Proc. Natl. Acad. Sci. USA
Vol. 93, pp. 8945–8950, August 1996
Biophysics

Unraveling principles of lead discovery: From unfrustrated energy landscapes to novel molecular anchors

PAUL A. REJTO AND GENNADY M. VERKHIVKER

Agouron Pharmaceuticals Inc., 3565 General Atomics Court, San Diego, CA 92121

Communicated by David Chandler, University of California, Berkeley, CA, May 13, 1996 (received for review December 22, 1995)

ABSTRACT **The search for novel leads is a critical step in the drug discovery process. Computational approaches to identify new lead molecules have focused on discovering complete ligands by evaluating the binding affinity of a large number of candidates, a task of considerable complexity. A new computational method is introduced in this work based on the premise that the primary molecular recognition event in the protein binding site may be accomplished by small core fragments that serve as molecular anchors, providing a structurally stable platform that can be subsequently tailored into complete ligands. To fulfill its role, we show that an effective molecular anchor must meet both the thermodynamic requirement of relative energetic stability of a single binding mode and its consistent kinetic accessibility, which may be measured by the structural consensus of multiple docking simulations. From a large number of candidates, this technique is able to identify known core fragments responsible for primary recognition by the FK506 binding protein (FKBP-12), along with a diverse repertoire of novel molecular cores. By contrast, absolute energetic criteria for selecting molecular anchors are found to be promiscuous. A relationship between a minimum frustration principle of binding energy landscapes and receptor-specific molecular anchors in their role as "recognition nuclei" is established, thereby unraveling a mechanism of lead discovery and providing a practical route to receptor-biased computational combinatorial chemistry.**

Concepts of Lead Discovery

Understanding the principles of molecular recognition is a long-standing problem in molecular biology (1, 2). Methods to discover novel lead molecules and assess their binding affinity and receptor specificity are of considerable utility in receptor structure-based drug design (3–7). Approaches that computationally screen data bases for complete inhibitors (8–15) are required to both assess the structure of the bound ligand–protein complex and reliably estimate the binding free energy. Each candidate is ranked on the basis of the best energetic orientation, evaluated by criteria such as packing density, electrostatic complementarity, molecular mechanics force field energy, and empirical solvation free energy corrections (8–11). These scoring functions, which are approximate for binding affinity, often are unable to distinguish between the structures of native and nonnative ligand–protein complexes. A detailed description of ligand–protein association involves a delicate balance between van der Waals and electrostatic interactions, solvation effects, and conformational entropy, resulting in a highly frustrated energy landscape of molecular recognition with many energetically similar but structurally different local minima, which makes reliable structure prediction difficult (16, 17). Hence, even if a thermodynamically complete and accurate energy function suitable for rigorous predictions of binding affinity were available, it would not solve the equally important problem of structure prediction in lead discovery. Thus, computational approaches to discover complete ligands are restricted both by the ambiguity of structural prediction and limitations of binding affinity estimates.

Insights into the principles of molecular recognition have emerged from experimental combinatorial chemistry, where natural selection is used to discover receptor-specific ligands (18, 19). Combinatorial screening of random peptide libraries for ligands that specifically bind to streptavidin manifested a recurrent consensus tripeptide sequence (20). Subsequent structural studies revealed a unique orientation of this motif and a consistent interaction pattern with the receptor (21). A unique pair of receptor-specific anchor residues are responsible for peptide recognition by human class I major histocompatibility complex molecules and also stabilize the protein in its biologically functional conformation (22–24). As a result of these insights, present efforts in receptor-biased combinatorial chemistry focus on identification of the groups critical for recognition, which are intolerant to substitution, followed by design of chemical libraries based on this core motif (25, 26). Studies of molecular recognition by streptavidin (20, 21), major histocompatibility complex molecules (22–24), antibodies (27–31), immunophilins (32–34), and SH3 domains (35–37) have revealed rather general principles of the primary molecular recognition event, which occurs when a relatively rigid portion of the receptor active site recognizes receptor-specific anchor motifs of the ligand. Primary molecular recognition of the FK506 inhibitor by the FK506 binding protein (FKBP-12) is fulfilled by the pipecolinyl moiety of FK506, which anchors the ligand into a single binding mode and positions the remainder of the inhibitor relative to it (32–34). Core motifs were used as structural anchors for tailoring lead fragments into potent peptide hybrids of the FK506 inhibitor (34); a similar strategy was used to develop nonpeptidic analogs of the angiotensin peptide hormone (30) and nonpeptidic cycloanalogs that bind with streptavidin (38). In all cases, the minimal recognition motifs are intolerant to mutation; a variety of peripheral groups that interact with flexible portions of the protein active site then provide increased binding affinity and confer ligand specificity.

