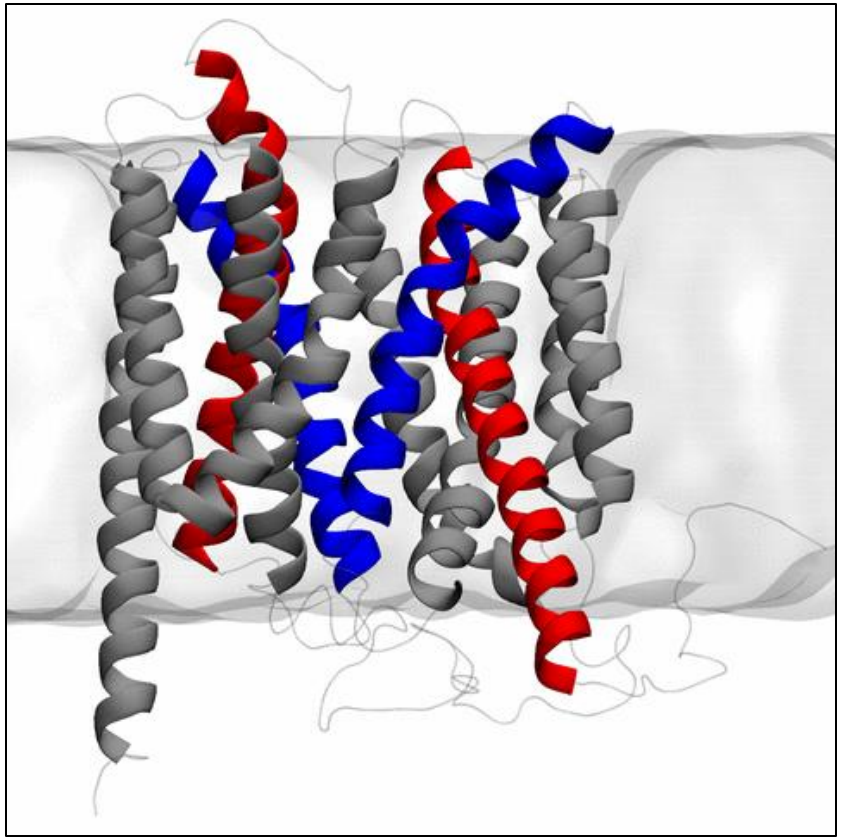
Characterizing Structural Transitions of Membrane Transport Proteins at Atomic Detail



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NCSA Blue Waters Symposium
for Petascale Science and Beyond
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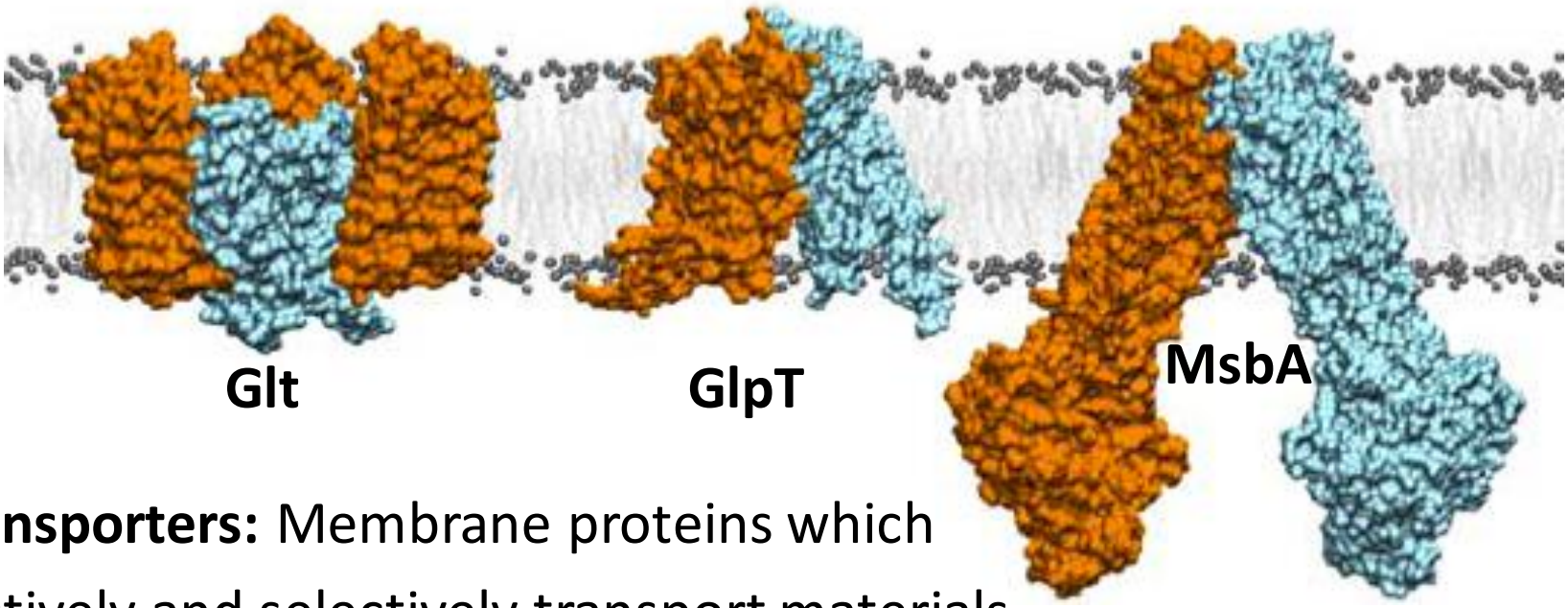
Outline

- **Introduction**
  - GlpT transporter
  - Transport cycle thermodynamics
- **Methodology**
  - Empirical search for reaction coordinates using nonequilibrium simulations
  - Iterative path-finding algorithms and free energy calculations
- **Reconstructed thermodynamic cycle of GlpT**
  - Free energy profile along the cycle
  - Global and local conformational changes and their coupling

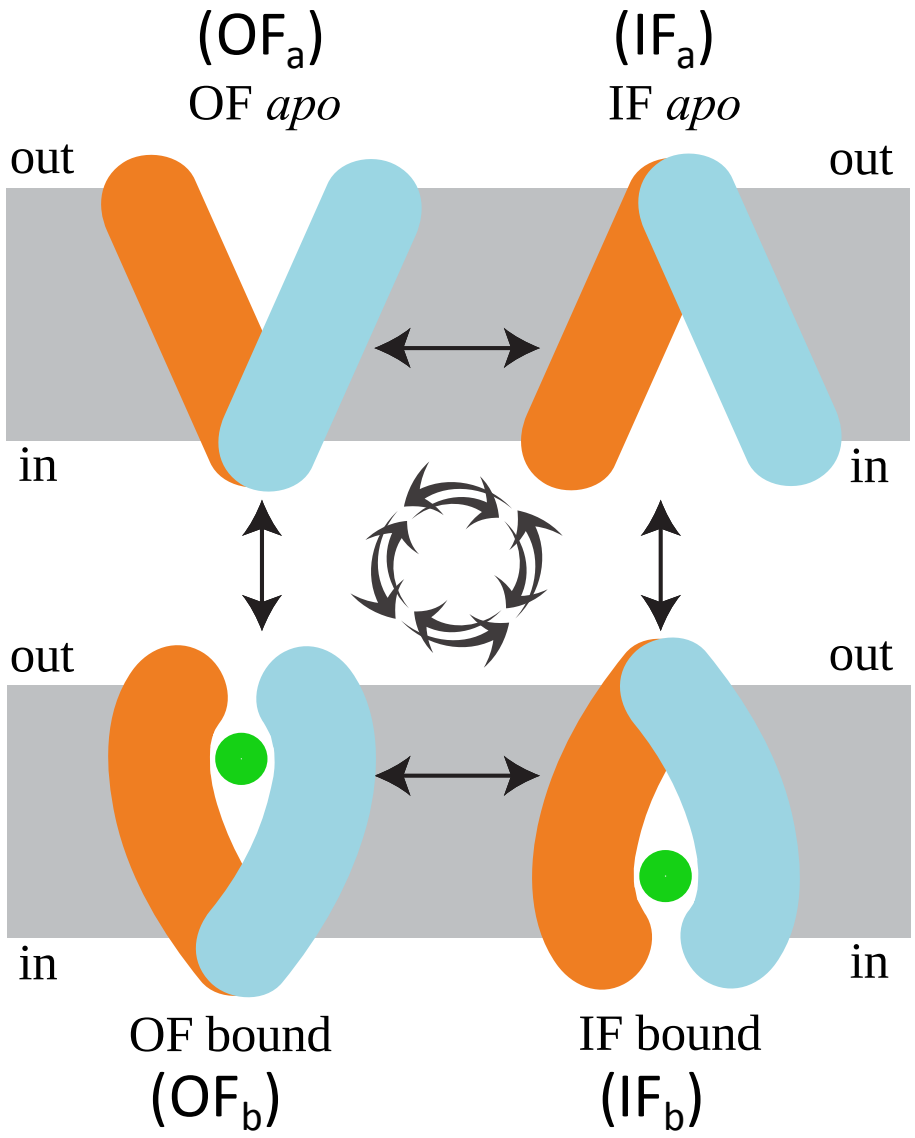
Membrane Transporters



- **Transporters:** Membrane proteins which actively and selectively transport materials (proton, ions, small molecules) across cell membranes.
- **Active transport**: Pumping substrates against their concentration gradient (from low to high concentration).
- **Source of energy:**
  - metabolic energy, e.g. from ATP hydrolysis (**primary**).
  - electrochemical gradient of an ion (**secondary**).

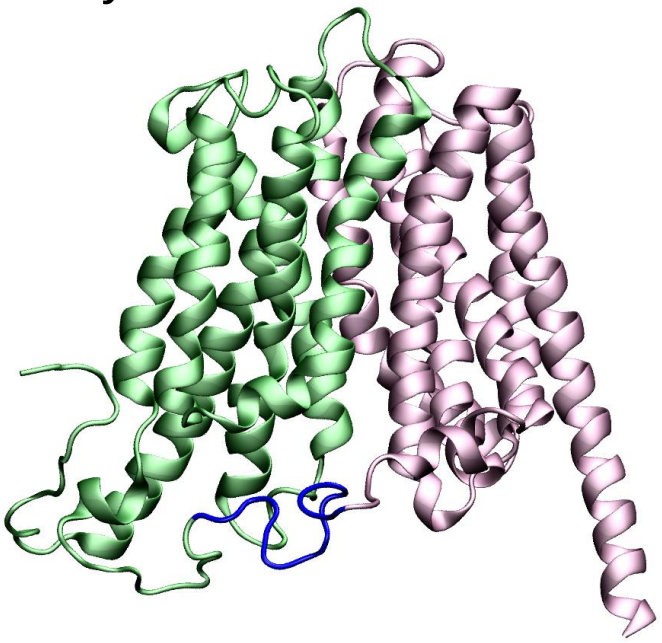
Alternating-Access Mechanism

- Membrane transporters rely on **large-scale conformational changes** to alternate between inward-facing (**IF**) and outward-facing (**OF**) states to pump the substrate against its concentration gradient, without being open (having the binding site accessible) to both sides of the membrane simultaneously.

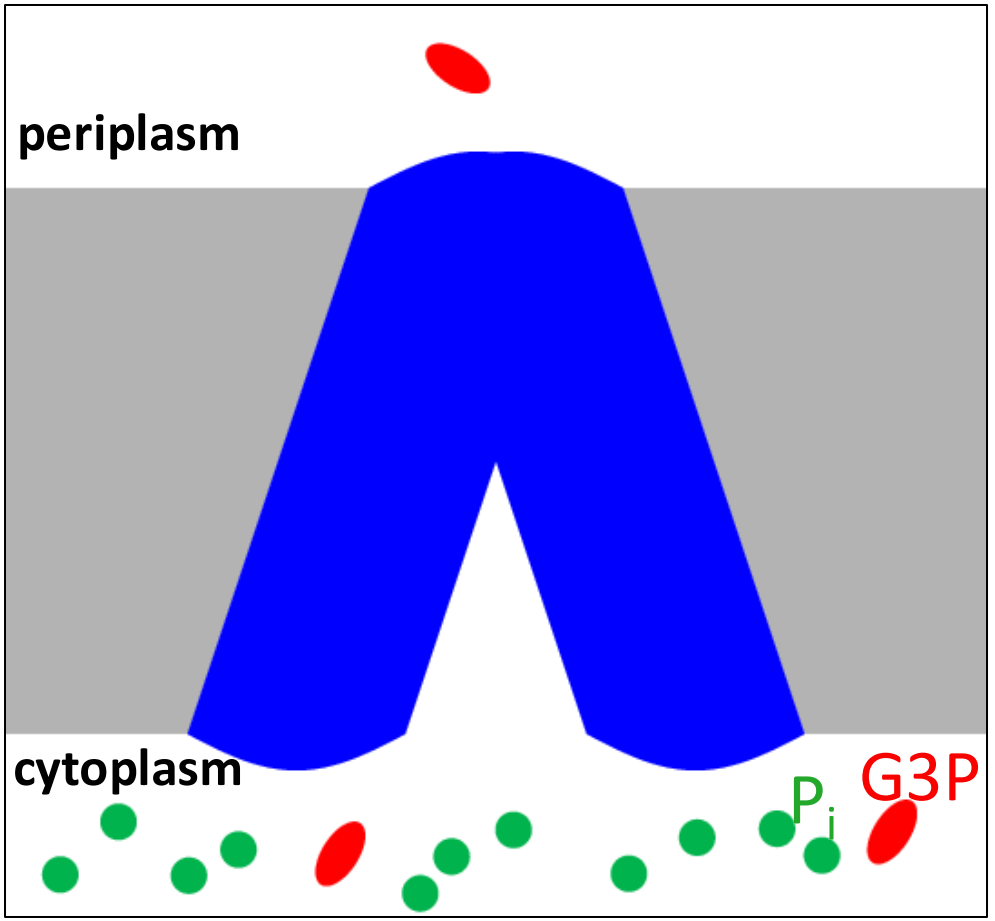


Glycerol-3-phophate (G3P) transporter (GlpT)

- Major facilitator superfamily (MFS)
- Secondary active transporter
- Crystalized in the IF state.

