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Enzyme Active Site Loop Revealed as Gatekeeper for Cofactor Flip by Targeted Molecular Dynamics Simulations and FRET-kinetics

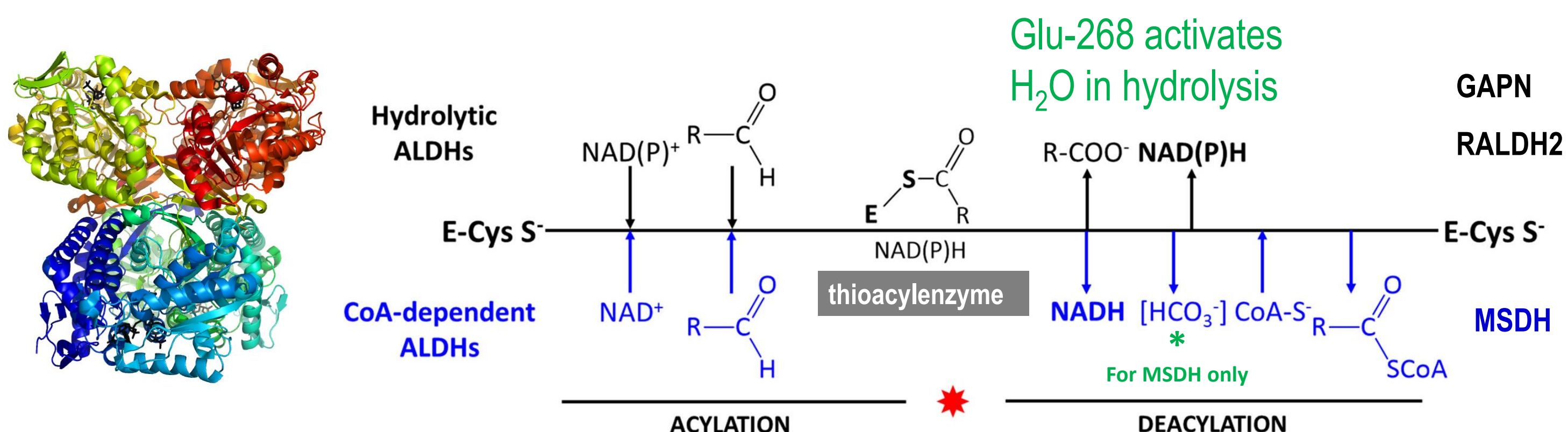
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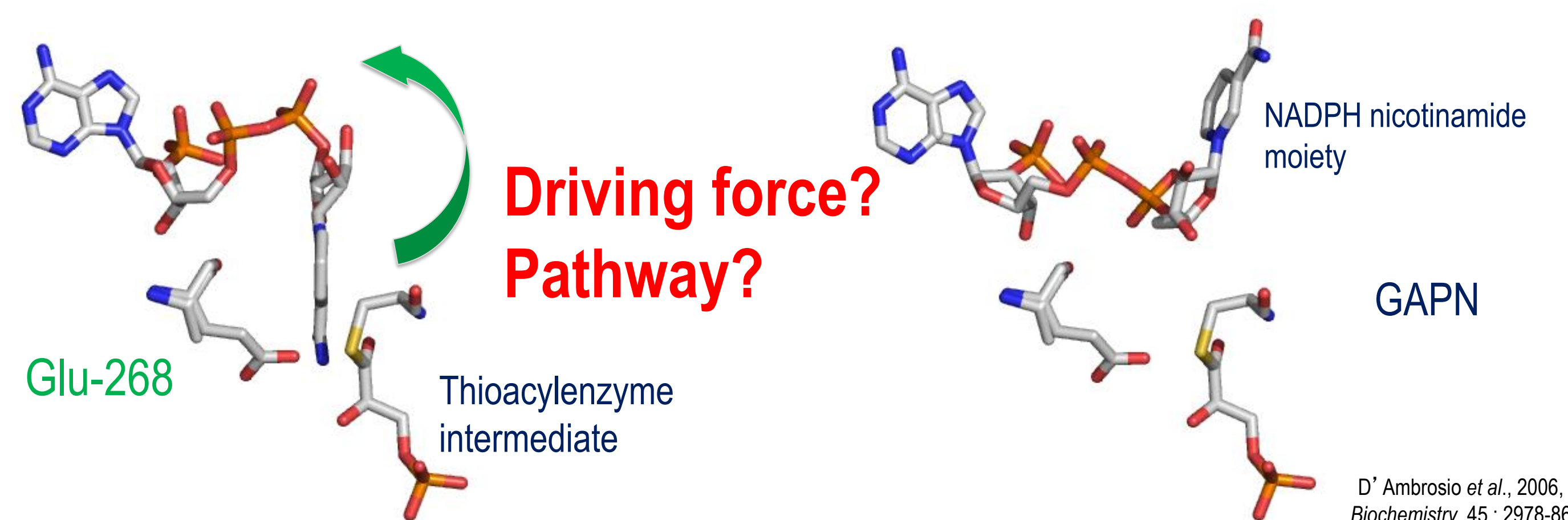
The context

