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EM by EM: High-Efficiency Epitope Mapping using High-Throughput Electron Microscopy

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The mapping of binding sites between antibodies and their target antigens is a fundamental step in the discovery and development of new therapeutics, vaccines, and diagnostics. Many therapeutic monoclonal antibodies (mAbs) recognize conformational epitopes of target antigens, which are often represented by membrane proteins, receptors, or multi-subunit proteins. While technological advances such as B cell cloning, deep sequencing, and phage display have greatly increased the ability to produce large numbers of mAbs, high-throughput mAb epitope mapping techniques have not kept pace. Historically, NMR and X-ray crystallography have been used to provide high-resolution information of these interactions. Despite their proven successes, these technologies are often limited by the size of the targets or by failures obtaining labeled samples or diffracting crystals, as is the case for many flexible and glycosylated targets. In addition, epitope-mapping analyses involving other methodologies, such as Hydrogen-Deuterium Exchange (HDxMS), site directed mutagenesis, two-hybrid/phage display, or peptide array screening are often challenging, time and labor intensive, or require large amounts of sample (Figure 1).

We have recently implemented an efficient and robust platform to perform epitope mapping of antibody-antigen interactions using high-throughput Electron Microscopy (EM) and single particle analysis. This method is applicable to proteins in the range of 100kDa to 4MDa as well as both symmetric and asymmetric complexes. The entire procedure is depicted in Figure 2. Briefly, purified antigens are incubated with individual high-affinity Fabs (pM-nM range) and purified by Size Exclusion Chromatography (SEC). The resulting complexes are then deposited on freshly glow-discharged EM grids and subjected to either negative staining or Cryo-EM preparation. The prepared grids are then imaged on a Transmission Electron Microscope (TEM), either manually or, more efficiently, using Leginon, an automatic data collection software [1]. Following data collection, the resulting images are used to produce a dataset of about 25,000-100,000 particles. This dataset is then processed using the Relion and Eman2 software packages [2,3] to obtain reference-free 2D classes and 3D reconstructions of the antigen-antibody complexes. Lastly, the crystal structures or homology models of Fabs and antigens are computationally docked into the 3D reconstructions, allowing for the identification of the residues at the binding interface. This entire process can be run as a user-supervised or automated process, and can be completed within a few hours on a high performance computer cluster.

Our platform for epitope mapping has a unique advantage over traditional methods, namely that only microgram quantities of sample are needed. In addition, this technology does not require samples at high concentrations, protein labeling, or crystallization.

Here, we present this pipeline applied to the characterization of the homotrimeric human serine protease HtrA1, implicated in the progression of age-related macular degeneration (AMD). A number of inhibiting mAbs against HtrA1 were also identified. Using the described technologies for epitope mapping we determined that the anti-HtrA1 antibodies bind to the surface-exposed loops B and C of the

catalytic domain, suggesting an allosteric inhibition mechanism (Ciferri et al., 2015).

In summary, we described a high-throughput approach for identifying epitopes on antigens of interest. This technology enables the rapid and accurate determination of epitope interactions and serves as an important tool for antigen design, selection of therapeutic targets, and vaccine development. Although this method is still under development for smaller and conformationally heterogeneous targets, it can efficiently complement existing approaches. Future efforts will focus on streamlining the preparation of mAb-antigen complexes for Cryo-EM analysis to improve the obtained resolution and obtain greater insights into antigen-antibody recognition.

