Supplemental Information for Wnek et al:

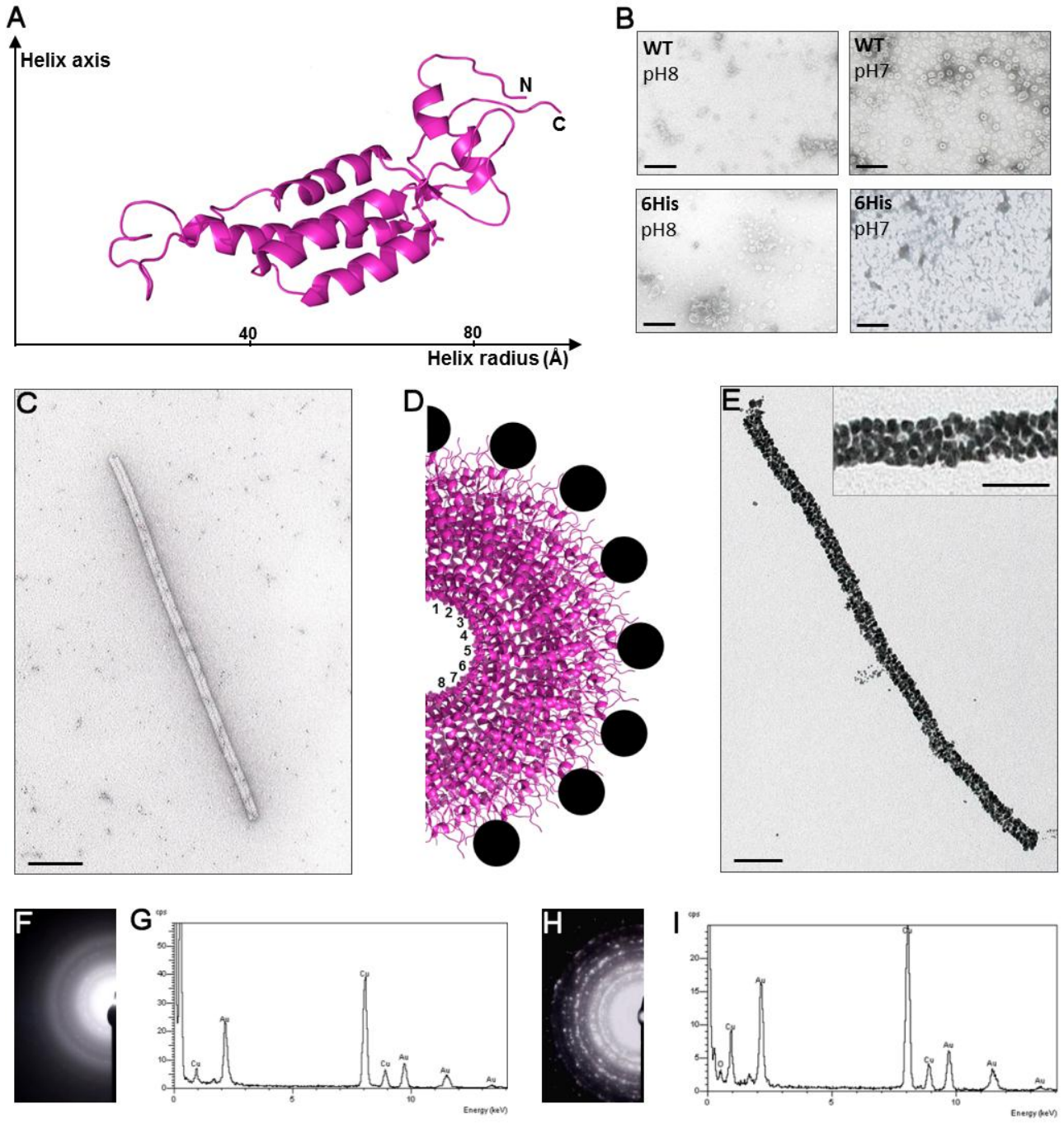
Mutagenesis, cloning and expression of TMV CP

