

*Dedicated to my beloved parents,*

*Mr. Udoi Bhaskar Puzari (Deta)*

*and*

*Mrs. Sangeeta Puzari (Maa)*

*for bestowing me with unconditional love,  
blessings, endless support and encouragement*

*In loving memory of my grandmother (Aita)*

*Late (Mrs.) Amiya Sharma*

## DECLARATION BY THE CANDIDATE

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I hereby declare that the thesis “Development of Analytical Methods for Identification of Indian Snake Venoms and Indian Red Scorpion Venom” being submitted to Department of Molecular Biology and Biotechnology, Tezpur University, Tezpur, Assam in partial fulfillment for the award of the degree of Doctor of Philosophy in Molecular Biology and Biotechnology, has previously not formed the basis for the award of any degree, diploma, associateship, fellowship or any other similar title or recognition. Due to unavailability of proper facilities in Tezpur University, the following experiments/sample analyses were carried out at other institutes:

1. LC-MS/MS analysis of protein samples were performed at National Centre for Cell Science, NCCS, Pune 411007, India.
2. *In vivo* experiments and biophysical characterisation of gold nanoparticles are performed at Institute of Advanced Study in Science and Technology (IASST), Guwahati 781035, India

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## TEZPUR UNIVERSITY

### CERTIFICATE OF SUPERVISOR

This is to certify that the thesis entitled “**Development of Analytical Methods for Identification of Indian Snake Venoms and Indian Red Scorpion Venom**” submitted to the School of Sciences, Tezpur University in requirement of partial fulfilment for the award of the degree of Doctor of Philosophy in Molecular Biology and Biotechnology is a record of research work carried out by Ms. Upasana Puzari under my supervision and guidance. All help received by her from various sources have been duly acknowledged. No part of this thesis has been submitted elsewhere for award of any other degree.

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**(q)** Absorbance spectrum for NnV spiked rat plasma detection by AuNP-FPAb conjugate. Absorbance curves correspond to plasma samples containing 0.125-2 ng/ $\mu$ L NnV; **(r)** Calibration curve for NnV spiked rat plasma detection at concentrations 0.125-2 ng/ $\mu$ L; **(s)** Absorbance spectrum for KV spiked rat plasma detection by AuNP-FPAb conjugate. Absorbance curves correspond to plasma samples containing 0.125-2 ng/ $\mu$ L KV; **(t)** Calibration curve for KV spiked rat plasma detection at concentrations 0.125-2 ng/ $\mu$ L; **(u)** Absorbance spectrum for NkV spiked rat plasma detection by AuNP-FPAb conjugate. Absorbance curves correspond to plasma samples containing 0.25-4 ng/ $\mu$ L NkV; **(v)** Calibration curve for NkV spiked rat plasma detection at concentrations 0.25-4 ng/ $\mu$ L; **(w)** Absorbance spectrum for RvV spiked rat plasma detection by AuNP-FPAb conjugate. Absorbance curves correspond to plasma samples containing 0.125-2 ng/ $\mu$ L RvV; **(x)** Calibration curve for RvV spiked rat plasma detection at concentrations 0.125-2 ng/ $\mu$ L; **(y)** Absorbance spectrum for EcV spiked rat plasma detection by AuNP-FPAb conjugate. Absorbance curves correspond to plasma samples containing 0.25-4 ng/ $\mu$ L EcV; **(z)** Calibration curve for

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EcV spiked rat plasma detection at concentrations 0.25-4 ng/ $\mu$ L;  
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## ABBREVIATIONS

Abbreviation	Full form
<b>3D</b>	Three-dimensional
<b>3FTx</b>	Three-finger toxin
<b>20WBCT</b>	20-min whole blood clotting test
<b>AB-</b>	Avidin-biotin micro enzyme-linked
<b>microELISA</b>	immunosorbent assay
<b>AChE</b>	Acetylcholinesterase
<b>ACN</b>	Acetonitrile
<b>APase</b>	Aminopeptidase
<b>AFM</b>	Atomic force microscopic
<b>ANOVA</b>	Analysis of variance
<b>ASPro</b>	Aspartic protease
<b>ATP</b>	Adenosine triphosphatase
<b>ASA</b>	Commercial equine anti-scorpion antivenom
<b>AuNP</b>	Gold nanoparticle
<b>BLASTp</b>	Protein-protein BLAST
<b>BPP</b>	Bradykinin potentiating peptide
<b>BSA</b>	Bovine serum albumin
<b>β-BuTx</b>	β-bungarotoxin
<b>cDNA</b>	Complementary Deoxyribonucleic Acid
<b>ChE</b>	Cholinesterase
<b>CP</b>	Custom peptide
<b>CCSEA</b>	Committee for Control and Supervision of Experiments on Animals
<b>CMYK</b>	Cyan, magenta, yellow and black
<b>CRISP</b>	Cysteine-rich secretory protein
<b>CSL</b>	Commonwealth Serum Laboratories
<b>CTL</b>	C-type lectin
<b>CVF</b>	Cobra venom factor
<b>DMF</b>	dimethyl formamide
<b>DNase</b>	Deoxyribonuclease
<b>DTT</b>	Dithiothreitol

