Epitope Binning: A Critical Step in Antibody Development

Direct Assay

The global antibody market has been on a growing trend and is predicted to reach USD $652 million by 2026 from USD $393 million in 2021, at a CAGR of 10.6% during the forecast period[1]. This is enabled by and a reflection of recent advancements in recombinant antibody technologies, allowing scientists to understand the structures and functions of these protein molecules for the precise design and manipulation of antibodies for different applications. Antibodies thus are not limited only to naturally occurring in the body as part of the immune system to fight against foreign pathogens but prove useful in fundamental research, diagnostics, and disease therapeutics.

In the antibody development process, the most suitable monoclonal antibodies (mAbs) are selected based on characterizations of specificity, stability, and overall biophysical properties. Epitope binning is a critical step in which a library of mAbs specific to a given antigen are grouped into "bins" based on the epitopes they bind to. Conventional assays, whether in the sandwich, premix, or tandem format, usually involve pairwise testing of the mAbs to determine whether the two block the same or a closely-related epitope (same bin) or occupy different epitopes (different bins). The binning process becomes time- and materials-consuming as the number of mAbs to be characterized increases. To overcome this challenge, epitope binning via the direct assay in the tandem format with FOPPR can accommodate up to four mAbs in one experiment run, enabling this characterization process to be more time- and material-efficient for scientists.

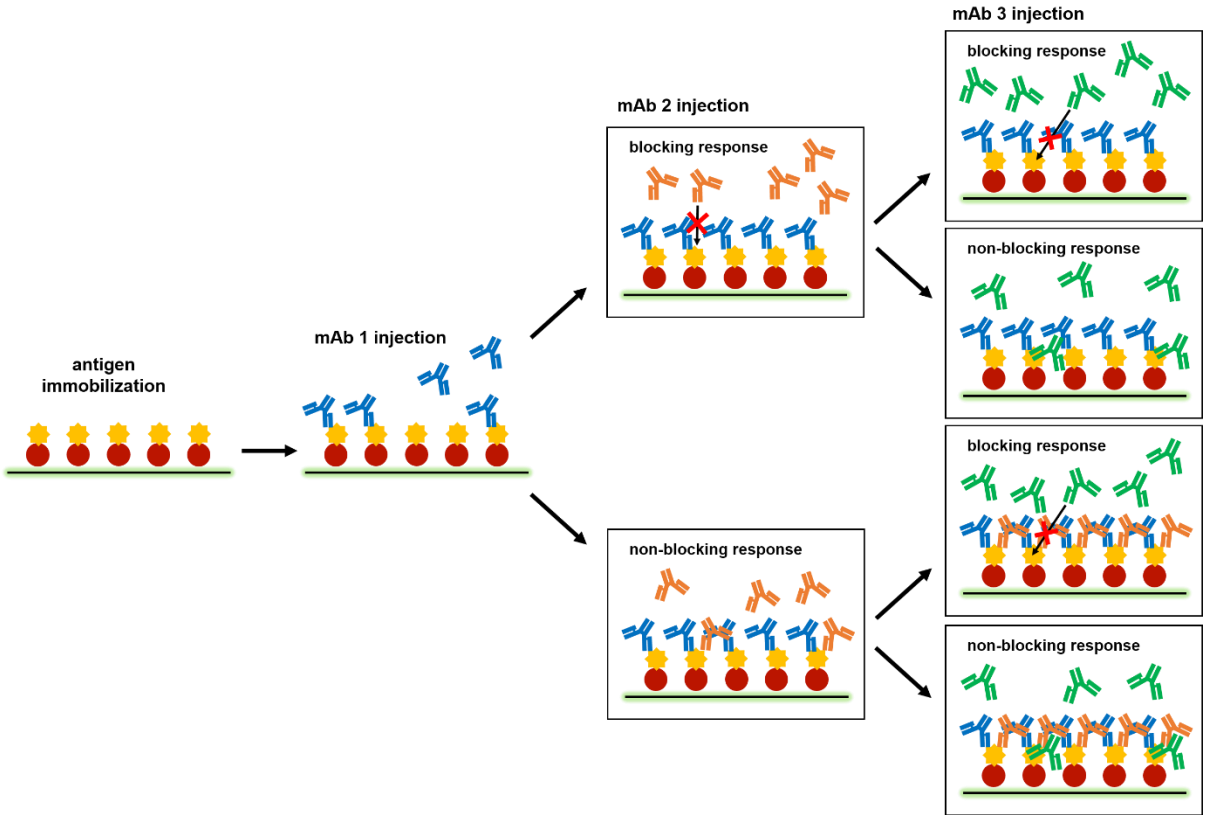
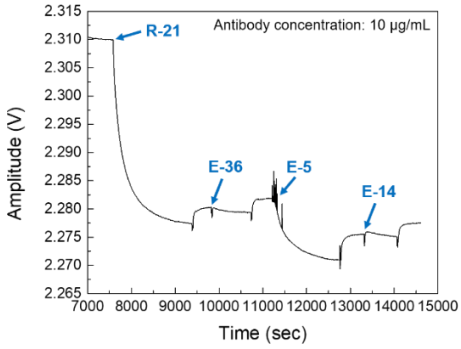
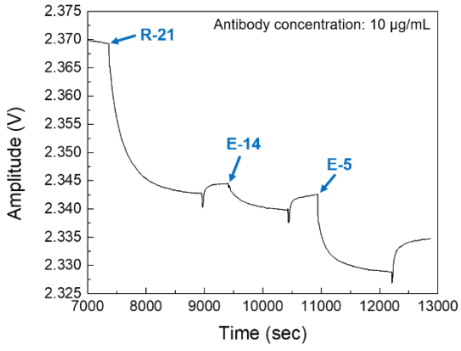


