Joint Research Initiative: CSIR-IGIB

Interfacing chemistry with biology

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The attempt to create a NCL-IGIB joint research initiative is an unique endeavor where scientists exchange knowledge and expertise while working towards achieving common scientific goals. Biology and chemistry are two complementary areas of science where the insight gained from one is useful in the context of the other. The principal goal of this joint research initiative (JRI) is to blend the knowledge and competence of biology and chemistry which form the core expertise areas of the two Institutes and to forge seamless cooperation at the interface of disciplines. With the advent of the new era in biology in the post genome-sequencing period, it is being increasingly appreciated that fundamental discoveries in modern biology would not only require interdisciplinary cross-talk but also the right blend of knowledge generated from other disciplines like chemistry. It was, therefore, necessary to create a seamless team of scientists at the interface areas of chemistry and biology.

Biocatalysis and biosynthesis

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Oxygenases are useful for the production of many industrially important molecules. Screening of an effluent treatment plant (ETP) sludge metagenomic library identified two clones encoding proteins, B1 and B2, with similarity to putative flavin monooxygenases from Mesorhizobium loti and Sphingomonas wittichi, respectively. The deduced amino acid sequences show only 20% identity, but both have a paired Rossman fold and a flavin monooxygenase (FMO) motif. B1 and B2 appear to be members of the flavin-containing monooxygenase and the Baeyer–Villiger monooxygenases subfamilies, respectively. When expressed in *Escherichia coli*, the two clones produced activities that oxidized indole to a mixture of indigo and indirubin pigments. These results suggest that B1 and B2 have potential as a biocatalyst in indigo/indirubin production

Several microorganisms were screened (isolated and from culture collection) for Oxido-reductase activity. Several fungal strains were found capable to bring about the stereo- and regiospecific hydroxylation on isoprenoids in specific terpenoids and steroids. The soil isolated versatile fungal strains, *Mucor piriformis*, *THV-S13*, and several other fungal strains could able to reduce the double bond in carvones in stereo-specific manner with quantitative yields.

Mathematical modelling and analysis of the steady-state and dynamic effects of posttranscriptional gene regulation by microRNA

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The expression of proteins is regulated at several levels, and the concentration of each regulator is itself regulated. MicroRNA (miRNA) are short, single stranded RNA molecules that regulate gene expression at the post transcriptional level. miRNA are generally believed to decrease target protein level, but recently some studies have reported 'unexpected' increase in target protein level due to presence of miRNA. miRNA are known to be dynamically regulated, and one particular class of miRNA called intronic miRNA are thought to be co-regulated with their 'host' genes. Therefore it is important to understand their effects both in steady state and dynamic models. We believe that such mathematical analysis will lead to a better understanding of mechanism of action of miRNA and the pathways regulated by miRNA.

We developed a comprehensive model of miRNA effects considering multiple steps in the regulation process. Using methodology from chemical engineering science, we formulated

Mathematical modelling and analysis of the steady-state and dynamic effects of posttranscriptional gene regulation by microRNA

the steady state equation for relative regulated protein concentration in terms of four dimensionless numbers composed of combinations of individual reaction rate constants. We identified conditions for the intuitive repressive action as well as the unintuitive activating effect of miRNA and established quantitative criteria for regulatory effect of miRNA, and established operating diagrams that specify the relative protein concentration in terms of the dimensionless numbers.

Stochastic simulations of miRNA systems showed that these dimensionless numbers are also sufficient to predict the relative noise in the steady state target protein distribution. We used the model to make falsifiable predictions about the exact mechanism for unintuitive experimental observations, effect of protein degradation, the nature of target protein distribution and relative noisiness.

miRNA effects had been incorporated in dynamic models of biological processes by reformulating the equations through the addition of at least one additional reaction (miRNA-mRNA binding) and one additional reactant (miRNA). In collaboration with researchers from IGIB, we developed a facile yet effective method to incorporate intronic miRNA effects in dynamic models without changing the number of reactants or reactions. This was achieved through the modification of the target protein formation rate as a function of the concentration of the host protein, which we showed was a reasonable proxy for the miRNA concentration and its effect. We modified a widely-used cell cycle kinetics model to incorporate dynamic effects of intronic miRNA, and showed that simulation results obtained from this modified model are in better agreement with experimental for cell cycle kinetics. We tested this approach in pathways with positive and negative feedback regulation of the target protein, and showed the general applicability of our method for incorporating intronic miRNA mediated dynamic effects in models for regulation of gene expression.

