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Im Rahmen des Projektseminars

Besprechung neuerer Arbeiten aus Angewandter Physik LVA Nr. 374.008

spricht

Herr Sebastian Diethör

über

Investigating the CD40-CD40L complex by AFM-based SFMS and a novel sample preparation method to catch IgG from serum

CD40 and CD40 ligand (CD40L) are members of the tumour necrosis factor (TNF) family and its respective receptors (TNFR), which play a significant role in a wide area of regulatory processes in the immune system. In immunological pathways, the stimulation of CD40 on antigen-presenting cells (APCs) is triggered by helper T-cells via CD40L, found as a trimer in T-cell membranes.

In the first part of our study, we investigated the CD40-CD40L interaction with single molecule force spectroscopy (SMFS), an AFM-based method, highly adaptive to study the binding interactions between proteins and their ligand in physiological conditions. For our experiments, CD40L was coupled to the AFM tip and measurements were performed on CHO cells transfected with the transmembrane CD40 protein. By detecting single bond ruptures between CD40 and CD40L, we were able to determine the interaction forces and characterize parameters describing the energy landscape, while with further analysis, kinetic parameters such as the dissociation constant K_D could be determined. In addition, we focused on double and triple bond formations and were able to determine a 3:3 molar ratio of the CD40-CD40L complex.

In the second part of our study, SMFS experiments were carried out with the Sars-Cov-2 spike protein and human IgGs. Here, we tested a novel sample preparation method, allowing to catch human IgGs directly from blood serum with the help of protein G. First experiments showed specific bond ruptures between the Sars-Cov-2 spike protein and serum antibodies, whereas no interaction was detected on negative control samples. In future, this sample preparation might become a useful method for quick antibody detection and characterization.

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