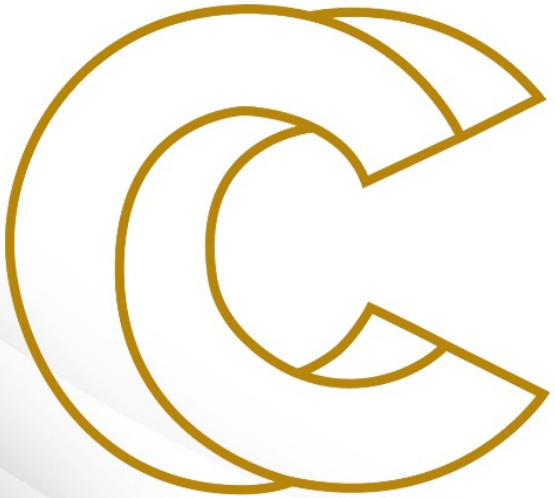
Sabancı Üniversitesi

TÜBİTAK



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Generating Mutant Protein Structures Using Advanced Techniques

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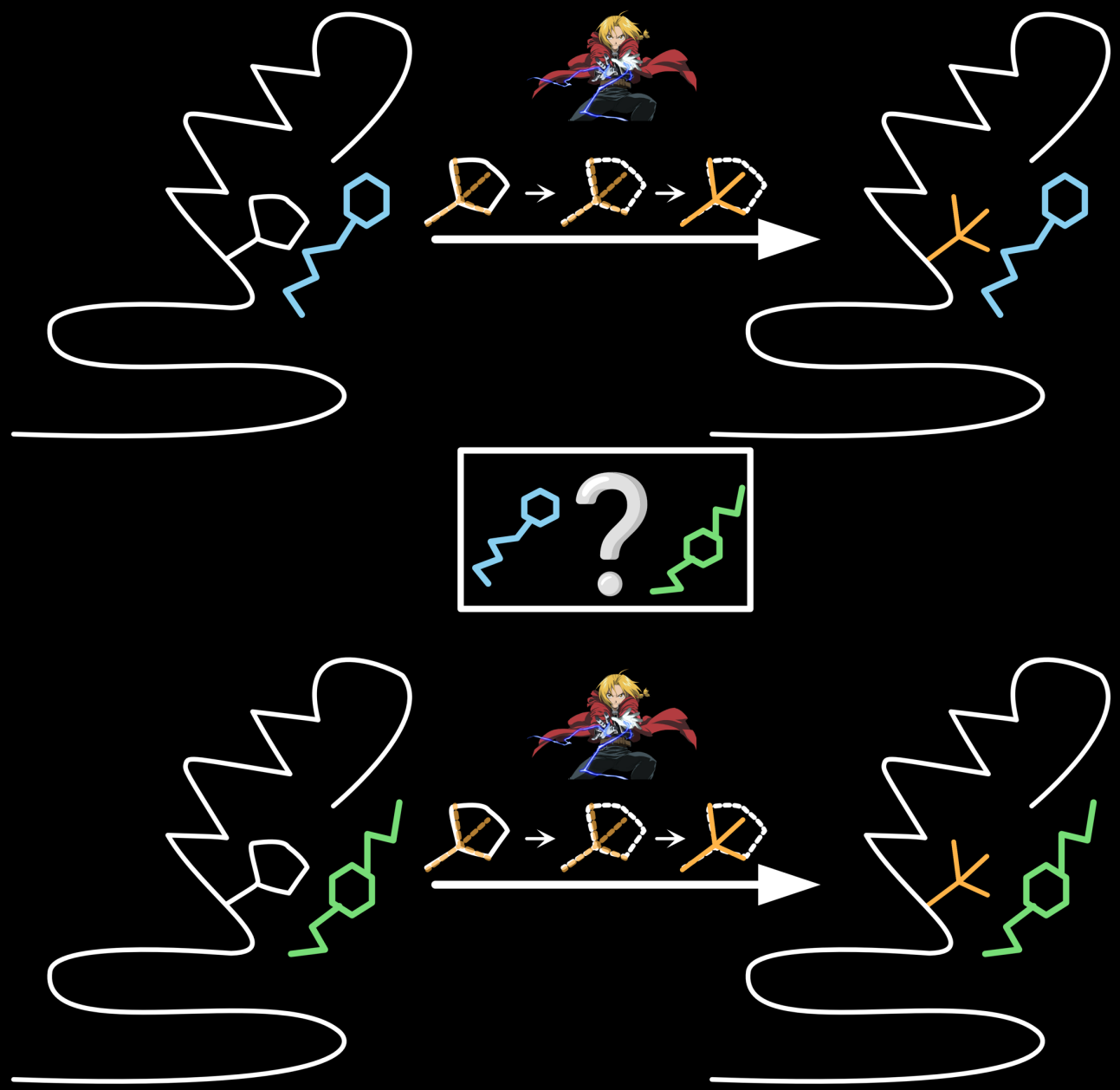
Free Energy Perturbation (FEP)



