# ENERGY ANALYSIS OF THE COMPLEX FORMATION OF AROMATIC MOLECULES IN AN AQUEOUS SOLUTION

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PACS 34.20.Gj, 82.20.Wt, 82.30.Nt


The energetics of noncovalent interactions at the self-association of aromatic molecules with various structures and charges has been analyzed. Twelve different molecules have been examined. A method to compute the contributions made by various physical factors to the total Gibbs energy has been developed. The contributions given by hydrogen bonds and entropic factors were found to be always favorable, whereas the contributions made by van der Waals, electrostatic, and/or hydrophobic effects may be stabilizing or destabilizing, depending on the specific system under consideration. The issues concerning the factors that stabilize/destabilize the stacking of aromatic molecules in the solution and their relative importance have been elucidated.

## 1. Introduction

The interaction between aromatic molecules is one of the most widespread interactions in natural and synthetic molecular systems. In aqueous solutions, the interaction between aromatic rings results in the formation of their piles (stacks) owing to the ring flatness and because the hydrophobic contribution is favorable. Stacking is known to be the key factor of nucleic acid stability [1], at the binding of DNA with ligands [2], at the formation of supermolecular structures from aromatic domains [3], and at the molecular recognition [4].

Important information concerning the stacking can be obtained using a thermodynamic (energy) analysis, which, in the case of aromatic systems, has been the subject of investigations for many years [4, 5]. Nowadays, owing to the application of well-developed techniques, a high measurement accuracy has been attained for the fundamental thermodynamical parameters–the Gibbs free energy $\Delta G$, the enthalpy $\Delta H$, and the entropy $\Delta S$. The research practice demonstrates that the analysis of those parameters often demands that the contributions of individual physical factors to the molecular complexation reaction should be singled out; it is the so-called problem of Gibbs total energy decomposition into its components [5]. The contributions of various physical factors to the total Gibbs energy at the stacking of aromatic molecules can be calculated with the help of various techniques developed well enough by various authors (see below). However, there are at least two fundamental problems, when those data are used in the thermodynamic analysis.

1. *The analysis of experimentally measured Gibbs free energies, enthalpies, and entropies can be insignificant, if, as a rule, systems with different structural or physical features are compared.* It is associated with the fact that the physical factors responsible for the total energies of noncovalent interactions between molecules are coupled with one another by the phenomenon of enthalpy–entropy compensation [6]. Therefore, any conclusion concerning the interrelation between the known peculiarities of systems under consideration and their general thermodynamic parameters can be invalid.

2. *The magnitudes of theoretically calculated Gibbs free energies, enthalpies, and entropies for specific physical factors can also be insignificant.* As a rule, the semiempirical approaches based on a parametrization (the so-called "force fields") are usually used to calculate the interaction energies in complex systems. However, the calculated energies depend on the initial structure, force-field parameters, constraints, and technique of molecular simulation that are used. Although the variations of calculated energies can be predicted correctly, it hardly provides any guarantee that the calculated energies correspond to the actual situation in a solution. The key reason is that the contributions of individual physical factors to the total stacking energy cannot be measured independently. Therefore, in the general case, a comparison between different contributions to the total energy is unreliable. This means that the questions "Which physical factors stabilize/destabilize the stacking of aromatic molecules in the solution?" and "Which is their relative importance?" cannot be answered unambiguously now. In the literature, for instance, a long dis-

cussion continues concerning the issue "Which forces (van der Waals, electrostatic, or hydrophobic) or interaction types (with the solvent or intermolecular ones) prevail at the stacking of aromatic molecule?" [7, 8].

We believe that those problems of thermodynamic analysis can be partially overcome, if the technique for the evaluation of energy contributions given by main physical factors (the free energy partition) satisfies the following requirements:

1. Summation of independently calculated energy terms reproduces the experimentally measured interaction energy to within reasonable error limits. In this case, the energy values calculated for different physical factors can be used in the comparative analysis.

2. Calculations should be carried out for a set of molecular systems different by their structure and charge. If the technique demonstrates a good agreement with experiment for one system only–or for a number of systems structurally belonging to the same type, as it often takes place–its extension on other systems will always be doubtful, with no guarantee that the calculated energies have any reason at all.

3. Calculations must be executed with the use of the same technique and the same set of parameters (restrictions) for every system under investigation. Otherwise, it may turn out no more than an artificial fitting of calculation results, which makes the calculated energies less reliable.

There are a few reports in the literature dealing with the partition of the total energy in various molecular systems: protein–protein [9], protein–ligand [10], ligand–DNA [11, 12], and ligand–ligand [8, 13, 14]. However, none of those researches satisfied the requirements given above. Those calculations were based on the usage of the total Gibbs energy [11], or they were carried out either for one system [8–10, 12] or not taking a solvent into account [13, 14]. Recently, we have made a successful attempt to develop and to apply a technique that would satisfy all three requirements given above, when considering the complexation of aromatic ligands with DNA [15]. This work aimed at adapting our technique to solve the problem of complexation reaction energy partition for aromatic molecules, different by structure and total electrical charge, in an aqueous solution. Aromatic compounds are known to form column-like aggregates ("sandwiches" or "stacking complexes") in the solution (see reviews [16, 17])–this process is named self-association or dimerization–and can serve as a prototype of stacking interactions (or $\pi$-$\pi$ interactions) between other aromatic systems.

## 2. Methods

### *2.1. General approach to energy partition*

The reactions of noncovalent formation of a self-associate (a dimer complex $X_2$) from two $X$-molecules give rise to a dynamic equilibrium between $X$ and $X_2$ in the solution,

$$X + X \overset{\Delta G_{\text{total}}}{\longleftrightarrow} X_2. \tag{1}$$

Reaction (1) is characterized by the equilibrium complexation constant $K$ and the total Gibbs free energy $\Delta G_{\text{total}}$ which can be measured experimentally. The Gibbs energy is composed of contributions made by various physical factors. All known main contributions to $\Delta G_{\text{total}}$ in reaction (1) can be summed up in an expression that resolves $\Delta G_{\text{total}}$ in terms of energy components given by different physical factors:

$$\Delta G_{\text{total}} = \Delta G_{\text{vdW}} + \Delta G_{\text{el}} + \Delta G_{\text{hyd}} +$$

$$+\Delta G_{\text{HB}} + \Delta G_{\text{entr}}, \tag{2}$$

where the subscripts denote the energy contributions from van der Waals (VdW), electrostatic (el), and hydrophobic (hyd) forces, hydrogen bonds (HB), and specific factors, mainly of entropic nature (entr). It should be emphasized that partition (2) of $\Delta G_{\text{total}}$ into "different physical factors" is conditional, because all the enthalpic components in this equation have the mutual electromagnetic origin. A more detailed description of the physical meaning for each term in Eq. (2) is given below.

Equation (2) is a cornerstone of the methodology developed in this work. If calculations satisfy the three requirements stated in Introduction, a deeper analysis of each energy component in Eq. (2) gives answers to the principal questions "Which physical factors stabilize/destabilize the stacking of aromatic molecules in the solution?" and "Which is their relative importance?"