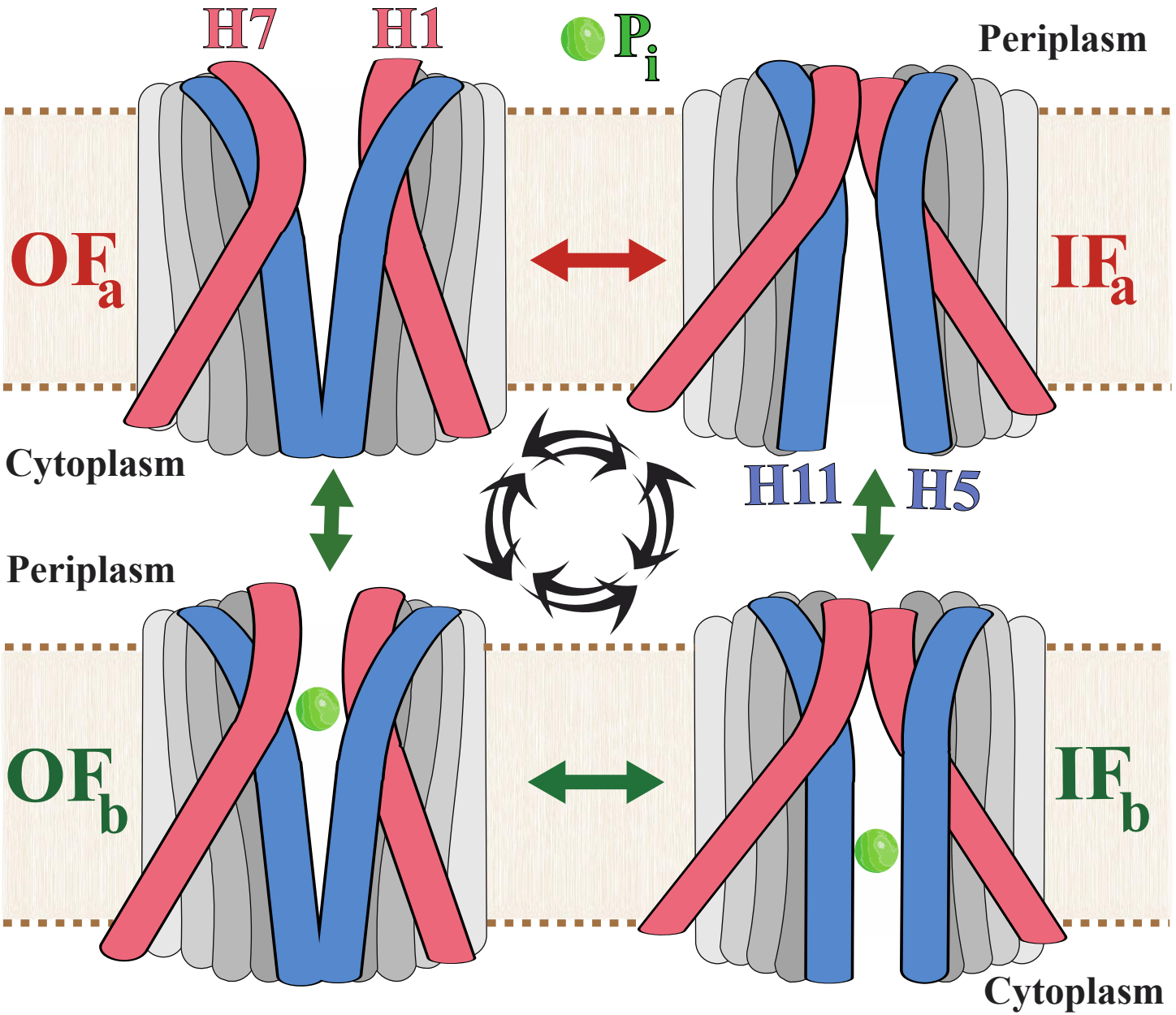


Huang, *et al.*, ***Science*** **301**, 616 (**2003**).



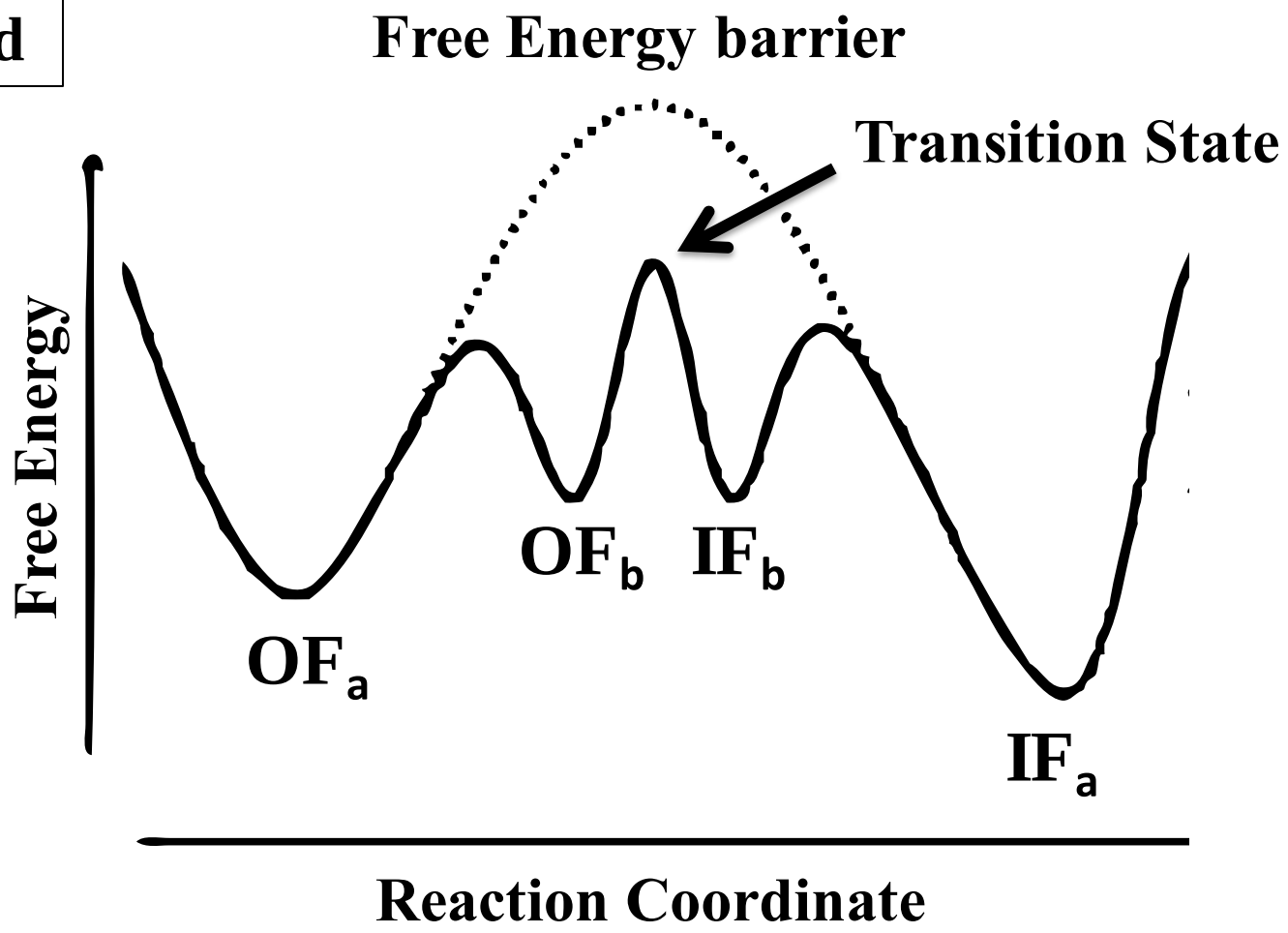
- GlpT transports **G3P** using $\mathbf{P_i}$ gradient.
- $\mathbf{P_i}$:$\mathbf{P_i}$ exchanger (in the absence of organic phosphate)
- **Rate-limiting step:** IF-OF interconversion.

