The main stages in drug discovery research are (a), the identification of small molecules that bind to the target (hit id), (b), characterisation of how the hits bind to the target, developing ideas for how the hits may be improved (hits to leads) and (c), the optimisation of the hits to have suitable drug-like properties (lead optimisation). Over the past ten years, the determination of protein structures by X-ray crystallographic methods has had an increasing impact on all three of these stages. Such crystal structures provide exquisite detail on the binding site and mode of interaction. However, this is a necessarily static image of the protein-ligand interaction. It can also take some effort to obtain a suitable protein-ligand complex, so crystallography is not a routine method for compound screening. A series of complementary biophysical methods based on NMR spectroscopy or Surface Plasmon Resonance (SPR) have been developed to support drug discovery. These include screening of compounds (including low molecular weight fragments) using SPR or NMR, locating the binding site (and sometimes orientation of binding) of ligands using NMR and characterising the kinetics (on and off rates) of binding using SPR. In this presentation, we will discuss the use of a range of biophysical methods in support of drug discovery research. This will include examples of fragment screening, binding to large protein-protein interaction targets and using on and off rates to rationalise the different in vivo properties of two structurally similar compound series.

Keywords: drug discovery, NMR spectroscopy, surface plasmon resonance

Flavopiridol binding to P-TEFb (CDK9/cyclin T1)

Flavopiridol is currently in Phase II clinical trials for the treatment of chronic lymphocytic leukemia, the most common of the leukemias. Flavopiridol, derived from a compound originally isolated from the bark of a tree Dioscyxylum bineciferum that grows in East Asia, was recognised as having anti-cancer properties as long ago as 1992. It appeared to be a non-specific inhibitor of the cyclin dependent protein kinases (CDKs). More recent work has demonstrated that flavopiridol is a potent inhibitor of P-TEFb, CDK9/cyclin T1, with Ki 3 nM, >10 more potent than its activity against other CDKs. CDK9 regulates transcription through modifications of transcriptional repressors and the C-terminal tail of RNA polymerase. Flavopiridol inhibits transcription therby leading to a decrease in the mRNA levels of many proteins involved in growth and signal transduction that have short lifetimes, including several antiapoptotic proteins. Following improvements in administration protocols to overcome scavenging by serum, flavopiridol has given encouraging results in clinical trials. We have recently solved the structure of CDK9/cyclin T1 in complex with flavopiridol. Flavopiridol binds to the ATP site of CDK9 and induces unanticipated structural rearrangements in the glycine rich loop of the protein kinase that bury the inhibitor. These provide a rationale for the strong inhibition of CDK9. Comparison of the mode of action with those for other protein kinase inhibitors will be discussed.

Keywords: flavopiridol, CDK9, protein kinase structure