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Screen 100,000 drug candidates *per hour*

~ up to one million compounds a day!

A review of Ultra High Throughput & Increased Flexibility in Bead-based Combinatorial Chemistry and Biology using *COPAS™ BIO-BEAD* Flow Sorting Technology

Introduction

Multi-well plate-based ultra high throughput screening (uHTS) platforms can presently test up to 200,000 compounds per day using 384 and 1536 well plates for biochemical or cellular assays. Increasing the well density of multi-well plates has been a major avenue for increasing compound screening assay speed and capacity while reducing assay cost. However, physical limitations of liquid handling are being reached in the ever smaller wells of 1536 and 6144 formats.

Alternative ways to achieve higher speed and assay miniaturization, which started to emerge a decade ago, are bead-based combinatorial chemistry and on-bead assays. In the early days of bead chemistry, MS and NMR structure determination on single beads was not yet developed, and as a result, elaborate systems for tracking bead history reduced the speed and flexibility of this technology. In addition, picking and dispensing of beads was done manually, thereby slowing and introducing errors into the reading of results from large bead collections.

Today, both NMR and MS structure determination are possible on single beads. In addition, beads with higher capacity (porous) and beads with superior physiological compatibility are now available.

COPAS Flow Sorting

This paper reviews advancement of the third enabling technology: the rapidly evolving '***biology on a bead***' concept combined with **COPAS** automated sorting technology for fast and accurate selection of beads from large combinatorial libraries (Fig. 1 & 3).

COPAS™ BIO-BEAD flow sorting allows researchers to achieve routine screening speeds of 100,000 compounds per hour or – if needed – up to 1,000,000 compounds in a day. When used in combination, these innovations have facilitated the develop-ment of more sophisticated bead-based combinatorial assays offering higher throughput.

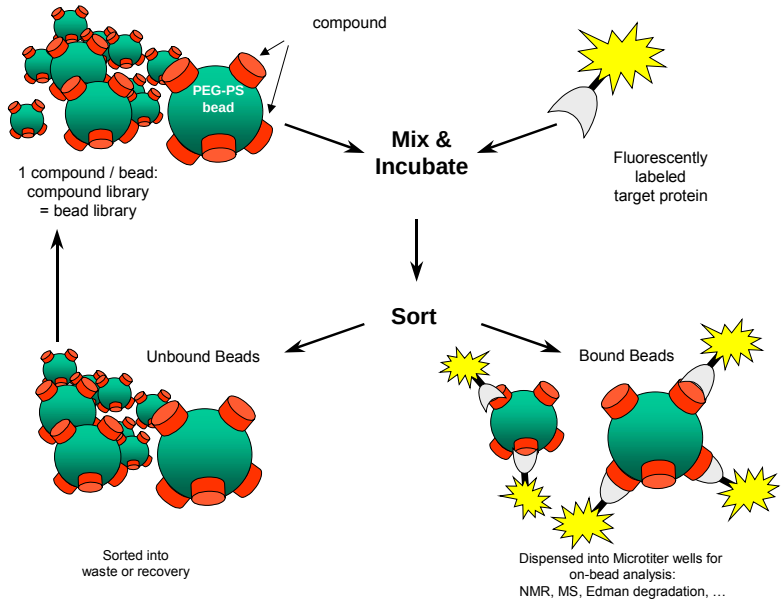

Figure 1: Assay Principle

Traditional Plate Method: 384 wells @ 20 ul/each = 20 liters/million cmpds.

COPAS Bead-based Method: 20-40 ml *total* / million compounds
… faster and utilizes 500-1,000x smaller volume of target & reagents

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APPLICATION NOTES

An Introduction to COPAS™ Instrumentation

(*Complex Object Parametric Analyzer and Sorter)*

COPAS flow sorting has refined the principles of flow cytometry to accommodate objects larger than individual cells. It provides a gentle, non-destructive sorting mechanism permitting sorted objects to be collected intact and undamaged, and then dispensed into multi-well plates for further analysis or observation.

Figure 2: COPAS with automatic plate handler

This technology has been utilized extensively for the viable sorting and dispensing of small **model organisms** such as *C. elegans* (nematode), *D. melanogaster* (fruit fly), and *D. rerio* (zebra-fish) larvae as well as *Arabidopsis thaliana* seeds. Organisms are sorted at a rate of over a 100,000 per hour, or over one thousand times faster than manual picking.

The *COPAS* line of instruments have flow cell diameters of up to one millimeter, which allows flow sorting of **beads with diameters up to 500 microns**, a size **large enough to allow single bead structure identification.** These instruments provide an accurate reading of two physical parameters for each bead: size and optical density of the bead.

In addition, *COPAS* can simultaneously read two fluorescent emission wavelengths emitted from the bead: green, yellow, and/or red are possible. These signals can come from tags such as recombinant protein tagged with fluorescent proteins such as GFP, YFP or DS-red. By setting the user-defined gatings, sorting and dispensing subsets of beads from the library may be done by utilizing any combination of the four aforementioned parameters.

