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Education

University of Hyderabad, Hyderabad, MSc 1997, Chemistry
Tata Institute of fundamental Research, PhD 2002, Physical Chemistry

Research Training

Jul 1997 – Nov 2002: Ph. D. Thesis, Tata Institute of Fundamental Research, Mumbai, India.
Advisor: Prof. N. Periasamy.

Nov 2002 – May 2003: Visiting fellow, Tata Institute of Fundamental Research, Mumbai, India.
Advisors: Prof. N. Periasamy and Prof. G. Krishnamoorthy.

May 2003 – Jan 2008: Post-doctoral research scientist, Department of Biological Sciences, Columbia University. New York, USA. Advisor: Prof. Julio M. Fernandez.

Professional Experience

Feb 2008 – present: Reader, Department of Chemical Sciences, Tata Institute of Fundamental Research, Mumbai, India.

Current Research Activities:

Mechanical forces play a central role in ubiquitous phenomena such as protein degradation, cell-adhesion, tissue organization, and muscle function in multi-cellular organisms. The key players in these phenomena are protein molecules, which act as mechanosensors and communicate the surrounding dynamic microenvironment within the cell. Hence, studying the mechanical response of these biomolecules would provide a wealth of information about their structure, function, and chemistry.

We use state-of-the-art atomic force microscope (AFM) to probe single molecules. By using this novel technique, we can apply stretching force to a single protein molecule, measure its mechanical response and study protein mechanics.

Previous Research Activities:

At Columbia University, New York, USA

We used single-molecule force spectroscopy techniques based on a custom-built AFM to study protein mechanics and chemical reactions. We were involved in the development of data acquisition protocols and software related to AFM. We also used general molecular biology, protein engineering, and protein expression and purification techniques to construct polyproteins (proteins covalently linked in tandem) based on titin immunoglobulin-like domain (I27) for the following projects.

- *Ligand-binding modulated mechanical stability of dihydrofolate reductase (DHFR).* DHFR is an enzyme that catalyzes the reduction of folate in the presence of the cofactor NADPH. Using force spectroscopy, we showed that the mechanical stability of DHFR increases from 27 pN to 80 pN upon ligand binding. This result also supported the view that protein translocation and protein degradation *in vivo* are 'mechanical' processes in which substrate proteins are mechanically stretched to unfold.
- *Controlled unravelling and accelerated refolding of proteins by disulfide bonds.* Disulfide engineering in polyproteins led to more detailed understanding on how proteins respond to mechanical forces. These single-molecule experiments measured an important parameter, contour length per amino acid (4.0 Å), which is an essential quantity for the determination of the size of a protein in protein mechanics. This study also suggested that extracellular proteins and cell-surface receptors are only partially unravelled under mechanical stress because the disulfide bonds interrupt unfolding and they also accelerate the refolding by acting as folding nuclei.

- *Force accelerated chemical reactions and transition state structure.* Engineered disulfide bonds in proteins are mechanically stretched and reduced with various reducing agents. Stretching forces applied to single disulfide bonds accelerated reduction reaction (S_N2 reaction) by lowering the activation energy. In addition, force-clamp spectroscopy determined the details of the transition state (TS) such as the disulfide bond elongation (0.30 – 0.45 Å) at the TS.
- *Mechanical unfolding pathways of the enhanced yellow fluorescent protein (EYFP).* Mechanical unfolding of EYFP identified two transition states for unfolding. Circular permutants (mutants of a protein which have different N and C termini compared to wild-type but with same structure) of EYFP allowed to decipher the two unfolding pathways of EYFP.

At Tata Institute of Fundamental Research (TIFR), Mumbai, India:

Ph. D. thesis titled “*Fluorescence Spectroscopy and Dynamics of Organic Molecules in Complex Systems*” encompasses the following two major projects. (Thesis advisor: *Prof. N. Periasamy*)

1. Theory and development of time-resolved area normalized emission spectroscopy (TRANES), an analytical technique, to determine the heterogeneity of complex systems such as a probe distribution in biological membranes, mixed solvents, and excited-state dynamics of photo-activated systems.
 2. Supramolecular assemblies of Porphyrins, Cyanines, and template-based aggregation using poly-lysine. Investigation of their optical, structural properties using absorption, fluorescence, dynamic light scattering (DLS), and atomic force microscopy (AFM).
- Solid-phase synthesis and purification of truncated human telomeric DNA sequences which form novel quadruplexes, and their structural characterization using nuclear magnetic resonance (NMR). (Advisor: *Prof. R. V. Hosur*)
 - Protein purification and characterization of Lactoperoxidase (LPO), an important peroxidase, using absorption, fluorescence and circular dichroism spectroscopy. (Visiting student research programme (VSRP), May-July 1996. Advisor: *Prof. D. V. Behere*)

