Title: Yeast Dual-Affinity BioBricks: Progress Towards Renewable Whole-Cell Biosensors

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Abstract:

Point-of-care (POC) diagnostic biosensors offer a promising solution to improve healthcare, not only in developed parts of the world, but also in resource limited areas that lack adequate medical infrastructure and trained technicians. However, in remote and resource limited settings, cost and storage of traditional POC immunoassays often limit actual deployment. Synthetically engineered biological components ("BioBricks") provide an avenue to reduce costs and simplify assay procedures. In this article, the design and development of an ultra-low cost, whole-cell "renewable" capture reagent for use in POC diagnostic applications is described. Yeast cells were genetically modified to display both single chain variable fragment (scFv) antibodies and gold-binding peptide (GBP) on their surfaces for simple one step enrichment and surface functionalization. Electrochemical impedance spectroscopy (EIS) and fluorescent imaging were used to verify and characterize the binding of cells to gold electrodes. A complete electrochemical detection assay was then performed on screen-printed electrodes fixed with yeast displaying scFv directed to *Salmonella* outer membrane protein D (OmpD). Electrochemical assays were optimized and cross-validated with established fluorescence techniques. Nanomolar detection limits were observed for both formats.

Keywords:

Electrochemical ELISA, Yeast surface display, Point-of-care diagnostics, Single chain variable fragment antibody, Electrochemical Impedance Spectroscopy, gold binding peptides

1. Introduction

Early and accurate diagnosis is critical for effective treatment and disease surveillance. Molecular diagnostic testing [1] offers greater accuracy and sensitivity compared to classical microbiological culture techniques, but entails methods and equipment that often restrict use in developed countries. Conversely, the lack of medical infrastructure in developing countries forces reliance on syndromic detection that frequently leads to misdiagnosis and incorrect treatment. Point-of-care (POC) technology incorporating highly sensitive, yet affordable and portable, biosensing devices can provide the functionality of an advanced laboratory in all settings and may be a key to faster and better healthcare.

Currently, protein-based affinity molecules such as polyclonal or monoclonal antibodies (mAbs) [2], [3], aptamers, antigen binding antibody fragments (Fab), single chain antibodies (scFv), and single domain (camelid) antibodies [4]–[6] remain the cornerstone of modern POC immuno-diagnostics. Major barriers in developing these immunoassays include production cost, complexity in reagent production and purification, assay assembly, controlled storage condition requirements, and limited shelf life [4], [5]. Many low cost POC testing platforms are becoming available, but typical costs are still a few dollars per test (with exceptions) [5], [6]. While these are revolutionary in many settings, tests that are under $1 or even $0.10 would be even more valuable in countries with minimal disposable income for health infrastructure and care. The limiting factor in reducing cost is still the production, purification, and application of the primary affinity molecule. A promising solution to these barriers, as well as the main focus of this article, is the engineering of display yeast cells (synthetic biological constructs in the vein of “BioBricks” [7]) for efficient incorporation into POC immunoassays.

Molecular display solves multiple issues common to current affinity assays (Fig. 1). Namely, this method ensures 1) that antibodies are correctly folded and displayed at high density on the cell surface [8], 2) that these antibodies are in the optimal orientation for affinity capture [9], and 3) that the construct has excellent stability with a potential shelf-life of years without need for refrigeration [10]. Further functionality can be added by inserting metal affinity proteins such as gold binding peptide (GBP) or 6×Histidine tag (His6) into the expression vector allowing the yeast cells to tightly adhere to the metallic surfaces of common transducers without additional reagents, simplifying the purification and deposition processes. Thus after antibody insertion or library screening, yeast cultures may be directly used to create functionalized tests strips in a single binding and washing step, rather than the multistep process typical of classical assays. Hence, this technique can provide stable affinity surfaces on metallic transducers with optimal antibody orientation and binding environment for antigen capture and detection, while at the same time keeping antibody production costs low and simplifying assay assembly. Furthermore, renewing of BioBrick-based capture reagent based on classical regeneration techniques can further reduce the assay cost.

