

AMERICAN  
ASSOCIATION FOR THE  
ADVANCEMENT OF  
SCIENCE

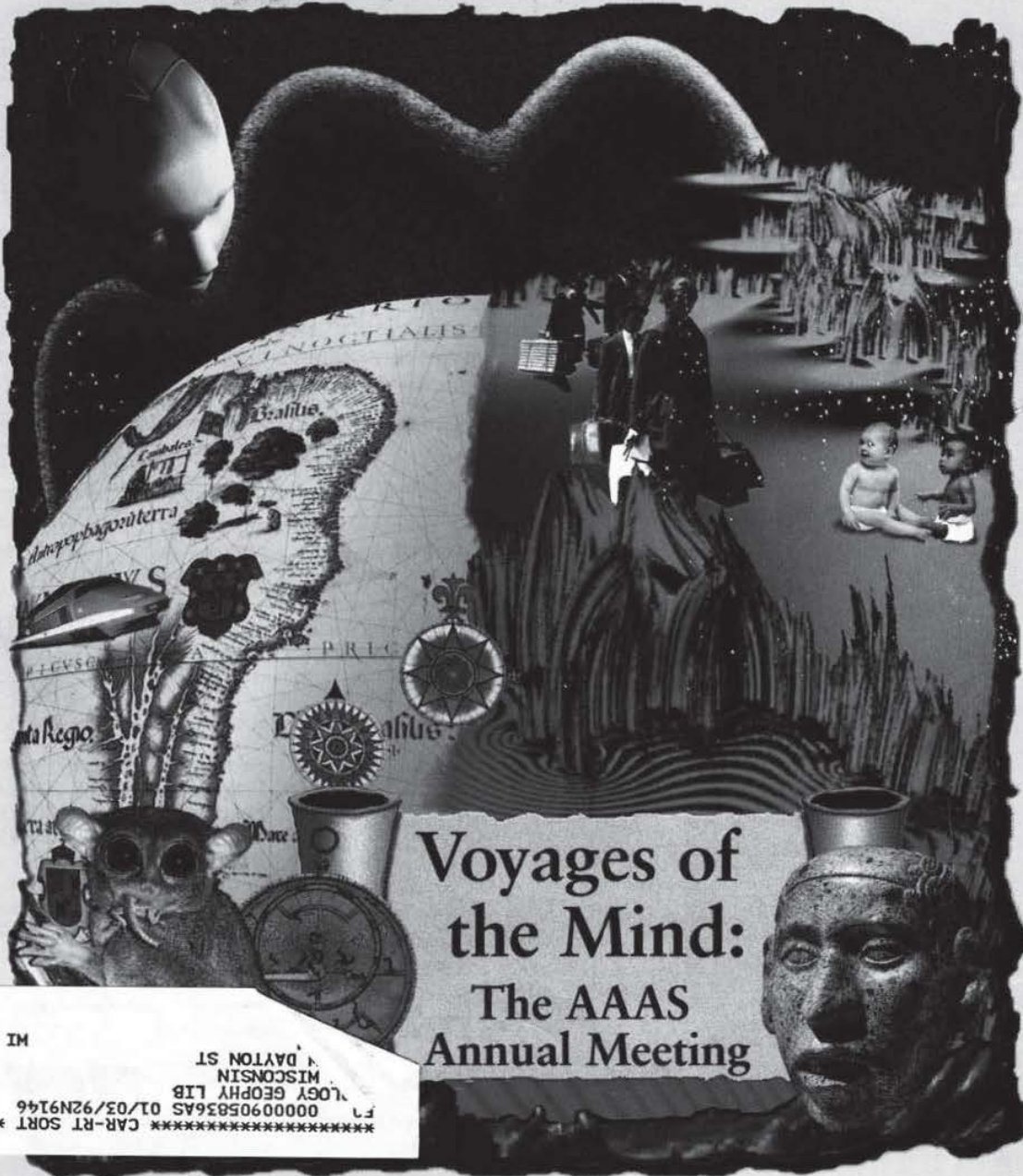
# SCIENCE

15 NOVEMBER 1991  
VOL. 254 ■ PAGES 909-1080

\$6.00

NOV 19 1991

University of Wisconsin  
Madison



## Voyages of the Mind: The AAAS Annual Meeting

MI 53706

DAYTON ST  
MISCONSIN

LOGY GEOPHY LIB

\*\*\*\*\* CAR-RT SORT \*\* CRO3  
00000905836AS 01/03/92N9146

	915 This Week in <i>Science</i>
<b>Editorial</b>	917 The Land of the Dammed
<b>Letters</b>	918 Scientific Misconduct Investigations: Who Should Conduct Them?: D. F. KLEIN ■ Phylogeny and Diversity: C. KRAJEWSKI ■ Extinction "Hot Spots": N. MYERS ■ Toxic Waste Cleanup: D. W. WOLF AND L. E. GREER ■ PCBs in the Environment: P. BOFFETTA AND H. VAINIO; G. W. GRIBBLE
<b>ScienceScope</b>	927 Brighter prospects for solar telescope; planning an Antarctic evacuation; etc.
<b>News &amp; Comment</b>	928 No Meeting of the Minds on Asbestos ■ Consensus Report Draws Fire From Both Extremes ■ "Third Wave": Roiling the Waters 931 Scientific Sleuths Solve a Murder Mystery 932 HUGO Takes on Role as Marriage Broker 933 Hughes Investigators Rile NIH Peer Reviewers 934 <i>Briefings</i> : Questions Raised on Math Rankings ■ Rating University R&D (cont.) ■ Catching Some (Cosmic) Rays ■ Students Thwart USDA Pest Plan ■ An Ice Cap on the Hottest Planet? ■ Primateologist Band Together ■ Relaying Science to the People
