# 05-Molecular Modelling and Design for Proteins and Drugs

The influenza virus neuraminidase is a surface antigen of the virus. Its best characterised role in the life cycle of the virus is facilitating the release of progeny virions from the surface of infected cells. Inhibitors of neuraminidase do not prevent infection or single cycle replication of virus in tissue culture. However they do prevent multi-cycle replication and could therefore be expected to have an effect on the course of the infection in animals.

Based upon the three-dimensional structures of neuraminidases from different strains of human and animal influenza viruses, and of their complexes with substrate (sialic acid) and putative transition state analogues, a number of tightly binding inhibitors of the enzyme have been designed and synthesised. The structures of these compounds complexed to the enzyme have been determined, and show that the designed molecules generally bind as predicted by the design process. The compounds show antiviral activity in an animal model of influenza.

**PS–05.03.10** THE DESIGN OF POTENTIAL DRUGS FOR THE TREATMENT OF DIABETES: A QSAR STUDY. K.A. WOODS*, L.N. JOHNSON, *Laboratory of Molecular Biophysics, University of Oxford, Rex Richards Building, South Parks Road, Oxford OX1 3QU, England;* G. CRUCIANI, *Laboratorio di Chemiometria, Dipartimento di Chimica, Universite di Perugia, Via Eece di Sotto 10, Perugia, Italy.*

The primary goal in any drug design project is to predict the activity of new compounds. Design methods have evolved to study the comparative properties of ligands.

The 3-dimensional structure of the receptor is often not known and information regarding ligand-receptor interactions is therefore unavailable. In such cases a method for finding relationships between the ligands is known as Principal Component Analysis (PCA) (Wold, S., Esbensen, K., Geladi, P., Chemometrics and Intelligent Laboratory Systems, 1987, 2, 37-52). PCA is used to build a model that describes how certain selected physical or chemical properties are interrelated. This results in a clustering of similar and different ligands which may be useful in the search for new compounds by indicating regions of structure that have not been previously explored.

When the activity coefficients for a series of ligands are known, a useful drug-design method involves the determination of Quantitative Structure/Activity Relationships (QSAR) (Martin, Y., *Quantitative Drug Design*, 1978, Marcel Dekker, New York). A new computer-aided QSAR methodology employs Generating Optimal Linear PLS (Partial Least-Squares) Estimations (GOLPE) (Baroni, M., Costantino, G., Cruciani, G., accepted to Quantitative Structure-Activity Relationships, 1992), an advanced procedure aimed at obtaining optimal PLS regression models with a very high predictive ability.

We have used a PCA and QSAR/GOLPE study to explore and predict new compounds in a search for an inhibitor of glycogen phosphorylase. We report the results of such a study based on a selected database of inhibitors of glycogen phosphorylase, where both the 3-dimensional structure of the ligand bound complexes and the kinetic coefficients of binding are known.

**PS–05.03.11** THE STRUCTURES AND BIOLOGICAL PROPERTIES OF THREE INSULIN MUTANTS

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Diabetics may benefit in the near future from the mutant insulins prepared by genetic engineering, which have long and rapid-acting effects. The site-specific mutagenesis of individual amino acids has an important role in altering molecular assembly, increasing their biological activity and improving the therapeutic properties. The human insulin mutants (1) HB10D/PB28D, (2) TA8H/SB9D/TB27E and (3) HB10D were designed for the above purpose. Their associative states deduced from osmometry are 1.6, 1.1 and 2.2 respectively at 1mM of concentration, and (1) and (3) have double the biological potence of human insulin. Their crystal structures have been determined by X-ray analysis, the solutions have been obtained using the molecular replacement methods. The crystallographic parameters are listed below:

| mutant | space group | cell parameters | Reso(Å) | R-f |
|---|---|---|---|---|
| (1) | C2 | a=66.0 b=46.5 c=45.0 β=128.79° | 2.1 | 0.19 |
| (2) | $P2_12_12_1$ | a=b=51.9 c=89.7 | 2.0 | 0.20 |
| (3) | $P2_13$ | a=b=c=113.2 | 3.0 | 0.21 |

There are two molecules in the asymmetric unit of structure (1) which are organised in the crystal as dimers disposed about the two fold crystal axis. The roles of B10D and B28D in the dissociation of insulin and in the interactions of insulin with its receptor will be discussed.

Surprisingly, structure (2) which was designed as a monomeric insulin, crystallised as a dimer with two dimers in the asymmetric unit. This unexpected aggregation state may be explained by the high concentration of (2) used to grow crystals. The details of the affects of the mutated residues on the molecular stability and the receptor binding sites will be given.

Structure (3) has an unusual aggregation of six dimers forming a dodecamer through zinc coordination with B5 His imidazoles around the three fold crystallographic axis. The implication of producing a more stable aggregation state opens a new possibility for combining traditional pharmaceutical formulation and protein engineering to alter insulin's physico-chemical properties.

**PS–05.03.12** GLUCOSE ANALOGUE INHIBITORS OF GLYCOGEN PHOSPHORYLASE: THE DESIGN OF POTENTIAL DRUGS FOR DIABETES. K.A. WOODS*, E.P. MITCHELL, L.N. JOHNSON, *Laboratory of Molecular Biophysics, Rex Richards Building, University of Oxford, South Parks Road, Oxford, England;* G.W.J. FLEET, M. ORCHARD, J.C. SON, C. BICHARD, *Dyson Perrins Laboratory, University of Oxford, South Parks Road, Oxford, England;* N. OIKONOMAKOS, D. LEONIDAS and A. PAPAGEORGIU, *The National Hellenic Foundation, 48, Vas. Constantinou Avenue, Athens, Greece.*

Glucose is known to be a weak inhibitor of glycogen phosphorylase (GP) and helps to control blood glucose levels by binding to phosphorylase at the catalytic site, resulting in a conformational change which stabilises the inactive T state of the enzyme, and promotes glycogen synthesis. It has therefore been suggested that inhibition of GP by glucose-analogues may help shift the balance, between glycogen synthesis and glycogen degradation, in favour of glycogen synthesis in both the muscle and liver. By exploiting the knowledge of the crystal structures of T state rabbit muscle GP*b* and the glucose-enzyme complex it is hoped to design an inhibitor that will be more effective than glucose.

  