For these constructs to be useful, however, they must be combined with a signal transduction method, the choice of which greatly affects the overall assay performance. Common detection techniques for POC biosensors include, but are not limited to plasmonic [11], spectrophotometric [11], chemiluminescent [12], [13], electrochemical [14], [15], and magnetic sensing [16], [17] methods. Spectrophotometric detection is commonly used with enzyme-linked immunosorbent assays (ELISA), but it can be limited by interference by absorption, turbidity, sample color and auto-fluorescence in biological samples [18], [19]. Furthermore, spectrophotometric detection usually requires a bulky and expensive imager. In keeping with the theme of cost-effective, yet highly sensitive biosensors, electrochemical detection is an obvious choice for use with a whole-cell assay. It easily interfaces with electronics and requires only simple metal electrodes as the

transducers, which can be screen printed to reduce costs. In a typical electrochemical sandwich immunoassay the antigen-antibody complex is quantified by measuring the activity of an antibody's labeled enzyme. The enzymatic conversion of substrate into an electrochemically active species occurs at the electrode-liquid interface and the detected analytical signal relates to the surface concentration of the bound species [20]. Because it is an interfacial rather than bulk solution phenomenon, electrochemical detection has inherent advantages over the spectrophotometric method, including higher sensitivity, wider dynamic range, and lower detection limits (as low as attomolar concentrations for biomarkers under ideal conditions) [21]. Hence, electrochemical detection combined with genetically modified yeast holds great promise for making accurate, portable, and affordable POC biosensors a reality.

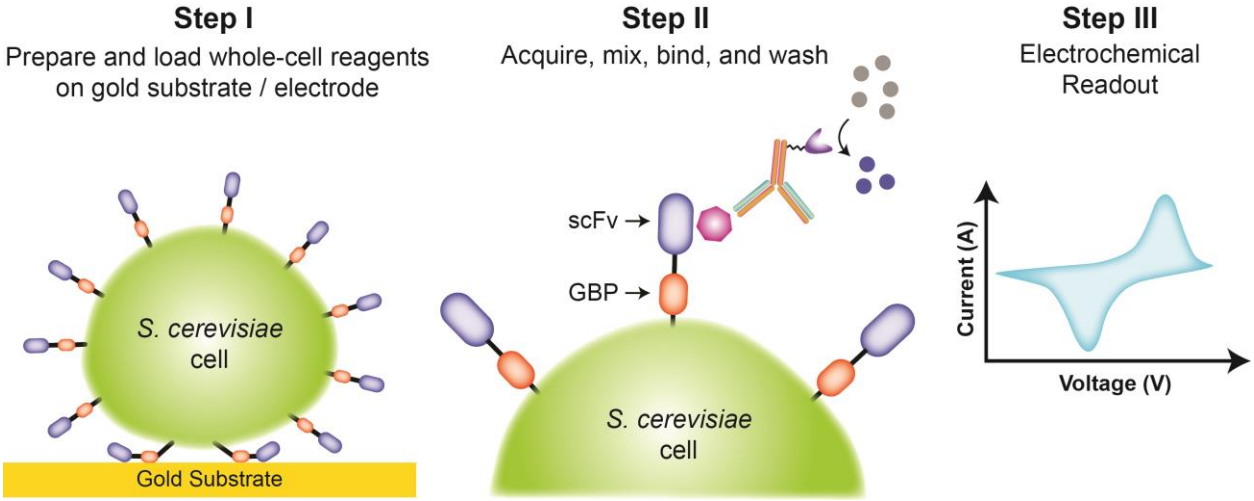

Figure 1: Schematic illustration of "whole-cell" display of affinity molecules. Step I: After induction, yeast cells are spun and washed and then directly affixed to metal electrodes. Step II: Sample is mixed with detection reagents and applied to the prepared electrode surface and, after incubation, the sample is washed with buffer containing the final detection substrate and redox active molecules. Step III: Detection is carried out on a laboratory or mobile potentiostat.

Here, we propose and demonstrate how yeast molecular-display can be used to produce pre-assembled, dual-affinity BioBricks. Genetically engineered yeast capable of single chain or single domain antibody surface-display have been previously reported [22], and have multiple advantages over classic technologies, since living systems are inherently self-replicating and are often resistant to harsh environmental conditions [10], [22]. Thus there are practical and cost advantages to such constructs. For instance, standard capture antibodies needed to produce ~100 chips may cost as much as $350 and require complex surface deposition, whereas the same number of chips may be made from yeast for less than $2 (see supplemental S1). Moreover, whole-cell systems hold the promise of cold-chain free storage and longer shelf lives in the field [9], [23], [24]. Therefore, integration of BioBrick technology into assay design has the potential to significantly reduce the cost of mass producing detection molecules, as growing a culture of *S. cerevisae* is far less expensive than current *in vivo* and *in vitro* antibody production techniques [25]. Recently, a POC platform to detect *E. histolytica* antigen using nano-yeast-scFv with label-free electrochemical detection has been reported in the literature [22], [26]. These data support the use of yeast reagents for low-cost assays, yet leave a number of directions to be further explored and optimized. First, current approaches still require surface functionalization and complex assay preparation. Second, while label-free methods are simple, they often lack specificity and do not benefit from amplification. Thus, there remains continued need for further research and development of whole-cell reagents.

