NanoLuc luciferase may not be as "nano" as thought

Jana Horáčková[1,2], Michal Nemergut[1,2], Daniel Pluskal[1,2], Sérgio Marques[1,2,3], Veronika Blechová[1,2,3], Jan Tulis[1,2], Jiří Damborský[1,2,3], Zbyněk Prokop[1,2,3], Yves Janin[4], Martin Marek[1,2,3], and David Bednář[1,2,3]

[1] Loschmidt Laboratories, Department of Experimental Biology, Faculty of Science, Masaryk University, Brno, CZ
[2] Loschmidt Laboratories, RECETOX, Faculty of Science, Masaryk University, Brno, CZ
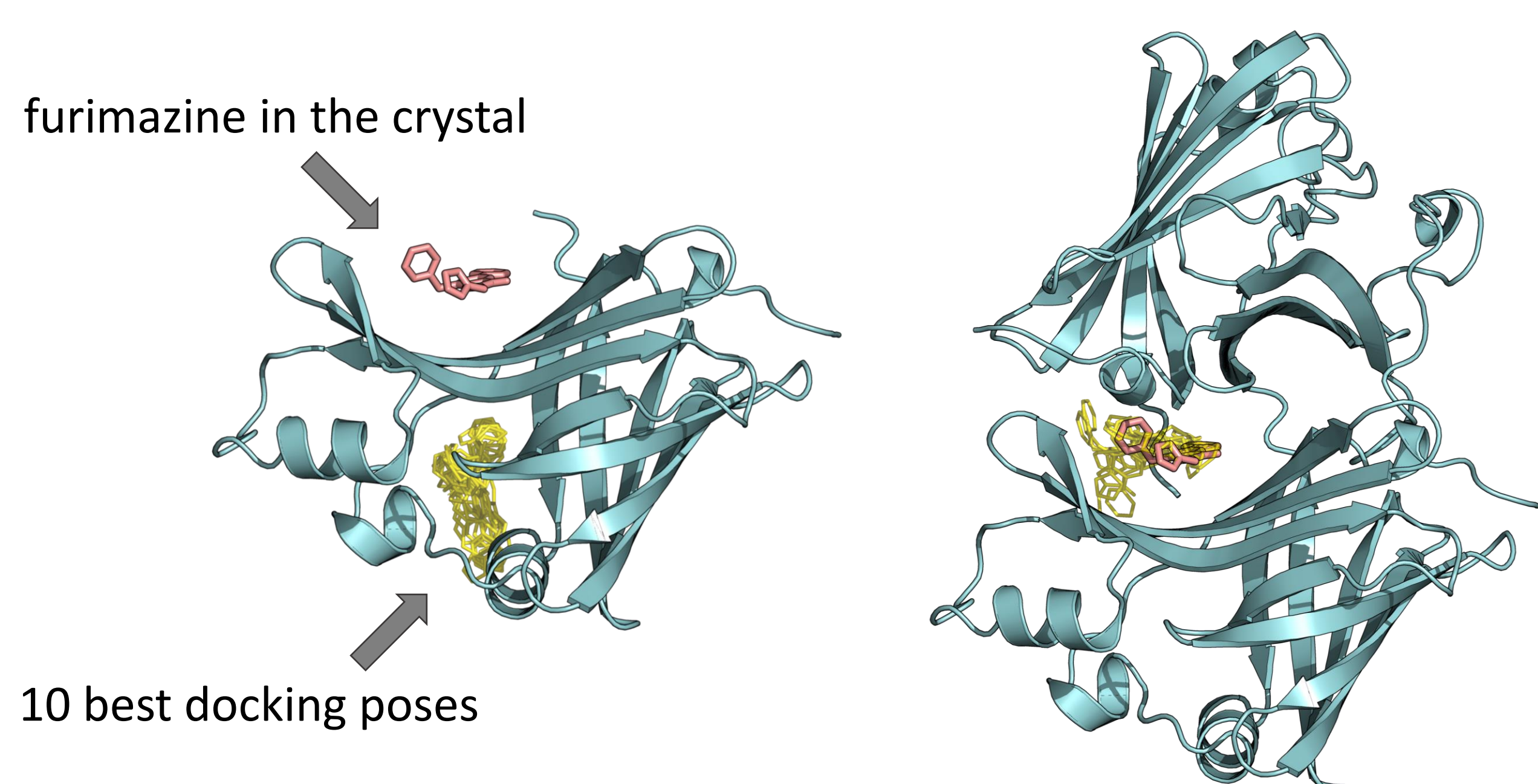[3] International Clinical Research Centre, St. Anne's University Hospital Brno, CZ
