

# Practical Course on Molecular Dynamics and Trajectory Analysis

## Episode 4: Advanced simulations and free energy

Jordi Villà i Freixa

Universitat de Vic - Universitat Central de Catalunya  
Facultat de Ciències, Tecnologia i Enginyeries (FCTE)

*jordi.villa@uvic.cat*

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# Episode objectives

- Apply advanced sampling and free energy techniques.
- Design robust protocols for complex systems.
- Evaluate convergence and reproducibility.

# Advanced techniques map

- Highly stable thermostats and barostats.
- Multiscale integration and constraints.
- Enhanced sampling: umbrella, metadynamics, REMD.
- Free energies: FEP, TI, BAR/MBAR.

## Control variables

$$\beta = \frac{1}{k_B T}, \quad \lambda \in [0, 1].$$

- The variable  $\lambda$  bridges the initial and final states.
- $\beta$  defines the statistical weight in the ensemble.

# Data requirements

- Trajectories long enough for each window.
- Independent sampling across windows or replicas.
- Energy reports and collective variables.

# General workflow

- ① Strict preparation and equilibration.
- ② Define CVs and windows.
- ③ Production and convergence checks.
- ④ Post-process with robust estimators.

# Advanced ensembles

- NVT for temperature control.
- NPT for realistic pressure and density.
- NP $\gamma$ T for surface tension in membranes.

# Langevin equation

$$m\ddot{\mathbf{r}} = -\nabla U(\mathbf{r}) - \gamma m\dot{\mathbf{r}} + \sqrt{2\gamma mk_B T} \mathbf{R}(t).$$

- Controls temperature via friction and noise.

# Nosé-Hoover chain

$$\dot{\eta} = \frac{1}{Q} \left( \sum_i \frac{p_i^2}{m_i} - N_f k_{\text{B}} T \right).$$

- Allows canonical sampling with less noise.

# Energy equipartition

$$\langle K \rangle = \frac{N_f}{2} k_B T.$$

- Check temperatures per subgroup (water, solute).

# Temperature diagnostics

- Speed histogram vs Maxwell-Boltzmann.
- Drift monitor for non-equilibrated systems.
- Watch for jumps when parameters change.

# Types of barostat

- Isotropic: box scales equally in all directions.
- Anisotropic: allows independent scaling.
- Monte Carlo barostat: discrete volume changes.

## Volume fluctuations

$$\kappa_T = \frac{\langle V^2 \rangle - \langle V \rangle^2}{k_B T \langle V \rangle}.$$

- Compare with experimental values if available.

# Compressibility and stability

- Excessive values signal poor convergence.
- Adjust barostat frequency and damping.

# Surface tension

$$\gamma = \frac{L_z}{2} \left( P_{zz} - \frac{P_{xx} + P_{yy}}{2} \right).$$

- Relevant for membranes and lipid bilayers.

# Pressure control

- Log volume and density per window.
- Avoid overly aggressive couplings.
- Stabilize before long sampling runs.

# Geometric constraints

- SHAKE/LINCS fix fast bonds.
- Allow larger integration steps.

# Verlet integrator

$$\mathbf{r}_{t+\Delta t} = \mathbf{r}_t + \mathbf{v}_t \Delta t + \frac{1}{2} \mathbf{a}_t \Delta t^2.$$

- Conserves energy in short integrations.

# Scale separation (RESPA)

$$U = U_{\text{fast}} + U_{\text{slow}}.$$

- Integrate slow forces with larger steps.

# Mass repartitioning

- HMR: increases hydrogen mass for stability.
- Enables  $\Delta t$  of 4–5 fs in well-constrained systems.

