Journal of Biomolecular NMR, 3 (1993) 261–269
ESCOM

J-Bio NMR 122

NMR structure-based drug design

Stephen W. Fesik

Pharmaceutical Discovery Division D-47G, AP9 Abbott Laboratories, Abbott Park, IL 60064, U.S.A.

Received 3 March 1993
Accepted 17 March 1993

Keywords: Drug design; Molecular modeling; 3D database searching; Heteronuclear multi-dimensional NMR

SUMMARY

NMR is a useful tool for rapidly determining the conformations of receptor-bound ligands and identifying those protions of the ligand in contact with the receptor. In addition, the complete 3D structures of receptors and ligand/receptor complexes can be obtained using recently developed heteronuclear multi-dimensional NMR techniques. This NMR-derived structural information is potentially useful for aiding in the design of improved pharmaceutical agents. Approaches for utilizing the NMR-derived structural information along with the computational tools that facilitate this process are discussed.

INTRODUCTION

The discovery of new pharmaceutical agents is a difficult task. In addition to binding tightly and specifically to its target site, the new chemical entity must be chemically and metabolically stable, be relatively nontoxic, be able to get to the site of action, and be synthetically and economically feasible to prepare. The drug discovery process begins by identifying a lead compound that possesses the desired pharmacological activity, followed by the synthesis and biological testing of several analogs in search of the ideal drug candidate. In practice, new leads are generally either a natural hormone or enzyme substrate, or they are found by randomly screening natural products or chemical libraries. The final drug molecule is discovered by fine-tuning these initial leads by making use of structure–activity relationships. This process is time consuming and often requires the synthesis and testing of several hundred compounds.

Another approach for discovering new pharmaceutical agents is structure-based drug design, in which the 3D structure of a receptor or ligand–receptor complex is used to generate new leads and guide lead optimization. Using structures obtained from X-ray crystallography, this approach had aided in the discovery of novel inhibitors for HIV protease (Bures et al., 1990; Des Jarlais et al., 1990; Erickson et al., 1990), thymidylate synthetase (Appelt et al., 1991; Varney et al., 1992), carbonic anhydrase (Baldwin et al., 1989), and purine nucleoside phosphorylase (Ealick et al., 1991). In principle, NMR spectroscopy could also be used to obtain structural information for

designing new drug molecules (Fesik, 1989, 1991). In this perspective, the potential of using NMR structures for drug design is discussed with emphasis on the type of relevant structural information that can be obtained by modern high-resolution NMR techniques and the computational tools that are available for utilizing the NMR-derived stuctural information.

NMR STRUCTURES OF BOUND LIGANDS

The NMR spectra of drug molecules by themselves in solution are relatively simple; however, in most cases it is difficult to derive any useful structural information for drug design from these NMR spectra. In the absence of their receptors, drug molecules are generally flexible and exist in several rapidly interconverting conformations, making it extremely difficult to correlate conformation and biological activity. In contrast, the 3D structure of a drug when bound to its biological site of action would be much more useful in the design of new molecules. There are several NMR methods that can be used to derive this structural information (Griffey and Redfield, 1987; Fesik et al., 1990b; Otting and Wüthrich, 1990; Fesik, 1991). For weakly bound ligands, transferred nuclear Overhauser effects (NOEs) can be measured for ligands that exchange rapidly from the bound to the free state and used to determine the conformation of a ligand when bound to a macromolecule (Albrand et al., 1979; Clore et al., 1981; Clore and Gronenborn, 1982). However, the transferred NOE experiment can be applied only to the study of weakly bound ligands that rapidly exchange on and off the enzyme. Unfortunately, many of the interesting leads bind tightly to their target proteins and cannot be studied by transferred NOE experiments.

To determine the conformation of tightly bound ligands, isotope-editing (Bendall et al., 1981; Otting et al., 1986; Bax and Weiss, 1987; Griffey and Redfield, 1987; Rance et al., 1987; Fesik et al., 1988) or heteronuclear 3D NMR techniques (Fesik and Zuiderweg, 1988; Marion et al., 1989; Kay et al., 1990a) could be applied, allowing the selective detection of protons attached to an isotopically labeled drug molecule when bound to its receptor protein (Fesik et al., 1988, 1990a, 1991a; Petros et al., 1991, 1992a; Weber et al., 1991). Alternatively, the proton NMR signals of an unlabeled ligand can be selectively observed when bound to an isotopically labeled receptor by using isotope-filtering techniques (Wider et al., 1990; Ikura and Bax, 1992; Petros et al., 1992b).

