The 21st century opened with the complete sequencing of the human genome, providing useful tools, new subjects, and opportunities for medical advance. Single nucleotide polymorphisms (SNPs), genetic mutations of single nucleotide bases, are the most prevalent type of DNA sequence variation found at a frequency of 0.5-10 per every 1000 base pairs in the human genome. SNPs are of great interest because they are the mutations that cause the overwhelming majority of genetic diseases and they can also serve as genetic markers. Recent research has revealed a close relationship between the SNPs and drug metabolism, spotlighting the SNPs as a potential indicator for drug action. There have been increasing demands for high-throughput SNP detection.

SNPs are silent. Nucleotides of both mutant and wild-type genes are completely intact with respect to chemical structure and form a normal Watson-Crick base pair, making it very difficult to detect SNPs without sequence information. However, when a wild-type gene is mixed with a mutation gene, SNPs are emphasized as mismatched base pairs in the resulting heteroduplex (the heteroduplex method developed by Myers et al.). This heteroduplex method opened up a way to directly arrest the silent SNPs as mismatched base pairs, providing chemists with a challenge to develop a strategy to detect mismatched base pairs within a large amount of genome sequences. In my thesis work, we worked to develop a strategy for rational design of small molecules that bind to mismatched base pairs and, moreover, to apply this chemical approach to a SNP scanning system.

Repair enzymes normally recognize mismatched DNA sites in nature. Uracil-DNA glycosylase recognizes mismatched uracil in DNA via a “nucleotide-flipping” mechanism, in which a uracil is flipped out and trapped by the enzyme pocket and the resulting “hole” at the flipped out uracil site is simultaneously occupied by a lysine residue from the enzyme. The recognition system is very complicated on the molecular level but can be represented as more general “push-pull” mechanism. This realization inspired us to propose a conceptually new approach for DNA mismatch recognition.

Our strategy is quite simple and based on rational “push-pull” chemistry. We hypothesized that the dimeric form of aromatic intercalating agents, possessing hydrogen bonding groups fully complementary to mismatched bases, can simultaneously recognize both mismatched nucleotide bases within the base stacks of a DNA duplex (Figure 1).

![Figure 1. Molecular design of a ligand selectively binding to mismatched base pair. Mismatched bases are colored red. Designed bis-intercalator is colored green.](image)
One of the mismatched bases is captured by one side of the hydrogen-bonding intercalator and the resulting hydrogen-bonded pair is stabilized within the $\pi$-stack of DNA. The other half of the dimer fills the hole and binds stably to the other mismatched base. It is very difficult to design a ligand possessing a huge hydrophobic pocket to flip out the mismatched nucleotide base, but in our strategy this difficulty is circumvented by utilizing the DNA $\pi$-stack itself as an enzyme pocket mimic. This small organic molecule (<500 Da) is a sophisticated mimic of the naturally occurring enzyme (>10,000 Da).

First, we synthesized a small ligand called dimeric naphthyridine (DNP), which is the dimeric form of a hydrogen bonding intercalator fully complementary to guanine (G), and investigated its binding to a G-G mismatch. G-G mismatches are one of the most stable mismatched base pairs. Quantitative binding analysis showed that DNP strongly binds to G-G mismatches with a very low dissociation constant (53 nM) and selectively binds to G-G mismatches over other mismatched and normal base pairs with more than 360-fold higher affinity.

To determine the mode of the binding of DNP to G-G mismatched base pairs, we analyzed the 1D and 2D NMR spectra of the complex. The 2D-NOESY experiments clearly showed that each intercalator of DNP simultaneously recognizes a guanine base in the G-G mismatch within the DNA $\pi$-stack. Structure-activity and energetic studies also clarified the mode of the binding, revealing that cooperative capture of both G-bases is indispensable for mismatch recognition. Based on these observations, we concluded that our conceptually new strategy is very appropriate for the design of mismatch-binding molecules.

Finally, we applied these results to the development of a mismatch-detecting sensor that can be applicable to high-throughput SNP scanning. We covalently immobilized the designed G-G mismatch binding ligand, DNP, on a gold-chip surface. When mismatched DNA is captured by immobilized DNP, the mass change induces a change of surface plasmon resonance (SPR) on the gold surface, because SPR is directly related to the total molecular weight of biomolecules on gold surface. The accuracy of this system was demonstrated by a marked SPR response obtained only for synthetic DNA containing a G-G mismatch while other mismatches produced only a weak response. Finally, with this system, we succeeded in detecting mismatched base pairs in heteroduplexes produced from real biological samples, specifically a 652 base pair PCR product of the human HSP70-2 gene. This SPR based method is suitable for high-throughput scanning of SNPs, because it is simple, inexpensive, and reproducible. This sensor can be used repeatedly without any special handling, each run of the assay finishes within 30 min, and this assay does not require any labeling of the DNAs.

In summary, my thesis work provided a new strategy to design mismatch-binding ligands that bind with high affinity and selectivity. The chemistry described here provides useful information for the development of related ligands that bind to the other mismatches in
DNA. The SPR sensor-chip that makes use of this chemical strategy can be a promising system for SNP scanning in the future, post genome era.