PROBING STRUCTURAL CHANGES IN HIV-1 NEF UPON LIPID MEMBRANE ASSOCIATION

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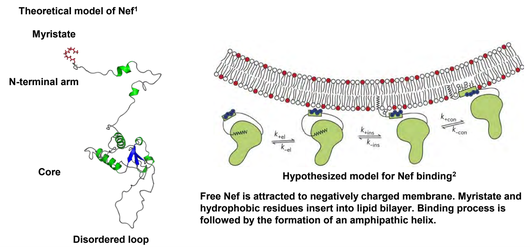
INTRODUCTION

HIV-1 Nef is a 25 kDa accessory protein that is critical for viral replication and progression to AIDS. Nef is cotranslationally myristoylated, localizing it to cellular membranes which is required for Nef functions. It has been postulated that Nef undergoes a significant conformational transition upon lipid association, although no structural details have been characterized. Here, we have used lipid vesicles, with hydrogen exchange mass spectrometry (HXMS) to investigate structural changes within Nef as a result of lipid association.



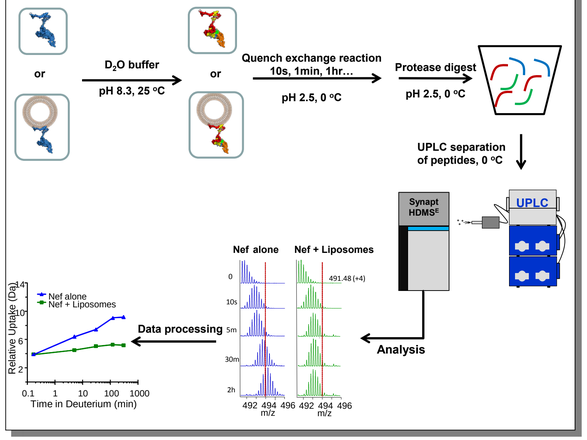
METHODS

Full-length myristoylated Nef (strain SF2) was overexpressed in *E. Coli* transformed with a duet vector coding for both C-terminally 6x his tagged Nef and N-myristoyltransferase. Nef was purified using nickel affinity chromatography and myristoylation was confirmed by electrospray mass spectrometry.

Liposomes were prepared using a 3:2 ratio of POPC and POPG. Lipids were dried with nitrogen, lyophilized over night and resuspended in buffer containing 20 mM Tris and 100 mM NaCl, pH 8.3. Resuspended lipids were extruded (Avanti Polar Lipids, AL) to form unilamellar vesicles 100 nm in diameter. Nef and liposomes were left to incubate for 1 hour prior to labeling.

Continuous labeling experiments[3] were carried out at room temperature. Peptide exchange samples were analyzed using a Waters nanoACQUITY with HDX technology[4] coupled to a Waters Synapt G2 equipped with an ESI source. To reduce the amount of lipids being introduced to the mass spectrometer, two C-18 VanGaurd™ pre-column traps were placed ahead of a UPLC BEH column. During separations, the maximum acetonitrile concentration was limited to 85 percent, to reduced lipids introduction into the mass spectrometer.

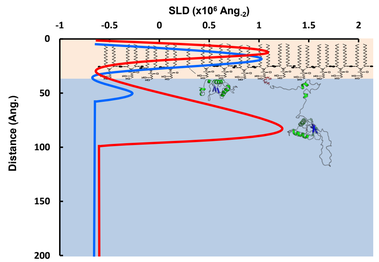
All samples were digested prior to RP-UPLC separation. Peptides were identified using Waters PLGS version 2.5 and deuterium incorporation was analyzed using Waters DynamX 2.0.



RESULTS AND DISCUSSION

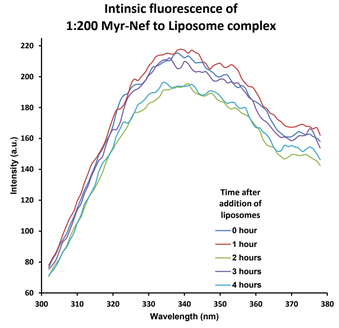
1

Neutron reflection measurements suggest significant conformational change in Nef when in the presence of lipid membranes.



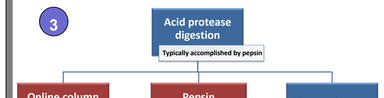
2

Intrinsic fluorescence data shows that Nef gradually associates with lipid vesicles over several hours.



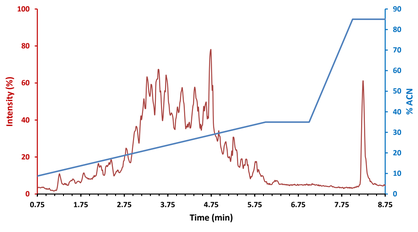
3

Several digestion and quench conditions were tested to optimize digestion with lipids.



