HOW TO FRAGMENT A POLYPEPTIDE? AN *ab initio* COMPUTATIONAL STUDY OF PAIR INTERACTIONS BETWEEN AMINO ACIDS AND LIGAND-AMINO ACIDS IN PROTEINS

Vojtěch KLUSÁK[a1], Petr DOBEŠ[b], Jiří ČERNÝ[c] and Jiří VONDRÁŠEK[a2,]*

[a] *Institute of Organic Chemistry and Biochemistry, Academy of Sciences of the Czech Republic, v.v.i., Flemingovo nám. 2, 166 10 Prague, Czech Republic; e-mail: [1] klusak@uochb.cas.cz, [2] jiri.vondrasek@uochb.cas.cz*

[b] *University Hospital Brno, Department of Internal Medicine – Hematooncology, Jihlavská 20, 625 00 Brno, Czech Republic; e-mail: petr.dobes@marge.uochb.cas.cz*

[c] *Institute of Biotechnology, Academy of Sciences of the Czech Republic, v.v.i., Vídeňská 1083, 142 20 Prague 4, Czech Republic; e-mail: jiri.cerny@img.cas.cz*

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Dedicated to Dr. Zdeněk Havlas on the occasion of his 60th birthday.

To determine reasonably which amino acid side chain contributes significantly to the stability of a protein or to the stability of a protein–ligand complex is not a straightforward task. We suggest a partial but systematic solution of the problem by a specific fragmentation of a protein chain into blocks of single amino acid side chains with their corresponding backbone part. For such systems of building blocks, we have calculated the stabilisation/interaction energies by means of correlated *ab initio* calculations. We have shown that a reasonable way to treat an amino-acid residue composing the protein is to break the homonuclear C–C bond between the C_α atom and the C(O) carboxyl carbon. The reference data obtained by the RI-MP2 method with the cc-pVDZ basis set were compared with RIDFT, RIDFT augmented by the dispersion term, SCC-DFTB-D and Hartree–Fock calculations. The results clearly show the failure of those methods lacking an appropriate treatment of the correlation energy. The DFT methods augmented by the empirical dispersion term on the other hand describe the interaction in good agreement with the reference method.

Keywords: Pair interactions; Amino acids; Protein stabilisation; Peptides; Correlated *ab initio* methods; DFT; Protein–ligand interaction; *Ab initio* calculations; Ligand design; Protein engineering; Protein models.

Proteins are spatial arrangements of linear chains of only twenty amino acids synthesised on a ribosome in a living cell. The formation of a protein is governed by several principal features. The first is a particular linear sequence of amino acids, which is crucial for the structural determination, as

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postulated by Afinsen[1]. Particularly, there are physical interactions of the amino acids in the sequential and spatial context that determine the structure[2] of the folded protein and its biological function. The other features comprise the interactions with solvent molecules, the pH and the ionic composition of the solvent.

To determine unambiguously the contribution to the stability coming from an amino acid is substantial for our understanding of the structure and function of a protein, but it is a complicated and not straightforward task. Structural studies can help to determine important amino-acid residues which play a crucial role in the protein structure stabilisation and function[3]. Interestingly, it is apparent that these residues are usually in contact with quite a large number of other residues, often from sequentially distant parts of the protein chain[4]. This can lead to an assumption that the importance of a residue is connected not directly with the quantity of contacts which it can maintain but with the overall strength of the interactions. It is widely accepted that besides hydrogen bonding that can bring a substantial attraction, other various types of non-covalent interactions can also play a decisive role[5]. Structural database studies, for example, have shown an abundance of π···π and (or) X–H···π interactions in proteins[6–8] and also in DNA[9].

It has recently been described that the interactions of the amino acids inside the hydrophobic core of a protein bring surprisingly high and important enthalpic stabilisation. Moreover, a peptide bond in a stacking arrangement with an aromatic residue is another surprisingly strong binding partner for the aromatic amino acid side chain[10,11].

