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ARTICLE

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Split trehalase as a versatile reporter for a wide range of biological analytes

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Abstract

In health care, biosensors are envisioned as universal diagnostic devices with AAAA characteristics (i.e., available for anything, anywhere, anytime, to anyone). Despite numerous attempts to develop such a diagnostic device, none have managed to fulfill all four criteria and be commercialized. Glucometers, the most successful class of biosensor currently marketed monitor blood glucose concentrations. Their performance in clinical samples, including sensitivity and specificity, has been optimized and they are small and relatively inexpensive. We aimed to develop a technology that uses this existing biosensor, but adds versatility in detection of a wide range of analytes. Herein, we report the periplasmic trehalase of *E. coli* as a novel split enzyme reporter capable of converting a wide variety of analytes into glucose. Conditional complementation of trehalase fragments induced by detection of analytes, resulting in trehalose hydrolysis and glucose production, was used to detect antibodies and bacterial cells. We also demonstrated retention of split TreA activity in undiluted clinical samples. In conclusion, a trehalase-based biosensor platform offers a versatile and convenient method for point-of-care applications as it does not require sample preparation or handling and can be integrated with existing glucometers or sensors.

KEYWORDS
antibodies, bacteria, biosensor, glucose detection, reporter, split enzyme, trehalase

1 | INTRODUCTION

Although biosensor technologies are omnipresent in our daily lives, the long-standing promise to develop a technology that relays meaningful health-related signals to the user and has an integral role in health care has never been achieved. Many biosensor devices have been developed (optical, electrical, electrochemical, and mass-based); however, few have made it into clinical practice or into our homes. The main reasons are their poor performance with clinical samples, insufficient sensitivity or/and specificity, expensive supporting equipment, or high cost of production (Sin, Mach, Wong, & Liao, 2014). Regardless, the market is expected to adopt an Anything-Anywhere-Anytime-Anyone (AAAA) biosensor if it truly met those criteria.

To fulfill the "Anyone" requirement, the biosensor needs to be extremely user-friendly (even for unskilled users) which requires non-invasive sampling (e.g., pin-prick of blood, tears, or saliva) with minimal sample handling. Moreover, it needs to be quantitative and compatible with emerging telehealth-based health care. For the "Anywhere" requirement, it must be independent from expensive equipment, be robust, and portable. To address the "Anytime" requirement, it needs to be accessible, inexpensive and fast, whereas for the "Anything" requirement, it needs to detect various analytes in a sensitive and specific manner. However, most biosensors are optimized for a specific analyte or group of analytes, which restricts their applicability and prevents broad implementation.

So far, the most successful class of biosensor currently marketed, meeting only the first three "A requirements," is the glucometer (measures blood glucose concentrations). This device is composed of a glucose-specific

oxido-reductase (glucose oxidase or dehydrogenase) and an electrochemical transducer that converts enzyme activity into an electrochemical signal (Montagnana, Caputo, Giavarina, & Lippi, 2009). However, a technology that added versatility (i.e., "Anything") to the glucometer by increasing the type of analytes detected would have important advantages.

The solution could come from a mid-twentieth century discovery that fragments of ribonuclease and beta-galactosidase can re-assemble into functional complexes (similar to protein complementation) (Richards, 1958; Ullmann, Jacob, & Monod, 1967). More recently, it was reported that fragments of a protein could be conditionally re-assembled when attached to interacting protein pairs (Johnsson & Varshavsky, 1994), offering a direct method to convert the interaction of two proteins to activity of the protein.

For successful creation of a split-reporter protein, several criteria must be met. Each fragment by itself should not exhibit activity, the affinity of the fragments in the absence of fused interacting proteins should be negligible, and the re-assembled split-protein must provide an easily measurable output. Despite their inherent simplicity, identification of potential split reporter proteins, and their appropriate dissection sites is limited. Split reporter proteins currently available include luciferase (Paulmurugan & Gambhir, 2003; Remy & Michnick, 2006), fluorescent proteins (Fan et al., 2008; Hu & Kerppola, 2003; Tchekanda, Sivanesan, & Michnick, 2014), beta-lactamase (Galarneau, Primeau, Trudeau, & Michnick, 2002), protease (Wehr et al., 2006), and others (Aranko, Wlodawer, & Iwai, 2014; Camacho-Soto, Castillo-Montoya, Tye, & Ghosh, 2014; Chelur & Chalfie, 2007; Dochow, Krumm, Crowe, Moore, & Plemper, 2012; Engelen & Merkx, 2017; Freudenthal, Gakhar, Ramaswamy, & Washington, 2010; Griss et al., 2014; Guo et al., 2016; Mabe, Nagamune, & Kawahara, 2014; Massoud, Paulmurugan, & Gambhir, 2010; Merkx, Golynskiy, Lindenburg, & Vinkenborg, 2013; Muller, Kries, Csuhai, Kast, & Hilvert, 2010; Nirantar, Li, Siau, & Ghadessy, 2014; Pelletier, Campbell-Valois, & Michnick, 1998; Scarabelli et al., 2017; Slaska-Kiss, Timar, & Kiss, 2012; Tafelmeyer, Johnsson, & Johnsson, 2004; Yu, Griss, Schena, & Johnsson, 2017). Notwithstanding, none has fulfilled AAAA criteria when used in diagnostic testing, due to aforementioned reasons.

