CaMKII protein supply for distinct conformations - Enabling early hit evaluation and rational drug design

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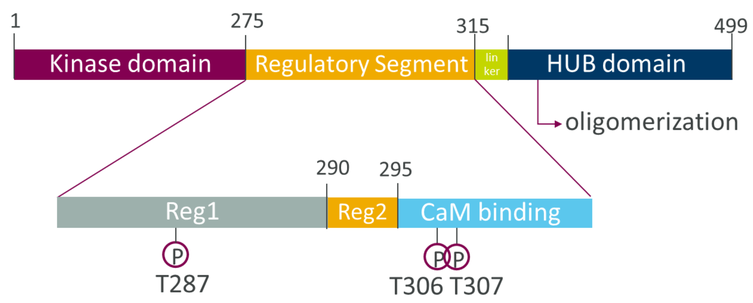
Abstract

Conformational changes regulating the activity of Ca^{2+}/calmodulin-dependent protein kinase II (CaMKII) make the design and optimization of small molecule inhibitors of the kinase more complex. Therefore a detailed characterization of the binding mode to different forms was aimed for. We describe here how protein supply for multiple inactive and active forms enabled those biophysical and structural studies.

Introduction

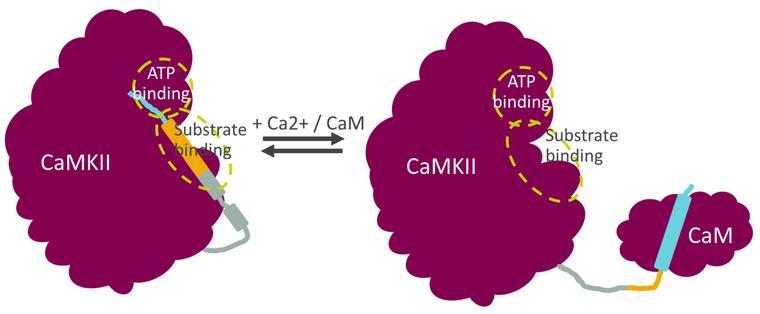
Domain organization



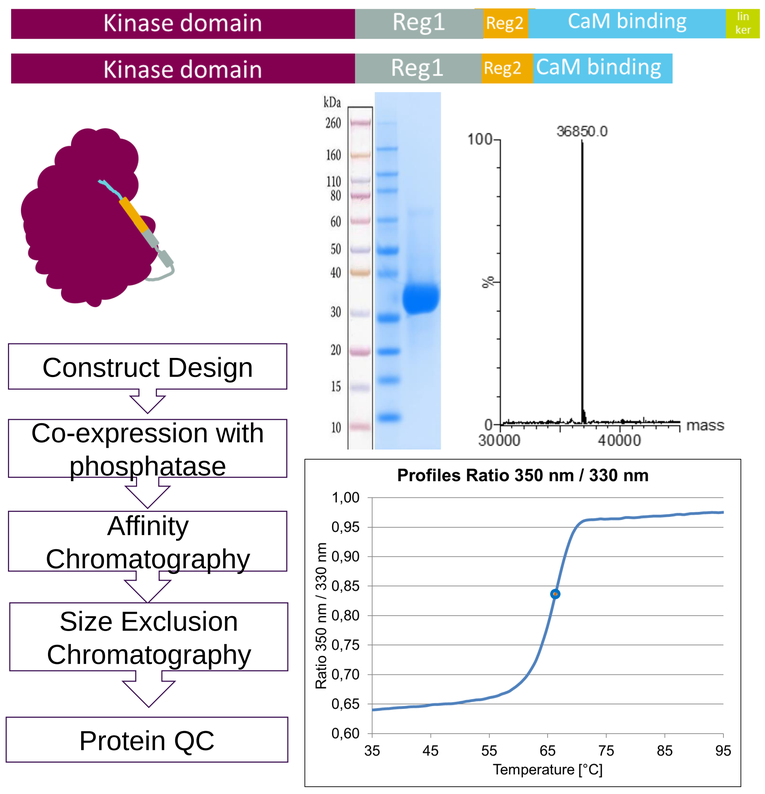
Protein status	Conformation / Activity
Unphosphorylated	Catalytic inactive conformation, CaM binding competent
T287 phosphorylated	autonomous active
T306/T307 phosphorylated	Catalytic inactive, CaM binding blocked
Ca^{2+} / CaM bound	Catalytic active

