

# Use of Photochemical Crosslinking and Mass Spectrometry to Determine the Contact Points between DNA and Proteins Contained in a Functional Subset of a Proteome

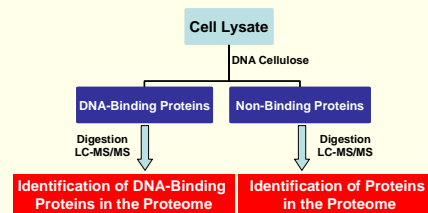
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## Overview

- Purpose:** Use the DNA-binding capability of proteins as an initial separation for a proteomic study along with photochemical crosslinking to determine amino acids involved in binding DNA
- Methods:**
  - DNA cellulose for affinity separation
  - Aerobically and anaerobically grown *E. coli*
  - Photochemical crosslinking of proteins to DNA
  - Identification of proteins and crosslinks via LC-MS/MS
- Results:**
  - Total of 540 proteins observed, 232 DNA-binding
  - DNA-binding sites characterized for three proteins

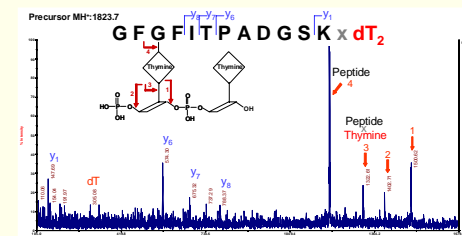
## Scheme for Identification of DNA-Binding Proteins



## Summary of Proteins Found

- 540 Total Proteins Observed from *E. coli***
  - 232 Proteins found to be DNA-binding
  - 52 out of 56 ribosomal proteins observed
- Interesting Findings from Comparative Study**
  - 36.1 KD Hypothetical protein bound only in aerobic lysates
  - Alcohol dehydrogenase present and strongly DNA-binding when expressed under aerobic conditions
  - Pyruvate dehydrogenase complex bound strongly to DNA cellulose in anaerobic samples

## MALDI-TOF-TOF MS/MS of Crosslinked Peptide from CSP-C



High energy CID (1 keV) appears to mainly fragment the oligonucleotide, yielding a characteristic pattern but little peptide sequence information.

## Conclusions

- Effective Use of Function-Based Separation**
  - 540 proteins observed from *E. coli*
  - 232 DNA-binding proteins identified
  - Observed changes in protein binding when proteins expressed under different growth conditions
- Protein Structure Elucidated**
  - DNA-binding site characterized for 3 proteins
  - Amino acids found to be crosslinked are in close proximity within crystal structure

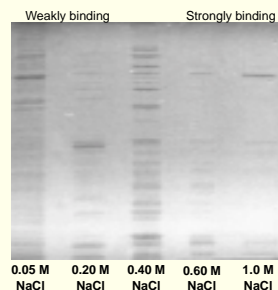
## Introduction

- 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>1</sup>.
- Using a large, generic DNA sequence may lead to the binding of and the subsequent identification of more proteins than a specific DNA sequence.
- Photochemically crosslinking DNA-binding proteins to their substrates is an established procedure for characterizing protein-nucleic acid interfaces<sup>2,3</sup>.
- Photocrosslinking combined with mass spectrometry has proven to be useful in the characterization of contact points between DNA and DNA-binding proteins<sup>4</sup>.

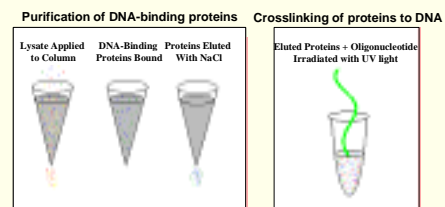
## Future Work

- Function-Based Separation**
  - Isotopic labeling for quantitative results
  - Different substrates for affinity separation
- Photochemical Crosslinking**
  - Variation of oligonucleotide substrates
  - Purification of the crosslinked peptides with IMAC (immobilized metal affinity chromatography)

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

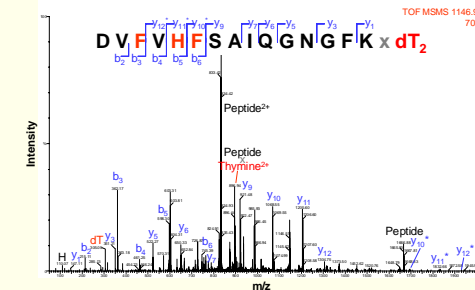


## Methods



Proteins digested in-solution with trypsin  
Nucleotides digested with nuclease P1

## ESI-Q-TOF MS/MS of Crosslinked Peptide from CSP-C



Due to variation in the position of the crosslinked nucleotide tag, both modified and unmodified versions of some y ions can be seen in this spectrum.

## Acknowledgments

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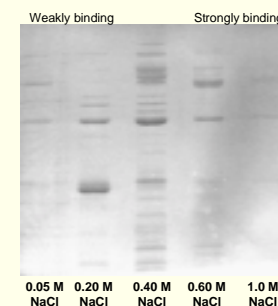
## References

- Bane, T.K., Leblanc, J.F., Lee, T.D., and Riggs, A.D. *Proceedings of the 49th ASMS Conference on Mass Spectrometry and Allied Topics*, Chicago, 2001.
- Shelter, M.D. (1980) *Photochem. Photobiol. Rev.* 5, 105-197.
- Meisenheimer, K. and Koch, T. (1997) *Crit. Rev. Biochem. Molec. Biol.* 32, 101-140.
- Doneanu, C.E., Galken, P.R., and Barofsky, D.F. *Proceedings of the 50th ASMS Conference on Mass Spectrometry and Allied Topics*, Orlando, 2002.
- Schindelin, H., Jiang, W., Inouye, M., Heinemann, U. *Proc Natl Acad Sci USA*, (1994) 5119.

## 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.
- Protein fractions were digested in-solution with trypsin.
- Oligonucleotide dT<sub>20</sub> was used for photochemical crosslinking.
- Tandem mass spectra were obtained on a Finnigan LCQ Classic ion trap, a Micromass Q-TOF Ultima Global, and an Applied Biosystems 4700 Proteomics Analyzer (TOF-TOF).
- Both Sequest and Mascot were used for data analysis.

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

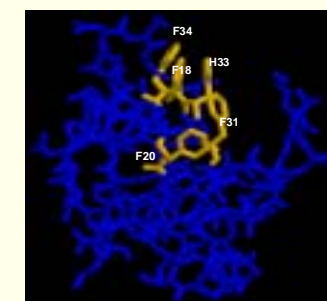


## Crosslinked Peptides Observed

- Cold Shock Protein C (CSP-C)**
  - GFGFITPDDGSK x dT<sub>2</sub>
  - GFGFITPDDGSK x dT<sub>3</sub>
  - DV FVHFSAIQNGGFK x dT<sub>2</sub>
  - DV FVHFSAIQGN x dT<sub>2</sub>
- Cold Shock Protein A (CSP-A)**
  - GFGFITPDDGSK x dT<sub>2</sub>
- Ribosomal Protein L24**
  - VGFRFEDGK x dT<sub>2</sub>

Amino acids in red correspond to those that were found to be crosslinked to the oligonucleotide tag via MS/MS fragmentation. Aromatic amino acids are most likely to be crosslinked due to their base stacking interactions with the DNA.

## Crystal structure from CSP-A<sup>5</sup>



Crosslinked amino acids found in CSP-C are highlighted in yellow.