Transport cycle thermodynamics



Transport cycle thermodynamics

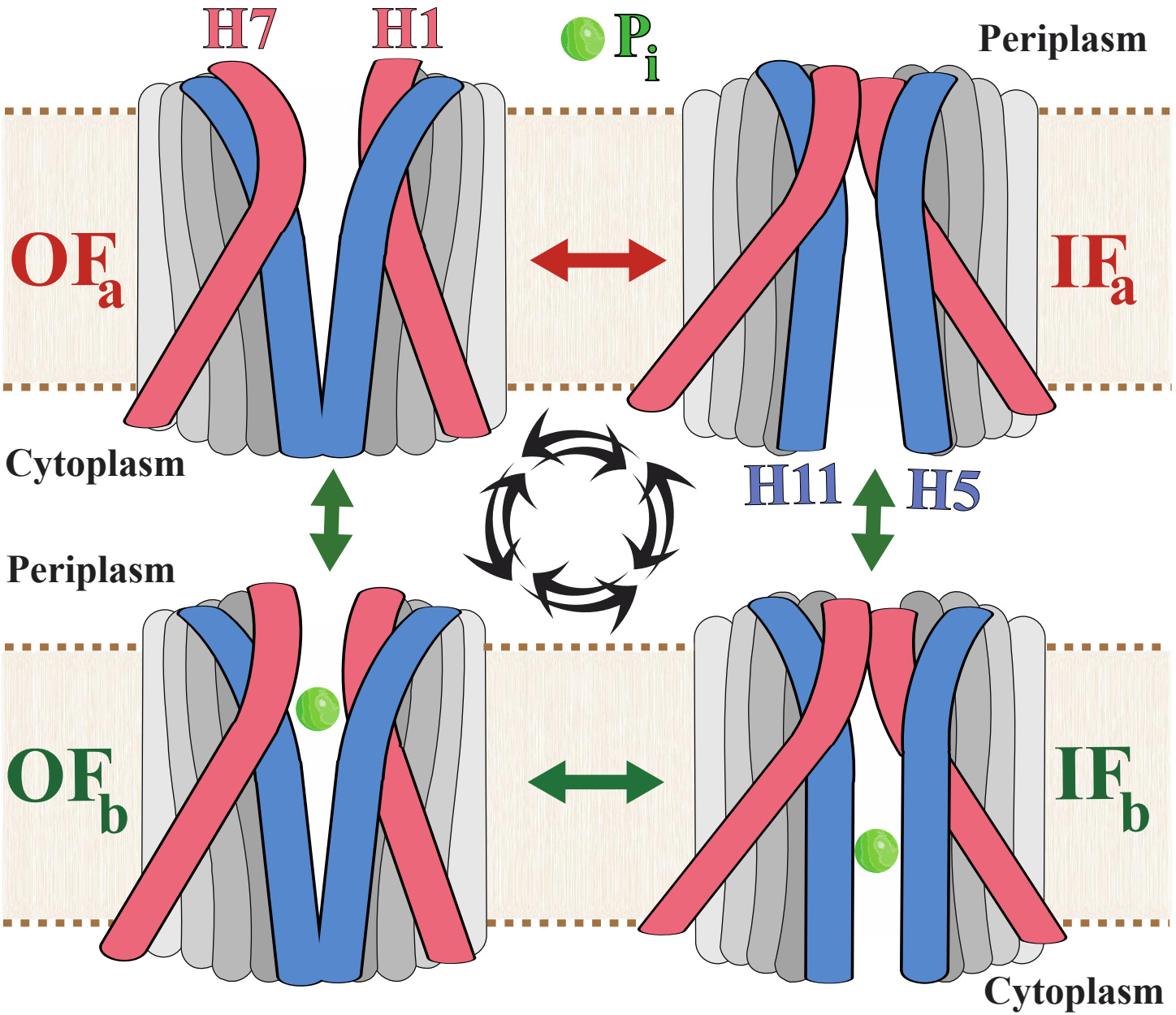
a: *apo*
b: bound



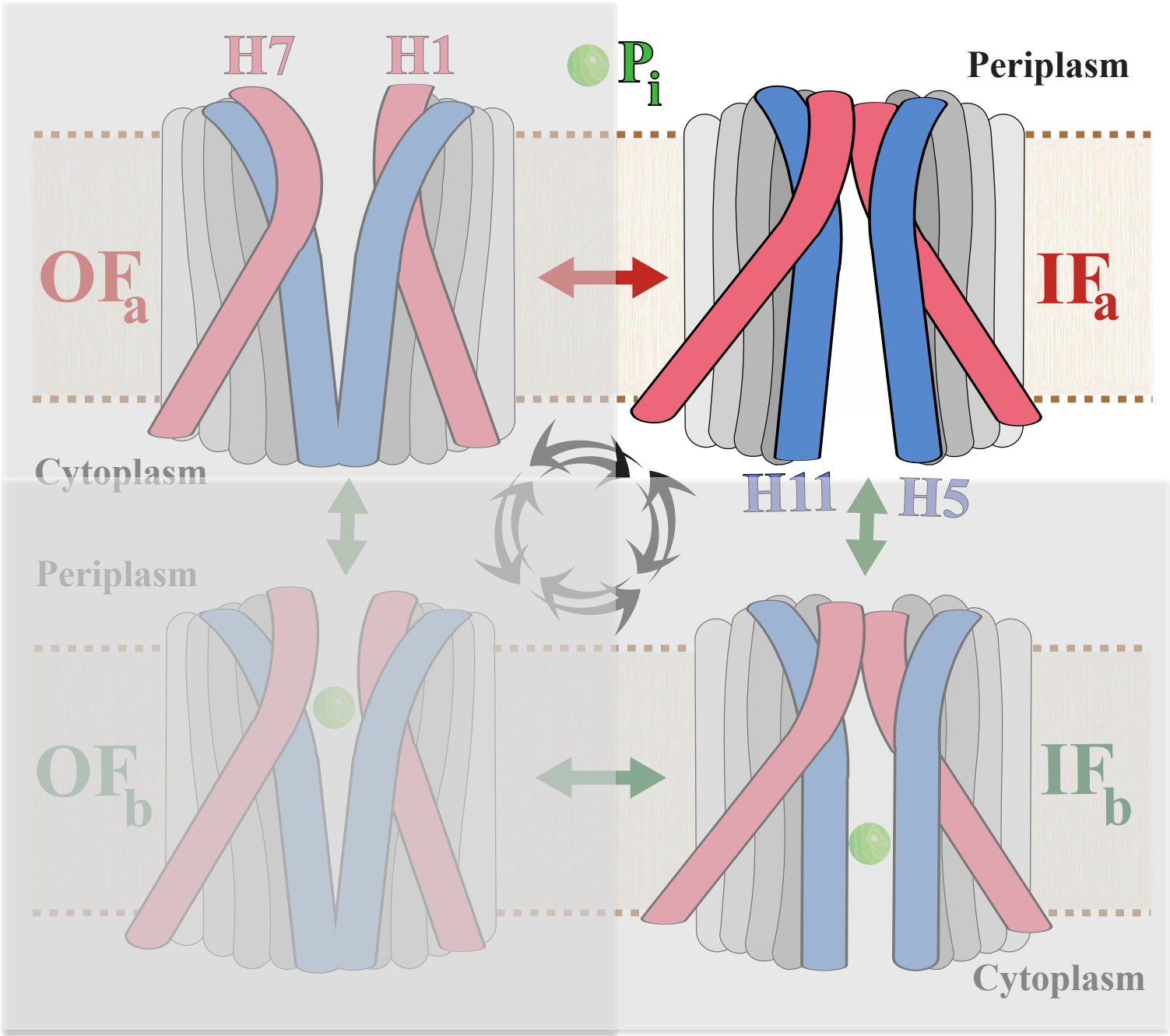
Lemieux, *et al.*, *Curr. Opin. Struct. Biol. 1***4**, 405 (2004).

Law, *et al.*, *Biochemistry* **46**, 12190 (2007).

Full thermodynamic cycle



the only available crystal structure



Key Challenge:

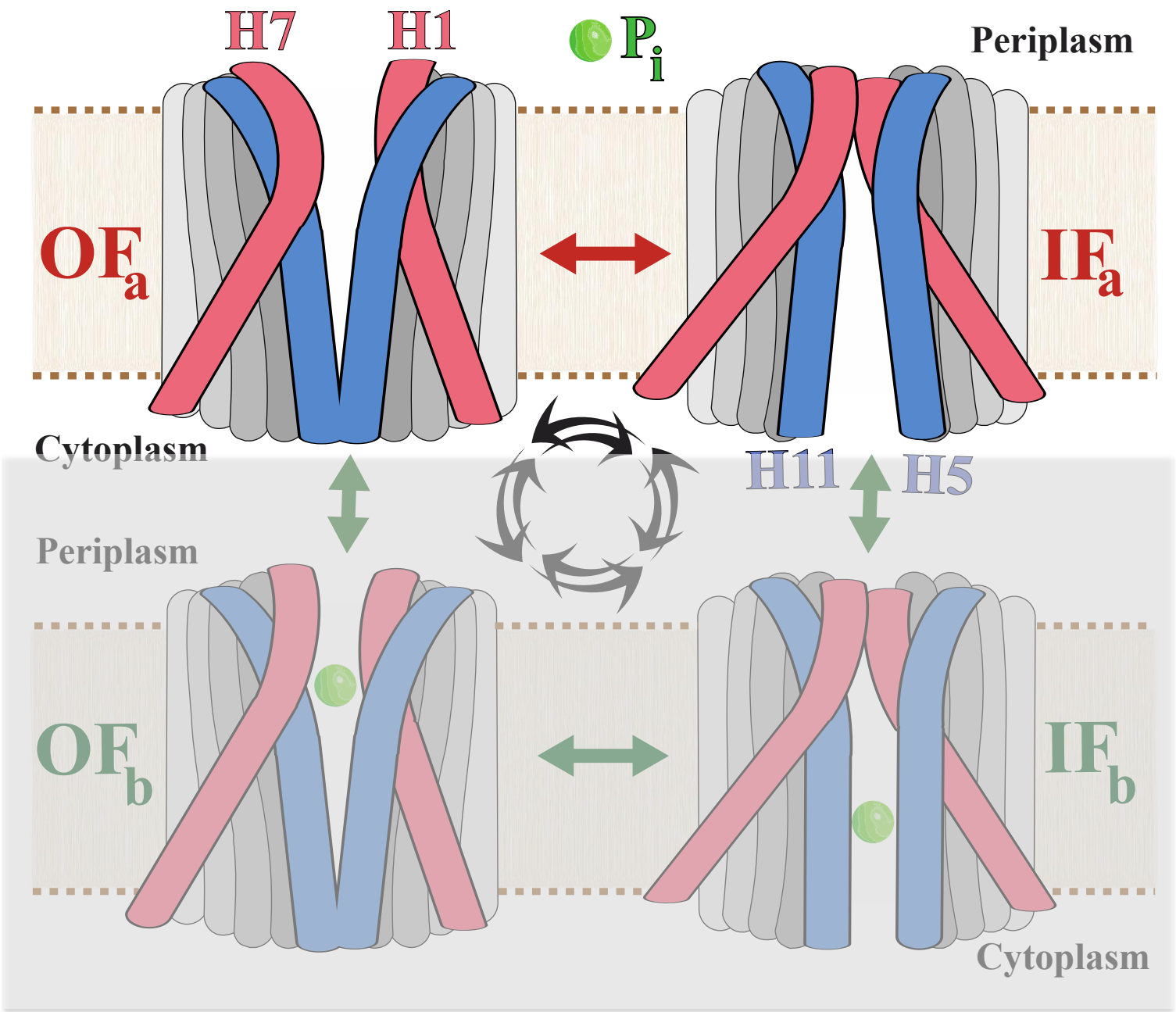
- **Slow dynamics**
  - Timescale gap between feasible all-atom molecular dynamics (MD) simulations and actual functionally relevant biomolecular processes.

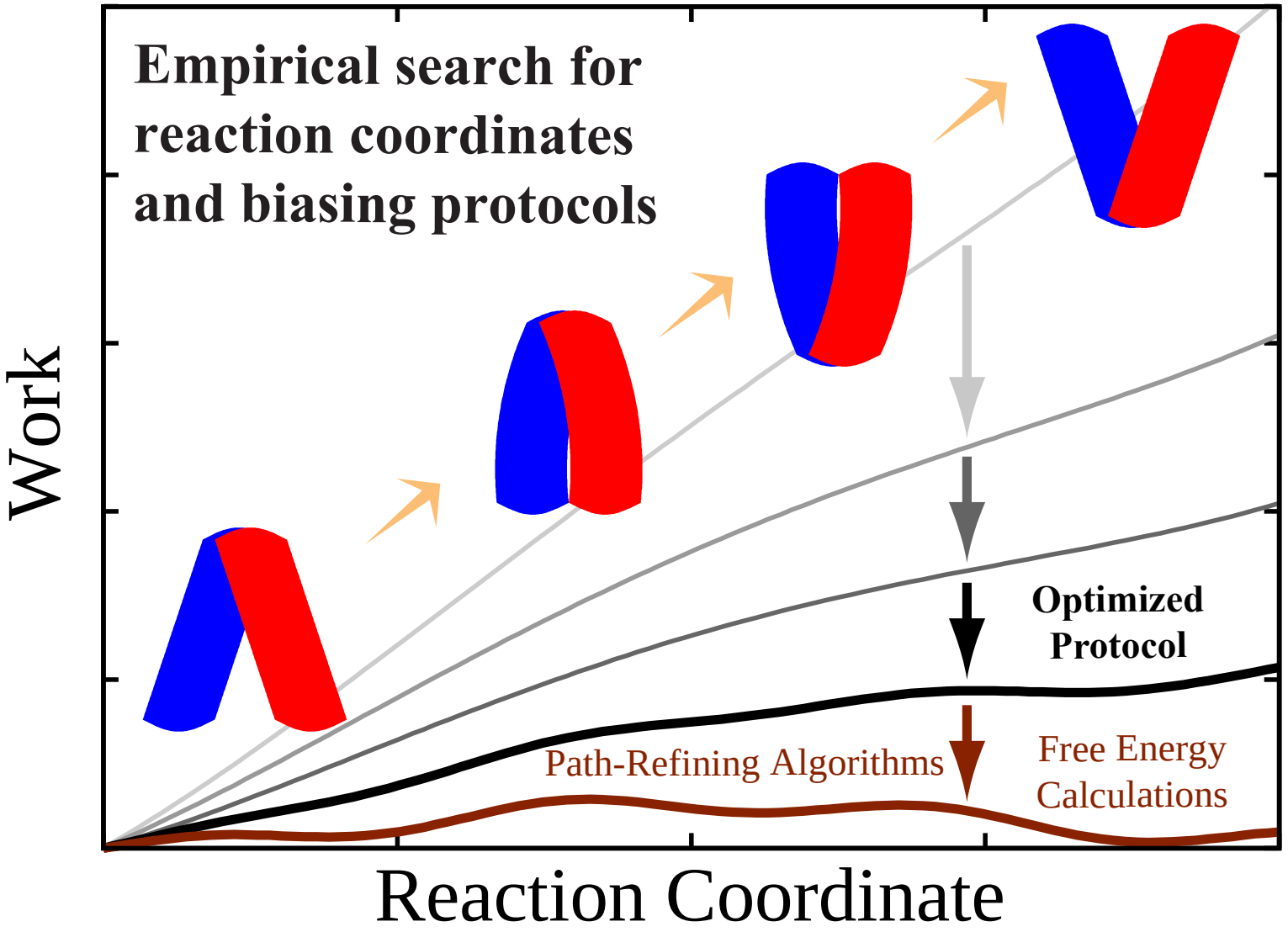
Sampling Strategies:

- **Long simulation**
  - application-specific computers
- **Multiple-copy simulations**
  - distributed computing
- **Enhanced sampling**
  - biased/adaptive simulations

Loosely-coupled multiple-copy algorithms (petascale computing)

Step 1: $OF_a \leftrightarrow IF_a$





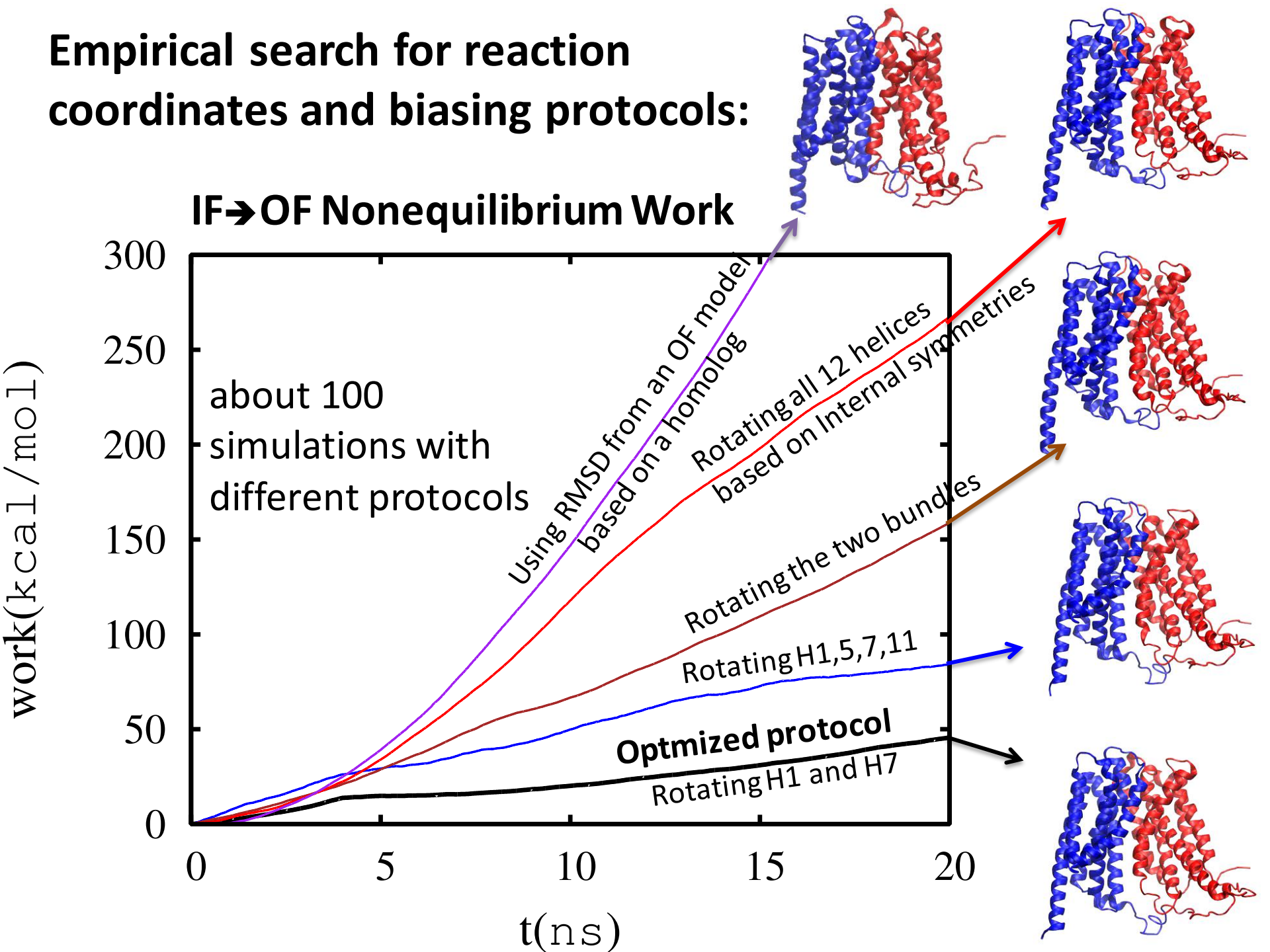
Theory/Method:

Moradi et al., **CPL 518** 109 (**2011**)
Moradi et al., **JCP 140** 034114,5 (**2014**)
Moradi et al., **JCTC 10** 2866 (**2014**)

Application:

Moradi et al., **PNAS 106** 20746 (**2009**)
Moradi et al., **NAR 41** 33 (**2013**)
Moradi et al., **PNAS 110** 18916 (**2013**)

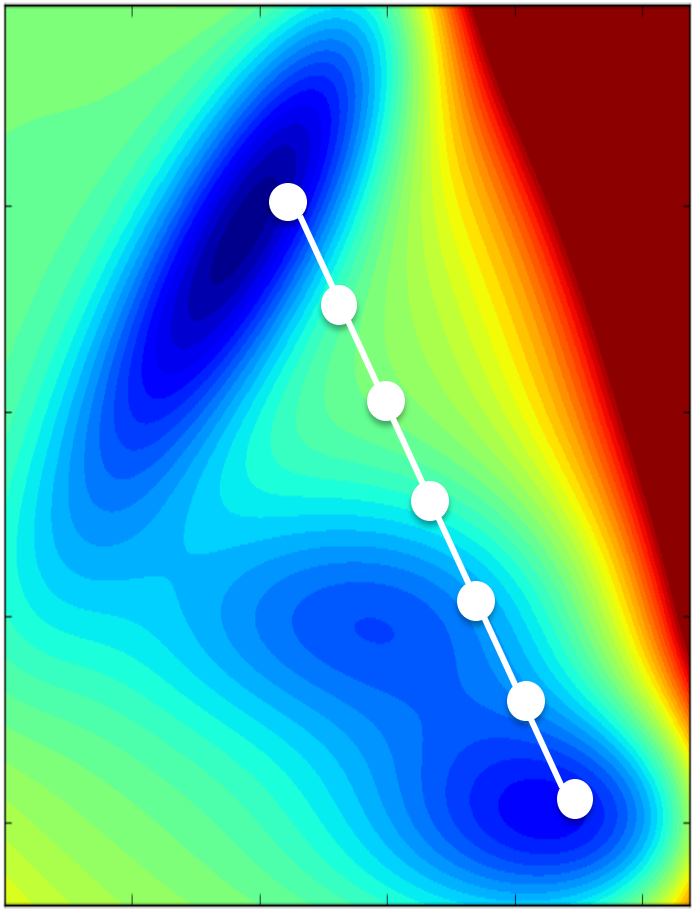
Empirical search for reaction coordinates and biasing protocols:



Path-finding algorithms

- String Method (finding approximate minimum free energy pathways on high-dimensional spaces)
  - **A pathway is represented by a "string"**, i.e., an ordered series of images $\{\xi_i\}$ connecting reactant and product regions.
  - **The string is iteratively updated** according to some ``rule'' until converges to a stationary solution:

$$\xi(s) \parallel \mathbf{g}^{-1}(\xi)\nabla_{\xi}F(\xi)$$

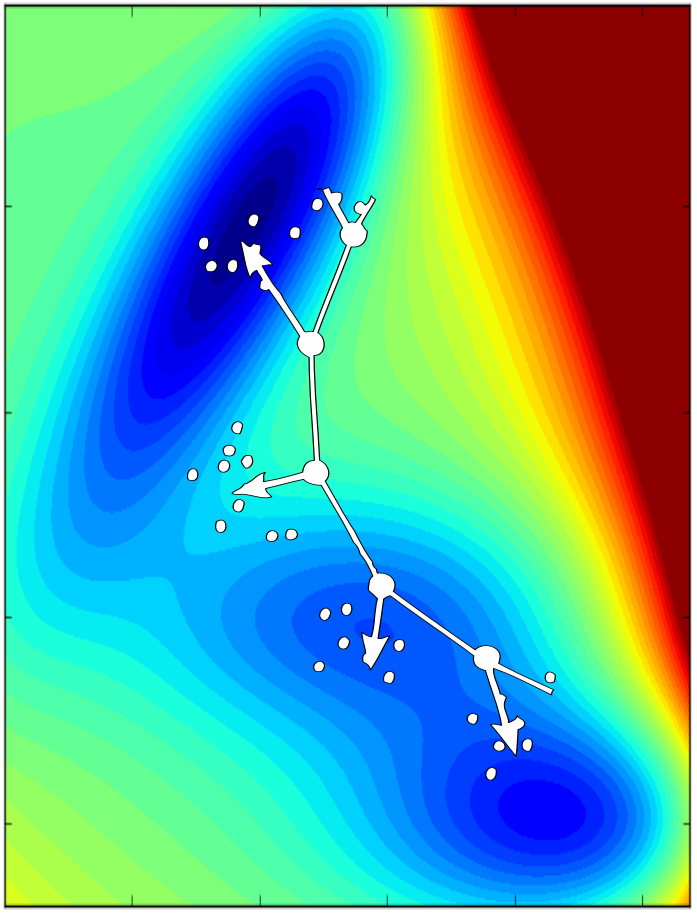


- Maragliano, Fischer, Vanden-Eijnden, and Ciccotti J. Chem. Phys. 2006, 125, 024106.
- Ren, Vanden-Eijnden, Maragakis, and E J. Chem. Phys. 2005, 123, 134109.
- Vanden-Eijnden and Venturoli; J. Chem. Phys. 2009, 130, 194103.

Path-finding algorithms

- String Method with Swarms of Trajectories (SMwST):
  - For each image **tens of copies** are launched:
  - Start with an initial string $\{\xi_i\}$
  - (1) Restrain M copies of each image at the current ξ_i
  - (2) Release the restraint
  - (3) Update the centers: $\xi_i = \left\langle \xi_i^t \right\rangle$
  - (4) Reparametrize

Pan, Sezer, and Roux
J. Phys. Chem. B 2008, 112, 3432−3440.



Collective variables: $\{Q\} = \{Q_1, Q_2, \ldots, Q_{12}\}$

Number of replicas: 50 X 20 = 1000

Simulation time: 1 ns/replica

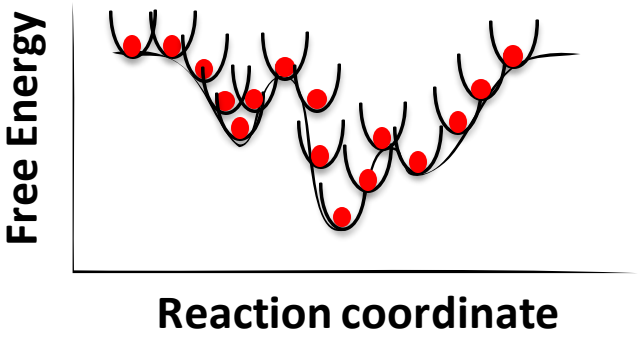
Free energy calculations

- Bias-exchange umbrella sampling **(BEUS)**

  (Loosely coupled multiple-copy MD)

  – Umbrella sampling

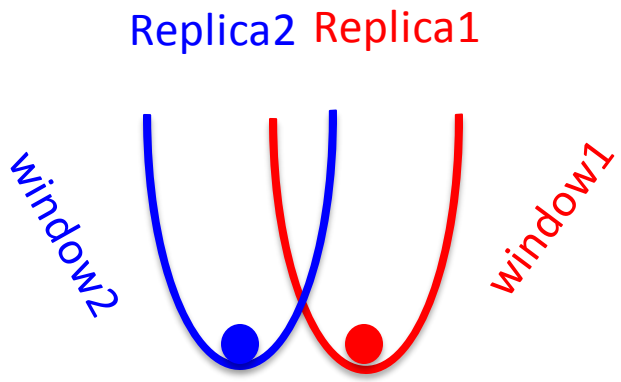
$$U_B(x_i^t) = \tfrac{1}{2} k(x_i^t - x_i)^2$$



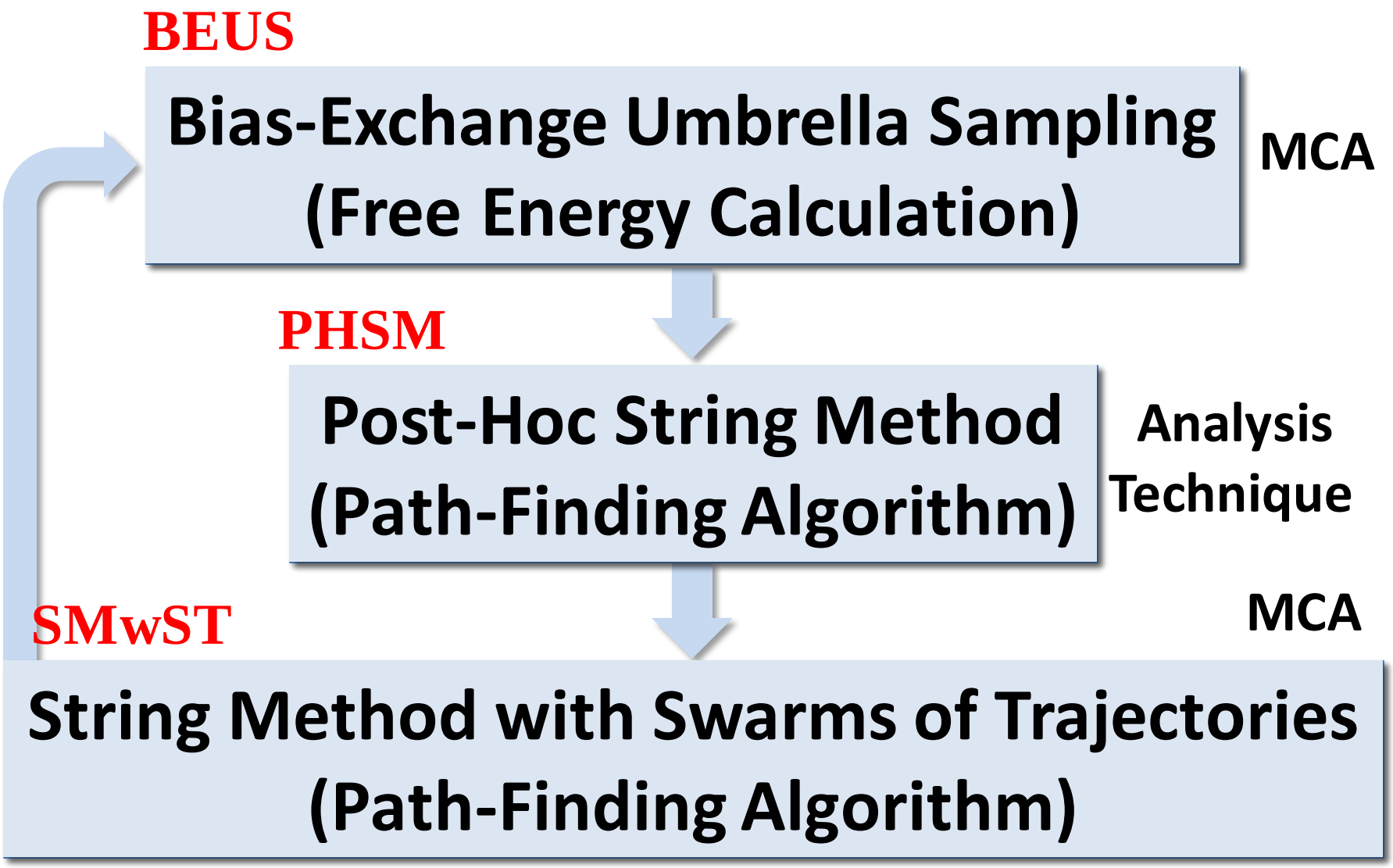
  – Replica-exchange MD

$$p(\boldsymbol{x}_1\boldsymbol{x}_2 \rightarrow \boldsymbol{x}_2\boldsymbol{x}_1) = \min\left(1, \frac{\pi_1(\boldsymbol{x}_2)\pi_2(\boldsymbol{x}_1)}{\pi_1(\boldsymbol{x}_1)\pi_2(\boldsymbol{x}_2)}\right)$$

