New technology automates sorting of large, bead-based combinatorial chemistry libraries.

K. Ver Donck[1], L. Bols[1], R. Bongaarts[1], P. Van Osta[1], J. Brouwer[2], R. M. J. Liskamp[2], J. Geysen[1], D. Perrault[3], K. Kalutkiewicz[3], R. Pulak[3] ~ Union Biometrica[1,3], (Geel, BE[1]; Somerville, MA[3]), Utrecht University (Utrecht, NL[2])

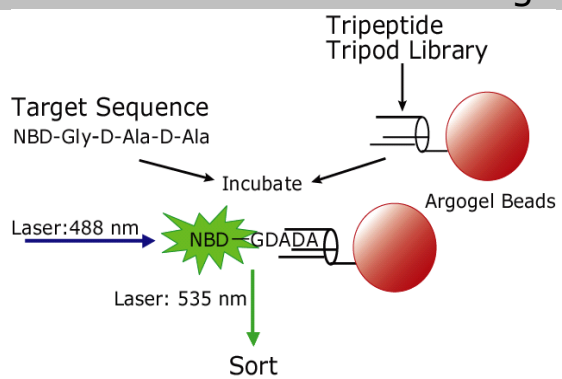
1. Introduction

The COPAS™ flow sorter offers a novel, fast assay platform that extends the limits of HTS to >100,000 compounds / hour. COPAS™ sorters can measure bead sizes and up to 2 colors of bead fluorescence-emission wavelengths. Furthermore, the COPAS™ can sort out bead subpopulations according to pre-set size and fluorescence criteria from a large population of unspecified beads. The instruments can handle bead sizes from 50 to 500 microns and read the typical green, yellow and red labels commonly used in today's fluorescence-based assays. Applications for bead-based drug discovery include: protease substrate discovery, ligand discovery, 1 bead 2 compounds applications, removing autofluorescent beads from compound libraries and removing damaged beads from library bead collections.

We used this technology with a chemically synthesized random peptide library on beads in a binding assay. The purpose was to identify short peptides that bind the carboxyl-terminal target of proteoglycan-precursors that form the bacterial cell wall. A fluorescently-tagged target peptide was used to identify those beads, with peptides synthesized on the bead surface, that bind to this target (peptide-peptide interactions). The selected beads were individually dispensed into wells of multi-well plates for further validation, retesting and subsequent compound analysis. Different beads bound different amounts of target under the tested conditions as determined by their levels of fluorescence. Edman degradation of the peptide on each selected bead revealed the binding sequence.

Our research illustrates how COPAS technology platform brings utility to bead-based assays and allows for the reconsideration of using these approaches in the drug discovery process.

3. Mode of action of binding



2. Methods: Binding Assay

Target: dipeptide end of peptidoglycan precursor

- •Normally cross-linked to form bacterial cell wall
- •Binding to compound may prevent cross-linking
- •Result: no cell wall -> bacterial disruption

Mode of action of the binding

- •Tripod chains encapsulate peptidoglycan end
- •Library: consists of variable tripeptide constituents on tripod fingers on Argogel (160 mm)
- •Principle: multiple weak interactions of tripeptides bind end of proteoglycan precursor and block cross-linking

