Computational model for small molecule TNFα inhibitors informed by structural proteomics

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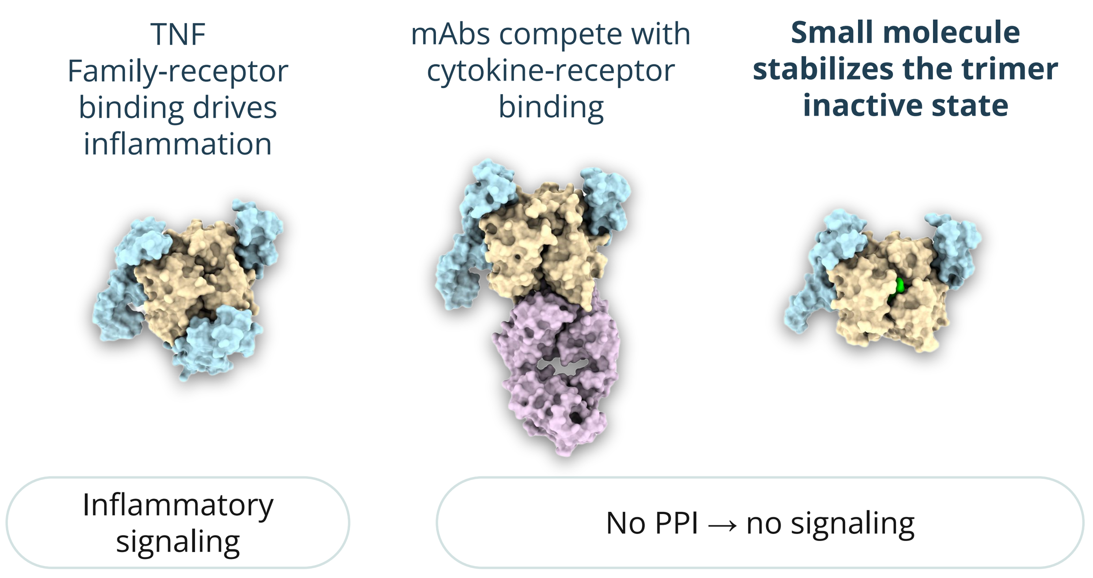
Background

Biologic like effect in a pill - via asymmetric stabilization



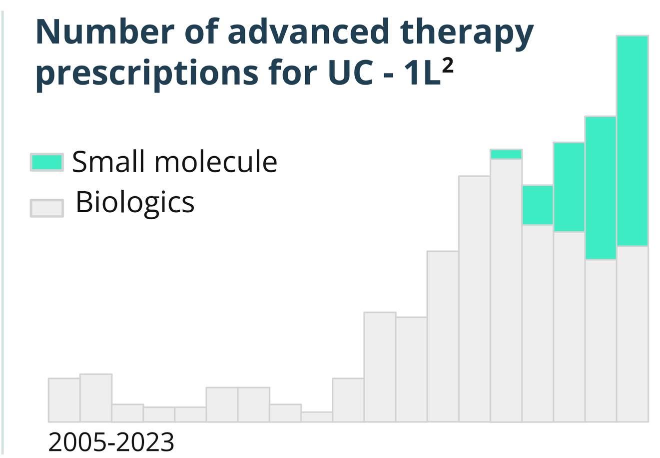
Small molecules are the patient's choice

Oral | Lower COGs | No Immunogenicity | Brain penetrant | No cold storage

75%

of patients on injectables would switch to an oral with similar safety & efficacy