Activity of CaMKII is regulated by complex mechanisms[1] and involves major conformational changes[2]. In order to understand small molecule inhibitor interaction, we established protein supply for the different forms of CaMKII.

Conformational changes



Protein Supply Inactive Conformation

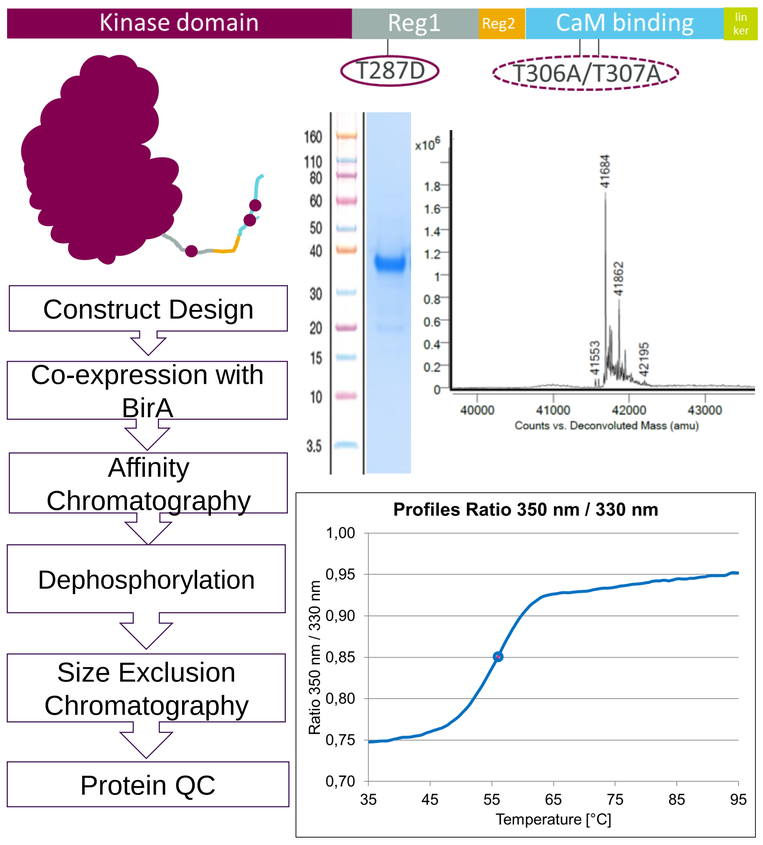


Recombinant protein from *E.coli* is inactive due to its phosphorylation status. Dephosphorylation of this protein enabled a highly productive structural campaign as well as SPR studies early in a drug development project.

