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Docking Studies on the Complexed and Uncomplexed FKBP12 Structures with Bound and Unbound Ligands: An Implication of a Conformational Selection Mechanism for Binding

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Abstract

Docking of FK506, rapamycin, and L-685,818 into their receptor, FKBP12, suggests that unlike the respective structures determined by X-ray crystallography, the uncomplexed FKBP12 structures determined by NMR may not be directly usable to identify high affinity ligands by docking studies for computational drug screening. In view of the resolution of the experimentally determined structures of FKBP12 and relatively small difference of the receptor binding sites between the complexed and uncomplexed states, it is unclear if the conformational induction mechanism is relevant to the binding of FKBP12 with its ligands. Alternatively, we advocate a conformation selection mechanism fundamentally akin to a mechanism proposed by Burgen. This mechanism better explains the experimental and calculated results for the binding of FKBP12 with FK506. It emphasizes that both guest and host select their most compatible preformed conformers to effect binding, and that the observed free energy of binding is a sum of the free energy change in complexation of the two most compatible conformers and the free energy changes in conversion of the Boltzmann-weighted principal conformers to the most compatible conformers. Conceptually, this mechanism represents one physical or nonphysical path of a thermodynamic cycle that is closed by the other path represented by the conformational induction mechanism, which can also be physical or nonphysical; it provides a theoretical means to estimate the affinity of the guest to the host with the experimentally available 3D structures of the two partners.

Keywords: Recognition, induced-fit, key-lock, SYSDOC, computational drug screening

Introduction

The protein FKBP12 is a soluble cytosolic receptor with high affinity for immunosuppressants such as FK506 [1-4]. The FK506-FKBP12 complex inhibits calcineurin phosphatase activity and consequently arrests one signal transduction pathway associated with transcriptional control in T lymphocytes [5]. Structural studies of FKBP12 and its ligands and complexes focused on development of further improved immunosuppressants have been extensively reported [4,6-15]. Recently, the ligand-free FKBP12 structure in the solid state

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has also been determined at 1.7 Å resolution (from Brian M. McKeever of the Merck Research Laboratories) and at 2.3 Å resolution [11] by X-ray crystallography.

The major conformation of FK506 alone in chloroform or in the solid state is found to be strikingly different from the corresponding bound conformation in water or in the solid state. The *cis*-amide bond in the first is changed to a *trans*-amide bond in the latter [16]. For FKBP12 itself, significant conformational changes between the complexed and uncomplexed states were observed in residues 35-45 and 78-96 by the X-ray and NMR studies [11-13], although fluorescence, CD, FTIR and calorimetry data all suggest that FKBP12 undergoes only subtle structural alterations upon ligand binding [17].

To study the conformational change that occurrs in the binding pocket of FKBP12 upon ligand binding, we first calculated distances between the beta-carbon atoms of the residues that constitute the receptor binding pocket in the complexed and uncomplexed receptor structures (see Materials and methods). This pocket is reportedly composed of the side chains of Y26, F36, F46, F48, W59, Y82, and F99 [13,18]. Selection of the beta-carbon atom as a reference point was because the displacement of the beta-carbon atom reflects (i) rotations about the psi and phi torsions, which partly govern the backbone conformation of the residue being examined and (ii) the motion of the side chain. We found that the binding pockets of the ligand-free FKBP12 structures in the solution state were slightly different from those of the ligand-bound proteins in the solid state, based on the distances between the beta-carbon atoms (see Table 1). This finding was consistent with the root mean square deviations (RMSDs) of the residue structures in the binding pocket between the two states (see Table 2). Such RMSDs range from 0.4 Å to 1.1 Å.

However, it was unclear whether the slight conformational differences of the binding site between the ligand-bound crystal structures and the ligand-free solution structures were real, because the RMSDs for the first 21 ligand-free NMR structures themselves are 1.4 and 2.5 Å for backbone heavy atoms and all non-hydrogen atoms excluding residues 83-90, respectively [15]. It was also unclear if the ligand-free crystal structure of FKBP12 was different from the ligand-bound crystal structure, because the two structures were determined at 1.7 Å resolution, whereas their RMSD was just 0.7 Å (see Table 2).