Herein, we describe a novel, versatile analyte detection platform based on the protein complementation principle designed to overcome many of the limitations identified in existing split reporters. The core of this platform is a glycolytic enzyme trehalase (TreA), present in the periplasmic space of *E. coli*, that catalyzes hydrolysis of trehalose into two glucose molecules that could be measured with a commercial glucometer. In this study, we characterize, for the first time, TreA as a split reporter enzyme and provide evidence that this platform can detect a wide variety of analytes, including antibodies, bacteria, protein-protein interactions, and protein aggregation.

2 | MATERIALS AND METHODS

2.1 | Materials

Monoclonal antibodies (anti-HIS-tag mAb and anti-HA-tag mAb; 1 mg/ml) were purchased from MBL Corporation (Woburn, MA), whereas rabbit polyclonal anti-HIV p24 polyclonal serum was purchased from Abcam (Toronto, Canada) (ab63913), and bovine IgG (12.8 mg/ml) was purchased from Sigma-Aldrich (Oakville, Canada).

2.2 | Bacterial strains

All plasmid manipulations and amplifications were performed in *E. coli* strain DH5α (New England Biolabs, Whitby, Canada) whereas protein expression was done in a *TreA* knock-out of *E. coli* strain BL-21 (DE3) (New England Biolabs), named BL-21ΔTreA, constructed by targeted chromosomal gene knockout system using red recombinase (Murphy, 2011). The entire TreA gene was replaced with Tn5 (*aph*) type II (kanamycin resistance) using the following primers: FOR: 5′-TATGGACAGCAAGCGAACCG-3′; and REV: 5′-TCAGAAGAACTCGTCAAGAAG-3′). *Staphylococcus aureus* and *Streptococcus epidermidis* isolates were provided by the Canadian Bovine Mastitis Milk Quality Research Network (CBMQRN). All strains were grown in LB broth at 37 °C.

2.3 | Plasmids

The gene for periplasmic Trehalase (*TreA*) was amplified from *E. coli* strain BL-21. During PCR amplification, the secretion peptide was replaced with a 6HIS-tag, and *NcoI* and *AvrII* restriction sites were introduced at 5′ and 3′ ends of the gene, respectively, to clone the amplified fragment in pETDuet vector (Novagen). Furthermore, TreA N (66 aa long) and C terminal (456 aa long) fragments tagged with the 6HIS or HA tag at the N- or C-terminals were generated using the same strategy. Primer sequences used for generation of all constructs are shown (Table 1).

Coding sequences for HIV p24 (AC: KJ925006.1) (Ganser-Pornillos, von Schwedler, Stray, Aiken, & Sundquist, 2004) and bankvole PrP (residues 23–230; Accession No. AF367624) were synthetized by Geneart (Thermo Fisher Scientific, Ottawa, Canada). The coding sequence for *S. aureus* binding peptide SA5-1 (VPHNPGLISLQG) (Rao, Mohan, Gao, & Atreya, 2013), the coiled-coil peptides (EI: EIAALEKEIAALEKENAALEWEIAALEK; KI: KIAALKEKIAALKEKNAALKWKIAALKE) (Aronsson et al., 2015) and Protein G (residues: 270–324; AC: P19909 TYKLILNGKTLKGETTTEAVDAATAEKVFKQYANDNG VDGEWTYDAATKTFTVTE) (Wilton, Tunnicliffe, Kamatari, Akasaka, & Williamson, 2008) were incorporated as oligonucleotide linkers. All coding sequences introduced at the N-terminus of fragments were cloned between NcoI and SalI restriction sites, whereas coding sequences introduced at C-terminus were cloned between BamHI and AvrII.

2.4 | Protein expression, HIS-tag purification, and buffer exchange

Proteins were expressed in BL-21ΔTreA by induction with 0.5 mM of isopropyl-β-D-thiogalactoside (IPTG) (UBP Bio, Aurora, CO) for 3 hr at 37 °C. Bacterial lysates were prepared by harvesting 10 ml of induced bacterial colonies at 3,000×*g* at 4°C for 10 min. Pellets were washed with PBS, re-suspended in 800 µl of 6M guanidinium buffer (6M Guanidinium-HCl; 25 mM Imidazole; PBS 1×) and sonicated using five