Both experimental and computational evidence suggests that molecular anchors need not be tight-binding fragments. A reported derivative of the pipecolinyl moiety is a weak binding ligand (39). Although a specific tripeptide consistently appears among millions of candidates that bind streptavidin, this motif makes a relatively small number of interactions in the binding site of the protein (21). The computed differences in binding interaction energies between different binding sites of the Fab of the monoclonal antibody B13I2 with its primary recognition tripeptide were not greatly different (31). While structural stability of the anchor portion of the antigen is critical for recognition, favorable interactions with the binding site of the antibody are provided by peripheral parts of the peptide.

The binding free energy of ligand–protein complexes is dependent on the composition and structure of the entire ligand molecule and is controlled by the resulting thermody-

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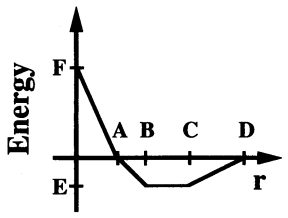


FIG. 1. The piecewise linear functional form for both the steric and hydrogen-bond interaction terms. The total intermolecular energy is given by a pairwise sum of these terms over all ligand and protein heavy atoms. The units of energy are arbitrary.

namics (7). However, the primary molecular recognition event, determined by a small anchor portion of the ligand acting as a receptor-specific "recognition nucleus," may not be driven exclusively by binding affinity. To fulfill its functional role, we suggest that a receptor-specific molecular anchor must satisfy both the thermodynamic requirement of the relative energetic stability of a favorable binding mode and its consistent kinetic accessibility. The kinetic requirement, as measured by the criterion of structural consensus in multiple docking simulations, implies that the molecular recognition energy landscape of molecular anchors must have a reduced frustration, which gives rise to a large number of conformations that can consistently proceed to the single binding mode (40, 41).

Molecular Recognition Model

Simple energy models coupled with extensive conformational search have shown their robustness in protein structure prediction (42). The requirements of a simple molecular recognition model for reliable structural prediction of ligand–protein complexes were also recently elucidated (43). The energetic model includes intramolecular energy terms for the ligand, given by torsional and nonbonded functions (44), and intermolecular ligand–protein interaction terms consisting of steric and hydrogen bond contributions calculated from a piecewise linear potential summed over all protein and ligand heavy atoms (Fig. 1). The parameters of the pairwise potential depend on the four different atom types: hydrogen-bond donor, hydrogen-bond acceptor, both donor and acceptor, and nonpolar. Primary and secondary amines are defined to be donors, while oxygen and nitrogen atoms with no bound hydrogens are defined to be acceptors. Hydroxyl groups are defined to be both donor and acceptor, and carbon atoms are defined to be nonpolar. The ligand and protein atoms interact via steric and hydrogen bond-like potentials (Table 1), which have the same functional form, with an additional three-body contribution to the hydrogen bond term (45). The parameters (Table 2) were refined to yield the experimental crystallographic structure of a set of ligand–protein complexes as the global energy minimum (46). No explicit tuning for the FKBP-12 protein or any of the fragments analyzed in this study was performed, and no assumptions regarding either favorable ligand conformations or any specific ligand–protein interactions were made. The ligand conformations and orientations are searched by a simulated evolution algorithm (46) in a rectangular box that encompasses the binding site obtained from the structure of the crystallographic ligand–protein complex with a 2.0-Å cushion added to every side of this box. The ligand bond distances and bond angles, as well as the torsional angles of nonrotatable bonds, were obtained from the crystal structure of the bound ligand–protein complex and held fixed during the docking simulations.

Table 2. Parameters of the atomic pairwise ligand–protein potentials

Interaction type	A*	B*	C*	D*	E†	F†
Steric	3.4	3.6	4.5	5.5	−0.4	20.0
Hydrogen bond	2.3	2.6	3.1	3.4	−2.0	20.0

*A, B, C, and D are in Å.
†E and F are in arbitrary energy units.

Results

The topology of binding energy landscapes may be characterized by studying the inherent structure, or local potential energy minima, of the underlying energy landscape (47–49). Consistent structural prediction of a single binding mode is the signature of an unfrustrated energy landscape, and therefore of potential molecular anchors, while structural prediction of many different binding modes is characteristic of rough energy landscapes with many alternative low energy states and a high degree of frustration.