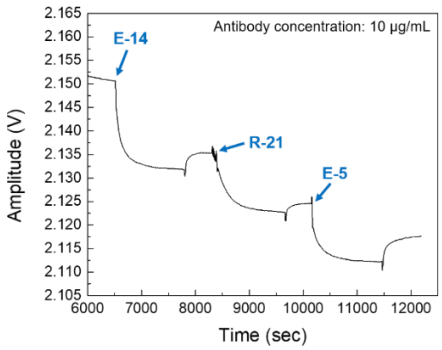
Fig. 1 Experiment scheme with the direct assay in the tandem format with FOPPR.

For brevity, the experiment scheme in Fig. 1 demonstrates only three mAb injections. The antigen is first immobilized onto the sensing surface. The first mAb is then introduced to the sensing surface at a saturating concentration, followed by a second mAb. The second mAb can produce a blocking or a non-blocking response. Following the second mAb, a third mAb is introduced to the sensing surface. This third mAb can similarly produce a blocking or a non-blocking response. Based on the blocking and non-blocking patterns, experiments with different mAb injection orders can be designed and carried out to correctly bin the mAbs.

Table 1 shows the sensorgrams for a series of epitope binning experiments to determine the bins of four IgG mAbs, E-5, E-14, E-36, and R-21. The binding kinetics and affinity between the antigen and each mAb were analyzed prior to the epitope binning experiments to determine the injection order of the mAbs (data not shown). Since R-21 has the highest affinity of the four mAbs, it was injected first, followed by E-36, E-5, then E-14. Injection of E-36 did not produce an obvious binding curve. This indicates that binding of E-36 to the antigen was blocked by R-21 and infers R-21 and E-36 have the same or a closely related epitope. Injection of a third mAb, E-5, produced a visible binding curve. E-5 was thus determined to bind to a different epitope than R-21 and E-36. Injection of a fourth mAb, E-14, did not produce an obvious binding curve. It was blocked by either R-21, E-36, or E-5. Two more experiments were performed to determine the epitope bins for E-5 and E-14. Based on the blocking and non-blocking patterns, the relative epitope orientation of the mAbs are determined and can be drawn into an epitope map, as seen in Fig. 2.

Table 1 Sensorgrams of a series of epitope binning experiments via the direct assay in tandem format with FOPPR for IgG mAb E-5, E-14, E-36, and R-21.

