

ENGINEERING PROTEIN DYNAMICS: DIVERGENCE OF ENZYME ACTIVITIES AT THE ENZYME CLASS LEVEL

Scan me!

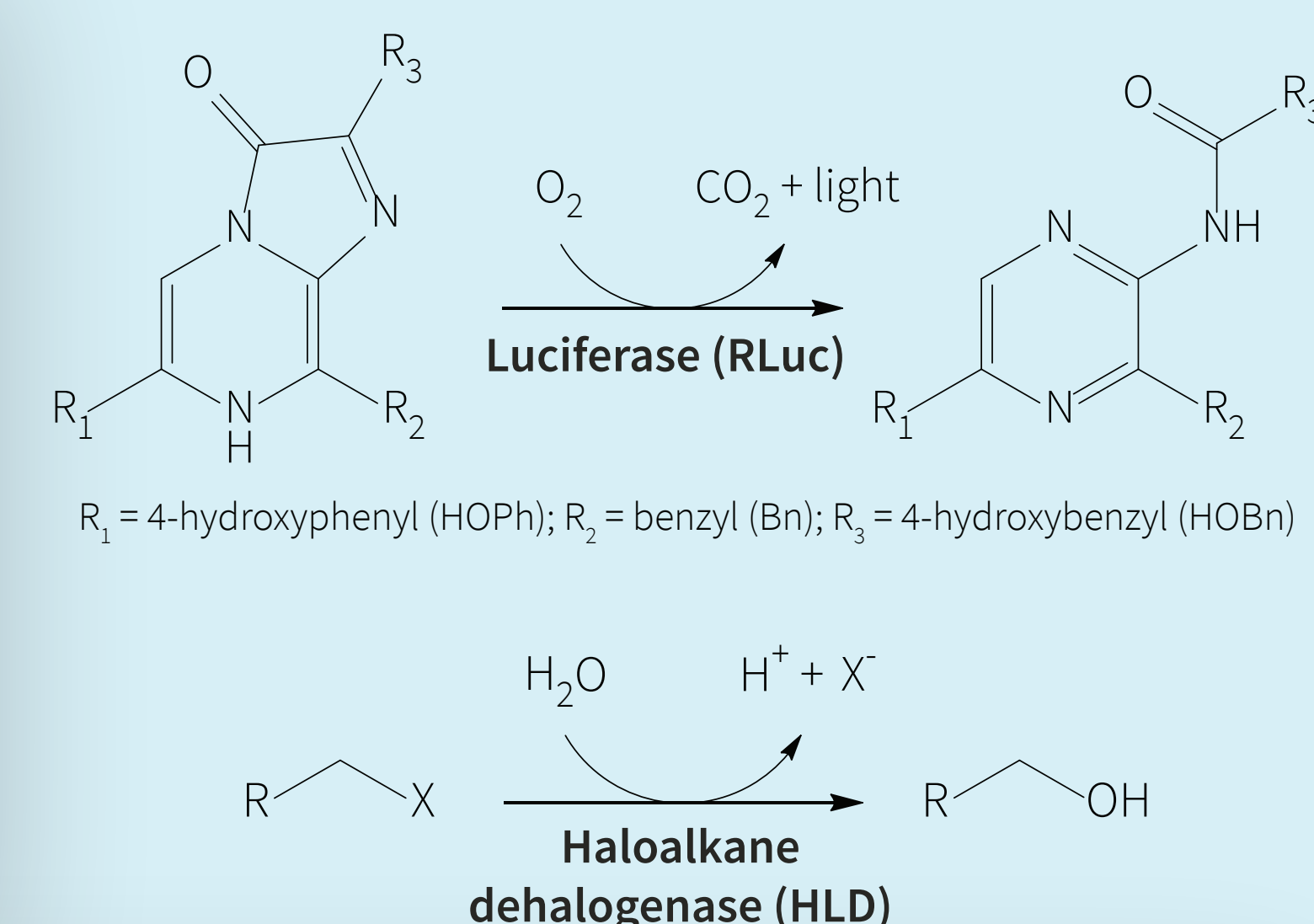


Toul M.^{1,2}, Schenkmyerova A.^{1,2}, Pinto G. P.^{1,2}, Marek M.^{1,2}, Hernychova L.³, Planas-Iglesias J.^{1,2}, Liskova V. D.^{1,2}, Pluskal D.², Vasina M.^{1,2}, Emond S.⁴, Dörr M.⁵, Chaloupkova R.², Bednar D.^{1,2}, Prokop Z.^{1,2}, Hollfelder F.⁴, Bornscheuer U. T.⁵, Damborsky J.^{1,2}

