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Signal processing approach to probe chemical space for discriminating redox signatures

Mijeong Kang[a,b], Eunkyoung Kim[a,b], Shuo Chen[c], William E. Bentley[a,b], Deanna L. Kelly[c], Gregory F. Payne[a,b,*]

[a] Institute for Bioscience & Biotechnology Research, University of Maryland, College Park, MD 20742, USA
[b] Fischell Department of Bioengineering, University of Maryland, College Park, MD 20742, USA
[c] Maryland Psychiatric Research Center, University of Maryland School of Medicine, Baltimore, MD 21228, USA

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ABSTRACT

The discovery of discriminating molecular biomarkers often couples omics for data acquisition with advanced information processing methods for data analysis. Here, we move information processing upstream for data acquisition and report that a sample's "chemical space" can be actively probed using a tailored sequence of redox-based input signals. Specifically, we use a redox-active iridium salt (K_3IrCl_6) and an oxidative pulse-redox-relaxation input sequence to probe serum samples for chemical information. Optical and electrical output responses are collected simultaneously and analyzed using signal metrics that are sensitive to component and concentration dependent chemical information. We use the example of schizophrenia to illustrate the potential of this signal processing approach to rapidly discover discriminating signatures using simple and inexpensive instrumentation. These studies indicate that redox-probing provides an orthogonal measurement approach to accelerate biomarker discovery and further suggest a simple means to supply chemical information for the internet-of-things in medical and consumer applications.

1. Introduction

Currently there is great excitement in applying artificial intelligence and machine learning to omics-acquired archival data to reveal discriminating molecular signatures for complex and poorly understood diseases (Aebersold and Mann, 2016; Clarke et al., 2008; de Castro and Priego-Capote, 2018; Kim et al., 2016b; Libbrecht and Noble, 2015; lik and Chinnaiyan, 2017; Quinones and Kaddurah-Daouk, 2009; Xia et al., 2009). While there are advantages to the use of omics as a "front end" to generate archival data rich in granular molecular detail, there are also limitations. First, omics methods tend to be slow, expensive, and rate limiting. Second, the quality of omics data can be reduced by sampling and measurement errors, and a loss of context-dependent information (e.g., a sample's pH and non-covalent binding interactions) (Feist and Hummon, 2015; Tabb et al., 2010; Weis, 2005). Third, omic discoveries may not be directly translatable to routine clinical practice and point-of-care analysis if specialized, complex or expensive methods are required (Chan et al., 2015; Hall et al., 2011; Ptolemy and Rifai, 2010). Here we report an alternative "front end" that enlists advances in signal processing (not analytical chemistry) to probe "chemical space" for global redox-based chemical features. This signal processing approach does not aim to replace traditional omics approaches for generating granular details of chemical composition, but rather this approach aims to access orthogonal systems-level redox information using simple, sensitive and inexpensive instrumentation.

To demonstrate the potential of this signal processing approach, we use schizophrenia as our initial clinical example. Schizophrenia is a complex poorly understood mental health disorder that lacks simple objective measures (Vargas, 2014). Currently, a diagnosis of schizophrenia is based on clinical assessments by mental health professionals which can be subjective, slow and require that the disease has progressed to a stage of rather severe symptom manifestations (Chan et al., 2015; Perkins et al., 2014; Weickert et al., 2013). There is considerable interest in developing blood tests to provide objective measures that enable clinicians to accelerate diagnosis and to monitor treatment efficacy. One of the most successful approaches was the development of multiplexed serum immunoanalysis that measures 51 serum analytes (Wehler and Preskorn, 2016). While the focus on specific chemical biomarkers is important for researchers unraveling the underlying pathophysiological mechanisms, this multiplexed immunoanalysis has been less successful for clinicians presumably because of the complexity, cost, low specificity and high false positives (Bahn et al., 2011;

* Corresponding author at: Institute for Bioscience & Biotechnology Research, University of Maryland, College Park, MD 20742, USA.
E-mail address: gpayne@umd.edu (G.F. Payne).

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Wehler and Preskorn, 2016).

Our signal processing approach is fundamentally different from conventional approaches in two ways: we focus on global measurements (not measurement of individual molecules); and we focus on measurements that can be made simply, at low cost and with instrumentation that can be used at/near point-of-care. Specifically, our approach relies on simple electrochemical measurements of global redox-based chemical features from serum. Emerging research indicates that schizophrenia is linked to redox dysregulation and oxidative stress (Bitanihirwe and Woo, 2011; Emiliani et al., 2014; Hardingham and Do, 2016) and this suggests that appropriate redox-measurements can access relevant information (Flatow et al., 2013; Kim et al., 2016a; Schiavone and Trabace, 2017). Our goal is to devise a method to probe the redox information in serum sample in such a way that we can generate an information-rich data stream and then to enlist the speed, power and convenience of microelectronics and signal processing to discern robust signatures from this data stream.

This signal processing approach resembles the electrophysiologist's use of electrodes to reveal global features of neuronal communication without focusing on the granular details of the individual ions responsible for the measured currents. One fundamental difference however, is that electrophysiologists measure ion flows, while the charged particles "flowing" through redox reactions are electrons. But while the charge carriers in redox biology are molecular in nature, they often act at global levels. For instance, biology tends to use somewhat generic reductants NAD(P)H, antioxidants (e.g., glutathione) and signaling molecules (e.g., H_2O_2) that can engage numerous pathways simultaneously. Further, inflammatory responses (e.g., oxidative burst) generate reactive species that act non-specifically (Dickinson and Chang, 2011; Nathan and Cunningham-Bussel, 2013) while indications that numerous pathologies are linked to redox dysregulation and oxidative stress suggest a more global phenomenon (Barnham et al., 2004; Baynes and Thorpe, 1999; Finkel and Holbrook, 2000; Manda et al., 2015; Markesbery, 1997; Seddon et al., 2007; Varga et al., 2015; Yao and Keshavan, 2011). Thus, while redox activities may share features of molecularly specific mechanisms (e.g. receptor-mediated signaling pathways), they also share features of the more global mechanisms of neuromuscular electrical signaling. The underlying hypothesis of this work is that redox probing can access global redox activities to gain systems-level information.

