



# Electrostatic potential calculation for biomolecules - creating a database of pre-calculated values reported on a per residue basis for all PDB protein structures

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**ABSTRACT.** STING and JavaProtein Dossier provide a collection of physical-chemical parameters, describing protein structure, stability, function, and interaction, considered one of the most comprehensive among the available protein databases of similar type. Particular attention in STING is paid to the electrostatic potential. It makes use of DelPhi, a well-known tool that calculates this physical-chemical quantity for biomolecules by solving the Poisson Boltzmann equation. In this paper, we describe a modification to the DelPhi program aimed at integrating it within the STING environment. We also outline how the “amino acid electrostatic potential” and the “surface amino acid electrostatic potential” are calculated (over all Protein Data Bank (PDB) content) and how the corresponding values are made searchable in STING\_DB. In addi-

tion, we show that the STING and <sup>Java</sup>Protein Dossier are also capable of providing these particular parameter values for the analysis of protein structures modeled in computers or being experimentally solved, but not yet deposited in the PDB. Furthermore, we compare the calculated electrostatic potential values obtained by using the earlier version of DelPhi and those by STING, for the biologically relevant case of lysozyme-antibody interaction. Finally, we describe the STING capacity to make queries (at both residue and atomic levels) across the whole PDB, by looking at a specific case where the electrostatic potential parameter plays a crucial role in terms of a particular protein function, such as ligand binding. BlueStar STING is available at <http://www.cbi.cnptia.embrapa.br>.

**Key words:** Electrostatic potential of biomolecules, DelPhi, STING, Protein structure analysis

## INTRODUCTION

<sup>Java</sup>Protein Dossier (<sup>J</sup>PD; Neshich et al., 2004) is a new concept database and visualization tool for both structures deposited at the Protein Data Bank (PDB; Berman et al., 2000) and those derived either by models or experiments, but not yet available from PDB. <sup>J</sup>PD is a part of the STING (Neshich et al., 2003, 2005a) environment which provides one of the most comprehensive collections (Neshich et al., 2005b) of physical-chemical parameters describing protein sequence, structure, stability, function, and interaction with other macromolecules.

Electrostatics is believed to play a pivotal role in regulating interactions between biological macromolecules, such as proteins and nucleic acids (Radic et al., 1997; Sheinerman et al., 2000). Among other aspects, the electrostatic contribution to the (de)solvation process has proved to be markedly important in many biological phenomena. For many applications, that contribution is modeled as a dielectric linear response to the electric field generated by the charge borne by the biomolecular system. Consistent with this model, the Poisson-Boltzmann equation (PBE) has proved to be able to provide quantitative estimates of the electrostatic interaction energy of biomolecules (Honig and Nicholls, 1995).

In addition to 311 other descriptors, <sup>J</sup>PD encompasses also the description of some electrostatic features, providing the numerical value of the mean electrostatic potential (EP) at each residue and at some relevant atoms, as well as the potential over the molecular surface, as described in what follows.

### Poisson-Boltzmann equation and biomolecules - STING variation

The EP is calculated by solving the PBE within a finite difference scheme by using the DelPhi program (Columbia University) with a few *ad hoc* modifications.

PBE is used in the context of the continuum approximation of matter (Cramer and Truhlar, 1999). In this model, molecules and solvent are treated as media that react linearly and uniformly to the electrostatic field generated by some kind of source, typically the charge distribution on the molecules themselves. The detail of the description of the solute

molecules is greater than the one of the solvent. Biomolecules are described at the atomic level, where each atom has its own partial charge, located in its center, and radius. Usually, but since the latest version of DelPhi this is no longer mandatory (Rocchia et al., 2001), all atoms belonging to a biomolecule have the same, low, dielectric constant, that is, they react with the same intensity to a preexisting electric field. The Connolly-Richards molecular surface (Connolly, 1983; Eisenhaber et al., 1995; Rocchia et al., 2002) is then built, being the surface generated by a solvent molecule that rolls over the solute. The space volume outside the molecular surface is treated as a uniform, high dielectric medium, with a typical dielectric constant value of 80. A suitable space-filling routine (Eisenhaber et al., 1995) assigns the remaining space regions to the dielectric constant of the closest solute atom. Usually, the concept of dielectric constant is used to describe the collective behavior of matter and does not go down to the atomic level of description. However, in this context, dielectric constant is used to describe any linear response of the medium that has not been modeled explicitly. For example, covalent bonds cause a charge imbalance which is already taken into account explicitly in the partial charges, and the dielectric constant should not account for this. In contrast, some researchers are interested in getting a quick idea of what is the effect of exposed mobile side chains, and they prefer to use a large dielectric constant for these side chains rather than averaging over the different conformers of the molecule. By default, the dielectric constant of solute molecules is set to be equal to 2, accounting for electronic polarizability.