Curricular/Academic Achievements

- Gold Medal in Bachelor of Science (B.Sc.).
- All India Merit Scholarship for post graduation (M.Sc.), Govt. of India (1995-1997).
- Visiting Student’s Research Programme (VSRP) Scholarship from Tata Institute of Fundamental Research, India (1996).
- Gold-Medal in M.Sc.(Chemistry) from University of Hyderabad (1997).
- National CSIR fellowship for Junior Research fellowship and Lecturership by the Council for Scientific and Industrial Research, HRD group, Govt. of India (1997).
- Best Poster Award in the “Trombay Symposium for Radiation and Photochemistry”, at BARC, Mumbai India (1999).
- Travel grant from Sarojini Damodaran International Fellowship Programme in 2001 to attend the international conference, 'Methods and Applications in Fluorescence: Spectroscopy, Imaging and Probes', which was held in Amsterdam, The Netherlands (2001).
- Platform presentation of “Single Molecule Force and Fluorescence studies of the Enhanced Green Fluorescent Protein (EGFP)” at the 48th annual meeting of the Biophysical Society, Baltimore, Maryland, USA (2004).

Research Publications

1. Vasudha Aggarwal, S. Rajendra Kulothungan, M.M. Balamurali, S.R. Saranya, Raghavan Varadarajan, and **Sri Rama Koti Ainarapu***, Ligand modulated parallel mechanical unfolding pathways of Maltose Binding Proteins (MBPs). *J. Biol. Chem.*, (In press).
2. **Sri Rama Koti Ainarapu**, Single-molecule force-clamp spectroscopy: Probing transition states of mechanically activated chemical reactions, *ISRAPS Bulletin* Vol. 21 (2009) p 32-39.
3. Lorna Dougan, **Sri Rama Koti Ainarapu**, Georgi Genchev, Hui Lu and Julio M. Fernandez, A Single-Molecule Perspective on the Role of Solvent Hydrogen Bonds in Protein Folding and Chemical Reactions, *Chem. Phys. Chem.*, Vol. 9 (2008) p 2836-2847.

