CHAPTER I

INTRODUCTION

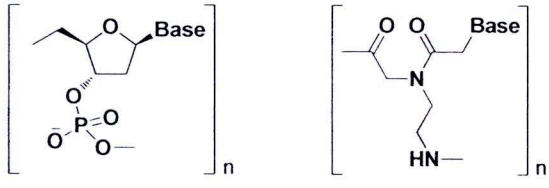
Rationale for the study

Currently, cancer is one of the most deathliest diseases in Thailand. For example, Colorectal and Cervical cancers are the cancer that usually found in patients. Presently, Prostate cancer and Ovarian cancer cause the highest fatality among male and female, respectively [1, 2]. Generally, Treatments of Prostate cancer [3, 4] and Ovarian cancer [5] are relatively complicated due to there are no sign of any symptoms in early stage cancer; therefore, it is difficult to identify and diagnosis. Once, cancers start growing and spreading, they rapidly transport through blood system and invade to other organs leading to fatality [3, 4, 5]. Thus, the development of cancer detection has tremendously increased over the past few years for better and effective treatments. Nowadays, diagnosis of cancers can be achieved by various methods, such as a blood test by monitoring the elevation of protein type cancer antigen 125 (CA 125) for Ovarian cancer [6, 7] and prostate-specific antigen (PSA) [8] for Prostate cancer. Actually, a blood test is normally useful for follow-up the progressive of cancer; however, it has not shown to be an effective method because of low accuracy and sensitivity [3, 4, 5]. Thus far, none of any detection tools can detect any cancers in early stage.

It has been known that genetic mutations lead to abnormal activities in cell ,such as irregular of protein productions and uncontrollable cell growth, in which cell finally evolutes to cancer; consequently, the interest for single-stranded DNA-based diagnostic tests has been rapidly growing. To identify a cancer at the early stage, detection of one base mutation in gene becomes considerably significant; therefore, a diagnostic test of single nucleotide polymorphism (SNP) [9] have been prominently developed for a high-throughput fashion with minimum cost; however, maintaining high detection sensitivity [10, 11].

To improve detection sensitivity, several approaches have been utilized such as changing solid supports from microarray slides to micro/nanoparticles [12, 13, 14]. In addition to modify solid support, detection probes can be replaced from single-stranded DNA (*ss*DNA) probe to DNA mimics. Peptide nucleic acid (PNA), for example, was introduced by Nielsen and coworkers in 1991. Replacement of phosphate backbone with an *N*-(2-aminoethyl)glycine (*aeg*) unit in PNA (Figure 1) [15] causes decreasing of an electrostatic force between DNA and PNA probe making PNA becomes ideally bimolecular detection tools.

In 2007, the PNA probe was developed for detection of Single Nucleotide Polymorphism (SNP) employing Q sepharose, ion-exchange resin, as solid support combine with MALDI-TOF mass spectrometry. The concept of this invention was originally based on charge difference between *D*-prolyl-2-aminocyclopentanecarboxylic acid PNA (*acpc*PNA) (neutral charge) and DNA (negative charge). When *acpc*PNA hybridized with complementary DNA, *acpc*PNA:DNA duplex was elerctrostactically adsorbed on Q sepharose and unhybridized *acpc*PNA could easily separate from *acpc*PNA:DNA complexes by simple washing. This novel method was rapid and inexpensive with detection limit at 1pmol/30 μL [16].



(a) **(b)**

Figure 1 Structures of (a) DNA, (b) *aeg*PNA (Base: A, C, G, T)

To enhance detection sensitivity of advanced functional probes, nanotechnology is one of the most attractive approaches for biomolecule applications with high sensitivity in which requires small amounts of sample and short preparation times compared to traditional diagnostic methods [17]. Particularly, magnetite nanoparticle particles (MNPs)

have received much attention due to its superparamagnetic behavior, nanometer size, high surface area, and inert to oxidation reaction [18]. Additionally, ease of surface modification of particles becomes a good choice to obtain any desired biocompatible entities enhancing interaction between the particles and biological species. Inspired by the unique properties of MNPs, development of a novel probe based on nanoparticles solid support (Figure 2) in order to enhance selectivity and sensitivity was initially explored.