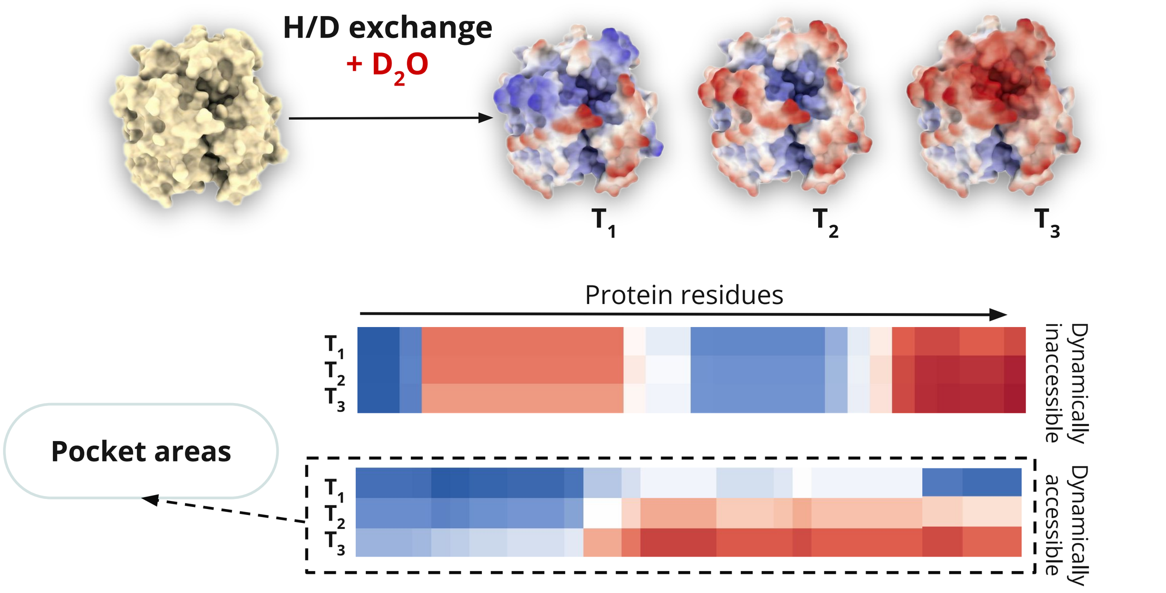
— J&J business review Dec 2023;
2 Elford, A.T. et al. (2025)



- Tumor Necrosis Factor alpha (TNFα) is a central cytokine in inflammation, immune regulation, and cell death. Dysregulated TNFα signaling underlies a broad range of autoimmune, inflammatory, and oncologic diseases.
- All approved TNFα inhibitors are monoclonal antibodies that block ligand receptor interactions. While clinically effective, therapeutic antibodies are limited to injections, high production costs, immunogenicity risks, and poor penetration into certain tissues, including the central nervous system.
- Small molecule drugs could overcome these challenges by enabling oral administration, expanding indication space, and reducing immunogenicity. However, development of small molecule TNFα inhibitors has been challenging due to lack of obvious druggable pockets in static structures, as TNFα functions as an oligomer with dynamic, hidden allosteric sites.
- To address this challenge, we applied the AIMS™ platform, an integrative approach combining structural proteomics and computational sciences. Here, molecular dynamics enhanced sampling simulations, pocket detection algorithms, and Hydrogen Deuterium Exchange Mass Spectrometry (HDX-MS) were used.