Similarly to the intramolecular interactions between amino acids, ligand binding is a process for which the balance of the various amino acid interactions is crucial. Therefore, an understanding of the origins of selectivity and intermolecular stabilisation is essential for further rational ligand modifications and a fine-tuning of the binding properties between a ligand and its receptor. It is also known that the interaction of a ligand with aromatic residues[12] can provide a strong energetic (enthalpic) stabilisation, which is most likely one of the key factors determining ligand recognition.

Currently, there are many fragment-based computational *ab initio* methods to treat complex biomolecules efficiently and at the highest possible level of accuracy. To mention the most important ones we have to refer to fragment molecular orbital method (FMO)[14–16], divide and conquer methods (DAC)[18,19], adjustable density matrix assembler method (ADMA)[20] and molecular fractionation with conjugate caps (MFCC)[21,22] – also well suited for total energy calculation. The recent review by Gordon et al.[23] and the

paper by He and Merz[24] provide the comparison of all of the utilised schemes and methods quite comprehensively and show that various methods of fragmentation could be used with reasonable accuracy. The partitioning scheme applied in this work is fully justified by the recent progress in this area.

There is still need for simple and intuitive fragmentation scheme at a decent computational level which would be based on agreement with accurate *ab initio* calculations that would reasonably evaluate and map the mutual interactions between amino acids in a protein or interactions between ligands and proteins. The application of high-level *ab initio* methods can ultimately provide reliable and relevant information about the real importance of a particular amino acid in a structure or function. In recent years, these methods have been successfully used for the gas-phase systems with accuracy challenging the experimental results[25]. Although the best methods can not be routinely used for the whole protein, they can describe the interactions between structurally or functionally important parts and establish a measure for similar cases.

We have a long history of testing computational methods, basis sets and parameters to evaluate the interaction energies of noncovalent interactions in biomolecules. More specifically, the utilisation of perturbation methods and specific basis sets (the RI-MP2/aug-cc-pVDZ basis sets) was tested for various sorts of interactions including the interaction between charged amino acids[26], the model of the peptide bond – aromatic ring interaction[27], the interaction between proline and tryptophane[28] and the interaction between the side chains of the amino acids[29].

We have suggested a partial but systematic solution of the problem for peptides, proteins and their complexes with ligands. As was already mentioned a calculation of the interaction energies between the fragments of the system can provide a good understanding of a system with reasonable accuracy. However, common separation (fragmentation) of the protein chain into amino acids breaks the peptide bond. Neglecting the peptide bond interaction with the side chains or ligands could be crucial[10,11]. In this study, we have addressed a set of drawbacks and advantages connected with a different kind of protein-chain fragmentation and its effect on the overall stabilisation, constructed as the sum of the contributions from the individual fragments.

It is worth mentioning here that the decomposition of a protein molecule into regions suitable for QM treatment in the QM/MM methods would also suit the purposes of this study. The QM region is usually cut out of the

enzyme/protein molecule and it is a question of chemical intuition as to where to place the boundaries.

MATERIAL AND METHODS

Fragmentation

The introduced fragmentation method for proteins or peptides is based on splitting a system into fragments of a dimension suited for a demanding but reliable theoretical description. The method of choice for the theoretical description is the correlated *ab initio* quantum chemical method.

