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The molecular basis of urokinase inhibition: from the nonempirical analysis of intermolecular interactions to the prediction of binding affinity

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Abstract Urokinase-type plasminogen activator (uPA) is a trypsin-like serine protease that plays a crucial role in angiogenesis process. In addition to its physiological role in healthy organisms, angiogenesis is extremely important in cancer growth and metastasis, resulting in numerous attempts to understand its control and to develop new approaches to anticancer therapy. The α-aminoalkylphosphonate diphenyl esters are well known as highly efficient serine protease inhibitors. However, their mode of binding has not been verified experimentally in details. For a group of average and potent phosphonic inhibitors of urokinase, flexible docking calculations were performed to gain an insight into the active site interactions responsible for observed enzyme inhibition. The docking results are consistent with the previously suggested mode of inhibitors binding. Subsequently, rigorous ab initio study of binding energy was carried out, followed by its decomposition according to the variation–perturbation procedure to reveal stabilization energy constituents with clear physical meaning. Availability of the experimental inhibitory activities and comparison with theoretical binding energy allows for the validation of theoretical models of inhibition, as well as estimation of the possible potential for binding affinity prediction. Since the docking results accompanied by molecular mechanics optimization suggested that several crucial active site contacts were too short, the optimal distances corresponding to the minimum ab initio interaction energy were also evaluated. Despite the deficiencies of force field-optimized enzyme-inhibitor structures, satisfactory agreement with experimental inhibitory activity was obtained for the electrostatic interaction energy, suggesting its possible application in the binding affinity prediction.

Introduction

The growth and spread of cancer cells depends significantly on the extracellular matrix (ECM) quality. Destruction of this unique protein scaffold allows cancer cells not only to grow but also to move, to leave the site of their origin and spread throughout the body invading distant locations. The crucial step of this process, called metastasis, is the proteolysis of extracellular matrix proteins. Several proteases, such as matrix metalloproteinases (MMPs) and serine proteases (e.g., plasmin, urokinase) are involved directly or indirectly in ECM disintegration [1, 2].

The major role in degradation of structural EMC proteins is played by urokinase-type plasminogen activator (uPA)—a trypsin-like serine protease which activates plasminogen, a broad spectrum executioner protease. A form of active plasminogen, plasmin, directly hydrolyzes structural ECM proteins like fibrin, fibronectin or laminin [3, 4]. More importantly, plasmin is also involved in partial activation of MMP-3 which, in turn, activates several MMPs (e.g., MMP-1, MMP-7, MMP-8, MMP-9) that degrade structural proteins [5–8]. It also modifies pro-urokinase to the form which gives mutual activation of both proteases resulting in the strong proteolytic force focused on a cancer cell membrane surface.

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Angiogenesis process is regulated by a mechanism similar to metastasis that involves the uPA/plasmin system activity. High concentration of active urokinase along with its receptor (CD87) has been found at the leading edge of growing endothelial cells which form the blood vessels. As the growth of the blood vessel network is important for health body development, it is unwanted during tumor growth and progression. If the cancer angiogenesis could be prevented when the tumor is about 3 mm^3, it would lead to its death because of the high level of toxic metabolites and the lack of oxygen and nutrition [9].

Due to its importance in tumor growth and development, uPA is an attractive molecular target in anticancer therapy. The molecules able to inhibit the proteolytic activity of urokinase constitute promising anticancer agents. Among several groups of low molecular weight, uPA inhibitors (for recent review see [10]), our attention has been paid to the phosphonic analogues of amino acids [11]. α-Amino-alkylphosphonate diphenyl esters are known as slow binding, essentially irreversible inhibitors of serine proteases [12]. They are highly specific toward target protease and stable in physiological conditions. In contrast to other synthetic serine proteases inhibitors, aromatic esters of α-aminophosphonates do not react with cysteine proteases and proteasome threonine proteases. Although several phosphonic inhibitors toward different serine proteases have been synthesized (e.g., toward chymase, trypsin or elastase [13–15]), only a few papers describe their application as uPA inhibitors [16–18].

