known inhibitor-enzyme complex [6]. In this structure-based design we have already found 100x better inhibitor of mitochondrial nucleotidase and some other promising compounds.

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Keywords: Nucleotidase, Structure-based drug design

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Control of the capacity for structural polymorphism of a T=3 capsid protein

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Rabbit Hemorrhagic Disease virus (RHDV) is the causative agent of a highly infectious disease of domestic and wild rabbits. RHDV is the prototype strain of the genus *Lagovirus* within the family *Caliciviridae*, a group of nonenveloped, icosahedral viruses. Because a cell culture able to support authentic RHDV has not been found, much of our understanding of these viruses, including their structure, has depended on self-assembled recombinant RHDV emptyVLP.

Caliciviruses are composed of 180 copies of a single capsid protein (CP). Atomic resolution structures of VLP of Norwalk virus [1], and native San Miguel sea lion virus (SMSV) [2] and feline calicivirus (FCV) virions [3] indicate that the virion consists of 90 dimers of the CP arranged with a T=3 symmetry. Each monomer contains three structural domains, an N-terminal arm (NTA), the shell (S), and a protruding domain (P). The P domain is highly flexible [4], which facilitates virus-host receptor interactions at the outermost surface [5].

We established the mechanism that allows VP60 (the RHDV CP) to switch among quasi-equivalent conformational states. In contrast to results with other caliciviruses, the VP60 molecular switch that controls its structural polymorphism (to acquire the three conformations required for a T=3 capsid) is contained in the NTA region, which faces the inner surface of the capsid shell. Mutants lacking the first 29 N-terminal amino acid residues lost the ability to acquire different conformations and assemble mostly into a T=1 capsid [6].

RHDV VLP structure has been solved to 7.0 Å resolution by three-dimensional cryo-electron microscopy. These studies allowed definition of the backbone structure of VP60 protein following flexible docking analysis of homologous models. Our quasi-atomic model was validated by insertion of foreign epitopes at the N- or C-terminal regions, as well as in predictable internal loops facing the outermost surface of protruding domain. These structural studies constituted the framework for use of RHDV capsids as a delivery system for B and T cell epitopes. The hypervariable region (HVR) of FCV capsid protein (HVR_{FCV}), which localizes at the outermost tip on its protruding domain, contains a neutralizing epitope. The inserted HVR_{FCV} epitope has the sequence GSGNDITTANQYDAADIIRN. Double insertion of this epitope at the VP60 N-terminal end leads to larger T=4 capsids, increasing the capacity for structural polymorphism of a CP. Previous manipulations to decipher the molecular basis of the inherent structural polymorphism of a capsid protein always led to loss of structural plasticity, and we are exploring this unprecedented discovery at finer structural and molecular levels

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Keywords: three-dimensional cryoelectron microscopy, structural polymorphism, capsid protein.

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Electron diffraction of submicron 3D protein crystals

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X-ray diffraction is still the leading technique for structure determination of three dimensional protein crystals. However, obtaining well diffracting protein crystals suitable for X-ray crystallography is still a major bottleneck. Small needle-like crystals are often obtained that cannot be optimized for X-ray diffraction. Recent results have shown that electron diffraction is more suitable for obtaining diffraction patterns from sub-micrometer sized protein crystals. However, radiation sensitivity still makes it difficult to obtain multiple electron diffraction patterns of a single crystal, limiting structural studies. This work discusses the use of a Medipix detector for data collection. The Medipix is a CMOS, electron counting pixel detector collecting virtually no background noise. The detectors high sensitivity allows diffraction rotation data to be obtained from a single protein crystal. This could make data collection of electron diffraction patterns suitable for solving three dimensional protein structures.

Keywords: protein nano-crystallography, electron diffraction, CMOS detector

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Structural analysis of *Rice dwarf virus in vitro* and *in vivo*

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Rice dwarf virus (RDV), member of the genus *Phytoreovirus* in the family *Reoviridae*, is an icosahedral double-shelled particle of approximately 70 nm in diameter. The core of the virus is composed of P3 proteins which encapsidate 12 segments of double-stranded RNA and transcriptional enzymes. The core is surrounded by the outer capsid shell, which consists of the P8 proteins as the major component and the P2 and P9 proteins as the minor components. The capsid structure of RDV including 120 copies of P3 and 780 copies of P8 proteins has been determined at 3.5 angstrom resolution by x-ray crystallography [1]. However, the structure and location of the P2 protein required for vector transmission remains unknown, because the specimen used for x-ray crystallography lacked the P2 protein during the sample preparation. Here, we used complete RDV virions with