Electrophoretic Mobility Shift Assay (EMSA) for 1\textsuperscript{st}, 2\textsuperscript{nd}, and 3\textsuperscript{rd} Finger of the Zinc Finger Protein Domain SP1

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Abstract — Zinc fingers describe a class of DNA binding proteins with a modular design \cite{1} in which single fingers can be assembled to form multi-finger arrangements to recognize any desired target sequence. Each individual finger binds preferentially to a specific DNA triplet “with defined three-base-specificity” \cite{2} p. 1. Naturally occurring protein-binding domains typically contain three fingers that bind to a DNA-binding site of a 9 base pair long DNA sequence (9-mer). The modularity of the fingers lends itself naturally to a broad variety of engineering applications. Protein/DNA hybrid structures have applications, for example, in the fabrication of nano-scale functional assemblies \cite{3}, but their primary application is as a versatile tool for designing DNA binding proteins for any target sequence on the human genome \cite{1} for the purpose of gene regulation and genome modification. Such designer zinc fingers have been successfully used to cure genetic diseases \cite{4} and to modify plant and animal genomes \cite{5, 6}. Other useful applications of ZFN have been reported e.g. \cite{7}.

Keywords: zinc finger nuclease (ZFN), cytotoxicity, SP1, binding spectra.

Practical application of engineered zinc fingers in humans is severely limited due to cytotoxic side effects caused by “off-target” binding site activities leading to cell death and apoptosis \cite{8}. To add to the challenge, recent findings indicate discrepancies and inconsistencies of results produced by various \textit{in-vitro} and \textit{in-vivo} assays \cite{2, 9}, which might be caused by evolutionary plasticity \cite{10} in which the binding capabilities of single fingers vary significantly due to the high malleability of their three-dimensional structure leading to changes in their binding preferences in various tissue conditions \cite{11}. Because of “our limited understanding of even simple DNA protein interaction” \cite{12} p.2500, limited knowledge of TF functions \cite{11} p.253 and lack of precise data to accurately predict binding recognition \cite{13}, \cite{14} p.144, progress is slow to systematically translate brilliant therapies from animal models e.g. \cite{4} into clinical endpoints.

To improve the current situation therefore, it is of vital interest to investigate the nature of “off-target” binding, to identify and eliminate the potential factors that prevent medical use and to gain insights from diverse sources for directing further research efforts and technological advances that will provide the means to create critical knowledge and technological breakthroughs with broad impact on research and society especially, because molecular biology today not only enables us to modify the human genome for curing inherited genetic diseases, but also will, in the foreseeable future, has the potential to replace damaged or aging tissues and organs. This is due to the unprecedented advances in the biomedical sciences of which, first of all stands out, our capability to induce the creation of stem cells from our own ordinary skin cells and then grow them in numbers to replace burned skin or entire organs.

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Materials and Methods

Sequence-specific DNA recognition is made by interactions of the amino acids in the α-helical region of SP1 (Fig. 1), by establishing specific hydrogen-bonds with the nucleotides in the DNA's major groove involving three neighboring base pairs on a DNA string. For SP1, the consensus target binding site is GG(G/T) G(C/A)G (G/T)GG in which the primary triplet for the 2nd finger is GCG e.g. [2, 15].

![Amino acid sequence and structure of zinc finger protein SP1.](image)

Sp1 protein is expressed using plasmid pPacSpl-516c provided by R. Tjian and purified by FPLC Mono S chromatography [16-18]. The DNA binding capability of the 2nd finger of Sp1 can be assessed by incubating the 64 P32-labeled double-stranded oligonucleotides (Fig. 1d) by performing electrophoresis mobility shift assays (EMSA).

![P32-labeled double-stranded oligonucleotide.](image)

ANN-series: AAA, AAC, AAG, AAT, ACA, ACC, ACG, ACT, AGA, AGC, AGG, AGT, ATA, ATC, ATG, ATT

CNN-series: CAA, CAC, CAG, CAT, CCA, CCC, CCG, CCT, CGA, CGC, CGG, CGT, CTA, CTC, CTG, CTT

GNN-series: GAA, GAC, GAG, GAT, GCA, GCC, GCG, GCT, GGA, GGC, GGG, GGT, GTA, GTC, GTG, GTT

TNN-series: TAA, TAC, TAG, TAT, TCA, TCC, TCG, TCT, TGA, TGC, TGG, TGT, TTA, TTC, TTG, TTT

Figure 3: The 64 nucleotide triplets for the 2nd finger are divided into four 16 triplet series.

