

David Montpeyó i Garcia-Moreno, Universitat Autònoma de Barcelona

Introductory concepts

Reversible phosphorylation has been proved to be one of the most important posttranslational modifications which can occur to a protein. A huge family of proteins, the protein kinases, are the responsible of these reactions, and due to his biological importance is one of the most studied family of proteins. In particular, PKA is the best understood protein kinase.

Computational methods can be used to make an approach of the kinetic mechanisms for a chemical reaction catalysed by an enzyme. There are two main groups of models used in this purpose. Quantum mechanical models are based in the Schrödinger equation and bring information of the molecular structure, the energy and bonding of a molecule. On the other hand, molecular mechanics models are based on classical mechanics; they are more simple and fast, but don't bring information about bonding nor electron distribution. QM/MM models are used in the case of enzymes, approaching the active site kinetic mechanism through QM and the structural part of the protein through MM models.

PKA, such as the other protein kinases, exhibit two different mechanisms for the phosphoryl transfer step. In the associative path (fig. 1a) the transfer of the γ -phosphoryl group and a proton occurs between the ATP (donor) and the substrate (acceptor). The other mechanism, known as dissociative path (fig. 1b), γ -phosphoryl group is also transferred from ATP to the substrate, but Asp166 accepts the substrate proton.

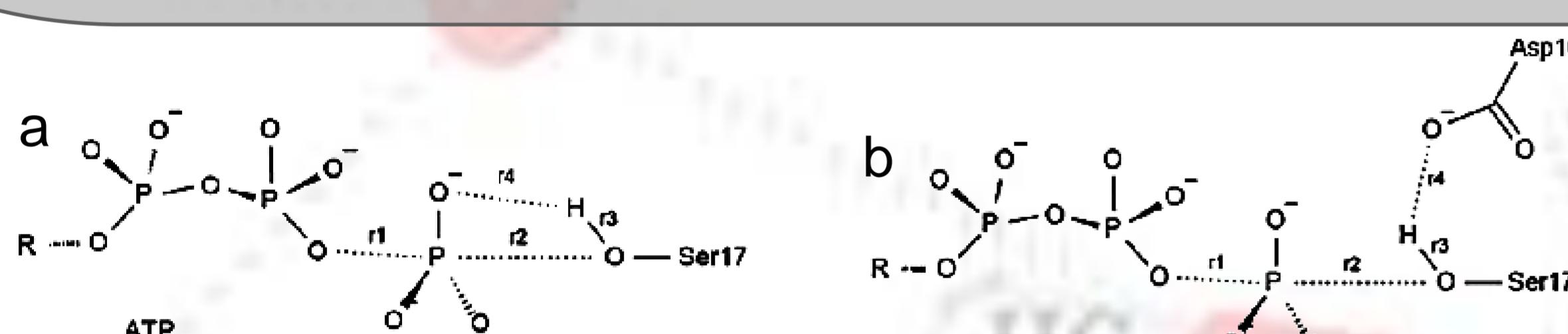
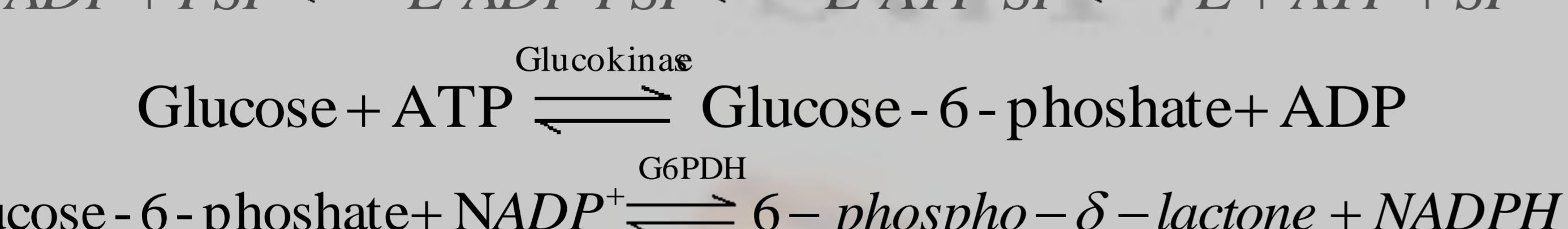
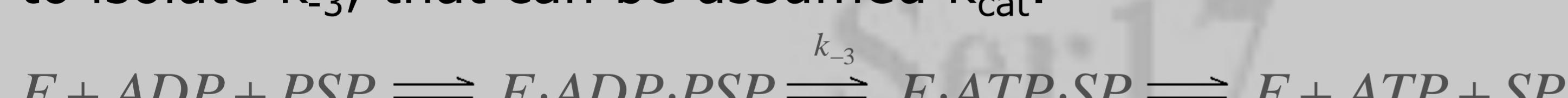


Fig. 1: Transition state of the γ -phosphoryl transfer step. a) Associative mechanism. b) dissociative mechanism.