It has been shown experimentally that the pipecolinyl moiety of FK506 acts as a molecular anchor (34). The following questions then arise: what distinguishes the region of FKBP-12 that recognizes the pipecolinyl portion of FK506, and can this moiety be identified computationally compared with other fragments that constitute the FK506 ligand? To address these issues, the FK506 inhibitor was fragmented into seven different pieces consisting of 14-heavy atoms each (Fig. 2), with one of the fragments chosen to encompass the critical pipecolinyl moiety (32–34). From multiple docking simulations in the FKBP-12 protein active site, all seven fragments are predicted to bind to the region where the pipecolinyl moiety is located when FK506 is bound to the protein, suggesting that this region of the binding site recognizes receptor-specific anchor fragments (Fig. 3). However, only the pipecolinyl fragment is consistently predicted to be in a single binding mode in this region by the docking simulations. The pipecolinyl fragment deeply penetrates the active site, surrounded by residues Tyr-26, Phe-36, Ile-56, and Tyr-82, and is located within 1.0 Å rms of its position in the FKBP-12–FK506 complex (Fig. 3*a*). When the fragments are held rigid during docking simulations, the energy of the pipecolinyl structure is the lowest of the seven fragments, but this moiety cannot be identified on an absolute energetic basis during flexible docking simulations (Fig. 4*a*). The extent of structural consensus from multiple docking simulations can be determined quantitatively by computing the average rms deviation of the resulting structures relative to the lowest energy structure (Fig. 4*b*). Importantly, the criterion of structural consensus does not require a reference crystal structure, so core motifs can be identified in the absence of *a priori* information regarding the crystal structure of the bound complex. The fragment containing the pipecolinyl moiety has significantly lower rms deviation (or higher structural consen-

Table 1. Pairwise atomic interaction types for the molecular recognition model

Ligand atom type	Protein atom type			
	Donor	Acceptor	Both	Nonpolar
Donor	Steric	Hydrogen bond	Hydrogen bond	Steric
Acceptor	Hydrogen bond	Steric	Hydrogen bond	Steric
Both	Hydrogen bond	Hydrogen bond	Hydrogen bond	Steric
Nonpolar	Steric	Steric	Steric	Steric

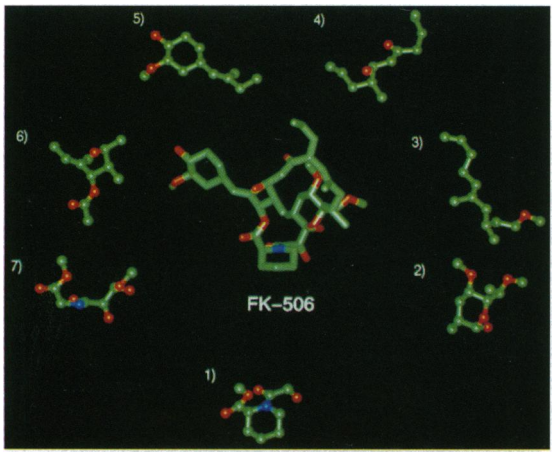


FIG. 2. A heavy-atom depiction of the FK506 inhibitor and the seven fragments generated from FK506, each of which consists of 14-heavy atoms. One of these fragments (fragment 1) incorporates the pipecolinyl moiety, while the remaining fragments incorporate other portions of the FK506 molecule. The number of internal degrees of freedom for the seven fragments are two (fragment 1), three (fragment 2), eight (fragment 3), eight (fragment 4), three (fragment 5), four (fragment 6), and five (fragment 7).

sus) than all other fragments. This feature of the binding energy landscape unambiguously distinguishes this moiety as a recognition nucleus from the other fragments in both rigid and flexible docking simulations. Therefore, the extent of structural consensus is suitable for searching libraries of chemical compounds for molecular anchors even when the structure of the bound complex is not known.

Multiple docking simulations were then performed to find novel core motifs for the FKBP-12 protein active site. To eliminate the bias of the absolute energy toward ligands with more atoms, the pipecolinyl moiety was seeded in a minimized set (50) of 1374 molecules consisting of 14-heavy atoms obtained from the Fine Chemicals Directory (MDL Information Systems, San Leandro, CA). Molecules with zero, one, two, three, or four rotatable bonds were examined. This range was chosen to encompass the number of rotatable bonds in the pipecolinyl moiety and to assure the reliability of the structural consensus measurements resulting from multiple docking simulations. While the absolute energy of the pipecolinyl moiety is about average for the screened molecules (Fig. 5*a*), the structural consensus for this core moiety is in the top 6% of all molecules and in the top 3% when compared with molecules with the same number of rotatable bonds (Fig. 5*b*). The computational time requirements are consistent with rapid data base searches: 1000 molecular fragments can be evaluated in ≈8 hr using a small network of 20 typical workstations such as a Silicon Graphics R4400 (Silicon Graphics, Mountain View, CA).