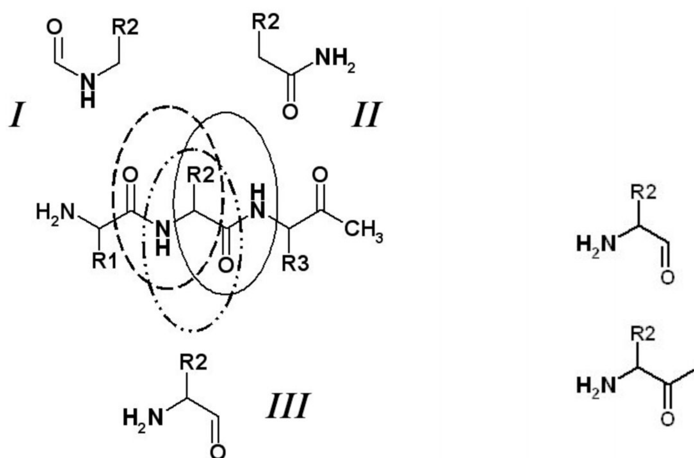
If a reliable *ab initio* method manages to describe the interaction of a particular amino acid with the whole protein or the interaction of the whole receptor with its ligand, it will also reflect the intramolecular polarisation that is lost owing to the cutting of the bonds between the composing fragments. Therefore, before using the fragmentation method for any particular protein structure, the losses when cutting the system and the way of cutting it to minimise the loss must be determined. The method, as described above, regards the interactions as additive ones. This is similar to the treatment of the methods of classical molecular mechanics, utilising empirical force fields. It should be kept in mind that this is only an approximation, neglecting cooperativity – a mutual polarisation of the groups.

There are three bond types present in a protein backbone, so there are three possibilities of how to break it. These are between the carbonyl carbon and amide nitrogen in the C(O)–N (CON scheme of partitioning) peptide bond, between the amide nitrogen and the tetrahedral carbon C_α in the N–C_α bond (CN scheme of partitioning), and between the C_α and the carbonyl carbon in the C_α–C(O) bond (CC scheme of partitioning, for details see Scheme 1A). We can generally expect that the best choice would be to cut the least polar bond, ideally a homonuclear bond between two tetrahedral carbon atoms. In the case of protein backbone cutting, it is the C_α–C(O) bond that is the least polar. Such a treatment of the protein chain, for example the CC scheme, does not strictly reflect the structure and composition of a single, isolated (monomeric) amino acid, but it has another advantage: it reflects the natural role of particular functional groups in the context of a protein structure more precisely. Besides the fact that the estimation of the interaction strength is thus more complex, there is an additional advantage in the involvement of the 'peptide bond' in the calculation – its dipole character (Scheme 1A).

Once a fragment is made, a terminal group must be added to saturate the bond cut. To make as few alterations to the physical properties of the particular functional groups of the native macromolecule as possible, a hydrogen atom or a methyl group is generally used to cap the termini of the fragments. In this study, we have tested both alternatives: the H atom as well as the methyl group on the C terminus in the CON scheme of partitioning (see Scheme 1B).

System Selection

An evaluation of the studied partitioning schemes requires a comparison of the whole system on the one hand and the fragmented system on the other. For this reason, the model system must be large enough for fragmentation but small enough to allow the interaction energy to be calculated by advanced *ab initio* methods. We have selected two model systems for the purpose of the study. The first one was a part of the structure of the protein rubredoxin from *desulfovibrio vulgaris* [code 1RB9] and the other one comes from the structure of the protein cycline-dependent kinase 2 (CDK2)[30,31]



SCHEME 1
The scheme of the fragmentation procedure. There are three types of bonds in the protein backbone: in the first case (*I*) it is the C_α–C(O) bond that is cut (CC), in the second (*II*) it is the N–C_α bond (CN) and in the third (*III*) it is the C(O)–N bond (CON) in a peptide bond (A). A possible way of compensating for the cut chain uses capping groups instead of hydrogen, e.g. the methyl group as shown in the (B)

with the ligand roscovitine. Both systems are shown in Fig. 1 along with their interaction schemes. For both systems, we chose a representative tripeptide cut out from the protein. In the case of rubredoxin, the tripeptide (Y4V5C6) interacts with the F49 phenylalanine residue inside the hydrophobic core of the protein. In the case of CDK2, the studied tripeptide (F82L83H84) interacts with a nucleotide-like ligand. In both cases, the interactions of F49 or the ligand with the backbone are more important than the interactions with the side chains of the tripeptide. For the Y4V5C6 tripeptide, the peptide bonds are in stacking and T-shape arrangements with the aromatic ring of the F49. This interaction is mostly of a dispersion origin and rather strong[11]. For the F82L83H84 tripeptide, there are two H-bonds between the tripeptide backbone (L83) and the ligand. This interaction features energy of both an electrostatic and dispersion character. Therefore, a non-balanced partitioning into distinct blocks can introduce a substantial error with a direct impact on the computed interaction strength.