Despite the general knowledge of a mode of α-amino-alkylphosphonate diphenyl esters binding to serine proteases [13] (derived from available crystallographic data [19]), the actual way this class of compounds interacts with urokinase has not yet been verified experimentally. Although S1 pocket residues that are crucial for binding have been identified [20], their participation in protein–ligand interactions is also not clear. To clarify these issues and to gain an insight into the source of inhibitory activity and selectivity of several known [17, 21] and one newly synthesized [22] uPA inhibitors, docking simulations were performed, followed by non-empirical analysis of the strength and physical nature of interactions taking place in uPA active site. It is worth mentioning that theoretical results presented herewith were also compared to experimental inhibitory activities obtained consistently under identical conditions [22]. Apparently, experimental measurements reveal Gibbs free energy of binding that, in turn, results from both enthalpic and entropic contributions, as well as desolvation effects which would require the use of elaborate simulation techniques [23]. Whenever the set of similar ligands is considered, their comparable characteristics in terms of desolvation, protein (ligand) reorganization energy and conformational entropy loss is frequently
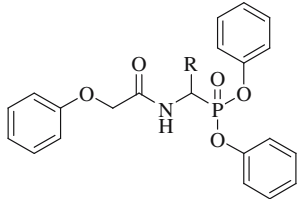
constant across the series and, thus, it tends to be negligible as long as relative values are taken into account. Accordingly, computationally expensive evaluation of binding free energy requiring use of empirical force fields can be replaced by the rigorous nonempirical quantum chemical calculations of a binding energy being the most pronounced contribution to binding affinity. The overall approach utilized here has already been successfully applied to two other classes of enzymes (i.e., leucine aminopeptidase and phenylalanine ammonia-lyase [24]) showing its potential for binding affinity prediction.
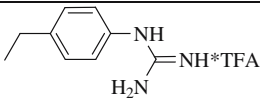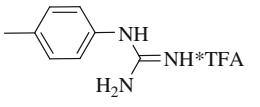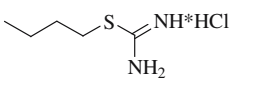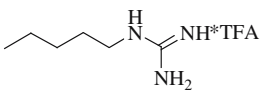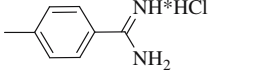
Materials and methods

Chemical synthesis of five α-aminophosphonates considered here and examination of their inhibitory activity towards human uPA (Table 1) was presented elsewhere [22]. Obtained experimental data apply to the racemic mixtures of these inhibitors. Noticeably, despite relatively minor changes in the structures of analyzed compounds, experimental IC$_{50}$ values span the range of five orders of magnitude (Table 1).

The structures of five inhibitors in R-enantiomeric form were initially optimized at the HF/6-31G(d) level of theory using Gaussian 03 program [25]. Crystal structure of urokinase was obtained from Protein Data Bank (accession code 1C5Y). The missing hydrogen atoms were built and further optimized using Tripos force field [26]. Docking calculations were performed with FlexX suite as implemented in Sybyl package [27]. During the docking procedure, active center was defined so as to include all residues within a radius of 20 Å from Ser195 residue. The receptor structure was treated as rigid, while inhibitor molecules were allowed to adjust their conformation to optimize their fit to the uPA active site. The resulting noncovalent uPA-inhibitor complexes were then optimized in conjunction with enzyme using Tripos force field. During optimization, both the active center of enzyme (defined as for the docking step) and inhibitor molecules were flexible. Criterion of convergence was energy gradient not greater than 0.05 kcal mol^{-1}/Å. The RMS deviation of final optimized complexes was found to be in the range of 0.51–0.64 Å (as calculated with respect to crystal structure and all non-hydrogen atoms of residues considered further in the calculation of binding energy; Fig. 1).

Protein–ligand complexes from the docking step were subjected to ab initio analysis of intermolecular interactions using a variation–perturbation approach [28] that allows the binding energy to be partitioned into physically relevant terms determining the actual nature of interactions. Following the variation–perturbation procedure, total stabilization energy at the second order Moller–Plesset level of theory

Table 1 The structures and inhibitory activities towards uPA of α-aminoalkylphosphonate diphenyl esters [22]

