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Purification and Evaluation of Manganese Superoxide Dismutase

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Outline:

- Background Information + Goals
- Methods
- Results
- Overall Findings
- Future Directions

Significance of p53

- Tumor suppressor protein mutated in over 50% of all cancers
- Stimulate genes involved in apoptosis
- Promote transcription of genes predicted to respond to oxidative stress

Significance of Manganese Superoxide Dismutase (SOD2)

- Mutations in SOD2 have been associated with several diseases including cancer
- Participates in the detoxification of reactive oxygen species (ROS)

P53 and SOD2 Interactions

Regulation of several cellular processes including apoptosis

P53 repression of SOD2 expression at the promoter level

Overexpression of SOD2 decreases p53-mediated induction of apoptosis

Goals of this Research

- The goal of this research is to evaluate the mechanism by which p53 and SOD2 interact
  - Develop purification protocols for both p53 and SOD2
  - Evaluate DNA binding affinity of SOD2 and p53
  - Conduct a series of assays to evaluate aggregation state of p53 and SOD2 under various conditions

SOD2/MnSOD

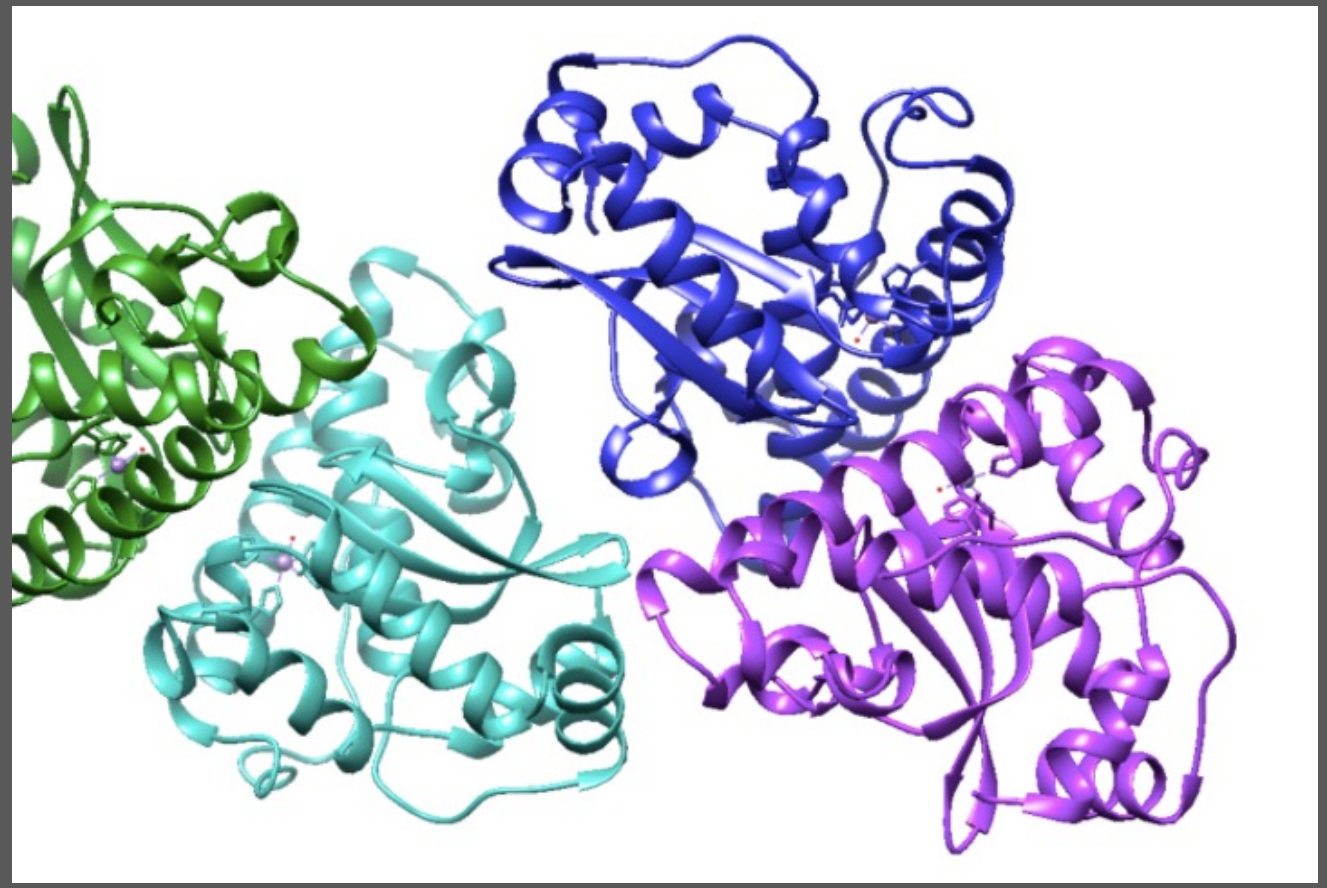


Figure 1. Quaternary structure of SOD2.

