Supporting Information

A Luminescence-Based Assay for Monitoring Changes in Alpha-Synuclein Aggregation in Living Cells

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Methods

Cloning: Expression vectors containing unfused N65 (pET-45b) and 66C (pRSF-1b) have been previously described.[1] A DNA sequence encoding WT-αSYN was cloned into a pET-45b vector (Novagen, 71553) containing the N65 sequence using 5' PshAI and 3' BglII restriction sites and was verified by DNA sequencing. Using the resulting WT-αSYN-N65 as a template, αSYN mutants were generated using site-directed mutagenesis (QuikChange Lightning Multi-Site Directed Mutagenesis Kit, Agilent, 210515) according to manufacturer protocols and were verified by DNA sequencing.

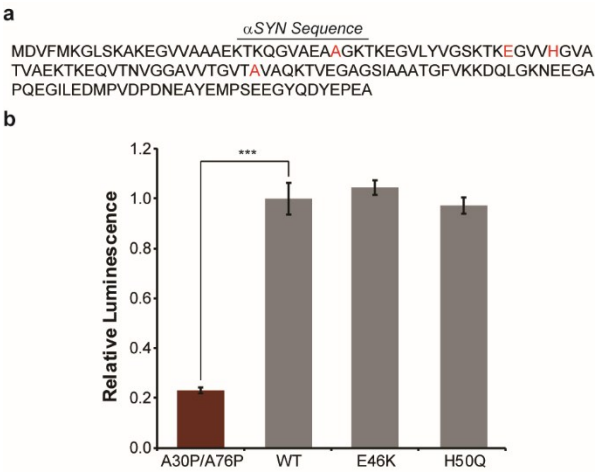
Preparation of EGCG stock: EGCG (Cayman Chemical, 70935) was prepared at 100 mM in DMSO.

Preparation of D-mannitol containing media: Flasks containing terrific broth (TB, 100 mL) liquid culture media were prepared according to manufacturer protocols. D-mannitol was added to the indicated final concentration. Control TB media was also prepared without D-mannitol. The resulting media was then autoclaved prior to use below.

Luminescence solubility assays: Assays were conducted as previously described.[2] BL21-Gold(DE3) (Agilent, 230132) cells were transformed with the indicated constructs described above and were grown to confluency overnight in TB (5 mL) with shaking at 37 °C in the presence of ampicillin (100 μg/mL) and kanamycin (50 μg/mL). The optical density of each culture was measured at 600 nm and cultures were diluted to an OD_{600} = 0.1 in TB or TB containing D-mannitol as indicated. The cells were then grown with shaking at 37 °C until an OD_{600} = 0.6 - 0.8 was reached. Cultures were induced with IPTG (0.2 mM) and EGCG was added where indicated. Cultures were incubated overnight at 16 °C with shaking. The next morning, the OD_{600} of each culture was measured and normalized to 3.0 in 1 mL. Cells were harvested by centrifugation at 700g for 10 min at 4 °C and resuspended in 200 μL of 1× Nluc assay buffer (50 mM MES pH = 6.0, 0.5 mM EDTA, 75 mM KCl, 1 mM 2-mercaptoethanol, and 17.5 mM thiourea). The cells were then mixed with an equal volume (200 μL) of 1× Nluc buffer containing 50 μM coelenterazine

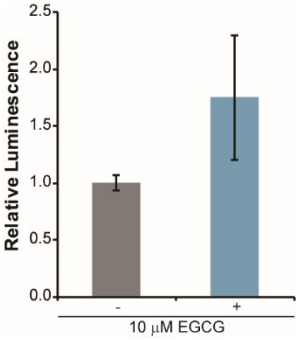
(GoldBio, CZ2.5, prepared as a 2.5 mM stock in acidified ethanol). The resulting assay mixtures were then loaded into a Corning 384-well assay plate (3824, white, low volume, flat bottom, 40 µL reaction volume). Luminescence intensities were determined using a SynergyH1 hybrid reader (BioTek) at 30 minutes after substrate addition.

Figure S1



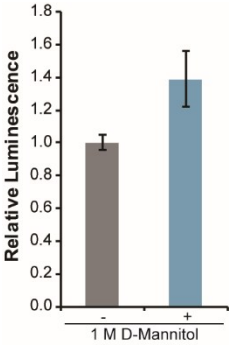
Mutations that increase oligomer formation lead to decreased luminescence. a) The amino acid sequence of αSYN is shown with mutation sites indicated in red. b) Luminescence from bacterial cells expressing the indicated mutant or wild-type (WT) αSYN fused to N65 in the presence of 66C. Error bars represent the standard deviation of two (A30P/A76P) or three (WT, E46K, and H50Q) biological replicates assayed in triplicate. *** indicates a p-value of <0.001.

Figure S2



Increasing the concentration of EGCG does not lead to a significant increase in luminescence relative to 1 μM EGCG (Fig. 3c). Error bars represent the standard deviation of three biological replicates assayed in triplicate.

Figure S3



Increasing the concentration of D-mannitol results in a decrease in luminescence relative to 0.5 M D-mannitol (Fig. 3d). Error bars represent the standard deviation of two biological replicates assayed in triplicate.

