SHARADA SIVARAMAN, Ph.D.

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OBJECTIVE:

A challenging position in research and development where I can apply my extensive knowledge of and experience in protein biochemistry.

HIGHLIGHTS:

- Over three years of experience in recombinant expression and purification of proteins (human and bacterial) in *E. coli*.
- Extensive experience in expression vector manipulation, molecular cloning and site-directed mutagenesis.
- Experience in directed evolution (DNA shuffling) to alter substrate specificity of aminotransferases.
- Experience in examining enzyme structure-function through enzyme kinetics.
- Established picomolar affinity and developed SAR of inhibition of an antibacterial drug target.
- Excellent scientific writing and presentation skills.
- Supervised the research of two undergraduate students.
- Good collaborative and communication skills.

RESEARCH EXPERIENCE:

Oct 2002-June 2006 **Postdoctoral Research, *University of California, Berkeley*.**
Advisor: Professor Jack F. Kirsch

- Engineered the *E. coli* tyrosine aminotransferase, a broadly substrate specific aminotransferase to narrow substrate specificity through DNA shuffling and random mutagenesis.
- Developed novel genetic selection methodology in *E. coli* to select for variants with altered aminotransferase activity.
- Expressed and characterized human tyrosine aminotransferase (hTATase), the enzyme responsible for the metabolic disorder, tyrosinemia type II.
- Discovered unique preference of human tyrosine aminotransferase for Tyrosine: $(k_{cat}/K_m)^{Tyr}$ / $(k_{cat}/K_m)^{Phe} \sim 10^4$
- Analyzed disease-causing hTATase mutants: effect of mutations on structure by urea denaturation and limited proteolysis.

1997-Aug 2002 **Graduate Research, *State University of New York at Stony Brook.***
Advisor: Professor Peter J. Tonge

- Initiated steady-state kinetic analysis of slow, tight binding inhibition (picomolar) of *E. coli*, enoyl-ACP reductase, FabI, by triclosan, a common biocide.
- Constructed FabI mutants: residues that are critical for triclosan binding and mutations that lead to triclosan resistance.
- Structure-function exploration: investigated the binding of triclosan to various FabI mutants. Extended the SAR study to triclosan analogues to systematically explore the contribution of functional groups on triclosan towards binding.
- Analyzed triclosan/analogues binding to WT and mutant FabIs – mutations correlated with increase in triclosan resistance. Identified molecular basis of high affinity.

- Validation of *E. coli* FabI as an excellent target for antibacterial interventions.

Summer 1996 **Visiting Students Research Program Fellow, *Tata Institute of Fundamental Research, Mumbai, India.***
Advisor: Professor G. Krishnamoorthy

- Investigated the presence of intermediates in the folding pathway of a mutant barstar protein through fluorescence quenching studies.

1995-1997 **Research Assistant, *Indian Institute of Technology, Mumbai, India***
Advisor: Professor C.P. Rao

- Synthesized protected monosaccharides, acetal derivatives of D-glucose.
- Complexation studies of protected saccharides with Co (II), Ni (II) and Cu (II) metal ions.

EDUCATION:

Oct 2002-June 2006 Postdoctoral Research Fellow, **University of California at Berkeley**

August 2002 Ph.D. in Biological Chemistry, **State University of New York at Stony Brook, NY**

1997 M.Sc. Inorganic Chemistry, **Indian Institute of Technology, Mumbai, India**

1995 B.Sc. Chemistry, **University of Madras, Madras, India**

SKILLS:

- Molecular biology: expression vector manipulation, PCR, DNA shuffling, error-prone PCR, site-directed mutagenesis, library cloning, genomic DNA manipulation, gel electrophoresis.
- Protein expression: heterologous expression of proteins (microbial and human) in *E. coli*, isolation from inclusion bodies, protein refolding.
- Protein purification: SDS-PAGE, Ion exchange chromatography, size-exclusion, affinity chromatography (His-tag/Ni-NTA, IMPACT system), hydrophobic interaction chromatography and FPLC.
- High throughput assay development using microtitre 96-well plate reader.
- Enzymology: Use of steady state enzyme kinetics, inhibition kinetics, coupled enzyme assays, binding constant determination by fluorescence and UV-VIS spectroscopy to determine enzyme-inhibitor interactions
- Basic skills in organic synthesis- bioorganic synthesis of substrate/analogs, purification by RP-HPLC.
- Antimicrobial susceptibility experiments for aerobically growing bacteria (MIC determination).
- Software: Insight II, Molscript, Rasmol, SWISS-MODEL, DeepView, PILEUP & PYMOL.

ACADEMIC HONORS AND AWARDS:

- Sigma Xi Award for Excellence in Research, May 2002.
- Travel Grant Award, American Chemical Society, Division of Medicinal Chemistry, April 2001.
- Travel Grant Award, American Society of Microbiology, June 2000.
- Sigma Xi Travel Award, SUNY at Stony Brook Chapter of Sigma Xi, May 2000.
- Visiting Students Research Program Fellowship, Tata Institute of Fundamental Research, Mumbai, India, May 1996
- Sister Helen Vincent Award for Outstanding Student in the Sciences, Stella Maris College, Madras, India, May 1995

PUBLICATIONS:

Sharada Sivaraman, Jacque Zwahlen, Alasdair F. Bell, Lizbeth Hedstrom and Peter J. Tonge; "Structure-Activity Studies of the Inhibition of FabI, the Enoyl Reductase from Escherichia coli, by Triclosan: Kinetic Analysis of Mutant FabIs". ***Biochemistry*** (2003) *42*, 4406-4413

Sharada Sivaraman, Todd J. Sullivan, Francis Johnson, Polina Novichenok, Guanglei Cui, Carlos Simmerling and Peter J. Tonge; " Inhibition of the Bacterial Enoyl Reductase, FabI, by Triclosan: A Structure-Reactivity Analysis of FabI Inhibition by Triclosan Analogues." ***J. Med. Chem.*** (2004); 47(3); 509-518.

Sharada Sivaraman and Jack F. Kirsch; "The narrow substrate specificity of human tyrosine aminotransferase, the gene deficient in tyrosinemia type II". ***FEBS Journal*** (2006) 273, 1920-1929.

PRESENTATIONS:

- **Sharada Sivaraman**, "Role of the Human Tyrosine Aminotransferase in Tyrosinemia Type II." Guest Lecture in the DeCAL Course "**L-Chem 101: Medicine and Life Systems"** Spring 2006, University of California, Berkeley.
- **Sharada Sivaraman** and Jack F. Kirsch "Narrowing the Substrate Specificity of a Broadly Substrate Specific Enzyme by Directed Evolution." Annual Biochemistry and Molecular Biology Research Conference & Retreat, Asilomar, January, 2005
- **Sharada Sivaraman** and Peter. J. Tonge "Inhibition of the Enoyl - ACP reductase from *E. coli*, FabI, by Triclosan". 222nd American Chemical Society National Meeting, Chicago, Illinois, August 2001.
- **Sharada Sivaraman**, Richa Rawat and Peter J. Tonge "Characterization of Putative Acyl-CoA Dehydrogenases FadE23/24". Gordon Research Conference on Tuberculosis Drug Development, New Hampshire, June 2001.

REFERENCES:

Professor Jack F. Kirsch
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Stony Brook, NY 11794-3400
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