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Validated determination of a protein structure by HR-HRPF combined with computational modeling

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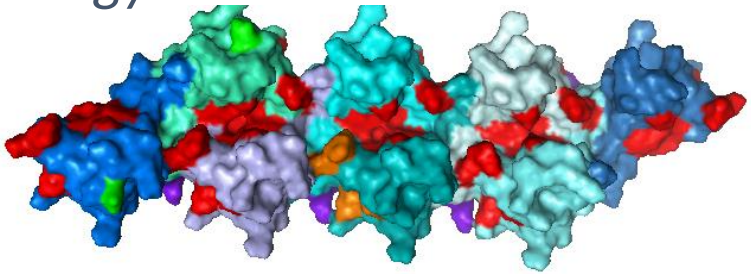
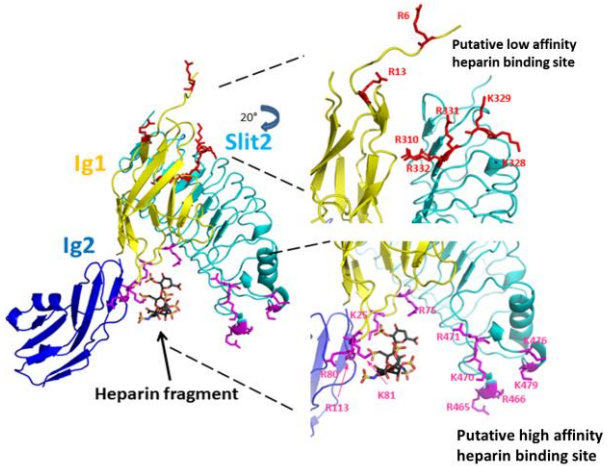
FCOI Statement: J.S.S. discloses a significant financial interest in GenNext Technologies, Inc., a growth-stage company seeking to commercialize benchtop HRPF to support the pharmaceutical industry

The Sharp Group

Developing new tools to address challenging problems in structural biology



Wang X. *et al* (2011) *Structure* **19:** 1138
Wang X. *et al.* (2013) *Progress in Molecular Biology and Translational Science*



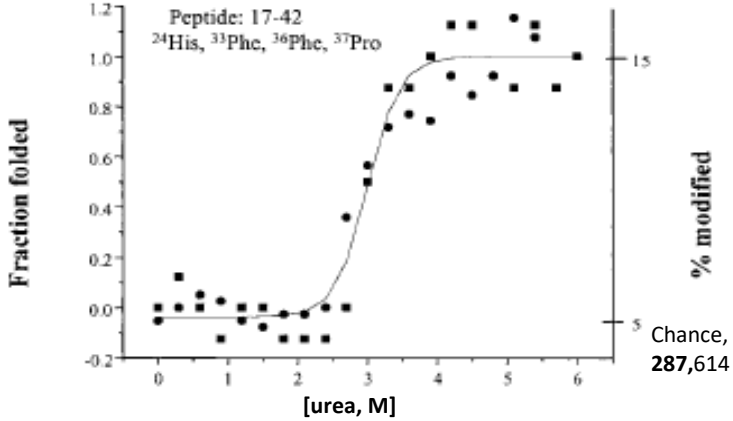
Li Z. *et al* (2015) *J Biol Chem* **290:** 10729
Zong C. *et al.* (2016) *J Am Chem Soc* **138:** 13059

- Structural biology is the backbone of our current molecular understanding of biology
- Numerous systems are highly challenging to study using traditional structural biology techniques
  - Dynamic systems
  - Heterogeneous systems
  - Large systems
  - Limited sample amounts
- Protein-carbohydrate complexes often fit into several of these categories simultaneously
  - Flexible
  - Dynamic oligomerization
  - Heterogeneous protein and/or carbohydrate ligand
  - Very large complexes
- The Sharp Lab focuses on developing and applying new tools to address these challenges in structural biology

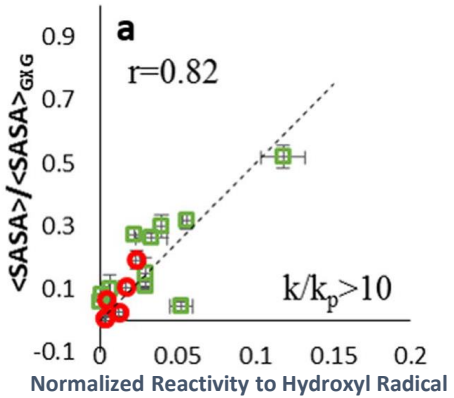
Foundation of Hydroxyl Radical Protein Footprinting

Why does it work?

