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Bluues server: electrostatic properties of wild-type and mutated protein structures

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ABSTRACT

Motivation: Electrostatic calculations are an important tool for deciphering many functional mechanisms in proteins. Generalized Born (GB) models offer a fast and convenient computational approximation over other implicit solvent-based electrostatic models. Here we present a novel GB-based web server, using the program Bluues, to calculate numerous electrostatic features including pKa-values and surface potentials. The output is organized allowing both experts and beginners to rapidly sift the data. A novel feature of the Bluues server is that it explicitly allows to find electrostatic differences between wild-type and mutant structures.

Availability: The Bluues server, examples and extensive help files are available for non-commercial use at URL: http://protein.bio.unipd.it/bluues/.

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1 INTRODUCTION

The structure, function (Fersht *et al.*, 1985), stability (Strickler *et al.*, 2006), protein–protein interaction (Sheinerman *et al.*, 2000) and small molecule binding (Szabo *et al.*, 1972) of a protein is largely dependent on its electrostatics. Electrostatics calculations are computationally impractical when modeling the solvent explicitly, e.g. by analyzing trajectories of a large number of solvent molecules in molecular dynamics simulations. Continuum solvent implicit models based on the Poisson–Boltzmann (PB) equation have been widely used (Davis and Mccammon, 1990; Fogolari *et al.*, 2002; Honig and Nicholls, 1995) and calculations are computationally achievable but are often very slow, especially for large molecules. When self- and interaction energies and forces are desired, the most widely used approach is based on the Generalized Born (GB) model (Bashford and Case, 2000; Koehl, 2006). GB approaches are a further approximation to PB methods but are considerably faster with calculations available in reasonable time, even for large molecules. Often the GB approach is benchmarked compared with PB methods due to the improved accuracy of the latter.

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Here, we present the Bluues server, a server that uses the program Bluues to perform electrostatic calculations for single-atomic structures with options for point mutations. The tool was conceived to yield accurate yet efficient models. Bluues, which is based on the GB model, was recently proven sufficiently accurate with respect to PB-based solvers (Fogolari *et al.*, 2012)). Bluues currently models: (i) GB radius of each atom; (ii) electrostatic solvation free energy; (iii) pH-dependent properties; (iv) pKa of all titratable groups; and (v) electrostatic potential at the surface of the molecule all in the order of minutes. Upon point mutation, delta values are calculated for the points listed above between the wild-type and mutant structures. It is implemented as a user-friendly web server with output such as downloadable files, molecular graphics and tables sorted by interesting characteristics.

2 SERVER OVERVIEW

Bluues requires as input a valid PDB identifier or a user-specified PDB file, from which it calculates a valid PQR protonation state file. Self made PQR files are also supported. The server contains two interfaces. One for analyzing a single-protein structure and the other for mutational analyses of the electrostatic features of two structures. The mutant protein can be derived from the server by point mutation of surface residues. For completeness the user can also supply their own wild type and mutant. All details about the methodologies used by the program Bluues are described in the reference paper (Fogolari *et al.*, 2012). By default the server executes the GB radii and surface potential calculations. The user has the option of altering the default parameters and executing surface area and pKa shifts calculations. These latter options will increase the computational return time.

The output consists of PDB, graph and text files which are described in more detail online. In summary, the radii and electrostatic potential for each atom are supplied by default. The pKa option gives data for titration curves on ionizable residues, pH-dependence on total charge and contribution to the free energy of folding data. The surface area option provides the solvent accessible surface area for the system in all chains, residues and atoms. The surface potential is displayed using the JMOL molecular viewer (Figure 1).

