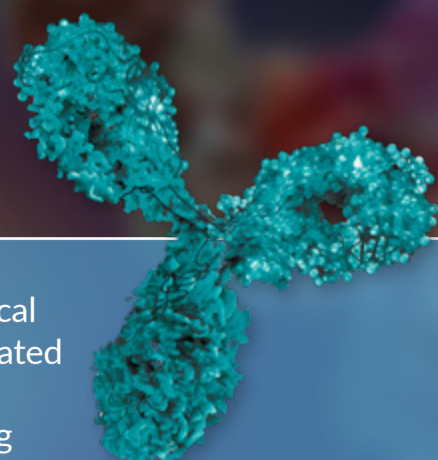
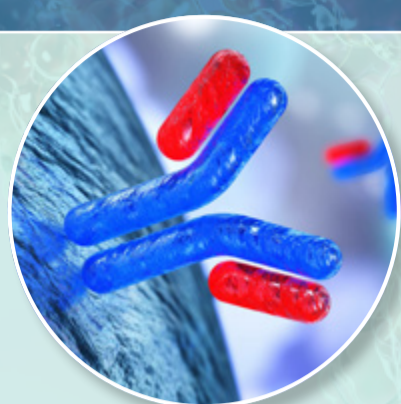


Monoclonal Antibody Characterization Methods



Monoclonal antibodies (mAbs) have been used for decades in clinical and basic research. The size of the global mAbs market¹ was estimated at nearly \$238 billion for 2023, and predicted to reach nearly \$495 billion by 2030. There are many techniques for characterizing mAbs. Each approach has specific uses and some limitations; careful analysis is necessary to select what best meets your needs. This infographic presents considerations for characterizing the structure, function, and purity of mAbs. For each consideration, its importance for mAb work, representative tools (with their utility and limitations), and representative vendors are listed.

Structure²



Importance

- Two complementarity-determining arms, with amino acid variability that dictates mAb specificity
- One leg (crystallizable fragment) that activates the immune system against the antigen

Representative vendor

- Shimadzu's quadrupole-TOF LCMS-9030-plus Protein Metrics software quantitates mAb structural heterogeneity

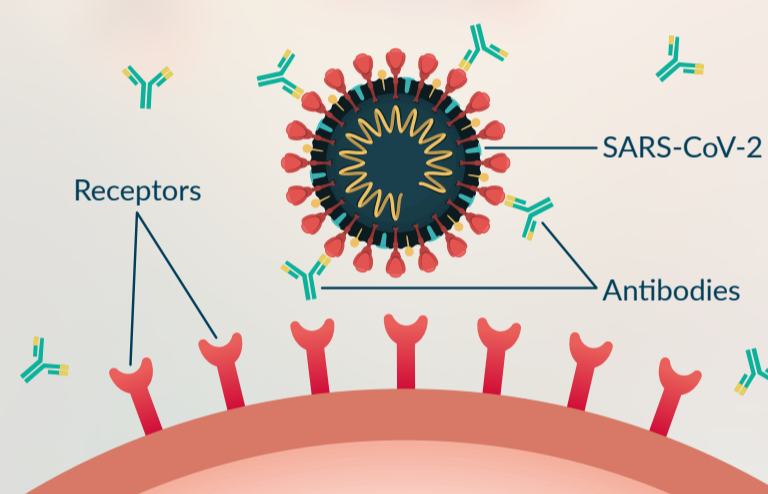
Representative tools

- **Primary structure:** MALDI-TOF MS
 - **Utility:** Peptide mass fingerprinting: accurate calculation of absolute mass
 - **Limitation:** Lower analytical sensitivity and resolution than electrospray ionization
- **Secondary structure:** Circular dichroism
 - **Utility:** Rapid determination of percent alpha helix content, and no mAb size limit
 - **Limitation:** Output spectrum does not correspond to only one possible structure
- **Tertiary structure:** 2D NMR
 - **Utility:** Atomic-level structural analysis of dynamic mAb conformations
 - **Limitation:** Challenging to differentiate between enantiomers

Function^{3,4}

Importance

- The equilibrium dissociation constant, K_D , is a quantitative metric of mAb binding to its target
- Cell potency evaluates downstream or off-target events



Representative tools

- **K_D :** Surface plasmon resonance
 - **Utility:** High sensitivity, label-free quantitation in crude samples
 - **Limitation:** No universal industry standards for using instrumental outputs
- **Off-target binding:** Peptide microarrays
 - **Utility:** Early-stage prediction of the mAb motifs responsible for off-target binding
 - **Limitation:** Performance validation—for many mAbs—remains a work in progress

Representative vendor

- WuXi Biologics evaluates mAb affinity parameters by surface plasmon resonance and fluorescence-activated cell sorting
- Cytosol's LSA platform evaluates the affinities, kinetics, and epitope specificities of mAbs

Purity^{5,6}

Importance

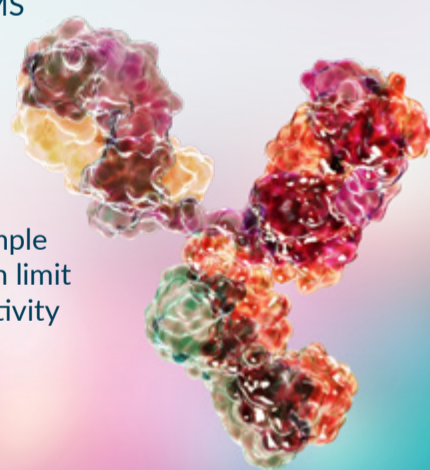
- Oxidation—mainly of methionine residues—is possible at any stage of mAb development and analysis
- mAb N-glycosylation is often heterogeneous and corresponds to mAb stability, safety, and therapeutic outcome

Representative vendors

- Nanotemper's Prometheus Panta platform simultaneously evaluates various biophysical metrics of the purity and stability of mAb formulations
- Creative Proteomics uses many platforms to provide comprehensive post-translational modification analyses and ensure the therapeutic efficacy of mAbs

Representative tools

- **Oxidation:** Capillary isoelectric focusing-electrospray ionization-MS
 - **Utility:** mAb separations based on isoelectric points
 - **Limitation:** Lack of automation
- **N-glycosylation:** Capillary zone electrophoresis-MS/MS
 - **Utility:** Absolute quantitation of mAbs in parallel with longer, less polar peptides
 - **Limitation:** Low sample loading capacity can limit the detection sensitivity



The structural and functional heterogeneity in mAb formulations—as well as their purity (generally referring to post-translational modifications)—are long-standing challenges. Furthermore, modern immunology research continues to question long-held perspectives, such as on the specificity of mAbs for therapeutic purposes.⁷ Ongoing efforts at establishing widely accepted characterization standards will be critical to maximizing the utility of mAbs in basic and clinical research.

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