



Advanced-Physics Powered module free energy perturbation

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Introduction

Free Energy Perturbation (FEP) is a fundamental computational technique in the field of drug discovery and molecular science. It plays a crucial role in estimating the binding free energies of protein-ligand complexes, providing valuable insights into the interactions between potential drug compounds and their target proteins. This technique is particularly valuable for understanding the energetics of protein-ligand binding, which is essential for designing effective drug candidates.

In drug discovery, one of the primary goals is to identify molecules that can effectively bind to a target protein, modulating its activity and thereby treating specific diseases. To achieve this, it is essential to accurately predict the binding free energy, which quantifies the strength of the interaction between the ligand (drug candidate) and the protein target. FEP calculations offer a physics-based approach for estimating these binding free energies, making them a powerful tool in this context.

The core concept behind FEP involves performing alchemical transformations of the ligand, transitioning it between different states within the protein's binding pocket. These transformations occur in discrete steps, represented by the parameter λ , which varies between 0 (unbound state) and 1 (bound state). By evaluating the energy changes associated with these transitions, researchers can estimate the ΔG change, providing insights into the energetics of protein-ligand binding.

FEP can also be used in lead optimization, where compounds are modified and mutated within the protein's binding pocket to assess their relative binding affinities. This comparative approach helps in selecting the most promising drug candidates for further development.

There are two primary categories of FEP calculations: relative binding free energy (RBFE) calculations and absolute binding free energy (ABFE) calculations. RBFE calculations are typically used when comparing the binding affinities of different ligands. In contrast, ABFE calculations are employed to determine the absolute binding free energy of a specific ligand to a protein.

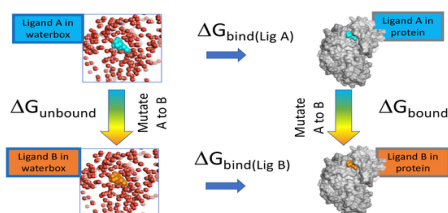


Figure 1. Protein Ligand Binding

To perform FEP calculations, researchers often use equilibrium simulations, where the system explores the ligand's various states and computes the associated free energy differences at each λ window. There is also a non-equilibrium approach, where rapid transitions between states are monitored to estimate the work required for these transitions.

Advantage of FEP

- FEP stands out as a remarkably precise technique capable of furnishing dependable predictions of binding affinity
- FEP emerges as a versatile approach, enabling the investigation of a diverse array of interactions between ligands and proteins
- FEP proves itself to be an efficient tool for the high-throughput screening of extensive compound libraries

Disadvantage of FEP

- FEP can be a time-intensive process, demanding several days or even weeks of computational resources
- FEP is a sensitive technique, and its accuracy can be compromised by errors in the chosen force field employed for system simulation
- Obtaining precise results with FEP may pose challenges when dealing with highly flexible ligands or ligands that interact with multiple binding sites on a protein

Applications of FEP in Drug Discovery

- 1. Identification of New Lead Compounds:**
 - FEP can efficiently screen large compound libraries to pinpoint molecules with the potential to bind to specific target proteins
- 2. Optimization of Existing Drugs:**
 - FEP allows for the fine-tuning of the binding affinity of existing drugs. Small structural modifications to drug molecules can be explored to enhance their efficacy
- 3. Understanding Mechanisms of Drug Action:**
 - FEP serves as a powerful tool to delve into the mechanisms of how drugs exert their effects

Relative free energy calculations in drug discovery

Relative free energy calculations in drug discovery involve the computation of the free energy difference between two molecular states, typically the binding of a ligand to a target protein in different environments or with different chemical modifications. This method is crucial for understanding the energetics of molecular interactions and predicting binding affinities, which is essential in drug design and optimization.

In drug discovery, the relative free energy change ΔG between two states A and B is calculated as follows:

$$\Delta G = G_B - G_A$$

Where:

- G_B is the free energy of state B (e.g., ligand bound to the target).
- G_A is the free energy of state A (e.g., ligand in solution).

How to Calculate Relative Free Energy?

The calculation is often performed using methods like Free Energy Perturbation (FEP) or Thermodynamic Integration (TI). These methods involve simulating the transition between the two states through a series of intermediate states, each represented by an ensemble of molecular configurations. The free energy change is then calculated by integrating the derivative of the Hamiltonian with respect to the transformation parameter.

