Supporting Information 1 Steady-state kinetic studies of the phosphorylation of LRRKtide catalyzed by wt LRRK2 (A) and the mutant G2019S (B). Initial velocities were measured as a function of LRRKtide at [ATP] = 500 (●), 250 (o), 125 (▼), 63 (▽), 31 (■), and 16 μM (□) μM. Each data point is the average of duplicate determinations. Each data set was globally fit to the equation reflecting random mechanism.

Supporting Information 2 Steady-state kinetic studies of the phosphorylation of PLK-peptide catalyzed by wt LRRK2 and the mutant G2019S. Initial velocities were measured as a function of [ATP] at [PLK-peptide] = 800 (●), 600 (o), 400 (▼), 200 (▽), 100 (■), 50 (□), and 25 (♦) μM for wt LRRK2 (A) and the mutant G2019S (B). Each data point is the average of duplicate determinations. The data set were globally fit to the equation reflecting random mechanism.

Supporting Information 3 Steady-state kinetic studies of the phosphorylation of LRRKtide^{S} catalyzed by wt LRRK2 and the mutant G2019S. Initial velocities were measured as a function of [LRRKtide^{S}] at [ATP] = 500 (●), 250 (o), 125 (▼), 63 (▽), 31 (■), and 16 μM (□) for wt LRRK2 (A) and the mutant G2019S (B). Each data point is the average of duplicate determinations. The data sets were globally fit to the equation reflecting random mechanism.

Supporting Information 4 Isotope exchange analysis for tG2019S-catalyzed LRRKtide phosphorylation as a function of time. Examples of exchange of radioactive ATP to PO_4-LRRKtide as a function of time under conditions of varied ADP/ATP (A), PO_4-LRRKtide/ATP (B), ADP/LRRKtdie (C), and PO_4-LRRKtide/LRRKtide (D). The concentrations of the varied reactants were maintained at a constant ratio of 20 while the other reactants were kept at 1 and 20 μM for the substrate and product, respectively.

Supporting Information 5 Isotope exchange analysis for tG2019S-catalyzed LRRKtide phosphorylation as a function of enzyme concentration. An example of initial exchange rate as a function of enzyme concentration under condition of varied PO_4-LRRKtide/LRRKtide while the concentration of ATP and ADP was kept at 1 and 20 μM, respectively.

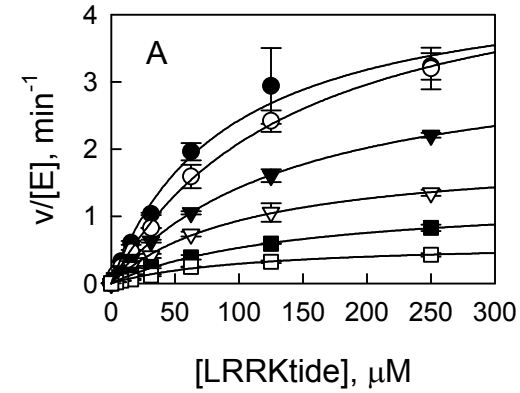
Supporting Information 6 Inhibition study of the mutant G2019S-catalyzed phosphorylation of LRRKtide by products ADP and PO_4-LRRKtide. A: Plot of initial velocities vs [LRRKtide] at [ADP] = 500 (•), 250 (o),125 (▼), 31 (■), and 0 μM (□) all at a fixed ATP concentration of 100 μM. B & C: ADP concentration dependencies of $(k_{cat})_{LRRKtide}$ and $(k_{cat}/K_m)_{LRRKtide}$ apparent values derived from analysis of the data of panel A. D: Plot of initial velocities vs [ATP] at [PO_4-LRRKtide] = 2.5 (•), 1.3 (o), 0.6 (▼), 0.2 (■), and 0 (□) mM all at a fixed LRRKtide concentration of 100 μM. E & F: PO_4-LRRKtide concentration dependencies of $(k_{cat})_{ATP}$ and $(k_{cat}/K_m)_{ATP}$ apparent values derived from analysis of the data of panel D.

