Extended habilitation report from assist. prof. Miroslav Rangelov PhD.

Introduction*

Ribosome is a complex molecular machine translating the genetic code of its provisional form - information RNA to proteins that are key building material for living cell and are catalysts in the metabolic pathways providing the exchange of matter and energy between living cell and inanimate nature. The key role of the bacterial ribosome makes it an important target for antibacterial agents. Indeed, a large number of clinically useful antibiotics target this complex translational ribonucleoprotein machinery.

The complete XDR atomic structure of the large ribosomal subunit showed no proteins within 18Å from the active site, revealing peptidyl transferase as an RNA enzyme (ribozyme). Reported structural and biochemical features of the universally conserved adenine residue A2486 (A2451 in Escherichia coli) in 23S ribosomal RNA led to the proposal of a mechanism of peptidyl transfer rRNA catalysis that implicates this nucleotide as the principal catalytic species. But *in vivo* and *in vitro* investigations that are made later showed, that the large ribosomal subunit with mutated A2451 has significant peptidyl transferase activity.

The elementary chemical reaction leading to peptide bond synthesis in the ribosome is an aminolysis reaction. During the elementary act of peptide bond formation the growing polypeptide chain, esterified to the cis-1,2-diol of tRNA 3`-terminal adenosine, reacts with the next amino acid in the ribosome peptidyl transfer center. For this reason, the aminolysis of esters was extensively studied both experimentally **[1o]** and theoretically **[2o]** as a model reaction for the formation of peptide bonds in living cells. Various computational studies on the mechanism of this reaction were based on small model systems. In order to simulate the ribosome interior at the peptidyl transferase center, these model studies are performed under vacuum or in aprotic solvents with low polarity.

---

* In this document references are organized in three different pools and are referred in text as numbers with suffixes, which describe if cited paper is from selected papers pool (suffix v), from additional papers pool (suffix g) or is paper not included in the competition or is from other authors.

Also small or larger molecules having both proton-donating and proton-accepting centers has been involved as catalyst of the process with questionable success.

The described in point **1)** Investigations are targeted on the catalytic possibility of very near surroundings of carbonyl carbon atom that undergoes aminolysis reaction during acyl transfer reaction in which the new amino acid residue was transferred from transport RNA to elongating polipeptide chain.

A lot of obscurities also exist in the mechanistic aspects of the ribosomal work. Because the translation of genetic code is acomplished by a large number of molecular movements, responsible for different acts on translational process, molecular dynamics simulation of the whole ribosome in physiologic solution will shed light on the very intimate mechanisms used in the work of such complicated molecular machine.

One of the major problems in molecular dynamics (MD) simulation of such large nucleoproteins is the uncompensated charge of phosphate groups. We developed heuristic method for their neutralisation which is applied on the ribosome.

Other problem is due to the limitations of X-ray methodology and because of it all available ribosome structures contain a lot of missing monomers and are unsuitable for molecular dynamics. For this reason we constructed a new whole ribosome structure (of E. coli) , starting from the most completed available PDB structures.

Algorithms and methodologies that we developed, while working on ribosome mechanism and whole ribosome molecular dynamical model, are used with success on a wide variety of tasks in the drug design field and some examples are described in point **2)**.

1) Investigation on the molecular aspects of the ribosome catalytic mechanism. [1v,2v,3v,4v]

An alternative catalytic effect on the reaction, catalysed by the ribosome, could come from the 2`-OH of the 3`-terminal adenosine of tRNA, which is always close to the reaction center. The crucial role of the vicinal 2`-OH on the catalytic activity of ribozymes was experimentally confirmed by the loss of peptidyl transferase activity of the ribosome with mutant 2`-deoxy-peptidyl-tRNA. **[1o(e)]** In an earlier work the

intramolecular catalytic effect of the vicinal OH group by the ammonolysis of monoacylated catechol has been modelled **[3o]** and after that we have continued to examine further details of that catalytic effects. Unfortunately even the simplest aliphatic model that includes vicinal hydroxyl group, has a very large conformational freedom, which led to the development and further optimization of a large number of software algorithms for automation of the enormous calculations, needed for these studies.
Some of that methodologies and algorithms are listed below:

1.1) Set of methodologies in the field of theoretical examination of conformationally flexible reaction routes. [1v]

The computational modelling of reaction mechanisms and energetics has become an important step in the investigation of different processes, occurring in biological systems **[4o]**, catalytic systems **[5o]**, organic synthesis **[6o]**, etc. The selection of the computational method **[7o]** and construction of the initial structures of the reactants, transition states, intermediates and products are often carried out after comparison with previous knowledge and chemical intuition. This approach becomes, however, inappropriate when the rigorous study of the reaction mechanism requires calculation of a large number of structures of the stationary points because of the size or flexibility of the modelled system. Here, we propose automated procedures for these first stages of a systematic computational investigation of reaction mechanisms that provide both computational efficiency and reliability.
The reported procedures are implemented in the software program suite MolRan.
The automated procedures for the first stages of a systematic computational investigation of reaction mechanisms include:

1.1.1) Methodology for selection of computational method and basis set based on statistical analysis of structural and energy data related to experimental values. [1v]

The initial step in a computational modelling is the selection of the computational method and the basis set that are appropriate for the

explored chemical system. In order to determine which method is reliable and at the same time computationally efficient, we developed an algorithm for exhaustive test of different computational method/basis set combinations and statistical analysis of the obtained structural and energetic data, which were compared with experimental values. By modelling the structure of formic acid and its dimer and of the ammonolysis of formic acid at different levels of theory, including about sixty combinations of 6 quantum-chemical methods and different basis sets and further statistical analysis of the obtained structural and energetic data, we defined B3LYP/6-31G** level as the least time consuming, yet still reliable method for correct description of the geometry of the modelled structures and the reaction energies.

