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# Antibody Engineering [and Discussion]

G. P. Winter and K. James

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# Antibody engineering

By G. P. Winter
*MRC Laboratory of Molecular Biology, Hills Road, Cambridge CB2 2QH, U.K.*

[Plate 1]

The antibody molecule is a therapeutic agent, designed by nature to bind to a wide range of antigen molecules and to trigger effector functions, such as complement lysis and cell-mediated killing. The genes encoding antibodies can be manipulated *in vitro*, allowing the binding sites for antigen and effector molecules to be dissected, and new properties to be engineered. The future for the application of engineered antibodies in medicine is reviewed in the context of the past century.

## Introduction

Protein engineering is the design and construction of new protein molecules for a specific purpose. It is possible to make small proteins by direct chemical synthesis, but it is much simpler to alter the DNA sequence of the gene and to express the altered protein in living cells. The idea of engineering proteins by altering the gene, was specifically mooted by Michael Smith and colleagues in 1978 (Hutchinson *et al.* 1978). They had shown that they could engineer a stop codon into one of the bacteriophage genes by priming on the single-stranded template of the bacteriophage $\phi$X174 with a mismatched synthetic oligodeoxynucleotide. Clearly, this opened the door to making changes in proteins at will, particularly for identifying roles of individual residues in proteins.

However, the idea lay dormant. It was not so simple to make synthetic primers, nor to express genes in cells. It is perhaps not surprising that the possibility of engineering proteins did not surface in the deliberations of the Spinks Committee in 1980. However, by 1982 synthetic oligonucleotides were becoming more widely available, owing to improved methods of chemical synthesis using the phosphotriester and the phosphoramidite approaches (for a historical review see Ohtsuka *et al.* 1982), and Michael Smith had introduced a simple method for making site-directed mutations in the bacteriophage M13 (Zoller & Smith 1982). In that year, for the first time, site-directed mutagenesis was used to change side chains in the active site of two bacterial enzymes, β-lactamase (Dalbadie-McFarland *et al.* 1982) and tyrosyl tRNA synthetase (Winter *et al.* 1982). Over the next three years, site-directed mutagenesis emerged as an important tool for kineticists and X-ray crystallographers to dissect the activities of their favourite protein, and the Royal Society Discussion meeting on the 'Design, Construction and properties of novel protein molecules', held in 1985, summarizes the state of the art at that time.

However, in addition to the fine-structure analysis of proteins by altering individual side chains, biologists were cutting and pasting entire protein domains by simple recombinant DNA techniques. At the Discussion Meeting in 1985, Michael Neuberger described how antibodies could be expressed in myeloma cells from the transfected gene, and how new properties could be engineered by cutting and pasting domains (Neuberger & Williams 1986). Since then,

site-directed mutagenesis has been used to dissect antibody functions and to engineer new properties.

Why is it imporant to engineer antibodies? Antibodies are all-purpose therapeutic agents. Antibodies are elicited on immunization of an animal with an antigen and after a few days, can be found in the serum of the animal. They bind to the antigen and *in vivo* earmark it for clearance from the serum by triggering a variety of effector functions. Nevertheless antibodies have never realized their true potential in therapy.

## Early serotherapy

The idea of using antibodies as protein therapeutics dates from their discovery in 1890: von Behring and Kitasato showed that resistance to diphtheria toxin could be transferred from one guinea pig to another by transferring some of the serum. The active component was called anti-toxin and subsequently became known as antibody (Behring & Kitasato 1890). Within a few years antibodies had been used to treat diphtheria in humans: horses were immunized and about 50 ml of crude horse serum was injected into the patient. For diphtheria, therapy was effective, in part because bacterial toxins are highly immunogenic and gave a good titre of neutralizing antibody.

In the late 1890s, attempts were made to treat cancer by serotherapy, and horses were immunized with human tumour cells. However, the therapy proved ineffective, presumably as antibodies directed against tumour-cell antigens comprised a small part of the overall serum. In general there were several pitfalls with serotherapy: horse antiserum was a crude preparation, not only of horse antibodies but other horse serum proteins, and its administration could give rise to complications such as immune complex disease and anaphylaxis, particularly with multiple infusions. The problems were lumped together as serum sickness (for review see Ratner 1943). Nevertheless, for diphtheria, serotherapy was a lifesaver and before the Second World War was the only treatment available for pneumococcal pneumonia.

The possibilities of clinical application prompted von Behring to become an entrepreneur, and industrial exploitation went hand in hand with the science. As professor in the University of Marburg, he set up a private clinic, 'Institute for Experimental Serotherapy' in Marburg. He helped fund his institute by selling the exclusive rights on his antibodies to Hoechst (Frankfurt), and later he invested the money from his Nobel prize to set up a company 'Behringwerke', which survives today as a subsidiary of Hoechst.

## Monoclonal antibodies

Over three quarters of a century later, in England, Cesar Milstein and Georges Kohler, working in the Medical Research Council's Laboratory of Molecular Biology in Cambridge, found a way of making monoclonal antibodies, antibodies that are exquisitely specific to a particular antigen (Köhler & Milstein 1975). They succeeded by taking the antibody-producing cells from the spleen of an immunized animal and fusing these cells with an immortal (myeloma) cell line. The hybrid cell (hybridoma) produces antibody of a single specificity, and antibodies of the required specificity were found by screening. The paper reported making monoclonal antibodies against sheep red blood cells. To Milstein this opened a whole host of scientific and practical possibilities. An immediate application would be to make antibodies




PHILOSOPHICAL TRANSACTIONS OF THE ROYAL SOCIETY B BIOLOGICAL SCIENCES

directed against cell-surface antigens, for example to tumour-cell markers in order to diagnose and treat cancer. Work along these lines began soon after.

John Newell of the British Broadcasting Corporation also saw the potential of monoclonal antibodies, and telephoned Milstein on the day the monoclonal paper was published; his report was carried on the World Service the next week. I quote from the cue in to his broadcast.

> Medical research workers at Cambridge have found a way to manufacture antibodies—the human body's natural chemical defenses against disease—on a huge scale, outside the body. It will be done by using specially grown cultures of hybrid cells, made by fusing one kind of living cell with another. John Newell (of our science unit) reports on a technique which sounds like science fiction, but has big applications, both in clinical medicine and in the drug industry.

Later in the broadcast Newell elaborated on the applications (Newell 1975).

> The need to boost patients' defenses by injecting antibodies is far from the only reason why doctors would like to see human antibody production lines set up. The drug industry which backs up the front line doctor needs them too. All sorts of tests used, to diagnose disease, or to decide on the best course of treatment, depend upon the use of absolutely pure antibodies of very precisely known types.

