be close to the ideal tail-on alignment through electrostatic molecules-surface interactions.



[1] Gajos, Katarzyna et al., Colloid Polym. Sci., **299**, 385 (2021)
[2] Gajos, Katarzyna et al., Applied Surface Science, **594**, 153439 (2022)
[3] Gajos, Katarzyna et al., Applied Surface Science, **518**, 146269 (2020)
[4] Gajos, Katarzyna et al., Molecules, **27**, 3672 (2022)