Poster Presentation

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Aglycosylated knob and hole Fc homodimers are anti-parallel

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Bispecific antibody and antibody-like molecules are of wide interest as potential therapeutics that can recognize two distinct targets. Among the variety of ways such molecules have been engineered is by creating "knob" and "hole" heterodimerization sites in the CH3 domains of two antibody heavy chains. The molecules produced in this manner maintain their biological activities while differing very little from the native human IgG sequence. To better understand the knob-into-hole interface, the molecular mechanism of heterodimerization, and to engineer Fc domains that could improve the assembly and purity of bispecific antibodies, we sought crystal structures of heterodimeric and homodimeric aglycosylated Fc fragments bearing "knob" and "hole" mutations. The structure of the knob-into-hole Fc was determined at 2.64Å. Except for the sites of mutation, the structure is very similar to that of the native human IgG1 Fc, consistent with a hetero-dimer interaction kinetic KD <1 nM. Homodimers of the "knob" and "hole" mutants were also obtained and their X-ray structures were determined at resolutions of 2.5Å and 2.1Å, respectively. Both kinds of homodimers adopt a head-to-tail quaternary structure and thus do not contain direct knob-knob or hole-hole CH3 interactions. By adding site-directed mutations at F241 and F243 in the CH2 domains, the head-to-tail arrangement was disfavored, leading to increases in both the rate and efficiency of bispecific (heterodimer) assembly.

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