TABLE 1 Primers used for cloning

	Primer	Sequence
pETDuet-HIS-TreA	6HIS-TreAFatg-F	tataccatggcacaccatcaccatcaccatgaagaaacaccggtaacaccaca
	TreARtaa-R	tatacctaggttaaggtgtgggttgtgcctct
pETDuet-HIS-N-ter	6HIS-TreAFatg-F	tataccatggcacaccatcaccatcaccatgaagaaacaccggtaacaccaca
	TreA-BamHI-N-R	taattcctaggtcaggatcccggaacatatttctcgccttc
pETDuet-N-ter-HIS	TreAFatg-F	tataccatggaagaaacaccggtaacacca
	TreA-TruncN-R	taattcctaggtcaatggtgatggtgatggtgcggaacatatttctcgccttc
pETDuet-HIS-C-ter	TreA-TruncC-F	taattccatggcacaccatcaccatcaccataatttcaccctgccgaaag
	TreARtaa-R	tatacctaggttaaggtgtgggttgtgcctct
pETDuet-C-ter-HIS	TreA-SalI-C-F	taattccatggcagtcgacaatttcaccctgccgaaag
	TreA-6HIS-R	tatacctaggttaatggtgatggtgatggtgaggtgtgggttgtgcctct
pETDuet-HA-N-ter	HA-TreA-N-F	tataccatggcatacccatacgatgttccagattacgctgtcgacgaagaaacaccggtaaca
	TreA-TruncN-R	taattcctaggtcaatggtgatggtgatggtgcggaacatatttctcgccttc
pETDuet-N-ter-HA	6HIS-TreAFatg-F	tataccatggcacaccatcaccatcaccatgaagaaacaccggtaacaccaca
	TreA-N-HA-R	tatacctaggtcaagcgtaatctggaacatcgtatgggtaggatcccggaacatatttctcgcc
pETDuet-HA-C-ter	HA-TreA-C-F	tataccatggcatacccatacgatgttccagattacgctgtcgacaatttcaccctgccgaaa
	TreA-6HIS-R	tatacctaggttaatggtgatggtgatggtgaggtgtgggttgtgcctct
pETDuet-C-ter-HA	TreA-TruncC-F	taattccatggcacaccatcaccatcaccataatttcaccctgccgaaag
	TreA-C-HA-R	tatacctaggtcaagcgtaatctggaacatcgtatgggtaggatccaggtgtgggttgtgcctc

5-s bursts (total of 30 s). Protein fragments were purified on Ni-NTA columns (Fisher Thermo Scientific) according to the manufacturer's instructions. Briefly, proteins were bound on columns of equilibrated Ni-NTA resin in the presence of 6 M guanidinium buffer (6 M guanidinium-HCl; 25 mM imidazole; PBS 1×), refolded on column during washing steps with Wash buffer (25 mM imidazole; PBS 1×) containing gradually decreasing guanidinium-HCl concentrations (6, 4, 3, 2, and 0 M, respectively) and eluted in Elution buffer (250 mM imidazole; PBS 1×).

Subsequently, samples were dialyzed against 1 L of sodium maleate buffer (50 mM, pH 6) or PBS (pH 7) with Snakeskin (Fisher Thermo Scientific) 7 kDa MWKO for 24 hr at 25° C to remove imidazole.

Bacterial lysates and purified proteins were separated on 10% SDS–page (Bis-Tris Acrylamide gel) and stained with Bio-Safe Coomassie blue G-250 (Bio-Rad, Mississauga, Canada).

2.5 | Complementation assays

Protein concentration was determined with a Qubit protein assay kit (Thermo Fisher Scientific). Antibody complementation assays, Leucine zipper assays and Ni-NTA complementation assays were performed in sodium maleate buffer (50 mM, pH 6). Antibodies were detected in 1 to 1 molar ratios with reagents. Bacterial complementation assays were performed in PBS (pH 7). For this, 0.2 ml of bacterial culture grown overnight was pelleted (OD_{600nm} = 1.2), washed three times with PBS, re-suspended in 10 µl of PBS (pH 7) and added to protein fragments.

For protein aggregation assays, rPrP fusions (rPrP-$TreA^N$ and rPrP-$TreA^C$) were purified separately on Ni-NTA columns under denaturing conditions, then mixed (1 to 1 ratio) and co-dialyzed against 1 L of 10 mM sodium phosphate (pH 5.8) with Snakeskin (Fisher Thermo Scientific) and 7 kDa MWKO for 24 hr at 25 °C to remove the denaturing agent. After dialysis, samples were centrifuged (10,000×*g* for 10 min) to separate precipitated from soluble proteins (including soluble dimers (Meier et al., 2003)). Supernatant was discarded and the pellet washed with PBS and re-suspended in 0.25 M trehalose.

All assays were performed with 5 µg of the $TreA^C$ fragment, whereas the concentration of $TreA^N$ fragment was adjusted for every assay (to maintain a 1 to 1 molar ratio). Assays were performed in 60 µl of final volume. Assays were incubated with 0.25 M solution of trehalose (Sigma) at 25 °C for at pH 6 for 1 hr or O/N at pH 7, as specified for each experiment.

Glucose concentrations were measured with glucometer strips (Aviva Accu-chek, Roche, Mississauga, Canada), a Benedict's reagent (Sigma) assay, or with a colorimetric enzymatic assay using glucose oxidase (0.26 U/ml; Sigma), horseradish peroxidase (0.2 U/ml; Sigma), and o-Dianisidine (0.5 mM; Sigma) in sodium maleate buffer (50 mM, pH 6). Absorbance (OD) was measured after 30 min (Ni-NTA, anti-HIS, and anti-HA), after 1 hr for Anti-HIV antibody assay, or 10 min (all other assays) of incubation at 450 nm of wavelength using a spectrophotometer (EnSpire, PerkinElmer, Waltham, MA) and Benedict's reagent assay (Sigma).

2.6 | Lyophilization of proteins

Proteins were mixed in 1 to 1 weight ratio with BSA and frozen at −80 °C for 30 min and then lyophilized O/N at −85 °C and 200 mTorr. Proteins were re-suspended in acidified sample (blood or milk). Samples were acidified by addition of 10 mg of citric acid, 61 mg of sodium citrate, and 0.189 g of trehalose.

