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► **To cite this version:**

Sophie Rahuel-Clermont, R Bchini, Sophie Barbe, S Boutserin, I. Andre, et al.. Enzyme Active Site Loop Revealed as Gatekeeper for Cofactor Flip by Targeted Molecular Dynamics Simulations and FRET-kinetics. Molecular Biophysics: ABC of the puzzle of Life ARBRE-MOBIEU Plenary Meeting., Mar 2019, Zagreb, Croatia. hal-02075703

**HAL Id: hal-02075703**

**<https://hal.univ-lorraine.fr/hal-02075703>**

Submitted on 21 Mar 2019

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

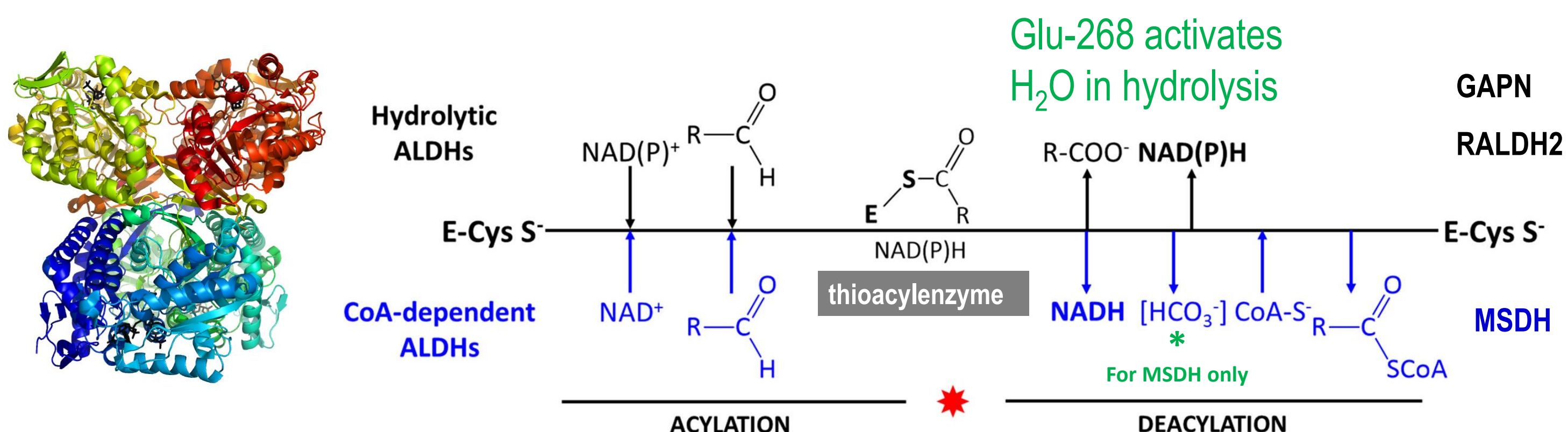
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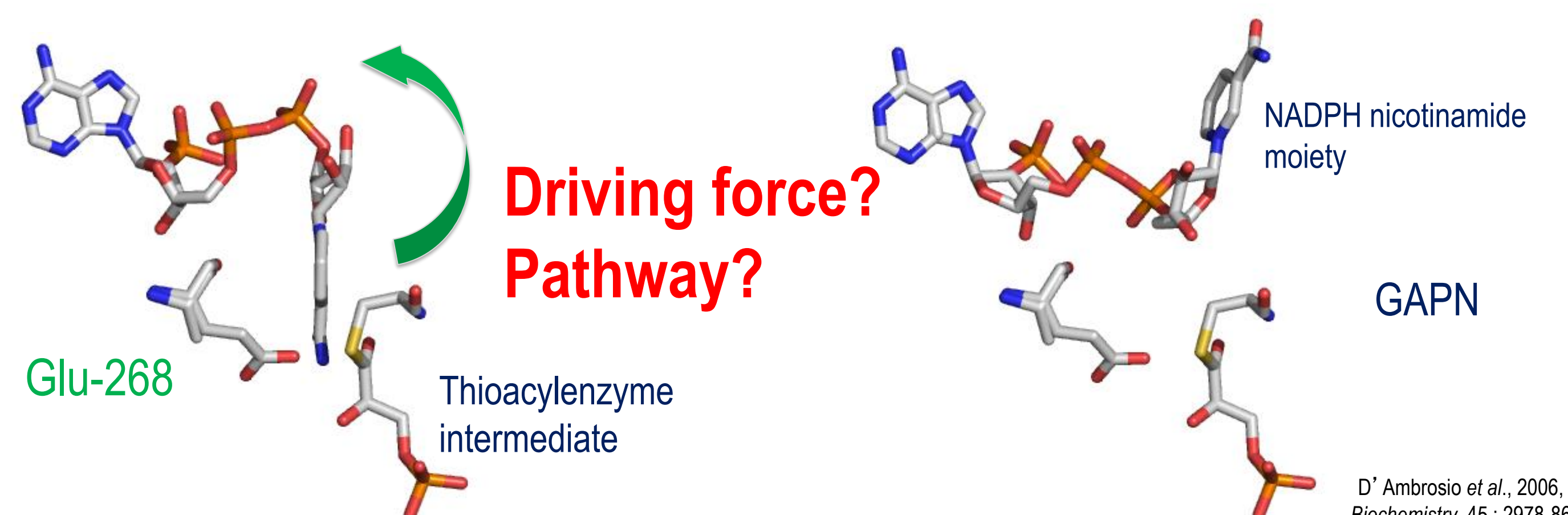
## The context

