RESEARCH WORK HIGHLIGHTS

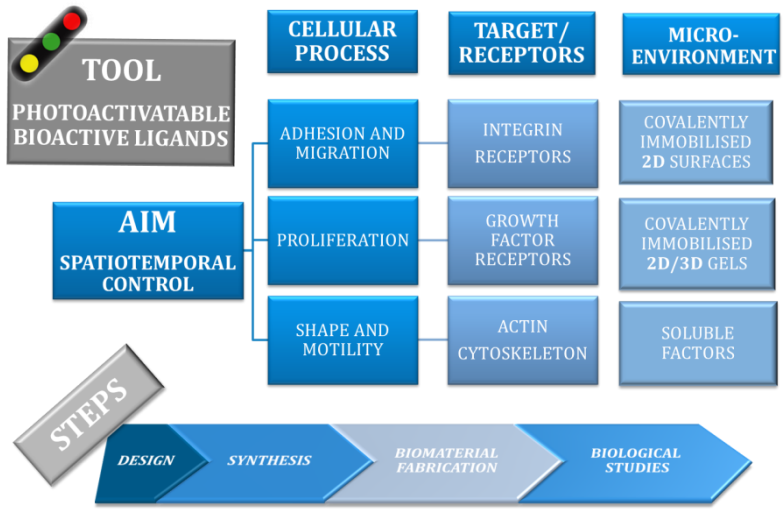
This document briefly describes all my research projects undertaken during my postdoctoral and doctoral studies. It concisely highlights the achievements and range of experiences gained along with the skills accrued.

POSTDOCTORAL RESEARCH WORK

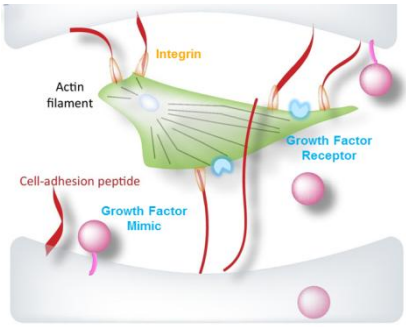
(Max-Planck Institute for Polymer Research, Mainz and Leibniz- Institute for New Materials, Saarbruecken)

The projects carried out during my postdoctoral stay dealt with complementary fields of photoactivatable molecules and biomaterials, at the interface to cell biology. My research focus was design, development and studies photoprotected bioactive ligands, which was tethered to biomaterials, to trigger cellular response upon activation with light at a defined time point and location.

Different Projects in a Nutshell



To understand and spatio-temporally regulate the interactions between the cells and synthetically modified biomimetic platforms containing cues for adhesion and growth..



Steps involved:

Design – Identification and insertion of photolabile protecting groups (PPGs) at selected positions of the peptide mimics so that initially the specific interaction with the membrane receptors is inhibited. Upon light exposure, PPGs could be selectively removed resulting in restoration of the bioactivity of the molecule at desired space and time. Design involved analyzing the reported crystal structure of the bioactive ligand and protein binding pocket and identifying target key interactions that could be restrained by PPGs.

