A Facile Two-Step Enzymatic Approach for Conjugating Proteins to Polysaccharide Chitosan at an Electrode Interface

NARENDRANATH BHOKISHAM,^{1,2} YI LIU,² HAIG PAKHCHANIAN,³ GREGORY F. PAYNE,^{2,3}
and WILLIAM E. BENTLEY^{2,3}

¹Biological Sciences Graduate Program - College of Computer, Mathematical and Natural Sciences, University of Maryland, College Park, 4066 Campus Drive, College Park, MD 20742, USA; ²Institute of Bioscience and Biotechnology Research, University of Maryland, College Park, 5115 Plant Science and Landscape Architecture Building, College Park, MD 20742, USA; and ³Fischell Department of Bioengineering, University of Maryland, College Park, Room 3122, Jeong H. Kim Engineering Building (Bldg. #225), College Park, MD 20742, USA

(Received 4 August 2016; accepted 26 October 2016; published online 14 November 2016)

Associate Editor Michael R. King oversaw the review of this article.

Abstract—Biological components are integrated with electronic devices to create microsystems with novel functions and chitosan, a naturally occurring biopolymer, can play a significant role as an interface material. Chitosan can be electrodeposited within confined geometries by cathodic charge and appropriate electrode design and proteins can be conjugated to chitosan. However, conjugation chemistries can be slow and chitosan, a polycationic polysaccharide, enables non-specific binding in biofabrication processes. There is a need to speed up the assembly process and reduce non-specific binding. Here, we have developed a two-step methodology that accelerates protein assembly, reduces background and increases specificity. We first “coated” the surface of chitosan with a Lys-Tyr-Lys (KYK) tripeptide in a slow step using tyrosinase-mediated conjugation chemistry and then conjugated proteins with C-terminal glutamine-tags to the saturating KYK tripeptide *via* transglutaminase. As a demonstration, we assembled a functioning two-enzyme bacterial metabolic pathway on an electrode chip. Results indicated a fivefold decrease in non-specific binding and an improvement in signal to noise ratio from 0.3 to 20. This transglutaminase-mediated approach is simple and quick, it requires no chemical reagents, no printing or stamping devices; it employs biological components and is biologically benign to the component parts—all characteristics of bio-fabricated devices.

Keywords—Biofabrication, Chitosan, Nonspecific binding, Transglutaminase.

INTRODUCTION

Biofabrication enables assembly of biological components including proteins, polysaccharides, DNA, cells etc. onto electronic devices to create microscale systems that mediate seamless interaction between the two.^{22,24,25,28} While biological components are naturally evolved with capabilities to ‘sense’ and ‘perceive’ (e.g., cells) or ‘act’ (e.g., enzymes, cells), biology’s molecular information flow is largely incompatible with electronics. Also, electronic devices are designed to process and communicate information to and from the user, typically not with the biological system. Biofabricated devices are developed by integrating biologic and electronic components and the devices have immense potential for bi-directional information transfer, affecting a wide range of fields from biosensing, lab on chip systems, bio-batteries and biofuel cells to personalized diagnostic devices.¹⁶ A second generation of biofabricated devices have been developed that not only combine the above mentioned characteristics but also display elements of ‘control’ and ‘actuation’¹² of biological components through electronic signals.

One of the critical components for integration of biological and electronic systems is the interface material. It is desired to functionalize the surface of electronic components with reactive groups that can easily interact with biological components¹⁶ and, importantly, in a way that precludes non-specific binding of materials at the electrode interface. Non-specific binding can be pronounced, eroding function and sensitivity in biosensors, for example. Various materials such as carboxy methylated-dextran, polyethylene glycol (PEG)-containing hydrogels,⁴ self-

Address correspondence to William E. Bentley, Fischell Department of Bioengineering, University of Maryland, College Park, Room 3122, Jeong H. Kim Engineering Building (Bldg. #225), College Park, MD 20742, USA Electronic mail: bentley@umd.edu

FIGURE 1. Enzymatic assembly of engineered proteins onto chitosan films. Left: a “one-step” tyrosinase mediated conjugation approach that utilizes mushroom tyrosinase to “activate” a tyrosine rich tag engineered onto the C-terminus of the assembled protein. Tyrosinase converts the phenolic functional group of tyrosine to an ortho-quinone which subsequently binds to the primary amine of chitosan (via Michael addition). Right: two-step approach utilizing the initial “coating” of chitosan films with Lys-Tyr-Lys (KYK) peptides using tyrosinase (as on left), followed by conjugation of proteins engineered with C terminal glutamine-rich tags onto the KYK peptides using a microbial transglutaminase.