3 | RESULTS

3.1 | Biosensor design

First, TreA (lacking a leader sequence) was split into two fragments: 66 aa N-terminal ($TreA^N$) and 456 aa C-terminal ($TreA^C$). The fragmentation point of TreA was placed into a 12 aa long region that was unresolved in a published crystal structure (PDB: 2JF4). No additional linkers were introduced into the sequence between the reporter and sensors. $TreA^N$ and $TreA^C$ were expressed and purified separately. Neither fragment had any detectable enzymatic activity during 24 hr of incubation with substrate, nor was there evidence of self-assembly (resulting in trehalase activity) when incubated together (Supplementary Figure S1A).

Conditional reassembly of fragments was tested by fusing HIS-tags (6HIS) to $TreA^N$ and $TreA^C$ and immobilizating them separately or together on Ni-NTA columns (Figure 1A). Co-immobilization of fragments on resin beads was sufficient to induce complementation, regardless of the N or C terminal position of the 6HIS-tag on either fragment. Trehalose was only hydrolyzed when $TreA^N$ and $TreA^C$ fragments were bound together on a column, whereas no glucose was detected when two fragments were separately bound on a column (Figures 1B and 1C). Elution of co-immobilized TreA fragments from the column abolished trehalase activity in the elution, confirming transient complementation (Supplementary Figure S1B).

In accordance with optimal pH of TreA (Gibson et al., 2007), the Ni-NTA resin complementation assay reacted faster at pH 6 then at pH 7, producing comparable amounts of glucose (output signal) in 1 hr at pH 6 and 18 hr at pH 7 (Figure 1D). Therefore, analyte detection assays were performed at pH 6 (unless the sensor that was incorporated into a detection assay required a neutral pH for proper function).

3.2 | Antibody detection

Antibody-dependent complementation of split TreA was demonstrated with anti-HIS and anti-HA monoclonal antibodies and anti-HIV (anti-p24) polyclonal serum (Figure 2A).

Incubation of 6HIS tagged $TreA^N$ (1.5 μM) and $TreA^C$ (1.5 μM) fragments with anti-HIS mAb (1.5 μM) induced complementation and subsequent trehalase activity, measured as glucose production (Figure 2C). The location of the 6HIS-tag antigen on either N or C-terminus only had minor impact on trehalase activity.

The TreA complementation occurred only when $TreA^N$ (1.5 μM) and $TreA^C$ (1.5 μM) fragments carrying the HA-tag (Anti HA specific TreA detection assay) were incubated with anti-HA mAb (1.5 μM) (Figures 2B and 2E). The time response of the anti-HA specific TreA detection assay was followed for 1 hr. The assay was incubated with or without Anti-HA, and glucose concentrations were measured every 5 min and the increase of glucose concentration was detected after 25 min (Supplementary Figure S2B).

Next, experimental sensitivity of antibody-mediated complementation was examined. Complementation of HA tagged TreA fragments was induced with decreasing concentrations of anti-HA mAb. Output glucose signal decreased proportionally with the analyte, with complementation detected even when the concentration of anti-HA was decreased five folds (Supplementary Figure S2A).

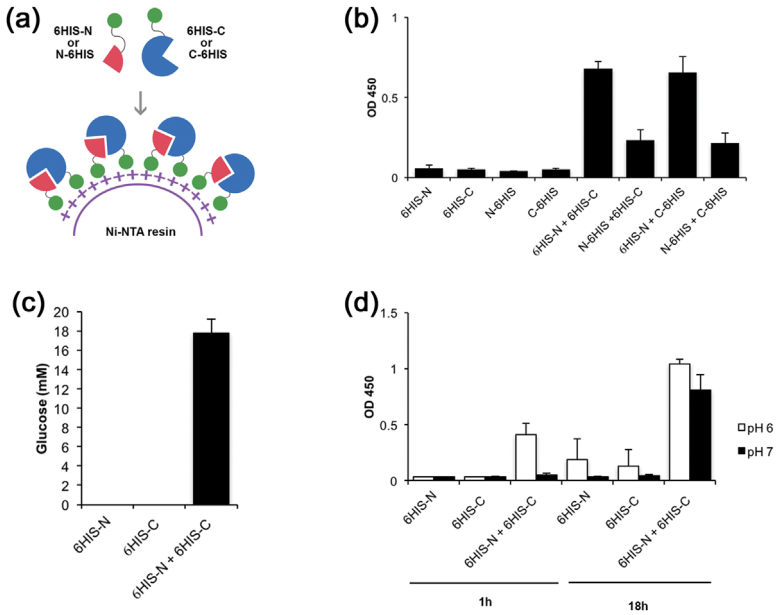

FIGURE 1 Complementation of TreA fragments by co-immobilization on Ni-NTA resin. (a) Schematic representation of the complementation of split TreA on Ni-NTA resin with the N-terminal fragment $TreA^N$ (red), C-terminal $TreA^C$ (blue), 6HIS tag (green), and the Ni-NTA resin (violet). (b) Recombinant $TreA^C$ and $TreA^N$ fragments, 6HIS-tagged at N, or C terminus, were immobilized on Ni-NTA resin, either separately or together ($n = 3$). Assay was incubated for 1 hr at pH 6. Trehalase activity and the resulting glucose concentration were measured by GOx-HRP assay after 30 min (c) or by accu chek glucose strips. (d) 6HIS tagged $TreA^C$ and $TreA^N$ fragments were immobilized on Ni-NTA resin, either separately or together at pH 6 or at pH 7. Trehalase activity was measured by GOx-HRP assay after 5 min ($n = 3$)