1.1.2) Methodology for the determination of all distinct conformations of transition states with large conformational freedom [1v]

Each rigorous theoretical investigation requires systematic study of all possible transition state structures and intermediates, belonging to different reaction paths, and identification of the structures, corresponding to the lowest barriers, in order to define the most energetically favourable reaction route. This search is particularly complicated in systems with large conformational freedom where it is important to check various possible conformations of the transition states and intermediates, in order to select among them a set of distinct structures with low energy, to be computed at the final stage by a chemically adequate but more time consuming computational method. For this reason a smooth and automated procedure for conformational search of transition state structures and stable species was developed. The overall advantage of this methodology is that the most energetically favourable reaction route among almost all possible conformations of participating species will be derived automatically from only one manually calculated reaction route with human defined conformations of species.

The methodology can be roughly divided on several autonomous main steps, which can be used also separately for other computational tasks.

1.1.2.1) Methodology for conformational search of transitional state structures as well as for stable species [1v]

The new sets of geometries were generated by stepwise variation of all dihedral angles not involved in the mathematical representation of atoms directly involved in transition state (i.e. atoms on which main parts of transition vector is placed). The generation of the different structures is performed by developed algorithm based on graphs theory representation of molecules.**[1v]**

1.1.2.2) Methodology for partial structure relaxation of large pools of generated structures [1v]

The conjugated gradient optimization procedure using analytical first derivatives was used. For this preliminary optimization we used modified version of MM force field supplemented by an exponential term for description of hydrogen bonds. During the geometry optimization of the structures at MM level the atoms from the cleaving/forming bonds are automatically kept fixed with respect to each other.**[1v]**

1.1.2.3) Methodology for determining and eliminating conformationally equivalent structures [1v]

The geometrical similarity of the structures is further estimated by numerical optimization of the geometry position of one molecule with respect to the other one by conjugated gradient quasi-Newton method. The gradients are calculated by means of Ridder polynomial extrapolation method and the RMSD distance between equivalent atoms, from the two molecules in the pair, serves as minimization function. This step of the procedure allows great

reduction of the number of structures to be treated at higher computational levels.**[1v]**

1.1.2.4) Methodology for connecting structures from different structure pools according to their geometry similarities [1v]

Due to the complexity of the transition vector of many of the transition structures and the variation of the atoms, over which the main components of the vector are positioned along the reaction coordinate, the IRC search often cannot reach the stable structures.

For this reason, in order to find the intermediates, to which the different transition structures will lead, and the intermediates, from which the transition structures could be obtained, a technique, similar to the procedure applied for elimination of the equivalent structures described above, is used. In the current procedure each couple of intermediate and transition state structures are positioned over each other as the structure of the intermediate is translated into the centre of the coordination system and the position of the transition state structure is set via translation and rotation. **[1v]**

The next step consists of variation of the structure of the transition state by enforcing over the atoms, the transition vector of the structure multiplied by a variation coefficient. Similarly to the equivalent structures search, the optimization is performed by conjugated gradient quasi-Newton method and the RMSD distance between equivalent atoms from the two molecules in the pair serves as minimization function.**[1v]**

1.1.2.5) Methodology for generation of unknown geometry of the transition states, based on pre-defined connectivity of the atoms involved in the reaction. [1v]

We reported an approach to the automatic construction of the initial structures of transition states with desired atomic connectivity (topology) but unknown geometry.

With this approach one constructs a sequence (complex) of points in the phase space of geometry coordinates, which allows identification of the point, corresponding to the global minimum, eventually obeying the imposed constrains. In the actual application the procedure results in locating the lowest energy transition state with desired connectivity by pre-definition of the forming and cleaving bonds in the hexagonal reaction ring and the covalent bonds in the reacting species. The minimizing function was defined from the modified MM field as implemented in the MolRan program suite as the terms, taking into account the formation of hydrogen bonds, were excluded. **[1v]**

The initial complex consisted of 2N/5 (N is the number of all degrees of freedom in the system) random geometries and it was optimized until the variation of the volume in the complex was 1×10^{-3}. Unlike other methods, used for finding of transition states structures, this approach is capable to find lowest energy transition state structure with pre-defined topology without initial guess of the structure. This method is particularly useful when the reaction system undergoes large unpredictable changes from reactants to products, or when the elementary reaction step includes cleavage/formation of more than one bond, where the applicability of most of the traditional approaches for transition state search is limited.**[1v]**

1.1.3) Methodology was developed for computational analysis which reveals a possibility for catalysis of proton donor or proton acceptor group according to its position relative to reaction center . [3v]

The algorithm leads to the construction of graphical maps representing catalytic effect of the probe molecule in its different position around atoms at which main components of transition vector are placed. Since the effect of a catalyst is to change the energy barrier of the rate determining reaction step, in order to estimate the catalytic effect of any factor on a studied process, it is necessary to calculate the difference in the energy change due to this factor for the reactants, on one hand, and for the transition states, on the other. The developed algorithm calculated that difference automatically by generating initial geometry, in which only position of probe molecule is fixed relative to selected atoms. It is followed by run of the calculation on quantum software package and read of the result of calculations. Graphical representation of such a difference can be regarded as a catalytic map and can be used in determining the influence of adjacent groups in enzymes and especially in the construction of theozymes or compuzymes. In addition, such maps provide a convenient tool to design drug molecules with functional groups oriented appropriately for the activation or inhibition of the modelled processes. **[3v]** In general, the catalytic maps, constructed in the way reported here, can be used to predict the optimal position of groups in hypothetic ribozyme or enzyme active sites. They can also help in the construction of new or in the modification of known drugs, targeting enzyme active sites, as well as, for the prediction of drug activity targeting known receptors.