In addition to the conformation of the bound ligand, isotope-aided NMR experiments can also be used to identify those portions of the ligand that are in close proximity to the receptor (Fesik et al., 1988, 1990a, 1991a; Wider et al., 1990; Petros et al., 1991, 1992a; Weber et al., 1991). One would expect that structural changes in such regions would lead to ligands with lower affinity. NMR methods have also been developed to study ligand–receptor complexes, using paramagnetic agents (Schmidt et al., 1982; Anglister et al., 1984; de Jong et al., 1988; Petros et al., 1990, 1992c; Fesik et al., 1991b). Techniques have been developed that rely on the change in longitudinal relaxation rates of the solvent-exposed protons of the bound ligand in the presence of a paramagnetic agent and have been used to define the solvent-exposed surface of immunosuppressants when bound to their target proteins (Fesik et al., 1991b; Petros et al., 1992c). Changes in these regions of the ligand should be more permissable.

NMR STRUCTURES OF RECEPTORS AND LIGAND–RECEPTOR COMPLEXES

Three-dimensional structures of drug receptors (e.g., Michnick et al., 1991; Moore et al., 1991)

and ligand–receptor complexes (e.g., Ikura et al., 1992; Theriault et al., 1992; Meadows et al., 1993) can also be obtained by NMR to provide additional, valuable structural information for drug design. Indeed, with the development of heteronuclear 3D and 4D NMR techniques (Fesik and Zuiderweg, 1988; Marion et al., 1989; Kay et al., 1990a, 1990b; Clore et al., 1991; Zuiderweg et al., 1991), larger and more precise NMR structures can be determined than previously possible (Clore and Gronenborn, 1991). New NMR methods allow the ^{1}H, ^{13}C, and ^{15}N resonances of receptor proteins to be rapidly assigned. In addition, many more NOEs (Clore et al., 1991; Meadows et al., 1993) as well as hetero- and homonuclear J-coupling constants (Montelione et al., 1989; Kay and Bax, 1990; Emerson and Montelione, 1992; Szyperski et al., 1992; Xu et al., 1992) can be obtained by using recently developed heteronuclear multidimensional NMR techniques. These studies require uniformly ^{15}N- and ^{13}C-labeled receptors, which can be readily obtained from either bacterial (Muchmore et al., 1989) or, more recently, mammalian cells (Hansen et al., 1992) that overexpress the protein of interest.

UTILIZING THE NMR-DERIVED STRUCTURAL INFORMATION

How can the NMR-derived structural information be used to design new pharmaceutical agents? From ligand–receptor NOEs, the functional groups of the ligand that are likely to be important for binding to the receptor can be identified, and the relative spatial orientation of these functional groups can be determined from the bound conformation of the ligand. This structural information about the ligand, which can be obtained in as little as 1–2 weeks by using the isotope-aided NMR methods discussed above, could be used to design analogs with different molecular frameworks that position the important functional groups of the ligand in their experimentally determined spatial orientation. These new analogs may bind more tightly to the receptor due to entropic considerations, may be more metabolically stable, or easier to synthesize. Portions of the ligand which are solvent-exposed are identified on the basis of a lack of ligand–receptor NOEs or paramagnetic-induced changes in the proton relaxation rates of the bound ligand. This information may be useful for identifying the functional groups of the ligand that can be modified without affecting binding affinity. These modifications could improve the physical properties of the ligand, aiding its transport to the biological site of action.

The complete 3D structure of the receptor or ligand–receptor complex provides additional information to aid drug design. By making use of NMR-derived structures, the steric and electronic properties of the receptor pocket can be mapped and used to design molecules that optimally fit into this site. From the NMR structure of a receptor–ligand complex, the interacting functional groups of the receptor and ligand are identified, leading to a better appreciation of the interaction energies. In addition, new functional groups on the receptor that are in close proximity to the ligand can be identified from the structure and used to design analogs with higher affinity or increased specificity that bind to this additional site.