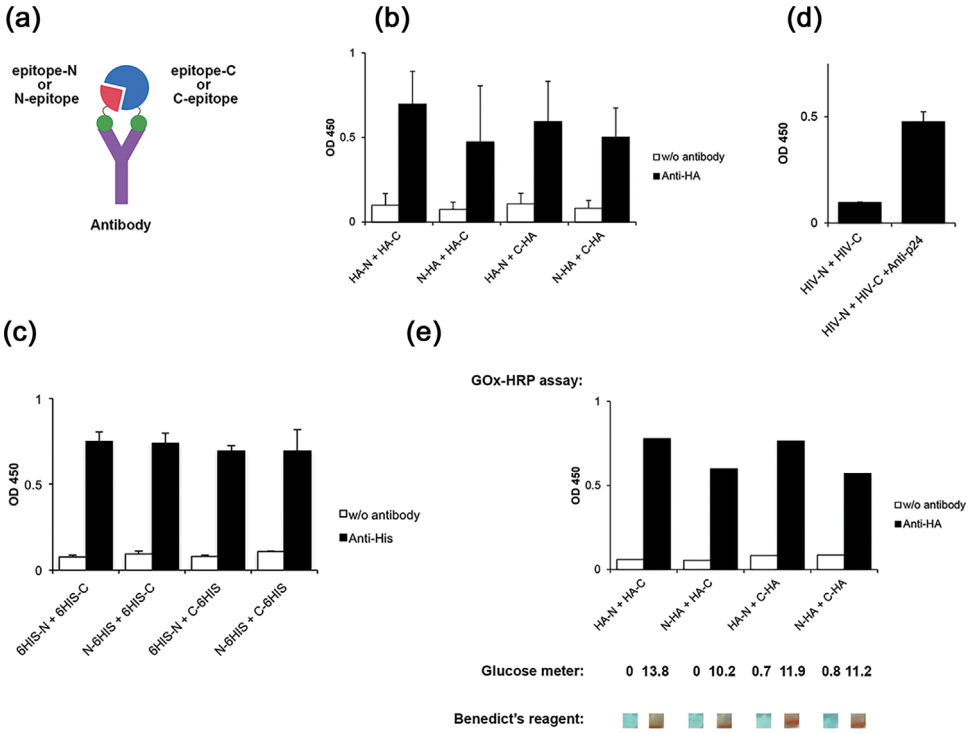

FIGURE 2 Detection of monoclonal and polyclonal antibodies with specific antigens fused to split TreA. (a) Schematic representation of the complementation of split TreA induced by antibodies with the N-terminal fragment $TreA^N$ (red), C-terminal $TreA^C$ (blue), peptide antigens (green) used in this study (HA, 6HIS, HIV (p24)) and the cognate antibodies (violet). (b) Trehalase activity after incubation of HA-tagged $TreA^C$ (1.5 μM) and $TreA^N$ (1.5 μM) fragments with or without anti-HA monoclonal antibodies (1.5 μM). (c) Trehalase activity after incubation of 6HIS tagged $TreA^C$ (1.5 μM) and $TreA^N$ (1.5 μM) fragments with or without anti-HIS monoclonal antibodies (1.5 μM) (Anti-6HIS assay). (d) Trehalase activity after incubation of $TreA^C$ and $TreA^N$ fragments fused to HIV capsid protein p24 (HIV) (1 μM of each) with or without anti-HIV (p24) rabbit antiserum (5 μl) (Anti-HIV assay). (e) Comparison of three measuring methods (GOx-HRP assay, glucometer, and benedict's reagent) for glucose detection. Assays were incubated for 1 hr at pH 6. Each assay was repeated three times. Glucose concentration was measured by GOx-HRP assay after 30 min for the HIS and HA assays and after 1 hr for the anti-HIV assay

Specificity of antibody-mediated complementation was tested by incubating 6HIS-tagged fusions of $TreA^N$ (1.5 μM) and $TreA^C$ (1.5 μM) with non-cognate anti-HA mAbs (1.5 μM) next to cognate anti-HIS mAbs (1.5 μM). Anti-HA mAb were unable to complement 6HIS-tagged TreA fragments, whereas anti-HIS mAb resulted in glucose production (Supplementary Figure S2C).

A complete antigenic protein, non-assembling mutant of HIV capsid protein p24 (Ganser-Pornillos et al., 2004), was fused to both $TreA^N$ and $TreA^C$ to investigate complementation by antibodies in a non-purified rabbit polyclonal hyper-immune antiserum. Fusion proteins (1 μM of each) were incubated with 5 μl of serum and increased glucose concentrations were detected only in the presence of anti-p24 serum (Figure 2D). Background glucose present in the non purified polyclonal antiserum was measured in parallel in absence of bioreagents and used as threshold value.