Once the system is physically modeled, the PBE is solved according to a finite difference scheme. Finite difference solutions to the PBE involve solving the equation in a lattice representation (Holst et al., 2000). This representation implies that charges and dielectric properties of the system are mapped on the lattice, that is, an ordered, in our case cubic, ensemble of so-called grid points where the EP is calculated. In the model, charge is mapped on the grid points closest to the atom center according to a tri-linear interpolation scheme (Rocchia, 2005). The dielectric constant, which is more generally a property of the “medium”, in turn is mapped on the mid-points (a mid-point is the central point between two adjacent grid points). In order to make it possible to handle different dielectric regions, each mid-point has to be associated with the dielectric constant of the region in which it is located. An important task in the mapping phase is the correct assignment of boundary grid points, located on the interface between different media.

In case of a low-charged system, PBE is usually linearized, leading to a simpler numerical problem. For a solution of a symmetric monovalent salt, the linearized PBE has the following form:

$$\vec{\nabla} \cdot [\epsilon_r(\vec{r}) \vec{\nabla} \phi(\vec{r})] = -\frac{1}{\epsilon_0} \left[ \rho^{fixed}(\vec{r}) - \frac{\epsilon_{solv} \kappa^2(\vec{r})}{4\pi} \phi(\vec{r}) \right]$$

where  $\epsilon_r(\vec{r})$  is the local relative dielectric constant,  $\epsilon_{solv}$  is that of the solvent,  $\rho^{fixed}(\vec{r})$  is the charge distribution over the solute.  $\epsilon_r(\vec{r})$  is the Debye parameter, which equals

$$\sqrt{\frac{8\pi e^2 C^{bulk}}{\epsilon_{solv} k_B T}}$$

if  $\vec{r}$  points to the solvent and 0 otherwise,  $C^{bulk}$  is the bulk concentration of the salt,  $k_B$  is the Boltzmann constant and  $T$  is the absolute temperature.

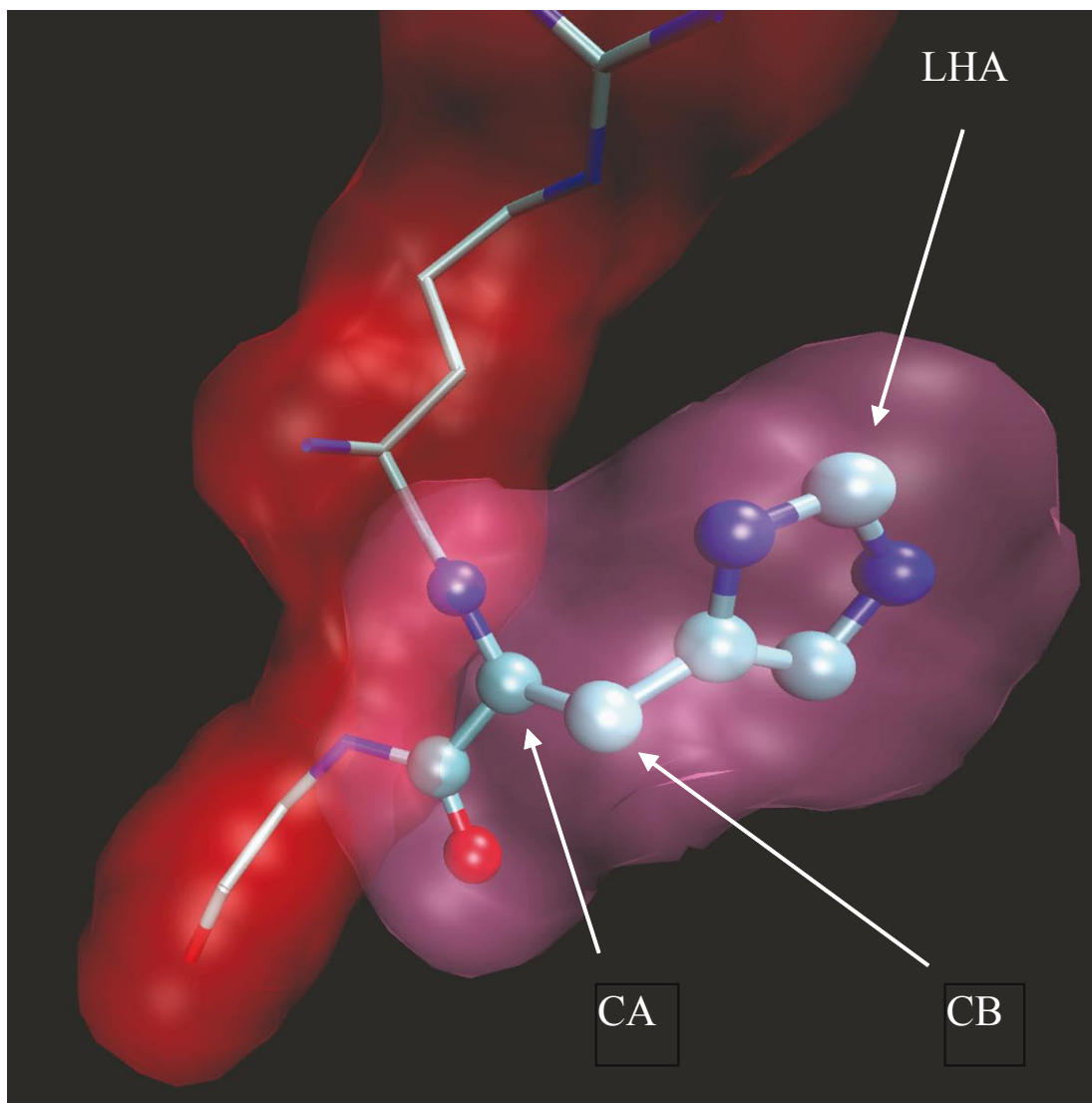
The above form of the equation is sometimes referred to as the strong form, in that the solving potential is required to be twice differentiable for the equation to hold in the traditional sense. The finite differences discretization process passes through the so-called weak formulation (Richards, 1977), which is less strict as far as the conditions on the derivatives of the solution are concerned.

