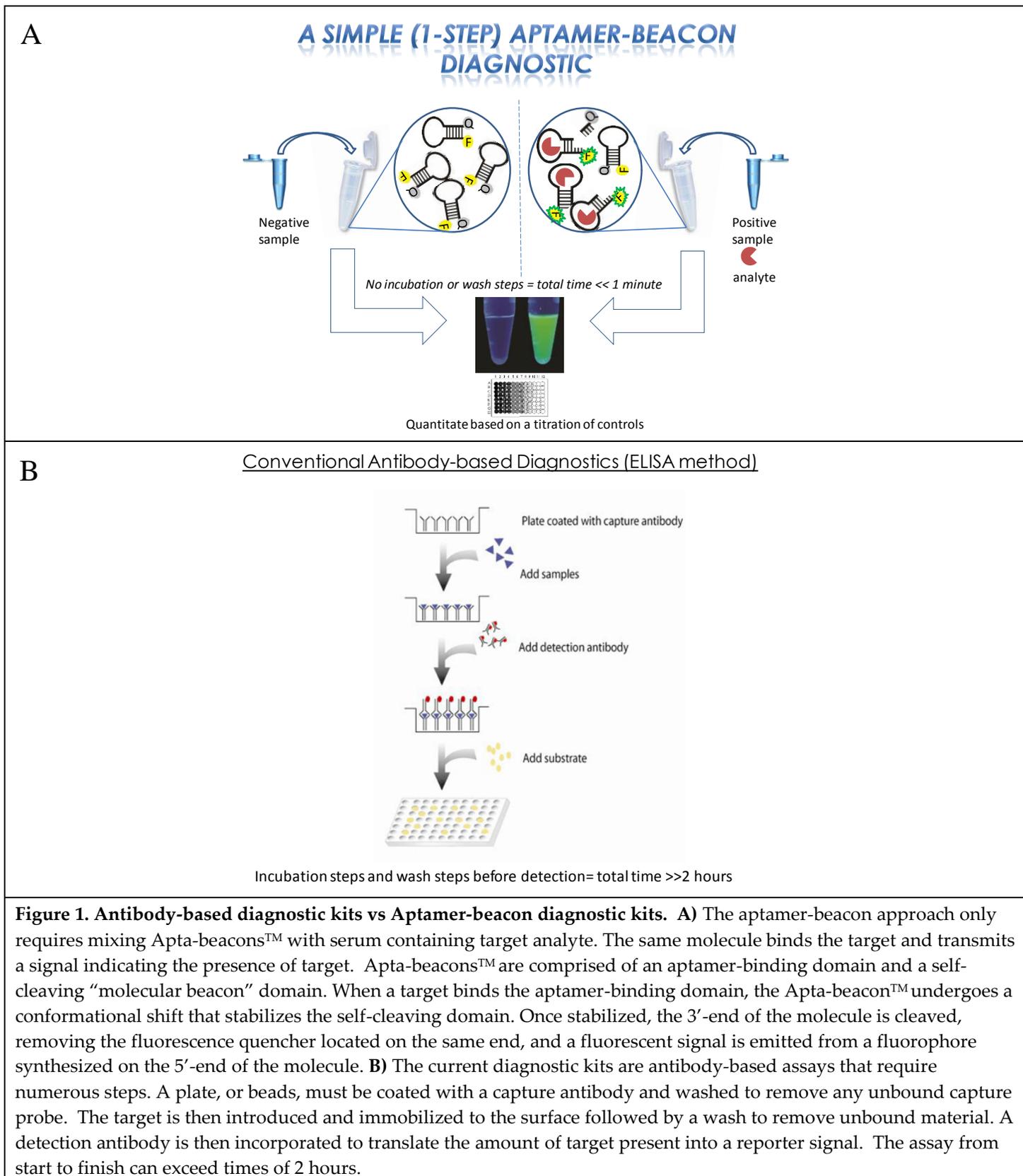Aptagen's aptamer-beacon (i.e. Apta-beacon™) translates a binding event in any sample containing antigens, targets, or biomarkers to a reporter signal in the form of a measurable fluorescent indicator. An Apta-beacon™ is a nucleic acid based molecule comprised of two functional groups: an aptamer-target binding domain and a self-cleaving ribozyme domain (**Figure 1**). The aptamer domain acts as the docking point for target interaction. Under target bound conditions, an Apta-beacon™ undergoes a conformational shift that stabilizes the ribozyme structure. Stabilization of the self-cleaving ribozyme domain results in a cleavage event on the 3'-end of the molecule, therefore, removing the fluorescence quencher dampening the fluorescent signal from the fluorophore synthesized on the 5'-end of the same molecule. This fluorescent signal can be easily detected using standard laboratory equipment with a fluorescence detector, such as a microplate reader or a fluorometer.

