FREE ENERGY CALCULATION
ANALYSIS OF SIMULATIONS

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Simulations yield *mechanical* observables: quantities that are immediately measurable in each configuration, like atomic distances, energies, velocities, pressure, dipoles, etc. By averaging over a representative *ensemble* of configurations, certain thermodynamic properties are obtained, like radial distribution functions, energies, temperature, pressure, dielectric constant, are found. Observing the time dependence of equilibrium fluctuations or the response to external disturbances, also *transport properties* as diffusion constant, viscosity, dielectric relaxation, can be 'measured'.

But thermodynamic quantities that involve *entropy*, as free energies, binding constants, thermodynamic potentials, partition coefficients, solubilities, pK's, phase diagrams, etc. cannot be derive from one simulation. How can we determine such quantities?

Can we distinguish the functionally relevant motions among the messy random fluctuations in proteins? Do simulations really help to understand and even predict function?

FREE ENERGY: BASIC RELATIONS-1

The *Helmholtz free energy* A is given by

$$A = -k_B T \ln Q$$

where Q is the NVT or *canonical* partition function:

$$Q = \frac{1}{N! h^{3N}} \int \exp[-\beta H(p,q)] dp\, dq$$

$$\beta = \frac{1}{k_B T}$$

This is an integral over phase space, which cannot be evaluated in simulations.

The *Gibbs free energy* G is given by

$$G = -k_B T \ln \Delta$$

where Δ is the NPT partition function:

$$\Delta = \frac{1}{N! h^{3N}} \int dV \int dp\, dq\ \exp[-\beta\{H(p,q) + pV\}]$$

This is also an integral over phase space (and over volume), which cannot be evaluated in simulations.

Relation between A and G:

$$G = A + pV$$

All thermodynamic quantities follow from the T, V, and composition dependence of A, or from the T, p, and composition dependence of G.

FREE ENERGY: BASIC RELATIONS-2

entropy S, *enthalpy* H, and *thermodynamic potential* μ_i:

$$G = H - TS$$

$$dG = V\,dp - S\,dT + \sum_i \mu_i\,dn_i$$

$$\left(\frac{\partial G}{\partial p}\right)_{n_i,T} = V$$

$$\left(\frac{\partial G}{\partial T}\right)_{n_i,p} = -S$$

$$\left(\frac{\partial G}{\partial n_i}\right)_{n_{j\neq i},p,T} = \mu_i$$

Additional relations:

$$\left(\frac{\partial G/T}{\partial 1/T}\right)_{n_i,p} = H \quad (\mathrm{Gibbs-Helmholtz})$$

$$G = \sum_i n_i\mu_; \quad (dG)_{p,T} = \sum_i \mu_i\,dn_i$$

$$\sum_i n_i\,(d\mu_i)_{p,T} = 0 \quad (\mathrm{Gibbs-Duhem})$$

Similar relations, with *internal energy* U $(H = U + pV)$,
for $G \to A$, $H \to U$, $p \to V$, $V \to -p$.

FREE ENERGIES FROM SIMULATIONS

It is *not* possible to evaluate integrals over space such as

$$Q = c \int_V \exp(-\beta V) d\boldsymbol{r}$$

because the full, very multidimensional, space is never sampled.

Free energy perturbation

It *is* possible to find ratios Q_1/Q_2 and hence *differences* in free energy $A_1 - A_2$ for two systems with slightly different potentials V_1 and V_2:

$$A_1 - A_2 = -k_B T \ln \frac{Q_1}{Q_2} = -k_B T \ln \frac{\int \exp(-\beta V_1)\, d\boldsymbol{r}}{\int \exp(-\beta V_2)\, d\boldsymbol{r}}$$

The latter ratio equals the *ensemble average* of $\exp[-\beta(V_1 - V_2)]$ over an equilibrium ensemble generated with potential V_2:

$$A_1 - A_2 = -k_B T \ln \frac{\int \exp[-\beta(V_1 - V_2)] \exp(-\beta V_2)\, d\boldsymbol{r}}{\int \exp(-\beta V_2)\, d\boldsymbol{r}} =$$
$$= -k_B T \ln \langle \exp[-\beta(V_1 - V_2)] \rangle_{V_2}$$

One can also take the inverse of the ensemble average of $\exp[-\beta(V_2 - V_1)]$ over an equilibrium ensemble generated with potential V_1:

$$A_1 - A_2 = -k_B T \ln \frac{\int \exp(-\beta V_1)\, d\boldsymbol{r}}{\int \exp[-\beta(V_2 - V_1)] \exp(-\beta V_1)\, d\boldsymbol{r}} =$$
$$= +k_B T \ln \langle \exp[-\beta(V_2 - V_1)] \rangle_{V_1}$$

This works only if $V_1 \approx V_2$, i.e., if the two ensembles overlap in configuration space.

