From Cofolding to FEP: Unveiling the Path to Absolute Antibody Affinities

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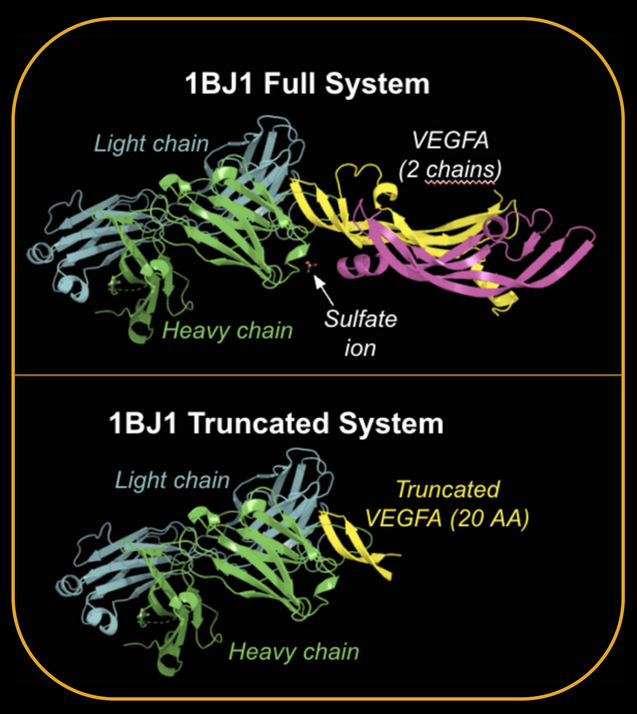
Pushing the Limits of Free Energy Calculations in Antibody Design

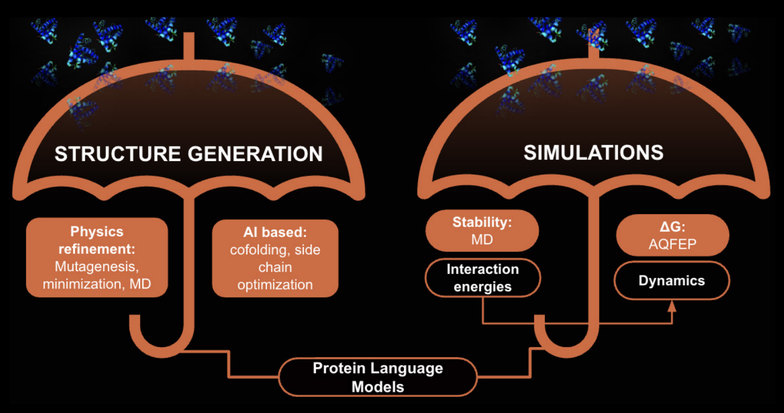
- The rapid evolution and structural diversity of antibody variable regions create profound challenges for accurate structure prediction and binding affinity estimation.
- Capturing the flexibility and conformational heterogeneity of CDR loops remains a major obstacle for current AI-based modeling approaches.
- Traditional free energy perturbation methods struggle to converge when applied to the large, dynamic configurational spaces characteristic of antibody-antigen systems.
- To address these gaps, we introduce AQFEP, a workflow that combines AI-driven structure prediction, deep learning-guided side-chain refinement, and enhanced alchemical sampling.
- This integrated strategy aims to enable robust affinity predictions and accelerate computational antibody design.

