Solution Dynamics of Human Guanylate Kinase, hGMPK

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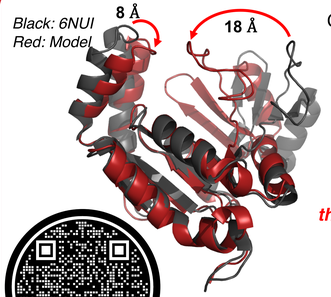
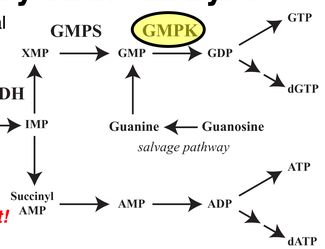
hGMPK: An Essential and Dynamic Enzyme



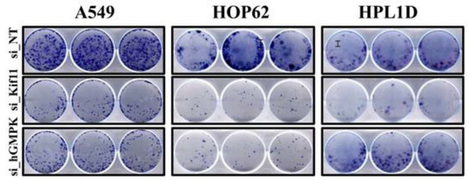
Scan to see GMPK in action!

Large amplitude rearrangement accompanies the reversible phosphorylation of GMP to GDP with ATP as a phosphoryl donor.

GMPK is essential to maintaining cellular GTP.

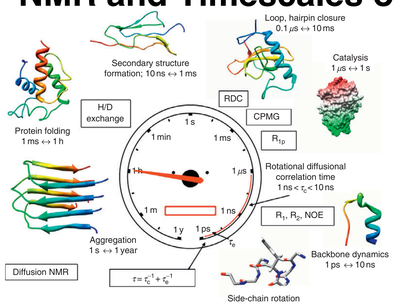


GMPK is a potential therapeutic target!

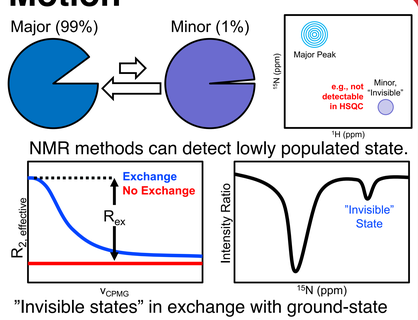


GMPK knockdown alters clonogenic potential of lung cancer cell lines but not HPL1D.

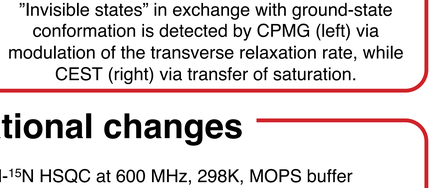
NMR and Timescales of Motion



NMR methods can probe the wide range of timescales of dynamics near physiological condition and at atomic resolution.



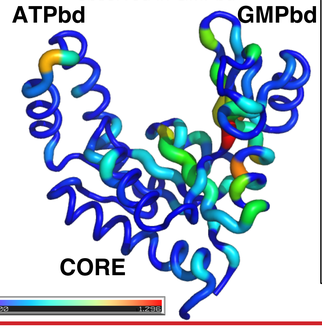
NMR methods can detect lowly populated state.



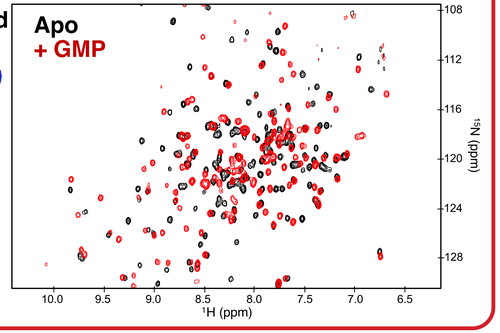
"Invisible states" in exchange with ground-state conformation is detected by CPMG (left) via modulation of the transverse relaxation rate, while CEST (right) via transfer of saturation.

