Developing a robust **vATPase** assay to identify novel therapies for **Alzheimer disease**

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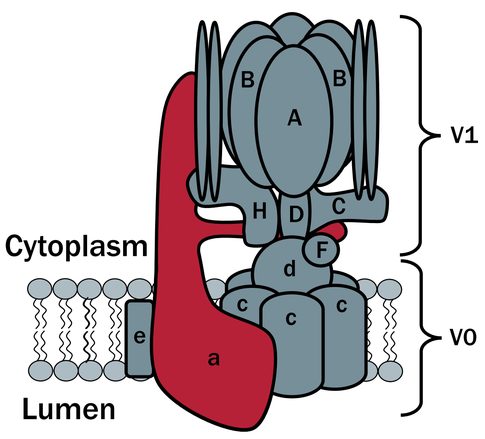
Abstract

- **Lysosomal dysfunction** is well known to cause Lysosomal Storage Disorders, the common cause of childhood neurodegeneration. However, more recently lysosomal dysfunction has also been implicated in diseases of aging, such as **Alzheimer disease (AD)**.
- The **vacuolar-ATPase (vATPase)** is an ATP dependant proton pump which maintains lysosomal pH. Dysfunction prevents protons from entering the lysosome, causing a rise in pH.
- There is both **genetic and functional data linking disrupted vATPase function to AD**. Mutations in the V0a3 subunit cause early onset AD[1], and Presenilin 1 familial Alzheimer disease causes a defect in vATPase processing that is central to pathogenesis[2].
- Boosting vATPase function is therefore a **potential therapeutic strategy** for AD. However, there are currently no good chemical starting points to achieve this. To identify **novel activators**, we have developed a high content imaging assay for lysosomal pH.
- This assay uses cells treated with a low concentration of the vATPase inhibitor bafilomycin A1 to mimic the lysosomal deacidification seen in AD, and pH-insensitive and sensitive **fluorophore-conjugated dextrans**, as markers for the lysosome and its pH.
- We have **validated the assay** using known inhibitors and activators of vATPase, and now intend to use it in a **high throughput screen**.

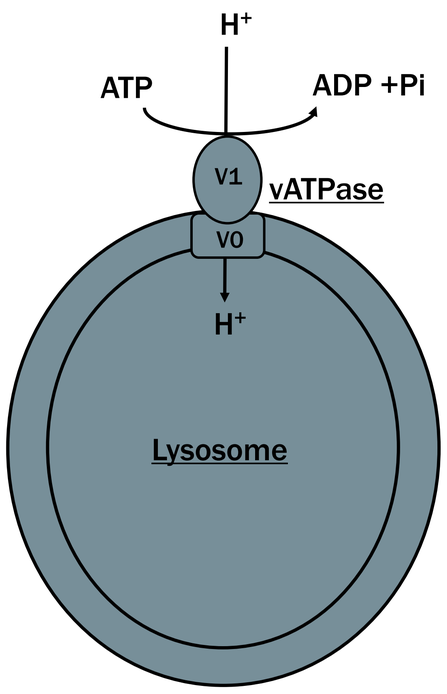
Background

vATPase Structure



The V0a subunit (in red) has been implicated in AD

vATPase in the lysosome



Methods

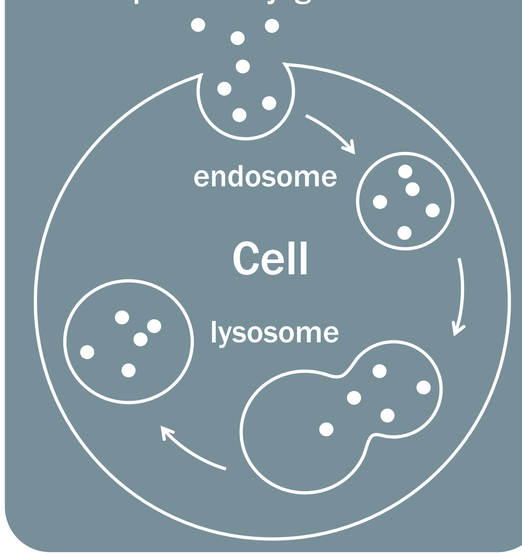
Cellular **vATPase activity assay** using lysosomal pH as a marker for activity.

Assay Components			
	Bafilomycin A1 inhibits vATPase, causing lysosomal deacidification	**Texas red** conjugated dextran used as a lysosomal marker	**FITC** conjugated dextran used as a marker for pH

- **24 hr pulse** — Add FITC, Texas red and treat with compound
- **24 hr chase** — Change media, treat with Bafilomycin, and re-add compound
- **30 min** — Stain with Hoechst and add HBSS+

Image cells on the Operetta to read fluorescence

How **dextrans** enter the **lysosome**



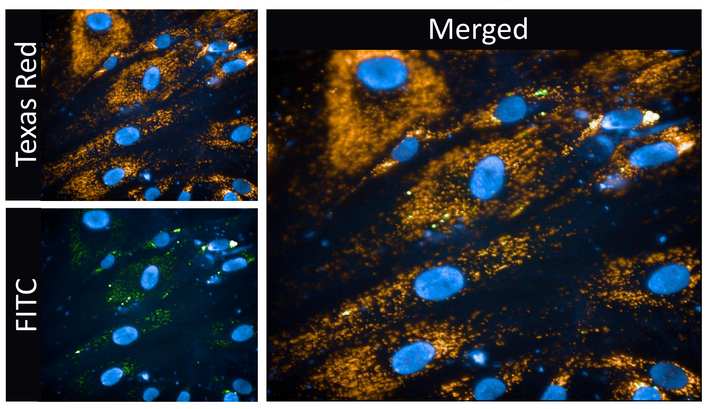
How are the images analysed?