Sensorgram	Note
	• R-21 and E-36 have the same or a closely related epitope • E-5 has a different epitope than R-21 and E-36 • E-14 is blocked by either R-21, E-36 or E-5
	• E-14 may have a closely related epitope to R-21 • E-5 has a different epitope than R-21, E-14, and E-36



- E-14 has a different epitope than R-21 and E-36

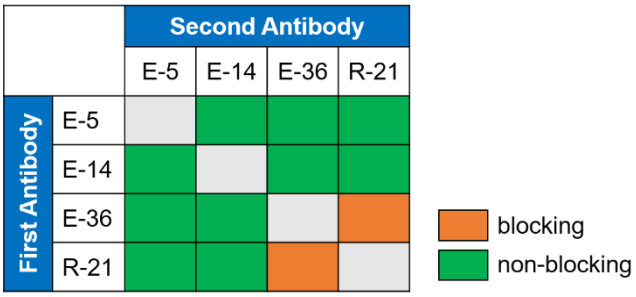
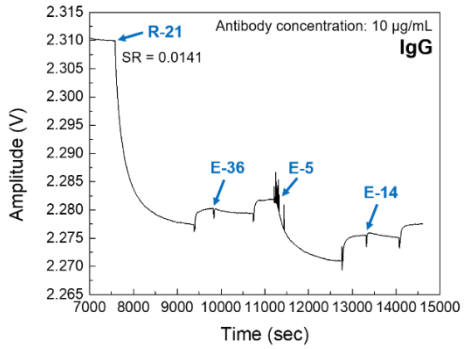
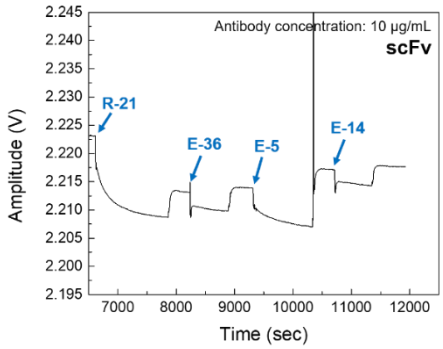
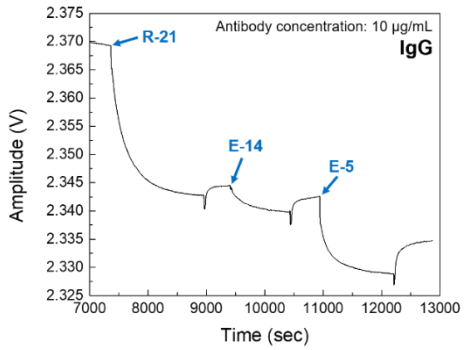
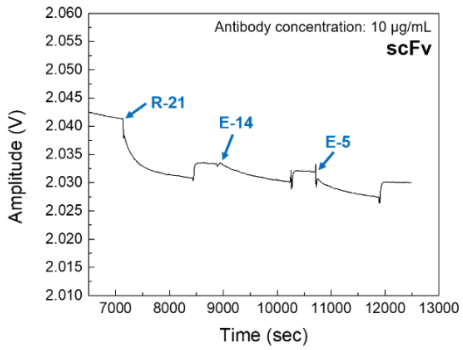
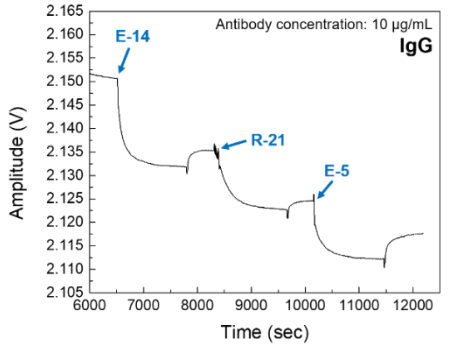
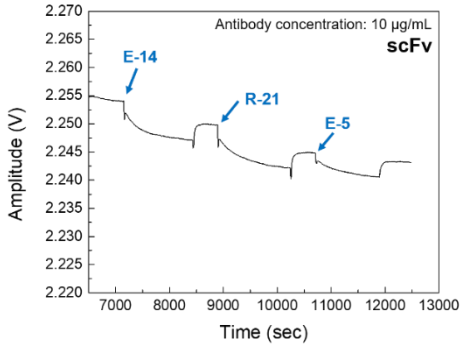


Fig. 2 Epitope map showing the blocking and non-blocking relations of IgG mAbs E-5, E-14, E-36, and R-21.

