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36

16



# Antibody engineering

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Antibody engineering has received a boost from the development of an *Escherichia coli* expression system that now allows the screening of libraries with bacteria or phages. These random selection techniques can be applied using knowledge obtained from new X-ray structures of recombinant antibody domains, and anti-peptide antibodies. The first crystal structure of an anti-idiotype complex has also been solved. Additionally, the engineering of binding sites for metals and haptens, and the design of new immunotoxins have been reported.

### Introduction

The antibody molecule, an immensely useful reagent in biology and medicine, has become a recent focus of interest for protein engineers, crystallographers, organic chemists and tumor biologists alike. Methods of gene technology, together with recent progress in expression technology, have opened the door to new approaches for the manipulation of such molecules. This review will focus on some of the key technologies and their development within the past year, as well as on the progress in areas directly related to antibody engineering.

## **Expression of antibodies**

238

infected insect cells [6,7,6]. Although the whole 7Santibody can be produced in these systems, the glycosylation pattern will probably be different from the native molecules. Additionally, developers of these systems will be required to match the enormous amount of empirical optimization work that has already gone into the largescale production of recombinant antibodies by mammalian cells, and it remains to be seen whether any of the new eukaryotic methods offer an advantage over mammalian expression hosts.

If the whole antibody and its glycosylation is not required, for example, in studies of antigen-binding or antibody structure, the ease and versatility of the recently developed functional Escherichia coli expression systems [8,9,10•] is unmatched by any of the eukaryotic systems. The handling of very large numbers of clones, which is necessary in all work with libraries, can only be carried out with bacterial systems at present. Various features of antibody expression in E. coli have recently been summarized [11•,12•]. Thus, only a brief account of some general considerations relevant for protein engineering will be given here. The strategy leading to expression of functional antibody fragments in E. coli necessitates secretion into the periplasm; correct folding in vivo requires the formation of the disulfide bonds in this oxidizing compartment and the simultaneous presence of approximately stoichiometric amounts of both the heavy and the light chain. The nascent chains probably act as folding templates for each other. To produce native fragments, two options therefore exist: either both chains making up the Fv fragment (or those making up the Fab fragment) must be co-secreted [8,9,10•]; or the two chains of the Fv fragment must be connected by a peptide linker so that a so-called single chain Fv is secreted [13•]. Single chain fragments had been produced previously by in vitro refolding from inclusion bodies in E. coli [14,15], but they can now be obtained in functional form by secretion [13•].

The exciting possibilities arising from the genetic modification of antibody genes, be it by site-directed mutagenesis, gene synthesis, random mutagenesis or work with DNA libraries, can only be taken advantage of if the modified antibody proteins can be expressed conveniently. In this context, it should be stressed that the screening of collections of mutants or of libraries for antibody function (i.e. antigen binding) requires that the antibody protein is produced in the native state by the production host. Clearly, the individual *in vitro* refolding of proteins isolated from the large number of different clones of a library is not practical.

Previously, functional expression of antibodies has been carried out mostly in myeloma or in Chinese hamster ovary cells. This is still the preferred method if the whole antibody is to be expressed — for example, in the investigation of effector functions which reside in the constant domains or which are even dependent on glycosylation of the constant domains [1,2,3]. A set of alternative eukaryotic expression systems has now become available in the form of yeast [4], plants [5•], and baculovirus-

### Abbreviations

CDR—complementarity-determining region; Mhr—myohemerythrin; PC—phosphorylcholine; PCR—polymerase chain reaction.

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Fv fragments may dissociate into  $V_L$  and  $V_H$  fragments at low concentrations, but the exact concentrations will vary from antibody to antibody, as the interaction energy of the hypervariable regions contributes to the domain association energy. Because dissociation reduces the availability of the binding site, labile Fv fragments must be stabilized by designing single chain Fv fragments, by introducing an intermolecular disulfide bond, or by chemical crosslinking between  $V_L$  and  $V_H$  [13•]. Some Fv fragments are perfectly stable, however, and it remains to be shown whether the peptide linker of single chain fragments may disturb certain antigen-binding sites.