[4] Structure et Instabilité des Génomes, Muséum national d'Histoire naturelle, INSERM, CNRS, Alliance Sorbonne Université, Paris, FR
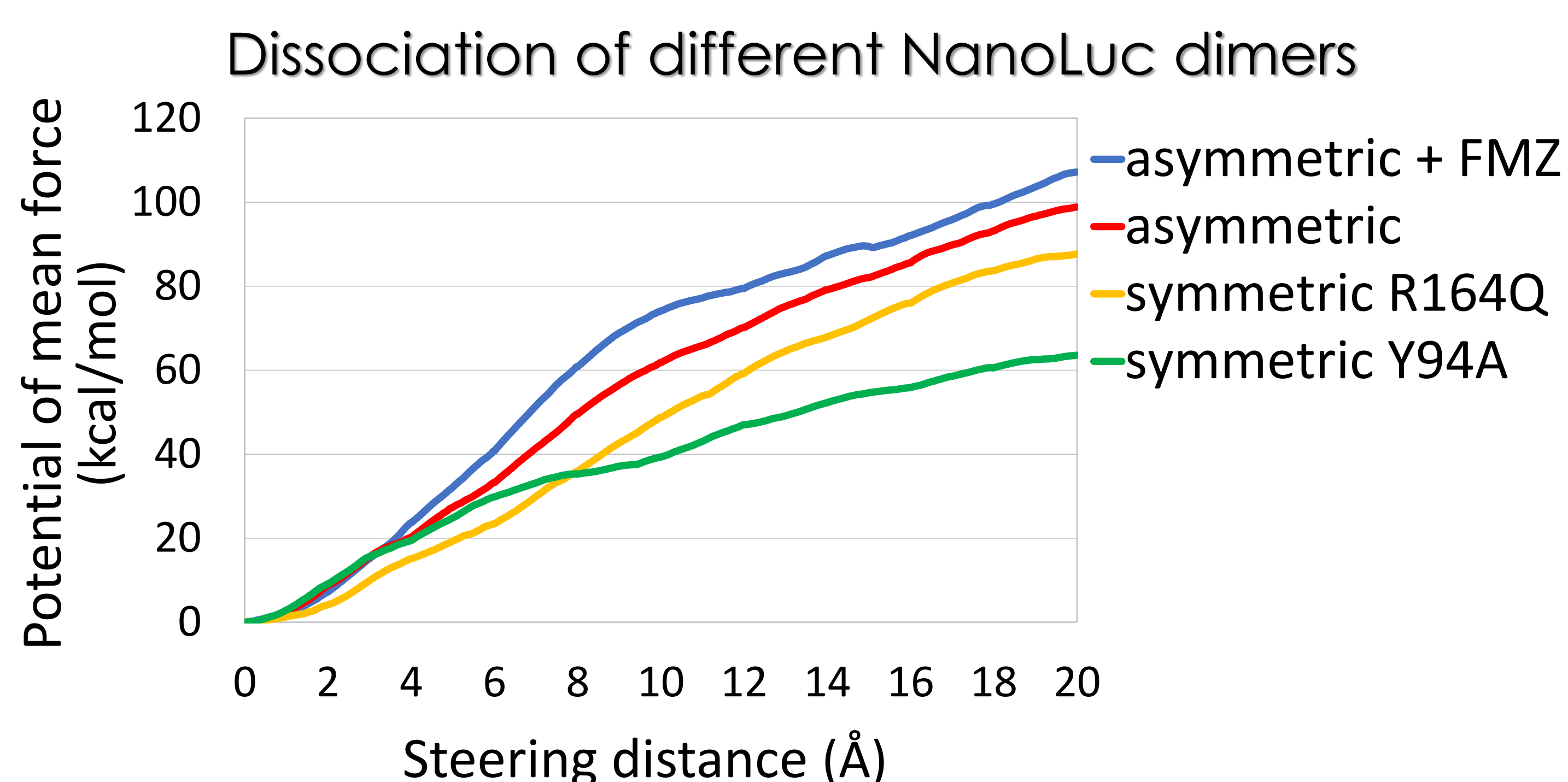
INTRODUCTION

NanoLuc is a bioluminescent enzyme of 171 residues engineered from OLuc, a deep-sea shrimp *Oplophorus gracilirostris* luciferase. NanoLuc utilizes furimazine (FMZ), an optimized analog of the natural substrate. The small size of NanoLuc is one of the important advantages of this luciferase [1].

