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Computer simulation based selection of optimal monomer for imprinting of tri-O-acetiladenosine in polymer matrix: vacuum calculations

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Abstract Molecularly imprinted polymers can be anticipated as synthetic imitation of natural antibodies, receptors and enzymes. In case of successful imprinting the selectivity and affinity of the imprint for substrate molecules are comparable with those of natural counterparts. The selection of the optimal functional monomer, monomer/template ratio as well as choosing of polymerization solvent is crucial determinants of the successful imprinting. In the present study the simulation approach to the development of molecular imprinting polymers for the extraction of new protein kinase ATP-competitive inhibitors is presented. By imprinting tri-O-acetyladenosine into polymer matrix the synthetic reproduction of adenosine triphosphate binding site to protein kinases can be fabricated and further used for adenosine triphosphate analogs screening in different sources. The optimized geometrical structure and energy of the pre-polymerization complexes of tri-O-acetyladenosine (template) with three different monomers—methacrylic acid, 3-vinyl benzoic acid and acrylamide in vacuum were calculated using hybrid quantum mechanical/molecular mechanical (QM/MM) approach. These calculations demonstrate that methacrylic acid forms the most stable complex with template, the next is 3-vinyl benzoic acid complex and the third—acrylamide one. The bond energies of the complexes are shown to increase monotonically as more monomers are linked to the template. The same conclusions are made from purely quantum self-consistent field calculations of pre-polymerization complex energy and structure. Hybrid calculation is shown to be effective and can substantially accelerate the development of the imprinting technology.

Keywords Molecular imprinted polymer · Molecular mechanics · NWChem package · Pre-polymerization complex · QM/MM method · Self-consistent field

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Introduction

Polymers imprinted with 3-O-acetiladenosine (TOAA), soluble in organic solvents analog of adenosine, can be used for selective recognition and extraction of ATP-competitive inhibitors from various natural and synthetic sources [1]. These compounds are capable of inhibiting the protein kinases, a large family of adenosine triphosphate (ATP)-dependent enzymes ubiquitously involved in cell signaling that controls a diverse set of cellular processes such as cell cycle progression, proliferation, metabolism, apoptosis and differentiation. Abnormal activity and deregulations of protein kinases are the etiology of a number of serious diseases, such as cancer, chronic inflammations and diabetes [2]. Nowadays it is recognized that inhibition of aberrant kinases activity has powerful therapeutic potential in treating the above mentioned diseases and there are considerable efforts to discover or design biological or small-molecule inhibitors as potential therapeutic agents [3].

The successful imprinting of the molecule-template is determined by the proper selection of a functional monomer capable of forming a stable monomer-template pre-

polymerization complex (PPC), proper selection of monomer/template/cross-linker ratio, reaction solvent and polymerization conditions. Computer technologies and existing simulation methods make it possible to reduce the research costs for selecting the above parameters and estimate their optimal magnitudes in terms of such physicochemical criteria as template-monomer bond energy both in vacuum and solution, geometry and topology of the spatial structure of the PPC and the imprinted polymer, and their temperature dependencies. The methods used widely for computer simulation of bio-molecular systems are molecular mechanics (MM), in which all interactions between the atoms are described by means of classical force fields, quantum mechanical method (QM) based on approximate solving of the Schrödinger equation for whole system and combined quantum/molecular mechanics approach (QM/MM) that combines quantum-mechanical calculations of one part of the system with molecular mechanics calculation of its remaining part [4].

The *in silico* selection of the best monomer for caffeine imprinting as well as evaluation of optimal template/monomer molar ratio was proposed by Farrington et al. [5]. The approach is based on MM modeling of pre-polymerization complex followed by sequential corrections by semi-empirical PM3 method. The main evaluation criterion here was the binding energy between the monomers and the template. The important point is that the template is bound to the monomer by intermolecular hydrogen bonds, which cannot be properly described by means of classical force fields. Semi-empirical methods result in underestimated bond energy and overestimated hydrogen bond lengths [6]. In the present paper, we propose to use the QM/MM approach for pre-polarization complex calculations in such a way that the regions containing hydrogen links are modeled by *ab initio* methods with 6–31 G** polarization basis set which includes polarization orbitals contributing appreciably to the hydrogen bonds [7], while the rest of the system is modeled using the MM approach with Amber force field that is effectively applied for modeling bio-molecular systems [8]. This approach enables one to accurately define the hydrogen bond parameters and avoid huge computer resources usage required in the case of modeling the whole system by purely QM methods. It is also intended to further use this approach for modeling the PPC-monomers solution as well as the process of polymerization. The suggested approach is applied for the investigation of tri-O-acetiyladenosine PPC with three different monomers: metacrilic acid CH_2 = $C(CH_3)COOH$ (MAA), 3-vinyl benzoic acid CH2 = CH-C_6H_4-COOH (TVB) and acrylamide CH_2 = $CHC(O)NH_2$ (ACR). The results obtained by QM/MM calculations are compared with calculations by self-consistent field (SCF). All calculations were carried out with NWChem- 6.0 [9] package using the resources of the Belarusian National Technical University's grid-site BY-BNTU.

MIP development with TOAA template

Protein kinases catalyze the phosphate group transfer from ATP to proteins-substrates. Malfunctioning of these enzymes may be a cause of several pathologies, such as inflammation, diabetes and some kinds of cancers. Drugs that are capable of inhibiting specific protein kinases are developed for treatment of these diseases [2, 3].

