1.3.4.3.5. *Neurological disorders*

Even a quick glance at Table 1.3.4.5 shows that crystallography contributes to new therapeutics for numerous human afflictions and diseases. Yet there are major gaps in our understanding of protein functions, in particular of those involved in development and in neurological functions. These proteins are the target of many drugs obtained by classical pre-crystal-structure methods. These proven drug targets are very often membrane proteins involved in neuronal functions, and the diseases concerned are some of the most prevalent in mankind. A non-exhaustive list includes cerebrovascular disease (strokes), Parkinson's, epilepsy, schizophrenia, bipolar disease and depression.

Some of these diseases are heart-breaking afflictions, where parents have to accept the suicidal tendencies of their children, often with fatal outcomes; where partners have to endure the tremendous mood swings of their bipolar spouses and have to accept extreme excesses in behaviour; where a happy evening of life is turned into the gradual and sad demise of human intellect due to the progression of Alzheimer's, or to the loss of motor functions due to Parkinson's, or into the tragic stare of a victim of deep depression. Human nature, in all its shortcomings, has the tendency to try to help such tragic victims, but drugs for neurological disorders are rare, drug regimens are difficult to optimize and the commitment to follow a drug regimen – often for years, and often with major side effects – is a next to impossible task in many cases. New, better drugs are urgently needed and hence the structure determinations of the 'molecules of the brain' are major scientific as well as medical challenges of the next decades. Such molecules will shed light on some of the deepest mysteries of humanity, including memory, cognition, desire, sleep *etc.* At the same time, such structures will provide opportunities for treating those suffering from neurodegenerative diseases due to age, genetic disposition, allergies, infections, traumas and combinations thereof. Such 'CNS protein structures' are one of the major challenges of biomacromolecular crystallography in the 21st century.

1.3.4.4. *Drug metabolism and crystallography*

As soon as a drug enters the body, an elaborate machinery comes into action to eliminate this foreign and potentially harmful molecule as quickly as possible. Two steps are usually distinguished in this process: phase I metabolism, in which the drug is functionalized, and phase II metabolism, in which further conjugation with endogenous hydrophilic molecules takes place, so that excretion *via* the kidneys can occur. Whereas this 'detoxification' process is essential for survival, it often renders promising inhibitors useless as drug candidates. Hence, structural knowledge of the proteins involved in metabolism could have a significant impact on the drug development process.

Thus far, only the structures of a few proteins crucial for drug distribution and metabolism have been elucidated. Human serum albumin binds hundreds of different drugs with micromolar dissociation constants, thereby altering drug levels in the blood dramatically. The structure of this important carrier molecule has been solved in complex with several drug molecules and should one day allow the prediction of the affinity of new chemical entities for this carrier protein, and thereby deepen our understanding of the serum concentrations of new candidate drugs (Carter & Ho, 1994; Curry *et al.*, 1998; Sugio *et al.*, 1999). Human oxidoreductases and hydrolases of importance in drug metabolism with known structure are: alcohol dehydrogenase (EC 1.1.1.1) (Hurley *et al.*, 1991), aldose reductase (EC 1.1.1.21) (Wilson *et al.*, 1992), glutathione reductase (NADPH) (EC 1.6.4.2) (Thieme *et al.*, 1981), catalase (EC 1.11.1.6) (Ko *et al.*, 2000), myeloperoxidase (EC 1.11.1.7) (Choi *et al.*, 1998) and beta-glucuronidase (EC 3.2.1.31) (Jain *et al.*, 1996). Recently, the first crystal structure of a mammalian cytochrome P-450, the most important class of xenobiotic metabolizing enzymes, has been reported (Williams *et al.*, 2000).

Of the conjugation enzymes, only glutathione S-transferases (EC 2.5.1.18) have been characterized structurally: A1 (Sinning *et al.*, 1993), A4-4 (Bruns *et al.*, 1999), MU-1 (Patskovsky *et al.*, 1999), MU-2 (Raghunathan *et al.*, 1994), P (Reinemer *et al.*, 1992) and THETA-2 (Rossjohn, McKinstry *et al.*, 1998). Tens of structures await elucidation in this area (Testa, 1994).

