# Computational Modeling of Phosphokinase Activity of Human 3'-Phosphoadenosine 5'-Phosphosulfate Synthase


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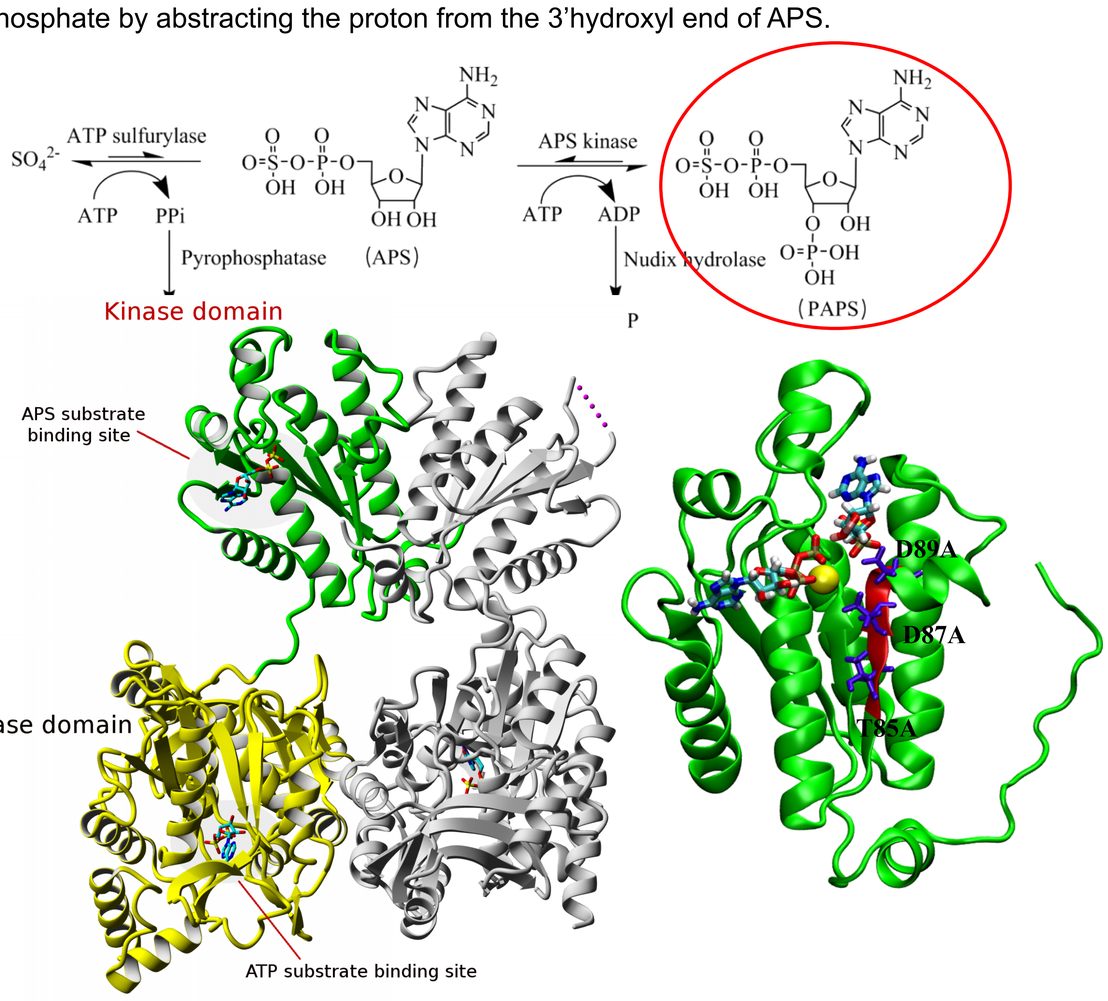
## Introduction

Human 3'-Phosphoadenosine 5'- Phosphosulfate Synthase (PAPSS) is a bifunctional protein consisting of N-terminal (1-260 aa) APS kinase domain and a C-terminal ATP sulfurylase domain. In mammals and higher organisms, the enzyme is the sole means for bio- integration of inorganic sulfate through its transformation into an activated, organic form.1 Although its overall mechanism and kinetics have been well studied in the past, more recent discoveries including the resolution of its crystal structure and research in its regulatory functions revealed previously unanticipated behaviors.2 As the ubiquitous sulfate donor in most biological systems, the product of the enzyme, PAPS, plays an essential role in ECM formation, embryonic development and biomolecule secretion.3 A potential phosphorylation/allosteric motif is located close in space to the APS binding site. Concretely the respective motif is "TLDGD" (residues 85-89) for PAPSS1 and PAPSS2b (Venkatachalam unpublished/proposed), and docking studies show that these residues might play a key role in positioning the magnesium ion and the ribose prior to the first step of the reaction, the nucleophilic attack on ATP-γ phosphate by abstracting the proton from the 3'hydroxyl end of APS.




*Human 3'-phosphoadenosine 5'-phosphosulfate Synthase*

## Methods

Since crystal structures of human PAPSS1 with various ligands and combinations have been resolved by X-ray diffraction to a resolution of up to 1.75 Å (PDBs: 1x6v, 2ofw, 1xjq, 1xnj), atomistic molecular dynamics simulations and ligand-docking can be applied to gain an understanding of the structural and functional consequences of specific residues in the conserved motif that is part of the substrate binding pocket of the kinase domain. Mutations in this motif have been studied experimentally and reported to alter the enzyme function, however, no molecular explanation has been reported so far. Homology modeling in YASARA allowed modeling of unresolved loops and *in silico* mutagenesis, ligand docking was performed in AUTODOCK and all complex structures were simulated using molecular dynamics simulations in GROMACS for at least 100ns using the AMBER99SB force field. MM-PBSA calculations were performed to calculate free energies.

