Simulation of a Complete Viral Shell

Applications

Viral shells are protein assemblies that protect the genetic material inside. The icosahedral structure of many animal and plant viruses allows internal pressures of more than 60 atm. These viral capsids behave extremely stable and highly elastic upon external forces and rupture of the shells occurs during indentations with an atomic force microscopy (AFM) tip of more than 30% in capsid height. Only a fundamental understanding of the capsid's mechanical properties and their distribution on the viral shells can finally answer the questions how viral assembly and infection proceeds, and what are the driving forces of the observed structural changes during maturation and capsid breakage.

We studied the mechanical properties of the fully solvated shells of Southern Bean Mosaic Virus (SBMV) and Human Rhinovirus (HRV) 16 on atomic length scale by extended force-probe (FP) molecular dynamics simulations. Both shells consist of 60 identical triangular subunits comprising the complete capsid. Structural units are built up from 5 subunits surrounding the 5-fold symmetry axes (pentamers) and 6 subunits around the 3-fold symmetry

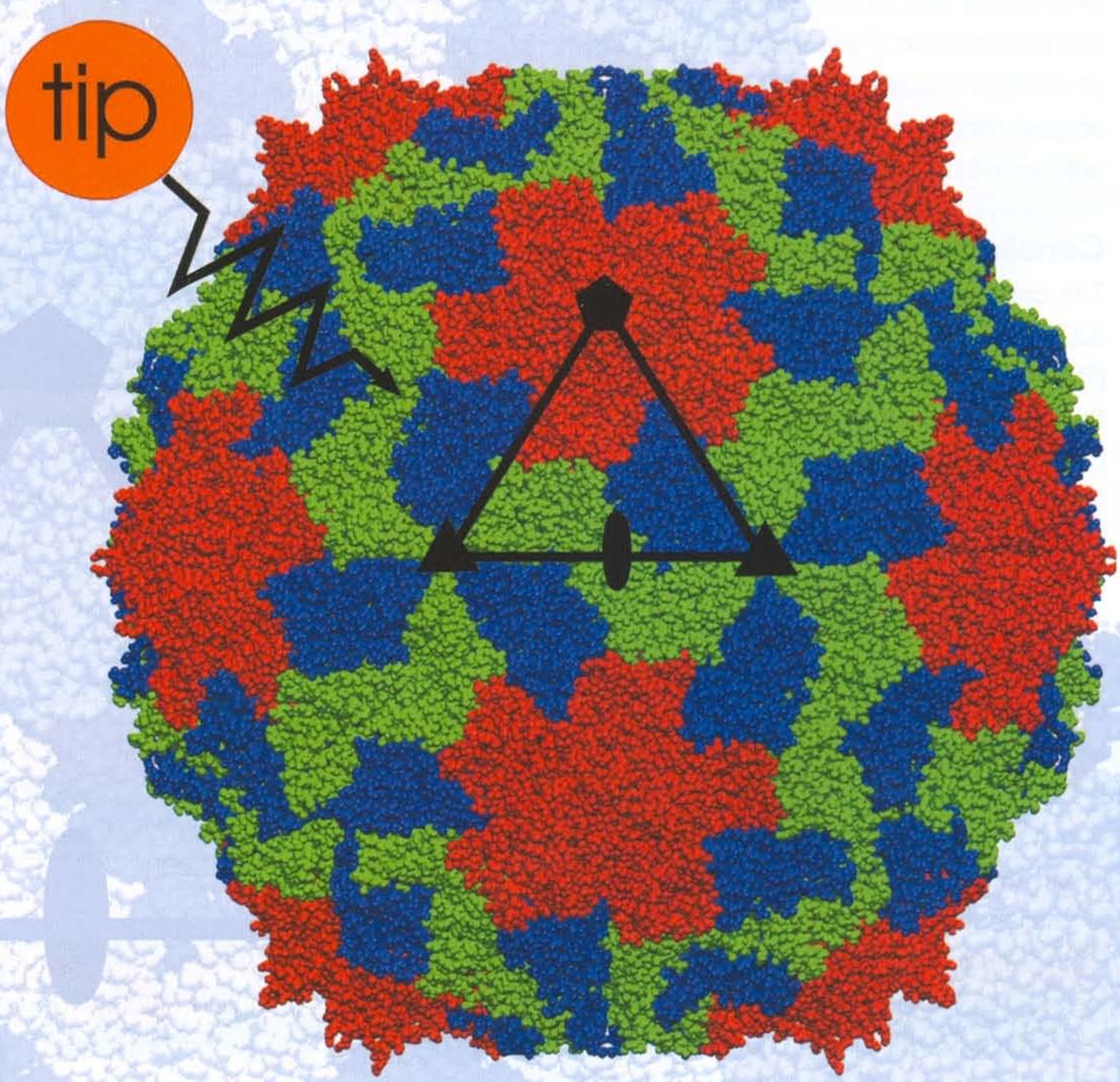

Figure 1: Shell of SBMV consisting of protein A (red), B (blue), C (green). The tip-sphere is shown as orange sphere. One subunit is marked (triangle), the black symbols denote the 5-, 3- and 2-fold symmetry axes.

axes (hexamers). All simulations were carried out on 64 processors of the Altix-SGI machines, using the software package GROMACS-4.0. To facilitate direct comparison with atomic force microscopy measurements, a Lennard-Jones sphere, which served as a model of the AFM tip, was attached to a "virtual" spring and pushed with different velocities towards and through the capsid protein at 19 different positions on the triangular subunit of the viral surface (Fig. 1). This simulation technique offers the unique opportunity to probe the mechanical properties of the capsids' internal surfaces. Thus, the tip-sphere was additionally placed inside the shells and pushed towards the inner surfaces of SBMV and HRV 16.

The simulation system of HRV 16 comprised over 4,200,000 atoms, the system of SBMV more than 4,500,000 atoms and are therefore two of the largest biomolecular systems simulated so far. In total, our simulations sum up to 0.5 μs in length which can only be handled with up-to-date supercomputer power.

Our simulations showed that the capsids of SBMV and HRV 16 behaved highly elastic upon indentation with the tip-sphere. Only the amino acids close to the indentation position deformed plastically, whereas the complete shell did not deform at all (Fig. 2).

A detailed picture of the spatial distribution of elastic constants that describe the stiffness of the material, and yielding forces needed to cause rupture and determine the shell's stability, was obtained for the surfaces of SBMV and HRV 16 (Fig. 3 and 4). An inhomogeneous distribution of both, elastic constants and yielding forces was found.

