Is Rigidity Conserved Across the Class A β-Lactamase Family?
Deepak Verma and Dennis R. Livesay
Department of Bioinformatics and Genomics, University of North Carolina, Charlotte, NC, 28223

Abstract
Antibiotic overuse has resulted in an evolved class of β-lactamase (BL) proteins that can hydrolyze extended spectrum antibiotics. BL, produced by bacteria, hydrolyzes the β-lactam ring of penicillin and chemically related structures, thus rendering the antibiotic ineffective. NMR and MD results have suggested that the TEM-1 class A BL structure is quite rigid. In this study, we characterize flexibility of 12 related Class A BL proteins, which include proteins with extended spectrum antibiotic resistance. We employ a Distance Constraint Model (DCM). The DCM is a computational modeling scheme that integrates thermodynamic and mechanical descriptions to compute Quantitative Stability/Flexibility Relationships (QSFR) of protein structure. QSFR results suggest that rigidity is constant throughout the BL Class A family, although many site-specific differences in flexibility and rigidity are also present. Interestingly, our results indicate the O-loop, which is important for substrate recognition and catalysis, is consistently marginally rigid. Taken together, our collective results provide a biophysical framework to characterize evolution within the class A β-lactamase family.

Overview of the Model
The DCM is based on a free energy decomposition scheme combined with constraint theory where microscopic interactions are represented as mechanical distance constraints.
- Starting with a native protein structure, an ensemble of conformations is generated from the fluctuating constraints, i.e., fluctuating number of hydrogen bonds (NHB) and torsional forces (Nnt). Covalent bonds are quenched.
- The free energy of a macrostate defined by \( (N_{HB}, N_{nt}) \) is computed using a hybrid mean-field approximation by Monte Carlo sampling, and is given by:
  \[
  G(N_{HB}, N_{nt}) = G(N_{HB}) + G(N_{nt}) + \text{interaction terms}
  \]
- The intramolecular H-bond energy \( (G_{HB}) \) is calculated by an empirical potential.
- Values of the free parameters \( (\alpha, \beta, \gamma) \) are determined by fitting to experimental heat capacity curves. The parameter \( \alpha \) describes the solvation H-bond energy. The others correspond to the energy \( (\beta) \) and entropy \( (\gamma) \) of a native torsion angle.

QSFR metrics
- In addition to thermodynamic quantities, the DCM provides a number of mechanical descriptions of protein flexibility that are appropriately ensemble averaged.
- The flexibility index (FI) characterizes backbone flexibility, where positive values indicate the # of excess degrees of freedom (DOF) and negative values indicate the # of redundant constraints.
- Cooperativity correlation (CC) plots describe all possible pairwise couplings within the protein, where blue indicates a residue pair within the same rigid cluster, red indicates a residue pair within the same correlated motion and white indicates no mechanical coupling.

Ω-Loop and Active Site Residues
- Flexibility within the Ω-loop is thought to be important for substrate recognition and catalysis.
- A majority of the Ω-loops across the β-lactamase family are identified as marginally (isostatic) rigid.
- The conserved isostatic nature suggests that rigidity is important for substrate-binding reproducibility, yet also allowing for motion that might be functionally required.
- Interestingly, the 8 active site residues are also isostatically rigid even though they occur throughout the β-lactamase sequence.
- Conversely, there are several other loops where flexibility is evolutionarily conserved across the family.
- Future work will attempt to correlate differences in active-site dynamics with antibiotic resistance.

Results & Conclusions
The DCM results predict that:
- All members of the Class A β-lactamase family have rigid backbones, punctuated by small flexible loops.
- The overall backbone flexibility/rigidity is mostly conserved across the family.
- QSFR descriptions reproduce evolutionary relationships. Examples:
  - TEM-52, TEM-1, SHV-2 and SHV-1, which act on extended-spectrum cephalosporins, are composed of one large rigid cluster and are evolutionary close to each other.
  - Carbapenemases SME-1 and NMC-A are evolutionarily close orthologs, and have similar flexibility properties.
  - Cephalosporinase L2, belonging to a different functional class 2a, is atypically flexible.
- Most of the characterized Ω-loops and active site residues across β-lactamase family are marginally rigid.

This work is supported by NIH R01 GM073082.