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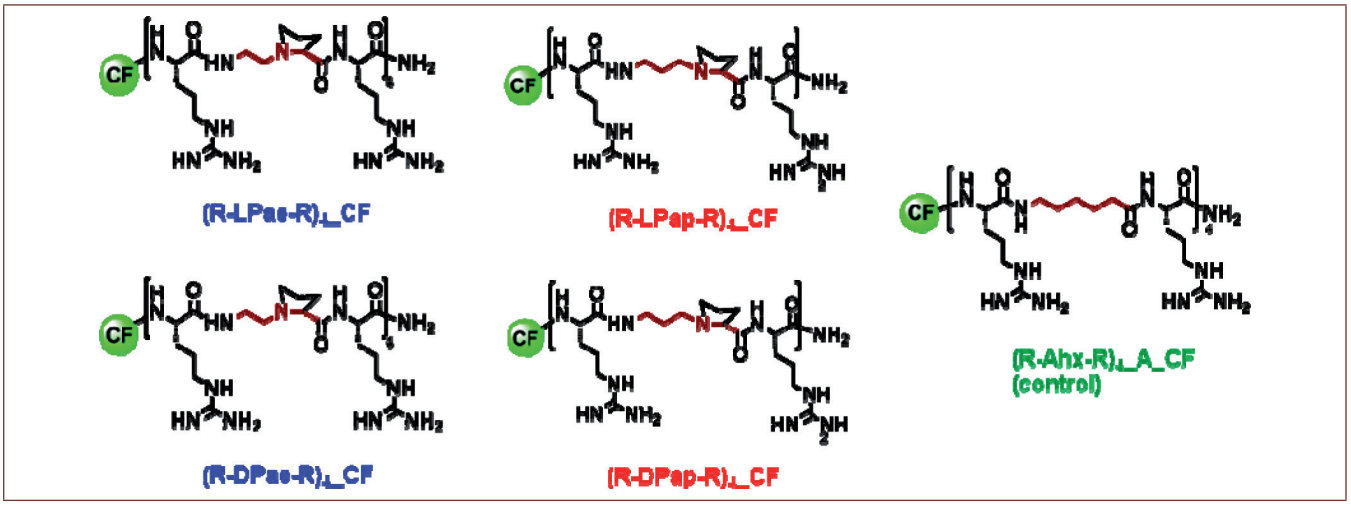
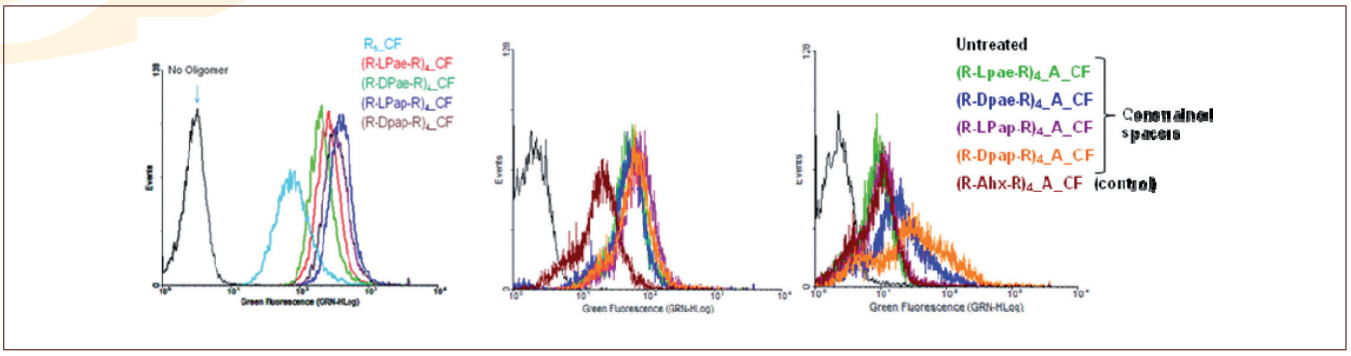
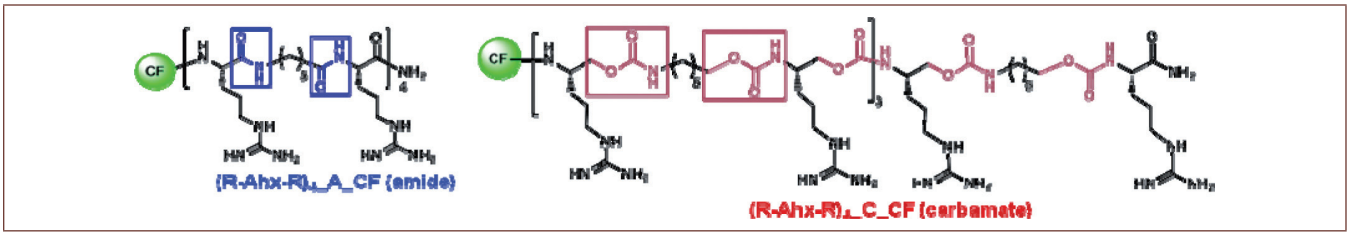
Two series of cationic peptides were designed and synthesized to include, among others, the 4-amino-*N*-(*N*-Boc-2-aminoethyl) prolyl synthon for conformational constraint. In addition to non-natural amino acids in the peptides, the amide linkage was also replaced by a **carbamate** linkage in selected designed peptides.



