

Biochemical Characterization of the Kinase Activity of DNA Repair Enzyme, PNKP from *C. elegans*

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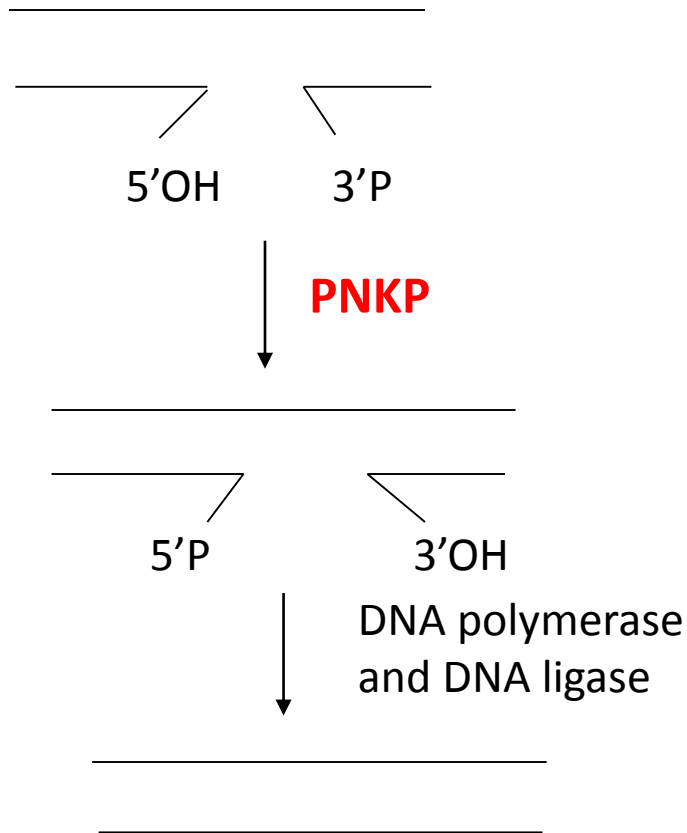


DNA DAMAGE AND REPAIR

- DNA damage: Change in the chemical structure of DNA
- Causes: Internal and External factors
- Main Focus: DNA strand breaks
- DNA Repair:
 - DNA polymerase and DNA ligase
 - PNKP (Polynucleotide Kinase/Phosphatase)

DNA DAMAGE AND REPAIR

➤ Possible outcomes from DNA damage

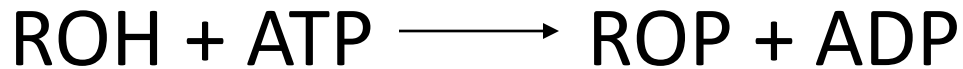


BACKGROUND

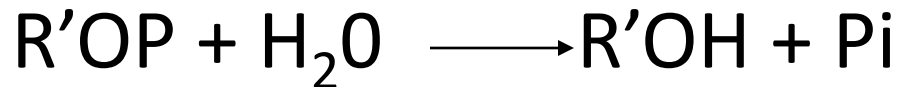
➤ PNKP Structure

- 3 Domains

➤ Kinase

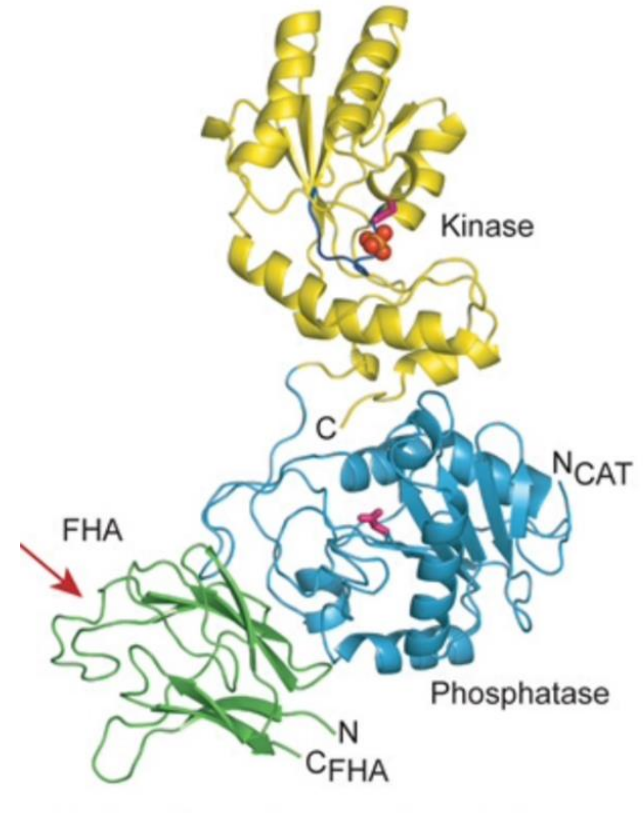


➤ Phosphatase



➤ FHA

Binds PNKP to other DNA repair enzymes



X-ray crystal structure of mouse PNKP (PDB: 1YJ5)