Supposing that the conformation modifications in a rigid chromophore of aromatic molecules $X$ are absent in the course of the complexation of the latter in an aqueous solution, the calculation of each component in Eq. (2) can be executed following the thermodynamic cycle (Fig. 1). The main feature of this cycle is the calculation of the free energy separately in

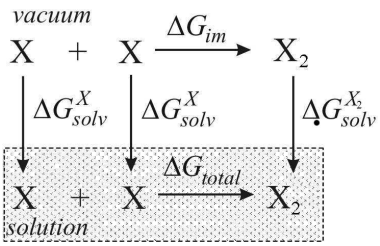


Fig. 1. Schematic representation of the thermodynamic cycle to calculate the energy components

vacuum (the intermolecular component $\Delta G_{\rm im}$) and in the aqueous phase (the solvation component $\Delta G_{\rm solv}$), with those components being coupled by the equation $\Delta G_{\rm total}{=}\Delta G_{\rm im}{+}\Delta G^{X_2}_{\rm solv} - 2\Delta G^{X}_{\rm solv}$. The latter relation can be simplified:

$$\Delta G_{\rm total} = \Delta G_{\rm im} + \Delta G_{\rm solv}, \qquad (3)$$

where the quantity $\Delta G_{\rm solv} = \Delta G^{X_2}_{\rm solv} - 2\Delta G^{X}_{\rm solv}$ reflects the contribution made by the interaction with a solvent.

Therefore, Eqs. (2) and (3) provide two strategies for the total energy partition, namely:

1) partition in terms of physical factors that are involved into the complexation process (Eq. (2)), and

2) partition in terms of interactions between molecules (in vacuum) and interactions with a solvent (Eq. (3)).

Generally speaking, the calculation procedures for each energy component in Eqs. (2) and (3) are well developed both at the *ab initio* and semiempirical levels (see review [18]). The application of *ab initio* methods requires that the considered static structures should be well studied, which confines the scope of consideration to rather simple molecules and makes the methods ineffective for studying the interaction with a solvent. Semiempirical methods, which are based on molecular dynamics (MD) simulations, have a limited accuracy, but give solutions to those problems and involve the aqueous environment and the actual energy averaging in time due to the thermal motion. According to our data, a unique successful attempt to resolve the total energy of the aromatic molecule stacking in a solution was made, while combining the *ab initio* and semiempirical approaches, although this task was solved only for a single type of relatively simple aromatic molecules of ferroquine and chloroquine [8].

In this work, we used a combination of various semiempirical approaches to demonstrate its capability to partition the total energy for a collection of molecules with different structures and charges.

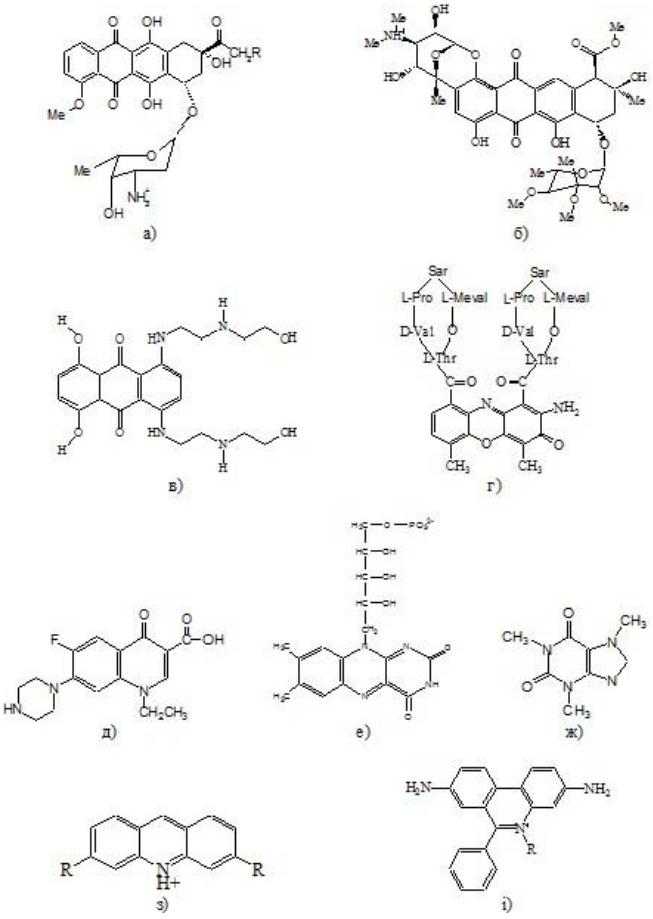


Fig. 2. Structures of molecules concerned: (*a*) daunorubicin (DAU, R=H) and doxorubicin (DOX, R=OH), (*b*) nogalamycin (NOG), (*c*) mitoxantrone (NOV), (*d*) actinomycin D (AMD), (*e*) norfloxacin (NOR), (*f*) flavin-mononucleotide (FMN), (*g*) caffeine (CAF), (*h*) proflavine (PF, R=$NH_2$) and acridine orange (AO, R=$N(CH_3)_2$), (i) ethidium bromide (EB, R=$CH_2CH_3$) and propidium iodide (PI, R=$(CH_2)_3N^+(CH_2CH_3)_2CH_3$)

### *2.2. Selection of molecules to study*

In this work, we studied aromatic molecules (hereafter, ligands, Fig. 2) which form self-associates by means of the $\pi$-$\pi$ stacking, which was confirmed by various experimental methods (see works [19, 20] and the references therein):

– antibiotics: daunorubicin (DAU), doxorubicin (DOX), nogalamycin (NOG), mitoxantrone (NOV), norfloxacin (NOR), and actinomycin D (AMD);

– mutagens: proflavine (PF), acridine orange (AO), ethidium bromide (EB), and propidium iodide (PI);

– nutritional molecules: caffeine (CAF) and vitamin flavin mononucleotide (FMN).

Within the context of the energy partition problem, the following reasons dictated the choice of those molecules.

1. The noncovalent intermolecular association comprises the basic molecular mechanism, by which aromatic compounds change their biological activity, if being in combination with one another [20]. It enhances the solubility of low-soluble compounds [21] and creates some supramolecular structures, which is important from both biological and technological viewpoints [22]. Therefore, it is important to know the contributions of various physical factors to the association energy in order to understand the mechanism that ensures the stability of such complexes in an aqueous medium.

2. For all those molecules, the self-associate structures and the magnitudes of Gibbs free energy were obtained under the identical-solvent condition and analyzed using the same methods. The corresponding results are quoted in the literature (see the references in Table 5), being high-quality initial data for the further analysis.

### *2.3. Molecular Dynamics*

The thermal dynamics of self-associates created by aromatic molecules was calculated with the use of the X-PLOR software package [23]. All structures of the complexes that were used in this work were obtained by minimizing the energy in an aqueous box with regard for (as initial constraints) induced proton chemical shifts and proton–proton cross-peaks of the Overhauser effect, as was described in work [24]. The geometry of complexes was optimized by minimizing the potential energy in the framework of the conjugate gradient method. While calculating the van der Waals and electrostatic interactions, we used a switching or shifting, respectively, function with a cutoff radius of 12 Å [25]. The atomic charges of all ligands were calculated using the Merz–Kollman method [26] at the level of the density functional theory (B3LYP functional) with the 6–31G* basis set. The parameters of noncovalent interactions corresponded to the MM3 force field [27].

At the first stage of energy minimization, the coordinates of ligand atoms were fixed to facilitate the water molecules to relax to their equilibrium positions. The second stage of energy minimization was carried out for fixed water molecules. The final stage of geometry optimization was carried out without imposing any restrictions on the motions of atoms in the system.

After the potential energy had been minimized, the MD procedure was executed according to the Verlet algorithm [28] at a constant temperature of 298 K. For a time step of 2 fs to be used, we restricted the motions of hydrogen atoms with the help of the SHAKE procedure [29]. In the course of MD simulation, the molecules in the external layer of the aqueous box were fixed to prohibit water molecules from escaping into external vacuum. The time of evolution for every system was selected to be 80 ps. The coordinates of every atom were registered every 1 ps.

