Development of a novel anthraquinone-derived fluorescent lysosomal probe

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Introduction

- Lysosomes present a unique intracellular environment, being comprised of lipid bilayer, containing a highly acidic interior, with several acid hydrolase enzymes.
- Partial or complete loss of any of the acid hydrolase enzymes often leads to impaired lysosomal function, leading to increases in both lysosome volume and number of lysosomes[1].
- Lysosomal dysfunction is recognised in several diseases, in addition to being the primary cause of over 50 lysosomal storage disorders. Therefore, the ability to visualise lysosomes is essential for both improving our understanding of diseases with lysosomal associated pathology and in development of new therapies to improve lysosomal function.
- Current fluorescent lysosomal probes have drawbacks to their use, including photobleaching and affecting cellular phenotypes by alkalising lysosomes[2]. The creation of novel lysosomal probes, without the undesirable characteristics, allows for increased imaging capabilities and assay potential.
- Preliminary work showed that anthraquinone derivatives can be used as cellular probes, showing strong fluorescence and chemical stability[3].

Aims and Objectives

- Test a series of far-red anthraquinone derivatives to determine the cellular toxicity of each molecule.
- Characterise the lead probe, AQ7, to confirm lysosomal localisation.
- Identify If AQ7 is shows pH sensitivity *in-vitro*.

AQ7 Cellular trafficking

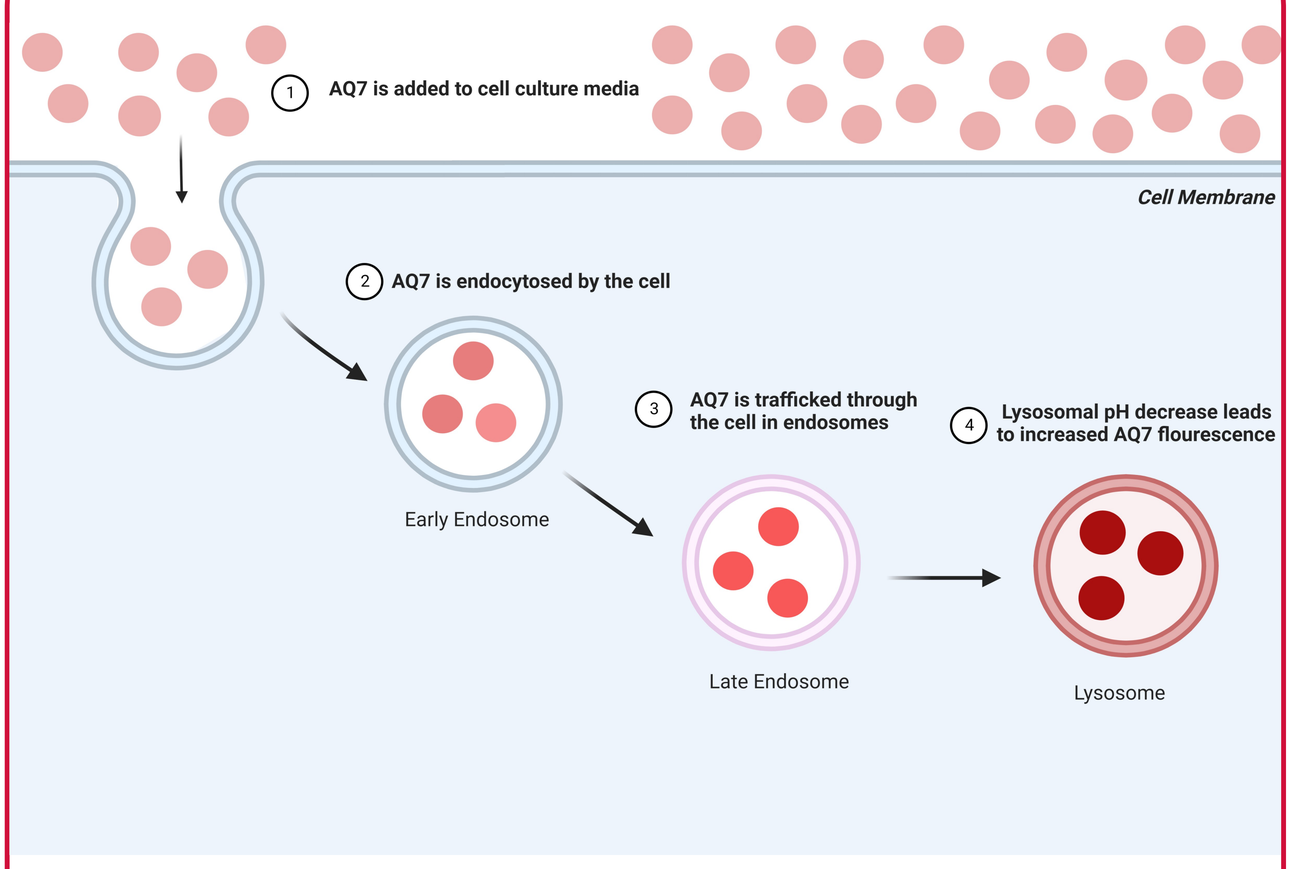

Figure 1. *Mechanism by which AQ7 enters cells and is trafficked through the endocytic system to lysosomes. Increasing pH from early endosomes to lysosomes leads to increasing fluorescence from AQ7.*