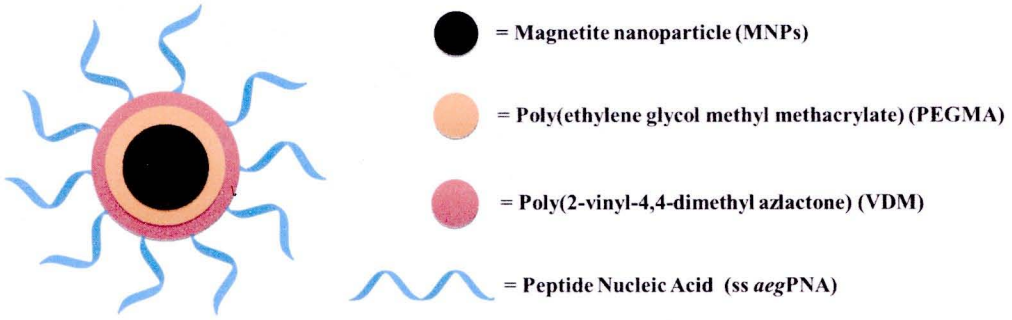


Figure 2 Conceptual design of *ss aeg*PNA oligomers-MNPs probe

Purpose of the study

1. To synthesize *ss aeg*PNA oilgomers-MNPs biomolecular probe
2. To investigate how length and steric of *ss aeg*PNA oligomers affect immobilization efficiency

