Functional and Biophysical Characterization of Cancer-Related PTP4A3 Mutations

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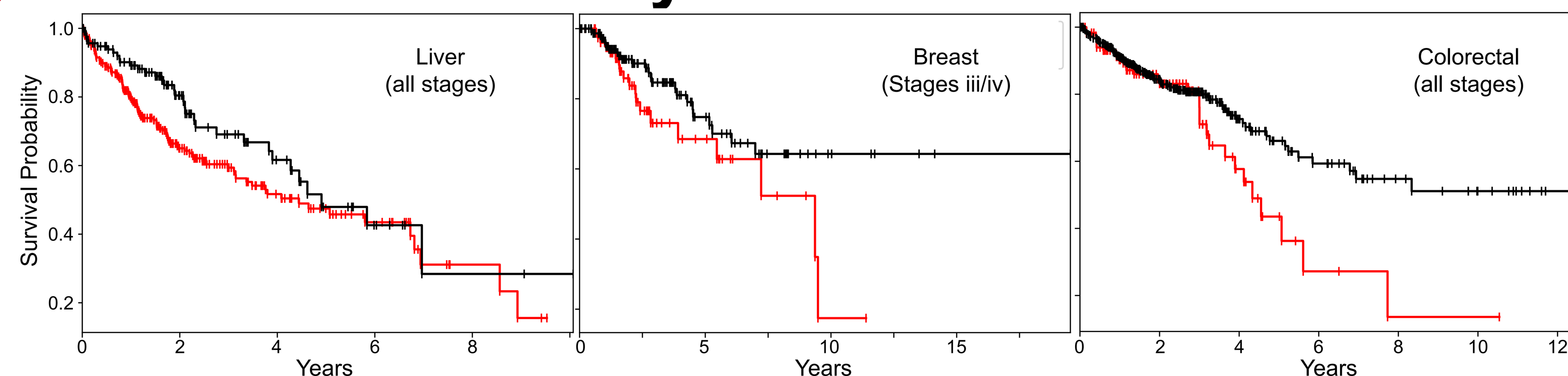
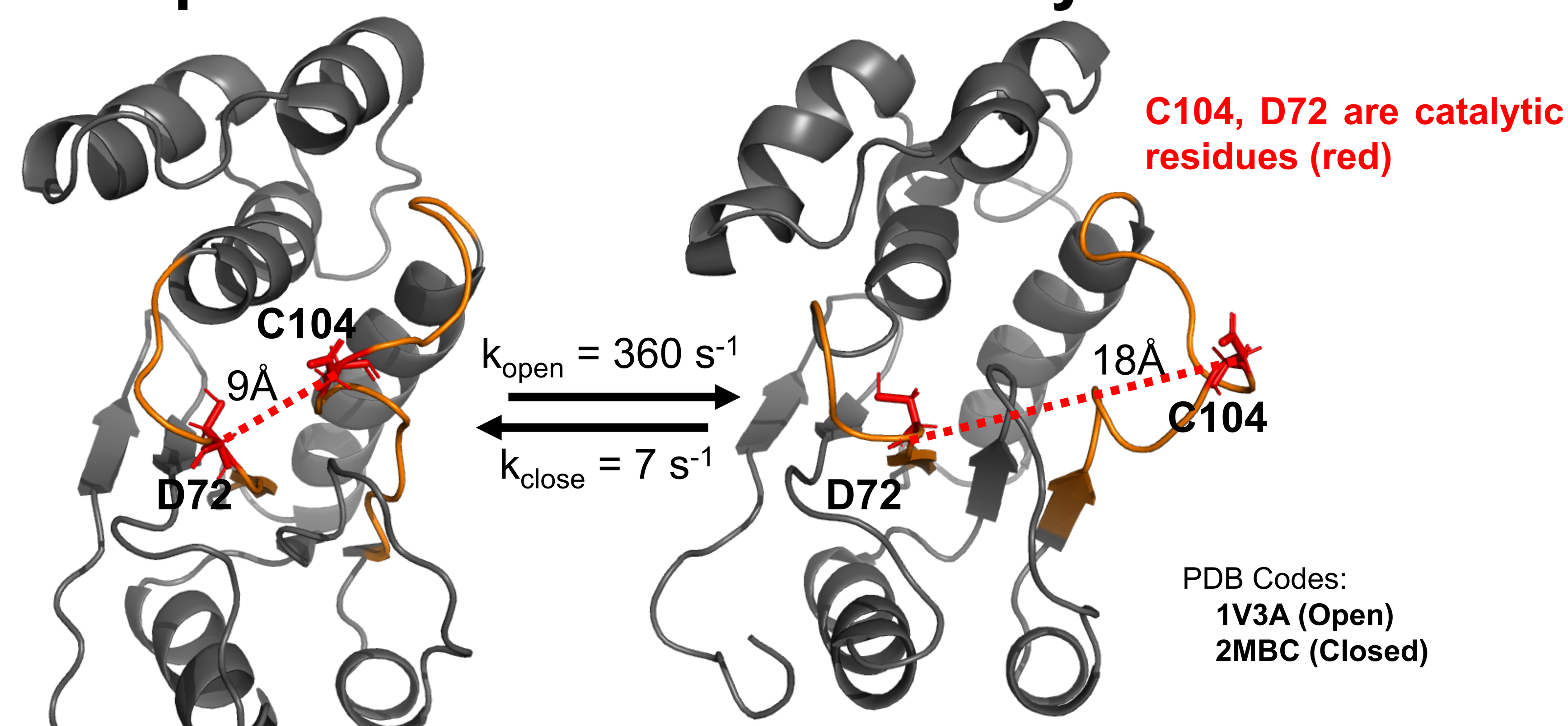
PTP4A3 is clinically-relevant



