High-Resolution HDX-MS Epitope Mapping: Characterizing the Nest1–CD47 Immune Checkpoint Interface

Marin-Lopez, Alejandro, et al. Science immunology 9.98 (2024). DOI: 10.1126/sciimmunol.adk9872

Our role: Creative Proteomics leveraged high-resolution HDX-MS to provide critical structural evidence that supported the mechanistic conclusions of the published study.

Executive Summary

Vector-borne pathogens exploit host immunity through protein–protein interactions (PPIs). In this case study, Nest1, a mosquito salivary factor, binds the human checkpoint receptor CD47 with high affinity and competes with SIRPα, suppressing cutaneous antiviral responses and enhancing Zika virus infectivity in human skin explants.

Creative Proteomics Hydrogen–Deuterium Exchange Mass Spectrometry (HDX-MS) provided localized, site-resolved interaction footprints and conformational changes that strengthened the mechanistic model by pinpointing buried regions on Nest1 and CD47 upon complex formation.

The Challenge

When a novel PPI is discovered (e.g., receptor–ligand or pathogen/vector factor–host target), teams typically need to answer:

- Where exactly is the binding interface? (epitope/paratope regions)
- Does binding induce conformational change or allostery?
- How does the interface relate to known competing ligands or therapeutic sites?
- Can we rapidly generate actionable structural hypotheses to guide mutagenesis, blocking strategies, or lead discovery?

Traditional structural methods can be slow or difficult for certain complexes. HDX-MS provides a fast, experimentally grounded route to map interfaces and dynamics in solution, providing structural context for downstream functional observations reported in the study.

Our Contribution (Creative Proteomics)

HDX-MS for Protein Interaction Interface Mapping

Creative Proteomics performed HDX-MS under controlled conditions to compare:

1. CD47 alone
2. Nest1 alone
3. CD47–Nest1 complex

What HDX-MS delivered in this project

- Protected (buried) segments upon complex formation, indicating binding surfaces
- Regions with increased exchange, supporting conformational rearrangements upon binding
- A solution-phase interaction map that complements screening and biophysical binding assays

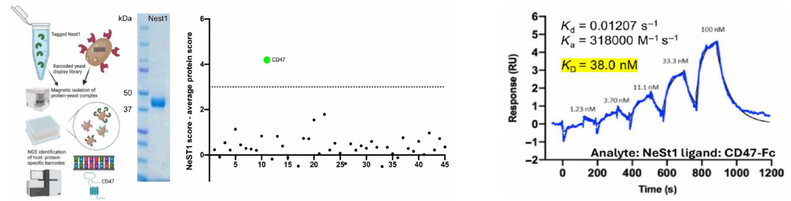
For Research Use Only. Not for use in diagnostic procedures.

Case Study Highlights

PPI Discover → Validate → Compete

A high-throughput extracellular interaction screen identified CD47 as the dominant Nest1-binding human target. Orthogonal assays confirmed a direct complex with nanomolar affinity (SPR Kd ~ 38 nM). Competition with CV-1 blocked binding, consistent with overlap near the SIRPα interface on CD47.

Value: Rapidly establishes a checkpoint-relevant PPI with actionable competition logic.

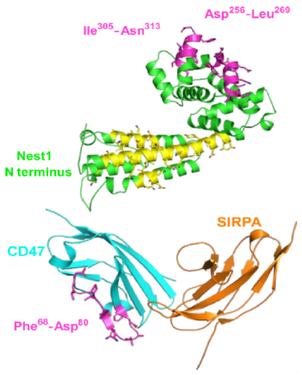


CD47 is the top Nest1-binding human receptor; SPR confirms nanomolar affinity (Kd ~ 38 nM).

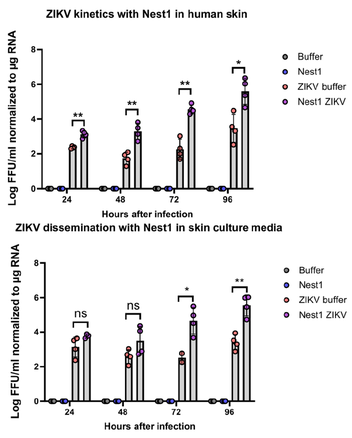
HDX-MS: Interface + Dynamics

HDX-MS comparing CD47, Nest1, and the complex revealed protected segments on both proteins upon binding (interface footprint) and increased exchange in specific Nest1 regions (binding-associated conformational change).

Value: Transforms binary 'Yes/No' binding data into structurally informed, site-resolved mechanistic hypotheses.



HDX-MS maps the Nest1–CD47 binding footprint and binding-associated conformational dynamics in solution.



Nest1 increases ZIKV replication in human skin explants (tissue and supernatant).

Biology: Immune Suppression → Higher Viral Replication

Nest1 reduced macrophage phagocytosis and dampened proinflammatory/IFN-linked responses. In human skin explants, Nest1 increased ZIKV replication kinetics, and RNA-seq supported suppression of innate antiviral programs in Nest1-treated conditions.

Value: Links molecular interaction → immune function → tissue-level infectivity.

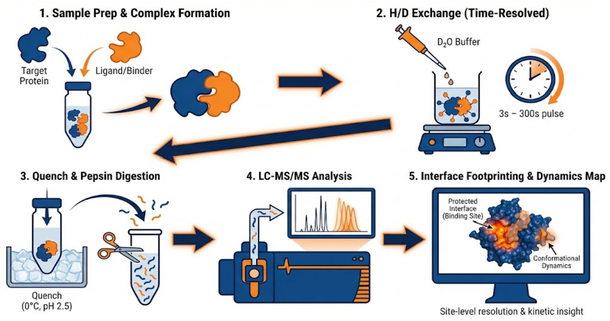
What Clients Can Achieve with Creative Proteomics HDX-MS

Use cases

- Epitope Mapping: For antibody–antigen, receptor–ligand, and protein–peptide interactions.
- Mechanism-of-Action (MoA): Assessing conformational dynamics and allostery.
- Rapid Lead Discovery: Guiding structure-based design and mutagenesis workflows.
- Competitive Interpretation: Distinguishing overlapping vs. allosteric binding sites.

Outputs

- Peptide-level deuterium uptake tables and differential plots
- Protected/exposed region mapping (interface footprints and dynamic regions)
- Clear experimental evidence to support mechanistic models and publication-quality figures



Why Choose Creative Proteomics

- Deep Coverage: Typically >90% sequence coverage for soluble domains under optimized experimental conditions.
- Time-Resolved: 3–300 s labeling captures both stable binding and fast dynamics.
- Low-Input Ready: Optimized for challenging targets, including membrane proteins (e.g., CD47).
- Actionable Deliverables: QC-backed, publication-ready interface and dynamics maps.

Need to localize a binding interface fast?

Creative Proteomics HDX-MS provides solution-phase, mechanism-ready interaction maps to accelerate discovery from hit validation → epitope mapping → actionable design.

Web: www.creative-proteomics.com

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