assembled monolayers of alkane thiols,³ chitosan-*N*-poly(ethylene oxide)²⁹ have been developed to lower non-specific binding of proteins and increase sensitivity. We are interested in chitosan, a deacetylated polysaccharide from chitin, as a facilitator of biological assembly onto microelectronic devices, such as biosensors, and MEMS devices. Because chitosan's solubility is easily controlled (via pH), it can be partitioned into various forms including hydrogels and coated on electrodes. In our work, chitosan enables electrode-based interrogation of cell signaling^{11,19,21} as well as conveyance of molecular information to and from electrodes.^{12,15}

Proteins and enzymes have been coupled to chitosan using a tyrosine based chemistry (see Fig. 1) mediated by tyrosinase.⁵ However, tyrosinase oxidation of phenolic groups to quinones can be slow.^{7,23} In addition, the abundance of positive charges due to the amine groups on the surface of chitosan, a property that makes chitosan viable in a multitude of applications, can also lead to nonspecific binding of proteins.⁶ The combination of both nonspecific binding and slow tyrosinase-mediated conjugation chemistry leads to a scenario of a low ‘signal to noise’ ratio for protein conjugation particularly when attempting rapid assembly. In our previous work, tyrosinase mediated coupling was shown to yield satisfactory ‘signal to

noise’, but conjugation reactions were carried out overnight.

In an attempt to accelerate this process and enhance ‘signal to noise’, we modified chitosan first using the tyrosinase-mediated coupling of a Lys-Tyr-Lys (KYK) tripeptide transforming the available primary amine to a pair of primary amines each on essentially a 4-carbon linker, then employed a microbial transglutaminase (mTG) to link proteins engineered with glutamine-rich tags²⁷ to peptide-chitosan complex. Importantly, mTG is a crosslinking enzyme with ~100 fold higher enzyme turnover number than tyrosinase.^{20,23} In this work, we specifically focus on the ‘signal to noise’ ratio of these two approaches to assemble proteins onto chitosan (see Fig. 1): the tyrosinase-mediated approach involving conjugation of proteins directly to chitosan and the two-step approach involving coating the surface of chitosan with KYK peptides using tyrosinase and direct conjugation of proteins to KYK peptides through transglutaminase. We further characterized the transglutaminase-catalyzed approach by assembling a two enzyme cascade onto gold chips coated with peptide modified chitosan. As model enzymes, we used *S*-adenosylhomocysteine nucleosidase (Pfs) and *S*-ribosylhomocysteine lyase (LuxS), both part of the activated methyl cycle in bacteria.¹⁰ While Pfs converts *S*-Adenosyl Homocysteine (SAH) to *S*-Ribosyl

Homocysteine (SRH), LuxS converts SRH to Homocysteine (HCY) and (*S*)-4,5-dihydroxy-2,3-pentanedione (DPD), a precursor to Autoinducer-2 (AI-2), a quorum sensing (QS) signal molecule. The net reaction rate is determined here by quantifying the level of homocysteine, an easily measured sulfhydryl-containing byproduct of LuxS. Using these methodologies, surfaces can be developed with tailored biochemical flux.

MATERIALS AND METHODS

Preparation of Chitosan Films in 48 Well Plates

We prepared chitosan films using 1.5% chitosan (Sigma Aldrich) solution at pH 5.6, dissolved in HCl. 200 μ L of chitosan solution was added to each well of the 48 well plate and vacuum dried at 65°C for 24 h. After incubation, we washed the films thrice with phosphate buffered saline (PBS; Sigma Aldrich), pH 7.5 and neutralized by immersing the films with 1 M NaOH (Sigma) for 45 min at RT. Later we washed the films again 3 $\times$ with PBS and used for experiments.

Electrical Deposition of Chitosan onto Gold Chips

We immersed clean gold chips in 1.5% chitosan solution and applied a current of 4 A/mm² for 2 min to each chip. Later we air dried the gold chips and used for experiments.

