EXPANDING THE DYNAMIC RANGE OF BIOMARKER DETECTION THROUGH MOLECULAR EQUALIZATION

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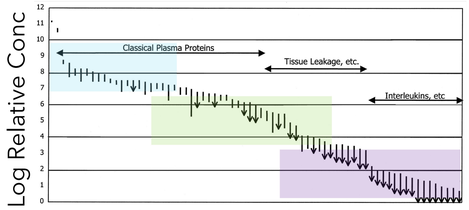
Clinical Need

- Blood-based quantification of protein biomarkers is a standard tool for the prediction, diagnosis, and monitoring of disease.
- The range of physiological concentrations of plasma **proteins span over 10 orders of magnitude**[1]

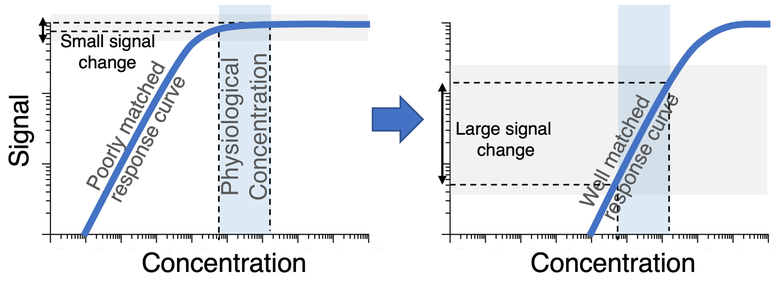


Example protein analytes in plasma

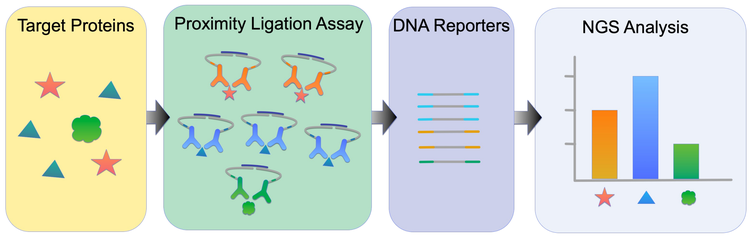
- Assays rely on different panels and dilutions to measure widely variant protein concentrations.
- Diluting causes inaccuracies due to "nonlinear dilution" from matrix effects and interferent molecules[2].
- There is **no existing technology** that can measure **nM** and **fM** proteins **simultaneously** from a **single sample**.

Our Solution

- **Tune the signal output of assays** by shifting the binding curve to match the physiological concentration of the analyte.



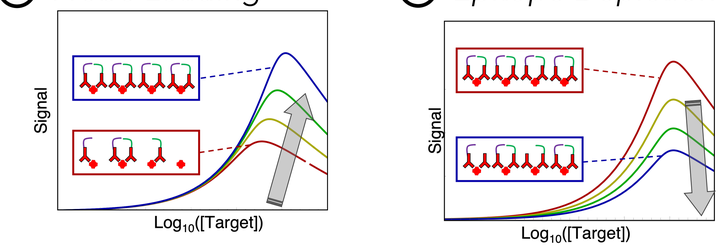
- Here we focus on tuning proximity-based assays[3] by modulating unlabeled and labeled detection antibody concentrations.



Our Solution (cont.)

Assay Tuning Mechanisms

① *Probe Loading* ② *Epitope Depletion*

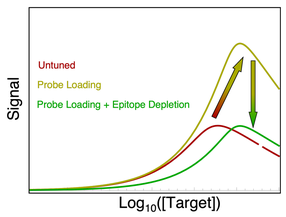


Assay Tuning is achieved with a two-pronged approach:

1. Probe loading: Increase signal output by increasing probe concentrations.

2. Epitope depletion: Decrease signal output by adding non-signal-producing agents, thereby decreasing available epitopes.