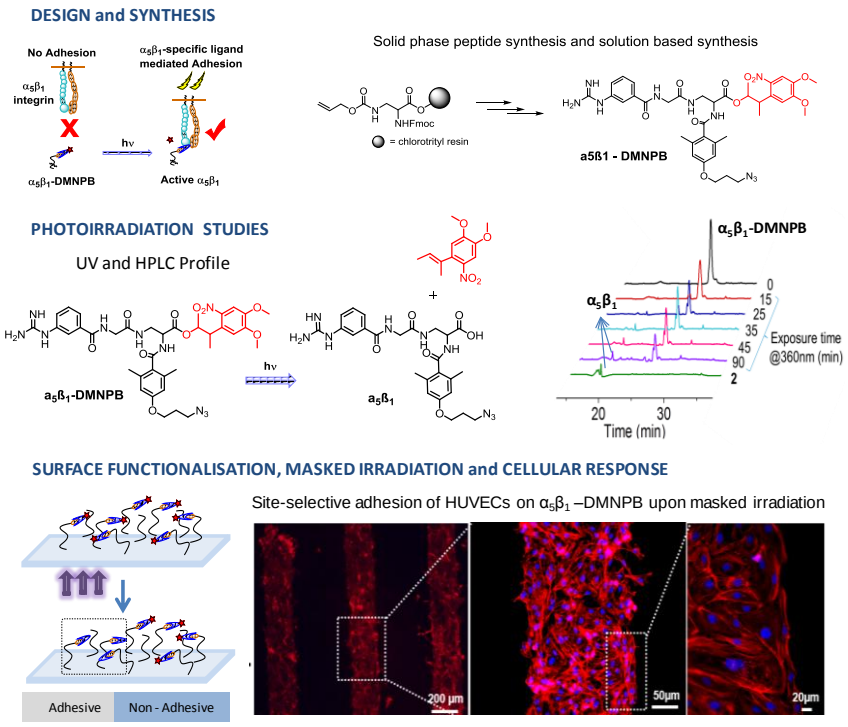
Synthesis and Photochemical Studies- Solid phase peptide synthetic method combined with solution based synthesis was carried out to obtain the designed photoprotected molecules. In order to evaluate the conversion of inactive to active bioactive species, irradiation studies were carried out at specific wavelengths using UV and HPLC.

Biomaterial fabrication - Biomimetic platforms on 2D functionalised glass surfaces / hydrogel platforms / 3D PEG based gels were prepared by tailoring the latent photoprotected molecules initially, which was later activated using cell friendly wavelengths (>360 nm) to generate migratory path for the cells.

Biological Studies - Cellular behaviour were studied on the biomimetic platforms with activated ligands with spatiotemporal control.

PROJECT 1 - Development of Photo-responsive Selective Integrin Antagonist for Spatiotemporal Control of Cell Adhesion and Migration

The integrin type $\alpha_5\beta_1$ is overexpressed in different kinds of tumors and has been identified as key component in mechanosensing (responsivity to mechanical stimuli such as force, stress, and strain due to substrate rigidity, topology, and adhesiveness) by the cells. Photoactivatable integrin-selective ligand was designed, synthesized, covalently bound to the model microenvironments and cellular behavior in response to changes in the activation of individual integrins was studied.

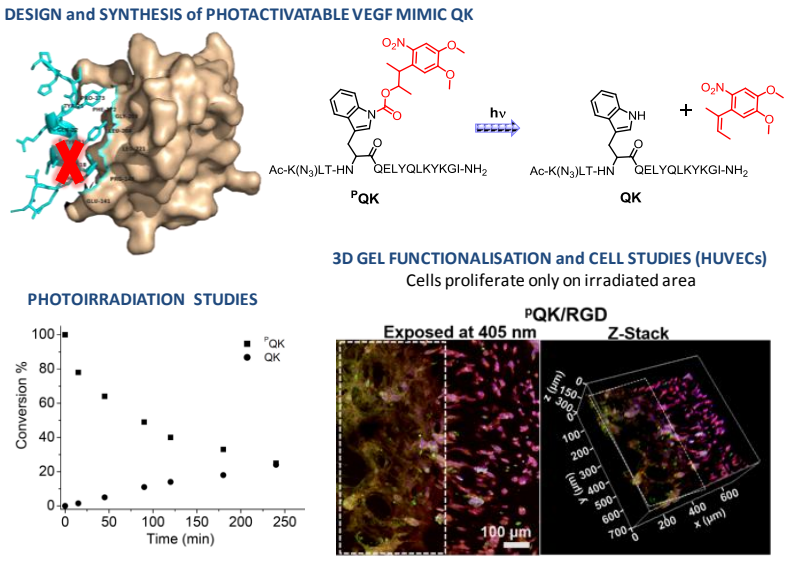


Masked irradiation of substrates modified with photoactivatable latent $\alpha_5\beta_1$-DMNPB (DMNPB is photolabile group displayed in red in figure below) allowed site-selective attachment of HUVECs at the photoactivated area due to exposed active $\alpha_5\beta_1$ integrin-selective ligand. Migration of HUVECs from the monolayer into $\alpha_5\beta_1$-activated lines in situ triggered using a scanning laser was also observed.

