Invited Lecture

Sialoglycan binding triggers Spike opening in a human coronavirus, and a high-resolution crystal structure of anti-FLAG M2 in complex with a FLAG peptide

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The talk consists of two parts, as described below:

The spike-mediated cell entry mechanisms of common cold betacoronaviruses are poorly understood. In contrast to the highly pathogenic SARS-CoV, SARS-CoV-2 and MERS-CoV, which engage proteinaceous receptors via the S1B domain, the spike protein of human betacoronaviruses HKU1 and OC43 have only been visualized in the fully closed, prefusion conformation. These viruses preferentially bind to α2,8-linked sialoglycans receptors via their spike S1A domain, and critically depend on these sugars for entry. We demonstrate using cryogenic electon microscopy (cryo-EM) and all-atom molecular dynamics simulations that 9-O-acetylated disialoside binding to the N-terminal domain S1A of the HKU1 spike protein allosterically triggers large-scale conformational changes in the S1B domain through intra-domain conformational changes and inter-domain crosstalk, priming the spike for subsequent membrane fusion steps. These results further our understanding of the cell entry mechanisms of common cold betacoronaviruses.

The FLAG-tag/anti-FLAG system is a widely used biochemical tool for protein detection and purification. Anti-FLAG M2 is the most popular antibody against the FLAG-tag, due to its ease of use, versatility, and availability in pure form or as bead conjugate. M2 binds N-terminal, C-terminal and internal FLAG-tags and binding is calcium-independent, but the molecular basis for the FLAG-tag specificity and recognition remains unresolved. Here we present an atomic resolution (1.17 Å) crystal structure of the FLAG peptide in complex with the Fab of anti-FLAG M2, revealing key binding determinants. Five of the eight FLAG peptide residues form direct interactions with paratope residues. The FLAG peptide adopts a 310 helix conformation in complex with the Fab. These structural insights allowed us to rationally introduce point mutations on both the peptide and antibody side. We tested these by surface plasmon resonance, leading us to propose a shorter yet equally binding version of the FLAG-tag for the M2 antibody.