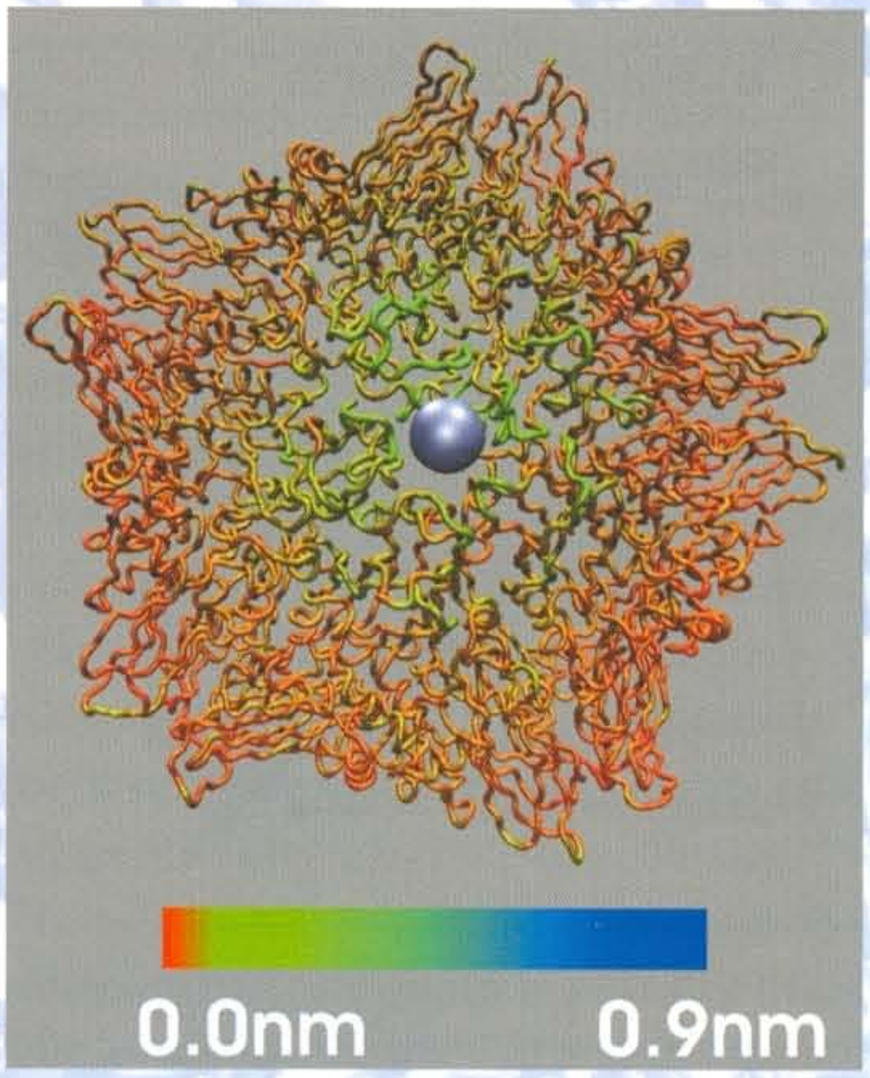

Figure 2: Indentation of the tip-sphere (grey) along the 5-fold symmetry axis of SBMV through the pentamer (shown as tube representation). The deformation of the amino acids is color-coded from red (no deformation) to blue (high deformation). The snapshot was taken at the yielding point at which the maximum force acting on the tip-sphere was obtained.

Our simulations showed a weak stiffness and stability, obtained as small elastic constants and yielding forces, respectively, at the subunit center of the inner and outer capsid surfaces of both shells, SBMV and HRV 16. The least stiff position of the HRV 16 capsid was found at the inner and outer pentamer center, as well as the inner pentamer center of SBMV. In contrast, the pentamer center on the outer shell of SBMV exhibited the largest elastic constant. The most stiff and stable position on the capsid of HRV 16 was found along the 2-fold symmetry axis. Large elastic constants and yielding forces along the 2-fold symmetry axis of SBMV were only found when the capsid was indented from the inside. On the outer capsid surface, the 2-fold symmetry axis exhibited a small stiffness and stability.

Although the assembly of subunits for SBMV and HRV 16 subunits is very similar, both capsids exhibit very different mechanical properties. The stiffness of hexamers and pentamers varies and depends on details on the atomic structure. Therefore no

