

GM070785 FINAL REPORT

A. Specific Aims:

The specific aims of the grant remained the same as in the initial proposal throughout the entire grant period.

B. Studies and Results

RNA viruses:

Our first-generation model of pariacoto virus (PaV) led to a new model for the assembly of icosahedral non-enveloped single-stranded RNA and DNA viruses (Devkota *et al.*, 2009). Protein-protein interactions are too weak to lead to capsid formation in the absence of RNA, because the N- and C-termini of the capsid proteins have large positive charges. We hypothesize that assembly is a two-step process. First, binding of these tails to RNA leads to the collapse of the genome in a process reminiscent of DNA condensation. Second, this collapse leads to high local concentrations of the protein globular domains that form the capsid, and this concentration is high enough to drive the relatively weak protein-protein interactions, yielding the mature virus.

We followed up on this hypothesis with a combined experimental and computational study on the condensation of nucleic acids by polyvalent cations. The experimental work was done in collaboration with Nick Hud, a professor in the School of Chemistry and Biochemistry at Georgia Tech, with financial support from this grant. We examined the condensation of double-helical DNA by polyvalent cations, in the presence and absence of integration host factor (IHF), a protein that helps to organize bacterial chromosomes. Tumpa Sarkar, a graduate student in the Hud laboratory, led the experimental work, while Anton Petrov, the lead postdoc on this grant, carried out coarse-grain simulations in the Harvey laboratory. We reported the results of that study in *Biochemistry* (Sarkar *et al.*, 2009).

We also developed an all-atom model for satellite tobacco mosaic virus (STMV). Alex McPherson's group (Univ of California, Irvine) had determined the structure of the capsid over a decade ago, using x-ray crystallography. That structure also revealed the icosahedrally averaged electron density for 30 RNA double helices, each containing 20 nucleotides; because of the averaging, the sequence cannot be determined for those double helices. In 2011 Susan Schroeder (Univ of Oklahoma) proposed a secondary structure for the genomic RNA inside the virus, based on chemical probing. We combined those data to develop an all-atom model for the mature virus, including every single amino acid and every single nucleotide. *This is the first all-atom model for any virus.* The paper has just appeared (Zeng *et al.*, 2012).

Second, we used the SHAPE probing technology to determine the secondary structures of the STMV RNA outside the virus. While analyzing the original experiments, we found that there are problems with the standard software that is used in other laboratories, so we have implemented our own analysis software. After establishing and verifying the SHAPE methodology – both experimental and computational – by replicating previous work on the P4/P6 ribozyme, we carried out studies on the STMV RNA, finding that the secondary structure of the free RNA is

very different from the structure inside the virus, determined by the Schroeder lab. Our results provide insights into the stability of the naked RNA, which is infectious and can travel through the tobacco plant without being encapsidated. Our results also shed light on possible mechanisms for viral assembly. The paper describing this work has just been submitted to *PLoS ONE* (Atavale *et al.*, submitted).

DNA bacteriophage:

We demonstrated that DNA torsional stiffness has no effect on the free energy cost of packaging double-helical DNA into phages, nor does it affect the final conformation (Rollins *et al.*, 2008). As part of our efforts to quantitatively describe the conformations of double-helical DNA inside bacteriophage, Anton Petrov, the postdoc on the project, developed a series of order parameters (Petrov *et al.*, 2009).

We had previously determined the effects of polyvalent cations such as magnesium, spermine, spermidine, and cobalt hexamine on the conformation of the DNA genome inside the virus, and after release into the host bacterium. We extended that work to explore the effects of polyvalent cations, viscosity, pulling forces and osmotic pressure on the thermodynamics and kinetics of genome release into free solution, or into the crowded environment of the host bacterium (Petrov *et al.*, 2011).

Other DNA Viruses:

Aravind Asokan (Univ of North Carolina) recently contacted us, asking for assistance in understanding an unexpected property of adeno-associated virus (AAV). This virus contains a DNA genome that is sometimes single-stranded, sometimes double-stranded, and sometimes a mixture of these, depending on sequence and the stage of the viral cycle. The Asokan laboratory examined the stability of the virus as a function of the kind of DNA inside it, using elevated temperatures to disrupt the virus. The empty capsid is quite stable, so it would be expected that viruses containing smaller genomes would be stable (they are) and that, as genome size is increased, the virus would become less stable, due to the DNA-DNA repulsive forces; this latter expectation is also true. Surprisingly, for a given genome size, viruses containing single-stranded genomes are less stable than those containing self-complementary genomes, even though double-stranded forms of the latter would be under much greater elastic and entropic pressure than the former. We have carried out a series of simulations on coarse-grained model viruses with single- and double-stranded genomes, concluding that confinement pressure is sufficient to inhibit the formation of extensive double-stranded regions, and that the gentle outward pressure of the partially double-stranded genome actually stabilizes the viral particles (Horowitz *et al.*, submitted).

Thermodynamics of Viral Packaging:

We analyzed the thermodynamics of DNA packaging into bacteriophage in a number of our simulations. Our calculated values for the free energy cost of packaging the phi29 genome were within 20% of the experimental values, and there are no free parameters in our model. This result provides strong support for the quality of our model. More important, we were able to decompose the free energy change into its enthalpic (electrostatic and elastic) and entropic components finding that about half the free energy cost is due to the entropic penalty of

packaging the DNA into the limited space inside the virus. To our surprise, that result generated some controversy, as two research groups had developed models for DNA packing in bacteriophage that assumed that there is no entropic cost.