High expression (red) in most cancers correlates with lower patient survivability[1]. There have also been studies showing that PTP4A3 is related to cancer aggressiveness, as in breast cancer. PTP4A3 is a critical drug target[2].

Loop motion is critical for catalysis



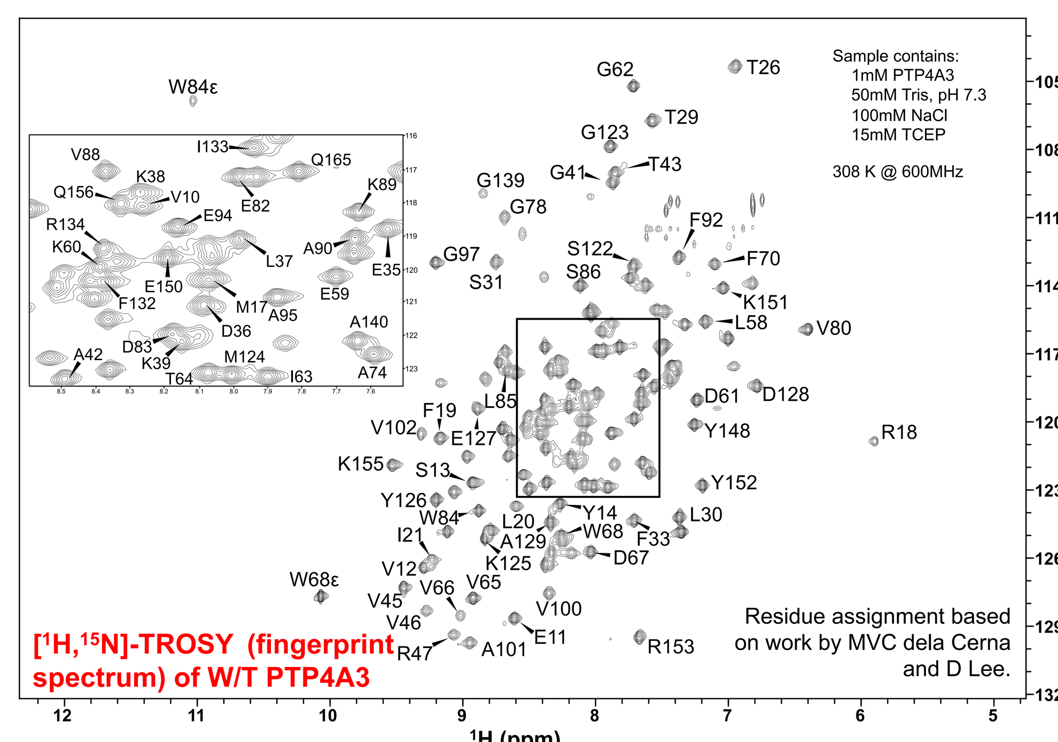
Catalytic residues are located in highly conserved loops (orange): CX_5R motif, which contains the active site cysteine, and the WPD loop, which has the aspartic acid. Both are required for catalysis. Phosphatase activity of PTP4A3 and other phosphatases are characterized by the opening (right) and closing (left) of the structure mediated by loop motion.[3]

