Docket #: S20-157

Methods and Compositions Related to Lanthanide-encoded Microbeads

Researchers at Stanford University have developed a bespoke method for multiplexing beads in bioassays that expands the possible coding space when compared to available bead barcoding technologies.

Multiplexed bioassays have drastically improved our understanding of complex biological process in recent years. In these assays, binding is measured between a single "bait" molecule and many "prey" molecules to provide a comprehensive understanding of interactome networks. While spatial arrays comprised the first generation of these technologies, they have been outpaced by multiplexed microbead assays which allow for many replicates per experiment, opportunities for quality control, and the ability to vary different probes and targets over multiple experiments. Spectrally encoded microbeads assays such as the Luminex platform use microbeads that are combined in ratiometric proportions with fluorescent or luminescent materials. However, these techniques have a limited possible coding space, are often ill-suited for solid-phase synthesis applications due to their incompatibility with harsh organic solvents, and have difficulty detecting low affinity interactions.

Stage of Development
Research-

in vitro

Stage of Research
The inventors have created a novel technology named MRBLES 2.0, an acronym for microspheres with ratiometric barcode lanthanide encoding. This technology involves ratiometrically incorporating lanthanide phosphors (Lns) to produce spectrally encoded beads. The previous iteration of MRBLES had complex production pipelines that required two-layer microfluidic devices with custom pneumatics

control hardware and resulted in slow and laborious bead generation. However, MRBLES 2.0 utilizes manual mixing of Lns and polymers followed by droplet generation using a single-layer parallel flow-focusing device that increases the throughput of the assay from ~6600 beads/hour generated to ~9,000,000 beads/hour. MRBLES 2.0 also allows for the localization of copolymers bearing functional groups used for bioconjugation to the surface of the hydrogel matrix during droplet generation, allowing this method of bead generation to be used in applications requiring bioconjugation. MRBLES 2.0 provides an innovative, high throughput method for the generation of barcoded beads that expands the possible coding space as well as allows for additional applications with the addition of bioconjugation capabilities.

Technology Reference
Stanford ref. no S20-157; CZ Biohub ref. no. CZB-164S; University of Copenhagen ref. no. 521-0730/20-7000

Applications

- Generation of multiplexed microbeads for use in bioassays using lanthanides (Lns) as barcodes
- MRBLES 2.0 is a broadly useful pipeline with potential applications in the detection of DNA hybridization, identifying protein-protein or protein/peptide interactions, and screening polymers for useful bioactivity

Advantages

- MRBLES use of lanthanides allows for a greater possible coding space given that Lns have narrow and well-separated emission spectra
- MRBLES 2.0 displays increased throughput of bead generation up to 9,000,000 beads per hour and requires less specialized equipment to create the microspheres
- Allows for bioconjugation using the addition of capture molecules on the surface of the hydrogel matrix during droplet generation

Publications

- Feng, Y., White, A.K., Hein, J.B., Appel, E.A., & Fordyce, P.M "MRBLEs 2.0: High-throughput generation of chemically functionalized spectrally and magnetically-encoded hydrogel beads using a simple single-layer microfluidic device." Microsystems & Nanoengineering (2020).

Patents

- Published Application: WO2021252735
- Published Application: 20230211307
- Issued: 12,702,963 (USA)

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