# Characterization of Profilin Binding Kinetics Using Ensemble Molecular Dynamics Simulations

Jocelyn Sunseri[1,2], David Gau[3], Partha Roy[3], David Ryan Koes[2]

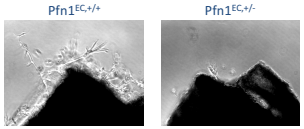
[1]Carnegie Mellon U-Pitt Joint Program in Computational Biology,
[2]Department of Computational and Systems Biology University of Pittsburgh,
[3]Department of Biomedical Engineering University of Pittsburgh

## Abstract

We investigate the dynamics of profilin binding, including differences in binding for the loading and recruiting subregions of VASP, the effect of binding site mutations on peptide affinity for profilin, and the effect of actin binding on profilin dynamics. Ensemble molecular dynamics simulations with bootstrapping of MM/PBSA and MM/GBSA-derived free energy calculations are used to robustly estimate binding affinities. The essential features of the interaction between profilin and poly-proline peptides is explored through statistical analyses of simulation data.

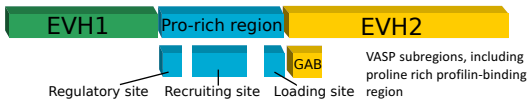
## Background

**Profilin (Pfn1)** is an actin-binding protein that is at the heart of regulation of actin dynamics in cells and an important downstream mediator of actin assembly triggered by Rho-GTPases. Pfn1 plays a critical role in angiogenesis.

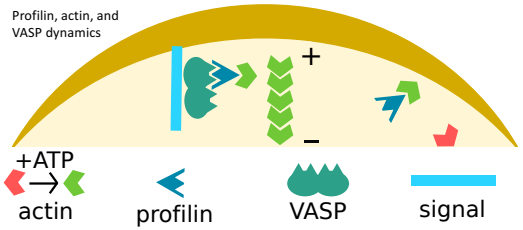


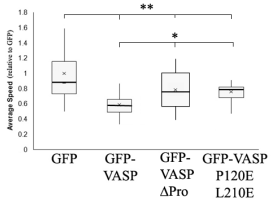

Heterozygous Knockout of Profilin-1 in Endothelial Cells Delays Sprouting Angiogenesis in Matrigel

**Vasodilator-stimulated phosphoprotein (VASP)** primarily serves as an actin filament elongation factor. It recruits G-Actin to the barbed end of a growing filament by binding profilin preferentially as part of an actin-profilin complex. It has several proline-rich subregions that have different functional roles.




VASP subregions, including proline rich profilin-binding region

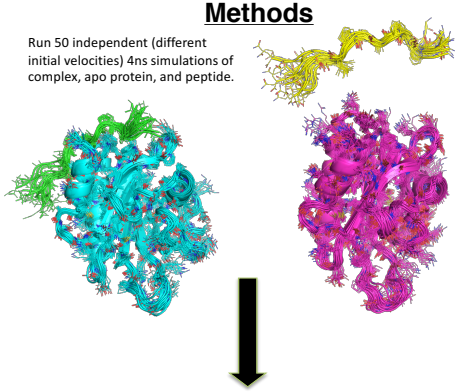


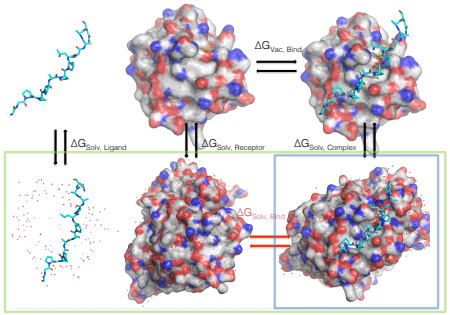

Modifications to VASP impact cell motility, underscoring its importance in actin dynamics.

## Methods

Run 50 independent (different initial velocities) 4ns simulations of complex, apo protein, and peptide.