Aldehyde dehydrogenases: NAD(P)-dependent dehydrogenases
Involved in detoxication and metabolic pathways (retinoic acid biosynthesis)



The question

Reduced nicotinamide moiety (NMNH) flipping is required for Glu-268 to activate the water molecule in the deacylation step



NADP⁺ in hydride transfer conformation → **No significant change of the protein structure** → NADPH in hydrolysis conformation

The strategy

Reduced nicotinamide moiety (NMNH) flipping

Impact on catalysis ?

→ Kinetic approach

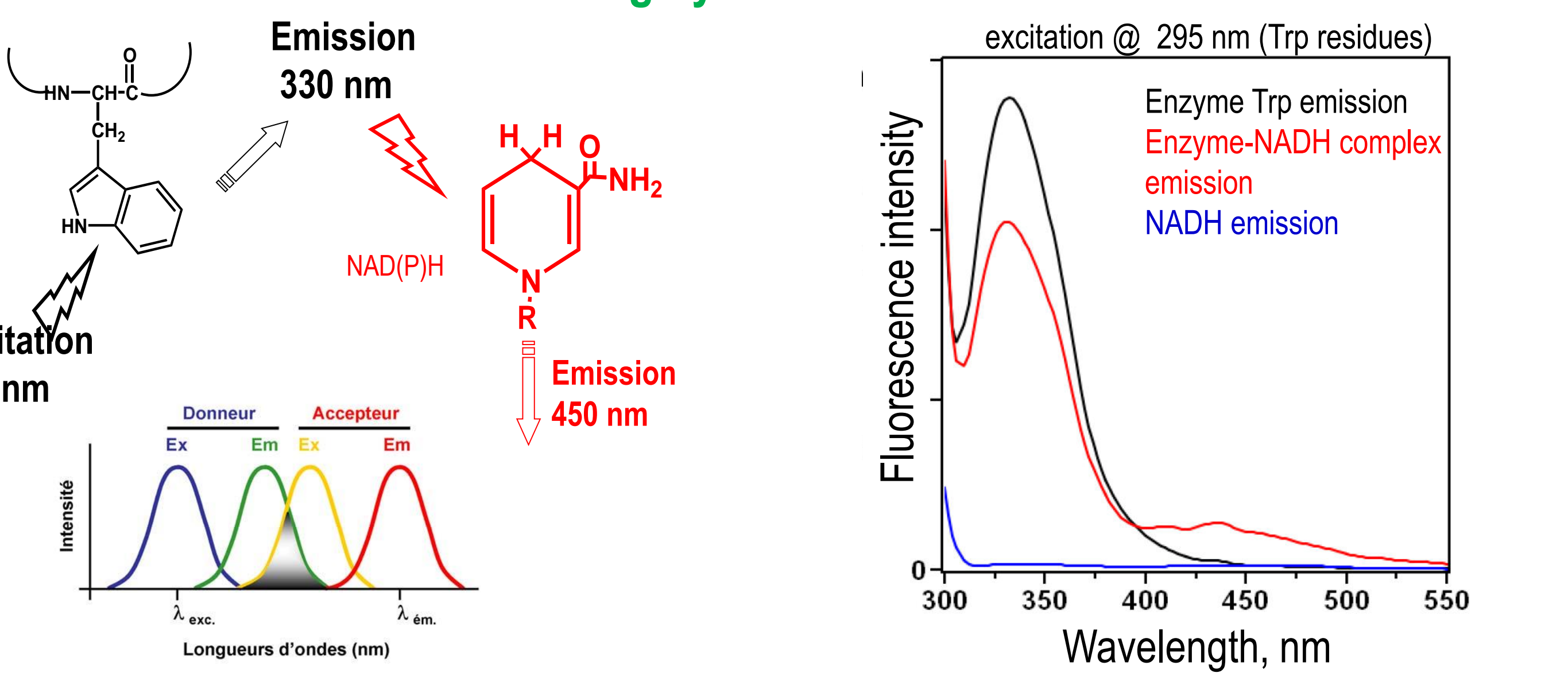
→ Intrinsic Trp/NADH FRET probe

Driving force ?

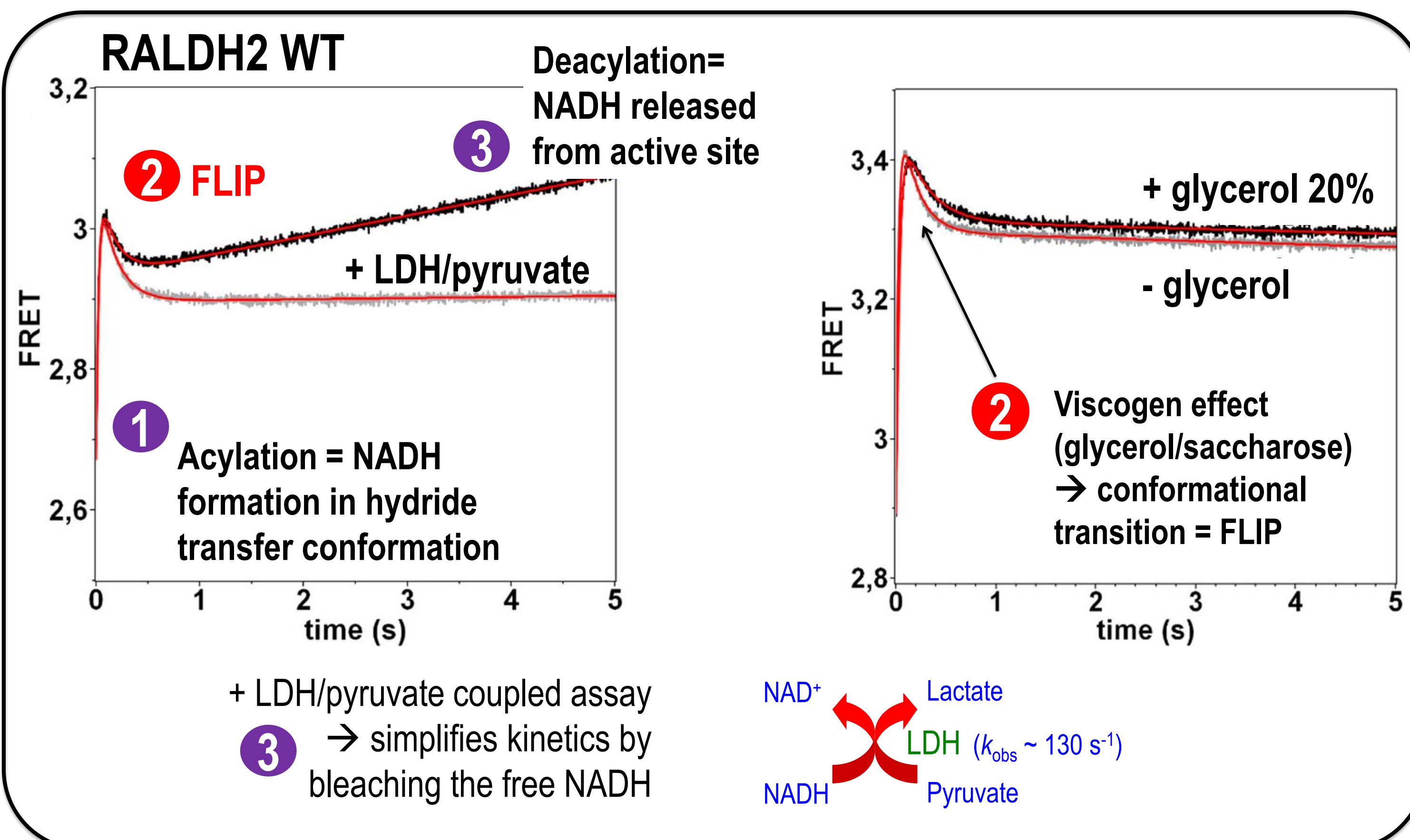
→ Identification of molecular factors allowing cofactor movement