Conclusions

- We have charactertsed a series of far-red flourescenct anthraquinone derivative molecules using high content imaging. The lead probe, AQ7, specifically locates to lysosomes, with no observable cellular toxicity at working concentrations. Additionally, we have shown that AQ7 fluorescence is pH sensitive, and can be used to monitor lysosomal pH *in-vitro.*
- AQ7 may be a superior live cell lysosomal marker for drug discovery than commercially available lysosomal probes.

Methods

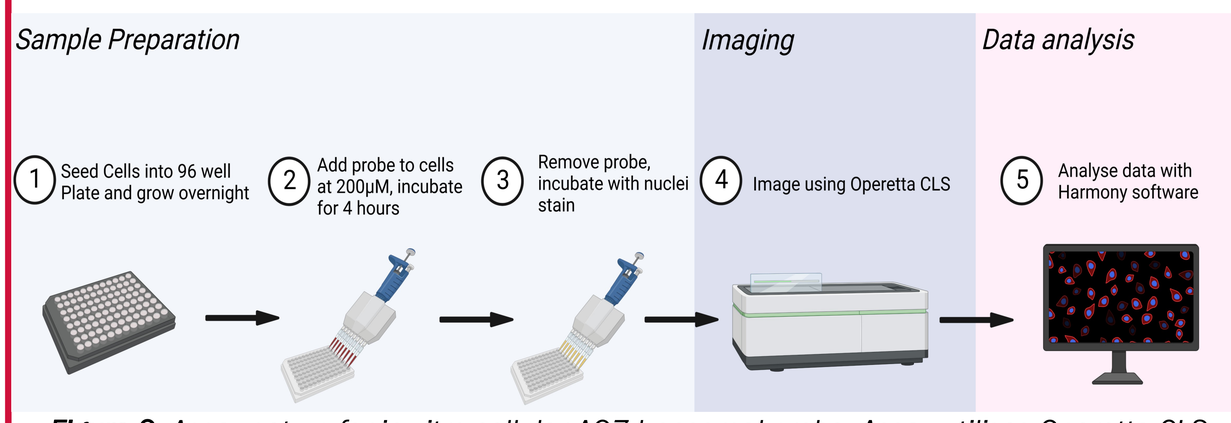

Figure 2. *Assay set up for in-vitro cellular AQ7 lysosomal probe. Assay utilises Operetta CLS high content microscopy in combination with Harmony data analysis software for data generation.*

Results

Anthraquinone derivative comparison

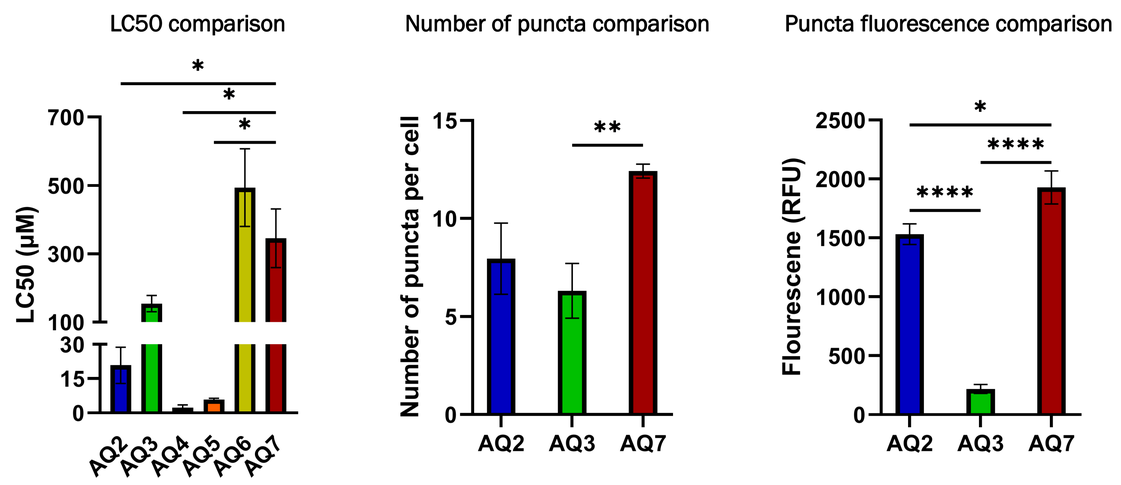

Figure 3. *Of the 6 anthraquinone derivates created, 3 were taken forward for in-vitro imaging. Comparison of both the number of puncta identified and the corresponding fluorescence intensity of puncta was shown to be highest in the cells treated with AQ7.*

