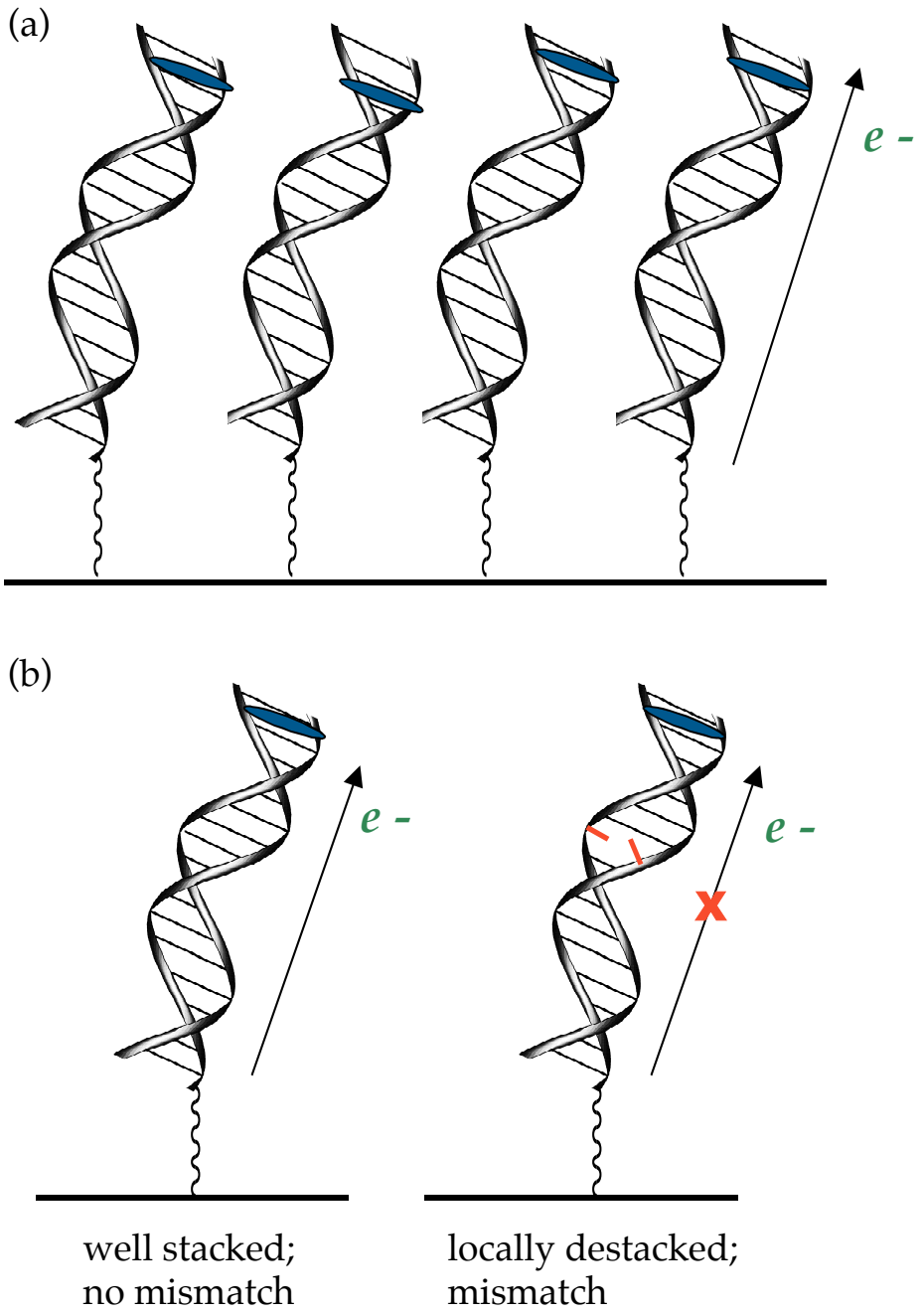
CHAPTER 11

Summary and Perspectives

In the past decade, the study of DNA-mediated charge transport has been of great interest. While the finer kinetic and mechanistic issues surrounding DNA-mediated charge transport chemistry are still being debated, it has become apparent that these reactions are extremely sensitive to DNA π-stacking (1-11). This sensitivity to base stacking provides the basis for mismatch discrimination by DNA-mediated charge transport. Mismatches are generally stacked in a DNA duplex but undergo somewhat greater dynamical motion than well paired bases (12-16). DNA charge transport detection, which depends on the electronic coupling within the base stack, is very sensitive to these motions. For example, a single CA mismatch inserted into a DNA duplex between a covalently attached photoinduced electron donor (ethidium) and a covalently attached intercalating acceptor ($Rh(phi)_2bpy'^{3+}$), significantly inhibits electron transfer based on the results of fluorescence quenching experiments (1).