In addition to IgG mAbs, epitope binning via the direct assay in the tandem format with FOPPR can also be used with scFv antibodies. ScFv antibodies have the advantages of better tumor penetration, more rapid blood clearance, lower retention times in non-target tissues, and reduced immunogenicity compared to IgG antibodies and consist of variable regions of heavy (V_H) and light (V_L) chains[2]. They can be expressed easily in E. coli, making improvements of antibody properties such as affinity and specificity more efficient. Table 2 displays the results of a series of scFv epitope binning experiments in parallel with results from IgG mAbs. The scFv antibodies in the three experiment runs were injected in the same order as the IgG mAbs. Sensorgrams from the scFv antibodies produced similar binding curve patterns as the IgG mAbs. The smaller binding curves for each scFv at the same saturating concentration of 10 µg/mL was due to the smaller molecular weight of the scFv fragment compared to the IgG mAb.

Table 2 Sensorgrams of a series of epitope binning experiments via the direct assay in tandem format with FOPPR for E-5, E-14, E-36, and R-21. Left column: IgG mAbs. Right column: scFv antibodies.

IgG	scFv
	
• R-21 and E-36 have the same or a closely related epitope • E-5 has a different epitope than R-21 and E-36 • E-14 is blocked by either R-21, E-36 or E-5	
	
• E-14 may have a closely related epitope to R-21 • E-5 has a different epitope than R-21, E-14, and E-36	
	
• E-14 has a different epitope than R-21 and E-36	

Finally, results from the epitope binning assays were compared with data obtained from ELISA, as shown in the heat map in Fig. 3. In the ELISA assay, the IgG mAbs were added first to the detection wells. The scFv antibodies were then added to compete with the IgG mAbs. The optical density reading of each IgG and scFv pair

was then normalized for comparison. Data with smaller values (red) indicate greater competition while data with larger values (blue) indicate smaller competition between the IgG and scFv antibodies.

Calculated Data		IgG			
		E-5	E-14	E-36	R-21
scFv	E-5	0.2495	0.1712	0.0049	0.0312
	E-14	0.3369	0.1810	0.0026	0.0367
	E-36	0.9315	0.7857	0.1129	0.5773
	R-21	0.3836	0.3389	0.0142	0.1113

Fig. 3 ELISA heat map of IgG mAbs and scFv antibodies E-5, E-14, E-36, and R-21.

Recombinant protein technologies have seen dramatic advancements in recent years. More efficient and simple-to-use to use tools are thus needed to accommodate to this rising trend. Epitope binning with FOPPR via the direct assay in the tandem format with FOPPR demonstrate a higher efficiency as each experiment can accommodate up to four mAbs. Furthermore, the binning assay can be applied to both IgG mAbs and scFv antibodies, helping researchers and scientists develop and optimize recombinant antibodies tailored to their specific needs. Finally, the data from the epitope binning assays were consistent with ELISA, a widely-used but time-consuming and more labor-intensive analysis tool.

Acknowledgements

Antibodies in this study were offered by AnTaimmu BioMed (ATBM). ATBM is a biotech company specializes in the development of novel antibodies for therapeutic drugs. During the COVID-19 outbreak period, ATBM screened antibodies targeted for the nucleocapsid protein of SARS-CoV-2, of which one antibody pair was developed into a lateral flow assay as the Vstrip COVID-19 Antigen Rapid Test. Additionally, dozens of antibodies targeted to the spike protein of SARS-CoV-2 were isolated, with several of exhibiting strong neutralizing activities. These antibodies demonstrate very high potentials for the treatment of COVID-19. ATBM continues its effort in developing high quality novel therapeutic and diagnostic antibodies for different cancers and infectious diseases.

References

[1] https://www.marketsandmarkets.com/Market-Reports/custom-antibody-market-164328301.html

[2] Ahmad et al. *Journal of Immunology Research* **2012** 980250.