do not have the knowledge needed for designing tighter binding protein complexes, and we rely on various combinatorial methods (Binz et al., 2005). The results presented by Sundberg and colleagues add greatly to the infrastructure necessary to build better, more precise methods for protein design purposes.

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A PRD1 by Another Name?

The extraordinary quality of the 7.3 Å map of the Bam35 virus (Laurinmäki et al., 2005; this issue of *Structure*) allows analysis of the relationship between lipid bilayer curvature and transmembrane protein location, and highlights its conserved structural features with membrane bacteriophage PRD1.

Membranes play key roles in biology by dividing the cell into distinct environments and controlling traffic between them. Membrane-containing viruses allow the application of the tools of structural biology to defined units of membrane structure.

The Bam35 structure (Laurinmäki et al., 2005) allows us to address a number of open questions in membrane structure, including the architecture of functional membrane assemblies, the influence of an intrinsic membrane protein on membrane structure, and the nature of the sites which mediate genome transfer. The choice of the Bam35 virus was propitious for Butcher and coworkers. The structure of the membrane virus PRD1 was recently determined to approximately 4 Å (Abrescia et al., 2004, Cockburn et al., 2004). The solution of the structure used a refined cryo-EM map (San Martin et al., 2002) originally determined by an author of this paper as her doctoral thesis project (Butcher et al., 1995). The hosts for Bam35 and PRD1 are believed to have diverged over a billion years ago, so the similarities between the two provide examples of conservation due to shared structural roles rather than in response to features of two quite distinct host environments. Consequently, the phenomena shown by the comparison of the structures are seen in an evolutionary context.

The crystallization of PRD1 resulted from a sustained effort (Bamford et al., 2002) driven by the realization that this virus would be a particularly advantageous target for structural biology. This virus contains its membrane within a protein shell so that its description as an "enveloped" virus ignores the most characteristic feature of the particle. The viral proteins surround the membrane rather than being enveloped within it as in the classical view of membrane viruses such as influenza virus. The issue of whether "enveloped" is ever an accurate description for a membrane virus particle remains an open question. The PRD1 structure provided a substantial advance in the structures of membrane assemblies (Abrescia et al., 2004; Cockburn et al., 2004).

The Bam35 structure assumes additional significance in the context of PRD1. PRD1 is a double-stranded DNA bacteriophage and the prototype member of the Tectiviridae. It infects gram-negative bacteria, such as *Escherichia coli* and *Salmonella enterica*. The mature virion has a molecular mass of 66 MDa and comprises over 20 distinct protein species. Protein P3 (43 kDa) is the major capsid protein (MCP) with 240 trimers arranged on an icosahedral lattice with pseudo-T=4 25 triangulation. Oligomers of protein P31 (14 kDa) occupy the icosahedral vertices. It associates with the trimeric P5 protein (34 kDa) and the receptor binding protein P2 (64 kDa) to form flexible spikes. P30 is an elongated protein that spans the capsid and is believed to function as a "tape measure" to fix the size of the virion. (Figure 1) Bam35 is another member of the Tectiviridae that shares its overall organization with PRD1, but infects gram-positive bacteria. Hence, the hosts are quite different. Infection must utilize different pathways.

Membrane Curvature. The influence of the transmembrane proteins on membrane curvature has been suggested since the structure of Sindbis virus was determined by cryo-EM and image reconstruction (Fuller, 1987). The description of the Bam35 revisits this issue in a much more rigorous way. The authors used a fitting of an optimized surface to the features bilayer to