TMV single-stranded RNA, used as a template in the reverse transcription, was isolated from the virus *Vulgare* strain (gift from Dr Marc van Regenmortel, University of Strasbourg, France) using phenol:chloroform extraction followed by ethanol precipitation. The reverse transcription reaction was then performed individually for the WT and 6His CP genes, using gene-specific reverse primers (see below), and RT products were amplified via PCR. PCR amplification of the WT and 6His genes was performed with an identical 5' CP gene-specific forward primer that contained *Nde*I restriction site (underlined): 5'-GGAATTCCATATGTCTTACAGTATCACTACTCC-3'. In addition, for the creation of WT coat protein, a 3' gene-specific reverse primer containing *BamH*I restriction site (underlined) was used (WT: 5'-CGCGGATCCTCAAGTTGCAGGACCAGAGGTCC-3'). For the creation of 6His mutant, a 3' gene-specific reverse primer containing *BamH*I restriction site (underlined), as well as the additional histidine codons (bold) was used (6His: 5'-CGCGGATCCTCA**ATGATGATGATGATGATG**AGTTGCAGGACCAGAGGTCC-3'). The WT and 6His genes were next digested with *Nde*I and *BamH*I restriction enzymes, and cloned into *Nde*I-*BamH*I–digested pET11c expression vector (Stratagene). Such created constructs (named pET11c-WT and pET11c-6His, respectively) were expressed in *E. coli* BL21-CodonPlus-(DE3)-RIPL (Stratagene). *E. coli* transformed with pET11c-WT or pET11c-6His were grown to mid-logarithmic phase (A_{600}=0.6-0.8) at 37°C, heat shocked at 50°C, and T7 transcription was induced with 1 mM IPTG. After overnight expression at 18°C, 1 ml samples were recovered, heated in loading buffer (50 mM Tris-HCl, pH6.8, 2% SDS, 20% glycerol, 1% β-mercaptoethanol, bromophenol blue) and analysed by SDS-PAGE. Proteins were stained with Coomassie brilliant blue to confirm their identity. The remaining cells were harvested, resuspended in lysis buffer (WT lysis buffer: 100 mM Tris-Cl, pH8, 1 mM EDTA, 50 μg/ml lysozyme, 100 μg/ml PMSF, 10 μg/ml *DNAse*I, 1% (v/v) Tween 20; 6His lysis buffer: 50 mM sodium phosphate, pH7, 100 mM NaCl, 50

μg/ml lysozyme, 100 μg/ml PMSF, 10 μg/ml *DNAse*I, 1% (v/v) Tween 20, 5% (v/v) glycerol, 1 mM imidazole) and subjected to ultrasonic lysis. For SEC, wild-type CP sample was loaded onto Superose 6 10/300 GL column (GE Healthcare) pre-equilibrated with SEC buffer (100 mM Tris-Cl, pH8), and eluted with the same buffer. For affinity chromatography, 6His mutant sample was loaded onto the HiTrap Chelating HP column (GE Healthcare) charged with Ni(II) solution. 6His protein was eluted with an increasing imidazole concentration in pH7 elution buffer (50 mM sodium phosphate, 100 mM NaCl, 5% glycerol, 1 M imidazole). Pure WT and 6His samples were dialysed against pH8 Tris-Cl buffer, which was followed by dialysis either against sodium phosphate buffer, pH7, for 'disk' formation and *in vitro* re-assembly or against sodium acetate buffer, pH5, for RNA-free helix formation, or distilled water for mass spectrometry analysis.

Nanowire formation and thermal annealing

WT and 6His VLPs were incubated with 2 nm GNPs (BBInternational) at a molar ratio of protein:GNP 10:1 or with 5 nm GNPs at a molar ratio protein:GNP 30:1. For UV photoreduction of gold ions, performed as described in [1], 10 μl of GNP-VLPs or WT VLPs were mixed with 2 μl of 2 mM $HAuCl_4$ and 2 μl of 2 mM Triton X-100 in a 1 cm quartz cuvette and exposed to 366 nm UV light. For electroless gold deposition, 6His-GNP-VLPs or WT-GNP-VLPs were incubated with 10 mM chloroauric acid and 6 mM hydroxylamine at a ratio GNP-VLPs:$HAuCl_4$:NH_2OH 4:1:1. Thermal annealing was performed after the electroless gold deposition onto the 2 nm GNP-VLPs, and was carried out on a nano-wire sample deposited onto a copper or silicon oxide TEM grid. The sample was heated in vacuum at an average rate of 10°C/min, until a desired temperature was reached. After 1 hour of annealing, the sample was slowly cooled to room temperature at a rate of 2.5°C/min.



Supplemental Figure 1. A. TMV coat protein structure and its position within the helix, with indicated helix axis, approximate size (in Å) and N- and C-termini, based on [2]. **B.** TEM of WT and 6His coat protein polymerisation into 'A-protein' (left) and 'disks' (right) at different pH ranges. All stained. Scale bars = 100 nm. **C.** WT VLP treated with 2 nm GNPs, stained with uranyl acetate, scale bars = 100 nm. **D.** View of a TMV VLP based on [3]. The CPs are shown as ribbon models and the sites of the his-tags are marked by black circles representing the 2 nm dia. GNPs to scale. **E.** 2 nm GNP-treated 6His VLP subjected to UV photoreduction, unstained. Inset shows a 2x magnification of the main image, scale bar = 50 nm. **F.** SAED pattern of the non-annealed nano-wire. **G.** EDX spectrum of the non-annealed nano-wire. **H.** SAED pattern of the annealed nano-wire. **I.** EDX spectrum of the annealed gold nano-wire.

References

[1] Zhou J, Gao Y, Lau J, Hamasaki T, Hu E and Dunn B 2007 Nanoscale assembly of gold nanowires templated by microtubules *J. Phys.: Conf. Ser.* 61 1352–6

[2] Namba K, Pattanayek R and Stubbs G 1989 Visualization of protein–nucleicacid interactions in a virus. Refined structure of intact tobacco mosaic virus at 2.9 A resolution by x-ray fiber diffraction *J. Mol. Biol.* 208 307–25

[3] Butler P J G and Klug A 1971 Assembly of the particle of tobacco mosaic virus from RNA and disks of protein *Nature New Biol.* 229 47–50