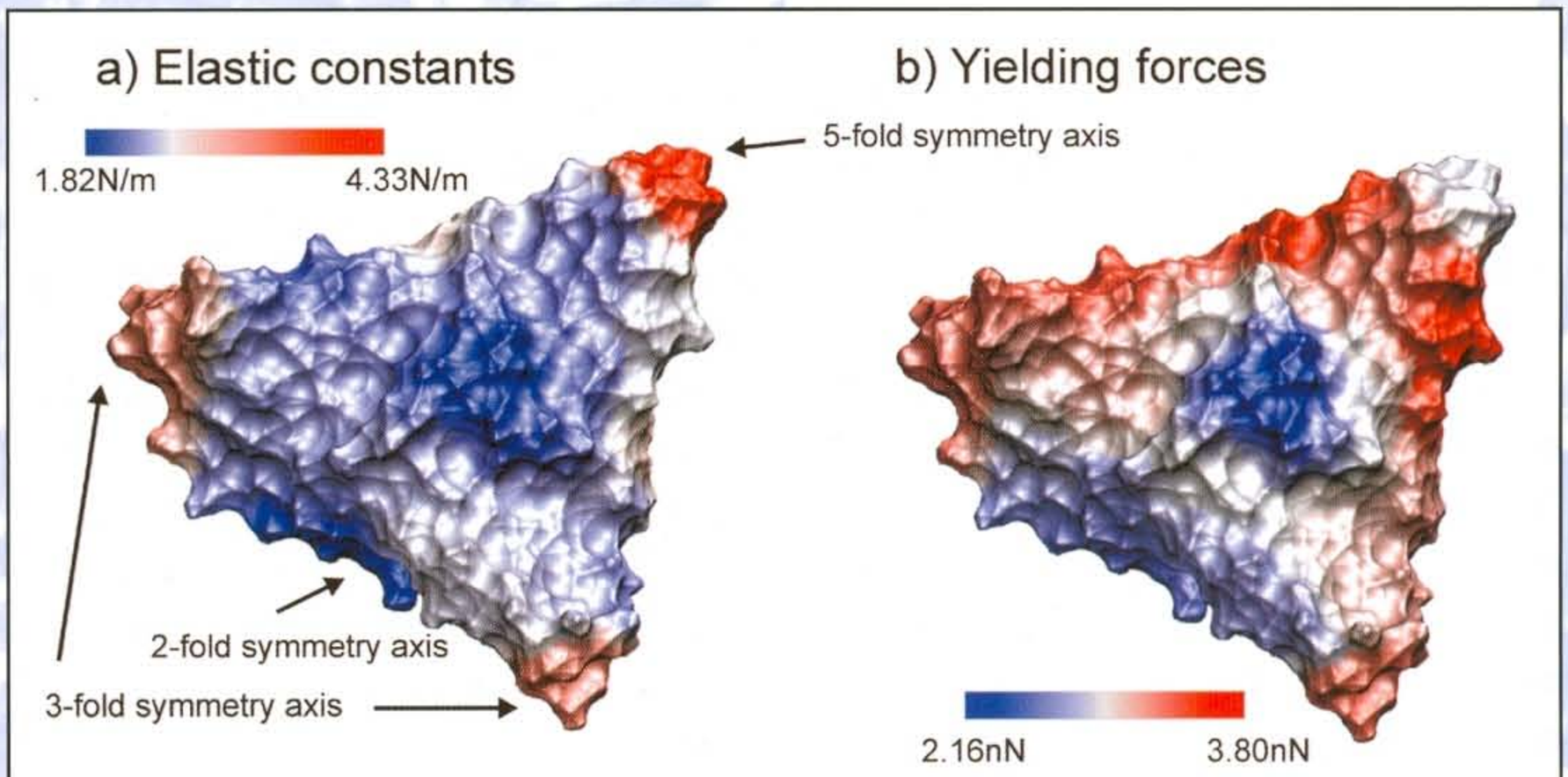

Figure 3: Subunit of SBMV. Color-coded distribution of elastic constants and yielding forces obtained from indentations of 19 positions on the outer subunit surface.