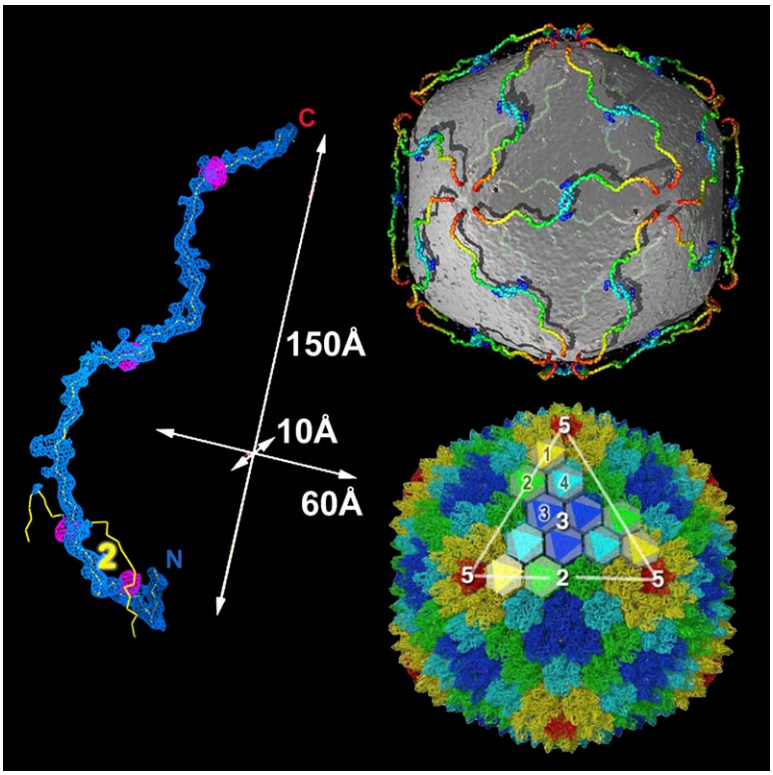

Figure 1. The Tape Measure Protein in PRD1

The electron density of a single copy of P30 is shown in royal blue on the left. The position of the icosahedral 2-fold (2) axis is marked in yellow. The positions of the P30 C (red) and N (blue) termini are shown next to the density. The dimensions of P30 are indicated on the adjacent arrows. The sixty copies of P30 are arranged as dimers to generate a framework (upper right) that positions the 240 copies of P3 (the major capsid protein) (lower right) and result in the properly assembled PRD1 capsid shell. The positions of the icosahedral 2-fold (2), 3-fold (3), and 5-fold (5) axes are marked with white numerals. The four quasi-equivalent trimers of the P3 protein are indicated with red numerals (1, 2, 3, and 4). The figure is adapted from Abrescia et al. (2004) with the gracious assistance of Dr. J. Cockburn.

calculate the curvature of the membrane. This method relies on the high quality of the data. Attempts to perform similar calculations on Semliki Forrest virus (Mancini et al., 2000) were less satisfactory. This may reflect the higher order dependence of curvature on the density that makes it more sensitive to noise. Nevertheless, this approach may become standard in the field as workers follow the authors' example and high-quality maps of membrane viruses become more common.

The authors' major improvement on previous work was in the investment of substantial effort in the refinement of the particle orientations that produced the final map. The initial orientations were determined by the polar Fourier transform method using previous PRD1 reconstruction as a starting model. Iterative refinement was used to achieve high resolution. The final reconstructions were corrected with the full contrast transfer function. The final map shows evidence of this attention to detail since Fourier shell correlation shows a 7.3 Å resolution using the relatively conservative FSC = 0.5 threshold. This may make the noise-sensitive curvature measure more reliable.

The authors show the same striking correlation of curvature with positions of trans-membrane spans for Bam35, PRD1, and Dengue virus. They suggest that this structural feature could function in the assortment of proteins during assembly of the membrane structure in a similar way as has been proposed for rafts (Briggs et al., 2003). This proposal casts the curvature of the membrane as an architectural feature of the virus that helps to define final structure. The modulation of curvature may reflect a lipid-protein interaction that clusters viral proteins within the host membrane and promotes assembly. Such a cooperative interaction could involve the tape measure protein (Figure 1). This peripheral membrane protein (presumably an analog of the PRD1 P30) has extensive N-terminal interactions with the trans-membrane complex. Lateral interactions between the major capsid protein (MCP) could promote the growth of capsid facets over the surface. This would accomplish the geometry-determining role ascribed to the tape measure protein in an elegant cooperation between the membrane and the protein components of the virion.

Protein Identification. One might expect that knowledge of the crystal structure of the similar PRD1 and the use of state-of-the-art biocomputing tools should simplify the identification of the sequences corresponding to the analogous proteins between these two phages. The reality is that Nature is more complex. Threading of the Bam35 sequences into the PRD1 protein structures gave clear answers for only the MCP. Other identifications were more equivocal. The identification of the tape measure protein, for example, was suggestive rather than conclusive. The cryo-EM map allowed the identification of the analogous structural function between the two phages on the basis of their structural similarity in the absence of conserved sequence characteristics. This is encouraging for future efforts in the field because it shows that cryo-EM maps provide insight into structure that extends beyond that of sequence analysis.

