Case Study

LENS^{ai}™ Epitope Mapping

BioStrand
a subsidiary of IPA

LENS^{ai} Epitope Mapping matches x-ray crystallography

Outperforms other epitope mapping technologies in benchmark study

Overview:

Epitope mapping remains a cornerstone of therapeutic antibody development, and the 2023 peer-reviewed study "Epitope mapping of monoclonal antibodies" (published in MABS, 2023, Vol. 15, No. 1, 2285285) offers a rare head-to-head comparison of leading technologies across five high-impact antibody-antigen pairs. Evaluating seven experimental methods—from peptide arrays to hydrogen-deuterium exchange—the study highlights both the strengths and limitations of traditional approaches. Building on this benchmark, we applied LENS^{ai} *in silico* Epitope Mapping to go head-to-head and analyze the results.

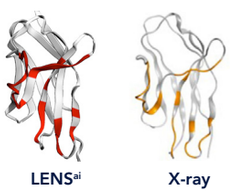
Challenge:

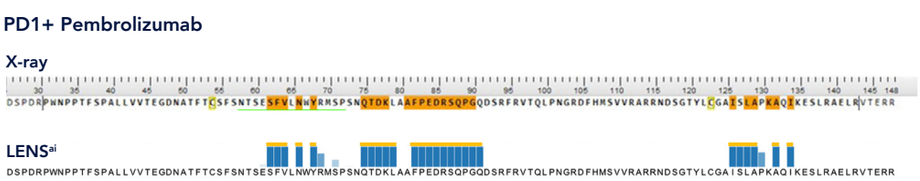
Traditional epitope mapping methods, such as x-ray crystallography and mass spectrometry, are time consuming, costly, and with feasibility that is highly dependent on the target type. Such challenges combined with the low-throughput nature of these technologies limit the utility of these methods to late-stage lead characterization or supporting IP filings. In contrast, *in silico* epitope mapping, which only requires sequences alone and is not reliant on physical material, offers a fast, high-throughput alternative that can be integrated earlier into the workflow, enabling more informed decisions and reducing risk. This case study examines the performance of LENS^{ai} *in silico* Epitope Mapping to demonstrate its potential for improving efficiency and confidence in the discovery process.

Background: a head-to-head comparison with x-ray crystallography and 6 additional technologies

The accuracy of LENS^{ai} Epitope Mapping is compared with x-ray crystallography, considered the gold standard, and six other methods (peptide array, alanine scan, domain exchange, hydrogen-deuterium exchange, chemical cross-linking, and hydroxyl radical footprinting) for epitope identification in five antibody-antigen combinations: Pembrolizumab+PD1, Nivolumab+PD1, Ipilimumab+CTLA4, Tremelimumab+CTLA4, and MK-5890+CD27.

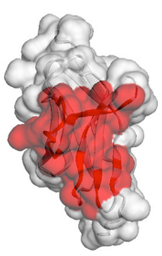
LENS^{ai} matched x-ray crystallography with exceptional accuracy

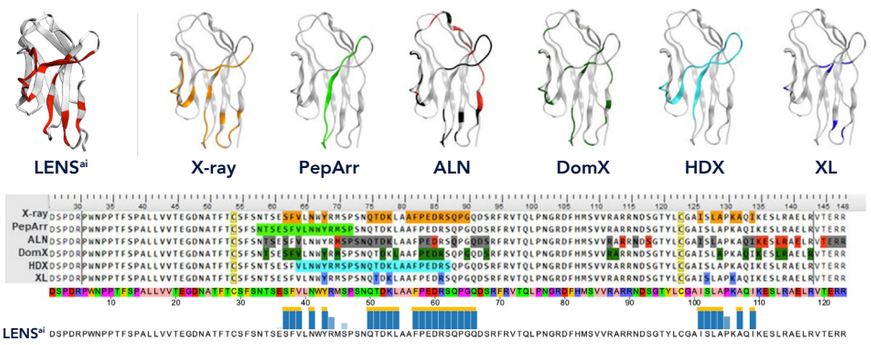




PD1 + Pembrolizumab

LENS^{ai} AUC = 0.80





AUC = 1 means a perfect prediction. AUC = 0.5 means no better than random guessing.

