# Par-4: an Attractive Target for Cancer Therapy

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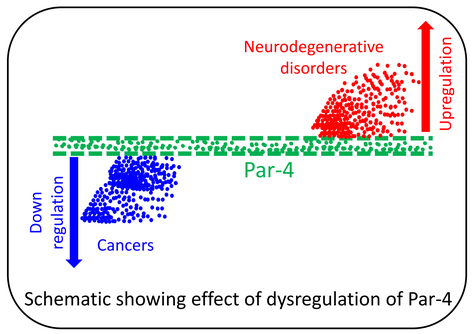
## Abstract

Lack of early diagnosis, cancer recurrence, metastasis, and adverse side effects are some of the major problems in the treatment of cancers. Par-4, a tumor suppressor protein, is an attractive target for cancer therapy as it selectively kills cancer cells. Cl-Par-4 is the active fragment of Par-4 that enters the nucleus and selectively induces apoptosis in cancer cells. It has also been reported that Par-4 increases the susceptibility of cancer cells to chemotherapy and reverses cancer recurrence. Further, Par-4 has been shown to play a dual role: inhibition of EMT (Epithelial-mesenchymal transition) as well as assistance in the reverse process, thereby lowering the chance of cancer metastasis. Because of these unique properties of Par-4, it offers an attractive target for developing anticancer therapy. However, so far only the C-terminal coiled-coil domain has been studied structurally. Here, we have optimized conditions that will be helpful in the structural determination of cl-Par-4 using NMR and X-ray crystallography.

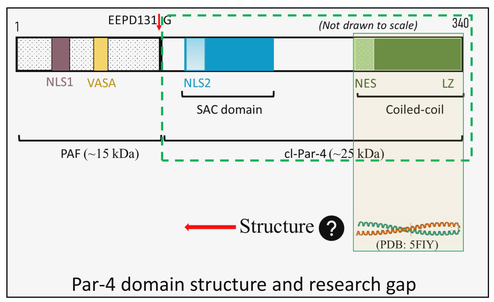
## Introduction

- Cancer: 2nd leading cause of death globally
- Prostate apoptosis response-4 (Par-4): tumor suppressor
- Downregulation of Par-4: reported in many cancers
- Caspase-cleaved Par-4 (cl-Par-4): is an active fragment
- Major obstacles in cancer T/t:
  - Lack of early diagnosis
  - Cancer recurrence
  - Metastasis
  - Adverse side effects
- WHY Par-4??- induces selective apoptosis in cancer cells
- Research gap: structure of cl-Par-4 is not known yet

*This study focuses on optimizing conditions for structure determination using solution NMR and X-ray crystallography*




Schematic showing effect of dysregulation of Par-4




Par-4 domain structure and research gap

## Objective

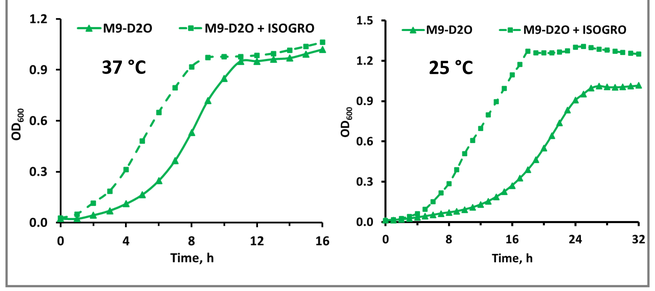
Optimize conditions for structural determination of cl-Par-4 tumor suppressor using solution NMR and X-ray crystallography

## Methods

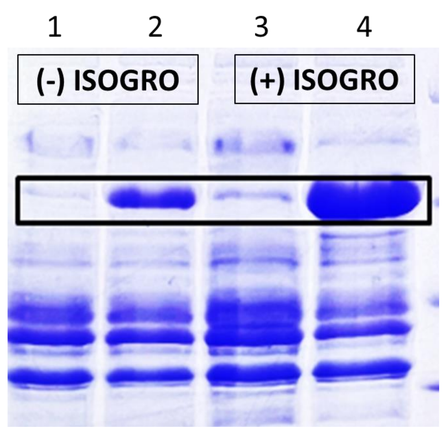
- Protein Expression: in BL 21 (DE3) *Escherichia coli*
- Growth medium: LB and modified M9 minimal media
- Expression vector: modified H-MBP-3C
- Purification: metal affinity chromatography using Ni-column
- *E. coli* growth pattern: monitored via $OD_{600}$ readings
- Test expression: using SDS-PAGE
- NMR: using TCI cryoprobe on 700 MHz spectrometer
- Crystal screening: vapor diffusion & microbatch-under-oil

