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Lipid Binding Domain Impact on HIV-1 Nef and Src-Family Kinase Hck U-SH3-SH2 Complex

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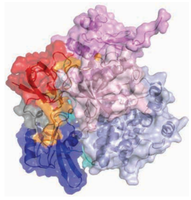
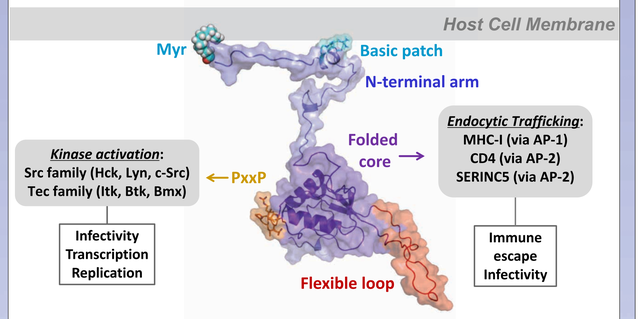
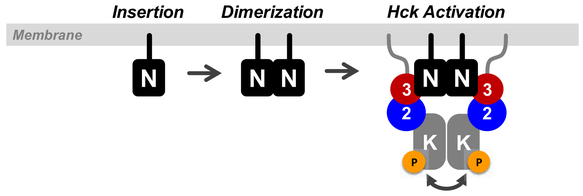


Background

HIV-1 Nef Hijacks Multiple Host Cell Pathways

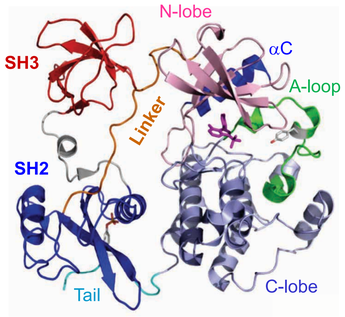


HIV-1 Nef activates Hck via domain displacement



Objectives

- Resolve the roles of the Hck regulatory domains (Unique, SH3, SH2) and cross-domain impact on Nef binding
- Determine the crystal structures and dynamic properties of the Hck regulatory region (U-SH3-SH2) as well as the full-length kinase in complex with Nef
- Identify additional intermolecular interactions between Hck and Nef
- Explore the mechanism of action of inhibitors of Nef-induced Src-family kinase activation and Nef dimerization



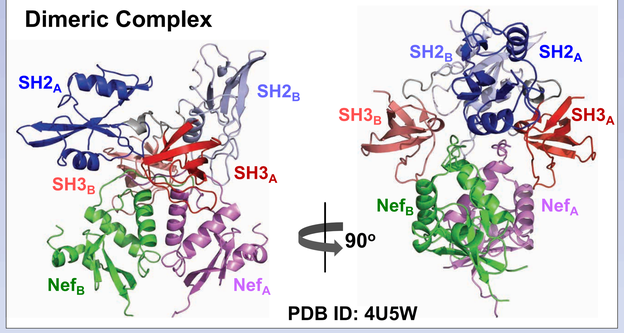
X-ray Crystal Structure of near full-length downregulated Hck (PDB: 1QCF). Note that the SH3 domain (red) engages the SH2-kinase linker (orange), while the SH2 domain (blue) binds to the tyrosine-phosphorylated tail (cyan). The SH3-SH2 domain assembly packs against the back of the kinase domain, away from the active site, to stabilize an inactive conformation. The small molecule inhibitor PP1 (magenta) maps the ATP binding site.

X-ray Crystal Structure of Nef in Complex with the Hck SH3-SH2 Domains

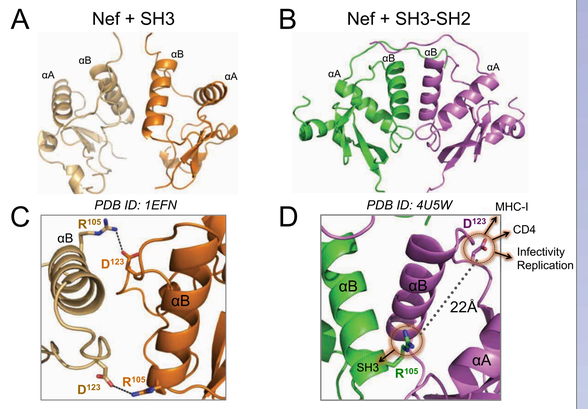
Monomeric Complexes



Dimeric Complex

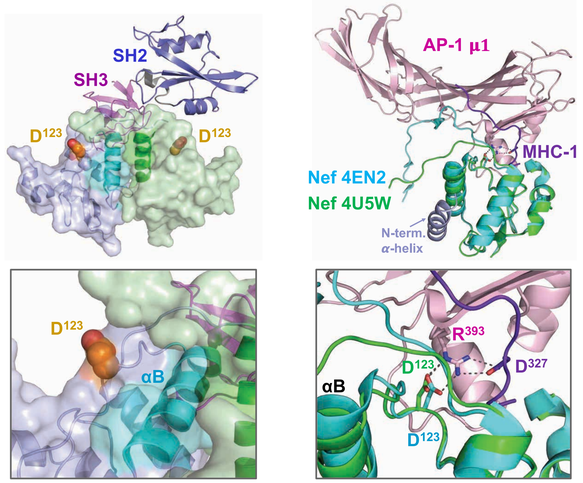


Hck SH3-SH2 Binding Stabilizes a Compact Nef Dimer and exposes Asp123 for interaction



In the Nef•Hck32 complex, binding of the SH3-SH2 ligand reorients and stabilizes a compact Nef dimer. X-ray structures of Nef dimers from Nef•SH3 (*A*) and Nef•SH3-SH2 (*B*) complexes. A Nef dimer contact observed in the Nef•SH3 complex structure shown in (*C*), involves reciprocal salt-bridge interactions between R105 and D123[1]. These R105:D123 salt-bridges have been shown to be required for Nef dimerization[2]. However, in the Nef-SF2:Hck SH3-SH2 complex structure R105 and D123 are separated by a distance of 22 Å as shown in (*D*), with R105 interacting with an SH3 domain residue and D123 assuming a solvent exposed position available for interaction with downstream effector proteins[3]. Importantly, Nef residue D123 has been shown to be important for downregulation of MHC I and CD4, and enhancement of virus infectivity and replication[2,4].