The methodology was demonstrated on the influence of proton donor group adjacent to the reaction center during ester ammonolysis of an acylated diol as a model reaction for peptide bond formation in the ribosome. In this case the analysis was performed using catalytic maps constructed after a detailed scanning of the available space around the reaction centers in different transition states, with a water molecule as probe molecule, acting as a typical proton donor. **[3v]**

- The catalytic maps of the influence of the adjacent proton donor group on the transition states for ammonolysis of acylated ethanediol, suggest that participation of the Ov center of the vicinal hydroxyl in a hydrogen bond with an adjacent proton donor can reduce the activation energy of the reaction steps by up to 7.2 kcal/ mol. **[3v]**

- The catalytic effect is most clearly pronounced for the rate-limiting step of the reaction, i.e. the first hexagonal transition state of the stepwise mechanism TS16. **[3v]**

- For all orientations of the adjacent group the hydrogen bond has a positive catalytic effect, i.e. the additional proton-donor group is only able to activate but cannot inhibit the process via the rate determining transition state. **[3v]**

- The modeling of a water molecule stabilized within an extended model of adenosine by hydrogen bonds to 2OH of ribose and the N3 atom of the nucleobase allowed us to conclude that such an adjacent proton donor center would facilitate a pro-S attack of the amino group by about 7 kcal/mol, while the effect in the case of a pro-R attack would be two times less then that. This result is in agreement with available experimental data. **[3v]**

1.1.4) The listed methodologies and algorithms are implemented in software code in the MolRan program suite [1v,3v]

MolRan is a graphical OpenGL based environment software aimed for visualization and generation of molecular geometries and analysis of the chemical properties and electronic structure information obtained by means of quantum-chemical calculations with specialized computational software, e.g., Gaussian or GAMESS. Various pre-defined z-matrix, Cartesian and fractional file formats (e.g., xyz, pdb, cif) are supported as in addition to a fully customized file reader that can extract and present structural data from text files, i.e., output files generated by computing

software. Various graphical representation tools are implemented, which allow the user to visualize the Hessian, gradients and other vectors, Newman projections along selected atoms, MD trajectories, RMS (root mean square) structures, etc. **[1v, 3v]**

The program also includes a set of tools for generating, loading, and displaying meshes generated on the base of different data, e.g., Van der Waals spheres, solvent accessible surfaces, meshes from loaded orbital coefficients and primitives producing AO or MO surfaces. In the last case the mesh could be also loaded from Gaussian03 cube file. The implemented tool allows mathematical modification of the generated/visualized meshes. A system of tools is available for constructing job files for conformational analysis and for analysis of the results obtained. The implemented file constructor can generate multiple job files with structures varied by selected structural parameters (interatomic distances, valence angles or dihedral angles) as the chosen variables can be altered synchronously or independently.**[1v, 3v]**

1.2) Modelling ribosome catalytic activity on amonolysis of diols [1v,2v]

The computational study was performed **[1v]** on the process of peptide bond formation in the ribosome investigating the influence of the vicinal OH group in monoacylated diols on the elementary acts of ester aminolysis. This is an alternative catalytic effect on the reaction, that could come from the 2‘ -OH of the 3’O terminal adenosine of tRNA, which is always close to the reaction center and its lack leads to ribosome inactivation.

We used model reaction as ammonolysis of 1-O-formyl 1,2-ethanediol and this selection of the reagents is determined by the presence in the formylethanediol molecule of a vicinal hydroxyl next to the reaction center analogously to the ribose ring in tRNA. We selected this reaction since it is a suitable model for elucidation of the mechanism of biosynthesis of peptide bonds in the ribosome, in connection with recent theoretical **[8o]** and experimental **[9o]** investigations. **[1v]**

The detailed results of our investigation include an exhaustive search for various possible conformations of the transition states, in which the vicinal hydroxyl group forms

intramolecular hydrogen bonds, resulting in stabilization of the observed structures or actively participates in the proton transfer, the so-called proton shuttle mechanism.

- The catalytic activation of the ester ammonolysis by a hydrogen bond from the vicinal hydroxyl via tetragonal transition states (occurring preferably via a concerted mechanism) was found to be rather modest, the free energy of activation being reduced by 5.2 kcal/mol only. The resulting free energy of activation, $\Delta G^{\#}$=44.3 kcal/mol, is still rather high, and for this reason the pathway via tetragonal transition states is unlikely to occur. The reason for this weak catalytic effect is the rather small proton transfer angles in the transition states, about 125-130°, much smaller than the optimal angle of 180°. The comparison with stabilization of the transition states via a hydrogen bond from an external water molecule suggests that the stabilization in the case of monoacylated ethanediol is close to the maximally possible stabilization of this type. **[2 v, 1v]**

-The other mechanism of catalytic activation of the ester ammonolysis; by participation of the vicinal hydroxyl in the proton transfer via hexagonal transition states does not affect considerably the stability of the transition states in the concerted mechanism and the second step of the stepwise mechanism.**[2 v, 1v]**

- Participation of the vicinal hydroxyl stabilizes considerably the first transition state of the stepwise mechanism; the Gibbs free energy of activation is reduced by 16.0 and 10.8 kcal/mol with respect to the referent value (structure without participation of vicinal hydroxyl group). The resulting free energy of activation, 33.5 kcal/mol, is significantly lower than the barriers calculated for the noncatalyzed reaction or the reaction occurring via the tetragonal transition states due to more favourable proton transfers in TS1 (6-membered). **[2 v, 1v]**

- A similar catalytic effect has been proposed for water-assisted aminolysis of 2-deoxy esters, **[10o]** where the OH group of water acts as a proton shuttle in the same way as the vicinal OH in monoacylated 1,2-ethanediol aminolysis modeled here. As we have shown, however, the proper accounting of the (translational and rotational) entropy of the free water molecule resulted in an increase of the free Gibbs energy of activation for the water-assisted process.

