ImprovedMaturation

Improved Maturation of HPV and Polyomavirus Capsids

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Citations:

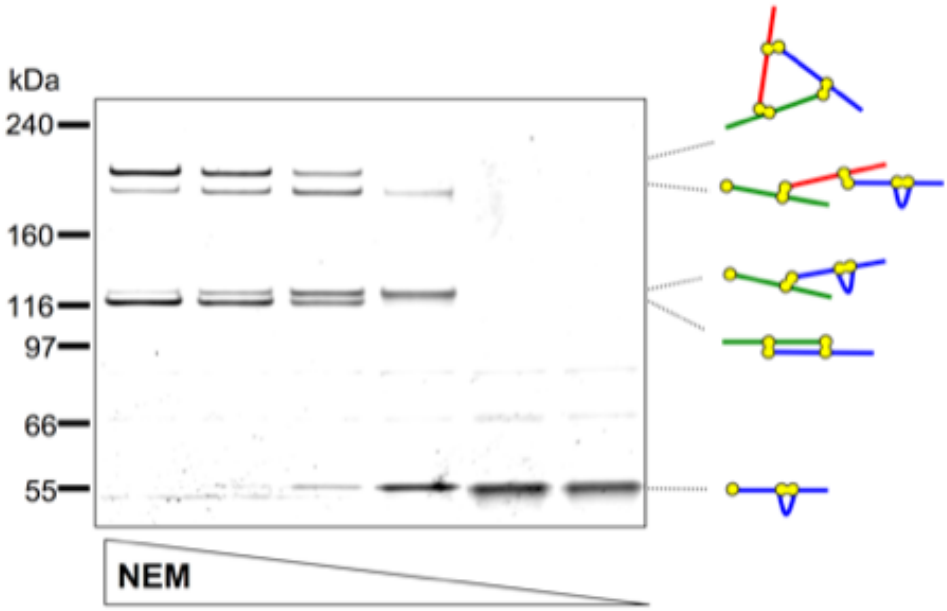
Giovanni Cardone[1*], Adam L. Moyer[2*], Naiqian Cheng[1], Cynthia D. Thompson[2], Israel Dvoretzky[3], Douglas R. Lowy[2], John T. Schiller[2], Alasdair C. Steven[1], Christopher B. Buck[2], and Benes L. Trus[4#] Maturation of the Human Papillomavirus Type 16 Capsid (2014) *mBio*5: e01104-14 PMID: 25096873

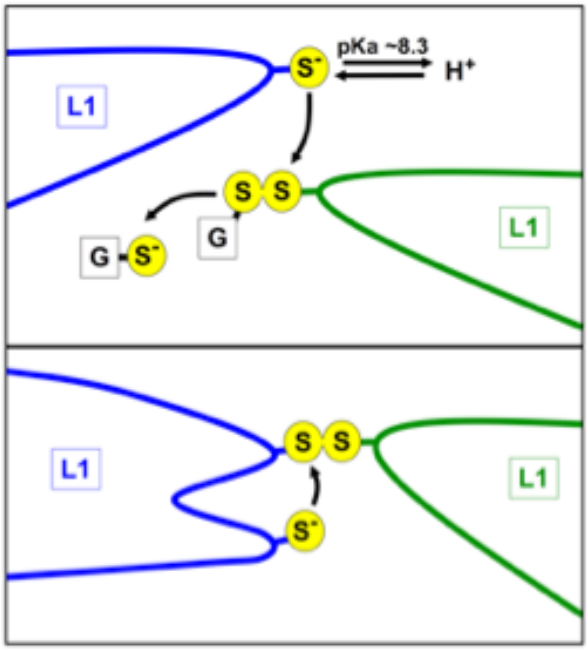
Buck CB and Trus BL The papillomavirus virion: a machine built to hide molecular Achilles' heels. In: *Viral Molecular Machines*(2012) Rossmann MG and Rao VB, eds. PMID: 22297524

Introduction: The HPV virion is reinforced by intermolecular disulfide bonds between neighboring L1 molecules. In the model proposed by Modis and colleagues (EMBO 21:4754) and modified by Wolf and colleagues (PNAS 107:6298), the disulfide bonding pattern reaches an endpoint of reciprocal dimers and ring trimers. Intermediates on the way to these endpoints are a topologically T-shaped dimer and an in-line “open” trimer. On SDS-PAGE gels, the open trimer migrates faster than the ring trimer, whereas the open dimer runs more slowly than the reciprocal dimer. Pseudovirion preps made using standard methods have varying ratios of each of the dimer and trimer species. In other words, pseudovirions often fall short of complete maturation. For reasons outlined below, the addition of a buffering agent to the cell lysate at the time of pseudovirion harvest improves the reliability and uniformity of pseudovirion maturation.