Conjugation of Lys-Tyr-Lys (KYK) Peptide to Chitosan Films

We prepared 1 mM KYK peptide (Sigma Aldrich) solution using Phosphate Buffered Saline, pH 7.5 (PBS, Sigma Aldrich). We added 100 μ L of KYK peptide solution along with mushroom tyrosinase (Sigma Aldrich) at a concentration of 50 U/mL in 50 mM potassium phosphate buffer, pH 6.5. We incubated the films with peptide solution at RT for overnight and later washed 3 $\times$ with PBS and used for experiments. 1 U of tyrosinase is defined as amount of enzyme required to change A_{280} by 0.001 per min at pH 6.5 at 25 °C in 3 mL reaction mix containing L-tyrosine.

Conjugation of Engineered Proteins Using Tyrosinase and Microbial Transglutaminase

We used proteins engineered with tyrosine¹⁷ (for one-step conjugation approach) and glutamines² (for

two-step conjugation approach). For one-step tyrosinase mediated conjugation of proteins to chitosan films, we added tyrosine engineered proteins and tyrosinase (dissolved in 50 mM potassium phosphate buffer, pH 6.5) at either 3.5 or 11 U/ μ L concentrations and incubated for 1 h at RT. For two-step transglutaminase mediated conjugation to peptide modified chitosan films, we added glutamine engineered proteins and incubated for 1 h at RT with 1.3 U/mL of mTG, purified from *Streptovorticillium mobaraense* (Ajinomoto) and dissolved in phosphate buffered saline, pH 7.5 (Sigma Aldrich). 1 U of mTG activity is defined as the amount of enzyme required to catalyze the formation of 1.0 μ mol of hydroxamate (product) per minute from Z-Gln-Gly and hydroxylamine (substrates) at pH 6.0 at 37°C.

Ellman's DTNB Assay

We prepared Ellman's DTNB stock solution containing 50 mM sodium acetate, 2 mM DTNB (Sigma Aldrich) using water. We prepared an homocysteine assay solution containing 100 μ L of 1 M Tris-HCl, pH 8, 50 μ L of DTNB solution and 800 μ L of water. We mixed 50 μ L of solution containing homocysteine with 950 μ L of assay solution and measured absorbance at 412 nm after at least 15 min.

RESULTS

Lys-Tyr-Lys Peptide Prevents Non-specific Binding to Chitosan

We compared the levels of non-specific binding of enzymes to chitosan films and peptide (KYK)-modified chitosan films (Fig. 2). We used *S*-Ribosylhomocysteine lyase (LuxS) as a model enzyme to measure non-specific binding of enzymes to chitosan. For this purpose, we engineered LuxS at its C terminus with either a penta-tyrosine tag (indicated as LuxS_Y) or a penta-glutamine tag (indicated as LuxS_Q). All proteins are engineered with hexahistidine tags at the N-termini.² As controls, we also used native LuxS (with N-terminal hexahistidine tag for purification) to measure non-specific binding. We note that both native and engineered LuxS's displayed similar enzyme activities per molecule (data not shown). For these experiments, chitosan films were cast into 48 well plates. To each well, we added 200 μ L of 1.5% chitosan solution, pH 5.6 and vacuum dried at 65 °C for 24 h. Dried films were later neutralized with 1 M NaOH for 45 min at RT. Post neutralization, chitosan films were washed 3 $\times$ with phosphate buffered saline (PBS, pH 7.4) and used for experiments. To modify

FIGURE 2. Non-specific binding profiles of LuxS, LuxSQ and LuxSY onto chitosan film and chitosan-KYK peptide films. Blocking protein bovine serum albumin (BSA; 70 µg/100 µL) was used; this level is the molar equivalent to 3.50 U/µL of Tyrosinase; 200 µg/100 µL of BSA is the molar equivalent to 11 U/mL of tyrosinase and 60 µM mTG. Error bars indicated with $n = 3$. * indicates $p < 0.001$ using student's t test.

