DISCOVERING SMALL-MOLECULE MODULATORS OF AUTOPHAGY FOR NEURODEGENERATION

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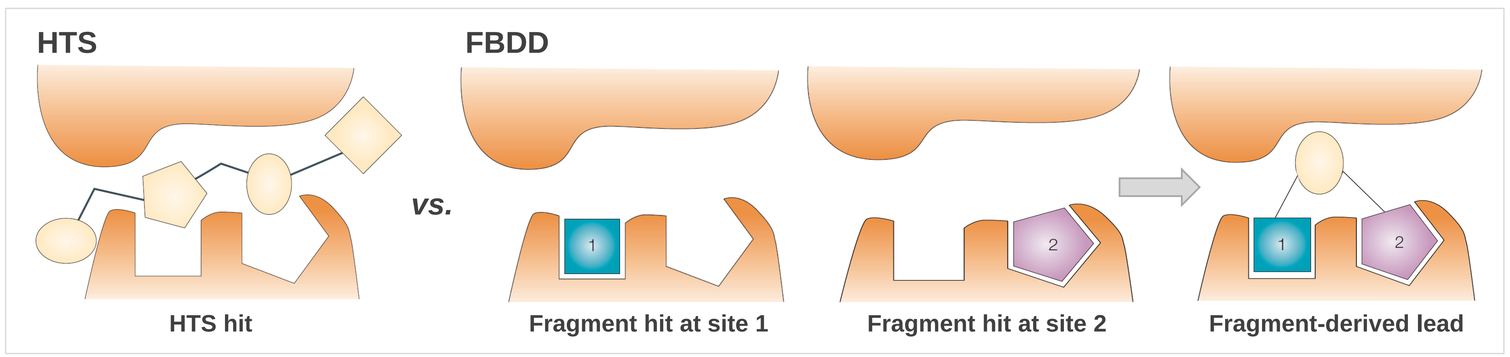
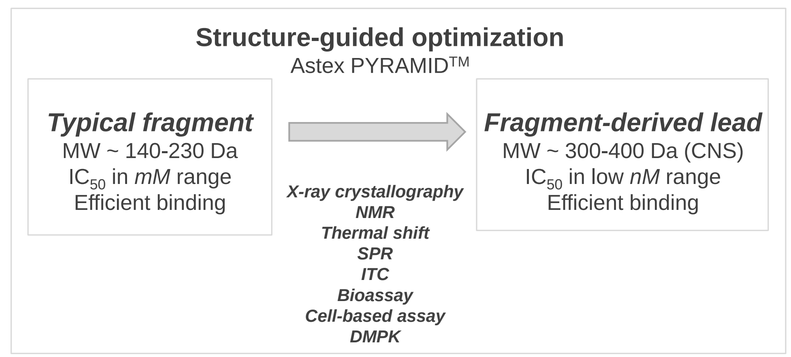
OUR DRUG DISCOVERY APPROACH

- Autophagy is a major protein degradation pathway with proven roles in protecting neurons against accumulation of aggregation-prone proteins and obsolete mitochondria. Genetics of human-disease and mouse-knockout studies highlight the connection between autophagy dysfunction and neurodegeneration, and therefore finding a way to augment autophagic-flux seems a promising therapeutic strategy (Rubinsztein DC, Bento CF & Deretic V. Journal of Experimental Medicine 2015; Bento CF et al. *Annual Review of Biochemistry* 2016).
- Fragment-based drug discovery (FBDD) has the potential to deliver potent, selective and CNS-penetrant small-molecules capable of inducing autophagy in the brain. For that purpose, the use of cell-based assays that accurately monitor the ability of small-molecules to modulate autophagy in a high-throughput- and unbiased-manner is instrumental.