4

Myristoylated peptides are extremely hydrophobic and elute much later than all other peptides.



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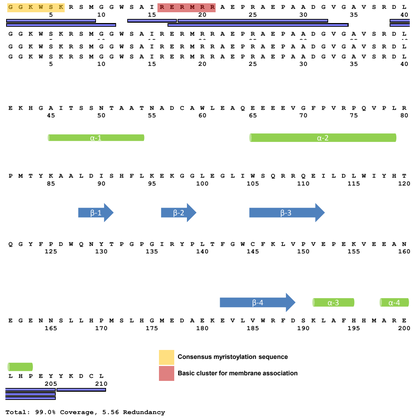
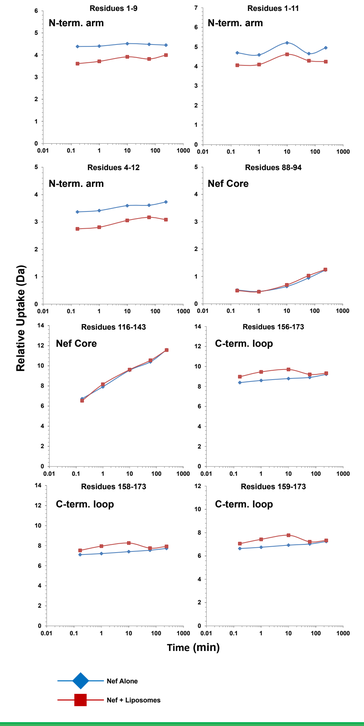


A 2:1(v:v) solution of Factor XIII (EC 3.4.23.18) and porcine pepsin (EC 3.4.23.1) each at 10 mg/ml was used in conjunction with a Gdn HCl denaturing quench buffer.

This efficiently digested Nef/lipid complexes and resulted in high peptide coverage and redundancy.

6

Deuterium uptake plots comparing the difference in overlapping peptides in the N-terminal arm, the protein core and the C-terminal disordered loop.



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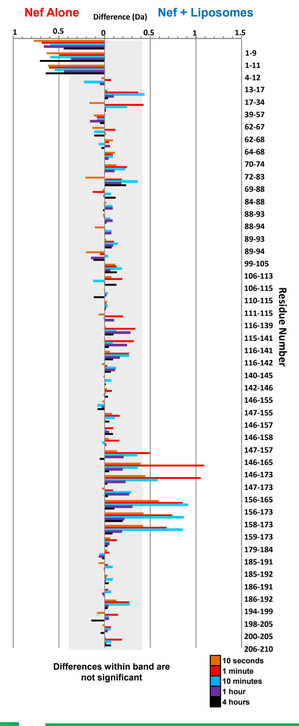
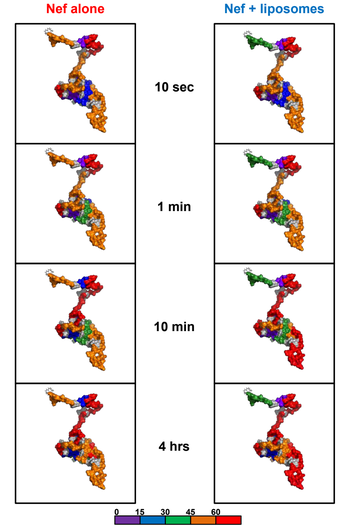
Difference plot highlighting the significant differences (>0.4 Da) between Nef alone and Nef with liposomes.



8

Deuterium levels mapped onto a theoretical model of Nef.



N-terminal arm incorporates less deuterium in the presence of liposomes, suggesting protection.

C-terminal disordered loop incorporates more deuterium in the presence of liposomes, suggesting deprotection.

REFERENCES

1. Geyer, M.; Peterlin, B.M. (2001) *Febs Lett.*, **496**, 91-95.
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3. Wales, T.E.; Engen, J.R. (2006) *Mass Spectrom. Rev.*, **25**, 1, 158-170.
4. Wales, T.E.; et al., (2008) *Anal. Chem.*, **80**, 17, 6815-6820.

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CONCLUSIONS

Using model lipid systems with HX MS provides valuable insight to a biologically relevant conformation of HIV-1 and suggests a significant change in structure when anchored to membranes. These results support previous fluorescence and neutron reflection data.

Nef incorporates deuterium differently upon association with lipid membranes.

The two main regions of difference are the N-terminal myristoylated peptides and the C-terminal disordered loop. Differences in uptake in the N-terminal residues suggest protection when anchored to lipids, while differences in the disordered loop suggest a more exposed and "open" conformation when associated with membranes.

Basic cluster region on the N-terminal arm also incorporates less deuterium when with lipids, again suggesting protection.