Two questions

The above considerations cast two fundamental questions. First, can the unbound FKBP12 structures accommodate FK506 as effectively as the bound ones? This question is critical to proposals of using directly the unbound FKBP12 structures as a host for identification of high affinity ligands by docking studies for computational drug screening [19]. The second question followed logically: which mechanism better describes the binding of FKBP12 to its ligands, a process of conformational induction (Koshland's Induced-Fit theory) [20] or conformational selection (a prototypic mechanism proposed by Burgen) [21]? In the conformational induction mechanism, a ligand binds initially to a less compatible conformation of a receptor and then induces the most compatible conformations of the two. In the conformational selection mechanism, ligand and receptor select their most compatible preformed conformer to effect binding, which consequently shifts progressively the equilibrium between the most compatible and less compatible conformers of both partners to the selected conformers. The second question is important to mechanistic studies of ligand-receptor interactions, especially computational studies of ligand-receptor energetics.

Materials and methods

Throughout this study, we examined the 22 uncomplexed FKBP12 NMR structures determined in water by Michnick *et al.* [13], because the RMSDs for the ones by Michnick *et al.* are smaller than those reported by Moore *et al.* [15]. The last two NMR structures by Michnick *et al.* are the optimized averages of the first 20 structures excluding and including electrostatic interactions during energy minimization, respectively [13]. We used these NMR structures as the uncomplexed receptor structures in solution, the ligand-free crystal structure of FKBP12 (from Brian M. McKeever of the Merck Research Laboratories) as the uncomplexed receptor structure in the solid state, the FKBP12 structures derived from the FK506-complex [9] and the complexes of its analog (rapamycin [10] and L-685,818 [4]) determined by X-ray crystallography as the complexed receptor structures in the solid state and the ligand structures taken from these crystal complexes as the bound ligand structures. Because the major receptor-free conformation of FK506 in chloroform [14] is identical to that in the solid state determined by X-ray crystallography [7], it is plausible that this conformation is also the major receptor-free conformation in water. We therefore used the receptor-free X-ray structure of FK506 as the major receptor-free conformation of FK506 in water.

Docking studies were carried out by employing the automated docking computer program SYSDOC [22,23] whose algorithm has been validated by independent experimental results [24,25]. This program systematically translates and rotates a guest in a putative binding pocket of a host to evaluate energetically favorable sites for each thermodynamically accessible conformation of the two. In this study, the translational and rotational increments of docking in a region (52 x 25 x 15 Å) enclosing the aforementioned binding pocket were 0.8 Å and 10° of arc, respectively. The docking results were further fine-tuned at 0.2 Å increment in the range of 1.2 Å and at 5° of arc increment in a 30° range of arc. The affinity of binding here was estimated from the potential energy of the complex relative to those of the composites in the free states. It was assumed that differences in entropy and solva-

Table 1. *Differences of the β–carbon distance between the FKBP12 structure derived from the FK506-FKBP12 complex and the corresponding structure in other conformations.*

	F48	F99	Y26	F46	Y82	D37	E54	I90
FKX	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0
RAX	0.0	0.0	0.1	0.0	0.2	0.2	0.1	0.3
LAX	-0.1	0.0	-0.1	0.0	-0.1	-0.1	-0.1	-0.2
UFX	0.1	-0.2	0.1	-0.1	0.3	0.7	-0.8	-0.3
KSO1	0.1	0.3	0.5	0.4	0.5	2.2	-0.5	1.8
KSO2	0.1	0.3	0.6	0.3	0.7	4.0	2.3	1.9
KSO3	0.2	0.3	0.6	0.3	-0.1	2.2	-0.3	0.8
KSO4	-0.2	0.4	0.4	0.1	0.5	2.7	1.2	1.7
KSO5	0.1	0.3	0.1	0.4	0.5	2.3	-0.6	1.2
KSO6	0.0	0.1	0.6	1.3	0.4	0.9	0.3	-0.8
KSO7	0.0	0.4	0.4	0.2	-0.1	2.6	-0.2	1.2
KSO8	0.2	0.2	0.4	0.1	0.2	2.2	1.5	0.7
KSO9	0.2	0.1	0.4	0.5	0.4	2.1	1.4	0.6
KSO10	0.0	0.4	0.5	0.0	0.8	3.0	-0.3	0.9
KSO11	0.0	0.4	0.5	0.0	0.4	1.8	-0.4	0.4
KSO12	0.1	0.3	0.5	0.4	0.4	1.9	1.2	1.8
KSO13	0.2	0.1	0.5	0.4	0.4	1.7	-0.5	1.2
KSO14	-0.3	0.4	0.6	0.1	0.6	2.5	-0.4	1.1
KSO15	0.0	0.2	0.6	0.1	0.4	1.6	1.2	0.7
KSO16	0.1	0.3	0.5	0.1	0.4	2.7	1.1	1.6
KSO17	0.1	0.2	0.5	0.2	0.1	2.8	-0.1	-0.7
KSO18	0.0	0.2	0.3	0.2	0.3	1.6	-0.5	0.1
KSO19	0.2	0.0	0.6	0.3	-0.3	2.2	1.2	0.6
KSO20	0.0	0.3	0.6	0.0	0.8	2.4	0.8	2.9
KSO21	0.1	0.3	0.6	0.3	0.5	1.9	0.2	1.1
KSO22	0.4	0.4	0.7	0.6	0.7	3.8	0.5	1.2