Assessing binding from structural perspective

1. Initial structure (Wild-type)
2. Different conformations of WT protein (molecular dynamics simulations)
3. Mutation energies (free energy perturbations)
4. Relative binding energies and comparison with experimental energies (thermodynamic cycles)
5. Relative binding energies, comparison of two drugs, (thermodynamic cycles)

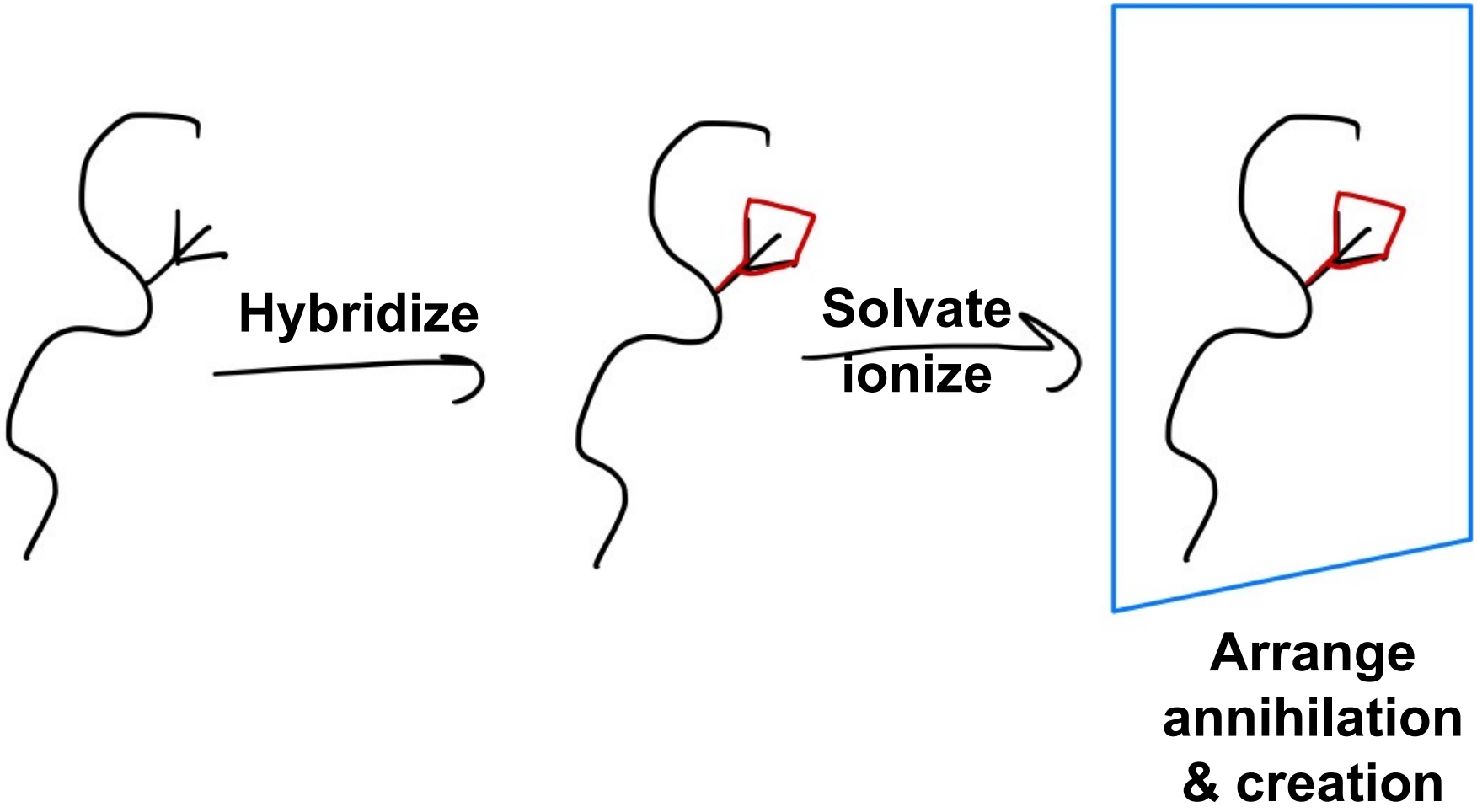
1. Initial structure (Wild-type)

