molecules or molecular conformations were treated) allowed for the first time the *a priori* differentiation of their therapeutic properties and led to the classification of psychotomic or sedative agents in the family of antidepressants. It is now possible from existing diagrams to predict the activity of new tricyclic compounds. The molecular electrostatic potential is computed using a monopole approximation[3] on the Van der Waals surface.

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Karma: a knowledge-based system for receptor mapping

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A combination of molecular modelling and quantitative structure–activity relationships (QSAR) is a powerful tool in the design of ligands and inhibitors, and provides a means to better understand ligand–receptor interactions[1,2]. This approach is most successful where the 3D structure of the receptor site is known. Karma (Kee assisted receptor mapping analysis) is an interactive computer-assisted rule-based drug design tool that utilizes real-time interactive 3D colour computer graphics (Silicon Graphics IRIS 2400T) with numerical computations (DEC VAX 8600) and symbolic manipulation techniques (Symbolics 3600 Lisp Machine) using the expert system software tool Kee (Knowledge engineering environment). Karma incorporates Qsar, conformational analysis (distance geometry and energy minimization), and graphics to generate a theoretical receptor site surface model[3]. Three-dimensional structures are input for the conformational analysis programs. Selected structures or distance matrices contain the geometric relations of the input structures and are used to generate the preliminary receptor surface model. An outline of the surface is obtained by the intersection of spheres, while details of the surface are generated using bicubic patches with the outline as the basis set. Bicubic patches form a continuous surface which may be reshaped interactively and have local density variations. The multiple visual cues of colour, texture and intensity represent simultaneously a number of receptor properties such as shape (i.e., cleft or hole), volume, hydrophilicity and hydrophobicity, and effectively summarize large amounts of numerical data. The deductions made by the KEE inference engine are based on Qsar equations, physicochemical parameters, kinetic data, and structural chemistry. Upon completion of the deduction phase, the characterized receptor model is displayed. The user may then manipulate and modify the model to test hypotheses and generate new rules. Modified models may be resubmitted to the inference engine to make additional deductions and detect inconsistencies. An integral part of Karma is user interation. By combining Karma's knowledge base with the knowledge and insight of the expert user, a more refined model may be built than permitted by either the user's or Karma's knowledge alone.

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Structural aspects of antigen–antibody recognition

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The antigenicity of a protein resides in discrete areas or epitopes of the molecule's surface that are specifically recognized by the binding sites or paratopes of antibody molecules. Although it is commonly assumed that paratopes always take the form of a pocket, there is evidence that shapes complementary between epitopes and paratopes can also take other forms. Exposed or protruding regions at the protein surface often correspond to antigenic sites which may consist of a series of overlapping epitopes that are distinguishable with appropriate monoclonal antibodies. Linear peptide fragments of a protein that binds to antibodies raised against the whole molecule are usually considered to represent continuous epitopes. However, it is possible that such peptides are antigenically active because they contain a short stretch of residues corresponding to a subregion of a larger discontinuous epitope. Discontinuous epitopes are made up of residues distant from each other in the sequence but brought together by the folding of the chain, and they may be more numerous in globular proteins than continuous epitopes. The following approaches have been used to delineate epitopes: 1) cross-reactivity studies between protein fragments and antibodies to the native protein; 2) cross-reactivity studies between native protein and antipeptide antibodies; 3) cross-reactivity studies among homologous proteins; 4) crystallographic study of antigen–antibody complexes. The approximate location of a limited number of epitopes has been established for only a dozen proteins of known tertiary structure. Attempts to correlate certain structural features of proteins with their antigenicity have been concerned mainly with continuous epitopes. Parameters that have

been considered are hydrophilicity, chain termination, mobility, accessibilty amphipathicity and evolutionary variability. Since all these properties of antigens are interconnected, it is probably futile to search for a single primary correlate to 'explain' antigenicity.

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Genetically engineered analogues of human α_1-antitrypsin

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The primary function of α_1-antitrypsin is the inhibition of neutrophil elastase, a protease capable of destroying connective tissue. A α_1-antitrypsin deficiency results in lung emphysema. The α_1-antitrypsin from lungs of individuals who smoke is partially inactivated due to oxidation of the Met residue at the active site of the protein, explaining the high incidence of emphysema associated with cigarette smoking. An ($Met^{358} \rightarrow$ Val) α_1-antitrypsin analogue which remains fully active as an elastase inhibitor but which is also resistant to oxidative inactivation has been constructed and characterized. The properties of other analogues with modified residues in the active site will also be described; the inhibition of thrombin and cathepsin G has been especially studied.

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Classical genetics and site directed mutagenesis in the study of the specific interaction with DNA of CAP, the cyclic AMP receptor protein in *E. coli* K12

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The catabolite gene activator protein (CAP), complexed with its allosteric effector, cyclic AMP (cAMP), plays a key role in gene expression in *E. coli*. It binds to specific DNA sites at or near promoters, where it stimulates the initiation of RNA synthesis. Three mutations that alter the DNA sequence specificity of the catabolite gene activator protein CAP have been isolated, as suppressors of mutations at positions 7 and 16 of the symmetrical CAP binding site that prevent the binding. All three mutations affect the same amino-acid of CAP, glutamic acid 181. It is proposed that it is this amino-acid of CAP that makes contacts with base pairs 7 and 16 of the symmetrical recognition site. The authors deduced the specific chemical interactions by which amino-acid side chains at position 181 interact with base pairs 7 and 16 and the precise alignment between structures of the CAP and DNA in the intramolecular CAP–DNA complex. Several predictions can be drawn from this model, that we are currently testing, by site directed mutagenesis of the *crp* gene coding for CAP.

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Probing the catalytic mechanism of glyceraldehyde-3-phosphate dehydrogenase by site-specific mutagenesis

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The glycolytic glyceraldehyde-3-phosphate dehydrogenase (GAPDH) is a tetrameric enzyme, with cooperative properties, requiring NAD^+ as cofactor. GAPDH catalyses the oxidative phosphorylation of D-glyceraldehyde-3-phosphate into 1,3-bisphosphoglycerate, through a series of steps, including several chemical intermediates[1]. The 3D structure at high resolution determined by X-ray crystallography is now available for the lobster muscle enzyme and for the *B.stearothermophilus* enzyme[2,3]. Kinetic, chemical and crystallographic studies have enabled the proposal of a plausible molecular model which could account for catalysis and explain substrate specificity. In particular, amino-acids which could play a role in the catalytic mechanism have been identified. This includes the binding of coenzyme NAD^+ in which the adenine moiety binds to Asp 32 and the nicotinamide moiety binds to Asn 313 and Tyr 317. The substrate 3-phosphate binds to Thr 179, Arg 231, and Arg 195. The inorganic phsophate binds to Ser 148, Thr 150 and Thr 208. The Cys 149 residue is implicated in the formation of the acyl intermediate and His 176 is implicated in acid–base catalysis. Recently, two fragments of 1500 and 4000 nucleotides in length, inserted in pBR 322 and containing the structural GAPDH genes of *E.coli* and *B.stearothermophilus*, respectively, have been selected by complementation of an *E.coli* GAPDH mutant[4]. A very high efficiency of expression of the two cloned genes was observed in *E.coli*. The sequence of the two inserts has been determined[5,6]. In order to confirm the catalytic mechanism of GAPDH, different mutants were generated, by site-specific mutagenesis. We have replaced the histidine 176 at the active site with an aspartine residue. We also obtained a double mutant