"Arbitrarily" Tunable Binding Curve



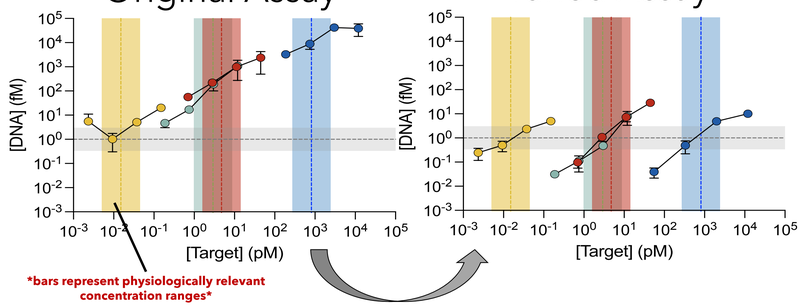
Using these tuning mechanisms, we can focus tune signal output on a concentration dependent manner:

- **Low concentration** analytes are tuned by probe loading
- **High concentration** analytes combine both probe loading and epitope depletion to shift response curve to physiological region

Results

Original Assay

Tuned Assay

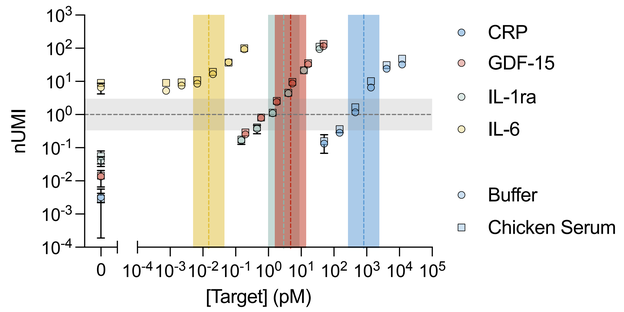


bars represent physiologically relevant concentration ranges

- In untuned assays, high concentration analytes will saturate signal of low concentration analytes.
- Tuning ensures equal representation of DNA signal output from each analyte regardless of relative concentration and have good signal resolution at physiological concentration.

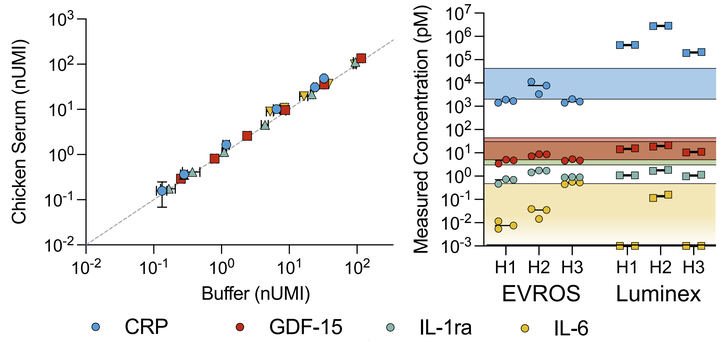
Results

Multiplexed Measurement Across 7 orders



Our tuned assay can **simultaneously measure 4 targets** important for cardiovascular disease (CVD) that present **over 7 orders of magnitude**. For example, small changes in **IL-6** at ~fM concentrations are discernable in a background of >10nM **CRP**!

Quantification in undiluted serum



Many engineered assays perform well in buffer but break down in serum samples. We successfully **quantify endogenous targets** in **100% human and chicken serum** and are comparable to gold standard, Luminex.

Conclusions

- Precise tuning of an ultrasensitive, multiplexed protein detection assay to normalize signal output of analytes of widely divergent concentrations.
- Simultaneously quantify proteins varying **over 7 orders of magnitude** (high nM to low fM) using only **5 µl** of **100% serum**.
- Offers the highest dynamic range to date.
- Enables quantitative protein biomarker detection without dilutions.

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References: 1. N.L. Anderson, *Mol. Cell. Proteomics MCP.* 1, 845-867 (2002) 2. N. Bolstad, *Bes Pract.Res.Clin Endocrinol Metab.* 27, 647-661 (2013) 3. R.Y. Nong, *Nat. Protoc.* 8(6):1234-48 (2013).