Viruses as Nanoparticles

**Structure of cowpea mosaic virus and its crystals.**

a) Left: A diagramatic representation of CPMV which shows the distribution of the two subunits that comprise the asymmetric unit, 60 copies of which form the icosahedral particle. The trapezoids in red and green represent the two domains of the large subunit clustered around the threefold symmetry axes and the blue trapezoid represents the small subunit clustered about the fivefold symmetry axes. Right: The folds of the two subunits.

b) Organization of five asymmetric units into the pentamer centered around a small hole at each fivefold axis.

c) Representation of the X-ray crystal structure of CPMV that highlights the EF-loop (in red) in the large subunit in which the cysteine-containing insert is made.

d) Left: A hexagonal crystal of CPMV. Electron micrographs of crystals thin sectioned perpendicular to the c axis (middle) and the a axis (right) showing the remarkably open lattice. Previous studies have shown that proteins with dimensions in excess of 50 Å can be reversibly soaked into the crystals. A typical crystal contains $10^{13}$ particles.
No reactivity with maleimide-labeled 1.4 nm Au nanoparticles ⇒ buried cysteine residues.
However, ethyl mercury phosphate can penetrate and react with buried -SH groups.
Unlike EMP, fluorescein reacts with the small subunit in WT.

Mutant allows for highly efficient reactivity due to surface-exposed CYS.

(five-residue insertion containing cysteine (GGCGG) was placed between positions 98 and 99 in the large subunit)
Hi-Res CryoSEM: Au-CPMV Conjugates

Cryo electron microscopy analysis of derivatized CPMV CYS mutant.

a) Three-dimensional reconstruction of CPMV particles at 29 Å resolution labeled with 1.4 nm nanogold clusters.

b) Difference electron density map generated by subtracting density computed with the native CPMV X-ray structure from the density shown in Figure 5a. Since the computed native CPMV density was made from only protein, the nucleic acid (shown in green) is visible in the difference map as well as the gold particles.

c) A pentameric section of the difference electron density map around the fivefold symmetry axis superimposed on the atomic model of CPMV showing that the gold is attached at the site of the CYS mutation.
(A) Subunit organization, (B) subunit ribbon diagram, and (C) space-filling model of the coat protein. The latter shows the exterior surface of the asymmetric unit with lysine side chain carbons in green and side chain nitrogen atoms in blue. Two lysine residues of the small subunit (S82, S38) appear to be exposed to solvent, whereas three in the large subunit (L99, L199, and L34) are similarly visible.
Lysine Reactivity-WT CPMV

In practice - only 60-70 modifications per virus - one lysine is uniquely reactive. Four lysines reactive under “forcing” conditions.
Cysteine Mutants II

(A) The atomic structure of the CPMV coat protein, with the sites of mutational insertion highlighted in red (B-C loop) and purple (E-F loop).

(B) Amino acid sequences corresponding to native and mutant CPMVs 1-4.

(C) A model structure of the entire particle shows the addition of a 5 residue insert (GGCGG) at the two positions of interest in the wild-type CPMV structure. The resulting mutant viruses correspond to 1 and 3. Note that the BC loop resides farther "up" on the protruding cap than the EF loop at each 5-fold axis of the icosahedral structure.
Cysteine Mutants II

Site-Specific Double Labeling of Mutant CPMV 3

(A) Synthetic routes; each dye attachment was followed by purification by size-exclusion chromatography.

(B) The uv-vis absorbance spectra of the indicated virus-dye conjugates.

(C) Denaturing protein gel showing specific labeling of small and large subunits, visualized directly under ultraviolet illumination: lane 1, 3-(S-F)$_{60}$; lane 2, 3-(S-F)$_{60}$-(N-F)$_{60}$.
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