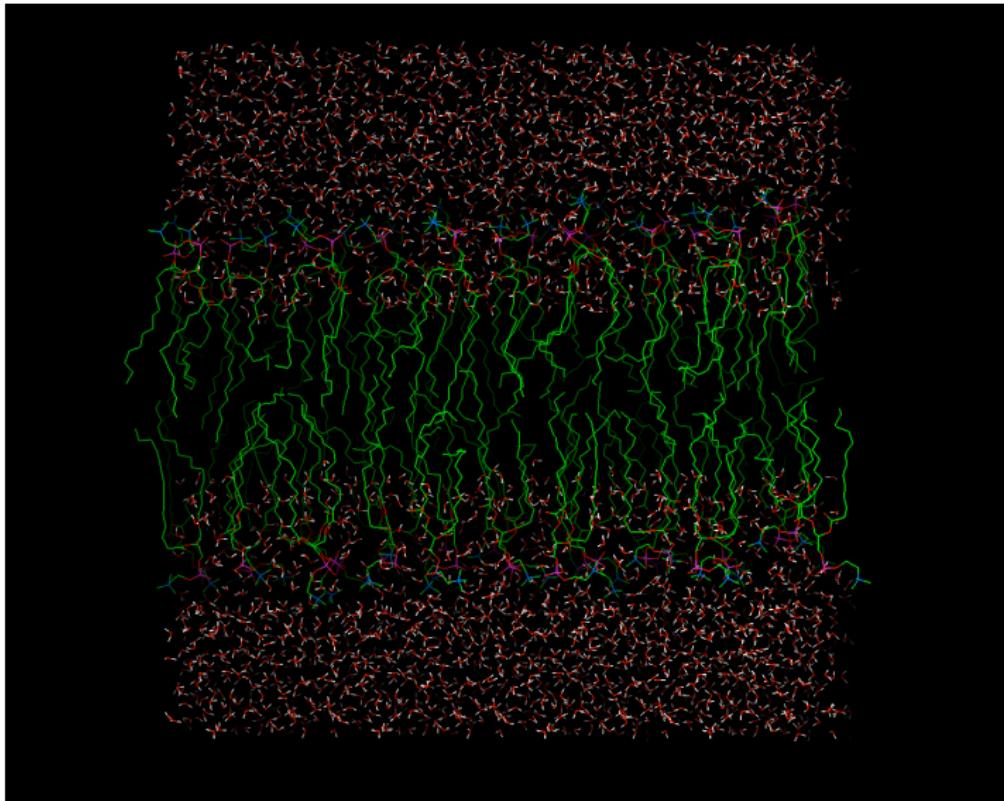
# Numerical stability

- Monitor total energy drift.
- Adjust  $\Delta t$  according to stiffness and temperature.

# Membranes and anisotropic systems

- Independent pressure scaling per axis.
- Control thickness and area per lipid.

# Lipid bilayer



# Explicit vs implicit solvation

$$\Delta G_{\text{solv}} = \Delta G_{\text{elec}} + \Delta G_{\text{np}}.$$

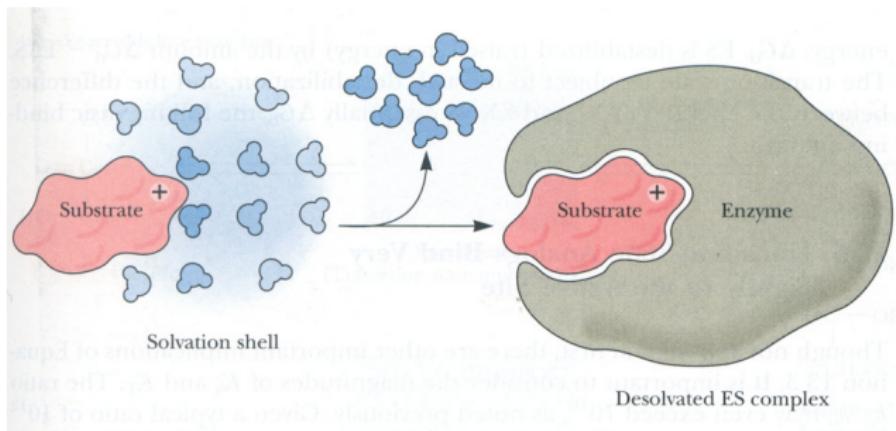
- Implicit models reduce computational cost.

# Ionic screening

$$\kappa^{-1} = \sqrt{\frac{\varepsilon k_B T}{2N_A e^2 I}}.$$

- The ionic strength  $I$  controls the screening.

# Desolvation energy



# Barriers and rare events

- Transitions with high  $\Delta G^\ddagger$  are rare.
- A bias potential accelerates sampling.

