The Experimental Method

In our experiments, monoclonal murine IgG2a (1mg/ml) (1, 2), in 10 mM Tris HCl buffer solution at pH 8.0, were mixed 2 : 1 with 10 nm colloidal gold particles. A 4 μl droplet of the suspension were deposited on a grid, blotted with filter paper and plunged into liquid ethane to produce a 50-70 nm vitrified film (3). In practice, the solvent water around the sample is frozen in place, and the sample is cryogenically protected. The usage of a liquid ethane (the melting point is $-188°$C) as a freezing agent prevent the frozen water from forming crystals of cubic ice. The plunging was performed in a climate chamber with humidity of about 80%. The grid was then moved into a Field Emission Gun (FEG) 200 KeV transmission electron microscope (Philips CM200) and kept cool at about the liquid nitrogen temperature (about $-195°$C).

A series of transmission Electron Microscope images was then produced, by tilting the specimen $\pm 60°$ degrees and acquiring the data at either every, or every second degree (2). The micrographs were recorded on a CCD detector at a magnification of 26715 times, thus resulting in a final pixel size of 5.24 Å. In order to minimize the radiation damage, the final total electron dose was kept below the threshold value of ~ 20 e/Å^2 and a post-image check was systematically performed to exclude visible damage during the data collection process.

The $2D$ pictures were then combined together to obtain the full $3D$ volume, using the principle of filtered back projection. To avoid numerical artifacts, which could dramatically affect the final reconstruction, the images need to be properly aligned. For this purpose, the 10 nm colloidal gold particles were used as a reference: their positions were measured for each tilted projection, and this information used to deduce the relative orientation of the planes. The $3D$ density was then further refined by applying the Constrained Maximum Entropy Tomography (COMET) image processing (4). The COMET implementation has been previously tested on the structurally well defined Adeno virus (4) and hnRNP in the Balbiani ring genes from dipteran of Chironomus Tentans (5) and it was shown to reduce the degenerative effect of noise, thus allowing more details to be included in the tomograms.

To validate the analysis we also created pseudo atomic models by making use of quantitative multi-resolution docking algorithms (6). High resolution maps from the crystal structure were docked into the tomograms, and display remarkably good agreement (see Fig. 1).

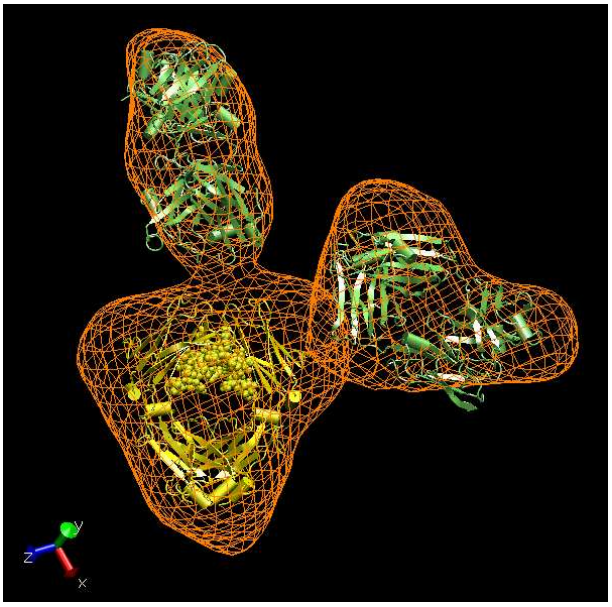

Figure 1: Pseudo atomic model of IgG. The (modified) crystal structure (green Fab; yellow Fc) is shown docked into the tomograms (orange wire frames) (2).

References

1. Okret, S., Wikstrom, A. C., Wrange, O., Anderson, B. & Gustafsson, J. A. (1984) *Proc. Natl. Acad. Sci. USA* **81**, 1609-1613.

2. Sandin, S., Öfverstedt, L.-G., Wikström, A.-C., Wrange, Ö & Skoglund, U. (2004) *Structure*, **12**, 409-415.

3. Adrian, M., Dubochet, J., Lepault, J. & McDowall, A. W. (1984) *Nature* **308**, 32-36.

4. Skoglund, U., Öfverstedt. L.-G., Burnett, R. M. & Bricogne, G. (1996) *J. Struct. Biol.* **117**, 173-188.

5. Wetterberg, I., Zhao, J., Masich, S., Wieslander, L. & Skoglund, U. (2001) *EMBO J.* **20**, 2564-2574.

6. Chacon, P. & Wriggers, W. (2002) *J. Mol. Biol.* **317**, 375-384.