MEETING REPORT

Twelfth annual conference of the Molecular Graphics Society. Interlaken, 7–11 June 1993

The twelfth annual conference of the Molecular Graphics Society (MGS) was held in Interlaken, June 7–11, 1993, with the theme *Molecular Graphics and the Design of Bioactive Compounds*. It gathered over 300 participants from the five continents, who enjoyed very much the site of Interlaken and the unique environment and infrastructure for such international meetings. As the scientific quality and the organization were in tune with the surroundings, it is no overstatement to assert that the conference was a total success.

The purpose of this report is to summarize the major presentations of this conference so as to give to our readers a flavor of the main trends of molecular graphics in 1993. However, this report should be considered *de facto* as a partial account, as it is of course impossible to present the main features of all the 8 invited lectures, 31 oral contributions, 91 posters, and 9 exhibitor stands. We will limit ourselves to what we consider as the key points and important developments presented in Interlaken on the rapidly expanding subject of molecular graphics design of bioactive species.

According to **M. Hann,** chairman of the MGS, "the aims of the Society are to promote and advance the computer-based science of molecular graphics and modeling." This is an interesting statement to emphasize as an introduction, as molecular graphics should be taken here in its broadest sense, encompassing the models which are at the basis of graphics, i.e., molecular modeling. As such, the main goal of the meeting was therefore to present and discuss the latest achievements in investigations dealing with computer modeling of the structures, functions and interaction mechanisms of bioactive systems. The purpose was therefore much broader than just "nice graphics" of molecular models, as an unwarned or innocent reader might have thought.

In a magistral opening lecture, **K. Müller** (Hoffmann-La Roche) presented an overview of the present capabilities and limitations of molecular graphics (MG) for structure design. He emphasized the improvements which should be made to the MG tools to allow them to evolve, namely: from qualitative graphics to quantitative design; from small, rigid, static conformations to large, flexible dynamic, folding systems; from ligand-guest molecules to receptor-host ensembles; from local energy minima in vacuum to free energies in the presence of solvent, obtained from wide sampling of configuration space; from cases and views to sets and overviews; and from computer-assisted studies on sequential machines to computer-automated investigations on parallel computers. In his opinion, MG techniques should be developed along these lines, though in many respects significant progress has already taken place. Turning then to applications, the speaker mentioned some recent important achievements, such as three-dimensional (3D) database searching for protein cavities able to bind substrates or pseudoreceptor modeling, i.e., the design of a ligand without knowing the receptor. Also worth mentioning are the use of templates to model receptors, and last but not least, the crucial importance of X-ray and NMR-based structural design. However, molecular modeling has also a place predicting and understanding the structural features of bioactive systems, whereas graphics is essential to visualize the structures and the binding characteristics of drugs.

The conference program was made of six half-day sessions, each featuring an invited speaker and 4–6 oral contributions. We shall review here the key points of these sessions.

MOLECULAR DESIGN I

The first session on molecular design was opened by **M. Hibert** (Marion Merell Dow), a medicinal chemist using molecular modeling to rationalize drug design, who showed how the old-fashioned key-lock models of drug-receptor interactions have been superseded by 3D structure matching. In particular, MG 3D models of all cloned G-protein coupled receptors (GPCR) have been built and analyzed. These models were defined using primary sequence comparisons, secondary structure predictions, and 3D homology building, taking *bacteriorhodopsin* (BR), whose structural features were determined in 1990 by R. Henderson, as a template. The GPCR systems are characterized by two regions responsible for binding to the membrane and coupling with hormone proteins, respectively. The ligand binding sites have been localized and, surprisingly, they have been found to be buried within the receptor at 20 Å from the surface. Similarly, all the residues likely to be responsible for receptor affinity, selectivity, stereospecificity, and effi-

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cacy have been identified. These results will undoubtedly provide the basis for structure–activity relationship studies and more rational drug design.

