Theor Chem Acc (2012) 131:1271
DOI 10.1007/s00214-012-1271-2

REGULAR ARTICLE

CompASM: an Amber-VMD alanine scanning mutagenesis plug-in

João V. Ribeiro · Nuno M. F. S. A. Cerqueira · Irina S. Moreira · Pedro A. Fernandes · Maria João Ramos

Received: 28 March 2012 / Accepted: 20 August 2012 / Published online: 30 September 2012

Abstract Alanine scanning mutagenesis (ASM) of protein–protein interfacial residues is a popular means to understand the structural and energetic characteristics of hot spots in protein complexes. In this work, we present a computational approach that allows performing such type of analysis based on the molecular mechanics/Poisson–Boltzmann surface area method. This computational approach has been used largely in the past and has proven to give reliable results in a wide range of complexes. However, the sequential preparation and manual submission of dozens of files has been often a major obstacle in using it. To overcome these limitations and turn this approach user-friendly, we have designed the plug-in CompASM (computational alanine scanning mutagenesis). This software has an easy-to-use graphical interface to prepare the input files, run the calculations, and analyze the final results. CompASM was built in TCL/TK programming language to be included in VMD as a plug-in. The CompASM package is distributed as an independent platform, with script code under the GNU Public License from http://compbiochem.org/Software/compasm/Home.html.

Dedicated to Professor Marco Antonio Chaer Nascimento and published as part of the special collection of articles celebrating his 65th birthday.

Electronic supplementary material The online version of this article (doi:10.1007/s00214-012-1271-2) contains supplementary material, which is available to authorized users.

J. V. Ribeiro · N. M. F. S. A. Cerqueira · I. S. Moreira · P. A. Fernandes · M. J. Ramos (✉)
REQUIMTE, Departamento de Química e Bioquímica, Faculdade de Ciências, Universidade do Porto, Rua Campo Alegre s/n, 4169-007 Porto, Portugal
e-mail: mjramos@fc.up.pt

Keywords Protein–protein interactions · Amber · VMD · MMPBSA · Software

1 Introduction

The association of proteins and the way they bind are a crucial topic in the study of living organisms. This importance stems from the fact that protein–protein interactions play a crucial role in the molecular recognition and cellular function. Mapping these interactions at the interface and revealing the key-stone residues will provide important insight into how these structures combine and how it is possible to manage them, as well as improving or inhibiting their association [1, 2].

One of the key features of these protein–protein interfaces is their sensitivity to mutations. This means that if we mutate a key interface residue by a residue alanine, there will be a significant variation in the protein–protein complex binding or association free energy. It has been defined in the literature that if the increase in the binding free energy is above 4 kcal/mol, then the mutated residue is extremely important and it is called a hot spot; if the energy increase upon mutation is between 2 and 4 kcal/mol, then this residue is relatively important for the protein–protein association and it is denominated a warm spot, and finally, if the mutations originate a binding free energy variation below 2 kcal/mol, then the residue is not particularly relevant for the interaction and it is termed a null spot [3–5].

Moreira et al. [3] have developed a protocol (schematized in Fig. 1), with low computational cost and high success rate that reproduces the quantitative free energy differences obtained from experimental mutagenesis procedures. This computational approach is transferable to any macromolecular complex and is a predictive model capable

of anticipating the experimental results of mutagenesis, thus capable of guiding new experimental investigations. It is based on the all-atom methodology MMPBSA (molecular mechanics/Poisson–Boltzmann surface area) [6] to probe protein–protein interactions by calculating free energies combining molecular mechanics and a continuum solvent.

There are several web servers already available to compute this protein–protein interaction [7]. Despite the large variety of available possibilities to study this kind of interactions, our method still proved to be better from the point of view of returning quantitative values of the binding free energy differences with molecular dynamics as the sampling method. The features contrast with the qualitative values and the analysis of only a few structures of other methods available. At the end of the Sect. 4, values from other approaches obtained for our case studies are also presented for comparison.

2 Methodology

The first stage of CompASM involves the relaxation and equilibration of the wild-type complex that is being analyzed. This can be accomplished by a minimization procedure only or by a molecular dynamics simulation in a continuum medium, using the Generalized Born model. Subsequently, only the relaxed wild-type complex is divided in several alanine mutated complexes that were previously defined by the user, depending on the study that is intended to be performed. To the wild-type and mutated complexes, it is then applied the MMPBSA script to calculate the respective binding free energy differences.

