# Drug Design Against Shifting Targets: A Structural Basis for Drug Resistance to a Influenza Virus Neuraminidase Variant

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Inhibitors of the influenza virus neuraminidase have been shown to be effective antiviral agents in man. Several studies have reported the selection of novel influenza strains when the virus is cultured with neuraminidase inhibitors in vitro. These resistant viruses have mutations either in the neuraminidase or in the viral haemagglutinin. Inhibitors, in which the glycerol side chain at the 6-position of Neu5Acen has been replaced by carboxamide-linked hydrophobic substituents, have recently been reported and shown to select neuraminidase variants.

The neuraminidase variant R292K modifies one of three arginyl residues which encircle the carboxylate group of the substrate. The structure of this variant with the carboxamide inhibitor used for its selection, and with other Neu5Ac2en analogues, is reported here at high resolution. Structural consequences of the mutation correlate with altered inhibitory activity of the compounds compared to wild-type neuraminidase.

The R292K variant of influenza neuraminidase affects the binding of substrate by modification of the interaction with the substrate carboxylate. This may be one of the structural correlates of the reduced enzyme activity of the variant. Inhibitors which have replacements for the glycerol at the 6-position are further affected in the R292K variant because of structural changes in the binding site which apparently raise the energy barrier for the conformational change in the enzyme required to accommodate such inhibitors. These data provide evidence that a general strategy for drug design when the target has a high mutation frequency is to stay as close as possible to the natural ligands of the target.