Compound		R	IC$_{50}$ [μM]
1	Cbz-(4-GuPhe)^P(OPh)$_2$		0.0065
2	Cbz-(4-GuPhg)^P(OPh)$_2$		0.532
3	Cbz-SArg^P(OPh)$_2$		0.76
4	Cbz-hArg^P(OPh)$_2$		0.78
5	Cbz-(4-AmPhg)^P(OPh)$_2$		160

consists of the electrostatic ($E_{EL}^{(1)}$), exchange ($E_{EX}^{(1)}$), delocalization ($E_{DEL}^{(R)}$) and correlation ($E_{CORR}^{(R)}$) components:

$$E_{MP2} = E_{EL}^{(1)} + E_{EX}^{(1)} + E_{DEL}^{(R)} + E_{CORR}^{(R)}$$

Well-established series of theoretical models described by gradually increasing accuracy and computational expense can be formed, giving the possibility of a reasonable development and validation of approximate models:

$$E_{EL}^{(1)} < E^{(1)} < E_{SCF} < E_{MP2}$$

In the above equation, $E^{(1)}$ denotes the first-order Heitler–London term calculated as $E^{(1)} = E_{EL}^{(1)} + E_{EX}^{(1)}$. All

Fig. 1 The components of uPA active site models A (large) and B (minimal) employed in non-empirical analysis of interaction energy. In ball-and-stick representation is an inhibitor molecule. Model A consists of all residues shown in a scheme; model B is composed of residues distinguished by green color

interaction energy terms mentioned here are evaluated using dimer-centered basis set to account for basis set superposition error (BSSE) by means of counterpoise correction scheme [29]. Binding energy was calculated with 6-31G(d) basis set using a modified version of the GAMESS-US program [30].

Urokinase binding pocket consisted of nine amino acid residues: Asp189, Ser190, Gln192, Val213, Gly216, Gly219, Cys220, Ala221, and Pro225. To reduce the size of a system, selected molecular fragments most distant from an inhibitor molecule were neglected (see Fig. 1 for details). In particular, the main chain amino group of Asp189 was replaced by a hydrogen atom. Since Pro225 residue interacts with an inhibitor mainly via its main chain atoms, only the latter were considered along with a main chain carbonyl group of the preceding residue (i.e., Lys224) as well as an amino group of the succeeding Gly226 residue. All broken bonds were saturated with hydrogen atoms.

The inhibitor structures were also simplified to include only the variable part of α-aminophosphonates considered in this study (i.e., the substituents listed in Table 1). All inhibitor molecules carried a positive charge (+1), whereas uPA residues were modeled as neutral species (except of negatively charged aspartate Asp189). Stabilization energy was then evaluated in a pairwise manner with total binding energy of particular inhibitors being the sum of two-body interactions.
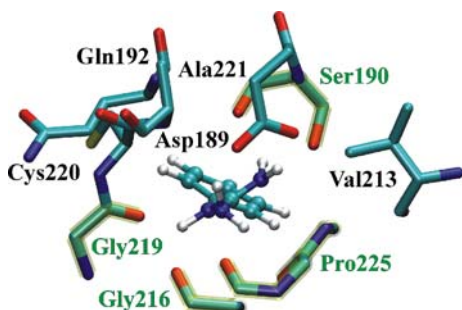
Results and discussion

Docking of the inhibitor molecules

Structures of one novel [22] and four already known [17, 21] urokinase phosphonic inhibitors (Table 1) were docked into the uPA active site using FlexX suite [27]. Selected models of protein–ligand complexes are presented in Fig. 2. Figure 3 shows the superimposition of inhibitor structures along with S1 pocket residues of uPA. The overall positioning of inhibitors is essentially similar. Slightly distorted orientation of phenyl rings from benzyloxycarbonyl group of Cbz-hArg^P(OPh)$_2$ can be explained by the greatest size and flexibility of a moiety occupying S1 pocket (Fig. 3).

