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Activity of Protein Kinase A Attached to Magnetic Beads

Kevin P. Lin, Mrugesh K. Parasa, and Tamara L. Kinzer-Ursem
Weldon School of Biomedical Engineering, Purdue University

ABSTRACT

Development of high throughput assays is a crucial step in developing more efficient techniques that aid in many important areas of research today such as drug development or identification of protein structure function relationships. Integration of high throughput assays into more research efforts could drastically decrease the time and cost it takes for a new drug to hit the market. Protein Kinase A (PKA) is an extensively studied protein as it is highly upregulated in cancer and is a hot spot for drug targeting. In this work, azide-tagged PKA is covalently attached to magnetic beads using azide-alkyne cycloaddition, a well-known click chemistry reaction that selectively and covalently links two compounds. Modified PKA is attached to magnetic beads and the activity of the covalently bound PKA is determined. Significant levels of PKA activity can open the door to development of more efficient drug screening processes. It is anticipated that the azide-PKA conjugated beads will have significantly more PKA activity than beads treated with non-tagged PKA since there is specificity in binding between the azide-tagged PKA and the magnetic bead. Additionally, preliminary data using an inhibitor assay and ATP gradient scale suggests that linked PKA has similar chemical properties with native state PKA subject to the same treatments.

KEYWORDS

Kinematics, Protein, Protein Kinase A, High Throughput