MOTIVATION

As the name suggests, NanoLuc is a small protein – at least in solution, it is true. However, from the crystal structure, it is not clear if NanoLuc is a monomer or a dimer. Moreover, luciferin-induced oligomerization was observed in dynamic light scattering experiments, which suggests NanoLuc might act as a dimer.

MOLECULAR DOCKING

furimazine in the crystal

10 best docking poses

ADAPTIVE SAMPLING

Furimazine unbinding

Eq. probability of bound furimazine

	monomer	dimer
ΔG^0 (kcal/mol)	1.51 ± 0.31	−1.18 ± 0.27
k_{off}/k_{on} (M)	12.25 ± 7.43	0.04 ± 0.05
K_D (M)	14.33 ± 18.85	0.16 ± 0.08

ΔG^0 – free energy of the bound state
k_{off}/k_{on} – ratio of unbinding and binding rate constants
K_D – dissociation constant

ADAPTIVE STEERED MD

Dissociation of different NanoLuc dimers

- asymmetric + FMZ
- asymmetric
- symmetric R164Q
- symmetric Y94A

Dimer dissociation

Eq. probability of associated dimer

associated

dissociated

	dimer + FMZ	dimer
ΔG^0 (kcal/mol)	−0.96 ± 0.49	0.02 ± 0.57
k_{dis}/k_{as} (M)	0.29 ± 0.17	2.35 ± 1.23
K_D (M)	0.28 ± 0.22	1.50 ± 1.39

