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Investigation of Disease-Causing Point Variants of VRK1

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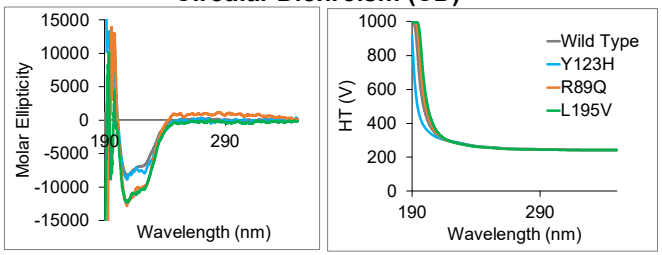
Abstract (Program Number 807.8)

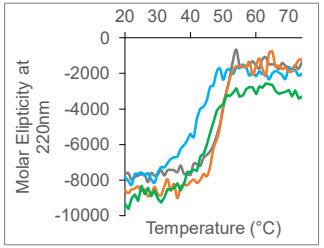
Vaccinia-related kinase 1 (VRK1) is a serine/threonine kinase that plays a variety of roles in transcription regulation and the cell cycle. Its substrates include histones, p53, and coilin. The kinase activity of the VRK1 protein is largely controlled by its C-terminal tail, which interacts with the enzyme active site. Several rare point mutations in VRK1 (including L195V, R89Q, and Y213H) are associated with neurodegenerative disorders, and questions remain as to how these mutations affect VRK1's intrinsic stability and activity.

For this *in vitro* study, mutations were generated in a His-tagged VRK1 kinase domain construct, and proteins were expressed in *E. coli* and purified. The mutant proteins' folding, and stability were analyzed by circular dichroism, and ligand binding was investigated using differential scanning fluorimetry. ATP hydrolysis kinetics were measured for wild-type, R89Q, and L195V mutants using ADP Quest activity assays. Protein modeling in PyMOL was also used to visualize the kinase and its associated changes.

Circular Dichroism (CD)



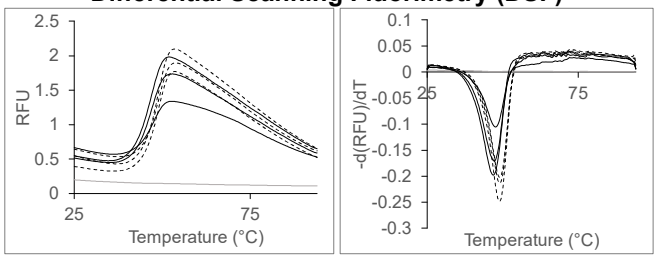
Representative wavelength scans and corresponding HT voltage for VRK1 variants. Protein samples were diluted to 0.05-0.1 mg/mL.



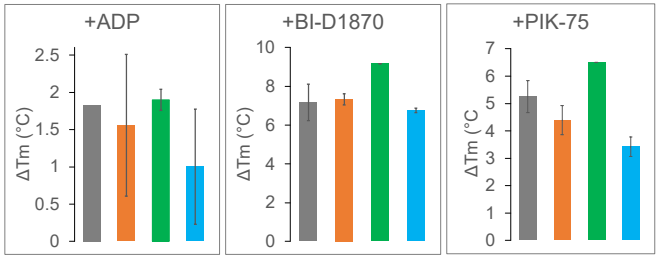
VRK1 Variant	Tm measured by CD (°C)	Tm measured by DSF (°C)
WT	48.8 ± 0.4	46 ± 1
R89Q	48.5 ± 0.3	45.4 ± 1
L195V	45 ± 1	38.8 ± 0.9
Y213H	41.6 ± 0.5	40 ± 1

Left: Representative thermal denaturation scans. Right: Comparison of melting temperatures (Tm) for VRK1 and variants. Scans in the left panel were fit to a 4PL function using MyCurveFit, and Tm ± error in the table are for a single experiment. DSF Tm ± error are the average and standard deviation for 3-6 independent experiments.

Differential Scanning Fluorimetry (DSF)

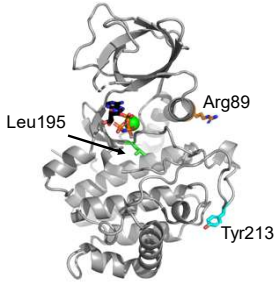


Representative DSF experiment with VRK1 WT with (dashed lines) and without (black, solid) ADP. DSF was run with 0.2-0.6 µM VRK1 and saturating ADP or inhibitor. SYPRO Orange dye fluoresces upon binding to unfolding protein, and fluorescence drops as the unfolded protein aggregates at high temperatures. Tm was calculated as the minimum of –dRFU/dT.

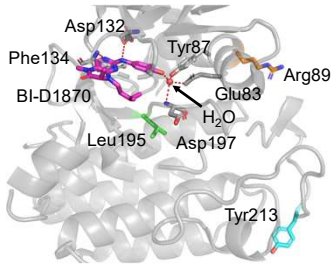


Average ΔTm values for 2-4 experiments. Error bars are the standard deviation for the average.

