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Immobilization of proteins on agarose beads, monitored in real time by bead injection spectroscopy

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A novel approach to real-time monitoring of protein immobilization resulted in the surprising finding that current immobilization protocols are far from optimized.

# Summary

This work introduces a novel tool for the examination and optimization of protein immobilization protocols, by measur- ing the rate and yield of coupling reactions, as they take place on the surface of agarose beads in a well-stirred microreactor. The power of the Bead Injection Spectroscopy (BIS) technique is demonstrated on examples of amino coupling reactions for albumin, ovalbumin, lysozyme, human IgG, ribonuclease A

and cytochrome C, using commercially available Aminolink1

agarose beads. It was found, surprisingly, that currently recommended protocols for reductive amination can be shortened from several hours to several minutes, and that, contrary to literature data, the yield of coupling is dependent on pH and the isoelectric point of the protein. In addition, leakage of immobilized ligands can be measured by direct spectroscopic interrogation of captured beads in situ. The methodology presented in this work documents that BIS is a useful tool for quality control of agarose-based chromato- graphic supports, as well as for the optimization of a wide variety of immobilization chemistries, as used for synthesis of chromatographic supports, immobilization of enzymes, and derivatization of biosensing surfaces.

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