Fingerprint spectrum of W/T PTP4A3

Human PTP4A3 was cloned into pET28a and expressed with ^{15}N stable isotope in *E. coli*. Protein is pure (>95%) and is well-folded as seen in fingerprint spectrum (right).

SEC profile shows high purity.

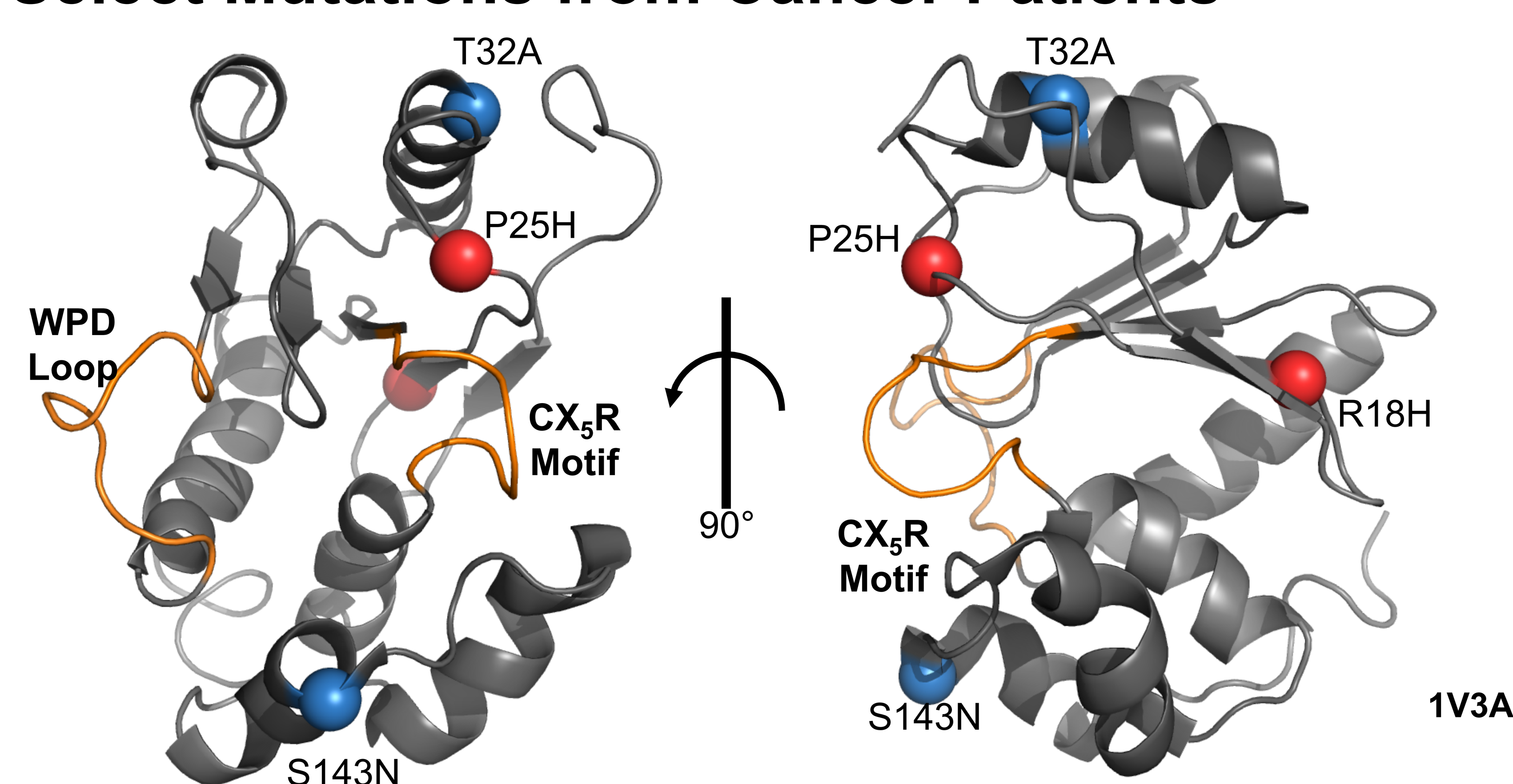
Superdex 75 16/60 Column
1 ml/min flow rate
Volume