- Rate of oxidation appears to primarily be a function of two factors
  - Chemical nature of oxidized residue
  - Average accessibility of oxidation target to the hydroxyl radical over the time of radical exposure
- General inherent reaction rates: sulfur-containing > aromatic > aliphatic > charged > polar > Ala > Gly
- Sequence context can influence inherent reactivity, especially for less reactive amino acids
- Changes in reaction rates in the same sequence reflects changes in average solvent accessibility

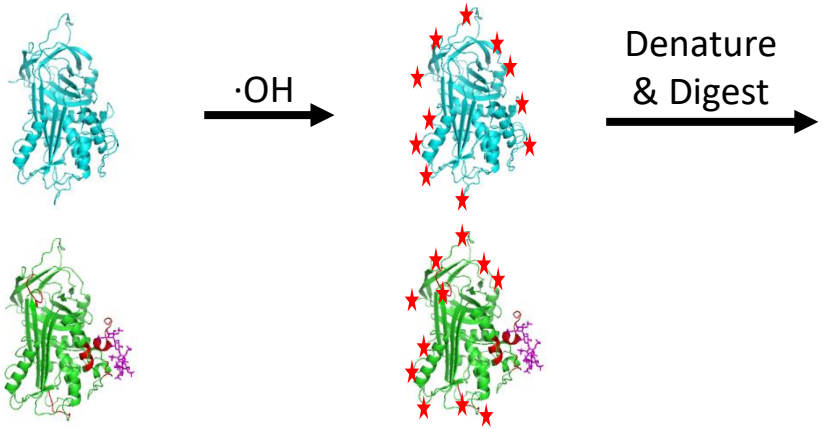


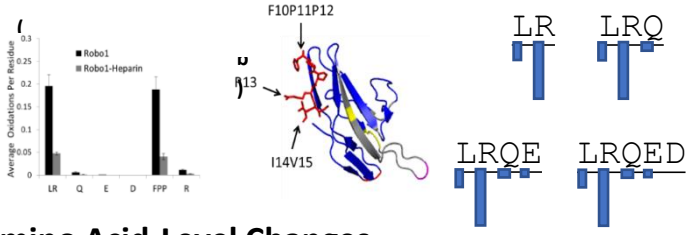
Chance, *Biochem Biophys Res Commun* **287,**614 (2001)



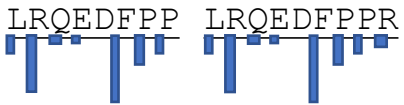
Xie *et al*, *Sci Rep* **7,**4552 (2017)

