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Predicting allostery and microbial drug resistance with molecular simulations

George A Cortina[1] and Peter M Kasson[1,2]

Beta-lactamase enzymes mediate the most common forms of gram-negative antibiotic resistance affecting clinical treatment. They also constitute an excellent model system for the difficult problem of understanding how allosteric mutations can augment catalytic activity of already-competent enzymes. Multiple allosteric mutations have been identified that alter catalytic activity or drug-resistance spectrum in class A beta lactamases, but predicting these in advance continues to be challenging. Here, we review computational techniques based on structure and/or molecular simulation to predict such mutations. Structure-based techniques have been particularly helpful in developing graph algorithms for analyzing critical residues in beta-lactamase function, while classical molecular simulation has recently shown the ability to prospectively predict allosteric mutations increasing beta-lactamase activity and drug resistance. These will ultimately achieve the greatest power when combined with simulation methods that model reactive chemistry to calculate activation free energies directly.

Addresses
[1] Departments of Molecular Physiology and of Biomedical Engineering, University of Virginia, Charlottesville, VA 22908, United States
[2] Science for Life Laboratory, Department of Cell and Molecular Biology, Uppsala University, Uppsala 75146, Sweden

Corresponding author: Kasson, Peter M (kasson@virginia.edu)

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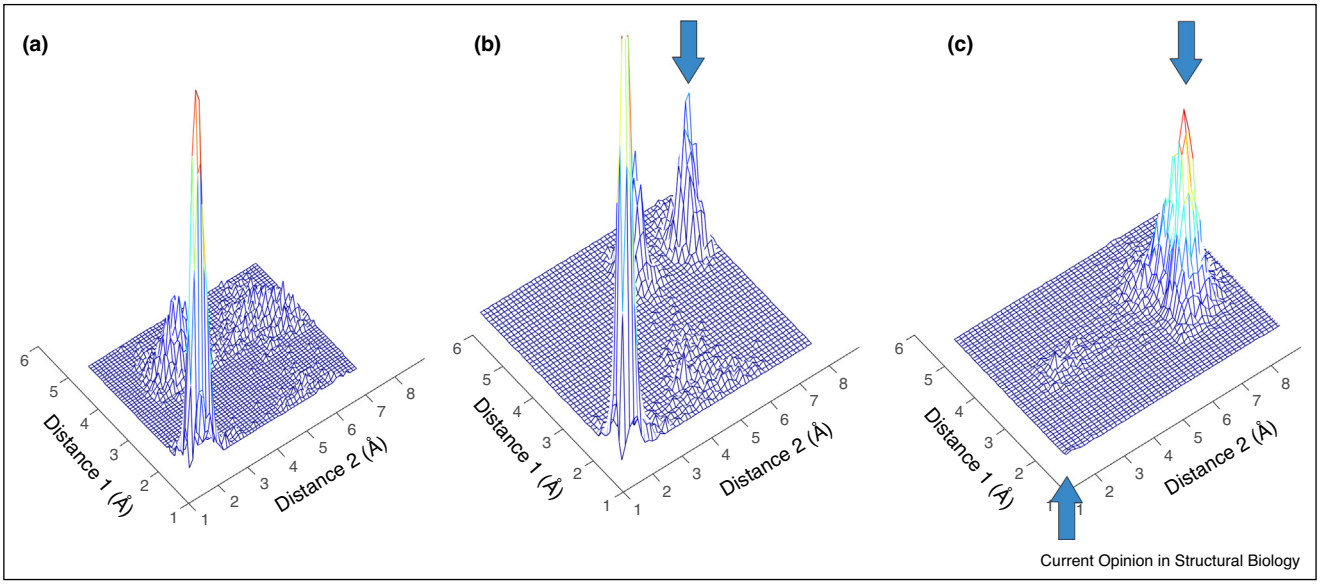
Beta lactamases provide the primary means of gram-negative bacterial resistance to the beta-lactam antibiotic classes that form a main part of the clinical armamentarium. Beta lactamase enzymes of classes A (the focus of this work), C, and D hydrolyze beta-lactam antibiotics in a manner analogous to that of serine proteases: a serine in the active site mediates acylation and opening of the beta-lactam ring to form a covalent acyl intermediate; this is then resolved via nucleophilic attack by a water molecule to form deacylated, inactive product and regenerate the enzyme. Because beta lactamases are under such selective pressure — they also provide resistance against microbial toxins — a wide variety of functional mutations have been identified, either clinically or in laboratory evolution experiments [1•,2–6]. Many of these mutations are allosteric in nature, yet predicting allosteric mutations prospectively has been challenging. The understanding required to make such predictions accurately would also yield deeper insight into the mechanisms of allosteric modulation in these well studied and clinically important enzymes.

