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Polarizable Water Molecules in Ligand−Macromolecule Recognition. Impact on the Relative Affinities of Competing Pyrrolopyrimidine Inhibitors for FAK Kinase

Benoit de Courcy,[†,‡,§] Jean-Philip Piquemal,[*,‡,§] Christiane Garbay,[†,⊥] and Nohad Gresh[*,†,⊥]

Université Paris Descartes, Laboratoire de Pharmacochimie Moléculaire et Cellulaire, U648 INSERM, UFR Biomédicale, 45 rue des Saints-Pères, 75270 Paris Cedex 06, France, UPMC Université Paris 06, UMR 7616, Laboratoire de Chimie Théorique, case courrier 137, 4 place Jussieu, F-75005 Paris, France, and CNRS, UMR 7616, Laboratoire de Chimie Théorique, case courrier 137, 4 place Jussieu, F-75005 Paris, France

Received July 21, 2009; E-mail: nohad.gresh@parisdescartes.fr; jpp@lct.jussieu.fr

Abstract: Using polarizable molecular mechanics (PMM), we have compared the complexation energies of the focal adhesion kinase (FAK) kinase by five inhibitors in the pyrrolopyrimidine series. These inhibitors only differ by the substitution position of a carboxylate group on their benzene or pyridine rings, and/or the length of the connecting $(CH2)_n$ chain ($n = 0-2$) while their inhibitory properties vary from micromolar to nanomolar. Energy balances in which solvation/desolvation effects are computed by a continuum reaction field procedure failed to rank the inhibitors according to their inhibitory potencies. In marked contrast, including energy-minimizing in the protein−inhibitor binding site limited numbers of structural water molecules, namely five to seven, ranked these energy balances conforming to the experimental ordering. The polarization energy contribution was the most critical energy contribution that stabilized the best-bound inhibitor over the others. These results imply that (a) upon docking charged inhibitors into the active site of kinases such as FAK, the presence of a limited number of structured water molecules is critical to enable meaningful relative energy balances and (b) accounting for an explicit polarization contribution within ΔE is indispensable.

1. Introduction

Focal adhesion kinase (FAK) is a protein tyrosine kinase which, subsequent to activation by autophosphorylation, initiates a cascade of protein−protein interactions. These result in signal transmission to the cell nucleus to trigger cell division and motility. FAK is found overexpressed in numerous cancers and constitutes an important target for the design of antitumor inhibitors.[1] Several related pyrrolopyrimidine FAK inhibitors were recently designed and synthesized by a group at the Novartis company, and the comparative affinities of these inhibitors were measured.[2] These compounds act as competitors of ATP, which is the natural substrate of kinases. Changes in the position of the terminal carboxylate group can affect dramatically the binding affinities of such compounds to FAK. This was exemplified in a series of five related derivatives, denoted as **16i**, **17g**, **17h**, **17i**, and **32**, whose affinities range from micromolar to nanomolar (Figure 1). It is essential to evaluate if theoretical computations are able to reproduce the ordering of their relative binding affinities. In this study, we investigate whether discrete water molecules could impact such affinities. In this connection, water molecules were found to be instrumental to understanding complex interactions within biomolecules. Thus, water networks were shown to be essential to model the observed electron transfer phenomenon in metalloenzymes.[3] Moreover, the critical role of highly structured water molecules in mediating protein−protein interactions[4] and in the thermodynamics of ligand−receptor interactions[5] was recently shown. To quantify their role, we apply state of the art polarizable molecular mechanics.[6]

2. Polarizable Molecular Mechanics (PMM) Procedure

The components of the energy balance correspond to the differences between the intermolecular kinase−inhibitor interaction energy *plus* the solvation energy of their complex on the one hand,

† Université Paris Descartes, U648 INSERM, UFR Biomédicale.
‡ UPMC Université Paris 06, UMR 7616.
§ CNRS, UMR 7616, Laboratoire de Chimie Théorique.
⊥ Current address: Université Paris Descartes, Laboratoire de Chimie et Biochimie Pharmacologique et Toxicologique, UMR 8601 CNRS, UFR Biomédicale, 45, rue des Saints-Pères, 75270 Paris Cedex 06 France.

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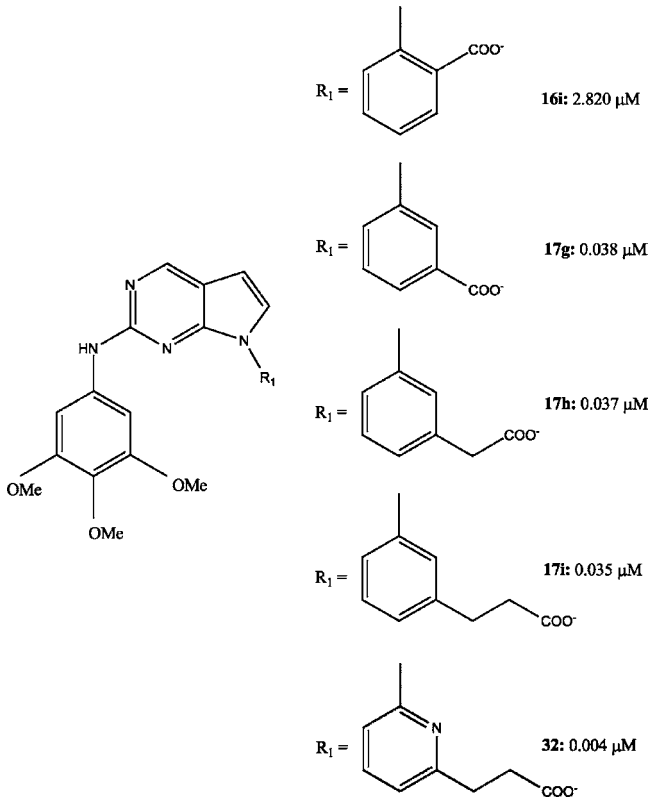

Figure 1. Molecular structures of the five pyrrolopyrimidine inhibitors **16i** to **32**. The values of their FAK inhibition constants (IC_{50}) are given in μM.

and the desolvation and conformational energy changes of both partners prior to complexation on the other hand. Such comparative energy balances involve small differences between large numbers, and it is essential that each component be computed as accurately as possible. Since ab initio quantum chemistry (QC) is intractable on large ligand−macromolecule complexes, it is necessary to resort to accurate molecular mechanics/dynamics (MM/MD) potential energy functions.

