SUMMERY OF THE THESIS WORK

The phosphodiester backbone makes DNA or RNA to behave as polyelectrolyte, the pentose sugar gives the flexibility, and the aglycones help in the self-assembly or the ligand-binding process. The hydrogen bonding, stacking, stereoelectronics and hydration are few of important non-covalent forces dictating the self-assembly of nucleic acids. The aim of my doctoral studies has been to explore the chemical nature of these non-covalent forces on the model nucleic acid system using the tools of NMR spectroscopy and computational chemistry.

The pH-dependent thermodynamics of mononucleosides-3',5'-bisphosphate (Figure 1, taking 1a–c as a mimic of trinucleoside diphosphate, and 1d as their abasic counterpart for comparison), clearly shows (Papers I and II) that by changing the electronic character of aglycone through protonation of nucleobase, the shift of North (N, C2'-exo, C3'-endo) ? South (S, C3'-exo, C2'-endo) pseudorotational equilibrium of the constituent sugar moiety (in absence of intramolecular base-base stacking interaction) is modulated by the interplay of stereoelectronic anmeric and gauche effects. This is further transmitted to tune the sugar-phosphate backbone conformation (ε<sup>+</sup> ? ε<sup>−</sup> equilibrium) in tandem as evident from pH-dependent cooperative shift of the (N,ε<sup>+</sup>) ? (S,ε<sup>−</sup>) equilibrium in
nucleotides (Figure 2). However, this aglycone promoted conformational transmission across the nucleotidyl wire depends upon the tunibility of aglycone (9-guaninyl > 9-adeninyl > 1-cytosinyl), thereby showing the cooperative interplay of constituent pentofuranose, nucleobase and phosphodiester moieties in controlling the intrinsic dynamics, hence the function, of nucleic acids.

The 3’-anthraniloyl adenosine (2c) and its 5’-phosphate (2d) [a mimic of 3’-teminal CCA\textsubscript{OH} of the aminoacyl-tRNA\textsuperscript{Phe}] binds to EF-Tu*GTP (Elongation Factor Tu complexes with Guanosine Triphosphatase) in preference over 2’-anthraniloyl adenosine (2a) and its 5’-phosphate (2b) (Figure 3), thereby showing (Paper III) that the 2’-endo sugar conformation is a more suitable mimic of the transition state geometry than the 3’-endo conformation in discriminating between correctly and incorrectly charged aminoacyl-tRNA by EF-Tu. This stereoelectronics dependent recognition switch (-2.9 kJ mol\textsuperscript{-1}) for EF-Tu binding is an important step, besides other recognition elements, during protein synthesis. The preferential stabilization of 2’-endo sugar conformation of 3’-anthraniloyl derivatives is further corroborated by the poorer transacylation of the anthraniloyl moiety from 3’- to 2’-position [$\Delta G^\circ < 0$ for stabilization of 3’-isomers 2c and 2d over 2’-isomers 2a and 2b respectively: $\Delta G^\circ_{2a} ? 2c = -1.2$ kJ mol\textsuperscript{-1} and $\Delta G^\circ_{2b} ? 2d = -1.7$ kJ mol\textsuperscript{-1}], which is cooperatively dictated by a balance of the tunable gauche effect of 2’- or 3’-anthraniloyl substitution. Thus, 3’-O-anthraniloyl adenosine derivatives have the potential to be useful as the EF-Tu recognition mimicry.

The 2’-OH distinguishes RNA from DNA both functionally as well as structurally. The NMR studies (Paper IV) on the 2’-OH mediated hydrogen bonding and hydration pattern in RNA at the nucleotide level showed (Figure 4): (i) The specific chemical nature of the vicinal 3’-substituent, geometrical factors like sugar pucker dependent spatial orientation of 2’-OH (proximity towards O3’) as well as the H-bond angle (non-linearity of $\angle$O-H…O
induced by attached sugar pucker) contribute to the overall strength of the weak non-linear intramolecular H-bonding (O-H2'-…O3') in adenosine (3) and adenosine 3'-ethylphosphate (4).

(ii) The presence of hydrophilic 3'-phosphate group in 4 compared to the 3'-OH in 3 causes a much higher water activity in the vicinity of 2'-OH (i.e. shorter exchange life time of 2'-hydroxyl proton with bound water, τ) in the former (τ2'-OH = 489 ms) compared to the latter (τ2'-OH = 6897 ms). Thus, the exposure of vicinal phosphate to the bulk water determines the overall availability of the bound water around its vicinal 2'-OH, thereby dictating the relative propensity of other inter- or intramolecular interactions.

The pH-dependent 1H NMR study with nicotinamide derivatives (5 and 6, Figure 5) shows (Paper V) the experimental strength of the intramolecular cation (pyridinium)-π (phenyl) interaction (-2.1 kJ mol⁻¹) in 5. It has been unequivocally demonstrated that this electrostatic interaction between pyridine and the neighboring aromatic phenyl moiety perturbs the pKₐ of the pyridine-nitrogen (∆pKₐ ~0.4, becoming more basic). The methyl protons of the linker 2,2-
dimethyloxazolidine moiety in 5 (but not in absence of neighboring phenyl group as in 6) is involved with CH (methyl)-π (phenyl) interaction (-0.8 kJ mol\(^{-1}\)), showing the electronically coupled nature of the pyridine-phenyl-methyl system. This edge-to-face electrostatic interaction between pyridinium and neighboring phenyl moiety is further supported by the \(T_1\) relaxation studies and \textit{ab initio} calculations.