Class A beta lactamases as model systems for allostery in well-structured proteins

Allostery can be broadly defined as perturbations to a molecule far from a binding or active site of interest that affect that site. These perturbations can take the form of allosteric ligands (other molecules) or allosteric mutations (changes to the reference molecule itself). Protein allostery is currently understood [7••] in terms of shifts to the conformational ensemble $P(\mathbf{x})$ to $P'(\mathbf{x})$, where $P(\mathbf{x})$ is used to denote the probability distribution of conformational states $\mathbf{x}$ across all position and velocity degrees of freedom. The simplest allosteric effect can be a structural change: in this case, the lowest free-energy state of an enzyme alters such that $P_{max}(\mathbf{x}) \neq P'_{max}(\mathbf{x})$, thus changing likely conformation [8]. Subtler shifts can manifest as population reweighting among minor (less populated) ensemble members [9] or emergence of a new minor population previously undetectable: in this case, $P_{max}(\mathbf{x})$ may not be altered (Figure 1). We outline four broad motional regimes to classify protein allostery: intrinsically disordered proteins [10,11], highly flexible proteins [12–14], proteins that undergo substantial but well-defined conformational changes [15–20], and proteins that maintain one general tertiary structure and may undergo small conformational changes within that overall structure [21]. Class A beta lactamases fall in the last category and are indeed relatively rigid. Therefore, one might expect them to be an easier target for predicting allosteric mutations. This, however, has been unexpectedly challenging, at least for predicting the relatively subtle changes to enzyme kinetics or substrate spectrum that are important for clinical resistance.

The main challenges in predicting beta-lactamase allosteric effects involve detecting subtle changes to the active-site conformational ensemble and correlating those to conformational fluctuations elsewhere in the protein. Since many class A beta lactamases have k_{cat} values

Figure 1



Two types of allosteric changes that can affect beta-lactamase function. Allostery can either change the relative probabilities of minor states in the acyl-intermediate ensemble without altering the minimum-free-energy state (panels **(a)**, **(b)**) or change the minimum-free-energy state of a protein conformational ensemble (panel **(c)**). Both of these can alter enzyme function and drug resistance. Illustrative examples from active-site distance distributions of CTX-M9. Arrows indicate previously minor states that have greatly increased in probability or major states that have been abrogated. Simulations are of CTX-M9 S281A (a), S220R (b), and A99K (c) from Ref. [26]; distances plotted are the 'oxyanion hole' hydrogen-bonding distances between the beta-lactam carbonyl oxygen and the backbone amide proton of Ser237 (distance 1) and that of Thr70 (distance 2).

ranging from $0.1\,s^{-1}$ to $1000\,s^{-1}$ [22–24], catalytically permissive conformations of the enzyme could easily be a small portion (e.g. 1%) of the overall equilibrium population without this equilibrium forming the major barrier for catalysis. However, such equilibria can still affect k_{cat} by reducing the steady-state concentration of enzyme–substrate or enzyme–intermediate complex in an active form [25,26••]. The chemical changes controlling substrate spectrum and resulting drug resistance are also relatively subtle. It is thus more difficult to predict how allosteric changes affect which beta-lactam drugs a given enzyme will hydrolyze, particularly gain or loss of catalytic ability for different beta-lactam drug classes for example penicillins, cephalosporins, carbapenems, and so on. This effectively becomes a problem of predicting the allosteric coupling between changes elsewhere in the protein, the functional groups on the drug, and the catalytic geometry.

A number of studies have characterized these interactions retrospectively, using either drug-resistant clinical isolates or resistance mutations identified by laboratory evolution studies and characterizing the location and catalytic effects of mutations [1,27–29]. Apart from the many mutations in the active site that affect class A beta-lactamase catalysis, the omega loop has been identified as having a particular effect on catalysis, even when the mutations lie in portions of the loop not contacting substrate [30,31,32,33,34•]. Intriguingly, a number of other allosteric mutations have been identified that are challenging either to rationalize or particularly to have predicted in advance.

Here, we discuss three broad sets of approaches to predicting allosteric effects on the resistance spectrum and enzymatic activity of class A beta lactamases: structure-based methods, methods based on classical molecular dynamics, and methods that explicitly represent reactive chemistry. While some of the methods we discuss include sequence phylogenetic data as well, methods primarily sequence-based in nature are not discussed here.

Structure-based methods for predicting allostery in beta lactamases

Most structure-based methods that have been applied to beta lactamases utilize some sort of network analysis: spatial connectivity, rigid-body analysis, or elastic network models. The fundamental idea behind network analyses of allostery, whether structure-based or otherwise, is that some sort of pairwise convolution holds for residue–residue interactions: if $f(i,j)$ and $g(j,k)$ describe the interaction of residues i with j and j with k, then $f*g$ describes the interaction of i with k via j. Different statistical measurements and assumptions can be used to build such pairwise edges, different convolution operators applied, and different approaches can be used to analyze the resulting networks for allosteric interactions, but this is the fundamental underlying principle. One