However, these possibilities were not seen by the National Research and Development Corporation (NRDC), which was at that time the only route available to MRC scientists to exploit their inventions. They advised the MRC as follows in October 1976: 'It is certainly difficult for us to identify any immediate practical application which could be pursued as a commercial venture ... and it is not immediately obvious what patentable features are disclosed in the *Nature* paper.' Despite the official pessimism, monoclonal antibodies were quickly applied for diagnosis and therapy. By 1980 it was clear that the NRDC had misjudged the commercial potential of monoclonal antibodies, and probably the patent possibilities as well. However, somehow Köhler and Milstein ended up taking the blame even from fellow scientists. I quote from the Spinks report, 'We are concerned that a lack of appreciation of NRDC, particularly by young scientists may continue to result in situations such as that which occurred over monoclonal antibodies, where patent protection was not sought early enough.'

Despite the rapid application of this technology, there was a cloud on the horizon. Milstein and Köhler had succeeded in making mouse monoclonal antibodies, but it proved practically difficult to make human monoclonal antibodies, let alone human monoclonal antibodies of the required specificity. Human monoclonal antibodies were required for therapeutic applications, as mouse monoclonal antibodies are recognized as foreign. In any case, for cancer applications, there is a fundamental problem in making human monoclonal antibodies in humans against human tissue. So if it is so difficult to make human monoclonal antibodies directly, why not make them indirectly? Why not turn mouse antibodies into human antibodies? Protein engineering offered just this possibility.

## Antibody expression

To engineer a protein, the gene must first be expressed. For antibodies, a simple approach was used. The antibody genes, along with the signals that drive their expression, were introduced into a myeloma cell. These cells are factories for antibody secretion, and from the 'transfectomas' antibody was secreted that was active in binding to antigen and in triggering effector functions (Neuberger 1983; Ochi *et al.* 1983; Oi *et al.* 1983). The antibody was




PHILOSOPHICAL TRANSACTIONS OF THE ROYAL SOCIETY B BIOLOGICAL SCIENCES

correctly folded and glycosylated, unlike the antibodies assembled in *Escherichia coli* at that time, which were produced in inclusion bodies and were refolded *in vitro* in poor yield (Cabilly *et al.* 1984). More recently antibody fragments have been secreted from *E. coli* and expressed in this way appear to be folded and antigenically active (Better *et al.* 1988; Skerra & Pluckthun 1988).

## Reshuffling antibody domains

The antibody is a Y-shaped molecule, consisting of two heavy chains and two light chains. Each chain is divided into a series of protein domains. At the tips of the antibody arms are located the variable domains. In different antibodies these have different sequences and are responsible for binding the different antigens. The rest of the antibody is made of a series of domains that are relatively constant in sequence, and are responsible for recruiting effector functions, such as complement-mediated lysis or phagocytosis. At the DNA level these protein domains are arranged as a series of separate exons: by cutting and pasting the recombinant genes it is relatively easy to take the variable domain of one antibody and attach it to the constant domains of another antibody.

By taking the variable domains from a mouse antibody of known specificity, and attaching them to the constant domains of human antibody, a simple chimeric antibody can be constructed in which the effector functions are entirely human (Boulianne *et al.* 1984; Morrison *et al.* 1984; Neuberger *et al.* 1985) (figure 1, plate 1). Such chimeric antibodies have at least two advantages over mouse antibodies. Firstly, the effector functions can be selected or tailored as desired. For example, of the human IgG isotypes, IgG1 and IgG3 appear to be the most effective for complement and cell-mediated lysis, and therefore for killing tumour cells. Secondly, the use of human rather than mouse isotypes should minimize the anti-globulin responses during therapy. Nevertheless it is likely that a chimeric antibody would provoke a greater immune response than a human monoclonal antibody.

## Building antigen-binding sites into human antibodies

Is it possible to reduce the mouse component even further? Could we make human antibodies by stealing only the antigen-binding site (rather than the entire variable domains) from the mouse antibody? If so, how would we identify the residues in the sequence of the mouse antibody that are required for antigen binding? One important clue came from a

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## Description of plate 1

Figure 1. A simple chimaeric antibody: a three dimensional model of an antibody with α-carbon trace marked to represent mouse variable domains (in red) and human constant domains (in white).

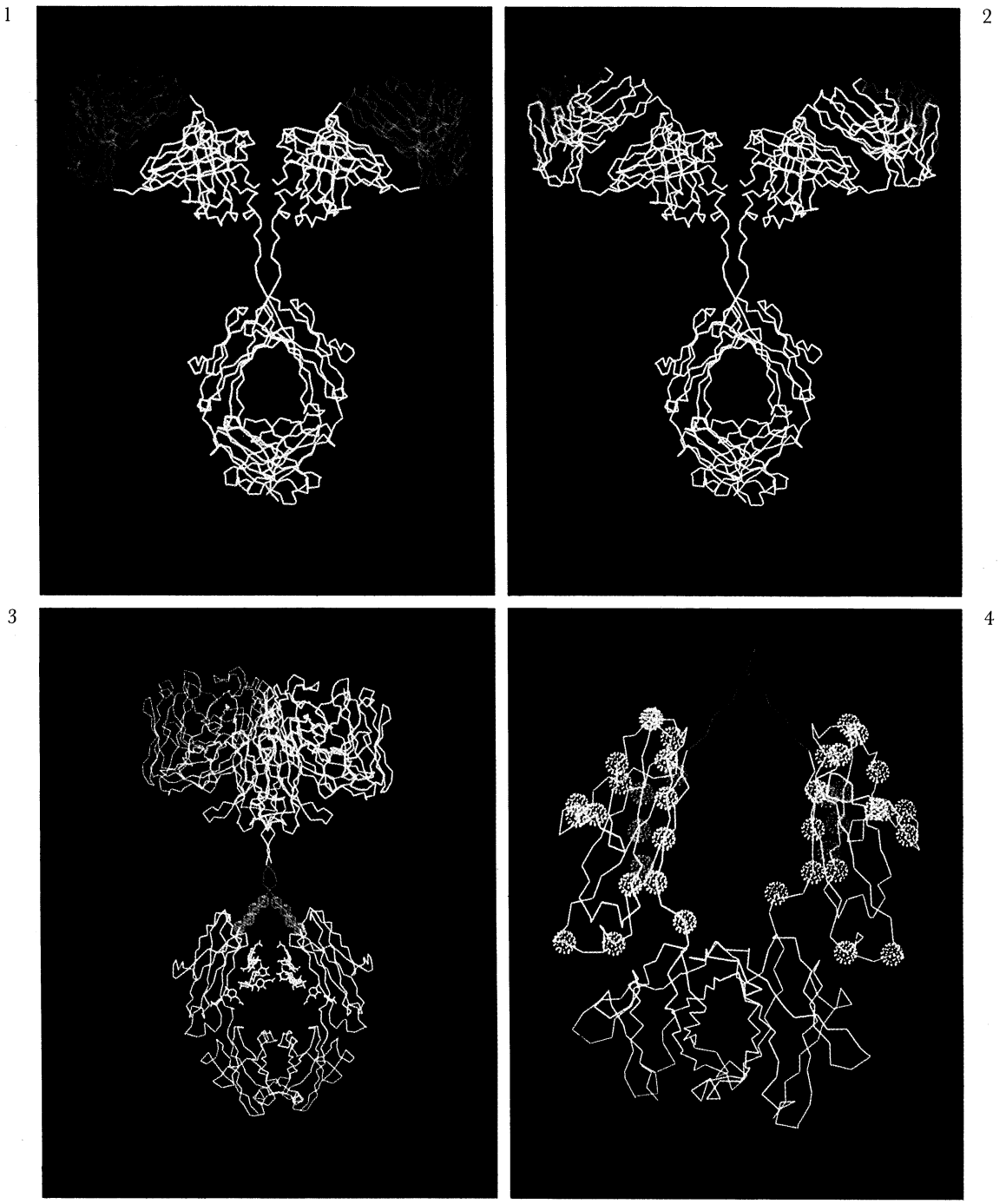
Figure 2. A reshaped human antibody: a three-dimensional model of an antibody with α-carbon trace marked to represent mouse CDRs (in red) in a human antibody (white).