BACKGROUND

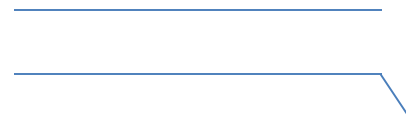
➤ Function

- hPNKP kinase substrate preference:
 - recessed 5'OH vs. blunt 5'OH



5'OH

Recessed End



5'OH

Blunt End

BACKGROUND

- Why is PNKP of interest?
- Plays a crucial role in DNA repair
- Target for design of novel cancer treatments

OBJECTIVES

➤ General Objective

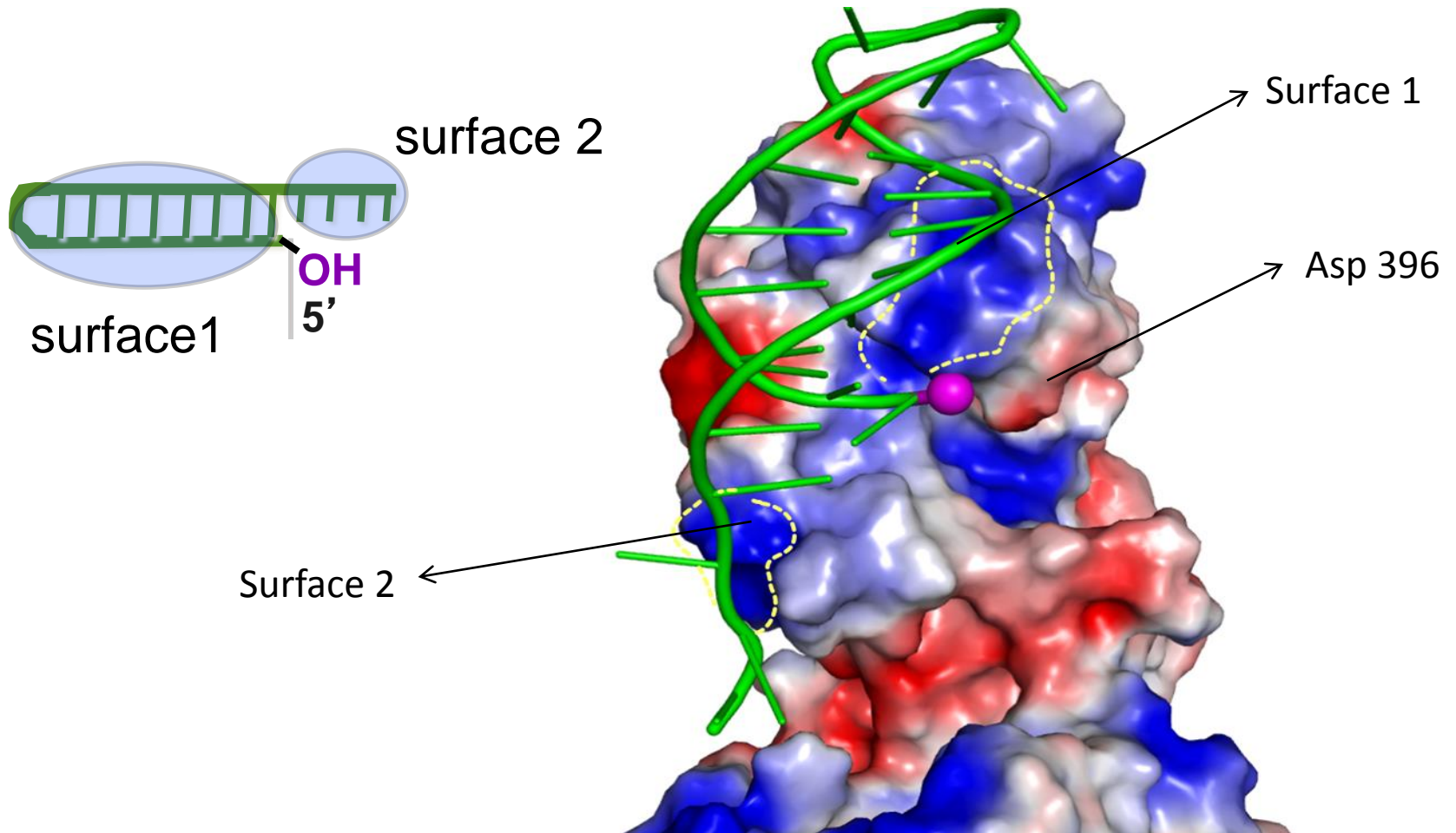
- Analyze the kinase activity of CePNKP in comparison to that of hPNKP to see if they are functionally similar