COMPUTATIONAL TOOLS FOR NMR STRUCTURE-BASED DRUG DESIGN

In order to utilize the NMR-derived structural information effectively, computational tools are required. In this section, some of these computational tools are described along with the NMR-derived structural information that is used by the different methods.

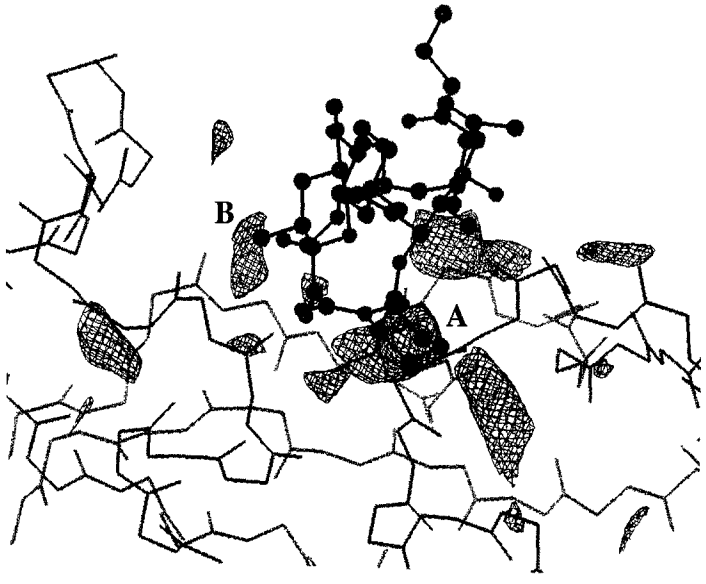

Fig. 1. NMR structure of the FKBP–ascomycin complex (Meadows et al., 1993) and low-energy (−3 kcal/mol) GRID contours (Goodford, 1985) generated with a methyl probe, using the NMR structure of the FKBP–ascomycin complex without the ligand. The ascomycin ligand is shown as a ball and stick model. The low-energy contours corresponding to hydrophobic binding sites are used by the pipecolinyl ring (A) and methyl group of the pyranose ring (B) of the ascomycin ligand.

Molecular graphics

There are several computer programs that can be purchased which will allow new molecules to be rapidly created and displayed. By manually altering the images on the computer screen, molecules can be designed that superimpose on the experimentally determined bound conformation of the ligand or that appear to fit into a receptor site and form favorable hydrophobic, electrostatic, or hydrogen-bonding ligand–receptor interactions. This process is aided by color- coding the different parts of the molecules, displaying the van der Waals' surfaces of the ligand and receptor site, and interactively checking for bad van der Waals' contacts while docking new ligands.

Although not a graphics program in itself, the GRID algorithm (Goodford, 1985) is a useful tool for locating and visualizing potential ligand binding sites. GRID calculates the interaction energies between a probe (e.g. methyl, carbonyl, hydroxyl) and the receptor protein. With a molecular modeling program one can display a contour plot of the interaction energies for the different probes along with the receptor protein. New analogs are designed by placing functional groups at the low-energy sites for the different probes and linking them together to form molecules that fit within the available surface of the receptor pocket, which can be visualized with GRID by contouring at a high-energy level (Goodford, 1985). An example of the GRID output is shown in Fig. 1. The NMR structure of the immunosuppressant, ascomycin (ball and stick), complexed to its target protein (FKBP) is shown (Meadows et al., 1993). In addition, the figure shows the contours corresponding to the favorable binding sites for a methyl probe generated by using the GRID program (Goodford, 1985) from the FKBP structure without the ligand. Low-energy contours corresponding to hydrophobic binding sites were observed in regions that are