The β–carbon distance was measured between the β–C of W59 and the β–C of other residues. A negative value indicates that the β–carbon distance of the individual conformer of FKBP12 is greater than that of the protein in the FK506-FKBP12 complex. FKX: FKBP12 in the FK506-FKBP12 complex determined by X-ray crystallography; RAX: FKBP12 in the rapamycin-FKBP12 complex determined by X-ray crystallography; LAX: FKBP12 in the L-685,818-FKBP12 complex determined by X-ray crystallography; UFX: the uncomplexed FKBP12 structure determined by X-ray crystallography; KSON: the Nth solution structure of FKBP12 determined by the NMR; KSO21: optimized average structure of the 20 NMR FKBP12 structures excluding electrostatic interactions; and KSO22: optimized average structure of the 20 NMR FKBP12 structures including electrostatic interactions.

tion energies between two analogous guest structures or two conformationally similar host structures can be neglected [22]. The relative potential energy difference is referred to hereafter as "binding energy" and is the simplified free energy change of binding; accordingly, the term "binding energy" is used cautiously. The potential energy was calculated according to equation 1 with the non-bonded, additive, all-atom force field parameters of TRIPOS [26] and the CHARMM template charges [27]:

$$E = \sum_{i<j} \varepsilon^*_{ij} k \left(\frac{r^{*12}_{ij}}{R^{12}_{ij}} - 2\frac{r^{*6}_{ij}}{R^{6}_{ij}} \right) + \sum_{i<j} \frac{q_i q_j}{\varepsilon_0 R_{ij}} \quad (1)$$

Table 2. *RMSDs of the non-H atoms of the residues in the binding pocket between the FKBP12 structure in the FK506-FKBP12 complex and other receptor structures.*

	Substructure I RMS Deviation (Å)	Substructure II RMS Deviation (Å)
FKX	0.0	0.0
RAX	0.3	0.4
LAX	0.1	0.1
UFX	0.4	0.7
KSO1	0.8	1.3
KSO2	0.8	1.2
KSO3	0.8	1.3
KSO4	0.8	1.2
KSO5	0.8	1.2
KSO6	1.0	1.2
KSO7	0.8	1.3
KSO8	0.7	1.2
KSO9	0.9	1.3
KSO10	1.0	1.4
KSO11	0.8	1.1
KSO12	0.7	1.2
KSO13	0.7	1.2
KSO14	0.9	1.3
KSO15	0.7	1.2
KSO16	0.7	1.2
KSO17	0.8	1.2
KSO18	0.7	1.0
KSO19	0.8	1.4
KSO20	0.9	1.5
KSO21	0.7	1.1
KSO22	0.6	1.2

See notes of Table 1 for structure nomenclature. Substructure I: Y26-F46-F48-W59-Y82-F99. Substructure II: Y26-F46-F48-W59-Y82-F99-D37-I90-E54.

where k = 1 when atoms i and j do not form a hydrogen bond, otherwise k = 0. The non-bonded cutoff for the van der Waals and electrostatic interactions was set to 8.0 Å in this study. A distance-dependent dielectric constant (ε_0 = r) was used in the Coulomb term.

Results and discussion

We first performed a control study to compare the SYSDOC-generated structures with the corresponding crystal complexes and to estimate the system error of the calculated binding energies by docking the bound FK506 and its analogs to each complexed receptor structure. The SYSDOC-generated, highest affinity complexes were in excellent agreement with the corresponding crystal complexes (see Figure 1). The small RMSDs between the crystal and SYSDOC-generated structures are listed in Table 3. The RMSD was obtained by first overlapping a set of receptor residues in the two complexes and then calculating the RMSD of the guest or host structure in the superimposed complexes. The system error of the calculated binding energies was estimated to be 9 kcal/mol by the assumption that the binding affinities of FK506 to the receptor structure derived from the three crystal complexes should be identical (see Table 3). With the SYSDOC program it is apparent from Table 3 that we were able to make a correct retrospective "prediction" that the three complexed receptor structures can tightly accommodate FK506, rapamycin and L-685,818 in their bound conformations.