To demonstrate the application of dual-affinity BioBricks, we have engineered yeast cells to detect invasive non-typhoid *Salmonella* (iNTS) antigen. iNTS is a significant pathogen worldwide [27]. In developed countries, iNTS is generally a disease of livestock with strong control measures to prevent disease in humans. In developing nations, especially sub-Saharan Africa, iNTS is a leading cause of human mortality and morbidity, with sequelae including sepsis syndromes, abscesses, osteomyelitis, and meningitis in young children and at-risk adults [27]. iNTS remains prevalent as a human pathogen in developing countries due to lack of robust sanitation infrastructure and poor surveillance measures. There is also a growing potential for nosocomial spread of iNTS, including highly antibiotic resistant strains with significant public health implications worldwide [27]. Thus, inexpensive POC diagnostics that directly detect antigen in patient samples or early bacterial cultures could be extremely useful in settings where iNTS is prevalent.

This research article lays the foundation for the development of a whole-cell based electrochemical immunosensor for detecting biomarkers, a format that represents and pushes the leading edge of truly mass-producible biosensor platforms. Specifically, this article describes: 1) production of advanced capturing and binding reagents derived from a yeast display method (based on the aga1-aga2 system) that allows expression of an scFv of interest in multiple orientations with respect to yeast surface, while co-expressing metal-affinity tags such as GBP and His6 for selective surface adduction; 2) verification of the yeast binding to the metallic surface and optimization of parameters for electrochemical detection; and finally 3) detection of *Salmonella* TM43-E10 surface antigen in a sandwich ELISA format where the antigen is specifically captured at the cell surface and detected both optically and electrochemically.

2. Materials and Methods

2.1 Construction of OmpD-scFv yeast display vectors. Yeast display vector pYD8 was constructed using pYD5 as starting material (pYD5= n-terminal aga2, vector kindly provided by K. Dane Wittrup, MIT, MA) [28] and canonical restriction sites (NheI and SalI at *N*- and *C*-terminals respectively) were introduced to facilitate insertion of cloned scFv fragments with respect to aga2 and His6 affinity-tags. OmpD-scFv cloned from pHAL 14 TM vector (kindly provided by Michael Hust, TU Braunschweig, Germany) by polymerase chain reaction (PCR) using primers with NheI added to the first 18 bp of the N-terminus of the scFv and a BamH1 site added to the C-terminal of the scFv. PCR product was then cleaned and ligated into pYD 6 yielding the following display construct **"Aga2 – $(G_4S)_3$ – V5 epitope – His6 – OmpD-scFv"**. The sequences of all vectors were confirmed using capillary population sequencing and all selected clones showed efficient expression (6,095%) as determined by cMYC or HIS6 display.

2.2 Insertion of Gold Binding Peptide. The GBP (sequence: MHGKTQATSGTIQS) was inserted in to the OmpD-scFv display system to enable the yeast bind to gold surface. The DNA sequence for GBP was cloned by PCR with both forward and reverse primer consisting of a BamHI site at the 5' end from the template [29]. The PCR product was then cleaved with BamHI and ligated in to pYD8 between V5 epitope and OmpD-scFv, and the resulting displayed protein was **"Aga2 – $(G_4S)_3$ – V5 epitope – GBP – $(G_4S)_3$ – His6 – OmpD-scFv"**

2.3 Biotinylation of *Salmonella* TM43-E10 Antigen. The lyophilized TM43 – E10 antigen (sequence: AC-CAAYAKSDRTNNQVKAASNLNAAGKNAEC-amide) was kindly provided by Michael Hust, TU Braunschweig, Germany. The peptide was biotinylated at the primary amine end using NHS-biotin chemistry (Biotinylation kit #20217, Thermo Fisher Scientific) and dialyzed overnight with MWCO 1000 membrane in 1 L PBS to remove the unreacted excess NHS-biotin from biotin – peptide conjugate.

2.4 Induction of scFv Expression. Transformed scFv expressing yeast were streaked and freshly grown on SD+CAA selective media plates, overnight at 30°C. Isolated colonies were then used to inoculate 5 mL of SR+CAA media and incubated for 1-2 days at 30°C then diluted and aliquoted as 1 OD_{600}/mL. To induce expression, 0.5 mL of this culture was then inoculated into 5 mL of S/Galactose and Raffinose+CAA media and incubated overnight at 20°C. Preparation of media is described in supplementary material (S2).