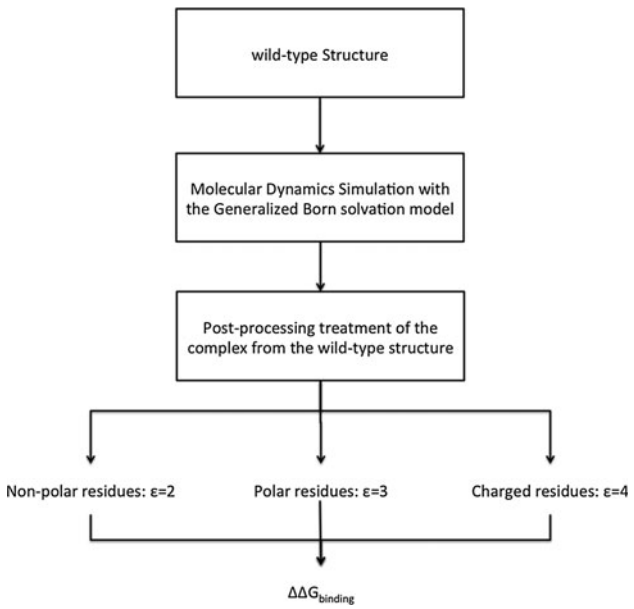

Fig. 1 General algorithm of the CompASM procedure [3]

To generate the structure of the mutant complex, a simple truncation of the mutated side chain is carried out, replacing carbon atom C_γ with a hydrogen atom, and setting the C_β–H bond direction to that of the former C_β–C_γ. The corresponding binding free energy can be calculated using the thermodynamic cycle shown in Fig. 2, in which ΔG_{gas} is the binding free energy between the two interacting partners in the gas phase, and ΔG_{solv}^{lig}, ΔG_{solv}^{recep}, and $\Delta G_{solv}^{complex}$ are the solvation free energy differences of the two binding partners and the complex, respectively. The binding free energy difference between an alanine mutant complex and a wild-type complex is defined as Eq. (1):

$$\Delta\Delta G_{binding} = \Delta G_{binding-mutant} - \Delta G_{binding-wildtype} \quad (1)$$

The binding free energy of two molecules in a complex is defined as the difference between the free energy of the complex and those of the respective monomers. On the other hand, the free energy of a protein–protein complex and its respective monomers can be calculated by Eq. (2), that is, by summing the internal energy (bond, angle, and dihedral), $E_{internal}$; the electrostatic and the van der Waals interactions, $E_{electrostatic}$ and E_{vdW}; the free energy of polar solvation, G_{polar} solvation; the free energy of nonpolar solvation, $G_{nonpolar}$ solvation; and the entropic TS contribution for the molecule free energy.

$$G_{molecule} = E_{internal} + E_{electrostatic} + E_{vdW} + G_{polar\,solvation} + G_{nonpolar\,solvation} - TS \quad (2)$$

The first three terms in Eq. (2) are calculated using the Cornell force field [8] with no cutoff. The electrostatic solvation free energy is calculated by solving the Poisson–Boltzmann equation with the Delphi software [9, 10], which has been shown to constitute a good compromise between accuracy and computing time.

For the energy calculations, CompASM attributes specific values to three internal dielectric constant values, which depend exclusively on the type of amino acid that is mutated. Therefore, for the charged amino acids (aspartic acid, glutamic acid, lysine, arginine, and histidine), a constant of 4 should be used, for the remaining polar residues (asparagine,

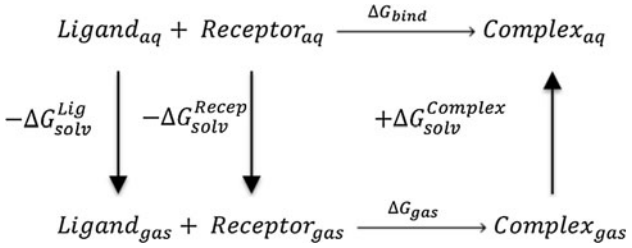

Fig. 2 Thermodynamic cycle used to calculate the binding free energy in the CompASM protocol

glutamine, cysteine, tyrosine, serine, and threonine) not ionized at physiological pH, the internal dielectric constant should be 3, and for the nonpolar amino acids (valine, leucine, isoleucine, phenylalanine, methionine, and tryptophan), the internal dielectric constant should be 2 [3]. The different internal dielectric constants account for the different degree of relaxation of the interface when different types of amino acids are mutated for alanine; the stronger the interactions these amino acids establish, the more extensive the relaxation should be, and the greater the internal dielectric constant value must be to mimic these effects.