***Publication*- Roshna V. Nair**, A. Farrukh and A. del Campo. A Photoactivatable α5β1-Specific Integrin Ligand. *ChemBioChem.*, 2018, **19**, 1280-1287.

PROJECT 2 - Spatiotemporal Control of Angiogenesis using Phototriggerable-VEGF mimetic peptide QK

The project intended to direct growth of blood vessels (angiogenesis) in 3D environment by controlled delivery of vascular endothelial growth factor (VEGF) mimic QK. Project intended to tackle fast clearance of GFs *in vivo* and lack of control of GF presentation to the cells in time and space.



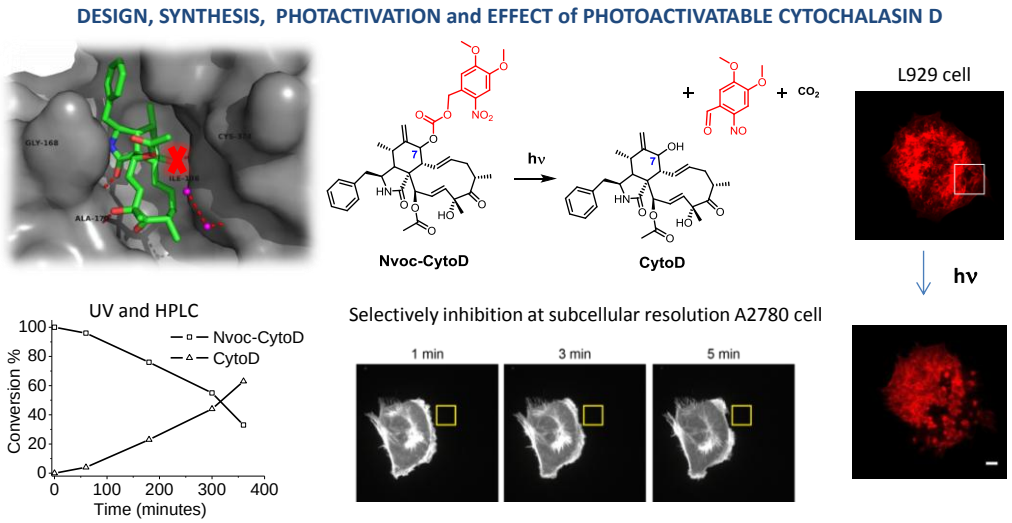
DMNPB protected QK was designed, synthesized and characterized for photolytic efficiency. Biomaterials platform for light-triggered angiogenesis in 2D and 3D cell cultures was achieved using phototriggerable QK at desired space and time.

This work resulted in 2 international collaborations.

***Publication*- Roshna V. Nair**, A. Farrukh and A. del Campo. Light-Regulated Angiogenesis via a Phototriggerable VEGF Peptidomimetic. *Adv. Healthcare Mater.* 2021, **10**, 2100488.

PROJECT 3 - Phototrigerrable regulation of actin inhibitor to regulate cell shape and motility

To control cytoskeletal dynamics, phototriggerable derivative of the potent actin disruptor was prepared that selectively interacts with individual cytoskeletal component i.e. *Actin Filaments*. Fungal metabolite Cytochalasin D (CytoD) permeates cell membranes and binds to actin filament with very high binding affinity for F-actin and blocks polymerization and the elongation of actin. Its extreme actin polymerization inhibitory nature necessitates its dosage control. Using photoactivable Nvoc-CytoD, delivery of active CytoD could be controlled with local precision and also at desired time period.



This work resulted in 10 collaborations with groups in Germany and abroad for various biologically relevant experiments.