The protein kinase active center consists of the ATP binding site and hydrophobic regions (Fig. 1, image adapted from [10]). ATP molecule is binding with kinase due to the creation of hydrogen bonds between its adenosine and amino acids of ATP binding site of kinase. That is why the main mechanism of protein kinase inhibiting (ATP-competitive mechanism) is prohibiting of ATP binding with the kinase active center by blocking this center with ATP-competitive inhibitors. At the molecular level, the mechanism of action of ATP-competitive inhibitors is based on the creation of coordinated hydrogen bonds between the inhibitor and the amino acids of protein kinase binding site which imitate the hydrogen bonds between ATP's adenine and protein kinase [2].

In particular, molecular imprinted polymers with cavities obtained using TOAA molecule as template and imitating ATP binding sites of protein kinases are proposed for recognizing and blocking of the appropriate inhibitors [1]. To fabricate such polymers, the molecular imprinting method based on functional monomer polymerization in the

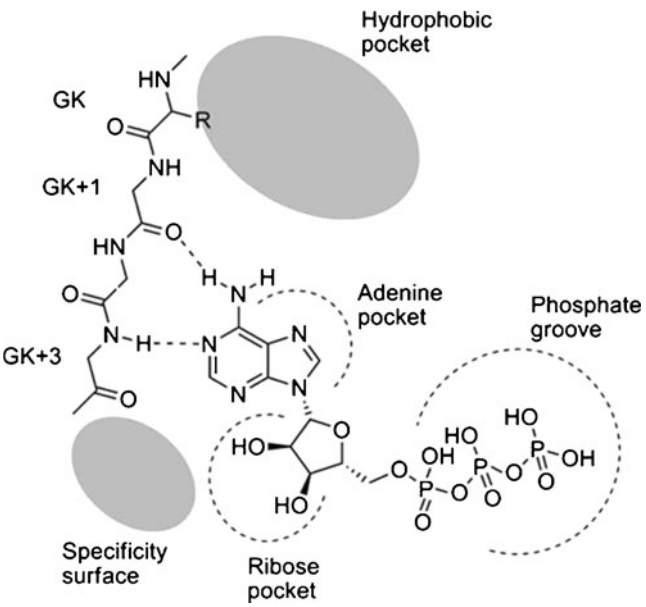

Fig. 1 Structure of ATP binding domain of protein kinases

presence of molecule-templates was applied. This method consists of several stages presented in Fig. 2.

At the first stage, the stable PPC is formed from the molecules of monomer and template solvates mixed in appropriate ratio in a solvent. At the second stage, the complexes polymerize in the presence of the linking agent. At the third stage, the template molecules are washed out from the polymer. Finally, the functional cavities remain in the polymer. They are capable of recognizing and binding specifically the molecules of the template analogs with similar molecular volume, shape and chemical functionality. It would be expected that the compounds de-mobilized in the TOAA imprints will be able to compete with ATP for the binding site and to inhibit the protein kinase by means of ATP-competitive mechanism.

General methodology of molecular modeling of imprinting process and *in silico* evaluation of imprinting efficiency

The suggested methodology includes sequential modeling of each stage of imprinting process and application of the molecularly imprinted polymers, which are shown above in Fig. 2.

At the first step of PPC formation the optimal monomer as well as optimal monomer/template ratio for efficient imprinting is evaluated by molecular modeling, the spatial structure of the complex and the corresponding energy and geometric parameters (size-shape factor) are computed.

Since the spatial structure of the PPC determines the shape of the imprint cavity imitating the binding site of the protein kinase, the monomers in PPC have to form bonds with the TOAA adenine only. To define the most probable spatial structure of the PPC, one should calculate the energies for all possible configurations and choose the structure

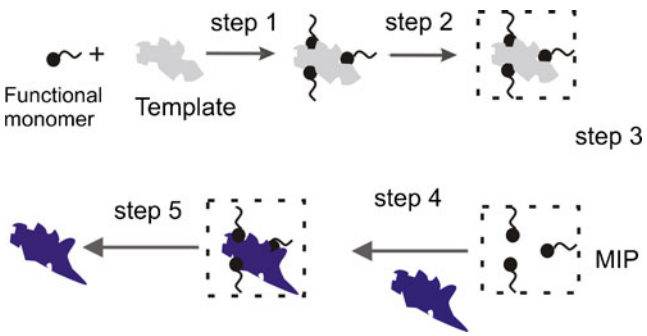

Fig. 2 The main steps of the imprinting process and application of the imprint: Step 1—formation of pre-polymerisation complex of functional monomer and template in solution; Step 2—polymerization in the presence of cross-linker; Step 3—washing out the template; Step 4—selective extraction of the molecules that fit the formed cavities by size, shape and functionality; Step 5—washing out the extracted molecules

Table 1 Energies of hydrogen bond between two water molecules (kJ mol^{-1})

Basis set	6–31 G	6–31 G**	Experiment
Energy	32.81	23.05	21

corresponding to the smallest energy. To do this, one should specify the initial coordinates of the atoms of the PPC with the monomer molecules placed around the template in such a way that hydrogen bonds could form between them. By optimization of the geometry of the molecular complex, it is possible then to find the local energy minimum corresponding to the most promising structure. Preliminary calculation of the pre-polymerization complex structure is performed for vacuum. Molar ratio determines the number of functional monomers attached to the molecule-template. The bonding energy of the PPC is calculated as the difference of the sum of the energies of the molecules constituting PPC and total PPC energy:

$$E_{bond} = E_{TAA} + n \cdot E_{mono} - E_{PPC}, \quad (1)$$

where n is the number of the attached monomers. E_{bond} value characterizes the PPC stability.