COPAS BIO-BEAD instruments are based on the same flow sorting principle but also can be configured with a UV laser for excitation of fluorophores typically used in the combinatorial chemistry lab.

Speeds of up to 50 beads per second or 180,000 beads per hour have been achieved. Dispensing may either be done into multi-well plates or into a bulk receptor.

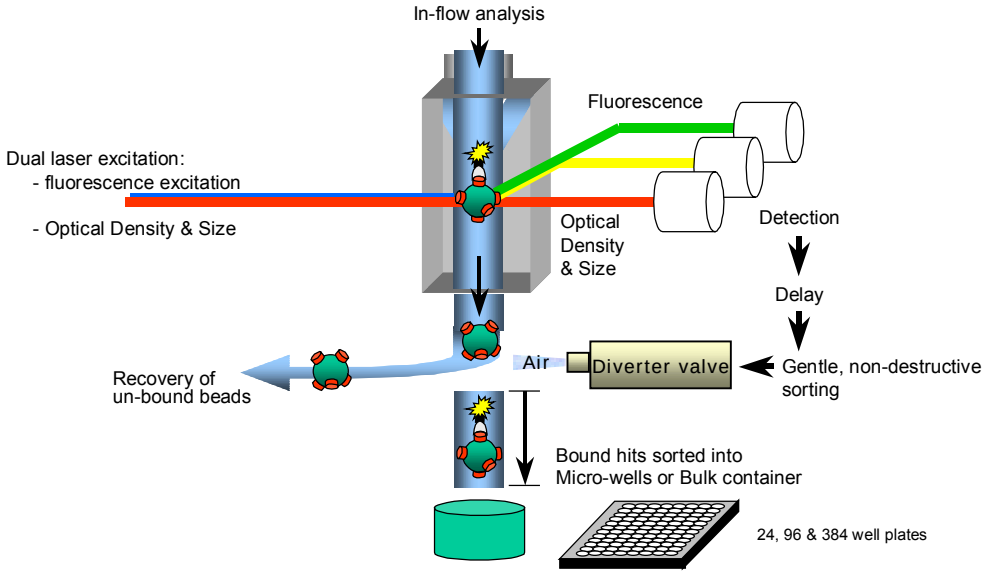
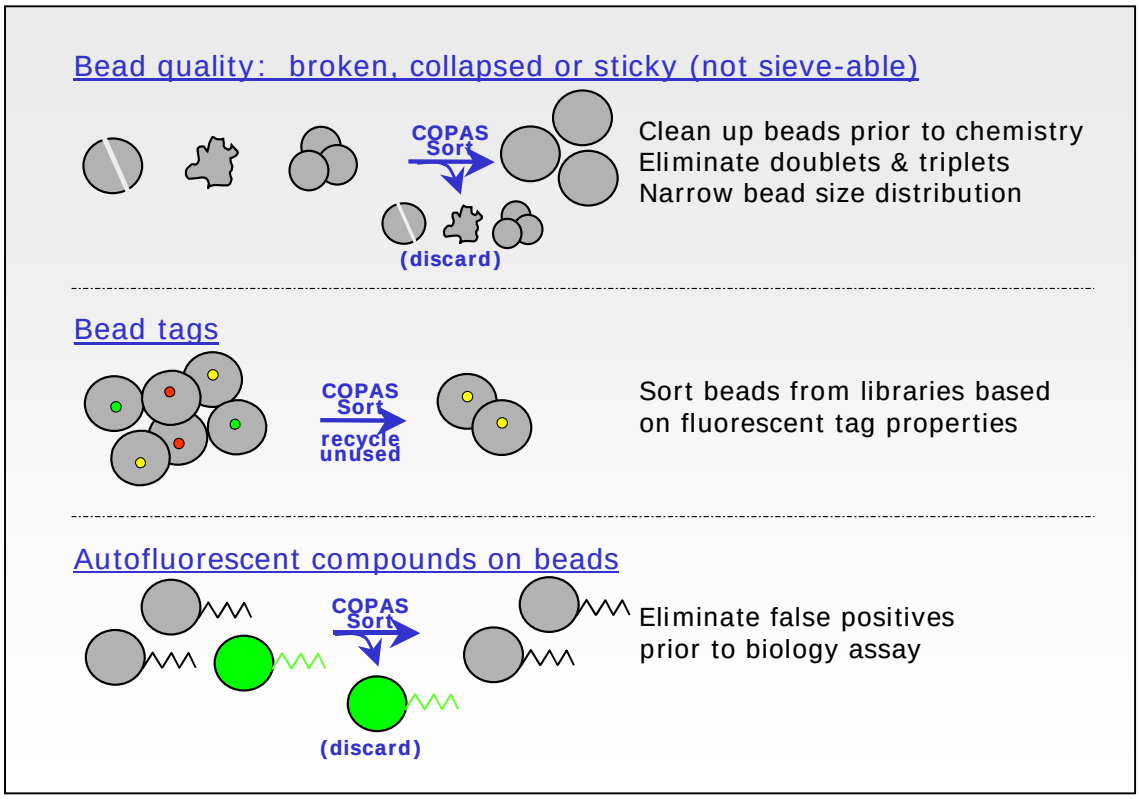