Publications-

1. **Roshna V. Nair**, S. Zhao, E. Terriac, F. Lautenschläger, J. H. R. Hetmanski, P. T. Caswell, A. del Campo. Possibilities and Limitations of Photoactivatable Cytochalasin D for the Spatiotemporal Regulation of Actin Dynamics. http://doi.org/10.26434/chemrxiv.12609545.v1
2. E. Latorre, S. Kale, L. Casares, M. Gomez-Gonzalez, M. Uroz, L. Valon, **Roshna V. Nair**, E. Garreta, N. Montserrat, A. del Campo, B. Ladoux, M. Arroyo, and Xavier Trepat. Active superelasticity revealed by three-dimensional epithelial sheets of controlled size and shape. *Nature 2018, **563**,* 203–*208.*
3. J. H.R. Hetmanski, H. de Belly, I. Busnelli, T. Waring, **Roshna V. Nair**, V. Sokleva, O. Dobre, A. Cameron, N. Gauthier, C. Lamaze, J. Swift, A. del Campo, T. Starborg, T. Zech, J. G. Goetz, E. K. Paluch, J.-M. Schwartz, and P. T. Caswell. Membrane tension coordinates rear retraction in durotaxis and 3D cell migration. *Dev Cell*, 2019, **51(4)**, 460–475.

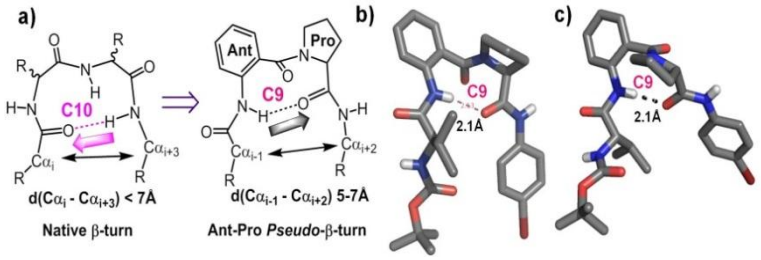
DOCTORAL RESEARCH WORK

(National Chemical Laboratory, University of Pune, India)

*My dissertation entitled "**Synthetic Oligomers with Heterogeneous Backbones Featuring α/β-Conjugated Building Blocks**" attempted the design and creation of assorted assemblies utilizing natural-unnatural α/β-conjugated (specifically aliphatic-aromatic) building blocks. The work involved design, synthesis, crystallization, extensive structural and conformational analysis of these three-dimensional compact folded assemblies known as ''foldamers'' using different solution state NMR studies, 2D-NMR analysis, molecular modelling and crystal structure analysis. The broad objective was acutely directed towards comprehending the folding behavior of biopolymers interplay of non-covalent interactions in orchestrating the structural preferences of compact three-dimensional assemblies.*

PROJECT 1 – Ant-Pro motif as a potent reverse turn mimic and β-hairpin initiator

Ant-Pro motif (Pro = $^{L/D}$proline and Ant = anthranilic acid), which is known to exhibit a nine-membered hydrogen-bonding pattern structurally akin to the natural β-turn found in natural peptides (Fig. a). In order to determine its sensitivity towards the structural perturbation, beta-branched amino acid like valine was substituted at N-terminal of the motif. Solid- and solution-state studies revealed the insensitivity of the turn segment towards structural and chirality modulation, establishing its potential as a robust reverse turn mimetic (Figure b,c).

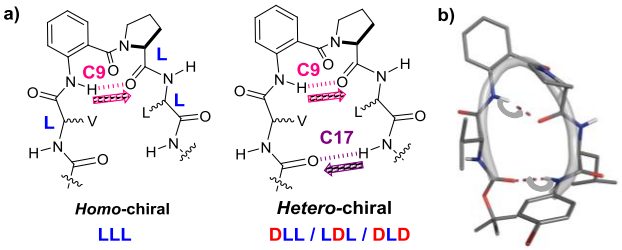


Ant-Pro based reverse turn mimic featuring their H-bonding patterns with their directions (highlighted with shaded arrows) (a), crystal structures of Boc-^{D}Val-Ant-^{L}Pro-p(C_6H_4)Br (b), and Boc-^{L}Val-Ant-^{L}Pro-p(C_6H_4)Br revealing stable reverse turn architecture.