The features of four molecules of size 14 heavy atoms with high structural consensus with the FKBP-12 protein are analyzed (Fig. 6). The predicted consensual binding mode of these molecular anchors is achieved by a combination of steric and hydrogen bond interactions in the protein active site. In particular, the unusual pattern of aromatic hydrogen bond interactions detected for the pipecolinyl moiety (33) is also apparent in these molecular anchors (Table 3). Key recognition elements of the FKBP-12 active site involved in interactions with the pipecolinyl moiety include Tyr-26, Phe-36, Ile-56, and Phe-99. Although the discovered anchors have a similar composition of hydrophobic and hydrophilic atoms, the number of hydrogen bonds varies from two, made by anchor 1, to five, made by anchor 4 (Table 3). It may be expected that the binding affinity of these molecules is different. Nevertheless, these molecules share the common feature of a high structural consensus in the FKBP-12 active site and are potential lead fragments. A rather diverse scaffold of hydrogen bonds and favorable steric complementarity anchors these leads in a unique orientation, and elaboration by a variety of synthetically feasible extensions could confer ligand specificity and enhance binding affinity.

A small, novel inhibitor of the FKBP-12 protein that consists of 17-heavy atoms was recently synthesized, and the crystal structure of the complex was solved (51). This inhibitor binds in the same location of the active site as the pipecolinyl core with a single binding mode, has modest binding affinity, and therefore may be regarded as another molecular anchor for the FKBP-12 protein. To further validate the ability of the structural consensus criterion to identify molecular anchors, this molecule was seeded into a minimized set of 984 17-heavy atom molecules taken from the Fine Chemicals Directory with between zero and four rotatable bonds. Once again, the absolute energy of the known molecular anchor is about average for the screened molecules, but the structural consensus is in the top 4% of molecules with the same number of rotatable bonds (Fig. 5 *c* and *d*).

The extent of structural consensus is a measure of the kinetic accessibility of the native binding mode, and high consensus presumably arises due to the presence of multiple kinetic routes in the binding energy landscape leading to the favorable binding mode. It has been shown in protein folding studies (52, 53) that kinetic accessibility is correlated with the relative energetic stability of the native structure compared with alternative local minima. An analysis of the binding energy

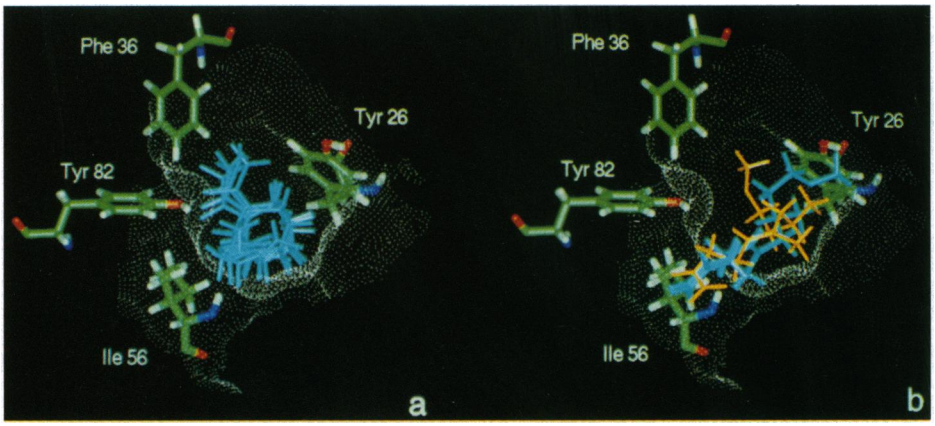


FIG. 3. (*a*) The structures of fragment 1 obtained from 100 flexible docking simulations were ranked by energy, and five representative structures (ranked 10, 20, 30, 40, and 50) are displayed. There is only a single binding mode (shown in blue). (*b*) Representative structures for fragment 2, which has the next best structural consensus, chosen as for fragment 1. There are two binding modes (shown in blue and orange).