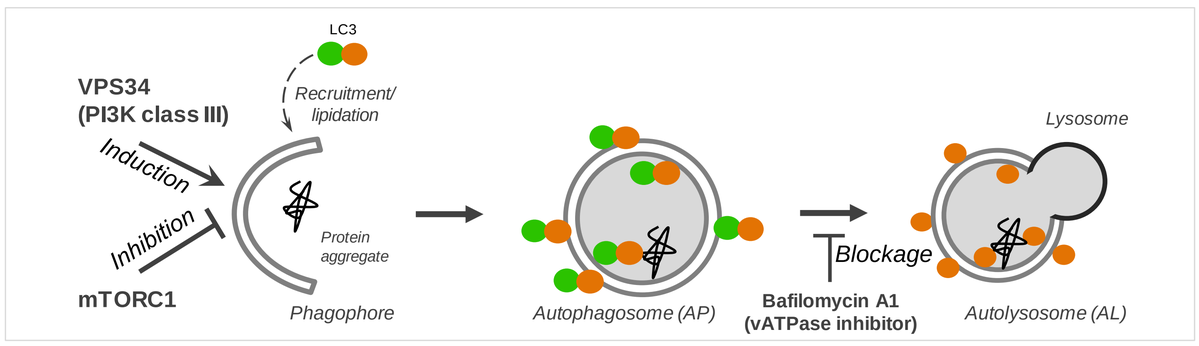


Adapted from Rees D *et al*. *Nature Reviews Drug Discovery* 2004

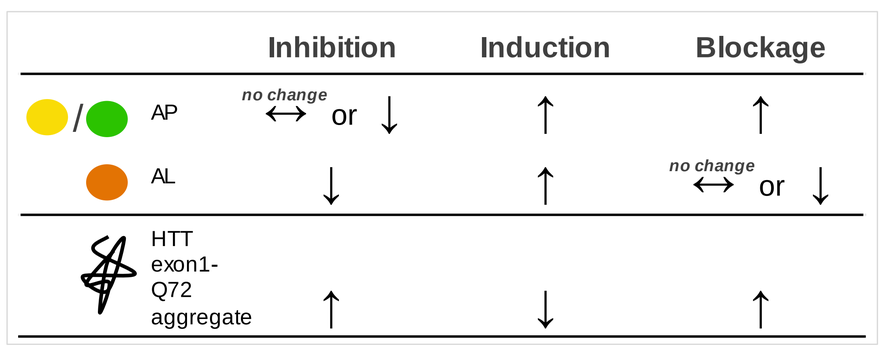
ASSAY DEVELOPMENT

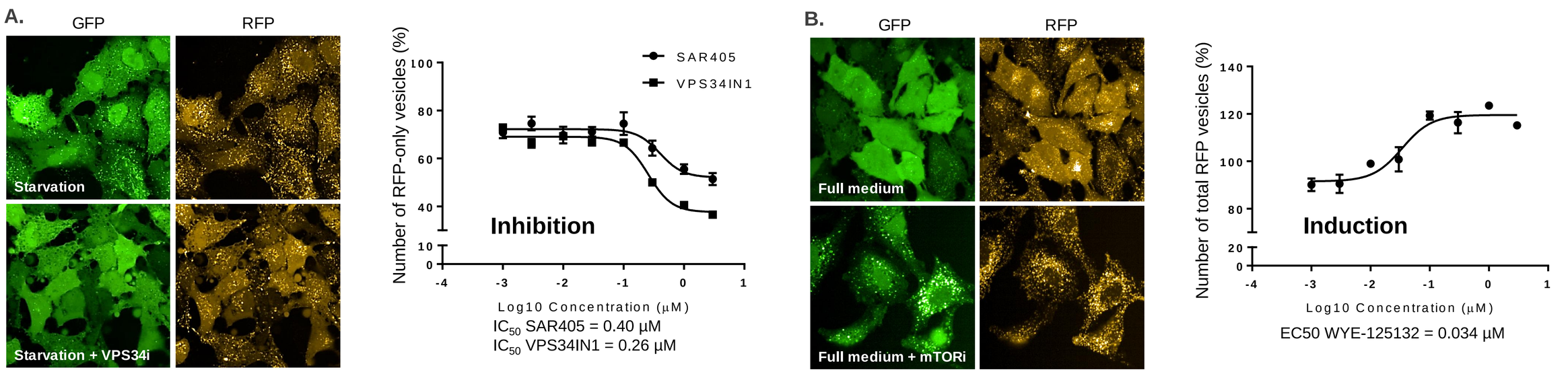
- High-content confocal imaging (Opera Phenix HCI, PerkinElmer™) of stable cell lines expressing *tandem* RFP-GFP-LC3 (Fig. A, B, C and D) or GFP-HTT-exon1-Q72 (Fig. E) was used for assay development and target validation. The RFP-GFP-LC3 cell-based assay exploits the properties of GFP and RFP; GFP fluorescence is quenched by the low pH of the lysosome when autophagosomes fuse with lysosomes, while RFP fluorescence is more stable in acidic compartments, which means that autophagosomes are labelled yellow (green and red merge) and autolysosomes are red only. Measurement of HTT-exon1-Q72 aggregates clearance rate was also used for studying autophagy and aggrephagy modulation by small molecules.