Significance of the study

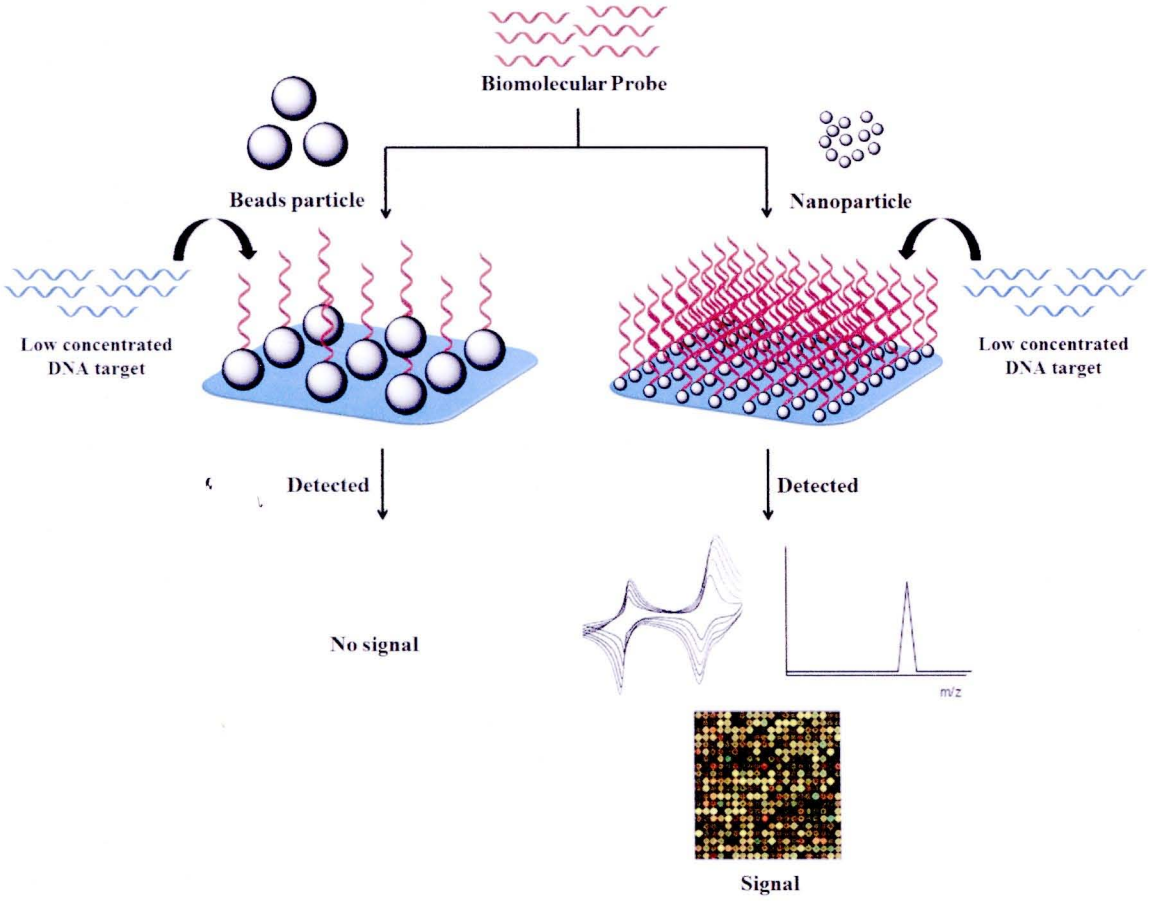


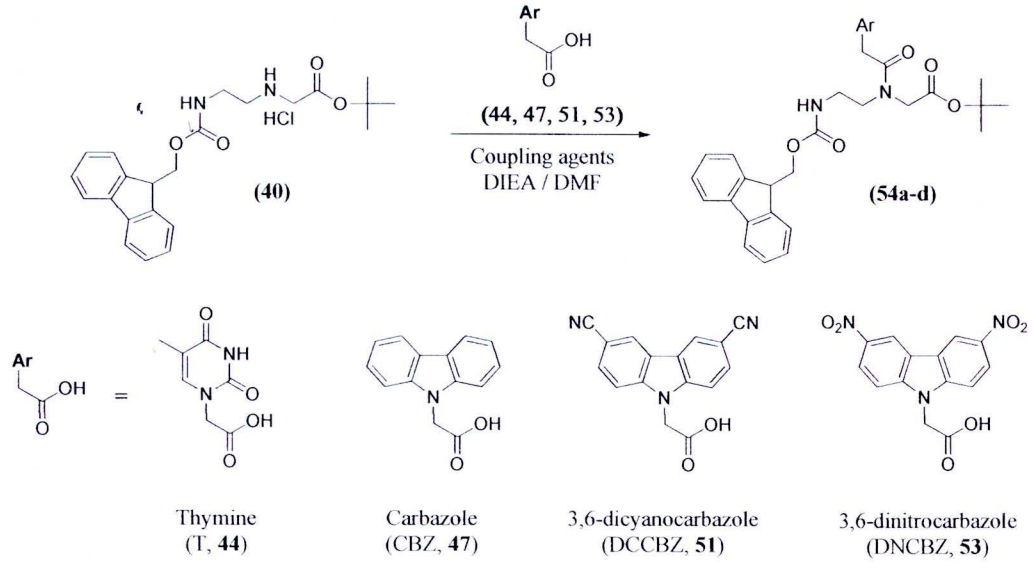
Figure 3 Ideal application of *ss aeg*PNA oilgomers-MNPs probe for DNA detection with high sensitivity

Incorporation of *ss aeg*PNA with MNPs should improve detection sensitivity and binding affinity (Figure 3). This novel probe can serve as nano biomolecular probes for medical diagnosis. Millions of different sequences of *ss aeg*PNA oligomers-MNPs probes can be placed on the small area and detection of the single mismatch of thousand target genes can perform in a high-throughput manner with short time could make the nanoprobes as robust, rapid and cheap medical analysis in near future. Importantly, early

detection of genetic related diseases can be detected and proper medications and treatments could be promptly and effectively performed for patients.

Scope of the study

Behavior of archetypal *ss aeg*PNA oligomers-MNPs bimolecular probes were investigated and this study was divided into two parts: (1) syntheses of *ss aeg*PNA oligomers, (2) immobilization of *ss aeg*PNA oligomers onto functionalized MNPs.