Fig. 1a illustrates our signal processing approach in which a sample (e.g., diluted serum) is actively probed for redox-based chemical information using tailored chemical and electrical inputs. The chemical input is an iridium-based redox mediator (Ir) that can exchange electrons with a wide range of components and report electron exchange by redox-state-dependent optical and electrical outputs (Kim et al., 2016a). In particular, Ir has high reactivities toward thiols (e.g., cysteine residues of proteins) which are expected to be important redox-active species in serum and targets of oxidative damage. The electrical input is a sequence of oxidative voltage pulses that serve to switch the inert reduced iridium (designated Ir^{RED}, colorless) into its oxidized state (designated Ir^{OX}, yellowish) which diffuses into and probes the sample for redox-dependent features (e.g., reactive free radicals, protective reductants, or oxidized proteins). Redox-probing essentially means that the Ir^{OX} "searches" the local environment for electron-rich molecular species from which it can extract electrons thereby switching the Ir^{OX} back to the Ir^{RED} state. As illustrated in Fig. 1b, the regenerated Ir^{RED} can again be re-oxidized at the electrode and thus this Ir-based redox-cycling serves to mediate the transfer of electrons from the sample to the electrode. Importantly, such redox-cycling substantially amplifies the current (a measure of the rate of electron-exchange at the electrode) and attenuates the optical absorbance associated with the colored Ir^{OX}. These electrical and optical output responses can be measured simultaneously using a perforated electrode in a spectroelectrochemical cell illustrated in Fig. 1a and Supplementary Fig. S1. Subsequent back-end processing of the multimodal output signals provides robust and

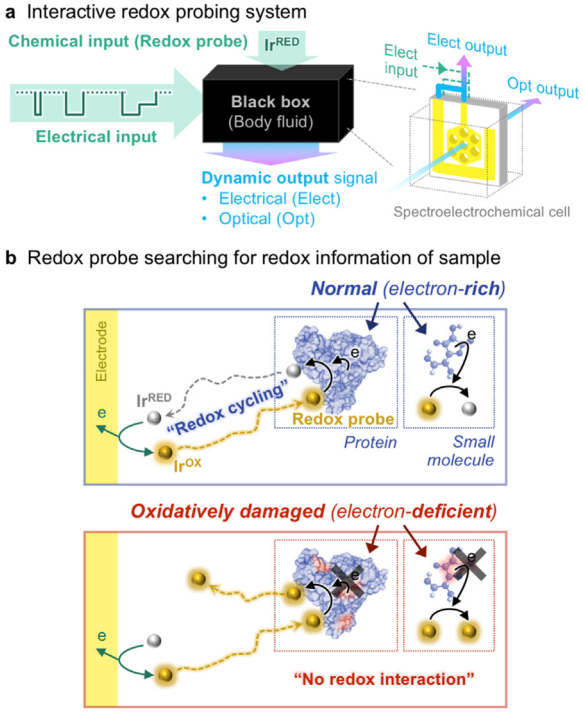

Fig. 1. Interactive redox probing for global chemical features. (a) Schematic illustrating that the sample is probed using a chemical input (Ir-based redox mediator) and an electrical input sequence, while the dynamic response of the sample is detected spectroelectrochemically by the simultaneous measurement of electrical and optical output signals. **(b)** Illustration of the Ir-based redox-cycling that serves to transfer electrons from reductants in the sample to the electrode: redox-cycling leads to an amplification of the electrical output (oxidative charge transfer) and an attenuation of the optical absorbance (due to the colored Ir^{OX}).

rich information (Liu et al., 2015) that would be available for analysis by big data methods to facilitate biomarker discovery (Ravi et al., 2017). Here, we show that our signal processing approach can access redox-based chemical features from clinical serum and yield discriminating signature patterns that correlate to clinical assessments of disease. We envision this signal processing approach should provide a simpler path to translation because of the simplicity, speed and convenience of the instrumentation. Further, this global discovery-based approach can complement more detailed omics based methods aimed at discerning the underlying molecular mechanisms of the disease.

2. Materials and methods

2.1. Materials

Iridium (K_3IrCl_6), glutathione (reduced), albumin (from human serum), uric acid, ascorbic acid, bilirubin and human serum were purchased from Sigma-Aldrich. Buffered solutions (0.1 M phosphate, pH 7.4) were prepared with deionized water (> 18 MΩ, Millipore).

2.1.1. Clinical serum samples

Blood serum samples were collected at the Maryland Psychiatric Research Center, University of Maryland School of Medicine with standard procedures as required by The State of Maryland Department of Mental Health and Hygiene (DHMH) and the University of Maryland School of Medicine. The sampled populations include individuals with a DSM-IV diagnosis of Schizophrenia or Schizoaffective Disorder and individuals without diagnosis. Serum samples were collected from the supernatant of centrifuged (at 3000 rpm) blood of each participant and stored frozen (−80 °C) before redox probing. Chemical analysis of the