Such entropy loss does not influence the free energy of the transition states in the case of catalysis by vicinal OH since the catalytic group is bound by a covalent bond to the reaction center. **[2v , 1v]**

- The vicinal 2'-OH of ribose, modeled here as ethanediol, is readily available, being next to the reaction center during the synthesis of each peptide bond. Catalysis, including an additional water molecule, would require the approach of an external water molecule to the reaction center to accomplish each reaction step. Thus, we consider the vicinal 2'-OH of the ribose as a realistic and relevant proton transfer mediator for the peptide bond formation in the ribosome. **[2v , 1v]**

- The proton shuttle role of the vicinal OH was found to provide stronger activation of the reaction of peptide bond formation than the activation via an additional hydrogen bond from this group. The more favorable structure of the transition states for ester aminolysis due to participation of the vicinal hydroxyl in the proton transfer results in a decrease of the free energy of activation by 16.0 kcal/mol, which corresponds to about 10^9-fold acceleration of the reaction with respect to the noncatalyzed process.**[2v, 1v]**

- Accounting for the influence of the environment on the reaction center by a polarizable continuum model (for ε from 5 to 80) resulted in further stabilization of the rate-determining transition state by 4 -5 kcal/mol. **[2v , 1v]**

1.3) Functional study of the mechanism of acyl migration between vicinal OH groups in monoformylated cis-tetrahydrofuran-3,4-diol as a model system for the acyl migration in amino-acylated tRNA [4v]

The acyl group migration in monoacylated diols or acylated carbohydrates is an important reaction both in living cells and in synthetic organic chemistry. While this reaction is undesirable for most chemical synthetic reactions due to reduction of selectivity, it is necessary for the living cell, where such chemical transformations are responsible for an acyl group shift in a wide variety of species, as lipids, acylated sugars, and nucleosides. One of the most interesting acyl migration reactions is that between vicinal hydroxyl groups in the amino-acylated tRNA, which is the substrate in peptide

bond synthesis in the ribosome. It is well known that in the cell such amino-acylated tRNA exists in two isomeric forms, 2-acylated and 3-acylated, with an equilibrium between them, but the ribosome can process only 3-amino-acylated tRNA. To shed light on this process, a density functional study for mechanism of the acyl migration between vicinal OH groups in monoformylated cis-tetrahydrofuran-3,4-diol was performed as a model system for the acyl migration in amino-acylated tRNA. [**4v**]

-Two alternative paths (stepwise and concerted) for formyl group migration between vicinal hydroxyl groups in tetrahydrofuran-3,4-diol were considered. The computational results suggest that the stepwise mechanism via an orthoester intermediate is preferred by 45–48 kJ/mol over the concerted mechanism, where migration of the formyl group, and the proton from the vicinal OH group occurs simultaneously via a four-atom transition state. Owing to the high conformational constraints in the THF ring, the steric hindrances can increase the energy of the transition states by 24 kJ/mol in the stepwise reaction path and by 37 kJ/mol in the concerted reaction path. [**4v**]

- The polarized continuum model (PCM) for four solvents evaluated the solvent effect on the reaction energetics. The calculated activation energy of the reaction in vacuum, 188 kJ/mol, is rather high. After taking into account the influence of the solvent, this energy is reduced, but only by 16 kJ/mol. **[4v]**

- Migration toward the ionized hydroxyl group was also modeled, which simulates the process in the presence of basic reagents. The results suggests that in the real systems the process is most likely catalyzed, by preliminary deprotonation of the vicinal OH group. Indeed, the calculations suggest that acyl group migration proceeds spontaneously when the vicinal OH group is deprotonated. [**4v**]

- Moreover, in all solvents considered, the intermediate formed is 15–30 kJ/mol more stable than the initial/final anion of the monoformylated THF-3,4-diol; i.e., such ionized orthoester species could be observed experimentally in the presence of a strong enough base.[**4v**]

2) Other activities concerning revelation of ribosomal action and applying developed methodology on other systems

As has been mentioned above the hydrogen bonds plays an important role in ribosomal catalytic activity. Formation of hydrogen bonds is exclusively used by the nature for arrangement of the structure of various biomolecules, molecular recognition and so on. Based on the knowledge on the active centers of key biomolecules, the hydrogen bonding ability of different functional groups is also used as a tool in the selective binding of drugs to their targets. In addition to the increasing the interaction between or within molecules, formation of hydrogen bonds can influence the reactivity of different active sites or can even change the reaction mechanism of chemical or biochemical processes. **[11o]**

Various hydrogen-bonded complexes of methanol with different proton accepting and proton donating molecules containing Cl, F, NH_2, OH, OR, and COOH functional groups have been modelled using DFT with hybrid B3LYP and M05-2X functionals. A general correlation was found between the binding enthalpy of the complex and the electrostatic potential at the hydrogen center, participating in the formation of the hydrogen bond. The calculated binding enthalpies of different complexes were used to clarify which functional groups can potentially form a hydrogen bond to the 2′-OH hydroxyl group in ribose, which is strong enough to block it from participation in the intramolecular catalytic activation of the peptide bond synthesis. Such blocking could result in inhibition of the protein biosynthesis in the living cell if the corresponding group is delivered as a part of a drug molecule in the vicinity of the active site in the ribosome. According to our results, such activity can be accomplished by secondary or tertiary amines, alkoxy groups, deprotonated carboxyl groups, and aliphatic fluorides, but not by the other modelled functional groups. **[1g]**

Metal ions (Na^+,K^+,Mg^{2+},Ca^{2+}) play important roles in the stability, folding, and functioning of nucleic acids. Their presence is essential for the structural integrity of the chromosomes of different living cells, including mammalian. These ions help maintain the spatial configuration of DNA and RNA not only by neutralizing the negative charges of the phosphate groups but also by mediation by some of them of the interactions

between parts of the macromolecules. Metal ions plays very important role in stabilization of the tertiary structure of RNA in the ribosome. **[7g,12o]**