chitosan films with KYK peptide, we incubated neutralized films with 1 mM Lys-Tyr-Lys (KYK) peptide and tyrosinase at 50 U/mL concentration overnight at RT.²⁷ Post incubation, films were washed 3× with PBS. In Fig. 2, to study levels of non-specific binding, we added 15 µM of native (LuxS) and engineered LuxS (LuxSY and LuxSQ) to empty and KYK peptide coated chitosan films. Additional controls consisted of enzymes mixed with 70 and 200 µg per 100 µL (10.6 and 30.3 µM, respectively) of bovine serum albumin (BSA, a common blocking agent). Post incubation at RT for 60 min, we washed the films 3× with PBS. We measured LuxS bound to films by measuring the enzyme activity; quantified by the sulfhydryl generation from homocysteine, a LuxS byproduct. We added 190 µL of 1 mM *S*-Adenosyl Homocysteine (SAH) and 10 µL of 20 µM enzyme *S*-Adenosyl Homocysteine nucleosidase (Pfs) and incubated the films for 60 min at 37 °C. Pfs converts SAH to SRH and SRH is converted to homocysteine (HCY) by LuxS. Since LuxS is the rate limiting factor in this two-step reaction, HCY generated by LuxS is directly proportional to amount of LuxS on the chitosan film.¹ After incubation, we measured HCY levels using Ellman's assay (sulfhydryl assay).

In the left most panel of Fig. 2, there was significant homocysteine generation (>0.01 µM/min/mm²) indicating significant non-specific binding to chitosan. There was no apparent difference due to the glutamine or lysine tags, nor was there a consistent influence due to BSA in the ranges of concentrations used (10–30 µM). Note that we co-incubated with BSA, we did not block with BSA first then add LuxS;

the effect of BSA here was to demonstrate the capacity of binding sites for LuxS and to mimic the addition of either the tyrosinase or the transglutaminase which were added at similar levels. Interestingly, we observed the HCY levels from the peptide modified chitosan film, on average, to be 5 times lower than the HCY levels from the empty chitosan films (p value <0.001). Again, there was no competitive advantage due to the addition of BSA at the conditions tested. Finally, we note both native and engineered LuxS were identical in their non-specific binding profiles. These results clearly demonstrated that addition of KYK peptide to the chitosan film reduced fivefold the quantity of enzymatic activity bound to the films. In separate experiments (not shown here), we found the peptide when incubated in solution with the LuxS enzyme without chitosan present had no influence on enzyme activity. In sum, our results are consistent with the hypothesis that less enzyme was non-specifically bound in the presence of the KYK peptide.

mTG Catalyzed Approach Significantly Improves 'Signal to Noise' for Enzyme Conjugation onto Chitosan

We next compared the one-step tyrosinase-mediated conjugation approach with the transglutaminase-catalyzed approach to measure the ratio between 'signal' from enzyme mediated specific conjugation and 'noise' from non-specific binding.

For the tyrosinase-mediated approach, we used tyrosinase to crosslink LuxSY to empty chitosan film. After the chitosan film was assembled and neutralized,

FIGURE 3. Comparison of tyrosinase mediated conjugation of tyrosine engineered LuxS (Lux_{SY}) to chitosan films with the two-step transglutaminase (mTG) catalyzed conjugation of glutamine engineered LuxS (Lux_{SQ}) to peptide-chitosan films. (a) Tyrosinase mediated conjugation of Lux_{SY} onto chitosan films. Controls indicated are for Lux_{SY} without tyrosinase. (b) Transglutaminase mediated conjugation of Lux_{SQ} to the chitosan-KYK peptide films. Controls include Lux_{SQ} incubated with chitosan-peptide films but without mTG. “S/N” indicates signal to noise ratio, where “Signal” is LuxS activity from enzyme mediated conjugation and “Noise” is LuxS activity from nonspecific binding. Error bars indicated with $n = 3$.

we added 15 μM of Lux_{SY} along with tyrosinase (dissolved in potassium phosphate buffer, pH 6.5) and incubated for 60 min at RT. In Fig. 3a, we used two tyrosinase conditions, 3.5 and 11 U/ μL . In previous work, we found 3.5 U/ μL was sufficient for coupling tyrosine tagged proteins to chitosan films^{12,26}; 11 U/ μL was the highest available concentration. After the 60 min incubation, we washed the chitosan film 3 $\times$ with PBS and measured the enzyme activity as described earlier. In both cases, LuxS enzyme activity was significantly lower than the control without tyrosinase indicating that nonspecific binding of Lux_{SY} to chitosan was surprisingly greater than with tyrosinase mediated conjugation. This reduction in activity of conjugated LuxS from chitosan films was due, in part, to a reduction of per unit enzyme activity of LuxS that is mediated by the tyrosinase itself (Fig. S1) and the potential interference of non-specific binding due to tyrosinase (as compared to BSA). Overall, the ratio of LuxS activity from tyrosinase mediated conjugation to non-specific binding was 0.33. We had previously demonstrated that tyrosinase linkage works well for two more stable proteins (GFP and

Pfs) relative to non-conjugation controls, however in those studies the conjugation times were between 12 and 16 h.^{18,19} At a conjugation time of 60 min, LuxS enzyme activity from specific conjugation was lower than the nonspecific binding controls.

