In order of importance, list of ten best papers of the candidate, highlighting the important discoveries/contributions described in them briefly (not to exceed 3000 words):

1. Kottur, J., and **Nair, D. T.** (2018) Pyrophosphate hydrolysis is an intrinsic and critical step of the DNA synthesis reaction. ***Nucleic Acids Res.*** 46:5875-5885.

   Time resolved crystallography was conducted to study the chemical reaction associated with DNA synthesis by DNA polymerases. The study showed that DNA synthesis reaction is a stepwise dissociative S_N2 reaction wherein the phosphodiester bond between the 3' –OH of the primer nucleotide and the a-phosphate of the incoming nucleotide triphosphate is formed first. This is followed by dissolution of the bond between the a-phosphate of the incoming dNTP and the bridging oxygen with the b-phosphate. The study also showed that, unlike a few high-profile studies in the recent past, two Mg^{2+} are sufficient for the DNA synthesis reaction. More importantly, the study showed that the hydrolysis of the pyrophosphate moiety is an intrinsic part of the DNA synthesis reaction. This observation provides an answer regarding a long standing question regarding the energetics of the DNA synthesis reaction catalyzed by DNA polymerases. It was generally believed that the free energy change of DNA synthesis by DNA polymerases is nearly zero. For the reaction to proceed in the forward direction, the pyrophosphate moiety is hydrolyzed by pyrophosphatase enzymes so that the free energy reaction for the two reactions together is highly negative. However, DNA synthesis by DNA polymerases is frequently conducted in vitro in many applications such as PCR and sequencing without the addition of pyrophosphatase enzymes. Our observation that the pyrophosphate moiety is also hydrolyzed by DNA polymerases provides an explanation for this decade's long conundrum in the field of DNA replication. The study was accorded breakthrough status by the journal Nucleic Acids Research.

2. Johnson, M. K., Kottur, J., and **Nair, D. T.** (2019) A polar filter in DNA polymerases prevents ribonucleotide incorporation. ***Nucleic Acids Res.*** 47:10693-10705.

   The presence of ribonucleotides in DNA can lead to genomic instability and cellular lethality. To prevent adventitious rNTP incorporation, the majority of the DNA polymerases (dPols) possess a steric filter. The dPol named MsDpo4 (*M. smegmatis)* naturally lacks this steric filter and hence is capable of rNTP addition. The introduction of the steric filter in MsDpo4 did not result in complete abrogation of the ability of this enzyme to incorporate ribonucleotides. In

comparison, DNA polymerase IV (PolIV) from *E. coli* exhibited stringent selection for deoxyribonucleotides. A comparison of MsDpo4 and PolIV led to the discovery of an additional polar filter responsible for sugar selectivity. Thr43 represents the filter in PolIV and this residue forms interactions with the incoming nucleotide to draw it closer to the enzyme surface. As a result, the 2' –OH in rNTPs will clash with the enzyme surface, and therefore ribonucleotides cannot be accommodated in the active site in a conformation compatible with productive catalysis. The substitution of the equivalent residue in MsDpo4- Cys47, with Thr led to a drastic reduction in the ability of the *Mycobacterial* enzyme to incorporate rNTPs. Overall, our studies evince that the polar filter serves to prevent ribonucleotide incorporation by dPols.

3. Nirwal, S., Kulkarni, D. S., Sharma, A., Rao, D. N. and **Nair, D. T**. (2018) Mechanism of formation of a toroid around DNA by the Mismatch Sensor protein. ***Nucleic Acids Res.*** 46:256-266.

   The DNA mismatch repair (MMR) pathway removes errors that appear during genome replication. MutS is the primary mismatch sensor and forms an asymmetric dimer that encircles DNA to bend it to scan for mismatches. The mechanism utilized to load DNA into the central tunnel was unknown and the origin of the force required to bend DNA was unclear. We show that, in absence of DNA, MutS forms a symmetric dimer wherein a gap exists between the monomers through which DNA can enter the central tunnel. The comparison with structures of MutS-DNA complexes suggests that the mismatch scanning monomer (B_m) will move by nearly 50 Å to associate with the other monomer (A_m). Consequently, the N-terminal domains of both monomers will press onto DNA to bend it. The proposed mechanism of toroid formation evinces that the force required to bend DNA arises primarily due to the movement of B_m and hence, the MutS dimer acts like a pair of pliers to bend DNA. We also shed light on the allosteric mechanism that influences the expulsion of ATP from A_m on DNA binding. Overall, this study provides mechanistic insight regarding the primary event in MMR i.e. the assembly of the MutS-DNA complex.

4. Surana, P., Vijaya, S. and **Nair, D. T.** (2014) RNA-dependent RNA polymerase of Japanese Encephalitis Virus binds the initiator nucleotide GTP to form a mechanistically important pre-initiation state. ***Nucleic Acids Res.*** 42:2758-2773.