AQ7 and LysoTracker green co-localisation

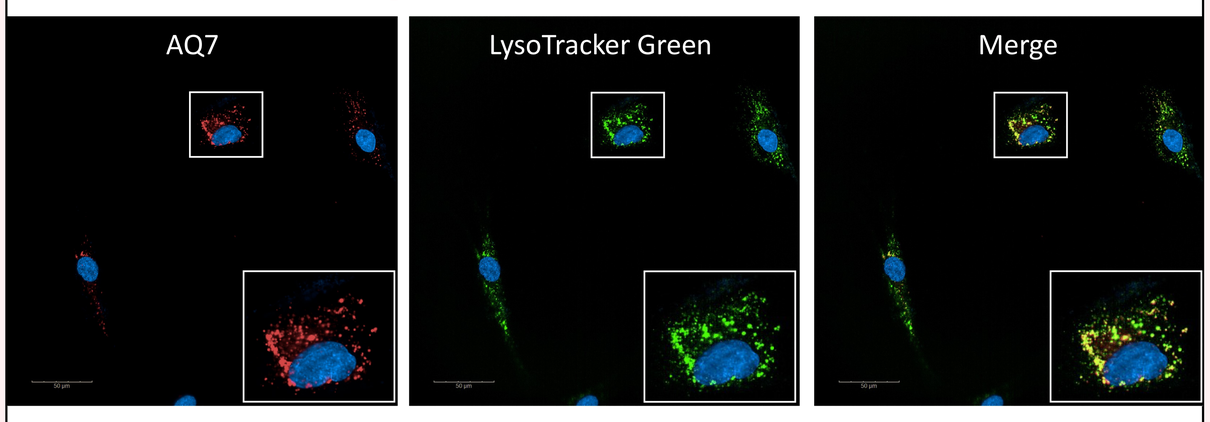

Figure 4. *Example images showing co-localisation between AQ7 and LysoTracker green, confirming that AQ7 is a lysosomal specific probe. Puncta analysis shows that an average of 94% of LysoTracker green puncta also had a corresponding overlapping puncta of AQ7.*

AQ7 pH sensitivity

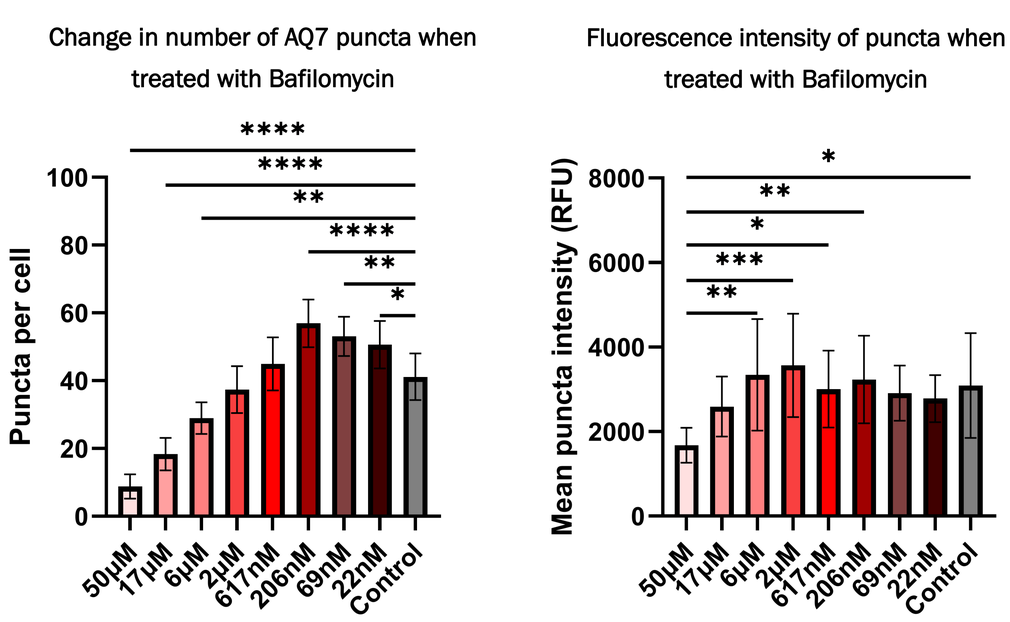

Figure 5. *Cells treated with increasing concentrations of a known lysosomal de-acidifier (Bafilomycin) showed less puncta of AQ7. Other than the highest concentration of Bafilomycin, no change in fluorescence intensity was observed, showing the pH sensitivity of AQ7.*

References

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