For this reason the interactions between sodium or magnesium ions and phosphate groups of the RNA backbone, represented as dinucleotide fragments in water solution, have been studied using *ab initio* Born Oppenheimer molecular dynamics. Results show that sodium ions have mobility higher than that of the magnesium ions. The coordination number of the sodium ions frequently changes in irregular intervals ranging from several femtoseconds to about 10 ps with the most frequently encountered coordination number five, followed by six. The magnesium ion is stable both as directly bonded to an oxygen atom from the phosphate group and as completely solvated by water. In both states the Mg^{2+} ion has exactly six oxygen atoms in the first coordination shell. Solvation of the terminal phosphate oxygen atoms by water molecules forming hydrogen bonds in different locations of the ions is also studied. The initial state (atom coordinates) of the dinucleotide are taken from a section with single-stranded RNA from an atomistic classical MD simulation of the ribosome in water solution. **[2g]**

Because the ribosome is mainly composed of RNA chains, that leads to a huge amount of negatively charged phosphate groups and mainly missing counter ions in the PDB X-Ray Structures. In nature they are neutralized by Na^{+}, Mg^{2+} ions and a limited amount of positively charged amino acid residues incorporated in the polypeptide chains. For neutralization of these groups, a heuristic algorithm for adding counter ions in big nucleoproteins such as the ribosome has been developed and the charged groups in the ribosome structure were effectively neutralized using mono and divalent ions, according to their environment. The algorithm places missing ions optimizing its type and position for best interaction with the nucleoprotein structure, leading to electro-neutral structure, which was further processed successfully by all atom molecular dynamics.

Using this algorithm, within the scope of the 7FP PRACE project, molecular dynamics simulation on all atom model of the whole *E. coli* ribosome was done and its model for drug design was prepared. **[13o]**

Algorithms developed during the work on ribosome mechanism and whole ribosome molecular dynamical model can be used with success on wide variety of task in the drug design field.

It is known that there is difference between crystal structure of proteins and their structure in solution. That structure differences are often small but functionally significant. It is shown that the RMDS values between NMR and crystal structures range from about 1.5 Å to about 2.5 Å, but the correlation between conformational deviations and residue type reveals, that hydrophobic amino acids are more similar in crystal and NMR structures than hydrophilic amino acids. Because the energy of a typical hydrogen bond (one of the main players in Interactions between drug and its target molecule) changes strongly with distance, this difference between crystal and solution structure of proteins is quite significant for good *in silico* drug activity evaluation in their active sites. Relaxation of crystal structure of proteins to the set of it solution conformations can be done by methods of molecular dynamics. On the other hand, there are often missing monomers in the XRD structures, mainly in the outer regions of globular structure, and they lead to cleavage in polypeptide chains, that can't be interpreted reasonably by most molecular dynamic software packages.

For all the above mentioned problems, the successful methodologies and algorithms mainly developed for simulations of large nucleoprotein of the ribosome, were extensively used for initial structures preparation for other software packages as well as for the analysis of the results, obtained from other software. These algorithms together with other software packages as GROMACS, COOT, CCP4, and MOE were used in series of works, where qualitative and quantitative estimation of interactions between drug like molecules and their receptor takes place:

1) Set of ligands was accomplished into an internal pocket formed by the activation segment and the P-loop of V599E mutant of B-Raf. It was established that the binding of the studied ligands to V599E B-Raf promotes an inactive conformation of the enzyme and they are promising candidates in the fight against B-Raf dependent carcinomas. **[3g]**

2) Molecular docking studies were performed to gain an insight into drug-like ligands binding with xanthine oxidase (XO). The studied morpholinediones derivatives exerting XO inhibition and anti-inflammatory effect are promising for future usage in the treatment of gout and other excessive uric acid production or

inflammatory conditions. Molecular docking was carried out into the salicylic acid active site of XO (PDB entry code 1FIQ) **[4g,5g]**

3) VV-Hemorphin-5 is an endogenous opioid peptide of the Hemorphin family with affinity at opioid receptors. A series of C-amide analogues have been synthesized, based on the structure of VV-Hemorphin-5, modified at position 1 and 7 by the un/natural amino acids. The peptide derivatives were evaluated for their anticonvulsant activity in three acute seizure tests in male ICR mice, the maximal electroshock (MES), the 6 Hz psychomotor seizure test, and the timed intravenous pentylenetetrazole (ivPTZ) infusion test. Their neurotoxicity was assessed in the rotarod test. Docking study results suggest that kappa opioid receptor binding could be the mechanism of action of peptide derivatives with anticonvulsant activity. The results suggest that incorporation of nonproteinogenic and/or natural amino acids at position 1 and 7 of the VV-Hemorphin-5 scaffold deserve further evaluation in models of epilepsy. **[5v]**

4) Phage-displayed single-chain variable fragment (scFv) antibodies obtain serious advantages that make them preferable tool for application in research, laboratory diagnostics and medicine. Recent progress in antibody engineering together with the microbial expression systems are milestones for design and production of antibodies for numerous applications. Variety of selection and screening strategies could be used to simultaneously derive many high-affinity scFv with different relevance. Importance of their diverse collection relies on high specificity and selectivity of antibodies, while offering distinct biological profiles in tissue distributions of unique molecules.

C1q is the recognizing first subcomponent of the classical complement pathway, which undergoes a conformation transition by its activation, exposing neo-epitopes. Naive phage display library is used to create a single-chain variable fragment (scFv) antibody, that mimics the globular head regions of native C1q (ghC1q). It is further used in *in-silico* modelling to characterise crucial for biological binding regions in C1q globular heads.