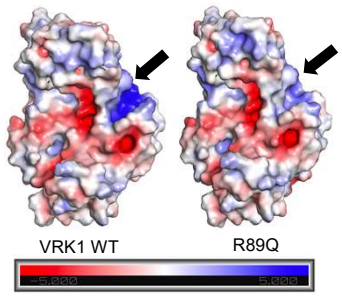
VRK1 Structure



Wild-type active state VRK1 (PDB ID 6ac9). AMP-PNP (black) is shown as sticks in the active site. Mutated residues are labeled.

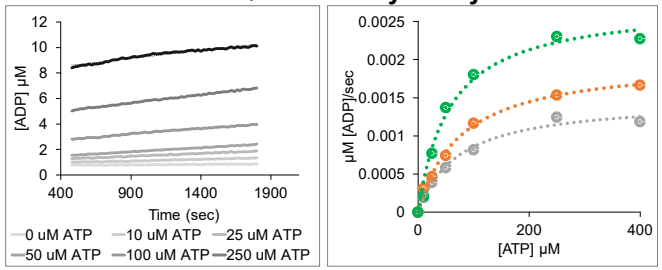


PDB ID 5uvf shows BI-D1870 (pink) in the active site of WT VRK1 (gray). H_2O is shown as a red sphere. Polar contacts to the inhibitor are shown as red dashed lines.

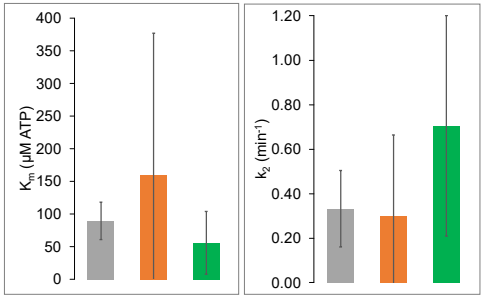


Electrostatic maps are shown for active state (6ac9) VRK1 WT and R89Q. Mutation was made in PyMOL and refined using 3Drefine and MolProbity. Maps were generated using the APBS Electrostatics plugin (red: negative surface area, blue: positive surface area). Black arrows show the mutation's location.

ADP Quest Activity Assay



Left: Representative ADP Quest experiment with WT VRK1. Assays were performed according to kit protocol, with 200 nM VRK1, varying [ATP], and no protein substrate. Signal may be due to ATP hydrolysis or autophosphorylation. Right: Example Michaelis-Menten plot of VRK1 mutants. Fit was determined using MyCurveFit.



k_2 and K_m, values for VRK1 variants. Bars and error bars represent the average of 2-3 Michaelis-Menten plots with R^2 of at least 0.80.

Conclusions

Circular dichroism shows VRK1 L195V and Y213H mutants have significantly lower Tm than WT and R89Q. However, all mutants have similar folded structure. This agrees well with previous work.[1-4]

A fluorescence-based assay was used to measure ATP affinity and hydrolysis. k_2 for the L195V mutant seems to be higher than for WT, but k_2 for the R89Q variant and K_m values are comparable. This does not agree with previous work,[1-4] but standard deviations are large and more trials are needed.

DSF was used to measure Tm in the presence and absence of known VRK1 inhibitors.[5] Higher ΔTm values measured with L195V and inhibitors suggest the mutant has a significantly more flexible active site than the wild-type enzyme.

The electrostatic maps created in PyMOL show a significant change in charge in the region around the mutation R89Q on the αC helix. This change, from very positive to slightly positive in the mutant form, may impact protein-protein interactions *in vivo*.

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References

1. El-Bazzal L, Rihan K, Bernard-Marissal N, et al. Loss of Cajal bodies in motor neurons from patients with novel mutations in VRK1. *Hum Mol Genet*. 2019;28(14):2378-2394. doi:10.1093/hmg/ddz060
2. Marcos AT, Martín-Doncel E, Morejón-García P, et al. VRK1 (Y213H) homozygous mutant impairs Cajal bodies in a hereditary case of distal motor neuropathy. *Ann Clin Transl Neurol*. 2020;7(5):808-818. doi:10.1002/acn3.51050
3. Martín-Doncel, E., Rojas, A.M., Cantarero, L. *et al*. VRK1 functional insufficiency due to alterations in protein stability or kinase activity of human *VRK1* pathogenic variants implicated in neuromotor syndromes. *Sci Rep* **9**, 13381 (2019). https://doi.org/10.1038/s41598-019-49821-7
4. Stoll M, Teoh H, Lee J, et al. Novel motor phenotypes in patients with VRK1 mutations without pontocerebellar hypoplasia. *Neurology*. 2016;87(1):65-70. doi:10.1212/WNL.0000000000002813h
5. Couñago, R.M., Allerston, C.K., Savitsky, P. *et al*. Structural characterization of human Vaccinia-Related Kinases (VRK) bound to small-molecule inhibitors identifies different P-loop conformations. *Sci Rep* **7**, 7501 (2017). https://doi.org/10.1038/s41598-017-07755-y