Common Approaches for Relative Free Energy Calculations

- **Free Energy Perturbation (FEP)**
 - FEP involves the transformation of one ligand into another while maintaining a smooth, continuous change in the potential energy
 - The integration of these changes yields the free energy difference

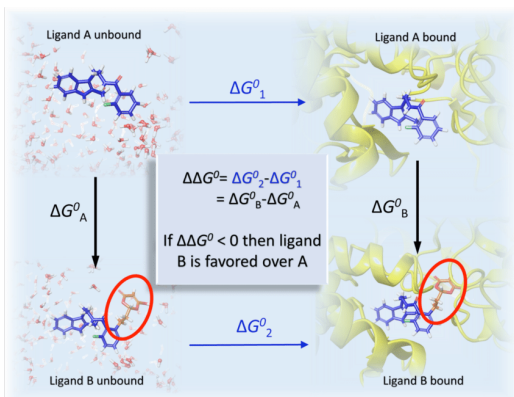


Figure 2. Relative free energy calculation

- **Thermodynamic Integration (TI)**
 - TI is based on the idea of gradually transforming one state into another, similar to FEP
 - The integration involves computing the ensemble average of the derivative of the Hamiltonian
- **Potential of Mean Force (PMF)**
 - PMF calculates the potential of mean force along a reaction coordinate, representing the free energy landscape of a system

Molecular Dynamics Simulations in Relative Free Energy Calculations

- **Role of Molecular Dynamics (MD)**
 - MD simulations provide a dynamic view of molecular systems, capturing movements and interactions over time
 - These simulations generate conformations and ensembles necessary for relative free energy calculations
- **Enhancements in Sampling Techniques**
 - Advanced sampling techniques, such as enhanced sampling algorithms (e.g., replica exchange, metadynamics), address the challenge of capturing rare events

Advantages of Relative Free Energy Calculations

- Accurate Binding Affinity Prediction
- Lead Optimization
- Understanding Binding Mechanisms
- Facilitates the screening of large compound libraries to identify potential drug candidates

Disadvantages of Relative Free Energy Calculations

- Computational Cost
- Convergence Issues
- Accuracy Dependence on Force Fields
- System Size Limitations
- Need for Expertise

Applications of Relative Free Energy Calculations

1. **Fragment-Based Drug Design**
 - Relative free energy calculations aid in optimizing fragment binding to develop potent lead compounds
2. **Protein Flexibility**
 - Assessing the impact of protein flexibility on ligand binding using relative free energy calculations provides valuable insights

Absolute free energy calculations in drug discovery

Absolute free energy calculations aim to quantify the stability and thermodynamics of molecular systems, specifically focusing on the binding of ligands to target proteins. Unlike relative free energy calculations that compare different states of the same system, absolute free energy calculations provide an absolute measure of the free energy change associated with a specific binding event.

How to Calculate Absolute Free Energy?

Calculating absolute free energy involves different methods depending on the approach chosen. The two common methods are the Molecular Mechanics/Poisson-Boltzmann Surface Area (MM/PBSA) method and the Quantum Mechanics/Molecular Mechanics (QM/MM) approach. Let's explore the formulas for each.

• Molecular Mechanics/Poisson-Boltzmann Surface Area (MM/PBSA):

- The absolute binding free energy (ΔG_{bind}) is calculated as the sum of molecular mechanics (MM) and Poisson-Boltzmann Surface Area (PBSA) components:

$$\Delta G_{bind} = \langle MM \rangle + \langle PBSA \rangle$$

a. Molecular Mechanics (MM) Component:

$$\langle MM \rangle = \langle EMM \rangle - T \langle SMM \rangle$$

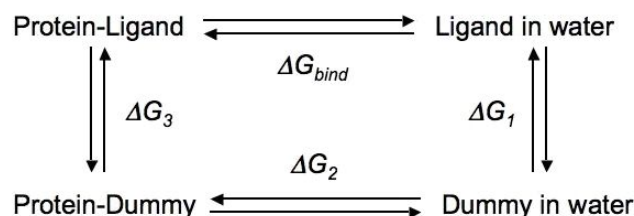
b. Poisson-Boltzmann Surface Area (PBSA) Component:

$$\langle PBSA \rangle = \langle G_{solv} \rangle - \langle G_{vac} \rangle$$

• Quantum Mechanics/Molecular Mechanics (QM/MM):