An answer to the first question

We then docked the bound FK506 and its analogs into the 22 uncomplexed receptor structures in solution. We found that none of these receptor structures was able to accommodate the bound FK506 and its analogs with comparable affinity to that achievable by the complexed receptor structures (see Table 4). Among the 22 solution structures, we found that the eighth and eighteenth solution structures were able to form a relatively low affinity complex with the bound FK506 and its analogs. However, the binding motif of these complexes was quite different from that of the corresponding crystal complexes. The allylic side chain of FK506 forms a face-to-face pi-pi interaction with the indole ring of W59 in the low affinity complexes, whereas the pipecolinyl ring moiety of FK506 interacts with the same ring in the corresponding crystal complexes. This implies that conformational adaptation of the 22 solution structures is required for effective binding.

We therefore answer the first question: the 22 uncomplexed solution FKBP12 structures cannot accommodate FK506 and its analogs as effectively as the complexed receptor structures in the solid state. We suggest that the uncomplexed solution structures may not be directly usable as a host to identify high affinity ligands by docking studies for computational drug screening, although they are useful in rational design of ligands according to receptor topology and in other applications (*vide infra*). Our answer is true in either case (i) that the conformational differences of the binding sites between the NMR determined uncomplexed and the X-ray determined complexed FKBP12 structures are indeed small but significant; or (ii) that such small conformational differences might be artifacts due to the resolution of