Thus, we have resorted to the sum of interactions between fragments ab initio computed (SIBFA) polarizable molecular mechanics procedure,[7] which has withstood comparisons with QC computations in a diversity of test cases, several of which were on complexes of over 100 atoms, and which has been applied to several protein−inhibitor complexes.[8] The intermolecular interaction energy is computed as a sum of five contributions: short-range penetration corrected multipolar electrostatic, E_{MTP*}, short-range repulsion, E_{rep}, polarization, E_{pol}, charge-transfer, E_{ct}, and dispersion, E_{disp}. Details on the formulation and calibration of these contributions are given in ref 7. E_{tor} is the torsional energy contribution, with the general expression $V = V_0/2(\cos(n\phi) + 1)$. The numerical values of V_0 are appended to Supporting Information S3. Bulk solvation energies ΔG_{solv} are computed using the Langlet−Claverie (LC) continuum reaction field procedure.[9] Its calibration is the same as in ref 8b.

2.1. Energy Minimizations. We use the X-ray structure of FAK kinase domain bound to a neutral pyrrolopyrimidine inhibitor (PDB id: 2ETM chain B) as a starting point (represented in Supporting Information S1). The energy minimizations (EM) are done on the internal coordinates using the Merlin package.[10] Consistent with our previous studies,[8] the protein backbone is held frozen, and the side chains of the recognition site residues are relaxed. The coordinates of the main-chain heavy atoms are taken from the PDB structure. Those of the C_β atoms and succeeding atoms are built using standard internal coordinates. As in our previous studies on proteins and peptides,[7] the multipoles and polarizabilities for the backbone atoms are those computed for the constitutive *N*-methylformamide fragment, and for pyrrolidone in the case of proline. The starting χ torsional angles are those derived from the PDB structure. The inhibitor is fully relaxed. EMs are done in the presence of the continuum reaction field procedure. The assumption of a rigid backbone is fully supported by recent X-ray structures of FAK, complexed with ADP [1MP8, 2IJM: ref 11], ATP [2IJM, 2J0L: ref 1] or with inhibitors in the pyrrolopyrimidine [2ETM], or the methanesulfonamide diaminopyrimidine series [3BZ3: ref 12]. The maximum fluctuations occur in the G-loop, known to be very flexible, with rms values <1.2 Å. Since none of the investigated inhibitors has arms than can reach such a loop, maintaining a rigid backbone appears appropriate.

2.2. QC Computations. QC energy-decomposition analyses on model complexes described below were done by the reduced variational space (RVS) procedure.[13] They were done with the CEP 4-31G(2d) basis set[14] using the GAMESS package.[15] Inclusion of correlation was done by the Moller−Plesset (MP2) procedure.[16]

3. Results and Discussion

We first present the results on the complete system, with and without structured water molecules. We then present a validation by parallel QC computations on smaller models.

3.1. Energy Balances in the Absence of Structural Water Molecules. Figure 2 gives a superimposition of the complexes of **16i** to **32** within the FAK recognition site and also of that of the neutral pyrrolopyrimidine inhibitor. This site comprises the side chains of the following residues: Arg426, Ala452, Lys454, Glu471, Cys502, Glu506, Arg508, Arg550, and Asp564. Table 1 reports the energy balances of the five complexes, denoted as '**a**'. It gives the intermolecular ligand−protein interaction energies, ΔE_{tot}, and their individual contributions which include E_{tor}, the continuum solvation energies, ΔG_{solv}, and the resulting energy balances $\Delta E_{fin} = \Delta E_{tot} + \Delta G_{solv}$. Each quantity is the difference between its value in the FAK complex (a) and its values resulting from energy minimization on uncomplexed FAK (b) on the one hand, and on the corresponding uncomplexed ligand (c) on the other hand. Energy minimizations of (b) and (c) were performed in the presence of continuum solvation. The separate (a), (b), and (c) values are reported in Supporting Information S2. E_1 denotes the sum of first-order contributions, E_{MTP*} and E_{rep}. Negative and positive values of these differences thus denote stabilization and destabilization,

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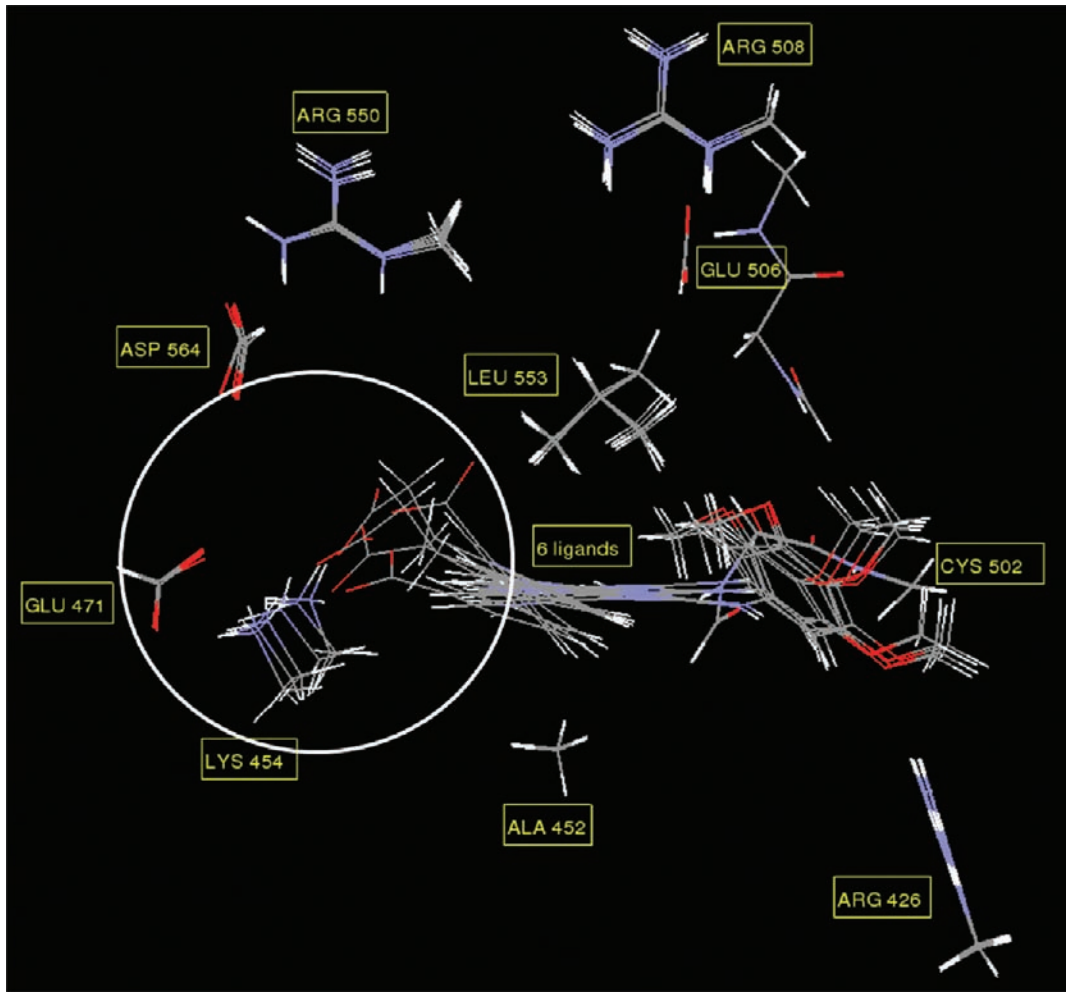