An alternative solution for the dissociation problem is the use of the Fab fragment, in which the constant domains provide additional interaction energy between the chains. The functional yield of the Fab fragment is lower than that of the Fv fragment, and the problem has been recognized to be caused by in vivo folding (A Skerra and A Plückthun, unpublished data). Yet, the Fab fragment may be useful when high stability is desired without taking recourse to Fv engineering, for example, when the Fv structure is unknown, or the linker undesired. The constant domains might also provide a desired spacing in some bifunctional molecules. The preferred fragment thus depends on the desired applications. The Fv fragment and its derivatives are the smallest fragments of the antibody that still contain the complete antigen-binding site. The use of these antibody fragments may be advantageous in structural studies and medical applications, where low antigenicity and small size is favoured. The small size should lead to improved tumor penetration and rapid clearance through the kidneys. The in vivo tumor targeting of a single chain Fv fragment has now been investigated. Its localization in the tumor tissue was found to be slightly better than that of a Fab fragment, and its clearance more rapid [16•]. The ramifications of these observations for improving the design and strategies in tumour targeting still need to be thoroughly evaluated.

least in some cases). Thus, this approach suffers from the necessity of careful controls, demonstrating in each case that antigen-binding is really a result of specific interactions. It must also be stressed that one of the hallmarks of antibodies is their selectivity, that is, their ability to bind to only one antigen (or a narrow family of antigens), and it is to be expected that this selectivity is impaired when only half of the antigen-binding site is provided by a single domain. Although this principal problem will remain, the technical challenge of altering the solubility characteristics of  $V_{\rm H}$  may be approachable by engineering the framework.

In an improved approach to obtaining expression libraries for screening of antigen-binding in E. coli, Fab fragments have been used [21..]. The vector for the library was a  $\lambda$ -ZAP derivative [25], into which an expression plasmid analogous to those originally reported [8,9,10•] was integrated. PCR amplification of the mRNA from the spleen cells of an immunized animal was used to generate independent libraries of just light chains or heavy chains, respectively. The DNA of the  $\lambda$ -phages was then cut with suitable restriction enzymes and the two libraries were mixed and religated. Thus, a combinatorial library of Fab fragments was obtained. Indeed, functional Fab fragments could be produced using this approach, and those with binding affinity could be detected by screening phage plaques with labeled antigen. Caton and Koprowski [22•] have used this methodology to characterize the murine immune response to influenza virus. The technique has also been applied to the cloning of human antibodies [26•,27•]. In these experiments, the source of the mRNA was peripheral blood lymphocytes from a donor previously immunized with tetanus toxoid and boosted with this antigen. Fab fragments recognizing the tetanus antigen were found on the phage plaques of the combinatorial library. It may be useful to put this method into perspective. The libraries reported so far have all used spleen cells from an immunized animal (or human peripheral blood lymphocytes) as the source of mRNA. The mRNA encoding the newly elicited antibody then constitutes up to a few percent of all mRNA in B cells. This explains why, in the combinatorial libraries, frequencies of positive antibodies as high as about one in a few thousand have been seen. In a crude approximation, this number should be the product of the probability of finding the appropriate light chain mRNA and heavy chain mRNA. This simple calculation suggests that finding a desired specificity in a 'naive' library (i.e. of an animal or a human not having had contact with the antigen recently) may be close to impossible, as the screening of libraries much larger than 10<sup>6</sup>–10<sup>7</sup> clones becomes impractical. Also, rather little is known about the actual diversity of the libraries. Problems would also arise if somatic mutation is required for a particular antibody to recognize its target with a binding constant sufficient to be detected in the screening. Yet, if a reasonable starting point could somehow be found with this method, plasmid mutagenesis might be used to select for increased affinities. In conclusion, this method might obviate the need for a cell fusion step in the pro-

# Libraries and screening

An important extension to the work on expressing defined antibodies is the development of expression libraries with the goal of establishing screening approaches in *E. coli*. Besides the strategies for the functional expression of antibody fragments in *E. coli* [8,9,10•,13•], the polymerase chain reaction (PCR) has proved to be a crucial component of a facile route to heterologous antibody expression libraries. A number of research groups have analyzed suitable PCR primers in the regions 5' and 3' to the variable domains [17•-20•,21••,22•,23•]. Techniques for using only one specific primer are also available [24•].

The first report investigating the possibility of screening for antigen binding in *E. coli* [17•] relied on the use of only  $V_H$  domains. The problem with  $V_H$  domains, however, is their tendency to aggregate and precipitate (at duction of monoclonal antibodies, but the first step of immunization still has to be carried out. It is also unlikely that the method will provide access to antibodies that a mouse would refuse to make, as the mRNA to be amplified would probably not be produced either. Clearly, more work needs to be done to define the limits.