4. **Sri Rama Koti Ainavarapu***, Arun P. Wiita, Lorna Dougan, Einar Uggerud and J.M. Fernandez*, Single-Molecule force spectroscopy measurements of bond elongation during a bimolecular reaction, *J. Am. Chem. Soc.*, Vol. 130 (2008) p 6479-6487.
Our mechanochemistry JACS paper has been highlighted in Research Highlights of Nature, 15 May 2008.
5. **Sri Rama Koti Ainavarapu***, Arun P. Wiita, Hector H. Huang, and J.M. Fernandez, A Single-Molecule Assay to Directly Identify Solvent Accessible Disulfide Bonds and Probe Their Effect on Protein Folding, *J. Am. Chem. Soc.*, Vol. 130 (2008) p 436-437.
6. Robert Szoszkiewicz, **Sri Rama Koti Ainavarapu**, Arun P. Wiita, Raul Perez-Jimenez, Jose M. Sanchez-Ruiz, and J. M. Fernandez, Dwell Time Analysis of a Single-Molecule Mechanochemical Reaction, *Langmuir*, Vol. 24 (2008) p1356-1364.
7. **Sri Rama Koti Ainavarapu**, Jasna Brujic, Hector H. Huang, Arun P. Wiita, Lewyn Li, Hui Lu, Kirstin Walther, Mariano Carrion-Vazquez, Hongbin Li, and J.M. Fernandez, Contour length and refolding rate of a small protein controlled by engineered disulfide bonds, *Biophys. J.*, Vol. 92, (2007) p 225-233.
8. Raul Perez-Jimenez, Sergi Garcia-Manyes, **Sri Rama Koti Ainavarapu**, and J.M. Fernandez Mechanical Unfolding Pathways of the Enhanced yellow Fluorescent Protein Revealed by Single Molecule Force Spectroscopy, *J. Biol. Chem.*, Vol. 281, No. 52 (2006) p 40010-40014.
9. Arun P. Wiita, **Sri Rama Koti Ainavarapu**, Hector H. Huang, and J.M. Fernandez, Force-dependent chemical kinetics of disulfide bond reduction observed with single molecule techniques, *Proc. Natl. Acad. Sci. USA*, Vol. 103, No. 19 (2006) p7222-7227.
Commentary on this article is reported in PNAS (2006), 103, p7533: "Covalent chemistry on distended proteins."
10. **Sri Rama Koti Ainavarapu**, Lewyn Li and J.M. Fernandez, Fingerprinting DHFR in single-molecule AFM studies, *Biophys. J.*, Vol. 91, No. 5 (2006) p 2009-2010.
11. **Sri Rama Koti Ainavarapu**, Lewyn Li, Carmen L. Badilla and J.M. Fernandez, Ligand binding modulates the mechanical stability of dihydrofolate reductase (DHFR), *Biophys. J.*, Vol. 89, No. 5 (2005) p3337-3344.
12. M.K. Singh, H. Pal, **Sri Rama Koti Ainavarapu** and A.V. Sapre, Photophysical Properties and Rotational Relaxation Dynamics of Neutral Red Bound to β -cyclodextrin, *J. Phys. Chem. A*, Vol. 108 (2004) p1465-1474.
13. C. Tiseanu, M.U. Kumke, **Sri Rama Koti Ainavarapu**, and V.I.Parvulescu. Lanthanides distribution in ZSM-5/MCM-41 hybrid materials, *J. Photochem. Photobiol. A: Chemistry*, Vol. 187, No. 2-3 (2007) p299-304.
14. **Sri Rama Koti Ainavarapu**, Jharna Taneja and N. Periasamy, Control of Coherence length and Aggregation size in the J-aggregate of Porphyrin, *Chem. Phys. Lett.* Vol.375, No. 1-2 (2003) p171-176.
15. N. Periasamy and **Sri Rama Koti Ainavarapu**, Time Resolved Fluorescence Spectroscopy: TRES and TRANES, *PINSA* Vol. 69, No. 1, (2003) p41-48.
16. **Sri Rama Koti Ainavarapu** and N. Periasamy, Self-Assembly of Template-Directed J-aggregates of Porphyrin, *Chem. Mat.*, Vol. 15, No. 2 (2003) p369-371.
17. Ira, **Sri Rama Koti Ainavarapu**, G. Krishnamoorthy and N. Periasamy, TRANES Spectra of Fluorescence Probes in Lipid Bilayer Membranes: An Assessment of Population Heterogeneity and Dynamics, *J. Fluor.*, Vol. 13, No. 1 (2003) p95-103.
18. **Sri Rama Koti Ainavarapu** and N. Periasamy, Time Resolved Area Normalized Emission Spectroscopy (TRANES) of DMABN Confirms Emission from Two States, *Res. Chem. Inter.* Vol. 28, No. 7-9 (2002) p831-836.
19. **Sri Rama Koti Ainavarapu** and N. Periasamy, Cyanine induced Aggregation in *meso*-tetrakis (4-sulphonatophenyl) Porphyrin Anions, *J. Mat. Chem.*, Vol. 12, No. 8 (2002) p2312-2317.
20. N. Periasamy and **Sri Rama Koti Ainavarapu**, Time Resolved Fluorescence Spectroscopy: TRES vs TRANES, *ISRAPS Bulletin* Vol. 12, No. 1-4 (2001) p26-29.
21. **Sri Rama Koti Ainavarapu** and N. Periasamy, Application of Time Resolved Area Normalized Emission Spectroscopy (TRANES) to multi-component systems, *J. Chem. Phys.*, Vol. 115, No. 15 (2001) p7094-7099.
Our TRANES work has been the part of Principles of Fluorescence Spectroscopy, 3rd edition, by J.R. Lakowicz, chapter 7, p237-276.
22. **Sri Rama Koti Ainavarapu** and N. Periasamy, TRANES Analysis of the Fluorescence of Nile Red in Organized Molecular Assemblies Confirms Emission from Two Species, *Proc. Indian Acad. Sci., (Chem. Sci.)* Vol. 113, No. 2 (2001) p157-163.

23. **Sri Rama Koti Ainavarapu**, M.M.G. Krishna and N. Periasamy, Time-Resolved Area-Normalized Emission Spectroscopy (TRANES): A Novel Method for Confirming Emission from Two Excited States, *J. Phys. Chem. A*, Vol. 105 (2001) p1767-1771.
24. **Sri Rama Koti Ainavarapu**, B. Bhattacharjee, N.S. Haram, Ranjan Das, N. Periasamy, N.D. Sonawane, D.W. Rangnekar, Photophysics of some styryl thiazolo quinoxaline dyes in organic media, *J. Photochem. Photobiol. A: Chemistry*, Vol. 137 No. 2-3 (2000) p115-123.
25. **Sri Rama Koti Ainavarapu** and N. Periasamy, Solvent Exchange in Excited-State Relaxation in Mixed Solvents, *J. Fluor.*, Vol. 10, No. 2 (2000) p177-184.
26. Prasanta K. Patel, **Sri Rama Koti Ainavarapu** and R.V. Hosur, NMR studies on truncated sequences of human telomeric DNA: observation of a novel A-tetrad, *Nucl. Acids Res.*, Vol. 27, No. 19 (1999) p3836-3843.