The Lee BioNMR group focuses on the use of NMR to study the relationship between the structure, function, and dynamics of biomolecules. Learn more about their research at BIONMR.ORG

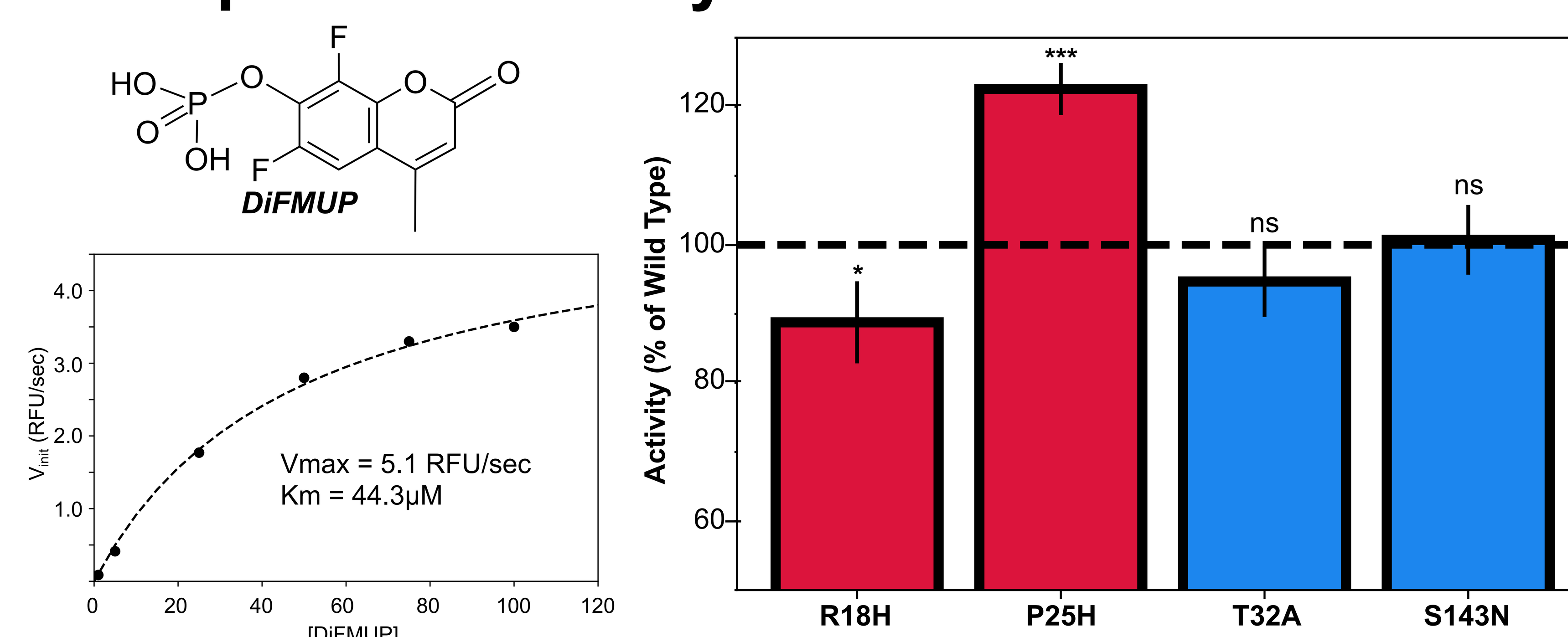
[1] Uhlen, et.al., *Science* 2017
[2] Wei, et.al., *Pharmacol Ther* 2018
[3] Jeong, et.al., *Biochemistry* 2014
[4] Raussens, et.al., *Anal Biochem* 2003

Select Mutations from Cancer Patients



I chose four cancer-associated mutations from TCGA, two are predicted to be damaging (red), and the other benign (blue) to protein function. Structurally, all of them are away from the active site (orange). ***My objective is to test their effects on stability, structure, and function.*** Mutations were introduced to the W/T plasmid by site mutagenesis and verified by sequencing (CGeMM).