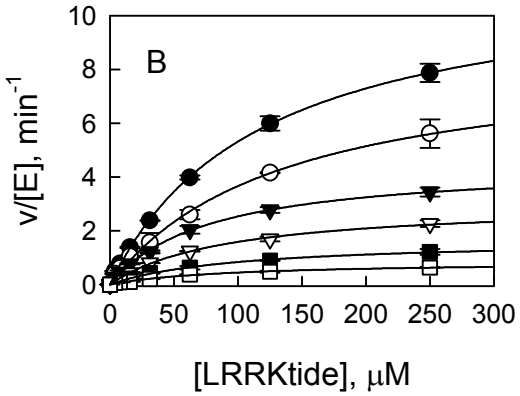
Supporting Information 7 pL-dependence of steady-state kinetic parameters of the mutant G2019S-catalyzed LRRKtide^S phosphorylation. A. pH (•) and pD (o) dependence of k_{cat} revealed an inverse SKIE of 0.65. B. pH (•) and pD (o) dependence of k_{cat}/K_m revealed an inverse SKIE of 0.56.

Supporting Information 8 pL-dependence of steady-state kinetic parameters of the mutant G2019S-catalyzed LRRKtide phosphorylation. A. pH (•) and pD (o) dependence of k_{cat} revealed a SKIE of 1.1. B. pH (•) and pD (o) dependence of k_{cat}/K_m revealed a SKIE of 1. **Supporting information 9** (a) Homology model of the kinase domain of LRRK2 between residues 1885-2138. (b) Conserved domains within this family of

kinases shown with overall surface representation of the LRRK2 kinase domain. (c) Structural details of the ATP binding site showing the Glycine rich loop, $Mg2^{+}$ atom and docked ATP molecule. D2017 from the DYG loop is shown to coordinate $Mg2^{+}$ and ATP in the DYG-in conformation. (d) Two spatially conserved hydrophobic spines in LRRK2 are shown overlayed with other kinases where these conserved domains have been observed. These include PKA, b-RAF, c-Abl kinases.

Supporting information Figure 10 (a) Superposition of LRRK2 model with PKA and their respective substrates. (b) calculated electrostatic potential (red and blue represent electrostatic potentials <−9 and >+9 kBT, respectively, where kB is the Boltzmann constant and T is the absolute temperature). LRRKtide is shown as stick representation.





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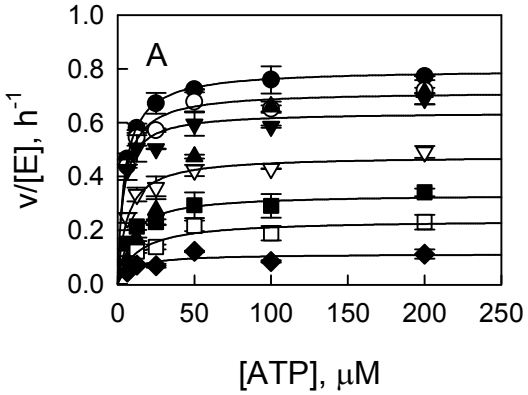