### *2.4. Calculation of the van der Waals energy*

Van der Waals (VdW) interactions are enthalpic by their nature. In this work, the VdW energy was calculated taking advantage of the Lennard-Jones potential which implicitly takes into account the dispersion, inductive, and orientation components, as well as a repulsion between atomic shells, being the most used one at the molecular simulation of interactions in an aqueous medium:

$$G_{\mathrm{VdW}} = \frac{A}{r^{12}} + \frac{B}{r^{6}}, \tag{4}$$

where $r$ is the distance between interacting atoms; and $A$ and $B$ are the repulsion and attraction parameters, respectively, which depend on the types of atoms and their chemical environment. The Lennard-Jones potential corresponds to the AMBER force field which is used at the simulation.

The potential $G_{\mathrm{VdW}}$ was calculated by analyzing the MD trajectories in the X-PLOR program, by averaging the system evolution within the last 40 ps.

### *2.5. Calculation of the electrostatic energy*

In this work, the term "electrostatic energy" stands for the energy of interaction between partial charges of ligand atoms, water molecules, and salt ions that are in a solution. The electrostatic energy $\Delta G_{\mathrm{el}}$ was calculated by solving the following nonlinear Poisson–Boltzmann equation (NPBE) with the use of the DelPhi computer program [30] which is widely used now to simulate electrostatic interactions in biomolecular complexes (for more details, see review [31]):

$$\nabla[\varepsilon(\mathbf{r})\nabla\varphi(\mathbf{r})] - \frac{8\pi^2 I}{kT}\sinh[\varphi(\mathbf{r})] + \frac{4\pi\rho_\phi(\mathbf{r})}{kT} = 0, \tag{5}$$

where $\varphi$ is the dimensionless electrostatic potential (in terms of $kT/e$-units) at a point which is described by the radius-vector $\mathbf{r}$; $k$ the Boltzmann constant; $T$ the absolute temperature; $\varepsilon$ the dielectric permittivity of the medium; $\rho_\phi$ the density of fixed charges, i.e. the charges

of the given molecule; and $I$ the macroscopic ionic strength of the solution (far from the given molecule). The value $I = 0.1$ M corresponds to the standard physiological conditions.

For a system simulated by NPBE (5), the quantity $\Delta G_{\rm el}$ can be calculated by integrating over the volume,

$$G_{\rm el} = \iiint\limits_{\infty} \left\{ \frac{\rho_\phi \varphi_\phi}{2} + \rho_\phi \varphi_M + \frac{\rho_M \varphi_M}{2} - \Big(\rho_M \varphi + \right.$$

$$\left. + kTc[2\cosh(\varphi) - 2]\Big) \right\} dV, \qquad (6)$$

where $\varphi_\phi$ and $\varphi_M$ are potentials induced by fixed and mobile (ionic) charges, respectively, so that $\varphi_\phi + \varphi_M = \varphi$; $c$ is the salt concentration; and $\rho_M$ is the density of mobile charges.

The NPBE involves a modification of the electrical properties of molecules in the nearest hydration sphere that takes place at the complexation, which makes this method the most applicable at researching the electrostatic interactions in aqueous solutions [30]. The hydration layer separates the region in the molecular volume, where the dielectric permittivity is low, from the solvent region with $\varepsilon_e = 80$. In the NPBE method, the solvent is specified implicitly, and the finite difference method is used to solve Eq. (5). The polarization of ligands was also taken into account implicitly by setting the internal dielectric permittivity of molecules and their complexes $\varepsilon_i = 4$. The values of VdW radii necessary to calculate the molecular surface correspond to the AMBER force field [32]. The technique used in this work to calculate $\Delta G_{\rm el}$ was described in more details in work [31].

### 2.6. Calculation of the hydrophobic energy

The hydrophobic stabilization of complexes is a result of the water displacement from the complex's volume into a free solvent. Therefore, the hydrophobic energy has mainly the entropic character.

The calculation of the hydrophobic contribution was based on a linear correlation between the hydrophobic dissolution energy and a variation of the solvent-accessible surface area (SASA) $\Delta A$ (see review [33]):

$$\Delta G_{\rm hyd} = \gamma \Delta A, \qquad (7)$$

where $\gamma$ is the microscopic surface tension coefficient. Different authors adopted different $\gamma$-values, although, in the last years, the majority of explorers used $\gamma =$ 50 cal/(mol $\times$ Å$^2$) (for review, see work [15]). It is important to emphasize that the coefficient $\gamma$ is most often determined from the distribution in an aqueous-organic phase [33]; in this connection, both the enthalpic components of the "water–water" interaction energy variation at the complexation and the entropic component of the hydrogen bond formation in water are already taken into account—however, partially.

The area of the surface accessible to a solvent was calculated, by using the GETAREA 1.1 computer program [34]. The SASA is defined as a geometric place of the center of a trial sphere with the radius equal to the van der Waals radius of water oxygen ($\approx$ 1.4 Å), when the sphere moves over the surface confined by van der Waals surfaces of the given molecule.

### 2.7. Calculation of the hydrogen bond energy

The energy of a hydrogen bond includes the van der Waals and electrostatic components, as well as specific factors of the quantum-mechanical origin. Often, when analyzing the complexation energetics, the H-bond is conditionally distinguished as a separate kind of interaction, which is characterized by a high specificity to the complex structure.

The variation of a hydrogen binding contribution at the complexation of aromatic molecules is governed by two phenomena:
1) formation of intermolecular H-bonds between ligands in the 1:1-complex, and
2) losses of H-bonds together with water losses owing to the ligand dehydration at the complex formation.

The number of H-bonds of the first type, $N_{\rm im}$, that are formed in the complex can be obtained from the calculated structure, and it can be verified using the literature data. The averaged energy of intermolecular H-bonds in an environment with a low dielectric permittivity (the complex) is equal to approximately $-9$ kcal/mol [35]. Hence, the energy contribution (enthalpic by its origin) of intermolecular H-bonds can immediately be estimated as $\Delta G_{\rm im} = \Delta H_{\rm im} = -N_{\rm im} \times 9$ kcal/mol.

The case of H-bonds of the second type is more complicated for the evaluation. In this work, in order to determine the energy of H-bonds between ligands and water, we calculated the average number of water molecules which form hydrogen bonds with hydrophilic atoms of molecules concerned (the hydration factor $N_{\rm solv}$) during the last 40 ps of the MD simulation. The presence of a hydrogen bond was registered, if the distance between the electronegative ligand atoms and the water oxygen or hydrogen atoms did not exceed 3.2 or 2.4 Å, respectively

[36]. The difference between an intermolecular H-bond and an H-bond with water is connected with a considerable negative entropic contribution to the free energy of the latter, owing to the loss of the translational and rotational degrees of freedom of water molecules engaged in an H-bond [35, 37]. The averaged Gibbs energy in the hydrogen binding with water is lower by absolute value than the enthalpy of an intermolecular H-bond in a medium with low dielectric permittivity, amounting to about −6 kcal/mol [35]. Hence, the corresponding Gibbs energy is $\Delta G_{\rm solv} = -\Delta N_{\rm solv} \times 6$ kcal/mol, and we obtain the following expression for the hydrogen bond energy:

$$\Delta G_{\rm HB} = -(9N_{\rm im} + 6\Delta N_{\rm solv}),\ \text{kcal/mol}. \tag{8}$$

However, in the scope of the given methodology, expression (8) cannot be used directly to calculate the H-bond energy in Eq. (2). First, the entropic component of the H-bond energy has already been taken into account, while calculating the hydrophobic contribution (see above). Second, it is well known that the electrostatic and (to a lower extent) van der Waals energies are the main contributors to the H-bond energy [38]. This means that the calculation of those energies by the technique described above already takes partially the enthalpic component of the H-bond energy into account. As was done in the previous work [15], we adopt that about 25% of the H-bond energy is underestimated, when calculating the van der Waals and electrostatic interactions. The average enthalpy of the formation of a hydrogen bond with water is very close to the energy of an intermolecular H-bond [35]. This means that the final expression for the hydrogen-bond component $\Delta\Delta G_{\rm HB}$, when being added into expression (2) instead of the term $\Delta G_{\rm HB}$, looks like

$$\Delta\Delta G_{\rm HB} = -0.25 \times 9(N_{\rm im} + \Delta N_{\rm solv}),\ \text{kcal/mol}. \tag{9}$$

It is worth noting that, although this method for the calculation of hydrogen bond energies is rather approximate, it was successfully applied by us earlier to partition the energies of complexation reactions between ligands and DNA [15], which gives grounds for its application in the considered case of the stacking of aromatic molecules as well. Moreover, the evaluation of the extra energy per one H-bond ($N_{\rm im} + \Delta N_{\rm solv} = 1$) by formula (9) brings about approximately 2 kcal/mol, which coincides with a similar estimation of the hydrogen binding energetics which can be determined experimentally for simple molecules [35].