## Window sampling (umbrella)

$$U_{\text{sesgo}}(\xi) = \frac{k}{2}(\xi - \xi_0)^2.$$

- Controls sampling in windows of the CV  $\xi$ .

# PMF from distributions

$$F(\xi) = -k_B T \ln P(\xi) + C.$$

- WHAM combines histograms from multiple windows.

# Replica exchange

$$P_{\text{acc}} = \min(1, \exp [(\beta_i - \beta_j)(U_j - U_i)]) .$$

- Temperature swaps to overcome barriers.

# Adaptive sampling

- Select new seeds based on uncertainty.
- Combine short simulations with iterative analysis.

# Collective variables

- Reduced coordinates that describe the transition.
- Must separate metastable states.

# Bias via Gaussian hills

$$V(s, t) = \sum_i w \exp \left[ -\frac{(s - s_i)^2}{2\sigma^2} \right].$$

# Well-tempered metadynamics

$$w(t) = w_0 \exp\left(-\frac{V(s, t)}{k_B T \Delta T}\right).$$

- Smooths the growth of the bias over time.

## Free energy reconstruction

$$F(s) \approx -\frac{T + \Delta T}{\Delta T} V(s, t \rightarrow \infty).$$

# Common pitfalls

- Poorly chosen CVs create artifacts.
- Deposition interval too short.
- Lack of sampling in key regions.

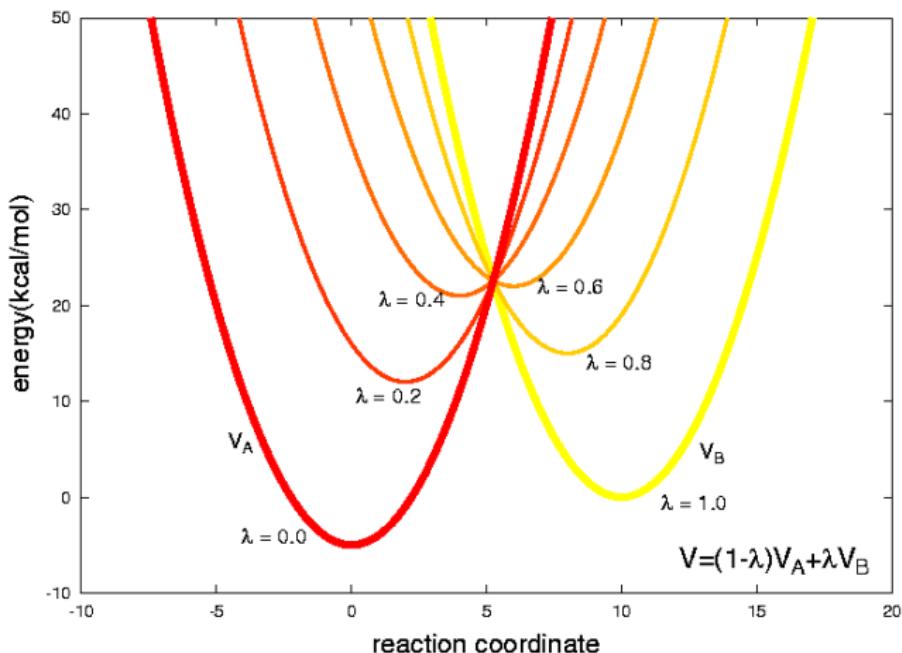
# Free energy perturbation

$$\Delta G = -k_B T \ln \langle \exp [-\beta \Delta U] \rangle_0.$$

# Thermodynamic integration

$$\Delta G = \int_0^1 \left\langle \frac{\partial U(\lambda)}{\partial \lambda} \right\rangle_\lambda d\lambda.$$

# FEP scheme



# Soft-core potentials

- Prevent singularities when turning off interactions.
- Ensure overlap of neighboring distributions.