Mapping the system on a lattice has some consequences, common to all of the finite difference schemes, of which the thorough user should be aware. First of all, the solution of the weak form nears the solution of the original equation only far away from the charged grid points. The grid potential at a charged site not only differs from the “strong” solution, where it should diverge, but its value depends quite strongly on the grid spacing and on the system position relative to the lattice. Therefore, shifting the system of fractions of Angstroms, as well as changing resolution, may very well result in quite different potential values at charged grid points. In the light of this reasoning, if the user wants to obtain potential values referring to individual atoms, it is advisable to use in the simulation a grid spacing that is a fraction of the smallest atom-to-surface distance in the molecular system (usually a value of 0.4 Å can be acceptable). Furthermore, as we already stated, the EP at each atom center is affected by the inaccuracy due to space discretization. One possible way to derive a more reliable atomic potential (AP) would be to average over many different system-to-lattice relative positions, but this would be very time-consuming; the strategy we adopt here is to define the AP as the average potential calculated at six points evenly distributed on the Van der Waals surface of the atom itself, or at a user-defined distance. In this way the contribution of the grid potential values at charged sites is reduced and the results are more reproducible.

Given our definition of AP, two quantities of interest can be further introduced: the amino acid EP and the surface amino acid EP.

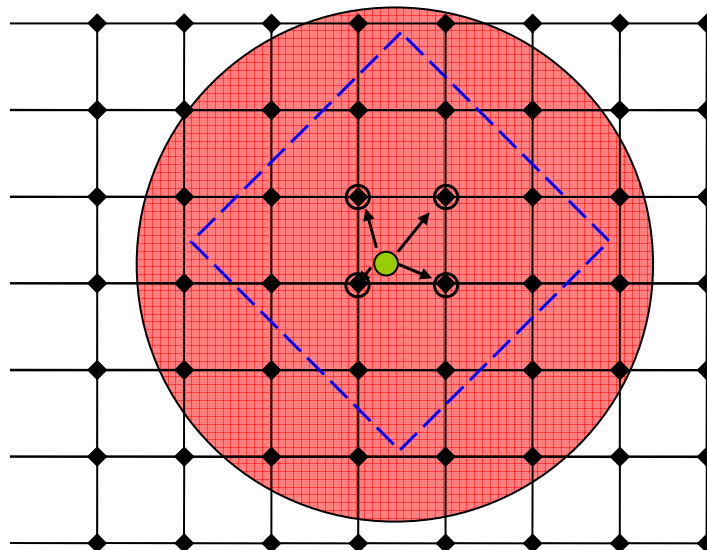
The amino acid EP is calculated as an average of the AP over each amino acid (Figure 1). From a numerical viewpoint, this average is closer to the value obtained by a numerical solver at a lower resolution, such as GRASP (Nicholls et al., 1991). The rationale for this is that when the grid spacing is large (Figure 2), several partial charges happen to be mapped on the same grid point, the net result looking very much like a spatial averaging.

The surface amino acid EP (Figure 3) is the average potential over the part of the molecular surface contributed by a given amino acid. Its derivation is somehow more complicated. The PBE solver that we used, i.e., DelPhi, has a routine that iteratively calculates the above-mentioned Connolly-Richards molecular surface (Connolly, 1983; Eisenhaber et al., 1995; Rocchia et al., 2002); the importance of this surface resides in the fact that the solvation contribution, better known as the reaction field energy, is generated by the polarization charge arising at the boundary between the molecule and the solvent. Therefore, all over the molecular surface there are points where the polarization charge is calculated, each of these points can be referred to an atom and, therefore, to a residue. Furthermore, it is important to note that, in this model, EP is a continuous variable across the molecular surface. Our claim of higher reliability is based on this last remark and on the fact that it is very unlikely to use PBE numerical solution at charged lattice points to calculate EP at the molecular surface.