Figure 3. Localization of the binding site for the high-affinity receptor (FcRI): the hinge region is marked in red, with the lower hinge region highlighted. The sugar residues on the inside face of the $CH_2$ domain are marked in yellow.

Figure 4. Localization of the binding site for the first component of complement C1q in the Fc fragment: the hinge is marked in red, and those positions critical for binding C1q are highlighted in red. Positions that are not involved in binding C1q are highlighted in yellow.




PHILOSOPHICAL TRANSACTIONS OF THE ROYAL SOCIETY B BIOLOGICAL SCIENCES




FIGURES 1–4. For description see opposite.



PHILOSOPHICAL TRANSACTIONS OF THE ROYAL SOCIETY B BIOLOGICAL SCIENCES

systematic alignment of the antibody sequences. The light-chain variable regions of different mouse antibodies are similar in sequence apart from three regions that are hypervariable. These sequences were proposed by Wu and Kabat to be the antigen binding regions and were termed complementarity determining regions or CDRs (for review see Kabat *et al.* 1987). The less variable regions (between the CDRs) were envisaged to be a scaffold, or framework, that supported the CDRs. The heavy-chain variable region also contains three CDRs. These ideas tied up nicely with the X-ray crystallographic structure of the antibody molecule. Each of the antibody variable domains consists of a β-sheet sandwich with a series of loops at each end, and the three CDRs correspond roughly to the loops at one end of the domain. The two sets of CDRs from heavy and light chain appear to fashion the antigen-binding site.

So if the CDRs encode the antigen-binding specificity, could we actually transfer the specificity of one antibody to another by transfering the CDRs? In the three-dimensional structure, this amounts to a loop transplant operation, in which the loops at the tip of one antibody are engrafted into the corresponding positions of another antibody (figure 2, plate 1). As these regions are hypervariable in sequence, to a first approximation such a reshaped human antibody might be indistinguishable from a genuine human antibody. In model experiments in 1986, we succeeded in transfering the binding site for a small hapten from a mouse antibody to a human antibody (Jones *et al.* 1986). We subsequently succeeded with a large protein antigen (lysozyme) (Verhoeyen *et al.* 1988). So now we wondered whether we could take a mouse or rat therapeutic antibody and cloak it in human form.

## Choosing effector functions for human antibodies

For clinical use, we need to chose the right effector mechanisms. Antibodies exert their therapeutic effect in at least four ways. The simplest way is by binding to the antigen. For example, antibody Fab fragments are effective in blocking the action of bacterial toxins such as diphtheria. However, for killing of cells or viruses, antibodies trigger complement-mediated lysis, phagocytosis and antibody-dependent cell-mediated cytolysis (ADCC): the antibody binds to antigenic determinants and marks them for the attention of these effector mechanisms via sites in the constant domains. Thus C1q, the first component of complement, binds to clusters of antibody (such as would form on a surface studded with antigenic epitopes), and triggers the complement lytic cascade. Likewise specialized effector cells, such as phagocytes or killer cells, can bind to antibody clusters through low-affinity receptors, and also to monomeric antibody by high-affinity receptors. This triggers phagocytosis or ADCC. The different antibody isotypes have different capabilities in complement and cell-mediated clearance. For example, IgM is effective in triggering complement lysis but not cell-mediated mechanisms. The human γ1 and γ3 isotypes are effective in both routes, but the human γ4 isotype is ineffective, and indeed may help to block the effector mechanisms. It is necessary to select the right isotype and effector functions for a therapeutic antibody.

## Serotherapy with a reshaped human antibody

We decided to reshape the rat antibody CAMPATH-1G in collaboration with Dr Herman Waldmann and his colleagues at the Department of Pathology in Cambridge (CAMPATH is a trademark of the Wellcome Foundation). This antibody is directed against human T and B




PHILOSOPHICAL TRANSACTIONS OF THE ROYAL SOCIETY B BIOLOGICAL SCIENCES

lymphocytes and was raised by immunizing rats with human lymphocytes. The particular antigen it recognizes is found on both T and B cells but not on the precursor stem cells. The rat CAMPATH-1G antibody had been used clinically with lymphoid malignancies, and showed marked reduction in lymphoid infiltration of blood and bone marrow and improvement of splenomegaly (Dyer *et al.* 1989). After some problems in reviving the antigenic specificity after transfering the rat CDRs to the human antibody, we finally succeeded in making a reshaped human antibody ($\gamma$1, $\kappa$), which was as effective as the rat antibody ($\gamma$2b, $\kappa$) in complement-mediated lysis, and more effective in ADCC (Riechmann *et al.* 1988*a*).

Recently this reshaped human antibody, termed CAMPATH-1H, has been used clinically by Dr Martin Dyer of the Department of Haematology in Cambridge to treat two patients with non-Hodgkin's lymphoma (Hale *et al.* 1988). The first patient was an elderly lady. Her lymphoma was treated for about four years with oral and intravenous chemotherapy, and splenic radiotherapy, but the disease progressed with increases in blood lymphocytes and spleen size, and she was treated with the rat CAMPATH-1G antibody for eight days. CAMPATH-1G induced fever, nausea and vomiting, and treatment was stopped on day eight when it resulted in a severe bronchospasm. The treatment did reduce the mass of the tumour and briefly cleared the blood and bone marrow of lymphoma cells. However, lymphoma cells soon reappeared in the circulation, and over the next few months the spleen size increased.