We next measured the analogous ‘signal to noise’ ratio for the two-step method. In Fig. 3b, we added 15 μM of Lux_{SQ} and 1.32 U/mL of mTG to the peptide modified chitosan film. As a control, we used Lux_{SQ} without mTG. We incubated the proteins at RT for 60 min and washed the films 3 $\times$ with PBS. After washing, we measured activity using Ellman’s assay. We observed (1) that, as in Fig. 2, there was minimal activity assembled onto the peptide modified chitosan films not treated with transglutaminase and (2) that enzyme activity from mTG-crosslinked proteins resulted in sixfold more activity than the tyrosinase-mediated case. Again, all conditions, concentrations, wash steps, etc., were identical. The only differences were the actual proteins assembled (Q vs. Y tag) and the enzymes used to crosslink (tyrosinase vs transglutaminase). In solution, just like tyrosinase, we observed a decrease in per unit enzyme activity of LuxS (Fig. S1)

FIGURE 4. Capacity for binding LuxSQ. Activities exhibited from different concentrations of LuxS conjugated with chitosan films using the two-step microbial transglutaminase approach. Controls indicated include LuxSQ without mTG. Error bars indicated with $n = 3$.

upon incubation with mTG, but this decrease was less than incubation with tyrosinase. Overall, the signal to noise ratio improved from 0.3 to 20 by switching from tyrosinase mediated conjugation method to mTG catalyzed conjugation method.

Characterization of Transglutaminase-Catalyzed Approach to Conjugation onto Chitosan

Having demonstrated that transglutaminase-catalyzed approach was potentially superior to the tyrosinase-mediated approach, we sought to further

characterize the transglutaminase-catalyzed methodology. That is, in Fig. 4 we varied the concentrations of LuxSQ added to the conjugation reactions. We prepared peptide modified chitosan films as described earlier and in addition, we also blocked the films with 5% BSA for 1 h. After blocking, we washed the films 3× with PBS to prep for subsequent enzyme assembly. We added varying amounts of LuxSQ to peptide-modified chitosan films and crosslinked the enzymes to films by mTG. The molar ratio between LuxS and mTG was constant at 1:3 for all cases. We discovered heuristically this was a good ratio for assembly. Con-

(a) Electrical deposition of chitosan to gold

(b)

FIGURE 5. (a) Schematic indicating electrical deposition of chitosan films onto gold chips.^{10,26} (b) Conjugation of LuxS and Pfs, representing a two enzyme metabolic pathway to peptide-chitosan films on gold chips and on microtiter plates. Controls include Pfs and LuxS enzymes incubated with KYK peptide chitosan films (as in Fig. 3) but without mTG. Error bars indicate $n = 3$.

trols consisted of identical experiments without mTG. After conjugation at RT for 60 min, we washed the films 3× with PBS and measured enzyme activity. We observed that there was a monotonic increase in HCY generated with increased LuxS_Q concentration. There were no significant differences among the negative controls, as expected.

Assembly of Two Enzyme Metabolic Pathway to Peptide Modified Chitosan on Chips

Having demonstrated the specificity of the two-step approach, we next assembled two enzymes onto peptide modified chitosan and mediated flux between the two enzymes. For this purpose, we used both enzymes Pfs_Q and LuxS_Q. That is, we generated Pfs engineered with penta-glutamine tag at C terminus (indicated as Pfs_Q) for this purpose. For conjugation, we used chitosan films both casted on plates as well as electrically-deposited chitosan films on gold chips and conjugated the KYK peptide to the chitosan films (Scheme in Fig. 5 and “Methods” section). Later, both films were blocked with 5% BSA for 60 min. After blocking, films were washed 3× with PBS and used for experiments. To demonstrate assembly onto gold chips, we first assembled green fluorescent protein (E_Q) using the transglutaminase-catalyzed approach (Fig. S2). To demonstrate assembly of the enzyme cascade, we first added varying concentrations of LuxS_Q to peptide modified chitosan using mTG (molar ratio of LuxS:mTG of 1:3). After a 60 min incubation, both the plates and chips were washed 3× with PBS. Under similar conjugation conditions, the second enzyme, Pfs_Q, was crosslinked to chitosan–peptide–LuxS_Q film (again with a molar ratio of Pfs:mTG of 1:3). After this second round of conjugation, plates and gold chips were washed again 3× with PBS and incubated with 1 mM SAH. After 2 h of incubation at 37 °C, the HCY generated was measured by Ellman’s assay. HCY levels generated from enzymes assembled to both plates and chips are indicated in Fig. 5b. Consistent with previous results, we found up to ~20 fold increases in biochemical flux through the assembled pathways. We also note that there were relatively minor differences due to the electrodeposited (denoted “On chips”) vs. the cast chitosan films (denoted “On plates”), although the deposited activities were somewhat higher. In all cases, the HCY generated from negative control experiments were insignificant.