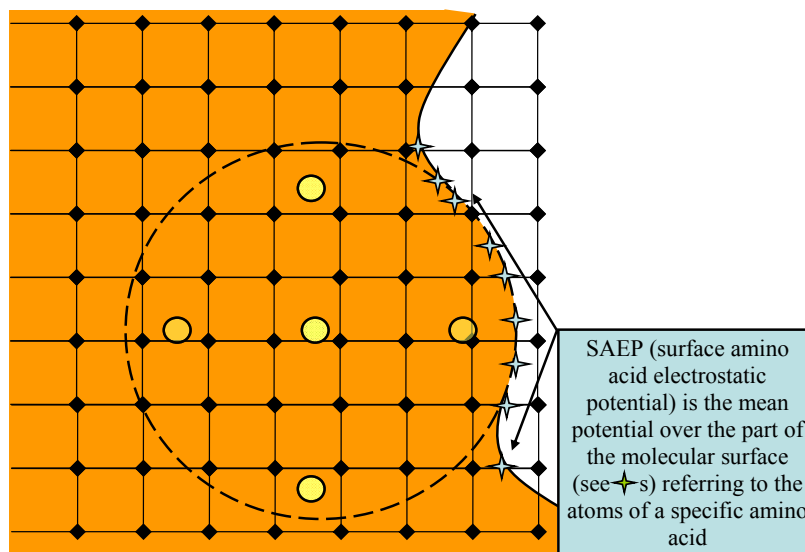


**Figure 1.** Representation of the key atoms considered in STING electrostatic potential database (CA = alpha carbon; CB = beta carbon; LHA = last heavy atom in side chain) together with the amino acid attributable molecular surface (presented as a violet color surface on the right part of the figure) over which the electrostatic potential is calculated.

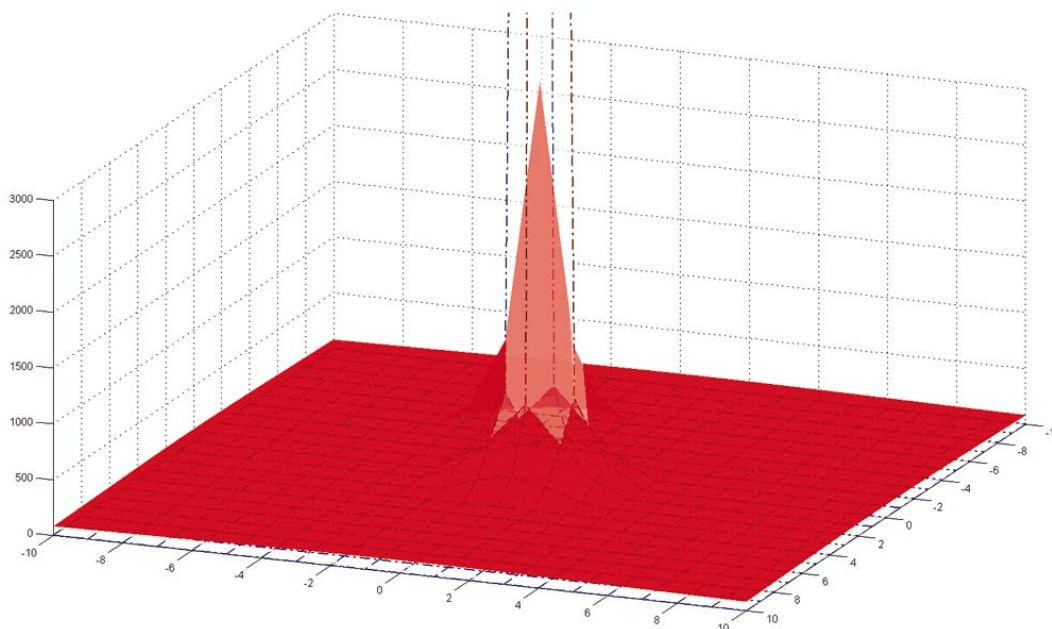
The innovative character of this approach resides in the fact that prior to the modifications we introduced into DelPhi, and then integrated into the STING environment, it was only possible to have EP reported at atom centers, with the inherent inaccuracy arising from the fact that most of the times a charge localizes in those positions (see Figure 4). In contrast, our novel definition of atomic potential virtually involves no charged grid points, leading to a higher reproducibility of the results. Furthermore, before the new features were introduced, there was no automatic way to link a point lying on the molecular surface to its parent atom. Thus, the task



**Figure 2.** Schematic 2-D representation of how the mapping on the grid of a charged atom is done. The Van der Waals volume, in red, is mapped on midpoints (here not represented for simplicity) between grid points. Partial charge, originally located in the atom center (green circle), is split to the nearest grid points. The equation is solved on the lattice. The potential values at charged grid points and nearest neighbors thereof (here enclosed in the blue dashed square) are the less reproducible.



