BS^3 Crosslinking Assay

Bis (sulfosuccinimidyl) suberate (BS^3) crosslinking was performed as described previously (Grosshans et al., 2001, 2002; Conrad et al., 2008). BS^3 is a membrane-impermeable agent, which selectively crosslinks cell-surface proteins to form high-molecular-mass aggregates. Because intracellular proteins are not modified, they retain normal molecular mass. This enables surface and intracellular pools of a particular protein to be distinguished by SDS–PAGE and Western blotting. Brain tissue containing mPFC were quickly sectioned as 400-μm slices with a Vibratome, and were incubated with BS^3 (1 mg/ml; Pierce Biotechnology, Rockford, IL, USA) in aerated ACSF (95% O_2 and 5% CO_2) at 4°C for 40 minutes with gentle agitation. The slices were then washed three times with ice-cold ACSF containing 20 mM Tris (pH 7.6) to quench the remaining BS^3 and the surface expression was determined by Western blot analysis.