2.5 Immunochemical and Fluorescent Assays. 100 µL aliquots of galactose induced cells were spun down in 96 well plates and resuspended in 15 µL of 1× PBS + 1% BSA and incubated at room temperature for 30 minutes to preblock. Biotinylated *Salmonella* TM43-E10 antigen and either streptavidin conjugated fluorescein isothiocyanate (FITC) for fluorescent or horseradish peroxidase (HRP) for immunochemical was then added sequentially to each well at a final volume of 20 µL to detect expressed scFv. Plates were rocked slowly for 2 hours at room temperature or overnight at 4°C, after which, yeast were pelleted and wells were washed 4× with 1× PBS. Finally, cells were washed 4× and measured for fluorescence (FITC/488) using flow cytometer (BD Accuri C6).

2.6 Preparation Electrode for Electrochemical Impedance Spectroscopy (EIS) and Electrochemical Sandwich ELISA. Screen printed electrodes for ELISA (DropSens DRP-250AT) and sputtered gold electrodes on glass slides for EIS were cleaned in a two-step procedure to remove organics and oxide layer present on the metal surface. First, the organics were removed by incubating the electrode in 50 mM KOH / H_2O_2 (3:1) solution mixture for 10 min, rinsed with deionized water and blow-dried. Next, the oxide layer on the gold electrode was electrochemically stripped in the presence of 50 mM H_2SO_4. The stripping was done by sweeping a potential between -0.4 V to +1.4 V at a rate of 100 mV/s for 12 cycles, rinsed with deionized water, and blow-dried. A bench-top potentiostat (CH Instruments 750E) was used for the electrochemical cleaning as well as all cyclic voltammetry and impedance spectroscopy measurements.

2.7 Preparation of Yeast cells for Electrochemical Impedance Spectroscopy and Electrochemical Sandwich ELISA. 100 µL of yeast cells per chip (1 OD_{600}) were spun down at 5,000 rpm for 5 min. The pellet was then washed three times by resuspending the cells in 200 µL of 150 mM phosphate buffer (pH 7.2) followed by centrifugation at 3,000 rpm for 3 min. The washed yeast cells were resuspended in 50 µL of 150 mM phosphate buffer (pH 7.2).

2.8 Electrochemical Impedance Spectroscopy. Due to their superior impedance spectra in the appropriate frequency range compared to that of screen printed metals, glass slides sputtered with 10 nm Cr/200 nm gold were used as the working electrodes in EIS experiments to measure the binding of yeast over time. With the slide affixed to a custom setup (Fig. 3a), which created 50 µL sealed wells, silver wire (Alfa Aesar #41390) and platinum wire (EMS Acquisition Corp #73500) were inserted from above that acted as reference and counter electrodes. An effective concentration of 1 mM of the redox marker Ferri/Ferro was made from equal parts potassium ferricyanide ($K_3[Fe(CN)_6]$) and potassium ferrocyanide ($K_4[Fe(CN)_6]$) from Spectrum (P1286, P1296) in a phosphate buffer (PB) of 150 mM potassium phosphate monobasic (#P285-500, Fischer Scientific) and potassium phosphate dibasic (#PX1570-1, EMD Millipore). Ferri/Ferro was then mixed with 1 OD_{600} of yeast cells and dropped into each well on top of the gold. Prior to EIS measurements, the redox potential for the Ferri/Ferro pair in each electrochemical cell was determined with cyclic voltammetry using a scan range of -0.3 V to +0.3 V at a rate of 100 mV/s. The voltages corresponding to the anodic and cathodic current peaks of the resulting voltammogram were averaged together to find the redox potential of Ferri/Ferro. The impedance of the electrochemical cell was then measured at approximately 4°C for six hours in 30-minute

intervals by applying a range of voltage tones from 100 kHz to 100 mHz with an amplitude of 5 mV centered around the previously measured redox potential. The impedance was measured again after washing the electrode with the PB solution to remove unbound yeast cells. The resultant data were then fitted to Randles equivalent circuit (Fig. 3b), which models the electrochemical interface between the surface of the working electrode and the solution [30].