Hck binding may structure Nef for interaction with MHC-I and AP-1



Nef•SH3-SH2 Complex D123 Exposed → **Nef•MHC-I•AP-1 Complex**

Superposition of the Nef core proteins in the complex with SH3-SH2 (PDB: 4U5W; *left panels*) and in complex with the AP-1/μ1 subunit (pink) and MHC-I tail peptide (purple); (PDB: 4EN2; Nef core from this complex in cyan). The Nef core proteins from these two independently solved crystal structures adopt remarkably similar conformations. Particularly important is the nearly identical positioning of Nef Asp123 in both structures (enlarged in lower panels), which form part of a hydrogen bond network with Arg393 from AP-1 and Asp327 from MHC-I that is critical to Nef-mediated downregulation of MHC-I[4,5].

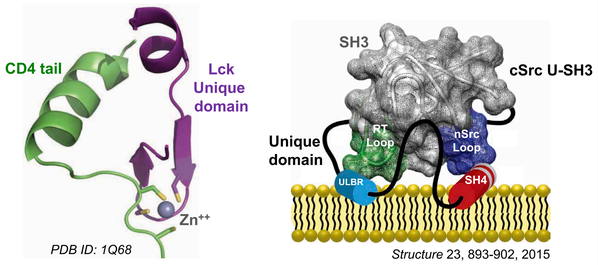
Nef and Hck Lipid-binding Regions Moonlight as Protein-protein Interaction Domains

HIV-1 Nef



Src-family kinases

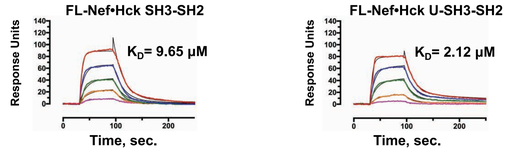




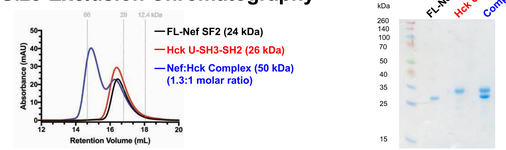
Top) Domain architecture of full-length Nef and Hck. Nef associates with membranes via the N-terminal arm by the insertion of the myristate moiety (Myr) and the association of a basic patch on the N-terminal α-helix. However, in the X-ray structure of the Nef•MHC-I tail•AP-1 μ1 complex, this helix packs against the Nef core (*previous panel; blue*). Domain structure of Hck includes a unique domain which is also myristoylated for membrane binding. While the Hck unique domain structure is not known, recent NMR data suggest regulatory interactions with the SH3 and Unique domains of Src[6,7,8] (*lower right*). In addition, the NMR structure of the Lck unique domain reveals a zinc finger clasp with the CD4 tail that is stabilized by hydrophobic contacts between the two helices[9] (*lower left*). These results suggest that the unique domain of Hck may also form a helix that contacts the N-terminal α-helical region of Nef.

Nef and Hck Lipid Binding Domains Enhance Nef•U-SH3-SH2 Protein Complex Formation

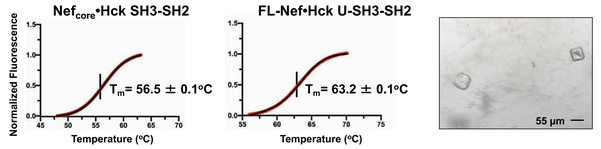
A. Surface Plasmon Resonance



B. Size-Exclusion Chromatography



C. Thermal Melt



D. Crystallization

A) Surface plasmon resonance (SPR) was conducted with full-length (FL) Nef covalently attached to the SPR chip surface and Hck SH3-SH2 and U-SH3-SH2 proteins as analytes. Inclusion of the Hck unique domain enhanced the affinity of the U-SH3-SH2 protein for Nef. *B*) Analytical size-exclusion chromatography demonstrates stable complex formation between FL-Nef and Hck U-SH3-SH2. *C*) Thermal melt analysis of purified Nef:Hck complexes demonstrates increased stability with the inclusion of the lipid binding domains in the FL-Nef•Hck U-SH3-SH2 complex. *D*) Crystallization of the FL-Nef•Hck U-SH3-SH2 complex.

Conclusions

- Nef$_{core}$•Hck SH3-SH2 crystallizes as a dimer of complexes in which Nef Asp123 is exposed to solvent for effector protein interaction
- Hck interaction may remodel the Nef structure for interaction with AP-1 and MHC-I as required for immune escape
- The Hck U-SH3-SH2 protein forms a stable complex with full-length Nef suitable for crystallization screening
- Increased binding affinity to Nef is observed with the inclusion of the Hck unique domain, suggesting additional contacts with Nef
- Inclusion of the Hck and Nef lipid-binding domains increases the thermal stability of the complex
- Successful determination of the X-ray crystal structure of the Nef•Hck U-SH3-SH2 complex will reveal the structure of the Nef and Hck lipid binding domains for the first time

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