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

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WORKSHOP ON CHALLENGES FOR HIGH SPEED CRYO ELECTRON TOMOGRAPHY

28th November 2018
Antibody complexes (not individuals) are important

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

Lu (2017) *Nat Rev Immunol* **18**:46
IgG1 antibody structure

2 x Fab

Fc

Antigen

Fab

C1 triggers innate immune response


Data collection

**NeCeN**

- FEI Titan Krios with:
  - Gatan Energy Filter
  - K2 direct electron detector
  - Volta Phase Plate (VPP)

- $\pm 70 \text{ e}^{-}/\text{Å}^2$ total dose (6-10 frames)
- Whole frame alignment$^{1,2}$
- IMOD$^2$ tomogram reconstruction

1. Li (2013) *Nat Methods* **10**:584
Tomogram of WT IgG1

- Antibody mostly in filaments
- Individual domains (50 kDa) are resolved
WT IgG1 forms fingerprint-like ridges

20 nm
Liposomes with anti-DNP IgG1 and C1

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

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

20 nm
More defocus could help – CTF Correction

-10 degrees tilt, (2.3 e⁻/Å², 23 e⁻/Å² 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

Rob de Jong
Frank Beurskens
Janine Schuurman

Universiteit Utrecht
Deniz Ugurlar
Piet Gros

Christoph Diebolder
Julio Ortiz
Ludovic Renault