Then the PPC atomic model with optimal structure is placed into a simulation cell, where the solvent molecules are uniformly distributed and the solvent density is specified. Simulation of the PPC stability is performed using the molecular dynamics method at various temperatures.

In simulation of the second stage (polymerization process), molecules of cross-linker are added to simulation cell in the amount corresponding to the specified linking coefficient. Then the geometry optimization, molecular dynamics and polymerization simulation are performed. As a result of this stage, one obtains a spatial structure of the MIP including the linking cavity shape, the accessibility of the linking cavities by template as well as the MIP-template binding energy.

At the third stage, the obtained MIP model containing molecules of template is introduced into solvent model for washing simulation. Simulation is performed using the

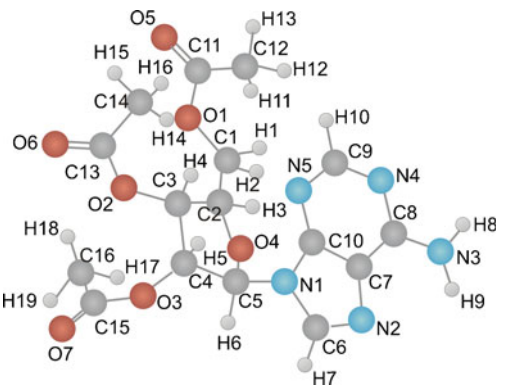

Fig. 3 TOAA molecule—whole molecule belongs to quantum region

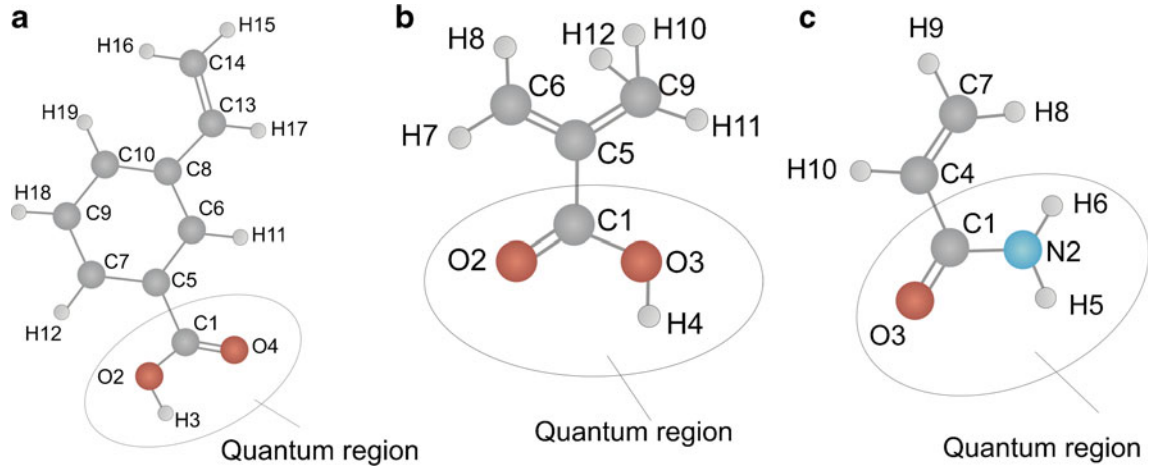

Fig. 4 Molecules of monomers: **a** TVB, **b** MAA, **c** ACR. Quantum regions are *circled*

