# Early-stage screen for small-molecule stabilisers to treat 55LCC-associated disorders

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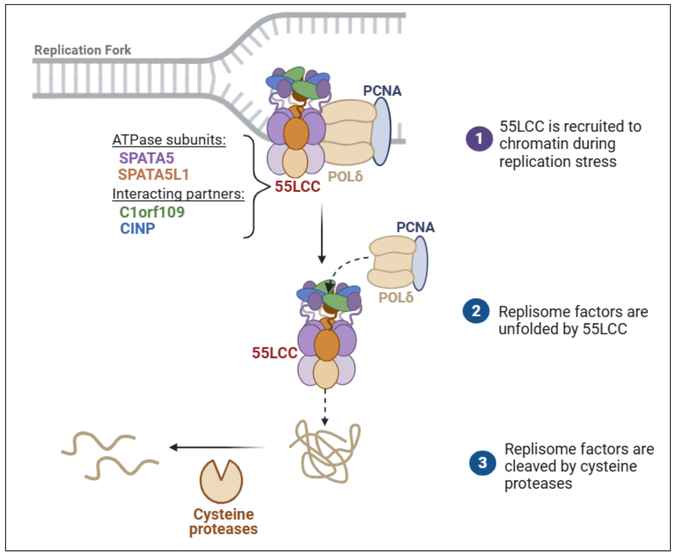
## Introduction

### 55LCC promotes genomic stability during DNA replication

- 55LCC (SPATA5–SPATA5L1–C1orf109–CINP) is an AAA+ ATPase with unfolding activity (1).
- It mediates replisome disassembly at stalled replication forks via a ubiquitin-proteasome-independent pathway.
- This promotes replisome proteostasis and fork progression.




- Mutations in SPATA5 destabilise 55LCC leading to severe childhood neurological disorders (2).
- Stabilising SPATA5 with small molecules may restore 55LCC integrity and function and these diseases.

### Project aims

- Optimise spectral shift assay to detect SPATA5-ligand binding
- Apply this assay to screen three small-molecule compounds and determine their binding affinity


## Acknowledgements

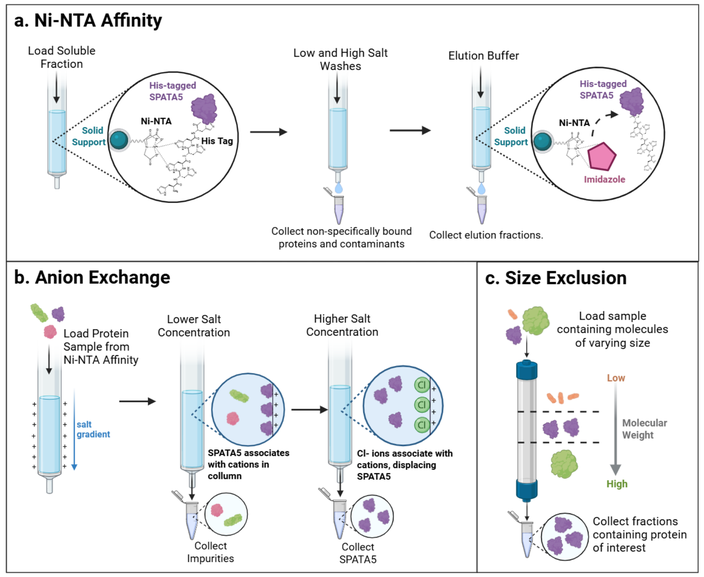
Many thanks to my supervisors Professor Elton Zeqiraj and Dr Martina Foglizzo for their support and guidance.