To extend the numbers of clones that can be screened, an enrichment system of candidate clones is required. A number of groups have reported variations of one basic idea, namely the use of the coat protein pIII of bacteriophage M13 for this purpose. The amino-terminal domain of this protein, which occurs at about five copies per phage particle, plays a role in the attachment of the phage to the pili of E. coli, whereas the carboxyl-terminal domain seems to be important for coat assembly. Smith and co-workers [28,29] showed that peptides inserted behind the signal sequence at the amino-terminus of the mature protein do not inhibit the infectivity of the phage. They have now extended this work by demonstrating that a library of random peptides can be generated and the whole bacteriophage can be adsorbed to an antibody bound to a solid phase which recognizes a few peptides in the library [30••]. The bound phage can be eluted, reinfected and thus enriched. After several cycles, epitopes for specific antibodies that closely resemble the known epitope were found. A very similar approach has also been reported by Cwirla et al. [31...].

be useful for libraries, in which each molecular species is represented by only a few copies, virtually every phage molecule displaying the correct ligand or receptor (e.g. antigen or antibody) would have to be bound in the first round and to be eluteable under mild conditions. Once it is lost, it can never be amplified again. Even so, these techniques look promising for the development of new efficient screening strategies.

## Antibody structure and flexibility

Current research on antibodies is at the exciting interface between rational engineering and random screening approaches. It is obvious, however, that structural information about natural and modified antibodies is a cornerstone for all engineering approaches. A number of new structures of antibody fragments have appeared within the last year, and some highlights will be discussed.

As an illustration of the general potential of the method, Devlin et al. [32••] used the same strategy to identify peptides that bound to avidin, a protein with no known affinity for peptides. Thus, this method provides a molecular biological equivalent of the mimotope strategy of Geysen et al. [33] developed in 1986. McCafferty et al. [34•] have now shown that the experiment can also be reversed and even a single chain Fv fragment can be inserted at the amino-terminus of the mature PIII protein without loss of infectivity. Phages carrying the Fv fragment can be enriched over phages not carrying the insert by antigen-affinity chromatography of whole phages. A further methodological extension of the strategy has been achieved by Bass et al. [35•], who have devised a means of attaching only single copies of PIII hybrids on the phage surface, by using a combination of a pIII hybrid-expressing phagemid and a helper phage (carrying the wild type pIII gene). This group applied the method to hormone-hormone-receptor interactions, although the strategy can also be used in antigen-antibody interactions. The expression of single copies of pIII hybrids eliminates any chelation effects that might occur on solid phase adsorption, as well as making it possible to select for point mutations, for further improving an already high binding constant.

A recent review on antibody structures with a special emphasis on antibody-antigen complexes has been presented [36]. The larger number of structures now available strongly suggests that there is no longitudinal transmission of information from the variable domains to the constant domains of the Fab fragment, because the 'elbow bend' (the angle between the long axis of the variable domain and the first constant domain in each chain) appears to be randomly distributed. This is seen most dramatically in the light chain dimer Mcg [37•], which crystallizes with dramatically different elbow bends under two different crystallization conditions. This independence of the variable domains from the constant domains is consistent with the finding that Fv fragments have the same antigen binding constant as the Fab fragment, or even the whole antibody [8,13•]. Simon and Rajewsky [38] further demonstrated this domain-independence by switching the variable V<sub>L</sub> and V<sub>H</sub> domains between the two chains and finding no change in antigen affinity. This result was even observed when a Fab fragment was produced with  $C_L$  in both chains. In this context, the function of the hinge region must also be discussed. Tan et al. [39•] have studied the influence of different hinge regions on complement activation, C1q binding and segmental flexibility (by measuring fluorescence emission anisotropy kinetics of bound hapten) in antibodies with the same antigen-binding sites. They found that segmental motion is correlated with the length of the 'upper hinge', but not the total hinge length, and that segmental flexibility is not correlated with the ability to activate the complement cascade.