The patient was then treated with the human CAMPATH-1H antibody over a period of 30 days. The blood and bone marrow were completely cleared of lymphoma cells (and lymphocytes) and the tumour was greatly diminished in size. After treatment, normal lymphocytes gradually reappeared in the circulation with no trace of the lymphoma cells. However, by day 100, lymphoma cells were again detected in the blood and the spleen started to increase in size. The patient was treated again with the CAMPATH-1H antibody for 12 days with similar therapeutic benefit. The spleen was then removed and the patient is in remission. The second patient with splenomegaly and lymphadenopathy was treated for 43 days and is in remission. No antibodies to Campath-1H could be detected in the serum of either patient at any stage during the treatment.

To recapitulate, we have been able to use a reshaped human antibody over an extended period to destory lymphocytes and a large mass of tumour in two patients. CAMPATH-1 is in itself immunosuppressive: thus the lack of anti-idiotypic antibodies is encouraging but does not strictly bear on the question of immunogenicity of reshaped human antibodies. Some answers are suggested by a model system (see below), but further clinical trials are needed with other reshaped antibodies, for example those directed against viruses, bacteria or tumour cells of non-lymphoid origin. Although we would expect to see an anti-idiotypic response to reshaped human antibodies (and perhaps even to genuine human monoclonal antibodies), a weak or delayed response may well pose no serious problems in the clinic.

## Simple chimeric versus reshaped human antibodies

The rationale behind making reshaped human antibodies, in which only the CDRs are mouse, rather than simple chimeric antibodies, in which the entire variable domain is mouse, is that the reshaped antibodies are less foreign, and should, hopefully, be less immunogenic in humans. Although therapeutic administration of mouse antibodies in humans can lead to an immune response against the variable domains of the immunizing antibody, it is not clear whether this




PHILOSOPHICAL TRANSACTIONS OF THE ROYAL SOCIETY B BIOLOGICAL SCIENCES

reflects the intrinsic antigenicity of the variable domain, or whether this is mediated by the foreign constant domains to which it is linked. However, we have shown recently that a recombinant mouse-human antibody, entirely mouse except for the β-sheet framework regions of the heavy chain variable region, was much more immunogenic in mice than the parent mouse antibody (M. Bruggemann, G. Winter, H. Waldmann and M. S. Neuberger, personal communication). Thus foreign components of the variable domain can be immunogenic even when attached to self constant domains. This suggests that in humans reshaped human antibodies, which have human framework regions, are likely to be less immunogenic than simple chimeric antibodies, which have mouse framework regions. However, it leaves open the question of the immunogenicity of the mouse CDRs in the reshaped human antibodies.

## Altering natural effector functions

Antibodies with natural effector functions clearly have a role in therapy, and it could be useful to identify the residues involved in effector functions, as this could allow us to engineer antibodies with altered effector properties. For example, by turning on and off certain functions (say complement lysis), the role of each function in killing *in vivo* could be dissected. Furthermore, it might be possible to design antibodies with improved effector functions by making changes at these sites, and also to design small peptides, proteins or organic molecules to inhibit or mimic these functions.

To date we appear to have located the regions of the antibody involved in binding to the human high-affinity receptor, and to C1q, the first component of complement. Previous work by Dennis Burton and his colleagues in Sheffield had suggested that the hinge region might be critical in binding to receptor. By making a series of point mutations in this region we have been able to confirm this hypothesis (Duncan *et al.* 1988). In fact we suspect that the receptor binds to the hinge and perhaps to some of the sugar residues that lie on the inside surfaces of the $CH_2$ domain (figure 3, plate 1). The C1q binding site was identified by making a scattering of mutations over the $CH_2$ domain of the antibody. Most mutations have little effect on C1q binding but there are three adjacent residues on a section of β-strand with their side chains pointing out into solution which appear to be critical (figure 4, plate 1). Indeed a small peptide, based on the sequence of this region, is able to inhibit complement lysis (Duncan & Winter 1988).

## Novel effector functions

As an alternative to using or enhancing natural effector functions, it is possible to link novel effector functions to the antibody; here the antibody is simply used as a targeting agent. Conjugates between antibody and toxins have been made by direct chemical cross-linking, for example linking into the hinge disulphides of the heavy chain (Vitetta & Uhr 1985). However, protein engineering should facilitate their construction, for example by introducing accessible sulphydryl groups at the end of the variable domain to hook up to toxins via a protein chemical linker. Alternatively, the reading frame of the antibody could be linked directly to that of the conjugate. Thus a model experiment, in which staphylococcal nuclease was linked to antibody Fab fragments, showed that such constructs can be secreted from myeloma cells (Neuberger *et al.* 1984).




PHILOSOPHICAL TRANSACTIONS OF THE ROYAL SOCIETY B BIOLOGICAL SCIENCES

A whole range of conjugates can be devised for therapy. For example, for tumour therapy, we could envisage attaching to an antibody with a specificity against tumour cells: (1) a potent drug, or cluster of drugs (perhaps in a liposome bag), (2) an enzyme that converts an inactive prodrug into an active drug, in the vicinity of the tumour, (3) radioisotopes or (4) bacterial or plant toxins. However, it is likely that immunoconjugates may prove more immunogenic than a natural antibody. For diagnostics, conjugates with radioisotopes have already proved their value in imaging tumours.

## Future directions in antibody engineering

I have suggested three main thrusts to pratical engineering: reshaping human antibodies for therapy, inproving natural effector functions and introducing novel effector functions. This work may bring about a renaissance for serotherapy. Full exploitation will require new technologies, for example in finding ways of improving antibody affinities and specificities, either by design or random change (Roberts *et al.* 1987; Allen *et al.* 1988). Recently it has been shown that antibody Fv fragments (heavy and light chain variable domains non-covalently associated together) can be secreted from myeloma cells or from *E. coli*, and that they retain their antigen-binding affinity (Riechmann *et al.* 1988*b*; Skerra & Pluckthun 1988). The possibility of working on Fv fragments secreted from *E. coli*, rather than complete antibodies secreted from myeloma cells, will dramatically facilitate the engineering of antigen-binding specificities. It should permit the rapid mutagenesis and screening of altered antibodies, and the small size of the fragments should facilitate both X-ray crystallography and nuclear magnetic resonance, allowing antibody-binding sites to be solved at atomic resolution more readily than hitherto. A larger database of solved structures would also help improve methods of structure prediction, and allow folding predictions to be tested directly. In the same way that the hybridoma monoclonal antibody has served as the raw material for protein engineering of therapeutic antibodies, I predict that the Fv fragment will prove to be raw material for altering affinity and specificity.

Finally, there is the possibility of turning antibodies into enzymes. As we might expect from theories of kinetics, if we could make binding sites for transition states, we could make an artificial enzyme. Catalytic antibodies have been made by immunizing animals with transition-state analogues and there are now several examples in which simple chemical reactions such as ester hydrolysis and ring closure can be catalysed by antibodies (for review see Lerner & Tramontano 1987). One problem with immunizing animals with the transition-state analogues is that the synthetic chemistry necessary to make the analogues can be difficult. There is an alternative approach: to design and build the catalytic site from first principles. In view of the underlying β-sheet framework, the design is simplified, and requires only the structures of the antigen-binding loops to be predicted. If this proves too difficult, then an antibody could be raised by immunizing with the substrate, and residues built into the loops to stabilize the transition state.