1BJ1 Fab Benchmark for Workflow Validation



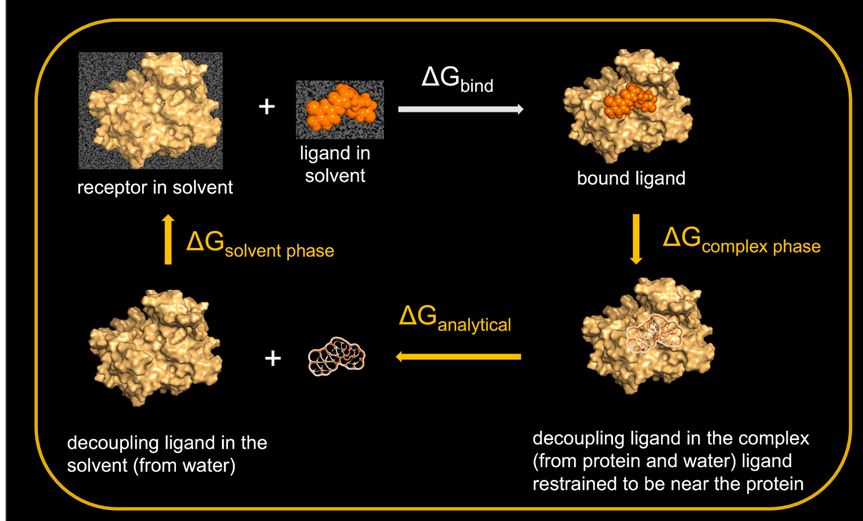
- The 1BJ1 Fab-antigen complex was selected as a representative Ab-Ag system based on its well-characterized structure and canonical binding interface.
- A benchmark dataset of 23 mutation combinations, including single- and multi-point variants relative to the wild-type, was generated for validation.
- Antigen truncation strategies (20, 50, full residues; continuous polymers vs restrained fragments) were tested, and their effects on convergence and accuracy were evaluated.
- Crystal and AI-generated models were refined through side-chain repacking and MD simulations, with unstable poses filtered prior to AQFEP setup.
- Absolute binding affinities were predicted using AQFEP with enhanced sampling, and triplicate runs confirmed reproducibility and consistency across models.

Optimizing Biologics FEP from Structure to Affinity



- Physics-based modeling generates complexes via mutagenesis, minimization, and local refinement.
- AI-based folding predicts antibody-antigen complexes to expand structural diversity.
- Deep learning-guided side-chain refinement performs mutagenesis and repacking to optimize binding site complementarity.
- MD simulations assess stability and filter out unstable candidates.
- AQFEP employs enhanced alchemical sampling with additional parameters to improve phase space overlap and ensure robust absolute affinity predictions.

AQFEP for Ab-Ag Absolute Binding Affinity



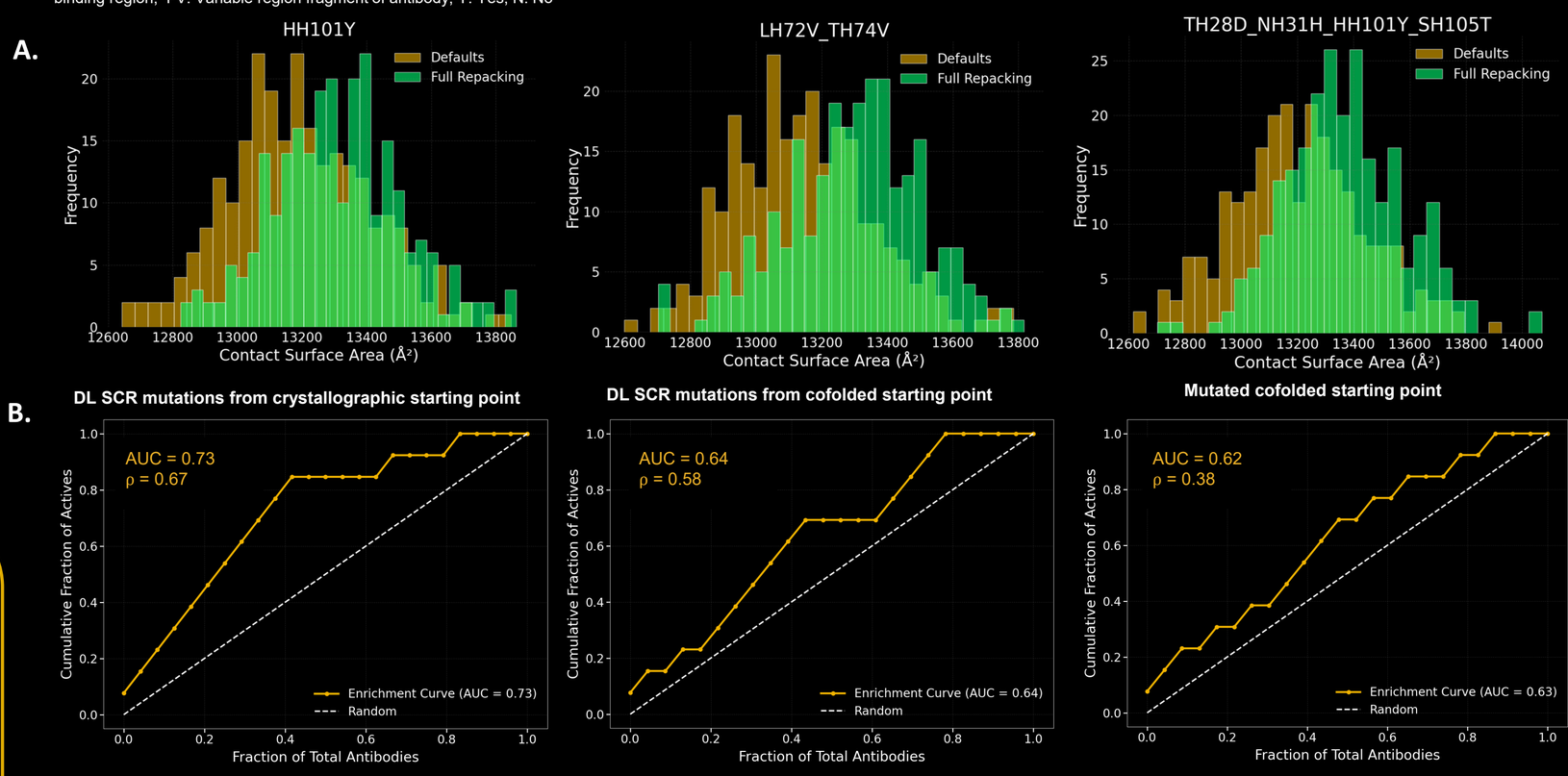
- AQFEP accelerates absolute binding free energy prediction by using a double-decoupling alchemical protocol with optimized short simulation times for high-throughput screening.
- Additional alchemical sampling and careful control of lambda windows improve phase space overlap while maintaining computational efficiency.
- The method leverages the quality of input poses and convergence checks via MBAR to deliver robust affinity estimates significantly faster than traditional AFEP approaches.