<b>Research News</b>	936 How Long Is the Human Life-span? 938 The Sound of One Dune Booming 939 Putting Einstein to the Test—in Space 941 AIDS: The Evolution of an Infection 942 Extinction Potpourri: Killers and Victims: Where to Run From a Mass Extinction? ■ Plate Tectonics as a Driver of Evolution ■ Looks Like the Yucatan Holds a Killer Crater
<b>News Reports</b>	944 What Next in the Gallo Case? 946 John Crewdson: Science Journalist as Investigator ■ Report Card on Crewdson's Reporting
<b>Perspectives</b>	953 Receptor-Induced Conformation Change of the Immunosuppressant Cyclosporin A: K. WÜTHRICH, B. VON FREYBERG, C. WEBER, G. WIDER, R. TRABER, H. WIDMER, W. BRAUN 954 Rusting of the Lock and Key Model for Protein-Ligand Binding: W. L. JORGENSEN
<b>Articles</b>	959 Changes in the West Antarctic Ice Sheet: R. B. ALLEY AND I. M. WHILLANS 963 Antigenic Diversity Thresholds and the Development of AIDS: M. A. NOWAK, R. M. ANDERSON, A. R. MCLEAN, T. F. W. WOLFS, J. GOUDSMIT, R. M. MAY
<b>Research Articles</b>	970 Electronic Correlation Effects and Superconductivity in Doped Fullerenes: S. CHAKRAVARTY, M. P. GELFAND, S. KIVELSON 974 Protein Hydration in Aqueous Solution: G. OTTING, E. LIEPINSH, K. WÜTHRICH
<b>Reports</b>	981 X-ray Damage to CF <sub>3</sub> CO <sub>2</sub> -Terminated Organic Monolayers on Si/Au: Principal Effect of Electrons: P. E. LAIBINIS, R. L. GRAHAM, H. A. BIEBUYCK, G. M. WHITESIDES 983 Scanning Tunneling Microscopy of Galena (100) Surface Oxidation and Sorption of Aqueous Gold: C. M. EGGLESTON AND M. F. HOHELLA, JR.

