PROTEOME WIDE PLASMA PROFILING USING ANTIBODY SUSPENSION BEAD ARRAYS

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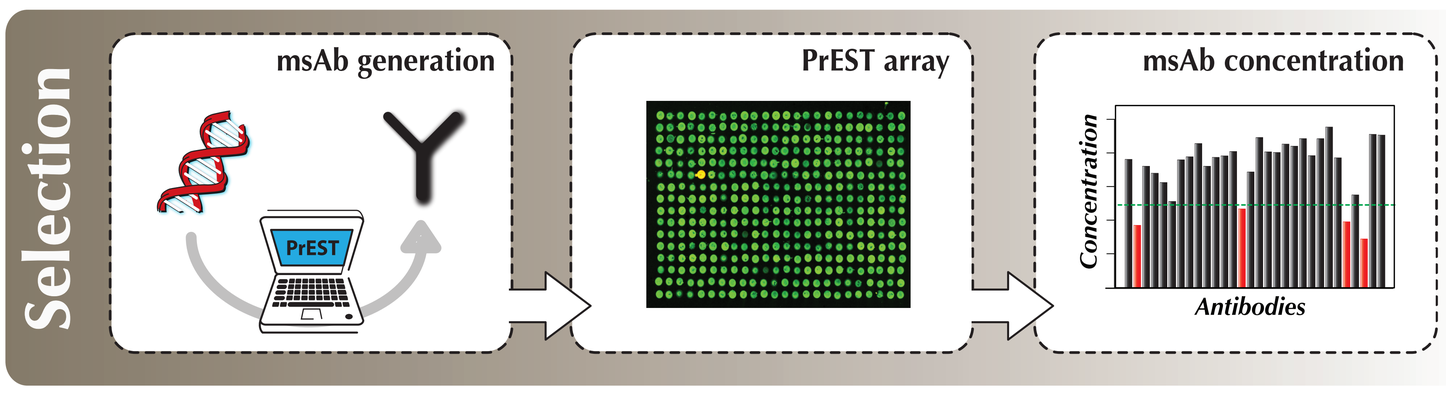
A newly developed antibody suspension bead array assay allows for a systematic analysis of the protein profiles of body fluids such as serum and plasma. This microtiter based assay uses antibody-coupled beads for a multiplexed analysis of minute amounts of directly labelled samples. As recent biomarker discovery projects emloying this assay have revealed, this suspension bead array gave rise to interesting protein profiles with significant differences between patient groups. While these and other affinity-based projects are dependent on a selection and availibility of functional affinity reagents, this requirement is met by the Human Protein Atlas project, where affinity purified mono-specific antibodies are produced in a high throughput manner. Our aim now is to perform an undirected proteome wide biomarker discovery, by employing all validated antibodies towards human proteins in this high-throughput plasma profiling.

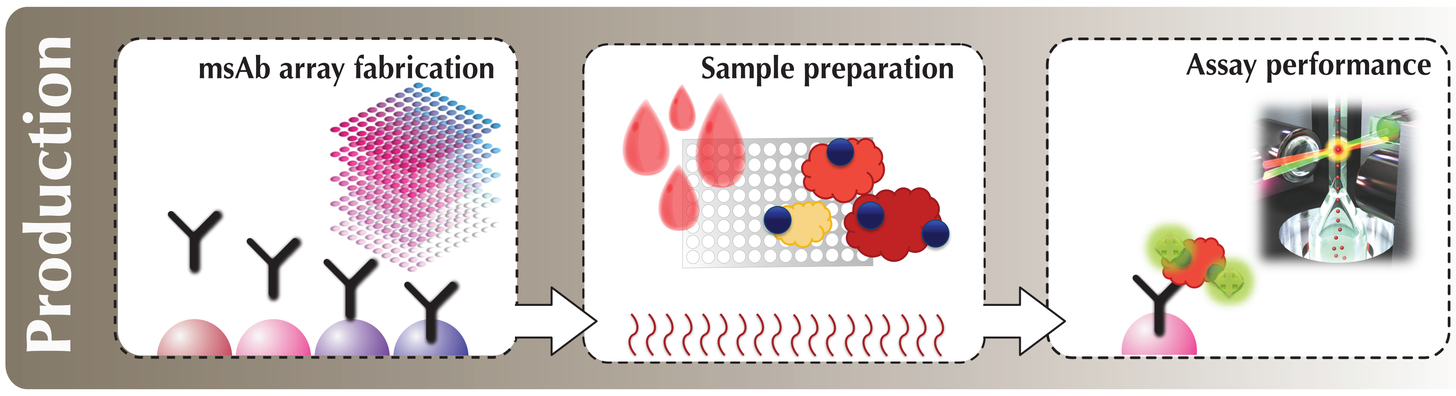
Plasma profiling procedure

1) The Human Protein Atlas project (HPA) produces monospecific antibodies in a high throughput fasion. For every gene, protein epitope signature tags (PrEST) are designed and produced recombinantly, immunized into rabbits and the antisera are affinity purified on the antigen.



