

Assay Development Tips for COOH Agile R100 Biosensor Chips

- Ambient compounds may contribute nonspecifically to biosensor response, decreasing the signal-to-noise ratio (SNR).
- Blocking and quenching steps are used with biosensors to reduce the contributions from background compounds that interfere with the measurement signal.
- Several aspects of biosensor immobilization can be optimized to drive down the effects of nonspecific interactions and increase the SNR for targeted detection, which is most helpful when sensing low concentrations of analyte with low signal.
- COOH Agile R100 biosensor chips deliver sensitive and specific responses with high SNR using effective blocking and quenching techniques.

ABSTRACT

Characterization of biomolecular interactions is the cornerstone of a wide range of diagnostic and therapeutic technologies. The design of the biosensor surface, including target immobilization, quenching of grafting sites, and blocking of the surface, are discussed in detail to improve the sensitivity and specificity of novel electronic-based biosensors. Interactions of buffers with the biosensor surface and target are also detailed. Further, the benefits of using FEB technology as a high-quality biosensor, and the corresponding blocking and quenching protocols on the Agile R100 system are examined.

INTRODUCTION

Whether using an ELISA, a Western blot, or immunohistochemical staining, immunoassays require blocking a surface to reduce nonspecific binding. Blocking then becomes a tradeoff between low background noise and high sensitivity (i.e. true positive rate) and specificity (i.e. true negative rate).¹ The optimal blocking reagent and technique for any given assay will be deduced empirically and may not be the definitive solution.²

Binding events trigger some change in a property of the system that can be detected, quantified, and translated into novel information about the molecules under study. Biomolecules interact with their surroundings using many covalent and noncovalent mechanisms with varying degrees of affinity or avidity. However, biology does not act in a vacuum; there are

always a myriad of unrelated proteins, nucleic acids, cells, ions, and other molecular complexes that intrude on the interaction of the targeted chemistry. The optimized design of the sensing system, including the choice of target, preparation of binding surface, and diluent contents, all affect the ultimate sensitivity and specificity of the biosensor by reducing the level of nonspecific interactions that produce a sensor response.³

Performance metrics of a biosensor come from its ability to immobilize targets (without destabilization or degradation) while maintaining binding site accessibility to target molecules (i.e. sensitivity of the biosensor) and rejecting nonspecific interactions with the targets and the sensor surface (i.e. specificity of the biosensor).² The most relevant interactions to the functionality of a biosensor are hydrophobic, electrostatic, polar, hydrogen bonding, and complex formation. All but complex formation are highly nonspecific, meaning many components in the biosensor cell are contributing to the overall sensor response. A high-quality biosensor must maximize the specific signal coming from the target and analyte pair and reduce the nonspecific signal coming from everything else. To accomplish this goal, there are several facets that can be optimized, including signal transduction, surface preparation, and diluent contents.

Field Effect Biosensing (FEB) technology integrates biologically active materials with a field effect transistor.⁴ Biosensors made with FEB technology couple a field effect transistor element to a biological recognition element (i.e. a target) that detects the interaction with an analyte. When the analyte binds to the target, the charge distribution at the surface changes, triggering a change in field effect transistor conductance. This value is directly read by measuring the source and drain current, effectively converting biological events (e.g. target and analyte interactions) into electrical signals, namely current and capacitance changes. The magnitude of the current and capacitance changes corresponds to the analyte concentration, and kinetic binding rates can be extracted for the characterization of the interaction with analyte. FEB tools require simpler blocking and quenching protocols because the electronic sensor signal is transmitted when the bound target interacts with a specific molecule, and therefore binding

kinetics are less affected by ambient biomolecules than optical systems.⁵

TECHNIQUES TO INCREASE SIGNAL-TO-NOISE

Signal Transduction

Agile R100 uses FEB technology that incorporates highly sensitive nanomaterials into the sensor cell to pick up every nanoscale interaction happening at the sensor surface. While this significantly improves sensitivity to binding events, it can also increase the noise from nonspecific interactions. Blocking and quenching steps become critical when the transduction element can detect every type of biomolecular interaction, and by incorporating simple blocking and quenching techniques, an FEB device can be an effective high-quality sensor with high SNR.

