

# A Proteomic Study of *E. coli* using the DNA-Binding Capability of Proteins

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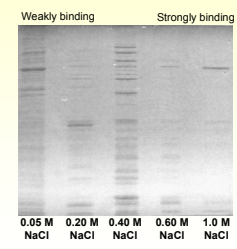
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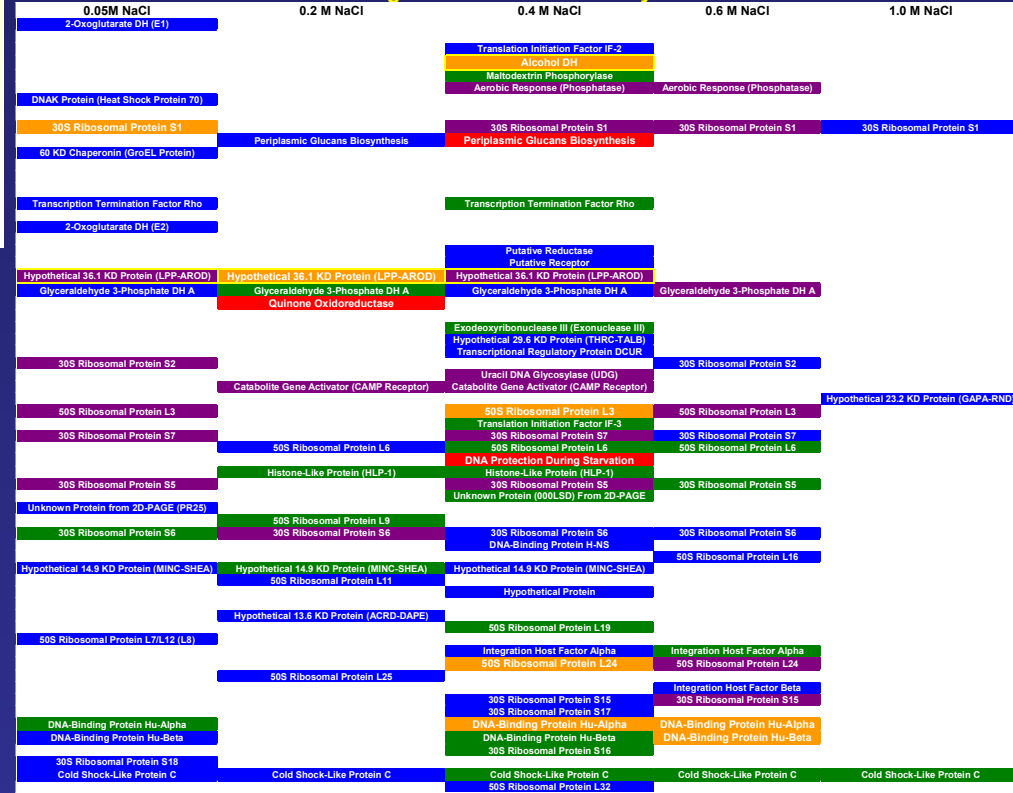
## Overview

- Purpose:** Explore use of the DNA-binding capability of proteins as an initial separation for a proteomic study
- Methods:**
  - DNA cellulose for affinity separation
  - Aerobically and anaerobically grown *E. coli*
  - Identification of proteins via LC-MS/MS
- Results:**
  - Total of 478 proteins observed, 127 DNA-binding
  - Changes in binding for different growth conditions
  - Hypothetical proteins and post-translational modifications

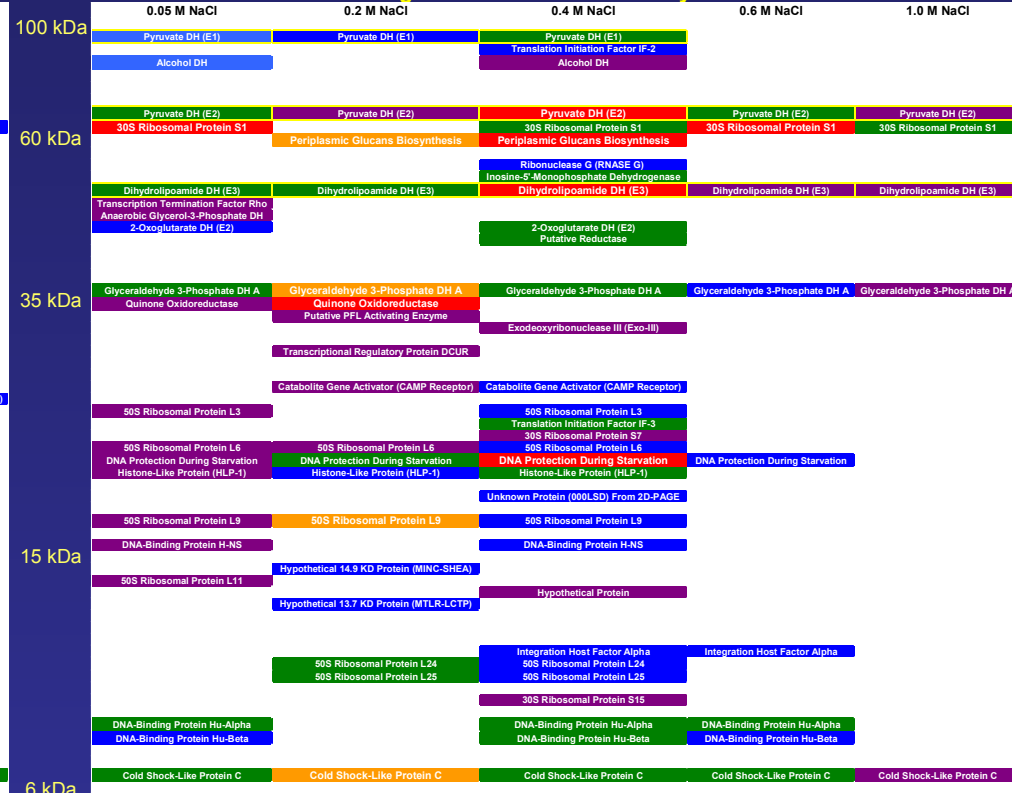
## SDS-PAGE of Aerobically grown *E. coli* DNA-Binding Fractions



