Antibody oligomerization and C1 binding on liposomes by single- and dual-axis phase plate tomography

Stuart C. Howes WORKSHOP ON CHALLENGES FOR HIGH SPEED CRYO ELECTRON TOMOGRAPHY 28th November 2018





Antibody complexes (not individuals) are important



Lu (2017) Nat Rev Immunol 18:46

- Avidity effects rather than changes in affinity often discriminate antigens
- Immune complexes directly neutralize/sequester antigens or interact with immune system

IgG1 antibody structure



Du (2007) J Biol Chem 282:15073

C1 triggers innate immune response



Hovland (2015) Atherosclerosis 241:480



Diebolder (2014) Science 343:1260

Data collection

NeCEN

- FEI Titan Krios with:
 - Gatan Energy Filter
 - K2 direct electron detector
 - Volta Phase Plate (VPP)
- \pm 70 e⁻/Å² total dose (6-10 frames)
- Whole frame alignment^{1,2}
- IMOD² tomogram reconstruction
- 1. Li (2013) Nat Methods **10**:584
- 2. Mastronarde (2017) J Struct Biol 197:102



Tomogram of WT lgG1

- Antibody mostly in filaments
- Individual domains (50 kDa) are resolved



WT IgG1 forms fingerprint-like ridges







Liposomes with anti-DNP IgG1 and C1



20 nm

Dual-axis tomograms

- Reduced missing wedge artefacts
 - Improved sampling
 - Greater radiation damage
- More robust alignment
- Suitable for initial model generation of C1-antibody complex



Scale 50nm

C1-IgG1 complex with flexible Fab domains

- Tight packing of C1 on surfaces made aligning/averaging single complexes challenging
- Features agree with average from solution C1-IgG1-RGY
- Variable Fab conformations observed below platform







Ugurlar (2018) Science 359:794-7

Preliminary dual-axis tomograms of IgG1

NeCEN

- Dose $\approx 35 \text{ e}^{-}/\text{Å}^{2}$ per tilt axis
- Tilt range $\pm 60^{\circ}$
- Slower data collection
 - Increased tracking frequency
 - More time conditioning VPP
 - Time for manually re-finding FOV





More defocus could help – CTF Correction



-10 degrees tilt, (2.3 $e^{-}/Å^{2}$, 23 $e^{-}/Å^{2}$ accumulated at start of exposure)

Summary

- WT anti-DNP IgG1 forms filaments on surfaces
- C1 complex binds IgG1 hexamers
- Reduced missing sampling from dual-axis sub-volumes helped with initial model generation
- Fab and Fc domains remain challenging to align and average



Acknowledgements



Roman Koning Thom Sharp Frank Faas Paul Parren Bram Koster Genmab

Rob de Jong Frank Beurskens Janine Schuurman



Christoph Diebolder Julio Ortiz Ludovic Renault



Universiteit Utrecht

Deniz Ugurlar Piet Gros