The Bluues server simply models point mutations using SCRWL (Krivov *et al.*, 2009) which uses a backbone-dependent rotamer library followed by a branch-and-bound search to remove steric clashes. This simple side chain replacement has been proven

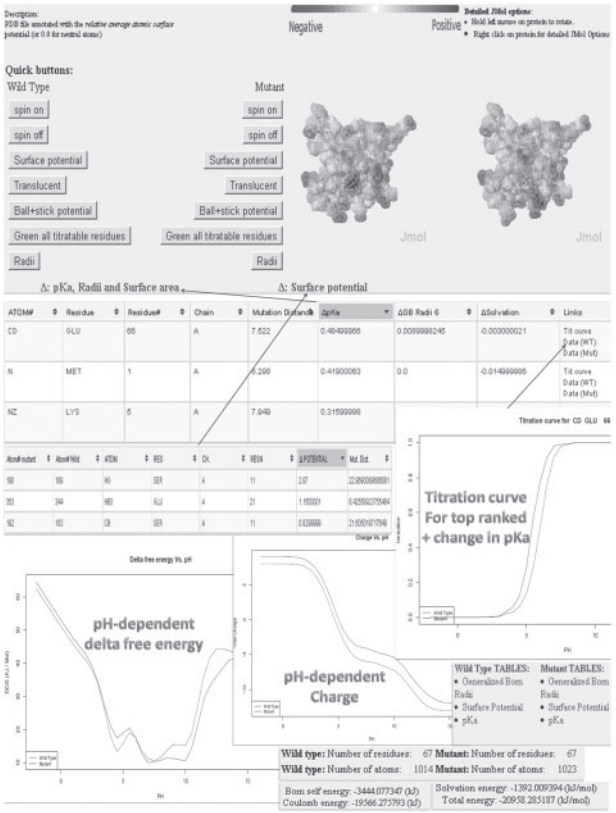

Fig. 1. Cold shock protein (Bc-CspB, PDB code 2ES2) mutation E3R is known to increase stability in mesophiles (Arginine in termophiles; Perl and Schmid, 2001). The Bluues server produces graphical and downloadable files, described online. The image above only shows the graphical aspects of Bluues. Both wild-type and mutated protein are shown in JMOL with the residue under mutation highlighted in black and actions implemented as buttons. Tables producing sorted ΔpKa and Δpotential are provided with corresponding distance to mutation (e.g. the E66 carboxylic group has the greatest positive pKa shift of 0.49). The corresponding titration curve can be examined for each pKa shift. Charge and pH-dependent folding free energy as a function of pH are also provided

successful for mutation (Wang *et al.*, 2008). Only residues in the PDB file which have a relative solvent accessibility, calculated by DSSP (Kabsch and Sander, 1983), above 40% are allowed to be mutated. This restriction was chosen because mutating buried residues may lead to unfolding (Wang *et al.*, 2008).

The server can analyze the effect of a particular mutation of interest with a very good accuracy, compared with more approximate methods (Fogolari *et al.*, 2012). After mutation both wild-type and mutant structures are converted to PQR format using the program PDB2PQR using CHARMM forcefield (Dolinsky *et al.*, 2007) and then processed with the Bluues algorithm. The output files are similar to those mentioned previously, except they are provided for both wild type and mutant. In addition, user-defined wild type and mutant can be uploaded to the server (PDB or PQR). This type of input could be useful, for example, in a setting where the user would prefer to perform a more comprehensive self-made mutant and wild-type model [e.g. homology modeling (Leonardi *et al.*, 2011)].

An important characteristic of the mutation output interface are the delta values provided between the wild type and mutant. These simply subtract the wild-type electrostatic features from the mutant ones. They are sorted in order of largest positive difference and may provide clues as to the most relevant electrostatic changes due to the mutation relative to the wild type. Examples of delta values include ΔpKa-values for ionizable groups and Δsurface potential. Moreover, atom distance from the mutation C–β atom is incorporated since long-range effects from the mutation may indicate important residues (Rajagopalan *et al.*, 2002), especially if they can be found to be conserved. In other words effects nearby the mutation site are expected while distant shifts may indicate an 'Electrostatic domino' effect highlighting distantly coupled residues.

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