IRUCTURE VI

NEW YORK—Two groups have recently reported solving the structure of a key immune system receptor, the FK506 binding protein (FKBP).

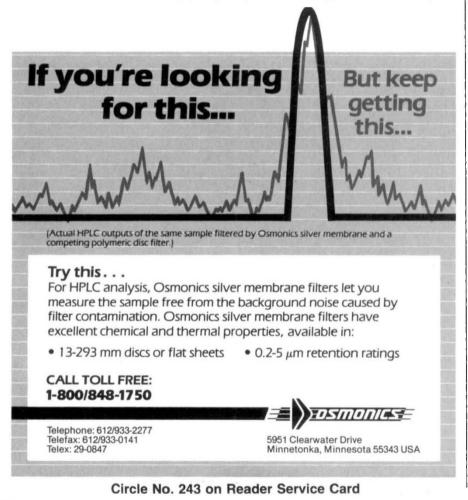
One determined the structure of FKBP alone, while the other determined the structure of the FKBP/ FK506 complex (FK506 is an immunosuppressive drug used in organ transplants). Both groups used NMR (nuclear magnetic resonance) spectroscopy, while one also disclosed their results using X-ray crystallographic methods. The differences in approach, and the competitiveness between the groups and the two journals in which these initial results appear, may generate a heated debate later on as to which technique (or combination of techniques) yields the correct set of structural data.

FK506, discovered in 1989 by scientists at Fujisawa Pharmaceutical (Osaka), is derived from a soil-borne fungus. The drug, which is not yet approved, was first used experimentally by transplant pioneer Thomas Starzl at the University of Pittsburgh Medical Center (PA). It has proved more effective than the current approved drug of choice, cyclosporin,

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Ribbon diagram of a single FKBP solution conformation.

in controlling rejection of transplanted organs, and with fewer sideeffects. But how FK506 works is unknown. The current belief is that FK506 binds to the FK binding protein of T cells and inhibits specific signal transduction pathways that lead to T lymphocyte activation. Thus, knowing the structural basis for molecular recognition of FK506 by the



immunophilin FKBP, and the biological consequences of the interaction, is key to developing still more effective FK506 analogs or other drugs that can inhibit the receptor interaction.

Both NMR spectroscopy and X-ray crystallography can be used to solve such protein-ligand structures. While crystallographic methods usually provide higher-resolution structural measurements, their use depends on obtaining good crystals. NMR, on the other hand, is performed with a protein in solution, which better represents its natural biological state. NMR measures subtle perturbations in the magnetic resonance of protons (the nuclear Overhauser effect [NOE]) to calculate proton distances. Probable models are then calculated using distance constraints. "The more constraints you have, the fewer the number of models will be," notes Manuel Navia of Vertex Pharmaceuticals (Cambridge, MA).

Reporting in the May 9 issue of Science, Stuart Schreiber's group at the Harvard University Department of Chemistry (Cambridge, MA) used NMR to calculate the probable FKBP structure. An accompanying paper, co-authored by Schreiber's team and John Clardy and colleagues at Cornell University (Ithaca, NY), presents X-ray data on the FKBP/FK506 complex. These data show differences in conformation of FKBP in the bound and unbound states. While the NMR data indicate a floppy structure (few NOE constraints indicate a poorly defined-and therefore flexiblestructure), the structure of the complex may, in fact, be conformationally restrained. The Harvard group assumed that the ligand binding in the FKBP/FK506 complex did not affect the native structure of FKBP.

The second set of data comes from Jonathan Moore and colleagues at Vertex. They also reported, in the May 10 issue of *Nature*, a flexible FKBP structure. Because of the competing publication in *Science*, *Nature* broke from its usual tradition and released an embargoed version of the Vertex results a week before the paper appeared.

The Vertex group intimated that they will be combining NMR and Xray crystallography results to better derive the FKBP structure. At the end of their report, they noted that "The X-ray structure of bovine FKBP has been solved by molecular replacement using the NMR structures presented here, and will be presented elsewhere." —Mark Ratner