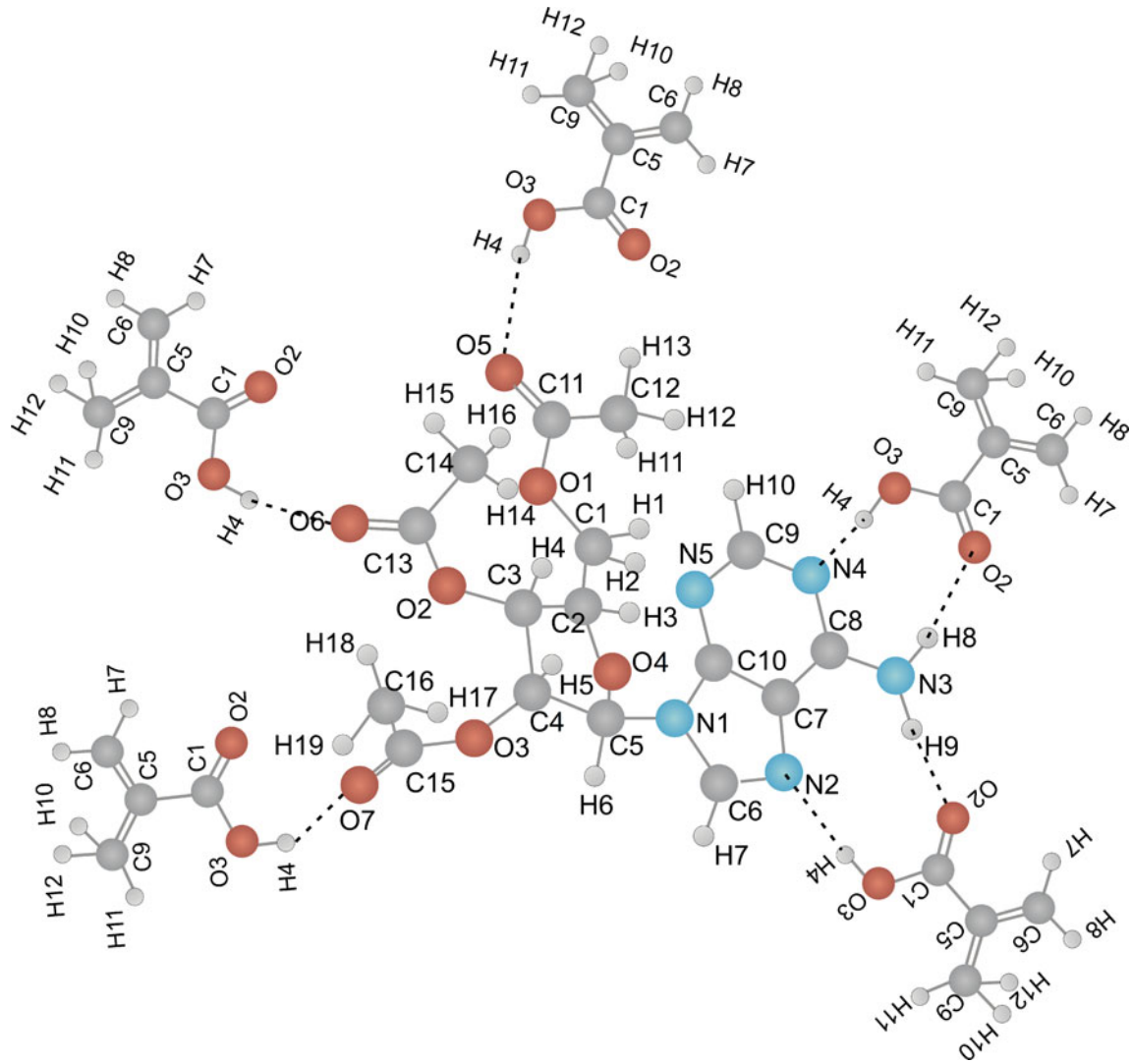

Fig. 5 TOAA molecule with five attached monomers of metacrylic acid. Hydrogen bonds are shown schematically

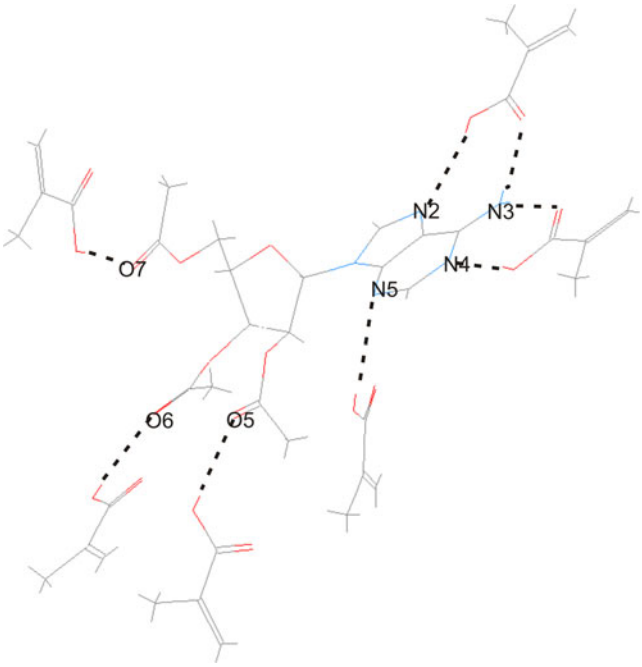

Fig. 6 Precise space structure of TOAA molecule with six attached monomers of metacrylic acid

molecular dynamics method at elevated temperature. As a result, the number of the molecular templates washed out from the imprint cavities is obtained as well as the porosity of the MIP. Thus, at the second and third stages, the area of selective surface of the polymer is defined.

At the last stage, the capability of the polymer to recognize the molecules of inhibitors is analyzed. With this aim in view, one optimizes the geometry of the imprinted polymer with the template molecule replaced by the molecule of inhibitor. Then the binding energy between the polymer and the molecule of inhibitors is determined, which characterizes the affinity of the imprinted cavities.

The complex analysis of the results obtained at every simulation stage enables selecting the functional monomer, solvent, monomer/template and monomer/cross-linker molar ratios that are best suited for the imprinting process.

In this study we concentrate on the first step of PPC molecular modeling.

Application of the QM/MM method for geometry optimization and calculation of binding energy of hydrogen bond complexes

Using the QM/MM simulation method implies that the system is divided into two regions: classical and quantum.

The total energy of the system is calculated as the sum of the energies of the classical and quantum regions plus their interaction energy. The NWChem algorithm for geometry optimization consists of the step-by-step minimization of the energies of classical and quantum regions using the limited-memory quasi-Newton method [11]. In this case, the interatomic bonds crossing the boundaries of the QM regions are replaced by the bonds with a fictitious hydrogen atom. Its radius vector is calculated as follows:

$$\vec{R}_H = (1-g)\cdot\vec{R}_{quant} + g\cdot\vec{R}_{classic}, \quad (2)$$

where $\vec{R}_{quant}$ is the radius-vector of the atom of the broken bond from the quantum region side, $\vec{R}_{classic}$ is the same from the classical region side, and g is the scale factor equal to 0.709 [11].

First, the geometry optimization of the quantum region with fictitious atoms is made. Then the electrostatic representation of the quantum region is calculated. When the classical region energy is minimized, the atoms of the quantum region and fictitious atoms are fixed. The cycle recurs until the convergence is achieved for both regions.

