



# Alchemical metadynamics: Enhancing configurational sampling in alchemical free energy calculations



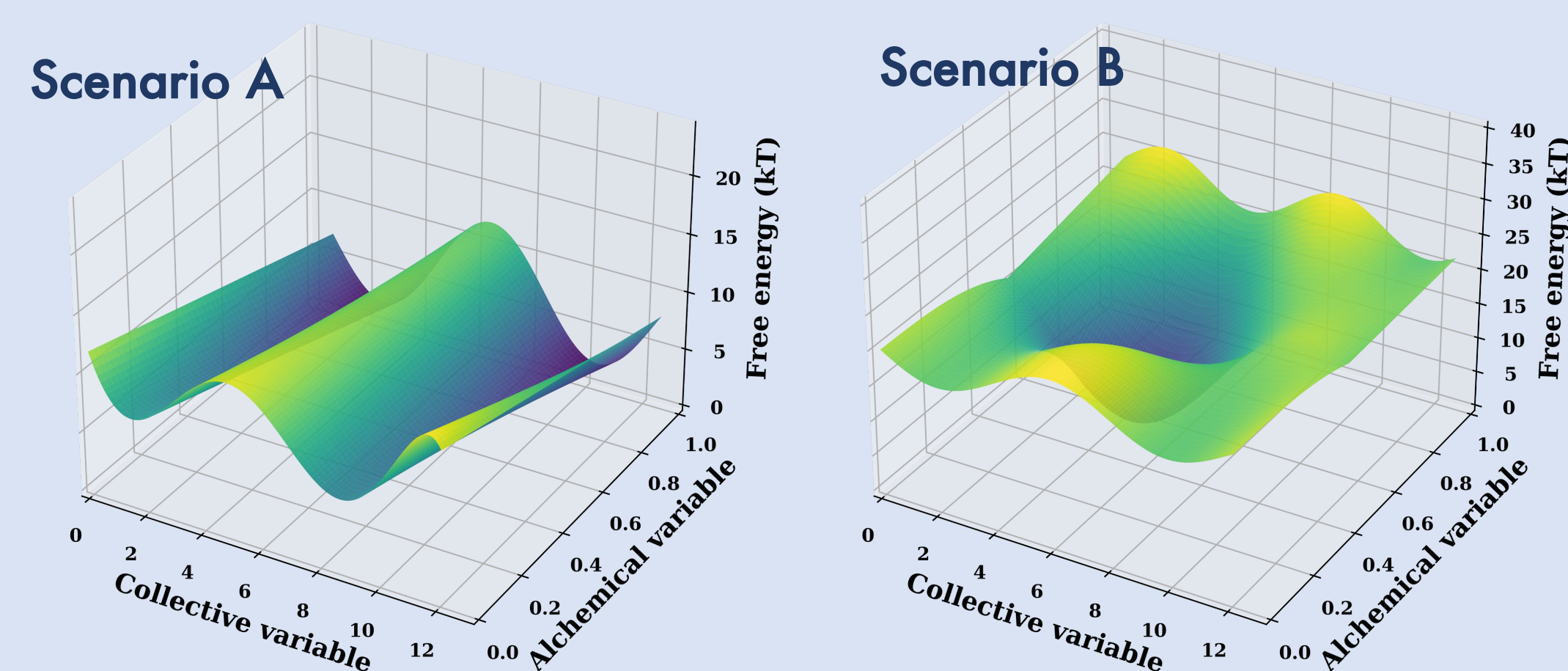
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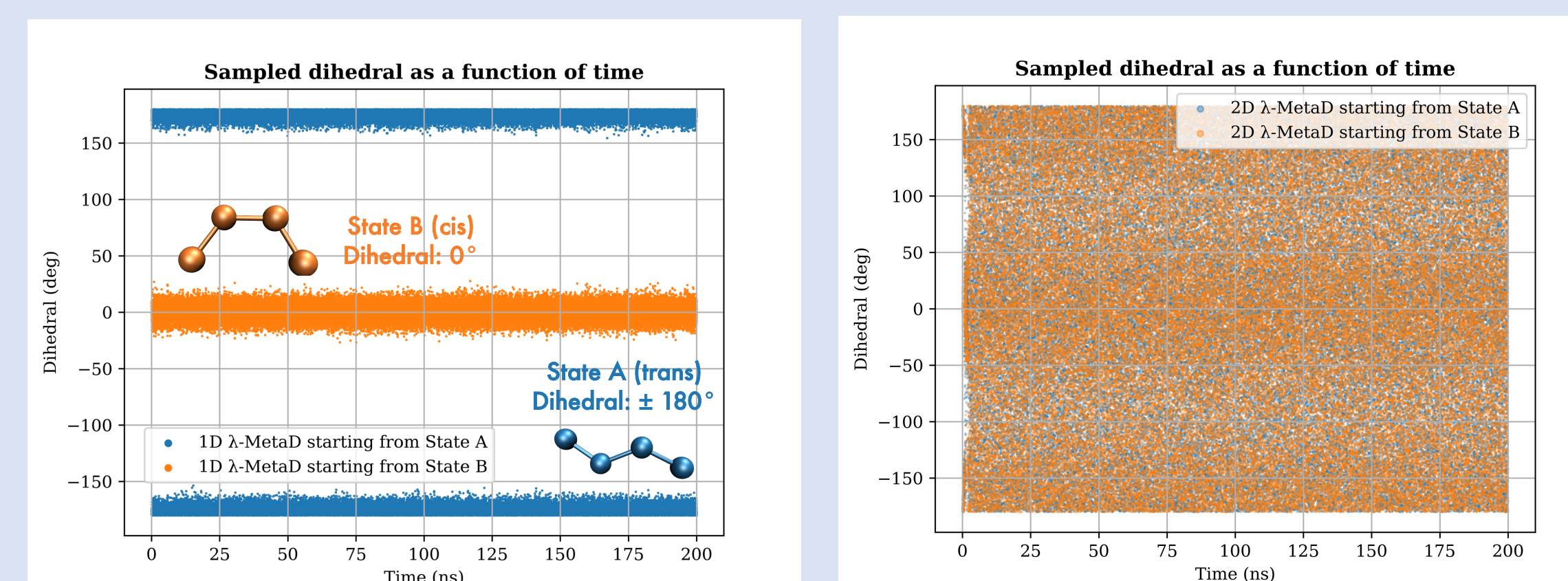
## Alchemical transformation is useful in free energy calculations but is limited in some cases