Antibody engineering holds out tremendous promise for therapy, diagnostics, fundamental structure/function studies and may even provide the building blocks for new enzyme activities.




PHILOSOPHICAL TRANSACTIONS OF THE ROYAL SOCIETY B BIOLOGICAL SCIENCES

## Exploitation of antibody engineering

At first sight it is surprising that the NRDC did not foresee the commercial potential of monoclonal antibodies and that the Spinks Committee, which included several eminent scientists, did not foresee protein engineering. Yet both of these possibilities were flagged clearly in the original scientific papers and, in the case of monoclonal antibodies, were even broadcast to the world by the BBC. Somewhere, somehow, the message was lost. Although it is likely that a scientist in his or her own field could see applications that would not be foreseen by bodies of civil servants or committees of other scientists, he or she would find it difficult to predict the timescale for application or to evaluate alternative approaches outside his own field. For example, if human antibodies are to be made for therapy by protein engineering, I can say confidently that reshaped human antibodies will probably prove superior to simple chimeric antibodies. However, other technologies might supersede the protein engineering approach, for example making human antibodies by *in vitro* immunization of human cells, or by cloning antibodies from the genomic DNA of individual human cells, or by making transgenic mice with a repertoire of human immunoglobulin genes, or even making patients tolerant to mouse antibodies.

Given the rapid advance of technology, it is essential to move inventions and new technology quickly to application, before they become outdated. At the MRC Laboratory of Molecular Biology in Cambridge, we have been evolving a strategy to ensure that we rapidly identify discoveries which can be applied, and then transfer the technology to companies. For example in the field of antibody engineering, and with the assistance of Celltech, a key patent was filed concerning the construction of antibodies with novel effector functions, and another on the construction of human antibodies by CDR graft. These patents are to be licensed widely to encourage the clinical application of a whole spectrum of engineered antibodies. At present, technology is being transferred to interested companies by paid consultancies with MRC scientists and through scientific collaborations: in the future we expect that the MRC Collaborative Centre will have an increasing role to play. To encourage inventors, the MRC has introduced an inventors awards scheme in which the inventor has a modest share in the royalties. So by encouraging the inventor and the consultant, by licensing the patents widely and welcoming direct contacts with industry, the MRC is taking antibody engineering to the market place. This approach, in which basic research is funded for is own sake and potential applications are seized upon and exploited, contrasts with the SERC Protein Engineering Club or the DTI LINK schemes, where companies help decide on the area of research at the earliest stage.

## References

Allen, D., Simon, T., Sablitzky, F., Rejewsky, K. & Cumano, A. 1988 Antibody engineering for the analysis of affinity maturation of an anti-hapten response. *EMBO J* **7**, 1995–2001.

Behring, E. von & Kitasato, S. 1890 Ueber das Zustandekommen der Diphtherie – Immunität und der Tetanus – Immunität bei Thieren. *Dt. med. Wschr.* **16**, 1113–1114.

Better, M., Chang, C. P., Robinson, R. R. & Horwitz, A. H. 1988 *Escherichia coli* secretion of an active chimeric antibody fragment. *Science, Wash.* **240**, 1041–1043.

Boulianne, G. L., Hozumi, N. & Shulman, M. J. 1984 Production of functional chimaeric mouse/human antibody. *Nature, Lond.* **312**, 643–646.

Cabilly, S., Riggs, A. D., Pande, H., Shively, J. E., Holmes, W. E., Rey, M., Perry, L. J., Wetzel, R. & Heyneker, H. L. 1984 Generation of antibody activity from immunoglobulin polypeptide chains produced in *Escherichia coli*. *Proc. natn. Acad. Sci. U.S.A.* **81**, 3273–3277.





PHILOSOPHICAL TRANSACTIONS OF THE ROYAL SOCIETY B BIOLOGICAL SCIENCES

Dalbadie-McFarland, G., Cohen, L. W., Riggs, A. D., Morin, C., Itakura, K. & Richards, J. H. 1982 Oligonucleotide-directed mutagenesis as a general and powerful method for studies of protein function. *Proc. natn. Acad. Sci. U.S.A.* **79**, 6409–6413.

Duncan, A. R. & Winter, G. 1988 The binding site for C1q on IgG. *Nature, Lond.* **332**, 738–740.

Duncan, A. R., Woof, J. M., Partridge, L. J., Burton, D. R. & Winter, G. 1988 Localisation of the binding site for the human high-affinity receptor on IgG. *Nature, Lond.* **332**, 563–564.

Dyer, M. J. S., Hale, G., Hayhoe, F. J. G. & Waldmann, H. 1989 Effects of CAMPATH-1 antibodies in vivo in patients with lymphoid malignancies: influence of antibody isotype. *Blood.* (In the press.)

Hale, G., Dyer, M. J. S., Clark, M. R., Phillips, J. M., Marcus, R., Riechmann, L., Winter, G. & Waldmann, H. 1988 Remission induction in non-Hodgkin lymphoma with reshaped human monoclonal antibody CAMPATH-1H. *Lancet* ii, 1394–1399.

Hutchinson, C. A., Philips, S., Edgell, M. H., Gillam, S., Jahnke, P. & Smith, M. 1978 Mutagenesis at a specific position in a DNA sequence. *J. biol. Chem.* **253**, 6551–6560.

Jones, P. T., Dear, P. H., Foote, J., Neuberger, M. S. & Winter, G. 1986 Replacing the complementarity-determining regions in a human antibody with those from a mouse. *Nature, Lond.* **321**, 522–525.

Kabat, E. A., Wu, T. T., Reid-Miller, M., Perry, H. M. & Gottesman, K. S. 1987 In *Sequences of proteins of* immunological interest. Washington: U.S. Department of Health and Human Services.

Köhler, G. & Milstein, C. 1975 Continuous culture of fused cells secreting antibody of pre-defined specificity. *Nature, Lond.* **256**, 495–497.

Lerner, R. A. & Tramontano, A. 1987 Antibodies as enzymes. *Trends biochem. Sci.* **12**, 427–430.

Morrison, S. L., Johnson, M. J., Herzenberg, L. A. & Oi, V. T. 1984 Chimaeric human antibody molecules: mouse antigen-binding domains with human constant region domains. *Proc. natn. Acad. Sci. U.S.A.* **81**, 6851–6855.

Neuberger, M. S. 1983 Expression and regulation of immunoglobulin heavy chain gene transfected into lymphoid cells. *EMBO J.* **2**, 1373–1378.

