

## **Modeling Prebiotic Catalysis with Adenylated Polymeric Templates: Kinetic Characterization of Assisted Phosphate Ester Cleavage and Oxygen Insertion Reactions**

### **Introduction**

A hypothetical scenario of prebiotic RNA world, where RNA served as a genetic template and possessed catalytic features necessary for self-replication, has long been proposed.<sup>1</sup> The discovery of catalytic RNA molecules (ribozymes) by Cech and Altman provided the impetus for an extensive discussion on the role of RNA, in the origin of life. This hypothesis assumes that all modern organisms are descendants of a prebiotic self-replicating moiety, which utilized RNA as both genetic and as well as catalytic material. However, due to lack of a credible mechanism for *de novo* nucleic acid template synthesis, difficulty in forming the glycosidic bond for the nucleotide synthesis under prebiotic conditions and inherent instability of RNA molecules, it is likely that life processes would have originated from a nucleic acid-like polymer possessing desired templating and catalytic features.<sup>2</sup> Therefore, identification and construction of a system that closely resembles RNA, capable of recognition and possessing catalytic domains, would shed more light on the origin of life processes. Numerous chemical and biochemical studies pertaining to the chemical origin of life, performed under simulated prebiotic conditions, have appeared due to pioneering efforts by Orgel, Miller, Ferris and others.<sup>3</sup>

### **Research Problem**

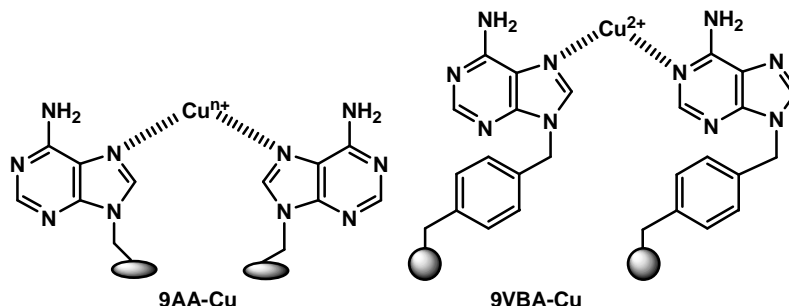
The thesis work describes the design of nucleic acid-like polymers containing non-hydrolyzable backbone and their catalytic potential in assisting chemical and biochemical transformations in the presence of metal ion cofactors. Inspiration for the mimicry of prebiotic catalysis was based on the studies related to the aggregation or entrapment of nucleobases on natural mineral surfaces, thereby providing cell-like enclosures and the discovery of metal-dependent catalytic activity of certain RNA motifs.

Our design strategy involves synthesis of insoluble cross-polymeric network containing multiple adenine residues as a mimic of aggregated nucleobases on mineral surface and metalation of the polymer to further invoke metal-nucleobase synergism for catalytic assistance. Two fundamentally important reactions were chosen to evaluate the catalytic potential of these polymeric matrices as an effort to catalyze phosphate ester hydrolysis and oxygen insertion between an inactivated C-H bond in aromatic systems.

### **Synthesis and characterization of Cu-metalated adenylated polymers**

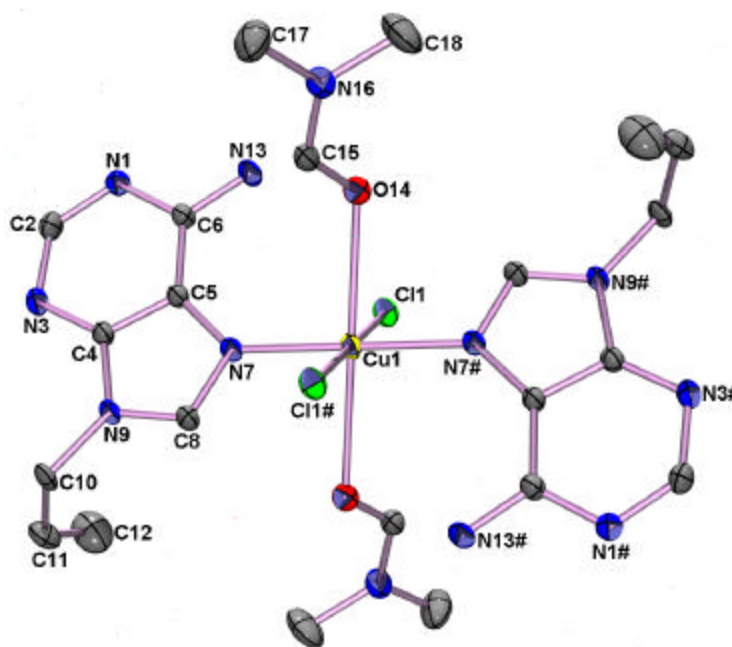
We have drawn inspiration from the nucleic acid analogues and the known metal-ion coordination ability of purine nucleosides and nucleotides, in constructing nucleic acid-like homo- and cross-polymers. We replaced the sugar residue at N9 position by an allyl (**9AA**) and vinylbenzyl (**9VBA**)