GMP induces conformational changes

Higher chemical shift perturbation observed in GMPbd

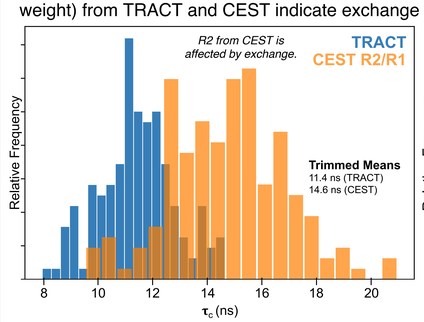


^{1}H-^{15}N HSQC at 600 MHz, 298K, MOPS buffer

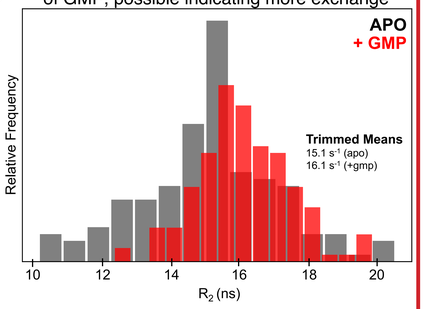


Relaxation parameters of hGMPK

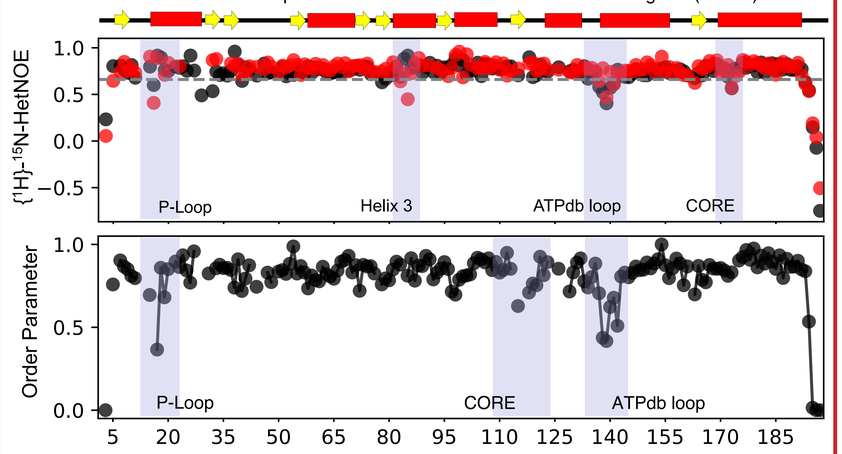
Correlation time (which is proportional to molecular weight) from TRACT and CEST indicate exchange

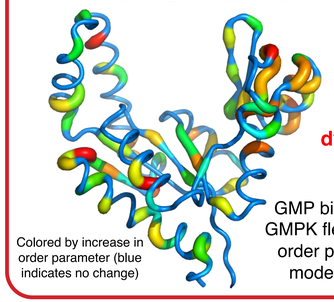


R_2 from CEST slightly increased upon addition of GMP, possible indicating more exchange



hetNOE and order parameters reveal fast motions and flexible regions (MOPS)



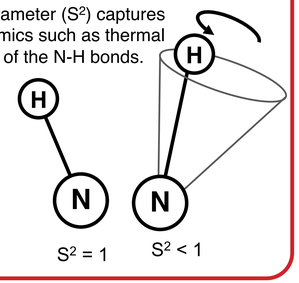


Colored by increase in order parameter (blue indicates no change)

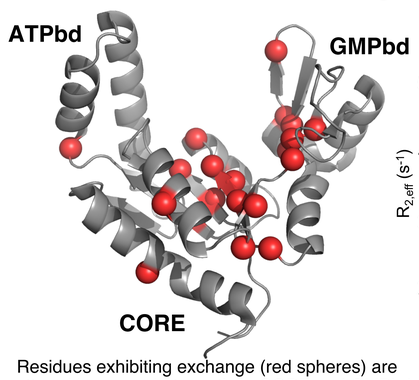
Role of internal dynamics in substrate recognition?

GMP binding increases GMPK flexibility based on order parameters from model-free analysis

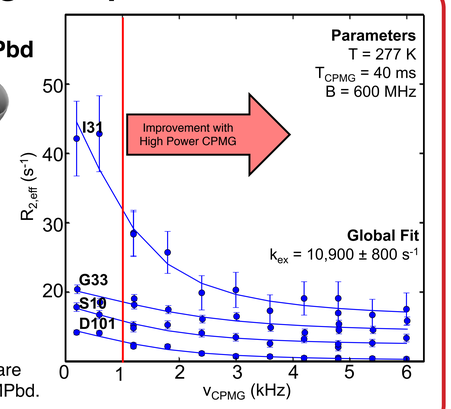
Order parameter (S^2) captures fast dynamics such as thermal motion of the N-H bonds.



CPMG detects exchange in apo hGMPK

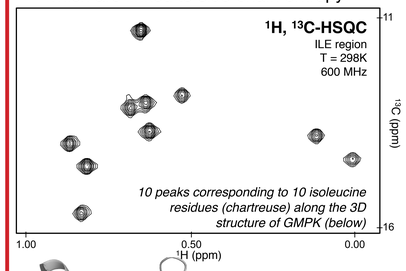


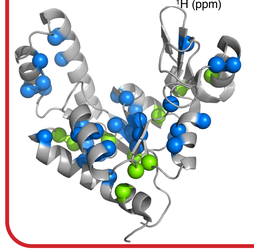
Residues exhibiting exchange (red spheres) are scattered in space, but mostly in CORE and GMPbd.

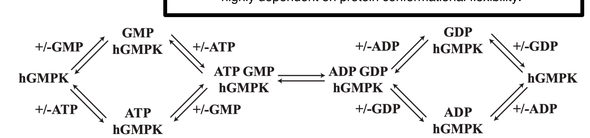


Future Directions

^{13}C labeling of methyl groups will allow for measurement of methyl order parameters which are linked to side-chain entropy.

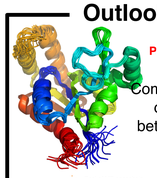






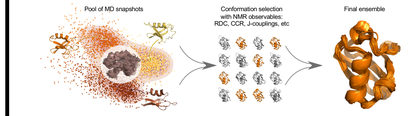
Relaxation parameters will be collected under various conditions (binary and ternary complexes) to understand the role of dynamics in catalysis. High-power CPMG and CEST will be applied to detect minor populations and conformational exchange.

Outlook and Significance



Proteins are dynamic, and their dynamic nature is critical to understanding their function.

Comprehensive characterization of GMPK dynamics will uncover relationships between timescales of motions and their role in catalysis and drug binding.



Functional ensembles can be generated from dynamics studies. These are relevant in drug discovery as drug binding kinetics is highly dependent on protein conformational flexibility.

Acknowledgements

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