Figure 2. Overlay of the five pyrrolopyrimidine inhibitors in their complexes with the FAK binding site (denoted as '**b**' in the text). For simplicity, the water molecules are not represented.

Table 1. Values (kcal/mol) of the FAK Inhibitor Interaction Energies and Their Contributions, of the Continuum Solvation Energies, and Resulting Energy Balances[b]

	Complexes **a** without water molecules.				
	16i	**17g**	**17h**	**17i**	**32**
E_{MTP*}	−21.5	−10.9	−30.6	−63.8	−54.6
E_{rep}	57.1	33.4	26.4	76.8	73.3
E_1	**35.6**	**22.5**	**−4.3**	**13.0**	**18.7**
E_{pol}	−12.1	9.5	7.7	−6.5	−8.1
E_{ct}	−1.8	−1.2	−0.1	−1.4	−1.3
E_{disp}	−52.7	−48.8	−45.7	−51.2	−50.0
E_{tor}	−3.3	−4.3	−8.6	−1.7	−5.6
ΔE_{tot}	**−34.2**	**−22.3**	**−50.9**	**−47.8**	**−46.3**
ΔG_{solv}	14.8	4.8	22.4	25.8	24.8
ΔE_{fin}[b]	**−19.4**	**−17.5**	**−28.5**	**−22.0**	**−21.5**
$\delta\Delta E_{fin}$	2.1	4.0	−7.0	−0.5	0.0
IC_{50} (μM)	2.820	0.038	0.037	0.035	0.004

[a] The experimental inhibitory potencies (μM) are also reported. See text for definitions. [b] $\Delta E_{fin} = \Delta E_{tot} + \Delta G_{solv}$.

respectively. The differences of $\delta\Delta E_{fin}$ values, taking the value for **32** as energy zero, is denoted δ. At this stage, the ordering of $\delta\Delta E_{fin}$ values does not correlate with the experimental ordering. Thus, **17h**, endowed with a micromolar affinity, stands out as the most stably bound complex, with a 7 kcal/mol preference over nanomolar compound **32**, and 6.5−11 kcal/mol preferences over the two other micromolar compounds **17i** and **17g**. This could possibly be due to an improper location of the energy minima of some of the less favored complexes. However, this possibility appears unlikely due to (a) the short lengths of the carboxylate-bearing chains consisting of 0−2 methylene groups; this limits the space available to the carboxylate group to optimize its binding to Lys454 methylammonium group; (b) the fact that all six complexes superimpose at the level of their three aromatic rings. The limited mobility of these rings is caused by their own interactions with the Cys502 backbone, Ala452 and Leu553 and the electrostatic interactions of the benzene−trimethoxy ring with the NH of the main chain of Glu506 and the side chain of Arg426. Such anchoring interactions further restrict the mobility of the terminal carboxylate groups. Inaccuracies in ΔE(SIBFA) and ΔG_{solv}(LC) were also ruled out on the basis of detailed comparisons between SIBFA and QC on model complexes extracted from the FAK-inhibitor complexes, and which will be reported in a separate paper.

3.2. Could Structured Waters Tip the Relative Energy Balances? While the ATP binding site of kinases features several hydrophobic residues, it encompasses three cationic and three anionic residues. In addition, each inhibitor in the investigated series binds FAK with a carboxylate group. The buildup of these charges could constitute an instance where Continuum procedures should be complemented by inclusion of a limited number of 'discrete' water molecules. This was recently exemplified in