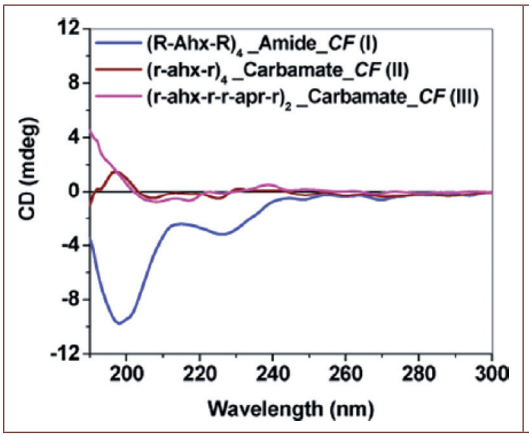
Cell uptake was studied in CHO-K1 and HeLa cells. The designed conformationally constrained peptides were shown to have better uptake properties than the control.

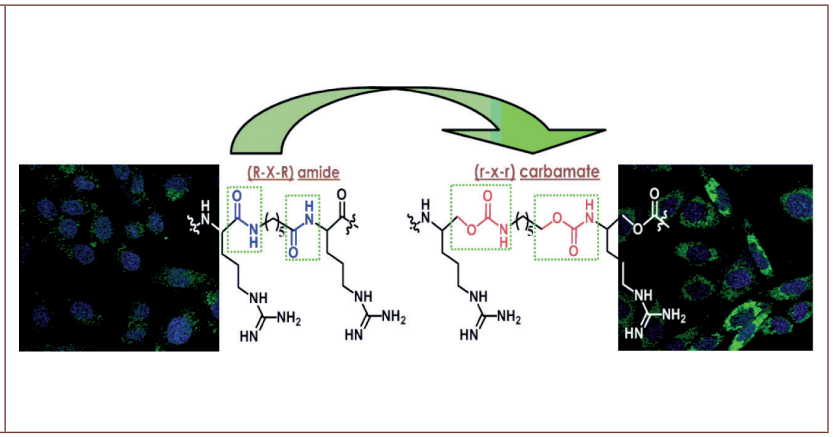


The unstructured carbamate-linked oligomers were found to be the best at cell penetration and enter cells predominantly by direct penetration, in contrast to the amide-linked oligomers, that get trapped in endosomes; cell penetration and delivery were evaluated using carboxyfluorescein as model cargo.