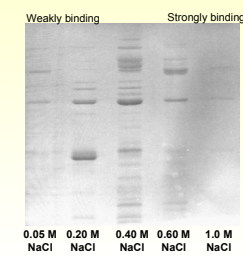
## DNA-Binding Proteins in Aerobically Grown *E. coli*



## DNA-Binding Proteins in Anaerobically Grown *E. coli*



## SDS-PAGE of Anaerobically Grown *E. coli* DNA-Binding Fractions



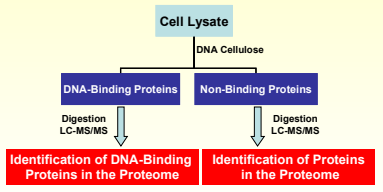
## Introduction

- Multidimensional chromatography techniques have led to the identification of 1500 proteins in a cell<sup>1</sup>, but the complexity of a whole cell lysate limits resolution.
- Employing a function-based separation prior to enzymatic digestion might yield protein identification plus protein function information.
- Proteomic studies of DNA-binding proteins have primarily been undertaken with specific DNA sequences on chip-based formats<sup>2</sup>.
- Using large generic DNA sequence may lead to the binding of and the subsequent identification of more proteins than a specific DNA sequence.

## Methods

- E. coli* was grown from a stock of strain BL21 purchased from Stratagene. Cells were cultured in LB broth under aerobic and anaerobic conditions.
- Single-stranded calf thymus DNA cellulose (~50 kb) was purchased from Sigma.
- Lysates were mixed with DNA cellulose for 10 minutes at 25 °C, washed with buffer, and centrifuged to separate the cellulose particles from the lysate. Bound proteins were eluted off with increasing concentrations of salt.
- Fractions were desalted prior to digestion with trypsin.
- Liquid chromatography was performed on a 0.17 mm column with Jupiter C<sub>18</sub> stationary phase.
- Tandem mass spectra were obtained on a Finnegan LCQ ion trap mass spectrometer.
- Both Sequest and Mascot were used for data analysis.

## Scheme for Identification of DNA-Binding Proteins



## Chart Key

Color of cell corresponds to the number of peptides used to identify the protein, which might be a rough indicator of the protein's concentration in each fraction.

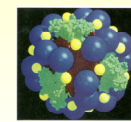
- 1 Peptide
- > 2 Peptides
- > 4 Peptides
- > 8 Peptides
- > 12 Peptides

For clarity, proteins identified with only one peptide in one fraction are not shown.

## Interesting Findings

- Hypothetical DNA-Binding Proteins**
  - 14.9 KD Protein from MINC-SHEA intergenic region bound to DNA cellulose under both conditions
  - 36.1 KD Protein from LPP-AROD intergenic region bound only in lysates grown under aerobic conditions
- Alcohol Dehydrogenase**
  - Only expected to be present in anaerobic cells, but was present and strongly binding under aerobic conditions
- Pyruvate Formate Lyase (PFL)**
  - Found in non-binding portions of the lysates with 4 times more peptides found in anaerobic *E. coli* than in aerobic. This result corresponds to the over-expression of PFL observed under anaerobic conditions in previous *E. coli* 2D-PAGE proteomic studies<sup>3</sup>.

## Pyruvate Dehydrogenase Complex



A model of the pyruvate dehydrogenase complex, based on the crystal structure of *E. coli* from *Acetobacter vinelandii*<sup>4</sup>.

- Bound strongly to DNA cellulose only from growth under anaerobic conditions
- Under aerobic conditions, this multienzyme complex oxidizes pyruvate prior to the citric acid cycle
- Under anaerobic conditions, the complex may change its specificity from NAD to NADP
- Perhaps the strong retention of the complex to the DNA cellulose is due to the shift to the binding of phosphates

## Post-Translational Modifications

- Total of 17 modifications observed, including:
  - Methylation (+14 Da)**
    - DNA-Binding Protein Hu-Beta
      - MNKSGLIKIAAGADISK
  - Acetylation (+42 Da)**
    - 30S Ribosomal Protein S5
      - Acetyl-AHEKQAGELEK
  - Phosphorylation (+79 Da)**
    - 30S Ribosomal Protein S1
      - DAATVNRKEDANFNINMAAEAFK
  - Methionine Loss (-131.2 Da)**
    - Uracil DNA Glycosylase
      - MANLTHWIVLAEEK

## Conclusions

- Effective Use of Function-Based Separation**
  - Over 470 proteins observed from *E. coli*
  - 17 Post-translational modifications found
  - Change in protein binding observed under different growth conditions
  - 104 Hypothetical/unknown proteins observed
- Future Work**
  - Multidimensional chromatography
  - Isotopic labeling for quantitative results (ICAT)

## References

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