group to generate a molecular framework containing multiple adenine residues in the polymeric matrix, connected by a non-hydrolyzable backbone. Such polymeric matrices, devoid of sugar residues and phosphate backbones, are expected to be more stable in routinely used reaction media. Copper metalation afforded a constellation of metal sites within the polymeric matrix (figure 1).

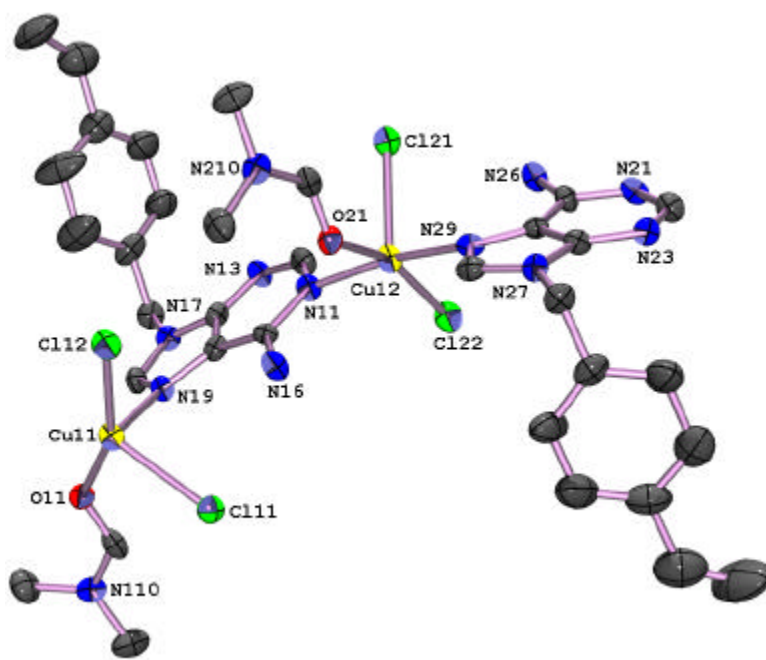


**Figure 1.** Allyl and vinylbenzyl-modified adenine polymers

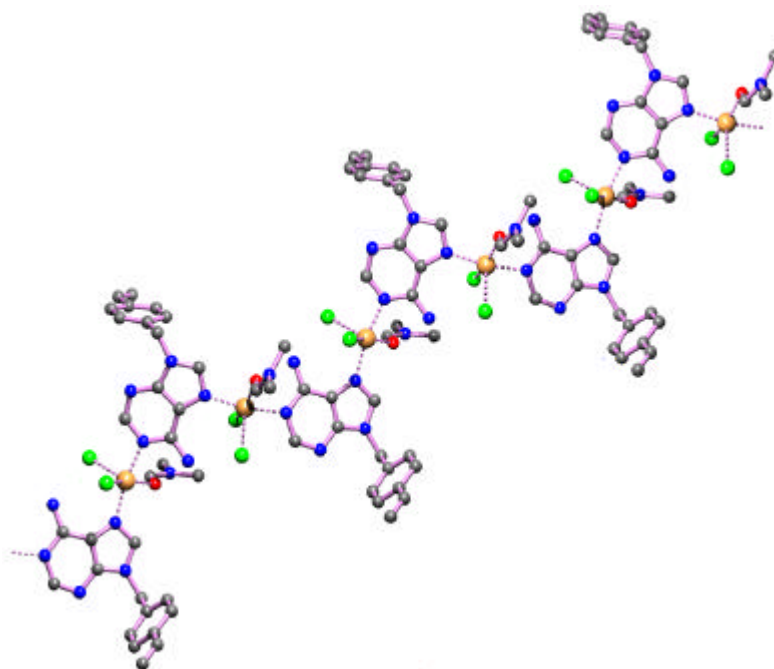
Metalated resins were characterized by EPR spectroscopy, TGA analysis and by magnetic susceptibility studies. X-ray crystal structure of **9AA-Cu** and **9VBA-Cu** monomer-metal complexes was determined to ascertain the coordination environment around the copper atom (Figure 2 and 3). A notable feature of **9VBA-Cu** complex is that it extends as a one-dimensional coordinated polymeric array assisted by metal centers (Figure 4). Such an extended framework bears a striking resemblance to the periodicity of single-stranded nucleic acids. In this case, modified adenine monomers appear to be tethered non-covalently through the participation of copper ions and this assembly extends in the solid-state solely by coordinating to the copper ions, which act in a fashion analogous to phosphodiester bridges in nucleic acids.