### *2.8. Calculation of the entropic contribution*

The total entropic contribution to the Gibbs free energy of the complexation of aromatic molecules is a sum of three main components,

$$\Delta G_{\rm entr} = \Delta G_{\rm tr} + \Delta G_{\rm rot} + \Delta G_{\rm vib}, \tag{10}$$

where $\Delta G_{\rm tr}$, $\Delta G_{\rm rot}$, and $\Delta G_{\rm vib}$ are the free energy changes for the translational, rotational, and vibrational degrees of freedom, respectively, at the complexation.

The components $\Delta G_{\rm tr}$ and $\Delta G_{\rm rot}$ are associated with the loss of three translational and three rotational degrees of freedom at the complexation. From the general point of view [9], the component $\Delta G_{\rm vib}$ corresponds to a variation of the vibrational energy at the complexation, which gives rise to the formation of new vibrational modes. However, it is evident that, in this case, the dominating contributors for molecules without massive side chains are rigid aromatic chromophores. Hence, one should expect that, at the self-association and the stabilization of a 1:1-complex by noncovalent forces, the molecules can possess residual vibrational motions in a complex. Earlier, such motions were found for the complexes of ligands with DNA and proteins [15]. Therefore, the distinguishing of components $\Delta G_{\rm tr}$ and $\Delta G_{\rm rot}$, which correspond to a total loss of degrees of freedom, in the structure of $\Delta G_{\rm entr}$ is conditional and could be valid, only we take additionally the energetics of residual molecular motions in complexes into account in Eq. (10). One of the probable approaches to the solution of this problem is related to the account of residual motions in the form of low-frequency vibrations [39]. Hence, the vibrational contribution to the association energy can be separated into two components:

$$\Delta G_{\rm vib} = \Delta G^{\rm I}_{\rm vib} + \Delta G^{\rm II}_{\rm vib}, \tag{11}$$

where $\Delta G^{\rm I}_{\rm vib}$ and $\Delta G^{\rm II}_{\rm vib}$ correspond to the energy changes of chemical bond vibrations (vibrations of the first kind) and mechanical vibrations (vibrations of the second kind), respectively. Since the classical vibration frequency is reciprocal to the mass, the vibrations of the first and second kinds can also be classed as high- and low-frequency ones, respectively.

The variations of the Gibbs free energies of translational and rotational degrees of freedom can be written down in the standard form:

$$\Delta G_{\rm tr} = \Delta H_{\rm tr} - T\Delta S_{\rm tr}, \Delta G_{\rm rot} = \Delta H_{\rm rot} - T\Delta S_{\rm rot}, \tag{12}$$

where $\Delta H_{\rm tr} = \Delta H_{\rm rot} = -\frac{3}{2}RT$ are the enthalpic equivalents of variations of the translational and rotational

degrees of freedom, respectively; $R$ is the gas constant; and $T$ is the absolute temperature.

The molar translational entropy can be found from the Sackur–Tetrode equation [40]

$$S_{\rm tr} = R\left[\frac{5}{2} + \frac{3}{2}\ln\frac{2\pi mkT}{h^2} - \ln\frac{N}{V}\right] \tag{13}$$

where $N = N_A = 6.02{\times}10^{23}$ mol$^{-1}$; $V = 10^{-3}$ m$^3$; $k$ and $h$ are the Boltzmann and Planck constants, respectively; and $m$ is the molecule mass.

With the help of Eq. (13), the expressions for the entropies of a self-associate $X_2$, $S_{\rm tr}^{X_2}$, and a free molecule $X$, $S_{\rm tr}^{X}$, can be written down, which allows an expression for a variation of the translational entropy to be obtained:

$$\Delta S_{\rm tr} = S_{\rm tr}^{X_2} - 2S_{\rm tr}^{X} =$$

$$= -R\left[\frac{5}{2} + \frac{3}{2}\ln\frac{\pi mkT}{h^2} - \ln\frac{N}{V}\right]. \tag{14}$$

The expression for the molar rotational entropy also follows from classical statistical thermodynamics [40]:

$$S_{\rm rot} = R\left[\frac{3}{2} + \frac{1}{2}\ln\pi I_x I_y I_z + \frac{3}{2}\ln\frac{8\pi^2 kT}{h^2} - \ln\sigma\right], \tag{15}$$

where $I_x$, $I_y$, and $I_z$ are the inertia moments with respect to the main inertia axes; $\sigma$ is the parameter of symmetry which equals 1 for nonsymmetric complexes. The moments for the ligands studied in this work were calculated with the help of the X-PLOR computer program.

The expressions for the entropy and the enthalpy of vibrations of the first kind (vibrations of chemical bonds) in the harmonic approximation follow from the classical statistical thermodynamics [40]:

$$S_{\rm vib}^{I} = \frac{1}{T}\sum_{j=1}^{3N-6}\left\{\frac{h\nu_j}{e^{h\nu_j/kT}-1} - kT\ln\left(1-e^{-h\nu_j/kT}\right)\right\},$$

$$H_{\rm vib}^{I} = \sum_{j=1}^{3N-6}\left(\frac{h\nu_j}{e^{h\nu_j/kT}-1} + \frac{h\nu_j}{2}\right), \tag{16}$$

where $N$ is the number of atoms, and $\nu_j$ are the frequencies of normal modes calculated with the help of the Gaussian03W software package and the PM3 method.

Hence, the variations of thermodynamical parameters in the self-association reaction look like

$$\Delta S_{\rm vib}^{\rm I} = S_{\rm vib}^{X_2} - 2S_{\rm vib}^{X}, \Delta H_{\rm vib}^{\rm I} = H_{\rm vib}^{X_2} - 2H_{\rm vib}^{X},$$

$$\Delta G_{\rm vib}^{\rm I} = \Delta H_{\rm vib}^{\rm I} - T\Delta S_{\rm vib}^{\rm I}. \tag{17}$$

The expressions for the variations of thermodynamic parameters of the second-kind (low-frequency) vibrations were obtained earlier for the complexation of ligands with DNA [15]. They can be applied to the self-association reaction, provided that residual rotational motions of molecules in the complexes are insignificant, and the vibrations are harmonic:

$$\Delta G_{\rm vib}^{\rm II} = \Delta H_{\rm vib}^{\rm II} - T\Delta S_{\rm vib}^{\rm II}, \Delta H_{\rm vib}^{\rm II} = RT,$$

$$\Delta S_{\rm vib}^{\rm II} = R\ln\frac{kT}{h\nu_r} + R. \tag{18}$$

The parameter $\nu_r$ in Eq. (18) is the classical frequency of mechanical oscillations along the coordinate axes $r \in (x, y, z)$,

$$\nu_r = \frac{1}{2\pi}\sqrt{\frac{2K_r}{m_{\rm red}}}, \tag{19}$$

where $K_r$ is the force coefficient, and $m_{\rm red}$ is the reduced mass of interacting molecules which is determined from the relation $\frac{1}{m_{\rm red}} = \frac{1}{m} + \frac{1}{m} = \frac{2}{m}$.