Based on the nucleotide sequence of an inhibitory scFv A1, a structural 3D model of resulting polypeptide chain is generated, as homology model according to

alignment on the deposited crystallized scFv PDBs. The resulting structure is used to find conformational and electrostatic similarities between homology structure and native human C1q globular heads. This similarity helps to clarify regions from C1q molecule crucial for generation of SLE (Systemic Lupus Erythematosus) autoantibodies as SLE pathogenesis is suspected to be associated with conformational or sequential neo-epitopes in ghC1q chains. The presented model will be further helpful giving insights into new scFv binding properties that represent a step toward understanding the recognition mechanisms of C1q and their biological pathogenesis targets. **[6g]**

3.) Future plans

- In the future, work on current projects will be continued, and the accumulated experience in the fields of computational chemistry and drug design will be used for revealing mechanistic aspects and properties of biomolecules of interest for the laboratory.
- We would apply for reasonable project funding from European and National programs in order to provide computational resources for constructing a whole working molecular model of the prokaryotic and human ribosome and its difference will be used for development of new antibiotics as well as spotting possible chemical modifications in existing ones in order to circumvent bacterial antibiotic resistance.

Such models will be exploited to derive 16S rRNA functional variability and results will be used in the field of microbiology and microbial ecology, where nowadays definition of microbial species is tightly connected with its ribosomal 16S subunit sequence.

- A long standing experience in the field of HPLC and its use in chemical analysis of complex matrices and in chemical kinetics will be used for the needs of the group and also in the field of archaeology and phytochemistry.
- Development of new software for solving difficult chemical tasks will continue.
- Effort will be made to attract young people in these fields as diplomants and doctorants.

Selected papers (group V) :

[1v] Rangelov, M.A., Petrova, G.P., Yomtova, V.M., Vayssilov, G.N., Hierarchical approach to conformational search and selection of computational method in modeling the mechanism of ester ammonolysis, *Journal of Molecular Graphics and Modelling* 29(2), (2010). 246-255

[2v] Rangelov, M.A., Petrova, G.P., Yomtova, V.M., Vayssilov, G.N., Catalytic role of vicinal OH in ester aminolysis: Proton shuttle versus hydrogen bond stabilization, *Journal of Organic Chemistry* 75(20), (2010). 6782-6792

[3v] Rangelov, M.A., Petrova, G.P., Yomtova, V.M., Vayssilov, G.N.**,** Determination of the optimal position of adjacent proton-donor centers for the activation or inhibition of peptide bond formation - A computational model study, *Journal of Molecular Graphics and Modelling* 30, (2011). 10-14

[4v] Rangelov, M.A., Vayssilov, G.N., Petkov, D.D., Quantum chemical model study of the acyl migration in 2′(3′)- formylnucleosides, *International Journal of Quantum Chemistry* 106(6), (2006). 1346-1356

[5v] Todorov, P., **Rangelov, M.**, Peneva, P., Todorova, N., Tchekalarova, J.**,** Anticonvulsant evaluation and docking analysis of VV-Hemorphin-5 analogues, *Drug Development Research* 80(4), (2019). 425-437

References from group "G" cited in the text :

[1g] Kolev, S.K., Petkov, P.S., **Rangelov, M.A.**, Vayssilov, G.N., Density functional study of hydrogen bond formation between methanol and organic molecules containing Cl, F, NH 2 , OH, and COOH functional groups, *Journal of Physical Chemistry A*, 115(48), (2011), 14054-14068

[2g] Kolev, S., Petkov, P.S., **Rangelov, M.**, Vayssilov, G.N., Ab initio molecular dynamics of Na+ and Mg2+ countercations at the backbone of RNA in water solution *ACS Chemical Biology*, 8(7), (2013). 1576-1589

[3g] Mavrova, A.T., Dimov, S., Yancheva, D., **Rangelov, M.**, Wesselinova, D., Tsenov, J.A., Synthesis, anticancer activity and photostability of novel 3-ethyl-2-mercapto-

thieno[2,3-d]pyrimidin-4(3H)-ones , *European Journal of Medicinal Chemistry*, 123, (2016). 69-7921

[4g] Smelcerovica,A., **Rangelov, M.**, Smelcerovic, Z., Veljkovic, A., Cherneva, E., Yancheva, D., Nikolic, G.M., Petronijevic, Z., Kocic, G., Two 6-(propan-2-yl)-4-methyl-morpholine-2,5-diones as new non-purinexanthine oxidase inhibitors and anti-inflammatory agents, Food and Chemical Toxicology 55, (2013). 493-497

[5g] Šmelcerović,Z., **Rangelov, M.**, Cherneva, E., Kocić, G., Stojanović, S., Jevtović-Stoimenov, T., Petronijević, Z., Yancheva, D., Inhibition mechanism and molecular modeling studies of the interactions of 6-(propan-2-yl)-3-methyl-morpholine-2,5-dione with xanthine oxidase , *Bulgarian Chemical Communications*, 47(3), (2015). 783-7

[6g] Rangelov, M. A.; Todorova, N. H.; Tsacheva, I. G., In silico investigation of single-chain variable fragment (scFv) antibody, structurally similar to native C1q globular heads , *Bulgarian Chemical Communications,* 49 (E), (2017), 50-53

[7g] Kolev, S.K., Petkov, P.S., **Rangelov, M.A.**, Trifonov, D.V., Milenov, T.I., Vayssilov, G.N., Interaction of Na+, K+, Mg2+ and Ca2+ counter cations with RNA, *Metallomics,* 10(5), (2018). 659-678

Other references:

[1o]

(a) Rodnina,M.V. Beringer,M., Wintermeyer, W., Mechanism of peptide bond formation on the ribosome, *Quart. Rev. Biophys*. 39, (2006). 203–225.

(b) Zhong, M.H. Strobel,S.A. , Synthesis of isotopically labeled P-site substrates for the ribosomal peptidyl transferase reaction, *J. Org. Chem*. 73, (2008). 603–611.