- For QM/MM calculations, the absolute binding free energy is determined by quantum mechanical calculations for the active site (QM) and molecular mechanics calculations for the surrounding environment (MM):

$$\Delta G_{bind} = \langle QM \rangle + \langle MM \rangle$$



$$\Delta G_2 \equiv 0$$

$$\therefore \Delta G_{bind} = \Delta G_3 - \Delta G_1$$

Quantum Mechanics/Molecular Mechanics (QM/MM): Quantum Mechanics/Molecular Mechanics (QM/MM)

• Integration of Quantum Mechanics

- QM/MM approaches combine quantum mechanical calculations for the active site (QM) with molecular mechanics calculations for the surrounding environment (MM)
- QM region typically involves the ligand, active site residues, and any critical chemical bonds

• Advantages of QM/MM

- Enables a more accurate representation of electronic effects and chemical reactions in the active site
- Suitable for systems where electronic structure effects play a significant role

Advantages of Absolute Free Energy Calculations

- Provides a direct measure of the binding affinity, facilitating accurate predictions of ligand binding strengths
- Enables a detailed analysis of specific molecular interactions contributing to the binding affinity
- Guides lead optimization efforts by quantifying the impact of chemical modifications on binding affinity

Disadvantages of Absolute Free Energy Calculations

- Absolute free energy calculations are computationally intensive, often requiring high-performance computing resources
- Results may be sensitive to the choice of methods, force fields, and simulation parameters, influencing accuracy
- Achieving convergence in simulations is challenging and may require extensive sampling to obtain reliable results
- Conducting accurate absolute free energy calculations necessitates expertise in both computational chemistry and molecular dynamics simulations

Figure 3. absolute free energy calculation

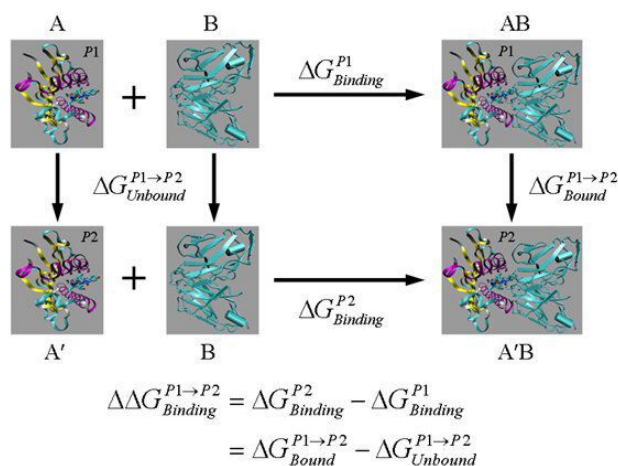


Figure 4. Thermodynamic cycle for Free energy calculation

FEP with Thermodynamics Integration (FEP-TI)

Free Energy Perturbation with Thermodynamic Integration (FEP-TI) is a computational approach that combines two powerful techniques, Free Energy Perturbation (FEP) and Thermodynamic Integration (TI), to calculate the free energy difference between two states of a molecular system. This method is widely used in the field of computational chemistry, particularly in the context of drug discovery.

Thermodynamic Integration (TI):

- **Purpose:** TI is another computational method used to calculate free energy differences between two states of a molecular system
- **Process:**
 - TI involves smoothly perturbing the system from one state to another, and then integrating the change in a thermodynamic property, such as internal energy, to obtain the free energy difference
 - This is done by running simulations for different values of the perturbation parameter and numerically integrating the derivatives of the free energy

FEP with Thermodynamic Integration (FEP-TI):

FEP-TI is a computational method that combines the strengths of Free Energy Perturbation and Thermodynamic Integration. It is employed to calculate the free energy difference between different states of a molecular system, providing valuable insights into the thermodynamics of molecular interactions.

• Integration of Techniques:

- FEP-TI combines the stepwise perturbation strategy of FEP with the numerical integration approach of TI
- The free energy change is calculated at each step of the FEP transformation, and these values are integrated to obtain the overall free energy difference

• Process:

- The system is transformed from the initial state to the final state through a series of small steps, as in FEP
- At each step, the free energy change is calculated using statistical mechanics equations, and these values are integrated along the perturbation path using the principles of TI

• Significance:

- Accuracy:** FEP-TI is known for providing accurate estimates of free energy differences, especially in cases where the transformation involves significant structural changes in the molecular system

$$\Delta A_{\text{FEP-TI}} = \sum_{i=0}^{n-1} \Delta A_{i\rightarrow i+1}$$

- $\Delta A_{\text{FEP-TI}}$: Overall free energy change combining FEP and TI.
- n : Number of steps in the perturbation.