OBJECTIVES

➤ Specific Objectives

- Determine if the recessed or blunt DNA end is preferred by CePNKP
- Compare level of preference between CePNKP and hPNKP
- Test how CePNKP binds its DNA substrate by analyzing point mutants at proposed DNA binding sites

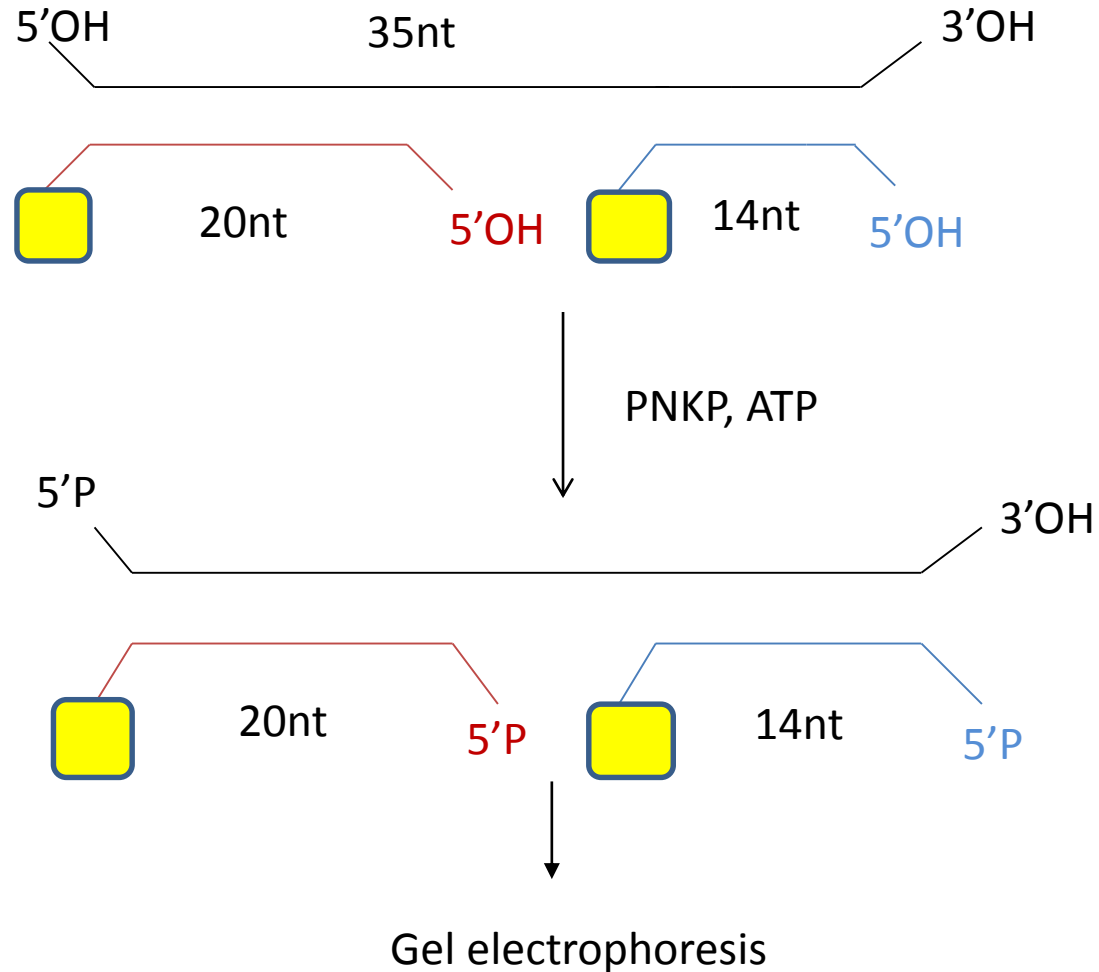
KINASE INTERACTION WITH SUBSTRATE



METHODS

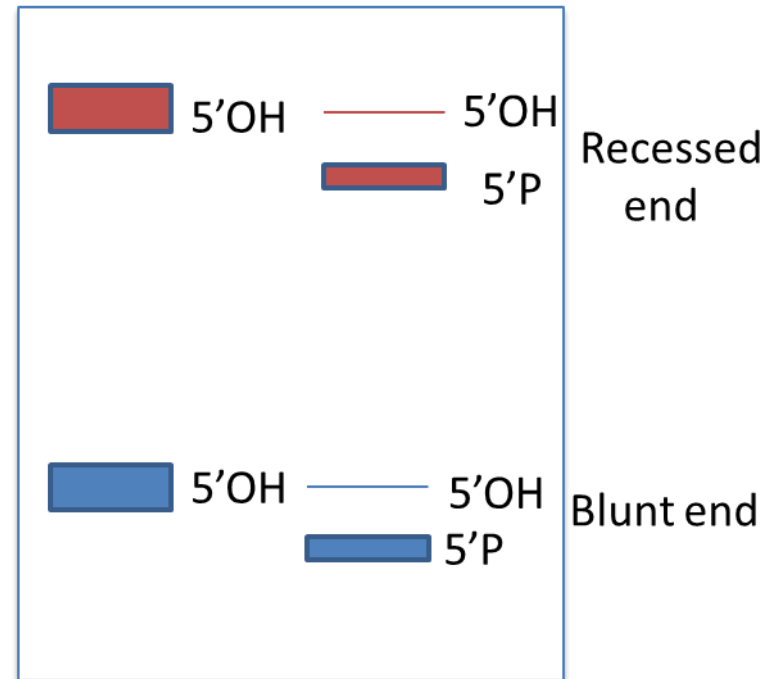
- Kinase assays were carried out on model DNA substrate in order to analyze the kinase activity of CePNKP and hPNKP
- Results were analyzed by polyacrylamide gel electrophoresis
- Fluorescence was detected