→ Targeted Molecular Dynamics

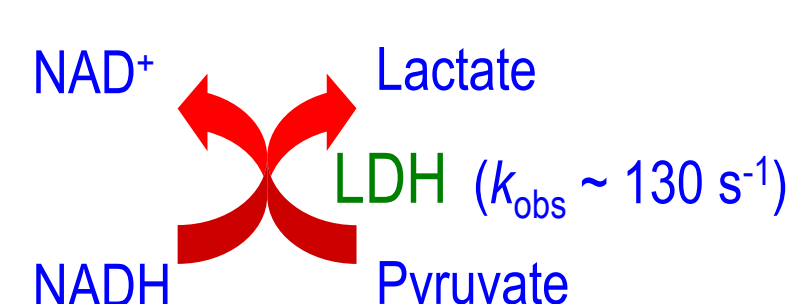
NMNH conformation monitoring by FRET



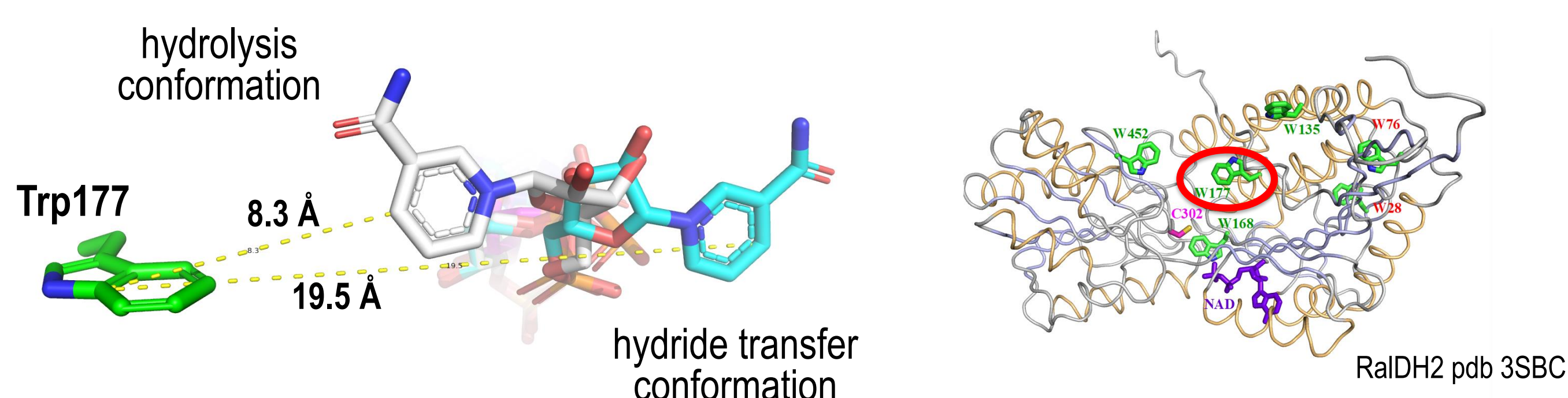
Rapid kinetic monitoring of the flip using stopped flow