Neuberger, M. S., Williams, G. T. & Fox, R. O. 1984 Recombinant antibodies possessing novel effector functions. *Nature, Lond.* **312**, 604–608.

Neuberger, M. S., Williams, G. T., Mitchell, E. B., Jouhal, S. S., Flanagan, J. G. & Rabitts, T. H. 1985 A hapten-specific chimaeric IgE antibody with human physiological effector function. *Nature, Lond.* **314**, 268–270.

Neuberger, M. S. & Williams, G. T. 1986 Construction of novel antibodies by use of DNA transfection: design of plasmid vectors. *Phil. Trans. R. Soc. Lond.* A **317**, 425–432.

Newell, J. 1985 *Living factories – new drugs from hybrid cells.* BBC External Services Central Talks and Features, 12 August 1975, no. L673.

Ochi, A., Hawley, R. G., Hawley, T., Shulman, M. J., Traunecker, A., Köhler, G. & Hozumi, N. 1983 Functional immunoglobulin M production after transfection of cloned immunoglobulin heavy and light chain genes into lymphoid cells. *Proc. natn. Acad. Sci. U.S.A.* **80**, 6351–6355.

Ohtsuka, E., Ikehara, M. & Soll, D. 1982 Recent developments in the chemical synthesis of polynucleotides. *Nucl. Acids Res.* **10**, 6553–6570.

Oi, V. T., Morrison, S. L., Herzenberg, L. A. & Berg, P., 1983 Immunoglobulin gene expression in transformed lymphoid cells. *Proc. natn. Acad. Sci. U.S.A.* **80**, 825–829.

Ratner, B. 1943 *Allergy anaphylaxis and immunotherapy.* Baltimore: Williams & Wilkins.

Riechmann, L., Clark, M., Waldmann, H. & Winter, G. 1988*a* Reshaping human antibodies for therapy. *Nature, Lond.* **332**, 323–327.

Riechmann, L., Foote, J. & Winter, G. 1988 Expression of an antibody Fv fragment in myeloma cells. *J. molec. Biol.* **203**, 825–828.

Roberts, S., Cheetham, J. C. & Rees, A. R. 1987 Generation of an antibody with enhanced affinity and specificity for its antigen by protein engineering. *Nature, Lond.* **328**, 731–732.

Skerra, A. & Pluckthun, A. 1988 Assembly of a functional immunoglobulin Fv fragment in *Escherichia coli. Science, Wash.* **240**, 1038–1040.

Verhoeyen, M. V., Milstein, C. & Winter, G. 1988 Reshaping human antibodies: grafting an antilysozyme activity. *Science, Wash.* **239**, 1534–1536.

Vitetta, E. S. & Uhr, J. W. 1985 *Immunotoxins.* In *Annual review of immunology* (ed. W. E. Paul, C. G. Fathman & H. Metzger), vol 3, pp. 197–212. Palo Alto, California: Annual Reviews.

Winter, G., Fersht, A. R., Wilkinson, A. J., Zoller, M. & Smith, M. 1982 Redesigning enzyme structure by site-directed mutagenesis: tyrosyl tRNA synthetase and ATP binding. *Nature, Lond.* **299**, 756–758.

Zoller, M. J. & Smith, M. 1982 Oligonucleotide-directed mutagenesis using M13-derived vectors: an efficient and general procedure for the production of point mutations in any fragment of DNA. *Nucl. Acids Res.* **10**, 6487–6500.





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*Discussion*

K. James (*Department of Surgery, University of Edinburgh Medical School, U.K.*). I feel that the work being done by Dr Winter and others involving the engineering of mouse antibodies is of extreme importance and is exercising the minds of a number of research groups. Apart from the attempts to develop therapeutics for treating cancer, there are also a number of other areas where such antibodies could be important, e.g. in the fields of autoimmunity, transplantation and the treatment of infectious diseases. I feel, however, that the human approach as such should not be neglected. There are obvious important examples where mice don't mount the appropriate immune response, such as in the development of antigens of the Rhesus D complex and to histocompatibility antigens. As emphasized in Dr Winter's paper, there are problems with respect to human monoclonal antibody production. Apart from the difficulty in obtaining suitable immune lymphocytes, there are problems with respect to the stability of the cell lines and the levels of antibody they secrete. A technology exists, however, to get round a number of these problems. In collaboration with other workers in the United States we have attempted to get over the instability and antibody expression problems by combining molecular biology and cell immortalization procedures. We have cloned and expressed the genes for a complete human antibody molecule in mouse cell lines together with promoter and enhancer sequences. We have thus immortalized the genes and also succeeded in obtaining constructs that secrete antibody at a much higher level than the parental line. There have been recent reports of similar approaches used by workers in Japan who have also switched the isotype of the immunoglobulin. It is my belief that work in this area should proceed in parallel with the engineering approaches being used by Dr Winter and others.

G. Winter. I agree with Dr James that there are other areas where human or reshaped antibodies could be important, particularly for the treatment of infectious disease. My group is concerned at present with reshaping antibodies with potential in virus and bacterial disease. Also I agree that there are certain circumstances where it may be opportune to rescue human monoclonal antibodies from an unstable myeloma by recombinant DNA techniques.