DISCUSSION

In this study we compared two different biofabrication approaches for the assembly of proteins onto

chitosan films. First, a tyrosinase-mediated approach involving conjugation of tyrosine engineered proteins to chitosan was examined for specificity and non-specific binding. In previous work, we demonstrated assembly of tyrosinase linked proteins on chitosan¹⁷ but did not address the non-specific interactions between the enzyme (Pfs) and chitosan. In addition, our previous studies focused on Pfs and GFP, which are more stable than LuxS. The two-step approach here involving tyrosinase mediated coating of chitosan film with Lys-Tyr-Lys tripeptide followed by direct conjugation of glutamine engineered proteins to peptide modified chitosan film using transglutaminase was simple, and exhibited far less background activity.

The reasons for *reduced* nonspecific binding is confounding given that we modified the surface of chitosan with peptides containing more positively-charged free amines. Beyond charges on the surface, there might be other factors at play that influence the extent of non-specific binding of proteins to chitosan. The thickness of the modified chitosan films and the method of deposition of these films have been reported to play a role in nonspecific binding. Specifically, reduced binding was attributed to reduced mobility of the different chitosan films.²⁹ In our case, the addition of tripeptide might have reduced the mobility of the chitosan films resulting in reduced non-specific binding. Also, poly-lysine molecules, owing to their charge, have been shown to bind to metal oxides.¹⁴ There is a remote possibility that tripeptides containing lysines bound to the surface of gold, preventing non-specific binding of proteins to gold, however, given the thickness of the films, it is quite unlikely to have played a major role. Analogously, should the peptide bind both gold and chitosan, reduced mobility could be envisioned.

Alternatively, proteins have been known to undergo refolding upon contact with a charged surface.¹³ In this study, since the non-specific binding is reported indirectly through enzyme activity on the surface of the biosensors, LuxS, the rate limiting factor in the two enzyme QS pathway might have refolded upon contact with a tripeptide leading to reduced enzyme activity. Perhaps this was a contributing factor. In either case above, additional experiments would be needed to evaluate the mechanistic causality of the reduced non-specific binding.

In sum, the two-step assembly process entails an extra step of modifying chitosan films with peptides prior to crosslinking with engineered proteins, thereby increasing the overall fabrication time. However, by switching from the use of empty chitosan films to peptide-modified films for crosslinking, we were able to transition from the low k_{cat} tyrosinase to mTG for final assembly. In biosensor and other fabrication processes, the assembly of the enzyme onto the biosensor surface

is the crucial time sensitive step. Here, we were able to accelerate crosslinking from 12 to 1 h through the use of mTG and peptides. Also, given the relatively low requirement for mTG (1.32 U/mL), we believe crosslinking times can be reduced even further. Parenthetically, since the peptide modified films are relatively stable, the peptide modification of chitosan might be done ahead of time and kept ready for enzyme crosslinking.

From a biofabrication standpoint, we have displayed construction of a two enzyme metabolic pathway on chips wherein enzymes/biomaterials can be specifically crosslinked within a short duration across a wide concentration range of 15 μ M to 15 nM and with relatively minimal nonspecific binding at all concentrations. We envisage using this methodology for the fabrication of biomaterials in bioMEMS devices, sensors and lab chip devices, some of which have already been shown.^{11,12,26} In general, by combining the pH-controlled solubility of chitosan with peptide-mediated reduced non-specific binding, peptide-chitosan complexes created here using transglutaminase are likely to find utility in antigen-antibody and immunodetection assays and other sensing applications on chips and within devices.