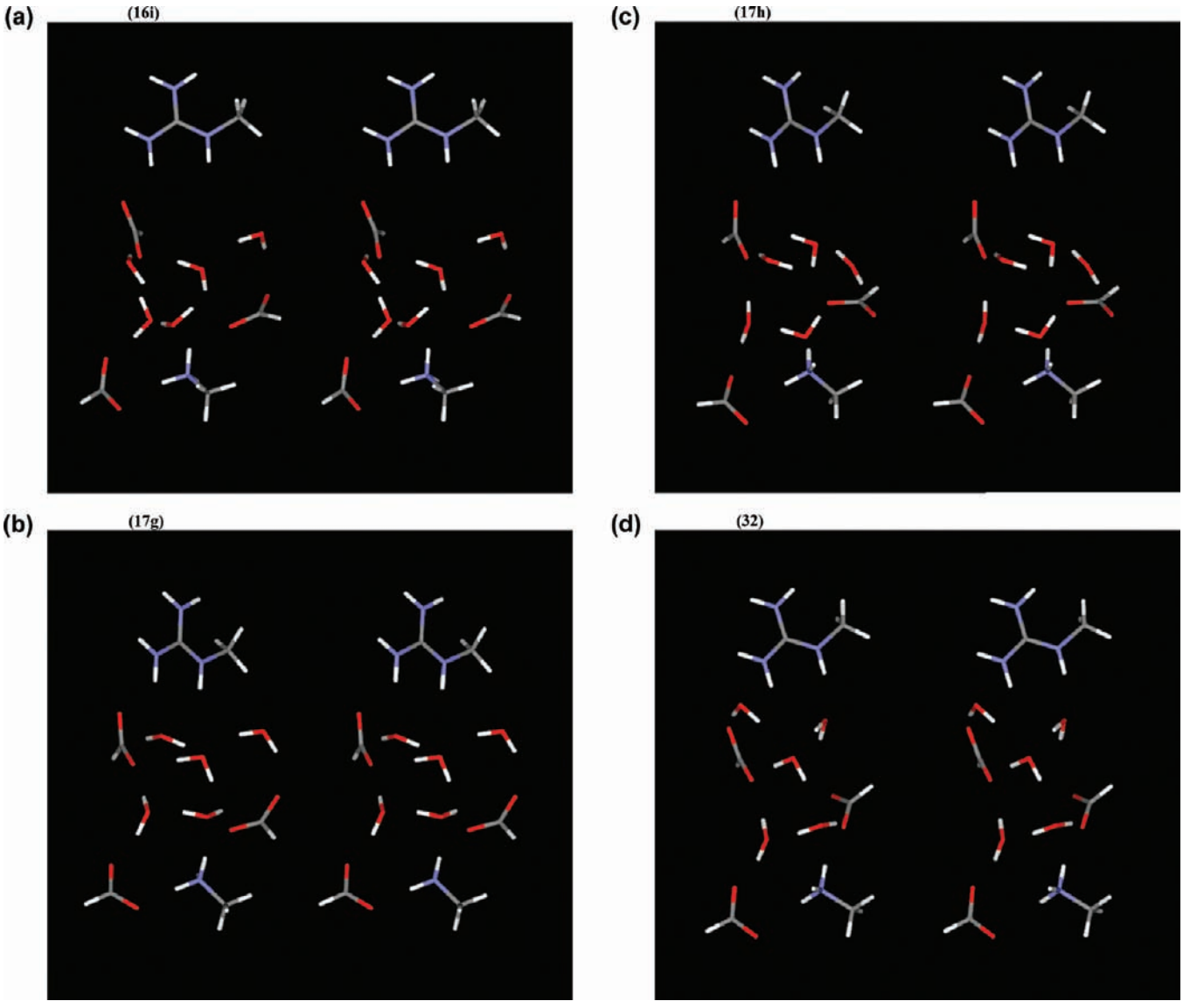

Figure 3. Stereo representations of the $\mathbf{c}_w$ complexes of ligands **16i**, **17g**, **17h**, and **32**.

ref 17. The presence of structural water molecules in protein cavities was demonstrated by low *B* thermal factors in X-ray crystallography,[18] NMR,[19] by MD simulations,[20] and, very recently, by QM/MM computations.[21] In view of their importance, the GOLD docking algorithm was recently modified to account for them in docking studies.[22] Classification rules for structured water molecules in protein binding sites (PBS) were put forth by Barillari et al.[23a] and Amadasi et al.[23b] Such studies were performed with 'classical', nonpolarizable potentials. By contrast, several studies have emphasized the essential role of polarization energy, E_{pol}, in liquid water[24] as well as in water clusters.[25] Therefore E_{pol} is anticipated to be critical as well in highly charged protein cavities, such as in FAK kinase. Such issues have prompted us to investigate: (a) whether structured water molecules can impact the relative energy balances of competing inhibitors and (b) the differential weights of the individual energy contributions of the energy balances.

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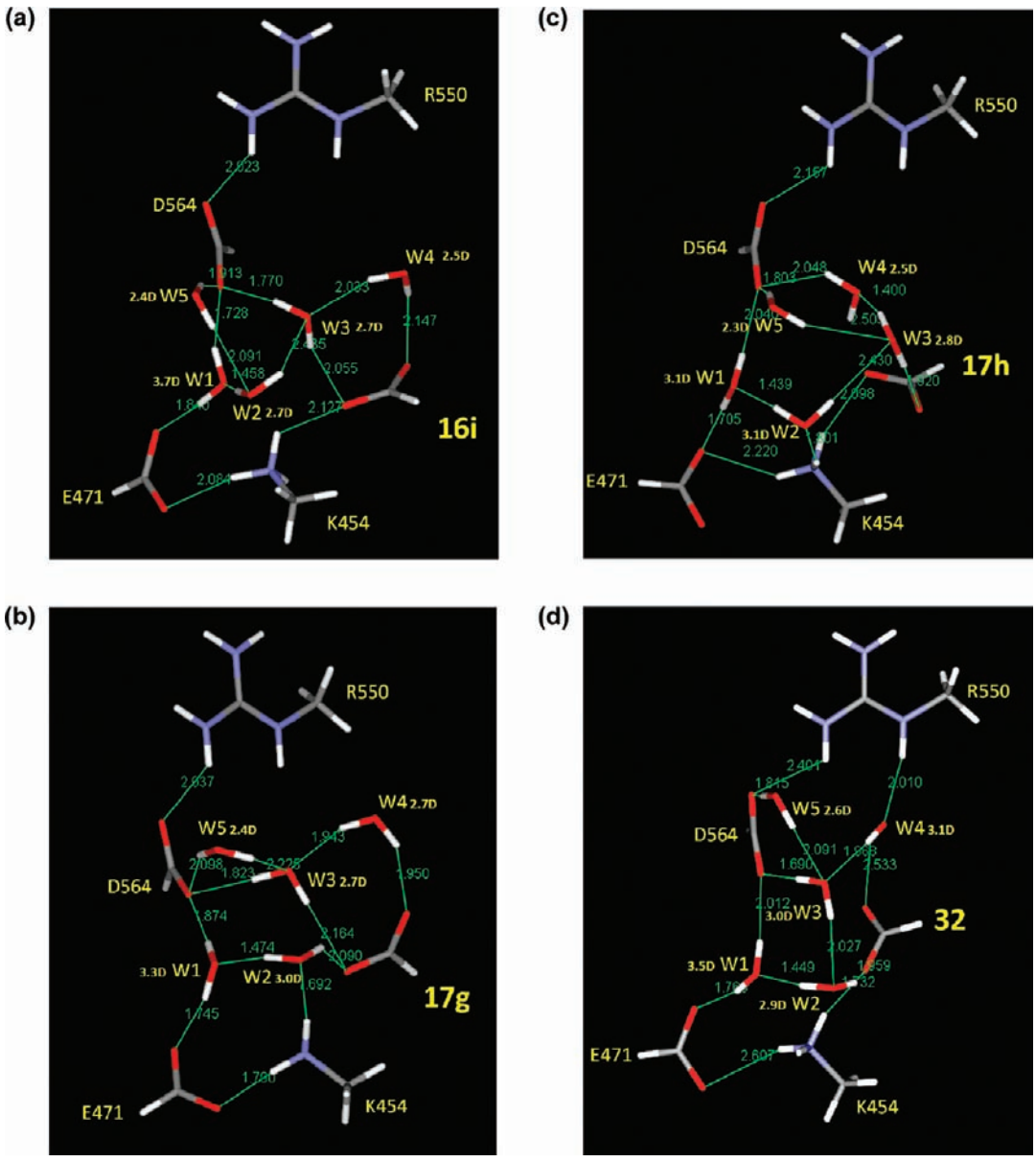

Figure 4. Representations of the interactions involving the inhibitor carboxylate group of **16i**, **17g**, **17h** and **32** with the vicinal FAK ionic amino acids, and the discrete water molecules (denoted as '$\mathbf{c}_w$' in the text). The water dipole moments are shown in rectangles.

