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CRISPR-powered Biosensing Platform for Quantitative Detection of Alpha-fetoprotein by a Personal Glucose Meter

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Abstract

Alpha-fetoprotein (AFP) is an important protein biomarker of liver cancer, as its serum levels are highly correlated with the progression of disease. Conventional immunoassays for AFP detection rely on enzyme-linked immunosorbent assay analyses with expensive and bulky equipment. Here, we developed a simple, affordable, and portable CRISPR-powered personal glucose meter biosensing platform for quantitative detection of the AFP biomarker in serum samples. The biosensor takes advantage of the excellent affinity of aptamer to AFP and the collateral cleavage activity of CRISPR-Cas12a, enabling sensitive and specific CRISPR-powered protein biomarker detection. To enable point-of-care testing, we coupled invertase-catalyzed glucose production with the glucose biosensing technology to quantify AFP. Using the developed biosensing platform, we quantitatively detected AFP biomarker in spiked human serum samples with a detection sensitivity of down to 10 ng/mL. Further, we successfully applied the biosensor to detect AFP in clinical serum samples from patients with liver cancer, achieving comparable performance to the conventional assay. Therefore, this novel CRISPR-powered personal glucose meter biosensor provides a simple yet powerful alternative for detecting AFP and potentially other tumor biomarkers at the point of care.

Graphical Abstract

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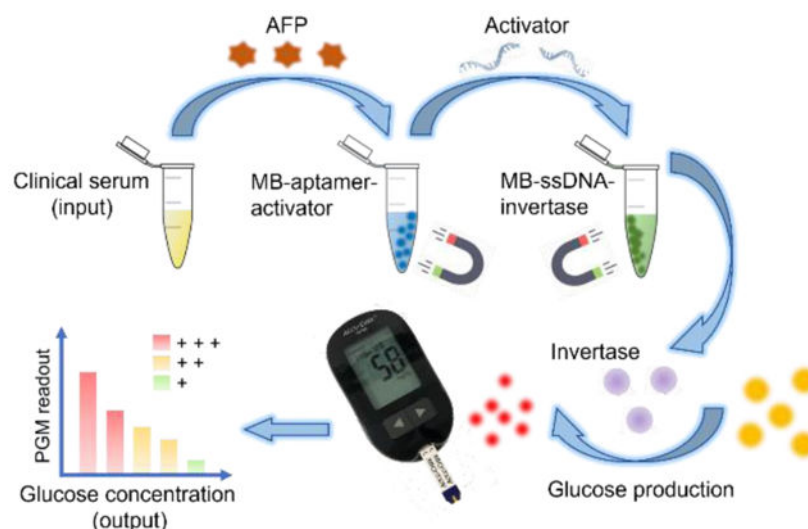
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Credit Author Statement

Zhengyang Jia: Conceptualization; Methodology; Investigation, Data collection; Formal analysis; Validation; Writing-original draft, Reviewing & Editing. **Ziyue Li:** Investigation, Data collection; Formal analysis; Reviewing & Editing. **Changchun Liu:** Conceptualization; Methodology; Funding acquisition; Project administration; Supervision; Reviewing & Editing. All authors reviewed, discussed, and contributed to the final manuscript and approved it to be published.

Declaration of interests

The authors declare that they have no known competing financial interests or personal relationships that could have appeared to influence the work reported in this paper.



Keywords

CRISPR-Cas12a assay; personal glucose meter; aptamer; alpha-fetoprotein detection; point-of-care diagnosis

1. Introduction

Liver cancer is the fifth most common malignancy and the third most common cause of cancer death globally.[1] Alpha-fetoprotein (AFP) in serum is recognized as an important protein biomarker for liver cancer assessment.[2] Owing to their remarkable sensitivity and specificity, immunoassays (e.g., enzyme linked immunosorbent assays (ELISAs)) are considered the standard method for AFP detection for conventional clinical diagnostic applications.[3] However, such antibody/antigen-based immunoassay systems typically require arduous procedures, sophisticated laboratory instruments, and highly trained operators, which severely limits their application for rapid screening of liver cancer in the population. Therefore, there is unmet need to develop a simple, affordable, and portable diagnostic tool for the identification, intervention, and treatment of liver cancer.[4–6]

Recently, the clustered regularly interspaced short palindromic repeats (CRISPR)-CRISPR-associated protein (CRISPR-Cas) biosensing system has attracted considerable interest for developing novel molecular diagnostic technologies for different diseases due to its simplicity, high sensitivity, and specificity.[7–10] However, much of the current focus is on developing nucleic acid-based diagnostic tools for point-of-care testing.[11–13] To extend the detection application of CRISPR technology beyond nucleic acids, a few research groups have proposed to combine the trans-cleavage activity of CRISPR-Cas12a with specific aptamers to detect non-nucleic acid targets (e.g., polypeptides, proteins, and metal ions). [14–16] Aptamers are short single-stranded DNA or RNA molecules that can be easily hybridized with the complementary CRISPR-targeted DNA sequence.[17] The presence of specific analytes results in the structural conformation of hybridization, allowing CRISPR-targeted DNA to be released, and therefore trigger the initiation of the CRISPR-Cas12a

reaction for signal output. Importantly, aptamers for a specific analyte can be rapidly and largely synthesized *in vitro*. [18,19] Despite significant progress, most systems still rely on the detection of fluorescence signal, which requires highly specialized instrument set-ups and personnel.