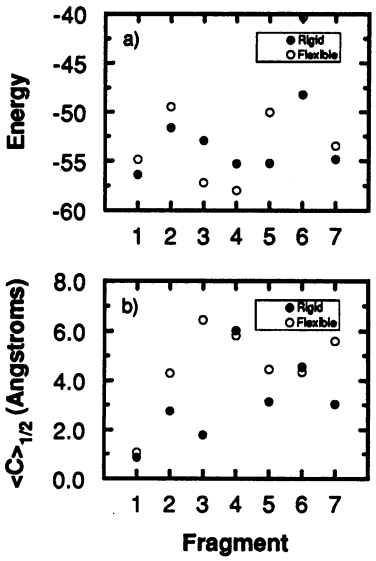


FIG. 4. (*a*) The energies of the lowest energy structures of the seven fragments for rigid (●) and flexible (○) docking simulations. The fragments are numbered as in Fig. 1. (*b*) The consensus value $\langle C \rangle_{1/2}$ of the seven fragments for rigid (●) and flexible (○) docking simulations, defined to be: $\langle C \rangle_{N/M} = \Sigma_{i=2}^{N} 1(N - 1)\sqrt{\Delta r_i^3}$. The structures are ordered in terms of energy and the lowest energy structure defines the reference. rms differences $\sqrt{\Delta r^2}_i$ between the reference structure and structure i are computed for the N lowest energy structures out of a total of M simulations. For each FK506 fragment, a total of $M = 100$ simulations were performed, $N = 50$, and the ratio $N/M = 1/2$.

spectra reveals that the two known molecular anchors, which have a high structural consensus, also have a pronounced stability gap between the native binding mode and structurally different binding modes (Fig. 7). Thus, high structural consensus is associated with relative energetic stability, and this energy gap ensures the thermodynamic stability of the native binding mode. By contrast, fragments of average energy and average structural consensus have little or no energy gap (Fig. 7) and are unlikely to be structurally stable molecular anchors.

Discussion and Conclusions

By applying the criterion of structural consensus, we have demonstrated that two different molecular anchors of FKBP-12 can be distinguished from random compounds of

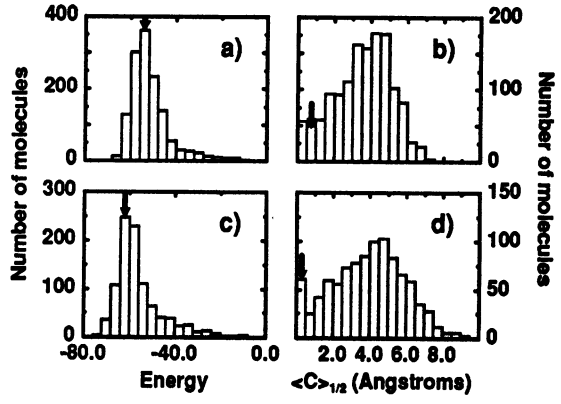


FIG. 5. The energy (*a*) and consensus (*b*) of 1374 molecules consisting of 14-heavy atoms and the energy (*c*) and consensus (*d*) of 984 molecules consisting of 17-heavy atoms, obtained from 20 docking simulations of each compound. The energy and consensus of the pipecolinyl acid residue is marked by arrows in (*a*) and (*b*), while in (*c*) and (*d*), the energy and consensus of the 17-heavy atom inhibitor are similarly marked.

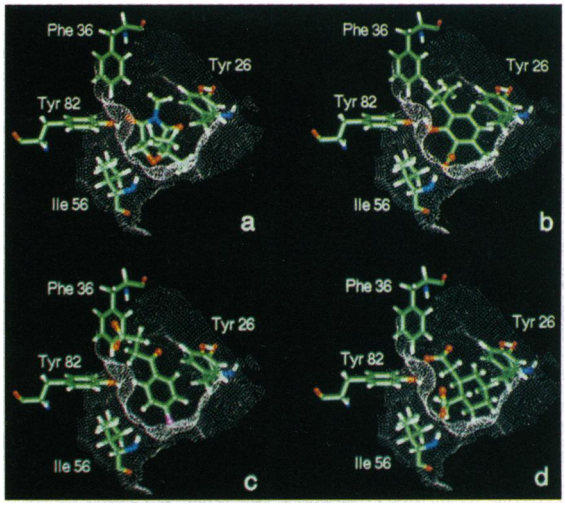
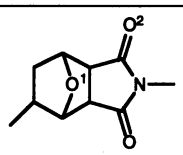
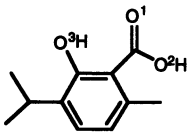
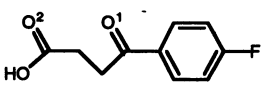
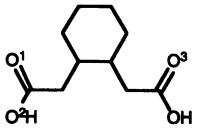