A limited number of water molecules were located on complexes limited to the sole ionic ends of the protein side chains in the PBS and the terminal ligand carboxylate, denoted as '**c**'. For each of the five complexes, we considered first a network of five water molecules. SIBFA energy minimizations along with Monte Carlo searches were done on their positions. The resulting energy-minimized structures were in turn exported to the actual FAK complexes and subjected to an additional cycle of EM, first in the absence of continuum solvation and then upon reprocessing in its presence. The outcome of EM resulted in complexes '$\mathbf{a}_w$'. The coordinates of the $\mathbf{a}_w$ complexes are given as Supporting Information S2. The backbone H_α atoms have the coordinates generated by the Insight software (Accelrys Inc., 9685 Scranton Road, San Diego, CA 92121-3752) on the basis of the PDB heavy-atom coordinates.

From each $\mathbf{a}_w$ complex, the ligand and the residues making up the PBS along with the waters were subsequently extracted, yielding complexes $\mathbf{b}_w$, from whence the smallest complexes $\mathbf{c}_w$ are themselves extracted.

In Figure 3, a−d are stereo representations of complexes $\mathbf{c}_w$ of ligands **16i**, **17g**, **17i**, and **32** with five water molecules. Panels a−d of Figure 4 are mono representations along with the relevant intermolecular distances and the values of the water dipole moments.

A short description of the water arrays is as follows. All complexes have water W_1 donating its hydrogens to Glu471 and Asp564, and accepting a proton from W_2. W_1 is a highly conserved water molecule, encountered in such a position in all FAK DFG-in[26] X-ray structures. W_1 is also endowed with the highest dipole moment, in the range 3.1−3.7 D in the different complexes (see Figure 4).

In the complex of **16i**, W_2 donates its second proton to W_3, and it accepts a proton from W_5. W_3 is involved in three additional interactions. It donates a proton to one ligand carboxylate O and its second proton to Asp564, and it accepts

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a proton from W_4. W_4 donates its second proton to the second ligand carboxylate O. W_5 donates its second proton to Asp564. One Asp564 carboxylate O thus accepts three protons from W_1, W_3 and W_5, while in all complexes the second carboxylate O accepts a proton from Arg 550.

In the complexes of **17g**, **17i**, and **32**, W_2 donates its second proton to one ligand carboxylate O, while in the complex of **17h**, and similar to that of **16i**, it interacts with it indirectly through W_3.

In the complex of **17g**, W_3 donates a proton to the W_1-bound Asp564 carboxylate O and the other to a ligand carboxylate O, and it accepts a proton from both W_4 and W_5. W_4 donates its second proton to the second ligand carboxylate O, and W_5 donates its second proton to the W_1-bound Asp564 carboxylate O.

In the complex of **17h**, W_2 donates its second proton to W_3. W_3 accepts a second proton from W_5, and it donates one proton to one ligand carboxylate O, and the other to W_4. Both W_4 and W_5 donate a proton to the W_1-bound Asp564 O.

In the complexes of **17i** and **32**, W_2 donates its second H to one ligand carboxylate O, and accepts a proton from W_3. W_3 donates is second proton to the W_1-bound Asp564 O and accepts a proton from both W_4 and W_5. W_4 donates its second proton to one ligand carboxylate O and accepts a proton from Arg550. W_5 donates its second proton to the Arg550-bound Asp564 O.

3.2.1. Comparative Energy Balances. The energy balances are reported in Table 2a. For consistency, the energy of FAK prior to complexation was energy-minimized in the presence of five structural water molecules and in the presence of the continuum reaction field. Each isolated ligand was itself energy-minimized in the presence of four inner-shell water molecules and of the continuum reaction field: one water bridges the NH nitrogen connecting the two rings and the nitrogen in the six-membered ring ortho to it. The other three waters hydrate the carboxylate group. Additional waters around this group were found, as the outcome of EM, to bind in the second shell (not shown). As for complexes **a**, we report in Supporting Information S3 the separate values of the energy-minimized intermolecular FAK−ligand interaction energies and of the separately optimized intramolecular energies of FAK +5W and of the ligand. For completeness, the balances subtract the stabilization energy of an energy-minimized water tetramer in the presence of the continuum solvation, which includes the tetramer ΔG_{solv} solvation energy. Such a value, common to all ligands, is −48.3 kcal/mol in the context of SIBFA.

Taking the complex of **32** as energy zero, the ordering of the energy balances is in kcal/mol:

32		**17i**		**17h**		**17g**		**16i**
0.0	<	2.3	<	8.0	<	10.4	<	12.3

The ordering in relative energy values $\delta\Delta E$ conforms to the experimental ordering, in marked contrast to the ordering of the **a** complexes.