Publications-

1. V. H. Thorat, T. S. Ingole, K. N. Vijayadas, **Roshna V. Nair**, S. S. Kale, V. V. E. Ramesh, H. C. Davis, P. Prabhakaran, R. G. Gonnade, V. G. Puranik, P. R. Rajamohanan and G. J. Sanjayan. The Ant-Pro Reverse-Turn Motif: Structural Features and Conformational Characteristics. (***Invited article for special issue on foldamers***) *Eur. J. Org. Chem.*, 2013, 3529-3542.
2. K. N. Vijayadas, **Roshna V. Nair**, R. L. Gawade, A. S. Kotmale, P. Prabhakaran, R. G. Gonnade, V. G. Puranik, P. R. Rajamohanan and G. J. Sanjayan. Ester vs. amide on folding: a case study with a 2-residue synthetic peptide. *Org. Biomol. Chem.*, 2013, **11**, 8348-8356.

With the intention to establish the minimum criteria for β-hairpin formation, different "α-β-α-α" tetramer sequences were synthesized with alternating chirality comprising both natural (L) and unnatural (D) analogues of Val, Pro and Leu residues. Four sequences of R_1-$^{L/D}$Val-Ant-$^{L/D}$Pro-$^{L/D}$Leu-R_2 (R_1 = Piv and R_2 = NHMe) were designed *i.e.* LLL, DLL, LDL and DLD with varying chirality pattern at α-amino acids. Their folding propensities were explored using solid and extensive NMR solution state studies, which revealed heterochirality favors hairpin conformation.



Intra-molecular H-bonding patterns with their directions (highlighted with shaded arrows) of the *pseudo*-β-hairpin featuring Ant-Pro reverse turn revealing consequence of stereochemical alteration on *pseudo*-β-hairpin nucleation (a), and crystal structure of Boc-^{D}Val-Ant-^{L}Pro-^{L}Leu-p(C_6H_4)Br (b).

Publication- **Roshna V. Nair**, A. S. Kotmale, S. A. Dhokale, R. L. Gawade, V. G. Puranik, P. R. Rajamohanan and G. J. Sanjayan. Formation of Pseudo-β-hairpin Motif Utilizing Ant-Pro Reverse Turn: Consequences of Stereochemical Reordering. *Org. Biomol. Chem.*, 2014, **12**, 774-782.

PROJECT 2 – Investigations of conformational pre-disposition of -α/β_n-oligomers

The project aimed to investigate the effect of constitutional ratio variation of residues on the conformational disposition of the oligomers of sequentially repeating *homo*-chiral and *hetero*-chiral -Pro-Ant-Ant- [α/β/β] units. Ant-Pro 1:1 sequential repeats are known to adopt a compact, rigid right-handed helical secondary structure displaying 1→2 forward turns. On the other hand, Ant-Ant repeats are well established sheet inducers due to the hybridization induced planarity of the aromatic rings and constant amide dihedral angles (ω). In addition to these, chirality of amino acids/stereocontrol is known to introduce severe effects on the structure formulation.

Structural studies revealed a unique zipper like folded architecture (in figure below) featuring a large inter-residual hydrogen bond spanning 26 atoms for the *hetero*-chiral R_1-($^{L}\alpha\beta_2{}^{D}\alpha\beta_2)_n$-$R_2$ (n=2) *i.e*. Piv-^{L}Pro-$(Ant)_2$-^{D}Pro-$(Ant)_2$-NHMe (center); whereas, the corresponding *homo*-chiral repeats R_1-($^{L}\alpha\beta_2)_n$-R_2 (n=2) exhibited a helical disposition (top right). In order to explore and establish the non-covalent forces responsible for the remote hydrogen-bond, further studies were undertaken.