A serious technical problem confounds the analysis of L1 disulfide bonding. Under denaturing conditions, L1 is prone to a type of disulfide shuffling that rearranges the *inter*molecular disulfides that stabilize the capsid into *intra*molecular disulfides within individual L1 molecules. This results in a collapse of L1 into a monomeric form. Since disulfide shuffling requires an initial deprotonation of a free sulfhydryl (pKa ~8.3), alkaline conditions promote disulfide shuffling, while acidic conditions block shuffling. Chemical alkylation of sulfhydryl groups also blocks collapse to monomers.

A different technical problem confounds analysis of virions from skin warts. In a fraction of wart virions, the C-terminus of L1 is clipped off. Ring trimers of clipped L1 can easily be mistaken for open trimers of full-length L1. It’s important to run a reduced control to see how much of the L1 is clipped.





The boxed G represents glutathione

Improvement of capsid maturation:

1. Follow the standard or revised production protocol.
2. Make 1 M ammonium sulfate. Adjust pH to 9.0. Sterile filter.
3. After adding detergent (Triton X-100 or Brij58) to the cell suspension, add 1/40thvolume of 1 M ammonium sulfate (25 mM ammonium sulfate final concentration). Unbuffered cell lysates rapidly acidify to pH ~6. Addition of ammonium buffers the lysate toward more neutral pH (usually pH ~7. 5). The neutral conditions promote disulfide shuffling. In this case, the disulfide shuffling reaction allows an L1 cysteins decorated with glutathione to be attacked by free a cysteine residue of a neighboring L1 molecule (see Figure). Thus, buffering the lysate facilitates the ultimate formation of the desired intermolecular L1 disulfide bonds.
4. Allow capsids to mature overnight in the buffered lysate then purify as usual.

Non-reducing SDS-PAGE analysis of L1 disulfide crosslinking:

1. Dilute about 500 ng (SYPRO Ruby staining) or 1 ng (Western) of purified virions (in Optiprep) to a total of 19uL in 10 mM sodium phosphate pH 6.5.
2. Add 1 uL of 200 mM N-ethylmaleimide (NEM, Pierce #23030) dissolved in 10 mM sodium phosphate pH 6.5. The half-life of NEM in aqueous solution is short so it's important that NEM be made fresh from powder immediately prior to use. The final concentration of NEM in this step should be 10 mM. NEM is most effective in the pH 6-7 range. Note that the traditionally used alkylating agent iodoacetamide is inhibited by SDS (Galvani (2001) *Electrophoresis*22:2058). NEM is therefore preferable to iodoacetamide.
3. Incubate the initial non-dentaturing alkylation reaction for 10 minutes at room temperature.
4. Add 10 uL of 3x Nupage Load Dye (Invitrogen #NP0007) containing 10mM NEM. Note that Nupage load dye is alkaline and therefore facilitates disulfide shuffling in the absence of NEM. Although NEM remains effective in Nupage Load Dye, if an absolute block against shuffling is desired, a neutral load dye (see recipe below) should be used instead of Nupage load dye.
5. Incubate the denaturing alkylation reaction for 10 minutes at room temperature, followed by 10 minutes at 65°C.
6. Load 20uL of the final mixture in a NuPAGE Tris-Acetate 3-8% 1.5mm x 10-well gel in a Novex Mini-Cell, filled with 800mL of NuPAGE Tris-Acetate running buffer. (All from Invitrogen)
7. Run gel at 150V for ~1 hour, as directed, until the dye front approaches the foot of the gel.
8. Remove gel from plastic casing, cut off the dye front, and stain using SYPRO Ruby protein stain (Invitrogen) using either the overnight (increased speckling but slightly higher sensitivity) or microwave protocol, as directed by Invitrogen. Coomassie can also be used, but quantitation of band intensity is much better with the fluorescent imaging. If performing a Western blot, add 10 mM 2-mercaptoethanol to the running buffer. This will serve to break the disulfide bonds in the dimer and trimer species, thereby facilitating their egress from the gel.

<u>3x Neutral Load (1mL)</u>

300 uL 20% Lithium Dodecyl Sulfate

200 uL 1 M Tris 6.8

120 uL 200mM N-ethyl maleimide stock (in 10mM phosphate pH 6.5)

25 uL 0.5 M EDTA

300 uL glycerol

55 uL 1% BPB