The molecular basis of urokinase inhibition by α-amino-alkylphosphonate diphenyl esters consists in formation of a covalent bond between phosphorus and Ser195 hydroxyl oxygen atoms. Subsequently, the resulting transtition state with pentacoordinated phosphorus atom is decomposed by departure of phenyl rings (as in the case of other serine proteases [13, 19]). To avoid any bias in our docking simulation we considered only noncovalent uPA-inhibitor complexes. Noticeably, the final mode of protein–ligand binding strongly supports the possibility of a covalent modification of urokinase that follows the initial formation of an enzyme-inhibitor complex. In particular, the phosphorus–oxygen distance (corresponding to the reaction coordinate for a nucleophilic attack of serine hydroxyl on the phosphorus center) differs within the range of 3.4–3.8 Å. One of the phosphonyl oxygen atoms is close to apical position in
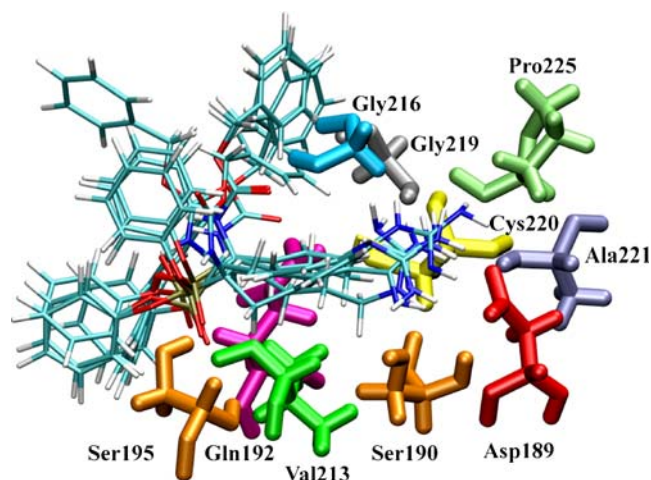

Fig. 3 The comparison of an arrangement of inhibitors within uPA active. Amino acid residues form S1 pocket that binds the variable part of inhibitor molecules

pentavalent phosphorus forming trigonal bipyramidal transition state by which phosphonylation reaction occurs [13].

Phosphonate oxygen atom is directed toward oxyanion hole formed by main chain amide groups of Gly193, Asp194, Ser195. In case of known trypsin structures and theoretical models of uPA-phosphonate inhibitors, these residues interact with an inhibitor molecule via hydrogen bonds [18, 19]. Although the respective distances obtained for docked complexes are too large to allow for such an interaction, it can be justified by a noncovalent character of analyzed models resulting in the greater separation between phosphorus atom and Ser195 residue. Nonetheless, hydrogen bonds can be observed between amidino and guanidino moieties occupying

Fig. 2 The α-aminoalkyl-phosphonate diphenyl esters docked in the active site of urokinase. **a** Cbz-(4-GuPhe)^P(OPh)$_2$; **b** Cbz-hArg^P(OPh)$_2$; **c** Cbz-(4-AmPhg)^P(OPh)$_2$; **d** Cbz-SArg^P(OPh)$_2$
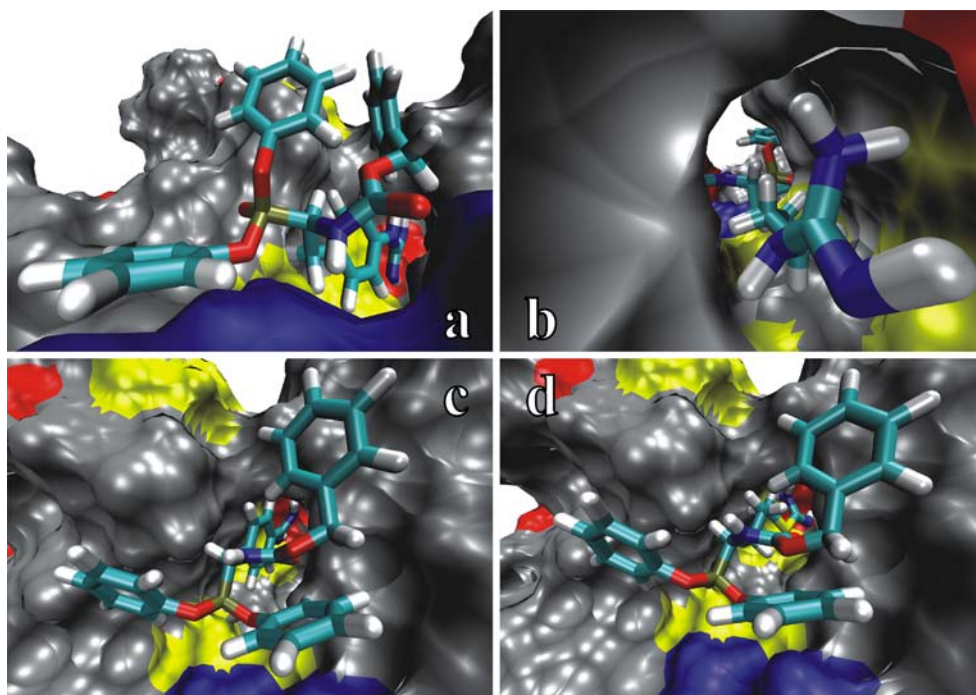
S1 pocket and oxygen atoms from Ser190 hydroxyl as well as Gly216 and Gly219 carbonyl groups.