It closely corresponds to the ordering in relative E_{pol} values:

32		**17i**		**17h**		**16i**		**17g**
0.0	<	2.2	<	14.2	<	28.8	<	35.8

The latter ordering is partly counteracted by the inverse ordering in relative E_1 values:

17g	>	**16i**	>	**17h**	>	**17i**	>	**32**
−27.1		−14.1		−3.6		−1.1		0.0

Table 2. Values (kcal/mol) of the FAK Inhibitor Interaction Energies and Their Contributions, of the Continuum Solvation Energies, and Resulting Energy Balances[a]

Table 2a

	Complexes $\mathbf{a}_{w5}$ with Five Water Molecules				
	16i	**17g**	**17h**	**17i**	**32**
E_{MTP*}	18.1	−34.1	−68.2	−24.5	−21.6
E_{rep}	29.0	68.4	126.0	84.8	82.9
$\boldsymbol{E_1}$	**47.1**	**34.3**	**57.8**	**60.3**	**61.4**
E_{pol}	0.2	7.2	−14.4	−26.4	−28.6
E_{ct}	9.7	5.8	2.2	8.1	8.1
E_{disp}	−41.5	−49.8	−55.2	−53.5	−53.1
E_{tor}	−1.5	1.4	−2.3	−5.0	−5.5
$\boldsymbol{\Delta E_{tot}}$	**14.1**	**−1.1**	**−11.8**	**−16.6**	**−17.6**
ΔG_{solv}	32.4	45.7	54.0	53.1	51.8
$\Delta H_{4w}{}^{b}$	−48.3	−48.3	−48.3	−48.3	−48.3
$\boldsymbol{\Delta E_{fin}}{}^{c}$	**−1.8**	**−3.7**	**−6.1**	**−11.8**	**−14.1**
$\delta\Delta E_{fin}$	12.3	10.3	8.0	2.3	0.0
IC_{50} (μM)	2.820	0.038	0.037	0.035	0.004

Table 2b

	Complexes $\mathbf{a}_{w6}$ with Six Water Molecules				
	16i	**17g**	**17h**	**17i**	**32**
E_{MTP*}	23.7	−10.3	−40.2	−42.1	−38.2
E_{rep}	26.1	67.0	96.9	102.9	100.1
$\boldsymbol{E_1}$	**49.8**	**56.7**	**56.7**	**60.8**	**62.0**
E_{pol}	3.0	7.0	−11.8	−30.9	−33.4
E_{ct}	10.4	4.0	5.3	6.4	6.4
E_{disp}	−43.2	−52.1	−52.2	−56.5	−56.1
E_{tor}	−4.7	−2.4	−6.0	−7.1	−8.0
$\boldsymbol{\Delta E_{tot}}$	**15.2**	**13.2**	**−7.9**	**−27.3**	**−29.1**
ΔG_{solv}	36.2	26.8	49.8	57.5	55.8
$\Delta H_{4w}{}^{b}$	−48.3	−48.3	−48.3	−48.3	−48.3
$\boldsymbol{\Delta E_{fin}}{}^{c}$	**3.1**	**−8.3**	**−6.5**	**−18.2**	**−21.6**
$\delta\Delta E_{fin}$	24.7	13.3	15.1	3.4	0.0
IC_{50} (μM)	2.820	0.038	0.037	0.035	0.004

Table 2c

	Complexes $\mathbf{a}_{w7}$ with Seven Water Molecules			
	16i	**17g**	**17h**	**32**
E_{MTP*}	43.9	6.4	−9.8	−29.1
E_{rep}	12.1	50.8	72.0	91.9
$\boldsymbol{E_1}$	**56.0**	**57.2**	**62.2**	**62.8**
E_{pol}	2.2	1.5	−14.9	−38.2
E_{ct}	12.1	3.9	6.0	8.4
E_{disp}	−39.9	−48.4	−46.8	−55.5
E_{tor}	−4.0	−2.2	−6.5	−7.8
$\boldsymbol{\Delta E_{tot}}$	**26.3**	**12.0**	**0.1**	**−30.4**
ΔG_{solv}	22.0	18.0	31.9	53.6
$\Delta H_{4w}{}^{b}$	−48.3	−48.3	−48.3	−48.3
$\boldsymbol{\Delta E_{fin}}{}^{c}$	**0.0**	**−18.3**	**−16.4**	**−25.1**
$\delta\Delta E_{fin}$	25.1	6.8	8.7	0.0
IC_{50} (μM)	2.820	0.038	0.037	0.004

[a] The experimental inhibitory potencies (μM) are also reported. See text for definitions. [b] Stabilization energy of an energy-minimized water tetramer in the presence of continuum solvation. [c] $\Delta E_{fin} = \Delta E_{tot} + \Delta G_{solv} + \Delta H_{4w}{}^{b}$.

In order to complete the solvation of the ligand carboxylate group, a sixth and a seventh water molecule were next introduced in succession and energy-minimized. This was done as follows. Using computer graphics, W_6 was first introduced in complexes $\mathbf{a}_w$ in two possible modes, upon donating a proton to one or to the other ligand carboxylate oxygen. It was energy-minimized in three steps: (a) EM was done first on the sole six