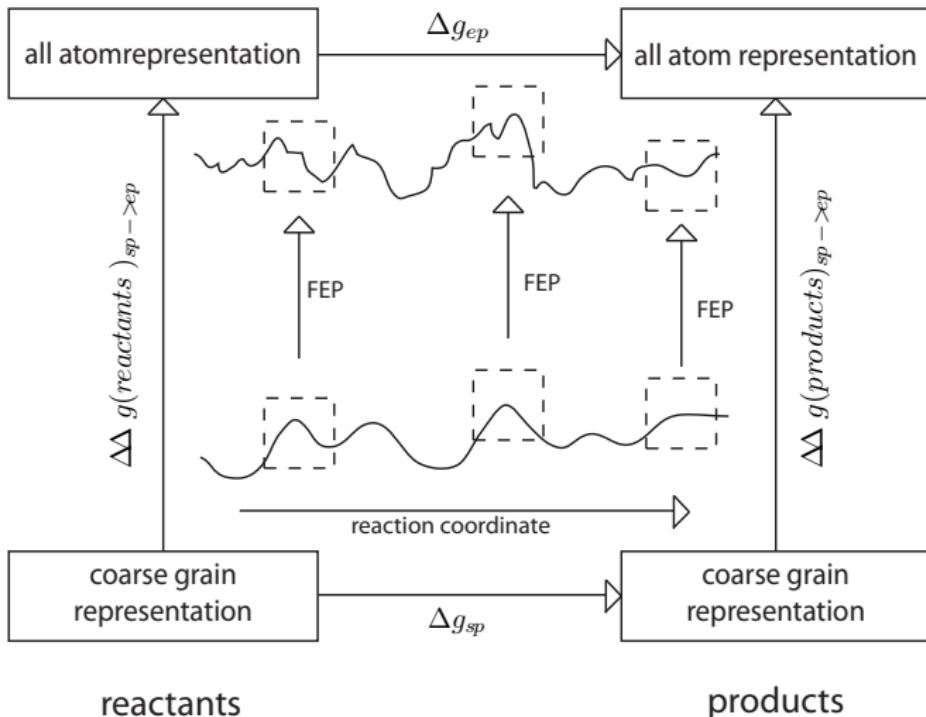
## $\lambda$ convergence

- Increase window density where changes are rapid.
- Monitor histograms of  $\partial U / \partial \lambda$ .

# Double decoupling

- Turn off ligand interactions in solvent and complex.
- The difference yields the binding  $\Delta G$ .

# Thermodynamic cycle



## Relation to affinity

$$\Delta G_{\text{bind}} = k_B T \ln K_d.$$

- Apply standard-state corrections when appropriate.

# Cycle closure

$$\sum_{\text{ciclo}} \Delta G_i = 0.$$

- A deviation indicates systematic errors.

# Error propagation

- Window errors add in quadrature.
- Report confidence intervals.

# Jarzynski equality

$$\Delta G = -k_B T \ln \langle e^{-\beta W} \rangle.$$

# Crooks relation

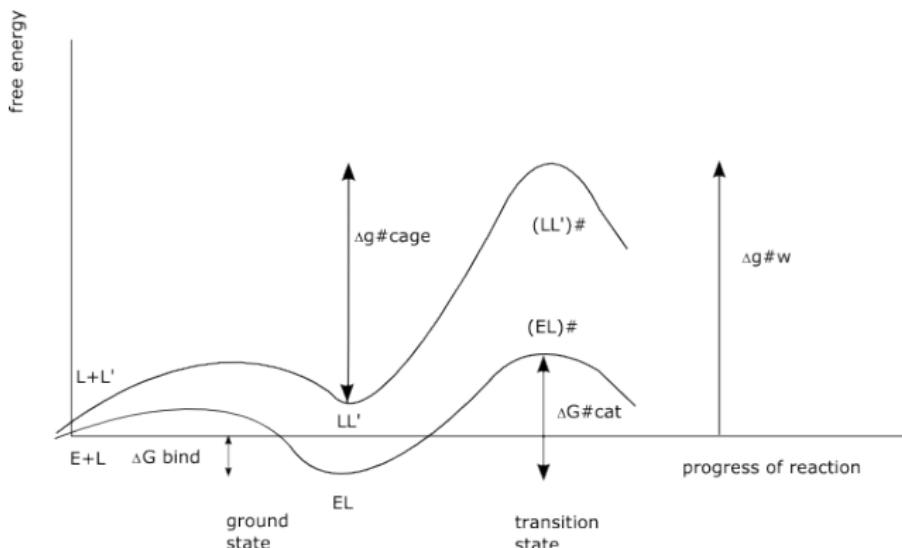
$$\frac{P_F(W)}{P_R(-W)} = e^{\beta(W - \Delta G)}.$$

# Steered MD

$$W = \int_0^\tau \mathbf{F}_{\text{pull}}(t) \cdot d\mathbf{x}.$$

- Work depends on the pulling speed.