**Figure 2.** POVray rendered, ORTEP diagram showing the asymmetric unit of **9AA-Cu** complex. Hydrogen atoms are excluded for clarity. Gray: carbon



**Figure 3.** POVRAY rendered, ORTEP diagram showing the asymmetric unit of **9VBA-Cu** complex. Hydrogen atoms are excluded for clarity. Gray: carbon

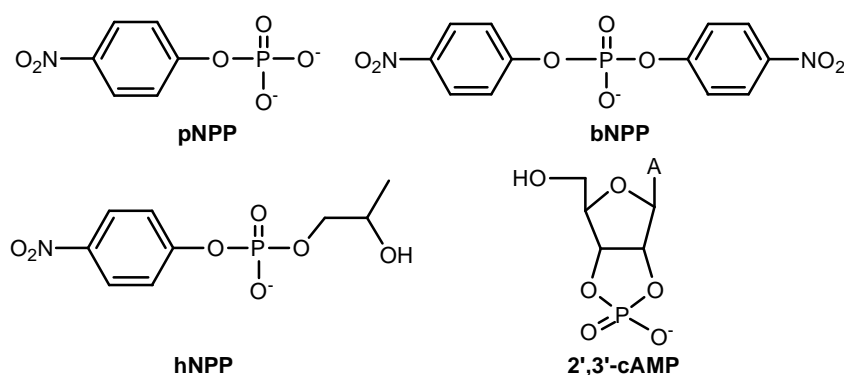


**Figure 4.** POVRAY diagram showing polymeric structure of **9VBA-Cu** complex. Hydrogen atoms are excluded for clarity. Gray: carbon, blue: nitrogen, green: chlorine, red: oxygen, copper: copper

### Kinetic evaluation of phosphate ester hydrolysis

Enzyme catalyzed phosphate ester (P-ester) hydrolysis is of considerable importance owing to its central role in key cellular processes.<sup>4</sup> Different classes of enzymes such as phosphatases, cyclic phosphodiesterases, nucleases and ribozymes catalyze this reaction. Most phosphate ester cleaving enzymes are metalloenzymes and display remarkable dependence for a wide array of metal ion cofactors.

We have employed model phosphate ester substrates to evaluate P-ester hydrolase activity of our adenylated polymers (Figure 5). Due to the heterogeneous nature of the catalyst, hydrolytic reactions were performed under heterogeneous conditions. Adenylated templates followed typical Michaelis-Menten saturation kinetics under turnover conditions. Significant rate accelerations were observed for the hydrolysis of activated phosphate esters, catalyzed by the templates, as compared to the uncatalyzed reaction. Two million-fold acceleration of bNPP hydrolysis by one of the templates is at par with many previously reported rate enhancements for this substrate by employing metalated ligands or by the direct addition of metal ions. Adenylated polymers also catalyzed the hydrolysis of unactivated phosphodiester 2',3'-cAMP, with appreciable rate enhancement and regioselectivity.