(c) Simonović, M., Steitz, T.A., Cross-crystal averaging reveals that the structure of the peptidyl-transferase center is the same in the 70S ribosome and the 50S subunit, *Proceedings of the National Academy of Sciences of the United States of Americ*a, 105 (2), (2008). 500-505.

(d) Schroeder, G.K., Wolfenden, R., The rate enhancement produced by the ribosome: An improved model, *Biochemistry*, 46 (13), (2007). 4037-4044.

(e) Weinger, J.S., Parnell, K.M., Dorner, S., Green, R., Strobel, S.A., Substrate-assisted catalysis of peptide bond formation by the ribosome, *Nature Structural and Molecular Biology*, 11 (11), (2004). 1101-1106.

(f) Dorner, S., Panuschka, C., Schmid, W., Barta, A., Mononucleotide derivatives as ribosomal P-site substrates reveal an important contribution of the 2′-OH to activity, *Nucleic Acids Research*, 31 (22), (2003). 6536-6542.

(g) Zhu, Y., Mao, Z., Gao, C., Aminolysis-based surface modification of polyesters for biomedical applications,*RSC Advances*, 3(8), (2013). Pages 2509-2519

(h) Zeng, S.a, Liu, J.a, Anankanbil, S.b, Chen, M.a, Guo, Z.b, Adams, J.P.c, Snajdrova, R.c, Li, Z., Amide Synthesis via Aminolysis of Ester or Acid with an Intracellular Lipase, *ACS Catalysis* 8(9), (2018). 8856-8865

[2o]

(a) Štrajbl, M., Florián, J., Warshel, A., Ab initio evaluation of the potential surface for general base-catalyzed methanolysis of formamide: A reference solution reaction for studies of serine proteases, *Journal of the American Chemical Society*, 122 (22), (2000). 5354-5366

(b) Trobro, S., Åqvist, J. Mechanism of peptide bond synthesis on the ribosome, *Proceedings of the National Academy of Sciences of the United States of America*, 102 (35), (2005). 12395-12400.

(c) Trobro, S., Åqvist, J. Analysis of predictions for the catalytic mechanism of ribosomal peptidyl transfer, *Biochemistry*, 45 (23), (2006). 7049-7056.

(d) A. Gindulyte, A. Bashan, I. Agmon, L. Massa, A. Yonath, J. Karel, The transition state for formation of the peptide bond in the ribosome, *Proceedings of the National Academy of Sciences of the United States of America*. 103 (2006). 13327–13332.

(e) Montecinos, R., Gazitúa, M., Santos, J.G.,The effect of the electrophilic group on the hierarchy of nucleofuges in the aminolysis reactions of thiol- and dithiocarbonates with secondary alicyclic amines: A kinetic and theoretical study, *New Journal of Chemistry*, 43(16), (2019). Pages 6372-6379

(f) Teotia, M.Email Author, Tarannum, N., Chauhan, M., Soni, R.K.,Structure-based rational design, synthesis, crystal structure, DFT and molecular docking of 1,4-benzene dicarboxamide isomers with application as hardeners, *New Journal of Chemistry*, 43(21), (2019). 7972-7983

[3o]

(a) Rangelov, M.A., Vayssilov, G.N., Yomtova, V.M., Petkov, D.D.,Theoretical study of the o-OH participation in catechol ester ammonolysis, Organic and Biomolecular Chemistry, 3 (5), (2005) 737-744.

[4o]

(a) Blomberg, M.R.A., Siegbahn,P.E.M., Quantum chemistry as a tool in bioenergetics, *Biochim. Biophys. Acta* 1797, (2010). 129–142.

(b) Baná˘s,P., Jure˘cka,P., Walter,N.G., ˘Sponer,J., Otyepka,M., Theoretical studies of RNA catalysis: hybrid QM/MM methods and their comparison with MD and QM, *Methods*, 49, (2009). 202–216.

(c) Dudev,T., Lim,C., Metal binding affinity and selectivity in metalloproteins: insights from computational studies, *Ann. Rev. Biophys.*, 37, (2008). 97–116.

(d) Gao,J., Ma,S., Major,D.T., Nam, K., Pu,J., Truhlar, D.G., Mechanisms and free energies of enzymatic reactions, *Chem. Rev*., 106, (2006). 3188–3209

(e) Huggins, D.J., Biggin, P.C., Dämgen, M.A., Essex, J.W., Harris, S.A., Henchman, R.H., Khalid, S., Kuzmanic, A., Laughton, C.A., Michel, J., Mulholland, A.J., Rosta, E., Sansom, M.S.P., van der Kamp, M.W. , Biomolecular simulations: From dynamics and mechanisms to computational assays of biological activity, Wiley Interdisciplinary Reviews: *Computational Molecular Science* ,9(3),(2019). Article number e1393

(f) Cupellini, L., Corbella, M., Mennucci, B., Curutchet, C., Electronic energy transfer in biomacromolecules, Wiley Interdisciplinary Reviews: *Computational Molecular Science*, 9(2),(2019). e1392

[5o]

(a) Morokuma,K., Theoretical studies of structure, function and reactivity of molecules—a personal account, *Proc. Jpn. Acad. B*, 85, (2009). 167–182.

(b) Loffreda,D., Delbecq,F., Vigné,F.,Sautet,P., Fast prediction of selectivity in

heterogeneous catalysis from extended brønsted-evans-polanyi relations: a theoretical insight, *Angew. Chem., Int. Ed.* 48, (2009). 8978–8980.

(c) Zinovjev, K., Tuñón, I., Reaction coordinates and transition states in enzymatic catalysis, Wiley Interdisciplinary Reviews: *Computational Molecular Science*, 8(1), (2018).e1329

[6o]

(a) T. Mineva, E. Sicilia, N. Russo, Density-functional approach to hardness evaluation and its use in the study of the maximum hardness principle, J. Am. Chem.Soc. 120 (1998) 9053–9058.