Surface Preparation

Immobilize

The surface of a COOH Agile R100 biosensor chip consists of a graphene layer supported on a silicon chip with targets immobilized across the graphene surface. The target may be attached to the sensor surface with standard carbodiimide crosslinker chemistry (N-Ethyl-N'-(3-dimethylaminopropyl) carbodiimide hydrochloride [EDC] and N-Hydroxysulfosuccinimide [sNHS]).⁵ FEB sensors are able to crosslink with any target such as antibodies, antigens, nucleic acids, aptamers, proteins, lipids, or other free amine containing biomolecule via an amide linkage (Figure 1A).

EDC and sNHS are used in conjunction to activate the carboxyl groups that have been placed on the surface of the graphene. The EDC and sNHS chemicals are ideal because they are water soluble, work at a pH that many biological molecules are stable in, and have been thoroughly tested for more than 50 years.⁶ The EDC converts carboxyl groups into amine-reactive intermediates. While the sNHS is not necessary, it greatly enhances crosslinking efficiency by increasing the stability of the reactive intermediate.⁶ sNHS is well-suited for water soluble experiments, while N-hydroxysuccinimide (NHS) is best used with organic solvents.

Immobilize Target

There are many factors to consider when selecting an appropriate target for an Agile R100. First and foremost, the target must be able to bind to the sensor surface using a free amine moiety. However, if a free amine is not available, commercial crosslinkers may be employed to adapt the binding chemistry. Another consideration for the ideal target is whether the free amine site is near the binding region of the

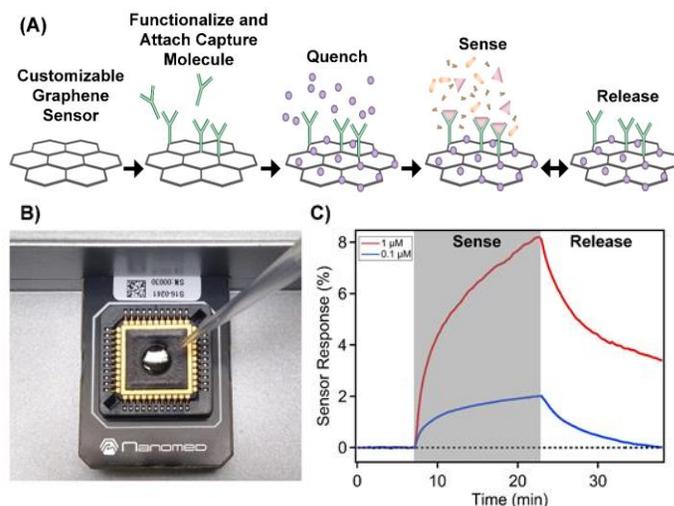


Figure 1: (A) The schematic of Agile R100's working principle. The blank FEB surface, the FEB surface immobilized with targets, blocking and quenching solutions to prevent nonspecific binding, analyte association, and analyte dissociation in assay buffer. (B) Agile R100 as a singleplex assay. (C) An example of sensor response (percentage change from baseline) with respect to time for Agile R100. Two concentrations of analyte interact with targets on Agile R100 (two experiments superimposed in one graph). Analyte is added at 8 min and released at 23 min, causing a return to baseline.

analyte. For example, with antibodies, the analyte binding only happens in a limited location, the complementarity determining region (CDR). The CDR must be left available for binding and not obscured by the sensor surface due to an unfavorable target binding orientation. Without an engineered tag on the target, random amine binding will not allow for every molecule on the surface to have the CDR region available. As an ensemble, sufficient targets will be bound across the entire sensor in the correct orientation to contribute to the overall sensor response, and sensor response is not negatively affected. Directional crosslinking to control target orientation may be necessary for a given target and analyte pair.

Blocking and Quenching

The goal of blocking and quenching (on an Agile R100 as well as on other biosensors) is to increase the SNR by physically blocking nonspecific interactions without interfering with desired and specific binding interactions. Such nonspecific interactions can arise from diluent or sample components binding to the target immobilization chemistry if there are unreacted binding sites still available. In addition, large charged molecules in the diluent may also adsorb onto the sensor surface and induce a sensor response. These scenarios are resolved using a blocking agent to react with all available EDC/sNHS active sites not bound by the target, and a quenching agent to passivate the entire biosensor surface, shielding it from undesirable adsorption effects. In Agile R100, the respective blocking and quenching chemicals are called Quench 1 and Quench 2, and blocking and quenching are

performed after target immobilization and before the addition of analyte in assay buffer (Figure 1A). Performance metrics of Quench 1 and Quench 2 have been described in detail using a monoclonal antibody and cytokine interaction in phosphate buffer saline (PBS) and simulated serum.⁷

