Crystallization of Recombinant HIV-1 Nef

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Negative factor (Nef) is a 27 kDA myristoylated protein of the primate immunodeficiency viruses (HIV and SIV). The protein is crucial in the pathogenesis of HIV and therefore an attractive target for basic research but also for drug discovery and vaccine development.

Nef has a two-domain structure consisting of a folded core domain and of a more flexible anchor domain. Nef has not been crystallized before as whole protein: only the core domain (alone or with Fyn SH-3 kinase) has been crystallized successfully[1,2]. The structure of the anchor-domain has been determined only by NMR-method.

In this work, we have expressed HIV-1 Nef as non-myristoylated GST-tagged fusionprotein in *E. coli.* Protein was purified by glutathione-sepharose column chromatography and GST-tag was cleaved by overnight treatment with bovine thrombin. Resulting Nef-concentrate was diafiltrated to water and concentrated to concentration of 10-18 mg/ml. Crystallization screens with polyethylene glycols and isopropanol in RT produced small crystals. The first experiments to identify the crystals by SDS-PAGE gave promising results of these being the first crystallized full-length Nef.

The crystallization parameters are still further optimized in order to produce crystals suitable for x-ray crystallography.

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