

## **Higher order structures of Adalimumab, Infliximab and their complexes with TNF $\alpha$ revealed by electron microscopy**

Tran, B.N.<sup>1</sup>, Chan, S.L.<sup>2</sup>, Ng, C.<sup>3</sup>, Jian, S.<sup>4</sup>, Correia, I.<sup>5</sup>, Radziejewski, C.<sup>2</sup> and Matsudaira, P.<sup>4</sup>

<sup>1</sup> National University of Singapore, Singapore, <sup>2</sup> Operations Science & Technology Biologics AbbVie Bioresearch Center Worcester MA, United States, <sup>3</sup> AbbVie Operation Singapore, Singapore, <sup>4</sup> Department of Biological Sciences National University of Singapore, Singapore, <sup>5</sup> R&D Global Protein Sciences AbbVie Bioresearch Center Worcester MA, United States

Adalimumab and Infliximab are recombinant IgG1 monoclonal antibodies that bind and neutralize human tumor necrosis factor alpha (TNF $\alpha$ );. Here, we report the structures of (1:1), (1:2), (2:2) and (3:2) of Adalimumab-TNF $\alpha$ ; and Infliximab-TNF $\alpha$ ; complexes using negative stain EM and cryo EM. The (2:2) complex structures of Adalimumab-TNF $\alpha$ ; and Infliximab-TNF $\alpha$ ; show diamond-shaped profiles and the 2D class averages reveal distinct orientations of the Fab domains, indicating different binding modes by Adalimumab and Infliximab to TNF $\alpha$ ;. The (3:2) complexes are more complicated but retain features recognized in the (2:2) complexes. Preliminary cryo-EM analysis of (3:2) individual antibody molecules, while each antibody molecule binds to two molecules of TNF $\alpha$ ; trimer.