1. Introduction

Alpha-synuclein is a protein that is abundant in the human brain and is found mainly at the tips of nerve cells (neurons) in specialized structures called presynaptic terminals. Recent efforts have shown that alpha-synuclein is the major constituent of Lewy bodies, protein aggregates that are pathological hallmarks of Parkinson’s disease. Given the importance of Parkinson’s disease, there is a critical need for assays capable of quantifying alpha-synuclein aggregation in living systems. We hypothesize that our recently discovered self-assembling nanoluciferase (NanoLuc) fragments can be employed to generate a sensitive luminescence-based assay for alpha-synuclein aggregation in living cells.

2. Alpha-Synuclein

Considered an intrinsically disordered protein, alpha-synuclein aggregates to form insoluble fibrils in pathological conditions characterized by Lewy bodies, such as Parkinson’s disease, dementia with Lewy bodies and multiple system atrophy. These disorders are known as synucleinopathies.

3. Nanoluciferase

Nanoluciferase (NanoLuc) is a newly developed small monomeric luciferase reporter with the brightest bioluminescence reported to date. In our sensor, NanoLuc is utilized as a novel ultrasensitive bioluminescent probe for protein aggregation studies. The N65/E66C split site is highlighted in red.

4. Protein Solubility Sensor Design

Based on the Protein Solubility Assay Schematic above, non-aggregated Alpha Synuclein will spontaneously reassemble with the C-term of nanoluciferase. Reassembled nanoluciferase will produce luminescence.

5. Molecular Cloning

Molecular cloning is employed to produce recombinant DNA for the sensor. The DNA to be cloned (gene encoding alpha-synuclein) is isolated from an organism of interest and treated with restriction enzymes to generate smaller DNA fragments. The fragments are then ligated into a plasmid vector (pET-21a) to generate recombinant DNA molecules and is introduced into a host organism (E. Coli). During reproduction, the recombinant DNA molecules are replicated along with the host DNA. This single cell is grown out exponentially to generate a large amount of bacteria, each of which contain copies of the original recombinant molecule. The resulting bacterial population and the recombinant DNA molecule are "clones."

6. Plasmid Construction

The alpha synuclein insert was amplified using standard High Fidelity Taq Polymerase protocol. The insert was analyzed by gel electrophoresis and successful ligation was confirmed by sequencing. Two mutants of highly contrasting aggregation levels (A30P/ A76P and E46K), were created with site directed mutagenesis.

7. Expected Results

The graph depicts how alpha synuclein mutation affects oligomerization and inclusion formation, enabling the selection of mutants with different effects. Because the A30P/A76P mutant displays the lowest level of aggregation, we expect it to produce the brightest luminescence signal. Likewise, the E46K mutant displays the highest level of aggregation and is expected to produce the lowest luminescence signal in mammalian cells.

8. Luminescence Assay Results

The differences in the luminescence signals between the mutant and wild type alpha synuclein are evident. However, E46K is producing the brightest signal while A30P/A76P is producing the weakest. This trend is opposite from what we expect. We hypothesize that the alpha synuclein may be expressed in the periplasm rather than the cytoplasm, allowing for inconsistent results. This issue may be resolved by expressing 66C nanoluciferase in the periplasm as well.

9. Future Direction

We will continue to optimize our alpha-synuclein assay aggregation assay. In the long term, we will utilize this approach to screen for inhibitors of alpha-synuclein aggregation.

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