Research project proposal

In order to determine the internal equilibrium constant for the phosphotransfer step, a V_o assay shall be done in order to isolate k_3 , that can be assumed k_{cat} .



NADPH formation in function of time can be monitored by absorbance variation at 340 nm. At different PSP concentrations we find different V_o , and k_{cat} can be calculated.

$$k_3 \approx k_{cat} = \frac{V_{max}}{[E]} \quad K_{eq \text{ int}} = \frac{k_3}{k_{-3}} \quad \Delta G_{PT} = -RT \ln K_{eq}$$

As in previous studies k_3 has been found to be about 500 s^{-1} , values of k_3 higher than 500 s^{-1} would corroborate an endergonic profile for the γ -phosphoryl group transfer.

Phosphotransfer thermodynamics

For the last decades the kinetic mechanism of PKA has been widely studied. One of the research lines on that field is focused on the kinetic mechanisms of the phosphoryl transfer step, through QM/MM modelling. It is still not clear whether the Gibbs free energy of this step is either positive or negative, as contradictory data exist on that aspect.

A 1993 [1] study suggested that this phosphotransfer step must exhibit an exergonic based on the fact that its internal equilibrium constant was assumed to be 100. This bibliographic review seeks to refute that hypothesis due to an erroneous procedure while calculating this constant, as the mentioned study did not proportion enough information to allow the internal equilibrium calculation.

More experimental research should be done to determine the equilibrium constant of that particular step, and a procedure is suggested on that purpose.

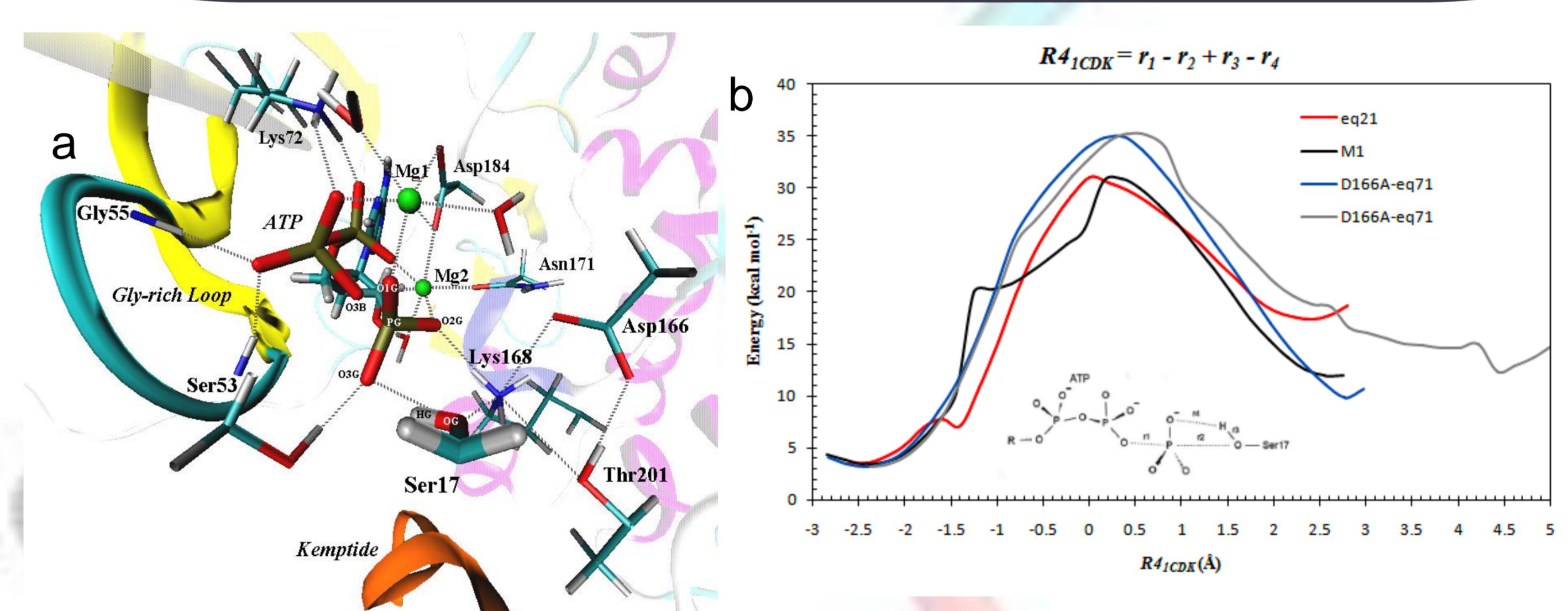


Fig. 2: a) Structure of the active site of the PKA catalytic domain. b) and c) Endergonic energy profile for γ -phosphoryl transfer step. b) is from Mireia Garcia et al. (unpublished), from Autonomous University of Barcelona and c) is from Gheng, Y. [2] et al. 2004.