Here, we report a simple, low-cost, and portable CRISPR-powered personal glucose meter (PGM) biosensing platform for quantitative detection of AFP in serum samples. The biosensor couples the excellent affinity of aptamer to AFP with the collateral cleavage activity of CRISPR-Cas12a, enabling sensitive and specific protein biomarker detection. To eliminate the need for expensive optical detection equipment, we further integrated invertase-catalyzed glucose production with the glucose biosensing technology to quantify AFP biomarker, analogous to home-based diabetes testing. Further, we demonstrated and validated the clinical utility of the CRISPR-powered PGM biosensor by determining AFP levels in clinical serum samples from patients with liver cancer. Due to its accuracy, portability, and cost-effectiveness, the developed biosensor provides a promising approach for screening and detecting AFP and potentially other tumor biomarkers at the point of care.

2. Experimental Section

2.1 Materials and Reagents

Magnetic beads (MBs) coated with streptavidin (Dynabeads M-280 Streptavidin) were purchased from ThermoFisher Scientific. All oligonucleotides, AFP aptamer (APT273), and CRISPR RNA (crRNA) used in this work (Table S1) were synthesized by Integrated DNA Technologies, Inc. with high-performance liquid chromatography purification. LbaCas12a, NEBuffer r2.1, and nuclease-free water were purchased from New England Biolabs Inc. Invertase (from baker's yeast), Tris(2-carboxyethyl) phosphine hydrochloride (TCEP), 4-(N-maleimidomethyl) cyclohexane-1-carboxylic acid 3-sulfo-N-hydroxysuccinimide ester sodium salt (Sulfo-SMCC), sucrose, ultrafiltration tubes (Amicon Ultra 0.5 mL), bovine serum albumin (BSA), human serum albumin (HSA) and IgG from human serum were purchased from Sigma-Aldrich. Buffers (phosphate buffered saline (PBS), pH=7.4; Tris-HCl, pH=8.0, and MES, pH=5.5) and magnet racks (DynaMag-PCR Magnet) were purchased from ThermoFisher Scientific. AFP was obtained from Lee Biosolutions, Inc. Serum samples from healthy individuals were obtained from Innovative Research, Inc. Serum samples from patients with liver cancer were obtained from Discovery Life Sciences. Human AFP ELISA kits were purchased from RayBiotech, Inc. PGMs (Accu-Chek Aviva Plus) and test strips were purchased from Roche Diagnostics Co., Ltd. All chemicals and reagents were of analytical grade and were used as received without further purification.

2.2 Preparation of Aptamer–Activator Duplex-modified MBs

An aptamer, termed APT273, was used as the AFP biomarker in this work due to its excellent specificity according to previous literature [20]. APT273–activator duplex modified MBs (termed MB-aptamer-activator) were prepared based on previous methods [15] with slight modifications. The mixture of APT273 (20 μ M) and DNA activator (20 μ M) in nuclease-free water was heated at 90°C for 10 min and then gradually cooled down (5°C/min) to room temperature to obtain the APT273–activator duplex. Following the removal of

storage buffer, the purified streptavidin MBs (0.4 mg) were redispersed in 0.4 mL of 1% Tris-HCl buffer containing APT273–activator duplex (1 μ M) and then stirred mechanically for 60 min in an ultrasonic bath. Excess APT273–activator duplex was removed using 6 cycles of magnetic separation (nuclease-free water $\times$ 6). APT273–activator duplex-modified MBs (MB–aptamer–activator) with a final concentration of 10 mg/mL in nuclease-free water were stored at 4°C for subsequent analyses and experiments.

2.3 Chemical Design of Invertase-modified MBs

Invertase was modified and immobilized to streptavidin MBs using a chemical coupling reaction as previously reported [21] with slight modifications. Briefly, 7 μ L TCEP solution (60 mM) was added to 20 μ L 1 $\times$ PBS containing 1 nmol biotin-DNA-thiol (single-stranded DNA (ssDNA) linker) and left to react at room temperature for 2 hours. Meanwhile, SMCC (0.25 mg) and invertase (3 mg) were dissolved in 100 μ L 10 $\times$ PBS and stirred (500 rpm) at room temperature for 1 hour. The mixture was then centrifuged for 6 cycles (8,000 rpm, 2 min) using an ultrafiltration tube (molecular weight cut-off = 100 k) and 1 $\times$ PBS as washing buffer to remove excess reactants. The purified invertase and activated ssDNA linker (1 nmol) were redispersed in 0.3 mL 1 $\times$ PBS and then stirred at room temperature for 2 days (500 rpm) to prepare biotin-ssDNA-invertase. Excess ssDNA linker was removed using 6 cycles of ultrafiltration as described above. Following the removal of storage buffer, a purified streptavidin MB (0.4 mg) was immediately delivered into 1 $\times$ PBS containing biotin-ssDNA-invertase and continuously stirred for 60 min in an ultrasonic bath. Finally, free biotin-ssDNA-invertase was washed off using 6 cycles of magnetic separation (1 $\times$ PBS $\times$ 6). Invertase-modified MBs (MB-ssDNA-invertase) with a final concentration of 20 mg/mL in 1 $\times$ PBS were stored at 4°C for subsequent analyses and experiments.