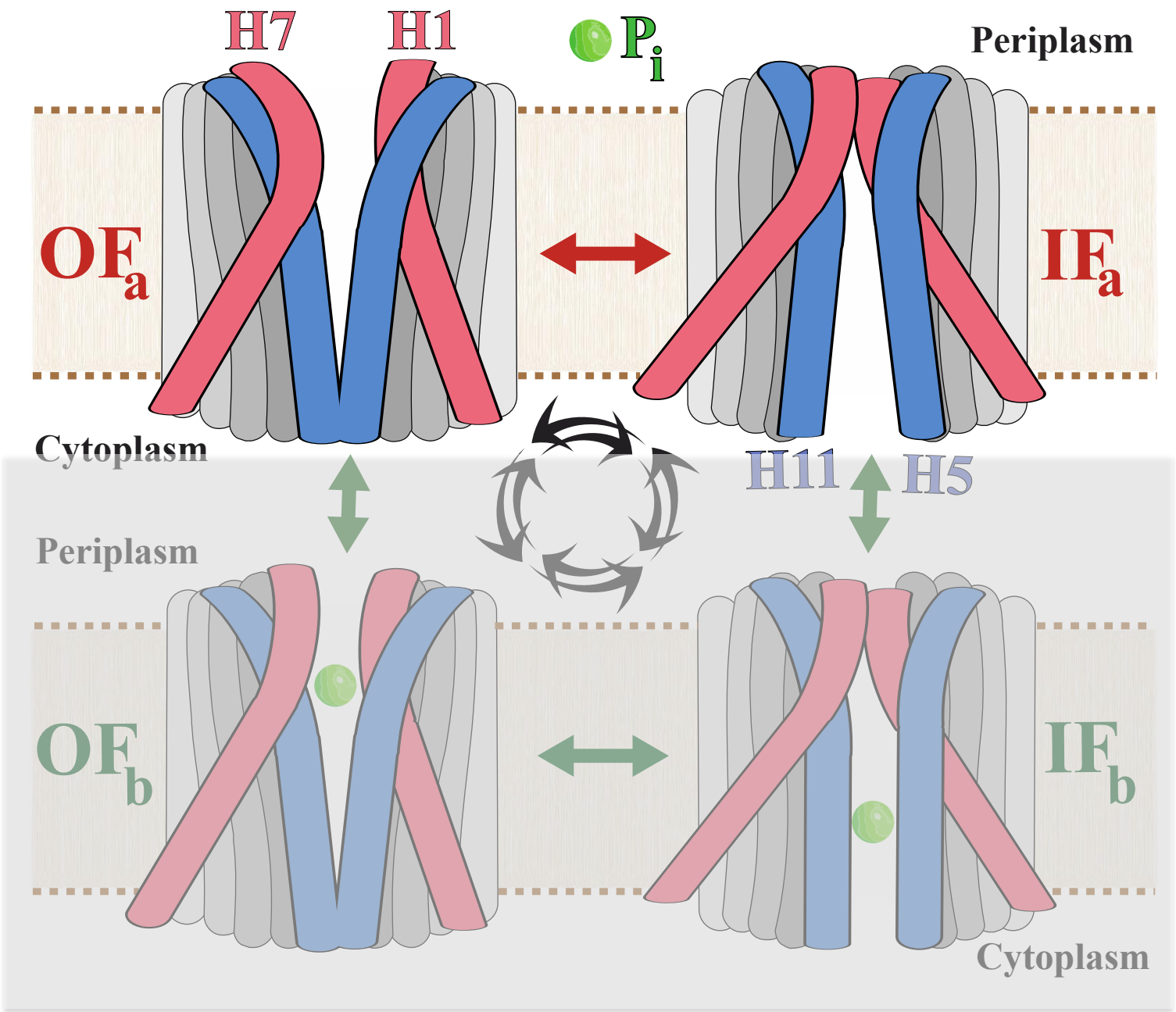
$$\min\left(1, \frac{e^{-\beta U_1(\xi_2)} e^{-\beta U_2(\xi_1)}}{e^{-\beta U_1(\xi_1)} e^{-\beta U_2(\xi_1)}}\right)$$



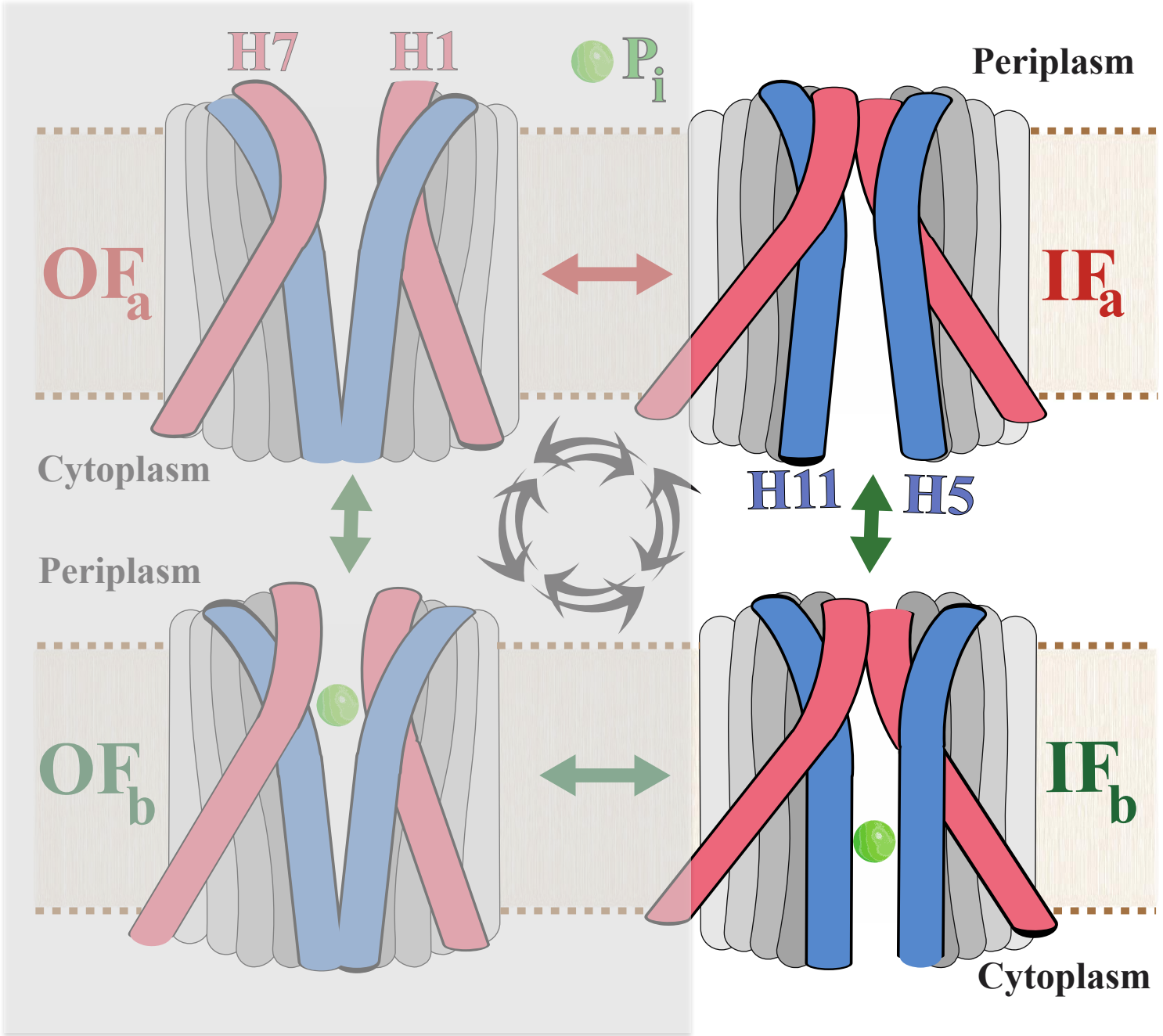
Iterative path-refining algorithms and free energy calculations