Protein Supply Active Conformation

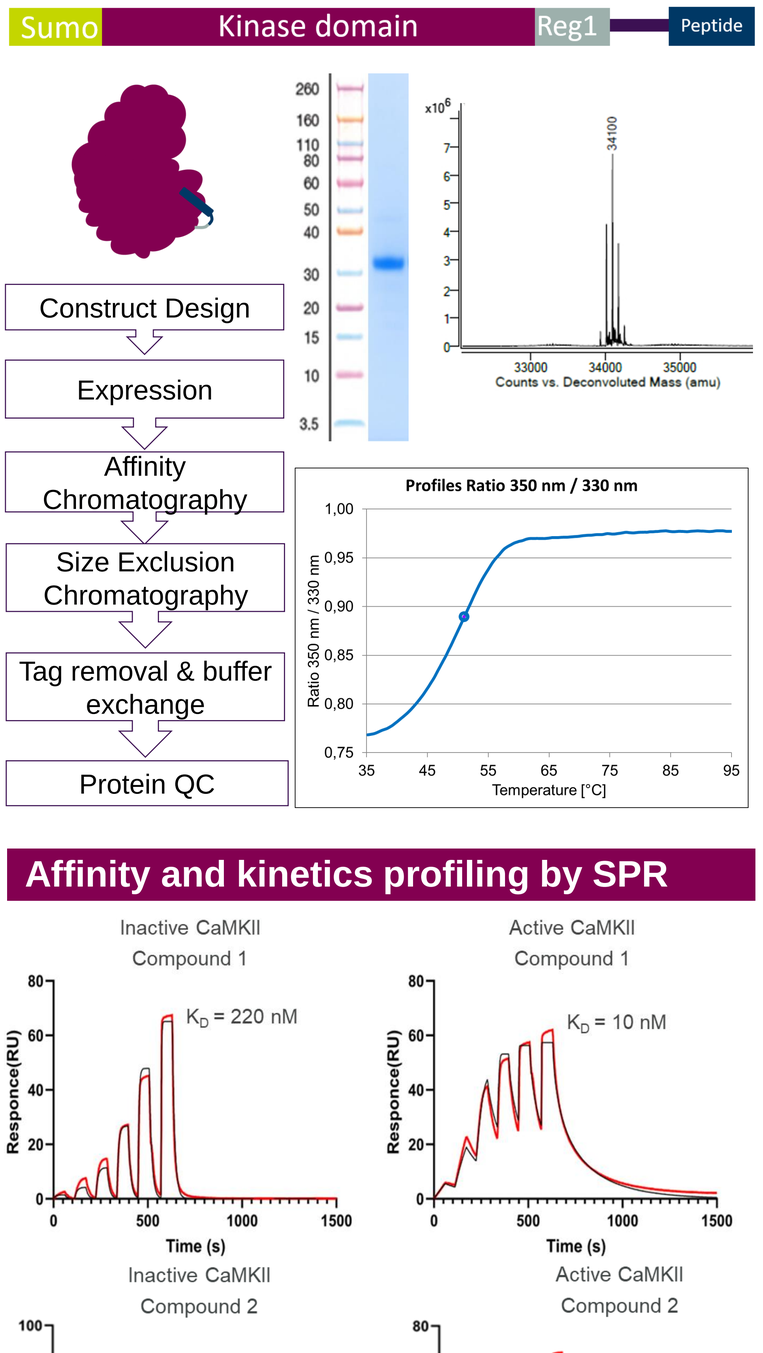
For Biophysics

Introducing a point mutation mimicking autophosphorylation of T287 results in autonomous kinase activity. The supply of this protein was key to kinetic profiling by SPR.