The amidino and guanidino nitrogens of inhibitors form salt bridges with oppositely charged carboxyl group of Asp189. Compared to the remaining residues located within S1 pocket, Asp189 is the only charged residue which explains strong preference for observed mode of P1 moiety binding. The presence of aspartate in S1 pocket is typical for trypsin-like serine proteases.

The docking results are consistent with the previously suggested mode of uPA inhibitors binding [13]. Moreover, available X-ray structures of uPA in complex with other classes of inhibitors support this conclusion as long as S1 pocket interactions are considered [31, 32]. This agreement could also be confirmed by similarity of an orientation of the docked complex of Cbz-(4-AmPhg)^P(OPh)$_2$ and the location of the same inhibitor in trypsin active site (as obtained from X-ray structure 1MAY, Fig. 4).

Toward the prediction of binding affinity

Interaction energy analysis was performed for uPA-inhibitor complexes derived from docking calculations. Due to the significant size of an active site model composed of nine residues (Fig. 1), binding energy was evaluated as a sum of pairwise interactions between respective inhibitor molecules and amino acid residues. Interaction energy was further partitioned allowing for the magnitude of particular binding energy terms to be examined. In addition to the study of a physical nature of total observed interaction, current analysis also aimed at the determination of uPA residues responsible for inhibitor specificity. Accordingly, the smaller active site model (referred to as B in contrast to full 9-residues model denoted by A) was derived by stepwise neglect of five residues (see Fig. 1) with minor
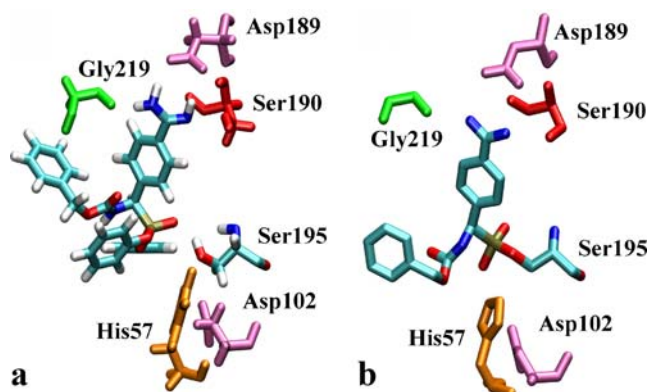

Fig. 4 The arrangement of **a** uPA and **b** trypsin active site (Ser195, His57, Asp102) and S1 pocket residues (Asp189, Ser190, Gly219) around the inhibitor (Cbz-(4-AmPhg)^P(OPh)$_2$). Trypsin structure with Cbz-(4-AmPhg)^P(OPh)$_2$ inhibitor is taken from Protein Data Bank (1MAY). Since it was solved by X-ray method, hydrogen atoms are not present in the PDB structure

contribution to overall ligand specificity. The results for active site models A and B are plotted in Fig. 5.

Remarkable correlation with experiment [22] was found for the first-order electrostatic energy (correlation coefficient for the relationship with experimental inhibitory activity is equal to 0.94; Table 2). The subsequent stabilization energy terms exhibit rather poor agreement with experimental data probably due to artificially shortened distances in force field-optimized complexes (see 'The quality of docking results'). However, interaction energy at MP2 level of theory is still in qualitative agreement with experiment suggesting that the overall model of inhibitors binding is reasonable.

Since several uPA residues were found to interact with all inhibitors to a similar extent, a limited-size model of uPA active site (model B) was constructed that does not include Asp189, Gln192, Val213, Cys220, and Ala221 residues. The quality of such an approximation was validated in terms of its ability to retain the initial correlation with experimental inhibitory activity (Fig. 5; Table 2). Noticeably, first-order electrostatic energy is able to reproduce experimental binding affinity with correlation coefficient 0.99 (Table 2). As regards the stabilization energy described by higher levels of theory, a similar decrease in respective correlation coefficients can be observed. Apparently, the cancellation of higher corrections to binding energy results in a good performance of electrostatic term. Exchange and delocalization components of stabilization energy are much more sensitive to the actual distance between interacting species and, thus, any errors in the latter are much more pronounced.