FIG. 6. Anchor fragments obtained from a search of the Fine Chemicals Directory for molecules consisting of 14-heavy atoms. A total of 20 docking simulations of each molecule into the FKBP-12 protein binding site were performed. These molecules have amongst them the ten best consensus values for a given number of rotatable bonds. *a*, zero rotatable bonds; *b*, one rotatable bond; *c*, three rotatable bonds; and *d*, four rotatable bonds.

similar size. We have shown that the basic requirement of a molecular anchor is the formation of a single binding mode that is both thermodynamically stable, compared with alternative binding modes, and kinetically accessible. Reminiscent of protein folding studies, molecular anchors may work as receptor-specific recognition nuclei (54) by providing not only relative thermodynamic stability of a favorable binding mode, but also ensuring the presence of dynamical routes or kinetic funnels leading to the native structure (55, 56). Molecules that meet these requirements and may serve as lead fragments in the drug discovery process represent a relatively small fraction of conceivable fragments, namely those that satisfy a minimum frustration principle. Randomly chosen molecular fragments of the same size and composition have rough energy surfaces

Table 3. Four novel molecular anchors obtained from the Fine Chemicals Directory using the criterion of structural consensus

Chemical structure	Donor	Acceptor	Distance, Å
	I56 NH	O^1	r(N-O)=3.10
	Y82 OH	O^2	r(O-O)=2.64
	I56 NH	O^1	r(N-O)=2.96
	I56 NH	O^2	r(N-O)=3.14
	Y82 OH	O^3	r(O-O)=2.57
	Y26 CeH	O^1	r(H-O)=2.24
	F36 CeH	O^1	r(H-O)=2.63
	Y82 OH	O^2	r(O-O)=3.06
	F99 CeH	O^1	r(H-O)=2.51
	F36 CeH	O^1	r(H-O)=2.40
	F36 CeH	O^2	r(H-O)=2.75
	I56 NH	O^3	r(N-O)=3.16
	Y82 OH	O^2	r(O-O)=2.73
	Y82 OH	O^1	r(O-O)=2.98

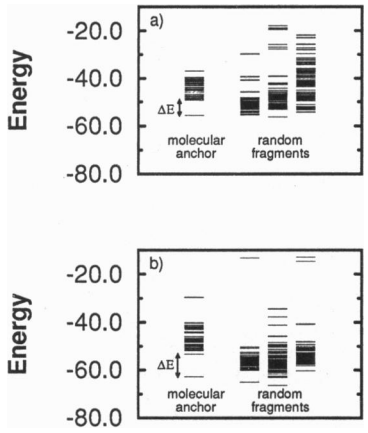


FIG. 7. (*a*) Energy spectra for the pipecolinyl core and three random fragments of size 14 heavy atoms and average energy and consensus. (*b*) Energy spectra for the 17-heavy atom inhibitor and three random fragments of the same size and average energy and consensus. Energies associated with the native binding mode are collapsed into a single value. This highlights that the stability gap between the native binding mode and alternative binding modes for molecular anchors is considerably larger than for random fragments.

with many alternative binding modes and are unable to be stable receptor-specific structural cores.

While standard screening techniques aimed at finding complete ligands properly focus on estimates of the binding affinity, molecular anchors need not be tight binding fragments, and binding affinity estimates therefore may not be discriminatory for screening virtual chemical libraries to find molecular anchors. The failure of the absolute energetic criteria to select molecular anchors may not be merely an artifact of the simple description of ligand–protein interactions employed in this study, but it may reflect more general molecular recognition rules that yield receptor specific molecular anchors. As in the nucleation mechanism suggested for reliably folding protein sequences (54), it may be important in the design of complete ligands that the relative stabilization energy be localized in a specific recognition nucleus. It is tempting to suggest that in early stages of evolution, proteins may have developed by natural selection to form first preorganized, rigid active sites that reduce frustration in the energy landscapes of molecular recognition with the anchor portion of their substrate. Nature may then have exploited the flexible portions of the protein active site to confer substrate specificity and tailor other properties. Molecular anchors, serving as receptor-specific recognition nuclei, may have provided the primary molecular recognition event at the relatively high temperatures corresponding to early stages of evolution.

The role of core fragments in binding is primarily structural; they act as thermodynamically stable and kinetically accessible platforms that are specific to a given receptor. Although molecules that have a strong binding affinity and a single binding mode need not necessarily contain an anchor, such molecules may be more susceptible to multiple binding modes, which complicates structure-based drug design. Computational discovery of core anchor groups with subsequent design of chemical libraries based on these cores provides a direct road to receptor-targeted combinatorial chemistry. Synthesis of lead molecules derived from anchors represents a strategy that is amenable to iterative chemical modifications, contrasting with lead discovery of natural products, where the inherent structural complexity of leads often precludes synthetic accessibility of chemical analogs. The approach proposed in this work may accelerate lead discovery and serve as a foundation for future drug discovery processes.