## Results

### ISOGRO shortens lag phase & enhances growth of *E. coli*

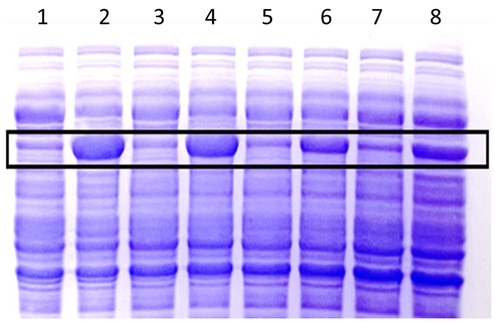



### ISOGRO improves protein yield in M9-$D_2O$



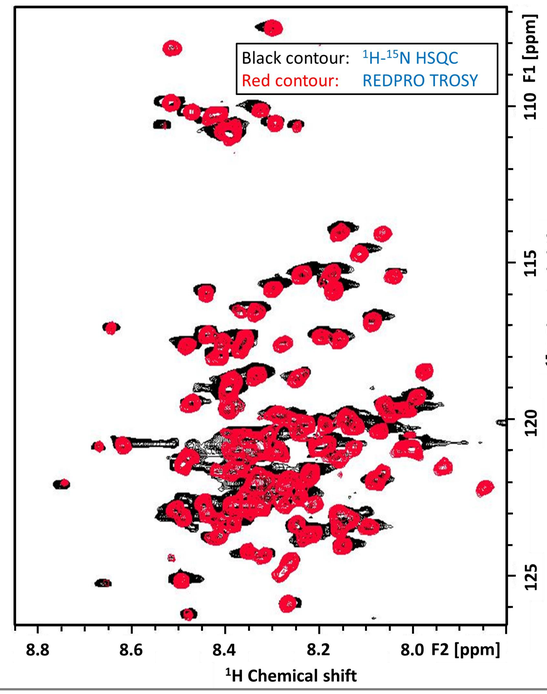

1 & 3: Uninduced; 2 & 4: Induced

### Induction at low OD improves protein yield in M9-$D_2O$




1, 3, 5 & 7: Uninduced;
2, 4, 6 & 8: Induced at 0.33, 0.47, 0.78 & 0.86 $OD_{600}$ values, respectively

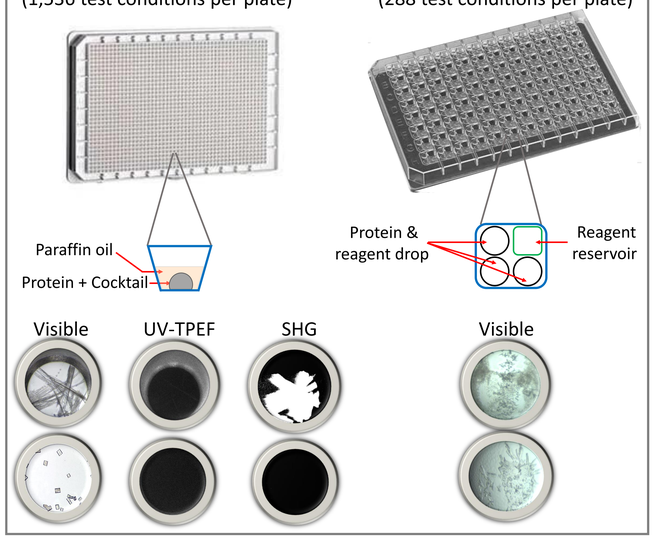
### REDPRO does not alter cl-Par-4 NMR spectrum



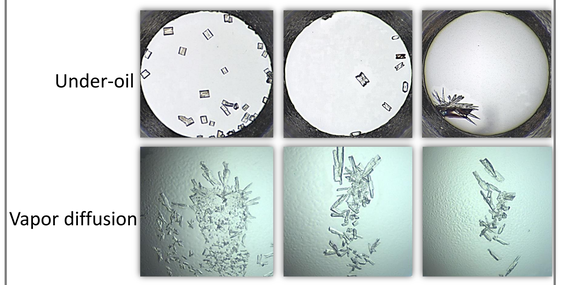

### High-throughput & complimentary imaging improve detection

Microbatch-under-oil
(1,536 test conditions per plate)

Vapor diffusion
(288 test conditions per plate)




### Possible cl-Par-4 crystal hits




## Conclusions

- ISOGRO supplement in $D_2O$-based medium
  - ✓ enhances *E. coli* growth
  - ✓ improves protein yield
- Induction at low OD ⟶ high protein yield in $D_2O$
- REDPRO strategy does not improve cl-Par-4 NMR spectrum
- High-throughput robotic techniques & complimentary imaging tools improve protein crystal screening

## Future works

- Determine the structure of the cl-Par-4 using solution NMR and X-ray crystallography

## Significance

- Knowing structure will help understand the mechanism of action, its interaction with other proteins, and in designing new therapeutics that would potentially target Par-4
- Development of either new drugs that mimic the protein's activity or therapeutics that target Par-4 will minimize adverse effects, and could possibly reverse cancer recurrence and lower the chance of cancer metastasis

## References

- *Biomolecules* **2021,** *11,* 386
- *Cell Death Dis* **2021,** *12,* 47
- *Cell Death Differ* **2017,** *24*, 1540-1547
- *WHO*, **2023**

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