Few observations relating yields from this enrichment procedure to binding constants have been reported for any of the experiments with filamentous phages. The optimal design of such hybrid proteins is yet to be elucidated and the problems of 'shedding' of pIII hybrids from the phage envelope, or irreversible binding of very tight complexes, have not yet been addressed. These questions are not merely academic because, in order to Poljak's group [40••] has solved the first crystal structure of an antibody-anti-idiotype complex. The first antibody (D 1.3) was directed against lysozyme and the sec-

ond antibody was selected to recognize 'private' epitopes on the first antibody, corresponding to residues in the hypervariable loops. Anti-idiotypic antibodies have been used as immunogens and vaccines, and the newly elicited immunoglobulin (directed against the anti-idiotypic antibody) often appears to share specificity with the first antibody. In an interesting application of this concept, antiidiotypic antibodies made against defined anti-ligand antibodies often recognize the same receptor as the particular ligand and have been used successfully in receptor cloning. Such observations have generated a picture among many immunologists that anti-idiotypic antibodies are the body's 'internal image' of the original antigen [41].

An inspection of the solved structure of such an antibody-anti-idiotype complex now requires modification of this view. Very little geometrical similarity between lysozyme and the anti-idiotypic antibody can be recognized, although part of the binding regions used by lysozyme and the second antibody overlap. If the surfaces interacting with the first antibody are examined more closely, an area with somewhat similar hydrophobicity and charge patterns to the second antibody can be discerned between lysozyme and the anti-idiotypic antibody, and both molecules form apparently rather stable complexes with D1.3. The deduction from this observation (which from a structural biology point of view may not be so surprising) is that the similarity between the antigen and anti-idiotype is limited to some critical contact areas in part of the binding pocket. Thus, the anti-idiotype approach may be an important tool for identifying receptors by known binding properties, but an anti-idiotype is apparently not a model for the antigen architecture, nor is the antibody directed against a ligand of a certain receptor a model for this receptor's structure or function.

ment. The structure of the V<sub>L</sub> domain in the V<sub>L</sub> dimer and in the Fab fragment is remarkably similar with the exception of some changes in the complementarity-determining region (CDR)3 loop (B Steipe, A Plückthun, R Huber, unpublished data). Furthermore, the association of the V<sub>L</sub> domain with another V<sub>L</sub> domain in the homodimer, is apparently similar to that of the V<sub>L</sub> and V<sub>H</sub> domains in the heterodimer. The particular crystal packing of the V<sub>L</sub> domain [45•] appears to tolerate loop exchanges and may be of use in building a structural database of CDR loops.

Crystallography, in conjunction with the properties and biochemical analysis of mutants, now made much easier by the E. coli expression system, will certainly give a new impetus to the field of antibody engineering. One of the central questions to be examined is the structure of the CDRs. From a comparison of the solved crystal structures, consensus CDR residues have been proposed (with the exception of CDR3 of the heavy chain) [46•]. It has been suggested that only a few critical residues determine the conformation of the loops [47]. Although a number of critical interactions have certainly been identified (e.g. with framework residue 71 in  $V_H$  [48]), a comparison of the Kabat Sequence Database with the sequences of antibodies with known structures shows that only a fraction of the typical lengths of the CDRs have actually been observed crystallographically. There have to be at least as many canonical structures as there are loops of different lengths, if not more.

Antibodies generated against peptides have often been found to recognize the whole protein. The structural basis of this crossreactivity has now been examined by the solution of the structure of an anti-peptide antibody with and without bound peptide [42•]. The peptide, in this case part of the carboxy-terminal  $\alpha$ -helix in myohemerythrin (Mhr), is bound as a type II  $\beta$ -turn to the antibody (although the free peptide in solution exists as a nascent helix) [43]. The antibody recognized apo-Mhr more efficiently than native Mhr, even though the former is probably largely disordered. The authors suggest that the native Mhr might be only recognized at all as a result of some conformational change induced by the experimental conditions. Thus, this peptide, when complexed to the antibody, is definitely not mimicking the structure of the holo-protein.

Martin et al. [49•] have combined the use of database searching with energetic evaluation of conformations using the program CONGEN [50] to achieve root mean square deviations of about 2Å (considering all atoms) in their predictions of variable domain structures. To refine such approaches further, a larger database of antibody structures is clearly needed. The structures of antibody combining sites have been analyzed from a different angle by Padlan [51], who has compiled the frequency of amino acids present in the sites with their solvent exposure. He proposes that the rather stable antibody framework can tolerate unusually exposed aromatic residues, which in turn contribute to antigen binding by both hydrophobic forces and hydrogen bonding. Mian et al. [52] have also reviewed these points and have tabulated the structural data of side chains that contact the antigen.