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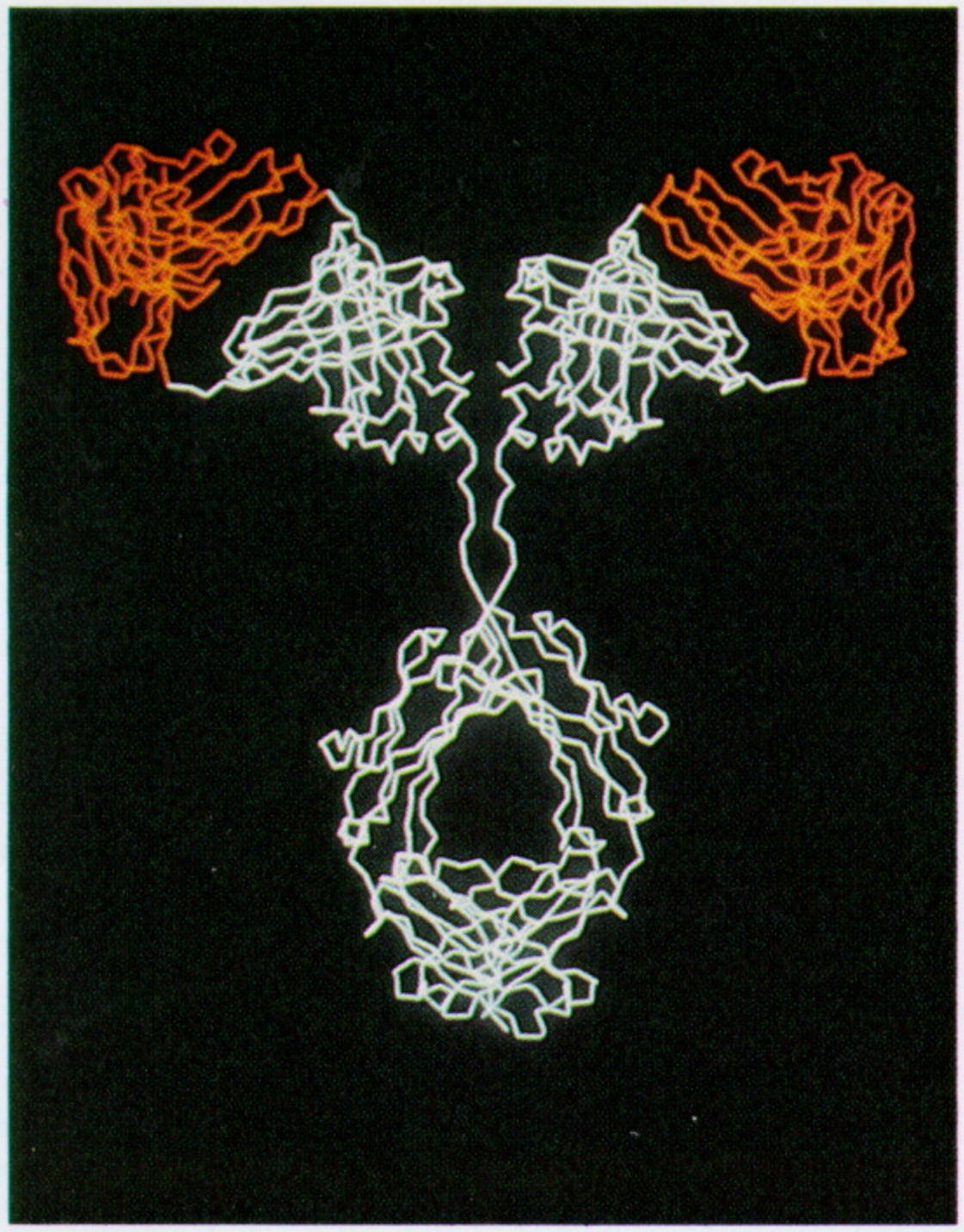

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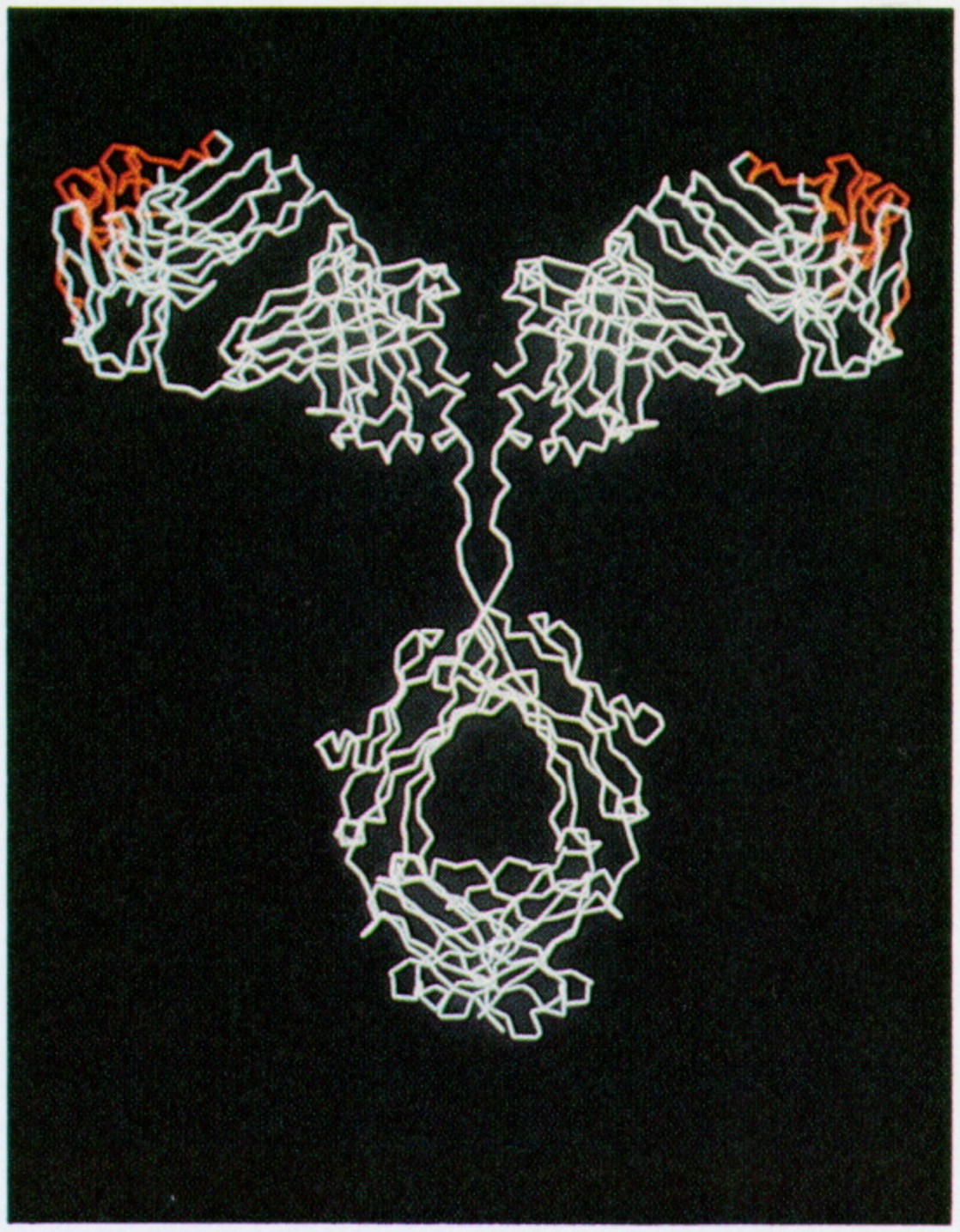