The quantity $K_r$ can be evaluated using the square-law approximation of the potential energy $U(r)$, provided that the oscillations along the $r$-direction are small:

$$U = U_0 + K_r(r - r_0)^2. \tag{20}$$

The calculation of the $U(r)$-dependence of the total energy of intermolecular interactions in a dimer was carried out, by using the X-PLOR software package. Then, the obtained $U(r)$-dependence was approximated by Eq. (20) to obtain the $K_r$-value. The further calculation of $\nu_r$ on the basis of Eq. (19) allows one to obtain the thermodynamic parameters according to Eqs. (18).

## 3. Results and Discussion

### 3.1. Analysis of the van der Waals energy $\Delta G_{\rm VdW}$

The results of calculations of the component $\Delta G_{\rm VdW}$ are presented in Table 1. One can see that the solvation energies $\Delta G_{\rm solv}^{X}$ and $\Delta G_{\rm solv}^{X_2}$ for a molecule $X$ and a dimer $X_2$, respectively, as well as the energy of intermolecular interactions in vacuum $\Delta G_{\rm im}$, are negative for each studied system. This fact evidences the attractive character of the VdW forces between interacting

molecules (solvent–ligand for the solvation energy, and ligand–ligand for the intermolecular one). However, the total variation of the solvation energy $\Delta G_{\rm solv}$ in reaction (1) is always positive and caused by the loss of favorable VdW contacts between a ligand and water molecules at the formation of a complex. This effect is well known as "desolvation" for the reactions where a ligand binds with DNA.

It is important to note that the solvation component, $\Delta G_{\rm solv}$, and the intermolecular one, $\Delta G_{\rm im}$, (see Table 1) have opposite signs, being close by absolute values. This results in a small total VdW energy $\Delta G_{\rm VdW}$, the sign of which depends on the type of interacting molecules. A similar conclusion was drawn earlier for the complexation of aromatic ligands with DNA (see work [15] and the references therein). As a result, two main conclusions can be made:

1. The total VdW energy $\Delta G_{\rm VdW}$ of the stacking of aromatic compounds is governed by a fine balance between the intermolecular interaction and the interaction with a solvent. Since the value of $\Delta G_{\rm VdW}$ is the difference of two large numbers, the analysis of the total VdW energy $\Delta G_{\rm VdW}$ is hardly significant: only the analysis of its solvation, $\Delta G_{\rm solv}$, and intermolecular, $\Delta G_{\rm im}$, components can have a physical sense.

2. However, the viewpoint that VdW forces give no contribution to the stabilization of the stacking of aromatic compounds is incorrect. As was demonstrated above, the component $\Delta G_{\rm im}$ provides an important contribution to the stabilization of dimer complexes. This fact is also confirmed by the results of quantum-mechanical calculations dealing with interactions between various aromatic compounds [13, 41, 42].

### *3.2. Analysis of the electrostatic energy* $\Delta G_{\rm el}$

The results of calculations of the component $\Delta G_{\rm el}$ are presented in Table 2. The solvation energies $\Delta G^{X}_{\rm solv}$ and $\Delta G^{X_2}_{\rm solv}$ have the same qualitative tendency that was described above for the VdW energy, namely, the favorable (due to attraction) character of interactions between $X$ and $X_2$, on the one hand, and the solvent molecules, on the other hand, is determined by ion-dipole and/or dipole-dipole interactions. It is important to emphasize that $\Delta G_{\rm solv}$ is a difference of two large numbers, i.e. $\Delta G_{\rm solv} = \Delta G^{X_2}_{\rm solv} - 2\Delta G^{X}_{\rm solv}$. Therefore, in contrast to the solvation VdW energy, the solvation electrostatic energy $\Delta G_{\rm solv}$ can be both positive and negative for various systems. In this case, there is no pronounced "desolvation" effect, which could be expected, if the favorable electrostatic, as well as VdW, interactions "water–ligand" at the complexation would be partially eliminated.

The analysis of $\Delta G_{\rm solv}$-values presented in Table 2 allowed us to reveal their correlation with the charges of interacting molecules. The $\Delta G_{\rm solv}$-value is always negative, if ligand molecules are charged. The largest values of $\Delta G_{\rm solv}$ are observed for dimers of FMN and PI molecules which are double-charged negatively and positively, respectively. This phenomenon can be explained, if one takes into consideration that the charge of a complex formed by two molecules with identical charges increases. This circumstance results in energetically favorable interactions with a solvent. This effect prevails over the unfavorable energy of desolvation to give the overall negative total energy $\Delta G_{\rm el}$. Such a behavior is similar to that observed earlier at studying the self-association of some aromatic ligands [8] and their complexation with duplex DNA [31, 43].

**T a b l e 1. Calculated components of the van der Waals energy (kcal/mol)**

| Compound | $\Delta G^{X}_{\rm solv}$ | $\Delta G^{X_2}_{\rm solv}$ | $\Delta G_{\rm solv}$ | $\Delta G_{\rm im}$ | $\Delta G_{\rm VdW}$ |
|---|---|---|---|---|---|
| AMD | -117.3 | –197.7 | 36.9 | –32.9 | 4.0 |
| DAU | –55.6 | –77.3 | 33.9 | –33.7 | 0.2 |
| DOX | –55.5 | –78.4 | 32.7 | –31.5 | 1.1 |
| NOG | –79.9 | –121.5 | 38.3 | –40.5 | –2.2 |
| NOR | –41.2 | –63.1 | 19.2 | –18.1 | 1.1 |
| NOV | –55.3 | –83.5 | 27.2 | –26.5 | 0.7 |
| AO | –31.4 | –45.4 | 17.3 | –15.6 | 1.7 |
| EB | –37.4 | –54.0 | 20.8 | –18.5 | 2.3 |
| PF | –23.2 | –36.5 | 9.9 | –12.0 | –2.0 |
| PI | –58.4 | –93.8 | 22.9 | –19.1 | 3.8 |
| CAF | –26.1 | –40.6 | 11.5 | –13.7 | –2.1 |
| FMN | –54.2 | –82.9 | 25.4 | –30.1 | –4.7 |

**T a b l e 2. Calculated components of the electrostatic energy (kcal/mol)**

| Compound | Charge | $\Delta G^{X}_{\rm solv}$ | $\Delta G^{X_2}_{\rm solv}$ | $\Delta G_{\rm solv}$ | $\Delta G_{\rm im}$ | $\Delta G_{\rm el}$ |
|---|---|---|---|---|---|---|
| AMD | 0 | –1724.9 | –3449.2 | 0.5 | 0.8 | 1.3 |
| DAU | +1 | –660.7 | –1328.1 | –6.7 | 9.9 | 3.1 |
| DOX | +1 | –797.4 | –1602.6 | –7.7 | 11.6 | 3.9 |
| NOG | +1 | –939.9 | –1885.9 | –6.1 | 11.2 | 5.1 |
| NOR | 0 | –276.6 | –551.5 | 1.6 | –1.3 | 0.3 |
| NOV | 0 | –591.7 | –1179.4 | 4.1 | –3.9 | 0.2 |
| AO | +1 | –165.2 | –341.7 | –11.2 | 12.2 | 1.0 |
| EB | +1 | –427.5 | –864.5 | –9.6 | 11.4 | 1.9 |
| PF | +1 | –379.8 | –770.5 | –10.9 | 12.3 | 1.4 |
| PI | +2 | –483.1 | –998.2 | –32.0 | 35.2 | 3.2 |
| CAF | 0 | –164.1 | –328.2 | 0.0 | 0.5 | 0.6 |
| FMN | –2 | –811.2 | –1651.3 | –29.0 | 36.4 | 7.4 |

The energy of electrostatic intermolecular interactions $\Delta G_{\rm im}$ can also be examined in terms of its correlation with the charges of interacting molecules. The $\Delta G_{\rm im}$-values are always positive and rather large, if the ligand molecules are charged; i.e. it is a simple effect of electrostatic repulsion. The highest $\Delta G_{\rm im}$-values were expectedly observed for FMN and PI dimers, which also corresponds to the largest energy $\Delta G_{\rm solv}$ for those systems.