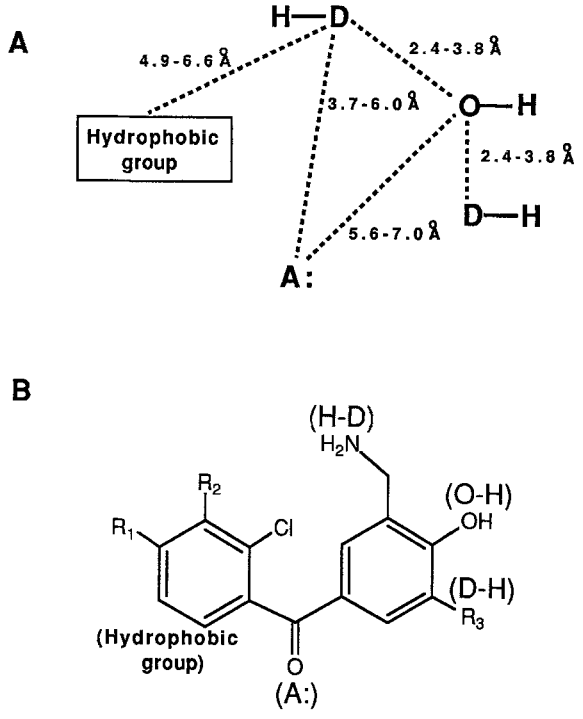

Fig. 2. (A) Schematic depiction of the substructures used in the geometric search for a nonpeptide inhibitor of HIV protease (Bures et al., 1990). The searching strategy was based on the X-ray crystal structure of a HIV protease–inhibitor complex (Erickson et al., 1990). The points were defined as a hydrogen bond donor (D), which could be either an OH, NH, or NH_2 group, a hydrogen bond acceptor (A:), corresponding to OH, =O. =NH, or =S, and a hydrophobic group. (B) Lead inhibitors found in the search.

used by the ascomycin ligand. As shown in Fig. 1, the pipecolinyl ring (Fig. 1A) and the methyl group of the pyranose ring (Fig. 1B) of ascomycin fit into hydrophobic pockets.

3D geometric searching

Another strategy for finding new leads is by searching databases containing the 3D structures of known compounds for molecules that fit particular substructural and geometric criteria (for a review see Martin, 1992). The 3D structures in the database are either experimentally determined (e.g., crystal structures from the Cambridge structural database (Allen et al., 1991)) or computationally generated, using rule-based programs such as CONCORD (Pearlman, 1987). The criteria used to conduct the search typically consist of points corresponding to atoms, projections from atoms, or the center of rings, lines of defined length between these points, and angles defined by three or four points. NMR-derived structural information could be useful for setting up the 3D database search. Knowledge of the functional groups that contact the receptor, obtained from protein–ligand NOEs, could be used to define the points in the search along with their particular chemical characteristics (e.g., hydrogen bond donor or acceptor, etc.), while the NMR-determined bound conformation of the ligand can define the relative distances and angles between these points (atoms). If the 3D structure of the receptor is known, a 3D database can be searched

using points corresponding to favorable binding sites and the relative distances and angles between these points. This input for 3D structure queries can be easily generated with the GRID program (Goodford, 1985), which is a useful complementary tool to 3D structure searching algorithms.

An example of a structure-based geometric search for a nonpeptidic lead inhibitor of HIV protease has been reported by Bures et al. (1990). Using the ALADDIN program (Van Drie et al., 1989), Bures and coworkers searched several 3D databases using the search criteria defined by an X-ray crystal structure of an HIV protease–inhibitor complex. The criteria used in the search consisted of a central hydroxyl group, two hydrogen bond donors, a hydrogen bond acceptor, and a hydrophobic group separated by the distances shown in Fig. 2A. From this 3D geometric search, a series of dibenzophenones (Fig. 2B) were found which moderately (10–100 μM) inhibited HIV protease and served as a starting point for lead optimization (Bures et al., 1990).

In addition to ALADDIN (Van Drie et al., 1989), which was used in the example described above, several other computer programs for 3D geometric searching are commercially available, including MACCS-3D (Moock et al., 1990), 3D SEARCH (Sheridan et al., 1989), ChemDBS-3D (Murall and Davies, 1990), SYBYL/3DB Unity (distributed by Tripos Associates, Inc.), and CAVEAT (Bartlett et al., 1989).