Two groups have now reported the solution of structures of antibody variable domains expressed in *E. coli*. Bhat *et al.* [44•] have compared the structure of the Fv fragment of the antibody D1.3 complexed to lysozyme with the Fab fragment complexed to the antigen. They found no dramatic differences in structure, although they were able to demonstrate a domain movement in the Fv fragment upon antigen binding. It should be noted that the smaller Fv fragment, now accessible through *E. coli* expression technology [8], gives rise to crystals of higher resolution than the Fab fragment. Glockshuber *et al.* [45•] report the solution of the structure of the V<sub>L</sub> domain of the antibody McPC603. Again, the resolution of the recombinant domains is significantly higher than that of the Fab frag-

# Antibody engineering: binding sites for metals and haptens

Metals are essential cofactors in many enzyme reactions and play an important role in the structure of numerous proteins. Metal ions may be bound either to a cofactor (such as heme) or directly to the protein. In the former case, it will usually be possible to generate metal-cofactor-binding antibodies by the immunization of an animal using the metal-cofactor as hapten [53,54•,55•], whereas there is no obvious procedure to elicit a metal ion-binding site directly in the immunoglobulin by immunization alone. Antibodies have been generated against metal-chelates (such as EDTA analogs) [53], but they only recognize the metal-chelate complex and not the naked metal. In order to introduce metals directly into antibody domains it is therefore necessary to employ the methods of protein engineering.

Metals play a role in numerous chemical reactions, most prominently, but not exclusively, in redox and hydrolytic reactions. Different metal ions 'prefer' different ligands and different geometric arrangements. Most experimental engineering work has been carried out with  $Zn^{2+}$ , as it is apparently bound exclusively by heteroatoms from amino acid side chains. The substitution of side chains is generally felt to be more easily achieved than the redesign of main chain loops, and other metal ions, such as  $Ca^{2+}$ , often use main chain carbonyl groups as ligands.  $Zn^{2+}$ is a central component of metalloproteases. The reaction mechanism of the best studied example, carboxypeptidase A, has been reviewed elsewhere [56]. body molecules. Recent examples include proteases and a synthetic four-helix bundle [64-66].

The rapid progress being achieved in the field of catalytic antibodies is primarily a result of the design of transition state analogs or other suitable immunogens for eliciting natural monoclonal antibodies capable of causing rate accelerations. Thus, most of the work falls outside the narrow definition of protein engineering and the scope of this review, but the interested reader is referred to some recent reviews presenting the progress of this field very coherently [67–70].

The examination of hapten-binding sites by protein engineering has now been reported for two phosphorylcholine (PC)-binding antibodies, which both have some catalytic activity in the hydrolysis of carbonate esters. Jackson *et al.* [71•] have studied the antibody S107 expressed in myeloma cells, and Glockshuber *et al.* [72•] have scrutinized the Fv fragment of the antibody McPC603 expressed in *E. coli.* It had been assumed that the mode of PC binding is very similar in these two antibodies, as they share sequence homology in the CDRs, but the effects of some analogous mutations were found to be dramatically different. As only the crystal structure of McPC603 is known, some assumptions about the similarity of all PC-binding antibodies might have to be revised.

The steady increase in the number of solved crystal structures of metalloproteins has made it possible to extract the geometry of various metal binding sites from some natural metalloproteins. A number of useful tabulations have appeared recently, highlighting various aspects of the distribution of ligands around the metal ion [57–61]. There is still a long way to go, however, before metalbinding sites can be engineered *ab initio*. At present, the most promising method relies on the assumption that functional metal-binding sites can be created if the amino acids making up a known metal-binding site in another protein are introduced at positions having similar geometric relationships in the new proteins. For instance, the Zn<sup>2+</sup>-binding site in carbonic anhydrase is made up of three His residues that are arranged on two neighboring strands of an antiparallel *β*-sheet. Because antibody domains are antiparallel  $\beta$ -barrels, a large number of such binding sites can be superimposed onto the antibody framework. The experiments described for antibodies [62•,63•] show that a number of potential  $Zn^{2+}$ -binding sites can be delineated, but so far only one of these has actually been produced experimentally. By fluorescence quenching, binding of Cu<sup>2+</sup> could be measured, and by competition,  $Zn^{2+}$  binding could be indirectly demonstrated. These experiments are first steps towards the successful use of metal ions directly bound to an antibody in catalysis (which has not yet been achieved). It should be born in mind that the geometry of the metal-binding with respect to the substrates is likely to be extremely critical and that many factors other than the presence of the metal contribute towards rate enhancement. In fact, it is by no means clear whether a perfectly positioned metal would cause a noticeable rate enhancement in hydrolysis by itself. Therefore, only in a few serendipitous cases will the scissile bond of the substrate be properly oriented and in the correct environment for an efficient metal-catalyzed hydrolysis reaction. It should be mentioned in this context that introduction of metal-binding sites by protein engineering has not been limited to anti-

