Supporting Information

Chemoselective surface immobilization of proteins through a cleavable peptide

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Experimental section

Peptide linker synthesis

The peptide, whose sequence is reported in Fig.1, was synthesized by solid phase method using Fmoc chemistry. Sieber Amide resin (213 mg, 0.72 mmol/g) was used as solid support. The following side chain protecting groups were used: Mmt, Cys; 2Cl-Trt, Tyr; Trt, Ser. Each amino acid was condensed by double coupling using HBTU/HOBt condensing agent. The last insertion was carried out with $(Boc)_2N\text{-}O\text{-}CH_2\text{-}COOH$. The deprotection of the side chains and the cleavage of the peptide from the resin were carried out by treatment with 1% 2,2,2-trifluoroacetic acid, 5% triethylsilane in DCM for 10 minutes.

PLP Oxidation

BSA (60 nmoles) was dissolved together with pyridoxal-5'-phosphate (12 μmoles) in 1.2 mL phosphate buffer (25 mM, pH 6.5) and incubated for 24 hrs at 37°C.

SPR analysis

All experiments were performed on a BIAcore X instrument (GE-Healthcare, Milwaukee, WI). Bis-Boc-protected peptide linker was immobilized onto a gold-coated sensorchip for SPR analysis (Xantech Bioanalytics, Dusseldorf, Germany) as follows: after measuring the RU response of the naked gold surface (starting baseline), the sensorchip was removed from the BIAcore apparatus, a drop (30 μL) of methanol containing the Bis-Boc-protected peptide linker (1 mM) was deposited

onto the gold surface and incubated for 16 hrs at 25 °C in a methanol-satured chamber. The excess of peptide not immobilized was removed by washing the gold surface with three alternate washing with deionized water and ethanol. Then, the sensorchip was re-inserted in the BIAcore apparatus and the new RU response was measured to evaluate the amount of peptide linker effectively immobilized onto the gold surface. The sensorchip was removed again and deprotection of Bis-Boc groups was achieved by a 16-hrs incubation at 25 °C in 1 M phosphoric acid pH 2 followed by extensively washing with deionized water. The sensorchip was finally re-positioned in the BIAcore apparatus and regenerated with 2 injection of 100 mM HCl followed by one injection of 50 mM NaOH and of 0.5% SDS in HBS-EP buffer (flow rate equal to 100 μL/min for 30 s). The fusion of PLP-BSA to the immobilized peptide linker was obtained by injecting the protein (50 μM in 100 mM anilinium acetate pH 4.5) at a flow rate of 10 μL/min for 5 minutes followed by the regeneration the sensorchip with an injection of 10 mM glycine pH 1.5 in HBS-EP buffer at the same flow rate for 4 minutes. The enzymatic cleavage was performed by injecting MMP-2 (0.02 μg/mL in HBS-EP buffer) at a flow rate equal to 2 μL/min for 50 minutes. Then, HBS-EP buffer alone was fluxed at the same flow rate for 2 hrs.