The p53-Mdm2 interaction: predicting helix mimetic side-chain modifications likely to increase inhibitor affinity

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Inhibitors targeting protein-protein interactions could theoretically be used to produce high-specificity pharmaceuticals with few side effects. Alpha-helices often participate in protein-protein interactions and where the interacting residues are all on one face of the helix it may be possible to mimic the helix structure using a rigid synthetic molecule (Wilson, 2009). Side chains of the inhibitor replace those of the natural helix. Knowing how best to change these side chains so as to optimize affinity and specificity is therefore of considerable importance.

The expense and time required for high-throughput screening makes it inaccessible to many academic researchers. We are developing a generic, *in silico* approach to side chain selection. Taking the structure of an inhibitor bound to the protein of interest as a starting point, the structure-based method uses ultra-high-throughput screening, clustering and docking to extract from a library of commercially available molecules compounds which could be used to add desirable side chains. Purchasable compounds are input in Daylight SMILES (2008) format. We have used the ZINC database (http://zinc.docking.org/) of Irwin and Shoichet (2005). Filter (OpenEye) is used with Daylight SMRIKS-based (2008) selection criteria to identify molecules compatible with the synthetic protocol of the inhibitor. Three-dimensional structures of these molecules are generated using Omega (OpenEye) and are then cleaved by ReCore (BioSolveIT) (Maass *et al.*, 2007) to yield the structures of the side chains that each molecule could be used to add to the inhibitor core scaffold. Ultra-high-throughput screening with ReCore removes side chains with no chance of fitting into the binding site. FlexX (BioSolveIT) (Gastreich *et al.*, 2006) then docks each side chain into the target protein to yield a score. A novel clustering method based on the k-means algorithm (see MacKay (2003)) has been developed which can be used to reduce the number of molecules which must be docked. We are also investigating using the structures of known inhibitors as part of the scoring and selection process.

Preliminary testing has focused on the interaction between the tumour suppressor protein p53 and the E3 ubiquitin ligase Mdm2. Mdm2 regulates p53 expression by binding to the alpha-helical p53 transactivation domain. p53 normally triggers cell cycle arrest or apoptosis in response to cellular stress but aberrant upregulation of Mdm2, which has been observed in roughly 7% of human tumours, causes this control to be lost (Murray and Gellman, 2007).

At the University of Leeds, a novel, robust method of p-oligobenzamide synthesis has been developed that enables a large variety of different side chains to be tested in many different combinations (Plante *et al.*, 2008). Following synthesis, oligobenzamides with side chains predicted by *in silico* methods to increase affinity will be tested by means of a Förster resonance energy transfer (FRET) assay using the yellow and green fluorescent protein hybrid proteins p53-YFP and Mdm2-GFP (Plante *et al.*, 2009).

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