Enzyme Coating of Trypsin on Electrospun Polymer Nanofibers for Efficient Protein Digestion in Proteomics

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For the rapid and efficient protein digestion for proteomic analysis, trypsin (TR) was immobilized and stabilized on electrospun polymer nanofibers (NF) via enzyme coating (EC) approach. After covalently attaching TR molecules on the NF (CA-TR/NF), the simple addition of crosslinking step (EC-TR/NF) resulted in a dramatic increase of both activity and stability of TR. EC-TR/NF showed increased stability than CA-TR/NF in rigorous conditions. By introducing superparamagnetic nanoparticles (NP) during electrospinning, NP entrapped nanofibers (NP-NF) were prepared that can be recycled via facile magnetic separation. Enzyme coatings of TR on magnetic nanofiber (EC-TR/NP-NF) still showed excellent performance, such as 4700 and 70 fold higher activity and stability, respectively, than those of CA-TR/NP-NF. EC-TR/NP-NF was employed for the digestion of a model protein, enolase. There was no decrease on the activity after 7 times of recycled uses. Highly active and stable enzyme coating approach with the combination of magnetically-separable NP-NF can be employed for many other enzymes, the applications of which are hampered by their poor stabilities.