2.4 Characterization of Modified MBs

The surface modifications of the MBs were analyzed and characterized using Fourier-transform infrared spectroscopy (FTIR). Infrared spectra were collected on an FTIR microscope (Nicolet Magna 560). MB samples with purification were lyophilized and measured as dispersions in KBr powder in diffuse transmission mode.

2.5 Detection of AFP Biomarker in Spiked Serum using a PGM

A total of 10 μ L AFP biomarker solution at various concentrations (10-fold serial dilution ranging from 100 μ g/mL to 0.01 μ g/mL) in PBS or human serum solution (5% human serum in 1 $\times$ PBS) was incubated with 10 μ L MB–aptamer–activator suspension at 37°C for 15 min. Following magnetic separation, 10.6 μ L supernatant was carefully collected and transferred into a new tube to prepare the CRISPR-Cas12a reaction system (final volume = 24 μ L), which contained 6 μ L diluent buffer containing LbCas12a (800 nM) and crRNA (1000 nM), 5 μ L MB-ssDNA-invertase (24 mg/mL) and 2.4 μ L 10 $\times$ NEBuffer r2.1. Subsequently, the mixture was placed in an incubator (37°C) under mechanical rotation for 1 hour. After magnetic separation, 20 μ L supernatant in the CRISPR-Cas12a reaction tube was carefully collected and then transferred into another tube to prepare the glucose-producing reaction system (final volume = 50 μ L) containing 20 μ L sucrose (1 M) and 10 μ L MES buffer. After incubation at 37°C for 30 min or 60 min, a total of 5 μ L of the mixed solution was collected and the glucose concentration was measured by a PGM.

2.6 Detection of AFP in Clinical Serum Samples

All experiments were carried out according to a protocol approved by the ethics committee at the University of Connecticut Health Center (IRB #08–310-1). To demonstrate practical application of the CRISPR-powered PGM biosensing platform, serum samples from liver cancer patients were detected following the protocol as described above. Each serum sample was filtered and diluted 20 times using 1× PBS before analysis. For methodology comparison, AFP concentration in clinical serum samples was quantified by an AFP ELISA kit, performed according to the supplier's protocol.

2.7 Fluorescence Detection of CRISPR Reactions

To detect the fluorescent signal intensity of CRISPR-Cas12a reaction products in real time, 2 μ M fluorescein molecule (FAM)-ssDNA-quencher probe (ssDNA-FQ) was used to replace MB-ssDNA-invertase in the CRISPR-Cas12a reaction system described above. The mixture was then immediately transferred into reaction tubes for incubation at 37°C and the intensity of FAM fluorophore was recorded every minute using the Bio-Rad CFX96 Touch Real-Time PCR Detection System (Bio-Rad Laboratories). After a 60-min incubation, endpoint fluorescence images of the CRISPR-Cas12a reaction products were acquired using the ChemiDoc MP Imaging System (Bio-Rad Laboratories).

2.8 Statistical Analysis

Three independent experiments were conducted to collect the experimental data. Statistical analysis was performed using the paired two-tailed Student's *t*-test. Significant statistical difference was defined as $p < 0.05$. GraphPad Prism 7.04 was used to analyze the data.

3. Results and Discussion

3.1 Design and Construction of the CRISPR-powered PGM Biosensor for AFP Detection

The workflow of AFP detection using a CRISPR-powered PGM biosensing platform is illustrated in Figure 1. This platform involves three main steps: i) AFP-induced activator release and magnetic separation from MB-aptamer-activator, ii) CRISPR-Cas12a activation by the released activator and cleavage of MB-ssDNA-invertase, and iii) glucose generation from sucrose hydrolysis caused by the released invertase and quantitative detection by the PGM. First, an activator with a partial complementary sequence of the AFP aptamer hybridizes with AFP aptamer modified with biotin. Then, the formed AFP aptamer-activator duplex is immobilized on the surface of the streptavidin MB by the specific reaction of streptavidin-biotin and forms the MB-aptamer-activator complex. In the presence of AFP biomarker, the AFP aptamer on the beads specifically binds with the AFP biomarker and changes the conformation of the aptamer-activator duplex, therefore leading to the release of the activator (Figure 1A). After the first magnetic separation, the released activator in the supernatant reacts with CRISPR-Cas12a reaction solution to specifically activate CRISPR-Cas12a. The activated CRISPR-Cas12a then non-specifically cleaves the ssDNA-conjugated invertase immobilized on the MBs (MB-ssDNA-invertase),[22] resulting in the release of free invertase (Figure 1B). Following the second magnetic separation, the released invertase is incubated with sucrose solution and hydrolyzes sucrose into glucose, which can be

quantitatively detected by a PGM (Figure 1C). Thus, the AFP biomarker can be detected by quantifying the generated glucose levels with a simple glucose meter, analogous to home diabetes testing.