The Amber force field included into the NWChem package was used for calculating the energy of the classical region, while for calculating the energy of the quantum region, the self-consistent field (SCF) method was used because of its fewer computer demands in comparison with density functional theory level. Selection of the atomic basis for SCF is determined by the presence of hydrogen bonds in the system and by the limitations on available computer resources.

Split valence basis sets (mainly 3–21 G or 4–31 G) are commonly used for *ab initio* calculations of the systems with hydrogen bonds. They are chosen because of the

Table 2 Binding energies of TOAA-monomers' prepolymerization complex after QM/MM optimization (kJ mol^{-1}). Atoms' numbering of TOAA are presented in Fig. 3

Number of monomers	Binding sites on template	MAA	TVB	ACR
1	N4,N3	66.69	58.5	46.00
	N2,N3	64.23	54.21	44.44
	O7	49.77	43.58	33.03
	O6	32.45	41.05	32.91
	O5	47.96	43.82	10.75
	N5	32.33	–	–
2	N4,N3,N2,N3	126.88	110.39	80.29
3	N4,N3,N2,N3,O7	174.88	149.63	110.50
	N4,N3,N2,N3,O6	172.57	147.36	110.39
	N4,N3,N2,N3,O5	171.58	148.87	111.31
4	N4,N3,N2,N3,O6,O7	220.73	187.15	139.71
	N4,N3,N2,N3,O5,O6	224.26	186.47	142.09
	N4,N3,N2,N3,O5,O7	221.14	186.59	140.96
5	N4,N3,N2,N3,O5,O6, O7	260.55	224.04	169.67
6	N4,N3,N2,N3,O5,O6, O7,N5	302.30	–	–

Table 3 Lengths of hydrogen bonds between TOAA and monomers (Å). Length of A—H···B bond is understood as the distance between A and B atoms

Number of monomers	Binding sites on template	MAA	TVB	ACR
1	(N4, N3)	(2.95, 2.98)	(2.88, 2.99)	(3.29, 2.97)
	(N3, N2)	(2.90, 2.99)	(3.09, 2.82)	(2.94, 3.33)
	O7	2.93	2.83	3.06
	O6	3.01	2.87	3.11
	O5	2.93	2.85	3.17
	N5	3.11	–	–
2	(N4, N3)	(2.96, 2.95)	(2.82, 3.03)	(3.29, 3.00)
	(N3, N2)	(2.92, 2.85)	(3.10, 2.85)	(2.93, 3.36)
3	(N4, N3)	(2.94, 2.94)	(2.80, 3.02)	(3.26, 2.99)
	(N3, N2)	(2.90, 2.83)	(3.06, 2.84)	(2.93, 3.33)
	O7	2.86	2.77	3.11
3	(N4, N3)	(2.98, 2.96)	(2.83, 3.03)	(3.26, 3.00)
	(N3, N2)	(2.91, 2.85)	(3.08, 2.83)	(2.93, 3.35)
	O6	2.90	2.86	3.14
3	(N4, N3)	(2.93, 3.02)	(2.82, 3.03)	(3.25, 2.99)
	(N3, N2)	(3.05, 2.91)	(3.07, 2.86)	(2.91, 3.33)
	O5	2.86	2.77	3.04
4	(N4, N3)	(2.95, 2.94)	(2.82, 3.03)	(3.20, 2.97)
	(N3, N2)	(2.91, 2.82)	(3.06, 2.82)	(2.93, 3.31)
	O6	2.85	2.84	3.18
	O7	2.88	2.79	3.01
4	(N4, N3)	(2.98, 2.96)	(2.86, 3.06)	(3.15, 2.93)
	(N3, N2)	(2.92, 2.86)	(3.07, 2.86)	(2.99, 3.41)
	O5	2.93	2.77	3.11
	O6	2.88	2.84	3.16
4	(N4, N3)	(2.92, 3.03)	(2.85, 3.04)	(3.28, 3.01)
	(N3, N2)	(3.05, 2.89)	(3.07, 2.86)	(2.93, 3.33)
	O5	2.88	2.78	3.29
	O7	2.86	2.81	3.18
5	(N4, N3)	(2.95, 3.04)	(2.87, 3.05)	(3.25, 3.02)
	(N3, N2)	(3.04, 2.88)	(3.07, 2.84)	(2.94, 3.29)
	O5	2.88	2.79	3.25
	O6	3.03	2.88	3.07
	O7	2.87	2.80	3.20
6	(N4, N3)	(2.84, 2.96)	–	–
	(N3, N2)	(2.95, 3.06)	–	–
	O5	2.96	–	–
	O6	2.94	–	–
	O7	2.98	–	–
	N5	3.21	–	–

necessity to compromise between the accuracy of calculations and the computational burden. Unfortunately, in such calculations the energies of hydrogen bonds are overstated, while their lengths (i.e., the distances between the heteroatoms) are slightly understated. Moreover, the stability of cyclic structures with hydrogen bonds appears appreciably overestimated. The inclusion of polarization orbitals into the basis set eliminates these disadvantages [3].

To compare the polarization and non-polarization basis sets applicability for description of hydrogen bonds, the geometry optimization and bond energy calculation of two water molecules was undertaken using NWChem package. The results for non-polarization basis set 6–31 G and polarization basis set 6–31 G** as well as the corresponding experimental data [3] are represented in Table 1.

According to the results presented, 6–31 G** basis set provides higher accuracy. Thus, it is advisable to use namely polarization basis sets 6–31 G* or 6–31 G** for *ab initio* calculations of the systems with hydrogen bonds. This is difficult to be implemented for purely quantum mechanical modeling of the systems in organic chemistry and biology usually having rather large amount of atoms but in QM/MM approach this obstacle becomes surmountable.