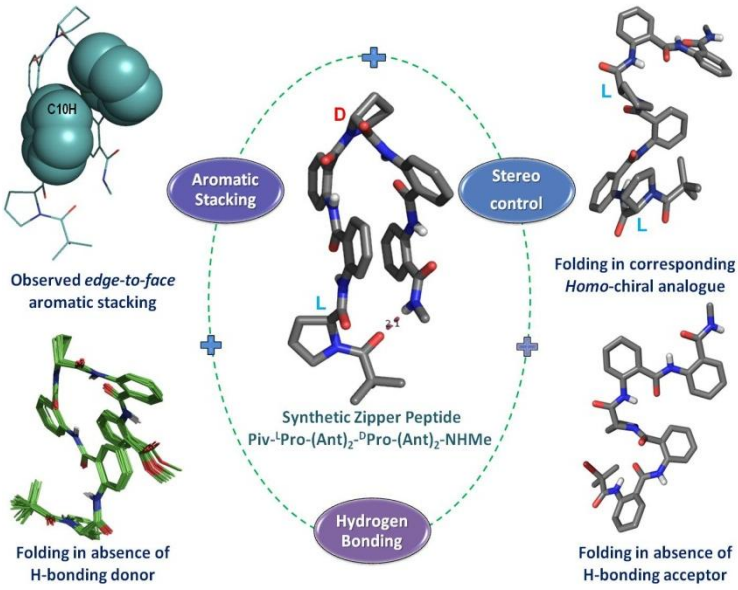


Figure - Unique zipper architecture orchestrated *via* the co-operative interplay of hydrogen bonding, aromatic stacking, and backbone chirality.

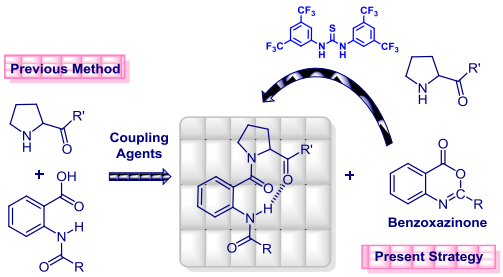
Publications-

1. **Roshna V. Nair**, S. Kheria, S. Rayavarapu, A. S. Kotmale, B. Jagadeesh, R. G. Gonnade, V. G. Puranik, P. R. Rajamohanan and G. J. Sanjayan. A Synthetic Zipper Peptide Motif Orchestrated *via* Co-operative Interplay of Hydrogen Bonding, Aromatic Stacking and Backbone Chirality. *J. Am. Chem. Soc.*, 2013, **135**, 11477-11480. (Won **NCL Research Foundation-NANAI NATU AWARD** - 2013 for **"Best Published Research Paper in Organic Chemistry"** and a **citation**)
2. S. Kheria, **Roshna V. Nair**, A. S. Kotmale, P. R. Rajamohanan and G. J. Sanjayan The role of N-terminal proline in stabilizing the Ant–Pro zipper motif. *New J. Chem.*, 2015, **39**, 3327-3332.

PROJECT 3– Local restraints in conformational proclivity of peptides

Synthesis and investigations of oligomer based on -Aib-Ant-^{L}Pro- repeats was attempted for structural investigation. Monomer unit synthesis *i.e*. -Aib-Ant-Pro- segment posed lots of challenges because of benzoxazinone formation as the major side product obtained on C-terminal activation of N_3-Aib-Ant-OH segment during coupling with H-^{L}Pro-OBn. Using Schreiner's (thio)urea in reaction provided a mild means for opening of azlactone moiety with amines successfully facilitated amide bond formation.

Publication- **Roshna V. Nair** and G. J. Sanjayan. (Thio)urea-Mediated Benzoxazinone Opening: Mild Approach Towards Synthesis of *o*-(substituted amido)benzamides. *RSC Adv.*, 2014, **4**, 7058-706.



Possible routes for the synthesis of the Ant–Pro dipeptide unit and thiourea-mediated benzoxazinone opening.

Review Articles based on Doctoral Research Work-

1. **Roshna V. Nair**, S. B. Baravkar, T. S. Ingole and G. J. Sanjayan. Synthetic Turn Mimetics and Hairpin Nucleators: Quo Vadimus? *Chem. Commun.*, 2014, **50**, 13874-13884. (**Feature Article, cover page**)
2. **Roshna V. Nair**, K. N. Vijayadas, A. Roy and G. J. Sanjayan. Heterogenous Foldamers from Aliphatic-Aromatic Amino Acid Building Blocks: Current Trends and Future Prospects. *Eur. J. Org. Chem.* 2014, **35**, 7763–7780.