PIN-ING IT DOWN: COOPERATIVE DESTABILISATION OF PIN1 TO TREAT CANCER

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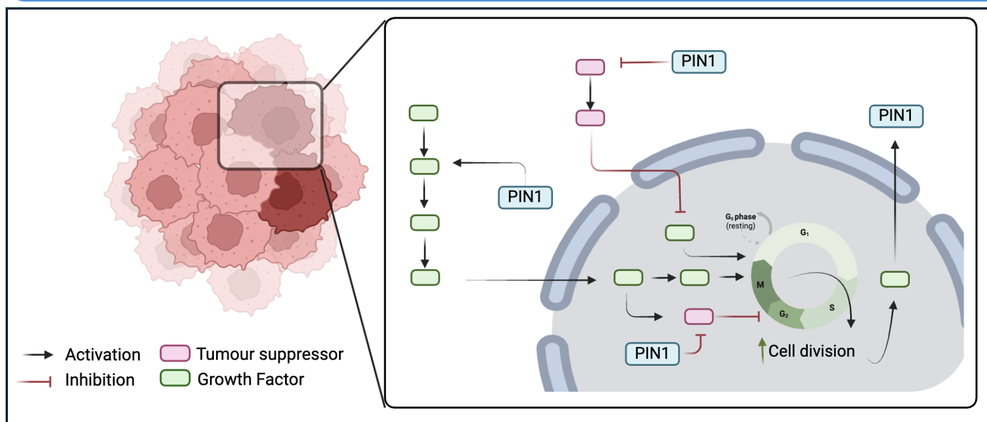
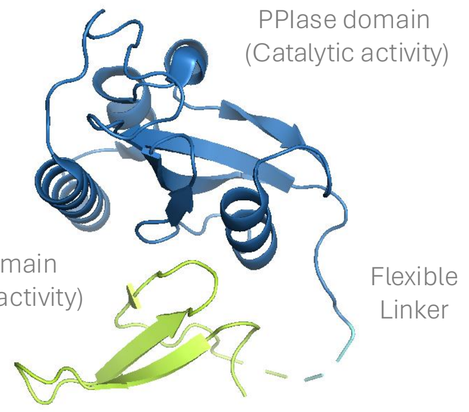
WHY TARGETING PIN1 FOR CANCER THERAPY?

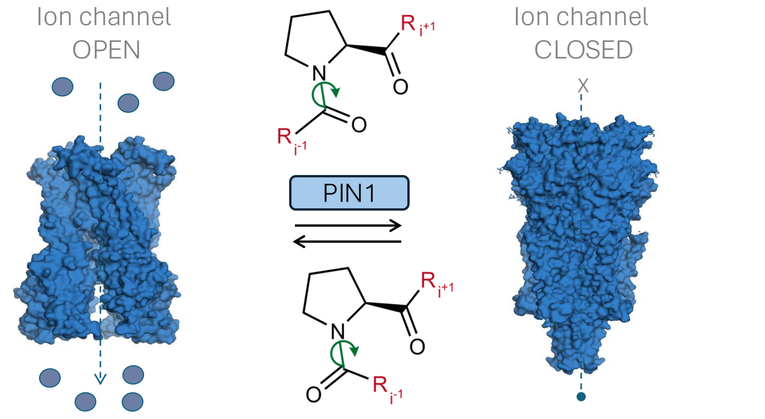


Pin1 is a hub protein involved in cancer progression, activating oncogenes or growth factors, and inhibiting those proteins that stop the cell cycle, tumour suppressors [1].