1. Fisher, E. (1894) *Ber. Dtsch. Chem. Ges.* **27,** 2985–2993.
2. Koshland, D. E., Jr. (1958) *Proc. Natl. Acad. Sci. USA* **44,** 98–104.
3. Wlodawer, A. & Erickson, J. W. (1993) *Annu. Rev. Biochem.* **62,** 543–585.
4. Appelt, K. (1993) *Perspect. Drug Discovery Des.* **1,** 23–48.
5. Kuntz, I. D. (1992) *Science* **257,** 1078–1082.
6. Clackson, T. & Wells, J. A. (1995) *Science* **267,** 383–386.
7. Miyamoto, S. & Kollman, P. A. (1993) *Proc. Natl. Acad. Sci. USA* **90,** 8402–8406.
8. Shoichet, B. K. & Kuntz, I. D. (1991) *J. Mol. Biol.* **221,** 327–346.
9. Shoichet, B. K., Stround, R. M., Santi, D. V., Kuntz, I. D. & Perry, K. M. (1993) *Science* **259,** 1445–1450.
10. Shoichet, B. K. & Kuntz, I. D. (1993) *Protein Eng.* **6,** 723–732.
11. De Voss, J. J. & Ortiz de Montellano, P. R. (1995) *J. Am. Chem. Soc.* **117,** 4185–4186.
12. Caflish, A., Miranker, A. & Karplus, M. (1993) *J. Med. Chem.* **36,** 2142–2167.
13. Eisen, M. B., Wiley, D. C., Karplus, M. & Hubbard R. E. (1994) *Proteins Struct. Funct. Genet.* **19,** 199–221.
14. Ring, C. S., Sun, E., McKerrow, J. H., Lee, G. K., Rosenthal, P. J., Kuntz, I. D. & Cohen, F. E. (1993) *Proc. Natl. Acad. Sci. USA* **90,** 3583–3587.
15. Li, Z., Chen, X., Davidson, E., Zwang, O., Mendis, C., Ring, C. S., Roush, W. R., Fegley, G., Li, R., Rosenthal, P. J., Lee, G. K., Kenyon, G. L., Kuntz, I. D. & Cohen, F. E. (1994) *Chem. Biol.* **1,** 31–37.
16. Cherfils, J. & Janin, J. (1993) *Curr. Opin. Struct. Biol.* **3,** 265–269.
17. Oshiro, C. M., Kuntz, I. D. & Dixon, J. S. (1995) *J. Comput. Aided Mol. Design* **9,** 113–130.
18. Gallop, M. A., Barrett, R. W., Dower, W. J., Fodor, S. P. A. & Gordon, E. M. (1994) *J. Med. Chem.* **37,** 1233–1250.
19. Gordon, E. M., Barrett, R. W., Dower, W. J., Fodor, S. P. A. & Gallop, M. A. (1994) *J. Med. Chem.* **37,** 1385–1400.
20. Delvin, J. J., Panganiban, L. C. & Delvin, P. E. (1990) *Science* **249,** 404–406.
21. Weber, P. C., Pantoliano, M. W. & Thompson, L. D. (1992) *Biochemistry* **31,** 9350–9354.
22. Fremont, D. H., Matsumura, M., Stura, E. A., Peterson, P. A. & Wilson. I. A. (1992) *Science* **257,** 919–927.
23. Matsumura, M., Fremont, D. H., Peterson, P. A. & Wilson. I. A. (1992) *Science* **257,** 927–934.
24. Madden, D. C., Garboczi, D. N. & Wiley, D. C. (1993) *Cell* **75,** 693–708.
25. Kick, E. K. & Ellman, J. A. (1995) *J. Med. Chem.* **38,** 1427–1430.
26. Weiss, G. A., Collins, E. J., Garboczi, D. N., Wiley, D. C. & Schreiber, S. L. (1995) *Chem. Biol.* **2,** 401–407.
27. Wilson, I. A. & Stanfield, R. L. (1993) *Curr. Opin. Struct. Biol.* **3,** 113–118.
28. Stanfield, R. L., Fieser, T. M., Lerner, R. A. & Wilson, I. A. (1990) *Science* **248,** 712–719.
29. Rini, J. M., Schulze-Gahmen, U. & Wilson, I. A. (1992) *Science* **255,** 959–965.
30. Garsia, K. C., Ronco, P. M., Verroust, P. J., Brunger, A. T. & Amzel, L. M. (1992) *Science* **257,** 502–507.
31. Friedman, A. R., Roberts, V. A. & Tainer, J. A. (1994) *Proteins Struct. Funct. Genet.* **20,** 15–24.
32. Van Duyne, G. D., Standaert, R. F., Karplus, P. A., Schreiber, S. L. & Clardy, J. (1991) *Science* **252,** 839–842.
33. Van Duyne, G. D., Standaert, R. F., Karplus, P. A., Schreiber, S. L. & Clardy, J. (1991) *J. Mol. Biol.* **229,** 105–124.
34. Ikeda, Y., Shultz, L. W., Clardy, J. & Schreiber, S. L. (1994) *J. Am. Chem. Soc.* **116,** 4143–4144.
35. Lim, W. A., Richards, F. M. & Fox, R. O. (1994) *Nature (London)* **372,** 375–379.
36. Feng, S., Chen, J. K., Yu, H., Simon, J. A. & Schreiber, S. L. (1994) *Science* **266,** 1241–1247.
37. Chen, J. K. & Schreiber, S. L. (1995) *Angew. Chem. Int. Ed. Engl.* **34,** 953–969.
38. Katz, B. A., Johnson, C. R. & Cass, R. T. (1995) *J. Am. Chem. Soc.* **117,** 8541–8547.
39. Holt, D. A., Konialian-Beck, A. L., Oh, H.-J., Yen, H.-K., Rosamus, L. W., Krog, A. J., Erhard, K. F., Ortiz, E., Levy, M. A., Brandt, M., Bossard, M. J. & Luengo, J. I. (1994) *Bioorg. Med. Chem. Lett.* **4,** 315–320.
40. Bryngelson, J. D. & Wolynes, P. G. (1987) *Proc. Natl. Acad. Sci. USA* **84,** 7524–7528.