- Alchemical states ( $\lambda$ -states) are a series of unphysical states connecting the desired end states.
- Traditional free energy methods such as Hamiltonian replica exchange (HREX) or expanded ensemble (EXE) could fail in the following two scenarios.
  - ✓ A: The free energy barrier is present for all  $\lambda$  states.
  - ✓ B: The system is stuck in a basin in the  $\lambda$  direction.



## Case 2 shows that 2D $\lambda$ -MetaD outperformed traditional methods in sampling and free energy calculations

- Task: Solvation free energy calculation of a 4-site model
- The two torsional metastable states are separated by a large free energy barrier ( $\sim 50$  kT).
- We performed both 1D and 2D  $\lambda$ -MetaD starting from each torsional state, with the latter biasing the dihedral angle.



- The lack of configurational sampling in the 1D simulations led to inaccurate free energy estimates, which was resolved in the 2D simulations.

	Starting from State A	Starting from State B
1D $\lambda$ -MetaD	$0.649 \pm 0.030$ kT	$-0.381 \pm 0.029$ kT
2D $\lambda$ -MetaD	$0.708 \pm 0.031$ kT	$0.694 \pm 0.031$ kT

## Case 3: It took 4 HREX simulations to calculate the relative methylation free energy $\Delta\Delta G_{syn+anti}^{dup/ns}$

- Process: Methylation of adenosine (A) to N6-methylated adenosine ( $m^6A$ ) in its isolated form and in a duplex.
- $\Delta\Delta G_{syn+anti}^{dup/ns} = \Delta G_{syn+anti}^{dup} - \Delta G_{syn+anti}^{ns}$  considers both *syn* and *anti* conformations of the methyl group.
- $\Delta G_{syn+anti}^{ns}$  and  $\Delta G_{syn+anti}^{dup}$  can be calculated from 4 HREX simulations. (See the thermodynamic cycle in the next column.)

$$\Delta G_{syn+anti}^{ns} = -\frac{1}{\beta} [\exp(-\beta\Delta G_{syn}^{ns}) + \exp(-\beta\Delta G_{anti}^{ns})]$$