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<sup>+</sup> 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

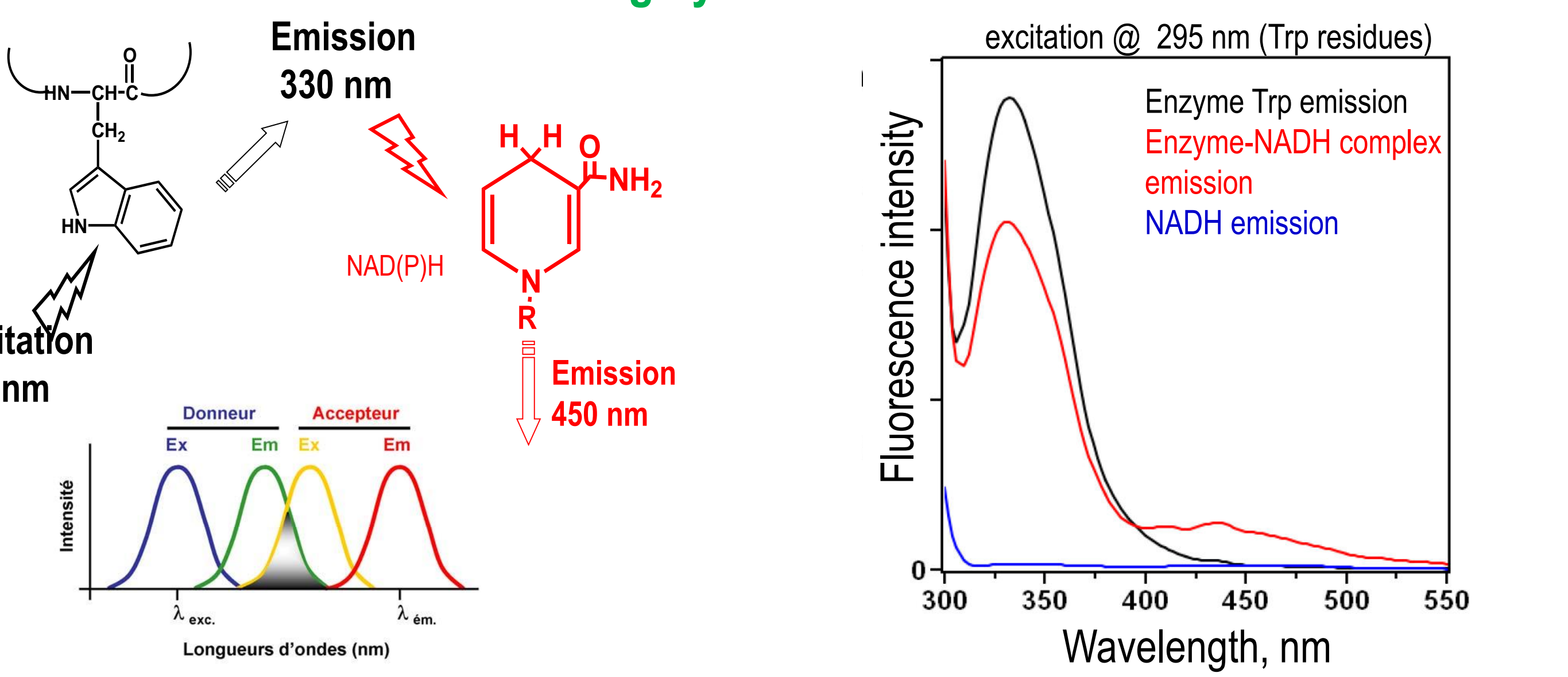
Driving force ?