In general, a conclusion can be drawn that the electrostatic solvation and intermolecular energies manifest the pronounced dependence on the charges of interacting molecules. On the contrary, the total electrostatic energy $\Delta G_{\rm el}$ is low; it is positive for the majority of studied systems and does not correlate with the type and charge of molecules. This means that the analysis of $\Delta G_{\rm el}$ has no physical significance for the stacking interaction between aromatic compounds; it was pointed out above in the case of the total VdW energy and had been reported earlier for the intercalation of ligands into DNA (see review [31]) and for the stacking of nitrogen bases [44].

The analysis of the results obtained for aromatic systems and presented in Table 2 allows a conclusion to be drawn that the electrostatic stabilization of self-associates is governed by the interaction with a solvent, whereas the VdW interaction always has the intermolecular nature.

### *3.3. Analysis of the hydrophobic energy* $\Delta G_{\rm hyd}$

The results of calculations for $\Delta A$ and $\Delta G_{\rm hyd}$ are presented in Table 3. For all examined systems, the hydrophobic contribution is energetically favorable and, by absolute value, is larger, on the average, than the VdW and electrostatic components in $\Delta G_{\rm solv}$ and $\Delta G_{\rm im}$, which affects the stabilization of complexes (see Tables 1 and 2, and the discussion above). This result agrees, in general, with the viewpoint [45] that the classical hydrophobic effect stabilizes the stacking of aromatic compounds in an aqueous solution, and it can be explained by a reduction of $\Delta A$ for interacting molecules at the formation of a complex (see Table 3).

**T a b l e 3. Calculated components of the hydrophobic and entropic energies ($\Delta G$, kcal/mol) and variations of the surface area accessible to a solvent ($\Delta A$, Å$^2$)**

| Compound | Hydrophobic | | Entropic | | | | |
|---|---|---|---|---|---|---|---|
| | $\Delta A$ | $\Delta G_{\rm hyd}$ | $\Delta G_{\rm tr}$ | $\Delta G_{\rm rot}$ | $\Delta G^{\rm I}_{\rm vib}$ | $\Delta G^{\rm II}_{\rm vib}$ | $\Delta G_{\rm entr}$ |
| AMD | –552.9 | –27.6 | 10.7 | 10.2 | –2.9 | –8.1 | 9.9 |
| DAU | –532.0 | –26.6 | 9.9 | 9.5 | –4.3 | –9.2 | 5.9 |
| DOX | –428.4 | –21.4 | 9.9 | 9.5 | –1.1 | –9.5 | 8.8 |
| NOG | –566.9 | –28.3 | 10.3 | 10.6 | –3.0 | –8.7 | 9.2 |
| NOR | –252.4 | –12.6 | 9.4 | 8.6 | –3.8 | –10.4 | 3.8 |
| NOV | –400.2 | –20.0 | 9.8 | 9.6 | –2.0 | –8.1 | 9.3 |
| AO | –283.1 | –14.2 | 9.3 | 8.1 | –3.6 | –8.9 | 4.8 |
| EB | –317.5 | –15.9 | 9.5 | 8.6 | –2.8 | –10.7 | 4.5 |
| PF | –234.8 | –11.7 | 9.1 | 7.5 | –3.0 | –10.4 | 3.2 |
| PI | –319.3 | –16.0 | 9.7 | 8.9 | –1.6 | –11.3 | 5.7 |
| CAF | –217.2 | –10.9 | 9.0 | 7.4 | –2.0 | –10.7 | 3.7 |
| FMN | –339.1 | –17.0 | 9.8 | 9.1 | –2.6 | –9.5 | 6.7 |

### *3.4. Analysis of the hydrogen bonding energy* $\Delta G_{\rm HB}$

The results of calculations of the component $\Delta G_{\rm HB}$ are summarized in Table 4. The average number of water molecules (the hydration factor $N_{\rm solv}$) that form H-bonds with a ligand molecule depends on the number of hydration sites and correlates qualitatively with the molecular mass of molecules concerned, being the largest for AMD, DAU, and DOX molecules, and the smallest for CAF and PF ones. As was expected, a change of the hydration factor, $\Delta N_{\rm solv}$, at the complexation is negative, which evidences the removal of water molecules and the corresponding loss of H-bonds with water (desolvation). This variation agrees with the behavior of the solvation component $\Delta G_{\rm solv}$, which was discussed above for the VdW, electrostatic, and hydrophobic interactions. Desolvation is partially compensated by the formation of intermolecular H-bonds, as it takes place in the case of antibiotic NOV ($N_{\rm im} = 2$), although the cooperative effect ($\Delta G_{\rm HB}$) of hydrogen binding is en-

**T a b l e 4. Calculated components of the hydrogen bonding energy ($\Delta G$, kcal/mol) and the hydration factors ($N$)**

| Compound | $\Delta N^{X}_{\rm solv}$ | $\Delta N^{X_2}_{\rm solv}$ | $\Delta N_{\rm solv}$ | $N_{im}$ | $\Delta G_{\rm HB}$ | $\Delta\Delta G_{\rm HB}$ |
|---|---|---|---|---|---|---|
| AMD | 11.8 | 21.5 | –2.1 | 0 | 12.8 | 4.8 |
| DAU | 12.4 | 19.2 | –5.6 | 0 | 33.7 | 12.6 |
| DOX | 12.8 | 23.4 | –2.2 | 0 | 13.4 | 5.0 |
| NOG | 10.8 | 16.5 | –5.1 | 0 | 30.5 | 11.4 |
| NOR | 4.6 | 7.5 | –1.8 | 0 | 10.6 | 4.0 |
| NOV | 8.8 | 14.3 | –3.3 | 2 | 1.6 | 2.9 |
| AO | 2.8 | 5.7 | 0.0 | 0 | 0.0 | 0.0 |
| EB | 5.9 | 10.6 | –1.2 | 0 | 7.3 | 2.7 |
| PF | 6.4 | 10.7 | –2.1 | 0 | 12.8 | 4.8 |
| PI | 1.6 | 2.4 | –0.8 | 0 | 5.0 | 1.9 |
| CAF | 1.5 | 1.1 | –1.9 | 0 | 11.7 | 4.4 |
| FMN | 3.2 | 4.4 | –2.0 | 0 | 12.2 | 4.6 |

F o o t n o t e: values for the number of intermolecular hydrogen bonds $N_{\rm im}$ were taken from the references in Table 5