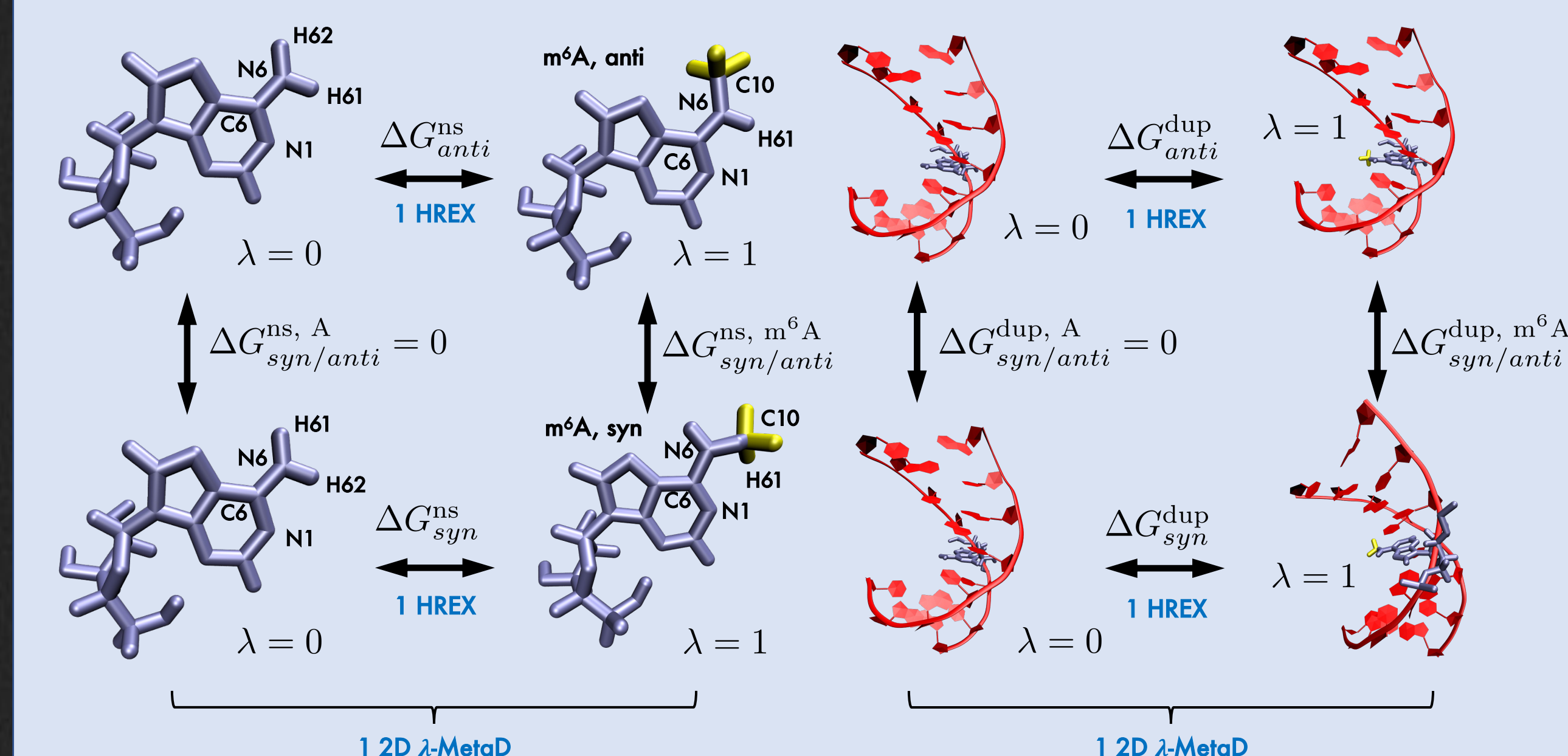
$$\Delta G_{syn+anti}^{dup} = -\frac{1}{\beta} [\exp(-\beta\Delta G_{syn}^{dup}) + \exp(-\beta\Delta G_{anti}^{dup})]$$

- Additional values can be calculated:

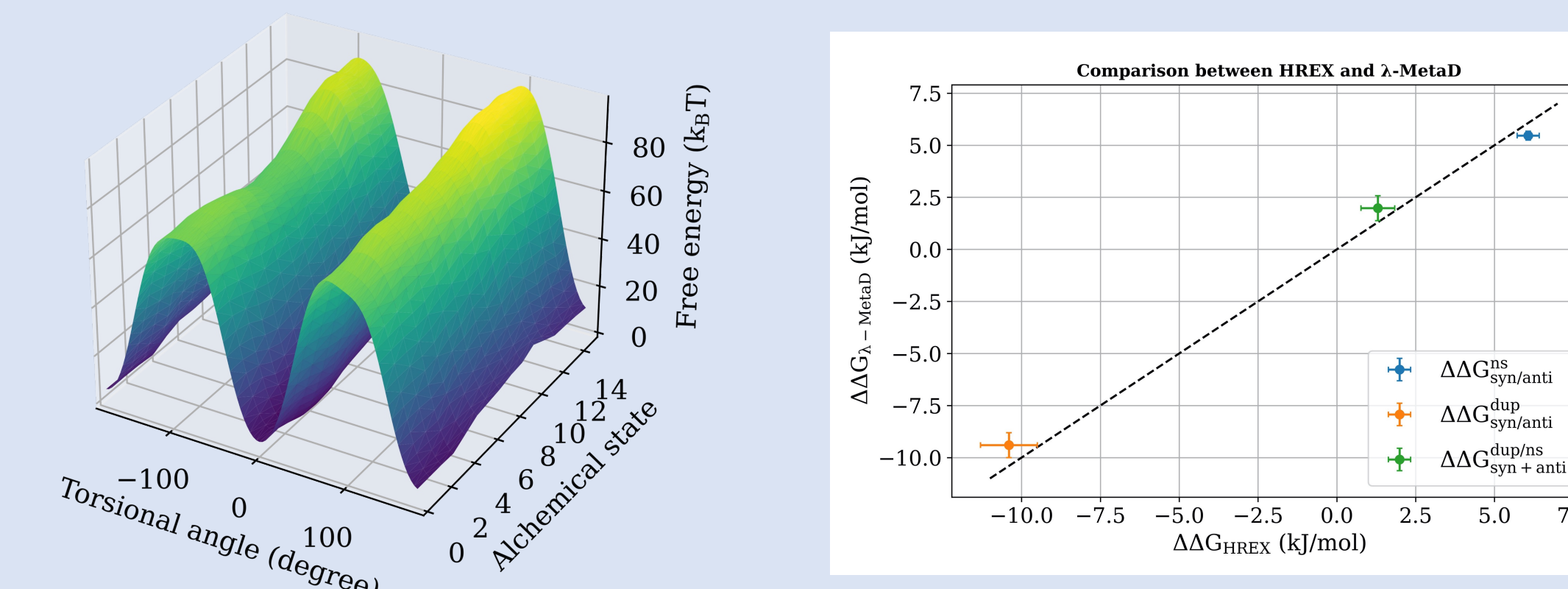
$$\Delta\Delta G_{syn/anti}^{dup} = \Delta G_{anti}^{dup} - \Delta G_{syn}^{dup}; \quad \Delta\Delta G_{syn/anti}^{ns} = \Delta G_{anti}^{ns} - \Delta G_{syn}^{ns}$$

## Case 3: It only took 2 $\lambda$ -MetaD simulations to recover consistent results, with additional information available

- All 3 values  $\Delta\Delta G_{syn+anti}^{dup/ns}$ ,  $\Delta\Delta G_{syn/anti}^{ns}$ , and  $\Delta\Delta G_{syn/anti}^{dup}$  can be calculated from 2D alchemical metadynamics.
- Configurational CV:  $\eta$ , the average of 3 torsions identified by the atoms N1-C6-N6-C10/H61/H62.



- 2D  $\lambda$ -MetaD additionally recovered the free energy profile along  $\eta$ , which was not available in HREX.



- $\lambda$ -MetaD can be further empowered when combined with ML-based methods that identify the optimal CVs.

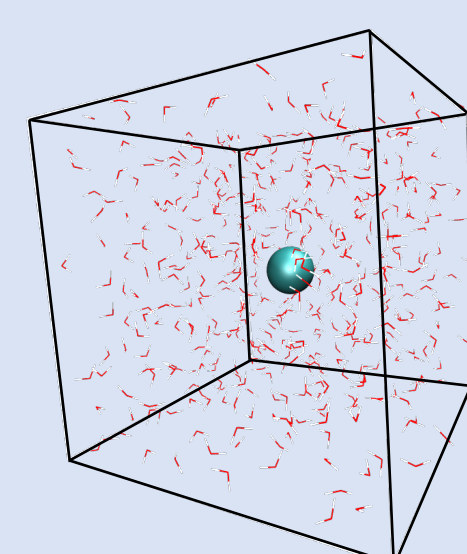
## Alchemical metadynamics (available in PLUMED 2.8!) aims to address the issues mentioned above

- In  $\lambda$ -MetaD, we introduced the alchemical variable ( $\lambda$ ) as an allowed collective variable in metadynamics.
- We tested our implementation by free energy calculations in 3 systems:
  - ✓ Case 1: Solvation free energy of an argon atom
  - ✓ Case 2: Solvation free energy of a 4-site system
  - ✓ Case 3: Relative methylation free energy of adenosine between the isolated and the duplex form  $\Delta\Delta G_{syn+anti}^{dup/ns}$

## Case 1 shows that 1D $\lambda$ -MetaD is essentially EXE with a different bias-updating scheme

- The solvation free energies estimated by EXE and 1D  $\lambda$ -MetaD are statistically consistent.

Solvation free energy of argon	
EXE	$-3.275 \pm 0.016$ kT
1D $\lambda$ -MetaD	$-3.284 \pm 0.010$ kT



## Check our JCTC paper, tutorial, and current work!

- We are developing **ensembles of expanded ensemble (EEXE)**, a new method combining the the working principles of EXE and HREX!
- Scan the QR code to
  - ✓ Learn more about EEXE
  - ✓ Check the tutorial and the JCTC paper of  $\lambda$ -MetaD
  - ✓ Connect and more! ☺