However, for the sake of flexibility, in order to allow the user to set other values that he might see fit to the dielectric constants, we have added a feature in which the values of any number of dielectric constants can be changed from the default ones mentioned above or even added if necessary. This introduces flexibility to the plug-in and allows the user to improve the quality of the results, if considered necessary.

The nonpolar contribution to solvation free energy due to van der Waals interactions between the solute and the solvent and cavity formation was modeled as a term that is dependent on the solvent-accessible surface area of the molecule. It was estimated using empirical relation (3),

$$\Delta G_{\text{nonpolar}} = A+ \tag{3}$$

where A is the solvent-accessible surface area that was estimated using the MolSurf program, which is based on the idea primarily developed by Michael Connolly. Constants α and β are empirical, taking the values 0.005 42 kcal Å^{-2} mol^{-1} and 0.92 kcal mol^{-1}, respectively. The entropy term, obtained as the sum of translational, rotational, and vibrational components, was not calculated because it was assumed, on the basis of previous work [11, 12], that its contribution to $\Delta\Delta G_{\text{binding}}$ is negligible.

The calculation of $\Delta\Delta G_{\text{binding}}$ is achieved applying several modules of the AMBER program [13], and despite the apparent simplicity, this kind of study can easily become cumbersome. Beyond the repetitive tasks, such as the generation of mutation structures and the input for the MMPBSA [12] calculation, the handling of a large amount of files can be a tricky job. Regarding these difficulties and combining the visual facilities provided by visual molecular dynamics (VMD) [14] with the extremely intuitive graphical user interface (GUI) and the AMBER molecular dynamics calculations, we propose a new VMD-AMBER plug-in, named CompASM, which allows even the non-expert user to perform easily Alanine Scanning Mutagenesis calculations.

3 Software description

CompASM is a versatile tool created to study protein–protein interfaces, allowing the user to skip the repetitive task of creating input files and generating all necessary structures, as well as providing new options and procedures to perform a computational alanine scanning mutagenesis experiment. The input file is based on an AMBER-format file. To maintain the functionality in almost all situations, we have divided the software in two main structures: a VMD plug-in GUI and an independent CORE.

The CompASM GUI was designed to drive the user through the different steps of ASM, allowing simultaneously all the freedom needed to treat all kind of structures that require different specifications. Beyond the ligand/receptor differentiation and mutation selection, this GUI allows the user to exclude or include non-protein structures (known as heteroatoms) as well as to insert their parameterization files (.mol2 and/or.frcmod files). Another facility presented by CompASM GUI is the molecular minimization/dynamics simulation set-up tab. Here, the user can set all variables to the values needed to submit a molecular dynamics simulation or just a minimization to AMBER. The user can add more variables directly in the input file following the instructions that pop up as one proceeds in the calculation. Another useful tool is the mutation selection by non-solvent contact area (NSCA-explained schematically in Fig. 3)-based selection (Fig. 3).

This is based on a "sasa measure" VMD command, and the residue is selected if the area that is in contact with another structure is larger than 40 Å^2 (more detail in supporting information). The final results are visualized in the VMD graphics window, using the same color scheme of the summary and the detailed tables in the GUI.