41. Bryngelson, J. D., Onuchic, J. N., Socci, N. D. & Wolynes, P. G. (1995) *Proteins Struct. Funct. Genet.* **21,** 167–195.
42. Yue, K. & Dill, K. A. (1996) *Protein Sci.* **5,** 254–261.
43. Verkhivker, G. M., Rejto, P. A., Gehlhaar, D. G. & Freer, S. T. (1996) *Proteins Struct. Funct. Genet.*, in press.
44. Mayo, S. L., Olafson, B. D. & Goddard, W. A., III. (1990) *J. Phys. Chem.* **94,** 8897–8909.
45. McDonald, I. K. & Thornton, J. M. (1994) *J. Mol. Biol.* **238,** 777–793.
46. Gehlhaar, D. G., Verkhivker, G. M., Rejto, P. A., Sherman, C. J., Fogel, D. B., Fogel, L. J. & Freer, S. T. (1995) *Chem. Biol.* **2,** 305–316.
47. Stillinger, F. H. & Weber, T. A. (1982) *Phys. Rev. A* **25,** 457–464.
48. Fukugita, M., Lancaster, D. & Mitchard, M. G. (1993) *Proc. Natl. Acad. Sci. USA* **90,** 6365–6368.
49. Verkhivker, G. M. & Rejto, P. A. (1996) *Proc. Natl. Acad. Sci. USA* **93,** 60–64.
50. Mohamadi, F., Richards, N. C. J., Guida, W. C., Liskamp, R., Lipton, M., Caufield, C., Chang, G., Hendrickson, T. F. & Still, W. C. (1990) *J. Comput. Chem.* **11,** 440–467.
51. Babine, R. E., Bleckman, T. M., Kissinger, C. R., Showalter, R., Pelletier, L. A., Lewis, C., Tucker, K., Moomaw, E., Parge, H. E. & Villafranca, J. E. (1995) *Bioorg. Med. Chem. Lett.* **5,** 1719–1724.
52. Sali, A., Shakhnovich, E. I. & Karplus, M. (1994) *J. Mol. Biol.* **235,** 1614–1636.
53. Gutin, A. M., Abkevich, V. I. & Shakhnovich, E. I. (1995) *Proc. Natl. Acad. Sci. USA* **92,** 1282–1286.
54. Abkevich, V. I., Gutin, A. M. & Shakhnovich, E. I. (1994) *Biochemistry* **33,** 10026–10036.
55. Pande, V. S., Grossberg, A. Y. & Tanaka, T. (1994) *Proc. Natl. Acad. Sci. USA* **91,** 12976–12979.
56. Ebeling, M. & Nadler, W. (1995) *Proc. Natl. Acad. Sci. USA* **92,** 8798–8802.