Table S1: Amino Acid Sequences for Constructs Used in This Work

Protein	Amino Acid Sequence
WT-αSYN-N65	MAHHHHHHVGTGSNDDDDKSPMDVFMKGLSKAKEGVVAAAEKTKQGVAEA AGKTKEGVLYVGSKTKEGVVHGVATVAEKTKEQVTNVGGAVVTGVTAVAQ KTVEGAGSIAAATGFVKKDQLGKNEEGAPQEGILEDMPVDPDNEAYEMPS EEGYQDYEPEAQISYASRGGGSSGGGELMVFTLEDFVGDWRQTAGYNLDQ VLEQGGVSSLFQNLGVSVTPIQRIVLSGENGLKIDIHVIIPYE
A30P-N65[a]	MAHHHHHHVGTGSNDDDDKSPMDVFMKGLSKAKEGVVAAAEKTKQGVAEA PGKTKEGVLYVGSKTKEGVVHGVATVAEKTKEQVTNVGGAVVTGVTAVAQ KTVEGAGSIAAATGFVKKDQLGKNEEGAPQEGILEDMPVDPDNEAYEMPS EEGYQDYEPEAQISYASRGGGSSGGGELMVFTLEDFVGDWRQTAGYNLDQ VLEQGGVSSLFQNLGVSVTPIQRIVLSGENGLKIDIHVIIPYE
A53T-N65[a]	MAHHHHHHVGTGSNDDDDKSPMDVFMKGLSKAKEGVVAAAEKTKQGVAEA AGKTKEGVLYVGSKTKEGVVHGVTTVAEKTKEQVTNVGGAVVTGVTAVAQ KTVEGAGSIAAATGFVKKDQLGKNEEGAPQEGILEDMPVDPDNEAYEMPS EEGYQDYEPEAQISYASRGGGSSGGGELMVFTLEDFVGDWRQTAGYNLDQ VLEQGGVSSLFQNLGVSVTPIQRIVLSGENGLKIDIHVIIPYE
G51D-N65[a]	MAHHHHHHVGTGSNDDDDKSPMDVFMKGLSKAKEGVVAAAEKTKQGVAEA AGKTKEGVLYVGSKTKEGVVHDVATVAEKTKEQVTNVGGAVVTGVTAVAQ KTVEGAGSIAAATGFVKKDQLGKNEEGAPQEGILEDMPVDPDNEAYEMPS EEGYQDYEPEAQISYASRGGGSSGGGELMVFTLEDFVGDWRQTAGYNLDQ VLEQGGVSSLFQNLGVSVTPIQRIVLSGENGLKIDIHVIIPYE
A30P/A76P-N65[a]	MAHHHHHHVGTGSNDDDDKSPMDVFMKGLSKAKEGVVAAAEKTKQGVAEA PGKTKEGVLYVGSKTKEGVVHGVATVAEKTKEQVTNVGGAVVTGVTPVAQ KTVEGAGSIAAATGFVKKDQLGKNEEGAPQEGILEDMPVDPDNEAYEMPS EEGYQDYEPEAQISYASRGGGSSGGGELMVFTLEDFVGDWRQTAGYNLDQ VLEQGGVSSLFQNLGVSVTPIQRIVLSGENGLKIDIHVIIPYE
E46K-N65[a]	MAHHHHHHVGTGSNDDDDKSPMDVFMKGLSKAKEGVVAAAEKTKQGVAEA AGKTKEGVLYVGSKTKKGVVHGVATVAEKTKEQVTNVGGAVVTGVTAVAQ KTVEGAGSIAAATGFVKKDQLGKNEEGAPQEGILEDMPVDPDNEAYEMPS EEGYQDYEPEAQISYASRGGGSSGGGELMVFTLEDFVGDWRQTAGYNLDQ VLEQGGVSSLFQNLGVSVTPIQRIVLSGENGLKIDIHVIIPYE
H50Q-N65[a]	MAHHHHHHVGTGSNDDDDKSPMDVFMKGLSKAKEGVVAAAEKTKQGVAEA AGKTKEGVLYVGSKTKEGVVQGVATVAEKTKEQVTNVGGAVVTGVTAVAQ KTVEGAGSIAAATGFVKKDQLGKNEEGAPQEGILEDMPVDPDNEAYEMPS EEGYQDYEPEAQISYASRGGGSSGGGELMVFTLEDFVGDWRQTAGYNLDQ VLEQGGVSSLFQNLGVSVTPIQRIVLSGENGLKIDIHVIIPYE
66C	MGGLSGDQMGQIEKIFKVVYPVDDHHFKVILHYGTLVIDGVTPNMIDYFG RPYEGIAVFDGKKITVTGTLWNGNKIIDERLINPDGSLLFRVTINGVTGW RLCERILALQGSELHHHHHH

Amino acid sequences of aggregating proteins are underlined. [a]The point mutations of αSYN are denoted in red.

References

1. J. Zhao, T. J. Nelson, Q. Vu, T. Truong and C. I. Stains, *ACS Chem. Biol.*, 2016, **11**, 132-138.
2. T. J. Nelson, J. Zhao and C. I. Stains, *Methods Enzymol.*, 2019, **622**, 55-66.