- PDB
- Docking?
- Alphafold3? (Nobel!!!)
  - Boltz-1
  - Chai-1
  - RosettaTTFold-AllAtom (Nobel!!!)

2. Different conformations of WT protein (molecular dynamics simulations)

- SASA
- Cpptraj
- Hydrogen bonds

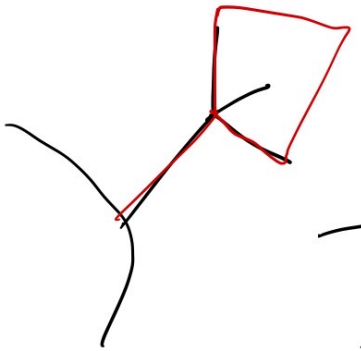
3. Mutation energies (free energy perturbations)



3. Mutation energies (free energy perturbations)

minimize → forward → backward

3. Mutation energies (free energy perturbations)



$\lambda = 0$ → Black × 1, Red × 0

$\lambda = 0.25$ → Black × 0.75, Red × 0.25

$\lambda = 0.5$ → Black × 0.5, Red × 0.5

$\lambda = 0.75$ → Black × 0.25, Red × 0.75

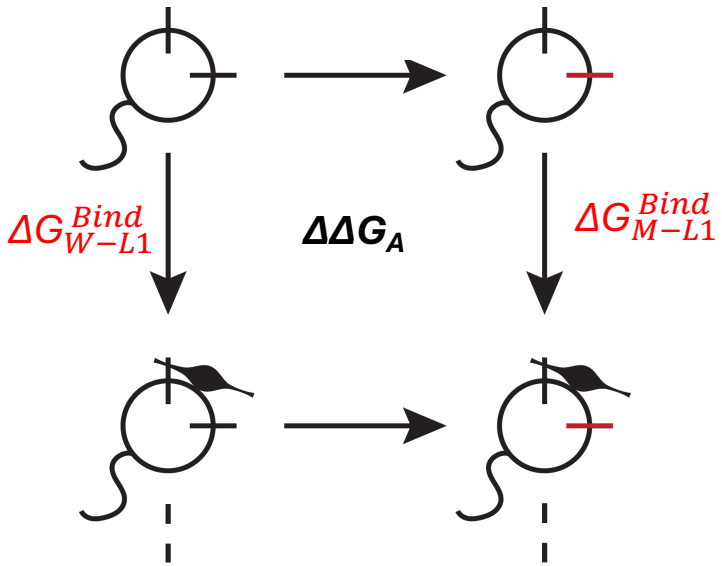
(vdW)

$\lambda = 1$ → Black × 0, Red × 1

3. Mutation energies (free energy perturbations)

Practice time!

