Quantum Electrochemical Spectroscopy (QES): A Novel AI-driven Spectroscopic Approach to Simplify Bioanalytical Development in Biomanufacturing and Synthetic Biology

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Introduction and Goals

- Manufacturing biopharmaceutical products, requires demonstration of process control from raw material through finished product.
- Typically, hundreds of assays are conducted with a diverse set of analytical techniques, each of which requires extensive development, bespoke reagents, complex sample preparation, expensive instrumentation and highly trained staff.

Goal: to simplify biopharmaceutical testing as well as early detection of process anomalies that may lead to batch failure

ProbiusMini QES Platform

Using molecular vibrations as units of biological information

- The Probius™ QES instrument performs a voltage scan of the sample at the nanoscale electrochemical interface to acquire the vibrational fingerprints of the molecules therein.
- QES translates a sample aliquot into a high-dimensional phenotypic signature, a digital twin, that can be re-analyzed and redefined in the cloud without the need of additional sample volume

MiniQES Reader

Simple operation and small benchtop footprint

QES Disposable Vial

Proprietary sensor enables QES without reagents

Bioanalytical Cloud Services

To enable classifiers, biomarker discovery, and analyte quantitation

Overall procedure

Small Sample Requirement:

- 2-20 ul of broth, extract or supernatant

Simple Data Acquisition:

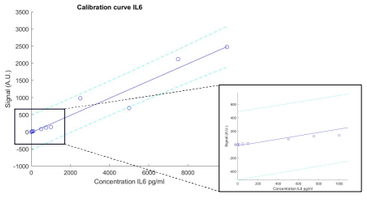
- 1 pipetting step, ~30-60 min
- No reagents or sample prep

Versatile Data Analysis:

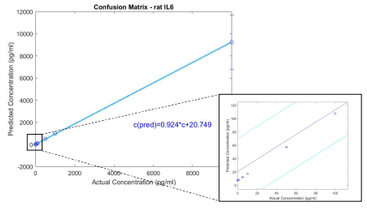
- Quantify Analytes & Classify Samples in the digital domain
- Build your own assay (ByOA)

1 Quantification of low abundance protein

Raw QES spectrum from rat recombinant IL-6 at 10pg/ml. QES spectra are generated by measuring vibrational response in frequency bins to incident electron energy, for a range of electronic energy values.



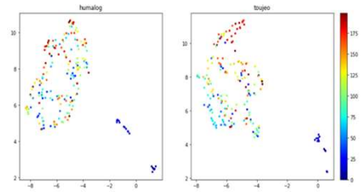
Calibration curve generated from raw spectra by identifying vibrational modes specific to target analyte (rrIL-6) and monotonically sensitive to its concentration in standards.
Dark blue line is least squares fit and dotted cyan line bounds 95% CI

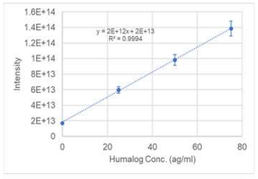


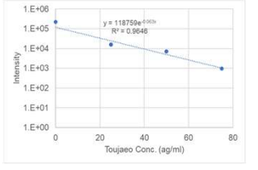
Prediction of concentrations of rat IL-6 in 'blinded' rat serum spiked samples
High concordance and high limit of detection is observed between predicted and actual values with a low CV < 5%
Dark blue line is least squares fit and dotted cyan line bounds 95% CI

2 High- sensitivity in protein isoform differentiation

- ✓ QES is used to *differentiate* and *quantify* long-acting (Toujeo/insulin glargine) and short-acting (Humalog/insulin lispro) in a mixture, where the two insulin-derived peptides differ in composition by *three amino acids* at positions B28, B29 and B30 of the B-chain.
- ✓ Both molecules can be *differentiated to the ag/mL level*, demonstrating the very high sensitivity of the QES approach.



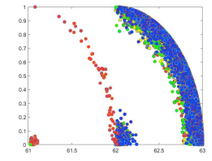


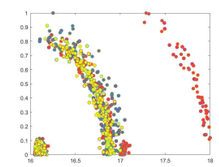


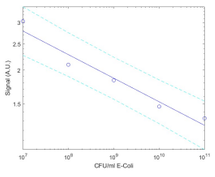
Top panel: aligned UMAP representations of Humalog (left) and Toujeo (right) in vibrational space at 50ag/ml.
Right panel: calibration curves characterizing signal intensity vs concentration for Humalog in a batch of Toujeo (top) and vice-versa (bottom)

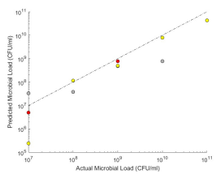
2 Fermentation process characterization

- ✓ QES generates a broad-spectrum signature of fermentation broth, acquired from 3 bioreactors and across 5 time points corresponding to bacterial life-cycle from a single measurement
- ✓ Spectral features can be used to assess batches and time points for conformance to process "normal"
- ✓ With suitable standard addition-based reference samples, a machine learning regression model can be trained to recognize organism-specific features, to characterize microbial load as a function of life cycle.









Top panel: Broad-spectrum QES features clustered by time points (left) and by batch (right), depicting time points and batches that are significantly different in broth chemistry

Bottom panel: Calibration curve for microbe loading in broth extract, with 95% C.I. in dotted cyan lines (left) and prediction efficacy of calibration model (right), as indicated by concordance to y=x dotted line. Colors correspond to batches

Conclusions

Developing new assays in the bioanalytical lab for pharmaceutical development is intensive and expensive due to the complexity of the assays and the cost to validate relevant reagent or sample preparation variables.

Here we demonstrate:

1. A simple and straightforward 1-step data acquisition model that can simplify assay development by eliminating the need for sample preparation or reagents
2. Despite being a simple and straightforward analytical method, *QES does not compromise the sensitivity or specificity* of the analyte detection,
3. QES can be leveraged to measure fermentation biochemistry in a broad-spectrum manner; with suitable reference data, the measurement data can be re-queried to get specific intelligence on microbe health, product concentration and end-product consumption, all from one measurement and with one instrument

QES can simplify assay development and testing without compromising sensitivity or specificity.

More Information

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