3.2 Surface Modification and Characterization of MB-ssDNA-invertase

Streptavidin MBs are selected to immobilize biotinylated ligands because of their high biotin binding capacity and surface area.[23] Construction of MB-ssDNA-invertase is carried out as illustrated in Figure 2A. Following activation by Sulfo-SMCC, invertase is covalently conjugated to biotin-ssDNA-thiol (ssDNA linker) via sulfhydryl-reactive crosslinker chemistry between the terminal thiol group in the ssDNA linker and the reactive maleimide group in the invertase molecules. Subsequently, the biotin-ssDNA-invertase is immobilized on the streptavidin MBs via the strong biotin–streptavidin interaction. We confirmed successful surface coating of ssDNA-invertase onto MBs by FTIR analysis (Figure 2B). In comparison with the non-modified streptavidin MB, the infrared spectra of MB-ssDNA-invertase displayed three intense absorption peaks at $1,090\text{ cm}^{-1}$, $1,050\text{ cm}^{-1}$, and 890 cm^{-1} , which can be assigned to the presence of phosphate backbone, deoxyribose C-O, and deoxyribose rings of DNA chains, respectively.[24] We observed two strong absorption peaks close to $1,620\text{ cm}^{-1}$ and $1,540\text{ cm}^{-1}$ for MB-invertase, which can be attributed to the vibration of amide bands of invertase.[25] We note that streptavidin was coated on the surface of non-modified MBs (streptavidin MBs). Therefore, we also detected these two feature peaks of amide bands in the spectra of non-modified streptavidin MBs, although to a lower extent (Figure 2B).

3.3 Analytical Performance of the CRISPR-powered PGM Biosensor

We optimized the concentration of the APT273–activator duplex for CRISPR-powered AFP detection. As shown in Figures S1–S4, the overall fluorescence signals improved significantly when we increased the concentration of APT273–activator from $10\text{ }\mu\text{M}$ to $30\text{ }\mu\text{M}$. However, we observed an elevated, non-specific fluorescence signal in 5% human serum without spiking AFP when $30\text{ }\mu\text{M}$ of APT273–activator was used. Consequently, we selected $20\text{ }\mu\text{M}$ of APT273–activator as the optimal concentration.

To evaluate the analytical performance of the CRISPR-powered PGM biosensing platform for AFP detection, we tested a ten-fold serial dilution of AFP biomarker ranging from $100\text{ }\mu\text{g/mL}$ to $0.01\text{ }\mu\text{g/mL}$ in a 5% human serum solution. First, we compared and evaluated the quantitative readout of the PGM for AFP biomarker detection at different catalytic reaction times, specifically 30 min and 60 min. As shown in Figure 3A and Table S2, the detection signal of the glucose meter increased as the incubation time extended from 30 min to 60 min. With an incubation time of 30 min, the PGM biosensor can detect $0.1\text{ }\mu\text{g/mL}$ AFP biomarker in serum samples, but not $0.01\text{ }\mu\text{g/mL}$ (or 10 ng/mL) because the generated glucose concentration was below the limit of detection of the PGM (20 mg/dL) (Figure 3, marked as “not detected” (n.d.)). When we increased the incubation time to 60 min, the biosensor’s readout increased significantly. After a 60-min incubation, the biosensor could consistently detect $0.01\text{ }\mu\text{g/mL}$ (or 10 ng/mL) of AFP biomarker in serum samples, which could be attributed to the increased glucose generation through the catalytic effect of invertase with the increased incubation time.[26] To determine the reproducibility of the

biosensor, we repeatedly detected AFP with a concentration of 1 $\mu\text{g/mL}$ in a 5% human serum solution at 60 min post catalytic reaction. The glucose biosensor showed excellent reproducibility and reliability with a coefficient of variation of 2.22% (Figure S5). In addition, we evaluated its specificity by testing different proteins, including BSA, HSA and IgG from human serum. As shown in Figure S6, we did not observe any non-specific signal for other proteins. Therefore, our CRISPR-powered PGM biosensor provides a simple, reliable, specific, and quantitative approach for AFP biomarker detection at the point of care without the need for complex equipment.

To quantify the AFP biomarker, we evaluated the relationship between glucose readouts and AFP concentrations through logarithmic fitting curves. We achieved quantitative detection of AFP biomarker within a dynamic range of 0.01–100 $\mu\text{g/mL}$ using the established formula $y = 41.32 + 7.46 \cdot \ln(x - 0.02)$ ($R^2 = 0.877$, Figure 4A) or $y = 80.73 + 22.53 \cdot \ln(x + 0.08)$ ($R^2 = 0.988$, Figure 4B) (y: concentration of glucose (mg/dL); x: concentration of AFP ($\mu\text{g/mL}$)) at 30 min or 60 min post glucose-producing reaction, respectively. As shown in Figure 4, we observed a strong positive correlation between the glucose signal and the concentration of spiked AFP, confirming the applicability of the designed PGM biosensor for the quantitative detection of AFP. However, the AFP concentrations and glucose readouts with a 60-min incubation (Figure 4B) exhibited better correlation ($R^2 = 0.988$) compared with that of the 30-min incubation reaction (Figure 4A). This result might be due to the kinetics of the invertase action. Indeed, previous studies have confirmed that a time lag occurs during the initial hydrolysis of sucrose, particularly under conditions of a high c(sucrose)/c(invertase) ratio.[26, 27] In addition, AFP levels in healthy individuals are typically below 25 ng/mL.[28] Thus, we selected the 60-min incubation time to further test clinical serum samples with our CRISPR-powered PGM biosensor because its higher detection sensitivity and better quantitative detection accuracy.