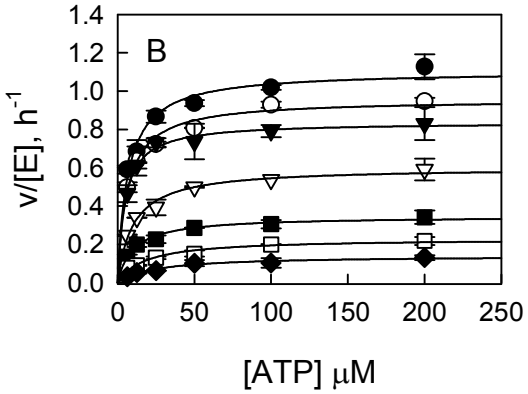

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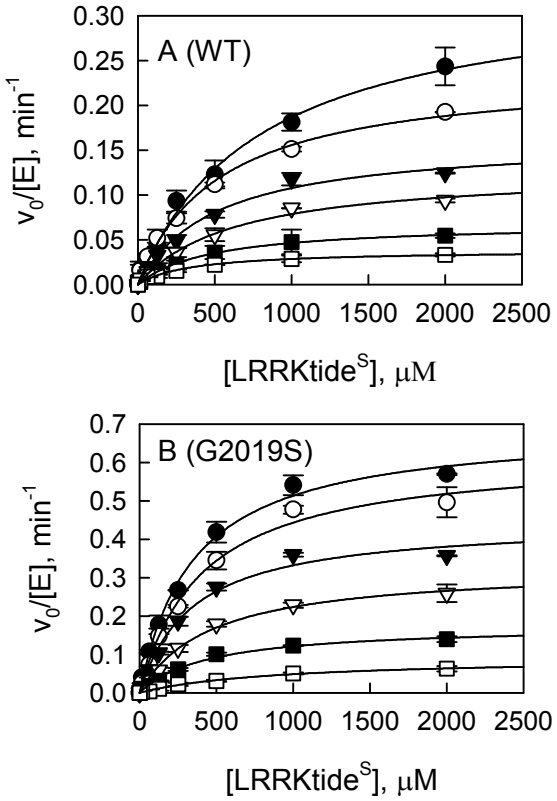

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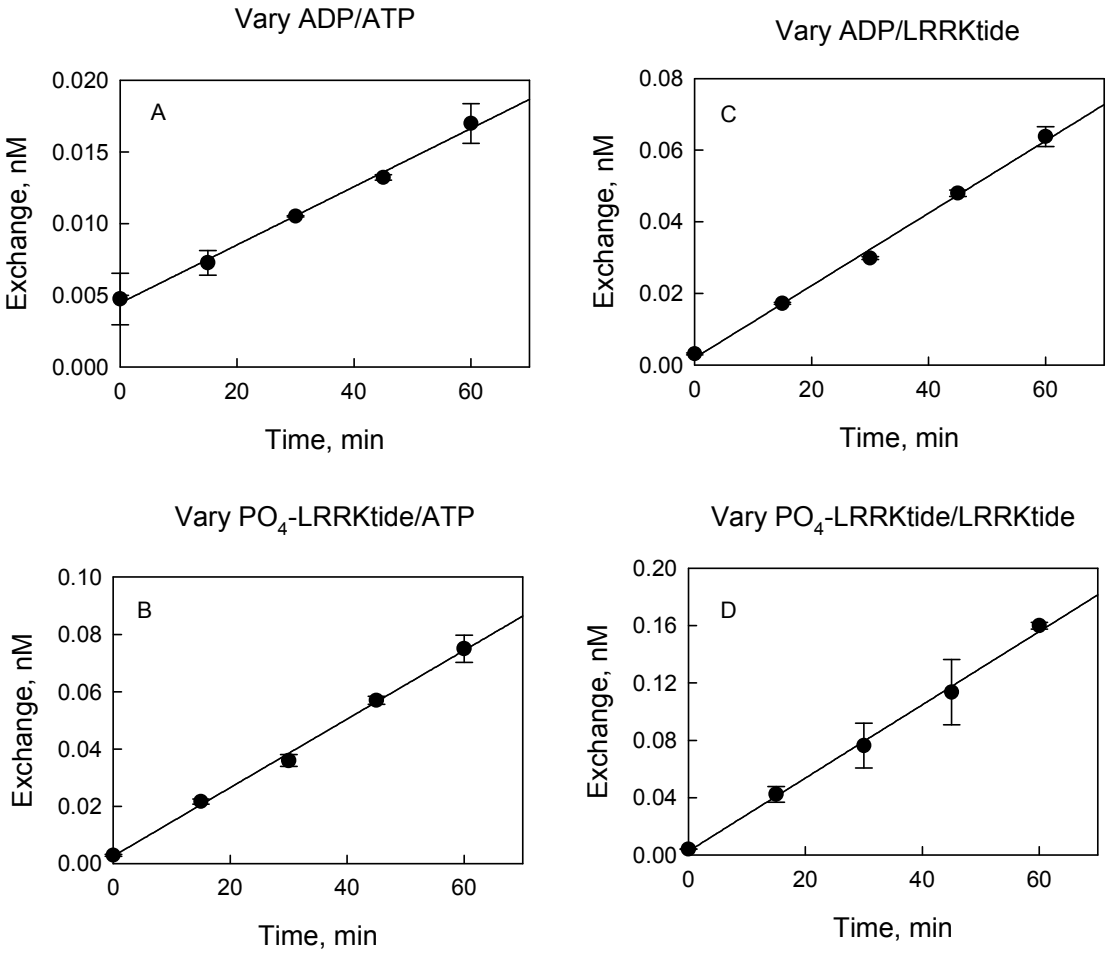