+ LDH/pyruvate coupled assay
→ simplifies kinetics by bleaching the free NADH

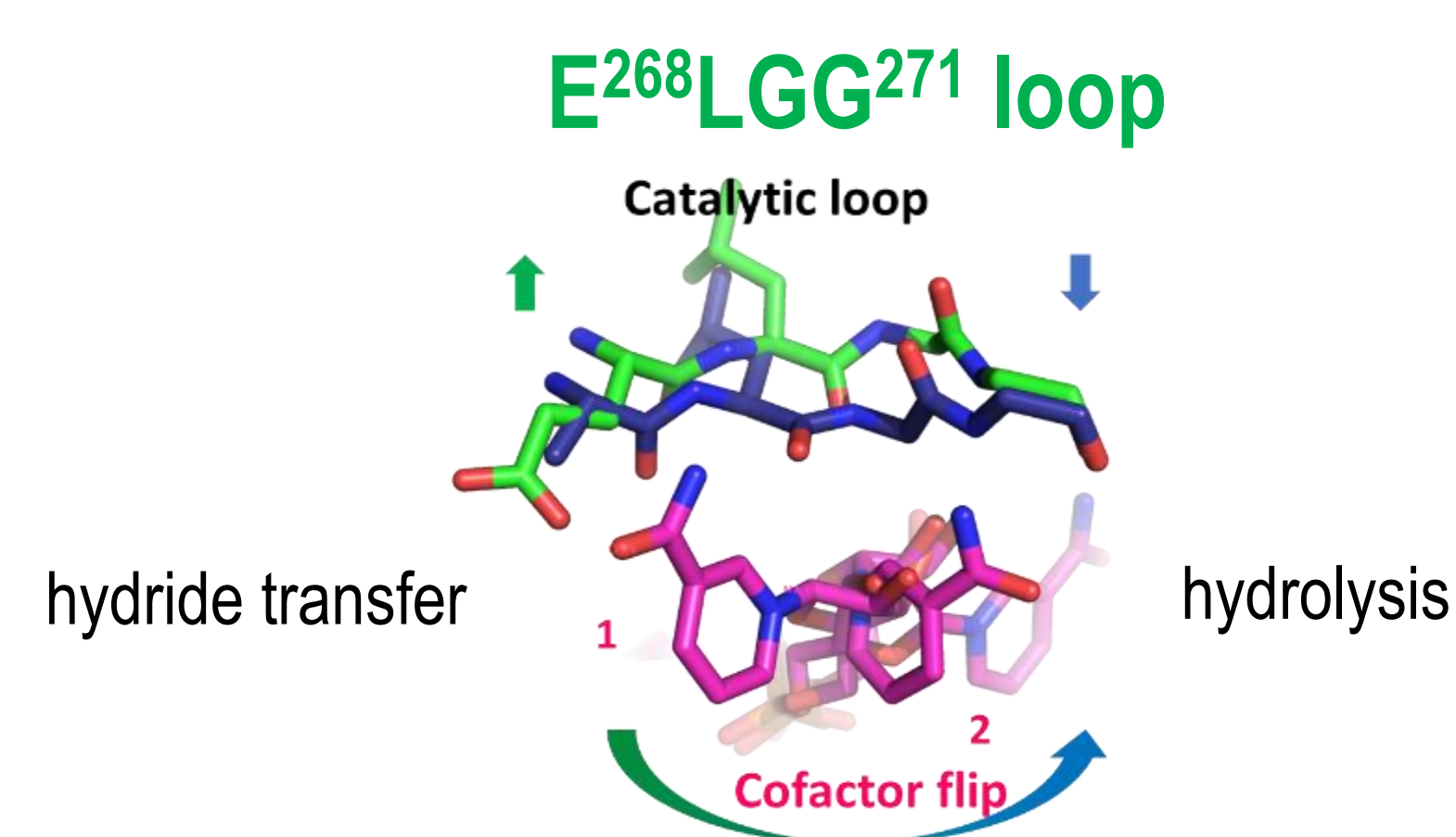
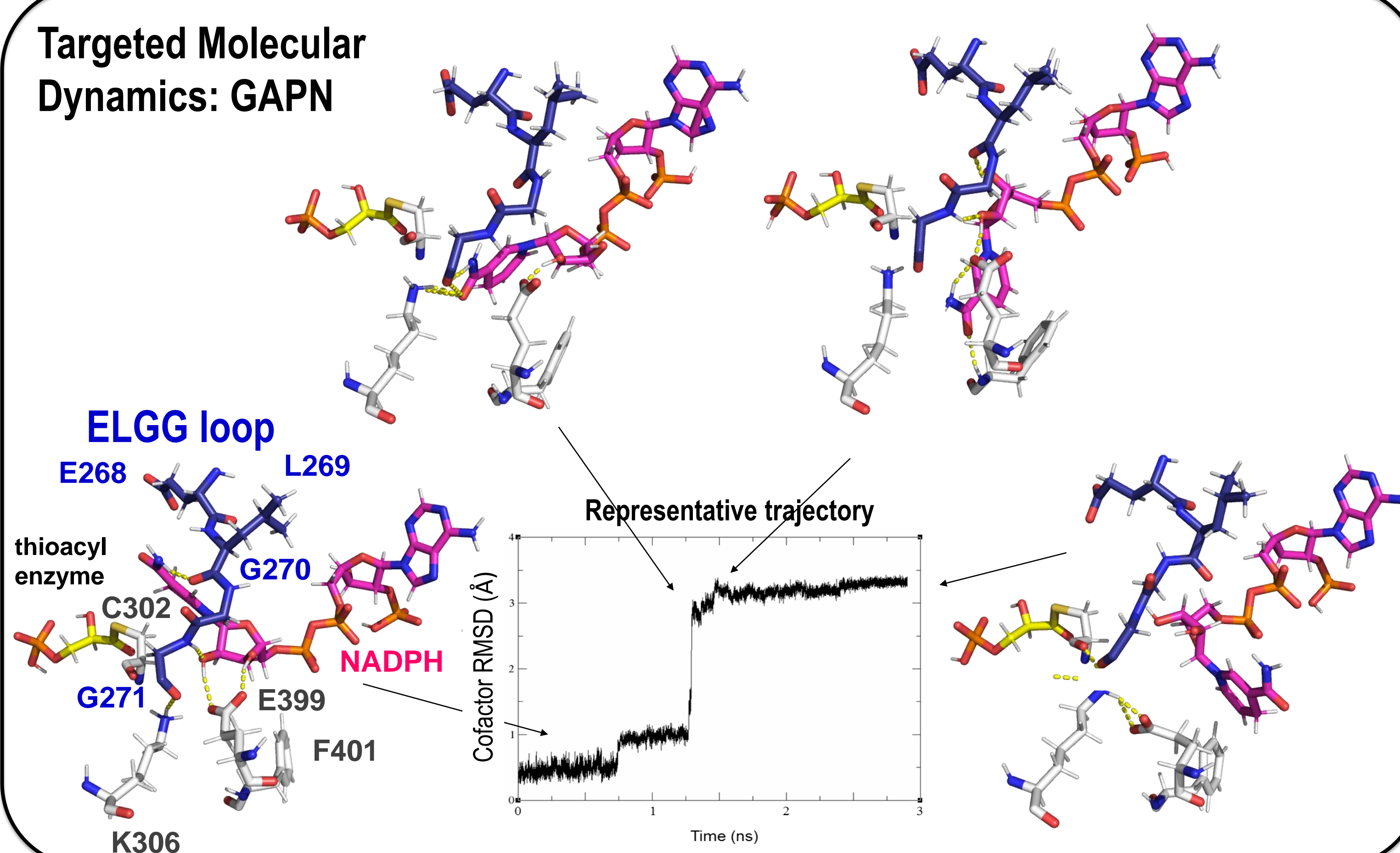


2 Trp177 is the specific probe of cofactor flipping



Conserved ELGG loop as a gatekeeper for cofactor flipping

Targeted Molecular Dynamics: GAPN



Hypothesis
Role of E²⁶⁸LGG²⁷¹ loop in flip control ?