The thermodynamic cycle of a single mutation and one ligand

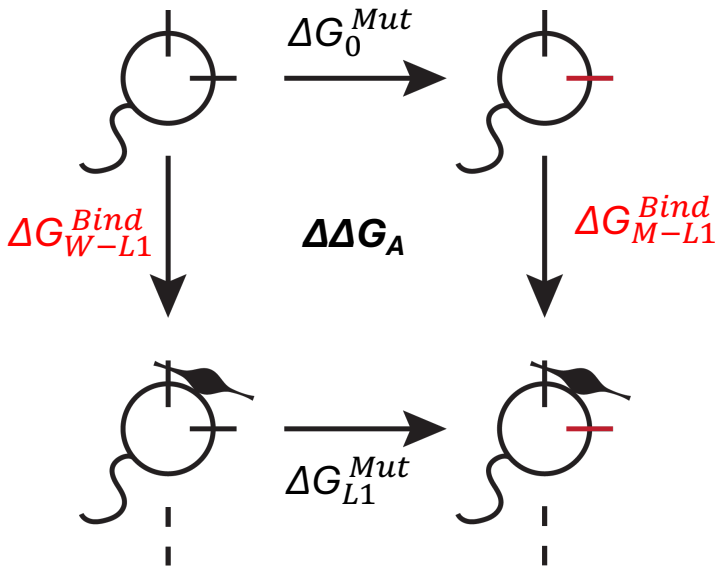


Experimental binding energies

- Calculated by $\Delta G = -RT\ln(K_D)$

$$\Delta G^{Bind}_{M-L1} - \Delta G^{Bind}_{W-L1} = \Delta\Delta G_A$$

The thermodynamic cycle of a single mutation and one ligand



Experimental binding energies

- Calculated by $\Delta G = -RT \ln(K_D)$

$$\Delta G_{M-L1}^{Bind} - \Delta G_{W-L1}^{Bind} = \Delta\Delta G_A$$

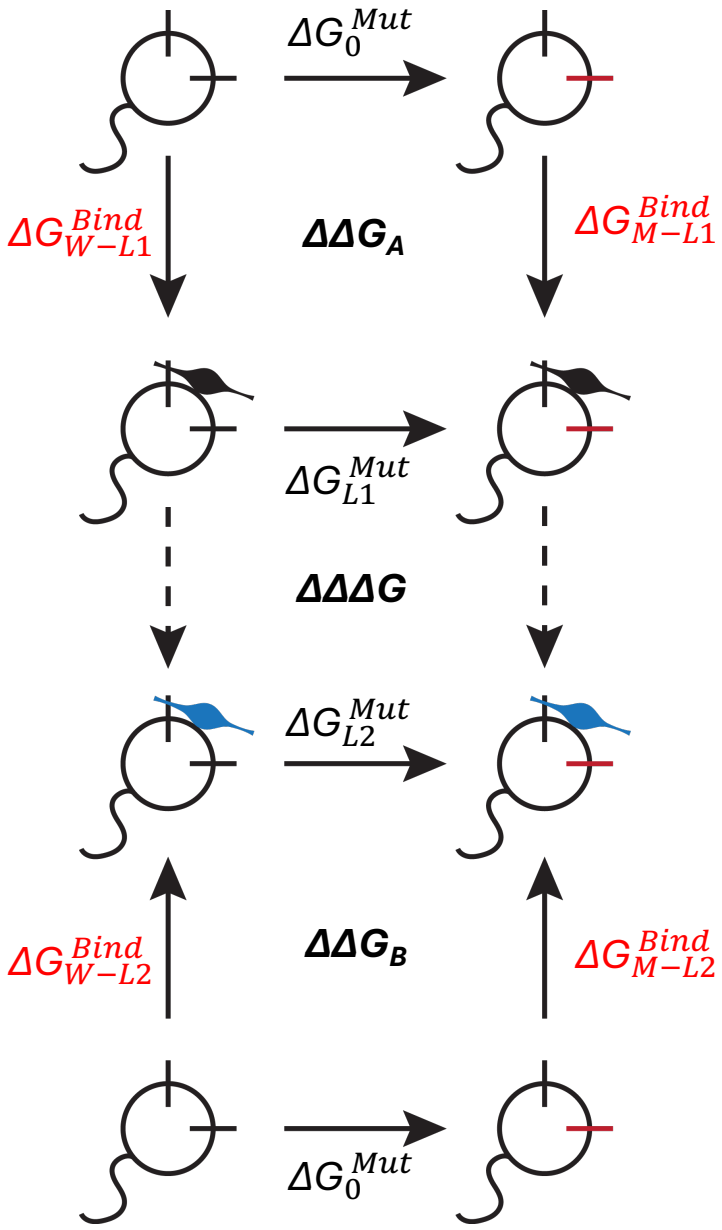
Computational mutation energies

- Calculated by FEP simulations
- Eight structures taken from 1000 ns long MD simulations
- Average of 3 FEP simulations

$$\Delta G_{L1}^{Mut} - \Delta G_0^{Mut} = \Delta\Delta G_A$$

A negative $\Delta\Delta G$ indicates that the ligand-binding is more favorable after the mutation.