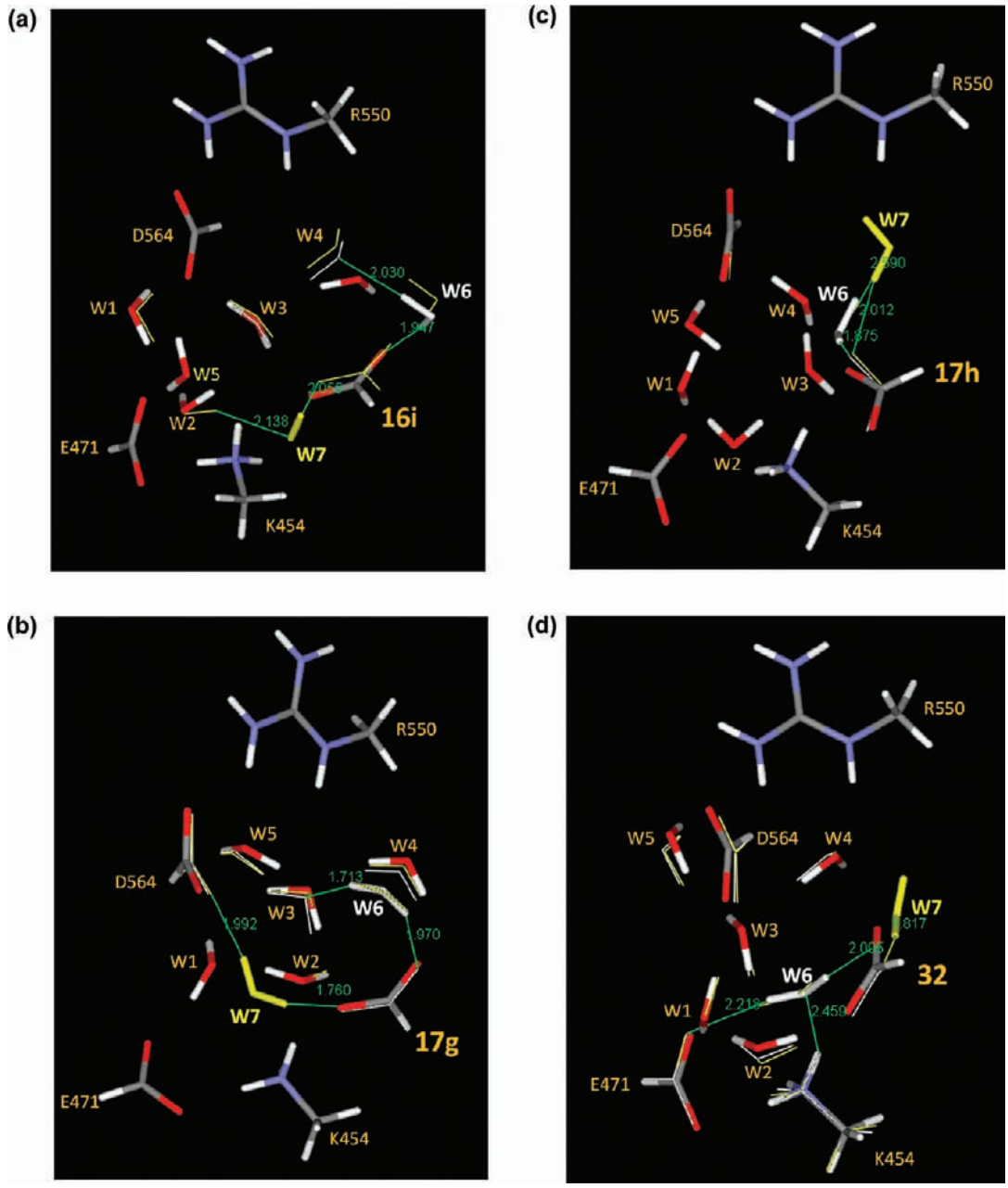

Figure 5. Superimpositions of the $n_w = 6$ and $n_w = 7$ complexes over the $n_w = 5$ $\mathbf{c_w}$ complexes of ligands **16i**, **17g**, **17h**, and **32**.

variables defining its position of approach, with a 2.0 Å distance restraint between one W_6 H atom and the bound O; (b) EM was resumed by removing the constraint; (c) the more stably bound complex of W_6, whether to O_1 or to O_2, was retained and submitted to the same EM protocol as with five molecules. W_7 was subsequently introduced and its position energy-minimized following the same succession of steps, its approach being enforced in step (a) toward the O_1/O_2 atom which is the farthest from W_6. The coordinates of the $\mathbf{a_w}$ complexes with six and seven structural waters are given as Supporting Information, S4 and S5, respectively. For $n_w = 7$, the computations were not done in the case of **17i**, on account of its close overlap with **32** observed with $n_w = 5$ and 6. For ligands **16i**, **17g**, **17h**, and **32**, superimpositions of the $\mathbf{c_w}$ complexes for $n_w = 6$ and $n_w = 7$ with the corresponding $n_w = 5$ ones are shown in Figure 5a−d. They are represented in thin lines and in white ($n_w = 6$) and yellow ($n_w = 7$) colors, W_6 and W_7 being highlighted using a stick rendering.

In the **16i** complex, W_6 donates its second proton to W_4, while W_7 donates a proton to the other ligand O as well as to W_2. In the **17g** complex, W_6 donates its second proton to W_3 and accepts a proton from W_2. W_7 bridges by its two protons one ligand O and Asp564, similar to W_3. In the **17h** complex, W_6 bridges one ligand O and the main chain of Asp564, from which it accepts the NH proton, while W_7 accepts the second W_6 proton. In the **32** complex, W_6 bridges one ligand O and Glu 471 and Lys 454. W_7 bridges the other ligand O and, as in the **17g** complex, the main chain NH of Asp564.

Very importantly, we observe that the overall ranking of the complexes of **16i**, **17g**, **17h**, **17i**, and **32** is preserved upon going from five to seven water molecules. While the values of δ increase for $n_w = 6$, they decrease concerning **17g** and **17h** for

Table 3. SIBFA and QC(RVS) Intermolecular Interaction Energies (kcal/mol) in Model Complexes **c** and $\mathbf{c_w}$

	E_{MTP^*}	E_C	E_{rep}	E_{exch}	E_{pol}	E_{pol}(KM)	E_{ct}(SIBFA)	E_{ct}(RVS)	ΔE(SIBFA)	ΔE(RVS)	E_{disp}	E_{corr}
						Complexes **c**						
16i	−206.0	−203.6	19.4	18.5	−21.5	−23.3	−2.6	−2.6	−210.8	−210.0	−5.9	−12.4
32	−194.0	−190.8	24.0	22.5	−24.6	−26.7	−2.5	−3.0	−197.0	−196.9	−6.7	−13.5
δ(**32**−**16i**)	**12.0**	**12.8**	**4.6**	**4.0**	−3.1	−3.4	0.1	−0.4	**13.8**	**13.1**	−1.2	−0.9
						Complexes $\mathbf{c_w}$						
16i	−348.1	−344.2	142.1	138.0	−48.5	−51.3	−18.4	−16.6	−272.9	−272.8	−40.0	−44.0
32	−352.4	−350.2	152.6	148.7	−63.1	−66.0	−21.0	−19.6	−283.9	−284.3	−42.4	−48.9
δ(**32**−**16i**)	**−4.3**	**−6.0**	10.5	10.5	**−14.6**	**−14.7**	−2.6	−3.0	**−11.0**	**−11.5**	**−2.4**	**−4.8**