THERMODYNAMIC INTEGRATION

If the potential is a function of an external parameter $V = V(\lambda)$, then the *derivative* of the free energy with respect to λ can be found:

$$\frac{dA}{d\lambda} = -\frac{k_B T}{Q}\frac{dQ}{d\lambda} =$$

$$= \frac{\int (\partial V/\partial\lambda)\exp(-\beta V)\, d\boldsymbol{r}}{\int \exp(-\beta V)\, d\boldsymbol{r}} = \langle \frac{\partial V}{\partial \lambda} \rangle_V$$

In this way the free energy of a state (potential V_1) can be found by integration from a reference state (potential V_0). Choose $V(\lambda)$ such that

$$V(\lambda = 1) = V_1; \qquad V(\lambda = 0) = V_0$$

Then:

$$A_1 = A_0 + \int_0^1 \langle \frac{\partial V}{\partial \lambda} \rangle_\lambda \, d\lambda$$

This can be done by numerically integrating the derivatives measured in a number of well-equilibrated intermediate points, or the λ can be slowly increased in a *slow-growth* method. Make sure that the growth is slow enough to proceed through equilibrium states: one check is to grow backwards from $\lambda = 1$ to $\lambda = 0$ as well; the two curves $G(\lambda)$ should be the same.

APPLICATIONS

Free energy of liquid
Take ideal gas as a reference state ($V_0 = 0$), for which the free energy is trivial, and integrate to full potential.

Free energy of solid
Take Einstein solid as a reference state (each atom bound to a lattice site as a harmonic oscillator), for which the free energy is simple, and integrate to full potential.

Free energy of binding ligand to macromolecule
'Remove' ligand from bound site by letting interactions disappear; do the same for ligand free in solution, and subtract the results (hard to get good statistics).

Relative binding constants of two ligands to protein
'Mutate' L$_1$ into L$_2$ while bound to the protein. Do the same for the ligands free in solution; subtract the results (complete the thermodynamic cycle). Gives differences in free energy of binding, and thus ratio of binding constants.

Relative binding constants of a ligand to two protein mutants
'Mutate' protein both in the presence and absence of the ligand. Subtract the results.

THERMODYNAMIC CYCLE

Example of a thermodynamic cycle:
Relative binding strength of two ligands L_1 and L_2 to a protein P

$$\begin{array}{ccccc} P+L_1 & \leftrightarrow & PL_1 & \qquad & \Delta G_1^0 = -RT\ln K_1 \\ \Delta G_L \downarrow & & \downarrow \Delta G_{PL} & & \\ P+L_2 & \leftrightarrow & PL_2 & & \Delta G_2^0 = -RT\ln K_2 \end{array}$$

We require

$$\Delta G_1^0 - \Delta G_2^0 = -RT\ln\frac{K_1}{K_2}$$

We compute ΔG_L and ΔG_{PL}. Note that both quantities have no meaning in the real world, as they refer to free energy differences between two different molecules! Also note that the computed quantities are differences, independent of the concentration used, so they can also be denoted by ΔG_L^0 and ΔG_{PL}^0.

The **thermodynamic cycle** looks like:

$$\begin{array}{rccl} & +\Delta G_1^0 & & \\ L_1 & \rightarrow & PL_1 & \\ -\Delta G_L^0 \uparrow & & \downarrow +\Delta G_{PL}^0 & \\ L_2 & \leftarrow & PL_2 & \\ & -\Delta G_2^0 & & \end{array}$$

$$\Delta G_1^0 + \Delta G_{PL}^0 - \Delta G_2^0 - \Delta G_L^0 = 0$$

or

$$\Delta G_1^0 - \Delta G_2^0 = \Delta G_L^0 - \Delta G_{PL}^0$$

PARTICLE INSERTION

The **thermodynamic potential** of a particle can be determined by the *particle insertion method* of Widom (B. Widom, J. Chem. Phys. **39** (1963), 2808–2812). Works only in dilute systems.

$$\mu_i = \left(\frac{\partial A}{\partial n_i}\right)_{V,T}$$

$$\mu_i = A(n_{i+1}, n_j, V, T) - A(n_i, n_j, V, T) = -k_B T \ln \frac{Q(n_{i+1})}{Q(n_i)}$$

$$= -k_B T \ln \frac{1}{(n_i+1)} \left(\frac{2\pi m k_B T}{h^2}\right)^{3/2} \frac{\int e^{-\beta V}\, d\boldsymbol{r}_1, \ldots, \boldsymbol{r}_{n_i+1}}{\int e^{-\beta V}\, d\boldsymbol{r}_1, \ldots, \boldsymbol{r}_{n_i}}$$

Now the total potential V can be split up into the interaction V_n between all particles without particle $n_i + 1$ and the *interaction* V_{int} between the extra $(n+1)$-th particle and the rest:

$$V = V_n + V_{int}$$

$$\mu_i = -k_B T \ln \frac{V}{(n_i+1)} \left(\frac{2\pi m k_B T}{h^2}\right)^{3/2} \frac{\int \left(\frac{1}{V} \int e^{-\beta V_{int}}\, d\boldsymbol{r}_{n+1}\right) e^{-\beta V_n}\, d\boldsymbol{r}}{\int e^{-\beta V_n}\, d\boldsymbol{r}}$$

($d\boldsymbol{r} = d\boldsymbol{r}_1, \ldots, \boldsymbol{r}_n$) The first terms are those of a free particle (ideal gas), and the second is an ensemble average:

$$\mu_i = \mu_i^{\mathrm{id.gas}} - k_B T \ln \langle e^{-\beta V_{int}} \rangle$$

The ensemble is generated *without* the extra particle, which is called the *ghost particle*, as its presence is not felt by the other particles.