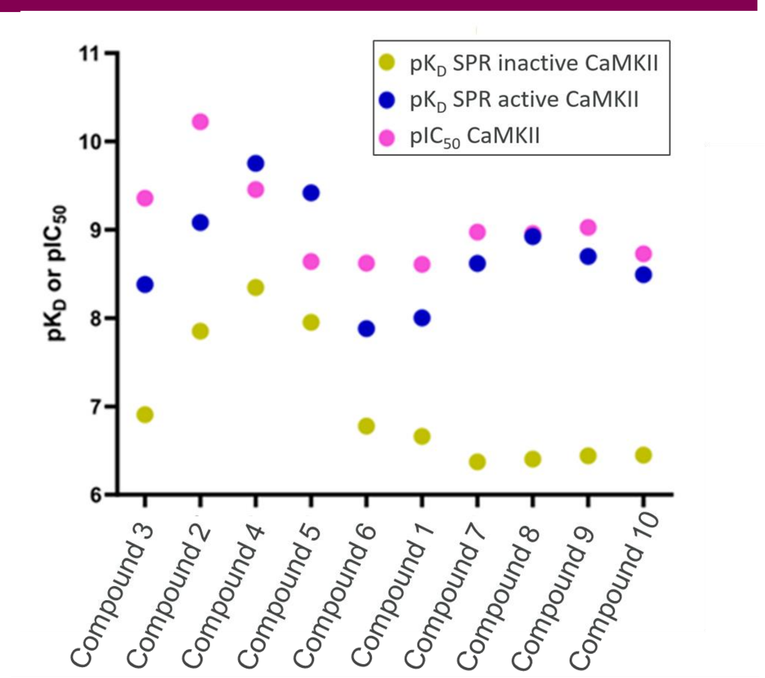


For Crystallography

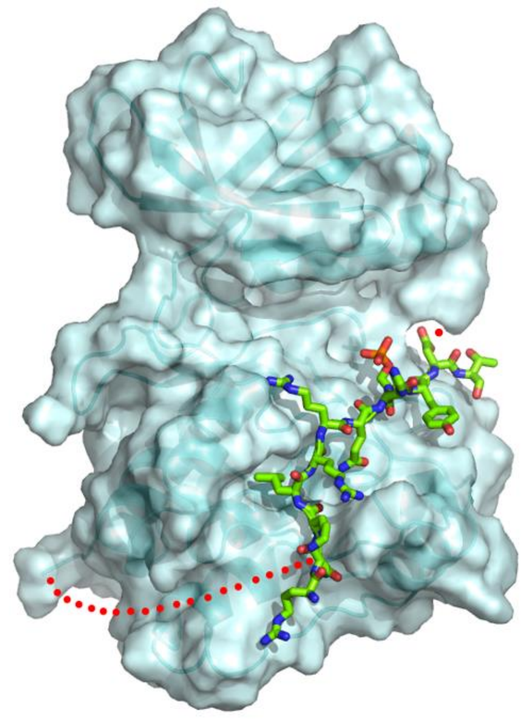
For a crystallizable active conformation, a shorter protein lacking the regulatory segment was needed. Production of this was possible by fusion of a solubilisation tag and a substrate peptide.



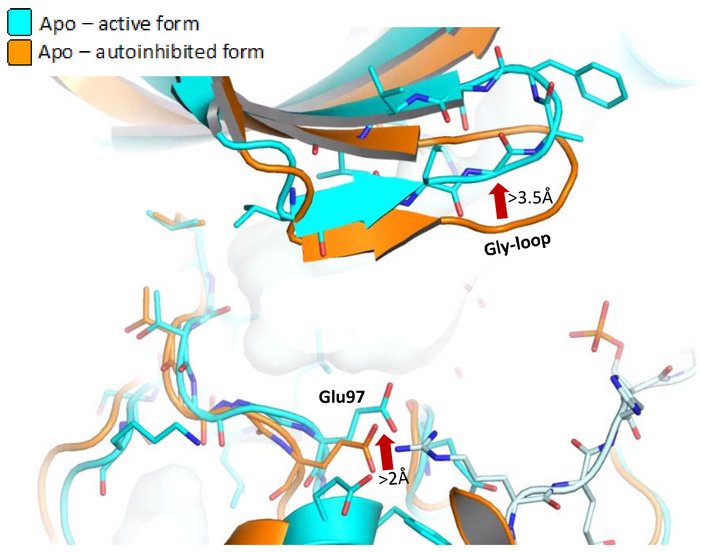
Affinity and kinetics profiling by SPR



Structural Biology



Fusion of a GluN2B peptide locks CaMKII in its active conformation.



Differences between active and inactive conformation like shifts of the Gly-loop and the D-helix affect ligand binding and are considered for inhibitor design.

Summary

Robust protocols for protein supply of inactive and active CaMKII were established and used for biophysical and structural studies. Correlation of observed binding mode in structures with binding kinetics in SPR are used for the design of potent and selective inhibitors for CaMKII.

References

1. Bayer et Schulman, Neuron, 2019, 103(3): 380-394
2. Rellos et al., PLoS Biol, 2010, 8(7) e1000426

Acknowledgements

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