A. Vedani (SIAT, Ettingen) presented the *YAK* package, which allows the construction of a peptidic pseudoreceptor (i.e., receptor model) around any single small molecule or molecular ensemble of interest (e.g., a pharmacophore). The peptide-growing algorithm is based on the directionality of ligand–receptor interactions. A proper force field, called *Yeti*, is used to describe this directionality, for which there is experimental evidence deducible from the Cambridge database. Suitable receptor residues are identified using a preference database and the molecular lipophilicity potential. This technique of pseudoreceptor modeling has been evaluated by modeling the active sites of metalloproteins.

Bacteriorhodopsin (BR) at work, an impressive 13-minute video, was then presented by **H. Grubmüller** (Universität München). The video displays a computer animation for the 13-14-*cis* model of the photochemical cycle of BR, which provides in condensed form detailed insights into the functioning of the light-driven, transmembrane proton-pump BR. The video is broken into two parts: the first introduces the structural elements essential for an understanding of the proton-pump process, whereas the second is devoted to the dynamics of the photocycle as obtained from molecular dynamics simulations covering all major known isomerization steps occurring during the process. The simulations are based on the refined structure of BR provided by R. Henderson, and solvent molecules have been taken into account. Though the author has correctly pointed out the danger of such animated representations (what you see is what you believe, but it is all based on model simulations), this video is a beautiful example of the important progresses witnessed recently in the use of scientific visualization techniques for both education and research purposes.

A very interesting MG development based on texture mapping was then presented by **M. Teschner** (Silicon Graphics). Texture mapping is well known to the computer graphics community as a technique used to project an image onto the surface of a 3D object, leading thus to much better realism and visual quality of rendered images. Modern graphics workstations offer support for real-time texture mapping, which means that new levels of performance may be reached in MG applications. Among several examples, the speaker showed that this technique leads to much better mappings of a physical property, e.g., the molecular electrostatic potential (MEP), onto an arbitrarily shaped envelope such as the molecular surface. For instance, it is possible to easily generate contour levels of MEPs on molecular surfaces, which greatly facilitates an accurate determination of the position of MEP minima or a quantitative discrimination between competing reactive sites.

STRUCTURE–PROPERTY CORRELATIONS

Several presentations were devoted to this fundamental concept in the design of bioactive compounds. In an invited lecture, **D. J. Abraham** (Virginia Commonwealth University) treated the problem of hydrophobic interactions and of the CoMFA (comparative molecular field analysis) tool using HINT, a homemade package allowing one to graphically and numerically quantify hydrophobic interactions between small molecules and proteins. After having reviewed the basic definitions and aspects of hydrophobic fields and hydrophobic effects, the speaker stressed the importance of these concepts for investigating the binding of drugs to proteins or receptors. In particular, he emphasized the connection between hydrophobicity and the well-known water-octanol log P partition coefficient proposed by Hansch, which represents a landmark in the studies of structure–property correlations. Various examples showing how hydrophobic effects influence the structures of drug–receptor systems were presented, and the speaker mentioned the importance of such effects on the function of hemoglobin. Finally, the efficiency of the HINT program was demonstrated by impressive results dealing with DNA–antibiotic interactions, the effect of mutations on allosteric activity, and HIV protease inhibitors.

E. A. Vorpagel (BIOSYM) reported on Apex-3D, an expert system aiming at the identification of pharmacophores within a diverse set of biologically active structures. This package uses advanced statistical techniques and 3D pattern-matching algorithms to assign probabilities and reliabilities to the identified pharmacophores. An interesting feature of this new tool in structure–property correlations is the introduction of charge-transfer complex formation into the description of ligand–receptor interactions. Among the examples presented by the speaker, were several activity classes, including antipicornavirus agents, angiotensin converting enzyme inhibitors, and allosteric hemoglobin modifiers.

GOLPE, an advanced variable selection procedure aiming at obtaining PLS (partial least-squares projection to latent structures) with the highest predictability, was reported on by **G. Cruciani** (Università di Perugia). The GOLPE procedure has the advantage of being usable in 3D-QSAR studies on the fields generated by CoMFA, as well as on any data sets obtained from similiarity indices. The performance of GOLPE was tested on a series of 36 glucose analogue inhibitor compounds whose X-ray structures had been determined when bound to the glycogen phosphorylase b enzyme.