Calculations

The geometries of the model systems (fragments of peptide chain, ligands) were taken from X-ray crystal structures, and the positions of the hydrogens were determined by the DFT/B3LYP/6-31** gradient optimisation of the complex in question (with the positions of the heavy atoms being kept frozen) using the Gaussian 03 program suite[32].

RI-MP2 Interaction Energy

It has been shown previously[11,12] how important the selection of the proper method is for the description of weak intermolecular interactions. The method in question has to cover the dispersion energy contribution, important for the interactions of aliphatic and aromatic amino acids. It has been proved that the interaction energy for small non-covalently bonded systems is well described by the RI-MP2 method with extrapolation to the complete basis set limit and corrections by the CCSD(T) term[33]. The extrapolation is based on calculations in the aug-cc-pVDZ and aug-cc-pVTZ basis sets. A comparison of the total interaction energy values obtained by this method showed that the RI-MP2/aug-cc-pVDZ values are on average very close to the CBS values corrected by the CCSD(T) term. All of the results of our studies mentioned previously have clearly shown that the application of the RI-MP2 method and aug-cc-pVDZ basis set works reasonably on a

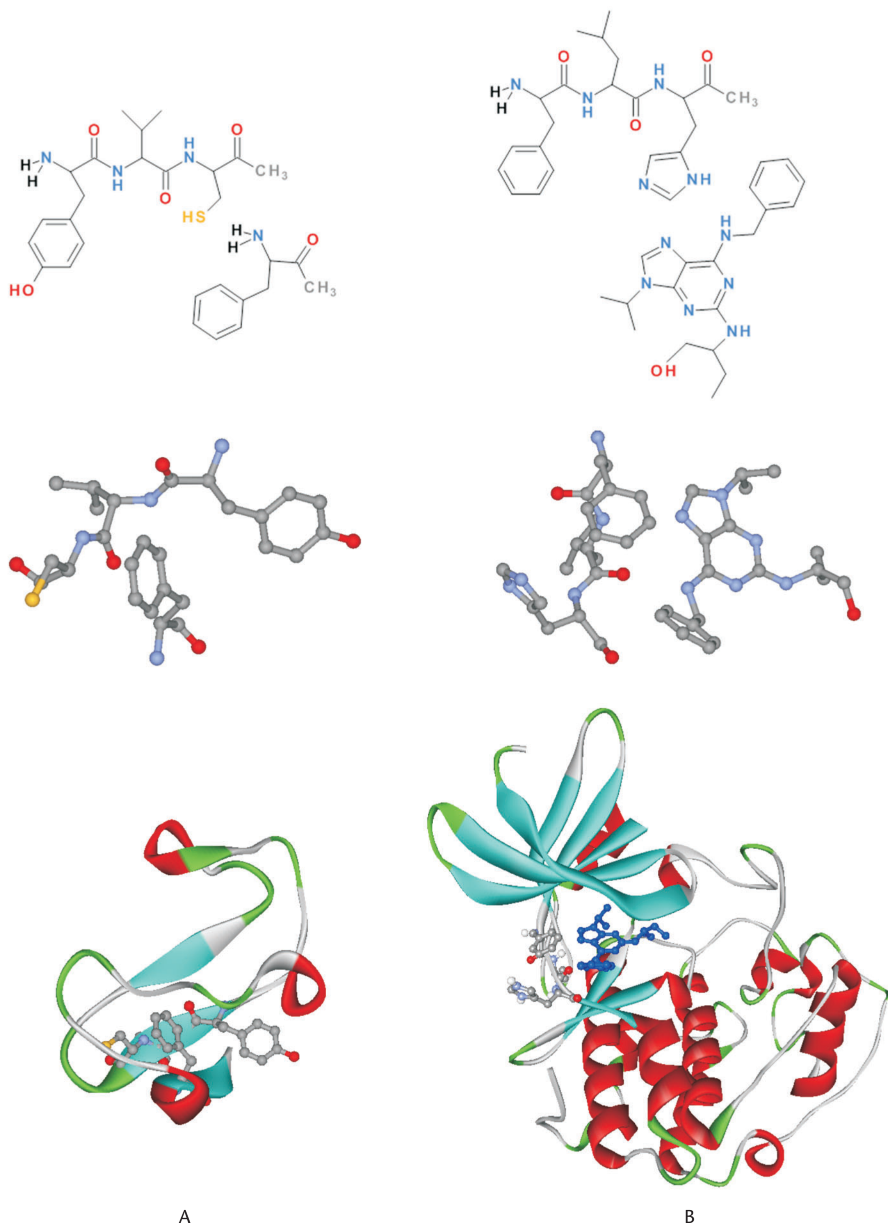


FIG. 1
A schematic view of the two model systems: a part of rubredoxin (PDB code 1RB9) (A) and a part of the complex of CDK2 and roscovitine (B), the structure based on unpublished X-ray data

broad range of systems and the decision to take the RI-MP2 values as the benchmark for our calculations is based on sound analysis and experience.

Therefore, for the purpose of this study, we have calculated the interaction energy at the RI-MP2/aug-cc-pVDZ level using the Turbomole 5.7 program suite[34]. For all of the calculations, the counterpoise corrections were made.

DFT Interaction Energy Calculations

SCC-DFTB-D

This method is based on a combination of the approximate self-consistent charge and of the density functional tight-binding method[35] with empirical dispersion energy (SCC-DFTB-D). The inclusion of an empirical dispersion term improved the major traditional deficiency of the DFT methods, namely the omission of the dispersion energy[36]. Another advantage of the SCC-DFTB-D method is its computational efficiency, which allows its use in MD simulations for small systems (up to 400 atoms). A more detailed description of this method can be found in reference[37].