Find cells with Hoechst
↓
Select lysosomes with Texas Red
↓
Read FITC intensity inside lysosomes

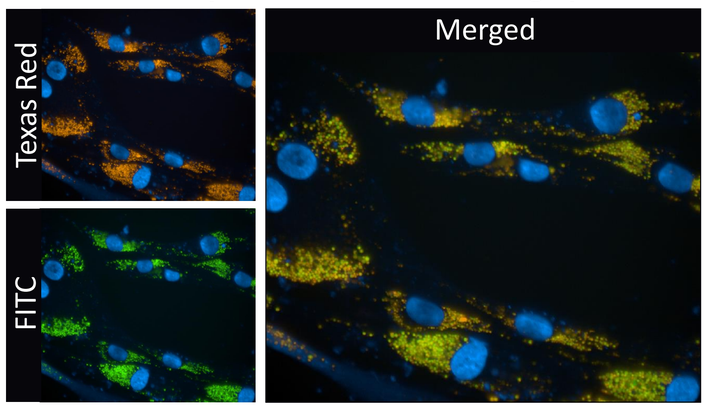
Operetta images

Examples of the images collected by the **Operetta** plate reader. These are then analysed using **Harmony** software.

Untreated cells with low pH

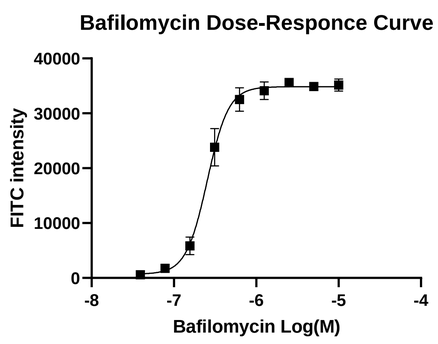


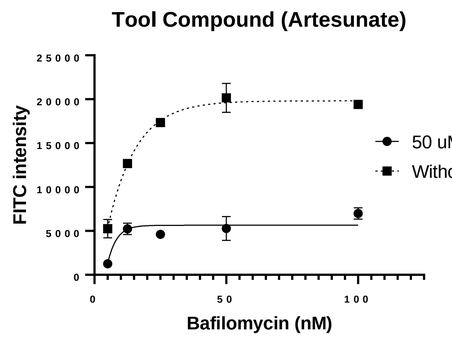
Bafilomycin treated cells with high pH

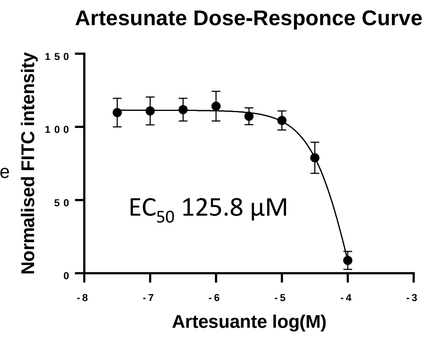


Assay Validation

The assay was validated with a **bafilomycin** dose-response curve, testing the bafilomycin response with and without the **tool compound artesunate**, followed by an artesunate dose-response curve.







Conclusion

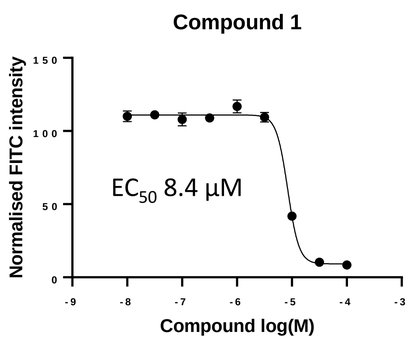
- A **vATPase cellular assay** was successfully established.
- We are now ready to apply for funding to run a **HTS**.
- In-house, we have found **compounds that activate vATPase**, with IC_{50} values of < 30 µM.

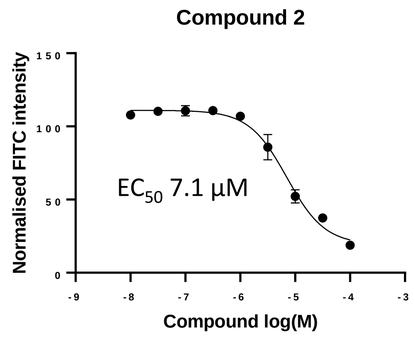
Future Research

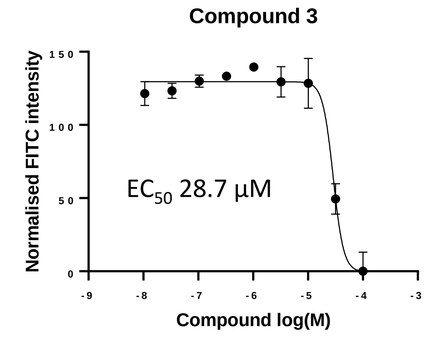
- To run a **high-throughput screen**
- I have developed an **in vitro assay** from the literature, I will be testing compounds to **validate** these results
- Look at **pH calibration**

Compound Dose-Response Curves

Dose-response curves for some of the compounds, with **EC_{50} values.**







References

1. Song Q, et al., (2020). The emerging roles of vacuolar-type ATPase-dependent lysosomal acidification in neurodegenerative diseases. Translational Neurodegeneration 9:17.
2. Lee J, et al., (2010). Lysosomal proteolysis and autophagy require presenilin 1 and are disrupted by Alzheimer-related PS1 mutations. Cell 141(7):1146-1158.