

# Painless Particles®

Global Newsletter  
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**B E A D S • A B O V E T H E R E S T™**

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## ISO-9002 Certification



BLI has been certified by TRA Certification as having a Registered Quality System—ISO 9002.

## The 1988 AACC Bead Booth Story (from our first AACC Meeting)

In 1988 when we had been in business only three months, we exhibited at this big show. We had no booth, so we rented a counter, some chairs, and a big, brown, cork bulletin board (like those used for poster sessions). We "put our name up"—not in lights, but with small white foam balls (like ping-pong balls) to represent microspheres. We stuck the balls on the board with push pins to spell out our name, BANGS LABORATORIES.

Our neighbors with their fancy booths and graphics were mightily amused at our primitive booth and I'm sure they wondered how long we would last. Some folks brought their friends by to see our "cute little sign." The smiles faded when they saw the traffic we attracted. We set the company record (it still stands) with over 400 leads generated!

Well, we are still showing - at our 15th consecutive AACC show this year - and still growing. Not bad for such a modest start!

## AACC 2002 Follow-Up

### OEM Lecture Series

This year, we were invited to give two lectures. Both were very well attended and resulted in many questions right after the lecture and continuing discussions at our booth. Here are the abstracts of the information shared. If you have any questions, please ask for more information.

### *COOH-functionalized silica microspheres: development, characterization, & application* by Kathryn L. Turner

The coupling of biomolecules to microspheres confers benefits including permanent attachment, favorable presentation of ligand, and suitability for automation. The unique properties of silica (low autofluorescence, low nonspecific binding) have further made such microspheres the solid phase of choice for a number of applications in the life sciences.

The objective of this project was to develop high quality, stable, and cost-effective functionalized silica microspheres. Silanization conditions were optimized to achieve high COOH functionalization. The level of COOH functionalization was assayed, and activity demonstrated through protein (streptavidin) binding. The performance of a binding capacity assay [conjugation of biotin-FITC] revealed activity comparable to that of streptavidin-coated polymeric microspheres. Functionalized silica microspheres are thus demonstrated to be well-suited for applications demanding a highly functionalized solid phase with negligible autofluorescence, e.g. for use as sensors and biomarkers, and in cell separation and flow cytometric assays.

### *QuantumPlex™ beads: a flexible system for multiplexed analyte detection* words and music by Nathan Foushee and Kathryn Turner

Bead-based multiplexing enables the researcher to detect and quantitate the presence of multiple analytes in a single sample. The primary benefits conferred by these types of assays include reduced cost (reagents, supplies, labor), time, and improved sensitivity and throughput.

Bangs Laboratories' QuantumPlex beads provide a platform that researchers may use to construct their own multiplexed assays. This lecture will demonstrate that the QuantumPlex beads, when conjugated to cytokine-specific antibodies, yield multiplexed assays with the ability to detect and quantitate cytokines of the researcher's choosing. QuantumPlex beads are suitable for use in a variety of applications, including studies of autoimmunity, allergy, tumor markers, SNPs, etc. Data include results from cytokine and soluble tumor marker analyses.

## United Way Day of Caring September 13th

In our area, the United Way sponsors a special day when employees from all sorts of organizations volunteer for special projects, like painting or fixing the inside or outside of some needy person's home. Bangs Labs folks have always been very willing to help out, and for the second year in a row, over 50% have volunteered. So, if you call us on September 13th and have slow service, you'll know why. We are very proud that so many stepped forward to help out some nice people who just need some assistance.

## "On the Road Again!"

Below is the BLI meeting/show schedule for the rest of 2002. If you will be attending any of these shows, please stop by or booth and meet our fabulous folks, a.k.a., *The Bangs Gang!*

❖ Society for Biomolecular Screening: September 23-26, The Hague, Netherlands. Meet us at Booth 1350. We'll be up front, near the lounge. Also, look for our poster in the "Novel Detection Technologies and Assay Formats" section. [www.sbsonline.com](http://www.sbsonline.com)

❖ GLIIFCA – Great Lakes International Imaging and Flow Cytometry Association: October 4-6, Detroit, MI. [www.cyto.purdue.edu/flowcyt/glifca/gliifca.htm](http://www.cyto.purdue.edu/flowcyt/glifca/gliifca.htm)

❖ Clinical Applications of Cytometry: October 13-16, Keystone, CO. Booth not yet assigned.

