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A tunable proximity assay that can overcome dilutional non-linearity

Researchers at Stanford have developed a tunable proximity assay with a wide dynamic range that can detect multiple analytes in a single sample.

Blood-based quantification of protein biomarkers is a standard tool for the prediction, diagnosis, and monitoring of disease. Physiological concentrations of different plasma proteins can vary considerably, spanning over 10 orders of magnitude. Current methods rely upon sample splitting and differential dilutions, which are vulnerable to issues arising from non-linear dilutions (NLD). NLD describes the phenomenon wherein measured concentrations of a given analyte deviate greatly from their expected values when measured at different dilutions. The effects of NLD can be dramatic, and there is currently no single assay that can quantify both low- and high-abundance proteins simultaneously from a single sample.

Stage of Research
The inventors have developed a tunable proximity assay, EVROS, that can overcome the problems arising from NLD. The EVROS method is based on paired oligonucleotide-tagged affinity reagent detection of target analytes; when two detection reagents simultaneously bind to the same target molecule, barcoded DNA strands can undergo a ligation reaction in the presence of a complementary "hybridization splint" DNA strand. Ligated and barcoded nucleic acids can be further amplified and detected by high-throughput sequencing (HTS). Importantly, EVROS introduces two independent tuning strategies to modulate the signal response curve of each analyte individually. First, a probe loading strategy ensures that similar signals are produced for all targets, regardless of abundance. Second, an epitope depletion strategy (i.e., introduction of a pool of depletant antibodies) shifts the binding curve of the detection reagents to match the physiological concentration range of the target. These two tuning mechanisms can be applied to many types of immunoassays. The inventors demonstrate the power of EVROS over the Luminex

approach in solid phase proximity ligation assay (spPLA) format to simultaneously quantify four different proteins with physiological concentrations ranging from low femtomolar to high nanomolar – a dynamic range spanning seven orders of magnitude in a single 5 µL sample of undiluted serum.

Stage of Development
Research – in vitro

Applications

- Multiplexed quantification of disparate analyte concentrations from small starting sample volumes (e.g., biomarker discovery, disease monitoring, blood testing in neonates or biobanked samples).

Advantages

- Tunable EVROS assay has a wide dynamic range, capable of determining the concentration of multiple analytes in a sample, even if the analytes are present at very different concentration levels
- EVROS requires only 5 µL of sample
- spPLA format includes polyclonal antibodies that are divided into pools for each target analyte (e.g. unlabeled depletant antibodies, detection antibodies, target capture antibodies), eliminating the need to screen multiple sets of monoclonal antibodies
- Affinity reagents can be combination of antibodies, aptamers and nanobodies

Publications

- Wilson BD, Soh HT. Re-evaluating the conventional wisdom about binding assays. 2020. Trends Biochem Sci.

Patents

- Published Application: WO2023154909
- Published Application: 20250180545

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