Volume Determination of Globular Proteins by Molecular Dynamics

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Molecular dynamics simulations of myoglobin and aspartate aminotransferase, with explicit solvent, are shown to accurately reproduce the experimentally measured molar volumes. Single amino-acid substitution at VAL39 of aspartate aminotransferase is known to produce large volumetric changes in the enzyme, and this effect is demonstrated in simulation as well. This molecular dynamics approach, while more computationally expensive than extant computational methods of determining the apparent volume of biological systems, is quite feasible with modern computer hardware and is shown to yield accurate volumetric data with as little as several nanoseconds of dynamics.

I. INTRODUCTION

Experimental techniques for determining the partial specific volume and partial specific adiabatic compressibility of proteins in solution have provided key insight into structural and catalytic events. The application of these methods has resulted in a broad base of knowledge about solvation effects, ligand binding and dissociation, the influence of protein domains on catalytic events, and protein folding pathways.

Theoretical methods offer the potential to link specific events to the thermodynamic observables that experimentalists measure in the course of their research. In particular, molecular dynamics (MD) can provide a powerful approach to correlating the molecular trajectories of proteins that may give rise to an experimentally measured molecular volume or change thereof.

The state-of-the-art method to date for determining the apparent volume of a protein is the method of “accessible surface area”, a method that employs a spherically-accessible surface integration of the proteins crystal structure. This method is attractive in that it is not computationally demanding (and is thus accessible to the typical computational equipment of an experimental lab), however it calculates volumes that can be significantly different from those measured in solution.

Prior research on the volume of small molecules has been performed by our group and the methodology presented here is an application of those techniques to protein systems. This work demonstrates the validity of the MD approach toward determining the apparent molecular volume of globular proteins.

II. METHODS

The apparent molecular volume of a protein is calculated as:

\[ V_p = V_{p+w} - V_w \]  

The NAMD molecular dynamics package uses a Langevin-Hoover hybrid method where a piston is coupled to the equations of motion for a particle in the isothermal-isobaric (NPT)ensemble.

A simulated annealing algorithm was implemented to perform a global energy minimization after making single amino-acid substitutions. The protein and water system is brought to a higher energy state and allowed to randomly walk through phase space as the system is cooled over a specific temperature schedule.

Post-simulation analysis consisted of calculating the
correlation time of the volume signal using a block-
averaging method in order to calculate an unbiased error
in the volume, as well as structural analysis of the equi-
librium structures (RMSD, Ramachandran plots) versus
their crystal structures.

III. RESULTS AND DISCUSSION

A. Horse-heart myoglobin

Preparatory simulation stages (NAMD) consisted of
constructing the solvated protein system and local en-
ergy minimization, followed by heating and equilibration
steps. Initial myoglobin structural coordinates were ob-
tained from the RCSB protein data bank (crystal struc-
ture entry 1DWR4), solvated with 10,796 TIP3P water
molecules and then minimized by the method of conjugate
gradients. The protein was not mutated in any way. The
system was then heated to 300 K over a period of
1.2 ns, followed by equilibration in the NPT ensemble for
0.2 ns.

A production NPT (300 K/1 atm/2.0 fs timestep) run
of 10.0 ns resulted in an average volume for the protein-
water system of 341,875 Å³; simulating the bulk water
alone over a trajectory of equal time duration yielded an
average system volume of 319,775 Å³. The difference of
these values gives an apparent molecular volume of wild-
type myoglobin corresponding to 22,100 Å³ or 0.747 cm³/
g. The correlation times of both volume signals were de-
termined by block-averaging and the signals were then
uncorrelated to provide an unbiased volume error of
±0.001 cm³/g. This computationally determined appar-
ent volume of 0.747 ± 0.001 cm³/g agrees precisely with
experimentally reported sound velocity measurements[1]
and is within the experimental error of that study. The
equilibrium protein structure in solution was aligned and
compared with the crystal structure of myoglobin and
while the overall RMSD was minimal, a local region of
amino acids near GLY80 was found to be displaced by
6.0 Å due to solvation.

B. Aspartate aminotransferase

The halozyme of E. coli aspartate aminotransferase
(RCSB entry 1ASM)[2, 3] a large dimer consisting of
identical 404 amino acid subunits complete with LYS258-
bound pyridoxal-5'-phosphate cofactors, was simulated
in the NPT ensemble with 58,361 water molecules. Both
native AspAT and it’s VAL39 mutant were simulated in
order to compare molar volumes. An average apparent
volume of 0.733 cm³/g was calculated at the end of a
0.5 ns run, a result that is in good agreement with the
experimentally measured value of 0.731 cm³/g. This single-
point mutation induced a large conformational change in
the dimer[4] and an associated change in the apparent
volume by -0.035 cm³/g, one of the largest molecular
volume changes observed due to a single residue mutation
relative to the native protein. Since the crystallographic
structure of the V39G mutant has not yet been eluci-
dated, manual alteration of the VAL39 side chain of the
1ASM crystal structure was performed to give the V39G
initial configuration. A simulated annealing algorithm
was developed and performed on the mutant to help fa-
cilitate the adoption of its new equilibrium conformation
prior to performing production NPT runs.

IV. CONCLUSIONS

The application of molecular dynamics for studying
the volume of globular proteins can accurately model ex-
perimental data. The methods presented can offer in-
sight into structural protein studies since conformational
changes can be examined and correlated with experi-
mentally observed volume changes in solution. The volumet-
ric contribution of various regions of a protein (including
the more difficult case of a single-point mutation) can be

![FIG. 2: Volume signals for the solvated myoglobin system
vs. the bulk water. After initial equilibration the volumes
fluctuate about their average values for the 10 ns trajectory
the difference of which gives an apparent molecular volume of
0.747 cm³/g.](image)
FIG. 3: Van der Waals representation showing the steric effects of the valine (V39), tyrosine (Y70 on the second subunit of the dimer) and isoleucine (I37) residues of interest for the V39G mutant active site. Substitution of the valine for a glycine reduces the steric interactions leading to a large scale conformational change as well as a drastic change in catalytic activity. The same triad can be seen in the distance to the left on the opposite side of the dimer.

elucidated through use of this practical and consistent methodology.

Work is currently in progress to resolve the separate Coulombic and Van der Waals contributions to the apparent volume. Hybrid Monte Carlo (HMC) methods are also being developed to more efficiently explore the phase space of folding intermediates and to allow the use of additional potential energy terms.

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