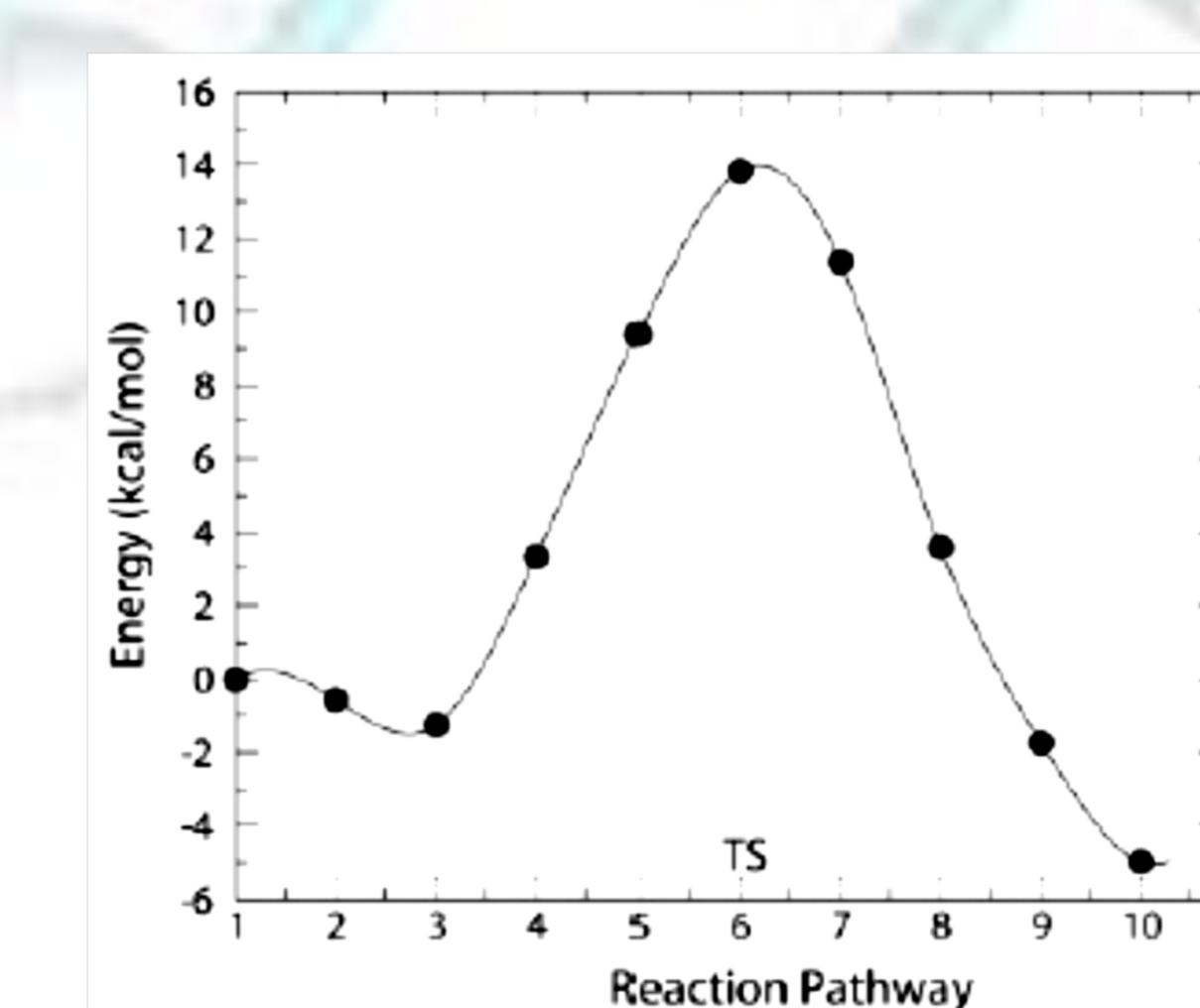


Fig. 3: Exergonic energy profile for γ -phosphoryl transfer step. [3]

Conclusions

It seems that there is no evidence to assure that the Gibbs free energy profile for the γ -phosphoryl group transfer reaction is clearly, contrarily to what was stated in 1993 [1] and 2007 [3] by Susan Taylor and her team. An experimental procedure is suggested to uncover such an unknown.

References

- [1] Adams, J. and Taylor, S. *Protein Science*, 1993. 2: 2177-2186
- [2] Cheng, Y. et al. *J. Am. Chem. Soc.* 2005. 127: 1553-1562
- [3] Valiev, M. et al. *J. Phys. Chem.* 2007. 111: 13455-13464