**T a b l e 5. Calculated components of the total energy (kcal/mol)**

| Compound | $\Delta G_{\mathrm{VdW}}$ | $\Delta G_{\mathrm{el+HB}}$ | $\Delta G_{\mathrm{hyd}}$ | $\Delta G_{\mathrm{entr}}$ | $\Delta G_{\mathrm{total}}$ | $\Delta G_{\mathrm{exp}}$ | $\lvert\Delta G_{\mathrm{total}} - \Delta G_{\mathrm{exp}}\rvert$ |
|---|---|---|---|---|---|---|---|
| AMD | 4.0 | 6.0 | –27.6 | 9.9 | –7.7 | –4.3 [20] | 3.4 |
| DAU | 0.2 | 15.8 | –26.6 | 5.9 | –4.7 | –3.9 [20] | 0.8 |
| DOX | 1.1 | 8.9 | –21.4 | 8.8 | –2.5 | –4.4 [20] | 1.9 |
| NOG | –2.2 | 16.5 | –28.3 | 9.2 | –4.7 | –5.1 [20] | 0.4 |
| NOR | 1.1 | 4.3 | –12.6 | 3.8 | –3.5 | –2.9 [46] | 0.6 |
| NOV | 0.7 | 3.1 | –20.0 | 9.3 | –6.9 | –6.1 [20] | 0.8 |
| AO | 1.7 | 1.0 | –14.2 | 4.8 | –6.6 | –5.1 [19] | 1.5 |
| EB | 2.3 | 4.6 | –15.9 | 4.5 | –4.4 | –3.4 [20] | 1.0 |
| PF | –2.0 | 6.2 | –11.7 | 3.2 | –4.4 | –3.8 [20] | 0.6 |
| PI | 3.8 | 5.0 | –16.0 | 5.7 | –1.4 | –2.5 [19] | 1.1 |
| CAF | –2.1 | 4.9 | –10.9 | 3.7 | –4.4 | –1.5 [20] | 2.9 |
| FMN | –4.7 | 12.0 | –17.0 | 6.7 | –2.9 | –3.3 [20] | 0.4 |

ergetically unfavorable for the examined complexes of aromatic compounds.

### *3.5. Analysis of the entropic contribution*

The results of calculations of the component $\Delta G_{\mathrm{entr}}$ are presented in Table 3. Expectedly, the energies that correspond to the losses of the translational, $\Delta G_{\mathrm{tr}}$, and rotational, $\Delta G_{\mathrm{rot}}$, degrees of freedom are unfavorable, whereas those associated with the formation of new vibrational modes of chemical bonds, $\Delta G^{\mathrm{I}}_{\mathrm{vib}}$, and mechanical vibrations, $\Delta G^{\mathrm{II}}_{\mathrm{vib}}$, are favorable. It is important that all the four entropic components are close by absolute value to the experimental energy $\Delta G_{\mathrm{exp}}$ measured at the formation of a complex (see Table 5). Therefore, the account of those components, while analyzing the stacking interaction energy between aromatic compounds, is obligatory.

The total contribution of all entropic factors $\Delta G_{\mathrm{entr}}$ turns out positive owing to the energetically unfavorable losses of the translational and rotational degrees of freedom.

### *3.6. Analysis of the total dimerization energy*

In Table 5, the experimental energy $\Delta G_{\mathrm{exp}}$, the total calculated energy $\Delta G_{\mathrm{total}}$, and the contributions of various physical factors involved into the complexation of considered molecules to the total energy are presented. Since the quantity of $\Delta\Delta G_{\mathrm{HB}}$ has no special physical meaning, and the electrostatic component is the major contributor to the hydrogen binding energy, those two contributions were combined in Table 5 as $\Delta G_{\mathrm{el}} + \Delta\Delta G_{\mathrm{HB}} = \Delta G_{\mathrm{el+HB}}$.

It is necessary to determine whether the correspondence between experimental and theoretical values is satisfactory. Though it is difficult to calculate the error of the calculated absolute energies precisely, we may expect that the error for the total energies would amount to a few kcal/mol. The previous researches also confirmed that an error of about a few kcal/mol is typical, when using the implicit solvent model (the NPBE method) with charged ligand molecules [8], in analogy with our case. The difference $|\Delta G_{\mathrm{exp}} - \Delta G_{\mathrm{total}}|$ between the experimental and theoretical free energies for each molecular system is quoted in Table 5. The main result of our analysis is the fact that there is a satisfactory agreement (to within a few kcal/mol) between the theory and the experiment for each considered system. In particular, the average deviation of calculation results from experimental ones amounts to $|\Delta G_{\mathrm{exp}} - \Delta G_{\mathrm{total}}| = 1.3$ kcal/mol and the average spread of experimental values is $\left|\Delta G_{\mathrm{exp}} - \overline{\Delta G_{\mathrm{exp}}}\right| = 1.0$ kcal/mol. The maximal mismatch is observed for the antibiotic actinomycin D, which may be associated with an extra influence of massive pentapeptide lactone rings (see Fig. 2,*d*), which was not taken into consideration in the energy analysis. In general, this means that the methodology used in this work "traces" the experiment, and that the analysis of the dependence of energy components on various physical factors is significant from the physical viewpoint. It is also important to note that the algorithm applied in this work to partition the energy is based on various methods independent of one another: nonempirical (the NPBE and Eq. (10)), semiempirical (the force field for VdW energies) and empirical ones (Eqs. (7) and (9)). This methodology is free of any systematic error which can be inserted, if only one method is used (e.g., using only MD in energy calculations).

From Table 5, one can see that, among four contributions to the total energy, the hydrophobic one dominates in all the systems under consideration, being also ener-

getically favorable. The main source of the destabilization is a combined contribution of the electrostatics and the hydrogen binding, as well as the loss of degrees of freedom $\Delta G_{\rm entr}$. However, as was discussed above, the analysis of the total energy does not answer the questions "Which physical factors stabilize/destabilize the stacking of aromatic molecules?" and "Which is their relative importance?". Let us illustrate the answer to those questions, by using two different molecular systems as an example. Note also that the contribution of hydrogen bonds and entropic factors is always unfavorable for all systems concerned.

*DAU dimer.* Here, the main stabilization is provided by the intermolecular VdW interactions $\Delta G^{\rm im}_{\rm VdW}$ and, to a less extent, by the hydrophobic interactions $\Delta G_{\rm hyd}$ and the electrostatic interactions with a solvent $\Delta G^{\rm solv}_{\rm el}$. The VdW interactions with a solvent $\Delta G^{\rm solv}_{\rm VdW}$ and the electrostatic intermolecular ones $\Delta G^{\rm im}_{\rm el}$ are energetically unfavorable.

*PI dimer.* Here, the main stabilization is provided by the electrostatic interaction with a solvent $\Delta G^{\rm solv}_{\rm el}$ and, to a less extent, by the hydrophobic interactions $\Delta G_{\rm hyd}$ and the intermolecular ones $\Delta G^{\rm im}_{\rm VdW}$ (two latter ones contribute in the almost 1:1 ratio). The VdW interactions with a solvent $\Delta G^{\rm solv}_{\rm VdW}$ and the electrostatic intermolecular ones $\Delta G^{\rm im}_{\rm el}$ are energetically unfavorable.

In general, the stabilizing and destabilizing factors depend on the specific molecular system under consideration, They can be of the van der Waals, electrostatic, or hydrophobic nature. The results of this work elucidate the role of various physical factors involved in the stabilization of the stacking of aromatic molecules.

## 4. Conclusions

1. The technique has been developed to calculate the free energy of the stacking of aromatic compounds. The free energy is considered to be contributed by a number of physical factors. It is important to note that this technique takes into account the most complete collection of physical interactions in a solution: van der Waals, electrostatic, hydrophobic, and hydrogen bonding (intermolecular and with a solvent) ones, as well as the energy equivalent of the loss and the appearance of degrees of freedom at the complexation. Our approach combines nonempirical, semiempirical, and empirical methods, so that any systematic error that may arise, if only one calculation technique is used, becomes substantially reduced. Another characteristic feature of the method proposed is the successful verification with the experimental data obtained for 12 molecules with different structures and charged states. This means that the magnitudes of calculated components of the total energies are significant, and their analysis has a physical sense. According to our data, there is no analogous technique now to analyze the energies of stacking interactions between aromatic molecules.