k_{dis}/k_{as} – ratio of dissociation and association rate constants

CONCLUSIONS

NanoLuc dimer is likely the functional unit.
Furimazine stabilizes the NanoLuc dimer.
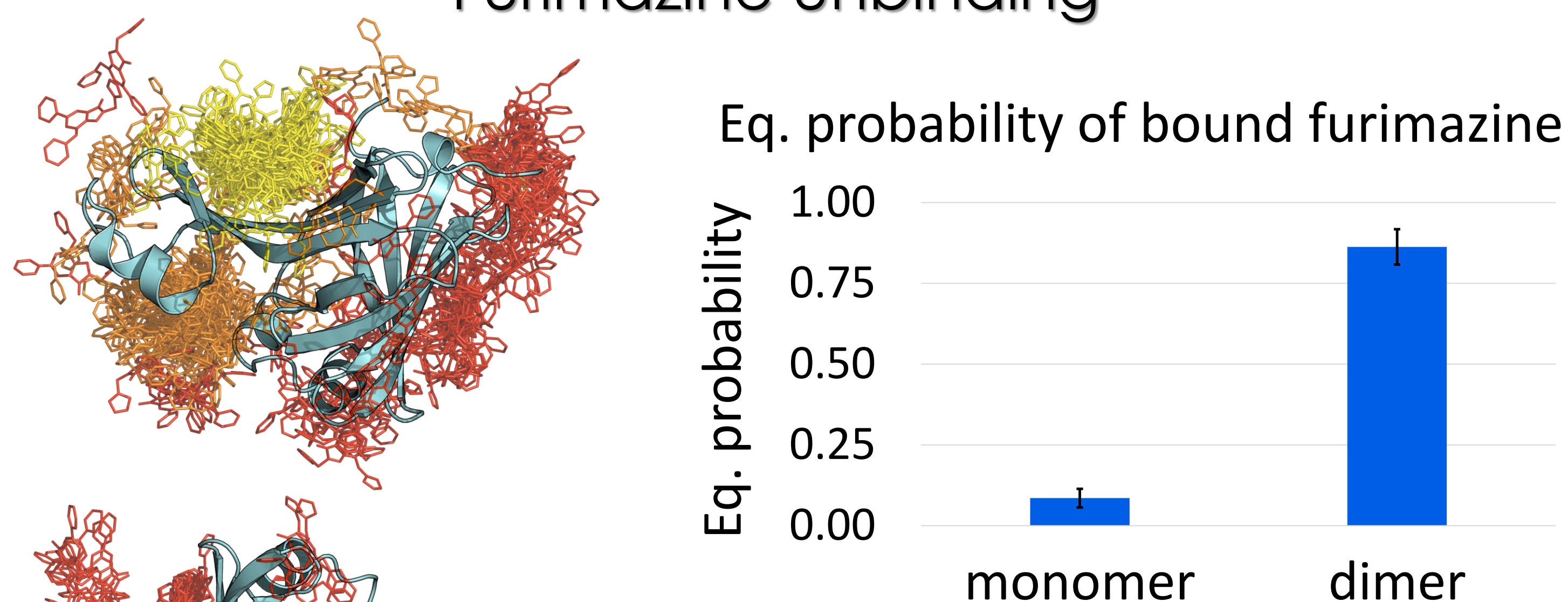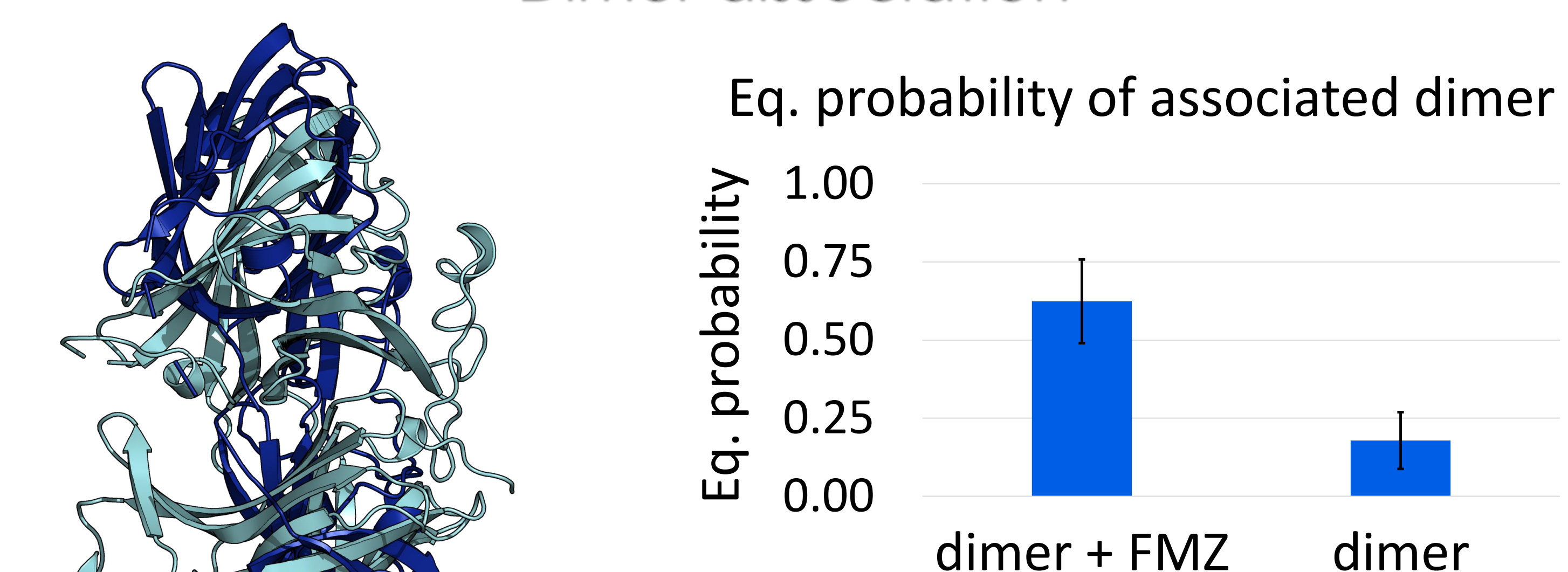Asymmetry contributes to dimer stability.

REFERENCE

[1] England, C. G.; Ehlerding, E. B.; Cai, W. NanoLuc: A Small Luciferase Is Brightening Up the Field of Bioluminescence. *Bioconjug. Chem.* **2016**, *27* (5), 1175–1187. https://doi.org/10.1021/acs.bioconjchem.6b00112.

ACKNOWLEDGMENT

MUNI | RECETOX LŁ metacentrum cesnet FNUSA ICRC