The CompASM CORE is an independent set of procedures that load the structure and perform the algorithm proposed by Moreira et al. [3]. This CORE handles the files organization, performs the MMPBSA calculations, and returns an output file (ASM.out) with all the information necessary to evaluate the final results. To improve the speed and make the best use of the computational resources, we parallelized the slower procedure, the MMPBSA calculation, running each independent calculation in different CPU cores. In the CORE's procedures, the molecular dynamics simulation is performed in sets of 1/10 of the total time requested by the user. In each set, the program checks if the coordinates from the structure (backbone only) are stabilized, evaluating several parameters of the linear regression of the root-mean-square deviation (RMSD) calculated by the ptraj AMBER tool. The simulation is considered stabilized if the slope of the straight, the standard deviation and the correlation factor are in agreement with the values set by the user. For more information, please see the supporting information of this document. This software was developed in TCL/TK as the programming language, and it is

Fig. 3 Scheme representing the non-solvent contact area (NSCA). This area intends to represent by which amount each residue is buried in the protein surface. All surface areas are evaluated by the "sasa measure" command present in the visual molecular dynamics (VMD) software

available for the Amber 8 and 9 versions, requiring the DELPHI package. The application of this software using higher versions of Amber is not presently possible, only due to the inexistence of the DELPHI package in the MMPBSA protocol (i.e. not available in the Amber 10 package).

4 Results

The data resulting from CompASM are displayed in a simplified table and in a more detailed table are displayed all the values obtained from the MMPBSA calculation, including the NSCA values. All this data can be analyzed interactively in a VMD window, coloring the residue depending on the obtained score. Figure 4 shows the results obtained from CompASM concerning the protein–protein interface study of immunoglobulin complexed with an egg lysozyme, as an example.

In this section, we describe the validation process that was used to evaluate the performance of the CompASM software in the determination of the hot, warm, and null spots of three different protein–protein complexes. The results of this validation process are shown in Table 1. The structures analyzed are immunoglobulin complexed with an egg lysozyme (1VFB) [15]; complexes that mediate bacterial cell division (1F47) [16] and human immunoglobulin IgG complexed with the C2 fragment of streptococcal protein *G* (1FCC) [17]. Table 1 shows the values of NCSA, the experimental value of $\Delta\Delta G_{\text{binding}}$ of each mutation and the $\Delta\Delta G_{\text{binding}}$ evaluated by CompASM. All calculations involved molecular dynamics simulations using the default values of the CompASM GUI (Fig. 4) and modifying only the MMPBSA frequency to achieve low

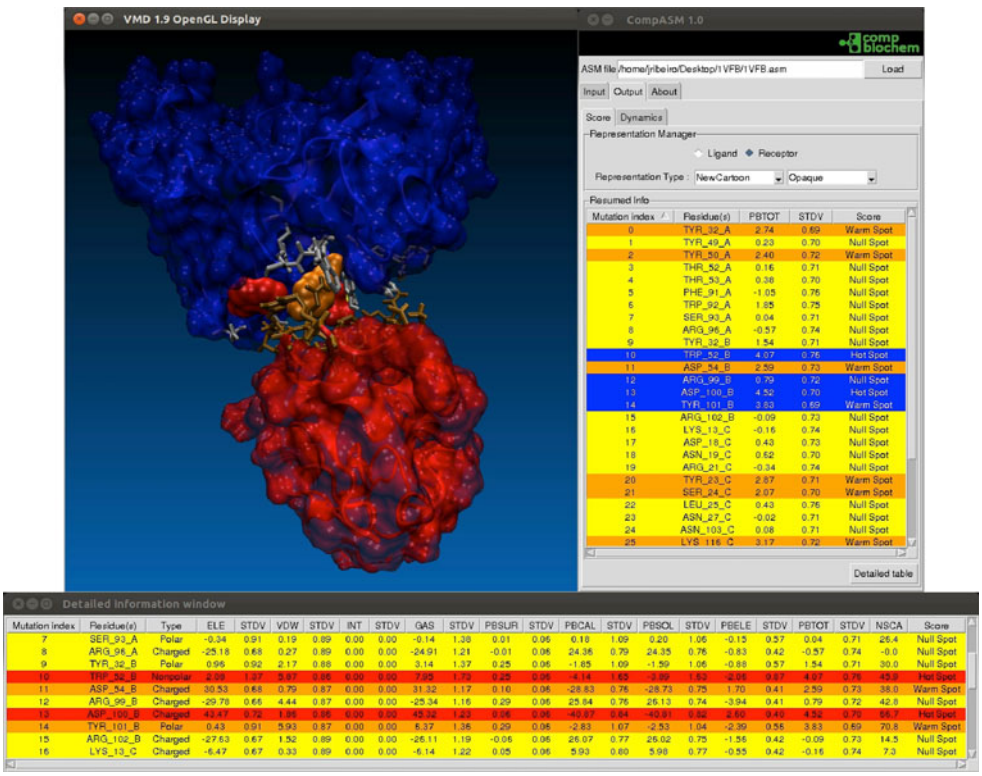

Fig. 4 Results of the protein–protein interface study of immunoglobulin complexed with an egg lysozyme (detailed results in supporting information)