The carbamate oligomer successfully delivered a covalently linked therapeutic peptide in mammalian cells.

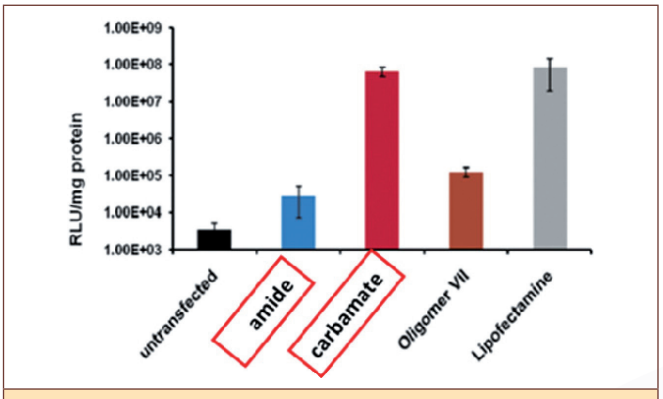




Delivery of siRNA (siGlo) as well as a plasmid DNA and its expression was successfully achieved by simple complexation with the carbamate oligomer.

Delivery of was achieved comparable to the delivery by lipofectamine.

Cell viability assays indicate low cytotoxicity of the oligomers.



Transfection of plasmid pMIR-Report™ Luciferase in CHO-K1 cells with designed oligomers

Bioactive natural products as possible chemotherapeutic agents

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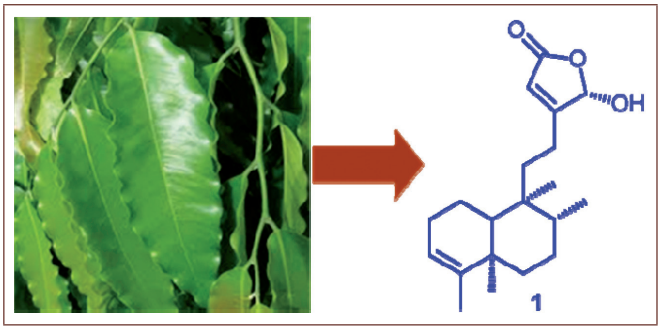
The present endeavour aims to bring together the potential to integrate the strengths of chemistry and biology so as to make a considerable and unique contribution towards the search for new bioactive natural products from Indian medicinal plants as possible chemotherapeutic agents. The objectives of this study are:

- To explore therapeutically significant molecules from Indian medicinal plants against a screen of zebrafish,
- To develop a small library of phytomolecules from Indian medicinal plants and their semisynthetic analogues and
- To develop a structure-activity-relationship (SAR) model for prediction of biological activities of natural products and their analogue.

As part of these initiatives, two plants, *Polyalthia longifolia* var. pendula and *Parthenium hysterophorus* were selected for and undertaking systematic chemical examination for the isolation of bioactive molecules from different parts of these two plants. Chemical examination of *Polyalthia longifolia* var. pendula led to the isolation of a clerodane diterpene which was identified as 16α-hydroxycleroda-3,13(14)*Z*-15,16-olide (**1**) on the basis of spectral data. Compound (**1**) has shown some remarkable bioactivities and at present, some semi-synthetic analogues are being generated based on this scaffold to further explore their bioactivities. The process for the isolation of compound (**1**) from *P. longifolia* var. pendula has been optimized for filling patent(s). Also, two more novel diterpenes have been isolated and currently their structure elucidation and bioactivities are being pursued.



Chemical examination of *Parthenium hysterophorus* has led us to isolation of abundant sesquiterpene lactone, parthenin (**2**). Further, chemical examination of the plant led to the isolation of four novel sesquiterpenes which are being identified and their bioactivities will be taken up.