General HRPF Workflow

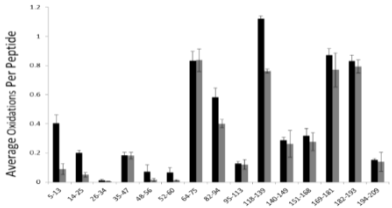




Amino Acid-Level Changes
Structural Models

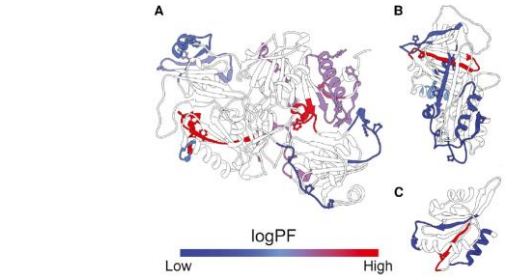


LC-MS

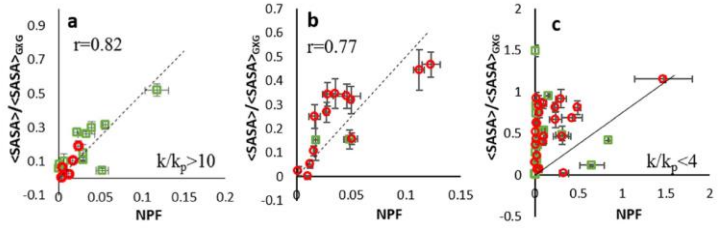


Peptide-Level Changes

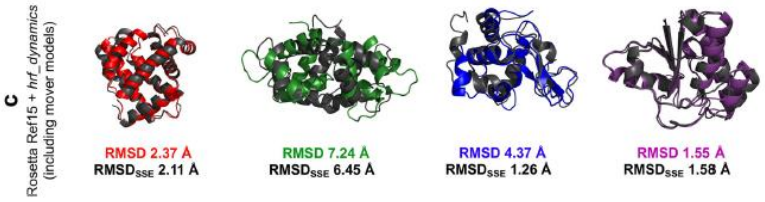
Protein Structure Determination by HR-HRPF: Highlights



Huang *et al* (2015), *Biophys J* **108:** 107; Kaur *et al* (2015), *Mol Cell Prot* **14:** 1159



Xie *et al* (2017), *Sci Rep* **7:** 4552



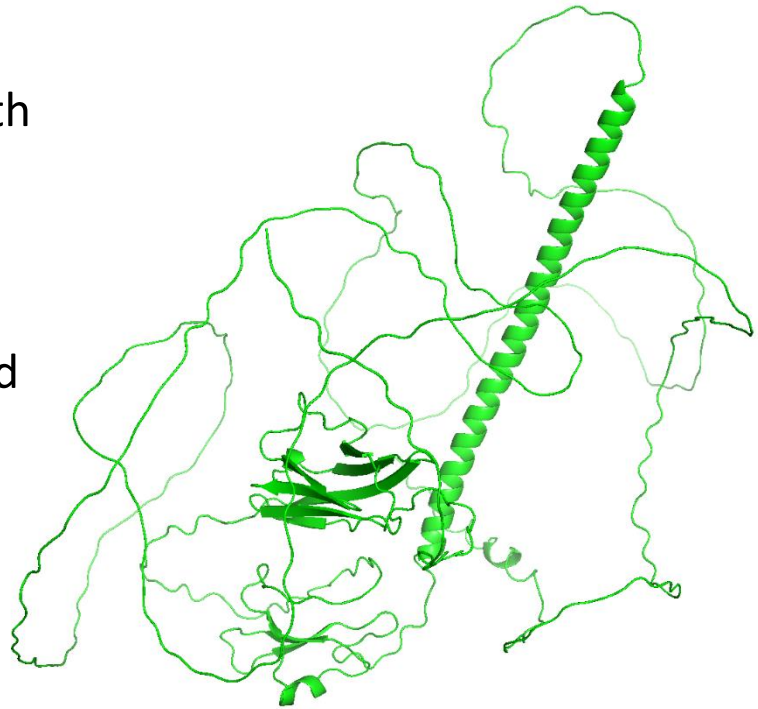
Aprahamian *et al* (2018), *Anal Chem* **90:** 7721; Biehn and Lindert (2021), *Nat Commun* **12:** 341

- Definition of protection factor (apparent oxidation rate normalized by the free amino acid reactivity with ·OH); correlation of protection factor with structural contacts and fractional SASA; demonstrated on proteins of known structure
- Observation that normalization by free amino acid reactivity is more accurate for the more reactive amino acids; development of denatured:native comparisons for determination of inherent reactivity rates; generation of empirical conversion factors to determine structure based on SASA; demonstrated ability to use SASA to select accurate homology models; examined MD simulations to include flexibility; demonstrated on proteins of known structure
- Found that most reactive amino acids (W,Y,F,H,L) gave most robust results; incorporated dynamics to improve predictions; used optimized conical neighbor count for cheap and robust correlations with HR-HRPF data; demonstrated on proteins of known structure