STRUCTURE-BASED DESIGN (NMR SPECTROCOPY)

Recently, due to the pioneering developments of Kurt Wüthrich and others, multidimensional NMR techniques have been shown to lead in many cases to the determination of the 3D structure of biological macromolecules. **H. Kessler** (Technische Universität München) reported on that subject, and demonstrated how such techniques facilitate the extraction of homo- and heteronuclear J-coupling constants for isotopically labeled and nonlabeled molecules by using various procedures such as COSY, DISCO, or HETLOC. A new technique based on the use of J-coupling constants as experimental constraints in molecular dynamics (MD) calculations was presented, and the speaker stressed its powerful aspects, though the analysis of the results may be a difficult task. The example of bioactive cyclic pentapeptides was taken: this procedure leads to a considerable reduction of the conformational space, and only a few allowed confor-

mations are obtained which contain the NOE-derived structures. For linear peptides made of up to 50 amino acids, the situation is somewhat different as they may adopt many possible structures which are very sensitive upon environmental effects, which means that the MD simulations must explicitly include the solvent. Several eloquent examples of the progresses recently achieved in structural determinations from NMR measurements were then presented, ranging from the sequential assignment of the P13 domain of mannose permease protein to the conformations of o-glycopeptides and cyclosporin A.

In the same session, **M. J. J. Blommers** (CIBA-GEIGY) presented the results of NMR studies of an unnatural oligonucleotide duplex, a member of a new class of drugs which form stable and specific hybrids with complementary natural nucleic acids. As they are resistant to nucleases, such compounds are promising targets in the treatment of human disorders like viral and bacterial infections, and cancer as well. Both NMR studies using mainly NOE intensities and simulated annealing MD calculations based on the NMR restraints show that the oligonucleotide duplex adopts a structure close to that of the B-DNA double helix.

Combined molecular modeling and ^{1}H NMR studies of opiates and β-casomorphins were reported on by **W. Brandt** (Martin-Luther Universität, Halle), in an attempt to derive structure–activity relationships for these species. The results lead to a uniform model for the drug–receptor interactions, which in turn provides coherent relationships between the structure and opioid activity of the β-casomorphins. This study is a further example of the very valuable synergy between NMR and modeling and simulation techniques which may be efficiently used today in order to design novel bioactive compounds.

STRUCTURE-BASED DESIGN (X-RAY CRYSTALLOGRAPHY)

The other essential experimental technique leading to the tertiary structure of proteins is X-ray crystallography, and as such, it deserved a full session at this Interlaken meeting. This session was opened by **T. L. Blundell** (Birbeck College) who gave a very interesting talk on protein structure and drug design. To illustrate the importance of the design of novel active compounds, the speaker took the example of the acute intermittent porphyry illness which struck king George III, with the consequence that he intermittently lost his mind and overtaxed his overseas subjects which among other things contributed to the American revolution. Had an active drug against prophyry existed by that time, then the face of the new world would be somewhat different today! Actually, to understand the origin of this illness, it is necessary to know the structure of the enzyme responsible for haem synthesis, namely porphobilinogen deaminase, and this requires the use of powerful techniques such as X-ray crystallography. By taking several other examples, the speaker showed that X-ray analysis has already defined the 3D structures of many proteins that are targets of drug design, including enzymes, receptors, and carrier and DNA-binding proteins. The topographies of their binding sites, often defined by structural studies on ligand complexes, provide a guide to drug discovery. In this process, computer graphics techniques represent an important tool for visualization of the complex macromolecular structures. According to the speaker, comparative modeling allows one to define protein subfamilies, e.g., anion binding proteins; and superfamilies, e.g., aspartic proteinases and renins; and this is very useful for solving the inverse folding problem: From the knowledge of the 3D structure of a protein, how can one predict all the sequences which adopt the same fold?

K. Gubernator (Hoffmann-La Roche) presented modeling results based on 3D structure information about serine hydrolases and their inhibitors: thrombin, betalactamase, lipase, and acetylcholinesterase. After having stated that the chemical function of bioactive systems is dictated by their structure, the underlying concept being sequence, the speaker showed how a detailed study of the catalytic mechanisms of these serine enzymes, based on their X-ray structures and on the conformations of several complexes with substrates, substrate analogues, and inhibitors, revealed a common stereo-electronic mode of action. In particular, a model of the specificity pocket of thrombin suggests that the driving force for protein–inhibitor interaction is hydrophobicity rather than a binding mechanism involving the fragments responsible for the catalytic functionalities. These results provide a firm basis for the rationalization of structure–affinity relationships and the design of novel potent inhibitors.