RI-DFT

All of the DFT calculations were made using the TPSS meta-generalised gradient functional[38] and the TZVP basis set as implemented in the Turbomole 5.7 program suite. TPSS is a pure DFT functional, for which the RI approximation[39] was used to obtain a reasonable computational efficiency. The interaction energy was obtained as the difference of the energy of the complex and the energies of the monomers. The triple-zeta basis set is sufficiently large to keep the BSSE small (~1 kcal/mol), and no counterpoise corrections were made. The dispersion energy, which is not covered by DFT methods, was included as an empirical correction[36] and abbreviated as RI-DFT-D.

RESULTS AND DISCUSSION

The final interaction energy values obtained by the different partitioning schemes for both systems are shown in Tables I and II. The reference values obtained by the RI-MP2 method are the interaction energies between the whole Y4V5C6 tripeptide and the F49 residue in rubredoxin, and the F82L83H84 tripeptide and roscovitine in the case of CDK2 (column 2, row 5 in Tables I and II).

The interaction energy was determined as –12.2 kcal/mol for the first system (Y4V5C6···F49) and –17.6 kcal/mol for the second system (F82L83H84··· roscovitine). This energy serves as a reference value for a further comparison between different schemes of partitioning as well as for the evaluation of the methods utilised. The values of the total interaction energies, which depend on the scheme of partitioning, on the RI-MP2 level show relatively small differences, which indicates that almost all of the types of the artificial cutting of the polypeptide chain are acceptable. The two cases manifest another similar trend: the largest loss of energy and non-negligible underestimation comes from the cutting of the peptide bond between the C(O)–N atoms, e.g. the CON partitioning (2.2 kcal/mol for rubredoxin and 1.2 kcal/mol for roscovitine). The largest overestimation was observed by breaking

TABLE I
The stabilisation energies for different partitioning schemes in rubredoxin

