SHEDDING LIGHT ON THE SECRETS OF NANOLUC: ITS MECHANISM AND ALLOSTERIC BEHAVIOUR

Jana Horáčková[a], Michal Nemergut[a,b], Daniel Pluskal[a], Tereza Šustrová[a], Jan Tulis[a], Tomáš Bárta[c], Racha Baatallah[d], Glwadys Gagnot[d,e], Veronika Nováková[a,b], Marika Majerová[a,b], Karolína Sedláčková[a,b], Sérgio M. Marques[a,b], Martin Toul[a,b], Jiří Damborský[a,b], Zbyněk Prokop[a,b], Yves L. Janin[f], Martin Marek[a,b], David Bednář[a,b]

[a]Loschmidt Laboratories, Department of Experimental Biology and RECETOX, Faculty of Science, Masaryk University, Brno, Czech Republic; [b]International Clinical Research Center, St. Anne's University Hospital Brno, Czech Republic; [c]Department of Histology and Embryology, Faculty of Medicine, Masaryk University, Brno, Czech Republic; [d]Unité de Chimie et Biocatalyse, Institut Pasteur, UMR 3523, CNRS, Paris, France; [e]Université Paris Cité, Paris, France; [f]Structure et Instabilité des Génomes, Muséum National d'Histoire Naturelle, INSERM, CNRS, Alliance Sorbonne Université, Paris, France

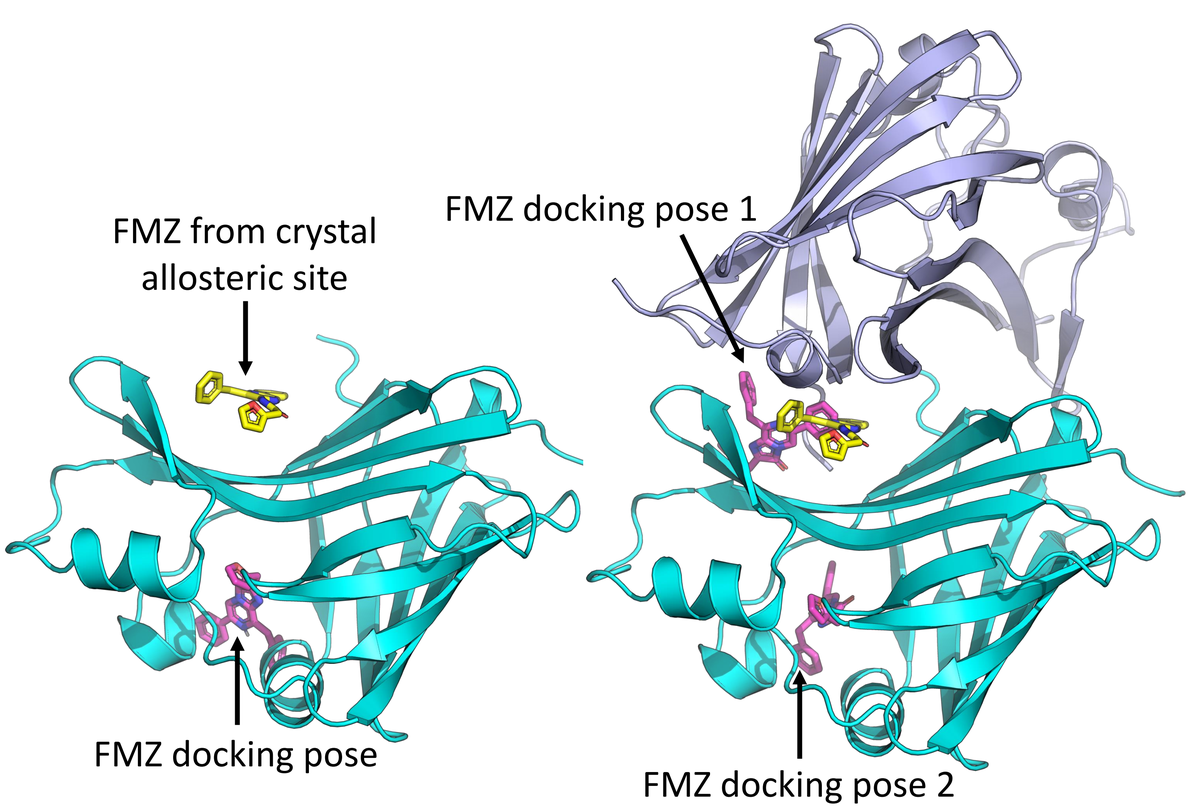
INTRODUCTION

NanoLuc is a small but very bright bioluminescent enzyme. Therefore, this luciferase is widely used in biotechnology and biomedicine. NanoLuc was designed in 2012 by mutating a luciferase from deep-sea shrimp *Oplophorus gracilirostris* [1]. NanoLuc utilizes furimazine (FMZ), an optimized analog of coelenterazine (CTZ), the substrate of *Oplophorus* luciferase.