<b>Abbreviation</b>	<b>Full form</b>
<b>EcV</b>	<i>Echis carinatus</i> venom
<b>ECL</b>	Enhanced Chemiluminescence
<b>EDC</b>	1-Ethyl-3-(3-dimethylaminopropyl) carbodiimide
<b>EIA</b>	Enzyme immunoassay
<b>EIS</b>	Electrochemical impedance spectroscopy
<b>ELAA</b>	Enzyme-linked aptamer assay
<b>ELISA</b>	Enzyme-linked immunosorbent assay
<b>ExPASy</b>	Expert Protein Analysis System
<b>FELISA</b>	Fluorogenic enzyme-linked immunosorbent assay
<b>Fmoc</b>	9-fluorenylmethoxycarbonyl
<b>FPAb</b>	Purified polyclonal snake venom toxin-specific antibody formulation
<b>FTIR</b>	Fourier-transform infrared spectroscopy
<b>GC</b>	Glutaminy cyclase
<b>GQDs</b>	Graphene quantum dots
<b>HAP</b>	High-abundance protein
<b>HCL</b>	Hydrochloride
<b>HMG CoA</b>	Hydroxymethylglutaryl-coenzyme A
<b>Hya</b>	Hyaluronidase
<b>HRP</b>	Horse radish peroxidase
<b>HSS-Abs</b>	Hemorrhagic species-specific antibodies
<b>IgG</b>	Immunoglobulin G
<b>ISFET</b>	Ion-sensitive field-effect transistor
<b>K<sub>D</sub></b>	Dissociation constant
<b>KLH</b>	Keyhole Limpet Hemocyanin
<b>KSPI</b>	Kunitz-type proteinase inhibitor
<b>KV</b>	<i>Bungarus caeruleus</i> venom
<b>LAAO</b>	L-amino acid oxidase
<b>LC-MS/MS</b>	Liquid chromatography-tandem mass spectrometry
<b>LFA</b>	Lateral flow assay
<b>LMMPT</b>	Low molecular mass peptide toxins
<b>LoD</b>	Limit of detection

<b>Abbreviation</b>	<b>Full form</b>
<b>LoQ</b>	Limit of quantitation
<b>LPP</b>	Lipolysis potentiating peptides
<b>LSPR</b>	Localised surface plasmon resonance
<b>mAb</b>	Monoclonal antibody
<b>MALDI-TOF</b>	Matrix-assisted laser desorption/ionization -Time of flight
<b>MBS</b>	Maleimidobenzoyl-Nhydroxysuccinimide ester
<b>MTV</b>	<i>Mesobuthus tamulus</i> venom
<b>MSI</b>	Match Precursor Intensity
<b>MUA</b>	Mercaptoundecanoic acid
<b>NCBI</b>	National Center for Biotechnology Information
<b>NEI</b>	North-East India
<b>NGF</b>	Nerve growth factor
<b>NHS</b>	N-Hydroxysuccinimide
<b>NnV</b>	<i>Naja naja</i> venom
<b>NkV</b>	<i>Naja kaouthia</i> venom
<b>NP</b>	Natriuretic peptide
<b>NSS-Abs</b>	Neurotoxic species-specific antibodies
<b>OIA</b>	Optical immunoassay
<b>OLP</b>	Ohanin-like protein
<b>PAb</b>	Purified polyclonal antibody
<b>PAbF</b>	Purified polyclonal scorpion venom toxin-specific antibody formulation
<b>PAV</b>	Commercial equine anti-snake antivenom
<b>PBS</b>	Phosphate buffered saline
<b>PCR</b>	Polymerase chain reaction
<b>PDB</b>	Protein Data Bank
<b>PDE</b>	Phosphodiesterase
<b>PIR</b>	Protein Information Resource
<b>PLA<sub>2</sub></b>	Phospholipase A <sub>2</sub>
<b>PLB</b>	Phospholipase B
<b>PSVPL</b>	Premium Serum and Vaccine Pvt. Ltd.
<b>PVDF</b>	Polyvinylidne fluoride

<b>Abbreviation</b>	<b>Full form</b>
<b>Qdots</b>	Quantum dots
<b>RGB</b>	Red, green and blue
<b>RGI-MDS</b>	Registrar General of India-Million Death Study
<b>RIA</b>	Radioimmunoassay
<b>RP-HPLC</b>	Reversed-phase high-performance liquid chromatography
<b>RT-PCR</b>	Reverse transcription polymerase chain reaction
<b>RvV</b>	Daboia russelii venom
<b>SDS-PAGE</b>	Sodium dodecyl sulfate-polyacrylamide gel electrophoresis
<b>SELEX</b>	Systematic Evolution of Ligands by EXponential
<b>SPI</b>	Serine protease inhibitor
<b>SPLP</b>	Serine protease-like protein
<b>SPPS</b>	Solid-phase peptide synthesis
<b>SPR</b>	Surface plasmon resonance
<b>SSAbs</b>	Species-specific antibodies
<b>SVDK</b>	Snake venom detection kit
<b>SVMP</b>	Snake venom metalloprotease
<b>SVSP</b>	Snake venom serine protease
<b>SVTLE</b>	Snake venom thrombin-like enzyme
<b>TBS</b>	Tris buffered saline
<b>TBS-T</b>	Tris buffered saline with 0.05% tween-20
<b>TEM</b>	Transmission electron microscope
<b>TEMED</b>	Tetramethylethylenediamine
<b>TMB/H<sub>2</sub>O<sub>2</sub></b>	3,3,5,5'-tetramethylbenzidine/hydrogen peroxide
<b>TiO<sub>2</sub></b>	Titanium dioxide
<b>UniProtKB</b>	Universal Protein Resource Knowledgebase
<b>UV-Vis</b>	Ultraviolet-visible
<b>VDET</b>	Venom Detection ELISA Test
<b>VEGF</b>	Vascular endothelial growth factor
<b>Vesp</b>	Vespryn
<b>WHO</b>	World health organization