Poster

Structural basis for NanoLuc luciferase action

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The widely used NanoLuc luciferase was engineered over ten years ago but the oxidative mechanism by which it generates blue photons remained unclear [1]. Here we decipher NanoLuc luciferase action through crystallographic, spectroscopic, and computational experiments. We show that imidazopyrazinone luciferins bind to an intra-barrel catalytic site but also to an allosteric site shaped on the enzyme surface [2]. Structurally, binding to the allosteric site prevents simultaneous binding to the catalytic site, and *vice versa*, through concerted conformational changes. We demonstrate that restructuration of the allosteric site by mutagenesis can boost the luminescent reaction in the remote active site. Mechanistically, an intra-barrel arginine coordinates the imidazopyrazinone component of luciferin, which reacts with O_2 via a radical charge-transfer mechanism, and then it also protonates the resulting excited amide product to form a light-emitting neutral species. Concomitantly, an aspartate, supported by two tyrosines, fine-tunes the blue color emitter to secure a high emission intensity. Thus, we reveal that NanoLuc, despite its structural dissimilarity, employs an analogous catalytic principle to generate blue photons, as we recently revealed for coelenterazine-powered *Renilla*-type luciferases [3].

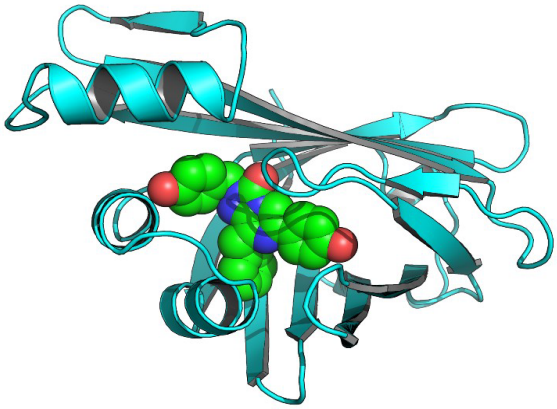

Figure 1. The co-crystal structure of NanoLuc luciferase complexed with substrate analogue azacoelenterazine.

[1] Hall M.P., Unch J., Binkowski B.F., Valley M.P., Butler B.L., Wood M.G., Otto P., Zimmerman K., Vidugiris G., Machleidt T., Robers M.B., Benink H.A., Eggers C.T., Slater M.R., Meisenheimer P.L., Klaubert D.H., Fan F., Encell L.P., Wood K.V. (2012) ACS Chemical Biology 7 (11): 1848-1857.

[2] Nemergut M., Pluskal D., Horackova J., Sustrova T., Tulis J., Barta T., Baatallah R., Gagnot G., Novakova V., Majerova M., Sedlackova K., Marques S.M., Toul M., Damborsky J., Prokop Z., Bednar D., Janin Y.L., Marek M. (2023) Nature Communications, 14 (1), art. no. 7864.

[3] Schenkmayerova, A., Toul, M., Pluskal, D., Baatallah, R., Gagnot, G., Pinto, G. P., Santana, V. T., Stuchla, M., Neugebauer, P., Chaiyen, P., Damborsky, J., Bednar, D., Janin, Y. L., Prokop, Z., Marek, M. (2023) Nature Catalysis 6: 23-38.

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