3.3 | Detection of antibodies through interactions with their variable and constant regions

Fusions of TreA were engineered to recognize and detect various regions of antibodies (Figure 3A). Protein G (pG) (Wilton et al., 2008), a virulence factor expressed in streptococcal bacteria that binds to constant regions (Fc) of IgGs, was introduced as a sensor into TreA fusions. Combinations of TreA fragment fusions (1.4 μM per fusion) carrying protein G and HA tag (variable-constant region binding assay [VC assay]), Protein G only (constant-constant region binding assay [CC assay]), or HA tags only (variable-variable region binding assay [VV assay]) were used to detect anti-HA mAb in antigen-specific or antigen non-specific manners (Figure 3B). Fusions of TreA carrying only protein G were inefficient in detecting anti-HA mAb raised in mouse cells, although when bovine IgG were used as an analyte, dimerization was more efficient (Figure 3C), indicating a species difference in capacity of IgG to bind two molecules of protein G.

Furthermore, experimental sensitivity of the CC assay was investigated. TreA fragments carrying protein G were used to detect decreasing concentrations of bovine IgG. The resulting output signal decreased in proportional manner compared to the concentration of the analyte (Supplementary Figure S3A). Time response of this assay was measured as well. As described for Anti-HA assay, glucose concentrations were measured every 5 min for 1 hr and the increase of glucose concentration was detectable after 25 min (Supplementary Figure S3B).

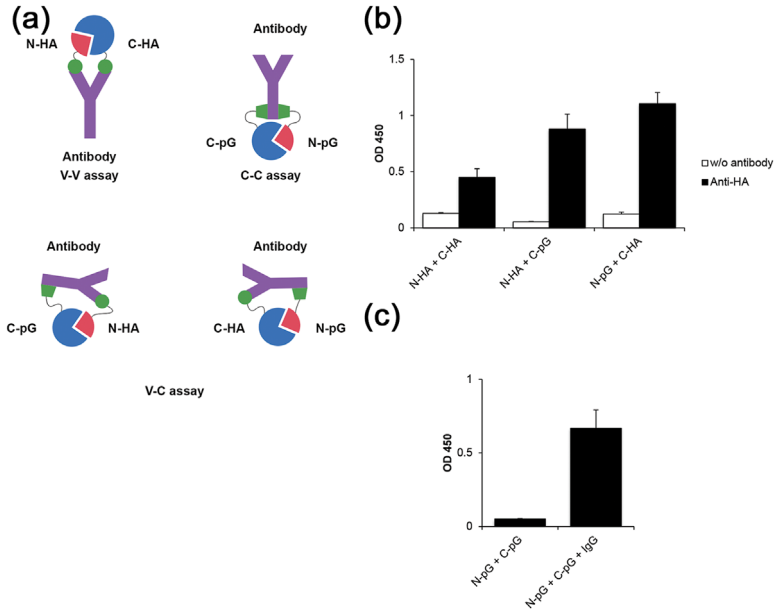

FIGURE 3 Detection of antibodies by binding the variable and constant regions with combinations of antigens and protein G fused to split TreA. (a) Schematic representation of the complementation of split TreA induced throughout interaction with different antibody regions (variable [antigen binding] and constant region) with the N-terminal fragment $TreA^N$ (red), C-terminal $TreA^C$ (blue), antigen (green), protein G (pG) (green), and the analyte (violet). (b) Trehalase activity after incubation of $TreA^N$ (1.4 μM) and $TreA^C$ (1.4 μM) fragments either both fused to HA-tag or one fused to Protein G and the other to HA-tag in the presence of cognate monoclonal antibodies ($n = 3$). (c) Trehalase activity after incubation of $TreA^N$ and $TreA^C$ fragments both fused to Protein G (1.4 μM of each) in the presence bovine IgGs. Assays were incubated for 1 hr at pH 6 ($n = 3$). Glucose concentration was detected by GOx-HRP assay after 10 min

3.4 | Direct whole-pathogen detection

Split TreA was applied to detect intact bacterial cells by incorporating peptide aptamers (Figure 4A). Specifically, *S. aureus* surface binding peptides (Rao et al., 2013) were placed at the N or C terminals of $TreA^N$ and $TreA^C$ fragments. Resulting TreA fusions (1.5 μM per fusion) were incubated with *S. aureus* or *S. epidermidis*. Trehalase complementation and a subsequent glucose increase was only detected when TreA fragments were incubated with *S. aureus*, whereas glucose concentrations were low when fusions were incubated with *S. epidermidis* (Figure 4B). Trehalase activity was higher when the peptide aptamer was fused to the N-terminus of both TreA fragments.