3D steric searching

Another approach for identifying novel lead compounds involves searching 3D databases for compounds that *sterically* fit into a receptor pocket. This approach requires a 3D structure of a receptor, which can be determined by NMR, as well as a database of 3D structures. An example of a computer program for 3D steric searching is the DOCK algorithm (Kuntz et al., 1982). With DOCK, the search for ligands with complementary surfaces to a receptor is accomplished in three stages. First, the receptor pocket is filled by a set of overlapping spheres that intersect with the receptor surface, providing an image of the space accessible to a ligand. Next, compounds from the database are represented by spheres that define the space occupied by the molecules. In the next step, the two sets of spheres corresponding to the shape of the ligand and receptor pocket are matched and scored on the basis of their degree of overlap. Improvements in the DOCK algorithm have allowed the scoring of ligands not only on the basis of steric criteria but also according to molecular mechanics interaction energies (Meng et al., 1992).

The DOCK algorithm has been shown to be a powerful tool to generate new leads from the 3D structure of the receptor. Indeed, several lead enzyme inhibitors active in the micromolar range have been found with DOCK (Kuntz, 1992). However, lead optimization has proved to be more difficult, owing to the many assumptions used in the 3D steric searches, such as the conformation of the ligand, neglect of entropy and solvent, and simplified evaluation of the interaction energies (Kuntz, 1992). Approaches for improving 3D steric searches by alleviating these difficulties are in progress.

3D structure generation

Another approach for finding new leads involves the piecing together of molecular fragments to *create* (rather than search) compounds that will bind to a receptor whose 3D structure is known (Moon and Howe, 1991; Nishibata and Itai, 1991; Böhm, 1992). The GROW algorithm (Moon and Howe, 1991) was originally designed for this purpose and automatically links amino

acids together to form peptides that could bind to enzymes. With GROW, an amide group is manually placed into an experimentally determined 3D structure of a receptor and expanded into a peptide by adding amino acid templates. Another program, LUDI (Böhm, 1992), identifies potential interaction sites from the 3D structure of the receptor, selects molecular fragments from a library of organic molecules to interact with these sites, and automatically links these pieces together with bridges selected from a library of molecular fragments.

CONCLUSIONS AND FUTURE PERSPECTIVES

NMR is a useful tool for rapidly determining the conformations of receptor-bound ligands and identifying those portions of the ligand in contact with its receptor. The complete 3D structures of receptors and ligand–receptor complexes can be obtained as a result of recent advances in heteronuclear multidimensional NMR techniques. With the help of improved computer graphics software and recently developed strategies for searching 3D databases, this NMR-derived structural information could be used to aid drug design, and further advances in both NMR and these computational tools will make this approach even more feasible. In the near future, NMR structures will be determined more rapidly by automating the assignment procedure and structure determinations, and 3D structures will be determined more precisely by including additional distance, angular, and chemical shift-derived restraints into the structure calculations. Thus, NMR structures of larger molecules and biomolecular systems that have previously been too difficult to study by NMR will become accessible. The computational tools for using the NMR-derived structural information will also improve by searching 3D databases more rapidly, by allowing more than one conformation of the molecules to be searched (Murall and Davies, 1990; Smellie et al., 1991; Güner et al., 1992), and by improving the *de novo* design strategies. In addition, better procedures for evaluating the modeled compounds will be developed that take entropy and solvent into consideration (Still et al., 1990; Kuntz, 1992). These advances, coupled with the improved capability to rapidly generate and test diverse molecules from extensive libraries, will make NMR structure-based drug design an important approach for discovering new pharmaceutical agents.

REFERENCES

Albrand, J.P., Birdsall, B., Feeney, J., Roberts, G.C.K. and Burgen, A.S.V. (1979) *Int. J. Biol. Macromol.*, **1**, 37–41.

Allen, F.H., Davies, J.E., Galloy, J.J., Johnson, O., Kennard, O., Macrea, C.F., Mitchell, E.M., Mitchell, G.F., Smith, J.M. and Watson, D.G. (1991) *J. Chem Inf. Comput. Sci.*, **31**, 187–204.

Anglister, J., Frey, T. and McConnell, H.M. (1984) *Biochemistry*, **23**, 1138–1142.