QM/MM modeling of pre-polymerization complex

In accordance with general discussion of QM/MM approach above we choose the quantum region of PPC including in it all atoms of the template molecule (Fig. 3) and the monomers' atoms which participate in the formation of hydrogen bonds with the template (Fig. 4). The classical region contains the rest of atoms of monomers.

Figure 5 shows the atomic model of TOAA molecule with possible locations of hydrogen bonds of TOAA with MAA monomers as example. Calculations of bond energies of PPC were made for template with 1, 2, 3, 4, and 5 linked monomers of all three types. PPC with six MAA monomers was studied too to clarify the role of N5 nitrogen atom as a site for monomer attachment (Fig. 6).

Bond energies of pre-polymerization complex calculated by QM/MM method for considered functional monomers attached to the different template atoms (binding sites) are presented in Table 2, where atoms' numbering from Fig. 3 is used. Table 3 shows the corresponding data for the lengths of hydrogen bonds between template and monomers (hydrogen bond length is defined as the distance between electronegative atoms participating in this bond). From the results presented it follows that there are three kinds of monomer-template hydrogen bond linking. The first one is strongest and includes creation of two hydrogen bonds between every monomer and pair of nitrogen atoms of the template (N2 and N3 or N3 and N4). Bonds with N3 atom are shorter then that with N2 and N4 for ACR and longer both for MAA and TVB.

The second kind of linking includes ordinary hydrogen bonds between monomers and O5 or O7 atoms of template. Third kind is weakest and realizes as ordinary hydrogen bond between monomer and O6 or N5 atoms of template.

Based on the energy values for PPC formation, one can conclude that MAA is the best functional monomer for producing PPC, then TVB, and ACR is the least.

It should be noted that the process of PPC creation can be inhibited by the creation of dimers both of functional monomers and template. The bond energies of the dimers of MAA, TVB, ACR and TOAA are 85.65 kJ mol^{-1}, 63.43 kJ mol^{-1}, 49.94 kJ mol^{-1} and 47.92 kJ mol^{-1} correspondingly. Monomers in these dimers are linked by two H-bonds (in notations of the atoms from Figs. 3 and 4 these are O2 H4′—O3′ and O2′ H4—O3 for MAA dimer, O4 H3′—O2′ and O4′ H3—O2 for TVB dimer, O3 H5′—N2′ and O3′ H5—N2 for ACR dimer and N2 H9′—N3′ and N2′ H9—N3 for the most stable "plane" TOAA dimer, where stroked and non-stroked symbols correspond to the atoms of the first and second monomers of dimer). The energies above exceed corresponding bond energies of PPC with TOAA template and single monomer (see Table 2), then, the creation of the dimers is preferable with respect to the creation of such PPCs. However, for PPC with several monomers the situation is opposite and PPC creation is more preferable (see Table 4). For example, the net bond energy of TOAA dimer and two MAA dimers (219.22 kJ mol^{-1}) is less significant then the net bond energy of two PPCs containing TOAA template with two MAA monomers (253.77 kJ mol^{-1}). The close investigation of these effects will be presented elsewhere simultaneously with the discussion of the role of the solvent molecules and cross-linkers in PPC formation in solutions.

In concern with these future studies of other steps of TOAA template MIP formation, it should be noted that the electrostatic interaction of template with monomers in vacuum is only one part of the effects which determine the PPC concentration in the initial solution, before polymerization. It depends on the entropy effects which are

Table 4 Comparison of the binding energies of the systems of dimers of TOAA, ACR, TVB and MAA versus doubled binding energies of corresponding PPCs (kJ mol^{-1}). Dimer of molecule A is designated as d-A

System of dimers	Bond energies' sum for dimers' system	2 PPC kind	2 PPC bond energies' sum
d-TOAA + d-MAA	133.57	(TOAA + MAA)*2	133.39
d-TOAA + d-TVB	111.35	(TOAA + TVB)*2	116.99
d-TOAA + d-ACR	97.86	(TOAA + ACR)*2	92.00
d-TOAA + 2* d-MAA	219.22	(TOAA + 2MAA)*2	253.77
d-TOAA + 2* d-TVB	174.78	(TOAA + 2TVB)*2	220.78
d-TOAA + 2* d-ACR	147.80	(TOAA + 2ACR)*2	160.59

Table 5 Mulliken charges of the hydrogen bonded atoms

Binding atom	TOAA + 5ACR		TOAA + 5MAA		TOAA + 5TVB	
	PPC	Isolated molecules	PPC	Isolated molecules	PPC	Isolated molecules
N4	−0.73	−0.66	−0.78	−0.66	−0.80	−0.66
h	0.38	0.32	0.42	0.37	0.43	0.37
r2	−0.80	−0.78	−0.64	−0.61	−0.65	−0.62
N3	−0.81	−0.77	−0.81	−0.77	−0.81	−0.77
H8	0.38	0.33	0.38	0.33	0.38	0.33
r1	−0.64	−0.60	−0.62	−0.57	−0.63	−0.58
N3	−0.81	−0.77	−0.81	−0.77	−0.81	−0.77
H9	0.38	034	0.38	0.34	0.38	0.34
r1	−0.64	−0.6	−0.62	−0.57	−0.62-	−0.58
N2	−0.64	−0.58	−0.69	−0.58	−0.70	−0.58
h	0.37	0.32	0.43	0.37	0.43	0.37
r2	−0.80-	−0.78	−0.64	−0.61	−0.65	−0.62
O5	−0.57	−0.53	−0.58	−0.53	−0.58	−0.53
h	0.37	0.32	0.41	0.37	0.42	0.37
r2	−0.79	−0.78	−0.63	−0.61	−0.64	−0.62
O6	−0.57	−0.53	−0.56	−0.53	−0.58	−0.53
h	0.37	0.32	0.40	0.37	0.41	0.37
r2	−0.80	−0.78	−0.62	−0.61	−0.64	−0.62
O7	−0.56	−0.52	−0.57	−0.52	−0.57	−0.52
h	0.37	0.32	0.41	0.37	0.42	0.37
r2	−0.80	−0.78	−0.63	−0.61	−0.64	−0.62