1.3.4.5. *Drug manufacturing and crystallography*

The development of drugs is a major undertaking and one of the hallmarks of modern societies. However, once a safe and effective therapeutic agent has been fully tested and approved, manufacturing the compound on a large scale is often the next major challenge. Truly massive quantities of penicillin and cephalosporin are produced worldwide, ranging from 2000 to 7000 tons annually (Conlon *et al.*, 1995). In the production of semi-synthetic penicillins, the enzyme penicillin acylase plays a very significant role. This enzyme catalyses the hydrolysis of penicillin into 6-aminopenicillanic acid. Its crystal structure has been elucidated (Duggleby *et al.*, 1995) and may now be used for protein-engineering studies to improve its properties for the biotechnology industry. The production of cephalosporins could benefit in a similar way from knowing the structure of cephalosporin acylase (CA), since the properties of this enzyme are not optimal for use in production plants. Therefore, the crystal structure determination of CA could provide a basis for improving the substrate specificity of CA by subsequent protein-engineering techniques. Fortunately, a first CA structure has been solved recently (Kim *et al.*, 2000), with many other structures expected to be solved essentially simultaneously. Clearly, crystallography can be not only a major player in the design and optimization of therapeutic drugs, but also in their manufacture.

1.3.5. Vaccines, immunology and crystallography

Vaccines are probably the most effective way of preventing disease. An impressive number of vaccines have been developed and many more are under development (National Institute of Allergy and Infectious Diseases, 1998). Smallpox has been eradicated thanks to a vaccine, and polio is being targeted for eradication in a worldwide effort, again using vaccination strategies. To the best of our knowledge, crystal structures of viruses, viral capsids or viral proteins have not been used in developing the currently available vaccines. However, there are projects underway that may change this.

For instance, the crystal structure of rhinovirus has resulted in the development of compounds that have potential as antiviral agents, since they stabilize the viral capsid and block, or at least delay, the uncoating step in viral cell entry (Fox *et al.*, 1986). These rhinovirus capsid-stabilizing compounds are, in a different project, being used to stabilize poliovirus particles against heat-induced denaturation in vaccines (Grant *et al.*, 1994). This approach may be applicable to other cases, although it has not yet resulted in commercially available vaccine-plus-stabilizer cocktails. However, it is fascinating to see how a drug-design project may be able to assist vaccine development in a rather unexpected manner.

Three-dimensional structural information about viruses is also being used to aid in the development of vaccines. Knowledge of the architecture of and biological functions of coat proteins has been used to select loops at viral surfaces that can be replaced with antigenic loops from other pathogens for vaccine-engineering purposes (*e.g.* Burke *et al.*, 1988; Kohara *et al.*, 1988; Martin *et al.*, 1988; Murray *et al.*, 1998; Arnold *et al.*, 1994; Resnick *et al.*,

1995; Smith *et al.*, 1998; Arnold & Arnold, 1999; Zhang, Geisler *et al.*, 1999). The design of human rhinovirus (HRV) and poliovirus chimeras has been aided by knowing the atomic structure of the viruses (Hogle *et al.*, 1985; Rossmann *et al.*, 1985; Arnold & Rossmann, 1988; Arnold & Rossmann, 1990) and detailed features of the neutralizing immunogenic sites on the virion surfaces (Sherry & Rueckert, 1985; Sherry *et al.*, 1986). In this way, one can imagine that in cases where the atomic structures of antigenic loops in 'donor' immunogens are known as well as the structure of the 'recipient' loop in the virus capsid protein, optimal loop transplantation might become possible. It is not yet known how to engineer precisely the desired three-dimensional structures and properties into macromolecules. However, libraries of macromolecules or viruses constructed using combinatorial mutagenesis can be searched to increase the likelihood of including structures with desired architecture and properties such as immunogenicity. With appropriate selection methods, the rare constructs with desired properties can be identified and 'fished out'. Research of this type has yielded some potently immunogenic presentations of sequences transplanted on the surface of HRV (reviewed in Arnold & Arnold, 1999). For reasons not quite fully understood, presenting multiple copies of antigens to the immune system leads to an enhanced immune response (Malik & Perham, 1997). It is conceivable that, eventually, it might even be possible for conformational epitopes consisting of multiple 'donor' loops to be grafted onto 'recipient capsids' while maintaining the integrity of the original structure. Certainly, such feats are difficult to achieve with present-day protein-engineering skills, but recent successes in protein design offer hope that this will be feasible in the not too distant future (Gordon *et al.*, 1999).

Immense efforts have been made by numerous crystallographers to unravel the structures of molecules involved in the unbelievably complex, powerful and fascinating immune system. Many of the human proteins studied are listed in Table 1.3.4.5 with, as specific highlights, the structures of immunoglobulins (Poljak *et al.*, 1973), major histocompatibility complex (MHC) molecules (Bjorkman *et al.*, 1987; Brown *et al.*, 1993; Fremont *et al.*, 1992; Bjorkman & Burmeister, 1994), T-cell receptors (TCR) and MHC:TCR complexes (Garboczi *et al.*, 1996; Garcia *et al.*, 1996), an array of cytokines and chemokines, and immune cell-specific kinases such as lck (Zhu *et al.*, 1999). This knowledge is being converted into practical applications, for instance by humanising non-human antibodies with desirable properties (Reichmann *et al.*, 1988) and by creating immunotoxins.