This thesis work describes an electrochemical assay based on charge transport through double stranded DNA-modified gold electrodes to exploit the sensitivity of DNA-mediated charge transport in routine mismatch detection (Figure 11.1) (17-23). Modified electrodes are prepared by self-assembly of prehybridized duplexes as described in the Appendix. These surfaces have been characterized by cyclic voltammetry, ellipsometry, radiolabeling of the duplexes, and AFM (17-19). We have found densely packed monolayers are formed when no potential is applied to the electrode surface and in the presence of high concentrations of Mg^{2+} (Chapter 8). The morphologies of our DNA surfaces are greatly affected by the flexibility and orientation of the organic linker between the duplex and the gold surface

Figure 11.1. (a) Schematic representation of a DNA-modified gold electrode with bound redox active DNA intercalators for use in electrochemical assays. (b) Schematic representation of the efficacy of charge transport in well matched and mismatched duplexes. In mismatched DNA, base stacking is locally perturbed at the site of the mismatch and electron transfer is shut off.



(Chapter 6), and the density of DNA duplexes on the surface can be reduced by taking advantage of the electrostatic repulsion between duplexes (Chapter 8). Thus we are starting to understand how these surfaces pack and some of the parameters that control surface morphology, although there are still questions to be answered. We have yet to observe densely packed surfaces with pieces of DNA longer than about 25 base pairs. Is this due to more flexibility or greater density of charge with longer lengths of DNA? The persistence length of DNA is about 150 base pairs and Mg^{2+} should shield the charge, so there are obviously other factors we do not yet understand.

We have nonetheless established that in well packed films of DNA duplexes, the DNA-mediated charge transport reaction can proceed using noncovalently bound intercalators such as methylene blue (MB^{+}) (Chapters 2-4) as the electrochemical probe of base stacking in DNA films. Importantly, in order to detect a mutation, the electrochemical probe must be an intercalator, which binds to DNA by insertion between base pairs, effectively becoming a part of the base stack and thus electronically coupled into the base stack of DNA (Chapters 2 and 4). This sensitivity to mismatches depends upon electronic coupling within the base pair stack rather than upon the thermodynamics of base pairing.

All of our experimental observations indicate that the path of electrochemical charge transport at DNA-modified surfaces seems best described as mediated by the base pair stack of DNA, however the detailed mechanism of this DNA-mediated charge transport reaction remains unknown and researchers are only just beginning to explore this area of study. There are several proposals in the literature concerning oxidative

charge transport in solution, as discussed more thoroughly in Chapters 1 and 4 of this thesis. Although some of the parameters thought to govern charge transport in those systems surely apply to reduction reactions at DNA films, it is clear that much more experimental as well as theoretical work needs to be done before a complete picture of the mechanism of DNA-mediated charge transport at DNA films can truly come into focus.

By coupling the electrochemical reduction of DNA-bound intercalators mediated by charge transport chemistry to catalytic reoxidation of the intercalator in solution, we can now achieve greatly enhanced selectivity and sensitivity (Chapters 2 and 3). Electrocatalysis essentially amplifies the absolute MB^+ signal as well as enhancing the inhibitory affect of a mismatch. Furthermore, due to its catalytic nature, the measured charge increases with increased sampling times; longer integration times provide even greater absolute signals as well as increased differentiation between fully complementary and mismatched DNA. In each experiment the surface-bound DNA is repeatedly interrogated. In duplexes containing mismatches, fewer MB^+ molecules are electrochemically reduced, so the concentration of active catalyst is lowered and the overall catalytic response is diminished (Chapter 5).