Figure 5. FEP-TI Formula

FEP-TI in Hit To Lead Optimization

FEP-TI is a valuable computational tool in the Hit to Lead stage, providing quantitative insights into the impact of chemical modifications on binding affinity. This information guides the iterative optimization of lead compounds, contributing to the rational design of drug candidates with improved pharmacological properties.

FEP-TI can be employed during the Hit To Lead Stage as follows:

FEP-TI is a valuable computational tool in the Hit to Lead stage, providing quantitative insights into the impact of chemical modifications on binding affinity. This information guides the iterative optimization of lead compounds, contributing to the rational design of drug candidates with improved pharmacological properties.

1. Hit Identification:

- During the hit identification stage, potential drug candidates with biological activity against a specific target are identified through high-throughput screening, virtual screening, or other methods
- Hits are chemical compounds that show initial promise in terms of their ability to interact with the target of interest

2. Lead Compound Selection:

- From the pool of identified hits, lead compounds are selected based on factors such as potency, selectivity, and other pharmacological properties
- Lead compounds serve as starting points for further optimization

3. Structural Optimization Process:

- Once lead compounds are identified, structural optimization is crucial to enhance their binding affinity, selectivity, and other desirable properties
- FEP-TI can be applied during this stage to guide structural modifications and assess the impact of chemical alterations on binding affinity

4. FEP-TI Process:

• Defining Perturbations:

- a. Identify specific chemical modifications or structural changes to the lead compound that you want to evaluate
- b. Examples include modifications to functional groups, side chains, or other regions of the molecule

• Setting Up Simulations:

- a. Use molecular dynamics simulations to model the behavior of the lead compound and its modified forms
- b. Run simulations for each perturbation, gradually transforming the lead compound into the modified forms

• Calculating Free Energy Changes:

- a. Apply FEP calculations at each step of the perturbation to estimate the free energy change associated with the chemical modifications
- b. This involves calculating the potential energy differences and ensemble averages for each perturbed state

• Thermodynamic Integration:

- a. Integrate the calculated free energy changes along the perturbation path using the principles of Thermodynamic Integration
- b. Obtain the overall free energy difference between the lead compound and the modified forms

5. Interpretation and Decision-Making:

- Analyze the FEP-TI results to understand how each structural modification impacts the binding affinity of the lead compound
- Make informed decisions about which modifications are likely to improve or maintain the desired pharmacological properties

6. Iterative Optimization:

- Based on the insights gained from FEP-TI, iteratively optimize the lead compound by further refining its structure
- Continue the cycle of simulations, FEP calculations, and decision-making to guide the iterative optimization process

7. Validation and Experimental Testing:

- Validate the predictions from FEP-TI through experimental testing, such as binding assays or other relevant experiments
- Use experimental data to refine the computational models and improve the accuracy of future predictions

8. Lead Optimization:

- Successful application of FEP-TI contributes to the lead optimization process, guiding the development of lead compounds with improved pharmacological properties



Figure 6. Drug Discovery Pipeline

Benefits of FEP-TI in Hit to Lead Optimization:**1. Quantitative Insights:**

- FEP-TI provides quantitative estimates of the impact of chemical modifications on binding affinity, allowing for a more rational and data-driven lead optimization process

2. Efficiency:

- Computational methods like FEP-TI can accelerate lead optimization by prioritizing modifications that are more likely to yield improvements in drug-like properties

3. Resource Savings:

- FEP-TI can help prioritize lead compounds and modifications, potentially saving time and resources compared to a purely experimental approach

4. Guidance for Synthesis:

- FEP-TI results can guide medicinal chemists in designing and synthesizing new compounds for experimental testing

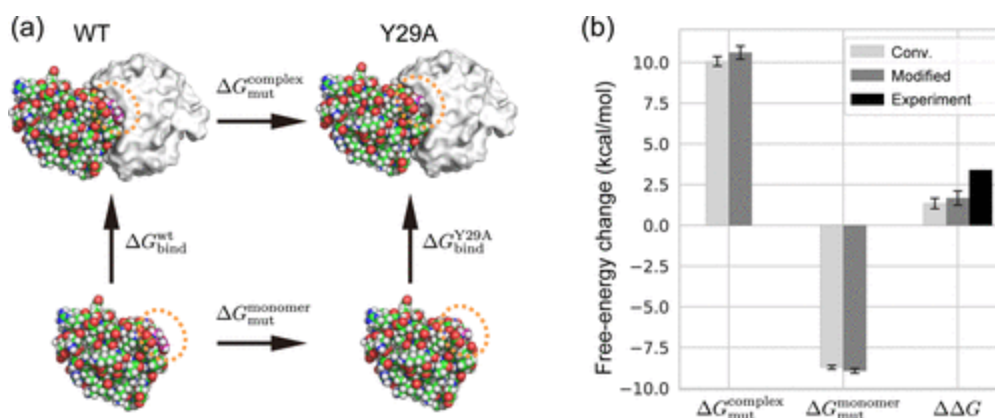


Figure 7. Thermodynamic cycle of the barnase-barstar binding in wild type (wt) and the Y29A mutant barnase (Y29A)