→ Identification of molecular factors

allowing cofactor movement

→ Targeted Molecular Dynamics

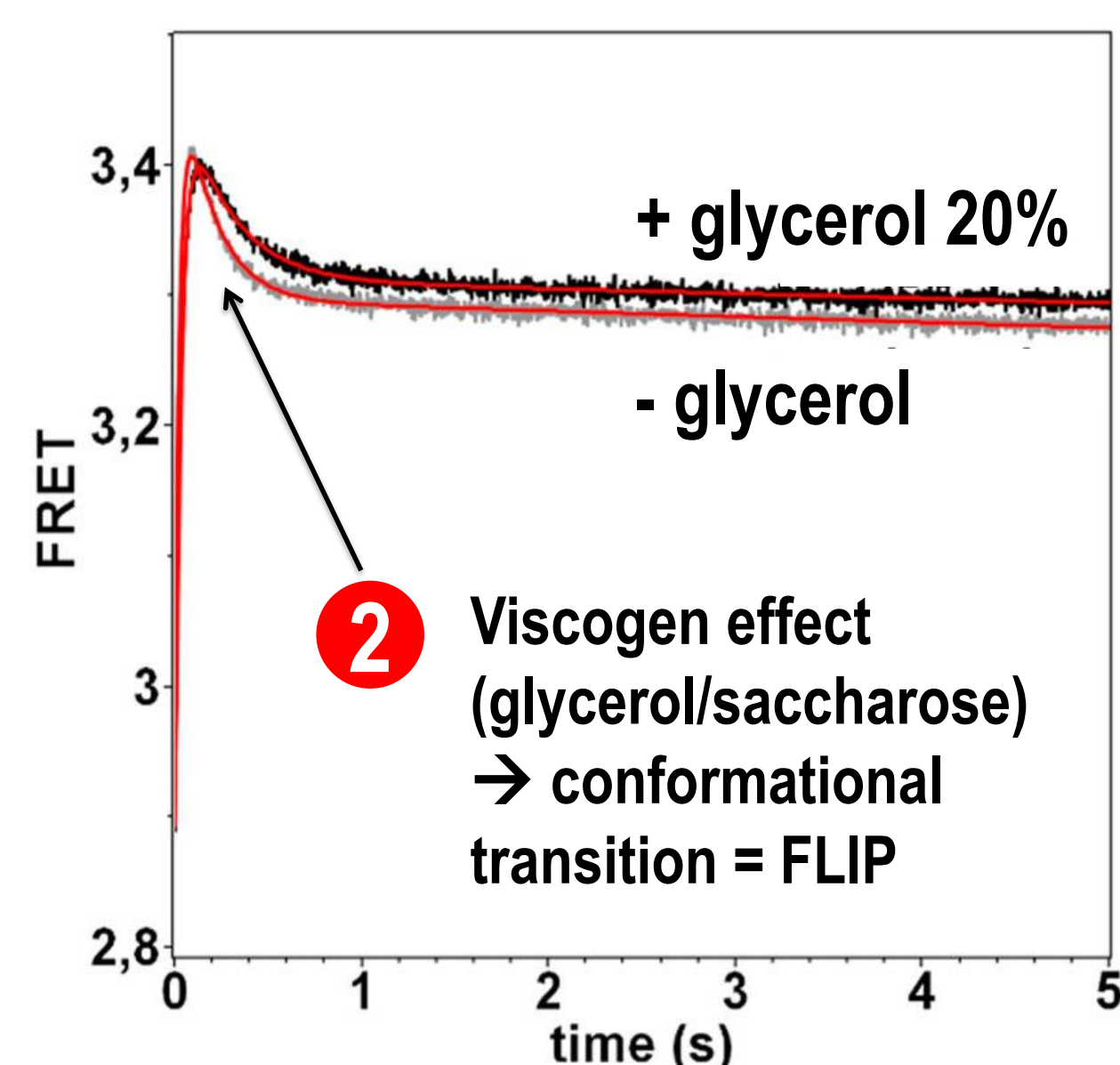
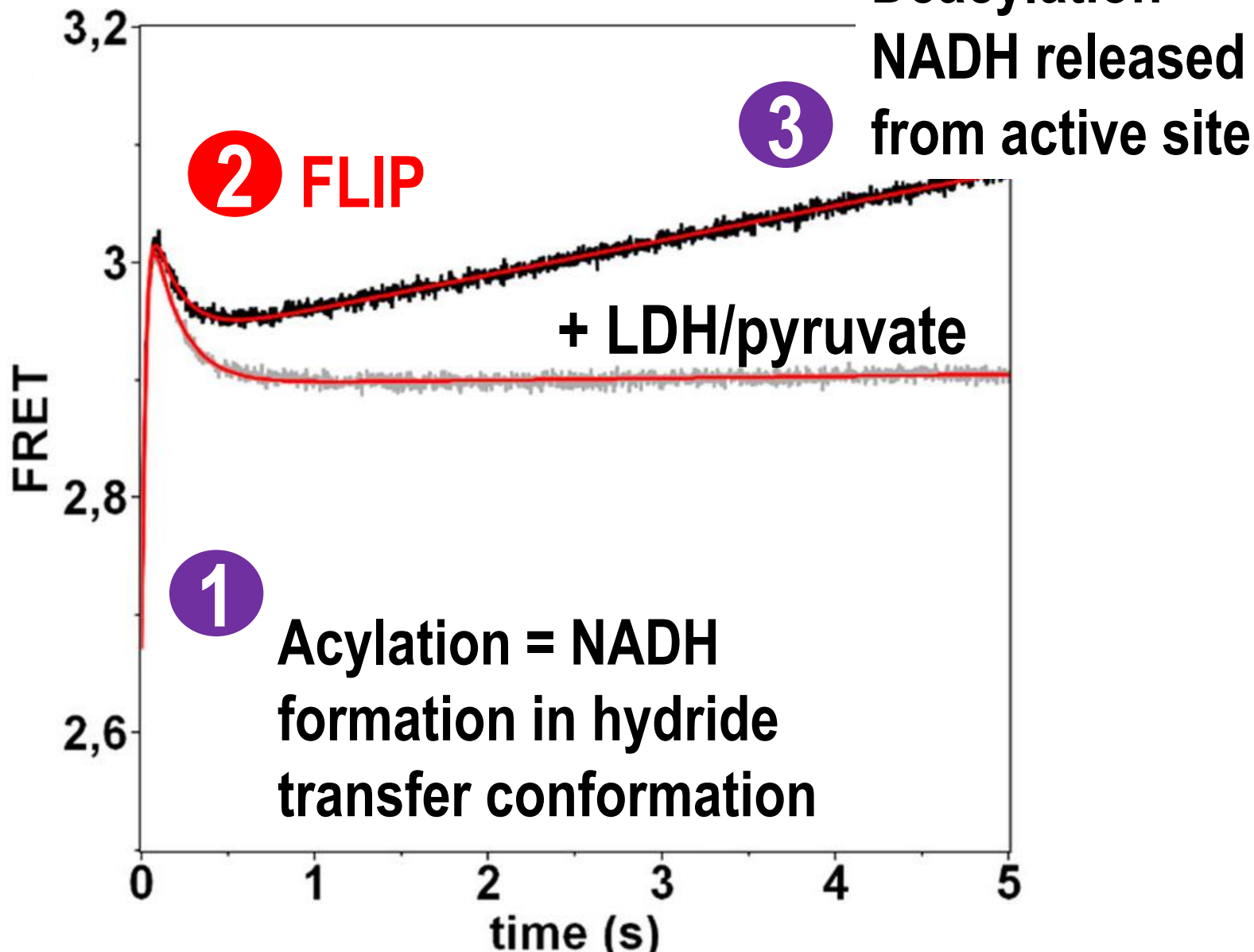
NMNH conformation monitoring by FRET