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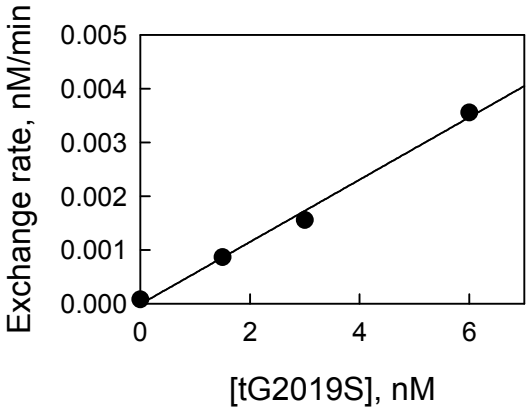

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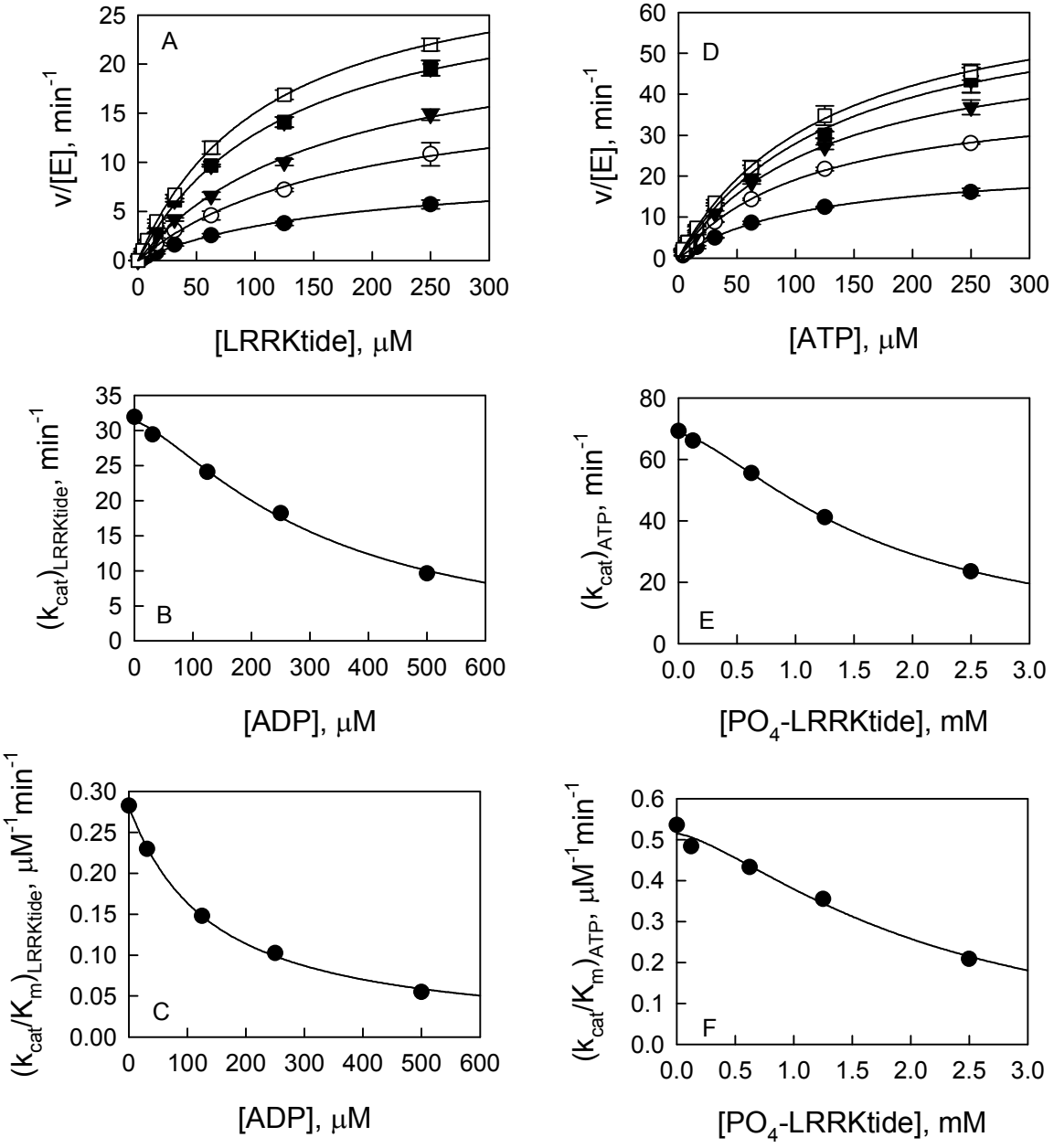