2.9 Electrochemical Sandwich ELISA. The cells were pipetted on to the electrode and incubated overnight at 4°C. The electrode was washed with 1× PBS, blocked with 10% BSA in PBS for 1 hour at room temperature and washed with 1× PBS. The electrode with immobilized yeast cells was then incubated with antigen of desired concentration for 1 hour at room temperature and washed with 1× PBS. A solution of alkaline phosphatase (ALP) conjugated detection antibody in PBS was added to the electrode and incubated for 1 hour at room temperature, and washed with 1× PBS. The presence of yeast cell-antigen-antibody was detected by addition of inactive electrochemical substrate *p*-aminophenyl phosphate (*p*APP) (SCBT.com #sc-281392) in glycine buffer (pH 10.4) and incubated for 10 min at room temperature. The conversion of *p*APP to *p*-aminophenol (*p*AP) by cleavage of a phosphate turns *p*APP into an electrochemically active molecule, which is detected by cyclic voltammetry by sweeping a potential between +0.3 V to -0.3 V at a rate of 25 mV/s for 10 cycles. The *p*AP concentration was determined by calculating the maxima at the anodic potential of the 10^{th} cycle. The current vs concentration was fitted with 4-parameter logistic function to calculate K_d.

3. Results and Discussion

3.1 Construction of Yeast Display System. Different methods were explored to optimize scFv affinity and surface binding. We created vectors for display of antibodies with altered gene orientations (i.e. $v_H - v_L \rightarrow v_L - v_H$), domain order or linker length, and metal binding tags (GBP and His6) were added for one-step enrichment and adduction to metal surfaces. Antigen binding experiments with displayed scFv confirmed that variable chain order and orientation of the scFv with respect to the aga2 cell-wall anchor (NH_2–scFv–Aga2 –COOH → NH_2–Aga2–scFv–COOH) can dramatically affect affinity of displayed antibodies for antigen, thus requiring either domain swapping or n-terminal expression of aga2 for OmpD-scFv to detect TM43-E10 (data not shown). This phenomenon may represent steric competition between the aga2 anchor and OmpD-scFv antigen binding cleft or alteration in protein folding leading to binding site changes. Repositioning of either the aga2 fusion site or swapping the variable domain order may support folding or orient antigen-binding motifs towards solvent, enhancing binding affinity. This may also represent a limitation of some display systems as good antibodies may be missed during yeast-display selection, but have activity if orientation or domain order were changed. The pYD8 vector was derived from pYD to facilitate n-terminal expression of the aga2 protein with metal binding peptide and scFv (Fig. 2). Standard cloning sites (BamHI/NheI/SalI) for transferring scFv from exogenous vectors were added. scFv were expressed as V_{heavy}—$(G_4S)_3$—V_{light}, where the variable chains (V_{heavy}/V_{light}) are separated by a glycine-serine linker.

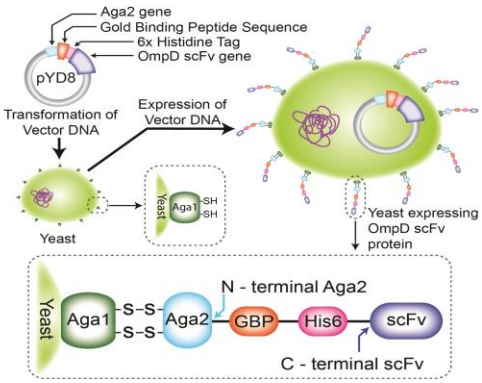

Figure 2: Schematic illustration of yeast display system to detect *Salmonella* TM43-E10 peptide.

3.2 Confirmation of Surface Binding with Electrochemical Impedance and Fluorescence Spectroscopy. The event of yeast binding to the metal surface via gold-binding peptide repeats was detected and quantified by EIS and fluorescent imaging. In principle, yeast cells binding to the gold will obstruct the surface and reduce the probability that redox active molecules will react and transfer electrons to the electrode [31] [32]. This decrease in reactivity on the surface thereby increases the charge transfer resistance (R_{ct}) seen at the working electrode interface. Figure 3c shows the Nyquist plots of impedance data for a typical yeast-binding event over time. The R_{ct} of this particular event, whose trend can be determined by inspection of the location of the dip in each Nyquist plot, increases with time, saturates at about 6 hours, and decreases after washing. The R_{ct} data for all EIS experiments were calculated from raw impedance data fitted to the equivalent circuit model as shown in Fig. 3b for all EIS experiments. Fig. 3d shows a comparison of the R_{ct} change over time for the control with only Ferri/Ferro solution and blank, GBP only, HIS only, and GBP and HIS yeast varieties. The difference in R_{ct} between T=0 and after washing represents the amount of cells specifically bound to the surface, while the change between T=6 hours and after washing represents the amount that non-specifically bound to the surface. The significant R_{ct} change of the blank yeast compared to the other yeast varieties implies that the binding seen before washing is most likely due to cells being held to the surface of the electrode by weaker non-specific forces such as gravity. Furthermore, it can be inferred from this data that, while all the yeast types seem to exhibit some level of non-specific binding when compared to the empty redox solution, only those yeast with GBP remain fixed to the electrodes in significant quantities after washing. To further verify the stability of immobilization and suitability for biosensing applications, fluorescent microscopic imaging (Fig. 3e) was performed on multiple washes of GBP yeast bound to the gold surface of the DropSens SPEs. Even after 5 consecutive washes with PBS, there is no notable decrease in the concentration of yeast cells bound to the surface. This tight binding suggests that GBP is an effective mechanism for yeast adherence to gold surfaces such as those used in electrochemical assays described below. The yeast cells displaying scFv were assay compatible for at least 4 months when stored in 1× PBS at 4°C. The stability can be increased to several months to years by lyophilizing the cells after harvest and hydrated before the assay procedure.