Scheme 1 Synthesis of Fmoc-*aeg*PNA monomers (54a-d)

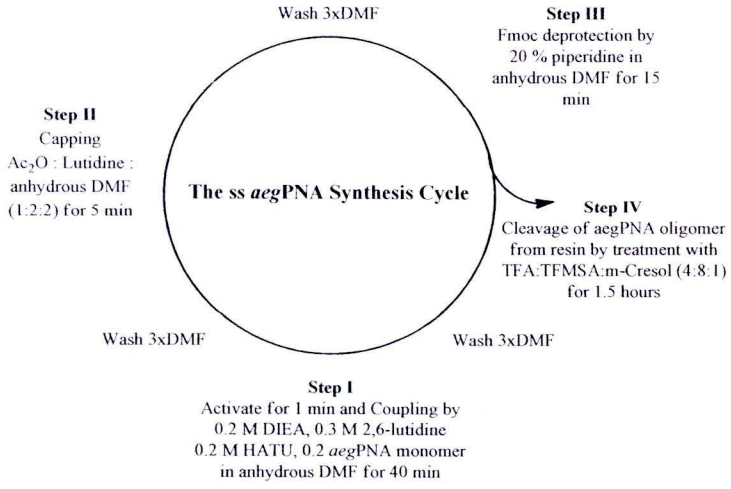
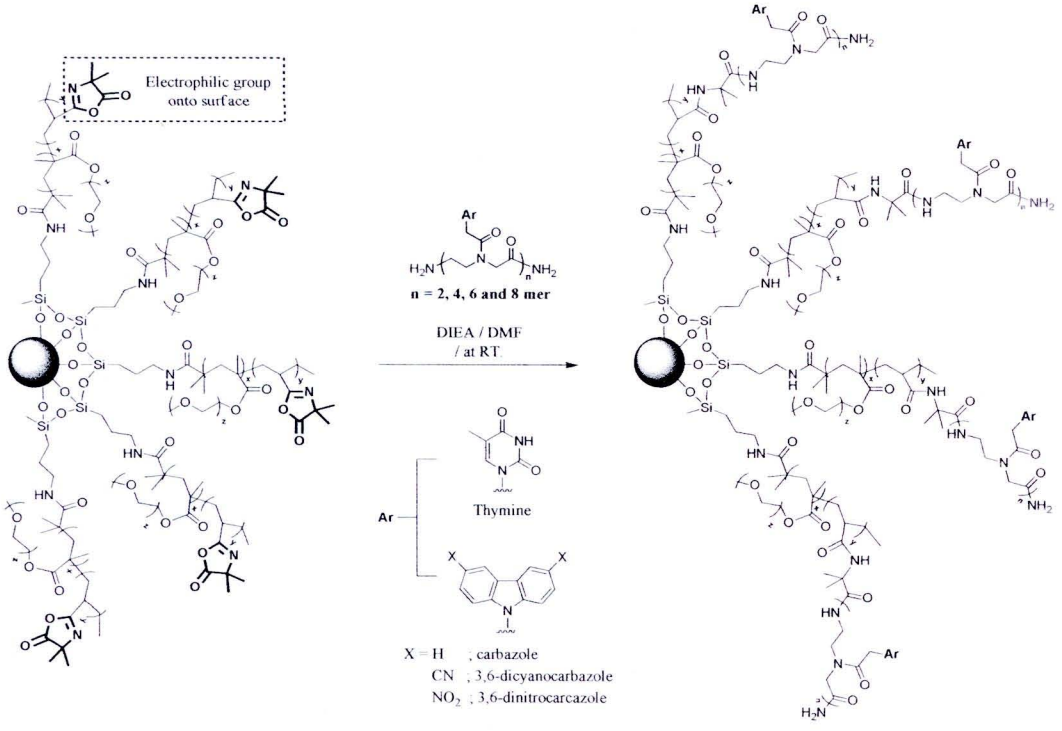


Figure 4 Synthesis of *ss aeg*PNA oligomers (56a-g)



Scheme 2 Immobilization of *ss aeg*PNA oligomers coated onto electrophilic MNPs