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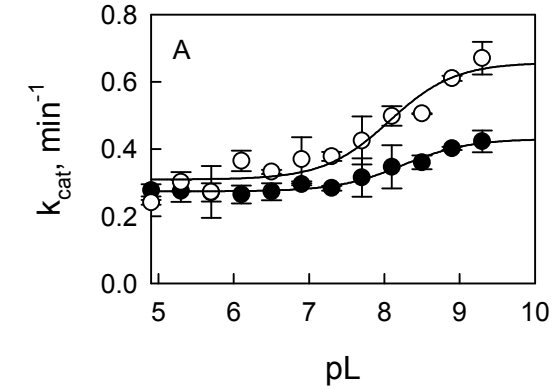

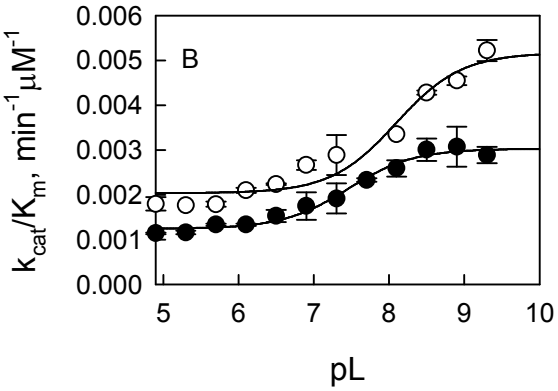

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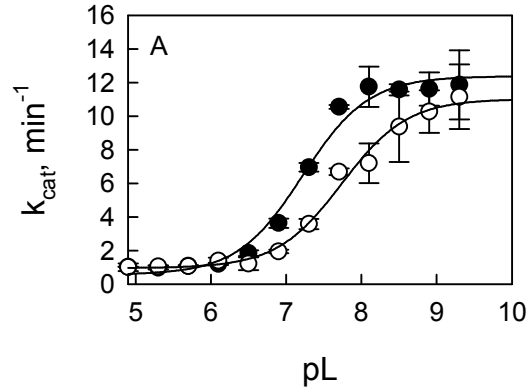
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