Using this electrocatalytic assay, all single base mismatches have been detected within DNA and DNA/RNA hybrid duplexes (Chapter 3). Furthermore, this technology is well suited to DNA chip based formats and mismatch detection has been demonstrated on electrodes as small as 30 μm in diameter. Mismatches can be detected even in very low concentration within a perfectly matched DNA film (Chapter 3), making it possible to sensitively

detect mutations in only a small number of cells. DNA-mediated charge transport chemistry is not restricted to mismatch detection, but rather any structural perturbation of the π-stack of DNA can be diagnosed. Several common base damage products (Chapter 3) as well as a conformationally constrained deoxyribose moiety (Chapter 7) are easily discriminated from native DNA using electrocatalysis. In fact, DNA-mediated charge transport chemistry should be applied as a general method to directly probe DNA base pair stacking.

We propose the application of this technology for the detection of mutations, lesions, and SNPs in chip based genetic analysis as well as structural analysis of DNA-protein complexes. DNA-mediated charge transport can provide a sensitive and practical method of genetic screening for known gene sequences (Figure 11.2). As demonstrated here, all single base mismatches and many common single base lesions are detected conveniently without manipulation of conditions for testing or deconvolution of data through computation. Furthermore, given amplification of the signal through electrocatalysis, the need for additional amplification of test samples using the polymerase chain reaction may be obviated.

This mismatch detection strategy is based on entirely different properties of DNA mismatches than available technologies. Our approach exploits the exquisite sensitivity of DNA-mediated electron transfer to stacking perturbations. Lesions to the base stack effectively block the charge transfer pathway to intercalating probe molecules (Figure 11.1). Consequently, this assay reliably reports on single base changes even under strongly hybridizing conditions. Several features of this new approach

provide important advances over existing hybridization based technology, including i) an insensitivity of the detection of single base mismatches to AT or GC sequence content, ii) the detection of all mismatches (including some with comparable thermodynamic stability to Watson-Crick pairs), and iii) electrochemical detection with noncovalently bound intercalating probes avoids expensive and cumbersome fluorescence or radioactive tagging of biological samples. We have not yet systematically varied the DNA length on the electrode surface, but an 8 x 8 array of 15mers would be sufficient to assay for a typical gene (100,000 base pairs) with no redundancies.

The detection of mutations based on DNA-mediated electron transfer therefore provides an alternative method to hybridization based assays. The ability to detect mutations within intact duplexes would greatly simplify the analysis of multiple test sequences at an addressable array. Analyses could be performed under strongly hybridizing conditions, allowing both native and mutated test strands to bind to the probe sequences, regardless of the overall base composition of the individual addresses in the array (Figure 11.3). In addition, electrochemical detection methods are better suited for the development of inexpensive, portable devices than the sensors currently available employing high resolution confocal microscopy (5).

However, electrochemical detection presents new challenges for the fabrication of functional DNA "chips" on electrode surfaces. The reproducibility and effectiveness of the assay presented here requires tightly packed films that inhibit the diffusion of intercalators into the monolayer. Furthermore, the applicability of our potentiometric and amperometric methods have only been preliminarily proven to detect point mutations

Figure 11.2. Schematic representation of our proposed approach to mismatch diagnosis by electrochemistry.