- **3D target model:** intense red indicates epitope
- **Blue bars:** height indicates the confidence score
- **Orange line:** indicates hitting the epitope threshold

LENS^{ai} Epitope Mapping assigns a confidence score (0–1) to each amino acid (AA) in the target. Residues with scores above a set threshold (orange line) are classified as part of the predicted epitope. LENS^{ai} confidence scores are visualized as blue bars. The model's ability to distinguish epitopes from non-epitopes is measured by the AUC (Area Under the Curve), which plots the True Positive Rate against the False Positive Rate.

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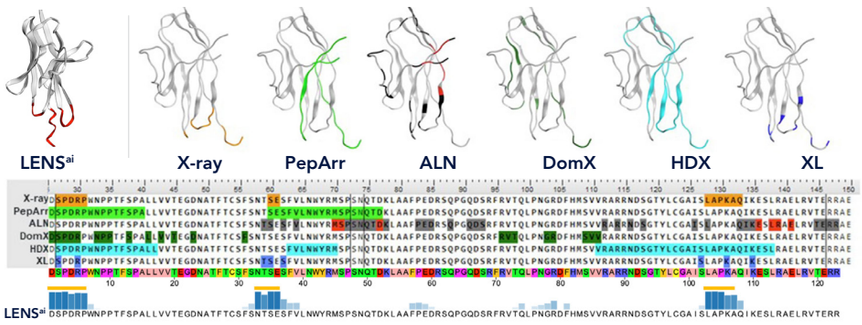
The benchmark comparison:

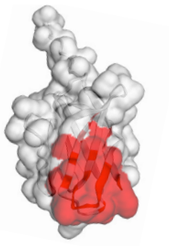
PD1 + Nivolumab

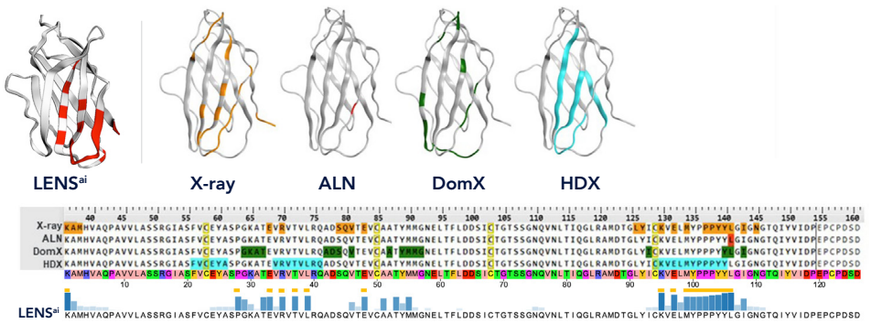
LENS^{ai} AUC = 0.79



CTLA4 + Tremelimumab

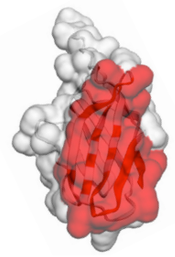
LENS^{ai} AUC = 0.83

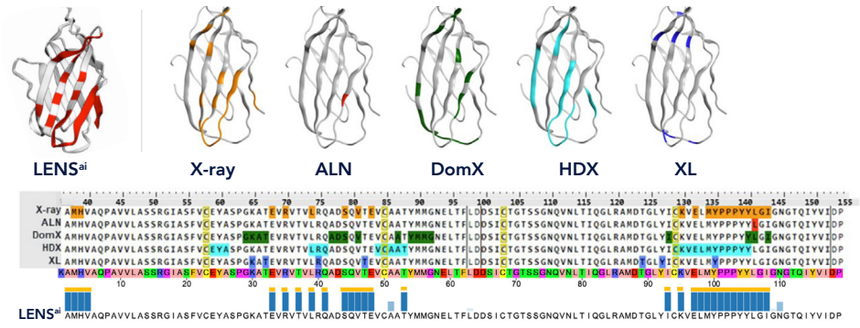




CTLA4 + Ipilimumab

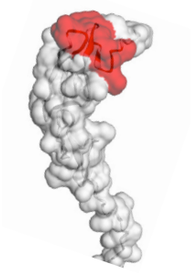
LENS^{ai} AUC = 0.84

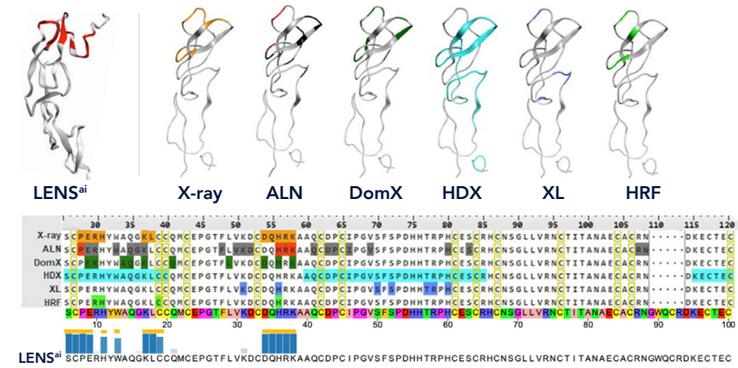




CD27 + MK-5890

LENS^{ai} AUC = 0.89





Method:

The epitope identified by x-ray crystallography is set as ground truth. The following standard metrics are used to quantify epitope prediction accuracy of the different methods:

The True Positive Rate:*

$$TPR = \frac{TP}{(TP+FN)}$$

measures the proportion of residues being part of the true epitope that are correctly identified

The False Positive Rate:

$$FPR = \frac{FP}{(FP+TN)}$$

is the proportion of residues not being part of the true epitope that are incorrectly predicted as part of it

Precision:

$$TP = \frac{TP}{(TP+FP)}$$

measures how many of the residues predicted to be part of the epitope are correctly predicted

*(Recall or Sensitivity)

AUC (Area Under the Curve) plots the True Positive Rate against the False Positive Rate. Measures the ability to distinguish epitopes from non-epitopes.
AUC = 1 means perfect prediction. AUC = 0.5 means no better than random guessing.

Implementation:

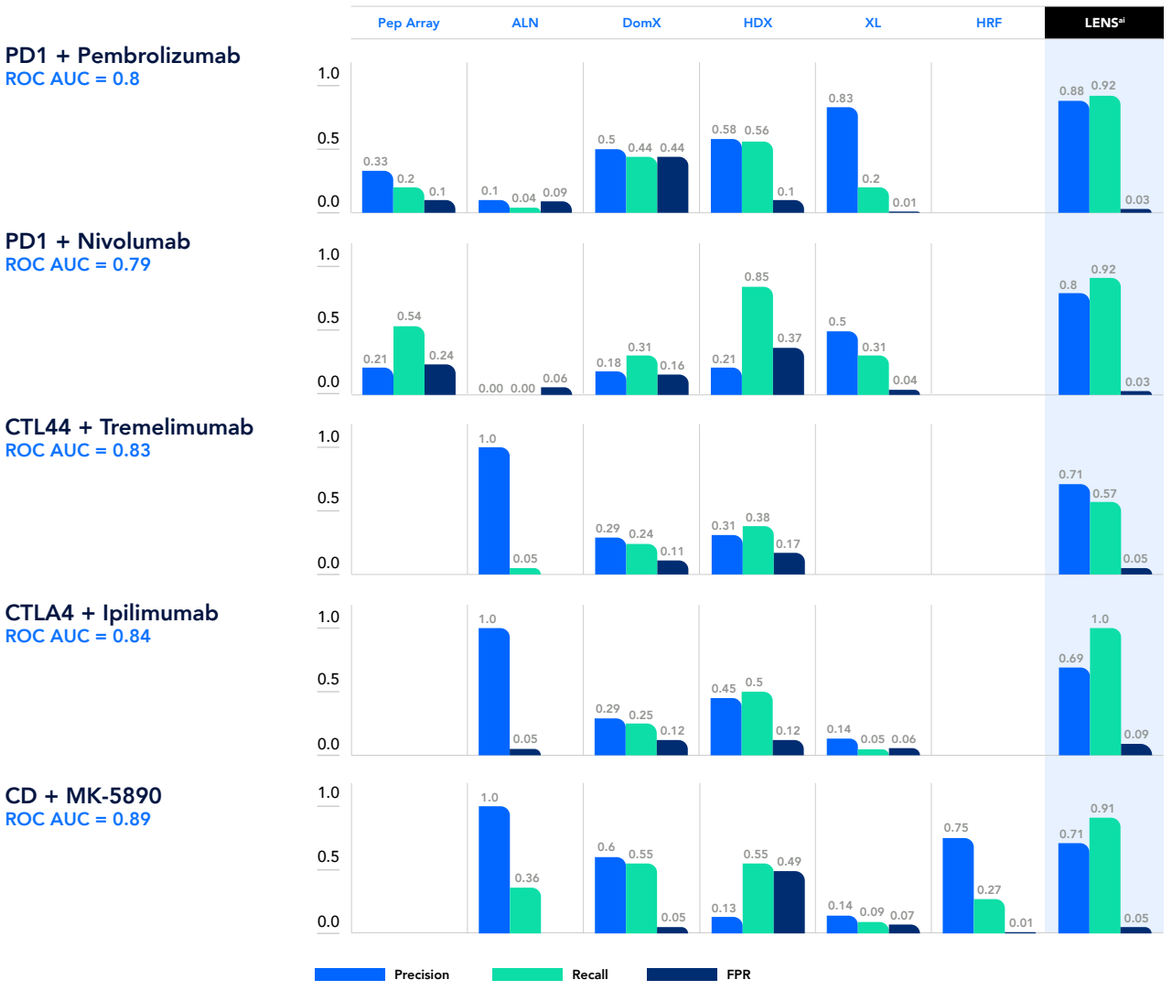
LENS[ai] Epitope Mapping overcomes the limitations of traditional methods—delivering early-stage, high-throughput predictions from sequence alone, with greater speed, scalability, and flexibility across target types.

	LENS[ai]	X-ray Crystallography	Pep Array	ALN	DomX	HDX	XL	HRF
Input	✓ Sequences	Physical Material	Physical Material	Physical Material	Physical Material	Physical Material	Physical Material	Physical Material
Workflow Stage	✓ Flexible – all stages	Late	Early-Mid	Late	Mid	Late	Late	Late
Timeline	✓ Hours/Days	Months	Weeks	Weeks	Weeks	Weeks	Weeks	Weeks
High-Throughput	✓ Yes	No	Yes	No	Partial	Partial	Partial	No
Target Types	✓ Most	Limited	Limited	Limited	Limited	Limited	Limited	Limited

Comparison:

LENS[ai] clearly outperforms all wet-lab based methods and shows a near x-ray crystallography performance. LENS[ai] can accurately identify true epitope residues (high recall or sensitivity) while minimizing wrong predictions (high precision and low FPR).

A well-performing classifier for epitope mapping should accurately identify true epitope residues (high recall) while minimizing wrong predictions (high precision and low FPR).



Conclusion:

LENS[ai]™ Epitope Mapping was evaluated against various wet-lab methods, using epitopes determined by x-ray crystallography as the ground truth. LENS[ai] demonstrated superior performance, achieving near x-ray accuracy. It consistently identified true epitope residues with high sensitivity while maintaining high precision and minimizing false positive rates. Unlike traditional methods, LENS[ai] requires only the target and antibody sequences as input and delivers results within hours, enabling high-throughput application. By providing accurate epitope mapping early in the discovery and development workflow, LENS[ai] accelerates decision-making and reduces risk.

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