Method	RI-MP2	HF	RI-DFT/TZVP	RI-DFT+disp	SCC-DFTB-D
CON	–10.0	9.8	5.8	–8.0	–14.2
CN	–13.7	5.3	2.0	–11.8	–16.0
CC	–13.1	5.7	2.3	–11.2	–15.4
CONMet	–12.7	10.7	6.0	–10.1	–16.6
Y4V5C6	**–12.2**	5.8	3.1	–9.7	–14.5

TABLE II
The stabilisation energies for different partitioning schemes in roscovitine

Method	RI-MP2	HF	RI-DFT/TZVP	RI-DFT+disp	SCC-DFTB-D
CON	–16.4	2.6	–4.0	–14.7	–14.5
CN	–19.8	–0.8	–7.3	–18.0	–16.9
CC	–17.4	1.9	–4.9	–15.4	–16.6
CONMet	–17.4	3.6	–3.6	–15.5	–16.3
F82L83H84	**–17.6**	0.9	–3.0	–14.9	–15.6

the N-C_α bond, the CN partitioning (1.5 and 2.2 kcal/mol, respectively). The reason for such overestimation can be mesomeric effect brought by the missing side chain on the C_α which can influence the electron properties of

the peptide bond. The CC partitioning yields better results (differences of 0.9 and 0.2 kcal/mol, respectively) and preserves all the important properties of the amino acid in the context of the polypeptide chain, e.g. the existence of the peptide bond and the presence of the side chain adjacent to the amide. The best correspondence between the reference energy value and the values calculated as a sum of particular contributions was obtained in the CONMet partitioning with the methylated C terminus. Unfortunately, this scheme would be misleading in some cases. For instance, it can establish artificial interactions (Fig. 2), as for example the one between the capping methyl group and the aromatic ring of the phenylalanine residue, which does not correspond to a real situation. It is obvious that such an artefact can cause quite significant errors and that these non-native contacts can provide a wrong description of the stabilisation.

One of the goals of this study was to compare various methods for the calculation of the stabilisation energy. It is quite clear that the Hartree–Fock level of description is simply deficient (column 3 in both Tables I and II), mainly because the prevailing character of the studied interactions is of a dispersion origin. All of the results for rubredoxin exhibit strong repulsion at the HF level. The situation is slightly different for the values obtained for the F82L83H84···roscovitine complex. The interaction is a mixture of hydrogen bonds and dispersion attraction, and therefore the interaction energy values are not so high in terms of repulsion. In one case, energy even shows moderate attraction. A systematic deviation between the results for the systems studied was obtained utilising the RI-DFT methodology. (For

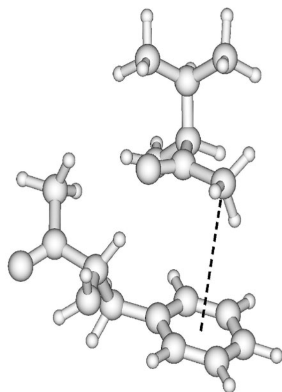


FIG. 2
An artificial CH···π bond between the capping methyl group and the aromatic ring of Phe30. The case of rubredoxin

comparison, see the work of Dobeš et al.[40].) For rubredoxin, there was not a single case of attraction regarding the scheme of partitioning. This is unlike the case of the interaction energies obtained for the F82L83H84···roscovitine complex. All of the RI-DFT values show attraction. This can be explained by the fact that if the system contains hydrogen bonds as well as dispersion interactions, it can result in final attraction, because the polar interactions are described properly. The RI-DFT method improved with empirical dispersion (London dispersion) is the first method for which we have obtained qualitative agreement with the reference method. Nevertheless, all of the values obtained systematically underestimate the total interaction energy. In the given set of structures, there is no significant improvement when a larger basis set is used (unpublished results, Supporting Materials). Nevertheless, the method preserves the trends observed for the RI-MP2 values. The DFT-TB-D values provide stronger interaction in comparison with the reference RI-MP2 method in the case of rubredoxin tripeptide interacting with the F49. The opposite is true in the second studied system – roscovitine with the CDK2 tripeptide. For both systems, the overall trend is roughly preserved in terms of the energies for the individual fragments (across fragments and across different partitioning schemes).