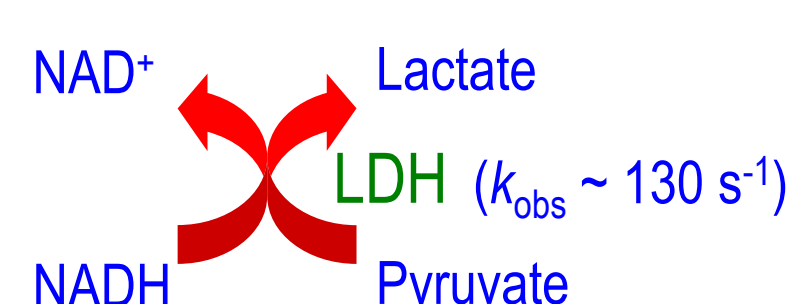
## Rapid kinetic monitoring of the flip using stopped flow

RALDH2 WT

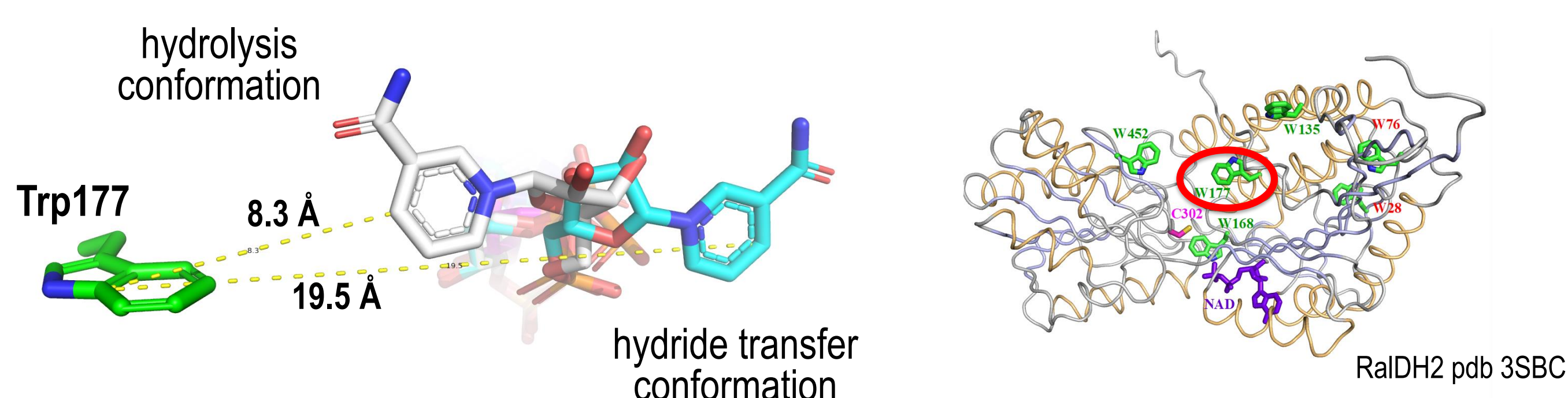
Deacylation = NADH released from active site