3.4 Clinical Validation of the CRISPR-powered PGM Biosensor for AFP Detection in Serum Samples

After evaluating and optimizing the analytical performance of the CRISPR-powered PGM biosensor, we further investigated its clinical application to detect AFP biomarkers in 11 clinical serum samples, including eight samples from liver cancer patients and three samples from healthy individuals (Figure S7). Before testing clinical serum samples with the biosensor, we used a conventional ELISA system to detect and quantify AFP biomarkers in clinical serum samples (Figure 5A). We quantified the AFP concentration in each clinical sample based on the standard curve from the ELISA kit (Figure S8). For the biosensing detection, we diluted individual serum samples 20-fold in $1 \times \text{PBS}$ before analysis using the above-established workflow. To ensure reliable testing results, we detected each sample with the biosensor three times. We recorded the glucose readouts from various samples after the 60-min incubation reaction (Figure 5B, C, and S7). As expected, for serum samples from healthy controls, the CRISPR-powered PGM biosensor did not detect glucose signal (marked as n.d.) (Figure S7). On the contrary, all eight serum samples from patients with liver cancer exhibited significantly high glucose signals (Figure S7). We calculated and quantified the AFP concentration in each patient sample by referring to the established fitting curve (Figure 5C). As shown in Figure 5D, the biosensor demonstrated comparable

performance to the conventional ELISA method. Therefore, our CRISPR-powered PGM biosensor provides a promising simple, low-cost, and portable alternative for quantitative detection of AFP and potentially other tumor biomarkers.

4. Conclusion

In this study, we developed a simple, low-cost CRISPR-powered PGM biosensing platform for quantitative detection of AFP biomarkers in human serum samples by combining aptamer-based molecular recognition, CRISPR-Cas12a cleavage reaction, and glucose biosensing technology. The biosensing platform is capable of quantitatively detecting AFP biomarkers in serum samples within a range of 100 $\mu\text{g/mL}$ to 0.01 $\mu\text{g/mL}$ with a detection sensitivity of less than 0.01 $\mu\text{g/mL}$. Further, we tested clinical serum samples and validated the clinical utility of our platform for AFP detection, achieving comparable performance to the conventional ELISA system. The developed CRISPR-powered PGM biosensing platform offers several advantages over the conventional approach. i) It is a simple and low-cost biosensing platform. We utilize glucose biosensing technology to quantify AFP biomarker, eliminating the need for an expensive optical detection instrument (e.g., microplate reader). ii) It is a sensitive and specific assay for protein biomarkers by CRISPR detection technology. By combining the excellent affinity of aptamer to AFP biomarker and collateral cleavage activity of Cas12a, the AFP biomarker can be specifically detected by CRISPR technology without the need for time-consuming antibody production in conventional antibody/antigen-based immunoassays. iii) It provides portable and quantitative detection. By taking advantage of existing PGM biosensing technology, the amount of AFP in serum samples can be directly quantified by a portable PGM. To further simplify the operation and improve the detection performance, we will integrate different detection modules into a microfluidic platform, enabling simple and portable biomarker detection.[29] Therefore, the CRISPR-powered PGM biosensor represents an alternative low-cost and portable biosensing platform for the detection of AFP and other tumor biomarkers at the point of care.

Supplementary Material

Refer to Web version on PubMed Central for supplementary material.

Acknowledgments

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Biographies

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Highlights

- A CRISPR-powered biosensor is developed to detect AFP biomarker.
- AFP in serum samples was quantified by a simple personal glucose meter.
- The biosensing platform shows a detection sensitivity of down to 10 ng/mL.
- The affordable biosensor is validated to detect AFP in clinical serum samples.