■ SCIENCE (ISSN 0036-8075) is published weekly on Friday, except the last week in December, by the American Association for the Advancement of Science, 1333 H Street, NW, Washington, DC 20005. Second-class postage (publication No. 484460) paid at Washington, DC, and additional mailing offices. Copyright © 1991 by the American Association for the Advancement of Science. The title SCIENCE is a registered trademark of the AAAS. Domestic individual membership and subscription (51 issues): \$82 (\$47 allocated to subscription). Domestic institutional subscription (51 issues): \$150. Foreign postage extra: Mexico, Caribbean (surface mail) \$50; Other countries (air assist delivery) \$95. First class, airmail, student and emeritus rates on request. Canadian rates with GST available upon request, GST # 1254 88122. Change of address: allow 6 weeks, giving old and new addresses and 11-digit account number. Postmaster: Send change of address to Science, P.O. Box 2033, Marion, OH 43305-2003. Single copy sales: \$6.00 per issue prepaid includes surface postage; Guide to Biotechnology Products and Instruments, \$20. Bulk rates on request. Authorization to photocopy material for internal or personal use under circumstances not falling within the fair use provisions of the Copyright Act is granted by AAAS to libraries and other users registered with the Copyright Clearance Center (CCC) Transactional Reporting Service, provided that the base fee of \$1 per copy plus \$0.10 per page is paid directly to CCC, 27 Congress Street, Salem, Massachusetts 01970. The identification code for Science is 0036-8075/83 \$1 + .10. Science is indexed in the *Reader's Guide to Periodical Literature* and in several specialized indexes.

■ The American Association for the Advancement of Science was founded in 1848 and incorporated in 1874. Its objectives are to further the work of scientists, to facilitate cooperation among them, to foster scientific freedom and responsibility, to improve the effectiveness of science in the promotion of human welfare, to advance education in science, and to increase public understanding and appreciation of the importance and promise of the methods of science in human progress.

10 is located on the opposite side of the ring plane. For the polypeptide backbone the structural rearrangement upon binding to the receptor is reminiscent of the inversion of a glove, whereby the hydrophobic exterior edges formed by the N-methyl groups in free CsA are replaced by a polar surface of amide protons and carbonyl oxygens.

The conformation of the receptor-bound CsA indicates the possibility of hydrogen bonding with the receptor protein. For the amide proton of Abu<sup>2</sup> the presence of hydrogen bonding is directly supported by the observation of slowed exchange with the solvent (10). Hydrogen bonding is also compatible with <sup>1</sup>H-<sup>1</sup>H nuclear Overhauser effects (NOEs) observed between CYP and the residues 1 to 3 and 9 to 11 of CsA (10, 11). The dramatic global rearrangement of the polypeptide backbone conformation in CsA, which enables recognition by hydrogen bonding, is particularly remarkable for a cyclic compound, which has a greatly reduced accessible conformation space when compared with a corresponding linear polypeptide. Considering the important role of molecular modeling in drug design, it is instructive to note that molecular dynamics calculations that started from the crystal structure or the solution structure of free CsA and that used different potential functions to represent the solvent (15) gave no indication of an imminent major conformation change away from the starting conformation. Thus CsA may well end up as a textbook case to illustrate the importance of experimental studies with both free and receptor-bound effector molecules for understanding structure-function correlations as a guide to improved molecular design.

The NMR investigations of receptor-bound CsA were performed with combined use of isotope-labeling and heteronuclear NMR experiments. In an unlabeled system the large number of protons from the receptor protein would interfere with the observation of the resonance lines of the ligand. However, binary complexes are ideally suited for studies with efficient labeling schemes, because the two components can be labeled separately with <sup>13</sup>C or <sup>15</sup>N before complex formation and subsequently combined with unlabeled partner molecules. Suitably chosen heteronuclear editing schemes (16, 17) can then be used to separate the <sup>1</sup>H NMR lines of the two molecules in the complex. In particular, use of the so-called heteronuclear half-filters (17, 18) in 2D <sup>1</sup>H NMR spectra represents a valid alternative to the use of three or higher dimensional experiments for improved resolution in such systems. An intrinsic advan-

tage is that the 2D <sup>1</sup>H-<sup>1</sup>H NMR spectra can be recorded with sensitivity and digital resolution comparable to those of corresponding conventional 2D <sup>1</sup>H NMR spectra. With <sup>13</sup>C-labeled CsA a double-half-filter technique was particularly helpful (19) because it produced different subspectra that contained either exclusively intramolecular NOE cross peaks between different protons of CsA or different protons of CYP or exclusively intermolecular NOE cross peaks relating protons of CsA with protons of CYP. These techniques are generally applicable with binary or multicomponent molecular assemblies, primarily in systems with very stable receptor-effector complexes, and represent an attractive avenue for the use of NMR in conjunction with projects on drug design.