Appelt, K., Bacquet, R.J., Bartlett, C.A., Booth, C.L.J., Freer, S.T., Fuhry, M.A., Gehring, M.R., Herrmann, S.M., Howland, E.F., Janson, C.A., Jones, T.R., Kan, C.-C., Kathardekar, V., Lewis, K.K., Marzoni, G.P., Matthews, D.A., Mohr, C., Moomaw, E.W., Morse, C.A., Oatley, S., Ogden, R.C., Reddy, M.R., Reich, S.H., Schottlin, W.S., Smith, W.W., Varney, M.D., Villafranca, J.E., Ward, R.W., Webber, S., Webber, S.E., Welsh, K.M. and White, J. (1991) *J. Med. Chem.*, **34**, 1925–1934.

Baldwin, J.J., Ponticello, G.S., Anderson, P.S., Christy, M., Murcko, M.A., Randall, W.C., Schwam, H., Sugrue, M.F., Springer, J.P. and Gautheron, P. (1981) *J. Med. Chem.*, **32**, 2510–2513.

Bartlett, P.A., Shea, G.T., Telfer, S.J. and Waterman, S. (1989) In *Molecular Recognition: Chemical and Biological Problems*, (Ed., Roberts, S.M.) Royal Society of Chemistry, London, pp. 182–196.

Bax, A. and Weiss, M.A. (1987) *J. Magn. Reson* **71**, 571–575.