2. The analysis of the calculated energies gives the answers to the questions "Which physical factors stabilize/destabilize the stacking of aromatic molecules in the solution?" and "Which is their relative importance?" The stabilizing and destabilizing factors were found to depend on the specific system under consideration. They can have the van der Waals, electrostatic, or hydrophobic origin. At the same time, the contributions of hydrogen bonds and entropic factors are always unfavorable.

We express our gratitude to the anonymous Referee for the constructive remarks.

1. P. Yakovchuk, E. Protozanova, and M.D. Frank-Kamenetskii, Nucleic Acids Res. **34**, 564 (2006).
2. D.E. Graves and L.M. Velea, Curr. Org. Chem. **4**, 915 (2000).
3. T.F.A. De Greef, M.M.J. Smulders, M. Wolffs, A.P.H.J. Schenning, R.P. Sijbesma, E.W. Meijer, Chem. Rev. **109**, 5687 (2009).
4. E. Meyer, R.K. Castellano, and F. Diederich, Angew. Chem. Int. Ed. **42**, 1210 (2003).
5. N. Lane and T.C. Jenkins, Quart. Rev. Biophys. **33**, 255 (2000).
6. H.-J. Schneider, Angew. Chem. Int. Ed. **48**, 3924 (2009).
7. S.L. McKay, B. Haptonstall, and S.H. Gellman, J. Am. Chem. Soc. **123**, 1244 (2001).
8. E. Buisine, K. de Villiers, T.G. Egan, and C. Biot, J. Am. Chem. Soc. **128**, 12122 (2006).
9. S.Yu. Noskov and C. Lim, Biophys. J. **81**, 737 (2001).
10. B. Jayaram, K.J. McConnell, S.B. Dixit, and D.L. Beveridge, J. Comput. Phys. **151**, 333 (1999).
11. I. Haq, Arch. Biochem. Biophys. **403**, 1 (2002).
12. W. Treesuwan, K. Wittayanarakul, N.G. Anthony, G. Huchet, H. Alniss, S. Hannongbua, A.I. Khalaf, C.J. Suckling, J.A. Parkinson, and S.P. Mackay, Phys. Chem. Chem. Phys. **11**, 10682 (2009).
13. S. Tsuzuki, K. Honda, T. Uchimaru, and M. Mikami, J. Chem. Phys. **125**, 124304 (2006)
14. D. Mackie and G.A. DiLabio, J. Phys. Chem. A **112**, 10968 (2008).
15. V.V. Kostjukov, N.M. Khomytova, and M.P. Evstigneev, Biopolymers **91**, 773 (2009).
16. M.P. Evstigneev, V.P. Evstigneev, and D.B. Davies, J. Chem. Phys. **127**, 154511 (2007).

17. R.B. Martin, Chem. Rev. **96**, 3043 (1996).
18. D.L. Mobley and K.A. Dill, Structure **17**, 489 (2009).
19. M.P. Evstigneev, D.B. Davies, and A.N. Veselkov, Chem. Phys. **321**, 25 (2006).
20. M.P. Evstigneev, V.P. Evstigneev, A.O. Lantushenko, Yu.V. Mukhina, and D.B. Davies, Biophys. Chem. **132**, 148 (2008).Mykhina Yu.V., Davies D.B.
21. R. Sangvi, D. Evans, and S.H. Yalkowski, Int. J. Pharm. Sci. **336**, 35 (2007).
22. A.K. Tewari and R. Dubey, Bioorg. Med. Chem. **16**, 126 (2008).
23. T. Brunger, *X-PLOR: A System for X-ray Crystallography and NMR* (Yale University Press, Yale, 1992).
24. M.P. Evstigneev, Yu.V. Mukhina, and D.B. Davies, Mol. Phys. **104**, 647 (2006).
25. R. Brooks, R.E. Bruccoleri, B.D. Olafson, D.J. States, S. Swaminathan, and M. Karplus, J. Comput. Chem. **4**, 187 (1983).
26. E. Sigfridsson and U. Ryde, J. Comput. Chem. **19**, 377 (1998).
27. N.L. Allinger, J. Am. Chem. Soc. **99**, 8127 (1977).
28. L. Verlet, Phys. Rev. **159**, 98 (1967).
29. J.-P. Ryckaert, G. Ciccotti, and H.J.C. Berendsen, J. Comput. Phys. **23**, 327 (1977).
30. W. Rocchia, E. Alexov, and B. Honig, J. Phys. Chem. B **105**, 6507 (2001).
31. V.V. Kostjukov, N.M. Khomytova, D.B. Davies, and M.P. Evstigneev, Biopolymers **89**, 680 (2008).
32. W.D. Cornell, P. Cieplak, C.I. Bayly, I.R. Gould, K.M. Merz, D.M. Ferguson, D.C. Spellmeyer, T. Fox, J.W. Caldwell, and P.A. Kollman, J. Am. Chem. Soc. **117**, 5179 (1995).
33. K.A. Sharp, A. Nicholls, R.F. Fine, and B. Honig, Science **252**, 106 (1991).
34. R. Fraczkiewicz and W. Braun, J. Comput. Chem. **19**, 319 (1998).
35. G.I. Makhatadze and P.L. Privalov, Adv. Protein. Chem. **47**, 307 (1995).
36. A.V. Teplukhin, V.I. Poltev, and V.P. Chuprina, Biopolymers **31**, 1445 (1991).
37. T.V. Chalikian, Biopolymers **70**, 492 (2003).
38. P.A. Kollman and L.C. Allen, Chem. Rev. **72**, 283 (1972).
39. V.V. Kostjukov, N.M. Khomytova, and M.P. Evstigneev, Khim. Fiz. **28**, 26 (2009).
40. A. Maczek, *Statistical Thermodynamics* (Oxford University Press, Oxford, 1998).
41. C. Alhambra, F.J. Luque, F. Gago, and M. Orozco, J. Phys. Chem. B **101**, 3846 (1997).
42. H. Sun, Y. Zhao, Z. Huang, C. Wang, Y. Wang, and F. Li, J. Phys. Chem. A **112**, 11382 (2008).
43. M. Baginski, F. Fogolari, and J.M. Briggs, J. Mol. Biol. **274**, 253 (1997).
44. R.A. Friedman and B. Honig, Biophys. J. **9**, 1528 (1995).
45. W. Blokzijl and J.B.F.N. Engberts, Angew. Chem. Int. Ed. **32**, 1545 (1993).
46. M.P. Evstigneev, K.A. Rybakova, and D.B. Davies, Biophys. Chem. **121**, 84 (2006).

Received 28.05.10.
Translated from Ukrainian by O.I. Voitenko

ЕНЕРГЕТИЧНИЙ АНАЛІЗ КОМПЛЕКСОУТВОРЕННЯ АРОМАТИЧНИХ МОЛЕКУЛ У ВОДНОМУ РОЗЧИНІ

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Р е з ю м е

Представлено аналіз енергетики нековалентних взаємодій при самоасоціації 12 ароматичних молекул, різних за структурою та зарядом. Розроблено методику обчислення внесків різних фізичних чинників у повну енергію Гіббса. Виявлено, що внески водневих зв'язків та ентропійні чинники завжди сприятливі, тоді як ван-дер-ваальсівські, електростатичні та (або) гідрофобні взаємодії можуть бути стабілізуючими чи дестабілізуючими чинниками залежно від досліджуваної системи. Аналіз, який проведено у даній роботі, дає відповідь на питання: які чинники стабілізують/дестабілізують стекінг ароматичних молекул у розчині та яка їх відносна важливість.