Next-generation bi-specific biologics

Overall aim:

Development of a cell-based display method for high-throughput discovery of affinity proteins using directed evolution

VINNOVA

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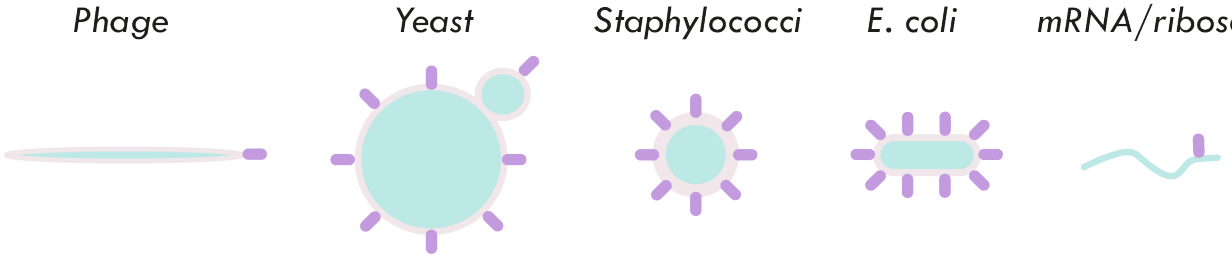
Why E. coli?



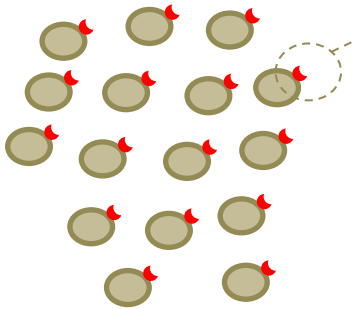
E. coli

- *Very large libraries*
- *Well investigated organism – large "toolbox"*
- *Option to use both FACS and MACS*

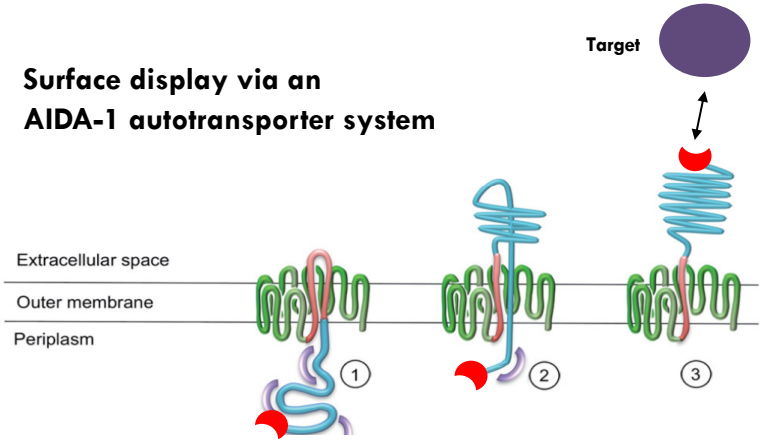
Directed evolution and microbial display

Library of *E. coli* cells

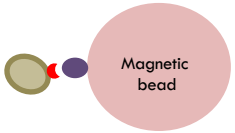
- Each displaying a protein library member



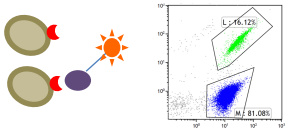
Surface display via an AIDA-1 autotransporter system



Enrichment of target binding clones

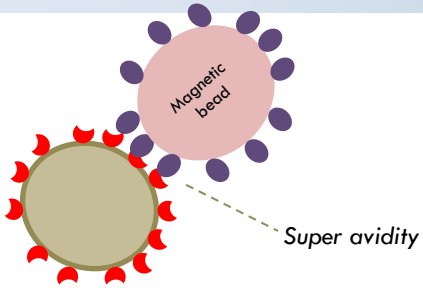


Magnetic-Activated Cell Sorting (MACS)



Fluorescence-Activated Cell Sorting (FACS)