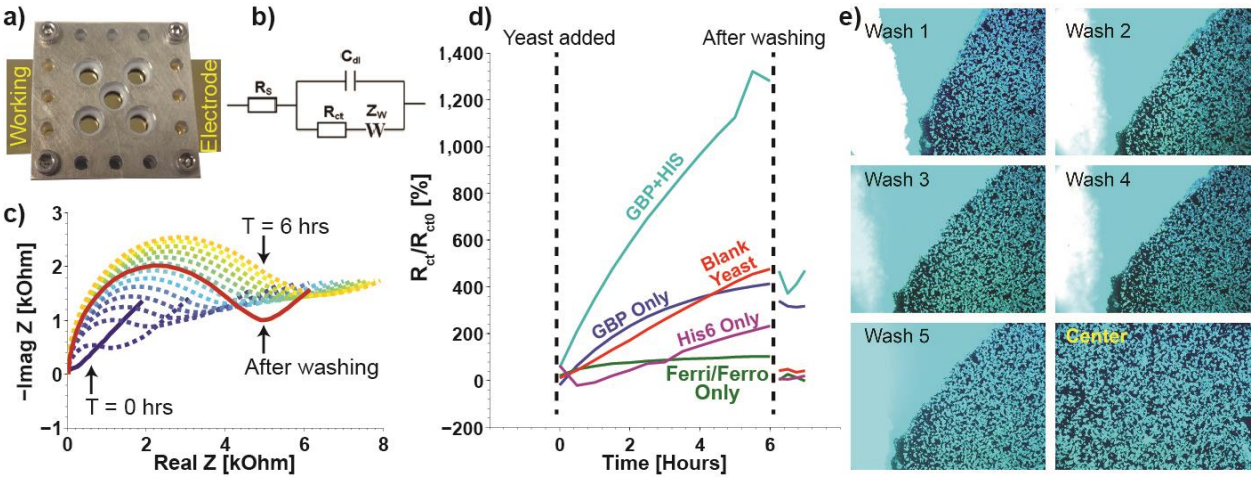

Figure 3: Electrochemical impedance spectra of yeast cells bound to gold surface. a) Photograph of custom EIS measurement setup, b) Equivalent circuit model, c) Nyquist plot showing yeast cells binding on gold surface over time, d) Normalized R_{ct} percentage of control and different yeast constructs, and e) Fluorescent microscopic images showing the stability of yeast cells bound to gold surface after five washes.

3.3 Electrochemical Sandwich ELISA. In recent years, ELISA combined with electrochemical detection has extended the limit of detection by a factor of 10 with small biological samples [33] yet there has been no appreciable decrease in cost. In this work, the classical sandwich ELISA was modified to detect antigen by using an electrochemical technique (Fig. 4) with ALP and *p*APP as the enzyme—substrate pair. Although HRP is a more economical enzyme, running an electrochemical ELISA using HRP with *p*APP as the substrate [18] requires a mercury drop working electrode, which cannot be easily integrated into POC devices due to environmental hazard and small form factor requirement. There exist many possible choices of substrate to use with ALP since it hydrolyzes different types of orthophosphate from a wide variety of phosphate esters under alkaline conditions such as phenylphosphate, *p*-nitrophenylphosphate, 3-Indoxyl phosphate, and naphtylphosphate. However, not all substrates are compatible for POC device applications. For example, 3-Indoxyl phosphate requires fuming sulfuric acid to halt enzymatic reaction [34]. *p*APP is one of the more suitable substrates for ALP since its enzymatic product, *p*AP, can be detected at low redox potentials that are well distinguished from the redox potential of the *p*APP [19].