After three years of hard work, we were able to come up with a theoretical approach to evaluating the entropy penalty of confining an elastic polymer of persistence length P into a small space. We treated confinement between parallel planes separated by a distance d , into a cylinder of diameter d , and into a sphere of diameter d . Our approach confirmed earlier predictions about the asymptotic behavior of the entropy change in the limits of very small and very large d for some geometries. We were able to go further, providing exact values for the entropy penalty over all values of d/P . This work, which substantiates our earlier estimates of the role of entropy in viral packaging, represents a fundamental advance in polymer theory, and the paper reporting these results has just appeared (Harvey & Smyda, 2012).

Reviews:

We published several reviews during the grant period. One surveyed all our results on DNA bacteriophage (Petrov *et al.*, 2008), while a later review covered both DNA and RNA viruses (Harvey *et al.*, 2009). We published one review covering just our coarse-grain methods (Tan *et al.*, 2009), and we published a long, detailed review in *Methods in Enzymology*, covering all aspects of the methods and a complete listing of the parameters. (Harvey *et al.*, 2011). Our most recent review raises questions about the global organization of RNA inside small icosahedral viruses (Harvey *et al.*, submitted).

C. Publications:

- G.C. Rollins, A.S. Petrov and S.C. Harvey, "The Role of DNA Twist in the Packaging of Viral Genomes," *Biophys. J.* **94**, L38-L40 (2008).
- A.S. Petrov and S.C. Harvey, "Packaging Double-Helical DNA into Viral Capsids: Structures, Forces and Energetics," *Biophys. J.* **95**, 497-502 (2008).
- B. Devkota, A. Petrov, S. Lemieux, M.B.Boz, L. Tang, A. Schneemann, J.E. Johnson and S.C. Harvey, "Structural and Electrostatic Characterization of Pariacoto Virus: Implications for Viral Assembly," *Biopolymers* **91**:530-538 (2009) doi:10.1002/bip.21168.
- S.C. Harvey, A.S. Petrov, B. Devkota and M.B. Boz, "Viral Assembly: A Molecular Modeling Perspective," *Phys. Chem. Chem. Phys.* **11**:10553-10564 (2009) doi:10.1039/B912884K.
- A.S. Petrov, C.R. Locker and S.C. Harvey, "Characterization of DNA Confinement Inside Bacteriophages," *Phys Rev. E.* **80**:021914 (2009) doi:10.1103/PhysRevE.80.021914.
- T. Sarkar, A. Petrov, J.R. Vitko, S.C. Harvey, I. Mukerji and N.V. Hud, "Integration Host Factor (IHF) Dictates the Structure of Polyamine-DNA Condensates: Implications for the Role of IHF in the Compaction of Bacterial Chromatin," *Biochemistry* **48**:667-675 (2009) doi:10.1021/bi8019965.
- Robert K.-Z. Tan, A.S. Petrov, B. Devkota and S.C. Harvey, "Coarse-Grained Models for Nucleic Acids and Large Nucleoprotein Assemblies", in Coarse-Graining of Condensed Phase and Biomolecular Systems (G.A. Voth, Ed., CRC Press, Boca Raton, FL), pp. 225-236 (2009).

- S.C. Harvey, A.S. Petrov, B. Devkota and M.B. Boz, "Computational Approaches to Modeling Viral Structure and Assembly," *Methods Enzymol.* 487, 513-543 (2011) PMID: 21187237.
- A.S. Petrov and S.C. Harvey, "Role of DNA-DNA Interactions on the Structure and Thermodynamics of Bacteriophages Lambda and P4," *J Struct Biol* 174, 137-146 (2011) PMID: 21074621.
- S.C. Harvey and M.R. Smyda, "The Entropic Cost of Polymer Confinement," *J Phys Chem B* 116, 10928-10934 (2012) doi:10.1021/jp302807r.
- Y.Y. Zeng, S.B. Larson, C.E. Heitsch, A. McPherson and S.C. Harvey, "The Structure of Satellite Tobacco Mosaic Virus," *J Struct Biol* 180, 110-116 (2012) doi:10.1016/j.jsb.2012.06.008.
- S.S. Athavale, J.J. Gossett, J.C. Bowman, N.V. Hud, L.D. Williams and S.C. Harvey, "In vitro Secondary Structure of the Genomic RNA of Satellite Tobacco Mosaic Virus", *PLoS ONE* (revised and resubmitted).
- A.S. Petrov, Scott S. Douglas and S.C. Harvey, "Effects of Pulling Forces, Osmotic Pressure, Condensing Agents and Viscosity on the Thermodynamics and Kinetics of DNA Ejection from Bacteriophage to Bacterial Cells: A Computational Study," *J Phys Condensed Matter* (submitted)
- E.D. Horowitz, K.S. Rahman, B.D. Bower, D.J. Dismuke, J.D. Griffith, S.C. Harvey and A. Asokan, "Biophysical Analysis of Adeno-Associated Viral Uncoating: Effect of Single-Stranded vs. Self-Complementary Genomes," *J Virol* (submitted).
- S.C. Harvey, Y. Zeng and C.E. Heitsch, "The icosahedral RNA Virus as a Grotto: Lots of Stalagmites. Any Stalactites?: " *J Phys Biol* (submitted)

D. Project-Generated Resources: None

E. Inventions and Patents: None