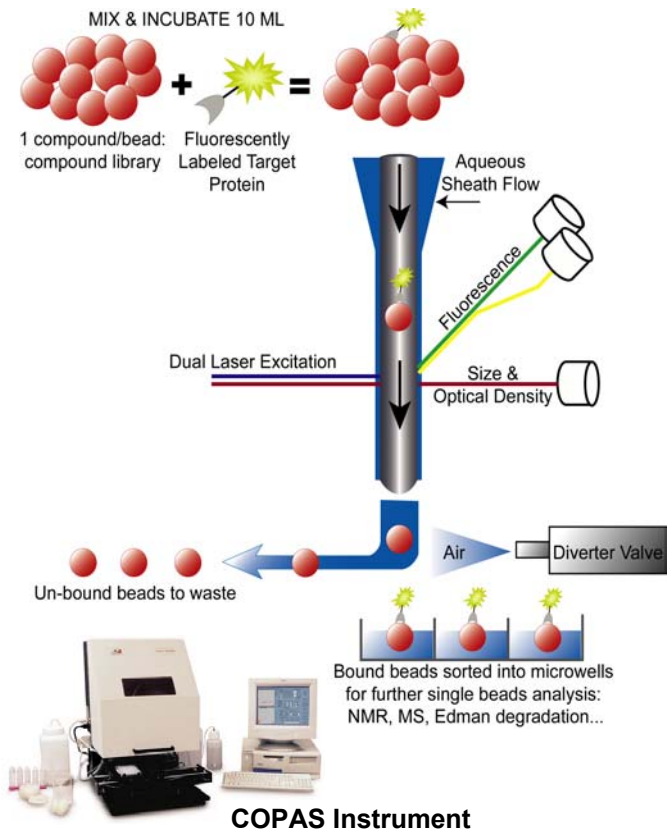
Batch Incubation

- •Buffer: 0.1N phosphate, pH 7.0
- •Incubate 8000 beads / experiment overnight at 20^{O}C
- •Test 3 concentrations of target: 0.5, 5, and 50 μM

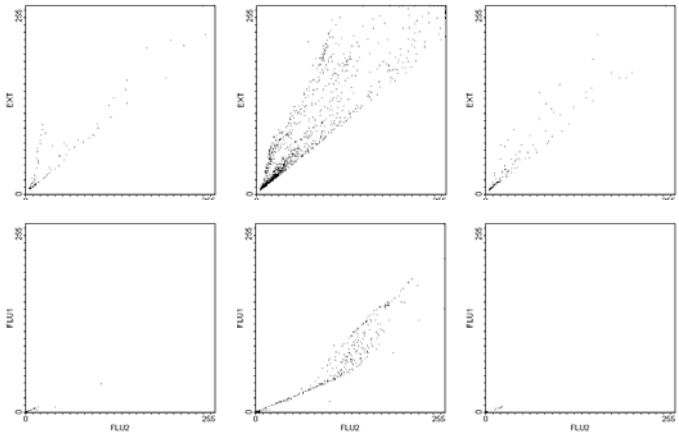
Method of analysis

- •On-bead fluorescence analysis & COPAS instrumentation

4. Bead assay principle



5. Negative Controls



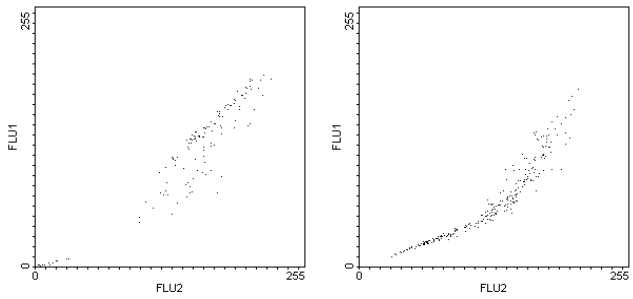
14
Beads + NBD-GDADA ~ 50 μM (washed)

13
Beads + NBD-GDADA ~ 50 μM (unwashed)

12
Bead library + buffer (washed)

The fluorescently-labeled target does not bind the Argogel beads non-specifically (leftmost bottom panel). The Argogel beads are not inherently fluorescent (rightmost bottom panel).