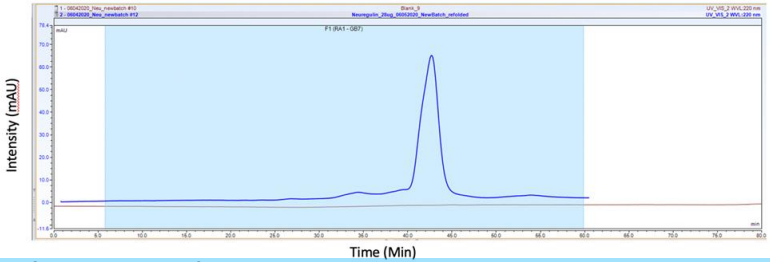
Immunoglobulin-like domain of human neuregulin 1

NRG1-Ig

- NRG1 is a signaling glycoprotein that interacts with tyrosine kinases
- Plays a key role in neuronal and cardiac development, regulation of synaptic plasticity
- Implicated in diseases including schizophrenia and some forms of cancer
- Many isoforms, including both soluble and membrane-bound
- Ig-like domain of NRG1 binds to heparan sulfate proteoglycans in the extracellular matrix
- ***Structure is currently unknown, and no near homologs of known structure***



AlphaFold model of full-length hNRG1
https://alphafold.ebi.ac.uk/entry/Q02297



Heterologous expression and purification of NRG-Ig

Sharp and Lindert Groups

HR-HRPF by multi-dose FPOP and LC-ETD MS/MS

Development and scoring of models by Rosetta with *hrf_dynamics* and mover dynamics

Prestegard Group

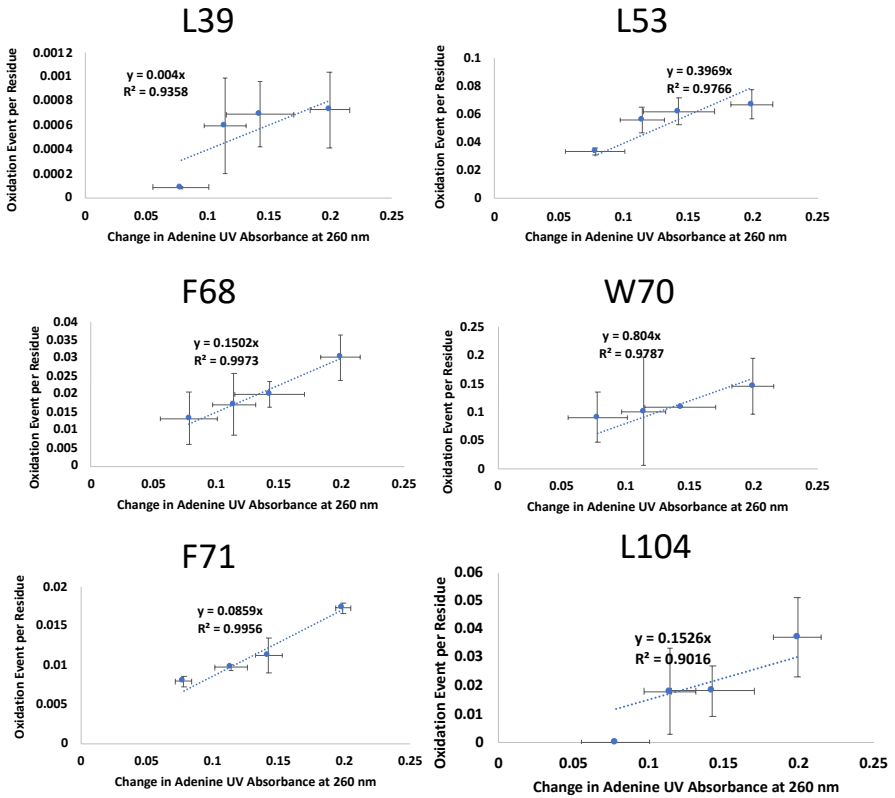
Multidimensional heteronuclear NMR

Resonance assignment and solution structure by NOE data

Comparison of independent models

Multi-Dose FPOP for HR-HRPF analysis

Measuring reactivity by FPOP



- HR-HRPF oxidation was measured at four different effective radical doses
- Reactivity measured by the slope of the regression of these data (b = 0)
- Reactivity measured for 20 amino acids in 118 amino acid construct
- R^2 of regression > 0.9 for all amino acids measured (mean R^2 = 0.971)
- Six amino acids used for *hrf_dynamics* scoring (W,Y,F,H,L)