ELECTRONIC SUPPLEMENTARY MATERIAL

The online version of this article (doi:10.1007/s12195-016-0472-5) contains supplementary material, which is available to authorized users.

ACKNOWLEDGEMENTS

N-B designed and performed the experiments, H-P assisted in performing the experiments, Y-L and G-F-P provided technical inputs and reviewed the manuscripts, N-B and W-E-B authored the manuscripts. Funding provided by the Defense Threat Reduction Agency (DTRA, HDTRA1-13-1-0037), the National Science Foundation (CBET 1160005, CBET 1264509, DMREF 1435957), and the R. W. Deutsch Foundation.

CONFLICT OF INTEREST

Narendranath Bhokisham, Yi Liu, Haig Pakhchian, Gregory F. Payne and William E. Bentley declare no conflicts of interests.

ETHICAL STANDARDS

No human studies or animal studies were carried out by the authors for this article.

REFERENCES

- ¹Bhokisham, N., *et al.* Data on biochemical fluxes generated from biofabricated enzyme complexes assembled through engineered tags and microbial transglutaminase. *Data Brief* 2016. doi:10.1016/j.dib.2016.07.005.
- ²Bhokisham, N., *et al.* Modular construction of multi-sub-unit protein complexes using engineered tags and microbial transglutaminase. *Metab. Eng.* 38:1–9, 2016. doi:10.1016/j.ymben.2016.05.004.
- ³Chaki, N. K., and K. Vijayamohan. Self-assembled monolayers as a tunable platform for biosensor applications. *Biosens. Bioelectron.* 17:1–12, 2002. doi:10.1016/S0956-5663(01)00277-9.
- ⁴Charles, P., *et al.* Reduction of non-specific protein adsorption using poly(ethylene) glycol (PEG) modified polyacrylate hydrogels in immunoassays for staphylococcal enterotoxin B detection. *Sensors* 9:645, 2009.
- ⁵Chen, T., H. D. Embree, L.-Q. Wu, and G. F. Payne. In vitro protein-polysaccharide conjugation: tyrosinase-catalyzed conjugation of gelatin and chitosan. *Biopolymers* 64:292–302, 2002. doi:10.1002/bip.10196.
- ⁶Chen, H., *et al.* Anti-HER2 antibody and ScFvEGFR-conjugated antifouling magnetic iron oxide nanoparticles for targeting and magnetic resonance imaging of breast cancer. *Int. J. Nanomed.* 8:3781–3794, 2013. doi:10.2147/IJN.S49069.
- ⁷Duckworth, H. W., and J. E. Coleman. Physicochemical and kinetic properties of mushroom tyrosinase. *J. Biol. Chem.* 245:1613–1625, 1970.
- ⁸Fernandes, R., and W. E. Bentley. AI-2 biosynthesis module in a magnetic nanofactory alters bacterial response via localized synthesis and delivery. *Biotechnol. Bioeng.* 102:390–399, 2009. doi:10.1002/bit.22078.
- ⁹Fernandes, R., V. Roy, H. C. Wu, and W. E. Bentley. Engineered biological nanofactories trigger quorum sensing response in targeted bacteria. *Nat. Nanotechnol.* 5:213–217, 2010. doi:10.1038/nnano.2009.457.
- ¹⁰Fernandes, R., *et al.* Electrochemically induced deposition of a polysaccharide hydrogel onto a patterned surface. *Langmuir* 19:4058–4062, 2003. doi:10.1021/la027052h.
- ¹¹Fernandes, R., *et al.* Biological nanofactories facilitate spatially selective capture and manipulation of quorum sensing bacteria in a bioMEMS device. *Lab Chip* 10:1128–1134, 2010. doi:10.1039/B926846D.
- ¹²Gordonov, T. *et al.* Electronic modulation of biochemical signal generation. *Nat. Nano* 9:605–610, 2014. doi:10.1038/nnano.2014.151. <http://www.nature.com/nnano/journal/v9/n8/abs/nnano.2014.151.html#supplementary-information>.
- ¹³Gray, J. J. The interaction of proteins with solid surfaces. *Curr. Opin. Struct. Biol.* 14:110–115, 2004. doi:10.1016/j.sbi.2003.12.001.
- ¹⁴Huang, N.-P., *et al.* Poly(L-lysine)-g-poly(ethylene glycol) layers on metal oxide surfaces: surface-analytical characterization and resistance to serum and fibrinogen adsorption. *Langmuir* 17:489–498, 2001. doi:10.1021/la000736+.
- ¹⁵Kim, E., T. Gordonov, W. E. Bentley, and G. F. Payne. Amplified and in situ detection of redox-active metabolite using a biobased redox capacitor. *Anal. Chem.* 85:2102–2108, 2013. doi:10.1021/ac302703y.
- ¹⁶Kim, E., *et al.* Chitosan to connect biology to electronics: fabricating the bio-device interface and communicating across this interface. *Polymers* 7:1, 2015.