#### REFERENCES

1. J. F. Borel, Ed., *Cyclosporin* (Karger, Basel, 1986).
2. S. L. Schreiber, *Science* **251**, 283 (1991).
3. H. R. Loosli et al., *Helv. Chim. Acta* **68**, 682 (1985).
4. H. Kessler, M. Köck, Th. Wein, M. Gehrke, *ibid.* **73**, 1818 (1990).
5. R. E. Handschumacher, M. W. Harding, J. Rice, R. J. Drugge, D. W. Speicher, *Science* **226**, 544 (1984).
6. G. Fischer, B. Wittmann-Liebold, K. Lang, T. Kiefhaber, F. X. Schmid, *Nature* **337**, 476 (1989); N. Takahashi, T. Hayano, M. Suzuki, *ibid.*, p. 473.
7. A. J. Koletsky, M. W. Harding, R. E. Handschumacher, *J. Immunol.* **137**, 1054 (1986); M. Kawamukai, H. Matsuda, W. Fuji, R. Utsumi, T. Komano, *J. Bacteriol.* **171**, 4525 (1989).
8. S. W. Michnick, M. K. Rosen, T. J. Wandless, M. Karplus, S. L. Schreiber, *Science* **252**, 836 (1991); J. M. Moore, D. A. Peattie, M. J. Fitzgibbon, J. A. Thomson, *Nature* **351**, 248 (1991).
9. G. D. Van Duyne, R. F. Standaert, P. A. Karplus, S. L. Schreiber, J. Clardy, *Science* **252**, 839 (1991).
10. C. Weber et al., *Biochemistry* **30**, 6563 (1991).
11. S. W. Fesik et al., *ibid.*, p. 6575.
12. K. Wüthrich, C. Spitzfaden, K. Memmert, H. Widmer, G. Wider, *FEBS Lett.* **285**, 237 (1991).
13. J. Kallen et al., *Nature* **353**, 276 (1991).
14. R. M. Wenger, *Angew. Chem. Int. Ed. Engl.* **24**, 77 (1985).
15. J. Lutz, H. Kessler, R. Kaptein, W. F. van Gunsteren, *J. Comput. Aided Mol. Des.* **1**, 219 (1987); J. Lutz, H. Kessler, H. P. Weber, R. M. Wenger, W. F. van Gunsteren, *Biopolymers* **29**, 1969 (1990).
16. R. H. Griffey and A. G. Redfield, *Q. Rev. Biophys.* **19**, 51 (1987).
17. S. W. Fesik, *Nature* **332**, 865 (1988).
18. G. Otting, H. Senn, G. Wagner, K. Wüthrich, *J. Magn. Reson.* **70**, 500 (1986); G. Otting and K. Wüthrich, *Q. Rev. Biophys.* **23**, 39 (1990); S. W. Fesik, J. R. Luly, J. W. Erickson, C. Abad-Zapatero, *Biochemistry* **27**, 8297 (1988); G. Wider, C. Weber, K. Wüthrich, *J. Am. Chem. Soc.* **113**, 4676 (1991).
19. G. Otting and K. Wüthrich, *J. Magn. Reson.* **85**, 586 (1989); G. Wider, C. Weber, R. Traber, H. Widmer, K. Wüthrich, *J. Am. Chem. Soc.* **112**, 9015 (1990).

## Rusting of the Lock and Key Model for Protein-Ligand Binding

WILLIAM L. JORGENSEN

ATOMIC-LEVEL KNOWLEDGE OF THE GEOMETRIES OF PROTEIN-LIGAND COMPLEXES has only been accumulating since the mid-1970s. About 50 x-ray structures have now been determined for peptides or proteins bound to enzymes or antibodies. The traditional notion of rigid lock and key complementarity received support from the early and numerous studies of complexes of proteolytic enzymes with small protein inhibitors (1) and from the first example of an antibody-protein complex (2). However, the idea has become increasingly challenged.