- One enzyme in the family of SOD enzymes
- Homotetramer binding one manganese per subunit
- Total molecular weight of 88 kDa

Methods

Methods for Production and Purification of SOD2

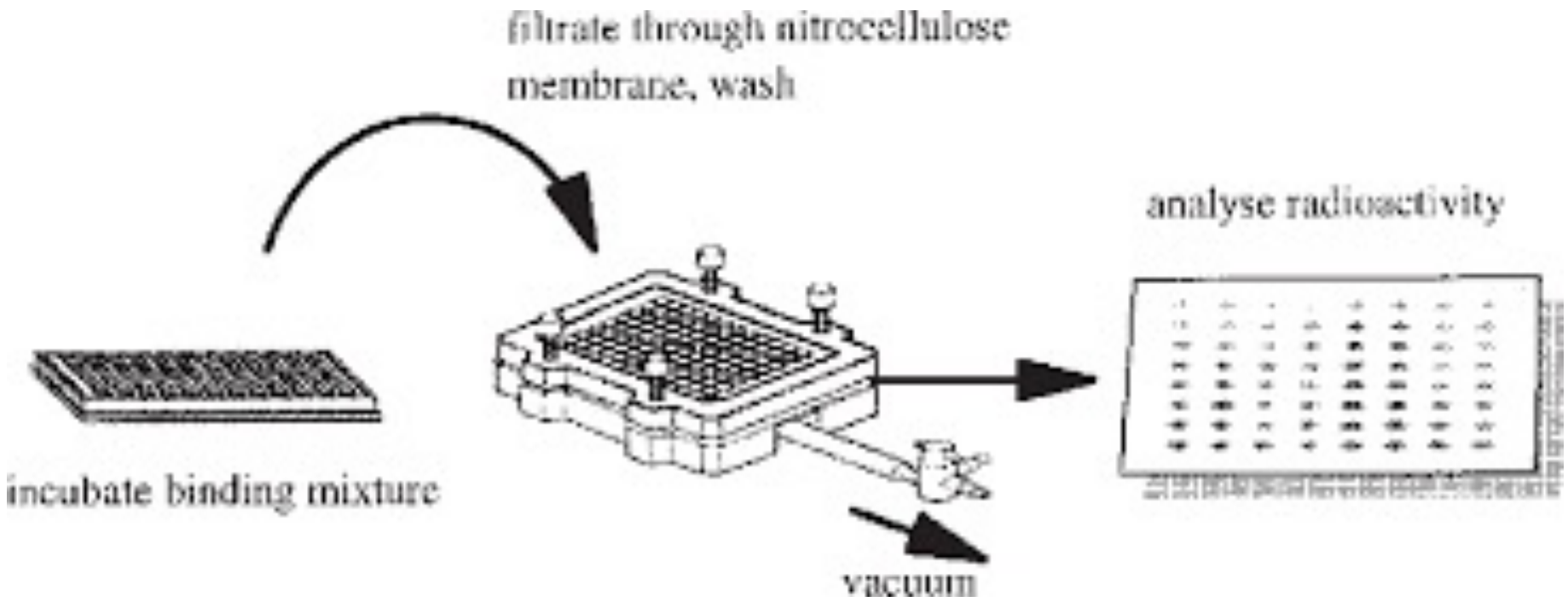
- Transformation of pSOD2 plasmid containing kanamycin resistance into BL21 cells to induce expression
- Extraction of SOD2 via sonification and cell lysis
- Purification Salting out and Dialysis
- Affinity Chromatography
  - Nickel Column
  - Heparin Column

Method for Evaluation of Purification by Identification of Protein of Interest

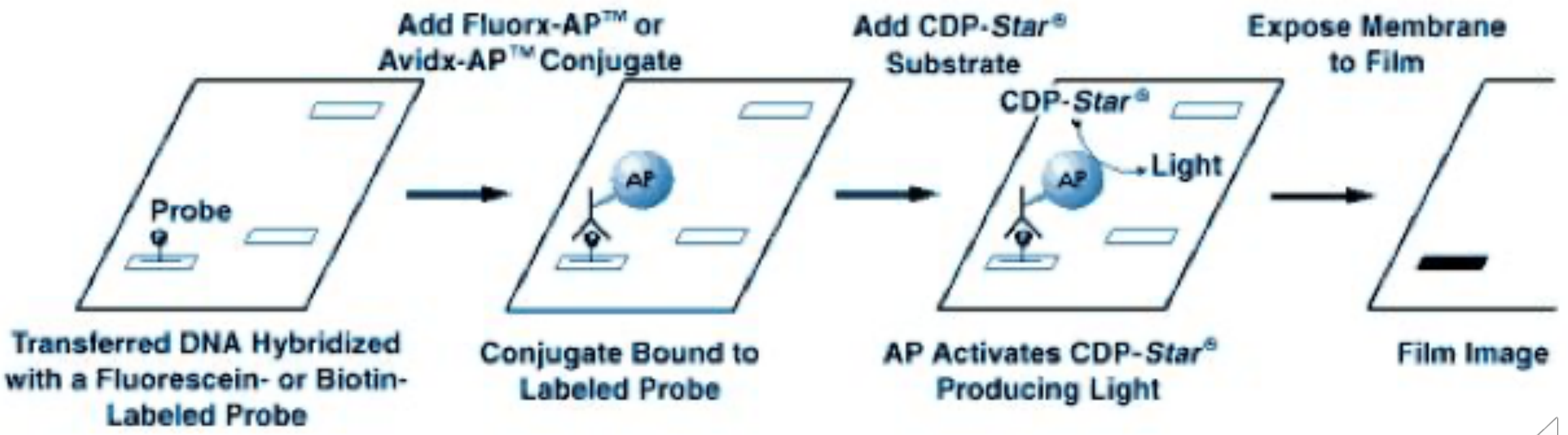
- SDS PAGE
- Western Blot

Method for Evaluation of SOD2 Interactions with DNA

Nitrocellulose Filter Binding Assay



Chemiluminescence Assay



Bovine Serum Albumin (BSA)