Rosetta with *hrf_dynamics* and mover

Calculated natural log of the protection factor (lnPF) using established relative intrinsic reactivity values and $slope_N$ values from Sharp group

↓

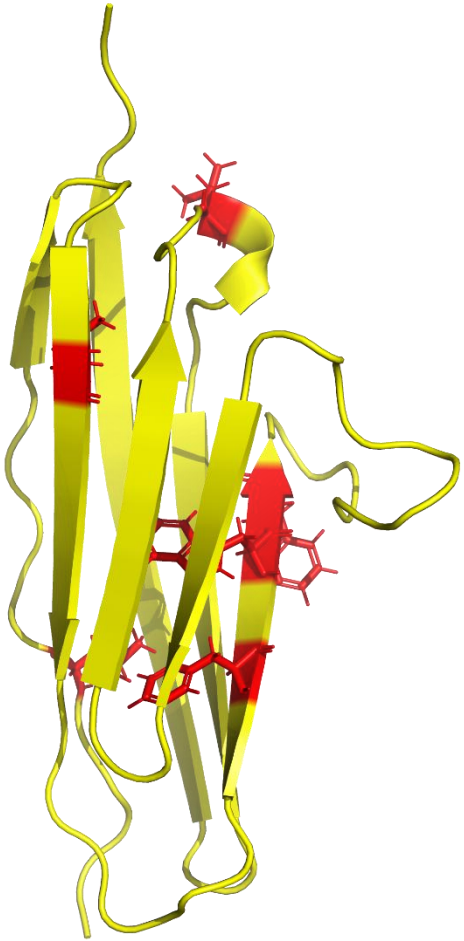
Generated 20,000 ab initio models + scored with Rosetta's score function named Ref15

↓

Scored models with our HRPF-guided score term, *hrf_dynamics*, then determined total score and identified top 20 scoring models

