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A simple procedure is described for patterning biotin on a glass substrate and then selectively immobilizing proteins of interest onto the biotin-patterned surface. Microcontact printing (μ CP) was used to generate the micropattern of biotin and to demonstrate the selective immobilization of proteins by using enhanced green fluorescent protein (EGFP) as a model protein, of which the C-terminus was fused to a core streptavidin (cSA) gene of Streptomyces avidinii. Fluorescence microscopy was used to visualize the pattern of the immobilized protein (EGFP-cSA), and surface plasmon resonance was used to characterize biological activity of the immobilized EGFP-cSA. As a proof-of-principle, we constructed Bacillus spores that displayed EGFP on the spore surface. The results suggest that this strategy is an effective way for fabricating biologically active substrates that are suitable for a wide variety of applications. [Our work was supported by the KOSEF through the Center for Ultramicrochemical Process Systems and by grant No. (R01-2006-000-11175-0) from the Basic Research Program of the Korea Science and Engineering Foundation.]