**NanoLuc luciferase may not be as “nano” as thought**

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NanoLuc is a recently developed commercially available bioluminescent enzyme for broad biotechnological and biomedical applications. One of the important declared advantages of NanoLuc is its small size of 171 amino acid residues compared to conventional luciferases from firefly (550 residues) and Renilla reniformis (312 residues). However, the experiments with NanoLuc conducted in our laboratory suggested that NanoLuc may homodimerize during the catalytic cycle, and thus the catalytically active bioluminescent system would be twice as large. Here we present a computational study employing molecular docking and enhanced sampling methods for molecular simulations to study substrate binding, protein dynamics, and dimer dissociation. These findings support the lab-based experiments and provide a bigger picture of the function of this widely used enzyme. Our findings suggest that while NanoLuc is a monomer in a substrate-free solution, in the presence of its substrate it is most likely a dimer, which should be considered when designing experiments with NanoLuc.