Engineering mNeon Mutations for the Development of a pH-Sensitive Biosensor With the Assistance of AI Tools

Tank, Anthony (School: North Toole County High School)

mNeon Green is a fluorescent protein that originates from Branchiostoma lanceolatum . The prior studies on mNeon are minimal, however, there are several studies indicating that fluorescent proteins in general, have an affinity for being biosensors. The primary goal of this study is to assess the potential of mNeon as a pH-sensitive biosensor. The secondary goal is to assess AI's ability to decrease engineering constraints (time and total protein mutations) for generating a library, impacting the research process. Artificial intelligence's current implementation in the biomedical fields are preliminary and not well-tested. The purpose of this project was to discover a mutation of mNeon that was pH sensitive and then compare the data to the AI generated estimate. First, I developed a code on Anaconda with Perplexity AI to generate fasta files of 8000 mutations. I ran the mutations through two Colab fold files (pH8 and pH4.5) to determine the rotation of the fluorescent protein mutations. I then compared the variants in both pHs using AI to determine which are most likely to be pH sensitive. To verify these findings, I performed the experimental component of the investigation. I isolated the DNA from mutated colonies, recorded their base fluorescence, added acid to the environment (decreasing the pH) and then retested the fluorescence. Statistical analysis was performed to determine which mutations were pH sensitive. The final step compared the experimental mutations to those predicted by AI.

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