Table 1 Results originated by CompASM

Protein	MMPBSA freq	Mutation	NSCA (Å^2)	Residue type	Experimental	ComPASM		Scores	
					$\Delta\Delta G_{exp}$ (kcal/mol)	$\Delta\Delta G_{ComASM}$ (kcal/mol)	STDV	Exp	CompASM
1VFB	67	TYR_32_A	65.00	Polar	1.30	3.20	0.89	Null spot	Warm spot
1VFB	67	TYR_49_A	20.60	Polar	0.80	0.39	0.89	Null spot	Null spot
1VFB	67	TYR_50_A	37.80	Polar	0.40	2.40	0.91	Null spot	Warm spot
1VFB	67	THR_53_A	15.00	Polar	−0.23	0.26	0.87	Null spot	Null spot
1VFB	67	TRP_92_A	48.30	Nonpolar	1.71	1.71	0.94	Null spot	Null spot
1VFB	67	SER_93_A	26.40	Polar	0.11	−0.10	0.90	Null spot	Null spot
1VFB	67	TYR_32_B	30.00	Polar	0.50	1.23	0.88	Null spot	Null spot
1VFB	67	TRP_52_B	45.90	Nonpolar	1.23	3.90	0.89	Null spot	Warm spot
1VFB	67	ASP_54_B	38.00	Charged	1.95	2.55	0.94	Null spot	Hot spot
1VFB	67	ARG_99_B	42.80	Charged	0.47	0.86	0.93	Null spot	Null spot
1VFB	67	ASP_100_B	66.70	Charged	3.10	4.47	0.92	Warm spot	Hot spot
1VFB	67	TYR_101_B	70.80	Polar	4.00	4.04	0.91	Hot spot	Hot spot
1VFB	67	ASP_18_C	27.90	Charged	0.30	0.51	0.97	Null spot	Null spot
1VFB	67	ASN_19_C	69.00	Polar	0.40	0.78	0.89	Null spot	Null spot
1VFB	67	TYR_23_C	12.10	Polar	0.80	2.84	0.91	Null spot	Warm spot
1VFB	67	SER_24_C	52.90	Polar	0.70	2.12	0.91	Null spot	Warm spot
1VFB	67	LYS_116_C	57.60	Charged	0.70	3.06	0.94	Null spot	Warm spot
1VFB	67	THR_118_C	42.70	Polar	0.80	0.19	0.91	Null spot	Null spot
1VFB	67	ASP_119_C	43.70	Charged	1.00	3.48	0.96	Null spot	Warm spot
1VFB	67	VAL_120_C	9.70	Nonpolar	0.90	−0.39	0.92	Null spot	Null spot
1VFB	67	GLN_121_C	108.10	Polar	2.90	2.53	0.91	Warm spot	Warm spot
1VFB	67	ILE_124_C	28.70	Nonpolar	1.20	0.09	0.92	Null spot	Null spot
1VFB	67	ARG_125_C	48.80	Charged	1.80	3.21	0.93	Null spot	Warm spot
1VFB	67	LEU_129_C	23.60	Nonpolar	0.20	−9.93	0.93	Null spot	Null spot
1F47	67	ASP_4_A	43.20	Charged	0.69	−1.60	0.8	Null spot	Null spot
1F47	67	TYR_5_A	63.30	Polar	0.86	4.21	0.74	Null spot	Hot spot
1F47	67	LEU_6_A	81.70	Nonpolar	0.92	1.59	0.78	Null spot	Null spot
1F47	67	ASP_7_A	22.30	Charged	1.73	−0.46	0.78	Null spot	Null spot
1F47	67	ILE_8_A	50.70	Nonpolar	2.50	3.12	0.77	Warm spot	Warm spot
1F47	67	PHE_11_A	77.00	Nonpolar	2.44	3.60	0.79	Warm spot	Warm spot
1F47	67	LEU_12_A	53.10	Nonpolar	2.29	2.21	0.79	Warm spot	Warm spot
1FCC	35	GLU_27_C	45.80	Charged	4.90	10.91	1.17	Hot spot	Hot spot
1FCC	35	LYS_28_C	122.30	Charged	1.30	2.40	1.22	Null spot	Warm spot
1FCC	35	LYS_31_C	56.10	Charged	3.50	4.83	1.20	Warm spot	Hot spot
1FCC	35	ASN_35_C	61.80	Polar	2.40	0.95	1.12	Warm spot	Null spot
1FCC	35	ASP_40_C	44.20	Charged	0.30	0.49	1.23	Null spot	Null spot
1FCC	35	GLU_42_C	26.00	Charged	0.40	0.34	1.23	Null spot	Null spot
1FCC	35	TRP_43_C	37.80	Nonpolar	3.80	−0.13	1.09	Warm spot	Null spot