$n_w = 7$ recovering values comparable to those of the $n_w = 5$ complexes. Nano- and micromolar ligands **32** and **16i** have the most and the least favorable $\delta\Delta E_{fin}$ values, respectively, while submicromolar ligands **17g** and **17h** have intermediate values. The relative values of E_1 have a tendency to equalize in the series, while E_{pol} retains its discriminatory role in the δ trends. E_{disp} is the contribution with the largest magnitude in absolute values but is not discriminatory. The larger affinity of **32** than of **17i** is due to the fact that with the discrete water molecules, the binding to FAK of the carboxylate is favored by the near coplanarity of the benzene ring of **17i** and of the pyridine ring of **32** with pyrrolopyrimidine. The corresponding torsion angles have amplitudes ≤30°. Coplanarity is stabilized by resonance and is more advantageous, upon FAK binding, for **32** than **17i**, since one H atom of the pentacyclic ring of pyrrolopyrimidine faces the electron-rich pyridine of **32** rather than the corresponding CH group of **17i**. With $n_w = 5-7$, but not with $n_w = 0$, some correlation could be observed between the E_{tor} trends and those of E_{pol}. Overall the ordering of the energy balances for $n_w = 5$, 6, and 7 is qualitatively consistent with the ordering of experimental inhibitory potencies of **16i**−**32**. However, (a) more detailed explorations of the energy surface and (b) inclusion of rotation/vibration entropy effects are mandatory before the present computational procedure could be used in a broader context toward more quantitative agreement with experimentation. Entropy effects are only embodied in the present treatment in the continuum solvation contribution ΔG_{solv}. Additional inclusion of rotation/vibration entropy effects could be anticipated to reduce the large E_{tot} differences which favor **32** and **17i**, since E_{tot} is of exclusive enthalpic nature. Issue (a) will be addressed upon integrating procedures to approach the global minimum, such as metadynamics.[27] Issue (b) could be addressed upon integrating procedures to sample in a statistical way the vicinity of the energy minima, as in the context of the 'mining minima' procedure.[28] Addressing such issues can become computer-intensive upon dealing with a large number of degrees of freedom, particularly in the framework of polarizable molecular mechanics. Work is in progress along these lines and will be reported in due time.

The discriminatory role of E_{pol} was confirmed by comparisons by parallel RVS/SIBFA computations on complexes **c** and $\mathbf{c_w}$, which were extracted in the course of EM bearing on complexes **a** and $\mathbf{a_w}$ with five structural water molecules. Such comparisons are reported in Table 3 concerning **16i** and **32**, which are respectively the least and the most active ligands in the series. In complexes **c**, both ΔE(SIBFA) and ΔE(RVS) strongly (>13 kcal/mol) disfavor nanomolar inhibitor **32** with respect to micromolar **16i**. This is due to the first-order contribution E_1, notably to E_C/E_{MTP^*}. A remarkable reversal of the trends occurs with complexes $\mathbf{c_w}$. The presence of the discrete waters now results into **32** giving rise to the most stable complex.

Such a Preference Is Due to the Polarization Energy Contribution. Table 3 shows very close numerical agreements between the individual SIBFA energy contributions and their RVS counterparts. This attests to the need for a proper separability of PMM potentials, without which it could be illusory to attempt to reproduce, let alone predict, the values of QC interaction energies.[7] The values of E_{disp}(SIBFA) and E_{corr}(MP2) are seen to also favor **32**, but by more modest amounts than E_{pol}. The larger magnitudes of E_{corr}(MP2) than those of E_{disp}(SIBFA) stem from large BSSE effects at the correlated level with the CEP 4-31G(2d) basis set.[29]

The impact of polarization translates in the very large magnitudes of the water dipole moments (Figure 4). Thus in the complex of **32**, three water molecules have μ values of ≥3.0 D, while the corresponding μ value amounts to approximately 2.4 D in liquid water and 2.7 D in ice-like structures.[25b,30] The 'iciest' water, denoted W_1 ($\mu = 3.5$ D), donates one proton to Glu471 and the other to Asp564. The position it occupies is found to be highly conserved in *all* FAK DFG-in high-resolution X-ray structures reported to date.[26]

16i and **32** differ by the manner the carboxylate group is linked to their common scaffold, namely, directly connected in *ortho* to the benzene ring in **16i**, and connected through a two-methylene chain meta to the pyridine ring in **32**. The present results illustrate the impact of these differing linkages on the water network that extends from the carboxylate ligand to Arg550.

Previous studies in the context of SIBFA have highlighted the essential role of polarization in stabilizing multiply H-bonded complexes,[25b] polycoordinated mono- and binuclear Zn(II) complexes.[31] Recently, a free energy simulation using the polarizable AMOEBA MM procedure quantified the role of polarization in the preferential binding to trypsin of the benzamidine ligand compared to diazamidine.[32] Along with ref 32 the results of the present study constitute to our knowledge

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the very first examples in which E_{pol} appears as the critical energy contribution in the ordering of energy balances of competing inhibitors. In the present study, its discriminatory role arises solely owing to the networks of 'icy' water molecules mediating the interactions between the ligand and the charged residues of the protein recognition site. Either one, or a small network of structured mediating water molecules were shown to be essential in another context, namely electron-transfer between a donor and an acceptor site in Cu-metalloproteins.[3] The ability of icy molecules to tip the relative energy balances of competing inhibitors was, furthermore, also recently observed in the case of a Zn-metalloenzyme, phosphomannoisomerase (manuscript in preparation).

Conclusions

The necessity of explicit polarization in molecular recognition is acknowledged in refs 7 and 33 and references therein. However, quantitative evaluations of its weight on the binding energies of competing ligands for a given target remain scarce. In addition, while 'discrete' water molecules are considered essential partners of the ligand−macromolecule complex,[4,5,21−23] there is a need to quantify their impact on the comparative energy balances.[34] The present study, bearing on the complexes of five pyrrolopyrimidine inhibitors to FAK kinase, showed that both factors are interwoven. These findings could have important implications for free energy calculations, and underline the critical need for an explicit polarization contribution. In addition, the present methodology could be applied to refine the positions of structural water molecules identified by X-ray crystallography.

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Supporting Information Available: Complete refs 2 and 12. **S1**: Representation of the PDB structure of the complex of parent pyrrolopyrimidine ligand with FAK; **S2**: coordinates of FAK and of its complexes with ligands **16i**, **17g**, **17h**, **17i** and **32** and with five structural water molecules; **S3**: complexes **a** and $\mathbf{a}_w$, $n_w = 5-7$ structural waters; details of the stabilization energies, given for each contribution, the intermolecular FAK−ligand interaction energies, and the intramolecular interaction energies of isolated FAK and ligand; the latter two energies were minimized with n_w and with four structural waters, respectively, and in the presence of the continuum reaction field; **S4**: coordinates of FAK and of its complexes with ligands **16i**, **17g**, **17h**, **17i** and **32** and with six structural water molecules; **S5**: coordinates of FAK and of its complexes with ligands **16i**, **17g**, **17h**, and **32** and with seven structural water molecules.This material is available free of charge via the Internet at http://pubs.acs.org.

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