3.5 | Protein-protein interaction and protein aggregation detection

The TreA detection assay was also used to detect protein-protein interactions and protein aggregation (Figure 5A). As an example of the former, complementary coiled-coil peptides with the leucine zipper motif, Ki, and Ei (Aronsson et al., 2015), were used to induce TreA

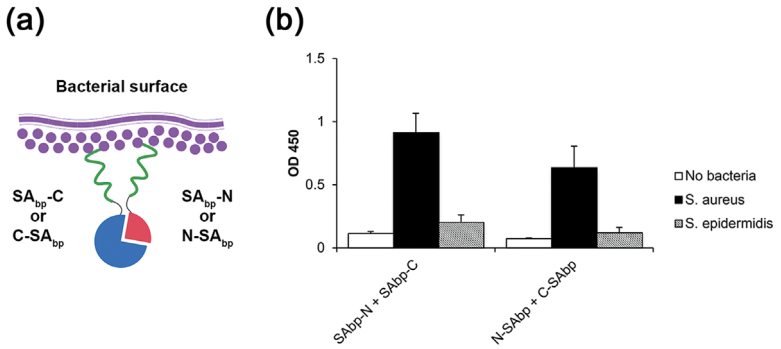

FIGURE 4 Detection of *S. aureus* whole cells with peptide aptamers fused to split TreA. (a) Schematic representation of split TreA complementation occurring on the pathogen's surface with the N-terminal fragment $TreA^N$ (red), C-terminal $TreA^C$ (blue), sensors (green), analyte (violet). (b) Trehalase activity after incubation of $TreA^C$ and $TreA^N$ fragments fused with *S. aureus* specific binding peptides (SAbp) (1.5 μM per fusion) in the presence of *S. aureus*, *S. epidermidis*, or in the absence of bacteria ($n = 3$). Assays were incubated O/N at pH 7. Glucose concentration was detected by GOx-HRP assay after 5 min. Samples containing only bacterial cell did not produce any signal (data not shown)

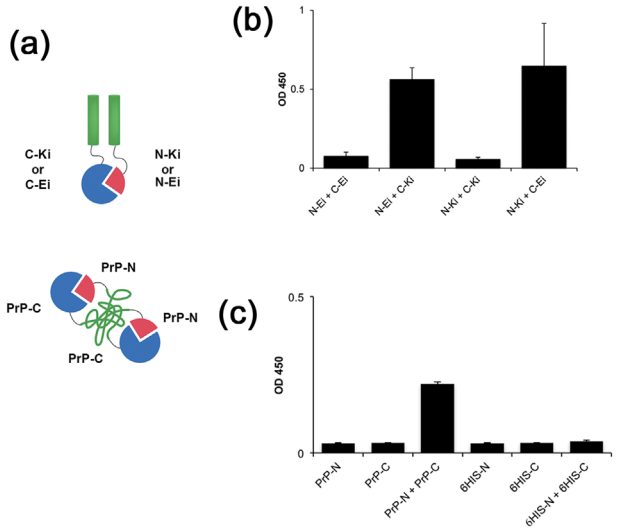

FIGURE 5 Detection of protein-protein interaction and protein aggregation by split TreA. (a) Schematic representation of split TreA complementation occurring upon protein-protein interaction and protein aggregation with the N-terminal fragment $TreA^N$ (red), C-terminal $TreA^C$ (blue), leucine zippers, or recombinant PrP (green), (b) Trehalase activity after co-incubation of $TreA^C$ and $TreA^N$ fragments fused to complementary leucine zippers (Ei; Ki) (1.5 μM per fusion) ($n = 3$). Assay was incubated for 1 hr at pH 6. (c) Trehalase activity after aggregation of $TreA^C$ and $TreA^N$ fragments fused to PrP induced by co-dialysis ($n = 3$). Glucose concentration was detected by GOx-HRP assay after 10 min

complementation. $TreA^N$ (1.5 μM) and $TreA^C$ (1.5 μM) with complementary heterodimzerizing peptides KI and EI or non-complementary zippers (Ei + Ei and Ki + Ki) were assayed for TreA complementation. Only combinations with complementary leucine zippers (Ki + Ei) led to increase in glucose concentrations after 25 min, whereas other combinations did not (Figure 5B and Supplementary Figure S4).

Leucine zipper TreA fusions were used as model to investigate sample matrix effects on TreA complementation assays and to explore the possibility of the bio-reagents to withstand lyophilization and subsequent resolubization (to enable long-term shelf life). The $TreA^N$-Ei and $TreA^C$-Ki fusions were purified and lyophilized separately in the presence of substrate. Next, fusions were re-suspended in whole bovine blood or bovine milk (acidified to pH 6 by addition of citric acid/sodium citrate powder). Glucose concentrations were measured over 3 hr; the glucose signal doubled in samples containing complementary fusions, demonstrating that neither lyophilization nor the presence of blood or milk inhibited TreA complementation (Supplementary Figure S5A and S5B).

Furthermore, $TreA^N$ and $TreA^C$ were fused N-terminally to recombinant bank vole prion protein ($rPrP^C$), which forms aggregates spontaneously under specific conditions. The rPrP-dependent aggregation was induced by co-dialysis of $TreA^N$ and $TreA^C$ fusions, and protein aggregates were isolated from soluble fusion proteins and incubated with substrate. Glucose production was detected only when the $TreA^N$ and $TreA^C$ rPrP fusions were co-aggregated, but not when they aggregated separately (Figure 5C). Furthermore, 6HIS-tagged TreA fusions showed no evidence of aggregation under the same conditions.

