

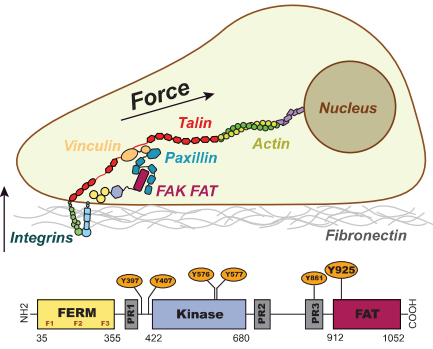
Mechanical force unlocks cryptic phosphorylation in FAK to sever cooperative paxillin binding

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Background



Focal Adhesion Kinase (FAK) is a focal adhesion protein that functions both as a force sensor and a kinase. It undergoes post-translational modifications that are essential for cell division, growth, and signal transduction.

Focal Adhesion Targeting (FAT) through localizes FAK to focal adhesions through its interaction with paxillin (LD2, LD4) and regulates cell adhesion, migration, and survival via Src-mediated phosphorylation of Y925.

Hypothesis: Mechanical forces are required to expose the cryptic Y925 binding site, allowing it to be phosphorylated by Src and thereby blocking cooperative paxillin binding.

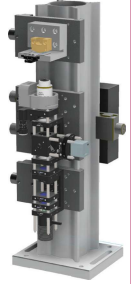
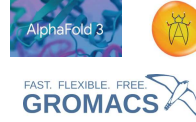
Methodology

Single Molecule Magnetic Tweezers (MT) Force Spectroscopy

- Use of tape head to apply forces upto 45 pN
- Resolution: ~1 ms, ~1 nm and <0.1 pN