❖ American Society for Human Genetics: October 15-19, Baltimore, MD. Meet us at Booth 324. [www.ashg.org/genetics/ashg/meet-2002/2002-info.htm](http://www.ashg.org/genetics/ashg/meet-2002/2002-info.htm)

❖ NIH Research Festival 2002: October 17-18. Bethesda, MD. Meet us at Booth 853.

❖ MEDICA: November 20-23, Düsseldorf, Germany. Booths not yet assigned.

❖ American Society for Hematology: December 6-10, Philadelphia, PA

❖ American Society for Cell Biology: December 14-18, San Francisco, CA. Meet us at Booth 717.

## The Bangs Gang

### More News of our Employees

**Robin Bryant** joined us in May – a December 2001 graduate from Ball State University, with a B.S. in Biology. She is already a great asset in Customer Service and you will be able to hear her wonderful smile and pleasant disposition over the phone.

**Chris Greathouse** joined Customer Service in July. A May graduate of Indiana State University, with a B.S. in Biology, he likes golf, fishing, and basketball. Of course, in Indiana and especially at ISU, it's mandatory to like "roundball." You will enjoy his friendly manner.

**Chris Ballas**, Ph.D., joined us in early July in the capacity of R&D Manager, with the mission of developing a range of new flow cytometry related products. He was chosen as a result of a 10-month long search, involving hundreds of applicants. We waited for just the right person, with the right set of talents and experiences. Keep your eyes peeled for exciting new developments from Chris's lab.

## P(articles)<sub>2</sub> = Particle Articles

❖ Lauer SA, Goldstein B, Nolan RL, Nolan J, "Analysis of Cholera toxin-ganglioside Interactions by Flow Cytometry," *Biochemistry*, **41**, 1742-1751 (2002). Work from John Nolan's group at Los Alamos (he was a lecturer at The Latex Course™ in June); involves self assembly of lipid bilayers on silica beads and FITC flow standards.

❖ Lauer SA, Nolan JP, "Development and Characterization of Ni-NTA-bearing Microspheres," *Cytometry*, **48**, 136-145 (2002). This article cites use of BLI's plain and COOH silica.



"WE'VE PROVEN, WITHOUT A DOUBT, THAT THIS PARTICLE HAS A NEGATIVE CHARGE. UNFORTUNATELY AN ACCELERATOR IN SWITZERLAND HAS PROVEN, WITHOUT A DOUBT, THAT IT IS A POSITIVE CHARGE."

If You Missed the Course, You Can Still Buy the Book...

## The Latex Course™ Book

### "Designing Microsphere-Based Tests and Assays"

Well, you missed the course (June 10-12 in Indianapolis), but you can still order the >500 page course book that all attendees received, for \$395 + shipping. We'll sell any extra copies while they last. Last year, we sold out. (For speakers, topics, biographies, and ordering information, visit our website.)

(Cartoon reprinted with special permission from Sidney Harris <SHarris777@aol.com> and [www.sciencecartoonsplus.com](http://www.sciencecartoonsplus.com).)



## Ask "The Particle Doctor<sup>®</sup>"

### Flow Question

**Q** : Help! My microsphere reagent has lost activity only one month after adsorbing IgG and blocking BSA. What happened? What can I do? (*Kathy Turner answered this one.*)

**A** : Your problem with reagent stability could be due to one or more of the following:

1. **Concentration of BSA in storage buffer** (too much BSA might cause competition between BSA and IgG molecules);
2. **Level of IgG coating** (perhaps using a higher concentration of protein will allow you to load more on the surface, making it less accessible to BSA molecules in the buffer);
3. **Use of BSA as a blocker** (other blockers might not compete as efficiently for the surface);
4. **Use of PBS buffer** (other buffers might provide improved storage stability).

Another factor to consider is that proteins tend to become more tightly adsorbed to surfaces over time. The loss of activity that you have observed may be caused by this phenomenon - as molecules become more tightly adsorbed, they undergo conformational changes that can reduce activity. If this is the case, using a higher IgG concentration might improve loading, and force molecules into a crowded, upright position.