Libraries of randomized oligonucleotides have been shown to contain oligonucleotide ligands that bind tightly to various target compounds other than complementary nucleic acid sequences [1-4]. These nucleic acid ligand biopolymers (i.e. aptamers) have structural specificity based on their shapes, and can be engineered to bind to many different compounds, proteins, and molecules (**Figure 2**). For the past 20 years, a variety of these tight-binding nucleic acid ligands have been developed as an attractive alternative to the conventional antibody[2]. Aptamers have captured the attention and interest of scientists across many disciplines within the biological sciences. One reason for the tremendous interest and excitement is that there is a practical advantage of an aptamer over an antibody. Unlike the time and costs associated for producing a protein-based antibody, an aptamer can be synthesized quickly and cheaply using an automated oligo synthesizer.

A growing body of literature supports the fact that aptamers exhibit high affinity binding against their cognate targets, and reinforces the notion that an aptamer can be developed against virtually any target. There are a variety of reports of aptamers that exhibit with binding affinities from the micromolar to the nanomolar range against various targets, and in some case picomolar [5]. In many cases, the binding affinity is observed to be much greater than that of monoclonal antibodies, lead compounds obtained from random peptide libraries, small molecule libraries, or natural product extracts [1, 2]. As a functional unit in Apta-beacons™, it is important that the aptamers exhibit affinities in the nanomolar ranges to properly functionalize the self-cleaving ribozyme at very low target concentrations. The Breaker laboratory at Yale University has been a pioneer in the field of engineering allosterically modulated ribozymes [6-8]. Allosteric ribozymes have been engineered to act like molecular switches turning "on" or "off" in the presence or absence of effectors. Ribozyme catalysis is modulated by rapid conformational changes imparted by an effector molecule binding to an aptamer domain apart from the catalytic site. Although they play different roles, ribozymes share a common characteristic with aptamers: they are both nucleic acids which exhibit structural plasticity along their phosphate backbone polymer chain, and because of this, it was soon realized that a ribozyme sequence can be modified to incorporate an aptamer sequence to allow for allosteric control [6,7]. The aptamer domain functions as an allosteric trigger by its target for subsequent ribozyme activity. Therefore, with the advent of aptamers and the discovery of ribozymes, it has been possible to design and construct allosteric ribozymes.
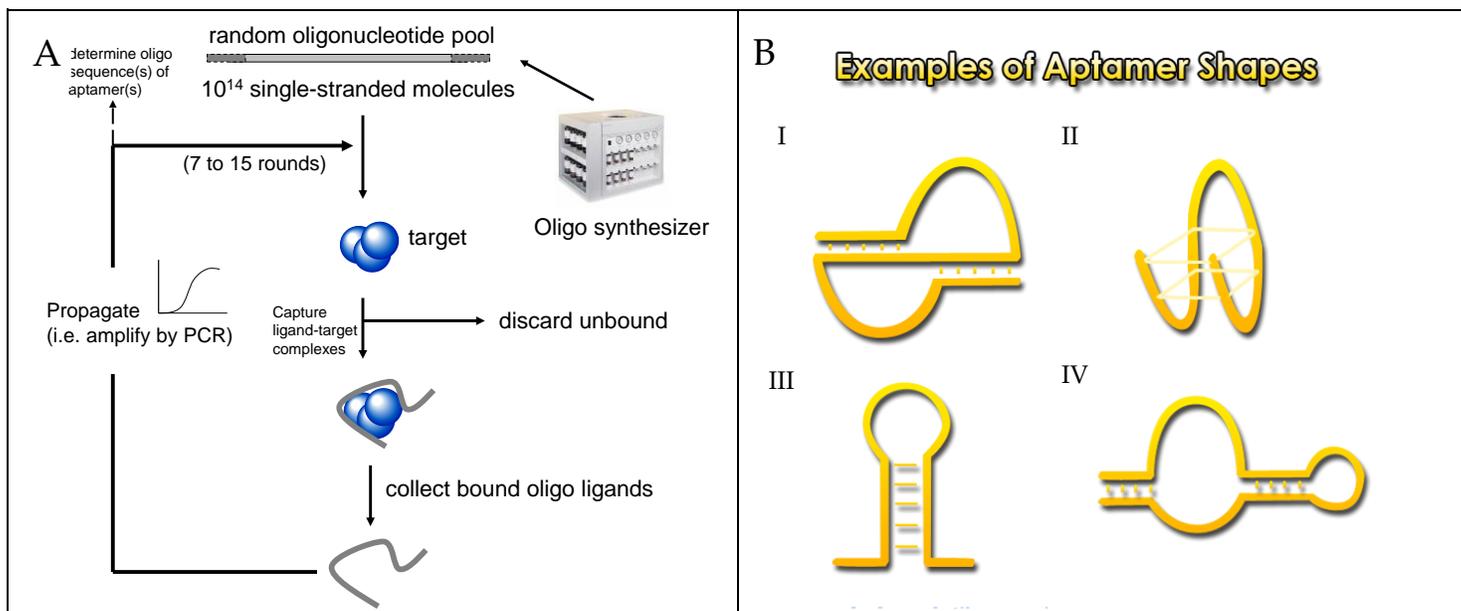
A

