# Crystallographic structures of NanoLuc give a glimpse of its intrinsic dynamics

Guillaume Gotthard*†[1], Joel Walker[2,3], Melissa Saidi[1], Julien Hiblot[4], and Antoine Royant[5]

[1]European Synchrotron Radiation Facility (ESRF) – European Synchrotron Radiation Facility – 71 Avenue des Martyrs, 38043 GRENOBLE CEDEX, France

[2]Promega Bioscience (Promega) – Promega Biosciences LLC, 277 Granada Drive, San Luis Obispo, California 93401, United States

[3]Promega Corporation – Promega Corporation, 2800 Woods Hollow Road, Madison, Wisconsin 53711-5399, United States

[4]Max-Planck-Institut for Medical Research (MPIMF) – Chemical Biology department, Jahnstrasse 29, D-69120 Heidelberg, Germany

[5]Institut de Biologie Structurale (IBS) – Université Grenoble Alpes, Commissariat à l'Énergie Atomique et aux Énergies Alternatives (CEA) - Grenoble, Centre National de la Recherche Scientifique - CNRS – 71, Avenue des Martyrs, F-38000 Grenoble, France

**Abstract**

Bioluminescence is a chemical process that is widespread in Nature. In many cases, it stems from the oxidation of a luciferin molecule that is catalyzed by an enzyme, dubbed luciferase, resulting in the emission of a visible light photon. NanoLuc is a small and bright luciferase derived from the catalytic domain of a shrimp luciferase that uses coelenterazine as substrate. The enzyme has been shown to exhibit a high tolerance for substrate modification, allowing the development of optimized substrates and red-shifted substrate analogues. Despite its paramount interest in the toolbox of cell biologists, its development has been hindered by the lack of structural information. While the recent determination of the crystallographic structure of the apo-enzyme has unveiled its structural organization, it has raised the question of the accommodation of substrates within the active-site. Our work provide a comparison of NanoLuc structures obtained in three different space groups, highlighting the dynamics of loops capping the active-site. In addition, we have determined the first crystallographic structure of NanoLuc in complex with an inhibitor of the luciferase activity. This structure gives a glimpse of the loop rearrangements and key residues that are necessary for substrate accommodation, shedding light for further engineering efforts.

**Keywords:** NanoLuc, crystallographic structure, protein dynamics

*Speaker

†Corresponding author: guillaume.gotthard@esrf.fr