The pH-dependent NMR studies of single stranded (ss) di-ribonucleotides (7a–f, Figure 6) demonstrates that \(pK_a\) of the ionized nucleobase can also be measured from the aromatic marker protons of the neighboring nucleobase in a electronically-coupled π system via intramolecular offset stacking [\textit{Paper VI}]. Similar studies with single stranded tri-ribonucleotides (8a and 8b, Figure 6) show [\textit{Paper VII}] that this electrostatic nearest-neighbor interaction propagates over ~6.8Å in a step-wise manner [from first to third nucleobase via the second, \textit{i.e. G ⇔ A ⇔ A} (or C)]. The further pH-dependent NMR studies of oligo-ssRNAs (9a–c, Figure 6) and oligo-ssDNAs (10a–c, Figure 6) with single ionization of 5’-(9-guaninyl) over a particular pH range titration show (\textit{Papers VIII} and IX) that the propagation of the interplay of nearest-neighbor electrostatic interactions (Figure 7) across the hexameric ssDNA chain is considerably less favourable (\textit{NMR detectable effectively up to the fourth nucleobase}) compared to that of the isosequential ssRNA (\textit{NMR detectable up to the sixth nucleobase residue}). It therefore shows that the stacking is more

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**Figure 6**

B = 9-Guaninyl (G); 9-Adeninyl (A); 1-Cytosinyl (C); 1-Uracilyl (U); 1-thyminyl (T)
easily perturbed in ssRNAs, upon the generation of 9-guanylate ion, compared to those in isosequential ssDNAs. Moreover, the pseudoaromatic character of the nucleobases in ssDNA is much less tunable compared to those in ssRNA. Each nucleobase across the oligomeric

![Model monomeric titrating site](image)

**Figure 7.** A schematic representation of the nearest-neighbor electrostatic interaction in hexameric ssRNA (9c), showing the pK_a perturbation of 9-guaninyl by the local microenvironment as well as demonstrating the nearest neighbor electrostatic interactions across the single stand.

single strand is engaged through a cascade of variable nearest neighbor electrostatic interactions, thereby cross-modulating each others pseudoaromatic character. This results into the creation of a unique set of aglycones in an oligo or polynucleotide, whose physicochemical properties are completely dependent upon the propensity and geometry of the nearest neighbor stacking interactions.

The dissection of relative strength of basepairing vis-à-vis stacking in a duplex shows (Paper X) that stability of DNA-DNA duplex weakens over the corresponding RNA-RNA duplexes with the increasing content of A-T base pairs, while the strength of stacking of A-T rich DNA-DNA duplex increases in comparison with A-U rich sequence in RNA-RNA duplexes.

THE PUBLICATION LIST

The thesis is based on the following original publications


*J. Am. Chem. Soc. 2003 (submitted)*

*J. Am. Chem. Soc. 2003 (submitted)*

*By the time of submission of this application, Paper X has been accepted and Paper IX is under review.*

The following original publications were not included in the thesis


XII. Acharya, P.; Thibaudeau, C. and Chattopadhyaya, J. An Energetic Correlation of *Ab initio* and NMR Studies of the 3'-gauche effect in 3'-substituted Thymidines.  

XIII. Zamaratski, E.; Trifonova, A.; **Acharya, P.**; Isaksson, J.; Maltseva, T. and J. Chattopadhyaya, Do the 16mer, 5'-GUGGUCUGAGGCC-3' and the 25mer, 5'-CGCCGAACUCGUAAGAGUCACCAC-3', Form a Hammerhead Ribozyme Structure In Physiological Condition? An NMR and UV thermodynamic study.  

XIV. Acharya, P.; Velikian, I.; Acharya, S. and Chattopadhyaya, J. Molecular modeling of 2'-OH Mediated Hydrogen Bonding in Ribonucleos(t)ides by NMR Constrained AM1 and MMX Calculations.  

XV. Review article: **Acharya, P.**; Issakson, J.; Pradeepkumar P.I. and Chattopadhyaya, J. Experimental Evidences Unequivocally Prove the Role of Stereoelectronics as One of the Major Forces Responsible for the Self-assembly of DNA and RNA.  
ACADEMIC AND PROFESSIONAL AWARDS

1. **1989-'91: National Merit Scholarship**, awarded by Department of Education, Govt. of West Bengal, India, during the study years of Higher Secondary School Examination (on the basis of Secondary School Examination results).

2. **1993-'96: National Merit Scholarship**, awarded by Department of Education, Ministry of Human Resource Development, Govt. of India, during the 2nd and 3rd year of studies of Bachelor Degree (on the basis of 1st year of Bachelor degree result) as well as during the Masters degree studies (1994-'96, on the basis of B. Sc. results).

3. **1997**: Awarded [Junior Research Fellowship](#) sponsored by Department of Science & Technology, Govt. of India for pursuing pre-doctoral research at the Department of Organic Chemistry, Indian Association for the Cultivation of Science, India.

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5. **2003**: Awarded [IUPAC Travel Grant for Young Scientist](#) to participate and present poster in the 39th IUPAC Congress held in Ottawa, Canada, during August 10-16, 2003.