Assay rationale



Possible scenarios





Figures A and B. The use of small-molecule inhibitors against the well-established autophagy regulators VPS34 (SAR405 by *Sanofi* and VPS34IN1 by Univ. of Dundee & *AstraZeneca*) and mTOR (WYE-125132 by *Wyeth* is given as an example, but other available mTOR kinase inhibitors performed similarly) enabled us to accurately measure autophagic-flux changes and validate the assay. VPS34 inhibitors consistently showed autophagy inhibition under starvation conditions, with a decrease in autolysosome numbers **(Fig. A)**, while mTOR inhibitory-chemotypes showed a dose-dependent increase in numbers of autophagosomes and autolysosomes in full medium conditions, indicating increased autophagic flux **(Fig. B)** (Z-factors > 0.5).

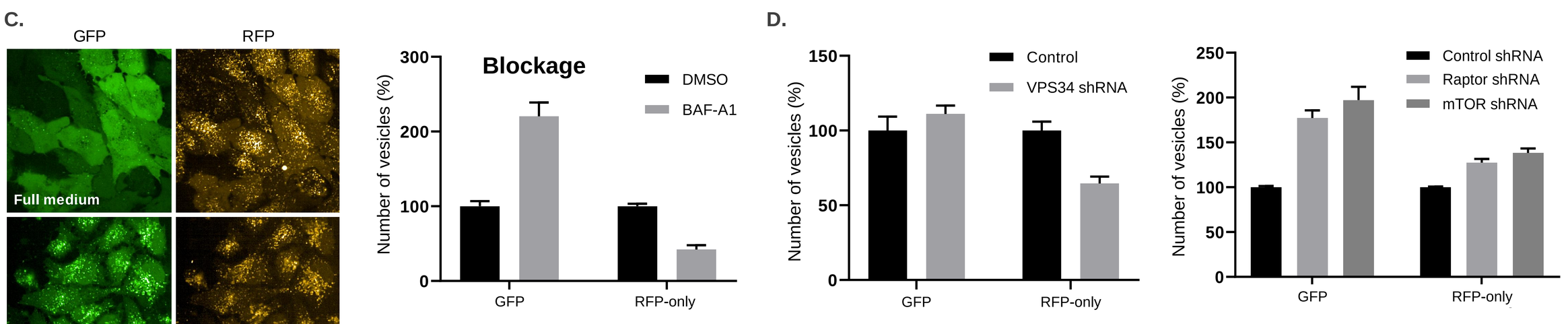


Figure C. The assay shows robustness in identifying autophagy blockers and distinguishing these from autophagy inducers (Fig. B), otherwise impossible with more conventional assays such as counting number of vesicles solely based on GFP-LC3 expression.

Figure D. Genetic-depletion of VPS34, mTOR or Raptor (mTORC1 subunit) confirmed on-target effects.

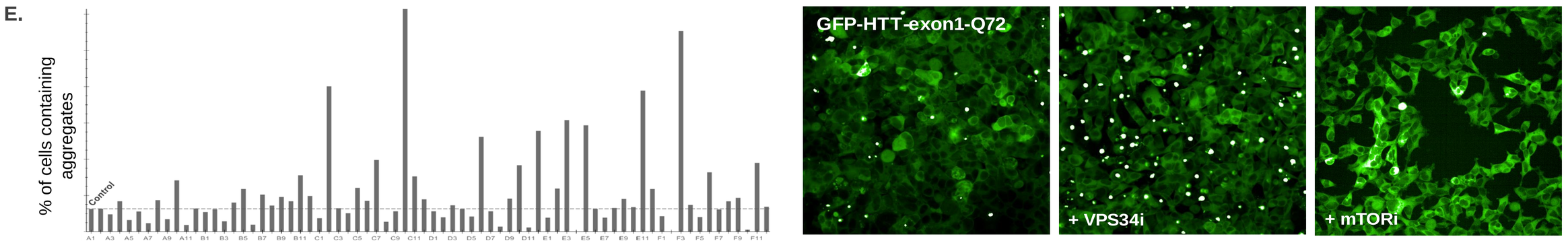


Figure E. Counting number of GFP-HTT-exon1-Q72 aggregates in cells also enables identification and validation of autophagy modulators. For instance, VPS34 and mTOR inhibition led to clear changes in the percentage of cells containing aggregates, which is compatible with a role in controlling the autophagic flux.

- The assays outlined here proved to be robust for identification and validation of genetic and pharmacological modifiers of autophagy, and therefore have the potential to enable the discovery of autophagy inducers, possibly capable of modulating pathological mechanisms associated with neurodegeneration.

 Poster presented at the 14th International Conference on Alzheimer's and Parkinson's Diseases, 2019. (Poster available to download from Astex's website at www.astx.com)