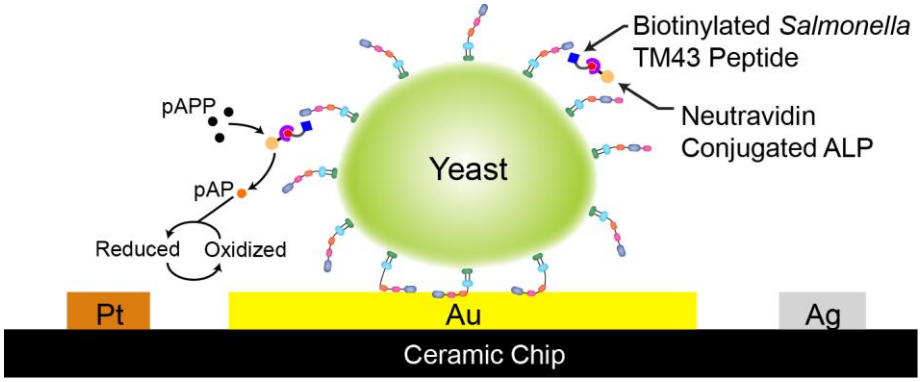

Figure 4: Illustration of electrochemical sandwich ELISA using whole-cell capture reagents

TM43-E10 antigen was detected in an electrochemical sandwich immunosorbent assay using dual affinity (GBP—OmpD-scFv) yeast BioBricks. First, bound yeast cells displaying OmpD-scFv

captures the biotinylated TM43-E10 complex, which will be subsequently sandwiched by the neutravidin conjugated ALP and used for electrochemical detection after adding the substrate *p*APP. Initial voltammograms obtained from cyclic voltammetry experiments run over the potential range of –0.3 V to +0.3 V at a scan rate of 25 mV/s with respect to the Ag – pseudo-reference electrode initially yielded a low signal-to-noise ratio (SNR), possibly due to endogenous phosphatase activity at the yeast cell surface (see supplementary S3). Sodium azide, a biocide and a preservative [22] was added to halt the cellular metabolism by inhibiting the adenosine triphosphate (ATP) synthesis and the yeast respiration is completely inhibited at 0.15 mM concentrations (~ 0.001%) [35]. It also reacts with carbonyl complexes similar to CO coordinated molecules, thereby inhibiting many yeast metabolites from contributing to unwanted background noise [36]. The yeast cells bound to the gold electrode were exposed to different sodium azide concentrations (0.05%, 0.1%, 0.2%, and 1%) and blocked with 1% BSA for 90 minutes. The sandwich ELISA was performed again on the treated cells and subsequent measurement of *p*AP formation showed improving SNR with increasing sodium azide concentration. At a constant antigen concentration (250 nM), cells treated with 1% sodium azide showed a 3.7× increase in current amplitude compared to untreated (control) cells (Fig 5a) (see supplementary S4). The inhibition efficiency of endogenous phosphatase activity by sodium azide is demonstrated (Figure 5b) by normalizing the 250 nM current response of both untreated and treated cells to unity. An 8-10% noise reduction was observed while comparing the difference in normalized current of the blank (no antigen) tests between untreated and 1% sodium azide treated cells.

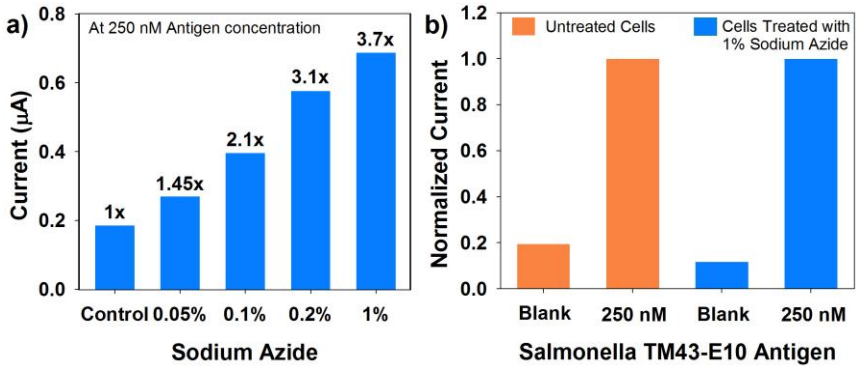

Figure 5: Effect of Sodium azide treatment in signal enhancement. a) Comparison of different sodium azide concentrations, and b) Comparison of normalized current intensity between treated and untreated cells.