Due to formation of ionic pair with positively charged inhibitor moieties, the strongest interaction was observed with the Asp189 residue. However, the strength of binding is similar in all these cases and Asp189 exclusion from model B does not affect the agreement with experimental data. Therefore, Asp189 residue is responsible rather for the overall positioning and strong binding of inhibitor molecules, while it appears to contribute little, if any, to substrate specificity.

The quality of docking results

The loss of correlation with experimental data for the first-order Heitler–London energy suggested the improper (i.e., too short) distances between the interacting monomers. Non-empirical analysis of interaction energy terms along the selected contacts revealed that the minimum energy distances from force field-optimized complexes are about 0.5 Å shorter than the corresponding ab initio values (see sample results in Fig. 6). As a result of artificially high exchange repulsion term, the first order interaction energy is overestimated and does not correlate with the experimentally determined inhibitory activity. It can be seen from Fig. 6 that exchange component of binding energy is the most distance-dependent
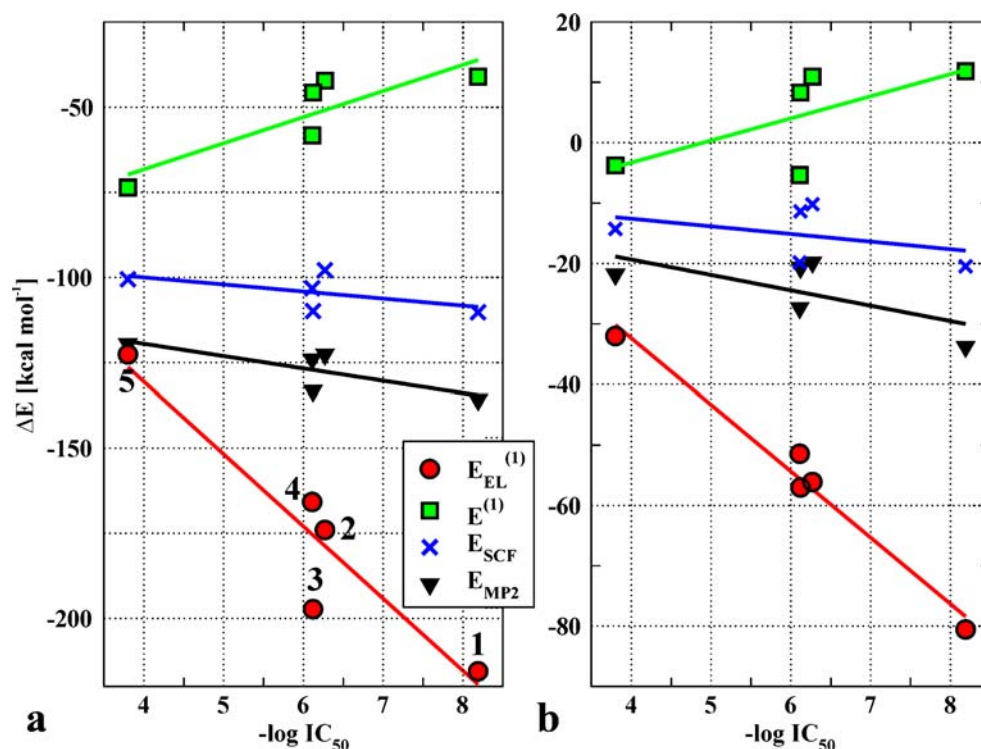
term. While the difference in MP2 interaction energies for
force field and ab initio optimized structures is equal to
4.2 kcal mol^{-1}, the corresponding value for exchange energy
is 15.6 kcal. This observation can be employed to explain
the sudden drop in correlation with experiment when going
from electrostatic to Heitler–London interaction energy.

Conclusions

The following conclusions can be derived from the results
described throughout this contribution:

– The complexes of urokinase-inhibitors obtained from
docking calculations are in agreement with generally
accepted mode of binding of serine protease inhibitors
as well as experimental data available for both uPA and
other serine proteases, such as trypsin.