The non-solvent contacting area (NSCA) is calculated by the CompASM program

standard deviation values. In the MMPBSA calculations of the 1VFB and 1F47 proteins, 50 structures were used, from the fourth and fifth nanosecond, respectively, of the simulation, and in the case of the 1FCC corresponding protein, 100 structures were used from the forth nanosecond. These same structures were also analyzed using well-known web servers available, and the positive predictive value or specificity P (Eq. 4), true positive rate or sensitivity R (Eq. 5), and the F measure test accuracy $F1$ (Eq. 6) values were calculated and shown in Table 2.

Table 2 Comparison of the values obtained using CompASM with those that result from the software/servers available

Abbrev.	ISIS	Promate	ROBETTA	KFC2-A	KFC2-B	HotPoint	CompASM
TP	1	0	7	5	6	6	8
TN	26	25	21	25	23	20	17
FP	2	3	7	3	5	8	11
FN	9	10	3	5	4	4	2
R	10	0	70	50	60	60	80
P	33	0	50	63	55	43	42
F1	15	0	58	56	57	50	55

TP true positive, *TF* true negative, *FN* false negative, *FP* false negative, *R* positive predictive, *P* negative predictive, *F1 F* measure

$$P = \frac{\mathrm{TP}}{\mathrm{TP} + \mathrm{FP}} \tag{4}$$

$$R = \frac{\mathrm{TP}}{\mathrm{TP} + \mathrm{FN}} \tag{5}$$

$$F1 = \frac{2\mathrm{PR}}{P + R} \tag{6}$$

In Eqs. (4), (5), and (6), TP corresponds to the correct computational prediction of the number of hot spots (TP—true positive), that is, when these residues are experimentally classified as hot spots; FP corresponds to the number of computationally predicted hot spots when these residues are experimentally classified as null spots (FP—false positive); and finally, FN corresponds to the number of computationally predicted null spots when these residues are experimentally classified as hot spots (FN—false negative). Therefore, the closer the values are to 100 the better they are, meaning in this case that the software is capable to predict correctly the numbers of hot spots and null spots.

To compare our software to those from web servers already available, we tested the same mutations in the proteins analyzed above using the following softwares: ISIS [18]; Promate [19]; Robetta [20]; K-FC2A and B [21]; and HotPoint [22].

Tables 1 and 2 demonstrate that the values resulting from CompASM are in the same range of those provided by experimental means, that is, the CompASM software was able to detect all the hot spots. This software was also sensitive enough to detect and to distinguish most warm- and null spots. The results are very close to those obtained by Moreira et al. [3] in which the methodology of CompASM was based on. The small differences result from the differences in the structures retrieved from the MD simulations and submitted to the MMPBSA calculations. CompASM has its own algorithm, which is used to control the molecular dynamics simulations. Once all the criteria are achieved, which the user can either set him/herself or accept the default, the MD simulation is stopped and the MMPBSA protocol starts. This algorithm is very useful because it makes the process straightforward and minimizes the computation time. Simultaneously, it allows the process to be reproducible. This makes it very handy not only for non-expert users, but also for more advanced users that can modify the criteria used in the CompASM GUI.