The basic principles underlying an empirical scale of conformational entropy for protein folding were then put forward by **M. J. E. Sternberg** (Biomolecular Modelling Laboratory of the Imperial Cancer Research Fund). As protein folding is favored by hydrophobic effects, this leads to an entropically favorable process since the burial of nonpolar residues allows the surrounding solvent to be less ordered. A major effect in the energetics of protein folding is therefore the loss of conformational entropy of the side chains. The consistency of a simple model for protein folding that includes side chain and main chain entropies, both derived from a new empirical scale (obtained from Boltzmann sampling over the rotameric states of selected proteins), hydrophobicity, and hydrogen bonding has been explored. This procedure allows one to rationalize the stability of site-directed mutations and to provide an additional tool for performing QSAR in drug design.

C. M. Venkatachalam (Molecular Simulations) has developed a computational algorithm for loop closure and applied it to the modeling of the hypervariable loops in antibodies. This algorithm involves random perturbations of the backbone phi, psi torsion angles of the loop regions forming the antigen binding site, followed by intelligent alterations to these variables in order to restore and maintain correct closure of the loop with respect to the rest of the protein. After a reasonable backbone conformation is obtained, the side chains are placed using a rotamer library, and the loop is annealed by energy minimization using the CHARMm force field.

Databases

The tremendous development of (bio)chemical databases and the possibility to carry out 3D substructure searching contributed to make this session one of the key points of the

conference. According to **P. Murray-Rust** (Glaxo Research), data in structural biology are being published at a rate which doubles every 18 months. This results in an information crisis due to the volume and complexity of the data and the diversity of methods, and there is a need for a change of culture. Among the solutions suggested by the speaker were the development of new standards, tools, networking, and cooperation. To vividly illustrate this topic and to demonstrate that standardization and cooperation are at present insufficient, he asked the provocative question: Would you get up into a plane built in collaboration by the software marketers? To underline the basic aspects of the present situation, the speaker mentioned that most of the time the data are fuzzy and therefore the conventional top-down approach in managing the information is out of date by the time it is completed. The solution is to actually build a new world of *bioinformatics*, this novel concept resting on interconnected elements such sequences, structures, diagrams, and abstracts. The most important point will be collaboration and communication between groups across the world, since most of the data will be in the public domain. It will be critical to provide navigation systems through the data since users cannot be expected to know where they are and how to use them. This formidable challenge requires a new set of tools, among which are: managed libraries of data, *de facto* data standards, flexible graphical interfaces, object-oriented processing languages, and transparent, high-bandwith communications for multimedia applications. According to the speaker, changing the technology is easy but changing the culture is difficult!

Several very good communications dealing with the problem of 3D pattern searching in databases of, e.g., drug molecules, were presented in this session, which emphasizes the importance of such developments for pharmaceutical industries. For example, **O. F. Güner** (Molecular Design Ltd.) addressed the problem of interactively performing conformationally flexible searches of databases of 3D structures. Indeed, according to the speaker, it is essential to take into account the flexibility of the structures, since patterns can be present with large conformational changes in the same 3D database. This feature has been implemented in three phases based on: screening of interatomic distance ranges, conformational fitting during the substructural search, and torsional fitting combined with calculations of nonbonded interactions. Impressive figures were reported concerning the performance of this procedure compared to traditional rigid searches.

IDEA, a program for 3D pattern searching in databases of drug molecules, was presented and discussed by its author, **G. Barnickel** (E. Merck). This package allows one to retrieve pharmacophoric patterns defined by atomic distances. Additional molecular features, such as hydrogen donors and acceptors, ring systems, and flexible chains, are treated as dummy atoms and are therefore accessible for retrieval within this approach. Furthermore, a representation of the electrostatic or hydrophobic field is used to allow nontopological information to be treated.