METHODS



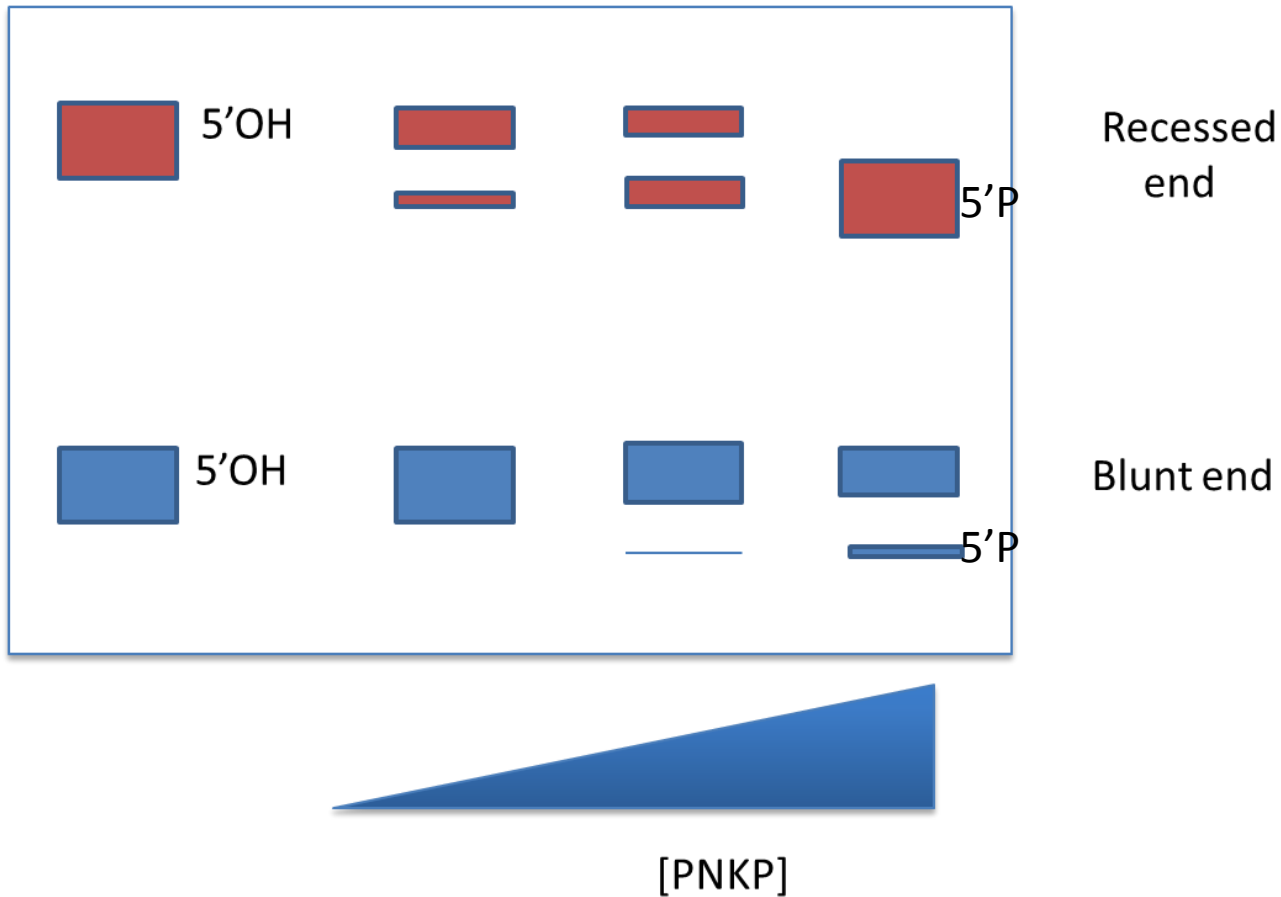
METHODS

➤ Electrophoresis of kinase assay



No reaction +Enzyme

EXPECTED RESULTS



EXPERIMENTS

- CePNKP vs hPNKP
- CePNKP WT vs mutants of surface 1 and 2

Positive control: hPNKP

Negative control: Buffer (no PNKP)

