

ΔFlucs: Brighter Photinus pyralis firefly luciferases identified by surveying consecutive single amino acid deletion mutations in a thermostable variant light up stem cell therapy for Huntingdon's disease *in vivo*

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The bright bioluminescence catalysed by *Photinus pyralis* firefly luciferase (Fluc) enables a vast array of life science research such as bio imaging in live animals and sensitive *in vitro* diagnostics. The effectiveness of such applications is improved using engineered enzymes that to date have been constructed using amino acid substitutions. We describe Flucs: consecutive single amino acid deletion mutants within six loop structures of the bright and thermostable × 11Fluc. Deletion mutations are a promising avenue to explore new sequence and functional space and isolate novel mutant phenotypes. However, this method is often overlooked and to date there have been no surveys of the effects of consecutive single amino acid deletions in Fluc. We constructed a large semi-rational ΔFluc library and isolated significantly brighter enzymes after finding ×11 Fluc activity was largely tolerant to deletions. Targeting an “omega-loop” motif (T352-G360) significantly enhanced activity, altered kinetics, reduced Km for D-luciferin,

altered emission colours, and altered substrate specificity for redshifted analog DL-infraluciferin. Experimental and *in silico* analyses suggested remodelling of the Ω-loop impacts on active site hydrophobicity to increase light yields. This work demonstrates the further potential of deletion mutations, which can generate useful Fluc mutants and broaden the palette of the biomedical and biotechnological bioluminescence enzyme toolbox. Lastly, we constructed a redshifted deletion mutant which allowed us to track primary stem cells implanted into rat brain models of Huntingdon's disease.

Speaker Biography

Amit Paul Jathoul completed his PhD at the age of 29 years from Cambridge University, United Kingdom. He is a SER CYMRUII Fellow of Cardiff University, United Kingdom. He has 12 publications including an article having been cited over 160 times, and is currently inventing/ developing new and exciting tools for bioluminescence imaging.

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