Table 2 Correlation coefficients of the relationship between experi-
mental inhibitory activity [22] (-log IC$_{50}$) and the interaction energy at
various levels of theory for a full (A) and limited-size (B) active site
models

Method	uPA active site model	
	A	B
E_{MP2}	0.79	0.68
E_{SCF}	0.58	0.42
$E^{(1)}$	−0.87	−0.69
$E_{EL}^{(1)}$	0.94	0.99

– Ab initio results indicate that some force-field opti-
mized enzyme-inhibitor structures contained shortened
intermolecular contacts due to the deficiencies of
empirical parameterization. This resulted in poor
correlation of the E_{SCF} and $E^{(1)}$ levels of theory
containing exaggerated short range exchange and
delocalization components. As these terms are of
opposite sign, they tend to cancel each other to a
significant degree explaining remarkably reasonable
correlation of electrostatic term $E_{EL}^{(1)}$ with experimental
results. This is consistent with conclusions obtained for
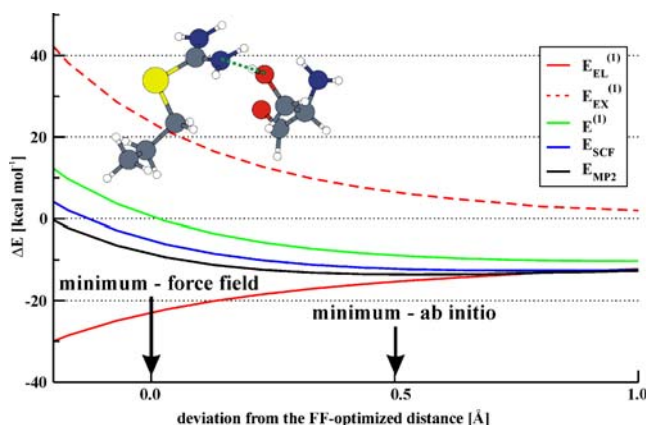other enzymatic systems [24].

Fig. 6 Binding energy terms for the Cbz-SArg^P(OPh)$_2$ inhibitor and
Ser190 interaction. The distance between monomers was sampled by
0.1 Å in both directions. The zero value corresponds to the distance
found in a final docked complex (i.e., force field-optimized structure).
The optimal force field and ab initio separations are denoted by
arrows

– Considering electrostatic interaction energy appears to be a reasonable approach for analysis of complexes with underestimated monomer separation.

– The electrostatic stabilization energy of several uPA inhibitors coincides with their inhibitory activity indicating negligible solvation and entropic contributions to the binding affinity.

– The minimal uPA active site model composed of the four amino acid residues (i.e., Ser190, Gly216, Gly219, Pro225) is sufficient to reproduce the results obtained for a starting model encompassing nine uPA residues.