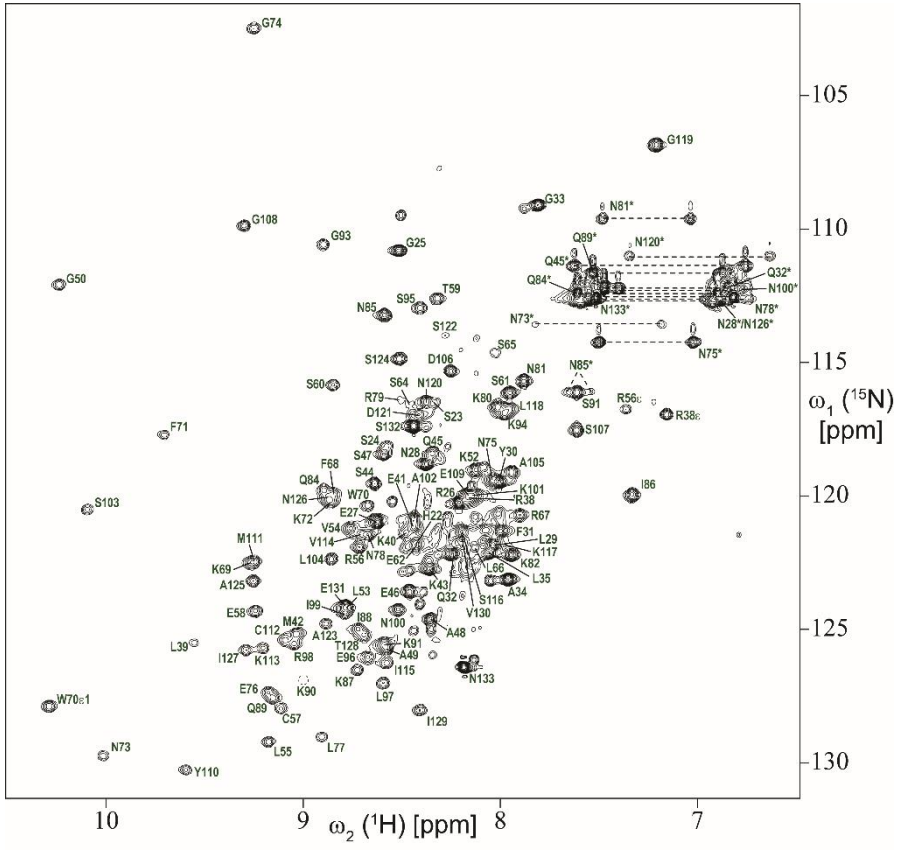
↓

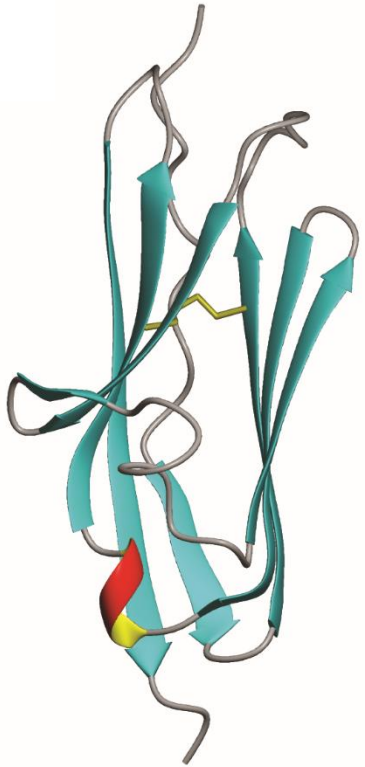
Generated 30 mover models for each of top 20 scoring models. Scored mover models with both Ref15 + *hrf_dynamics* and identified best scoring model from 20,600 models



Multidimensional NMR model

HSQC assignment





Comparison of HR-HRPF model and NMR structure

- After both groups independently determined their best model, groups shared information
- Models were judged by backbone RMSD from all amino acids, with the NMR model presumed to be accurate