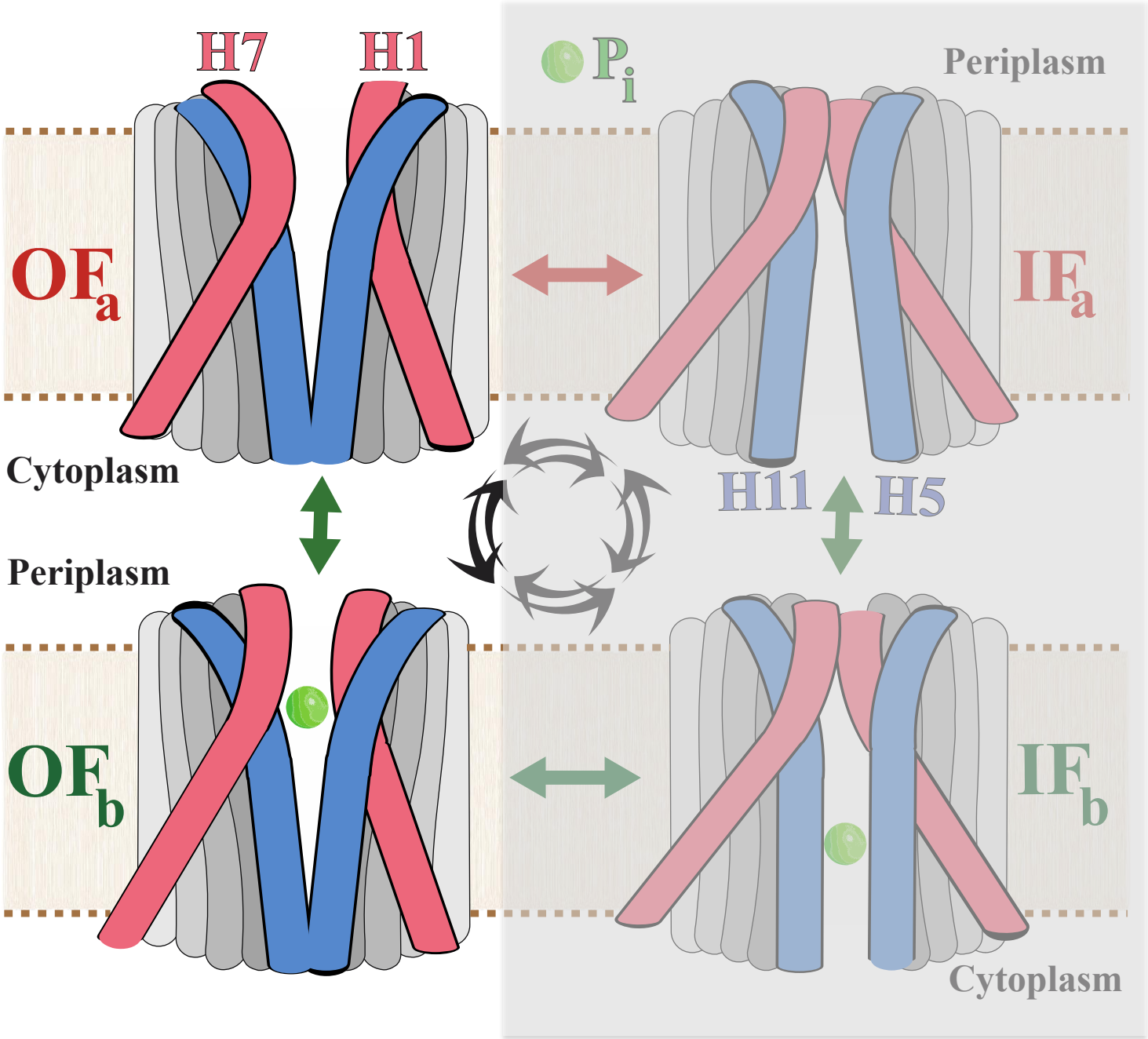
Step 1: $OF_a \leftrightarrow IF_a$



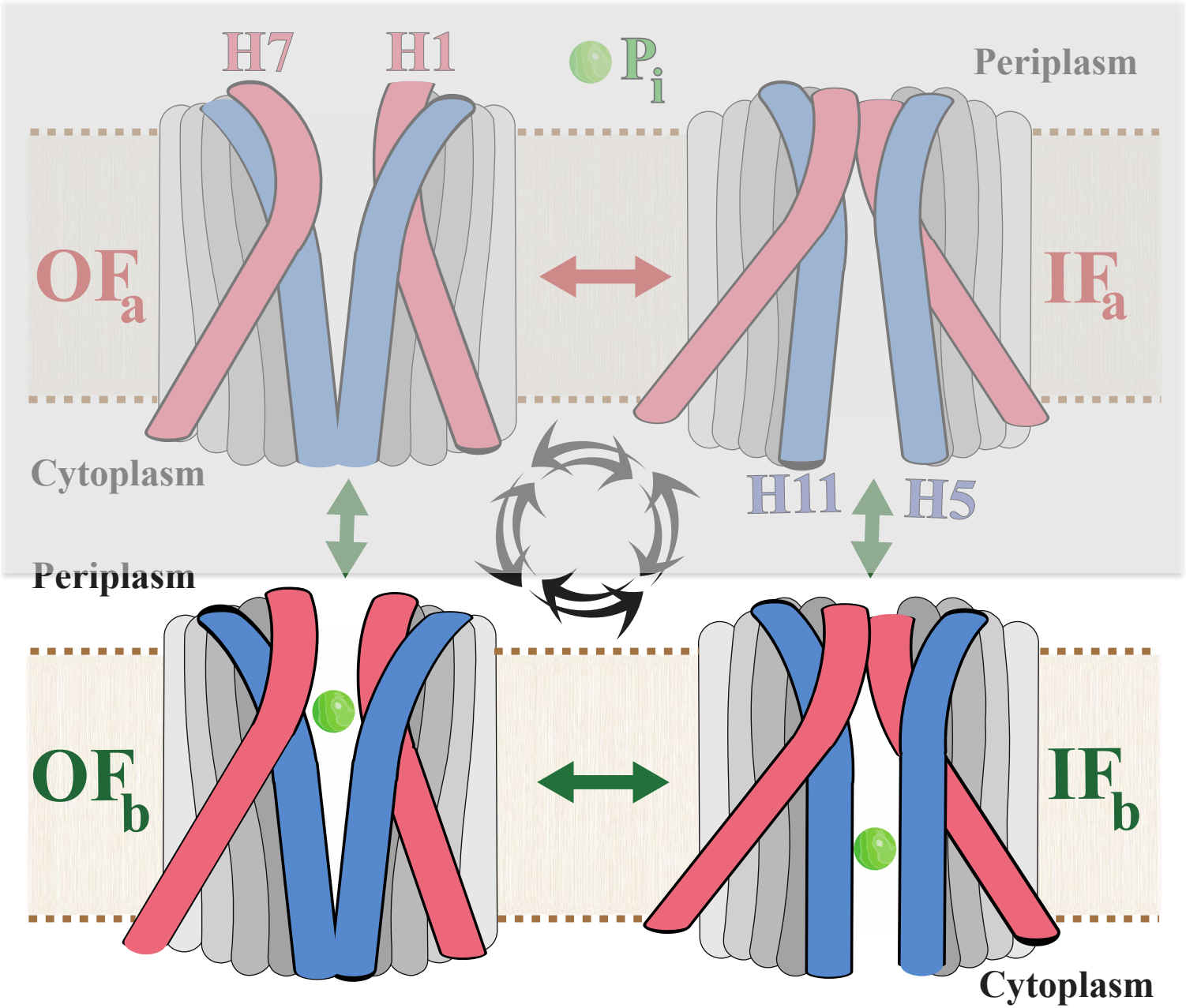
Step 2: $IF_a \leftrightarrow IF_b$



Step 3: $OF_a \leftrightarrow OF_b$

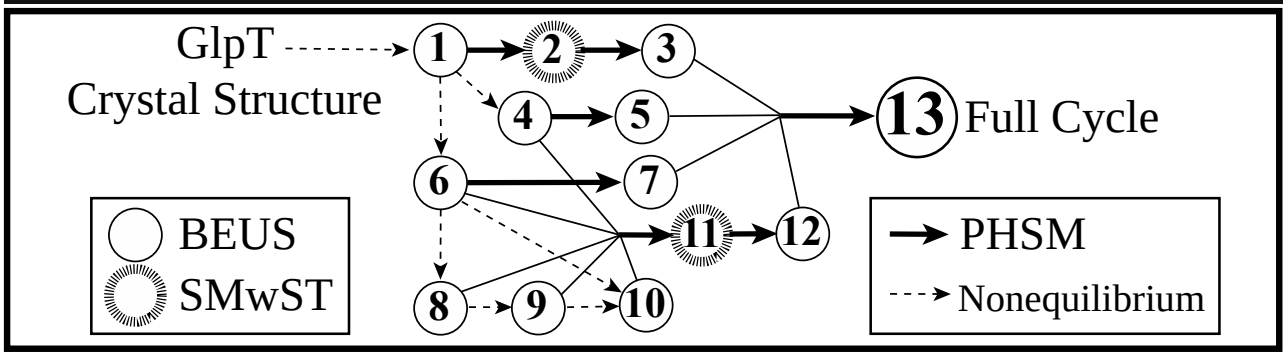


Step 4: $OF_b \leftrightarrow IF_b$

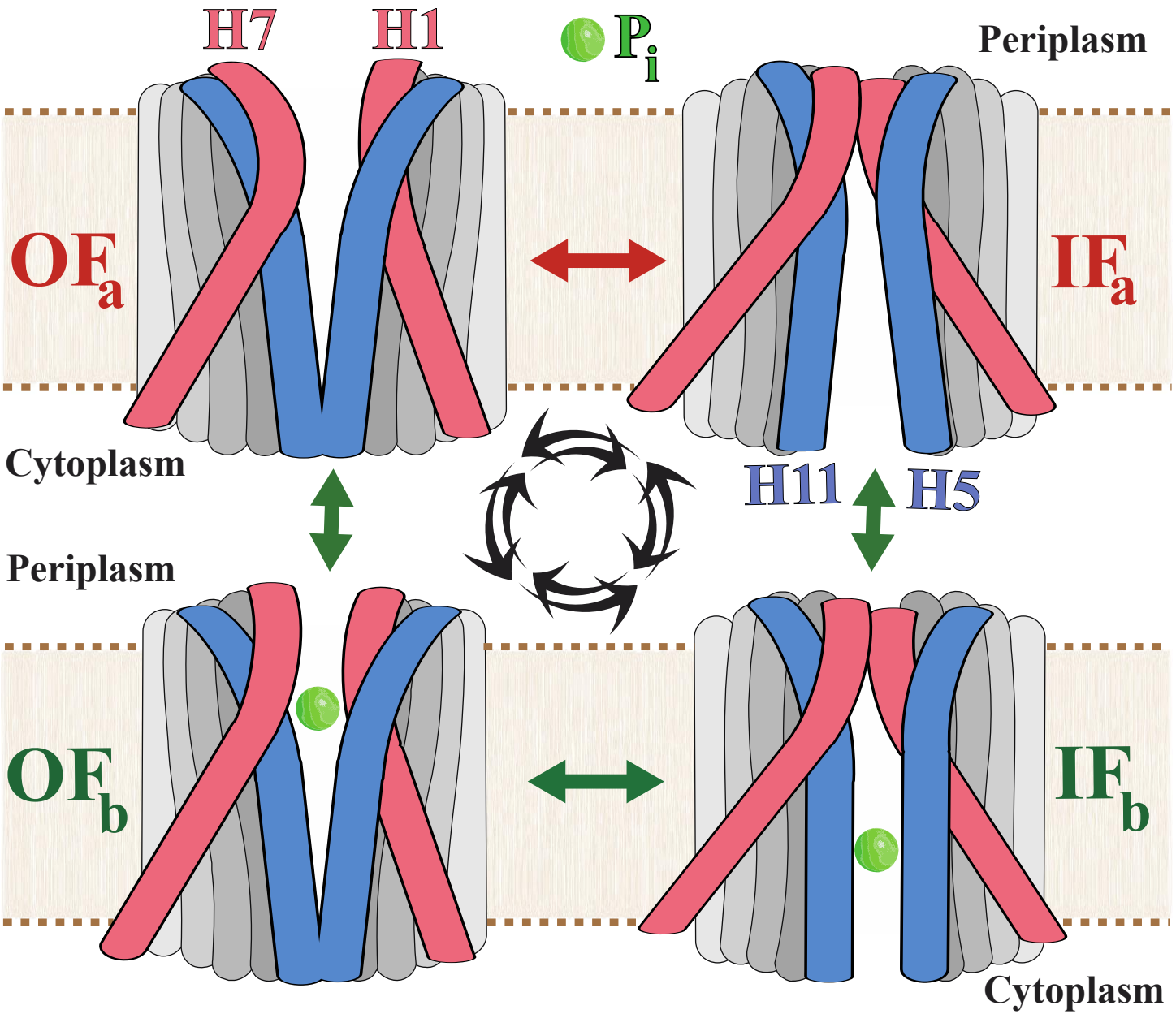


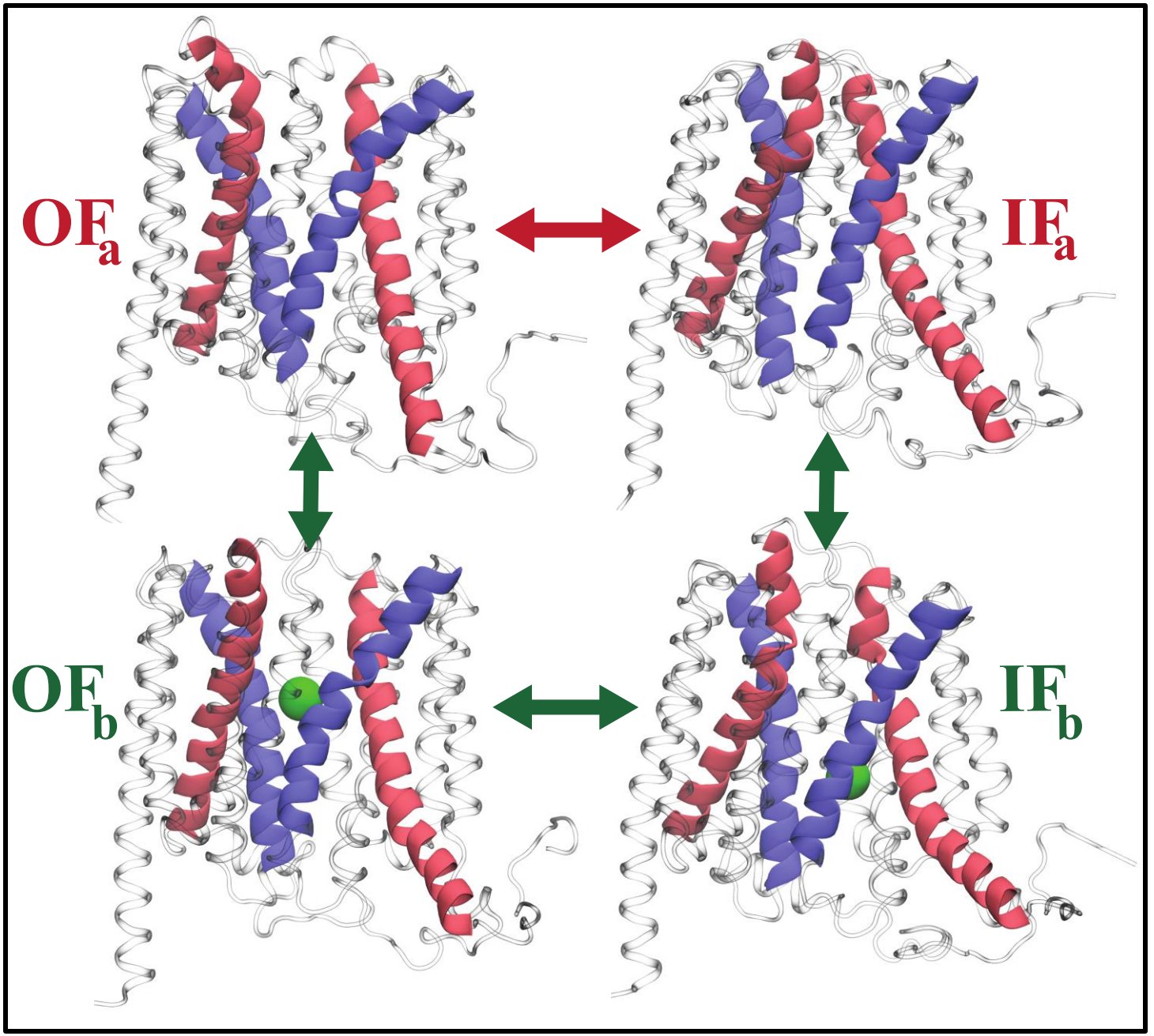
Simulation protocols

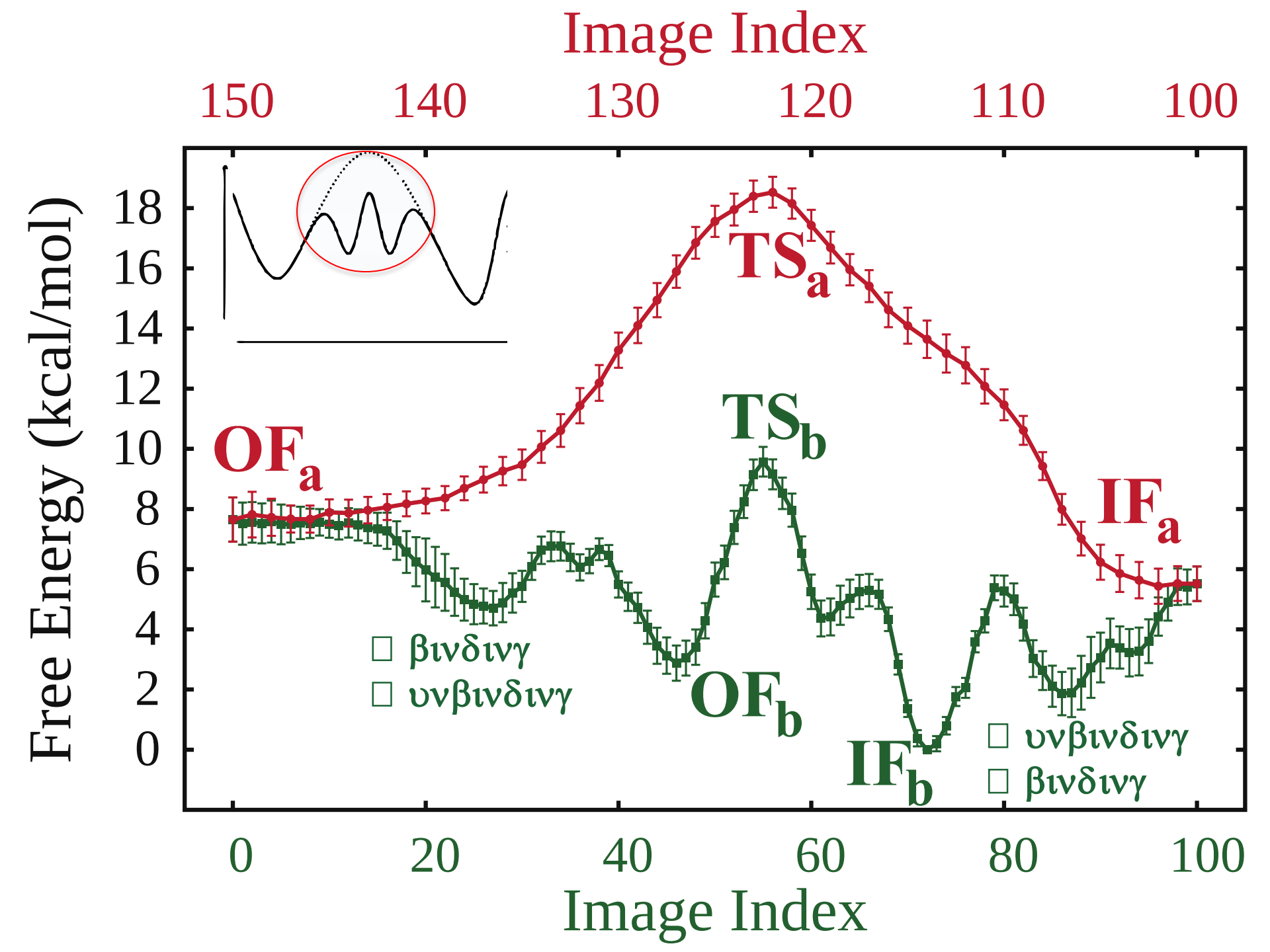
	Transition	Technique	Collective Variables	# of Replicas × Runtime		
1	$IF_a \leftrightarrow OF_a$	BEUS	(Q_1,Q_7)	12 × 40 ns	=	0.5 ms
2		SMwST	{Q}	1000 × 1 ns	=	1 ms
3		BEUS	{Q}	50 × 20 ns	=	1 ms
4	$IF_a \leftrightarrow IF_b$	BEUS	Z_{Pi}	30 × 40 ns	=	1.2 ms
5		BEUS	$(\{Q\}, Z_{Pi})$	30 × 40 ns	=	1.2 ms
6	$OF_a \leftrightarrow OF_b$	BEUS	Z_{Pi}	30 × 40 ns	=	1.2 ms
7		BEUS	$(\{Q\}, Z_{Pi})$	30 × 40 ns	=	1.2 ms
8	$IF_b \leftrightarrow OF_b$	BEUS	(Q_1,Q_7)	24 × 20 ns	=	0.5 ms
9		BEUS	Z_{Pi}	15 × 30 ns	=	0.5 ms
10		2D BEUS	$(\Delta RMSD, Z_{Pi})$	200 × 5 ns	=	1 ms
11		SMwST	$(\{Q\}, Z_{Pi})$	1000 × 1 ns	=	1 ms
12		BEUS	$(\{Q\}, Z_{Pi})$	50 × 20 ns	=	1 ms
13	**Full Cycle**	**BEUS**	**$(\{Q\}, Z_{Pi})$**	**150 × 50 ns**	**=**	**7.5 ms**
	Total Simulation Time					**18.7 ms**