## Key References

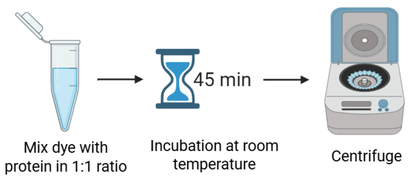

1. Krishnamoorthy, V., Foglizzo, M., Dilley, R.L., Wu, A., Datta, A., Dutta, P., Campbell, L.J., Degtjarik, O., Musgrove, L.J., Calabrese, A.N., Zeqiraj, E. and Greenberg, R.A. 2024., The SPATA5-SPATA5L1 ATPase complex directs replisome proteostasis to ensure genome integrity. Cell. 187(9), pp.2250–2268.e31.
2. Tanaka, A.J., Cho, M.T., Millan, F., Juusola, J., Retterer, K., Joshi, C., Niyazov, D., Garnica, A., Gratz, E., Deardorff, M., Wilkins, A., Ortiz-Gonzalez, X., Mathews, K., Panzer, K., Brilstra, E., van Gassen, K.L.I., Volker-Touw, C.M.L., van Binsbergen, E., Sobreira, N., Hamosh, A., McKnight, D., Monaghan, K.G. and Chung, W.K. 2015., 'Mutations in SPATA5 are associated with microcephaly, intellectual disability, seizures, and hearing loss', American Journal of Human Genetics, 97(3), pp. 457–464.


## Methods

### Purification of His6-tagged SPATA5

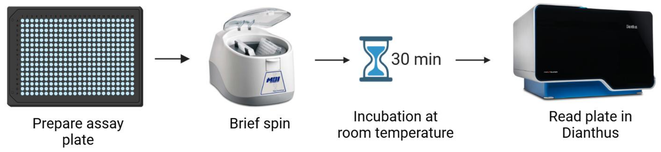



### Fluorescence labelling




SPATA5 labelled with RED-tris-NTA dyes to enable spectral shift detection

### Spectral shift assay optimization




- Selected optimal dye
- Validated ATPγS as positive control
- Assessed DMSO tolerance

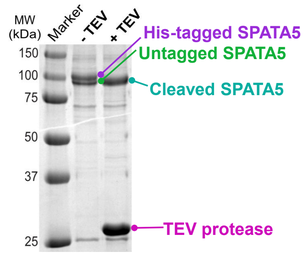
### Compound screening

Binding of small molecules quantified via dose–response spectral shift analysis.

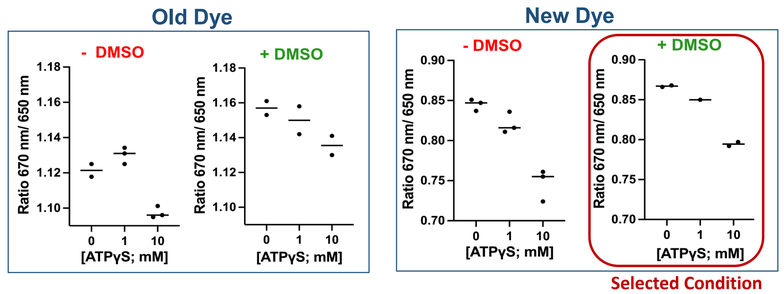
## Results

### Validation of purified SPATA5 by TEV protease cleavage

- Band at ~100 kDa confirms SPATA5
- TEV cleavage causes band shift, confirming presence of His6-tag



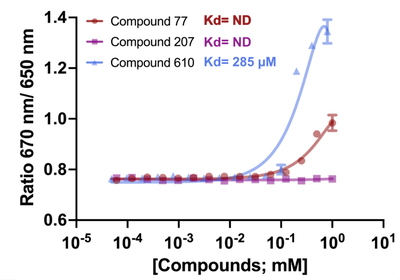

### Optimisation of spectral shift assay conditions for SPATA5




- ATPγS showed dose-dependent binding, validating the assay.
- DMSO affected signal with the old dye but had minimal impact with new dye.
- New dye showed a clearer and more consistent dose-dependent response.

### Identification of SPATA5-binding compounds

- Compound 610 generated a dose-response curve, enabling estimation of apparent Kd.
- Compound 77 showed binding but did not reach saturation.
- No binding detected for compound 207




## Conclusions & Future Directions

- ✓ Robust spectral shift assay established for detecting SPATA5-ligand interactions.
- ✓ Two potential binders (compounds 77 and 610) identified.
- ○ **Limitations:** DMSO tolerance, compound solubility, and limited replicates prevented precise affinity determination.
- ➢ **Therapeutic relevance:** Provides a foundation for developing SPATA5 stabilisers as potential therapeutics to rescue 55LCC function.
- ➢ **Future directions:** Expand compound screening to larger libraries and characterise interactions further via functional, kinetic, and structural studies.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