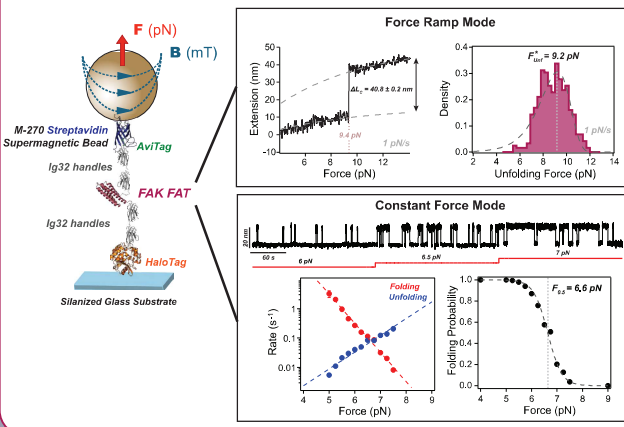
Molecular Dynamics (MD) Simulations

- Conventional MD simulations
- SMD force-extension simulations

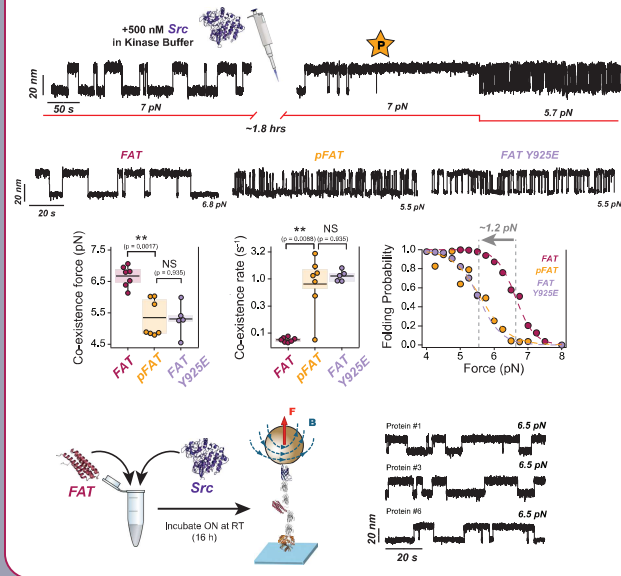


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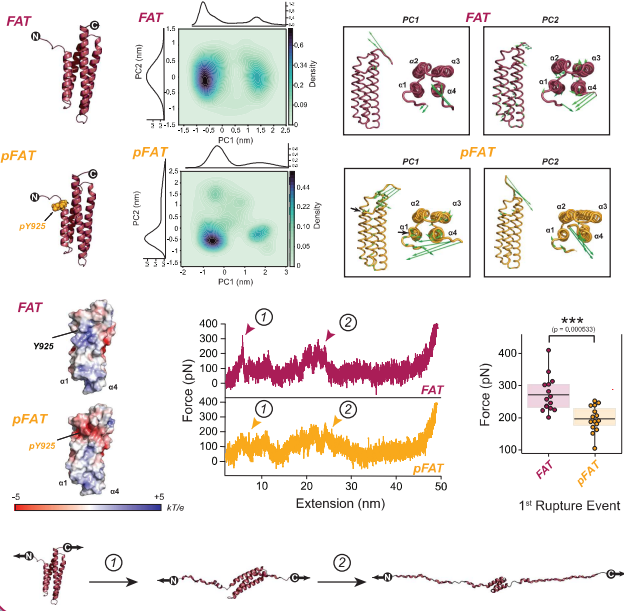
FAT Characterisation



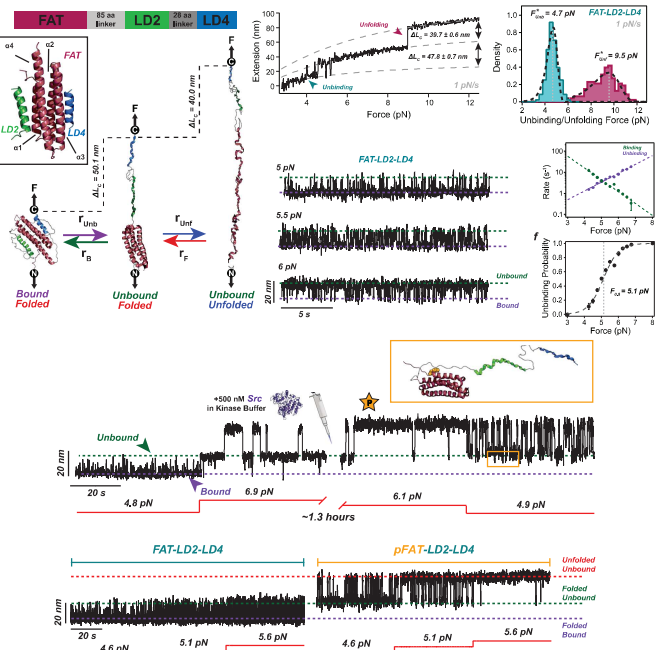
Force-induced FAT Phosphorylation



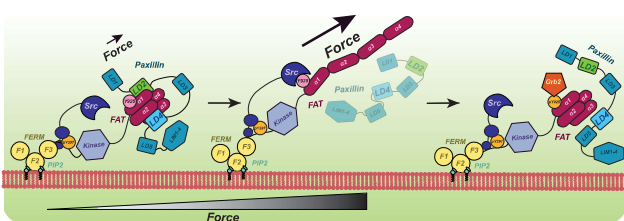
FAT and pFAT MD Simulations



FAT-LD2-LD4 Characterisation and Phosphorylation



Proposed mechanism for force-gated FAT phosphorylation



References

- [1] B. Deramondt, et al. FAK phosphorylation at Y925 regulates cross-talk between focal adhesion turnover and cell protrusion. *Mol Biol Cell* 22, 956-974 (2011)
- [2] J. Cooke et al. In vitro phosphorylation of the focal adhesion targeting domain of focal adhesion kinase by Src kinase. *Biochemistry*, 51, 2213-2223 (2012)
- [3] R. Tapia-Rojó et al. Enhanced statistical sampling reveals microscopic complexity in the talin mechanosensor folding energy landscape. *Nature Physics*, 19, 52-60 (2023)
- [4] R. Tapia-Rojó et al. Single-molecule magnetic tweezers to probe the equilibrium dynamics of individual proteins at physiologically relevant forces and timescales. *Nature Protocols*, 9, 1775-1806 (2024)

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