Docket #: S22-253

Kinase/Phosphataste Substrate Analysis and compositions Using Spectrally Encoded Microbeads

Researchers at Stanford have developed a multiplexed bead-based dephosphorylation assay to allow for the measurement of multiple dephosphorylation reactions in one experiment.

Post-translational modification involves the modification of proteins after they are translated from RNA and is an exciting area of study in biomedical science. Of these, phosphorylation is one of the most prevalent post-translational modifications which involves the addition of a phosphate to a protein substrate by kinases. This phosphate group can also be removed via phosphatases. Phosphorylation dynamics are a key regulatory mechanism in cells and is essential for cellular function. Understanding these dynamics is integral to the study of cellular processes in both health and disease. However, additional methods for the study of these dynamics in a high-throughput manner are needed.

Stage of Development
Research -

in vitro

Stage of Research
The inventors have developed the novel method MRBLE:Dephos. This method utilizes microfluidically produced hydrogel beads encoded with radiometric combinations of lanthanide nanophosphors (MRBLES). More specifically, this method quantifies binding of a fluorescently labeled protein or chemical group to 96 bead-bound peptides in parallel. The inventors have extended the MRBLES technique into the dephosphorylation space by producing a library of 96 Ser and Thr peptides on MRBLES with basic, acidic, or hydrophobic amino acids surrounding the

phosphorylation site. This library can then be used to investigate the effect of amino acid intrinsic properties on dephosphorylation kinetics of a protein and/or binding site of interest by incubating with phosphatase and then quantifying phosphorylation of each unique peptide using a binding reagent specifically for phosphorylated proteins.

Technology Reference Nos.

- CZ Biohub ref. no. CZB-260S
- Stanford ref. no. S22-253

Applications

- Evaluation of phosphorylation dynamics of many peptides in parallel
- Elucidate crosstalk between N-terminal and C-terminal residues surrounding phosphorylation sites of interest.

Advantages

- Peptides can be directly synthesized on MRBLES resulting in small reaction volumes and minimal reagent waste.
- Easily adaptable to other kinase assays.

Publications

- Hein, J.B., Nguyen, H.T., Garvanska, D., Nasa, I., Feng, Y., Lopez-Mendez, B., Davey, N.E., Kettenbach, A., Fordyce, P.M.*, Nilsson, J., "Global substrate identification and high throughput in vitro dephosphorylation reactions uncover PP1 and PP2A-B55 specificity principles." bioRXiv (2023) (pdf) (web) (OSF repository)

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