always large and tend to inhibit the associations. The association free energies are certainly less negative than the association energies. One important entropy effect comes from translation and rotation contribution to entropy which can be written as [12]:

$$S_{temp} = a + b \cdot \log M_{temp}, \quad (3)$$

where M_{temp} is the mass of template molecule and positive parameters a and b depend on the solvent nature. For vacuum calculations both parameters are definite constants. The association entropy is then:

$$\Delta S = S_{PPC} - S_{temp} - S_{mon} = -a + b \cdot \log\left(\frac{M_{temp} + M_{mon}}{M_{temp} M_{mon}}\right), \quad (4)$$

where M_{mon} is the mass of monomer in PPC. For any positive a and b $\Delta S < 0$ for $M_{mon} > 2$ and obviously one has $\frac{\partial \Delta S}{\partial M_{mon}} < 0$, then entropy contribution to free energy is positive and grows with M_{mon}. This clearly shows that the association is more inhibited by heavy monomers. One may say therefore that from the entropy point of view the TVB monomer is certainly unfavored by its mass.

Comparison of the results of QM/MM and SCF quantum mechanical modeling

NWChem package does not have the tool to describe all electronic properties of the system simulated by QM/MM method. That is why to study them for PPCs the SCF calculation of the whole system was undertaken for the geometry obtained after QM/MM optimization. Namely, Mulliken charges were calculated for all PPC atoms in this geometry as well as for isolated molecules of TOAA and monomers by SCF method with 6–31 G** basis set. These charges for atoms forming the template/monomer hydrogen bonds are given in Table 5. Capital letters designate the hydrogen bond atoms in the template, small letters designate

Table 6 Designation of the atoms of hydrogen bonds of PCC from the monomer side. Atoms' numbering is presented in Fig. 4 for all three monomers

Binding atom on monomers	ACR	MAA	TVB
r1	O3	O2	O4
r2	N2	O3	O2
h	H5	H4	H3

Table 7 SCF binding energies of PPCs after QM/MM optimization (kJ mol^{-1}). BSSE corrected values are set off in italics

Number of monomers	Binding sites on template	E_{bond} MAA	E_{bond} TVB	E_{bond} ACR
1	N4,N3	64.02 *(56.85)*	57.13 *(45.37)*	44.75 *(38.18)*
2	N4,N3,N2,N3	121.45 *(106.71)*	107.47 *(68.33)*	78.07 *(65.62)*
3	N4,N3,N2,N3,O7	166.11 *(145.33)*	144.93 *(123.30)*	107.01 *(89.36)*
4	N4,N3,N2,N3,O6,O7	210.28 *(182.11)*	180.58 *(153.53)*	136.08 *(113.87)*
5	N4,N3,N2,N3,O5,O6,O7	246.80 (*215.46*)	216.07 *(183.65)*	162.95 *(134.19)*

the same in functional monomers. Symbols *h, r1* and *r2* correspond to the atoms in monomers that are indicated in Table 6, where atoms' numbering from Fig. 4 is used. From the data in Table 5 it follows that during PPC formation the electronic density near H atoms participating in hydrogen bonds creation is decreased while its value near corresponding O and N atoms is increased. It is known that for H-bonded molecules the transfer of a small amount of electron density (0.01–0.03 elementary charge) from the proton acceptor (B) to proton donor side (A—H) of H-bond A—H B is characteristic [13]. This means that in the case of PPC creation this transfer is not localized in the vicinity of H-bonded atoms.

The bond energies of PPCs for geometries, obtained after QM/MM optimization, were recalculated by purely quantum SCF method using 6–31 G** basis set. The results of these calculations are given in Table 7. Comparison of them with the data in Table 2 shows that purely quantum calculation gives the values of PPCs' bond energies systematically reduced by several per cents with respect to QM/MM results.

We apply the SCF method with the same basis set as well for the geometry optimization of complexes, monomers and template for comparison with QM/MM results. The initial geometries of all systems were the same as for QM/MM optimization. The binding energies of SCF-optimized PPCs calculated in accordance with Eq. (1) are given in Table 8. Comparing them with data given in Table 2 one concludes that SCF optimization leads to decreasing of the binding energies of PPC with MAA and TVB monomers while that for ACR is increasing. These changes do not alter the conclusion about the order of monomers (MAA, then TVB, then ACR) in PPC stability provision ranking.

Figure 7 shows the binding energies versus the number of functional monomers in PPC, calculated by SCF and QM/MM methods.