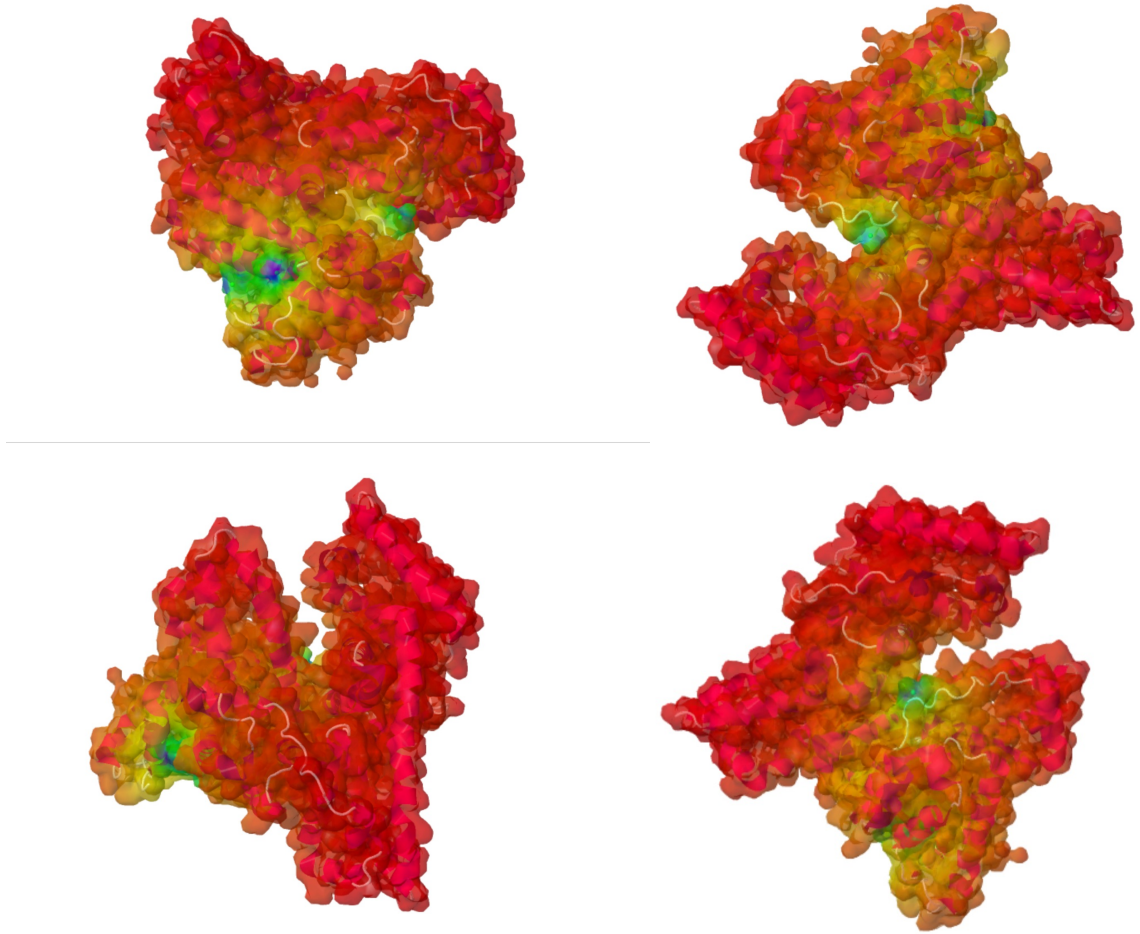
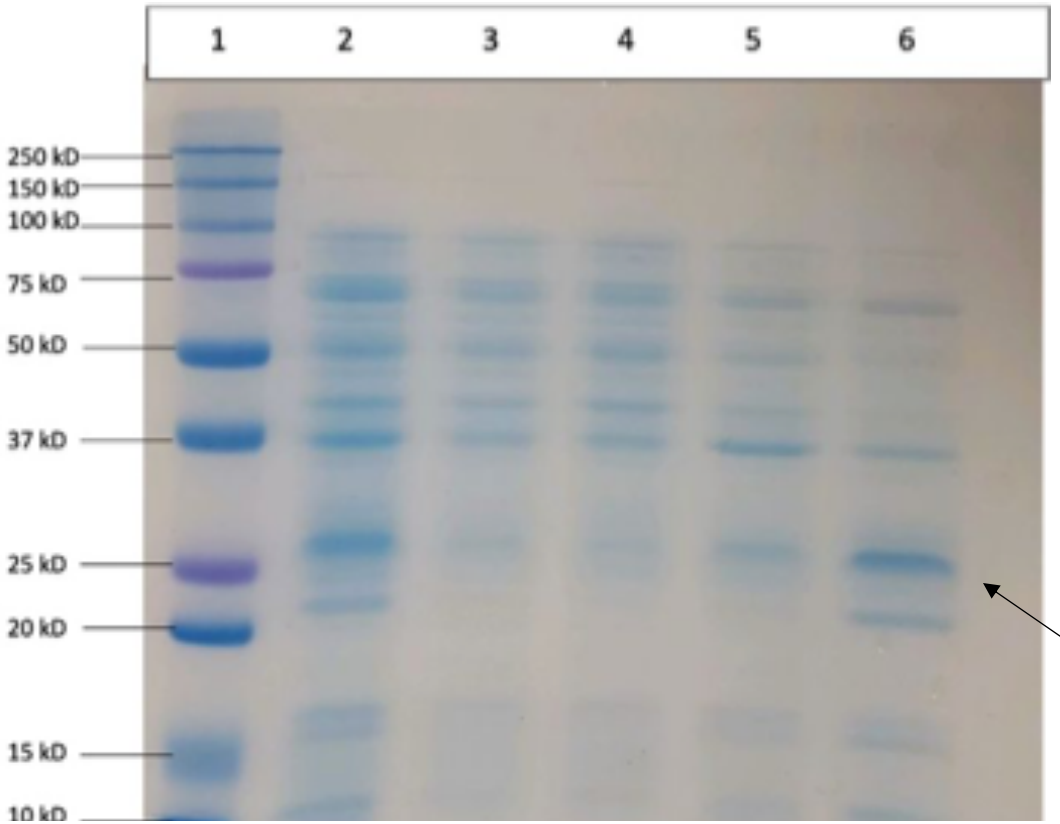