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



## 2 Trp177 is the specific probe of cofactor flipping

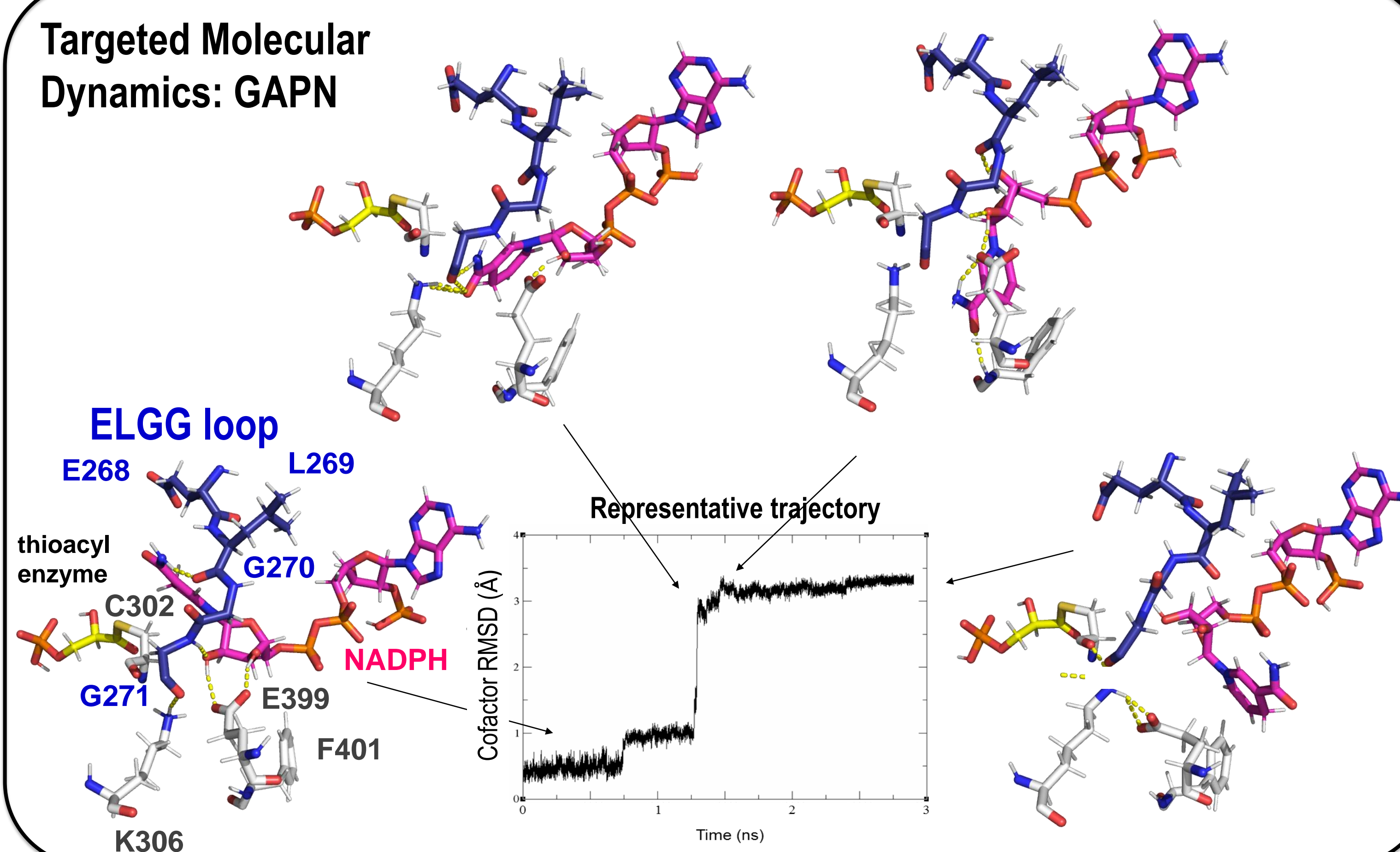


## References

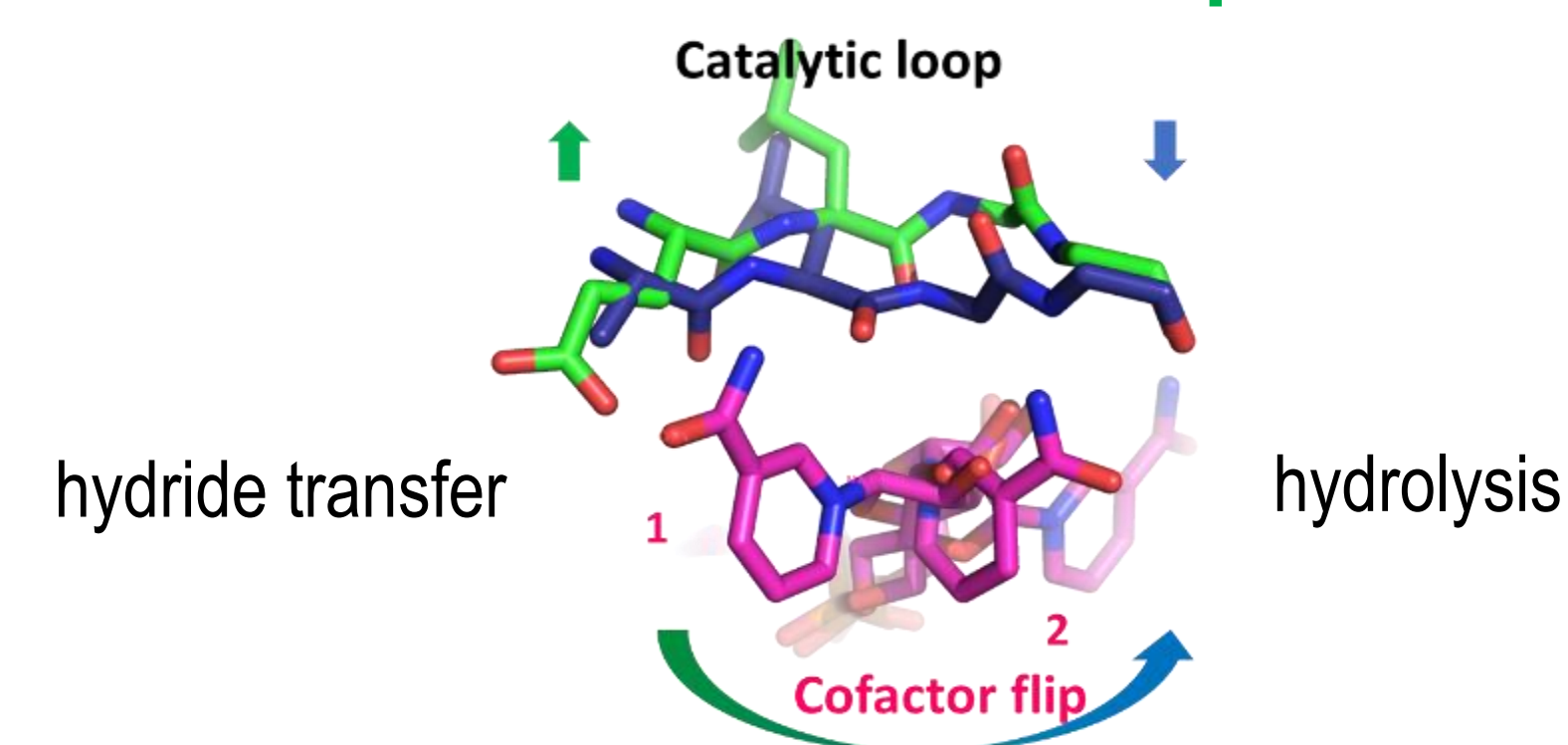
D'Ambrosio, K., Pailot, A., Talfournier, F., Didierjean, C., Benedetti, E., Aubry, A., Branlant, G., and Corbier, C. (2006) The First Crystal Structure of a Thioacylenzyme Intermediate in the ALDH Family: New Coenzyme Conformation and Relevance to Catalysis. *Biochemistry*, 45, 2978–2986  
Rahuel-Clermont, 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