RESULTS

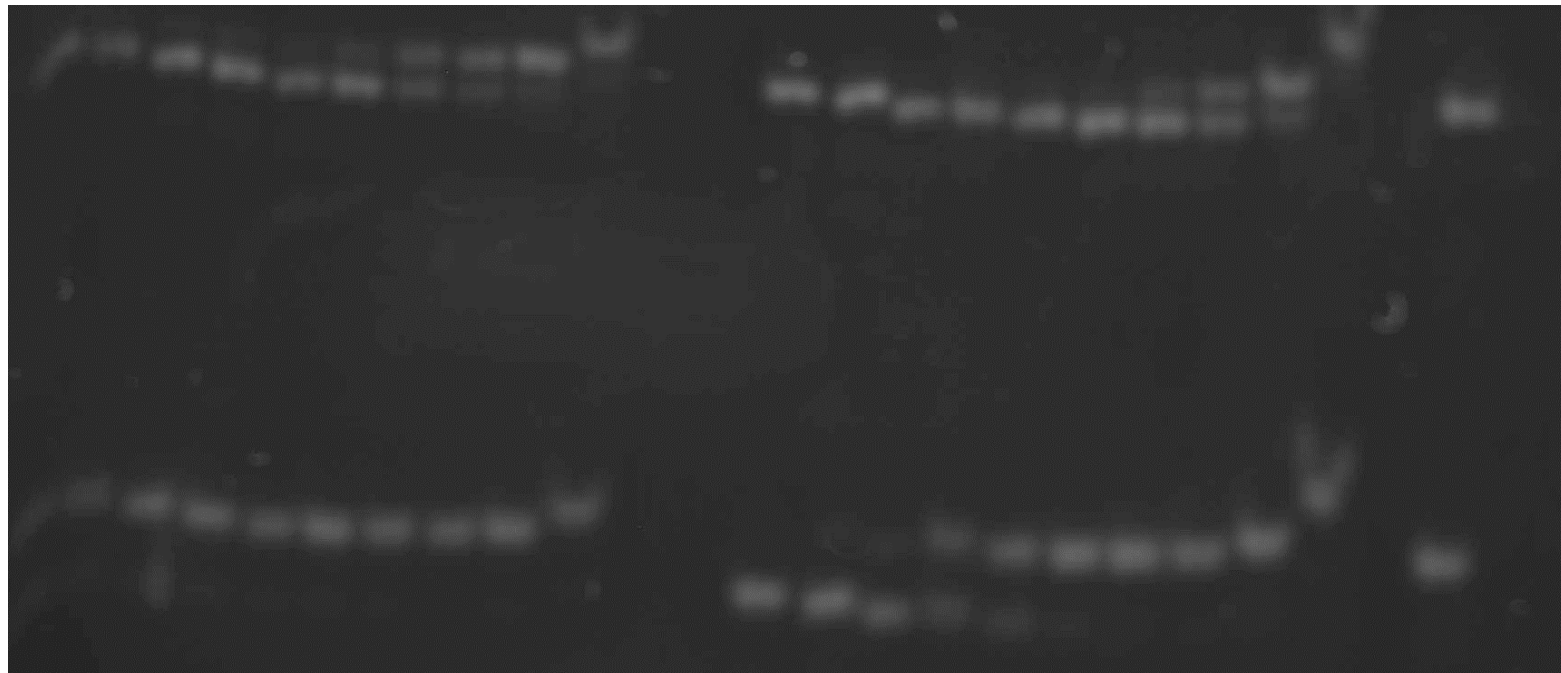
➤ CePNKP vs hPNKP

CePNKP

6.6

hPNKP

6.6



Recessed
End

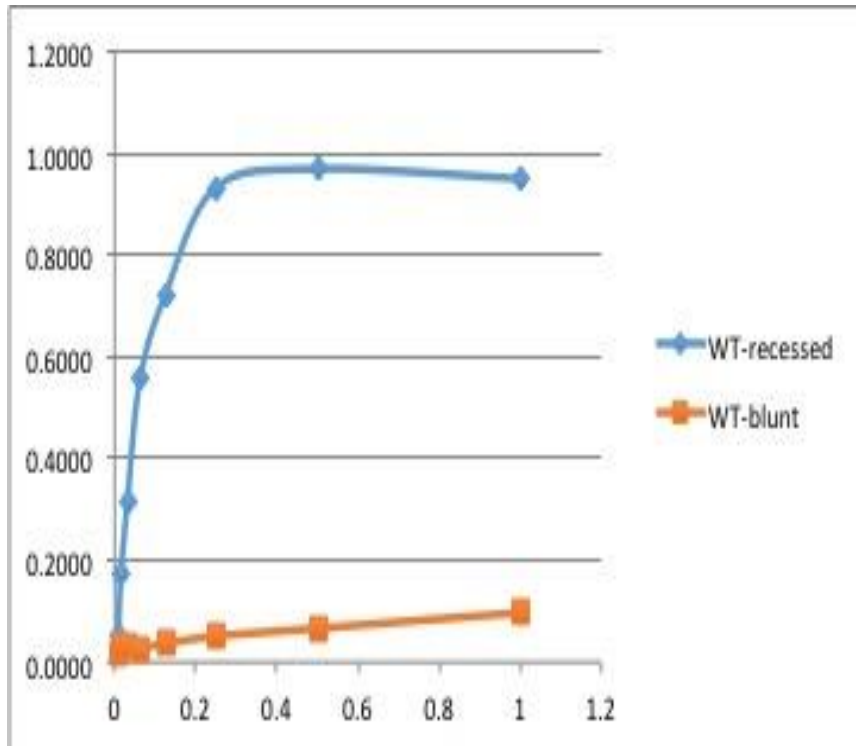
Blunt
End

[PNKP]



