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## Hydrophobic amino acids functionalized SPION as a powerful tool for insulin nanofibril bioseparation

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### Abstract

Superparamagnetic iron oxide nanoparticles (SPIONs) have garnered significant interest due to their unique superparamagnetic properties and high surface-area-to-volume ratio, which enhance molecular interactions and facilitate efficient absorption and penetration across various applications. A key feature of SPIONs is their ability to lose magnetization when the external magnetic field is removed, making them ideal for targeted applications and easy removal after use. In this study, SPIONs with an iron oxide core were synthesized via co-precipitation and functionalized with different hydrophobic amino acids to target insulin fibrils formed under stress conditions. Among them, SPIONs functionalized with optimum hydrophobicity were selected. Due to their iron content, these nanoparticles exhibit low toxicity and good biocompatibility with the human body, akin to the iron in hemoglobin. The behavior of the functionalized SPIONs with insulin fibrils was investigated using spectroscopy and fluorescence microscopy. Results demonstrated high selectivity of the SPIONs for insulin fibrils, effectively separating them while exhibiting no such affinity for native insulin. Additionally, the efficacy of SPIONs in the presence of a molecular crowding agent—mimicking the high concentration of fibrillated protein in cellular environments—was confirmed, further highlighting their potential for therapeutic applications.

**Key words:** Insulin, fibrils, hydrophobicity, superparamagnetic iron oxide nanoparticles, amino acid, bioseparation