4 | DISCUSSION

In this study, we engineered a novel, versatile biosensor platform, and demonstrated that this platform can be used to detect various types of molecules, for example, antibodies, whole pathogens, and inter-molecule interactions (protein-protein binding and protein aggregation). The platform is based on split enzyme complementation of an *E. coli* glycolytic enzyme trehalase (TreA), that hydrolyses trehalose into two molecules of glucose, which can be easily detected with a commercially available glucometer (Montagnana et al., 2009). Glucose detection is compatible with clinical samples and does not need additional handling or processing. In contrast with many other approaches (e.g., luminescence or fluorescence from GFP), a glucose signal is more compatible with existing detectors and with detection in biofluids from humans or animals. Moreover, the platform can be easily adapted for different applications by incorporating a sensor with specificity for another analyte of interest without redesigning the whole detection system.

We developed a novel and convenient approach for initial testing of novel split enzyme reporters by fusing polyhistidine tags (6HIS) to the TreA fragment termini and immobilizing them on a continuous binding surface of Ni-NTA resin beads. This platform efficiently detected monoclonal antibodies as well as polyclonal antibodies present in non-purified serum by fusing either peptide epitopes or whole-protein antigens to the split enzyme fragments. A positive signal (increased glucose concentrations) was detected in <30 min at room temperature with the GOx-HRP assay and with glucose strips, only when the bioreagents were interacting with cognate antibody. Successful conversion of the presence of antigen-specific antibody into a glucose signal (measured with a conventional glucometer) enables this biosensor platform to monitor host humoral immune responses (e.g., in response to an infection).

By incorporating immunoglobulin binding proteins that recognize the constant regions of immunoglobulins, the TreA platform detects total immunoglobulin concentrations. In addition, immunoglobulin binding protein and antigen fusions were combined to detect antigen-specific antibodies in a manner that required only one TreA fragment to be modified to result in a new test, simplifying antigen screening.

Next, we adopted this platform to detect whole bacterial cells, protein-protein interactions, and protein aggregation, simply by replacing the sensor component with protein elements specific to the analyte of interest. Fusions of TreA fragments with small peptide aptamers specific to components present on bacterial surface allowed us to detect bacterial cells (e.g., *S. aureus*). Heterodimerizing leucine zippers and PrP were used as models to demonstrate that this platform can be applied to monitor protein-protein interactions or protein aggregation, respectively.

In this study, we implemented previously described dimerization and complexation strategies to complement a split enzyme to monitor various analytes and interactions in parallel. We also described complementation strategies never before combined with split reporter enzymes, for example, surface binding and protein aggregation (PrP). In addition, heterodimerizing peptides were used to investigate effects of clinical sample composition. It was noteworthy that that TreA complementation was not severely impacted by the composition of real life/clinical samples,

in contrast to some other reporters (e.g., GFP). Further characterization and optimization of each biosensor assay is still needed.

A potential drawback of this reporter is the background signal given by free glucose normally present in blood and tissues, or potential trehalase activity in vertebrates. Fortunately, initial background glucose concentrations can be measured in advance or in parallel and subtracted from total glucose concentrations to determine de novo glucose production. In the presented experiment where anti-P24 antibodies were detected in non purified polyclonal HIV antiserum, the antiserum was tested separately and the detected glucose levels were used as the threshold. Trehalase activity is non-existent or extremely low in most biological samples from vertebrates (Arola et al., 1999 Yoshida, Mizukawa, & Haruki, 1993) as the only enzymes with trehalose hydrolyzing activities known to be present in vertebrates are restricted to the microvillus intestinal mucosa and renal brush-border membranes (Arguelles, 2014).

Currently, purification of the recombinant fusions presents another drawback, as it requires a refolding step in order to obtain functional proteins. This presents a limit on the size and type of the fusions that could be engineered in the future (e.g., fusions with disulphide bond might need specifically tailored refolding conditions). To some extent, this complication could be overcome by directly obtaining soluble proteins by for example, co-expressing them with chaperones (e.g., Chaperone Plasmid Set, Takara). Alternatively, different expression systems, such as insect or mammalian cells could be used to produce the sensor proteins. Whereas bacterial expression system have the advantage of yielding high amounts of proteins in a simple and cost-efficient way, more complex expression systems would allow for efficient expression of sensors that require post-translational modifications for proper function.

Current sensitivity of the assay requires up to 1 hr of incubation for a positive read-out (sufficient increase in glucose concentration). Nevertheless, sensitivity will be increased significantly with next-generation glucose meters (currently under development) able to detect micro-molar glucose concentrations (Taguchi, Ptitsyn, McLamore, & Claussen, 2014). By lowering the glucose concentrations required for detection, the quantity of the analyte necessary for signal detection by the biosensor will be lower as well, resulting in an increase of sensitivity of the assay. Furthermore, the sensitivity of the assay can be improved by increasing the catalytical efficiency of the trehalase throughout random or structure-inspired mutagenesis.

In conclusion, we presented herein a novel detection platform that will facilitate development of an inexpensive, one-step POC test to be used directly in the field, compatible with a wide range of analytes, and sample types.

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CONFLICTS OF INTEREST

The authors declare that they have no conflicts of interest with the contents of this article.

AUTHORS' CONTRIBUTION

The project was conceived and designed by JDB. The experimental work and data acquisition was done by MD. Data analysis and interpretation and writing of the manuscript and preparation of figures was done by MD and JDB.

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SUPPORTING INFORMATION

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