- ¹⁷Lewandowski, A. T., D. A. Small, T. Chen, G. F. Payne, and W. E. Bentley. Tyrosine-based, “Activatable Pro-Tag”: enzyme-catalyzed protein capture and release. *Biotechnol. Bioeng.* 93:1207–1215, 2006. doi:[10.1002/bit.20840](https://doi.org/10.1002/bit.20840).
- ¹⁸Lewandowski, A. T., *et al.* Towards area-based in vitro metabolic engineering: assembly of Pfs enzyme onto patterned microfabricated chips. *Biotechnol. Prog.* 24:1042–1051, 2008. doi:[10.1002/btpr.44](https://doi.org/10.1002/btpr.44).
- ¹⁹Lewandowski, A. T., *et al.* Protein assembly onto patterned microfabricated devices through enzymatic activation of fusion pro-tag. *Biotechnol. Bioeng.* 99:499–507, 2008. doi:[10.1002/bit.21580](https://doi.org/10.1002/bit.21580).
- ²⁰Lewis, K. B., D. C. Teller, J. Fry, G. W. Lasser, and P. D. Bishop. Crosslinking kinetics of the human transglutaminase, factor XIII[A2], acting on fibrin gels and γ -chain peptides. *Biochemistry* 36:995–1002, 1997. doi:[10.1021/bi961636z](https://doi.org/10.1021/bi961636z).
- ²¹Luo, X., *et al.* Biofabrication of stratified biofilm mimics for observation and control of bacterial signaling. *Biomaterials* 33:5136–5143, 2012. doi:[10.1016/j.biomaterials.2012.03.037](https://doi.org/10.1016/j.biomaterials.2012.03.037).
- ²²Mironov, V., *et al.* Biofabrication: a 21st century manufacturing paradigm. *Biofabrication* 1:022001, 2009.
- ²³Molloy, S., *et al.* Engineering of a bacterial tyrosinase for improved catalytic efficiency towards D-tyrosine using random and site directed mutagenesis approaches. *Biotechnol. Bioeng.* 110:1849–1857, 2013. doi:[10.1002/bit.24859](https://doi.org/10.1002/bit.24859).
- ²⁴Nakamura, M., S. Iwanaga, C. Henmi, K. Arai, and Y. Nishiyama. Biomaterials and biomaterials for future developments of bioprinting and biofabrication. *Biofabrication* 2:014110, 2010.
- ²⁵Wu, L.-Q., and G. F. Payne. Biofabrication: using biological materials and biocatalysts to construct nanostructured assemblies. *Trends Biotechnol.* 22:593–599, 2004. doi:[10.1016/j.tibtech.2004.09.008](https://doi.org/10.1016/j.tibtech.2004.09.008).
- ²⁶Wu, H.-C., *et al.* Biofabrication of antibodies and antigens via IgG-binding domain engineered with activatable pen-ta-tyrosine pro-tag. *Biotechnol. Bioeng.* 103:231–240, 2009. doi:[10.1002/bit.22238](https://doi.org/10.1002/bit.22238).
- ²⁷Yang, X., X.-W. Shi, Y. Liu, W. E. Bentley, and G. F. Payne. Orthogonal enzymatic reactions for the assembly of proteins at electrode addresses. *Langmuir* 25:338–344, 2009. doi:[10.1021/la802618q](https://doi.org/10.1021/la802618q).
- ²⁸Yi, L., *et al.* Biofabrication to build the biology–device interface. *Biofabrication* 2:022002, 2010.
- ²⁹Zhou, Y., *et al.* Chitosan-*N*-poly(ethylene oxide) brush polymers for reduced nonspecific protein adsorption. *J. Colloid Interface Sci.* 305:62–71, 2007. doi:[10.1016/j.jcis.2006.09.058](https://doi.org/10.1016/j.jcis.2006.09.058).