(b) F.P. Rotzinger, Treatment of substitution and rearrangement mechanisms of transition metal complexes with quantum chemical methods, Chem. Rev. 105 (2005) 2003–2037.

(c) Galabov,B., Nikolova,V., Wilke,J.J., Schaefer,H.F., Allen,W.D., Origin of the SN2 benzylic effect, *J. Am. Chem. Soc.,* 130, (2008). 9887–9896.

(d) Hu, P., Ye, J., Jiang, H., Which isomer is better for charge transport:: Anti - Or syn -?, *Journal of Materials Chemistry C,* 7(20), (2019). 5858-5873

[7o]

(a) Proynov, E., Kong, J., Assessment of a new hybrid functional on thermochemistry, metal–ligand bonding and reaction barriers, in: Theoretical Aspects of Catalysis, vol. 3, Heron Press, Sofia, 2009, pp. 1–9.

(b) Afzal, M.A.F., Hachmann, J., Benchmarking DFT approaches for the calculation of polarizability inputs for refractive index predictions in organic polymers, *Physical Chemistry Chemical Physics,* 21(8),(2019). 4452-4460

(c) Loskot, P., Atitey, K., Mihaylova, L., Comprehensive review of models and methods for inferences in bio-chemical reaction networks, *Frontiers in Genetics*,10,(2019). Article number 549

[8o]

(a) 2o (d)

(b) Wallin,G., Åqvist,J., The transition state for peptide bond formation reveals the ribosome as a water trap, *Proc. Natl. Acad. Sci. U.S.A.,* 107, (2010). 1888–1893.

(c) Rangelov, M.A., Vayssilov,G.N., Yomtova,V.M., Petkov,D.D., The syn-oriented2-OH provides a favorable proton transfer geometry in 1,2-diol monoester aminolysis: implications for the ribosome mechanism, *J. Am. Chem. Soc.* 128, (2006). 4964–4965.

[9o]

(a) [1o (a)]

(b) Changalov, M.M., Ivanova, G.D., **Rangelov, M.A.,** Acharya, P., Acharya, S., Minakawa, N., Földesi, A., Stoineva, I.B., Yomtova, V.M., Roussev, C.D., Matsuda, A., Chattopadhyaya, J., Petkov, D.D., 2′/3′-O-peptidyl adenosine as a general base catalyst of its own external peptidyl transfer: Implications for the ribosome catalytic mechanism, ChemBioChem, 6(6), (2005). 992-996

(c) Chattopadhyaya,J., Petkov,D.D., 2/3-O-peptidyl adenosine as a general base catalyst of its own external peptidyl transfer: implications for the ribosome catalytic mechanism, *ChemBioChem,* 6, (2005). 992–996.

(d) - [1o (b)]

[10o]

(a) Yang, W., Drueckhammer, D.G., Computational studies of the aminolysis of oxoesters and thioesters in aqueous solution, *Organic Letters*, 2 (26), (2000). 4133-4136.

[11o]

(a) Hynes, J. T., Klinman, J. P., Limbach, H.-H., Schowen, R. L., Eds. Hydrogen Transfer Reactions , *Wiley-VCH: Weinheim*, (2007).

(b) Testa, B., van de Waterbeemd, H., Folkers, G., Guy, R., Pharmacokinetic Optimization in Drug Research: Biological, Physicochemical, and Computational Strategies , *Wiley: Zurich, Switzerland*, (2001).

(c) Hirota, S., Lin, Y.-W., Design of artificial metalloproteins/metalloenzymes by tuning noncovalent interactions, *Journal of Biological Inorganic Chemistry,* 23(1), (2018). 7-25

[12o]

(a) Celander, D., and Cech, T., Visualizing the higher order folding of a catalytic RNA molecule. *Science* 251, (1991). 401−407.

(b) Sigel, A., Sigel, H., and Sigel, R., Structural and Catalytic Roles of Metal Ions in RNA , Vol.9, (2011) 101−124, *RSC Publishing:Cambridge, U.K.*

(c) Strick, R., Strissel, P. L., Gavrilov, K., and Levi-Setti, R., Cation-chromatin binding as shown by ion microscopy is essential for the structural integrity of chromosomes, *J. Cell Biol*. 155, (2001). 899−910.

(d) Levi-Setti, R., Gavrilov, K. L., and Rizzo, P. J., Divalent cation distribution in dinoflagellate chromosomes imaged by high resolution ion probe mass spectrometry. *Eur. J. Cell Biol.* 87, (2008). 963−976.

(e) Hud, N. V., and Polak, M., DNA-cation interactions: the major and minor grooves are flexible ionophores. *Curr. Opin. Struct. Biol*. 11, (2001). 293−301.

(f) Egli, M., DNA-cation Interactions: Quo Vadis? *Chem. Biol.* 9, (2002). 277−286.

(g) Draper, D. E., A guide to ions and RNA structure., *RNA*, 10, (2004), 335−343.

(h) Chen, S.-J., RNA folding: conformational statistics, folding kinetics, and ion electrostatics, *Annu. Rev. Biophys.* 37, (2008). 197−214.

(i) Klein, D. J., Moore, P. B., and Steitz, T. A., The contribution of metal ions to the structural stability of the large ribosomal subunit, *RNA*, 10, (2004). 1366−1379.

(j) Bashan, A., and Yonath, A,. The linkage between ribosomal crystallography, metal ions, heteropolytungstates and functional flexibility, *J. Mol. Struct.*, 890, (2008). 289−294

[13o]

(a) Rangelov, M.A.,Petkov, P.,Markov, S.,Vayssilov, G.N., Litov, L. “Algorithm for the ribosome structure neutralisation and development of force field for drug candidates,” *Journal of peptide science*, 20 (1), (2014) S52-S52