Diluent Buffer Contents

Assay buffer

Diluent components may also interfere with the specific signal on a biosensor by increasing the nonspecific noise and thereby decreasing the SNR. Analyte are carried in an aqueous solution or buffer, referred to as the assay buffer. Some analyte require specific buffer components, such as DMSO for small molecule drug candidates or bovine serum albumin (BSA) used as a carrier molecule to increase stability of cytokines. These additional buffer components may bind nonspecifically to the biosensor surface and can lead to an increase in noise. When the blocking and quenching protocols discussed here are employed on Agile R100, nonspecific molecules in assay buffer (such as DMSO and BSA) do not nonspecifically bind to the sensor surface and therefore do not significantly add to the overall noise of the sensor response.

The proposed blocking and quenching techniques prevent nonspecific binding of diluent components to the sensor surface, but the diluent components may also bind nonspecifically to the immobilized target. In complex analyte solutions (e.g. cell lysate or serum), adding detergents, such as a low concentration of Tween20, to the assay buffer may help to prevent nonspecific binding to the target with FEB technology and with other biosensing platforms. Such detergents should be appropriately blocked from inducing a nonspecific response to the sensor surface using the proposed blocking and quenching techniques with FEB technology.

Analyte

The analyte may nonspecifically bind to the sensor surface in addition to specifically binding with the target, thereby increasing noise of the biosensor. Commonly used in immunoassays such as ELISAs, animal serum albumins (e.g. bovine, chicken, mouse, horse, or human) in the assay buffer can help increase signal by passively absorbing to the sensor surface, thereby preventing the analyte from binding nonspecifically to the assay surface. However, additional blocking in this manner with FEB technology such as Agile R100 is not necessary due to the electronic modality of the binding readout as well as the blocking and quenching protocols discussed here.

ASSOCIATION AND DISSOCIATION

On an Agile R100 high quality biosensor, a calibration step normalizes the sensor response to a baseline by measuring the sensor response to assay buffer prior to the addition of analyte. When analyte is added, the sensor response relative to the

calibrated baseline is measured and reported as the percent change due to the analyte interacting with the targets (Figure 1B and 1C). Since the FEB sensor is highly sensitive to electrical changes in the surrounding solution, Agile R100 with FEB technology can accurately measure binding kinetics during the association and dissociation of target and analyte, which cause changes in the electrical properties of the surrounding solution near the graphene surface.

CONCLUSIONS

High-quality biosensors such as Agile R100 excel at capturing signal from the biomolecular interaction under investigation while rejecting background noise from all other sources, yielding high sensitivity and specificity. Crucial aspects of this task include robust target immobilization chemistry, maintenance of target functionality, quenching of active chemical moieties on the sensor surface, and passivation of the surface to minimize nonspecific adsorption. Chemistries have been developed for Agile R100 to successfully block nonspecific adsorption and quench unreacted surface compounds to increase the SNR. Using FEB technology, highly sensitive biosensing data is achieved, and using the developed blocking and quenching methods, highly specific interactions are acquired. Agile R100 uses FEB technology that links biology directly with electronics for the first time, providing superior accuracy, streamlined processes, and drastic cost savings.

REFERENCES

1. Gibbs J. Effective Blocking Procedures. *ELISA Tech Bull.* 2001;Corning In(Kennebunk, ME).
2. Zourob M. *Recognition Receptors in Biosensors.*; 2010. doi:10.1007/978-1-4419-0919-0.
3. Herold KE, Rasooly A. Biosensors and molecular technologies for cancer diagnostics. *Ser sensors.* 2012. doi:10.1118/1.4804051.
4. Lerner MB, Pan D, Gao Y, et al. Large scale commercial fabrication of high quality graphene-based assays for biomolecule detection. *Sensors Actuators, B Chem.* 2016;1-7. doi:10.1016/j.snb.2016.09.137.
5. Shao Y, Wang J, Wu H, Liu J, Aksay IA, Lin Y. Graphene based electrochemical sensors and biosensors: A review. *Electroanalysis.* 2010;22(10):1027-1036. doi:10.1002/elan.200900571.
6. D.G. Hoare DEKJ. A Procedure for the Selective Modification of Carboxyl Groups in Proteins. *J Am Chem Soc.* 1966;88(9):2057-2058.
7. Lerner M, Nokes J, Locascio L, Barron F. *Nonspecific Binding Is Prevented on Agile R100.*; 2016.