A SIMPLE (1-STEP) APTAMER-BEACON DIAGNOSTIC

Negative sample

Positive sample

analyte

No incubation or wash steps = total time << 1 minute

Quantitate based on a titration of controls

B

Conventional Antibody-based Diagnostics (ELISA method)

Plate coated with capture antibody

Add samples

Add detection antibody

Add substrate

Incubation steps and wash steps before detection= total time >>2 hours

Figure 1. Antibody-based diagnostic kits vs Aptamer-beacon diagnostic kits. A) The aptamer-beacon approach only requires mixing Apta-beacons™ with serum containing target analyte. The same molecule binds the target and transmits a signal indicating the presence of target. Apta-beacons™ are comprised of an aptamer-binding domain and a self-cleaving "molecular beacon" domain. When a target binds the aptamer-binding domain, the Apta-beacon™ undergoes a conformational shift that stabilizes the self-cleaving domain. Once stabilized, the 3'-end of the molecule is cleaved, removing the fluorescence quencher located on the same end, and a fluorescent signal is emitted from a fluorophore synthesized on the 5'-end of the molecule. **B)** The current diagnostic kits are antibody-based assays that require numerous steps. A plate, or beads, must be coated with a capture antibody and washed to remove any unbound capture probe. The target is then introduced and immobilized to the surface followed by a wash to remove unbound material. A detection antibody is then incorporated to translate the amount of target present into a reporter signal. The assay from start to finish can exceed times of 2 hours.

Figure 2. Schematic of aptamer development and their various structures A) A pool of 10^{14} random oligonucleotides is synthesized and introduced to a target molecule (eg. Protein). Unbound oligonucleotides are discarded while bound species are collected and propagated using PCR. This selection step is repeated numerous times before identifying the candidates in the pool with a DNA sequencer. **B)** Aptamers have structural specificity according to their shapes. The following are structural conformations of various aptamers: **I)** PseudoKnot (ligand for HIV-1 reverse transcriptase), **II)** G-quartet (ligand for thrombin), **III)** Hairpin (ligand for bacteriophage for T4 polymerase), **IV)** Stem loop/bulge (ligand for ATP).

Numerous molecular switches targeting an array of molecules, such as proteins and small inorganics, have been previously reported [9, 10]. **The fact that these molecules can be used in a solution-based assay allows for more sensitive detection compared to the current antibody-based assays.** As seen in **Figure 1**, the current diagnostic kits use an enzyme immunoassay that involves immobilizing the target to a surface, or beads, prior to analyte detection with a secondary antibody (i.e. ELISA). This multi-step approach is required to reduce background caused by non-specific antibody binding. Apta-beacons™, on the other hand, are developed using **S**ystematic **E**volution of **L**igand **E**nrichment (SELEX) [11] . This technique results in tight binders for a specific target, removing random RNA or DNA molecules that do not bind to the target while retaining those that do bind the target with varying levels of affinity. Monoclonal Apta-beacons™ with the highest affinities for a target are ultimately selected. The entire process allows for low background signals and a one-step assay to detect the presence of a target. The one-step approach utilized by Apta-beacons™ increases the assays potential to be point-of-care technology. Figure 1A depicts the simplicity of target detection using Apta-beacons™. Following target-binding to the aptamer domain, the samples are analyzed on a fluorometer and compared to the standards.

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