Investigating the allosteric regulation of the protein kinase A regulatory domain using time-resolved X-ray crystallography

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Protein kinases play a crucial role in mediating precise cellular responses to extracellular stimuli through phosphorylation of various substrates [1]. Consequently, protein kinase regulation is highly controlled within cells and has significant relevance to many important cellular processes [2]. Protein kinase A (PKA) is a prominent protein kinase, participating in the cyclic adenosine monophosphate (cAMP) signalling cascade [3]. The regulatory subunit of the PKA complex suppresses PKA activity in the absence of cAMP and activates PKA in the presence of cAMP. Regulation is achieved through conformational changes in the regulatory domain caused by cAMP binding [4].

However, in-depth understanding of how cAMP binding causes large conformational changes in the PKA regulatory subunit is unknown. Time-resolved X-ray crystallography is an ideal technique to expose such details by revealing the intermediate states of the conformational change. We aim to facilitate this by conducting soaking experiments using isolated regulatory subunit and a cAMP analogue, Rp, which promotes similar conformational changes upon binding [5]. The insights gained from these experiments could apply to many other kinase regulatory domains.

Currently, we have carried out a control cAMP soaking experiment, collecting data at SLS (Fig. 1). This initial experiment revealed cAMP soaking unsuccessful due to steric clashing with Tyr205 of another regulatory subunit because of crystal packing. Consequently, we have mutated Tyr205 to Ala205, and plan to repeat the soaking experiment.

**Figure 1.** Molecular model of PKA regulatory subunit. The cAMP binding pocket (yellow) is depicted containing glycerol. The tyrosine from another regulatory subunit (blue) packs into the substrate-binding region.