Deep Learning Boosts Accuracy for Wild-Type and AI Models

	Starting	Energy Minimized	Repacked	Antibody	Mutation Engine	Spearman correlation, ρ
1	X-ray	N	Y	FAB	DL SCR	0.67
2	AQC	N	Y	FV	DL SCR	0.58
3	AQC	N	Y	FAB	DL SCR	0.44
4	X-ray	N	N	FAB	DL SCR	0.43
5	AQC	N	Y	FV	AQC	0.38
6	AQC	Y	Y	FV	AQC	0.31
7	AQC	N	N	FV	AQC	0.26
8	AQC	Y	N	FV	AQC	0.16
9	AQC	Y	N	FV	DL SCR	0.15
10	AQC	Y	Y	FV	DL SCR	-0.34

Abbreviations:
AQC: AI-based Cofolding Algorithm, DL SCR: Deep Learning Side-Chain Refinement; Fab: Fragment antigen-binding region; FV: Variable region fragment of antibody; Y: Yes; N: No

- Deep learning–based side-chain refinement (DL SCR) outperformed cofolding-only models, achieving Spearman correlations up to **0.67**.
- Repacking significantly improved prediction accuracy; non-repacked structures showed lower correlations.
- Energy minimization alone without repacking led to degraded performance (ρ = 0.16 to – 0.34).
- Triplicate AQFEP runs achieved **>90% convergence**, confirming differences arose from structure preparation.



A. Contact surface area comparison between default and deep learning-repacked structures for three of the 23 1BJ1 variants. Deep learning side-chain refinement increases surface area and improves binding.

B. Enrichment plots across WT and 23 1BJ1 variants after deep learning side-chain refinement for both X-ray and cofolded starting structures. Strongest recovery of experimental actives is observed when X-ray structures are used as the starting point.

Conclusions and Future Directions

- A scalable workflow combining structure generation, deep learning side-chain refinement, and AQFEP enables robust absolute affinity prediction for antibody-antigen systems.
- Deep learning refinement expanded binding site surface area and improved predictive accuracy across crystallographic and AI-predicted structures.
- Validation on the 1BJ1 system (23 variants) demonstrated strong reproducibility (>90% convergence) and superior recovery of experimental actives.
- This approach accelerates computational antibody screening, reducing reliance on animal-based affinity maturation.
- Future work will integrate protein language models to guide sequence design and extend predictive screening across broader antibody libraries.

References

1. *Crivelli-Decker, J. E., et al. (2023). Machine Learning Guided AQFEP: A Fast & Efficient Absolute Free Energy Perturbation Solution for Virtual Screening. J. Chem. Theory Comput., 20 (16), 7188-7198*
2. *Jankauskaitė, J., et al. (2019) SKEMPI 2.0: an updated benchmark of changes in protein–protein binding energy, kinetics and thermodynamics upon mutation. Bioinformatics 35, 462–469*