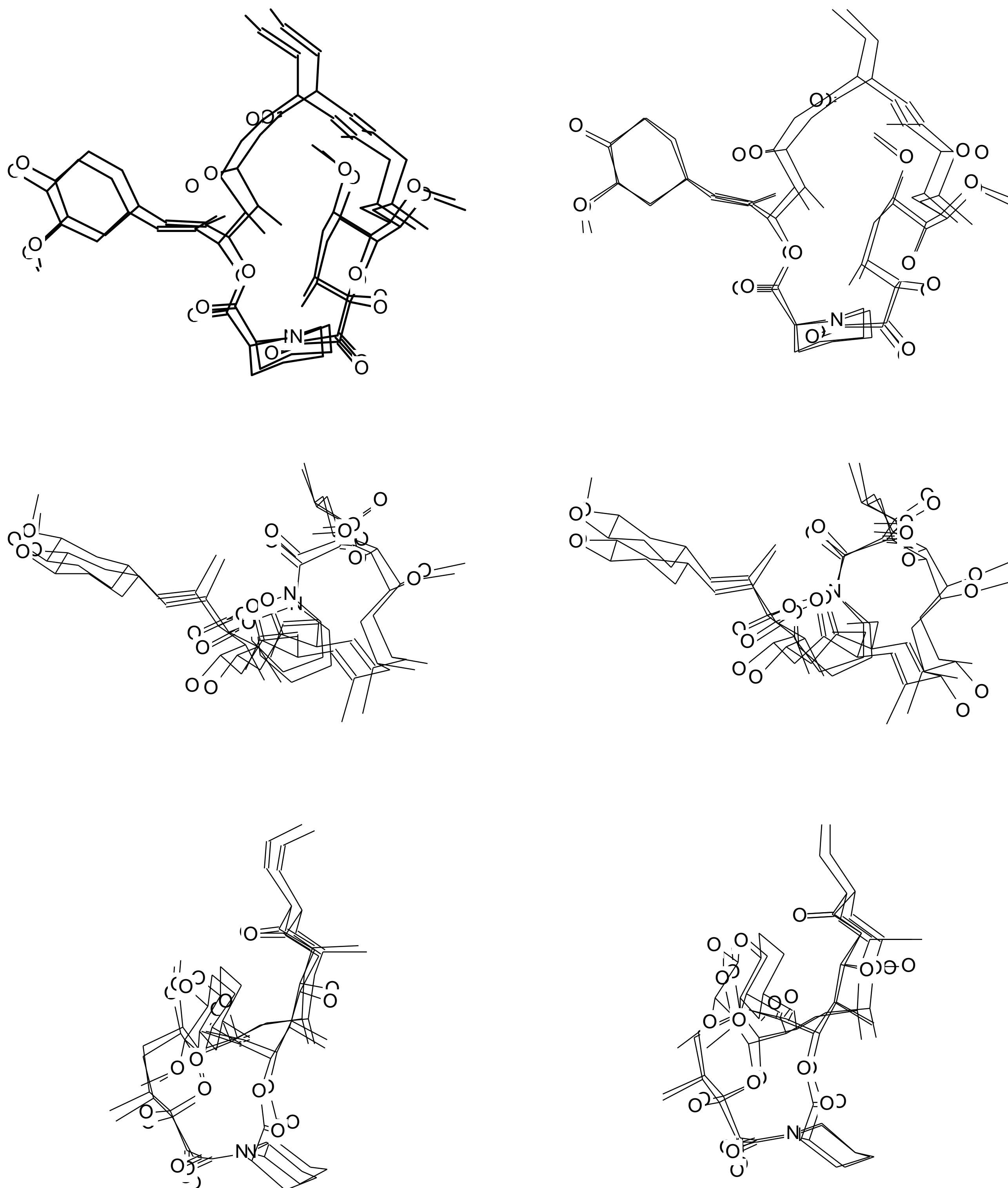
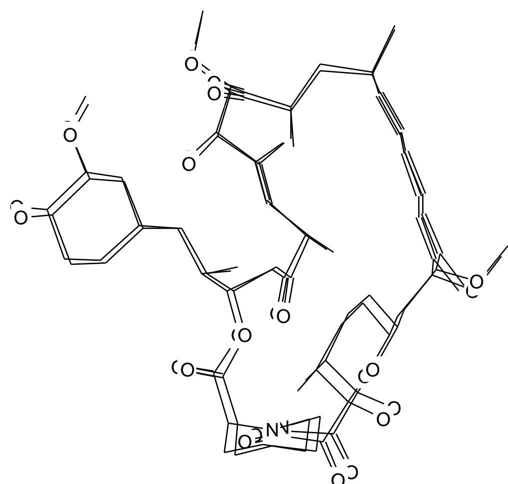
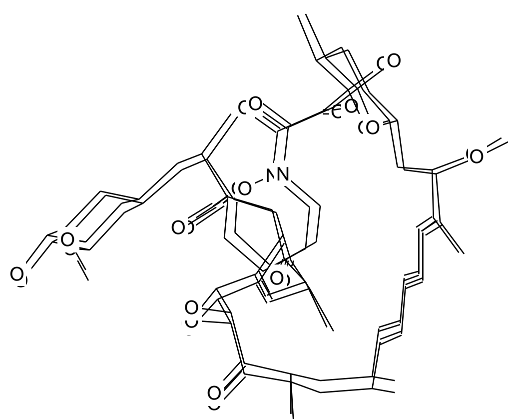


Figure 1a. *Representation of the RMSDs for the non-H atoms in the guest structure between the SYSDOC-generated and crystal complexes emphasizing the smaller deviation in the pipecolinyl ring portion which contacts with FKBP12 (FK506 in the FK5d-FKX complex: RMSD = 0.56 Å;* top*: face view,* middle*: top view,* bottom: *side view).*

Figure 1b. *Representation of the RMSDs for the non-H atoms in the guest structure between the SYSDOC-generated and crystal complexes emphasizing the smaller deviation in the pipecolinyl ring portion which contacts with FKBP12 (L-685,818 in the L-685,818-LAX complex: RMSD = 0.54 Å;* top*: face view,* middle*: top view,* bottom: *side view).*





