

A COMBINED STD-NMR SPECTROSCOPY/MOLECULAR MODELING PROTOCOL FOR STUDYING PROTEIN-LIGAND COMPLEXES

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A protocol for studying protein-ligand complexes by the combined use of Saturation Transfer Difference (STD)-NMR spectroscopy, molecular modeling, and CORCEMA-ST calculations will be described. Several protein-ligand pairs were used to develop the final protocol. Binding of the known glycosidase inhibitors kifunensine and salacinol to Golgi α -mannosidase II was studied by STD-NMR spectroscopy. Docking (AutoDock) in the active site of the enzyme (obtained by X-ray crystallography) followed by calculation of STD effects with CORCEMA-ST gave excellent correlation with the experimental data for the non-mobile portions of the ligands. Excellent correspondence with the X-ray crystal structures of the complexes obtained independently was also observed. Secondly, a system for which the crystal structure of the complex is not known was tested. UDP-galactopyranose mutase (UGM), the key enzyme involved in the biosynthesis of Gal f , in complex with its substrate UDP-Galp and its inhibitor UDP, was solved using the combined protocol. Excellent correlation between experimental and calculated STD effects was obtained. The results are consistent with insights gained from X-ray crystallographic analysis of the UGM structure alone and previous mechanistic studies, thus validating the model for the UGM- UDP-Galp complex and lending credence to the protocol.

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