method,[26] in which the binding positions of molecules, representing fragments, are determined by NMR. Fragments that interact closely are then linked to make a larger ligand.

But the real power of the method comes from more recent computational application of the method. In this approach, the binding of fragments is determined computationally, and the compounds derived from the mapping of such fragments are then synthesized and tested.[27–29] The accuracy of these computations is matched against the experimental results.

An extension of these methods is to use either the experimental or the computational method to identify binding sites that can then be probed by molecular docking procedures[30,31] to find compounds that will bind at a particular site. In all cases, the success of the methods is determined by the effect of the final compound on the biological system being studied. Examples of such procedures are found in other chapters in this book.

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REFERENCES

1. Jancarik, J.; Kim, S.-H. Sparse matrix sampling: a screening method for crystallization of proteins. *J. Appl. Crystallogr.* **1991**, *24*, 409–411.
2. Dvir, H.; Harel, M.; McCarthy, A. A.; Toker, L.; Silman, I.; Futerman, A. H.; Sussman, J. X-ray structure of human acid-beta-glucosidase, the defective enzyme in Gaucher disease. L. EMBO Rep. **2003**, *4*, 704–709.
3. Lieberman, R. L.; Wustman, B. A.; Huertas, P.; Powe, A. C., Jr.; Pine, W. P.; Khanna, R.; Schlossmacher, G.; Ringe, D.; Petsko, G. A. Structure of acid beta-glucosidase with pharmacological chaperone provides insight into Gaucher disease. *Nat. Chem. Biol.* **2007**, *3*, 101–107.
4. Stout, G. H.; Jensen, L. H. *X-Ray Structure Determination: A Practical Guide*, 2nd ed. New York, NY: Wiley & Sons, **1989**.
5. Blow, D. *Outline of Crystallography for Biologists*. New York, NY: Oxford University Press; **2002**.
6. Rhodes, G. *Crystallography Made Crystal Clear*. Burlington, VT: Academic Press; **2006**.
7. Wlodawer, A.; Minor, W.; Dauter, Z.; Jaskolski, M. Protein crystallography for non-crystallographers, or how to get the best (but not more) from published macromolecular structure. *FEBS J.* **2008**, *275*, 1–21.
8. Irving, J. A.; Whisstock, J. C.; Lesk, A. M. Protein structural alignments and functional genomics. *Proteins* **2001**, *42*, 378–382.
9. Chevrier, B.; Schalk, C.; D'Orchymont, H.; Rondeau, J. M.; Moras, D.; Tarnus, C. Crystal structure of *Aeromonas proteolytica* aminopeptidase: a prototypical member of the co-catalytic zinc enzyme family. *Structure* **1994**, *2*, 283–291.
10. Desmarais, W.; Bienvenue, D. L.; Bzymek, K. P.; Petsko, G. A.; Ringe, D.; Holz, R. C. J. The high-resolution structures of the neutral and the low pH crystals of aminopeptidase from *Aeromonas proteolytica*. *Biol. Inorg. Chem.* **2006**, *11*, 398–408.
11. Hendrickson, W. A. Stereochemically restrained refinement of macromolecular structures. *Methods Enzymol.* **1985**, *115*, 252–270.
12. Kleywegt, G. J.; Jones, A. T. Where freedom is given, liberties are taken. *Structure* **1995**, *3*, 535–540.
13. Engh, R. A.; Huber, R. Accurate bond and angle parameters for X-ray protein structure refinement. *Acta Crystallogr. D. Biol. Crystallogr.* **1991**, 47, 392–400; and Engh, R. A.; Huber, R. Bond lengths and angles of peptide backbone fragments. In: *Structure Quality and Target Parameters*, International Tables of Crystallography, Vol. F, Rossman, M. G.; Arnold, E.; Eds. Dordrecht: Kluwer, **2001**, 382–392.
14. Allen, F. H. An experimental approach to mapping the binding surfaces of crystalline proteins. *Acta Crystallogr. D Biol. Crystallogr.* **2002**, *58*, 380–388.
15. Petsko, G. A.; Ringe, D. *Protein Structure and Function*. London: New Science Press; **2004**.
16. Brunger, A. T. Free R value: a novel statistical quantity for assessing the accuracy of crystal structures. *Nature* **1992**, *355*, 472–474.
17. Matthews, B. W. *J. Mol. Biol.* **1968**, *33*, 491–497.
18. Ramakrishnan, C.; Ramachandran, G. N. Stereochemical criteria for polypeptide and protein chain configuration – part II. Allowed conformations for a pair of peptide units. *Biophys. J.* **1965**, *5*, 909–933.
19. Bernstein, F. C.; Koetzle, T. F.; Williams, G. J. B.; Meyer, E. F., Jr.; Brice, M. D.; Rogers, J. R.; Kennard, O.; Shimanouchi, T.; Tasumi, M. The Protein Data Bank. A computer-based archival file for macromolecular structures. *J. Mol. Biol.* **1977**, *112*, 535–547.
20. Berman, H. M.; Wetbrook, J.; Feng, Z.; Gilliland, G.; Bhat, T. N.; Weissig, H.; Shindyalov, I. N.; Bourne, P. E. The Protein Data Bank. *Nucleic Acids Res.* **2000**, *28*, 235–242.
21. Fitzpatrick, P. A.; Steinmetz, A. C. U.; Ringe, D.; Klibanov, A. M. Enzyme crystal structure in a neat organic solvent. *Proc. Natl. Acad. Sci. U.S.A.* **1993**, *90*, 8653–8657.
22. Mattos, C.; Ringe, D. Locating and characterizing binding sites on proteins. *Nat. Biotechnol.* **1996**, *14*, 595–599.
23. Allen, K. N.; Bellamacina, C.; Ding, C.; Jeffrey, C.; Mattos, C.; Petsko, G. A.; Ringe, D. The Cambridge Structural Database: a quarter of a million crystal structures and rising. *J. Phys. Chem.* **1996**, *100*, 2605–2611.
24. Mattos, C.; Bellamacina, C. R.; Peisach, E.; Vitkup, D.; Petsko, G. A.; Ringe, D. Multiple solvent crystal structures: probing binding sites, plasticity and hydration. *J. Mol. Biol.* **2006**, *357*, 1471–1482.
25. Ringe, X.; Mattos, X. Location of binding sites on proteins by the multiple solvent crystal structure method. In: *Fragment-Based Approaches in Drug Discovery*, Jahnke, W.; Erlanson, D. A.; Eds. Weinheim: Wiley-VCH; **2006**.
26. Shuker, S. B.; Hajduk, P. J.; Meadows, R. P.; Fesik, S. W. Discovering high-affinity ligands for proteins: SAR by NMR. *Science* **1996**, *274*, 1531–1534.
27. Guarnieri, F. Computational protein probing to identify binding sites. U.S. Patent 6735530, **2004**.
28. Clark, M.; Guarnieri, F.; Shkurko, I.; Wiseman, J. Grand canonical Monte Carlo simulation of ligand-protein binding. *J. Med. Inf. Model.* **2006**, *46*, 231–242.