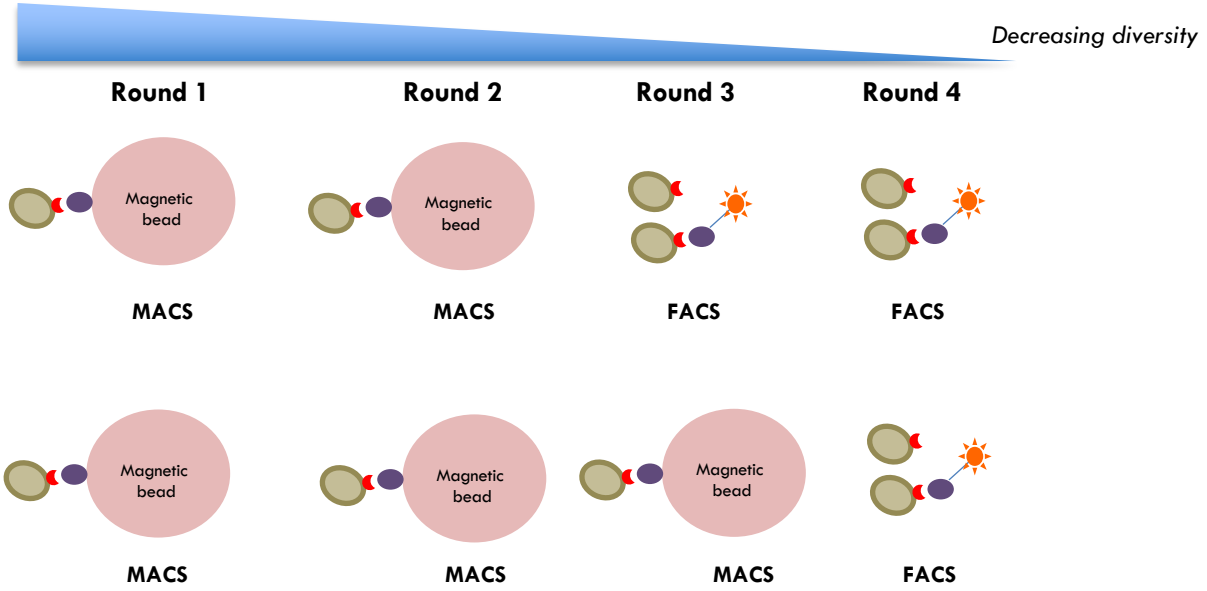
Selection of target-binding clones



MACS
- High throughput
- Low ”resolution”
- Capture of low affinity binders

FACS
- Lower throughput
- Very high “resolution”
- Isolation of highest affinity binders