Bendall, M.R., Pegg, D.T., Doddrell, D.M. and Field, J. (1981) *J. Am. Chem. Soc.*, **103**, 934–936.
Böhm, H.-J. (1992) *J. Comput -Aided Mol Design*, **6**, 61–78.
Bures, M.G., Hutchins, C.W., Maus, M., Kohlbrenner, W., Kadam, S. and Erickson, J.W. (1990) *Tetrahedron Comp. Meth*, **3**, 673–680.
Clore, G.M., Roberts, G.C.K., Gronenborn, A., Birdsall, B. and Feeney, J. (1981) *J Magn. Reson.*, **45**, 151–161.
Clore, G.M. and Gronenborn, A.M. (1982) *J. Magn Reson.*, **48**, 402–417.
Clore, G.M. and Gronenborn, A.M. (1991) *Science*, **252**, 1390–1399.
Clore, G.M., Kay, L.E., Bax, A. and Gronenborn, A.M. (1991) *Biochemistry*, **30**, 12–18.
de Jong, E.A.M., Claesen, C.A.A., Daemen, C.J.M., Harmsen, B.J.M., Konings, R.N.H., Tesser, G.I. and Hilbers, C.W. (1988) *J. Magn. Reson.*, **80**, 197–213.
DesJarlais, R.L., Seibel, G.L., Kuntz, I.D., Furth, P.S., Alvarez, J.C., Ortiz de Montellano, P.R., DeCamp, D.L., Babe, L.M. and Craik, C.S. (1990) *Proc. Natl. Acad. Sci. USA*, **87**, 6644–6648.
Ealick, S.E., Babu, Y.S., Bugg, C.E., Erion, M D., Guida, W C., Montgomery, J.A. and Secrist, J.A. (1991) *Proc. Natl. Acad. Sci USA*, **88**, 11540–11544.
Emerson, S.D. and Montelione, G.T. (1992) *J Am. Chem. Soc*, **114**, 354–356.
Erickson, J., Neidhart, D.J., VanDrie, J., Kempf, D.J., Wang, X.C., Norbeck, D.W., Plattner, J.J., Rittenhouse, J.W., Turon, M., Wideburg, N., Kohlbrenner, W.E., Simmer, R., Helfrich, R., Paul, D.A. and Knigge, M. (1990) *Science*, **249**, 527–533.
Fesik, S.W. and Zuiderweg, E R.P. (1988) *J. Magn. Reson.*, **78**, 588–593.
Fesik, S.W., Luly, J.R., Erickson, J.W. and Abad, Z.C. (1988) *Biochemistry*, **27**, 8297–8301.
Fesik, S.W. (1989) In *Computer-Aided Drug Design. Methods and Applications*, (Eds Perun, T.J. and Propst, C.L.) Marcel Dekker, New York, pp. 133–184.
Fesik, S.W., Gampe Jr., R.J., Holzman, T.F., Egan, D.A., Edalji, R., Luly, J.R., Simmer, R., Helfrich, R., Kishore, V. and Rich, D.H. (1990a) *Science*, **250**, 1406–1409.
Fesik, S.W., Zuiderweg, E.R.P., Olejniczak, E.T. and Gampe Jr., R.T., (1990b) *Biochem. Pharmacol.*, **40**, 161–167.
Fesik, S.W. (1991) *J. Med. Chem.*, **34**, 2937–2945.
Fesik, S.W., Gampe Jr., R.T., Eaton, H L., Gemmecker, G., Olejniczak, E.T., Neri, P., Holzman, T.F., Egan, D.A., Edalji, R., Helfrich, R., Hochlowski, J. and Jackson, M. (1991a) *Biochemistry*, **30**, 6574–6583.
Fesik, S.W., Gemmecker, G., Olejniczak, E.T. and Petros, A.M. (1991b) *J. Am. Chem. Soc.*, **113**, 7080–7081.
Goodford, P.J. (1985) *J. Med. Chem.*, **28**, 849–857.
Griffey, R.H. and Redfield, A G. (1987) *Q. Rev. Biophys.*, **19**, 51–82.
Guner, O.F., Henry, D.R. and Pearlman, R.S. (1992) *J. Chem. Inf. Comput. Sci*, **32**, 101–109.
Hansen, A.P., Petros, A.M., Mazar, A.P., Pederson, T.M., Rueter, A. and Fesik, S.W. (1992) *Biochemistry*, **31**, 12713–12718.
Ikura, M. and Bax, A. (1992) *J. Am. Chem. Soc.*, **114**, 2433–2440.
Ikura, M., Clore, G.M., Gronenborn, A.M., Zhu, G., Klee, C.B. and Bax, A. (1992) *Science*, **256**, 632–638.
Kay, L.E. and Bax, A. (1990) *J. Magn. Reson.*, **86**, 110–126
Kay, L.E., Clore, G.M., Bax, A. and Gronenborn, A.M. (1990a) *Science*, **249**, 411–414.
Kay, L.E., Ikura, M., Tschudin, R and Bax, A. (1990b) *J. Magn. Reson.*, **89**, 496–514.
Kuntz, I.D. (1992) *Science*, **257**, 1078–1082.
Kuntz, I.D., Blaney, J.M., Oatley, S.J., Langridge, R. and Ferrin, T E. (1982) *J. Mol. Biol.*, **161**, 269–288.
Marion, D., Kay, L.E., Sparks, S.W., Torchia, D.A. and Bax, A. (1989) *J. Am. Chem. Soc.*, **111**, 1515–1517.
Martin, Y.C. (1992) *J. Med. Chem.*, **35**, 2145–2154.
Meadows, R.P., Nettesheim, D.G., Xu, R.X., Olejniczak, E.T., Petros, A.M., Holzman, T.F., Severin, J., Gubbins, E., Smith, H. and Fesik, S.W. (1993) *Biochemistry*, **32**, 754–765.
Meng, E.C., Shoichet, B.K. and Kuntz, I.D. (1992) *J. Comput. Chem.*, **13**, 505–524.
Michnick, S.W., Rosen, M.K., Wandless, T.J., Karplus, M. and Schreiber, S.L. (1991) *Science*, **252**, 836–839.
Montelione, G.T., Winkler, M.E., Rauenbuehler, P. and Wagner, G. (1989) *J Magn. Reson.*, **82**, 198–204.
Moock, T.E., Christie, B.D. and Henry, D.R. (1990) In *Chemical Information Systems*, (Eds. Bawden, D. and Mitchell, E.M.) Chichester, pp. 42–49.
Moon, J.B. and Howe, W.J. (1991) *Proteins: Struct. Funct. Genet.*, **11**, 314–328.
Moore, J.M., Peattie, D.A., Fitzgibbon, M.J. and Thomson, J.A. (1991) *Nature*, **351**, 248–250.

Muchmore, D.C., McIntosh, L.P., Russell, C.B., Anderson, D.E. and Dahlquist, F.W. (1989) *Methods Enzymol.*, **177**, 44–73.