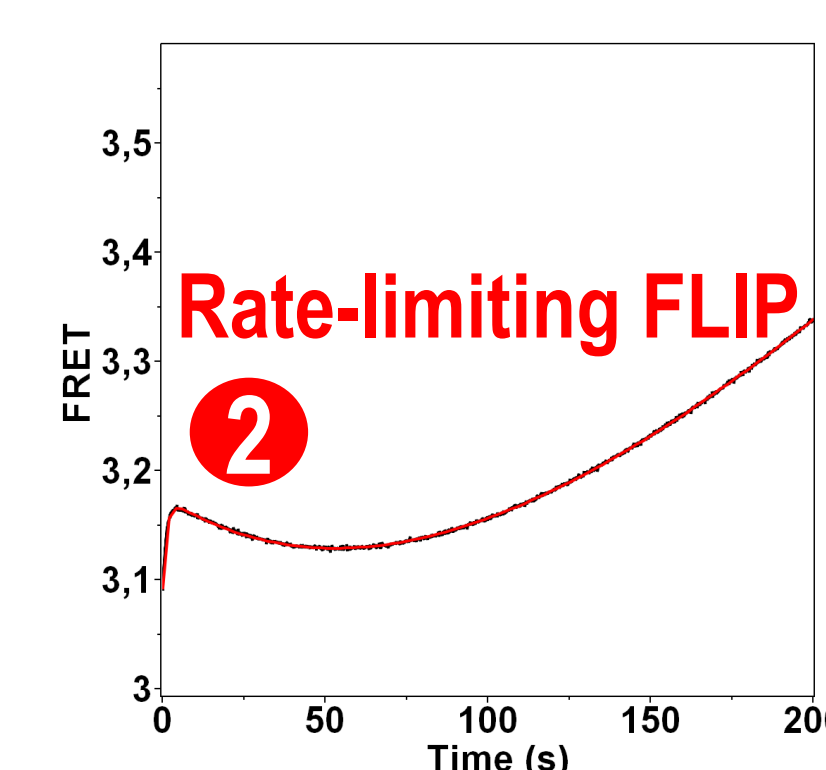
→ Mutation Gly271Ala

G271A mutation in ELGG loop drastically reduces the flip kinetics

RALDH2 G271A

- acylation remains non rate-limiting
- → allows flip to be measured
- □ □ of the flip rate constant

	k_{flip} (s ⁻¹)	k_{cat} (s ⁻¹)
WT	5	2
G271A	0,03	0,01



CONCLUSION

- Flip/catalytic efficiency relies on ELGG loop flexibility
- Cofactor flipping extends to all ALDH family including CoA-dependent aldehyde dehydrogenases

References

- D'Ambrosio, K., Pailot, A., Talfournier, F., Didierjean, C., Benedetti, E., Aubry, A., Branlant, G., and Corbier, C. (2006) The First Crystal Structure of a Thioacylenzyme Intermediate in the ALDH Family: New Coenzyme Conformation and Relevance to Catalysis. *Biochemistry*, 45, 2978–2986
- Rahuel-Clermont, S., Bchini, R., Barbe, S., Boutserin, S., André, I., and Talfournier, F. (2019) Enzyme Active Site Loop Revealed as a Gatekeeper for Cofactor Flip by Targeted Molecular Dynamics Simulations and FRET-Based Kinetics. *ACS Catal.* 9, 1337–1346