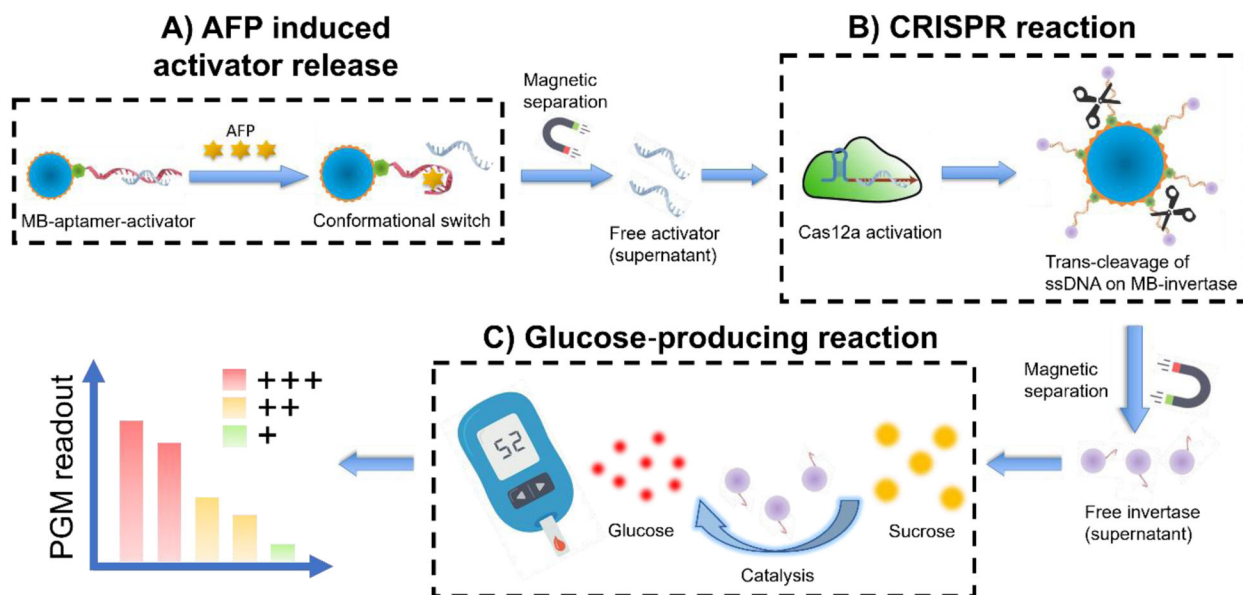


Figure 1. Schematic illustration of the working principle of the CRISPR-powered PGM biosensing platform for quantitative detection of AFP biomarker.

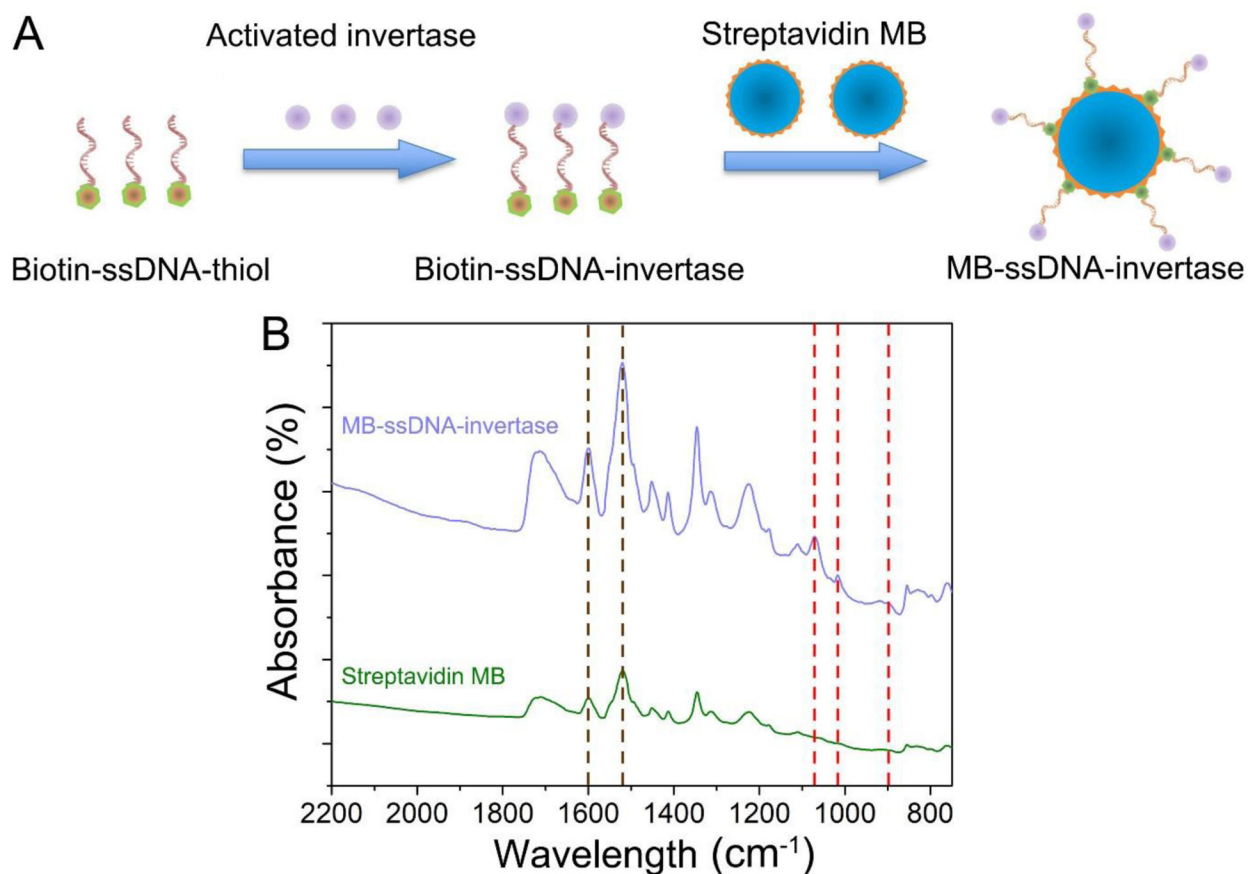


Figure 2.