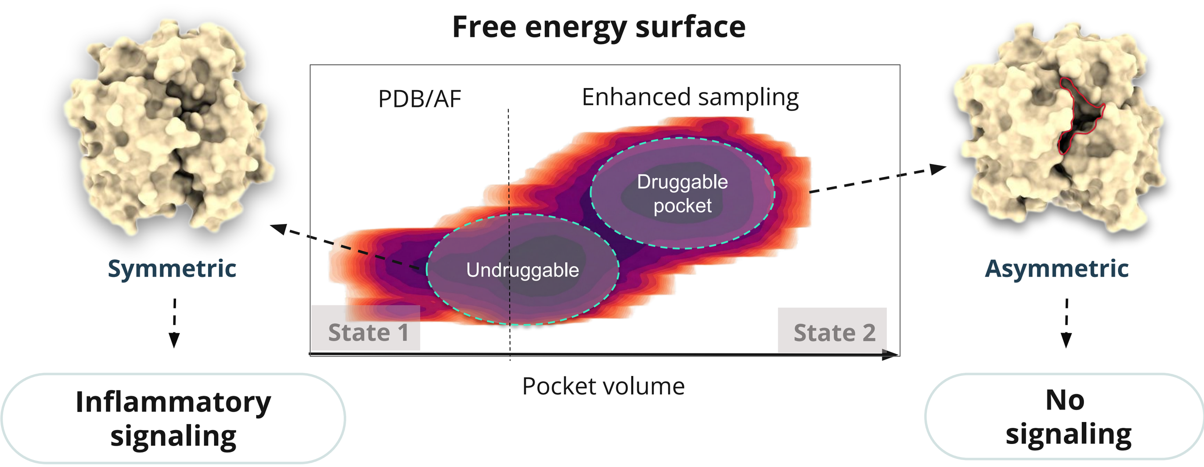
Retrospective validation - TNFα

HDX-MS identifies transient pocket



Apo HDX-MS reveals time-dependent deuterium uptake in specific regions, identifying dynamically accessible pockets in the protein.

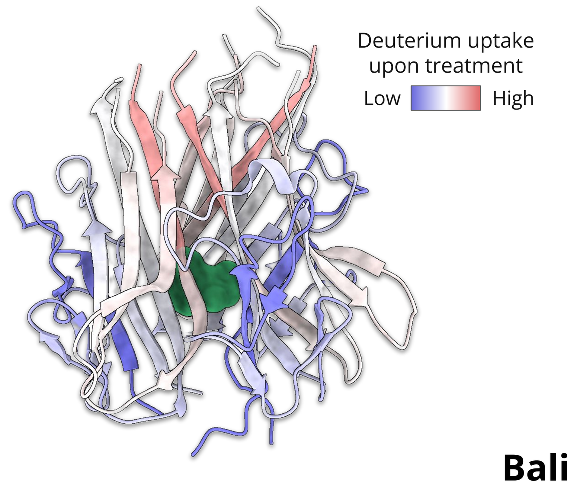
Metadynamics simulation validates druggable pocket



Metadynamics, performed by biasing the system along key collective variables, allows escape from local free-energy minima and enhances the sampling of otherwise rare conformations.

HDX-MS validates small molecule binding

Compound-induced asymmetric state validation using HDX-MS

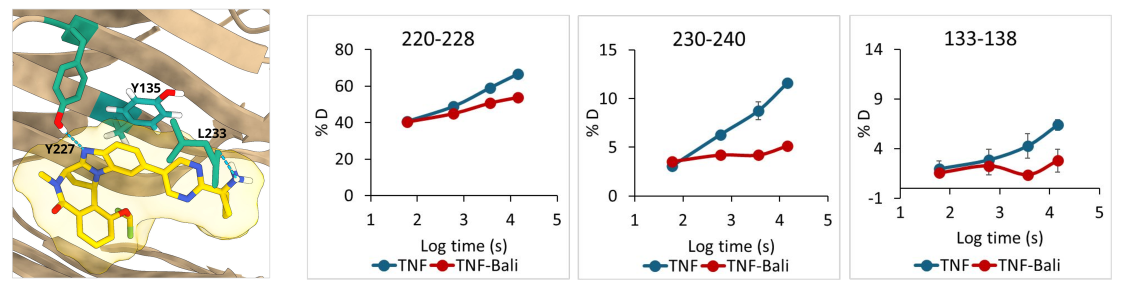


HDX-MS analysis of the apo and holo protein shows that asymmetric binding exposes the upper surface of the trimer, leading to increased deuterium uptake.

The compound allosterically stabilizes the asymmetric trimer, as evidenced by extensive regions of reduced deuterium uptake on the lower surface.

