Protein Tomography™—answering prevailing scientific questions in therapeutic-antibody research and development

Several methods are available for the study of antibody-antigen complexes. Present techniques, however, rely on indirect measurements of antibody binding, and do not reveal any information on what the antibody-antigen complex and possible associated proteins look like. Protein Tomography™ makes it possible, for the first time, to see antibodies free or bound to their target, both in solution and in tissue.

Today, there are approximately 200 antibody drugs in more than 350 clinical trials. The number of antibody targets has increased to over 70 described molecular targets in just a few years. As a result, many people in the industry are now looking into the next generation of therapeutic antibodies and preparing themselves for the second wave in the field. Obtaining structures and understanding the interaction between an antibody and its target on a molecular level is of vital importance in therapeutic-antibody development. Understanding the molecular mechanism of action facilitates the interpretation of preclinical data as well as clinical observations.

Unlike conventional protein-analysis methods, Protein Tomography allows the researcher to obtain visual evidence of protein interactions both *in situ* and *in vitro*, and effectively gives unique insights into the conformational dynamics of antibody-antigen binding. Protein Tomography addresses scientific questions such as whether two antibodies bind the same or different epitopes, the effects of antibody binding to the target structure versus function, the effects of antibody modification to the complex formation and the effects of ligand addition on antibody-binding properties.

Protein Tomography

Protein Tomography is a new visualization method developed by Sidec Technologies. It is the only method that can visually depict interactions

Table 1 | Protein Tomography versus conventional methods

	Visual 2D	Visual 3D	Protein localization	Protein conformation	In tissue	In cells	<i>In vitro</i>
Protein Tomography	x	x	x	x	x	x	x
Confocal microscopy and image analysis	x		x		x	x	
Mass spectrometry and proteomics			x				
Molecular imaging ^a	x		x		x		
Histology, immunohistochemistry, cytogenetics	x				x		
Electron microscopy	x		x		x	x	x
High-resolution structures, X-ray nuclear magnetic resonance spectroscopy		x		x			

^aPositron emission tomography, magnetic resonance imaging.

at the protein level in cells, in different tissues and in *in vitro* assays. The resulting three-dimensional (3D) tomograms can be compared to visualize target conformation, protein interactions and complex formation mechanisms in these different specimens. The ability of Protein Tomography to visualize protein structures directly in a cellular environment goes beyond the conventional methods used in biological validation studies such as X-ray crystallography and molecular imaging (Table 1).

Among other applications, Protein Tomography has successfully been used for the detection of receptor-ligand complex formation, visualization of antibody-antigen interaction sites and selection of therapeutic protein candidates.

How does it work?

Protein Tomography analysis requires only small quantities of sample material. After the sample preparation process, transmission electron microscopy is performed on cryo samples to generate tilt series of two-dimensional images (micrographs) taken at fixed angular increments.

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APPLICATION NOTES

Figure 1 | Tomogram of one of various conformations of IgG in solution. Ribbon structures of individual domains are docked in the image. The highest value of correlation coefficient is obtained when domains are properly oriented. Reprinted from reference 1 with permission from Elsevier. Image is courtesy of S. Sandin (Department of Cell and Molecular Biology, Karolinska Institute).

A low electron dose is used during this process to minimize radiation damage and preserve the biological structure of macromolecules. This, together with measures taken during sample preparation, ensures that the retrieved information optimally represents the actual situation in the sample.

After aligning the micrographs, 3D reconstruction is performed. The tomograms are reconstructed by conventional filtered back-projection technique followed by refinement by Sidec's unique algorithm. The algorithm acts as a mathematical filter on the preliminary 3D reconstruction to substantially improve the signal-to-noise ratio of the tomograms, thereby permitting 3D visualization of macromolecules at high resolution. The tomograms are then analyzed and evaluated.

In the realm of therapeutic-antibody research and development, there exist certain scientific questions that are of great value for the antibody-development process. How flexible is the antibody? What is the conformation of a target in resting, ligand-activated and antibody-inhibited states? These questions can be systematically addressed by Protein Tomography.

How flexible is the antibody in solution?

Protein Tomography was recently used to analyze an *in vitro* sample of mouse immunoglobulin G (IgG) monoclonals¹. Tomograms revealed the characteristic Y-shaped IgG molecules with three protruding sub-units, which were identified as two ellipsoidal Fab arms and a heart-shaped Fc stem. The fidelity of the findings were substantiated by docking X-ray crystalline structures from the Protein Data Bank to the tomograms and performing standard volumetric correlation analysis (**Fig. 1**). Protein Tomography added another dimension to our understanding by revealing evidence of the antibody's flexibility, information that could not be obtained by other methods. It was apparent from the results that the positions of the Fab arms relative to the Fc stem varied greatly from one molecule to another. This evidence of

Figure 2 | Different conformations of a receptor, its multimeric states and its interaction with antibodies can be studied using Protein Tomography.

the high flexibility of IgG in solution is of functional significance in its mechanism of action.

The study of antibody conformation and flexibility can have many other important applications in therapeutic-antibody research and development, such as when investigating the effect of modifications on the antibody during the antibody-development process.

What does the receptor on the cell look like in various states?

In a study with a company that creates and develops human antibodies for the treatment of diseases, Protein Tomography was used to study the conformation of the receptor in its resting state versus the antibody-inhibited state, *in situ* (**Fig. 2**). With the aid of primary and secondary markers (IgG and gold-labeled anti-IgG antibodies, respectively), we analyzed different samples of a receptor-overexpressing cell line with or without the addition of purified antibody. As the antibody-receptor complex in tissue is fixed before the gold-labeled secondary antibody is added, labeling does not affect the complex in question. Tomograms obtained gave insights to various receptor conformations in their cellular environment, thus greatly enhancing the understanding of the structure of the antibody's receptor and thus the antibody's mechanism of action. Furthermore, such studies could allow the visualization of the complex between the antibody and its receptor, providing insights into their interaction.

Conclusion

Protein Tomography is a valuable technology for 3D visualization and analysis of individual proteins, and their involvement in target complexes. In the field of therapeutic-antibody research, Protein Tomography can deepen our understanding of the structural conformation and dynamics of antibodies, their receptors and the mechanisms of antibody-receptor interaction. This will help the decision-making process, which is vital in the development of new candidates and the improvement of existing therapeutic antibodies.

1. Sandin, S., Ofverstedt, L.G., Wikstrom, A.C., Wrangé, O. & Skoglund, U. Structure and flexibility of individual immunoglobulin G molecules in solution. *Structure (Camb.)* **12**, 409–415 (2004).

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