Figure 2. Molecular electrostatic potential of BSA

Results

SDS-Page Gel Following Nickel Affinity Chromatography



Lane 1= Molecular Weight Standard

Lane 2= Dialyzed SOD2

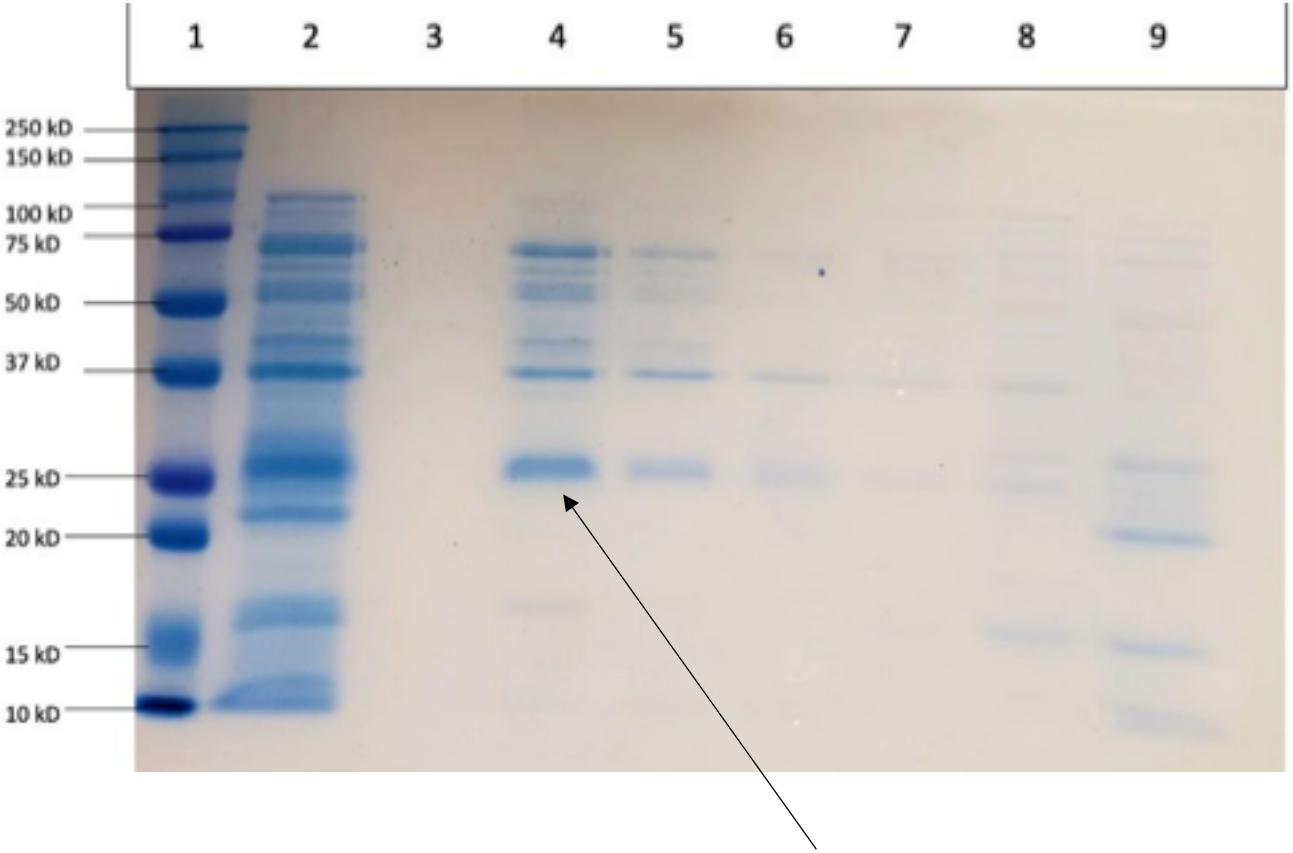
Lane 3= Flow Through

Lane 4= 20 mM Imidazole

Lane 5= 150 mM Imidazole

Lane 6= 300 mM Imidazole

SDS-Page Gel Following Heparin Affinity Chromatography



Lane 1= Molecular Weight Standard

Lane 2= Dialyzed SOD2

Lane 3= Flow Through

Lane 4= 10 mM Imidazole

Lane5= 25 mM Imidazole

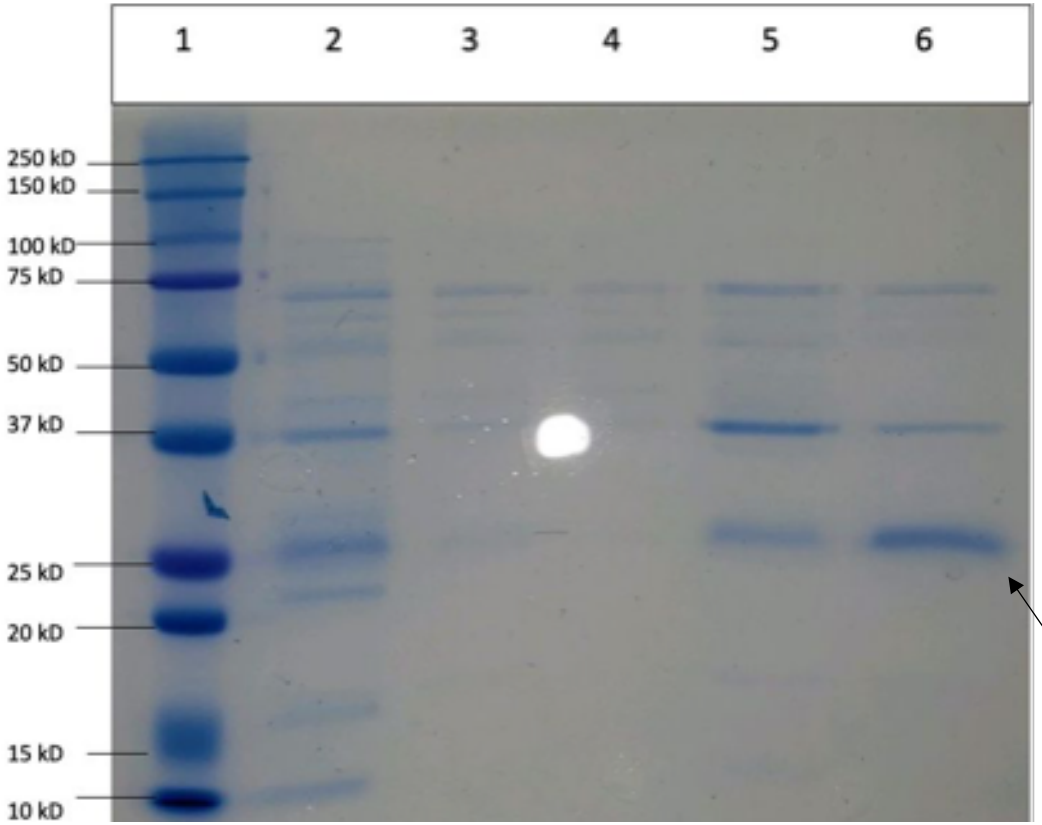
Lane 6= 50 mM Imidazole

Lane 7= 100 mM Imidazole

Lane 8= 250 mM Imidazole

Lane 9= 500 mM Imidazole

SDS-Page Gel Following Second Nickel Affinity Chromatography



Lane 1= Molecular Weight Standard

Lane 2= Dialyzed SOD2

Lane 3= Flow Through

Lane 4= 20 mM Imidazole

Lane 5= 150 mM Imidazole

Lane 6= 300 mM Imidazole

Nitrocellulose Filter Binding Assay (NCFBA)