To investigate, you might utilize a total protein assay to determine if the protein level is remaining constant (on the beads or in the supernatant). If it remains constant, this might indicate competitive desorption (replacement of IgG molecules with BSA molecules), or loss of activity due to conformational changes of protein. See our TechNote 205, *Covalent Coupling*, for references and suggestions on assaying beads for protein load and activity. Here are some other references:

- Puela, J.M., et al. 1995. Coadsorption of IgG and BSA onto sulfonated polystyrene latex: I. Sequential and competitive isotherms. *J Biomater Sci Polym Ed*, 7(3):231-240. PubMed ID: 7577826.
- Puela, J.M., et al. 1995. Coadsorption of IgG and BSA onto sulfonated polystyrene latex: II. Colloidal stability and immunoreactivity. *J Biomater Sci Polym Ed*, 7(3):241-251. PubMed ID: 7577827.
- Zalazar, F.E., et al. 1992. Parameters affecting the adsorption of ligands to polyvinyl chloride plates in enzyme immunoassays. *J Immunol Methods*, 152(1):1-7. PubMed ID: 1640104.

### Oligonucleotide Immobilization

**Q** : How many protein molecules can I adsorb onto 1µm microspheres?

**A** : If we assume that you are packing them tightly like a monolayer, then you should be able to calculate the number of protein

molecules per bead as follows:

- 1) From the Stokes diameter of the protein molecule, you can calculate that a spherical protein molecule would occupy an area (cast a shadow) of  $\pi d^2/4$ . If the diameter of IgG is 10nm, then its parking spot on a microsphere would be 78.5 sq. nm.
- 2) The surface area of a microsphere is  $\pi D^2$ . Then a 1µm (1000nm) microsphere has  $3.14 \times 10^6$  sq. nm. (or  $314 \times 10^4$  sq. nm.) of surface area.
- 3) You can therefore expect to be able to pack a maximum of  $\pi D^2 / \pi d^2/4 = 4 (D/d)^2$  molecules per sphere. In this case, it would be  $314 \times 10^4 / 78.5 = 4 \times 10^4$  IgG molecules on each microsphere.

Remember, different proteins will have different affinities for a bead surface. Also, more isn't always better, but depends on conformational changes and steric effects. *You* must test to determine how much adsorption is needed for best performance.

### CML Binding

**Q** : What size amine microspheres should I try for coupling reactions with NHS esters?

**A** : *Of course, the devil in me suggests that you try all the sizes!* But seriously, folks, for a proper response, we must ask you for more information: What do you want to do with the microspheres? What sort of assay or application do you have in mind? The size of bead is typically dictated by the application or assay format, etc. Size will impact bead handling, surface area (area for immobilization of biomolecule), settling times, etc. For example, flow cytometric tests and assays typically make use of beads that are ~2-8µm, strip tests typically require beads that are 0.1-0.4µm. Our 300 series of TechNotes describes a number of applications with usual bead sizes noted. Also see TechNote 402 (published article with a link to the publisher's website), which contains recommendations on bead sizes for a number of formats. (Of course, all our TechNotes may be downloaded from our website, [www.bangslabs.com](http://www.bangslabs.com).)

### Mail Bonding (Subscribers "do the 'write' thing!")

❖ "Thanks again for the information, Kathy. You have been really helpful and you are a joy to speak with on the telephone." (TJ, St. Louis) Thanks from Kathy (and all of us at BLI) for your kind words.

❖ "Thanks for the info. Your website is one of the best I've seen - easy to navigate and with very complete information." (KB, Los Angeles) Thanks for your comments. We try to keep it useful.

❖ "I just wanted to say that the Flash Red COOH beads are working like a charm, and thanks for everything." (AR, Albuquerque) Thanks.