Applications

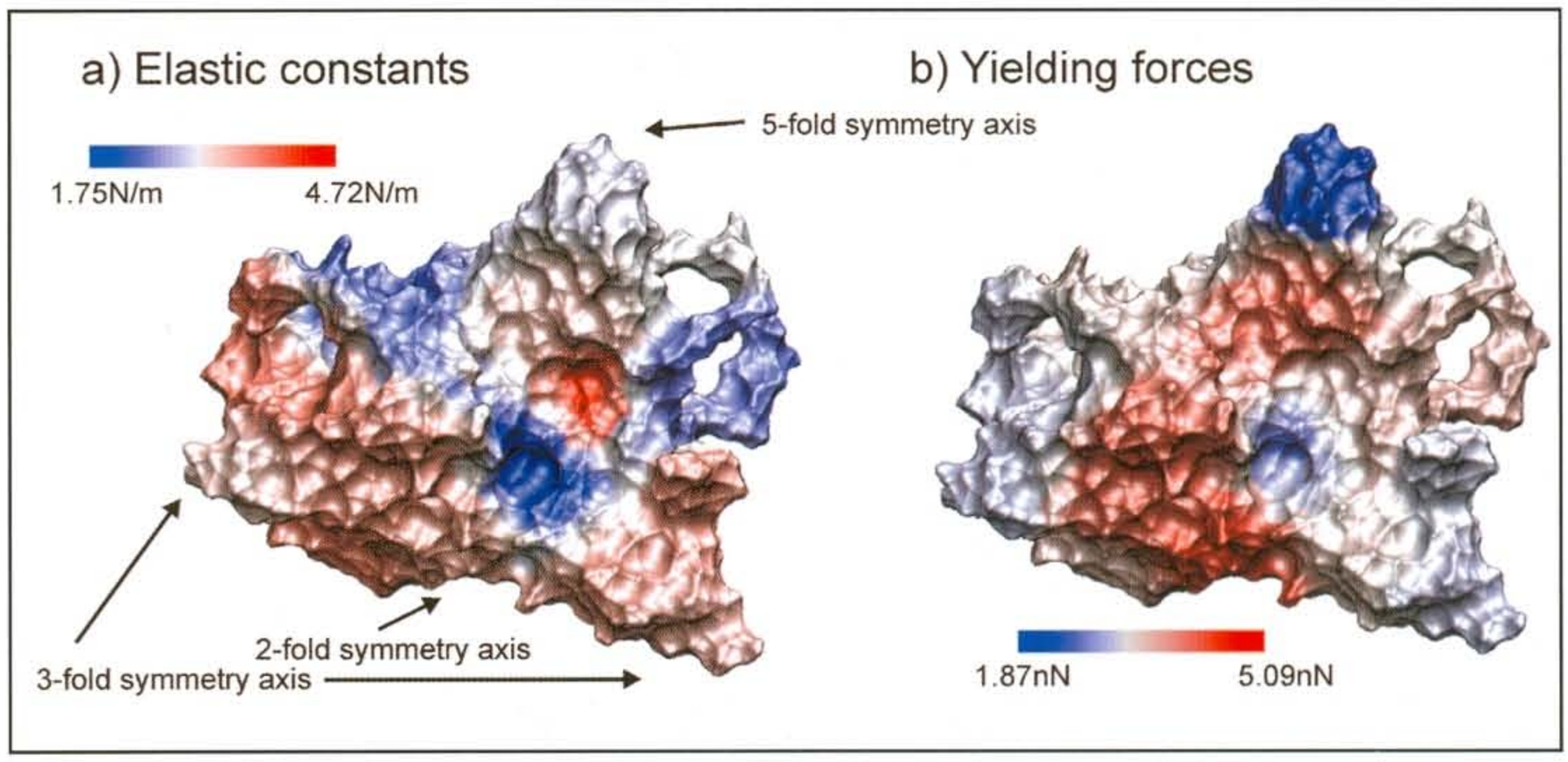

Figure 4: Subunit of HRV 16. Color-coded distribution of elastic constants and yielding forces shown as in Figure 2.

general picture for the distribution of mechanical properties on icosahedral capsids can be drawn. Our atomistic simulations clearly show that atomic detail cannot be neglected, because the atomic structure is a major determinant of the viral shell's mechanical properties, together with the geometrical arrangement of the subunits to an icosahedron.

The first step in viral infection of bean leaves with SBMV particles is the removal of the 180 calcium ions from the capsid structure. To study possible changes of the mechanical properties of SBMV without calcium compared to the coat protein with ions, the calcium ions were removed from the viral shell with subsequent equilibration of 32 ns with MD simulations. Subsequently, force-probe simulations were performed as described before for the capsid with ions. No significant change in mechanical properties was observed compared to the complete

capsid with ions, with the exception of a marked softening along the 5-fold symmetry axis. Due to the fact that the pentamer center exhibited the highest stability when ions were present in the structure but became the least stable capsid position of the surface of SBMV without calcium, we suggest that dematuration and, thus, capsid rupture might occur at the pentamer centers. Therefore, the pentamer center might act as a possible port for RNA release after calcium removal.

The next systems studied with force-probe simulations were three different structural capsid mutants of HRV 16. The first mutation was the removal of the pocket factor from the hydrophobic pocket in protein VP1. Docking of the ICAM-1 receptor to protein VP1 and emptying of the pocket was proposed to cause a destabilization of the capsid. The HRV 16 structure includes 12 zinc ions located at the pentamer center and block a possible channel for RNA release along the 5-fold symmetry axis. If the