MOLECULAR DESIGN II

The last session of the conference was again devoted to molecular design, and it was by no means less interesting than the first one. **P. A. Barlett** (University of California) underlined that 3D database searching has several purposes: searching for answers, with the aim to identify pharmacophores with some accuracy, which leads to time scales ranging between hours and days; and searching for ideas, with the aim to design new molecules and to identify templates or structural units. In the latter case, speed is a major component and the search should be achieved within minutes. This is the point which the speaker addressed, and to this end, he presented his well-known program CAVEAT, which searches 3D databases for structures containing a specific orientation. Indeed, the design of a molecule that conforms to a structural model is most efficiently approached by engineering a framework to enforce specific vector relationships among the bonds that link the functional units to it. Using CAVEAT, a structural database is preprocessed to determine the relationships between all pairs of relevant bonds in each molecule. The resulting CAVEAT database can then be searched rapidly to identify potential templates or linking fragments. As a complement to existing databases and as a source of templates, two databases of computed, minimized structures have been generated: TRIAD, which contains 411,000 tricyclic hydrocarbons, and ILIAD, which is made of 110,000 structures built up from linear chains of 5 units. The performance of this program package is impressive: a benchmark 3-vector CAVEAT search through the TRIAD database yields 750 hits that are screened and clustered into 6 district groups in less than 3 minutes on a SGI Indigo workstation.

R. A. Eades (Cray Research) introduced density functional theory, a rather new development in quantum chemistry which has been found to provide a very computationally cost effective method for the study of molecular systems. It was shown that the use of pseudopotentials leads to significant simplifications in electronic structure calculations by eliminating the need to treat core electrons and by replacing the strong electron–nuclear attraction by a soft one. A comprehensive study of molecular calculations comparing the use of pseudopotentials relative to all-electron basis sets has been carried out, and the results show good agreement as far as geometries, binding energies, and vibrational frequencies are concerned. These developments offer promising perspectives for accurate quantum chemical calculations of large systems such as drug-model receptor complexes.

The last speaker of the session, and incidentally of the Interlaken meeting, was **W. F. van Gunsteren** (ETH). His talk was devoted to the computer simulation of biomolecules and to the improvement in methodology for searching conformational space and predicting molecular properties. The speaker showed first how misleading MD simulations can be when performed using time-averaged structure factors. For example, the trajectories obtained for a cube of 9 carbon atoms are correct if one uses crystallographic data with 1-Å resolution, but a wrong solution is found when starting from 2-Å resolution data! Similarly, the speaker reported that MD simulations performed on α-cyclodextrin converge to an erratic solution when using low-resolution time-averaged structure factors, even though the final R value is excellent. Another topic related to MD simulations where one has to be very cautious deals with calculations of free energies using the procedure of the thermodynamic cycle: the example of azurin mutation taken by the speaker shows that, though the

structural features of the process are well reproduced, the free energy is significantly in error! This leads to the conclusion that the prerequisite conditions for an accurate calculation of free energies are: one has to make sure that the simulation is carried out for a system at equilibrium; the sampling of the configuration space must be adequate; and the molecular force field must be reliable; i.e., it should contain the essential energy components with the proper parameters. Finally, the speaker showed promising results obtained in MD simulations where searching and sampling are performed in four dimensions (4D), i.e., the usual 3D spatial variables plus an additional pseudo dimension allowing one to suppress the pole at origin due to the $1/r^n$ dependence of some potentials. From the results of several examples, including cyclosporin, it is seen that 4D MD simulations lead to improved results when comparing with 3D ones. Taking advantage of the uncoupling between the *xyz* spatial degrees of freedom and the fourth pseudo component, it should be even possible to calculate free energies using 4D MD simulations, which could possibly represent an improvement over the existing methodology of this important field.

This brief report has attempted to present the major aspects of the twelfth annual MGS conference. In addition to the high quality of the formal scientific presentations, this meeting was very successful because of the vast possibilities of mutual exchange and discussions it offered between the participants, most of them being eager, therefore, to take part in the thirteenth annual MGS conference, scheduled for 1994 in Australia.

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