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CLONE SELECTION FROM UN-PURIFIED SAMPLES

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Introduction

Optimal clone selection is a key step in the early process of protein production.

Most often, initial clone selection is performed on basis of a titer measurement only, followed by purifications and assessment of protein function of the purified protein.

In this poster, we introduce a novel method for clone selection of VHH producing E.coli., offering the advantage of including affinity data as well as titer, whilst performing the assay with un-purified cell supernatant.

Material

Assay Step 1: VHH antigen labelled with Alexa-488 (Thermo Fisher) was used as Indicator at constant concentration of 50 nM. For all 96 clones, the Indicator was premixed with a fixed volume of 10% VHH-containing non-purified E.coli cell supernatant in 90% PBS buffer.

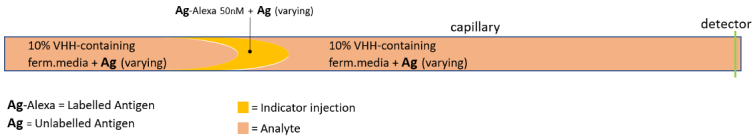
Assay Step 2: Cell supernatant (10% in 90% PBS) was pre-mixed with fixed amount of labeled VHH antigen (50 nM) in the presence of increasing amounts of unlabeled VHH antigen(15 – 2000 nM).

Experiments were performed on a Fida 1 system employing 480 nm LED detection using a standard capillary (Fida Biosystems).

Fida 1

Method

Assay principle of flow induced dispersion analysis (FIDA)



Flow Induced Dispersion Analysis (FIDA) is a capillary-based microfluidic method, exploiting that flow rate in the center of the capillary is faster than flow rates at the edges of the capillary. The resulting radial concentration gradients ant the front and the tail of the dispersion, results in diffusion of your chose indicator. Via Taylor equation and Stoke Einstein equation, this provides a direct “first principle” measurement of size (hydrodynamic radius).

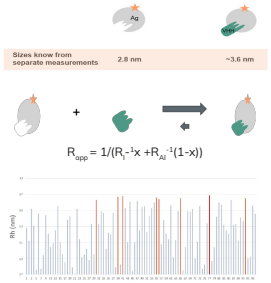
1 Ranking of clone performance based on titer and affinity

Fida 1 read-out is absolute size in nanometres.

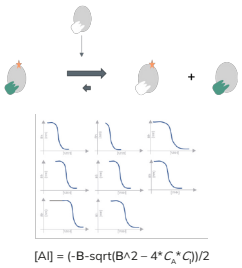
Size of 100% free antigen and size of 100% VHH bound antigen can be identified.

The size of fixed amount of labelled antigen with a fixed volume of cell supernatant from the various VHH-expressing clones, provides a measure of fraction bound of the labelled antigen. Increase in size can be driven by i) high expression levels and ii) strong affinities.

Selecting the top performers, is likely resulting in a mix of high expression level and strong affinity.



2 Deconvolution of titer and affinity



$[Al] = (-B-\sqrt{(B^2 - 4*C_A*C_l)})/2$

$B = C_A + C_l + Kd$

Reverse titration curves can are generated by adding increasing amounts of un-labelled antigen. The Fida 1 software includes a “reverse titration” module which models the measured sizes with well defined mathematical expressions.

Because the Fida 1 readout is absolute in nanometers, indicator size and complex size can be fixed in addition to fraction bound and known concentrations of un-labelled antigen. The result is 2 equations with 2 unknows (over-determined) which give very accurate assessment of VHH concentration as well as Kd.

Conclusions

The “1st principle” FIDA technology opens for fast and robust clone selection before any purification steps needs to be optimized. Selection criteria includes protein function (affinity) in addition to titer. The absolute readout of size in nanometres provides a solid basis for intuitive understanding your data as well as it provides fix-points when using the data for mathematical modelling.

In addition to measuring in un-purified matrices, the robustness of the assay also results in fewer fall positive than e.g. ELISA assays.

Not covered in this poster is the additional information in FIDA raw data about aggregation, conformational stability and other QC parameters. Please visit Fidabio.com for more information on sample QC.