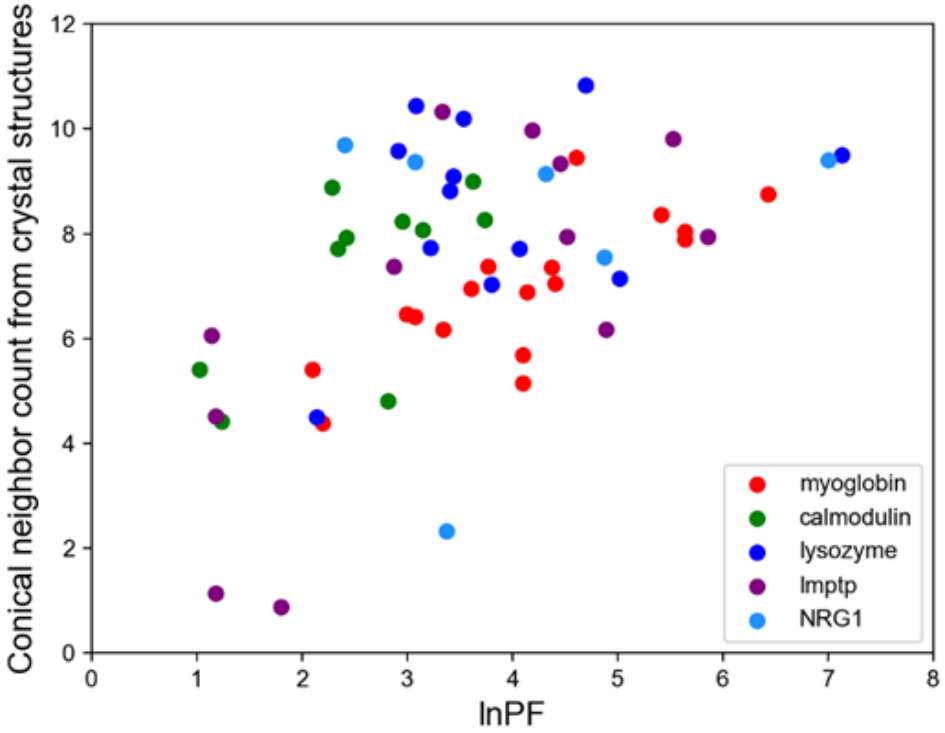
HR-HRPF

NMR

Backbone RMSD = 1.6 Å

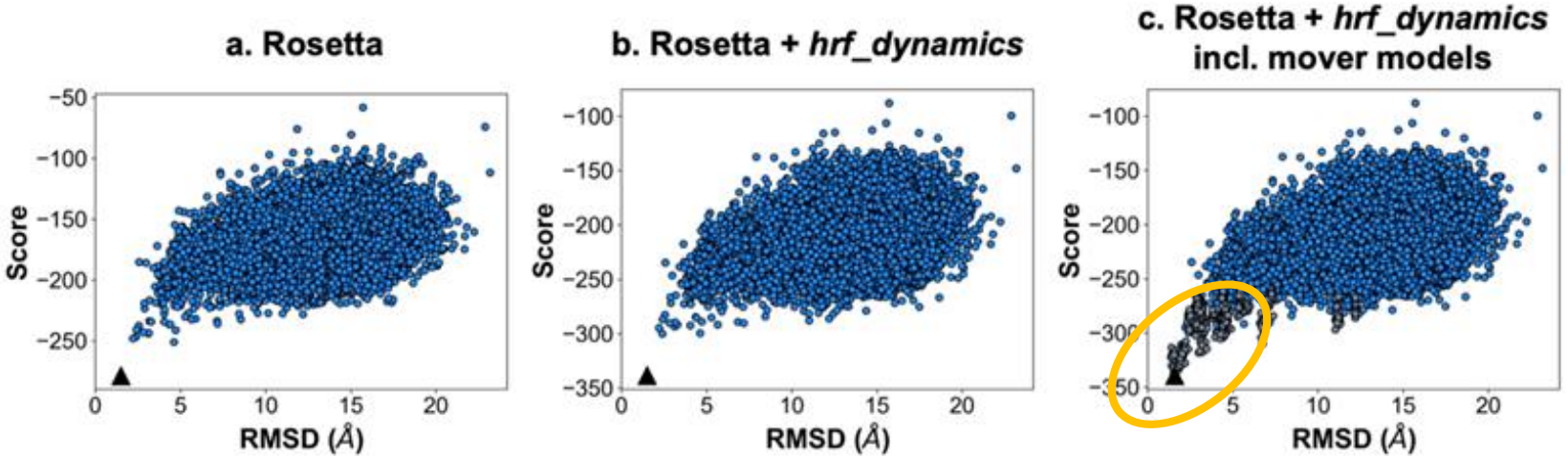
HR-HRPF correlation with conical neighbor count

Were data as reliable as those generated using proteins of known structure?



- Overlay of NRG1 data (cyan) with analysis of previously published data from Sharp (FPOP) and Kiselar/Chance (X-ray synchrotron) groups using proteins of known structure
- NRG1 data was comparably reliable to previously generated data
- No obvious evidence of bias in prior result reporting