The thermodynamic cycles of a single mutation and two ligands

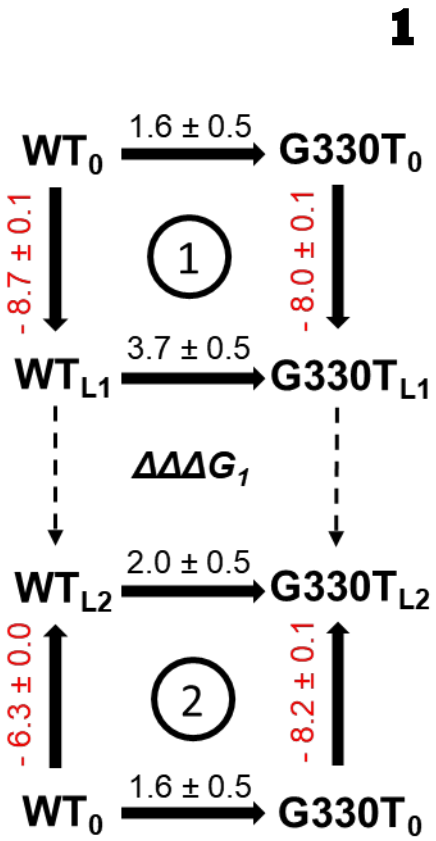


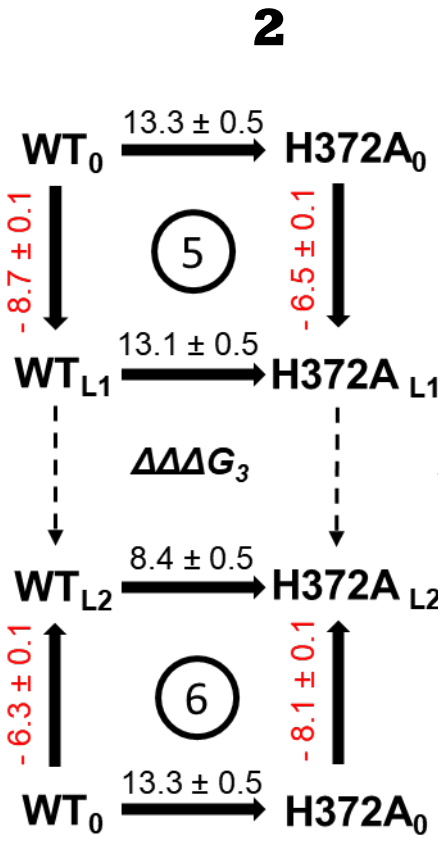
$$\Delta\Delta\Delta G = \Delta\Delta G_B - \Delta\Delta G_A$$

- A negative $\Delta\Delta\Delta G$ demonstrates that L_2 binds to the protein more favorable after the single mutation.
- $\Delta\Delta\Delta G$ is used to confirm our computational results against the experimental energies.

This is the keystone of our thermodynamic investigation; we assess the ligand preference of each mutation by using the $\Delta\Delta\Delta G$ energies.