Proposed Research

In the proposed research non-radioactive electrophoretic mobility shift assays (EMSA) is used to perform the 64 assays for each of the three fingers of Sp1. The 64 oligonucleotides (see Figure 1C) will be biotin-labeled. The advantages of biotinylation is that the process is rapid and specific that might result in more precise and repeatable data sets that can be employed for developing computational tools to design zinc finger domains and predict binding site recognition in various conditions. In addition, biotinylation can be used for teaching (undergraduate, graduate, PhD).

The proposed project has been approved by Brookhaven National Laboratory (BNL) for use of facilities and instruments at the Center of Functional Nanotechnology (CFN).
Future Research
Future projects focus on the application of technologies available at Brookhaven National Laboratory to accurately measure and capture signals produced by the cell-based assay using instruments at the Center of Functional Nanotechnology (CFN).

Project Plan
The project plan comprises four main phases: Installation of lab, preliminary testing and lab improvement, testing, and dissemination.

**Installation and Acquisition Phase:** Lab equipment and material (electrophoretic units, chemicals, measures, chemiluminescent detection imager, etc.) will be acquired.

**Evaluation Phase:** In this phase purified Sp1 protein and oligos are acquired to produce bandshifts, optimize lab protocols, adjust equipment and optimize lab routines. This phase usually is the most time consuming one.

**Testing Phase:** Bandshifts for each of three finger of Sp1 for up to the sixty-four possible binding triplets will be produced.

**Analysis and Dissemination Phase:** This phase involves the development of computational interfaces and tools for capturing, analyzing, and disseminating results, techniques, protocols and methods.

Project Timeline and Points of Evaluation

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        | Apr- | May- | Aug- | Nov- | Feb- | May- | Aug- | Nov- | Apr- |
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1      | Acquisition of material and lab installation |   |   |   |   |   |   |   |   |
2      | Acquisition of Sp1 protein and labeled oligos |   |   |   |   |   |   |   |   |
3      | Performing EMSA with Sp1 and 1-3 oligos |   |   |   |   |   |   |   |   |
4      | Protocol revisions and lab optimization |   |   |   |   |   |   |   |   |
5      | Performing EMSA with Sp1 and 1-64 oligos |   |   |   |   |   |   |   |   |
6      | Student Training/Workshop, Outreach, Publications and Conferences |   |   |   |   |   |   |   |   |
7      | Project report and result |   |   |   |   |   |   |   |   |
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*Figure 4: Project Timeline with points of Formative Evaluation.*
Formative Evaluation Points across the project timeline:

- **Assessment (Outcome 1a-c):** After material acquisition and lab installation there will be a quality assessment of lab equipment, materials and constructs in summer 2012.

- **Preliminary Tests (Outcome 1a-c):** Acquisition of purified Sp1 protein (GenScript) and biotin-labeled oligos (IDT).

- **Preliminary Tests (Outcome 1a-c):** First EMSA assays will be conducted. The goal is to produce bandshifts, develop a lab routine and improve lab protocols.

- **Module Development (Outcome 2):** Testing and potential adjustments of computational tools.

- **Workshops (Outcome 3a,b):** Participants will fill out an evaluation forms at trainings and workshops.

**Importance**

The outcome of the proposed work is an assay to determine the 64-rank-specific recognition code for single zinc fingers in a three finger domain. The Rank-Specific Recognition Assay (RSRA) allows determining the rank-specific recognition code for thousands of artificial and natural zinc finger domains.

**References**


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Professor Christian Bach serves as an Assistant Professor of Technology Management and Biomedical Engineering. His research interests include but are not limited to: intracellular immunization, induced Pluripotent Stems (iPS) cells, artificial transcription factors, and target Detection Assay.

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Professor Prabir Patra currently serves as the Assistant Professor of Mechanical Engineering and Program Director of Biomedical Engineering at the University of Bridgeport. He received his Master’s and Ph.D. degrees from Indian Institute of Technology (IIT) in Kharagpur, India. He has done his postdoctoral research at the University of Massachusetts Dartmouth and Rice University.

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