Protein Dynamics from Femtoseconds to Microseconds

Lennart Nilsson

Karolinska Institutet, Department of Biosciences and Nutrition, SE-14157 HUDDINGE, Sweden
Lennart.Nilsson@biosci.ki.se

Abstract.

Classical molecular dynamics simulations have been used to study a wide range of phenomena of importance in biochemistry/molecular biology. The validity of the results depends on several factors, one of which is sampling. Two applications making use of parallel computers to obtain good statistics will be discussed. The first concerns the dynamics of ultrafast macromolecular hydration, using as an example the sweet tasting protein monellin, for which we demonstrate that the experimentally observed time-dependent Stokes shift for the single Tryptophan residue is due mainly to intrinsic chromophore and protein motions, and not to any anomalous water dynamics. In contrast to these femtosecond-picosecond processes the second application deals with motions of secondary structure elements in proteins, occurring on a 10+ nanosecond timescale, requiring sampling extending to microseconds.

Ultrafast hydration of proteins

Time-resolved fluorescence spectroscopy is used increasingly to probe molecular motions at the aqueous interfaces of biological macromolecules and membranes. By recording the time variation of the fluorescence frequency, thermal atomic fluctuations in the vicinity of the chromophore can be probed. From such fluorescence Stokes shift (FSS) experiments, it has been inferred that water motions in the hydration layer are slowed down by 1–3 orders of magnitude. To provide a more secure foundation for the interpretation of FSS data, we use molecular dynamics simulations to examine the molecular origin of the FSS from a tryptophan residue in the protein monellin(1). Decomposing the FSS into its water and protein components, we find that the water component dominates the static FFS but decays rapidly. Thus, after a few picoseconds, the observable FSS decay essentially reflects protein dynamics, including the self-motion of the chromophore. Because of its collective nature, the FSS response is insensitive to the motion of individual water molecules. Collective water displacement by slowly fluctuating protein groups introduces a long-time tail in the water auto-correlation function, but this dynamic coupling is hardly manifested in the observed FSS. Our analysis reconciles FSS data with the picture of a highly dynamic hydration layer, derived mainly from magnetic relaxation dispersion

and simulation studies, and calls for a revision of previous interpretations of slow FSS decays in terms of hydration dynamics at biomolecular and other interfaces.

Long time dynamics in proteins

The variety of cellular functions performed by proteins of the thioredoxin superfamily is made possible by the wide range of redox potential associated with their active-site -Cys-X-X-Cys-motif. The determinants of these differences in redox potential are of considerable interest but are not well understood. *E. coli* Glutaredoxin 1 (Grx1) and 3 (Grx3) are important model systems with different redox properties, despite sharing the same -Cys-Pro-Tyr-Cys-motif, very similar overall structures and 33% sequence identity. Very long molecular dynamics simulations (0.25 μs) and electrostatic calculations provide a revised view of the reduced Grx1 active site, which now can be reconciled with biochemical and functional data. Comparison of this new model to Grx3 uncovers differences in the structure, dynamics and electrostatics of these active-sites. The influence of peripheral residues on the properties of the -Cys-X-X-Cys-motif is illustrated specifically with the effect of a Lys to Arg substitution.(2,3)

These long molecular dynamics simulations in explicitly reprented aqueous solution have been used to correlate protein flexibility, in the sidchains and in the backbone, with the stability of the protein structure and in complex formation. Stability estimates have been obtained using several different entropy estimators, based on order parameter analysis, quasiharmonic and covariance analysis. The effects of correlations between structural components are analyzed.

Methods

All simulations were setup, run and analyzed using the program CHARMM(4) (www.charmm.org) with the version 22 parameter set for proteins.(5) The standard setup is to use the leap frog integrator with a 2 fs timestep with SHAKE applied to constrain covalent bonds to hydrogen atoms. Non-bonded interactions are evaluated using a spherical cutoff with smooth truncation of energies and forces (FSHIFT) at 12Å.. The nonbonded pair list is generated with a 14Å cutoff and updated heuristically whenever any atom has moved more then 1Å (half the distance between the interaction cutoff and list generation cutoff).

Typical production runs were performed using 4-8 CPUs in parallel on a local GNU/Linux cluster of dual Intel Xeon 2.8GHz equipped PCs.

References

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