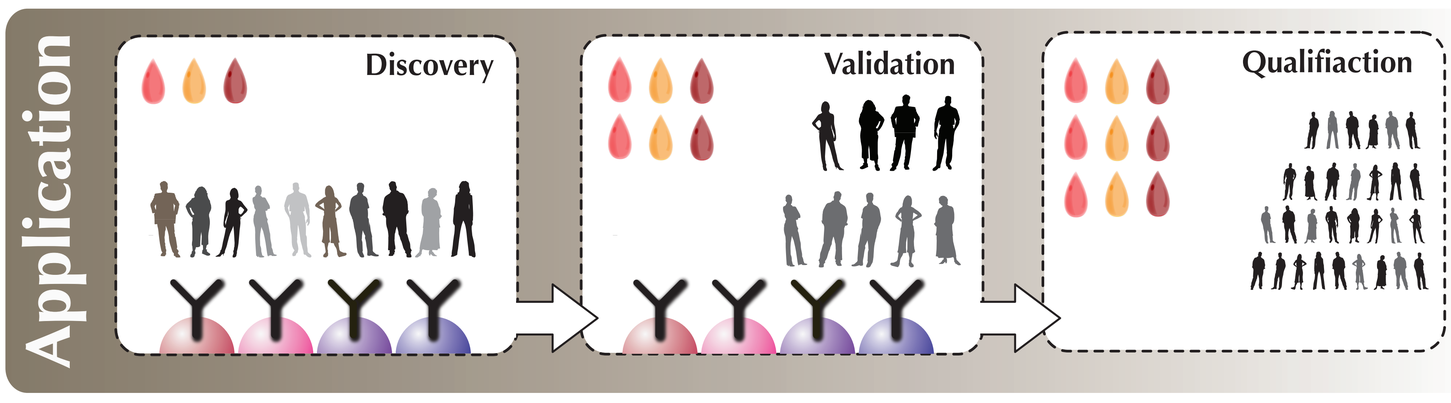
2) All HPA antibodies are validated through a systematic production pipeline, including a specificity test on a protein microarray. To be included into the suspension bead array platform, the antibodies also need to exceed a concentration limit of 0.05 mg/ml.

3) The antibody array in suspension offers a flexible alternative to planar arrays with a streamlined, purification free protocol. Antibodies are coupled to colour coded microspheres and plasma samples are directly labeled with biotin. A mild heat treatment of the samples improves the detectibility of the epitopes.



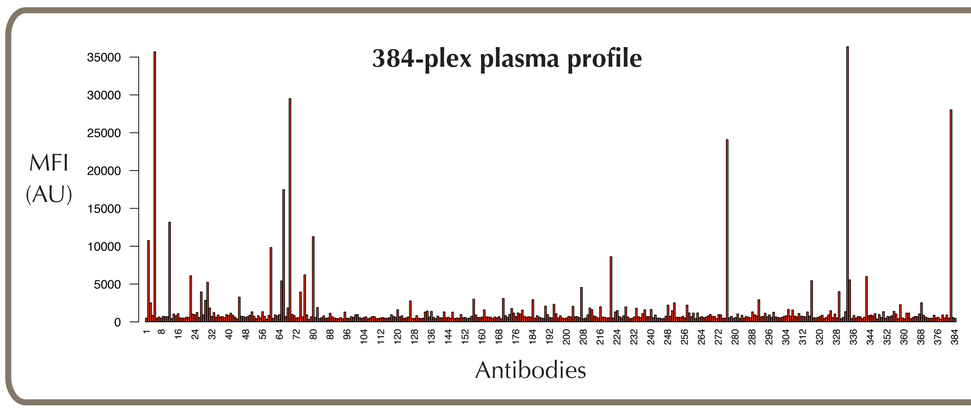
4) The multiplexed immunoassay results in binding profiles for 384 antibodies simultaneously. 384 samples can be measured within one experiment in a reproducible manner (%CV ± 10%). Spike in experiments have shown to detect proteins down into low ng/ml level, but true biological sensitivity is yet to be evaluated.

5) A multi-disease cohort has been combined of 384 plasma samples from patients representing various types of cancers, coronary heart diseases, neurodegenerative diseases and hormone related diseases. This cohort will be screened with 384-plex antibody arrays in a monthly fashion as a discovery phase.



6) Potential disease associated protein profiles will be validated in larger single disease cohorts consisting of hundreds of samples. To assess the potential of the target protein as a biomarker candidate, it will undego a qualifiaction phase in which thousands of samples are to be analysed with alternative methods.

Plasma profiling, one experiment
384 plasma samples, 0.1 µl each
384 antibodies, 1.6 µg each
150000 immunoassay data points



Example of a 384-plex bead array with antibodies from the HPA pipeline, profiling a plasma sample.

Acknowledgements
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