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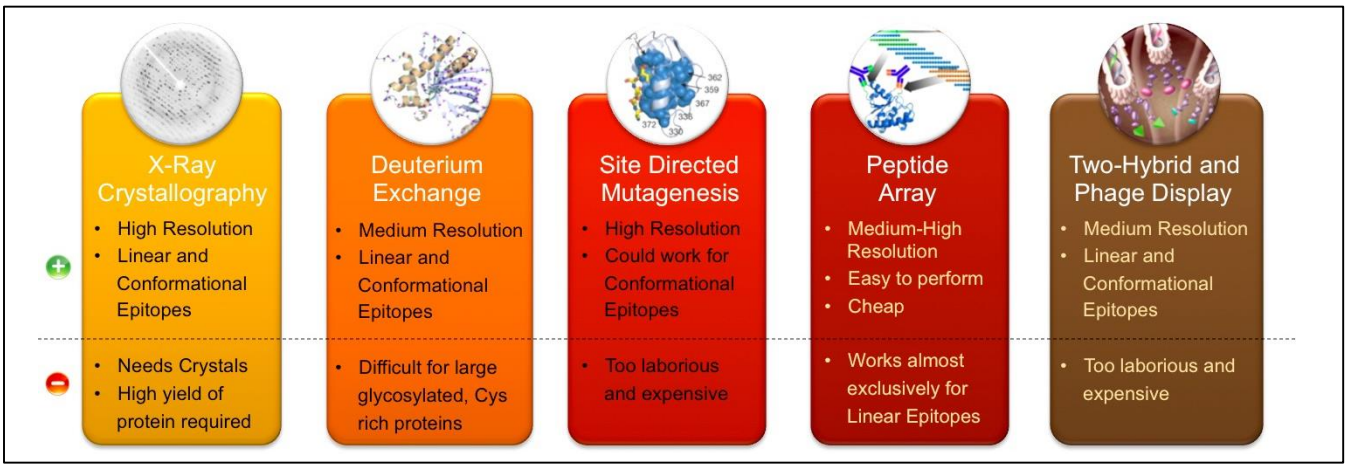

Figure 1: Common technologies available for Epitope Mapping. Advantages and disadvantages are indicated.

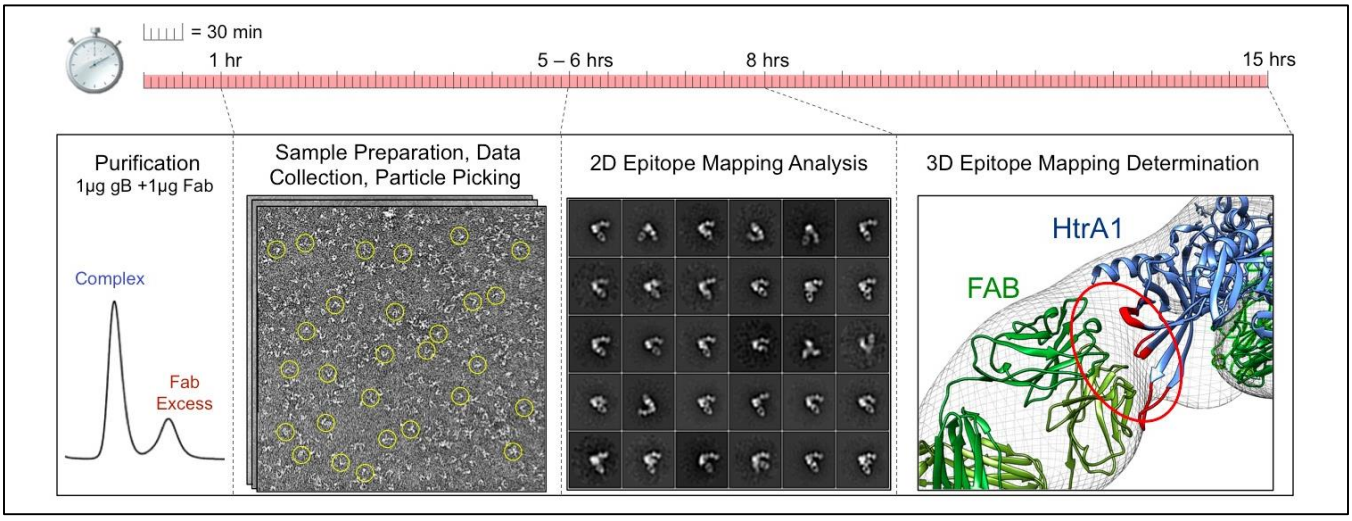

Figure 2: Schematic representation of the EM data collection and processing pipeline**.**

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