Figure 3: Schematic of ***COPAS*** flow sorting

Applications Using *COPAS BIO-BEAD* in Bead-based Combinatorial Assays

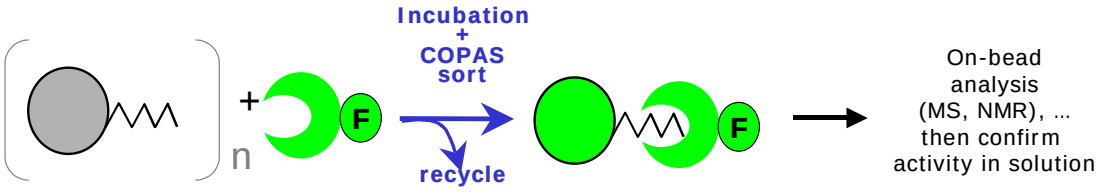
Figure 4: On-Bead (In-bead) Combinatorial Assays



Novel Trends in Combinatorial Assay Design

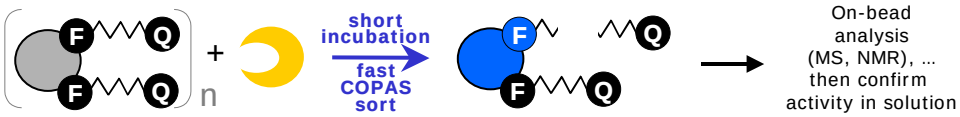
The very high speed at which beads can be automatically sorted and dispensed based on their size, optical density, and fluorescent characteristics facilitates the design of combinatorial experiments for HTS compound screening as well as for (HTS) assay development that can not be achieved by micro-well assays or manual picking. Researchers are utilizing this principle in different ways with *COPAS BIO-BEAD* instruments, some of which are reviewed below.

Binding Assays, kinetics and competition assays:



Beads in a library that have bound to a fluorescently labelled target protein can be sorted in a matter of minutes from the bulk of beads that do not bind to the target. (see also figure 1) By setting differential sort parameters, it is possible to sort strong from weak binders in a subsequent sort. An elegant example of this assay type has been the discovery of novel sulphated compounds that bind to the glycosaminoglycan binding site of fibroblast growth factor.

***On-bead enzyme substrate discovery*:**

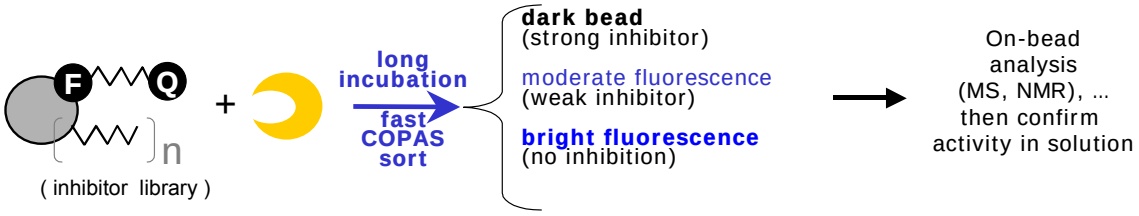


Using the enzyme *subtilisin* as an example, the team of Morten Meldal confirmed known (and discovered new) peptide sequences that bound to the active site of the enzyme and were cleaved. They synthesised a bead library of peptide sequences flanked by a pair of quenching fluorophores (FRAP). If a peptide on the bead is cleaved by the enzyme, then the two fluorophores separate and a bright fluorescent signal is generated inside the bead by the fluorescent tag that remains covalently linked to the bead. Using high speed ***COPAS BIO-BEAD*** sorting to achieve ultra short incubation times, the 'bright' beads from the library could be isolated fast enough so that they still contained enough intact peptide to allow on-bead structure elucidation of the peptide sequence after incubation. The entire cycle from library to structure elucidation was completed in one week, one hundred times faster and much more economical than previous cyclic testing of point mutation substrates.

***One bead – two compounds*:** *On-bead assay technology for protease inhibitor screens*

An even more sophisticated application of bead chemistry builds the assay within the bead library. In a first cycle of synthesis, a large batch of beads was treated so that a limited number of reactive sites in the bead are used to synthesise a FRAP quenched peptide known to be a substrate of the protease. This fluorophore-flanked peptide is the on-bead 'assay peptide' as described above.

In beads containing an effective protease inhibitor however, the degradation of the assay peptide will be slowed down and therefore the 'inhibited' bead remains 'darker' than 'un-inhibited' beads. Focusing on the darker beads in the library, ***COPAS BIO-BEAD*** proved to be a very flexible way to optimize incubation conditions and to screen for the dark beads at sorting speeds of 100,000 compounds per hour. Using this



In a second round of synthesis, subsets of the main batch were then treated with individual members of a library of potential inhibitors. When such a 'one bead – two compound' library is incubated with the protease, the majority of beads will become bright as the protease degrades the assay peptide and relieves quenching.

approach, inhibitors for *trypsin* and various *matrix metalloproteinases* have been identified in a few weeks.

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