After standardizing the basic assay parameters such as sodium azide treatment, incubation time, and wash steps, a standard calibration electrochemical assay was performed to determine the sensitivity, dynamic range, and dissociation constant (K_d) of the whole-cell assay. Figure 6 shows the comparison of calibration curves for both the electrochemical and optical assays. The K_d for electrochemical and optical assay were measured to be 155.92 ± 72.64 nM with a detection limit down to 8 nM antigen concentration and 51.71±6.87 nM, respectively (see supplementary S5 for data). A higher dynamic range was observed in the electrochemical ELISA format, which may be partially explained by the lower variability in optical imaging versus electrochemical amplification steps where more inter-test variation due to drift in parameters such as pH and temperature was observed. FACS data showed the least variability, however similar variability was noted when assays were probed enzymatically with HRP, again indicating the amplification step could introduce both new variation and new background (data not shown). Future experiments will more tightly control these parameters and explore new approaches to reduce noise and variability (e.g. by using only yeast-membrane fractions and testing other enzyme inhibitors). While the observed difference in affinity between optical and electrochemical methods likely represents variation in measurement conditions rather than intrinsic binding differences, differences from free-scFv

experiments are likely attributable to structural changes. Noting that Meyer *et al*. reported a K_d of 27 nM for the same TM43-E10 / anti OmpD–scFv pair based on surface plasmon resonance (SPR) measurements of free single chain antibodies (versus 51.71±6.87 nM derived from our FACS), the difference between our optically derived whole-cell affinity's and Meyer's data may be attributed to differences caused by scFv display and will be the subject of further evaluation and optimization in future work [37].

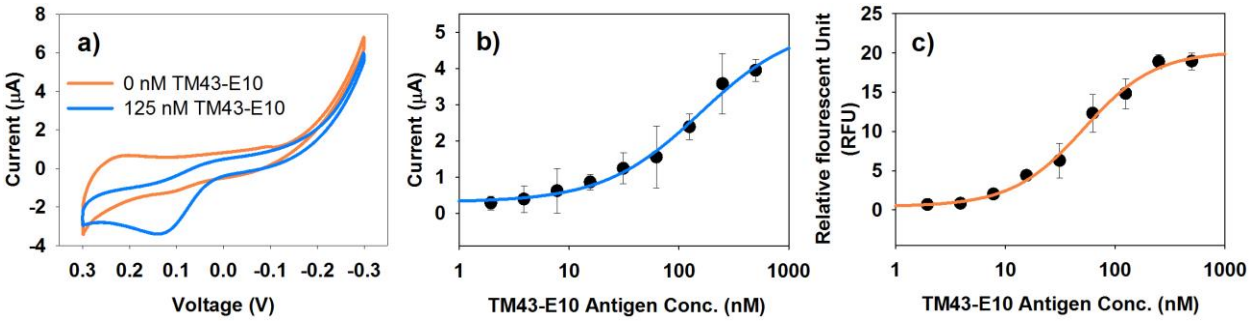

Figure 6: Experimental data of electrochemical ELISA to detect TM43-E10 antigen: a) selected cyclic voltammogram of control and 125 nM antigen concentrations b) electrochemical and c) optical detection of antigen captured by dual affinity yeast cells. The cells displaying scFv to *Salmonella* (anti-OmpD) were deposited, washed and treated with Sodium Azide to remove endogenous phosphatase activity on gold SPCE's. Experiments were conducted in triplicate with the average plotted above

4. Conclusions

This study further demonstrates the potential for whole-cell immune-capture reagents to serve as robust and low-cost alternatives to current biotechnologies. Here, OmpD surface-antigen was sensitively and specifically detected by yeast cells engineered to display anti-OmpD-scFv using the aga1-aga2 system. An integrated "whole-cell"-electrochemical ELISA revealed wide dynamic range with low nanomolar sensitivity for detecting *Salmonella* OmpD antigen. These data provide evidence for the simplicity and utility of whole-cell reagents in low-cost POC assays in general and point to future avenues for optimization and improvement. Sensitivity and specificity can likely be improved by affinity maturation of scFvs coupled to techniques to reduce non-specific binding and intrinsic enzyme activity from the yeast cell. Given the ease of reagent production and assay integration, we propose these methods present an opportunity to unlock the full potential of ultra-low cost "renewable" cell-based reagents, which when paired with low-cost sensors, can promote both local and global health.

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