Balinatunfib binding mode determination

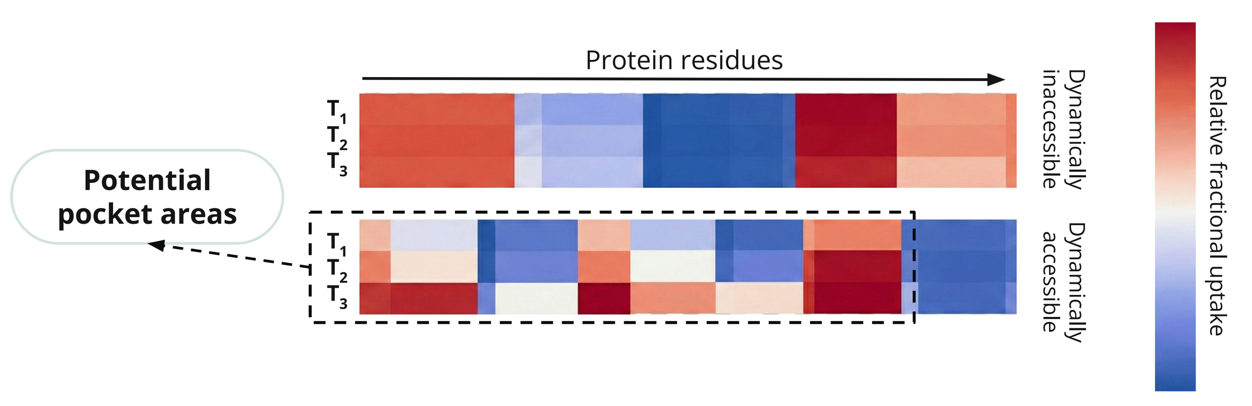
Peptides harboring known compound binding residues[2,3] display significant protection patterns.



Prospective discovery - TNF Family member

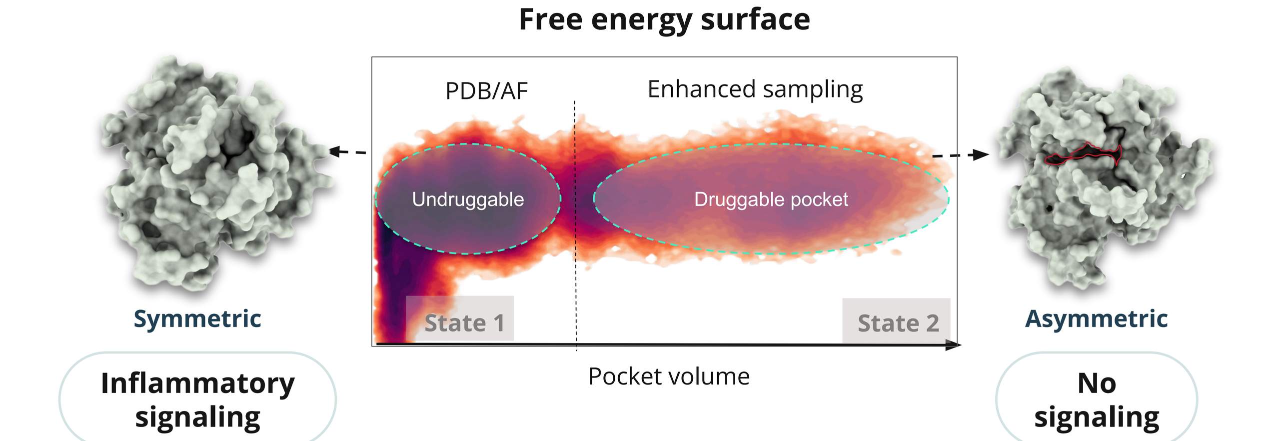
AIMS-Fold identifies novel druggable pocket

HDX-MS of family member identifies novel pocket



Apo state HDX-MS data reveal regions of dynamic deuterium uptake over time, indicating several potential novel pockets in the protein.

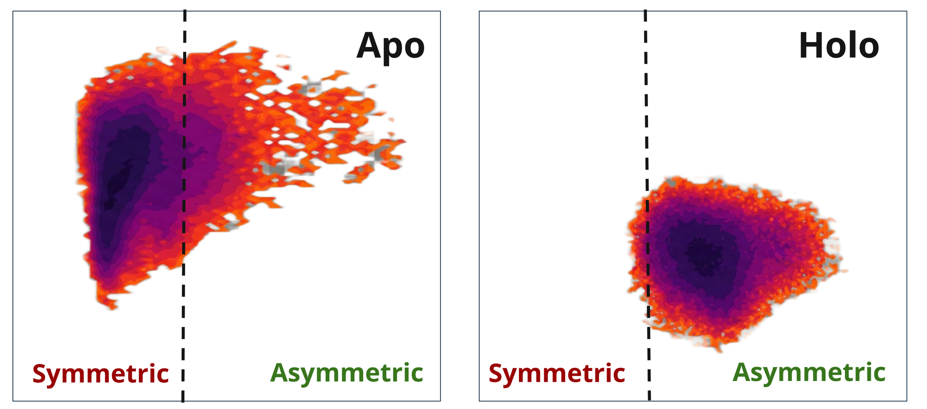
Metadynamics simulation validates druggable pocket



