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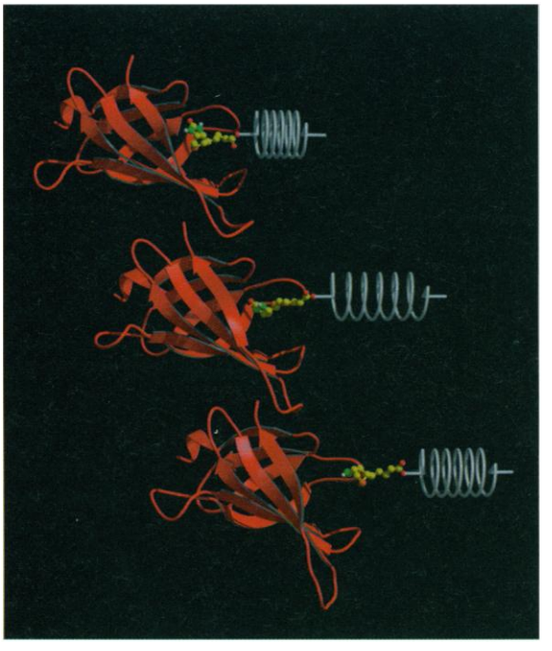
Bio-Molecular Dynamics Comes of Age

Herman J. C. Berendsen

Molecular dynamics (MD) as a computational technique for simulating the motion of atoms has been around since the late 1960s and has been applied to proteins since the mid-1970s. Limited computer resources and limited accuracy of available expressions for molecular force fields have for a long time fed doubts about the applicability of MD to real biological problems, but the tide is turning. Present-day computers, a thousandfold more powerful than those in the late 1970s, can simulate a system of tens of thousands of atoms as it evolves over times of nanoseconds (*1*). Thus, all of the necessary solvent molecules and long-range interactions can be included and most of the local fluctuations can be equilibrated to yield information on realistic time scales. Once a particular application has been validated by critical comparison with experiment, MD yields a wealth of insight into the atomic details of a biomolecular process.

Molecular dynamics methods have the advantage that they are not limited to equilibrium states but can be used to simulate nonequilibrium processes. Motions on a molecular scale, which are not often accessible to experiment, are sampled by MD. Hence, validation is generally indirect and subject to statistical traps. In 1994, Gaub's group succeeded in measuring the adhesion force between a single ligand and a receptor (*2*, *3*). Now, on page 997 of this issue, Grubmüller *et al.* (*4*) report on a simulation of the atomic force microscope (AFM) experiment by MD and find excellent agreement with experiment for the rupture force between ligand and receptor. The system studied is the binding of biotin (a vitamin with 16 heavy atoms) to streptavidin, a 159-residue protein that normally occurs as a tetramer with specific and strong binding to biotin. The tetramer was studied with AFM, along with the similar protein avidin and with biotin analogs; the simulations were performed on the smaller monomer in a complex with biotin and surrounded by a sphere of water, the total system comprising nearly 11,000 atoms.



Pulling ligands from receptors by computer. Simulation of molecular rupture as an AFM tip pulls the biotin ligand, causing unbinding and rebinding at different sites. The biotin is attached to a mechanically compliant agarose bead, shown as a spring. Careful computer pulling reveals binding pathways, identifies bottlenecks in the binding process, predicts rupture forces, and estimates free energies of binding. [Computer graphics: H. Grubmüller, University of Munich]

In the experiments, biotin molecules were attached to an agarose bead, and the rupture (or adhesion) force was 160 pN for avidin and 250 pN for streptavidin. To get an impression of the size of these piconewton forces, consider that a force of 300 pN (or much less in an aqueous environment) will rupture a hydrogen bond between two isolated water molecules, but more than 30,000 pN is needed to rupture a covalent carbon-carbon bond. Grubmüller *et al.* (*4*) simulated several pulling rates and found that their values could be accurately and linearly extrapolated to zero pulling rate, yielding the experimental force of 250 pN. It is most interesting that over a path of 9 Å, the ligand keeps sticking to the protein through continuous rearrangement of hydrogen-bonding networks. In the region where the rupture force (that is, the maximum free energy gradient) occurs, the free energy is dominated by enthalpic effects. It is therefore not surprising that the experimental rupture force for several ligands appears to be correlated with the enthalpy, rather than the free energy, of binding (*3*), although proper understanding of this observation needs some further thought.

The feasibility of following a molecular "unbinding" process within 1 ns is consistent with our own experience that local structural rearrangements in proteins, including those that involve water reorganization, can be reasonably probed in 1 ns. The full trajectory of configurations over 1 ns contains the necessary information to analyze the possible internal dynamics of a protein in solution. From such a trajectory it is possible to determine those collective degrees of freedom in which the molecules can really move. It turns out that 90% of the molecular displacement can be described by only a few (10 to 30) collective degrees of freedom. This reduces in principle the description of the mechanics of a protein to a few "essential" degrees of freedom and allows much more efficient probing of the available configurational space (*5*).

Not all motions are grasped in a nanosecond, however: Secondary structure elements, even isolated helices, are often slower to fold or unfold. But tens of nanoseconds (computationally feasible in a few years time) will suffice to bring such processes within reach. Further folding into tertiary structures is farther away and still beyond sight; MD is not a theoretical panacea ex-

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pected to solve the protein-folding problem by brute force.