TABLE III
The contribution of a particular amino acid to the overall stabilisation in rubredoxin

Method	Residue	HF	RI-DFT/TZVP	RI-MP2	RI-DFT+disp	DFTB-D
CON	4	3.1	1.7	–2.2	–2.0	–3.4
	5	3.1	1.9	–3.4	–2.8	–5.6
	6	3.6	2.2	–4.4	–3.2	–5.2
CN	4	3.2	1.8	–3.0	–2.9	–3.9
	5	0.0	–0.7	–6.2	–5.5	–7.5
	6	2.1	0.9	–4.5	–3.4	–4.6
CC	4	2.3	1.2	–1.8	–1.7	–2.9
	5	1.2	0.5	–2.6	–2.6	–2.6
	6	2.2	0.6	–8.7	–6.9	–9.9
CONMet	4	4.2	2.5	–2.7	–2.3	–4.3
	5	2.9	1.3	–5.6	–4.6	–7.1
	6	3.6	2.2	–4.4	–3.2	–5.2

We should stress here once again that we have chosen systems where the backbone is significantly engaged in the interaction. Therefore, it is not surprising that the interaction energies for the individual fragments are sensitive to the partitioning scheme. In Tables III and IV, we show details on the particular interactions between the amino acids and a ligand.

CONCLUSION

We have found that the description of a protein intramolecular interaction or protein–ligand decomposed into individual fragments of amino acids interacting within a protein or in a protein–ligand interaction is a reasonable way of describing the phenomenon. Such decomposition can provide information about the contributions of the individual residues to the overall stability or to the protein–ligand affinity. It is obvious that the best choice for reproducing the overall interaction energy is the CC bond-cutting scheme. We have also concluded that this partitioning is the most reliable with a hydrogen capping the C terminus, because it brings the smallest change to the electronic structure. This scheme of partitioning does not establish any artificial interaction in the terminal group and yields reasonable interaction

TABLE IV
The contribution of a particular amino acid to the overall stabilisation in roscovitine

Method	Residue	HF	RI-DFT/TZVP	RI-MP2	RI-DFT+disp	DFTB-D
CON	82	2.5	0.6	–4.4	–3.4	–4.7
	83	–3.7	–6.5	–9.4	–9.6	–6.8
	84	3.8	1.9	–2.6	–1.7	–3.0
CN	82	–1.1	–3.9	–9.5	–8.6	–7.6
	83	–3.6	–5.5	–8.2	–8.2	–6.7
	84	3.9	2.1	–2.1	–1.2	–2.6
CC	82	3.7	1.6	–3.1	–2.1	–3.6
	83	–4.8	–6.2	–8.1	–8.3	–6.7
	84	3.0	–0.3	–6.2	–5.0	–6.3
CONMet	82	3.8	1.2	–4.8	–3.7	–5.9
	83	–4.0	–6.7	–10.0	–10.1	–7.4
	84	3.8	1.9	–2.6	–1.7	–3.0

energies in comparison with the reference values obtained by the RI-MP2 method.

The other studied schemes yielded various results, but these results were systematically different albeit still reasonable. The best agreement was obtained for the CONMet partitioning scheme with the CH_3-capped fragments, but this scheme can make interactions which are artificial, and such results can be misleading.

RI-DFT with dispersion reproduces the trends well but systematically underestimates the values. DFT-TB-D, on the other hand, does not provide a systematic difference, but the values of the interaction energy are in reasonable agreement with the reference method. The actual values are overestimated in the case of rubredoxin and underestimated in the case of the roscovitine CDK2 complex.

The tested methods that do not describe the system properly in contrast to the RI-MP2 method are the HF and pure DFT methods. Both failed completely in describing the interactions with a prevailing dispersion character. On the other hand, the RI-DFT method can make sense for systems where a classical H-bond exists.

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