

# PATTERNED ASSEMBLY OF A GENETICALLY MODIFIED VIRAL NANOTEMPLATE VIA NUCLEIC ACID HYBRIDIZATION

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## Introduction / Outline:

The patterning of nanoparticles represents a significant obstacle in the assembly of nano-scale materials and devices. In this report, cysteine residues were genetically engineered onto the virion surface of *Tobacco mosaic virus* (TMV), providing attachment sites for metallic and fluorescent markers. To pattern these viruses, labeled virions were partially disassembled to expose 5' end RNA sequences and hybridized to virus specific probe DNA linked to electrodeposited chitosan. Electron microscopy and RNase treatments confirmed the patterned assembly of the virus templates onto the chitosan surface. These findings demonstrate that TMV nanotemplates can be dimensionally assembled via nucleic acid hybridization.



Figure 1. Visualization of TMV1cys. Background, electron micrograph of TMV (negatively stained). Bar represents 300nm. Foreground, structural model representing ~10% of a TMV1cys virion. Red space filling molecules show the location of the inserted cysteine residue.

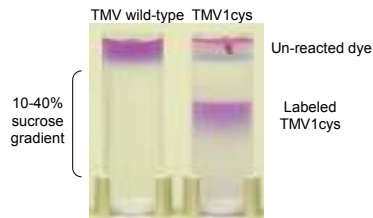


Figure 2. Specificity for the labeling of TMV1cys virions. 100ug of purified TMV wild-type or TMV1cys virus was incubated for 1 hr in 2 molar excess Texas Red maleimide. Dye conjugated virus was separated from the excess dye by sucrose gradient centrifugation.

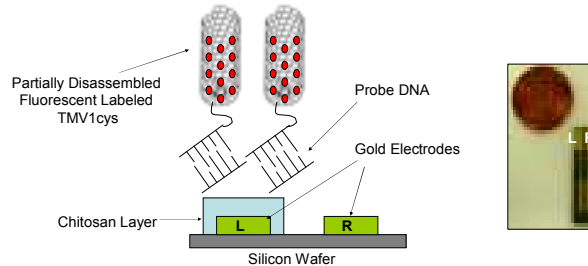


Figure 3. Diagram for the DNA probe-directed assembly of TMV1cys templates onto a conductive support. L and R represent left and right electrodes. Side insert shows actual chip.

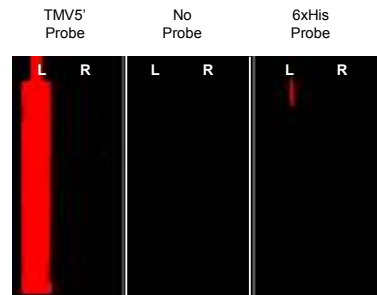


Figure 4. Attachment of Texas Red-labeled TMV1cys virions onto a gold patterned silicon chip using virus specific DNA oligo-hybridization. Each chip contains a left (L) and right (R) electrode. Chitosan is electrodeposited only on the left electrode of each chip. The right electrode on each chip carries no chitosan and functions as an internal control for non-specific binding. Chips were immersed overnight in a 20µg/ml solution of Texas Red labeled TMV1cys and rinsed 3 x 5min prior to examination by fluorescence microscopy. TMV5' Probe, DNA complementary to the 5' end genome sequence of TMV; No probe, chitosan only; and 6xHis Probe, probe DNA complementary to 6 histidine codons.

## Acknowledgments:

We wish to thank Tim Maugel in the Laboratory for Biological Ultrastructure for assistance with FE-SEM. This work was supported in part by DOE awards DE-FG02-02ER45975 & 76.

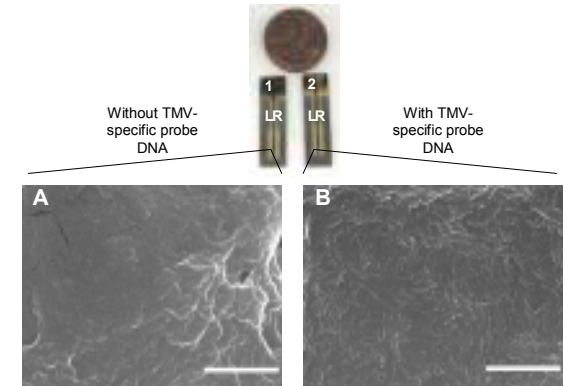


Figure 6. FE-SEM images of chitosan coated gold pattern silicon electrodes hybridized with TMV1cys nano-templates. Panel A shows a chitosan layer without TMV specific probe DNA. Note that no virus specific binding is observed. Panel B shows chitosan layer with TMV specific probe DNA. The virus can be seen densely packed across the surface of the electrode much like the pile of a carpet. This observation is consistent with the 5' end of the virus rod being tethered to the gold support. Bar represents 500nm.

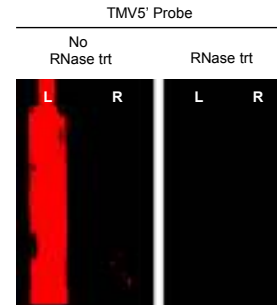


Figure 5. Hybridization of Texas Red labeled TMV1cys virions onto TMV 5' specific probe chips is directed through nucleic acid hybridization. Treatment of partially disassembled TMV1cys with RNase A prevents virus assembly onto the probe electrode.

## Conclusion:

In this study we have utilized several strategies to enhance the use of TMV as template for both the production and patterned assembly of nanoscale materials. First, the development of a genetically engineered TMV template provided a simple and direct means for the coupling of both inorganic and organic compounds to the virus surface. This was accomplished by inserting a cysteine residue onto the outer surface of the virus coat protein, providing an accessible thiol group that could be easily targeted as a covalent attachment site using standard conjugation chemistries. Second, we took advantage of the TMV evolutionary adaptation that permits removal of a few coat protein subunits from the 5' end of the viral RNA. Removal of these subunits via a simple centrifugation step allowed functionalized virus templates to be specifically addressed through nucleic acid hybridization. Finally, we utilized chitosan as an interface to direct the spatial assembly of the viral templates onto the surface of a gold-patterned silicon chip. Combined, these strategies provide the basis for further efforts to utilize viral templates in the construction of nanodevices.