The determination of the 7.3 Å resolution structure of the membrane virus, Bam35, is a tour de force that enables a substantial advance in our understanding of membrane assemblies. It shows the promise of cryo-EM in combination with X-ray crystallography for gaining a deeper understanding of membrane assemblies.

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Two Blades of the [Ex]Scissor

Boelens and coworkers (Tripsianes et al., 2005) present the structure of the heterodimeric complex of the C-terminal interaction domains of the human XPF/ERCC1 structure-specific endonuclease. The authors also provide new insights into the mechanism of XPF/ERCC1 by characterizing its DNA binding properties.

The integrity of the human genome is constantly threatened by environmental hazards such as ultraviolet (UV) radiation and chemical carcinogens, as well as endogenous toxins such as reactive oxygen species. To respond to the diverse types of DNA damage caused by these hazards, humans have developed sophisticated DNA repair mechanisms: nucleotide excision repair (NER), base excision repair, mismatch repair, and homologous recombination. Of these pathways, NER in particular repairs a broad spectrum of damage types, including bulky sunlight-induced cyclobutane pyrimidine dimers and 6-4 photoproducts.

The importance of a functional NER pathway for maintaining human health is highlighted in three hereditary autosomal recessive diseases: xeroderma pigmentosum (XP), cockayne syndrome (CS), and trichothiodystrophy (TTD). These diseases occur because of abnormalities in specific NER genes and their gene products. Patients with XP suffer from extreme photosensitivity and have an approximately 1000-fold higher incidence of squamous and basal cell carcinomas of the skin (Friedberg, 2001). Patients with CS and TTD have increased photosensitivity, but with much less likelihood for cancer progression, and also suffer other neurological and developmental disorders (Friedberg, 2001; Lehmann, 2003).

NER can be organized into three fundamental steps: (1) DNA damage recognition, (2) DNA damage excision, and (3) gap-filling synthesis and ligation. In this issue of *Structure*, Boelens and coworkers (Tripsianes et al., 2005) report on new insights into the second step of the overall NER process—damage excision. The authors describe biochemical and structural data for the human XPF/ERCC1 complex, a structure-specific endonuclease that cleaves a damaged DNA strand on the 5′ side of the lesion during the damage excision step of NER.

A major challenge to improving our understanding of the molecular mechanisms of NER is determining high-resolution 3D structures of the constituent proteins and the complexes they form. While much biochemical information has accumulated about the NER process (Riedl et al., 2003), it has been more difficult to obtain structural insight into the proteins functioning in DNA repair. This circumstance certainly has been true for the XPF/Rad1/Mus81/Hef structure-specific endonuclease family, although the 2005 publication year has witnessed a breakthrough with six reports describing structural information for members of the family. Remarkably, each study has brought its own unique advancement to the understanding of DNA damage excision.

Members of the XPF/Rad1/Mus81/Hef family possess a similar domain organization: a catalytic nuclease domain required for DNA cleavage and a helix-hairpin-helix (HhH) motif implicated in protein dimerization and DNA binding. Some family members also contain a helicase domain that may modulate interactions with DNA, although the function of the helicase-like remnant in human XPF, for example, is still ambiguous. The recent advances in structural characterization of XPF/Rad1/Mus81/Hef endonucleases have centered around these functional domains: (1) crystal structure determination of the archaeal Hef nuclease domain (Nishino et al., 2003), helicase domain (Nishino et al., 2005a), and HhH domain (Nishino et al., 2005b); (2) crystal structure determination of the crenarchaeal XPF homodimer alone and bound to dsDNA (Newman et al., 2005); (3) crystal structure determination of the central domain of human ERCC1 as well as the $(HhH)_2$ domain heterodimer of human XPF-ERCC1 (Tsodikov et al., 2005); and (4) the use of NMR cross-saturation experiments to map the interaction surface between human XPF and ERCC1 $(HhH)_2$ domains (Choi et al., 2005).