Phosphatase activity towards DiFMUP



Activity was tested with synthetic substrate, DiFMUP (left, top) which fluoresces at 450nm when hydrolyzed. Kinetic parameters for WT at 30°C are shown using different DiFMUP concentrations (left, bottom). End-point assays were done for mutants. As expected, R18H shows reduced activity, while T32A and S143N show activity at WT levels (dashed line). Interestingly, P25H shows increased activity. *As prolines are rigid, I hypothesize that the mutation increases protein flexibility allowing for increased activity.*

Acknowledgements

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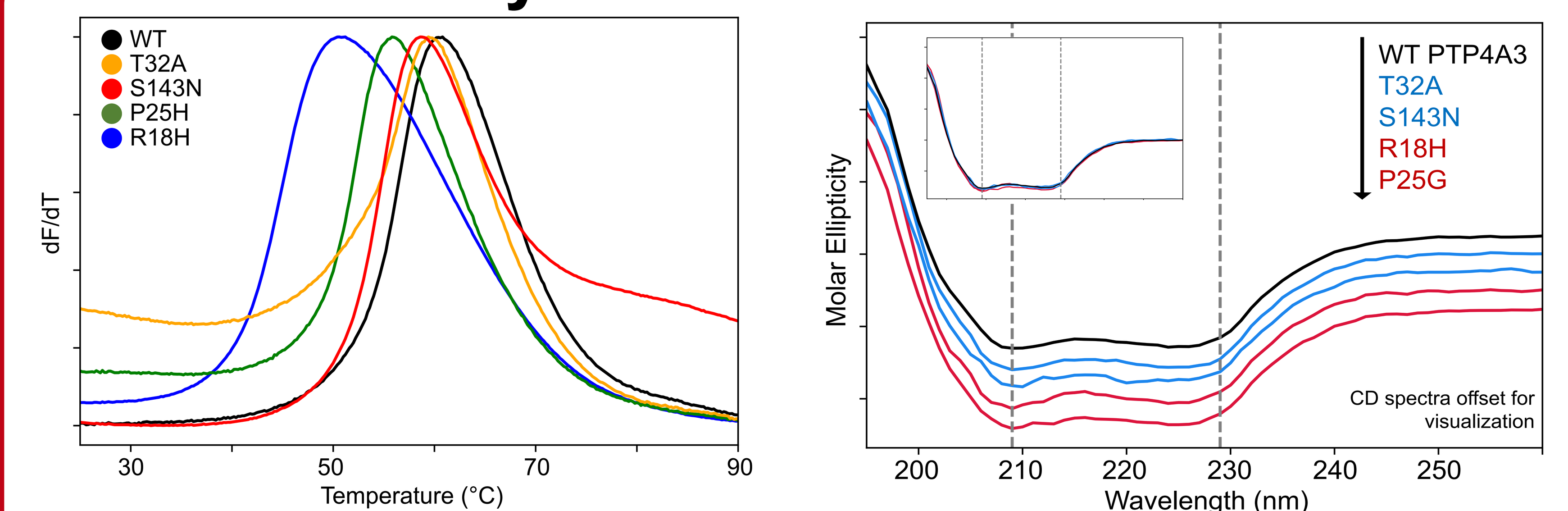
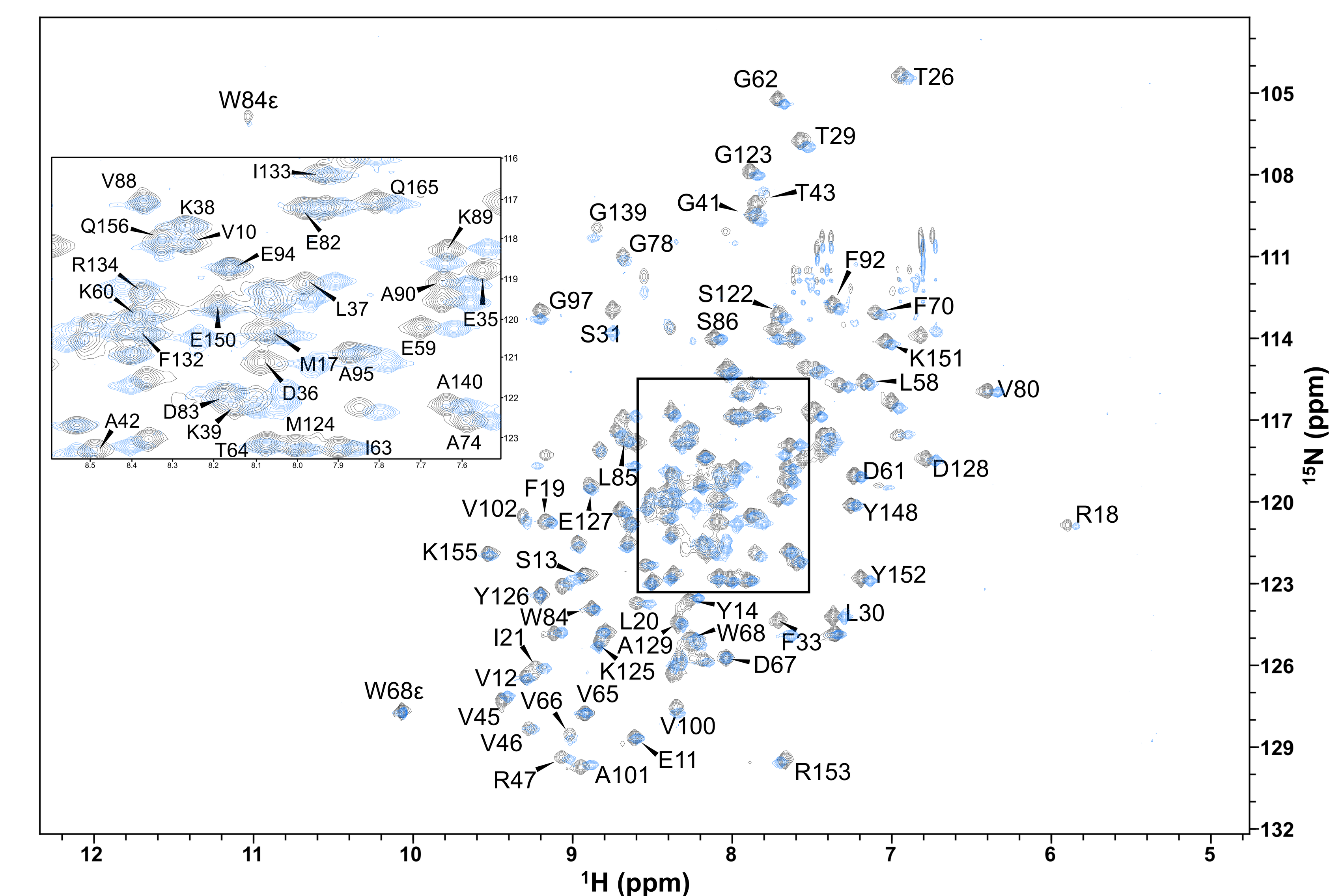
Protein Stability and Fold



Differential Scanning Fluorimetry shows reduced stability of PTP4A3 mutants with damaging mutations having lower T_ms (left). Meanwhile, circular dichroism profiles are very similar (right, inset show spectra without offset). Grey lines are guides for comparison. Secondary structures are similar to expected based on structure (not shown). Estimated alpha helix content ranges from 34-36%.[4]

Structural Perturbations by NMR



TROSY of mutant proteins were measured. Based on peak dispersion, all mutants are folded. Additionally, there are no major perturbations in the structure of PTP4A3 as shown for PTP4A3.T32A. Specifically, only a few residues exhibit chemical shift differences as expected for proteins with point mutations.

Conclusions and Future Direction

Mutations affected the biophysical properties of PTP4A3, as expected. Purified proteins are well-folded and have a conserved structure (NMR, CD), but have differing stability (DSF). Benign mutations did not affect activity as expected. While R18H reduced activity, it is interesting that P25H increased it. Further studies need to be done to validate effect of P25H. Other relevant parameters (Kcat, Kd, Km) will be determined. More mutations also need to be analyzed. Mutational studies may reveal allosteric networks involved in catalysis.