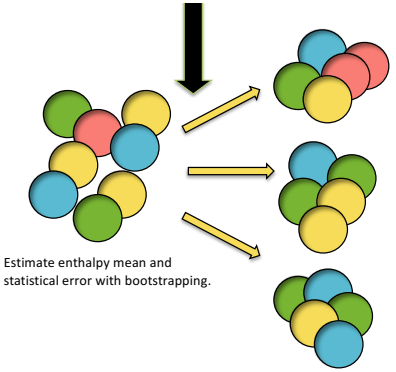


Estimate ΔH using MM/GBSA and MM/PBSA. Evaluate 1-traj (simulate complex-only) and 3-traj (simulations of complex, receptor, and peptide ligand) methods.




$\Delta G_{Solv,\,Bind} = \Delta G_{Vac,\,Bind} + \Delta G_{Solv,\,Complex} - (\Delta G_{Solv,\,Receptor} + \Delta G_{Solv,\,Ligand})$

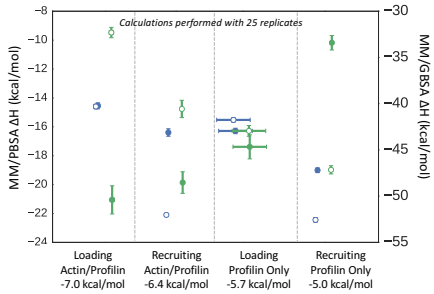
With the 1-traj method, ligand and receptor energies are extracted from a single simulation of the complex, unlike with 3-traj where the ligand and receptor structures are also simulated. $\Delta G_{solv}$ is computed using either the generalized Born and surface area (GBSA) or Poisson-Boltzmann and surface area (PBSA) methods.

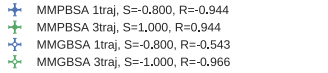


Estimate enthalpy mean and statistical error with bootstrapping.

## Results

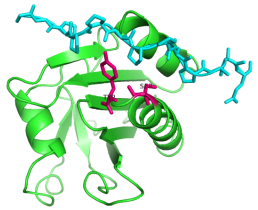
Comparison with VASP Experimental Results



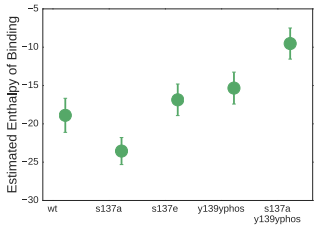

In a comparison of 4 methods (1-traj vs. 3-traj and GBSA vs PBSA), only 3-traj MM/PBSA correctly rankings the binding affinities of VASP loading and recruiting peptides to the profilin monomer and profilin-actin complex.

The other three methods exhibit a negative correlation with the experimentally determined affinities.

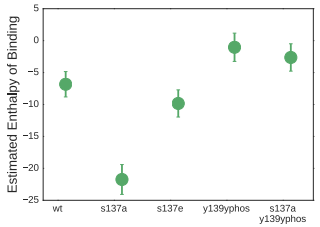


C-terminal profilin residues that are the subject of our mutagenesis/phosphorylation studies

Mutation Studies with Loading Region of VASP

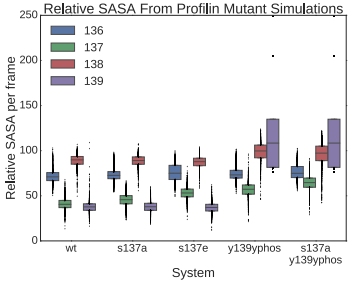



Mutation Studies with Recruiting Region of VASP




Phosphorylation of either s137 ors139 appears to disrupt binding of both the loading and recruiting region of VASP to varying degrees. However, the experimentally accessible phosphomimetic mutation s137e does not.

Relative SASA From Profilin Mutant Simulations




Phosphorylated 139 results in increased solvent accessible surface area (SASA) at the C-terminus, but has surprisingly little effect on the SASA of s137. It is therefore unclear if phosphorylation of 139 could promote the recognition of this residue by its associated kinase.

## Future Work

We will attempt to experimentally confirm the site-specific effects of phosphorylation on VASP-profilin binding dynamics. We will also attempt to rigorously bound the simulation length and replicate numbers required to provide an accurate, consistent estimate of binding enthalpy and provide a generalizable method of doing this for systems of different sizes and timescales.

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