Table 2 shows the number of TP, TN, FP, and FN values, as well as the accuracy of the different tested softwares.

Looking at the data resulting from CompASM, we can notice only 2 false negative values. There are, however, 11 false positives. In the majority of the complexes studied, an analysis of the dominant interactions suggests that van der Waals interactions and hydrophobic effects provide a reasonable basis for understanding binding affinities. In fact, the breakdown of Eq. (2) for all the cases studied with CompASM shows that the $\Delta\Delta E_{\mathrm{vdw}}$ values are almost all positive indicating that the van der Waals interactions are favorable to complex binding and that alanine mutation of the residues diminishes the vdW contacts at the interfaces. This is explained by the hydrophobic character of the interfaces.

To further examine the reliability and usefulness of CompASM, we have compared our predictions to the predictions of some of the more popular softwares that are available nowadays. The results are presented in Table 2 pointing to the fact that the methods Robetta, KFC2-A, -B, HotPoint, and CompASM have similar performances, with the latter giving slightly better values. However, in a subsequent work [23], we provide a full study documenting the results obtained with CompASM based on a large number of structures, which points out to its good performance generating the better values among other methods.

We notice that only two of the above-mentioned approaches, ours and Robetta's, provide quantitative values, which can be fully compared to the experimental ones, as opposed to qualitative values that classify the mutations between hot, warm, and null spots only. However, in order to compare the results obtained with CompASM to the values returned by other softwares, we translated our values to the binominal qualitative classification (null- and hot

spot) adopted by the tested softwares, which classify the residues in null spots if the $\Delta\Delta G$ values are below 2 kcal/mol and hot spots if the $\Delta\Delta G$ values are higher than 2 kcal/mol.

CompASM returns a value close to 100 (80) for the values of R (hot spots prediction over false negatives). However, as CompASM is a software with which we can obtain quantitative values, we know directly how close is the residue to be a null- or a hot spot by comparing the obtained $\Delta\Delta G$ with the reference barriers of 2 and 4 kcal/mol, respectively.

5 Conclusions

Computational alanine scanning mutagenesis [3] has proven to be an accurate means of detecting the residues that play an important role in protein–protein interfaces (hot spots). Here, we present a VMD plug-in, CompASM, which facilitates the application of this approach thus simplifying the study of protein interfaces. CompASM guides the user through all ASM steps, from the ligand/receptor selection to the molecular dynamics simulation, and provides the visualization of the final results in a VMD window. This program can run either in local machines or in a cluster (multicomputer system). The GUI package is multiplatform, and the CORE package works in a UNIX system (Mac OS and Linux).

The CompASM package is distributed as an independent platform, with script code under the GNU Public License from http://compbiochem.org/Software/compasm/Home.html.

Acknowledgments The authors would like to thank the FCT (Fundação para a Ciência e Tecnologia) for financial support (Grants SFRH/BD/61324/2009 and PTDC/QUI-QUI/103118/2008).