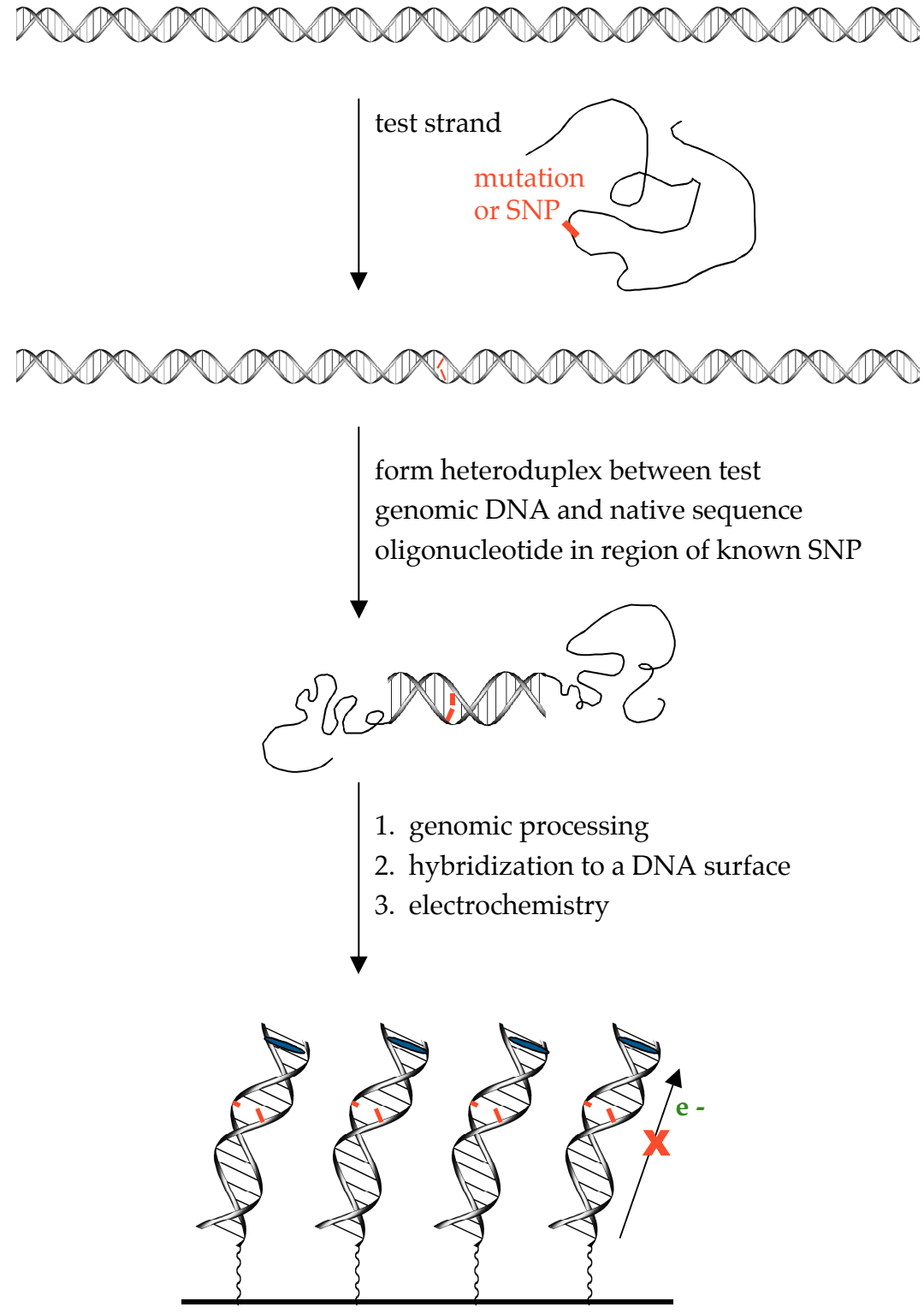
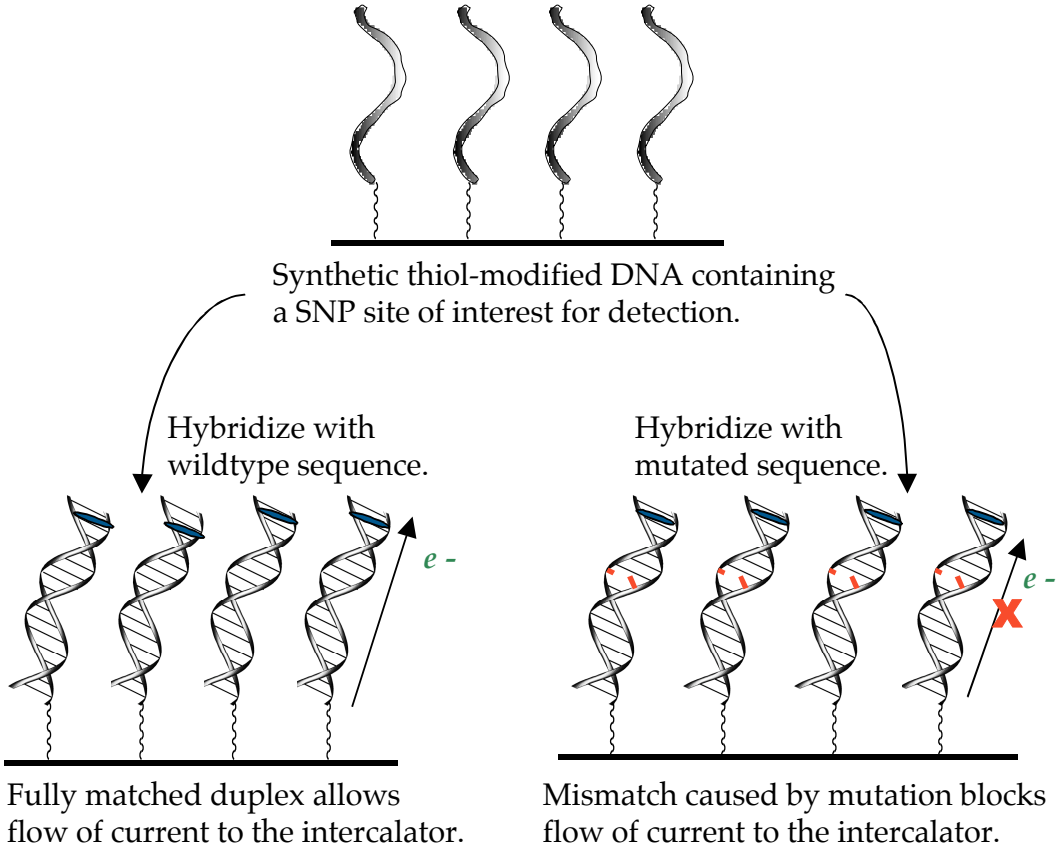