(A) Schematic illustration of the strategy to modify MBs with ssDNA-invertase and generate MB-ssDNA-invertase. (B) FTIR analysis to demonstrate successful modification of ssDNA-invertase on streptavidin MBs. The dashed lines indicate the infrared absorption of phosphate backbone ($1,090\text{ cm}^{-1}$, red), deoxyribose C-O ($1,050\text{ cm}^{-1}$, red), deoxyribose rings (890 cm^{-1} , red) and amide bands ($1,620\text{ cm}^{-1}$, and $1,540\text{ cm}^{-1}$, brown).

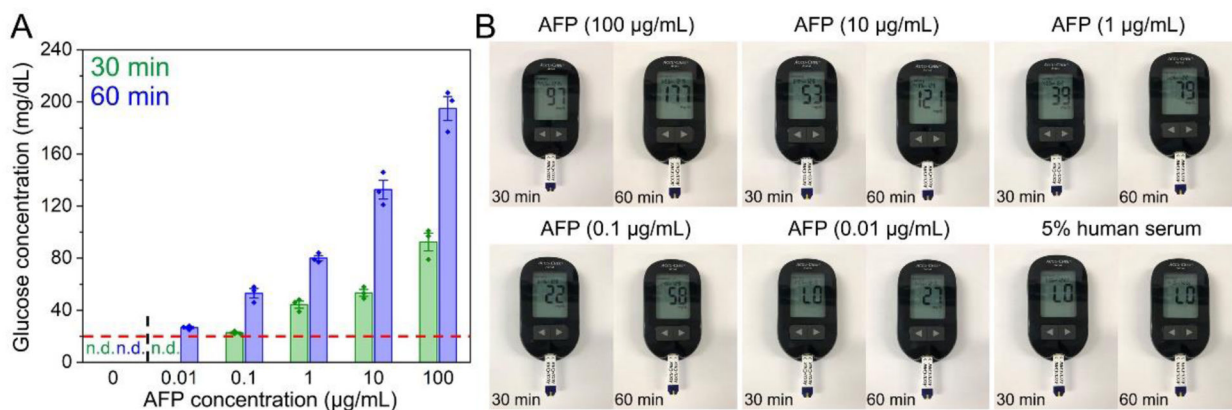


Figure 3. Relationship between the PGM readout and the serially diluted AFP biomarker in a 5% human serum solution. (A) Glucose concentration was measured by a PGM after a 30-min (green) or 60-min (blue) glucose-producing reaction. The time for AFP incubation and the CRISPR-Cas12a cleavage reaction was 15 min and 60 min, respectively. n.d. indicates “not detected.” Error bars represent $\pm$ standard deviation (s.d.) of three independent experiments. Red dashed line indicates the limit of detection of glucose concentration measured by the PGM (20 mg/dL). (B) Representative test results of serially diluted AFP using the CRISPR-powered PGM biosensing platform.

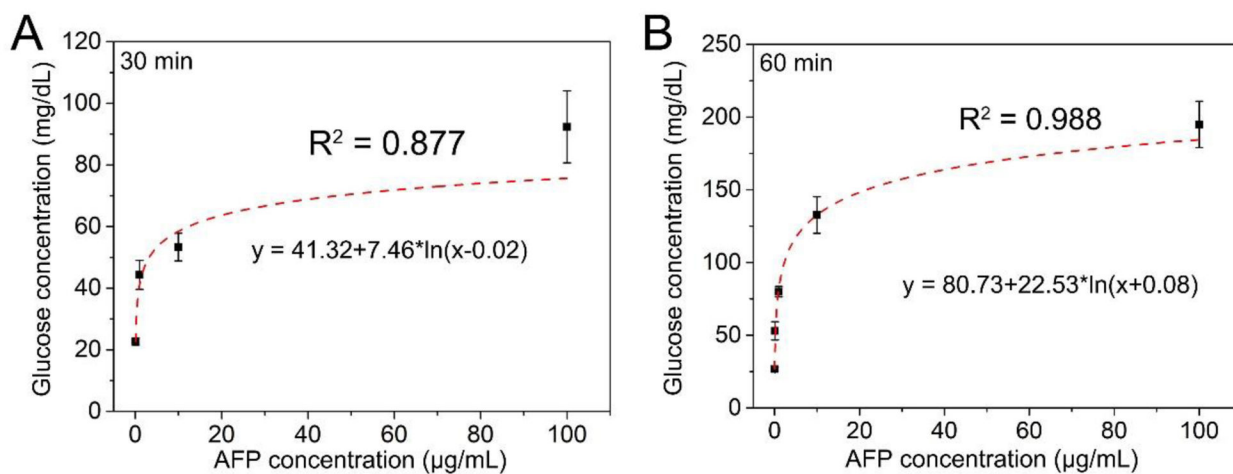


Figure 4. Logarithmic fitting of the measured glucose concentration vs. AFP concentration in 5% human serum solution after a (A) 30-min or (B) 60-min glucose-producing reaction. Error bars represent $\pm$ s.d. of three independent experiments.