It should be noted that when using the definition (1) for bond energy of PPC calculated at any quantum level of theory with finite basis set one has to take into account the basis set superposition error (BSSE) (see, for example, [13]). BSSE correction was not applied in geometry optimization because in NWChem package the algorithm of minimization of non-BSSE-corrected potential energy is realized both for QM/MM and SCF methods. However, this correction can be applied for calculation of binding energy of PPC in final optimal state if SCF method is used. Thus obtained values for QM/MM-optimized states are set off in Table 7 by italics. One can see that BSSE correction reduces the PPC binding energy, but MAA seems to be the most preferable in all cases. BSSE correction makes the difference between PPC bond energies calculated by QM/MM and SCF methods significant and they have to be analyzed in QM/MM implementation in NWChem package.

It is worth noting that the QM/MM energy calculation time is almost independent of the number of atoms in prepolymerization complex, while the SCF energy calculation time increases rapidly by increasing this number. In the cases considered here, the geometry optimization time does not change monotonically with the monomer number growth because the amount of optimization steps depends on how close the initial configuration is to the final one. Nevertheless, when the number of atoms in the system is greater than 90–100, the advantage of the QM/MM method becomes obvious. This is because the energy calculation time increases appreciably for the SCF method and becomes a critical factor even when the SCF method involves fewer steps of geometry optimization than the QM/MM.

Table 8 Binding energies of PPCs calculated by SCF optimization method (kJ mol^{-1})

Number of monomers	Binding sites on template	E_{bond} MAA	E_{bond} TVB	E_{bond} ACR
1	N4,N3	64.61	59.68	50.75
2	N4,N3,N2,N3	123.84	108.78	85.71
3	N4,N3,N2,N3,O7	171.89	147.34	116.76
4	N4,N3,N2,N3,O6,O7	223.93	185.5	148.16
5	N4,N3,N2,N3,O5,O6,O7	269.99	–	197.61

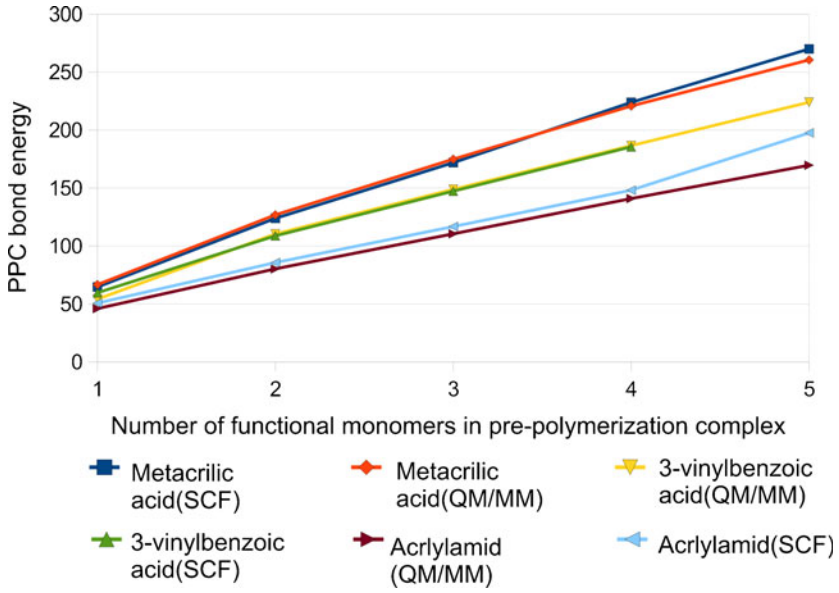

Fig. 7 Binding energies (kJ mol^{-1}) versus the number of functional monomers in PPC, calculated by SCF and QM/MM methods

Conclusions

The stability of the monomer/template pre-polymerization complex plays a crucial role in successful template imprinting and the resulting selectivity of the synthesized MIP with respect to the template and the template analogs.

Based on the energy values for PPC formation that are calculated by the QM/MM method, one can conclude that metacrylic acid is the best functional monomer for producing the pre-polymerization complex, then comes 3-vinyl benzyl acid, and acrylamide is the least suitable monomer of the three. The PPC bond energy increases as more monomers are added to the template. The same conclusions can be made on the basis of the results calculated by the SCF method despite minor quantitative distinctions.

However, increasing the monomer/template ratio leads to complex formation of a monomer with the oxygen of acetyl substitutes of ribose: O5, O6, O7, which is not specific for ATP bonding with protein kinase. Normally, in the synthesis of the molecular imprints by non-covalent imprinting method, the monomer in the reaction mixture is used in excess in order to shift the chemical equilibrium to PPC production. Our calculations show that when TOAA is used as ATP-substituent soluble in organic solvents, the extreme excess of the monomer can lead to fixing the redundant functional groups in the cavity and, as a result, to decreasing efficiency of the intended use of the polymer for recognizing and blocking the ATP analogs from natural and synthetic sources.

The efficiency of the QM/MM method in modeling small systems does not exceed that of the SCF method. The reason for it is that the quantum region includes more atoms than the classical region. Besides, the convergence rate is greatly influenced by initial conditions. Thus, there is no direct relation between the number of atoms in the system and the optimization time. However, for the system which consists of five molecules of 3-vinyl benzyl acid comprised of 142 atoms, QM/MM calculation time reduces dramatically as compared with SCF calculation time. As the system grows further (if a solvent or a polymer is added) it becomes more difficult to use the SCF method because the calculation time considerably increases. If the QM/MM method is used, only the classical region is to be added, as the interaction between molecules of solvent and polymer is due to covalent bonds and electrostatic forces satisfactory represented by bio-molecular force fields such as Amber. As a result the modeling time does not change sufficiently.

Thus, QM/MM modeling may be applied for rapid estimation of TOAA imprinting parameters thereby increasing the efficiency of developing the technology of polymers with TOAA imprints.

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