References

1. Liotta LA, Kohn EC (2001) Nature 411:375–379
2. Sheetz MP, Felsenfeld DP, Galbraith CG (1998) Trends Cell Biol 8:51–54
3. Danø K, Andreasen PA, Grøndhal-Hansen J, Kristensen P, Nielsen LS, Skriver L (1985) Adv Cancer Res 44:139–266
4. Andreasen PA, Kjøller L, Christensen L, Duffy MJ (1997) Int J Cancer 72:1–22
5. Birkedal-Hansen H, Moore WG, Bodden MK, Windsor LJ, Birkedal-Hansen B, DeCarlo A, Engler JA (1993) Crit Rev Oral Biol Med 4:197–250
6. Murphy G, Gavrilovic J (1999) Curr Opin Cell Biol 11:614–621
7. Nagase H, Suzuki K, Enghild JJ, Salvesen G (1991) Biomed Biochim Acta 50:749–754
8. Ramos-DeSimone N, Hahn-Dantona E, Sipley J, Nagase H, French DL, Quigley JP (1999) J Biol Chem 274:13066–13076
9. Folkman J (1995) Nat Med 1:27–31
10. Rockway TW, Giranda VL (2003) Curr Pharm Des 9:1483–1498
11. Oleksyszyn J, Subotkowska L, Mastalerz P (1979) Synthesis 985–986
12. Oleksyszyn J, Powers JC (1989) Biochem Biophys Res Commun 161:143–149
13. Oleksyszyn J, Powers JC (1994) Amino acid and peptide phosphonate derivatives as specific inhibitors of serine peptidases. In: Barret AJ (ed) Methods in Enzymology, Vol. 244. Academic Press, San Diego, pp 423–441
14. Oleksyszyn J, Powers JC (1991) Biochemistry 30:485–493
15. Oleksyszyn J (2000) Aminophosphonic and aminophosphinic acid derivatives in the design of transition state analogue inhibitors: biomedical opportunities and limitations. In: Kukhar VP, Hudson HR (eds) Aminophosphonic and aminophosphinic acids. Chemistry and biological activity. Wiley, Chichester, pp 537–558
16. Fastrez J, Jespers L, Lison D, Renard M, Sonveaux E (1989) Tetrahedron Lett 30:6861–6864
17. Joosens J, Van der Veken P, Lambeir A-M, Augustyns K, Haemers A (2004) J Med Chem 47:2411–2413
18. Joossens J, Van der Veken P, Surpateanu G, Lambeir AM, El-Sayed I, Ali OM, Augustyns K, Haemers A (2006) J Med Chem 49:5785–5793
19. Bertrand JA, Oleksyszyn J, Kam CM, Boduszek B, Presnell S, Plaskon RR, Suddath FL, Powers JC, Williams LD (1996) Biochemistry 35:3147–3155
20. Spraggon G, Phillips C, Nowak UK, Ponting CP, Saunders D, Dobson CM, Stuart DI, Jones EY (1995) Structure 3:681–691
21. Oleksyszyn J, Boduszek B, Kam C-M, Powers JC (1994) J Med Chem 37:226–231
22. Sieńczyk M Doctoral thesis (2006) Wrocław
23. Moreira IS, Fernandes PA, Ramos MJ (2007) Computational determination of the relative free energy of binding. In: Sokalski WA (ed) Molecular materials with specific interactions - modeling and design, series: challenges and advances in computational chemistry and physics, vol. 4. Springer, Berlin Heidelberg New York, pp 305–340
24. Dyguda E, Grembecka J, Sokalski WA, Leszczyński J (2005) J Am Chem Soc 126:1658–1659
25. Frisch MJ, Trucks GW, Schlegel HB, Scuseria GE, Robb MA, Cheeseman JR, Montgomery JR, Vreven T, Kudin KN, Burant JC, Millam JM, Iyengar SS, Tomasi J, Barone V, Mennucci B, Cossi M, Scalmani G, Rega N, Petersson GA, Nakatsuji H, Hada M, Ehara M, Toyota K, Fukuda R, Hasegawa J, Ishida M, Nakajima T, Honda Y, Kitao O, Nakai H, Klene M, Li X, Knox JE, Hratchian HP, Cross JB, Adamo C, Jaramillo J, Gomperts R, Stratmann RE, Yazyev O, Austin AJ, Cammi R, Pomelli C, Ochterski JW, Ayala PY, Morokuma K, Voth GA, Salvador P, Dannenberg JJ, Zakrzewski VG, Dapprich S, Daniels AD, Strain MC, Farkas O, Malick DK, Rabuck AD, Raghavachari K, Foresman JB, Ortiz JV, Cui Q, Baboul AG, Clifford S, Cioslowski J, Stefanov BB, Liu G, Liashenko A, Piskorz P, Komaromi I, Martin RL, Fox DJ, Keith T, Al-Laham MA, Peng CY, Nanayakkara A, Challacombe M, Gill PMW, Johnson B, Chen W, Wong MW, Gonzalez C, Pople JA (2004) Gaussian 03. Gaussian, Wallingford CT
26. Clark M, Cramer RD, Van Opdenbosch N (1989) J Comp Chem 10:982–1012
27. SYBYL 7.1, Tripos, 1699 South Hanley Rd., St. Louis, Missouri, 63144, USA
28. Sokalski WA, Roszak S, Pecul K (1988) Chem Phys Lett 153:153–159
29. Boys FS, Bernardi D (1970) Mol Phys 19:553–566
30. Schmidt MW, Baldridge KK, Boatz JA, Elbert ST, Gordon MS, Jensen JH, Koseki S, Matsunaga N, Nguyen KA, Su SJ, Windus TL, Dupuis M, Montgomery JA (1993) J Comput Chem 14:1347–1363
31. Zesławska E, Schweinitz A, Karcher A, Sondermann P, Sperl S, Sturzebecher J, Jacob U (2000) J Med Biol 301:465–475
32. Zesławska E, Jacob U, Sturzebecher J, Oleksyn BJ (2006) Bioorg Med Chem Lett 16:228–234