Different selection schemes possible

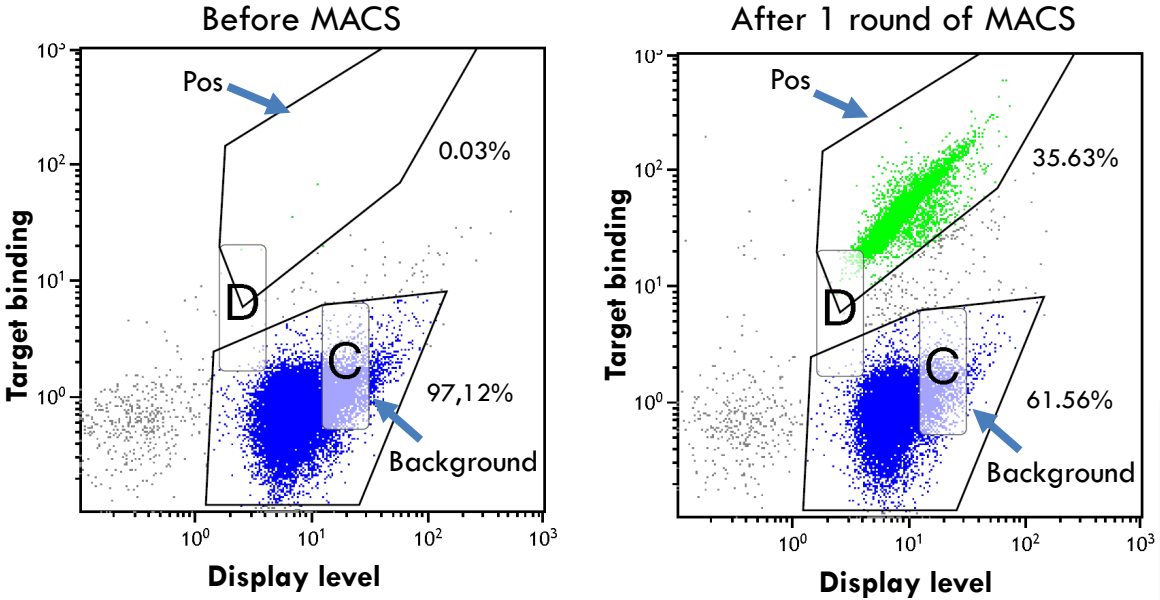


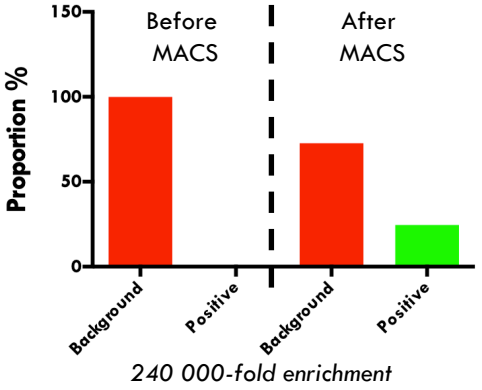
Input:
10^{10} library,
10 x coverage
=
10^{11} cells

- MACS in round 1 is "mandatory"
- FACS of 10^{11} cells would take ca. 1 000 hr

Optimization of MACS

MACS **1 : 1,000,000** (Pos : background). **10^{11}** cells in 100 ml



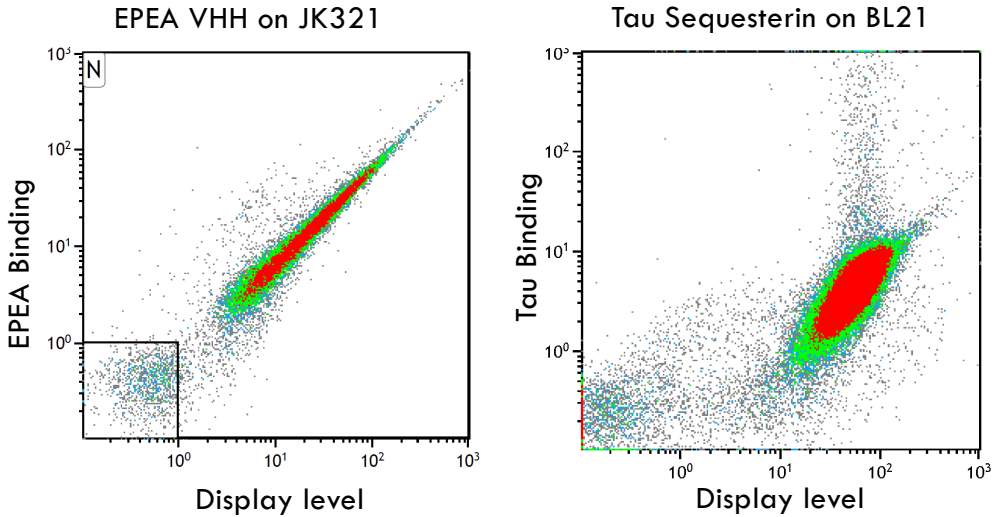


- Very efficient enrichment after single round of MACS

Aims of work packages during year 1- 3:

- ✓ *Increase display level – P/B (WP1)*
- ✓ *Optimize MACS (WP1)*
- ✓ *Automated MACS (WP1)*
- ✓ *Bead evaluation (WP1)*
- ✓ *Evaluate E. coli strain display of complex proteins (WP2)*
- ✓ *Assess E. coli strain electroporation (WP2)*

Efficient display of antibody fragments



- *E. coli* strain (JK231) identified that efficiently displays disulfide containing antibody fragments
- Established display of dimeric affibody Tau sequesterin on BL21