The interactions between chemokines and receptors, and the complicated signalling pathways within each immune cell, make it next to impossible to predict the effect of small compounds interfering with a specific protein–protein interaction in the immune system (Deller & Jones, 2000). However, great encouragement has been obtained from the discovery of the remarkable manner by which the immunosuppressor FK506 functions: this small molecule brings two proteins, FKB12 and calcineurin, together, thereby preventing T-cell activation by calcineurin. The structure of this remarkable ternary complex is known (Kissinger *et al.*, 1995). Such discoveries of unusual modes of action of therapeutic compounds are the foundation for new concepts such as 'chemical dimerizers' to activate signalling events in cells such as apoptosis (Clackson *et al.*, 1998).

In spite of the gargantuan task ahead aimed at unravelling the cell-to-cell communication in immune action, it is unavoidable that the next decades will bring us unprecedented insight into the many carefully controlled processes of the immune system. In turn, it is expected that this will lead to new therapeutics for manipulating a truly wonderful defence system in order to assist vaccines, to decrease graft rejection processes in organ transplants and to control auto-immune diseases that are likely to be playing a major role in cruelly debilitating diseases such as rheumatoid arthritis and type I diabetes.

1.3.6. Outlook and dreams

At the beginning of the 1990s, Max Perutz inspired many researchers with a passion for structure and a heart for the suffering of mankind with a fascinating book entitled *Protein Structure – New Approaches to Disease and Therapy* (Perutz, 1992). The explosion of medicinal macromolecular crystallography since then has been truly remarkable. What should we expect for the next decades?

In the realm of safe predictions we can expect the following:

(*a*) High-throughput macromolecular crystallography due to the developments outlined in Section 1.3.1, leading to the new field of 'structural genomics'.

(*b*) Crystallography of very large complexes. While it is now clear that an atomic structure of a complex of 58 proteins and three RNA molecules, the ribosome, is around the corner, crystallographers will widen their horizons and start dreaming of structures like the nuclear pore complex, which has a molecular weight of over 100 000 000 Da.

(*c*) A steady flow of membrane protein structures. Whereas Max Perutz could only list five structures in his book of 1992, there are now over 40 PDB entries for membrane proteins. Most of them are transmembrane proteins: bacteriorhodopsin, photoreaction centres, light-harvesting complexes, cytochrome bc_1 complexes, cytochrome *c* oxidases, photosystem I, porins, ion channels and bacterial toxins such as haemolysin and LukF. Others are monotopic membrane proteins such as squalene synthase and the cyclooxygenases. Clearly, membrane protein crystallography is gaining momentum at present and may open the door to atomic insight in neurotransmitter pharmacology in the next decade.

What if we dream beyond the obvious? One day, medicinal crystallography may contribute to:

(*a*) The design of submacromolecular agonists and antagonists of proteins and nucleic acids in a matter of a day by integrating rapid structure determinations, using only a few nanograms of protein, with the power of combinatorial and, in particular, computational chemistry.

(*b*) 'Structural toxicology' based on 'human structural genomics'. Once the hundreds of thousands of structures of human proteins and complexes with other proteins and nucleic acids have been determined, truly predictive toxicology may become possible. This will not only speed up the drug-development process, but may substantially reduce the suffering of animals in preclinical tests.

(*c*) The creation of completely new classes of drugs to treat addiction, organ regeneration, aging, memory enhancement *etc.*

One day, crystallography will have revealed the structure of hundreds of thousands of proteins and nucleic acids from human and pathogen, and their complexes with each other and with natural and designed low-molecular-weight ligands. This will form an extraordinarily precious database of knowledge for furthering the health of humans. Hence, in the course of the 21st century, crystallography is likely to become a major driving force for improving health care and disease prevention, and will find a well deserved place in future books describing progress in medicine, sometimes called 'The Greatest Benefit to Mankind' (Porter, 1999).

Acknowledgements

We wish to thank Heidi Singer for terrific support in preparing the manuscript, and Drs Alvin Kwiram, Michael Gelb, Seymour Klebanov, Wes Van Voorhis, Fred Buckner, Youngsoo Kim and Rein Zwierstra for valuable comments.