INTRODUCTION TO PIN1

Pin1 structure consists of two different domains, WW domain and a PPIase domain, which involves the catalytic domain, connected by a linker [2]. (PDB:6VAJ)





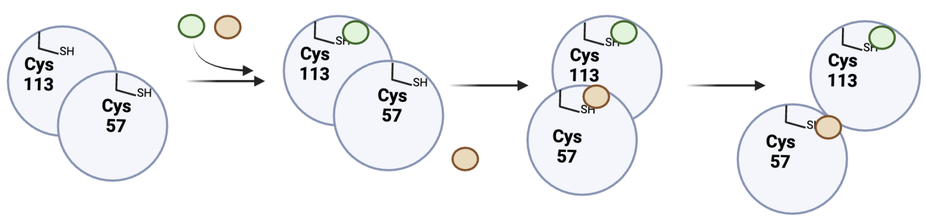
Pin1 catalyses the cis/trans isomerisation of a substrate that is phosphorylated on the Ser/Thr-Pro motif, thus inducing a conformational change. For instance, Pin1 proline isomerisation creates an open/close state of a neurotransmitter ion channel in the cell membrane [3]. (PDB:8C20 and 6Y1Z)

THE CURRENT CHALLENGE AND OUR OBJECTIVE

Pin1 is a difficult protein to target. Multiple small molecules have been developed to target either the PPIase domain or the WW domain. However, none have progressed toward clinical trials because of poor selectivity or potency.

We need
NEW AND NOVEL STRATEGIES
for targeting Pin1

Our objective, a cooperativity destabilisation with to covalent Pin1 inhibitors

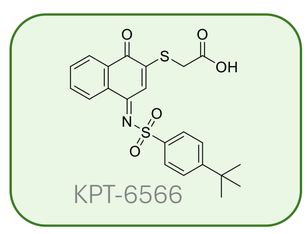


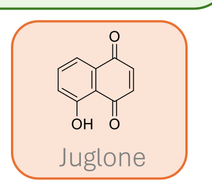
1. Pin1 has **two cysteines** in the PPIase Domain

2. **The cooperativity.** With the addition of two inhibitors, one binds, allowing the other inhibitor to bind

3. **The destabilisation.** When both bind, **we pin the protein into an inactive unfold state**

The inhibitors

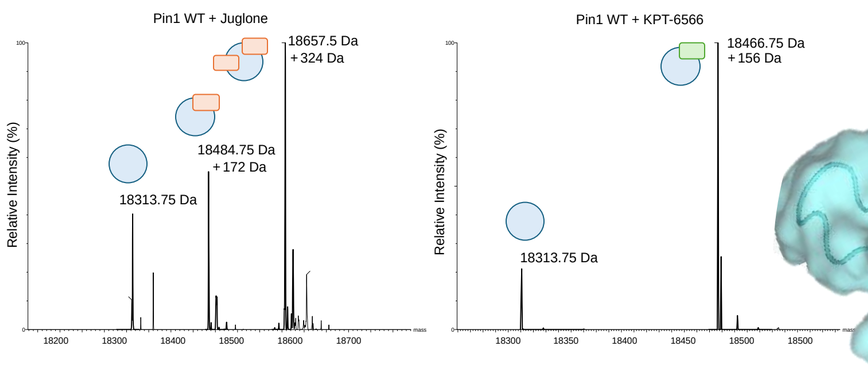




Exploiting useful features of two different ligands **to achieve MORE SELECTIVE AND POTENT inhibition.**