Murall, N.W. and Davies, E.K. (1990) *J. Chem. Inf. Comput Sci*, **30**, 312–316.

Nishibata, Y. and Itai, A. (1991) Tetrahedron, **47**, 8985–8990.

Otting, G., Senn, H., Wagner, G. and Wüthrich, K. (1986) *J. Magn. Reson.*, **70**, 500–505.

Otting, G. and Wüthrich, K. (1990) *Q. Rev. Biophys.*, **23**, 39–96.

Pearlman, R.S. (1987) *Chem Des. Auto News*, **2**, 1–7.

Petros, A.M., Müller, L. and Kopple, K.D. (1990) *Biochemistry*, **29**, 10041–10048.

Petros, A.M., Gampe Jr., R.T., Gemmecker, G., Neri, P., Holzman, T.F., Edalji, R., Hochlowski, J., Jackson, M., McAlpine, J., Luly, J.R., Pilot-Matias, T., Pratt, S. and Fesik, S.W. (1991) *J Med. Chem.*, **34**, 2925–2928.

Petros, A.M., Gemmecker, G., Neri, P., Olejniczak, E.T., Nettesheim, D., Xu, R.X., Gubbins, E.G., Smith, H. and Fesik, S.W. (1992a) *J. Med. Chem.*, **35**, 2467–2473.

Petros, A.M., Kawai, M., Luly, J.R. and Fesik, S.W. (1992b) *FEBS Lett.*, **308**, 309–314.

Petros, A.M., Neri, P. and Fesik, S.W. (1992c) *J. Biomol. NMR*, **2**, 11–18.

Rance, M., Wright, P.E., Messerle, B.A. and Field, L.D. (1987) *J. Am. Chem Soc.* **109**, 1591–1593.

Schmidt, P.G., Bernatowicz, M.S. and Rich, D.H. (1982) *Biochemistry*, **21**, 1830–1835.

Sheridan, R.P., Nilakantan, R., Rusinko, A., Bauman, N., Haraki, K.S. and Venkataraghavan, R. (1989) *J Chem. Inf. Comput. Sci.*, **29**, 255–260.

Smellie, A.S., Crippen, G.M. and Richards, W.G. (1991) *J. Chem. Inf. Comput. Sci.*, **31**, 386–392.

Still, W.C., Tempszyk, A., Hawley, R.C. and Hendrickson, T. (1990) *J. Am. Chem. soc.*, **112**, 6127–6129.

Szyperski, T., Güntert, P., Otting, G. and Wüthrich, K. (1992) *J. Magn. Reson.*, **99**, 552–560.

Theriault, Y., Logan, T.M., Meadows, R., Yu, L., Olejniczak, E.T., Holzman, T.F., Simmer, R.L. and Fesik, S.W (1992) *Nature*, **361**, 88–91.

Van Drie, J.H., Weininger, D. and Martin, Y.C. (1989) *J Comput.-Aided Mol. Des.*, **3**, 225–251

Varney, M.D., Marzoni, G.P., Palmer, C.L., Deal, J.G , Webber, S., Welsh, K.M., Bacquet, R.J., Bartlett, C.A., Morse, C.A., Booth, J., Herrmann, S.M., Howland, E.F., Ward, R.W. and White, J. (1992) *J. Med. Chem.*, **35**, 663–676.

Weber, P.L., Morrison, R. and Hare, D.J. (1988) *J. Mol. Biol.*, **204**, 483–487.

Weber, C., Wider, G., von Freyberg, B., Traber, R., Braun, W , Widmer, H. and Wüthrich, K. (1991) *Biochemistry*, **30**, 6563–6574.

Wider, G., Weber, C., Traber, R., Widmer, H. and Wüthrich, K. (1990) *J. Am. Chem. Soc.*, **112**, 9015–9016.

Xu, R.X., Olejniczak, E.T. and Fesik, S.W. (1992) *FEBS Lett*, **305**, 137–143.

Zuiderweg, E.R.P., Petros, A.M., Fesik, S.W. and Olejniczak, E.T. (1990) *J. Am. Chem Soc.*, **113**, 370–372.