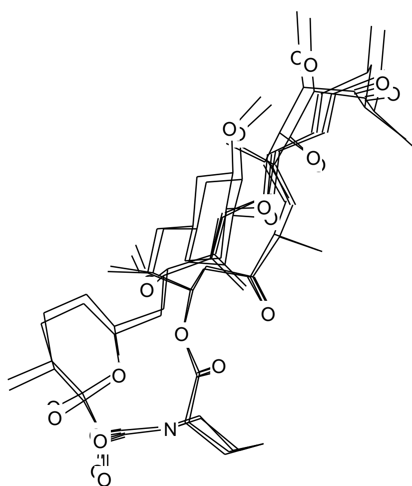
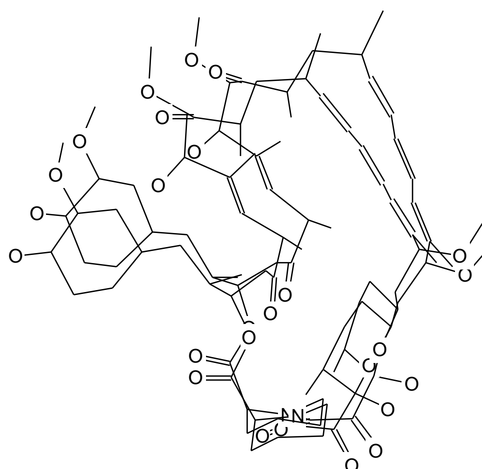
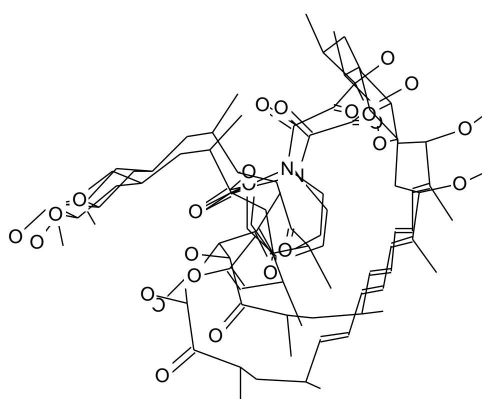


Figure 1c. *Representation of the RMSDs for the non-H atoms in the guest structure between the SYSDOC-generated and crystal complexes emphasizing the smaller deviation in the pipecolinyl ring portion which contacts with FKBP12 (rapamycin in the rapamycin-RAX complex: RMSD = 0.31 Å;* top*: face view,* middle*: top view,* bottom: *side view).*





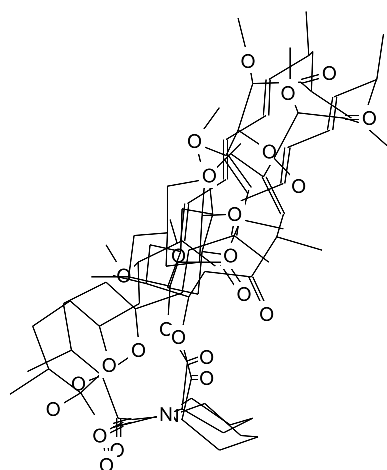


Figure 1d. *Representation of the RMSDs for the non-H atoms in the guest structure between the SYSDOC-generated and crystal complexes emphasizing the smaller deviation in the pipecolinyl ring portion which contacts with FKBP12 (rapamycin in the rapamycin-LAX complex: RMSD = 1.6 Å;* top*: face view,* middle*: top view,* bottom: *side view).*

Table 3. *RMSDs (Å) for the non-H atoms in the guest or host structures between the crystal and SYSDOC-generated complexes and the corresponding binding energies (kcal/mol).*

	FK506			RAPAMYCIN			L-685,818		
	guest	host	energy	guest	host	energy	guest	host	energy
FKX	0.6	0.0	-68	1.5	0.4	-40	0.3	0.2	-63
RAX	0.4	0.4	-61	0.3	0.0	-47	0.8	0.5	-54
LAX	0.4	0.2	-59	1.6	0.5	-44	0.5	0.0	-69

See notes of Table 1 for structure nomenclature. The selected residues of FKBP12 for superimposition were Y26, F36, D37, R42, F46, F48, E54, V55, I56, W59, Y82, H87, L97, and F99.

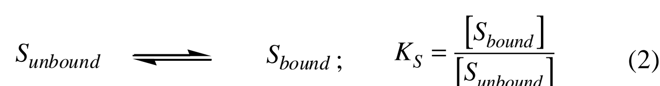
the NMR derived structures. Our answer is also important, since one could mistakenly assume that the uncomplexed structures are the same as the complexed structures for the docking studies because the conformational differences are so small and within the experimental errors. Further modification of the NMR structures is required for the docking studies. One proposed general approach to this problem is to "expand" the binding site of the uncomplexed solution structures by short molecular dynamics simulations on the structures initially filled with counter-ions and extra water molecules in the binding cavity [22].