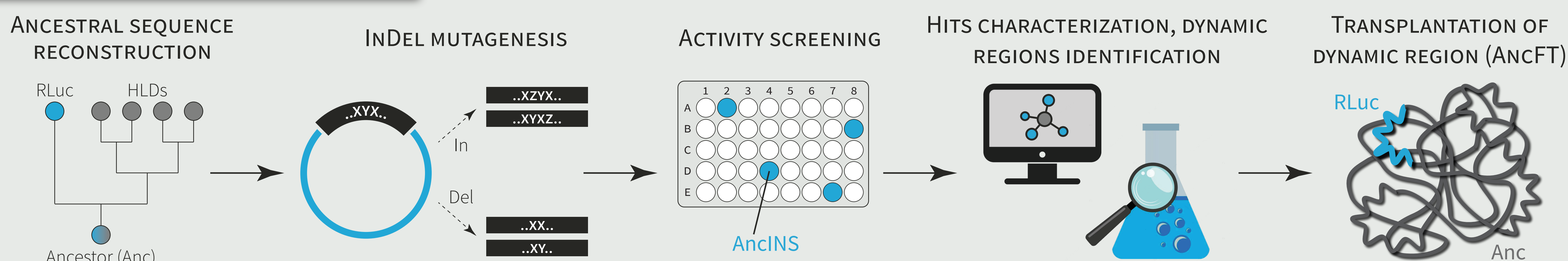
martintoul@mail.muni.cz

#1 INTRODUCTION

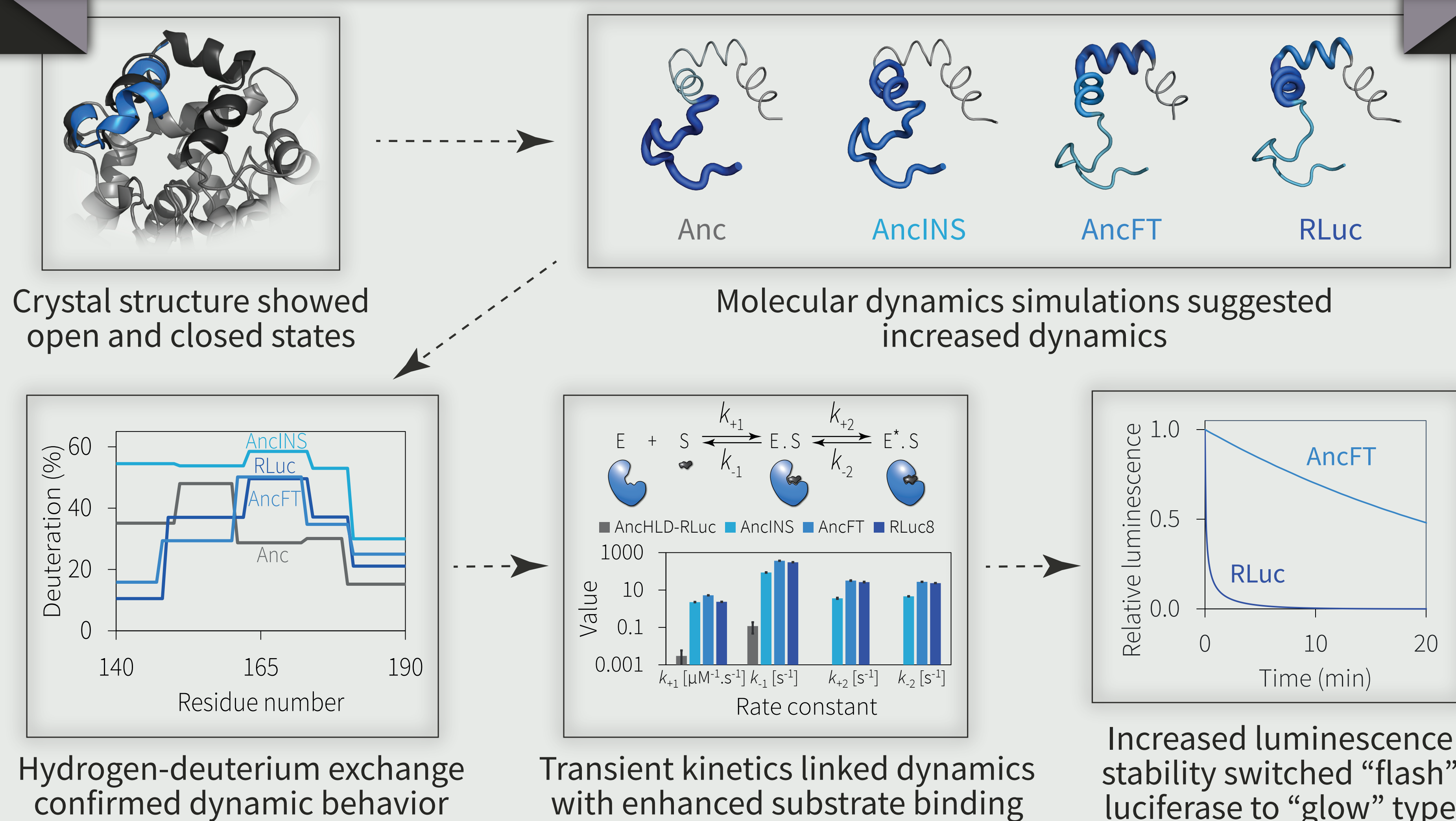
- Two enzyme groups – **luciferases** (oxidoreductase, EC 1.13.12.5) and **haloalkane dehalogenases** (hydrolase, EC 3.8.1.5) – functionally distinct but evolutionarily related¹
- A **stable ancestral protein**² exhibiting a dual promiscuous activity (starting point of evolutionary divergence)
- An urge to identify the driving force of the emergence of a new enzymatic function and understand the process of **evolution at the molecular level**
- Exploration of a novel technique for **insertion and deletion (InDel) mutagenesis**³



#2 WORKFLOW



#3 RESULTS



#4 CONCLUSIONS

- Alteration of dynamics demonstrated to be important for the evolution of the luciferase activity
- Engineered dynamics enhanced substrate binding and significantly increased bioluminescence half-life
- InDel followed by fragment transplantation is a valuable tool for the novel catalytic activity development

REFERENCES:

- (1) Loening AM et al. (2007) *J. Mol. Biol.* 374: 1017–1028. (2) Chaloupkova R et al. (2019) *ACS Catalysis* 9: 4810–4823. (3) Schenkmyerova A et al. (2021) *Nat. Commun.* 12: 3616.

Scan me!

