Once it was accepted that DNA carries genetic information, efforts were focused on deciphering the structure of the DNA molecule and the mechanism by which information stored in it is expressed to produce an observable trait. Discovery of double helical structure of DNA launched the era of molecular genetics. In this elegant design, nature stores all the genetic information that builds the characteristics necessary for life. Now, even after 60 years of the discovery of the double helix, this simple description of the genetic material remains true and has not been appreciably altered to accommodate new findings. In the late 1960s, it was discovered that eukaryotic DNA contained sequences that do not code for proteins, referred to as non-coding DNA (ncDNA) and thus appeared to have no significant evolutionary function. It was considered an evolutionary anomaly and dismissed as ‘junk DNA’. However, numerous studies have shown that the amount of non-coding DNA per genome is a more valid measure of the complexity of an organism than the number of protein-coding genes.

Chapter 1 comprises a general introduction about DNA, its structural diversity and polymorphisms. In addition to the canonical right-handed double helix, several non-canonical deoxyribonucleic acid (DNA) secondary structures have been discussed, including triplexes, hairpins, quadruplexes, Z-DNA and cruciforms. A brief description of single nucleotide polymorphisms (SNPs) is also given. SNPs do not occur randomly across genomic sequences but often occur in hotspots. This chapter also manifests association of chronic or prolonged inflammation with a number of diseases like cancer, heart attacks, diabetes, Alzheimer etc. and role of cyclooxygenase-2 (COX-2) in the production of proinflammatory prostaglandins. Application of antigen technology - Triplex Formation - in a regulatory region of a gene can block transcription initiation by inhibiting transcription factor binding or by interfering with formation of the initiation complex. Correlation of SNPs present at potential triplex forming sites in the regulatory region was also discussed.
Chapter 2 includes the details of various deoxyoligonucleotide sequences, and other chemicals used, along with the principles and procedures of the techniques adopted to perform various experiments.

Chapter 3 attempts to characterize human COX-2 gene with the help of various bioinformatics techniques and biological databases. It includes browsing and visualizing ontologies related to Human COX-2 gene using AmiGO database, identifying potential triplex targeting sites (pTTSs) overlapping SNPs in the human COX-2 promoter region using TTS Mapping software, extracting minor allele frequency from dbSNP database, comparing transcription factor binding sites at both alleles using Alibaba software and multiple sequence alignment of sequence of flanking region of human COX-2 gene with that of chimpanzee, orangutan, rat and horse. AmiGO illustrated that COX-2 have diverse functional traits like inflammation, angiogenesis, maintaining blood-brain barrier, response to various ions etc. Using TTS Mapping web-application, we found that just five out of thirty three pTTSs overlap with SNPs (rs20415, rs5270, rs20417, rs11567815 and rs57021644). rs11567815 was not considered for further studies since it is a rare variant having minor allele frequency less than 0.5%. In addition, no change in the transcription factor binding sites was observed for different alleles of rs57021644, thus it was not considered for further studies.

Chapter 4 deals with the biophysical and biochemical characterization of the oligonucleotides (COX-23 series, COX-29 series and COX-57 series), harboring the SNPs overlapping with the potential triplex forming sequences in the promoter region of COX-2 gene. In addition, triplex forming ability was investigated for transversion SNPs i.e. rs20417 (COX-57 series) and rs5270 (COX-29 series). We selected 57-mer oligonucleotide sequence containing ‘G’ allele of rs20417 SNP of human COX-2 promoter and termed it as COX-57G and the one having ‘C’ allele was named as COX-57C. Structural characterization of COX-57 series clearly reflects all the four oligonucleotides COX-57C, COX-57G, COX-57Cc and COX-57Gc exist as a mixture of intermolecular (bulge duplex with floating tails) and intramolecular (double hairpin as well as hairpin with floating tail) species, such that Tm of hairpin structure with floating tail was below 15°C and thus was not detected. In addition, variation of just
one base from C to G in COX-57C to COX-57G rendered a difference of 8°C in upper temperature transition and 5°C in lower temperature transition due to change from Watson-Crick base pairing in COX-57C to mismatched base pairing in COX-57G. Investigation of triplex forming ability carried out in presence of Na⁺ and Mg²⁺ (or K⁺ and Mg²⁺) ions, witnessed the triplex formation between TFO-22Pu and duplex of COX-57C • COX-57Cc, but not with duplex of COX-57G • COX-57Gc. Importantly, most part of TFO-22Pu exists naturally in the 3’- flanking region of COX-2 gene. This result gave way to our hypothesis that due to triplex formation at COX-57C duplex, SP-1 transcription factor is unable to bind with the duplex and thus resulting in significant reduction in COX-2 promoter activity. In addition, TFO-22Pu demonstrated structural transitions as a function of varying ionic composition of solution.

Gel electrophoresis studies carried out in varied oligomer and salt concentrations confirmed the predominance of intramolecular structural species in case of all the oligonucleotides of COX-29 series (COX-29C, COX-29G, COX-29Cc and COX-29Gc) and intermolecular structures by all the oligonucleotides of COX-23 series (COX-23A, COX-23G, COX-23Ac and COX-23Gc). Gel assay results were then correlated with that of thermal denaturation and circular dichroism. As far as triplex formation ability of COX-29 duplexes is concerned, gel electrophoresis results revealed that neither COX-29C duplex nor COX-29G duplex was able to form purine motif triplex with TFO-13Pu in presence of magnesium ions. Monophasic melting profiles further validated our results.

Chapter 5 describes the effect of flanking sequence on the structural heterogeneity of a set of designed GC-rich oligonucleotides having central A-tract with the help of various biophysical and biochemical techniques. A set of six oligonucleotides were designed such that each oligonucleotide has ‘GGCC’ on the 5’-side of central A₅-tract and 3’-side has 2G’s and 2C’s in different permutations and combinations. Gel electrophoresis studies revealed that TILT-13A, TILT-13C and TILT-13D existed as hairpin structure whereas TILT-13B, TILT-13E and TILT-13F were present in interlocked hairpin form. Salt concentration and Oligomer concentration dependence gels clearly depicted the structural transition of interlocked hairpin to bulge duplex in TILT-13B and TILT-13F, hairpin to interlocked hairpin in
TILT-13E and hairpin to bulge duplex in TILT13-C. The solution structure studies on the complementary sequences of all the six oligonucleotides (having central T$_5$-tract) revealed that all of them were present as hairpin form. T-counterpart of TILT-13B, TILT-13BT was designed i.e. the central A$_5$-tract of TILT-13B was replaced by T$_5$. It was found to exist as interlocked hairpin, thereby evidencing that the presence of A-tract is not an essential requirement for interlocked hairpin formation. In order to see the effect of length of flanking sequence TILT-13Bext, the extended version of TILT-13B was designed. Gel electrophoresis studies demonstrated the conformational equilibrium between bulge duplex and hairpin species, thereby, confirming the requirement of short flanking sequences for interlocked hairpin formation.

Chapter 6 summarizes the conclusions of the studies carried out to investigate the structural status as well as triplex forming ability of various potential triplex forming sites in COX-2 promoter region overlapping with SNPs. This study has been a small attempt towards gaining information of natural mechanism of gene regulation by triplex formation by one of the allele and thus making the native resistant to many diseases. Our study also focuses on the effect of flanking sequence on the structural heterogeneity of A-tract oligonucleotides.