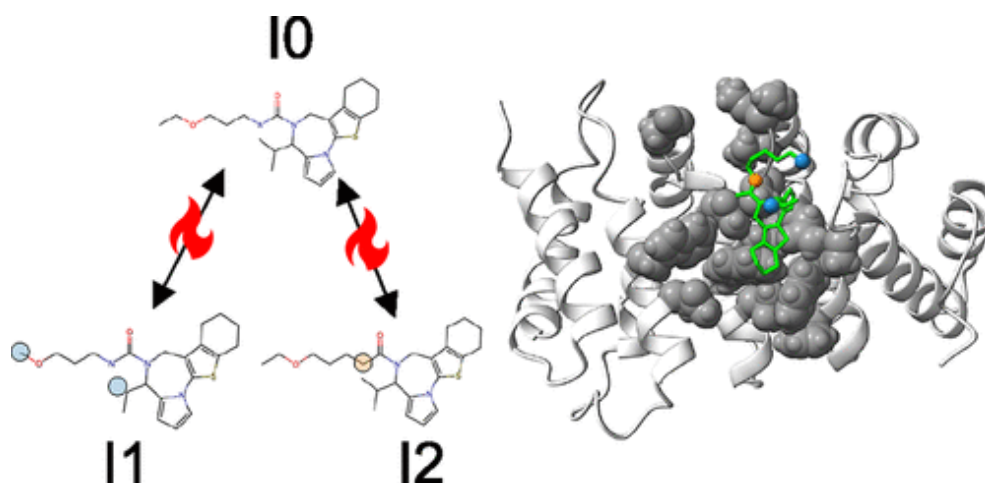


Figure 8. Hamiltonian Replica Exchange for Free energy calculation

FEP with Hamiltonian Replica Exchange (FEP-HRE)

Free Energy Perturbation with Hamiltonian Replica Exchange (FEP-HRE) is a computational method that combines two powerful techniques, Free Energy Perturbation (FEP) and Hamiltonian Replica Exchange (HRE), to enhance the efficiency and accuracy of free energy calculations in molecular simulations.

Hamiltonian Replica Exchange (HRE):

- **Purpose:** HRE is a technique used to enhance the exploration of conformational space in molecular simulations
- **Process:**
 - I. Multiple replicas (copies) of the system are simulated, each at a different Hamiltonian or energy scale
 - II. Periodically, exchanges are attempted between replicas with different Hamiltonians, allowing the system to explore different energy landscapes

FEP with Hamiltonian Replica Exchange (HRE):

FEP-HRE is a computational method that combines the principles of Free Energy Perturbation and Hamiltonian Replica Exchange. This integration aims to improve the accuracy of free energy calculations by enhancing conformational sampling in molecular simulations.

- **Integration of Techniques:**
 - I. FEP-HRE combines the stepwise perturbation strategy of FEP with the replica exchange approach of HRE
 - II. The incremental perturbation steps of FEP are performed in the context of multiple replicas with different Hamiltonians
- **Benefits:**
 - I. HRE helps to enhance the sampling of conformational space, which can be especially beneficial when studying complex systems with multiple energy minima
 - II. The combination of FEP and HRE aims to provide more accurate and efficient free energy calculations
- **Significance:**
 - I. **Accuracy:** FEP-HRE is designed to provide more accurate estimates of free energy differences, particularly in cases where the transformation involves significant structural changes in the molecular system
 - II. **Enhanced Sampling:** The use of Hamiltonian Replica Exchange facilitates enhanced conformational sampling, allowing the system to explore a broader range of configurations.

$$\Delta A_{\text{FEP-HRE}} = \sum_{i=0}^{n-1} \Delta A_{i \rightarrow i+1}$$

- $\Delta A_{\text{FEP-HRE}}$: Overall free energy change combining FEP and HRE.
- n : Number of steps in the perturbation.

Figure 9. FEP-HRE Formula