We then docked the bound FK506 and its analogs into the uncomplexed receptor structure in the solid state. We found that this structure was able to form a complex similar to the complexes determined by the X-ray crystallography. However, the affinity of this uncomplexed structure for FK506 was lower than those of the complexes generated by docking with the complexed receptor structures, but much higher than those of the complexes with the uncomplexed receptor structures (see Table 4). The result suggests that unlike the NMR structures, the uncomplexed crystal structure may be directly usable as a host for computational drug screening. In principle, one might also try to use the expansion method mentioned above, since the uncomplexed crystal structure may still not perfectly reflect the binding geometry when it is kept rigid in some docking procedures. Further studies are required to determine if the answer to the first question is generally relevant to other protein-ligand complexes.

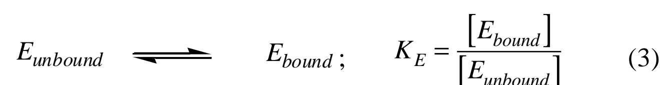
An answer to the second question

Surprisingly, we found that all the binding energies of the bound FK506 for the complexed receptor structures are about 20 kcal/mol lower than those of the respective rapamycin complexes (see Table 4). This result is at least qualitatively contradictory to the experimental finding of the enhanced binding of rapamycin (K_d = 0.2 nM) relative to FK506 (K_d = 0.4 nM) [3], since the binding energy difference by 20 kcal/mol reveals at least a difference of 10^5 in K_d. In addition, because the small conformational differences of the binding sites between the complexed and uncomplexed states as described earlier are within the errors of the experiments, it is still unclear if the conformational induction mechanism is involved in the binding of FKBP12 to its ligands, even though the docking studies imply that conformational adaptation of the uncomplexed receptor structures in solution is required for effective binding.

To address the second question with the above considerations, we advocate a conformational selection mechanism fundamentally akin to the mechanism proposed by Burgen [21]. We describe this mechanism from a thermodynamics perspective in order to avoid unnecessary confusion about Burgen's prototypic mechanism [28,29]. Our conformational selection mechanism emphasizes that both substrate (or ligand) and enzyme (or receptor) select their most compatible preformed conformers to effect binding, and that the observed free energy of binding is a sum of the free energy change in complexation of the two most compatible conformers and the free energy changes in conversion of the Boltzmann-weighted principal conformers to the most compatible conformers. The observed association constant can be calculated according to Eq. 5,



$$S_{unbound} \rightleftharpoons S_{bound}\,; \quad K_S = \frac{[S_{bound}]}{[S_{unbound}]} \qquad (2)$$



$$E_{unbound} \rightleftharpoons E_{bound}\,; \quad K_E = \frac{[E_{bound}]}{[E_{unbound}]} \qquad (3)$$

$$S_{bound} + E_{bound} \rightleftharpoons SE\,;$$

$$K_{SE} = \frac{[SE]}{[S_{bound}][E_{bound}]} \qquad (4)$$

Table 4. *The lowest binding energies (kcal/mol) of the SYSDOC generated FKBP12 complexes.*

	FK506 (bound)	FK506 (free)	Rapamycin	L-685,818
FKX	**-68**	> 0	**-40**	**-63**
RAX	**-61**	> 0	**-47**	**-54**
LAX	**-59**	> 0	**-44**	**-69**
UFX	**-9**	> 0	**-6**	**-9**
KSO1	> 0	> 0	> 0	-9
KSO2	> 0	> 0	> 0	> 0
KSO3	> 0	> 0	> 0	> 0
KSO4	> 0	> 0	-3	> 0
KSO5	> 0	> 0	-32	> 0
KSO6	> 0	> 0	> 0	> 0
KSO7	> 0	> 0	-19	> 0
KSO8	-40	-20	-40	-38
KSO9	> 0	> 0	> 0	> 0
KSO10	> 0	> 0	> 0	> 0
KSO11	> 0	-10	> 0	-30
KSO12	> 0	> 0	> 0	> 0
KSO13	> 0	> 0	> 0	> 0
KSO14	> 0	> 0	> 0	> 0
KSO15	> 0	> 0	> 0	> 0
KSO16	> 0	> 0	> 0	> 0
KSO17	> 0	> 0	> 0	> 0
KSO18	-35	-25	> 0	-29
KSO19	> 0	> 0	> 0	> 0
KSO20	> 0	> 0	> 0	> 0
KSO21	> 0	> 0	> 0	> 0
KSO22	> 0	> 0	> 0	-19

See notes of Table 1 for structure nomenclature. The bold number indicates that the binding motif is identical to that found in the crystal complex.