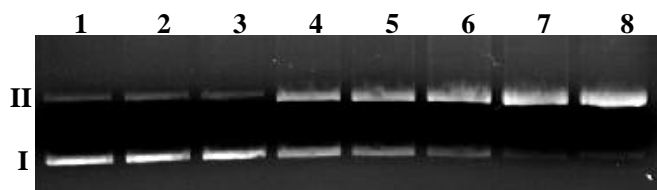


**Figure 5.** Model phosphate ester substrates

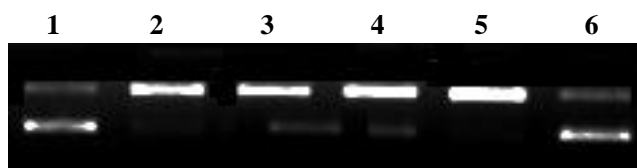
### Nuclease activity of polymeric templates

To extend the catalytic scope of these polymeric systems, we evaluated the nuclease activity of these constructs using supercoiled plasmid DNA as a natural macromolecular substrate. Cleavage of supercoiled plasmid DNA (pBR322) assisted by polymeric matrices were studied both in the absence and in the presence of exogenously added oxidants and reductants (thiols).

The interaction of supercoiled plasmid DNA (pBR322) and **9AA•Cu(I)** polymer containing divinylbenzene cross-linker revealed a complete conversion of supercoiled plasmid DNA (Form **I**) to nicked DNA (Form **II**) (Figure 6) in the absence of added activating reagent. While **9VBA•Cu(II)** polymers required exogenously added oxidants or reductants (thiols) to show cleavage activity (Figure 7). Preliminary mechanistic studies using radical scavengers indicated a possible role of copper-bound non-diffusible oxo species in the plasmid scission reaction.



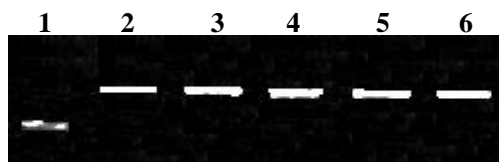
**Figure 6** Cleavage of supercoiled plasmid DNA, pBR322 by **9AA•Cu(I)** polymer. Polymer weight was 2  $\mu\text{g}$ . Lanes 1-3: pBR322; pBR322 + 100  $\mu\text{M}$   $\text{Cu}^{2+}$ ; pBR322 + unmetalated polymer after 6 h incubation, respectively. Lanes 4-8: pBR322 + **9AA•Cu(I)** polymer at 30, 90, 180, 270, 360 min, respectively



**Figure 7** Nicking of supercoiled plasmid DNA pBR322 assisted by **9VBA.Cu(II)** polymer (50  $\mu\text{g}$ ) in the presence of oxidant, oxone (100  $\mu\text{M}$ ) in a 3 min reaction (20  $\mu\text{L}$ , pH 7.5, 30°C). Lane 1: pBR322. Lane 2: pBR322 + **9VBA.Cu(II)** + oxone. Lanes 3-5: pBR322 in presence of radical scavengers, *t*-butyl alcohol, DMSO and D-mannitol (100 mM), respectively. Lane 6: pBR322 + oxone

### Recycling experiments

The most unique feature of the polymeric matrices is their facile recovery and reusability. The rarity of heterogeneous nucleolytic reagents and their possible reusability prompted us to explore our polymers for multiple nucleic acid cleavage reactions. The adenylated polymers were recycled for several consecutive reactions and each time a complete conversion of the supercoiled form **I** to the nicked form **II** was observed (Figure 8). This is a highly useful observation and it opens a vast area of chemico-biological explorations with insoluble chemical nucleases.