**Figure 3.** In order to temper the effects of numerical electrostatic potential values at charged grid points, we define the atomic potential as the average over the potential values interpolated at six points (light orange circles) evenly distributed over a geometrical sphere smaller or equal to the Van der Waals surface of the atom itself (dashed line). Due to the 2-D representation, two of these points overlap with the atom center (green circle). Each point on the molecular surface can be assigned to the closest atom of the solute. The potential at those points (indicated by turquoise stars) can then be calculated, averaged and assigned to the parental residue (SAEP).



**Figure 4.** The analytical 2-D potential (transparent, dash-dotted line) generated by a point charge, and the one obtained by solving the corresponding Poisson equation by finite difference (semi-transparent surface, red) are shown (data generated by Matlab®, The Mathworks, arbitrary units). The two potential profiles tend to coincide for large distances from the point charge. The symmetry induced by the square (cubic) lattice is such that along the axis directions the potential tends to differ from the spherically symmetric analytical one, by a larger amount. The second, more relevant difference is that, close to the point where the charge is located, the analytical potential diverges, whereas the finite difference solution assumes a finite value which depends on grid spacing. For more complex systems, that value would also depend on the boundary conditions for the equation.

of calculating the potential of the solvent-exposed part of a specific residue has been now made much easier. In addition, <sup>3</sup>PD also reports the EP values calculated and attributed to the alpha carbon atom and the last heavy atom (LHA) in the side chain of each amino acid.

### High throughput procedure for calculating electrostatic potential in STING

The STING script for calculation of EP values over the entire PDB starts with the IRIX OS version of the Reduce program (Word et al., 1999), which adds hydrogen atoms to the protein. Default values for the flags given to the program are: -HIS -NOADJust. Subsequently, the multi-chain proteins are processed so that the chains are separated and stored in temporary files. The DelPhi program is then run for each separate chain. The output files are stored, indexed by pdb id and chain id. Default parameters for the DelPhi run are: percentage filling = 80%, grid spacing = 0.4 Å, null ionic strength, probe radius = 1.4 Å, Stern layer width = 2.0 Å, internal relative dielectric constant = 2, and solvent relative dielectric constant = 80. For definition of the atomic electrostatic potential, a distance of 0.6 Å from the atomic center was chosen.

### Comparative studies of calculated values for electrostatic potential in STING and DelPhi

In order to better represent the novelty of data stored in STING\_DB relative to EP, we decided to compare the interesting quantities from the same source (namely: 1nmd PDB code, chains: B and C, residues: C\_697, B\_399 and B\_332, atoms: C $\alpha$ , C $\beta$  and LHA) but derived using the two different versions of the DelPhi program: before and after modification (Table 1).

**Table 1.** Numerical values (expressed in [kT/e]) for 3 residues (Lys\_697 of chain C, and Glu\_339 plus Asp\_332 of chain B) obtained by the two DelPhi versions: the STING one (in bold) and the previous, stand-alone one.

	EP@C $\alpha$		EP@LHA		EP@Surf		EP(AVG)	
Lys_C (697)	19.37	<b>19.36</b>	-110.51	<b>53.01</b>	NA	<b>3.26</b>	72.31	<b>35.41</b>
Glu_B (399)	8.31	<b>8.72</b>	-626.05	<b>-204.03</b>	NA	<b>-9.21</b>	-84.41	<b>-30.29</b>
Asp_B (332)	-20.23	<b>-19.93</b>	-527.15	<b>-199.14</b>	NA	<b>-11.37</b>	-122.60	<b>-66.96</b>

EP = electrostatic potential; C $\alpha$  = alpha carbon; LHA = last heavy atom; Surf = surface; AVG = average; NA = not available.

As the data clearly show, the EP values show the same tendency, except for two important aspects: a) the LHA EP for the Lys\_697 shows a very different behavior in the two calculations. One can clearly see that the earlier version of DelPhi provides EP values which are much more in accordance with the charge values than the STING version, where the environment EP plays a major corrective role. b) Surface values referring to single amino acids are not easily obtainable from the earlier DelPhi version. An obvious advantage of having pre-calculated values is that the user does not need to overcome the learning barrier of using DelPhi, and he/she can just read data already available in STING\_DB, reported in residue by residue fashion.