# Reaction coordinate



# Replica Exchange Solute Tempering (REST)

- REST uses 'OpenMMTools' to scale energies on a subset of atoms (OpenMM Cookbook REST).
- Define modified 'CustomBondForce', 'CustomAngleForce', 'CustomTorsionForce', and 'NonbondedForce', and swap replicas with 'ReplicaExchangeSampler'.
- Useful for the protein–ligand complex where only the ligand is heated while the environment stays stable.

# Guided umbrella sampling

- Apply a ‘HarmonicBias’ on a collective variable  $x(r)$  and collect histograms per window (OpenMM Cookbook Umbrella Sampling).
- From  $\Delta F(x) = -k_B T \log P(x)$ , reconstruct the free energy profile and identify barriers.
- Combine with `argon-chemical-potential.py` to validate over simple LJ potentials before applying it to real proteins.

# Pulling speed

- Slow pulls approximate quasi-equilibrium.
- Fast pulls require many repeats.

## Block averages

- Split the time series into equal blocks.
- Estimate mean and error from independent blocks.

# Statistical inefficiency

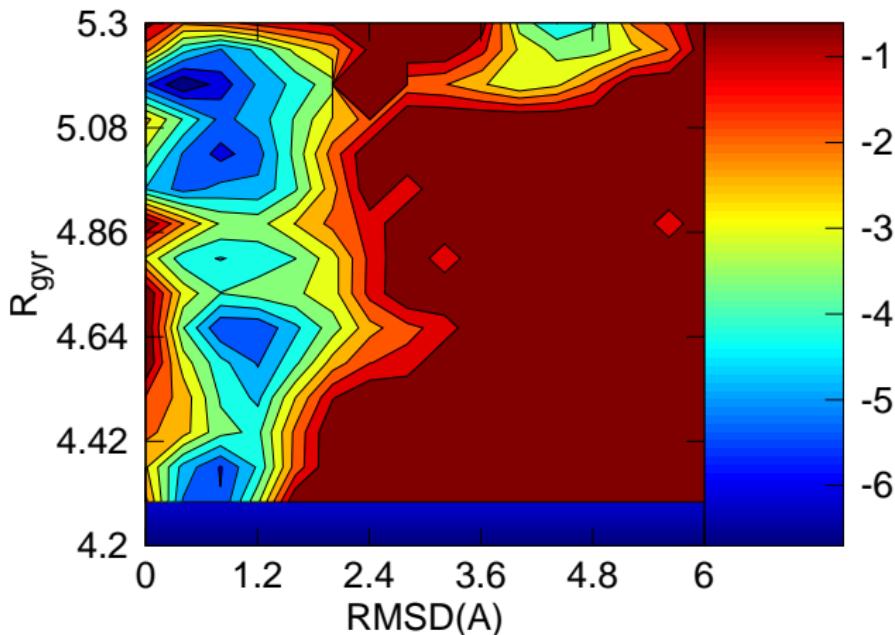
$$g = 1 + 2 \sum_{t=1}^{\infty} \rho(t).$$

- Reduces the effective number of samples.

# BAR/MBAR

- BAR minimizes variance between two states.
- MBAR generalizes to multiple windows.

# Free energy profile



# Convergence checklist

- Overlapping histograms between windows.
- Stable errors over time.
- Repeats yielding consistent results.

# Episode summary

- Reviewed advanced protocols and free energies.
- Emphasized convergence and statistical control.

# Best practices

- Document versions, seeds, and parameters.
- Automate analyses and checks.

# Common mistakes

- Insufficient windows during rapid changes.
- Lack of overlap between  $\lambda$ .

# Reproducibility

- Save scripts and seeding.
- Report uncertainty and initial conditions.

# Next step

- Dive into detailed trajectory analysis.

# References I