RESULTS

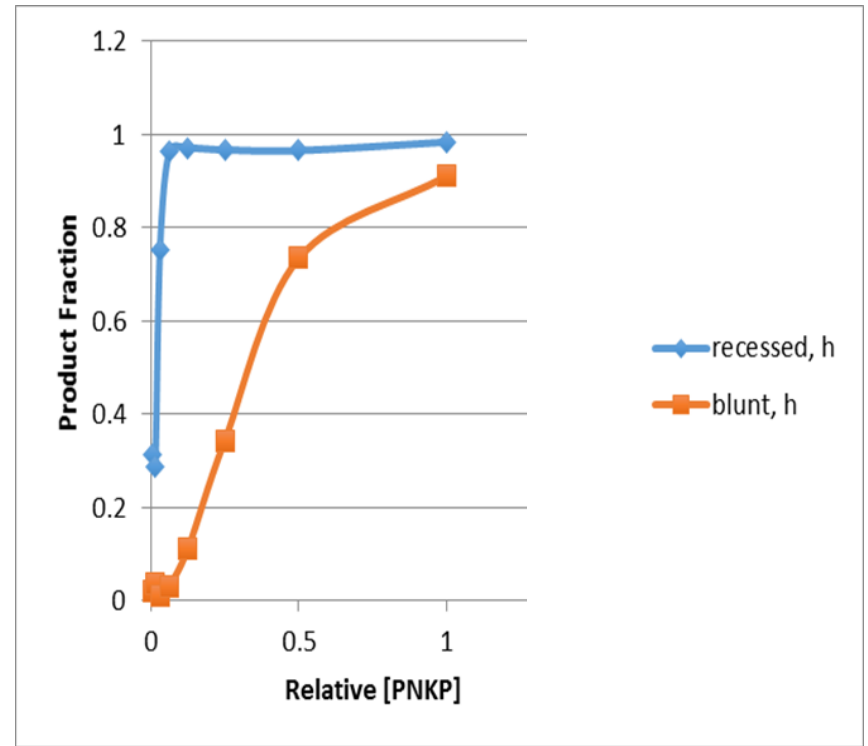
CePNKP



Product fraction = $P/P+R$

CePNKP prefers the recessed end to the blunt end

hPNKP

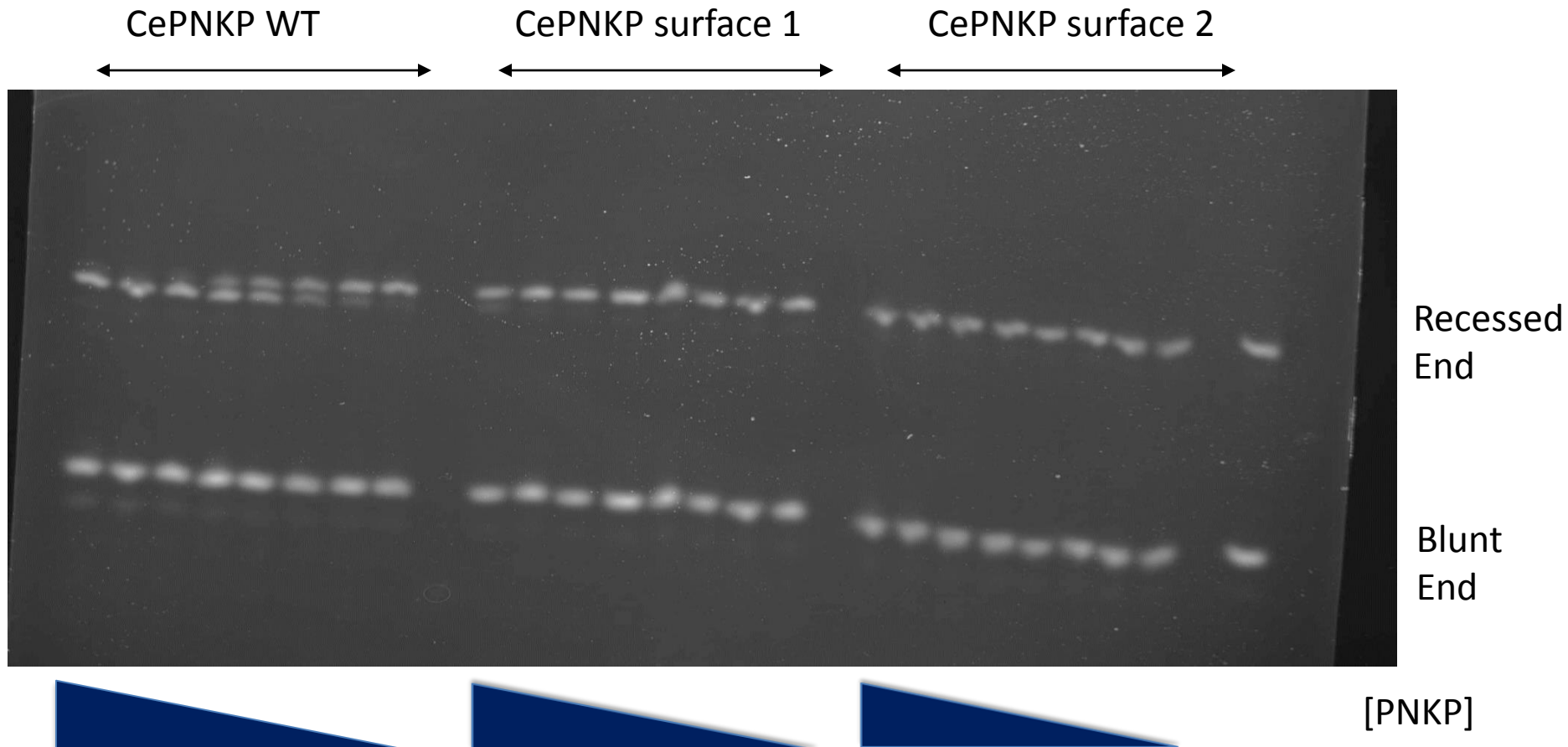


CePNKP prefers the recessed end to a greater extent compared to hPNKP

RESULTS




➤ CePNKP WT vs. CePNKP surface 1 vs. CePNKP surface 2