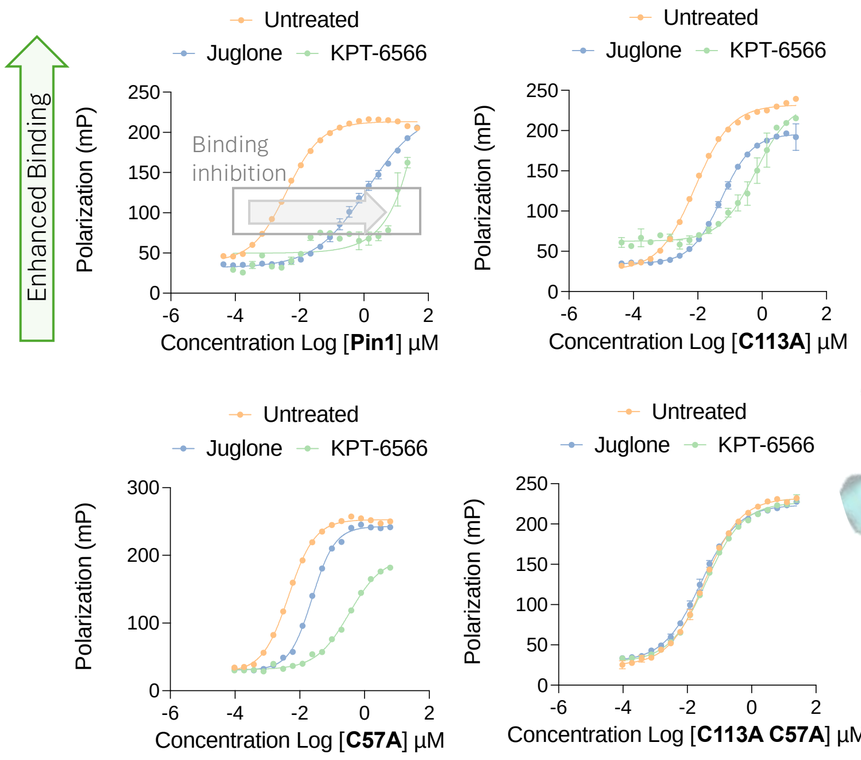
RESULTS

Juglone and KPT-6566 bind covalently to Pin1

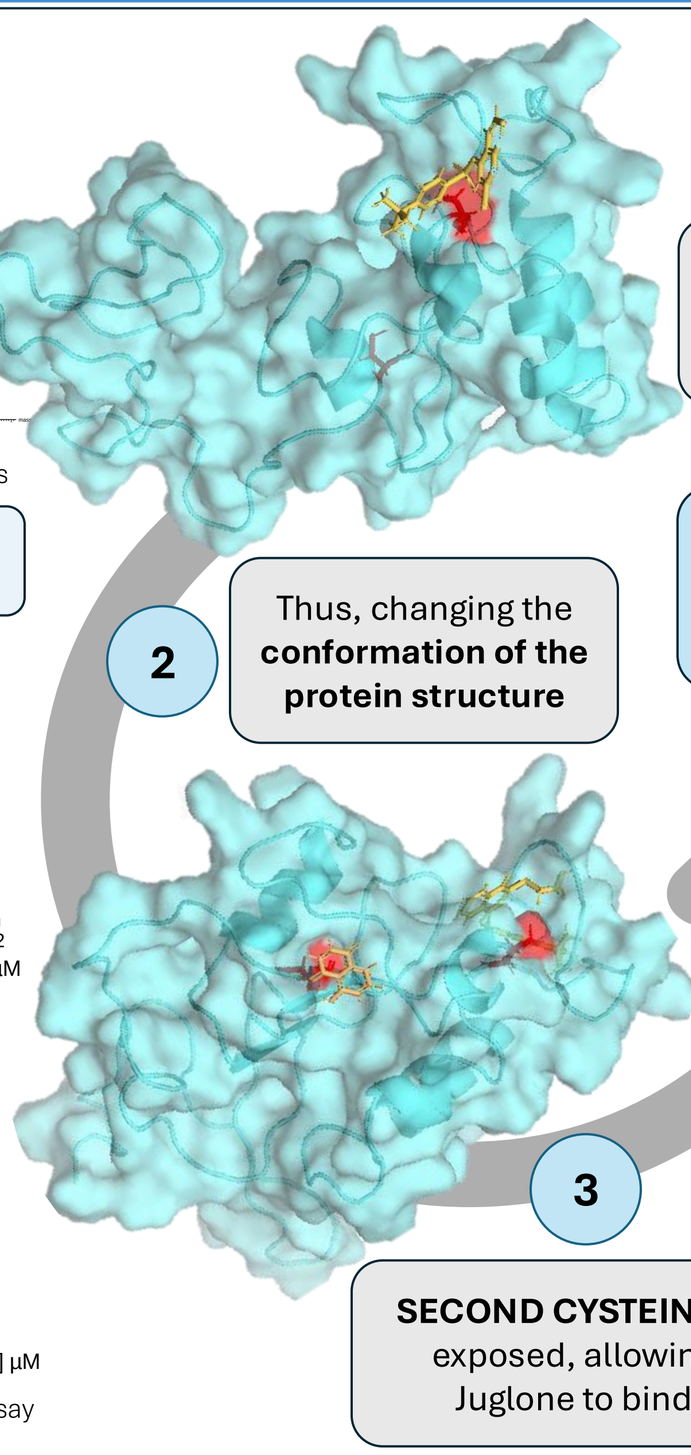


> Confirmed by Liquid Chromatography-Mass Spectrometry Analysis

Juglone and KPT-6566 bind to Cysteine 113 and Cysteine 57 of Pin1



> Substrate binding was assessed by Fluorescence Polarization Assay using a fluorescent substrate



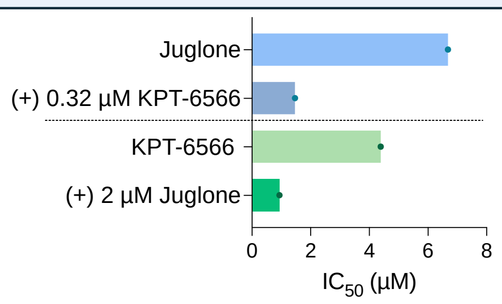
1 KPT-6566 binds to the **FIRST CYSTEINE**

2 Thus, changing the **conformation of the protein structure**

3 **SECOND CYSTEINE** is exposed, allowing Juglone to bind

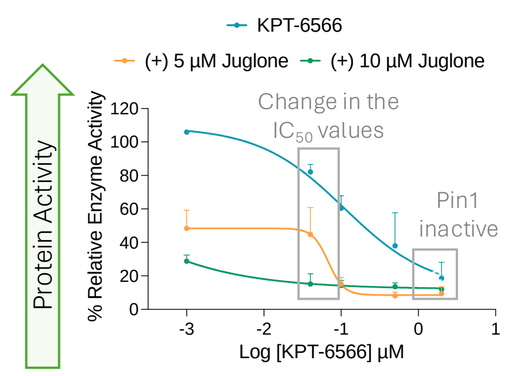
PIN-ING IT DOWN: PROTEIN DESTABILISATION

COOPERATIVE INHIBITION



> by Fluorescence Polarization Assay

Inactivation of Pin1 activity

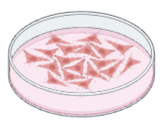


> By Protease-coupled Pin1 PPIase Assay

Pin1 DESTABILISATION

> Protein Stability was measured by Thermal Shift Assay using a dye that is fluorescent when binds inside the protein.

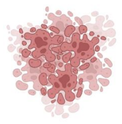
FUTURE RESEARCH



Cancer cells

(+) Dual Inhibition →

Pin1 inactivation by its targeted destabilisation →



Cancer cells dying

ADVANTAGES

↑ ↑

- Potency
- Selectivity

Promotes **protein degradation**

Offers a novel approach that can be applied to other proteins which are currently challenging to target using conventional methods

References

1. Stewart, R., Sharma, S., Wu, T., Okuda, S., Xie, G., Zhou, X.Z., Shilton, B. and Lu, K.P., 2024. The role of the master cancer regulator Pin1 in the development and treatment of cancer. Frontiers in Cell and Developmental Biology, 12, p.1343938. ; **2.** Lee, Y.M. and Liou, Y.C., 2018. Gears-In-Motion: the interplay of WW and PPIase domains in Pin1. Frontiers in Oncology, 8, p.469. ; **3.** Lummis, S.C., Beene, D.L., Lee, L.W., Lester, H.A., Broadhurst, R.W. and Dougherty, D.A., 2005. Cis–trans isomerization at a proline opens the pore of a neurotransmitter-gated ion channel. Nature, 438(7065), pp.248-252.; PDB codes for open and closed Pin1 states 7SA5.

Acknowledgements

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