LENSai™ Epitope Binning

BioStrand
a subsidiary of IPA

Validating LENSai *in silico* antibody epitope binning against classical wet lab binning

The Challenge:

Epitope binning is crucial in early stages of antibody discovery providing insights in the epitope landscape thereby facilitating the lead panel selection process. Technological advancements have led to a significant increase in the number of antibodies generated from discovery campaigns, highlighting the growing importance of *in silico* epitope binning algorithms in facilitating high-volume diversity-driven discovery efforts. However, they must meet two key criteria:
1. High accuracy — matching the results of classical wet lab binning procedures.
2. High scalability and throughput to support use in the early stages of a discovery campaign.

The Approach:

LENSai binning was performed on 29 Ab sequences directed against a transmembrane protein. Via an inter-annotator agreement analysis (Cohen's Kappa Test) using a majority consensus label mapping these results were compared with classical wet lab binning. The *in silico* clustering and wet lab binning were performed independently. The wet lab data were made available for comparison after results from both analyses were processed individually.

LENSai Epitope Binning

Using LENSai epitope binning 6 clusters were identified, with each cluster comprising antibodies sharing a similar target binding region.

Wet lab validation

Heatmap summarizing the classical wet lab epitope binning data. Bins are assigned based on the combined outcome of a sandwich assay containing the target monomer and a tandem assay containing the target dimer. BLI-based analysis of the 29 antibodies resulted in the identification of 6 non-overlapping bins, with 3 of them consisting of 2 sub-bins.
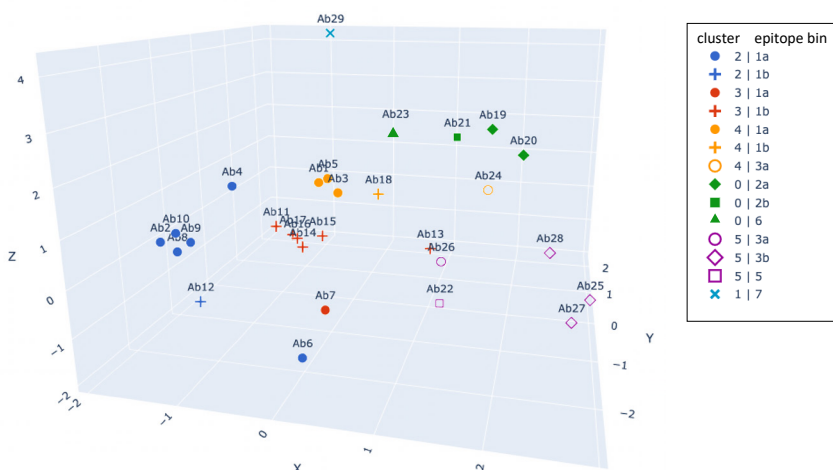
Program Summary

Scale:
29 antibodies

Type:
Blinded

Method:
Cohen's Kappa Test using a majority consensus label mapping

Superimposition of results

cluster	epitope bin
●	2 | 1a
+	2 | 1b
●	3 | 1a
+	3 | 1b
●	4 | 1a
+	4 | 1b
○	4 | 3a
◆	0 | 2a
■	0 | 2b
▲	0 | 6
○	5 | 3a
◇	5 | 3b
□	5 | 5
✕	1 | 7

In Silico
Clusters are the 'bins' predicted *in silico* and indicated by a color and associated number in the legend.

Wet Lab
Epitope bins are the bins determined by BLI-based wet lab screening and visualized with geometrical forms.

Before applying Cohen's Kappa Test, the labels of clusters and epitope bins are normalized by applying a majority consensus label mapping.

As this mapping is not reciprocal, especially if the number of bins and *in silico* clusters differ, it is applied in both directions for this validation case study.

Conclusion

Label mapping	κ
LENS^{ai} cluster > Wet lab bin	0.925
Wet lab bin > LENS^{ai} cluster	0.842

How to interpret Cohen's Kappa Coefficient (range -1 to 1):
> 0.8: Near perfect agreement

Near perfect agreement between LENS^{ai} *in silico* Epitope Binning and classical wet lab binning results. LENS^{ai} Epitope Binning matches *in vitro* competition assays with high confidence.

Contributing factors in the difference in results between *in silico* and *in vitro* methods

- Possible differences in glycosylation status of the target: full homogeneous glycosylation *in silico* versus potentially more heterogeneity *in vitro*.

- Subtle differences observed in wet lab data might be hard to interpret.

- Wet lab assays used both monomeric and dimeric target, while *in silico* binning was performed with monomeric target only.

- Steric hindrance is taken into account *in silico* and *in vitro* but methodology differs. Competitive behavior is main driver of wet lab binning.

- Difference in impact of antibody binding strength in *in silico* screens versus *in vitro* assays.