**"If you want to achieve excellence, you can get there today. As of this second, quit doing less than excellent work."** – Thomas Watson (1874-1956), Founder of IBM

**Technical References – See our website ([www.bangslabs.com](http://www.bangslabs.com)) for "downloadable" TechNotes and Product Data Sheets or ask for copies by mail or fax. We continually update and add new TechNotes and Product Data Sheets to our website.**

### Product-Specific TechNotes:

101. **ProActive® Microspheres** – Handling tips plus protocols for streptavidin, Protein A, and goat anti-Mouse coated microspheres.
102. **Magnetic Microparticles** – Data + handling tips for >11 varieties of superparamagnetic particles; COOH and NH<sub>2</sub>-modified, classical, encapsulated (low surface iron content), etc.
103. **Fluorescent/Dyed Microspheres** – Applications, fluorescence spectra, and product descriptions. Includes confocal microscopy standards.
104. **Silica Microspheres** – For immunoassays, nucleic acid capture, velocimetry (LDV, PIV), flat panel display spacers, and others.
105. **Microsphere Size Standards** – Data for 9 sizes (0.2-20µm), available singly or in kits, with certificates of analysis.
106. **Confocal Standards** – Using our three, bright, single-label 63nm fluorescent beads in confocal microscopy.

### Handling-Specific TechNotes:

201. **Working with Microspheres** – Choosing, cleaning, characterizing, coating beads, etc.
202. **Microsphere Aggregation** – Preventing, detecting, and reversing aggregation. Chemicals and equipment sources.
203. **Washing Microspheres** – Variety of methods for cleaning microspheres; advantages/disadvantages of methods; suppliers of equipment.
204. **Adsorption to Microspheres** – Adsorbing protein onto particles; use of "surface diluents" (blockers); recipes and references.
205. **Covalent Coupling** – Chemical attachment of proteins, nucleic acids, etc. to various types of surface-functionalized microspheres; recipes for buffers, blockers; miscellaneous coupling ideas, vendor information, and references.
206. **Equations** – For calculating particles/mL, area/g, "parking area", settling velocity @ 1G and in centrifuge, etc.
208. **Microsphere Sizing** – Various manual and automated methods are described and discussed, with references and supplier list.

**Flow Cytometry Standards?** See the "flow" portion of our website for lots of technical information about flow cytometry standardization in general and our expanding line of flow cytometry standards products in particular.

### Application-Specific TechNotes:

301. **Immunological Applications** – Review of commercial applications of microspheres.
302. **Molecular Biology** – Overview of purification and solid phase separation methods.
303. **Lateral Flow Tests** – Putting dyed particles on membranes so they will move properly.
304. **Light-Scattering Assays** – Turbidimetric and nephelometric applications of microspheres.

### Reprints:

402. **Microspheres, part 1: Selection, cleaning, and characterization, and part 2: Ligand attachment and test formulation** – LB Bangs & Mary Meza, *IVD Technology (in Medical Device & Diagnostic Industry)*, **17**, #3, 18-26, March, and #4, 20-26, April, 1995. (Note that you can download these papers at the IVDT website: [www.devicelink.com/ivdt/archive/95/03/009.html](http://www.devicelink.com/ivdt/archive/95/03/009.html) and [.../95/04/006.html](http://www.devicelink.com/ivdt/archive/95/04/006.html)).
403. **New Developments in Particle-Based Immunoassays** – Leigh B. Bangs, *Pure & Appl. Chem.*, **68**, #10, 1873-1879 (1996). Review of 40 years of diagnostic uses of microspheres – from LATs to biosensors.
405. **Applications of Magnetic Particles in Immunoassays** – Mary Meza, Ch. 22 (pp. 303-309) in *Scientific and Clinical Applications of Magnetic Carriers*, U. Häfeli, *et al*, Eds., Plenum Press, New York, 1997.
406. **Measuring Microsphere Binding Capacity** – JM Duffy, JV Wall, MB Meza, LJ Jenki, *IVD Technology*, **4**, #7, 28-34 (1988). (No reprints are available; you can download from our website.)
407. **Bead-based HTS Applications in Drug Discovery** – MB Meza, *Drug Discovery Today: HTS Supplement*, **1**, #1, 38-41 (2000).

**BLI Presentations and References** See our website for copies of the latest public presentations by the technical people at BLI and for publications that cite use of Bangs Beads or were authored by BLI personnel.

**See the "Hot Links" page at our website for help in locating equipment, instruments, etc.**

If you aren't able to locate answers to your microsphere application or handling/use questions (within our TechNotes, References, or Product Inserts), we invite you to call us directly, or to contact "The Particle Doctor®" through our website. Priority will be given to requests accompanied by chocolate chip cookies. (Just kidding – there is a high probability that, even without cookies, we will most likely get around to answering some questions eventually. They just might not be yours. [KIDDING!!!!])