Each replica consists of ~150,000 atoms


H7
H1
Pi
Periplasm
OFa
IFa
Cytoplasm
H11
H5
Periplasm
OFb
IFb
Cytoplasm


OFa
IFa
OFb
IFb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Free Energy (kcal/mol)
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IF$_a$
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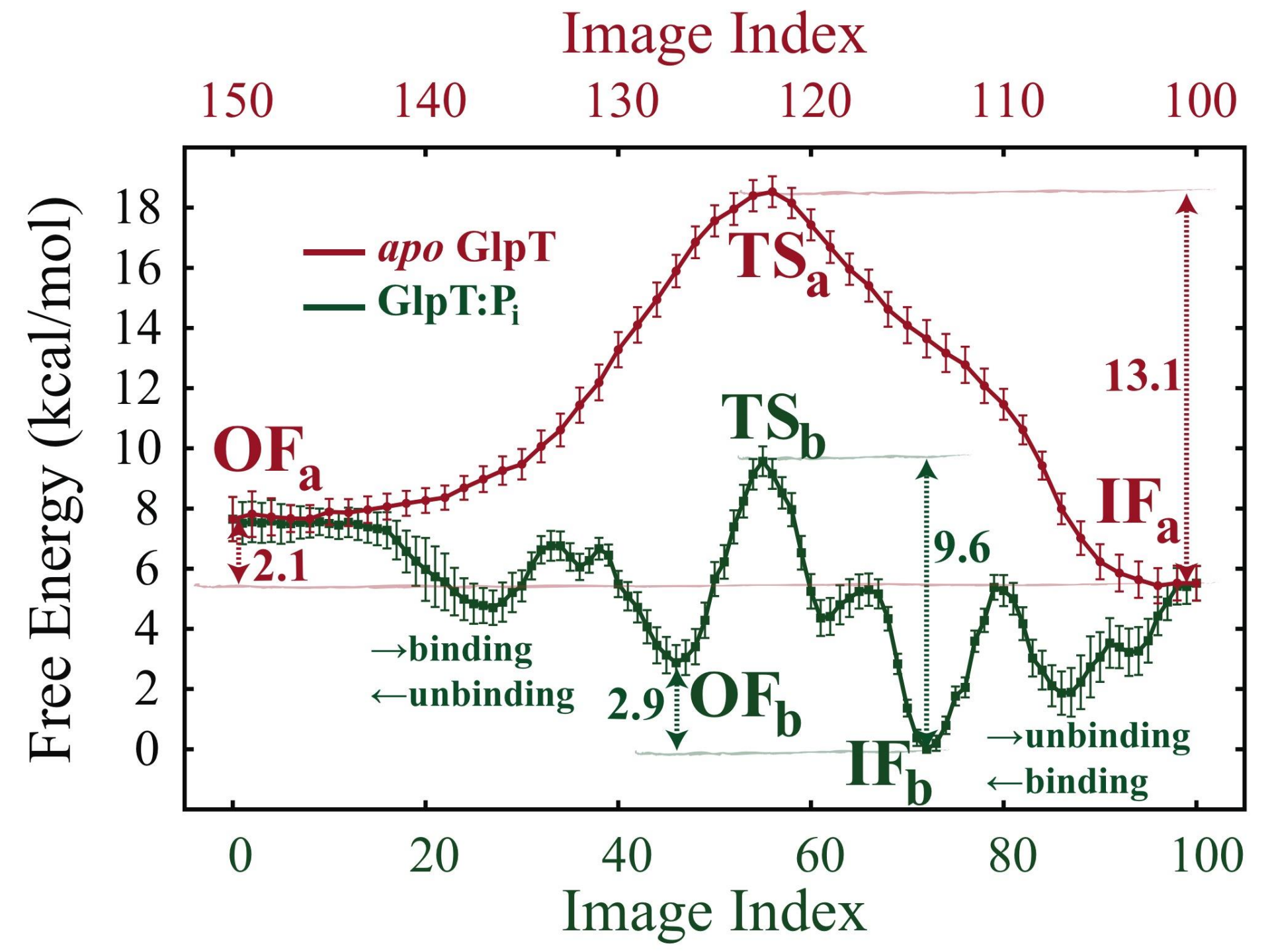
Image Index
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Free Energy (kcal/mol)
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IFb
OFb
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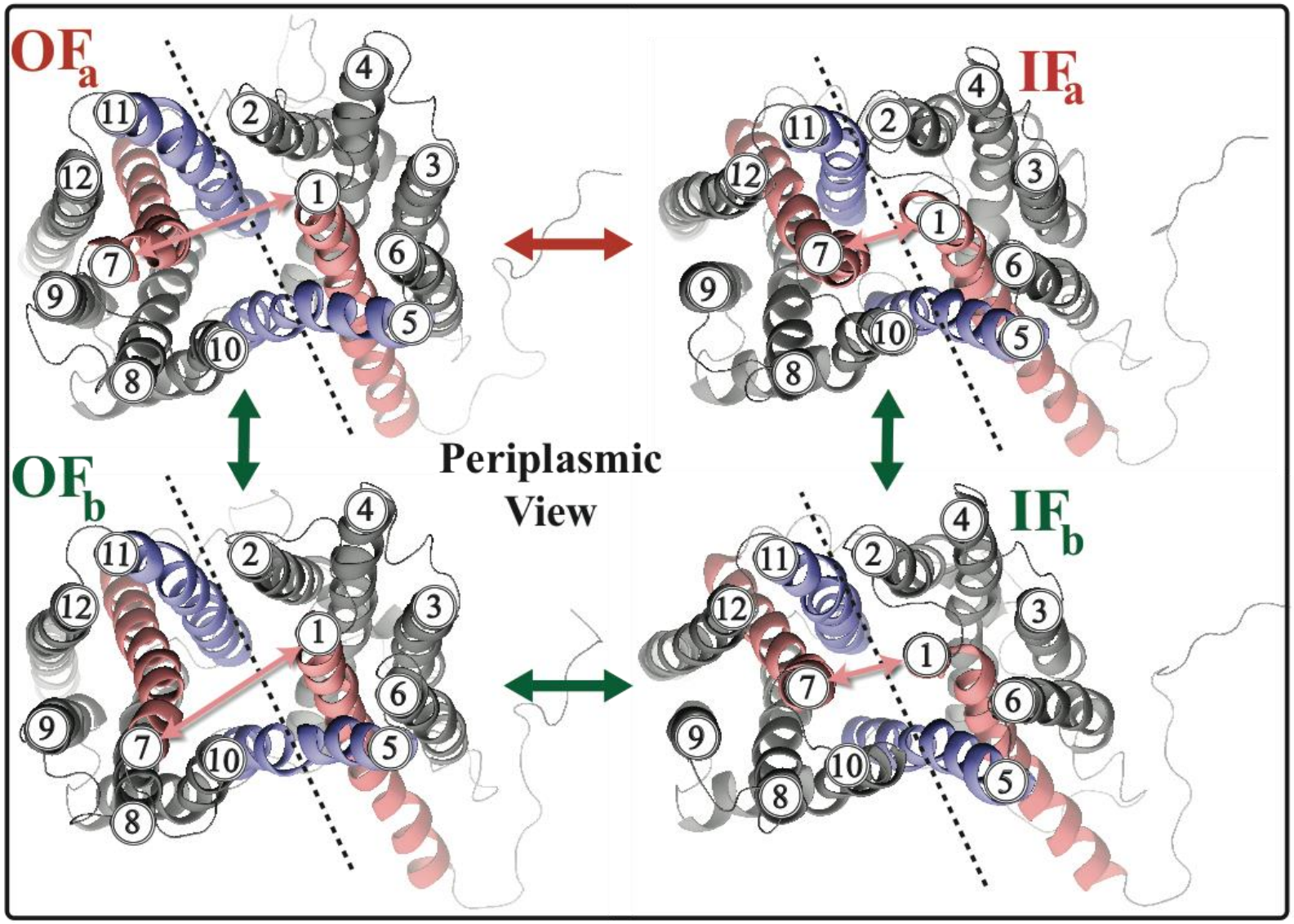
Image Index


OF$_a$
IF$_a$
OF$_b$
IF$_b$
Periplasmic
View
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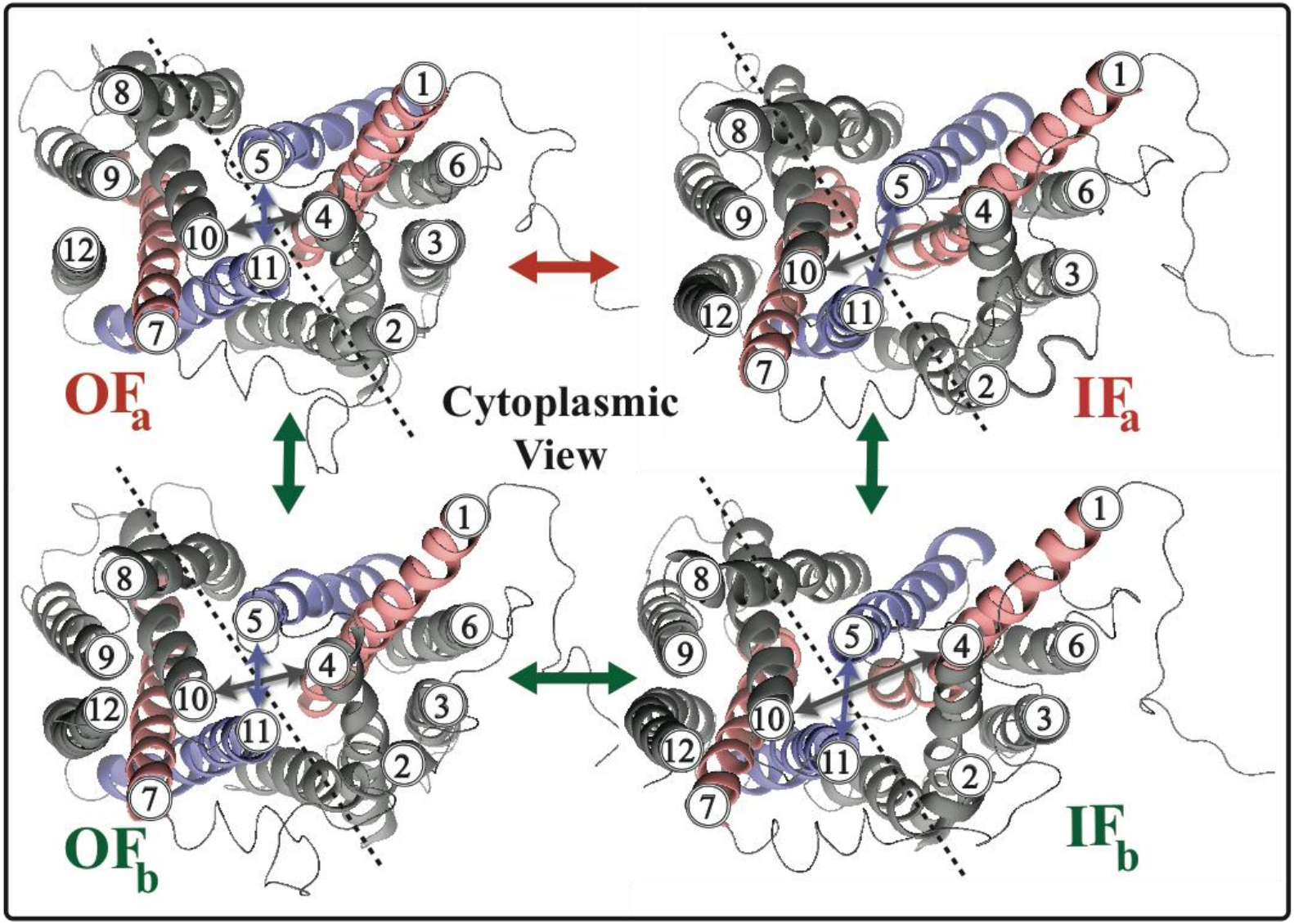
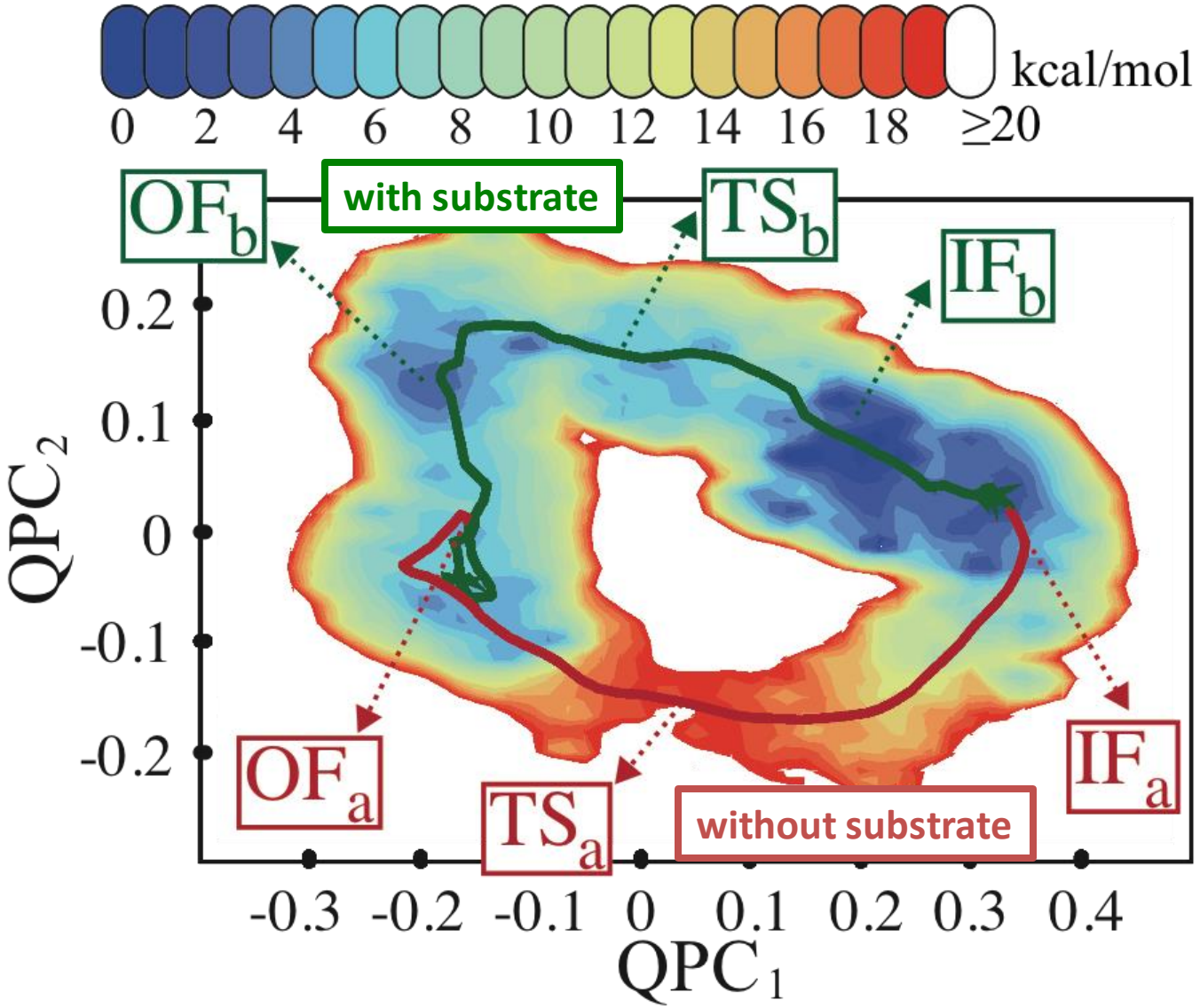