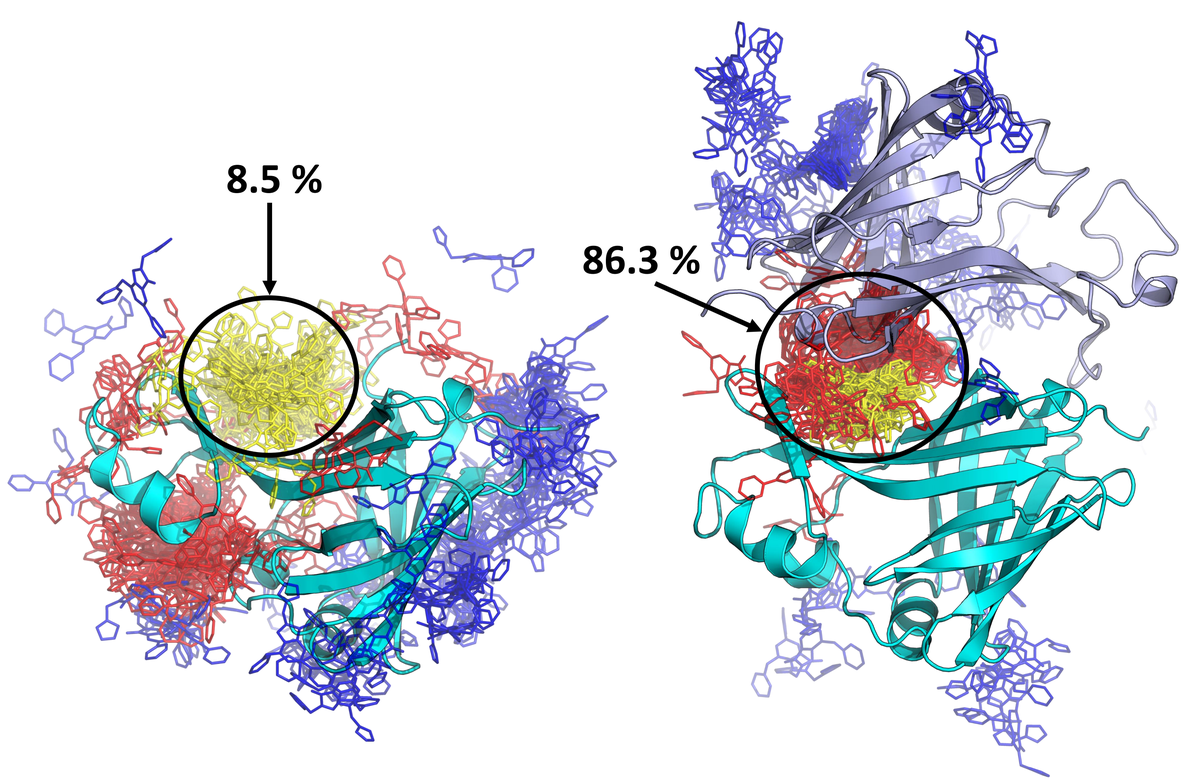
MOTIVATION

The mechanism of NanoLuc's light-emitting reaction has not been solved. However, it is vital for the development of future bioluminescent systems. In our crystal structures, we detected the catalytic site formed inside NanoLuc while in the "open" conformation. In contrast, the "closed" form of NanoLuc has an allosteric binding pocket formed on its surface [2].