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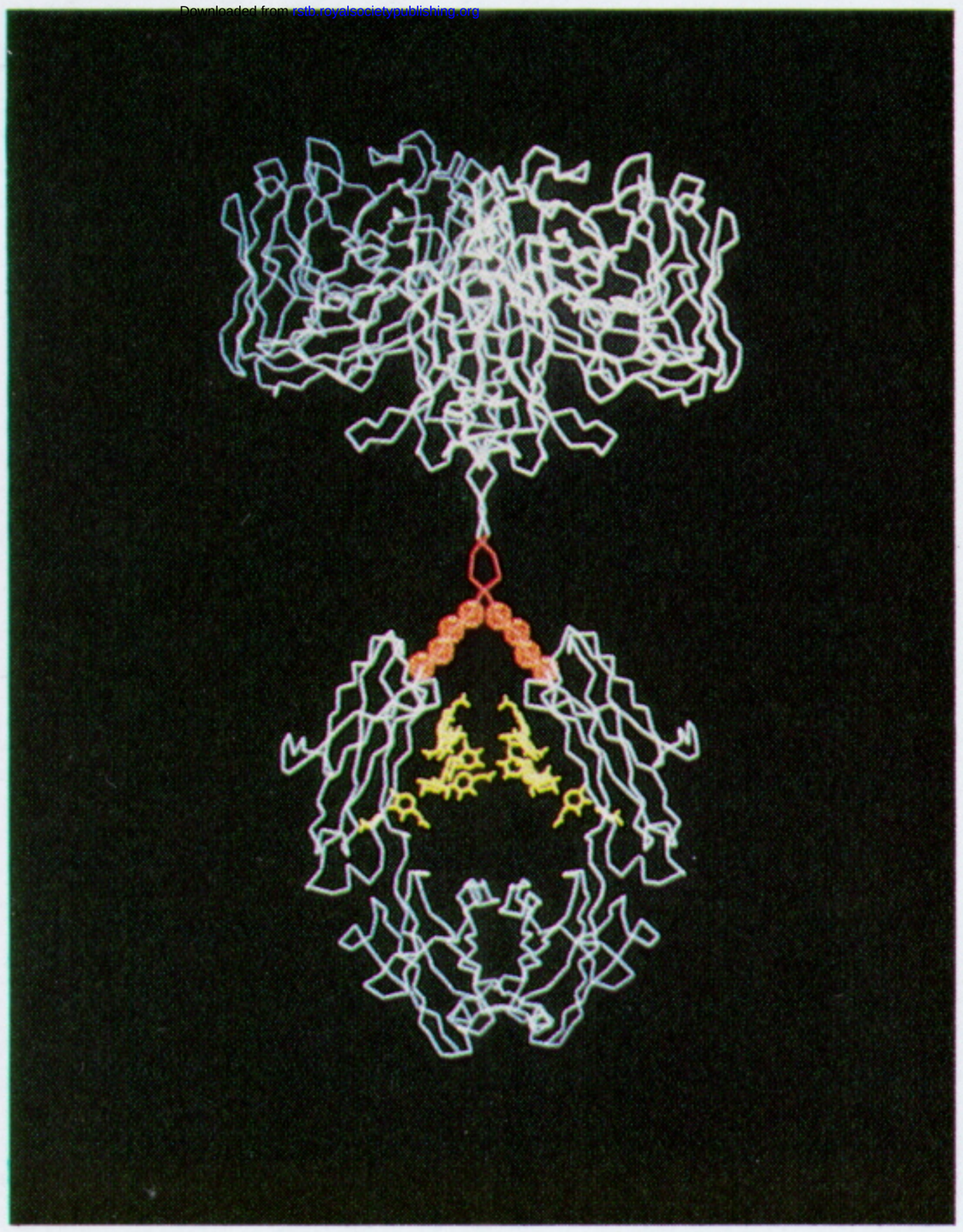

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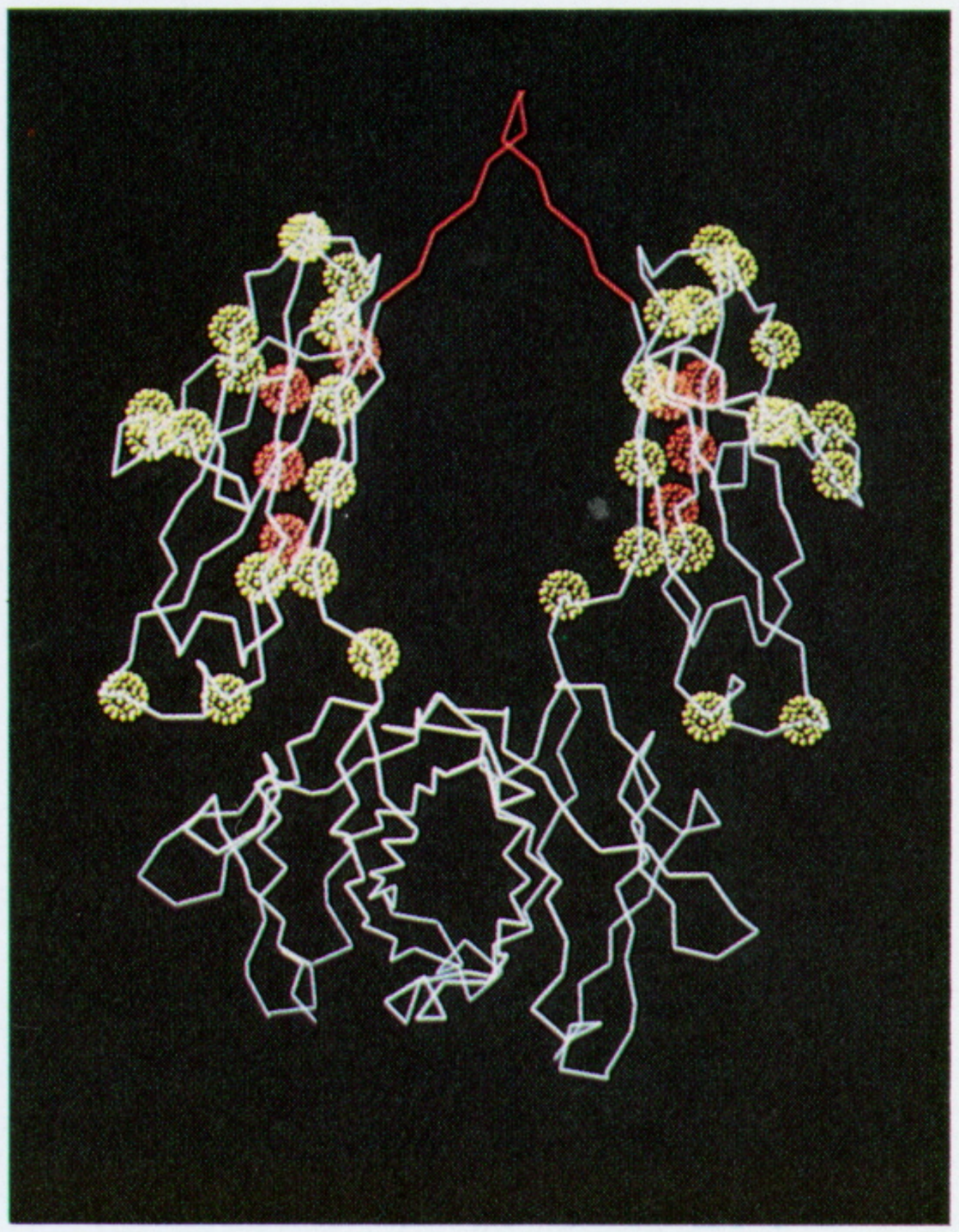



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FIGURES 1–4. For description see opposite.