

Despite growing understanding of the cellular and molecular basis of ARSACS pathology, no disease-modifying treatment has been approved. This project builds on our previous large-scale drug screen in ARSACS patient-derived motor neurons and aims to advance potential hits to a validated, well-characterized drug candidate.

The specific objectives of this grant were:

1. Confirmation screen, computational and biophysical validation
2. Functional and *in vivo* testing of the lead compounds

Using the Ca^{2+} -binding fluorescent probe Fluo-4 AM and a multi-well plate reader, we measured intracellular calcium flux as a proxy for neuronal activity (300 readings; 200 ms interval) in a high-throughput manner. We tested ~1000 FDA-approved small molecules to determine whether they could normalize neuronal hyperexcitability in iPSC-derived motor neurons from ARSACS patients. We identified potential hits from the primary calcium-flux screen and confirmed their efficacy in a secondary screen. We prioritized compounds with efficacy and minimal cytotoxicity and advanced them for computational structural docking against sacs1n. Of these, several compounds showed the strongest predicted binding affinity *in silico* and were carried forward for biophysical validation, including thermal shift assay (TSA) and surface plasmon resonance (SPR).

Based on biophysical validation of the lead compounds, we have advanced the top candidates to determine whether they can functionally reverse cellular phenotypes associated with sacs1n deficiency, including mitochondrial function and dynamics, cytoskeletal organization, neurofilament abnormalities, proteostasis, and neuronal excitability. We are drafting findings from our previous and current grant in parallel and expect to post a preprint with all findings before formal peer review is complete.

This project aims to identify small molecules that directly engage sacs1n in ARSACS. By combining high-throughput screening, computational prediction, and rigorous biophysical, functional, and *in vivo* validation, this work will move the field closer to identifying potential drug candidates for ARSACS.