## **Chimeric antibodies**

The term chimeric antibodies usually refers to the substitution of the constant domains with those from a different species. In most cases, human constant domains are fused to mouse variable domains to create antibodies less immunogenic in man than the (highly immunogenic) whole mouse antibodies. In two investigations, the immunogenicity of such chimeras was reported, one in mouse [73•], and the other in man [74•]. The immunogenicity in man seems to depend very critically on the particular mouse variable domain (AF Lobuglio et al., personal communication), but the immunogenic motifs are not at all understood. Winter's group [75] has developed the technique of grafting only the binding loops into the framework of human variable domains, to further decrease the immunogenicity of the antibody. It should be pointed out, however, that no evaluation of the immunogenicity of such CDR-grafted antibodies in man has been published, to the best of this author's knowledge, and it is not yet clear whether the surface created by the six CDRs themselves might not be immunogenic. Again, this immunogenicity may vary strongly among different antibodies.

In a broader sense, antibody chimeras also include molecules in which the constant domains have been sub-

stituted by non-antibody domains. Recent examples include fusions to the luminescent protein aequorin [76•], which might lead to the development of new sensitive analytical methods. Fusions of a single-chain Fv fragment to the *Staphylococcus aureus* Protein A [77•] that appear to preserve both antigen binding and binding to an IgG column have been reported. Fusions of a single chain Fv fragment to the carboxy-terminally linked *Pseudomonas* exotoxin [20•,78•,79•] and to the aminoterminally linked diphtheria toxin [80•] have also been reported. Again, both parts of the molecule seem to remain functional in the hybrid proteins after refolding *in vitro*.

### Conclusion

Although antibody engineering is still at an early stage, recent progress in expression, screening, structural chemistry and theoretical chemistry will probably act synergistically to speed up research in this area. However, in order to mimic the sophisticated engineering acquired by evolutionary forces in the design of antibodies, many more of nature's secrets of the antibody molecule need to be unravelled. 10. PLÜCKTHUN A, SKERRA A: Expression of Functional Antibody

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### 244 Pharmaceutical applications

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Random peptides were encoded at the amino-terminus of the pIII protein of a filamentous phage and thus presented on the phage surface. Phage displaying a certain epitope able to bind directly to avidin were enriched by repeated solid phase adsorption.

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 Method for Variant Proteins with Altered Binding Properties. *Proteins* 1990, 8:309–314.

Human growth hormone was expressed at the amino-terminus of the pIII protein of a filamentous phage, encoded in a phagemid in the presence of a helper phage, and thus presented in low copy number on the phage surface. It was shown that a tighter-binding variant of the hormone could be enriched after adsorption to solid phase-bound hormone receptor.

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Random peptides were encoded at the amino-terminus of the pIII protein of a filamentous phage and thus presented on the phage surface. Whole phage displaying a certain epitope can be enriched by adsorp• Hinge Region on Complement Activation, C1q Binding, and Segmental Flexibility in Chimeric Human Immunoglobulins. *Proc Natl Acad Sci USA* 1990, 87:162–166.

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  - BHAT TN, BENTLEY GA, FISCHMANN TO, BOULOT G, POLJAK RJ:
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### 246 Pharmaceutical applications

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A fusion between part of diphtheria toxin (at the amino terminus) and a single-chain Fv fragment (at the carboxy terminus) was constructed, refolded from inclusion bodies and shown to be cytotoxic to cells expressing the interleukin-2 receptor.

A fusion between Fv fragment (at the amino-terminus) and *Pseu*domonas exotoxin (at the carboxy-terminus) was constructed, refolded A Plückthun, Genzentrum der Universität München, c/o Max-Planck Institut für Biochemie Am Klopferspitz, D-8033 Martinsried, FRG.