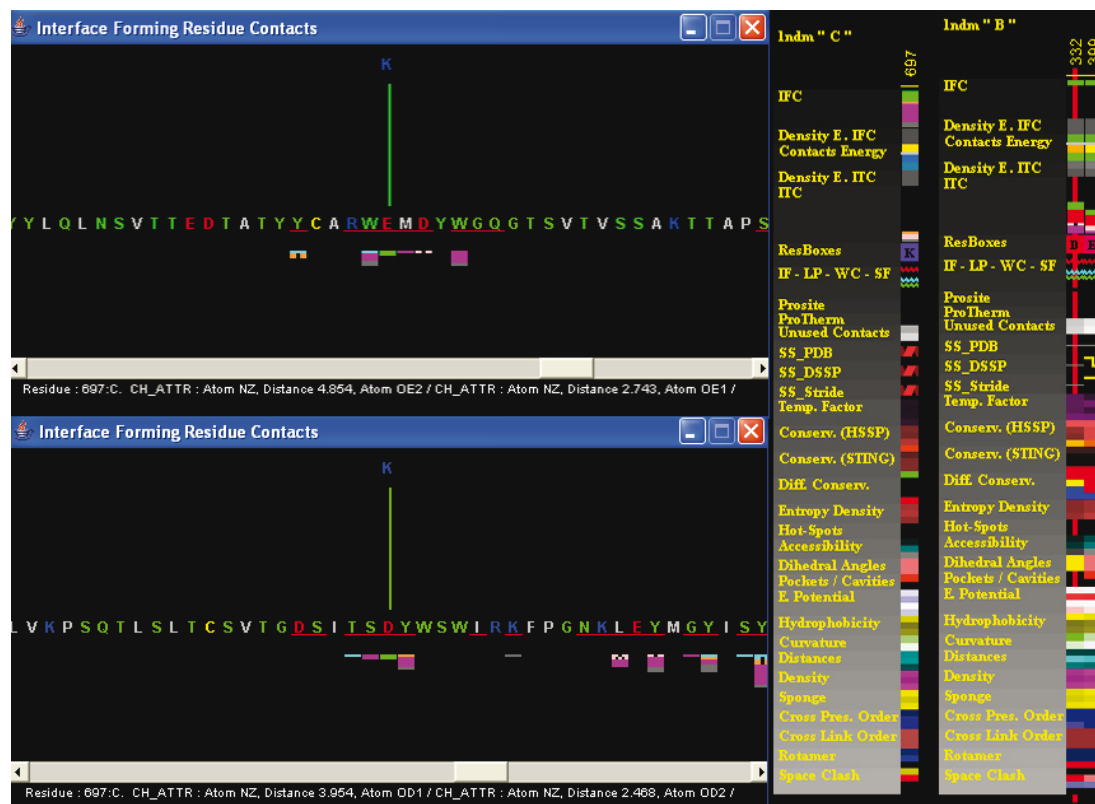
### Electrostatic potential at the antigen-antibody interface analyzed with the STING electrostatic potential

It was reported in the literature (Sinha et al., 2002) that some of the networked salt bridges present in HH26-HEL stand as very strong and play a major role in stabilizing the molecular system. We focused on interface contacts and electrostatic contributions toward binding. In general, EP factor, beyond helping binding, also limits the flexibility of the system, making the interface geometry very rigid. Therefore, we decided to use this particular case as a biologically interesting example where to apply our analysis.

The PDB file 1nmd.pdb was used to test how STING can perform in terms of how quickly a user can extract the information relative to the EP which is stored in the STING\_DB. In Figure 5, we show the information produced by the STING IFR contact module, showing the attractive electrostatic interaction between the Lys\_697 of the C chain (lysozyme) and both the Asp\_332 (the bottom left inset) and Glu\_399 (the upper left inset) of the B chain (antibody). On the right side of the same figure, we show the <sup>1</sup>PD content for the Lys\_697 of the C chain and next to it, on the right side, the content concerning two negatively charged residues of the B chain. The variation of the colors indicates existence of 4 values in the electrostatic potential



parameter line. If a user holds a mouse over this line, the numerical values for EP STING parameter show up (see Table 1, bold values).



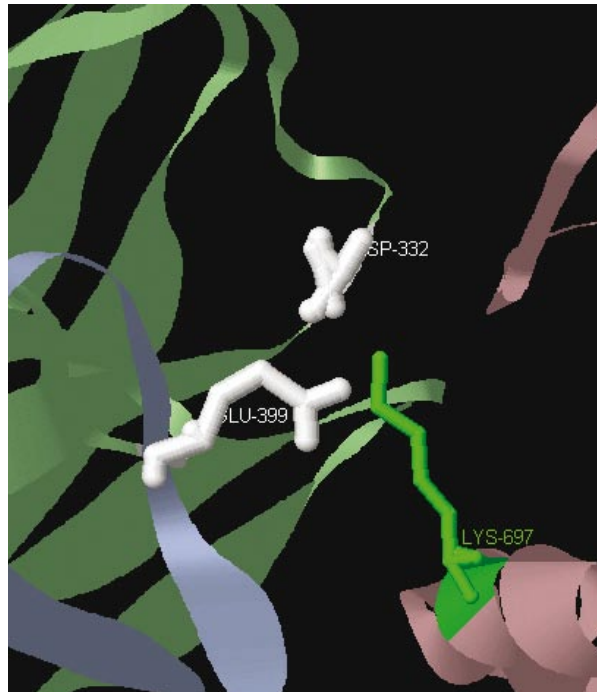
**Figure 5.** The STING IFR contacts (left) and 'PD images (right) for the 1ndm.pdb, B chain: Asp\_332 and Glu\_399, and for the C chain: Lys\_697. The electrostatic potential for these three residues is readily available. By using Sting\_Report, a user can also find how important and energetically aggregative these residues are.

