Constraining the kinetic parameters of signaling pathways through molecular-level modelling: isoform specific regulation of adenylyl cyclase.

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Background or Purpose
The enzyme adenylyl cyclase (AC) is a key actor in the signaling pathways that drive the cellular response to external stimuli. AC catalyzes the conversion of adenosine triphosphate (ATP) into cyclic AMP (cAMP), which acts as a secondary messenger within the cell. The ten known mammalian isoforms of AC (the membrane bound ACs 1 – 9, and soluble AC10) exhibit differing regulation by various effectors (Sadana and Dessauer, 2009).

The isoform AC5 is found within medium spiny neurons in the striatum, where, in response to activation of the D1 dopamine receptor, its activity is stimulated by Gαolf (Zhuang, 2000), while activation of the M4 muscarinic receptor results in its inhibition by Gαi. The downstream effects of intracellular cAMP concentration within neurons play an important role in reinforced learning, and it is thought that a better understanding of the processes involved could lead to novel therapies for the treatment of addiction.

In this work we use molecular modelling techniques to identify whether the interactions of Gαolf and Gαi with AC5 are competitive or non-competitive, and discuss the results in the context of constraining the parameters used in biochemical network models. Future applications of molecular modelling will also be discussed.

Methods
A combined molecular modelling and bioinformatics approach was employed to investigate the interaction of Gαi with AC5. Structural models of mouse AC isoforms 1 – 9 were generated using homology modelling and the electrostatic environment surrounding these proteins was compared. Protein Interaction Property Similarity Analysis (PIPSA) (Wade et al, 2001) allows a quantitative comparison of the interaction properties of proteins. Using this approach, along with the known isoform specific inhibition of AC1 AC5 and AC6 by Gαi (Sadana and Dessauer, 2009), we were able to identify the likely site of interaction of Gαi with these isoforms.

Results
PIPSA analysis of all membrane bound isoforms of AC identified three highly conserved regions of electrostatic similarity, namely the ATP binding pocket, the membrane facing exterior, and the Gαolf binding groove of the cytosolic C2 domain of AC. The high conservation of electrostatic environment surrounding the Gαolf binding site, suggests that the isoform specific regulation of AC by Gαi can not be explained by the altered affinity of Gαi to this region across different isoforms. In contrast, the pseudo-symmetric position on the C1 domain of AC was found to have conserved electrostatic properties within AC1, AC5 and AC6, which show divergence to all other isoforms.

Conclusions
The highly similar electrostatic environment surrounding the Gαolf binding site of each AC isoform suggests that Gαi does not inhibit AC through direct competition to Gαolf binding. Instead, our results imply that Gαi binds to an alternative position, remote to the Gαolf binding groove, suggesting the possibility of non-competitive inhibition.

References