OF$_a$
IF$_a$
Cytoplasmic
View
OF$_b$
IF$_b$

Distinct conformational transition pathways

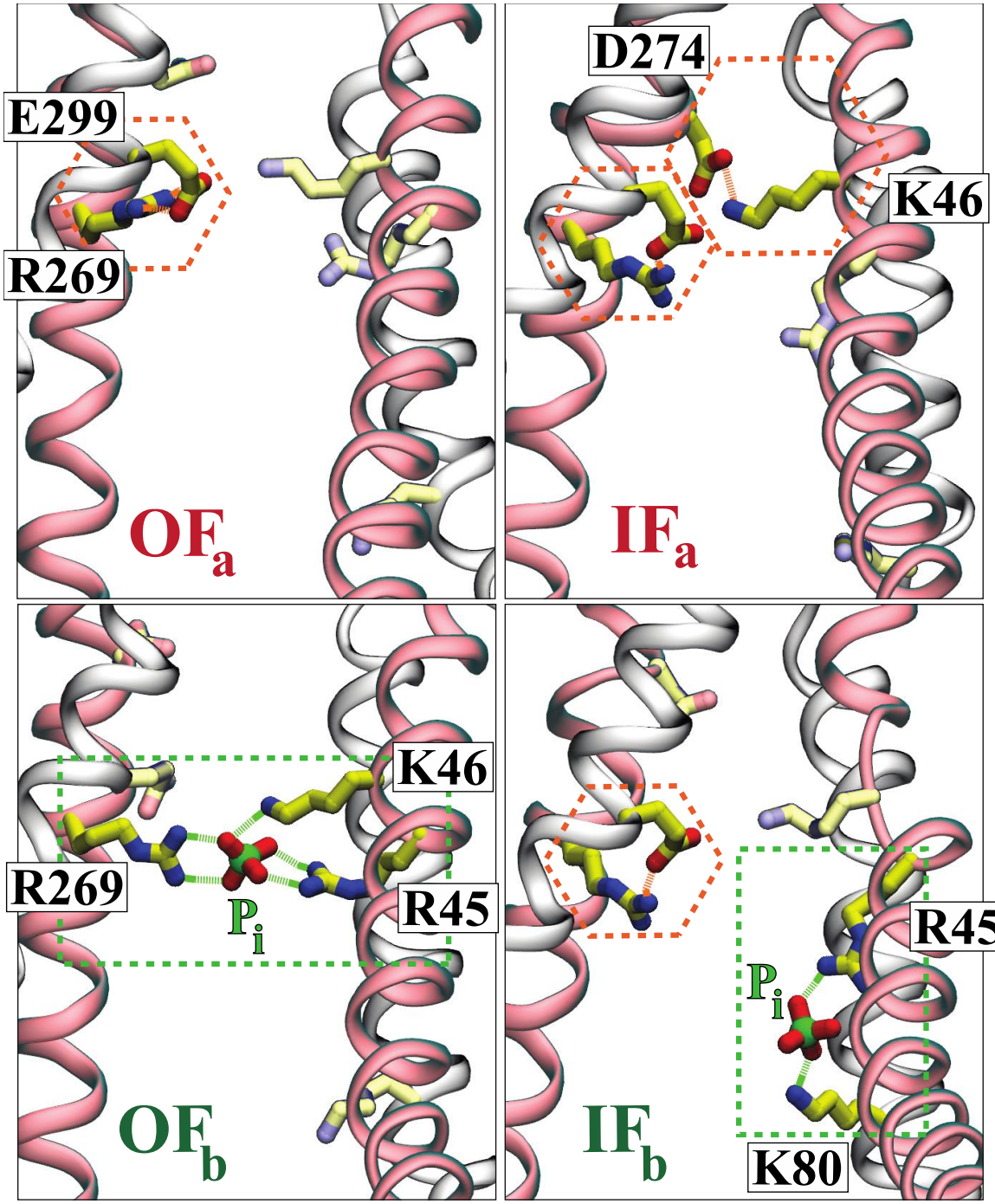
Quaternion-based principal components (**QPCs**) represent different modes of concerted motions of transmembrane helices.

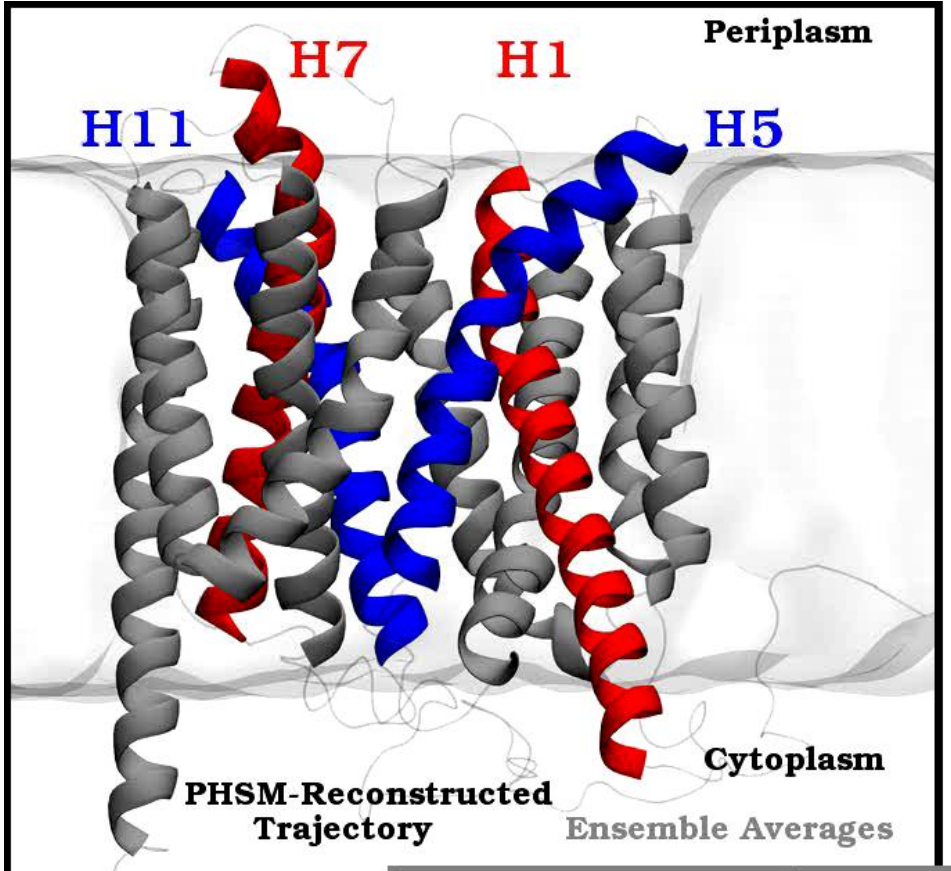


Characterizing protein local conformational changes within the lumen:

- Salt bridges stabilizing different conformations.
- Residues involved in binding.

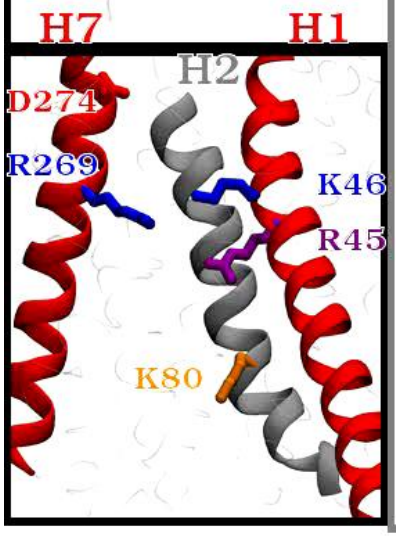
Conformational dynamics of the binding site

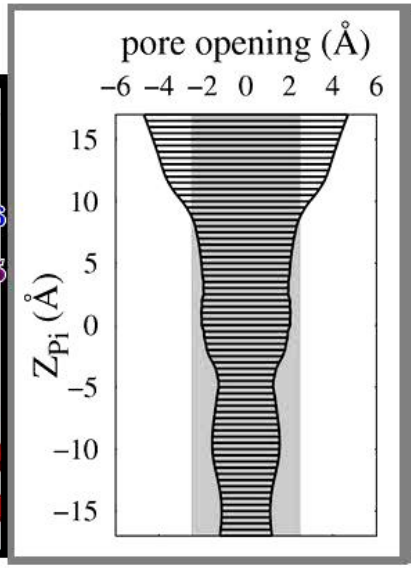


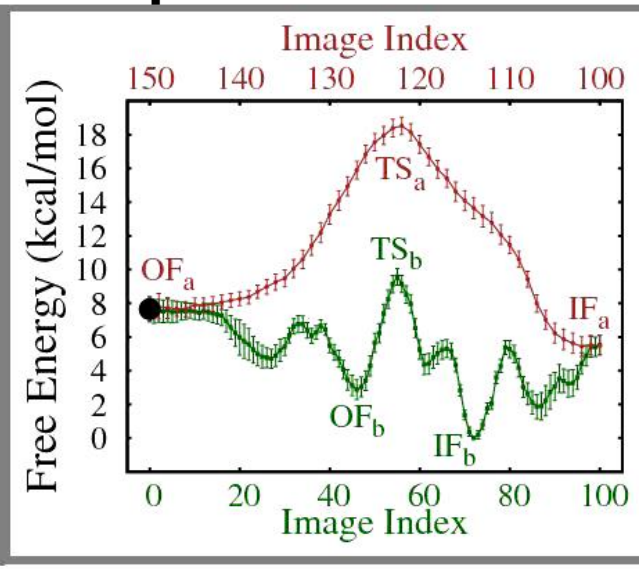


Outward-Facing

apo







Summary

- **Reconstructed thermodynamic cycle of GlpT**
  - **Alternating access** mechanism characterized (atomic level)
  - Substrate binding **lowers** the IF-OF transition **barrier**
  - Substrate binding **changes** the IF-OF transition **pathway**
  - **Coupling** between **local and global** conformational changes
- **Reconstructing transport cycles in membrane transporters using enhanced sampling techniques and petascale computing**

Moradi M., Enkavi G., and Tajkhorshid E., under review by *Nature Communication* (**2015**).

Acknowledgement

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NSF
NCSA
BLUE WATERS
SUSTAINED PETASCALE COMPUTING
XSEDE
Extreme Science and Engineering Discovery Environment

Rocker-switch model