6. Comparison of fluorescence levels



8
Library + NBD-GDADA ~ 50 uM (unwashed)

3
Library + NBD-GDADA ~ 50 uM (washed)

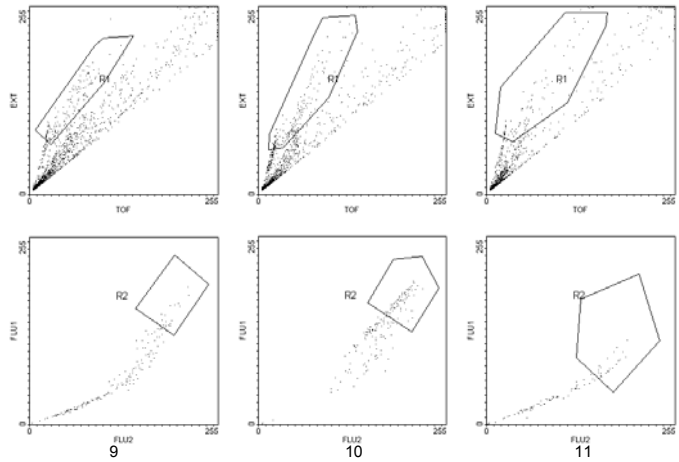
When comparing washed to unwashed beads:

•Some level of non-specific binding occurs between fluorescently-labeled target and the peptide library on the Argogel beads (comparing unwashed to the washed dot plots)

•There is a direct correlation between the concentration of fluorescently-labeled target & the amount of nonspecific binding.

•Our washing conditions are sufficient to reduce nonspecific binding.

7. Library sorting (Hit selection)



9
Library + NBD-GDADA ~ 50 μM (washed)

10
Library + NBD-GDADA ~ 5 μM (washed)

11
Library + NBD-GDADA ~ 0.5 μM (washed)

Lower panels indicate the relative distribution of fluorescence amounts for the library beads at each concentration of target. Polygon regions in lower panels indicate the sorted region of the distribution for each concentration of target.

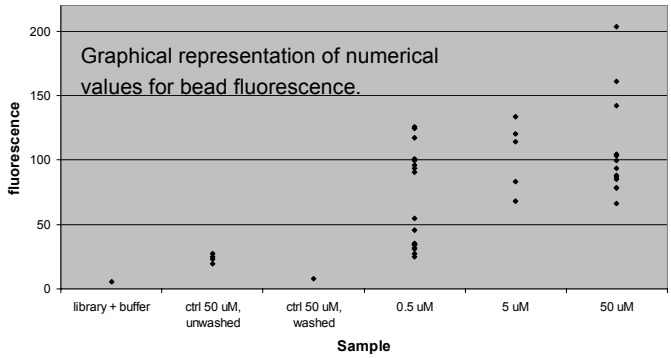
8. Sorted bead quantification

Fluorescent beads were viewed by fluorescence microscopy and intensity values of each bead displayed (in actual poster only)

Key to experiments:

•Exp 9 – from incubation with 50mm target
•Exp 10 – from incubation with 5mm target
•Exp 11 – from incubation with 0.5mm target
•Exp 14 – from control, Argogel beads and no target
•Exp 13 – control, Argogel plus 50mm target, unwashed
•Exp12 – control, Argogel plus 50mm target, washed

Numerical values for fluorescence can be collected from the data of the COPAS bead sorter.



9. Discussion

•Assay was designed to identify protein-protein interactions synthesized on the surface of small resin beads. Binding was detected using COPAS bead sorting and analysis technology.
•Control experiments indicate that resin used is not inherently fluorescent however, the fluorescently labeled target does bind to these Argogel beads, albeit weakly, as mild washing with buffer removes the binding.
•Data from resins carrying the peptide library indicate beads possess a greater amount of binding activity to the target protein than do the "naked" Argogel beads. Washing conditions that completely remove target from Argogel beads only reduces the level of binding to the peptide library beads. The nature of this binding is not understood.
•Selecting and collecting by sorting of beads with the highest levels of fluorescence after washing identified the tightest binders.
•Sorting is fast – approximately 15 minutes per analysis.
•Fluorescence level values can be determined in a number of ways. For example, using fluorescence image analysis instrumentation (microscope with CCD camera and appropriate software) or from the data collected by the COPAS instrument to allow the initial ranking of the strength of the binding interactions
•The sequences of the peptides on the beads is not presented at this time as further data needs to be collected.

Further information: www.unionbio.com or 617-591-1211