removal of the zinc ions could cause a structural change of the capsid that becomes able to release the RNA, remains unclear. Additionally, removal of protein VP4 was proposed as the first step in viral infection. Therefore, the mechanical properties of the HRV 16 capsid without pocket factor, VP4 and zinc ions were studied in order to investigate changes in elasticity and fracture behavior from which a possible port for RNA release could be proposed. All three capsid mutants were indented with the tip-sphere at the same 19 grip point positions.

We obtained modifications in the mechanical properties on the inner and outer surfaces of the three HRV 16 capsid mutants compared to the initial structure, whereas variations in elastic constants and yielding forces were small compare to the complete HRV 16 shell. The largest changes were obtained along the symmetry axes, e.g. the pentamer center became stiffer after removal of the pocket factor, the 2-fold symmetry axis of the capsid without zinc was strengthened. These variations in mechanical properties compared to the complete HRV 16 capsid were not strong enough to corroborate a possible pathway for RNA release. Therefore, the proposed mutations do not result in dematuration of HRV 16. We suggest that additional mutations or combinations of the studied mutations are necessary to destabilize the capsid structure and result in a release of the RNA. In contrast to SBMV which becomes infections after the removal of calcium in an alkaline environment, docking of external receptors to the capsid surface might also be necessary to cause RNA release of HRV 16.

To summarize, mechanical properties of viral shells were investigated on atomic length scales for the first time. The simulations show that small changes of the capsid structure can result in significant changes of mechanical properties from which possible RNA ports could be proposed.

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Biophys. J., in press, 2009

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