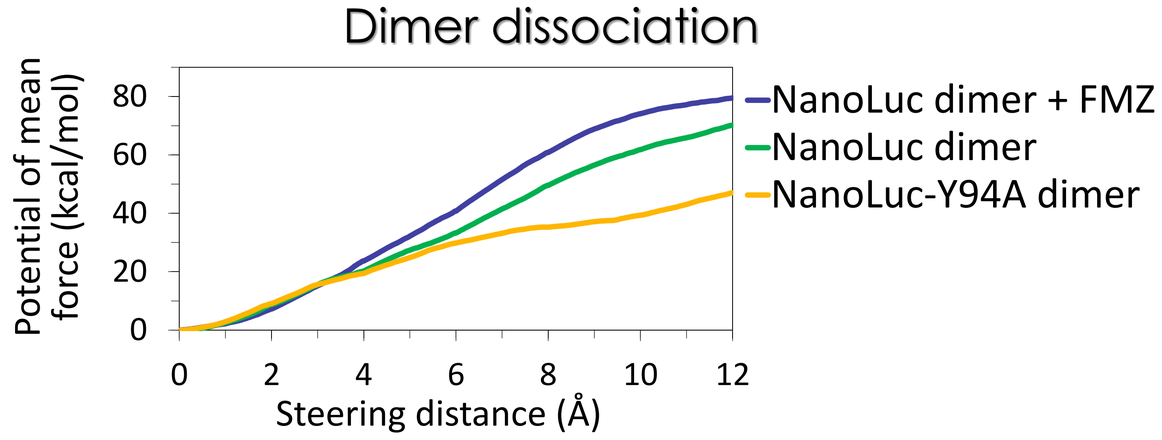
MOLECULAR DOCKING

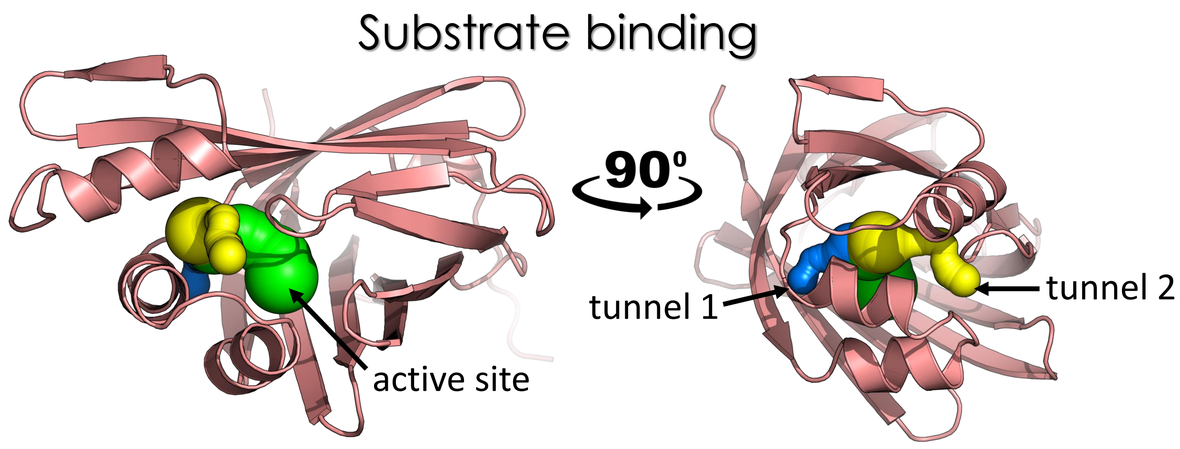


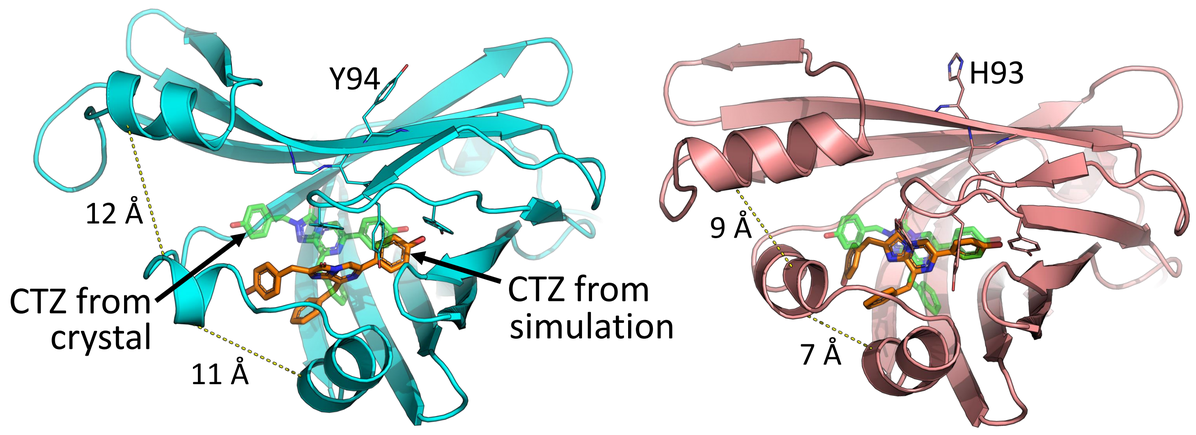
ADAPTIVE SAMPLING



ADAPTIVE STEERED MD







CONCLUSIONS

- Low affinity to the allosteric site in monomer
- Furimazine stabilizes the dimeric NanoLuc
- The active site is accessed through tunnel 1

REFERENCES

[1] Hall MP, Unch J, Binkowski BF, et al. Engineered Luciferase Reporter from a Deep Sea Shrimp Utilizing a Novel Imidazopyrazinone Substrate. *ACS Chem Biol*. 2012.

[2] Nemergut M, Pluskal D, Horackova J, Sustrova T et al. Illuminating the mechanism and allosteric behavior of NanoLuc luciferase. *Nature Communications* 2023.

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