Metadynamics simulation confirms the presence of a transient, accessible pocket in the asymmetric state, critical for ligand binding.

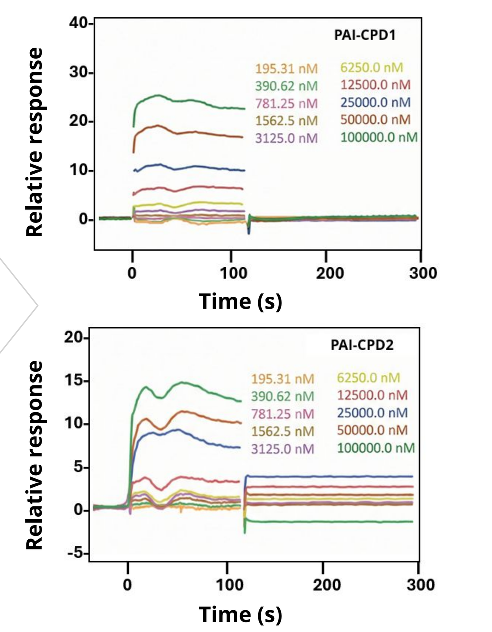
AIMS-Rank identifies novel hits

Computational ranking of asymmetric stabilizers

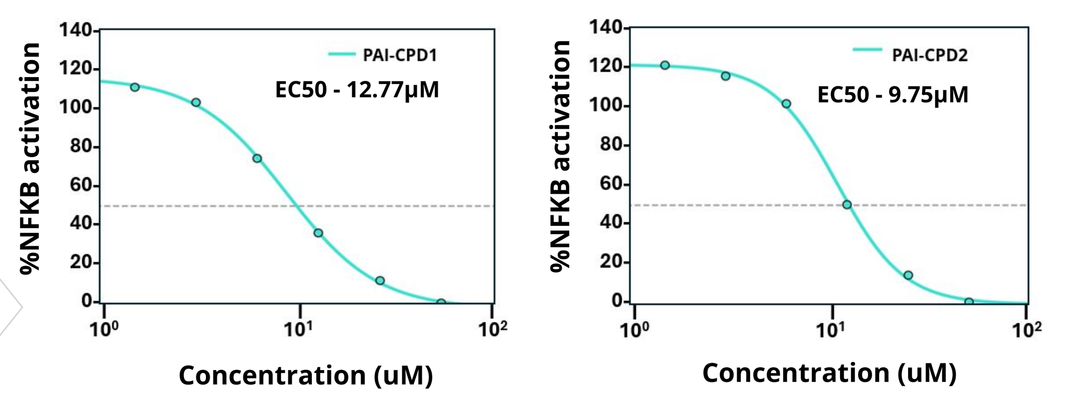


MD based virtual screening uncovers novel compounds that stabilize the asymmetric state.

SPR identifies binding hits



Hits modulate NF-κB activation in a dose-dependent manner



Selected hits were tested in an NF-κB reporter assay. The Y-axis shows %NF-κB activation relative to control across increasing compound concentrations.

Summary

Here, we demonstrate the use of the **AIMS™** platform, powered by HDX-MS and AI for the study of allosteric PPI inhibitors.

HDX-MS & simulations identify and validate a transient asymmetric pocket, providing a foundation for computer aided drug design of small-molecule alternative to antibody based therapies for TNF family members targets.

This approach enabled the rapid discovery of several **validated hits**, currently in hit-to-lead stage.

Acknowledgements

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References

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