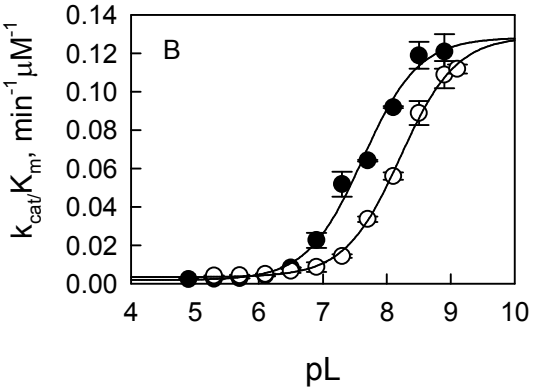
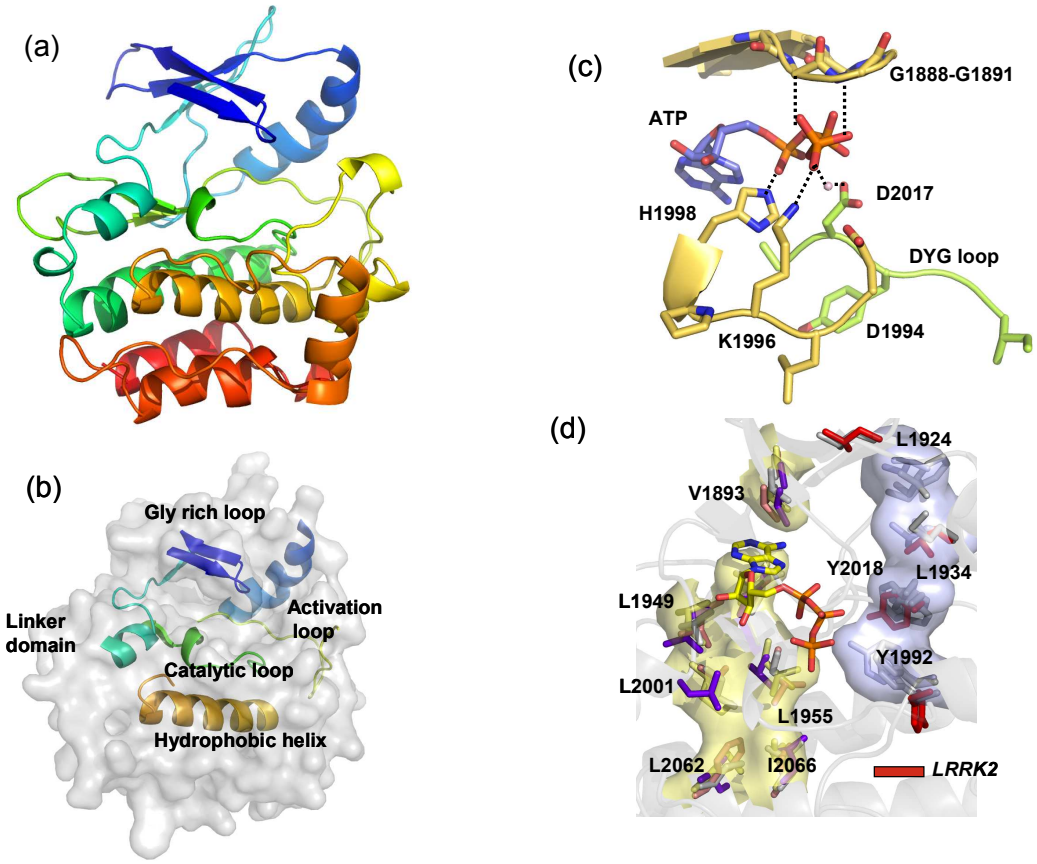
k_{cat}, min^{-1}
pL


B
k_{cat}/K_m, $min^{-1}\mu M^{-1}$
pL



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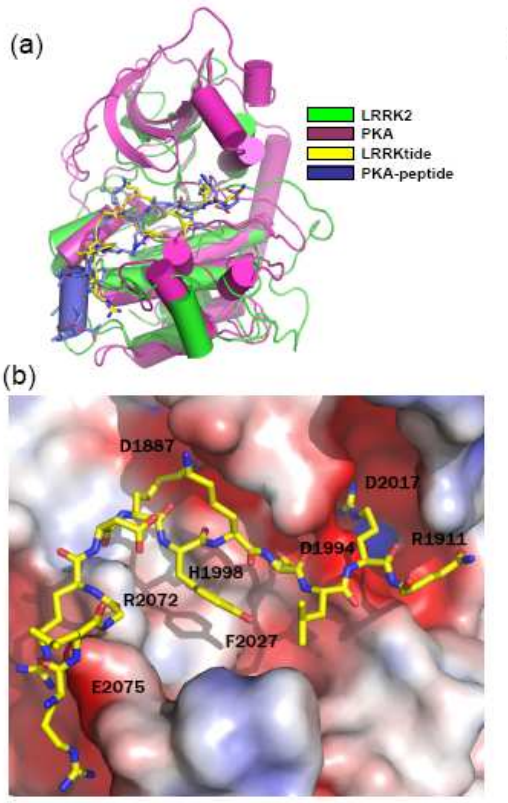

Supporting Information 10