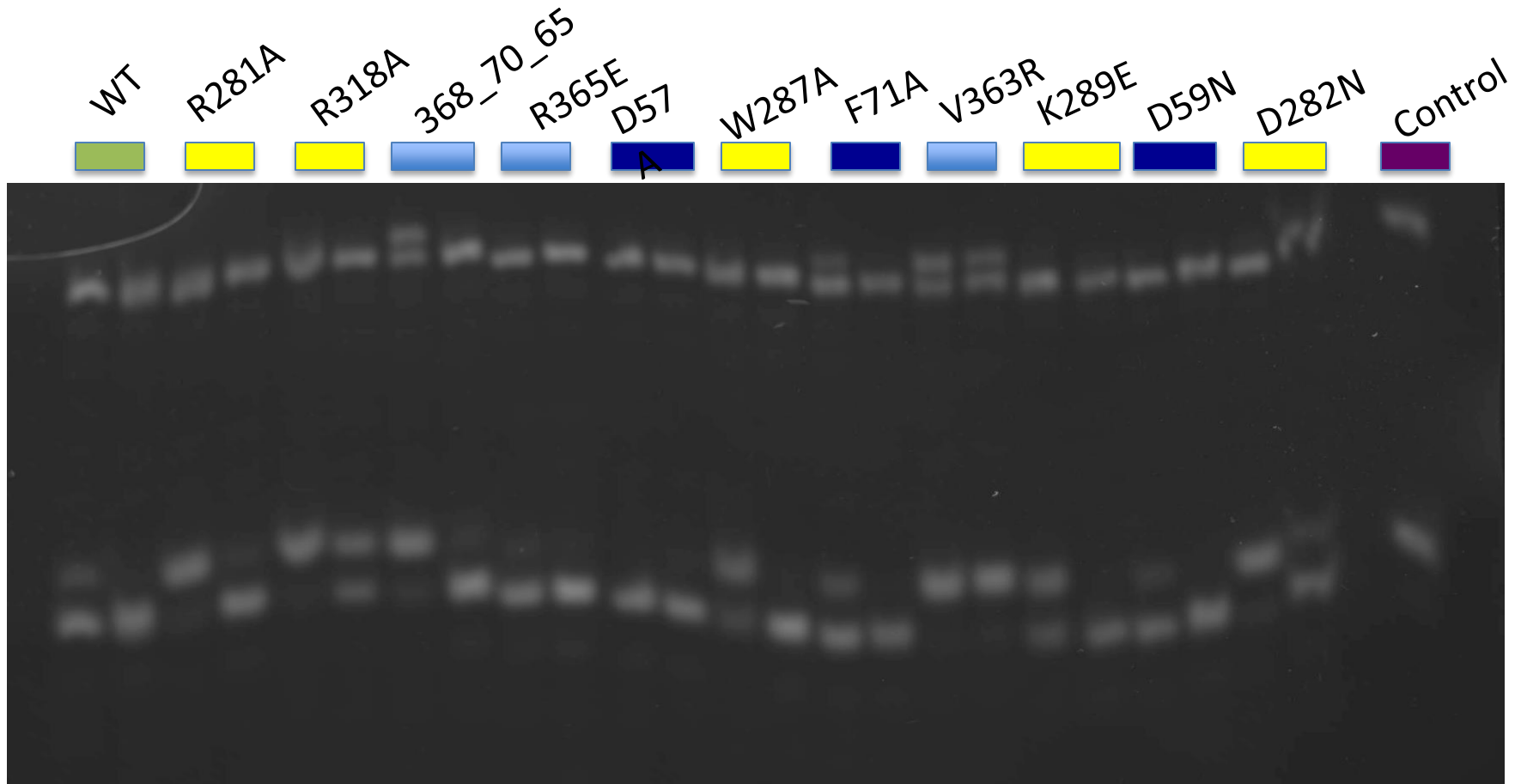
Result: No reaction was seen for
CePNKP mutants



RESULTS

➤ CePNKP WT and mutants

 Surface 1 mutants
 Surface 2 mutants
 Phosphatase domain mutants



CONCLUSIONS

- CePNKP and hPNKP prefer the recessed DNA end
- CePNKP prefers the recessed end more than hPNKP does at higher [PNKP]
- CePNKP mutants show activity
- Is CePNKP a good model for hPNKP?

ANTICIPATED SIGNIFICANCE

- The results of this project will help determine if CePNKP is a good model that can contribute to the development of hPNKP inhibitors
- hPNKP inhibitors may be developed into sensitizing drugs to improve efficiency of cancer treatments

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