References

1. Bordner AJ, Abagyan R (2005) Statistical analysis and prediction of protein–protein interfaces. Proteins 60(3):353–366. doi:10.1002/Prot.20433
2. Thorn KS, Bogan AA (2001) ASEdb: a database of alanine mutations and their effects on the free energy of binding in protein interactions. Bioinformatics 17(3):284–285
3. Moreira IS, Fernandes PA, Ramos MJ (2007) Computational alanine scanning mutagenesis—an improved methodological approach. J Comput Chem 28(3):644–654. doi:10.1002/jcc.20566
4. Ramos MJ, Moreira IS, Fernandes PA (2007) Hot spots—a review of the protein–protein interface determinant amino-acid residues. Proteins 68(4):803–812. doi:10.1002/Prot.21396
5. Moreira IS, Fernandes PA, Ramos MJ (2007) Hot spot occlusion from bulk water: a comprehensive study of the complex between the lysozyme HEL and the antibody FVD1.3. J Phys Chem B 111(10):2697–2706. doi:10.1021/Jp067096p
6. Kollman PA, Massova I, Reyes C, Kuhn B, Huo SH, Chong L, Lee M, Lee T, Duan Y, Wang W, Donini O, Cieplak P, Srinivasan J, Case DA, Cheatham TE (2000) Calculating structures and free energies of complex molecules: combining molecular mechanics and continuum models. Acc Chem Res 33(12):889–897. doi:10.1021/Ar000033j
7. Fernandez-Recio J (2011) Prediction of protein binding sites and hot spots. Wires Comput Mol Sci 1(5):680–698. doi:10.1002/Wcms.45
8. Cornell WD, Cieplak P, Bayly CI, Gould IR, Merz KM, Ferguson DM, Spellmeyer DC, Fox T, Caldwell JW, Kollman PA (1996) A second generation force field for the simulation of proteins, nucleic acids, and organic molecules (vol 117, pg 5179, 1995). J Am Chem Soc 118(9):2309–2309
9. Rocchia W, Sridharan S, Nicholls A, Alexov E, Chiabrera A, Honig B (2002) Rapid grid-based construction of the molecular surface and the use of induced surface charge to calculate reaction field energies: applications to the molecular systems and geometric objects. J Comput Chem 23(1):128–137
10. Rocchia W, Alexov E, Honig B (2001) Extending the applicability of the nonlinear Poisson–Boltzmann equation: multiple dielectric constants and multivalent ions. J Phys Chem B 105(28):6507–6514
11. Massova I, Kollman PA (1999) Computational alanine scanning to probe protein–protein interactions: a novel approach to evaluate binding free energies. J Am Chem Soc 121(36):8133–8143
12. Huo S, Massova I, Kollman PA (2002) Computational alanine scanning of the 1:1 human growth hormone–receptor complex. J Comput Chem 23(1):15–27
13. Case DA, Darden TA, Cheatham, Simmerling CL, Wang J, Duke RE, Luo R, Merz KM, Pearlman DA, Crowley M, Walker RC, Zhang W, Wang B, Hayik S, Roitberg A, Seabra G, Wong KF, Paesani F, Wu X, Brozell S, Tsui V, Gohlke H, Yang L, Tan C, Mongan J, Hornak V, Cui G, Beroza P, Mathews DH, Schafmeister C, Ross WS, Kollman PA (2006) Amber 9
14. Humphrey W, Dalke A, Schulten K (1996) VMD: visual molecular dynamics. J Mol Graph 14(1):33–38
15. Bhat TN, Bentley GA, Boulot G, Greene MI, Tello D, Dallacqua W, Souchon H, Schwarz FP, Mariuzza RA, Poljak RJ (1994) Bound Water-molecules and conformational stabilization help mediate an antigen-antibody association. Proc Natl Acad Sci USA 91(3):1089–1093
16. Somers WS, Mosyak L, Zhang Y, Glasfeld E, Haney S, Stahl M, Seehra J (2000) The bacterial cell-division protein ZipA and its interaction with an FtsZ fragment revealed by X-ray crystallography. EMBO J 19(13):3179–3191
17. Sauererikssson AE, Kleywegt GJ, Uhl M, Jones TA (1995) Crystal-structure of the C2 fragment of streptococcal protein-G in complex with the Fc domain of human-Igg. Structure 3(3):265–278
18. Ofran Y, Rost B (2007) ISIS: interaction sites identified from sequence. Bioinformatics 23(2):E13–E16. doi:10.1093/Bioinformatics/Btl303
19. Neuvirth H, Raz R, Schreiber G (2004) ProMate: a structure based prediction program to identify the location of protein–protein binding sites. J Mol Biol 338(1):181–199. doi:10.1016/J.Jmb.2004.02.040
20. Kortemme T, Baker D (2002) A simple physical model for binding energy hot spots in protein–protein complexes. Proc Natl Acad Sci USA 99(22):14116–14121
21. Darnell SJ, Page D, Mitchell JC (2007) An automated decision-tree approach to predicting protein interaction hot spots. Proteins 68(4):813–823. doi:10.1002/Prot.21474
22. Tuncbag N, Keskin O, Gursoy A (2010) HotPoint: hot spot prediction server for protein interfaces. Nucleic Acids Res 38:W402–W406. doi:10.1093/Nar/Gkq323
23. Moreira IS, Martins JM, Ramos RR, Fernandes PA, Ramos MJ (2012) Computational alanine scanning mutagenesis: a new method for hot-spot detection (Results to be published)