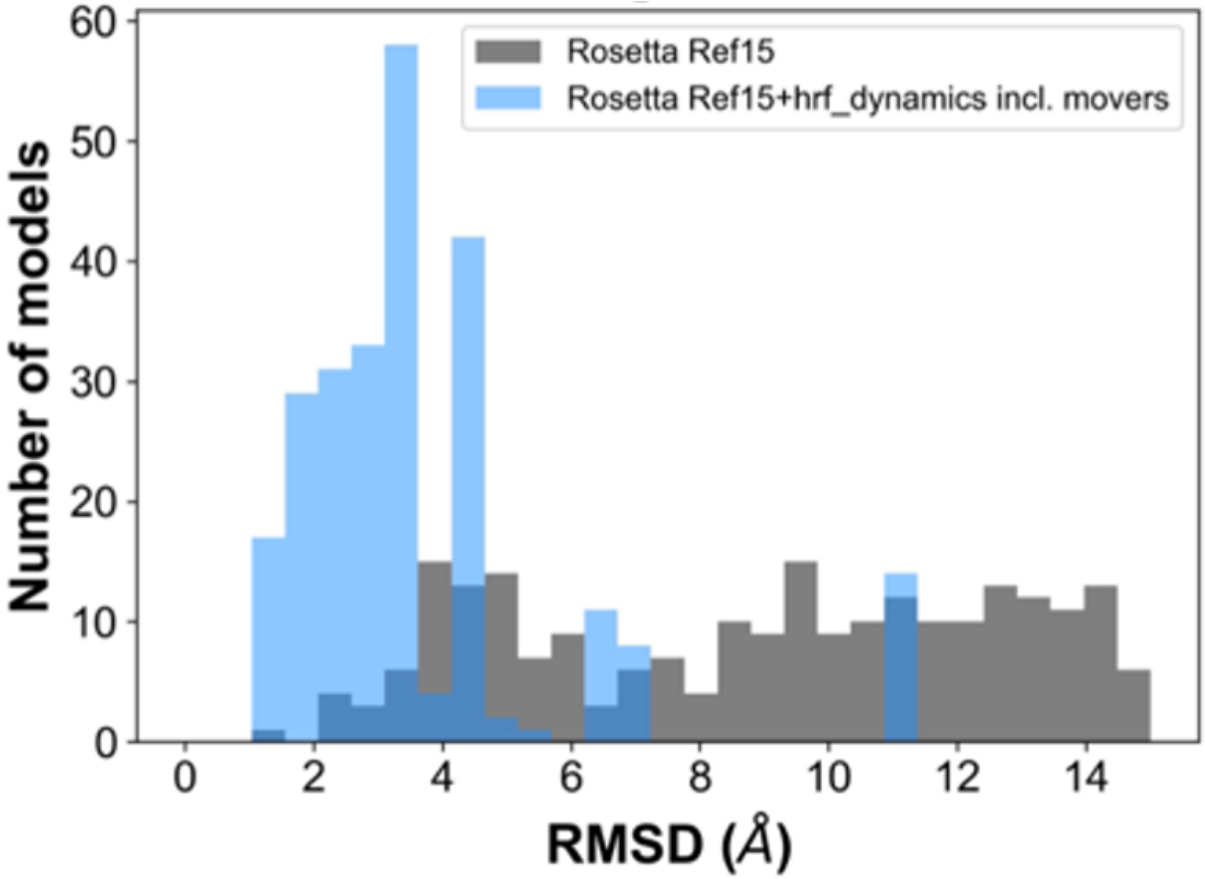
Improvements over Rosetta modeling alone



- Addition of *hrf_dynamics* term alone showed little improvement over Rosetta models alone
  - Score of best model improved, but accuracy of best model unchanged
- Inclusion of mover models scored with the *hrf_dynamics* term gave **great** improvement in robustness of modeling

RMSD distribution of top 250 scoring models

- Without *hrf_dynamics* and mover models, the top 250 scoring Rosetta models are evenly distributed between ~2 - 14 Å RMSD
- Addition of *hrf_dynamics* scoring term and mover models causes models to cluster around ~1.5 – 4.5 Å, with most below 3.5 Å RMSD



Conclusions

- First determination of unknown structure by covalent labeling mass spectrometry
- Determination of the structure by HR-HRPF and Rosetta modeling required much less sample, less time and no isotopic labeling
- Opportunities for use of HRPF-based modeling for larger multi-domain proteins of interest
- Work ongoing to investigate methods for including data from less reactive amino acids

Acknowledgements

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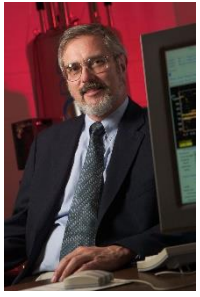

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