   Flaviviral RNA-dependent RNA polymerases (RdRps) initiate replication of the single-stranded RNA genome in the absence of a primer. The template sequence 5′-CU-3′ at the 3′-end of the flaviviral genome is highly conserved. Surprisingly, flaviviral RdRps require high concentrations of the second incoming nucleotide GTP to catalyze *de novo* template-dependent RNA synthesis. We show that GTP stimulates *de novo* RNA synthesis by RdRp from Japanese encephalitis virus (jRdRp) also. Crystal structures of jRdRp complexed with GTP and ATP provide a basis for specific recognition of GTP. Comparison of the $jRdRp_{GTP}$ structure with other viral RdRp-GTP structures shows that GTP binds jRdRp in a novel conformation. Apo-jRdRp structure suggests that the conserved motif F of jRdRp occupies multiple conformations in absence of GTP. Motif F becomes ordered on GTP binding and occludes the nucleotide triphosphate entry tunnel. Mutational analysis of key residues that interact with GTP evinces that the $jRdRp_{GTP}$ structure represents a novel pre-initiation state. Also, binding studies show that GTP binding reduces affinity of RdRp for RNA, but the presence of the catalytic Mn^{2+} ion abolishes this inhibition. Collectively, these observations suggest that the observed pre-initiation state may serve as a checkpoint to prevent erroneous template-independent RNA synthesis by jRdRp during initiation.

5. Sharma A., Kottur, J., Narayanan, N. and **Nair, D. T.** (2013) A strategically located serine residue is critical for the mutator activity of DNA Polymerase IV from *Escherichia coli*. ***Nucleic Acids Res.*** 41:5104-5114.

   The Y-family DNA polymerase IV or PolIV (*Escherichia coli*) is the founding member of the DinB family and is known to play an important role in stress-induced mutagenesis. We have determined four crystal structures of this enzyme in its pre-catalytic state in complex with substrate DNA presenting the four possible template nucleotides that are paired with the corresponding incoming nucleotide triphosphates. In all four structures, the Ser42 residue in the active site forms interactions with the base moieties of the incipient Watson–Crick base pair. This residue is located close to the centre of the nascent base pair towards the minor groove. *In vitro* and *in vivo* assays show that the fidelity of the PolIV enzyme increases

drastically when this Ser residue was mutated to Ala. In addition, the structure of PolIV with the mismatch A:C in the active site shows that the Ser42 residue plays an important role in stabilizing dCTP in a conformation compatible with catalysis. Overall, the structural, biochemical and functional data presented here show that the Ser42 residue is present at a strategic location to stabilize mismatches in the PolIV active site, and thus facilitate the appearance of transition and transversion mutations.

6. Kottur, J. and **Nair, D. T.** (2016) Reactive Oxygen Species Play an Important Role in the Bactericidal Activity of Quinolone Antibiotics. ***Angew Chem Int Ed Engl***. 55:2397-2400.
   Recent studies posit that reactive oxygen species (ROS) contribute to cell lethality by bactericidal antibiotics. However, this conjecture has been challenged and remains controversial. To resolve this controversy we adopted a strategy that involves DNA polymerase IV (PolIV). The nucleotide pool of the cell gets oxidized by ROS and PolIV incorporates the damaged nucleotides- especially 8oxodGTP- into the genome and this result in the death of bacteria. Using a combination of structural and biochemical tools coupled with growth assays, we show that selective perturbation of the 8oxodGTP incorporation activity of PolIV results in considerable enhancement of the survival of bacteria in the presence of the norfloxacin antibiotic. Our studies therefore, evince that ROS induced in bacteria due to the presence of antibiotics in the environment contribute significantly to cell lethality.

7. Narayanan, N. and **Nair, D. T.** (2020) Vitamin B12 May Inhibit RNA-Dependent-RNA Polymerase Activity of nsp12 from the SARS-CoV-2 Virus. ***IUBMB Life*** 72:2112-2120.
   SARS-CoV-2 is the causative agent for the ongoing COVID19 pandemic, and this virus belongs to the Coronaviridae family. Like other members of this family, the virus possesses a positive-sense single-stranded RNA genome. The genome encodes for the nsp12 protein, which houses the RNA-dependent-RNA polymerase (RdRP) activity responsible for the replication of the viral genome. A homology model of nsp12 was prepared using the structure of the SARS nsp12 (6NUR) as a model. The model was used to carry out *in silico* screening to identify molecules among natural products, or Food and Drug Administration-approved drugs that can potentially inhibit the activity of nsp12. This exercise showed that vitamin B12 (methylcobalamin) may bind to the active site of the nsp12 protein. A model of the nsp12 in

complex with substrate RNA and incoming NTP showed that vitamin B12 binding site overlaps with that of the incoming nucleotide. A comparison of the calculated energies of binding for RNA plus NTP and methylcobalamin suggested that the vitamin may bind to the active site of nsp12 with significant affinity. It is, therefore, possible that methylcobalamin binding may prevent association with RNA and NTP and thus inhibit the RdRP activity of nsp12. Overall, our computational studies suggest that methylcobalamin form of vitamin B12 may serve as an effective inhibitor of the nsp12 protein.