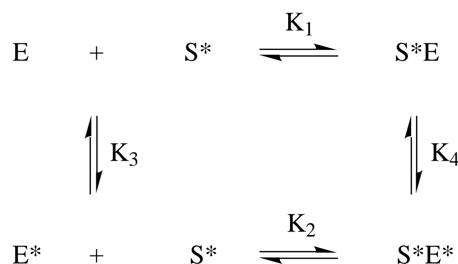
$$K_{observed} = \frac{[SE]}{([S_{bound}]+[S_{unbound}])([E_{bound}]+[E_{unbound}])} = \frac{K_{SE}}{\left(\frac{1}{K_S}+1\right)\left(\frac{1}{K_E}+1\right)} \quad (5)$$

where S_{bound} and E_{bound} are the most compatible conformers of the substrate (or ligand) and enzyme (or receptor), respectively; $S_{unbound}$ and $E_{unbound}$ are the Boltzmann-weighted major (or minor) but incompatible conformers of the substrate and enzyme, respectively; SE is the substrate bound enzyme complex; K_S and K_E are the equilibrium constants for the substrate's and enzyme's equilibriums between the bound and unbound conformers, respectively; and K_{SE} is the association constant of the complex SE.

With the conformational selection mechanism, we can now better explain the experimental and computational data on the binding of FKBP12. According to the above equations, the K_S for FK506 is much smaller than that for rapamycin, because, unlike rapamycin whose major receptor-free conformation in water is identical to the bound rapamycin [16], the major receptor-free conformation of FK506 in water is different from the bound FK506 and is presumably unable to bind tightly to FKBP12 (*vide infra*) [16]. Although the binding energy of FK506 is qualitatively lower than that of rapamycin, the smaller K_S for FK506 can reduce the observed binding affinity of FK506 insofar as a decreased binding of FK506 (K_d = 0.4 nM) relative to rapamycin (K_d = 0.2 nM) was observed [3].

To validate theoretically the assumption that the major receptor-free conformation of FK506 in water cannot bind tightly to FKBP12, we also docked this structure into the complexed and uncomplexed receptor structures. As assumed, we found indeed that this structure cannot bind to all the receptor structures used in this study with comparable affinity to that of the bound FK506 (see Table 4).

Further, to study the binding of FKBP12 with the conformation selection mechanism, we are now not confined by the resolution of the experiments. As described above, we don't know if the conformation induction mechanism is appropriate to the binding of FKBP12. But, with the following closed thermodynamic cycle constructed by the conformational induction (K_1 and K_4) and conformational selection (K_2 and K_3) mechanisms (the substrate is assumed rigid for simplicity), we know that the conformation selection mechanism is appropriate because the induced conformational change is no longer of concern.



where S* is the bound substrate (or ligand); E and E* are the uncomplexed and complexed enzymes (or receptors), respectively; and S*E and S*E* are the complexes consisting of the less and most compatible conformers, respectively.

Furthermore, we can use the conformational selection mechanism for theoretical calculations regardless of whether the path of K_2 and K_3 is physical or nonphysical. The uncomplexed average NMR structures or the uncomplexed crystal structures can presumably be used as the uncomplexed structure E. With the above mentioned expansion approach

to convert E to E* if necessary and the well-established free energy perturbation methods [30], we can theoretically estimate K_2 and K_3, and can therefore estimate the binding of E to S*, in this case, the binding of FK506 to FKBP12. With the conformational induction mechanism, on the other physical or nonphysical path of the closed thermodynamic cycle, the NMR structures would be useless, since it is technically difficult to calculate the free energy change for the perturbation from the S*E complex to the S*E* complex involving rotations of the ligand in the binding site as revealed importantly by the present docking studies. The difficulty is mainly a demand for excessive but probably unachievable sampling in molecular dynamics or Monte Carlo simulations to obtain converged free energy changes [30].

As a further conceptual extension, an unrelated, additional advantage of the conformational selection mechanism is that Emil Fisher's Key-Lock (or Template) mechanism [31] is explicitly included in the conformational selection mechanism. In the case when the most compatible conformers of the substrate and enzyme (S_{bound} and E_{bound}) are the Boltzmann-weighted principal conformers, $S_{unbound}$ and $E_{unbound}$ are then the Boltzmann-weighted minor conformers, the consequent large K_S and K_E result in $K_{observed}$ close or equal to K_{SE}. At this extreme, the conformation selection mechanism becomes the classical Key-Lock mechanism.

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