Figure 11.3. Strategy for the detection of mismatches in fully hybridized duplexes using DNA-mediated charge transport chemistry.



within heterogeneous biological samples. While the charge transport based method described here holds great promise for the detection of known mutations within defined sequences, hybridization based methods combined with algorithmic analyses may be better employed for sequencing assays. Overall, charge transport and hybridization based assays may ultimately provide complementary methods for the analysis of DNA sequence composition and abnormalities.

Charge transport through DNA-modified surfaces is also applicable to sensitive analysis of DNA-protein interactions and reactions. The results presented in Chapter 8 establish a new, sensitive assay for sequence specific protein-DNA interactions and reactions. DNA charge transport chemistry allows the rapid determination of structural perturbations in a DNA site associated with binding of a given protein. Charge transport chemistry also facilitates the real time monitoring of enzymatic reactions on DNA. DNA-modified electrodes are furthermore amenable to an array format and thus provide a practical tool for the selection and assay of proteins based upon their sequence specific interactions with DNA. Moreover the electrochemical assay on DNA monolayers provides a sensitive route to test for inhibitors of such protein-DNA interactions. Hence DNA charge transport not only provides a novel strategy for the structural analysis of how individual proteins bind DNA but also a remarkably sensitive tool in real time for DNA based proteomics. DNA-protein films may also offer a new approach to accessing the redox potential of bound metalloproteins as demonstrated with the base excision repair, FeS cluster containing enzyme MutY (Chapter 9).

In conclusion, the past five years of research in DNA-mediated charge transport chemistry have now confirmed that DNA-mediated charge transport can proceed over long molecular distances and that the reaction is sensitive to sequence as well as sequence dependent DNA structure (24). DNA-mediated charge transport has furthermore been demonstrated to proceed within *Hela* cell nuclei (25) as well as in the nucleosome core particle (26). DNA-binding proteins have been demonstrated to modulate long range charge transport chemistry (27-30). The electrochemistry experiments to sense mismatches and lesions in DNA described here also suggest that this chemistry might be valuable as a sensing device within the cell. Charge transport chemistry mediated by DNA seems to offer many opportunities to carry out a range of reactions from a distant position on the DNA helix. Thus, does long range charge transport similarly promotes chemistry at a distance within the cell? Do redox active proteins such as MutY (Chapters 9 and 10) participate in protein to DNA charge transport in vivo? It does seem possible that nature might access charge transport chemistry for efficient signaling or detection processes. The questions for future consideration have certainly presented themselves.

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