8. Narayanan, N. and **Nair, D. T.** (2021) Ritonavir may inhibit exoribonuclease activity of nsp14 from the SARS-CoV-2 virus and potentiate the activity of chain terminating drugs. ***Int. J. Biol Macromol***. 168:272-278.

   SARS-CoV-2is the causative agent for the ongoing COVID19 pandemic, and this virus belongs to the Coronaviridae family. The nsp14 protein of SARS-CoV-2 houses a 3′ to 5′ exoribonuclease activity responsible for removing mismatches that arise during genome duplication. A homology model of nsp10-nsp14 complex was used to carry out *in silico* screening to identify molecules among natural products, or FDA approved drugs that can potentially inhibit the activity of nsp14. This exercise showed that ritonavir might bind to the exoribonuclease active site of the nsp14 protein. A model of the SARS-CoV-2-nsp10-nsp14 complex bound to substrate RNA showed that the ritonavir binding site overlaps with that of the 3′ nucleotide of substrate RNA. A comparison of the calculated energies of binding for RNA and ritonavir suggested that the drug may bind to the active site of nsp14 with significant affinity. It is, therefore, possible that ritonavir may prevent association with substrate RNA and thus inhibit the exoribonuclease activity of nsp14. Overall, our computational studies suggest that ritonavir may serve as an effective inhibitor of the nsp14 protein. nsp14 is known to attenuate the inhibitory effect of drugs that function through premature termination of viral genome replication. Hence, ritonavir may potentiate the therapeutic properties of drugs such as remdesivir, favipiravir and ribavirin.

9. Kottur, J., Sharma, A., Gore, K. R., Narayanan, N., Samanta, B., Pradeepkumar, P. I. and **Nair, D. T.** (2014) Unique Structural Features in DNA Polymerase IV enable efficient bypass of the N^2-Adduct induced by the Nitrofurazone antibiotic. ***Structure*** 23:56-67.

The reduction in the efficacy of therapeutic antibiotics represents a global problem of increasing intensity and concern. Nitrofuran antibiotics act primarily through the formation of covalent adducts at the N^2 atom of the deoxyguanosine nucleotide in genomic DNA. These adducts inhibit replicative DNA polymerases (dPols), leading to the death of the prokaryote. N^2-furfuryl-deoxyguanosine (fdG) represents a stable structural analog of the nitrofuran-induced adducts. Unlike other known dPols, DNA polymerase IV (PolIV) from *E. coli* can bypass the fdG adduct accurately with high catalytic efficiency. This property of PolIV is central to its role in reducing the sensitivity of *E. coli* toward nitrofuran antibiotics such as nitrofurazone (NFZ). We present the mechanism used by PolIV to bypass NFZ-induced adducts and thus improve viability of *E. coli* in the presence of NFZ. Our results can be used to develop specific inhibitors of PolIV that may potentiate the activity of nitrofuran antibiotics.

10. Sharma, M., Narayanan, N. and **Nair, D. T.** (2020) The proofreading activity of Pfprex from *Plasmodium falciparum* can prevent mutagenesis of the apicoplast genome by oxidized nucleotides. ***Sci. Rep.*** 10:11157.

    The DNA polymerase module of the Pfprex enzyme (PfpPol) is responsible for duplication of the genome of the apicoplast organelle in the malaria parasite. We show that PfpPol can misincorporate oxidized nucleotides such as 8oxodGTP opposite dA. This event gives rise to transversion mutations that are known to lead to adverse physiological outcomes. The apicoplast genome is particularly vulnerable to the harmful effects of 8oxodGTP due to very high AT content (~87%). We show that the proofreading activity of PfpPol has the unique ability to remove the oxidized nucleotide from the primer terminus. Due to this property, the proofreading domain of PfpPol is able to prevent mutagenesis of the AT-rich apicoplast genome and neutralize the deleterious genotoxic effects of ROS generated in the apicoplast due to normal metabolic processes. The proofreading activity of the Pfprex enzyme may therefore represent an attractive target for therapeutic intervention. Also, a survey of DNA repair pathways shows that observed property of Pfprex constitutes a novel form of dynamic error correction wherein the repair of promutagenic damaged nucleotides is concomitant with DNA replication.