The thermodynamic cycles of the single mutations



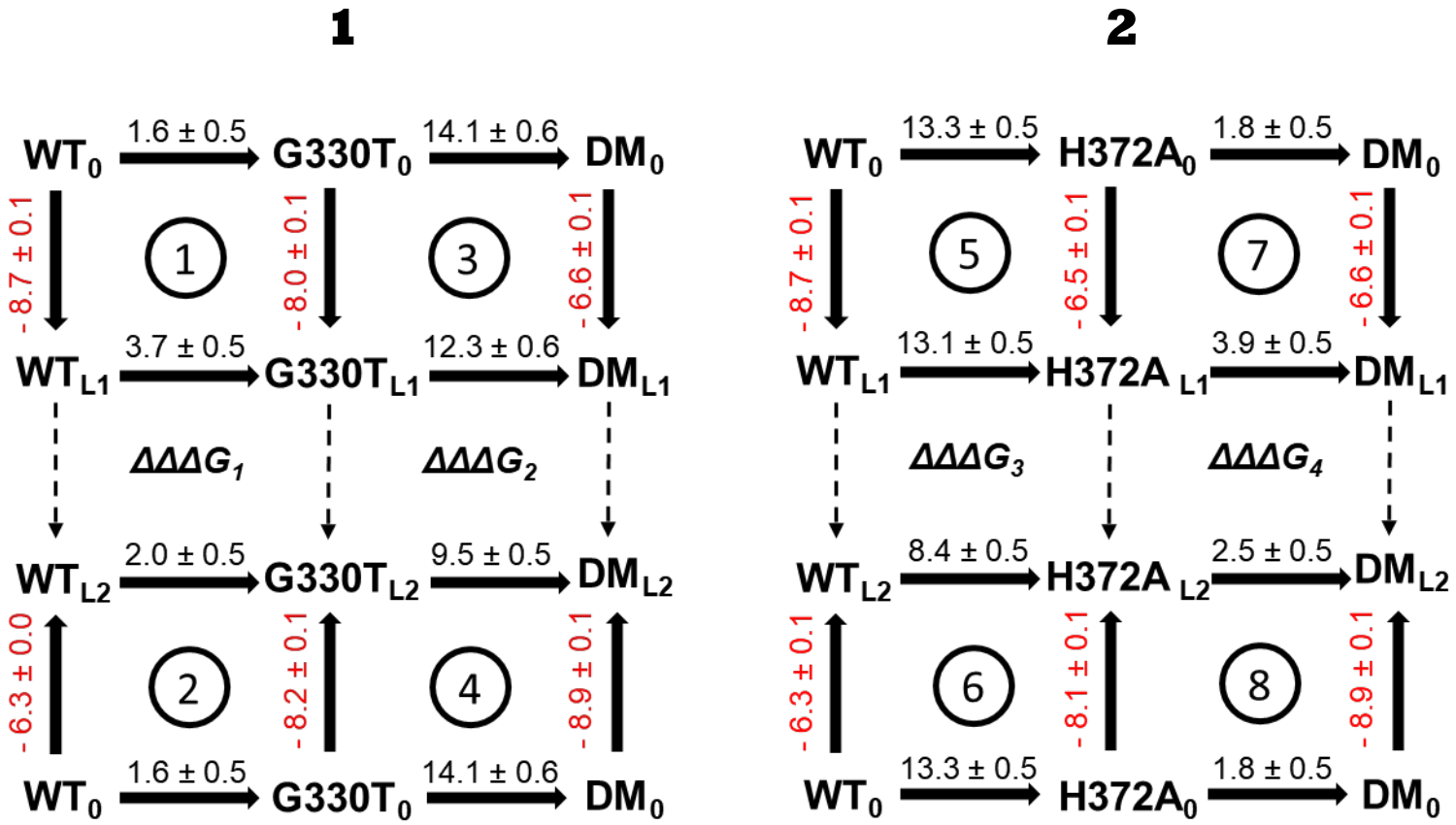


- All energies are in kcal/mol
- The binding energies lower than -7.0 kcal/mol lead to functional complexes
- The energy cost of G330T and H372A is different

WT_{L1} WT_{L2}
$G330T_{L1}$ $G330T_{L2}$
$H372A_{L1}$ $H372A_{L2}$
DM_{L1} DM_{L2}

Guclu et al., JCIM 2021.

The thermodynamic cycles of the mutations



- The G330T mutation is more functional as the prior mutation
- The mutation energies are additive or independent of each other
- The DM_{L2} complex is the most favorable form in a L_2 -rich environment.

WT_{L1} WT_{L2}
$G330T_{L1}$ $G330T_{L2}$
$H372A_{L1}$ $H372A_{L2}$
DM_{L1} DM_{L2}

Guclu et al., JCIM 2021.

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Thanks!

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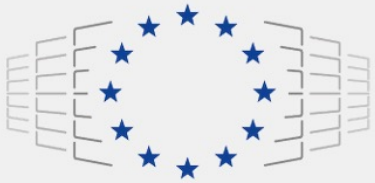
the European Union



EuroHPC
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