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POSTER ABSTRACT PRESENTATIONS

SESSION TITLE: COMPLEX AND EMERGING TECHNIQUES IN STRUCTURAL BIOLOGY

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# Abstract P-45: Structural Studies of Multispecific Antibody/Antigen Complexes by Cryo-EM

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**Background:** Multispecific antibodies are artificially engineered molecules designed to bind simultaneously to several (different) antigens. Potential advantages of generating viable multispecific antibodies include the identification of malignant cells coupled with the concurrent recruitment of immune cells and the blocking of complex viral escape mechanisms. The cross-over dual-variable immunoglobulin (CODV-Ig) has been proposed as a universal bispecific therapeutic format. Its unique antigen-binding fragment (Fab) architecture provides pM affinities for ligands, no positional effect in target binding and a stable self-supporting structure. However, the three-dimensional arrangement of the constant and antigen-binding fragments in the CODV-Ig format may play a role in its *in vivo* effects. To further understand the structure and function of multispecific antibodies based on the CODV-Ig format high-resolution structural information is required. Towards this, we use cryo-electron microscopy (cryo-EM).

**Methods:** We purified CODV-Ig both in an unbound state and in complex with a single antigen and validated sample quality using SDS-PAGE, Small Angle X-Ray Scattering (SAXS) and negative-stain electron microscopy (NSEM; Tecnai T12 and F20 microscopes at IBS, Grenoble). Data of sufficient quality for image analysis was obtained using a Titan Krios microscope (ESRF, Grenoble) equipped with a Quantum LS energy filter and K2 Summit direct electron detector.

**Results:** NSEM of CODV-Ig resulted in low-resolution structural models and suggested a preferential orientation of the antibody under negative-stain conditions. Close-to-optimal vitrification conditions for CODV-Ig and antibody-antigen complexes have been identified. Efforts are in progress to reduce the antibody's propensity to aggregation and aversion to conventional cryo-EM supports. Nevertheless, image processing of both CODV-Ig alone and in complex with antigens suggests very high flexibility and conformational heterogeneity.

**Conclusion:** Particle heterogeneity may require additional data to be collected, in order to have sufficient signal that will lead to well-defined classes. An additional strategy may involve efforts to immobilize the molecules as to obtain fewer and better-defined classes.

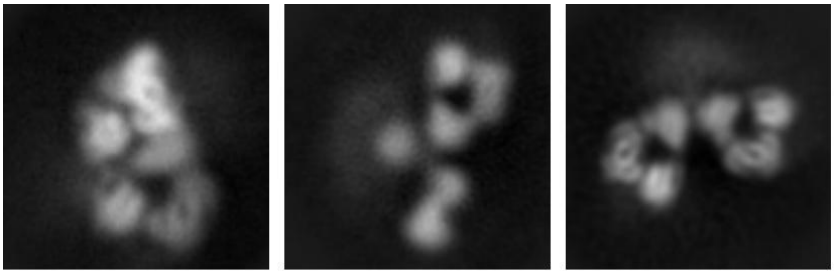

*Fig. 1. Representative classes from 2D classifications of an antigen-bound CODV-Ig cryo-EM sample. Ghost-like densities, a result of heterogeneous signal averaging, suggest great conformational heterogeneity.*

**Key Words**: antibody • cryo-EM • image processing

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