T20 DNA

T20 DNA

T20 DNA

1 µM BSA

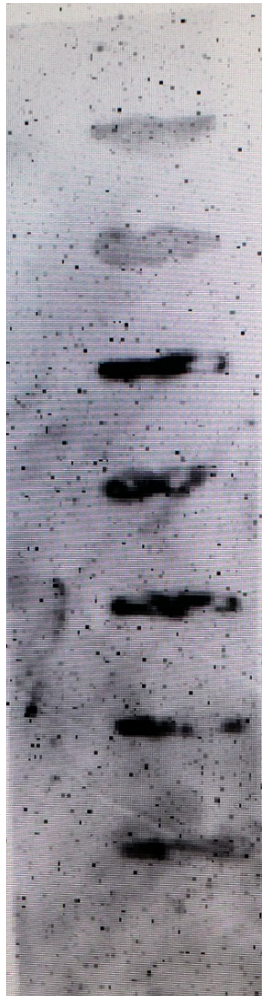
2 µM BSA

5 µM BSA

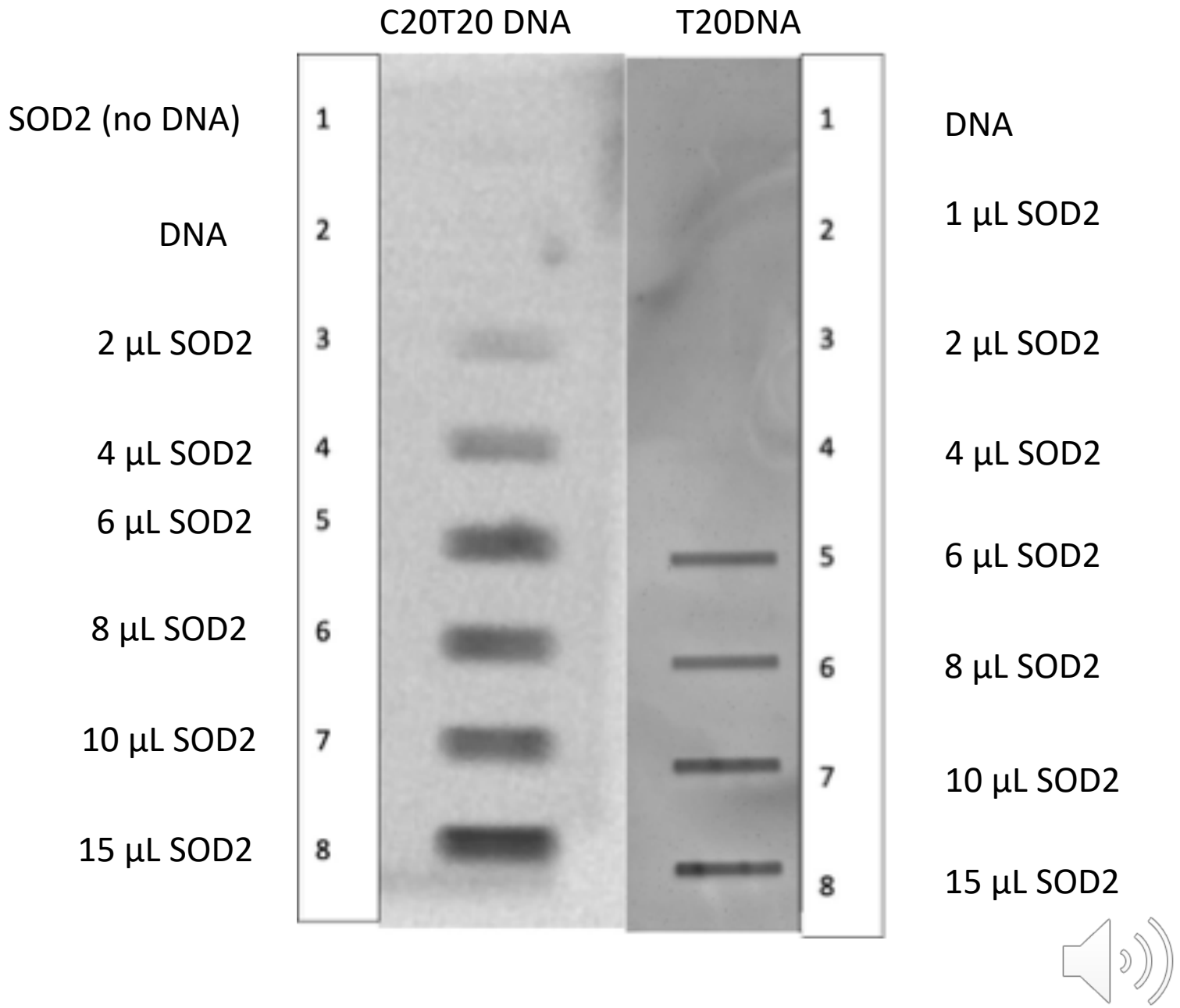
10 µM BSA

20 µM BSA

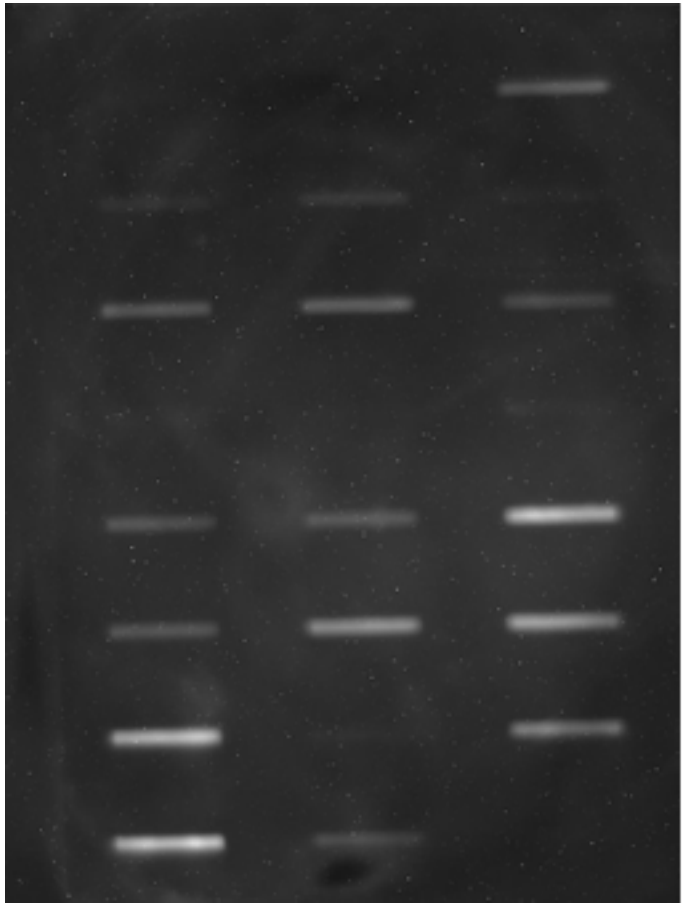
BSA (no DNA)

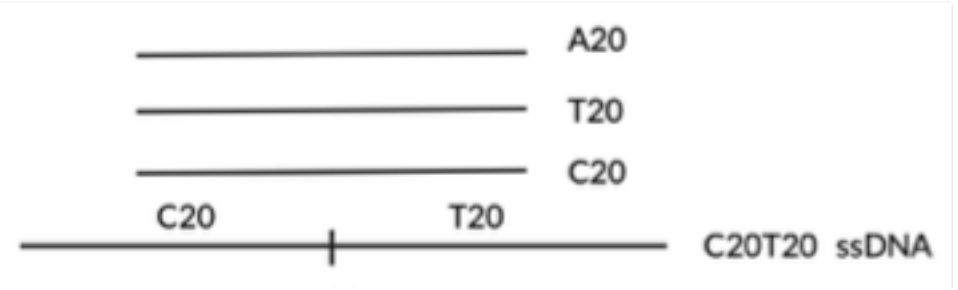


Nitrocellulose Filter Binding Assay (NCFBA)