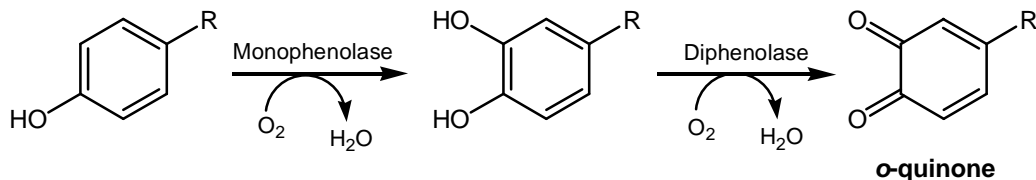


**Figure 8.** Recycle experiments for the cleavage of plasmid DNA by **9VBA.Cu(II)** polymer. Reaction conditions: concentration of DNA (9 ng/ $\mu\text{L}$ ) and MMPP (100  $\mu\text{M}$ ). Lane 1: pBR322. Lane 2: pBR322 + fresh **1** + MMPP. Lanes 3-6: recycle 1-4

### Phenol oxidation by **9AA•Cu(I)** adenylated polymeric template:

Copper-containing metalloproteins are involved in diverse array of oxidation reactions in biological systems.<sup>5</sup> As our polymeric matrix contains multiple copper residues, we became interested in evaluating tyrosinase-like activity of these templates towards phenol oxidation. Such studies were also intended to determine a functional role of adenine-copper complexes in acting as prebiotic

oxidases, albeit in the absence of redox cofactors used by the protein enzymes. Interestingly, **9AA•Cu(I)** adenylated polymer exhibited both monophenolase and diphenolase activity under Michaelis-Menten conditions with significant rate enhancement over uncatalyzed reaction for catechol, 4-*t*-butylcatechol, and 4-hydroxyanisole (Figure 9).



**Figure 9.** Monophenolase and diphenolase activity

## Conclusions

Adenylated polymers act heterogeneously to accomplish non-natural and natural phosphate ester hydrolysis and phenol oxidation reaction with appreciable rate enhancement over uncatalyzed reaction. These polymers also display excellent nuclease activity in the absence and in the presence of exogenous activating agents. Their prolonged and sustained reactivity, as demonstrated by recycle experiments indicate that they can retain metal-ion dependent catalytic property for long duration, without altered reaction rates and these polymers represent the first report on reusable chemical nuclease.

Interestingly, crystal structure of metalated vinylbenzyl adenine monomer is manifested in the form of an extended strand-like structure, which bears striking resemblance to single-stranded nucleic acids. Such metalated adenine containing polymers combine a scaffold for substrate binding and a nucleobase matrix for copper ion coordination, to produce a catalytically active nucleic acid-like polymeric template with a biological function. It is proposed that studies with such model constructs might provide further insight into primitive organization of nucleic acid constituents and to their cooperative interaction with metal ions for prebiotic catalysis.

Another intriguing possibility is that such metal-nucleobase assemblies could have functioned as short, prebiotic protonucleic acid templates for the recognition of other nucleobases and amino acids, for subsequent primordial oligomerization reactions. Our results reinforce previously reported catalytic activities of adenine, *N*-6-ribosyladenine, and related polymers and their potential role in prebiotic chemistry.<sup>6</sup>

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## Curriculum Vitae

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Synthesis of novel nucleic acid-like polymeric templates, derived from allyl and vinyl modified adenine nucleobase, and evaluation of their metal ion-dependent nuclease activity and oxygen insertion reaction. Thorough kinetic analyses have been undertaken to delineate the operating mechanism for the cleavage of activated and natural phosphate esters, and nucleic acids. A rare and notable feature of these templates is that they are amenable for multiple reaction cycles, with no apparent compromise in catalytic activity. Catalytic oxidation of phenols was also realized with one of these polymeric templates.

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## Publication List

### *From Thesis:*

- (1) **Srivatsan, S.G.** and Verma, S. (2000): Synthetic Dephosphorylation Reagents: Rate Enhancement of Phosphate Monoester Hydrolysis by Cu (II)-Metallated Adenine Nucleobase Polymers. *Chem. Commun.* 515-516.
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**Book Chapter:**

(15) Verma, S., **Srivatsan, S. G.** and Madhavaiah, C. (2004): Copper containing nuclease mimics: Synthetic models and biochemical applications: Artificial Nucleases 232 (Nucleic Acids and Molecular Biology Series), Ed. Zenkova, M. A., Springer-Verlag, Heidelberg, 13, 129-150.

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(1) **Srivatsan, S.G.** and Verma, S. (2000): Nucleobase polymers as enzyme mimetics. International Symposium on "Trends in Medicinal Chemistry and Biocatalysis", University of Delhi, January 26-29 (in India).

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