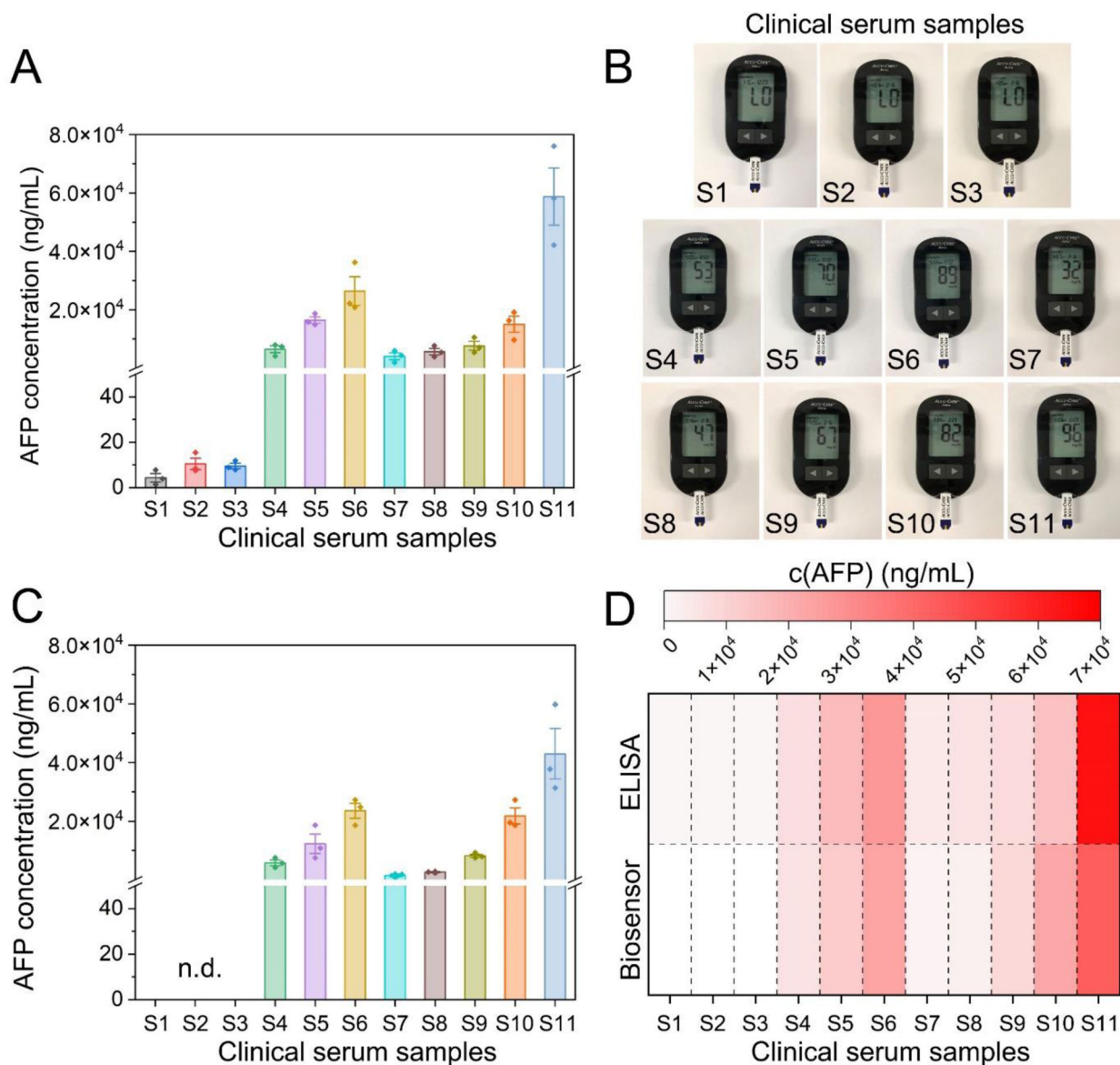


Figure 5. Quantitative detection of AFP biomarker in serum samples from patients with liver cancer (S4-S11) and healthy individuals (S1-S3) using the CRISPR-powered PGM biosensor. (A) Quantification of AFP concentration in the serum samples of patients with liver cancer and healthy individuals by using the ELISA method. Error bars represent $\pm$ s.d. of three independent experiments. (B) Representative AFP test results of the clinical serum samples from eight patients with liver cancer and three healthy individuals by using the biosensor. (C) AFP biomarker was measured and quantified by the biosensor after a 60-min glucose-producing reaction. AFP concentration in each clinical sample was calculated according to the logarithmic relationship between $c(\text{glucose})$ and $c(\text{AFP})$ (Figure 4B). n.d. indicates “not detected.” Error bars represent $\pm$ s.d. of three independent experiments. (D) Heat map showing the AFP concentration in clinical serum samples determined by ELISA and the

CRISPR-powered PGM biosensor. The presented concentrations are average values from three independent measurements.

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