According to the literature, those residues were shown to produce the largest EP contribution among all residues at the interface. Indeed, if we look at the STING\_Report for the residue Lys\_C (697) (not shown here) we can find that this residue is responsible for all salt bridges across the interface, and this is the largest interaction energy contribution. Figure 6A presents the 3-D constellation of Lys\_697 (in green) against the two negatively charged residues across the interface (in white color). In Figure 6B, we show both internal and interface contacts for Lys\_697.

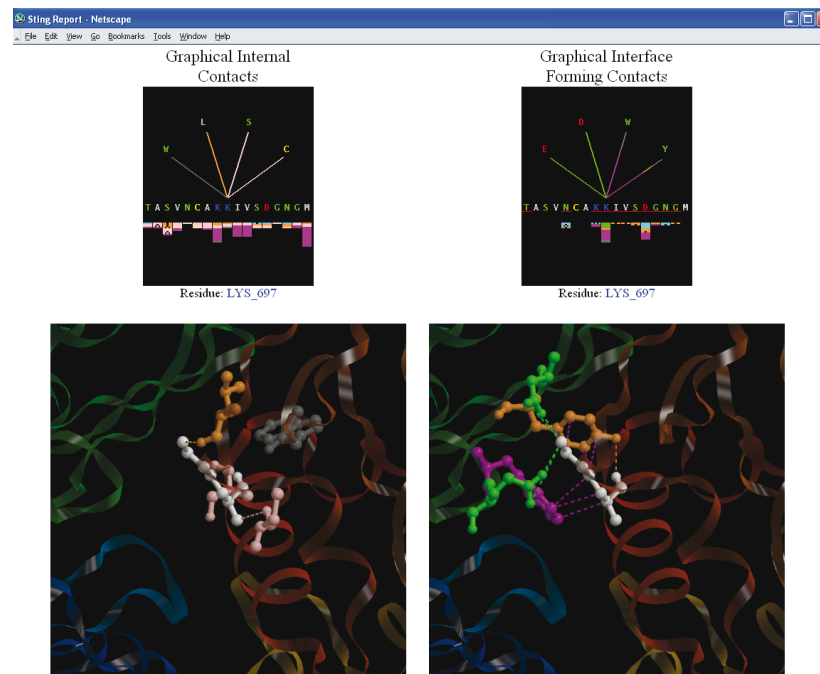
### Example of a STING\_DB query involving electrostatic potential values

A relational database query is a question about data, and the answer consists of a new relation containing the result. For example, we may want to find the ensemble of structures,

A



B



**Figure 6.** **A.** Jmol image from the STING environment: Lys\_697, in green, makes attractive electrostatic interactions with two negatively charged residues, in white, from the B chain in 1ndm.pdb. **B.** STING\_Report information in form of 3-D constellation of the Lys\_697 of the C chain in 1ndm.pdb.

selected from the whole PDB, that has the following characteristics: a) all structures that have a pocket (i.e., a depression in a protein surface, generally accessible to the solvent over its entire extent) with a volume ranging between 200 and 300 Å<sup>3</sup>, b) the residues forming such pocket would be highly conserved (with relative entropy, as calculated by the STING SH<sub>2</sub>Q<sup>s</sup> (Higa et al., 2006), being lower than 20), and c) the residues forming such pocket would also have an EP value for the surface portion attributable to that particular amino acid, positive or higher than 30 kT/e.

The biological meaning of this query (also described in Figure 7) would be to retrieve an ensemble of selected structures that could bind a negatively charged ligand. Clearly, such a search is possible due to the availability of pre-calculated data relative to EP. They were first stored in STING\_DB, and then, the flat (text) file was imported in a relational data base (STING\_RDB) from which complex queries can be made.