## Conserved ELGG loop as a gatekeeper for cofactor flipping

Targeted Molecular Dynamics: GAPN



E<sup>268</sup>LGG<sup>271</sup> loop



**Hypothesis**  
Role of E<sup>268</sup>LGG<sup>271</sup> loop in flip control ?

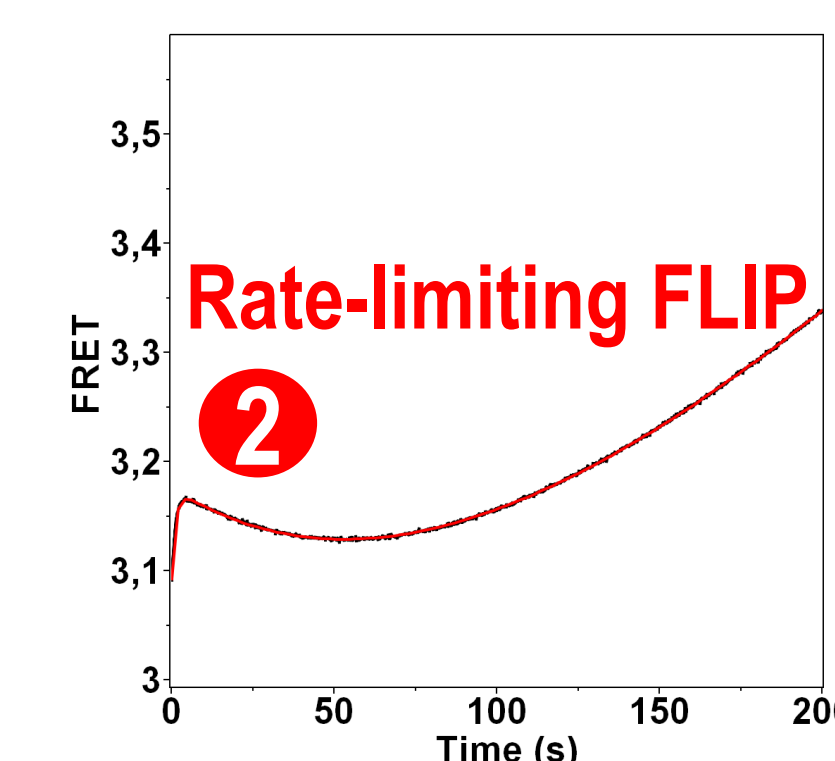
→ Mutation Gly271Ala

## 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 <sup>-1</sup> )	$k_{cat}$ (s <sup>-1</sup> )
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