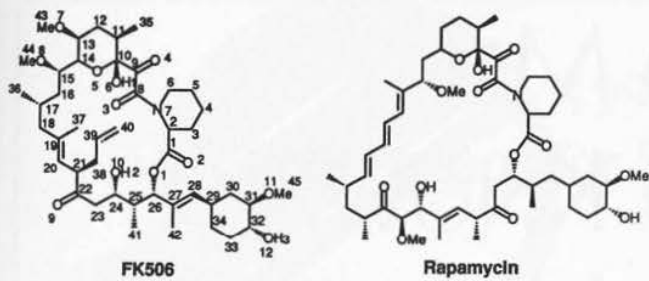
In fact, conformational changes for enzymes upon ligand uptake are well known and range from modest loop motions to hinge bending (3). The prototypical case of strong binding, streptavidin-

biotin, involves adjustments to streptavidin that include a loop flip (4), and the bear hug applied by human immunodeficiency virus type 1 (HIV-1) protease to a peptide inhibitor is a striking example of large-scale domain motions (5).

Recently, the effects of binding on ligand structure have received increased attention. Cases of profound conformational change have been provided by the determination of the structures of the immunosuppressive agents FK506 and cyclosporin A (CsA) complexed with their cytosolic binding proteins FKBP and cyclophilin. X-ray structures have been reported for the uncomplexed drugs and the FKBP-FK506 complex (6), and the structure of CsA bound to cyclophilin has been determined by two groups using multidimensional nuclear magnetic resonance (NMR) techniques (7).

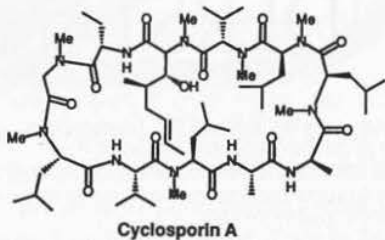
Both binding proteins are peptidyl-prolyl-*cis-trans* isomerases and have been shown to interfere with T cell signaling upon forming ternary complexes with their respective immunosuppressive agents and the protein phosphatase calcineurin (8). FK506 is a macrocyclic organic molecule with a critical  $\alpha$ -keto-homoprolyl subunit (upper right part of the structure) that acts as a transition-state surrogate (9), and CsA is a cyclic undecapeptide. Binding leads to substantial conformational change for FK506, including *cis-trans* isomerization

Department of Chemistry, Yale University, New Haven, CT 06511.



FK506

Rapamycin

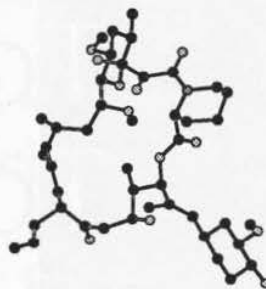


Cyclosporin A

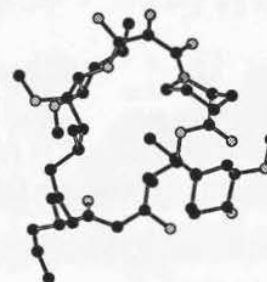
of the amide bond, concomitant repositioning of the homoproline ring, and inward disposition of the pyranose ring (top) toward the macrocycle. For CsA, the unbound structure has essentially been turned inside out to reach the bound conformation; the four intramolecular hydrogen bonds are lost, the 9,10-peptide bond isomerizes from cis to trans, and two sets of four side chains switch sides of the ring. These examples show that flexible ligands can undergo substantial geometrical distortions to achieve a suitable binding conformation.

For variety, nature has also provided rapamycin, an immunosuppressant that binds to FKBP and is closely related structurally to FK506, particularly in the C1-C14 binding domain. The bound and unbound structures of rapamycin are virtually identical, and the distortion of FK506 on binding to FKBP yields a binding domain that is superimposable on the rapamycin structure (10). The implicit greater preorganization of rapamycin might be thought to provide greatly enhanced binding relative to FK506; in fact, it only amounts to a factor of 2 in the binding constants.