The computation of atomic forces during a binding or unbinding process has more powerful applications than comparison with a rupture experiment. Forces indicate bottlenecks in binding pathways and hot spots in molecular conformations. Forces in the direction of an artificial constraint, averaged over local equilibria, are equivalent to the derivative of free energy profiles along the constraint and allow the reconstruction of such free energy profiles. Careful analysis of the pulling force, if the process is slow enough to ensure intermediate equilibration, should yield the free energy of ligand binding. The biotin-streptavidin binding free energy has already been determined from simulations by free energy perturbation techniques (*6*), but the pulling force method also predicts the probable path. If the inverse (binding rather than unbinding) can be modeled, pulling toward the binding site can provide automatic docking procedures with quantitative estimates of thermodynamic binding properties.

The use of average forces in a constraint direction to derive free energy profiles has been demonstrated in a completely different application: the prediction of water permeability through a lipid membrane (*7*). The solubility of water in a lipid membrane is so small that water permeability cannot be studied directly in MD simulations. But by constructing the free energy profile of water across the membrane and determining local diffusion constants, one can accurately compute the permeability. The free energy profile is integrated from the average force acting on water molecules constrained at a given depth in the membrane. In this way, MD simulations on a nanosecond scale can predict processes that occur on much longer time scales.

Biomolecular dynamics has come of age for two reasons. First, computers now allow routine nanosecond-scale simulations for systems of biologically relevant size, and thus, one can obtain at least local equilibrium and derive thermodynamic and transport characteristics. Second, methods have now become available to permit extrapolation to longer time scales and larger system sizes. In the near future, the link must be established between detailed MD and dynamical descriptions on coarser time and length scales, such as mesoscopic dynamics (*8*). This link will provide the "ab initio" understanding of macroscopic phenomena. The article by Grubmüller *et al.* is a milestone on this exciting path.

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When Proteins Receive Deadly Messages at Birth

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According to Mayan tradition, the first human beings turned out badly and the gods Tzakól, the Creator, and Bitól, the Former, quickly destroyed their creations. Since those days, quality control pathways have evolved to work at the molecular level, and now life cannot function properly without them. Proteins with errors can either be killed or cured: They are eliminated by proteolytic enzymes or repaired by molecular chaperones. Aberrant polypeptides expressed from defective mRNAs, however, are irreparable, and for such proteins, destruction is the only option. In this issue of *Science* (*1*), Keiler *et al.* describe a stunning new quality control mechanism for proteins in bacterial cells that serves precisely this function. By invoking a previously unknown ribosomal translation mechanism, nascent polypeptides, translated from truncated mRNAs lacking stop codons, receive short COOH-terminal peptide tags that target the resulting polypeptide fusions for degradation.

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This story emerged last year when Tu *et al.* (*2*) reported that a fraction of mouse interleukin-6 (IL-6) protein expressed in *Escherichia coli* received a novel COOH-terminal modification. These variants were truncated at the COOH-terminus to a different extent but contained an identical COOH-terminal peptide tag with the sequence AANDENYALAA, which is not encoded by the IL-6 mRNA. This new sequence, except for the first alanine, is encoded by a small (362 nucleotides) stable RNA, known as 10Sa RNA and expressed from the *ssrA* gene. A portion of 10Sa RNA can fold into a tRNA-like shape and can be charged with alanine (*3*). Thus, Tu *et al.* suggested that the added peptide tag is derived from this alanine residue and the translation product of 10 codons from the 10Sa RNA.

Sauer and co-workers realized that the added COOH-terminal tag resembled the degradation signal of an enzyme called tail-specific protease (Tsp; also known as Prc) (*4*) studied by their group. This enzyme, localized in the periplasm of *E. coli* cells, binds certain apolar COOH-terminal tails of substrates and cleaves proteins endoproteolytically (*5*). A cytosolic counterpart of the periplasmic Tsp protease, which also degrades proteins in a tail-dependent manner, appears to exist in *E. coli* cells, although it has not yet been characterized (*6*). Keiler *et al.* (*1*) inserted transcriptional terminators into the reading frames of one periplasmic and one cytosolic protein and observed that the resulting partial transcripts lacking stop codons were indeed translated with a COOH-terminal tag encoded by the 10Sa RNA. Remarkably, the periplasmic tagged fusion protein was degraded by Tsp, and the cytosolic protein by Tsp's cytosolic counterpart.

What is the biological relevance of this unusual tagging mechanism? Because of the action of nucleases, mRNAs may be truncated and lose their encoded stop codons at their 3′ ends. Translation of those messages can still proceed but comes to a halt when the ribosome reaches the 3′ end of the mRNA. In the absence of a stop codon, release factor cannot trigger the dissociation of nascent polypeptides from ribosomes, and all ribosomes engaged in translation of the same reading frame are stalled. Alanine-charged 10Sa RNA may come to the rescue (see figure). 10Sa RNA appears to combine both transfer and messenger RNA properties (I therefore suggest it be called a tmRNA). Keiler *et al.* (*1*) argue that the tRNA-like domain enables the ribosome to catalyze the next peptidyl transfer of the nascent chain to the 10Sa RNA–bound alanine. The defective mRNA can then be released, and the ribosome switches to the reading frame provided by the 10Sa RNA.