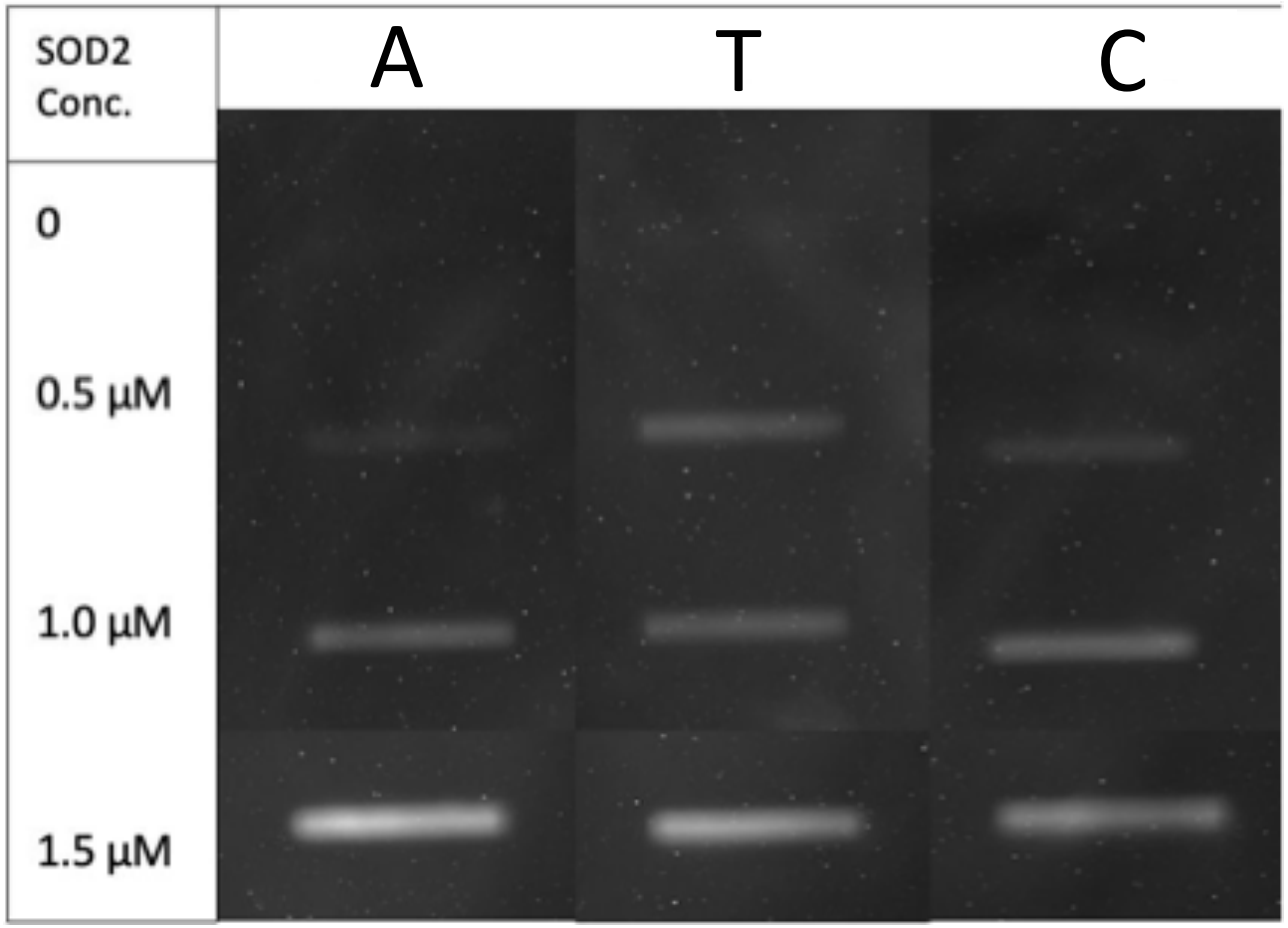
Nitrocellulose Filter Binding Assay (NCFBA)