#### SQL commands for STING\_DB search

```

SELECT Poc.PDB_Name, Poc.Chain_Id, Poc.RB_Number, Poc.Volume,
Con.Sting_Relative_Entropy, Poc. EP,
FROM Cavity_Isolation as Poc, Conservation as Con
WHERE Poc.PDB_Name = Con.PDB_Name AND Poc.Volume >= 200 AND
Poc.Volume <= 300 AND Con.Sting_Relative_Entropy < 20 AND
Poc.PDB_Name = (SELECT PDB_Name FROM Residue_Base
WHERE RB_Name = * AND PDB_Name = Poc.PDB_Name GROUP BY PDB_Name)
AND Poc.EP >= 30
GROUP BY Poc.PDB_Name limit 8;

```

**Figure 7.** An SQL query to find all the PDB files containing pockets with volume ranging from 200 to 300 cubic angstroms that are composed by residues whose relative entropy is less than 20. Also, the pocket formed by the amino acid ensemble has to have a surface portion of the electrostatic potential attributable to each of them that is greater than 30 kT/e.

#### Check for electrostatic potential data in STING\_DB for a given PDB file

In order to provide the user with the capability of verifying whether a given PDB structure was successfully processed during the high throughput STING\_DB update, we introduced in the STING version “Star” (Neshich et al., 2006), the quality assessment (QA) module. Consequently, if the user encounters a highly unlikely case where the EP data are non-existent for a given PDB structure, he/she may first opt to consult STING QA and to verify if that particular structure is included in the list of PDB files for which the procedure we implemented to calculate the four EP parameters could not be successfully completed. The reasons for such outcome (incomplete run for calculation of EP) may be various, most likely due to inconsistencies in nomenclature of atoms used by the authors of the deposited structure. In Figure 8, we show the

current (July, 2006) status of the QA module, reporting the percentage of the total number of PDB files that were found either empty (file created but no data calculated) or where the procedure was aborted during EP calculation (for unknown reasons) and no file was created.

Parameter	Eligible PDB Files	% of Missing Files	Number of Missing Files	% of Empty Files	Number of Empty Files
Accessibility and Interface Residue	37658	0.003	1	0.000	0
Cavity Complex	37658	2.440	919	0.003	1
Cavity Isolation	83306	1.103	919	0.001	1
Contact Energy Density Intrachain	36026	0.852	307	0.000	0
Contact Energy Density Interface	19165	3.726	714	0.000	0
Contact Map	36026	6.920	2493	0.000	0
Contacts	36026	0.000	0	0.000	0
Cross Link	36026	0.569	205	0.114	41
Cross Presence	36026	0.577	208	0.144	52
Curvature Complex	37658	2.616	985	0.643	242
Curvature Isolation	37658	0.860	324	14.945	5628
Density Sponge	36026	1.485	535	5.232	1885
Distances	36026	0.072	26	0.000	0
Electrostatic Potential	37658	2.512	946	0.000	0

Find missing file

1e7v: Missing - Size: 499013 bytes

1e8w: Missing - Size: 491087 bytes

1e8x: Missing - Size: 505106 bytes

1e8y: Missing - Size: 494222 bytes

1e8z: Missing - Size: 488856 bytes

1e90: Missing - Size: 487279 bytes

1ea0: Missing - Size: 1565333 bytes

Find empty file

**Figure 8.** The STING quality assessment indicating that 2.5% (equivalent to 946 PDB files to date) of total PDB files eligible to be processed for electrostatic potential data calculation have not been successfully completed and therefore will not show electrostatic potential data to the user.

## CONCLUSIONS

The EP is considered as one of the key factors influencing protein interactions. In the STING relational database, which is composed of more than 300 structural parameters concerning protein analysis, special attention was paid to the calculation of EP. Its value is calculated on a per atom basis and then reported for all eligible PDB files in a residue by residue fashion. Four pre-calculated categories are shown: 1) EP at the alpha carbon atom, 2) EP value at the LHA atom, 3) average EP value over all amino acid atoms, and 4) EP value averaged over the patch of the molecular surface that is attributable to that particular amino acid.

The main features that we outlined in this study can be summarized as follows:

- We integrated the DelPhi program within the STING environment.
- We improved specifically the how the EP is defined and calculated at atoms that carry the charge; as a consequence, we obtained more reproducible data.

- We stored the data on EP values in STING\_DB and STING\_RDB, enabling complex queries over the entire PDB.
- We made it possible to compare different protein structures at the same time, with respect to a user-defined range of values for the EP, which was not possible with the previous version of STING\_DB (which used flat files).
- The EP values in STING, obtained by DelPhi, were pre-calculated and consequently, the users can immediately start their analysis.

Apart from the above-mentioned features, STING\_RDB, together with its EP element, allows biologically relevant questions to be easily addressed now. We presented one test case in which an antibody-lysozyme interaction was analyzed from the platform of pre-calculated data available within STING\_DB.

## FUTURE DEVELOPMENTS

We expect to further improve both the quality of STING\_DB and its EP element, by constantly decreasing the percentage of PDB files for which our procedure has failed to generate usable output, as well as trying to improve the speed of the procedure and the algorithmic aspect in terms of obtaining more reliable data. Concerning EP calculation, particular attention needs to be paid to PDB files presenting non-standard residues or nomenclature, which could hinder correct radius and charge assignment to the structure.

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