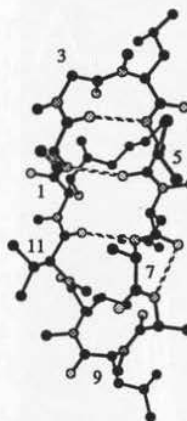
Although these examples are striking, they are not unprecedented. The binding of lysozyme by a  $F_{ab}$  fragment requires negligible distortion of either component (2). However, subsequent investigations suggest that this is not general. Complexation of the viral antigen neuraminidase by a  $F_{ab}$  fragment was found to displace some  $C\alpha$  positions in a binding loop by more than 1 Å from their locations in the free enzyme (11). More recently, an x-ray structure has been reported for a  $F_{ab}$  complex with a 19-amino acid homolog of the C helix of myohemerythrin (12). Unbound in water, the peptide shows no stable secondary structure, but the  $NH_2$ -terminal region of the peptide forms a type II  $\beta$  turn when bound to the antibody. Another salient example is provided by the expulsion of the heme from myoglobin upon binding to an antibody to apomyoglobin (13). Furthermore, the isolated S-peptide of ribonuclease A shows no helicity at 25°C but regains full helical character upon binding in the S-protein (14). In fact, most secondary structure in proteins can be considered to arise from bindinglike interactions with the remainder of the protein.



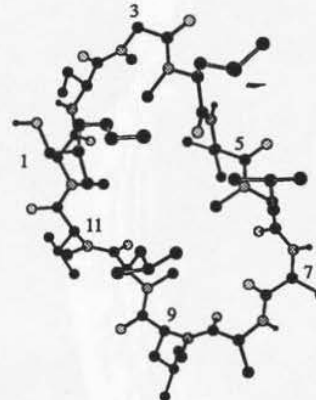
FK506 unbound



FK506 bound



CsA unbound



CsA bound

These examples confirm the reasonable expectation that flexible molecules distort to form optimal interactions with binding partners. A practical consequence is the frustration that will often accompany attempts to design drugs by analogy to the structures of flexible, unbound active substances.

#### REFERENCES

1. D. M. Blow, *Acc. Chem. Res.* **9**, 145 (1976); R. Huber and W. Bode, *ibid.* **11**, 114 (1978); S. J. Hubbard, S. F. Campbell, J. M. Thornton, *J. Mol. Biol.* **220**, 507 (1991).
2. A. G. Amit, R. A. Mariuzza, S. E. V. Phillips, R. J. Poljak, *Science* **233**, 747 (1986).
3. W. S. Bennett and R. Huber, *CRC Crit. Rev. Biochem.* **15**, 291 (1984).
4. P. C. Weber, D. H. Ohlendorf, J. J. Wendoloski, F. R. Salemme, *Science* **243**, 85 (1989).
5. M. Müller *et al.*, *ibid.* **246**, 1149 (1989).
6. G. D. Van Duyne, R. F. Standaert, P. A. Karplus, S. L. Schreiber, J. Clardy, *ibid.* **252**, 839 (1991).
7. C. Weber *et al.*, *Biochemistry* **30**, 6563 (1991); S. W. Fesik *et al.*, *ibid.*, p. 6574.
8. J. Liu, J. D. Farmer, Jr., J. Friedman, I. Weissman, S. L. Schreiber, *Cell* **66**, 807 (1991).
9. M. K. Rosen, R. F. Standaert, A. Galat, M. Nakatsuka, S. L. Schreiber, *Science* **248**, 863 (1990).
10. G. D. Van Duyne, R. F. Standaert, S. L. Schreiber, J. Clardy, *J. Am. Chem. Soc.* **113**, 7433 (1991).
11. P. M. Colman *et al.*, *Nature* **326**, 358 (1987).
12. R. L. Stanfield, T. M. Fieser, R. A. Lerner, I. A. Wilson, *Science* **248**, 712 (1990).
13. M. J. Crumpton, *Biochem. J.* **100**, 223 (1966).
14. P. S. Kim, A. Bierczynski, R. L. Baldwin, *J. Mol. Biol.* **162**, 187 (1982).