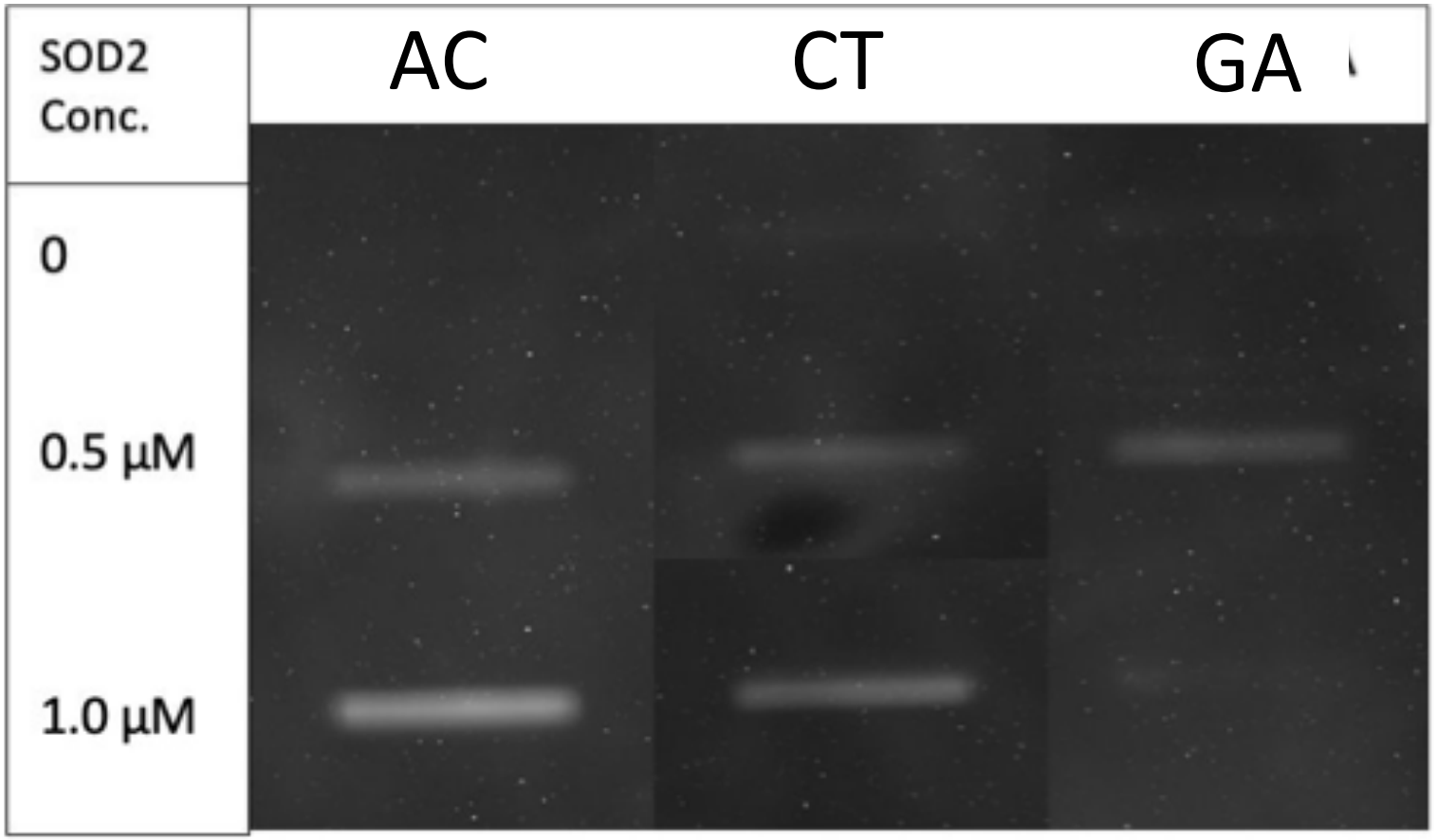


Oligonucleotides: A, T, C, AC, CT, GA

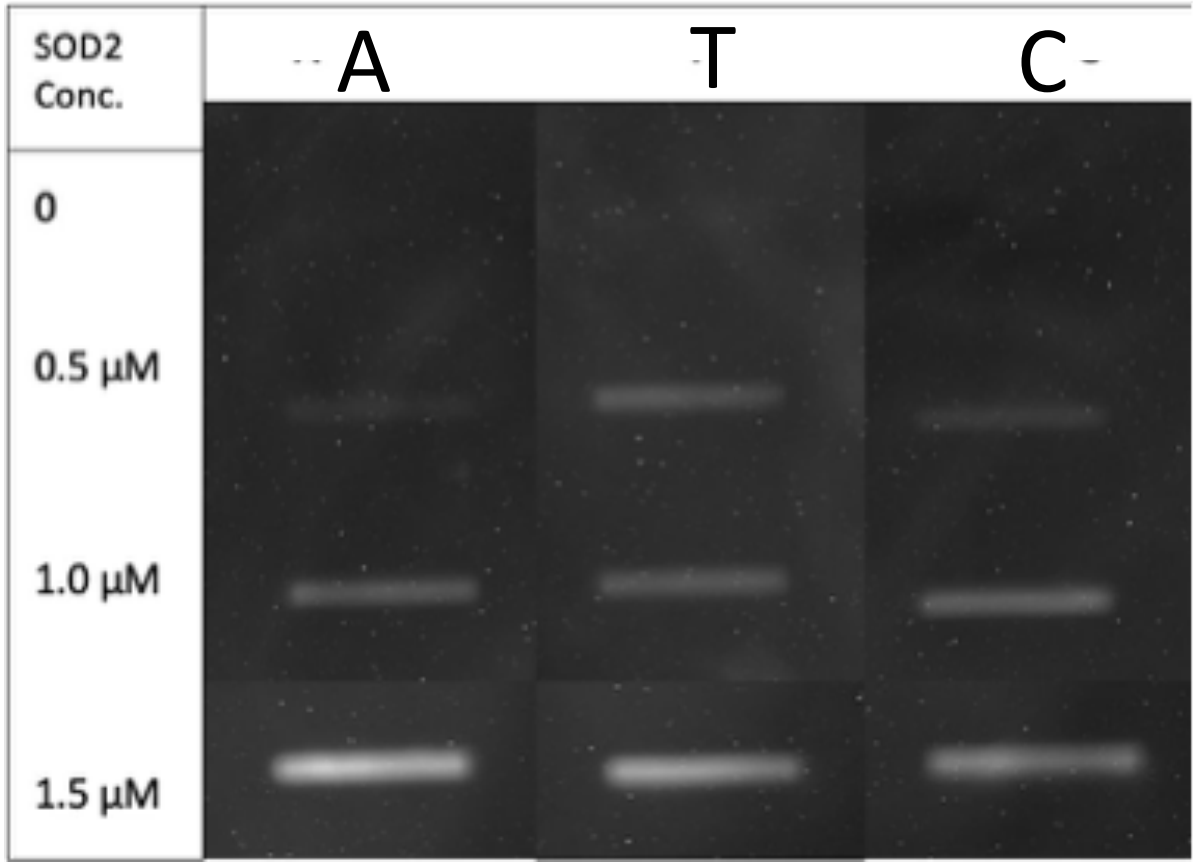
Nitrocellulose Filter Binding Assay (NCBFA)

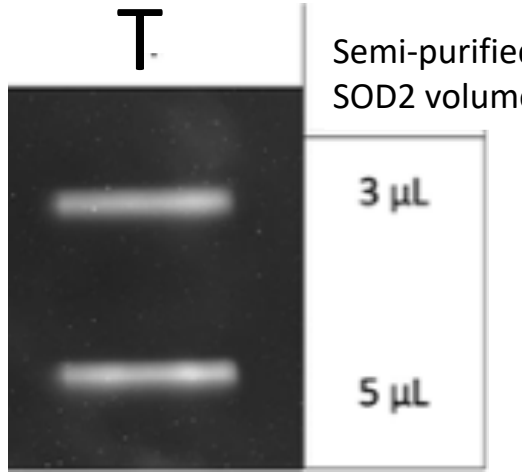


Nitrocellulose Filter Binding Assay (NCFBA)



Nitrocellulose Filter Binding Assay (NCFBA)



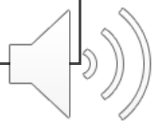


Overall Findings

Separation technique

SOD2 binding affinity to DNA

- Nonspecific binding- all oligonucleotides
- Qualitatively, comparable binding between oligonucleotides
- Concentration dependent binding affinity
- Semi-purified SOD2 showed comparable binding to commercial SOD2 of 1.5 µM



Future Directions

- Aggregation state of SOD2 under various conditions (concentration, salt, temperature, pH etc.)
- Quantitate binding affinity of SOD2 and DNA
- Interaction between SOD2 and p53

Thank you

Dr. Mascotti

Claudia Lenhart

Elizabeth Yirga

Thank you

Any Questions?
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References

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