## Objective

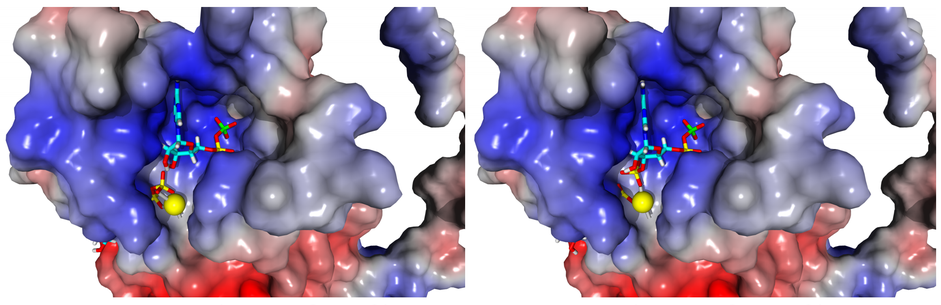
- Understanding how the three dimensional structure of PAPSS determines the enzyme function?
- Studying the roles of specific amino acid residues in the conserved motif in the dynamics of the enzyme in aqueous solution, the related quaternary arrangements of the enzyme and substrate binding.
- Making explicit structural and thermodynamic predictions for ligand-binding that can subsequently be tested experimentally.
- Understanding the structural and functional consequences of the proteins due to DNA mutations among various human populations.

## Results

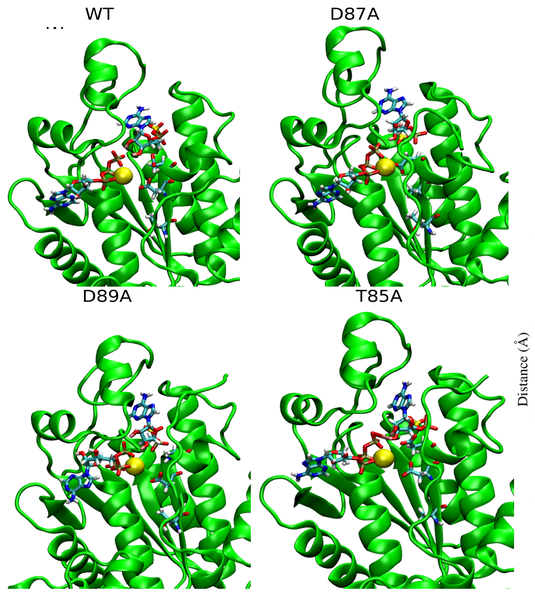
*Binding Energy Calculations*

| | WT (ATP+APS) kJ/mol | D87A(ATP+APS) kJ/mol | D89A(ATP+APS) kJ/mol | T85A(ATP+APS) kJ/mol |
|---|---|---|---|---|
| Van der Waal energy | -332.167 | -362 | -333.444 | -344.284 |
| Electrostatic energy | -714.176 | -1041.167 | -909.169 | -131.987 |
| Polar solvation energy (est)* | 488 | 488 | 488 | 488 |
| SASA energy | -32.72 | -32.121 | -31.882 | -38.801 |
| Binding energy | -591.663 | -947.28 | -807.091 | -637.072 |

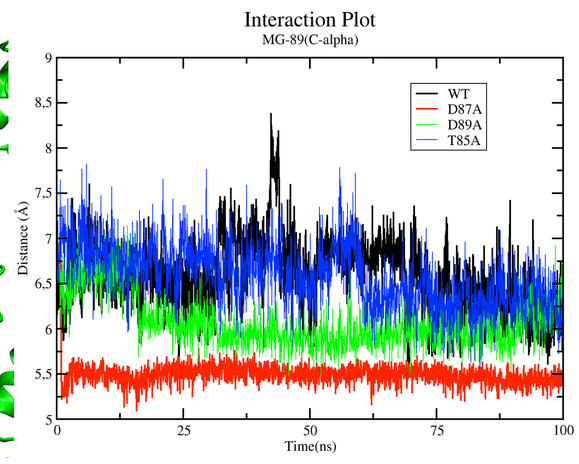
**Phosphate transfer from ATP to APS illustrated by molecular docking**



**Reactants** (ATP-MG-APS) **Products** (ADP-MG-PAPS)




**MD simulations :** WT and T85A mutant show a similar behavior for D87 and D89, where D87 seems to be involved in coordinating the magnesium ion while D89 is oriented towards the ribose. In D87A, however, D89 seems to replace the interaction of D87 with magnesium.




## Conclusions

Molecular docking, MD simulations and consequent MMPBSA calculations demonstrate the good binding for the ligand into mutants D87A and D89A that experimentally do not show any enzymatic activity. The binding affinity of the ligand is even higher for D87A and D89A compared to WT.

However, a deeper analysis of the binding pocket shows both residues involved in contacting a magensium ion or the ribose, and that these interactions as well as the side chain orientations change of the residues change in the mutants.

We propose that D87 and D89 might be involved in the reaction itself by either lowering a energy barrier, by properly placing the magnesium or directly participating in a transition state. Therefore we propose QM/MM calculations that could contribute to a better understanding of their actual role.


**References**

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