Subject: RE: complete model for VpNup133 beta-propeller domain
From: "Parthasrathy Sampathkumar" <psampath@einstein.yu.edu>
Date: 12/11/2013 6:52 AM
To: Seung Joong Kim <sjkim@salilab.org>

SJ,

I am Re-refining my xtal structure (mainly because I felt the difference between freeR and Rcryst was bit on the higher side for Refine26*) with new strategy. I will send an updated, final-final coordinates in a day or two. You could base your final calculations based on that. I was wanted to have the homology model, in the meantime, mainly to see if it could help to complete "short" disordered segments. If you have one sent it across.., if it is too much of trouble do not bother.

With regard to your questions:
1 and 2: I believe this is just an representation issue (with Refine26 I used DSSP based secondary structure assignment). I suggest that you should delete SS definition in the PDB file I sent.., and then open in Chimera to assign SS. This is will likely fix strand assignment issues. Let me know what happens.

question3: yes very N-term and very C-term are disordered, and B chain few more residues compared to A chain.

Here is complete sequence for 15133f11 (remember to replace Met with SeMET):

```
MSLTSNNKYGNTKILTETEKYSVTKLSTDLSFLPGSNGNNNTIDTHHFEGLVDTALQKALVND
LDHIYIWNYNSIQKDTPICKISLHDDYSVLSSPPICLFTSSISSTNNDTANYNNNASGNI
NSGKFNNGICIINKKNSQFLYFEDISTINNLYTKLSKSKAHVLDLKLKDNENITSTINCE
PSGIIIATSLGRVLFITIKDSTGKPKLELKQQLIKPQNSFFFRNLDSSKEIISLKKGPIV
GKGERLLYITTRGGSLQIWQLSINSKSFKRLEINIYEHVLDSLQDLYPFAHGTLAFLDSH
PIYSDTSSAHLTLASISNGNEIYYLMITVILDEKTNSFQIFSIYKLNTYFTKSTVDLNHK
PQLFIPNALDSIVSPTLSVYVLFNNAVVMTQISSKLDSSFPLRRKWEDIIRFNKDVEIIG
SGYSTDSIYVICKDMGVLKIASHSNNNQEGHHHHHH*
```

Bottom line: I will send update-final-final coordinates in by this Friday.

Thanks
Partha

From: Seung Joong Kim [sjkim@salilab.org]
Sent: Tuesday, December 10, 2013 9:03 PM
To: Parthasrathy Sampathkumar
Subject: Re: complete model for VpNup133 beta-propeller domain

Quick questions for VpNup133, some of them I asked two months ago, but I didn't get answer yet...

1. Multiple beta-strands are missing in the latest version (refine 26). They were In the previous version (refine 9), but now they are represented as a coil.

They are within the sequence of
"LSFLPGFEGLVDTAL", 81-107 (one beta-strand was there)

FLYFEDHVLDLKLKDN 193 - 224 (two beta-strands were there)

These should be the beta structures according to the secondary structure prediction.

2. Multiple beta-strands are shorter than the later version.
VGKGERLLYITTRGGSLQIWQLSIN 294-318 (two beta-strands are there)

3. According to SAXS data, the SAXS sample in solution covers "55-502", while the crystal structure covers from "61-497" (chain A), "62-497" (chain B). Also chain B has ~5 more residues than chain A. Do you think residues of 55-60 and 498-502 were disordered in crystal structure? I expect that these two regions to be disordered, but want to make sure.

4. What is the full sequence of the VpNup133?

TSNNKY
GNTKILTETEKYSVTKLSTDLSFLPGSNGNNNTIDTHHFEGLVDTALQKALVNDLDHIYI
WNYNSIQKDTPICKISLHDDYSVLSSPPICLFTSSISSTNNDTANYNNNASGNINSGKFN
NGICIINKKNSQFLYFEDISTINNLYTKLSKSKAHVLDLKLKDNENITSTINCEPSGIII
ATSLGRVLFITIKDSTGKPKLELKQQLIKPQNSFFFRNLDSSKEIISLKKGPIVGKGERL
LYITTRGGSLQIWQLSINSKSFKRLEINIYEHVLDSLQDLYPFAHGTLAFLDSHPIYSDT
SSAHLTLASISNGNEIYYLMITVILDEKTNSFQIFSIYKLNTYFTKSTVDLNHKPQLFIP
NALDSIVSPTLSVYVLFNNAVVMTQISSKLDSSFPLRRKWEDIIRFNKDVEIIGSGYSTD
SIYVICKDMGVLKIASH
SNNNQ

Correct? The observed MW from mass-spec is "51628 kDa", while the MW from the above sequence is "50270 kDa". I suspect that the above sequences are not complete, and there should be "a histag" attached as other samples have. Does VpNup133 has a histag? Can you forward the full sequence if you have?

Can you double-check all my questions? Models are coming soon.

- SJ

On 12/10/2013 12:48 PM, Parthasrathy Sampathkumar wrote:

> Hi SJ,
>
> Just wanted to check with you if had a chance to run these modeling.
>
> Thanks,
> Partha
>
> ---
>
> **From:** Seung Joong Kim [sjkim@salilab.org]
> **Sent:** Friday, December 06, 2013 4:51 PM
> **To:** Parthasrathy Sampathkumar
> **Subject:** Re: complete model for VpNup133 beta-propeller domain
>
> Hi Partha,

I'll do quickly this over the weekend. Sorry for the delay, I also have been to the SAXS beam line yesterday too. These days I have been so busy with many things. I'll provide the complete model on next Monday or so.

Thank you for the update, and yes, let's finish the Nup133 paper soon

- SJ

On 12/6/2013 1:38 PM, Parthasrathy Sampathkumar wrote:

> SJ,
>
> You might have already have this one: did you generate a complete model of VpNup133 beta-propeller domain using the xtal structure coordinates I sent earlier? If not, could you generate one (i.e. keep everything same, just simply fillup the disordered-missing residues) and send it across.
>
> I am Re-refining my structure with couple of new strategies, and send you an updated final coordinates next week.., and then we will quickly try to windup the Nup133 paper.
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> Thanks,
> Partha

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