# SUPPLEMENTAL INFORMATION

# Virus-mimicking nano-constructs as a contrast agent for near infrared photoacoustic imaging

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### *1. Fabrication of the optical viral ghosts (OVGs)*

The complete OVG fabrication protocol is provided in our previous report.[1] Barley leaves were mechanically inoculated with BMV to induce viral infection in their primary leaves. After 5 to 7 days post-inoculation, systemic leaves of the plant displayed viral infection. For OVG fabrication, well-infected leaves were collected, and BMV virions were purified according to established protocols.[2,3]

After collecting the purified BMV virions, they were disassembled into capsid protein (CP) and RNA by dialyzing them in disassembly buffer (0.5 M $CaCl_2$, 50 mM Tris HCl, 1 mM EDTA, 1 mM Dithiothreitol (DTT), 0.5 mM Phenylmethylsulfonyl Fluoride (PMSF), pH7.8)) for 24 hours. Following BMV disassembly, the RNA and dissociated BMV CP subunits were separated via high-speed centrifugation (90,000 RPM, 1 hour). Subsequently, we collected the supernatant solution, which pre-dominantly contained the CP subunits and some residual RNA. To remove the residual RNA, an intermediate re-assembly of BMV virions was performed in a reassembly buffer (50 mM NaCl, 50 mM Tris-HCl, 10 mM KCl, 5 mM MgCl2, and 1 mM DTT, pH7.2)) for 24 hours at 4 °C. After this step, all the residual RNA was packed within CP subunits into virions. These re-assembled BMV virions were removed from the sample by high-speed centrifugation (90,000 RPM, 1 hour). We collected the supernatant containing the purified excess CP subunits. ICG was added to purified suspension of CP subunits, and the mixture was then dialyzed against reassembly buffer (1 M NaCl, 50 mM NaAc, 1 mM EDTA, and 1 mM DTT, pH4.2) at 4 °C for 24 hours. Encapsulation of ICG by CP subunits is a self-assembly process mainly governed by electrostatic interaction between negatively charged ICG molecules and positively charged CP subunits.

For PAI experiments, we prepared three different OVG samples where ICG at increasing concentrations of 10, 20 or 40 μg $ml^{-1}$ was used. The ratio of CP to ICG concentration in all samples was fixed at 4:1 by increasing the concentration of CP subunits as ICG concentration changed from 10 to 40 μg $ml^{-1}$. Stock solutions of OVGs suspended in BMV buffer solution

with initial ICG concentrations of 10 μg $ml^{-1}$ (represented as the 10S sample in the subsequent text), 20 μg $ml^{-1}$ (represented as 20S) and 40 μg $ml^{-1}$ (represented as 40S) were loaded in Silastic® tubing (inner diameter: 1.47 mm, outer diameter: 1.97 mm). The 20S solution was diluted by a factor of 4 (represented as 20S/4), and 40S solution was also diluted separately by factors of 4 and 10 (represented as 40S/4 and 40S/10, respectively), and loaded into the Silastic tubes. In this manner, we were able to evaluate the effect of the number density of OVGs on the resulting PA signals.

***2. Morphological characterization and absorbance measurements***

The morphology and physical stability of OVGs were characterized by transmission electron microscopy (TEM, Tecnai 12) 15 days post construction. OVGs were placed on 200 mesh carbon-formvar grid (FCF200-Cu-50), and negatively stained with 1% uranyl acetate for approximately 5 minutes. The operating voltage for the electron gun was ~120 kV. The absorbance measurements of OVG suspensions were performed using a UV-VIS spectrophotometer (Cary 50, Varian, Inc.).

***3. Photoacoustic imaging system***

We present a schematic of the PA system used for imaging the tissue phantom containing OVGs (Fig. S1). The details of this dark-field illumination system with full details are reported in a previous publication.[4] Briefly, a tunable Ti:sapphire laser (LT-2211A, LOTIS TII) was pumped by a Q-switched Nd:YAG laser (LS-2137/2, LOTIS II). The laser system provided light pulses of < 15 ns pulse duration, with a 10 Hz pulse repetition rate. Subsequently, 5% of the energy of each pulse was split from the laser beam, and measured by a photodiode to compensate for shot-to-shot fluctuation of laser energy. The fluctuation in the photodiode signal is assumed to be representative of the fluctuations in the energy of the incident laser beam. We compensate for this energy fluctuation in the PA images in order to remove intensity fluctuation related artifacts.

The laser light passed through a conical lens to form a ring-shaped illumination, and then focused onto the sample using an optical condenser. The light travelled down through an optical condenser, and the ultrasound focal zone of the transducer was focused on the sample.

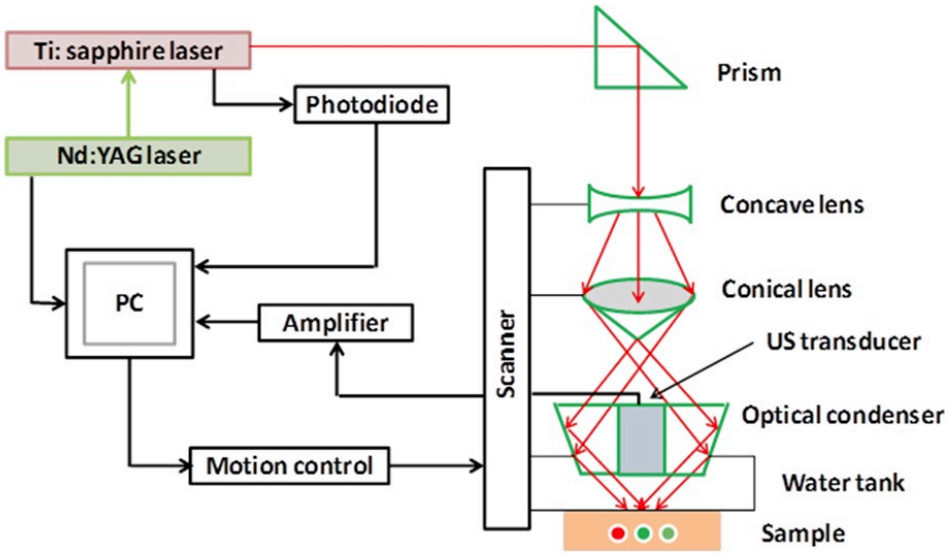


**Fig. S1** Photoacoustic imaging system.

We used a 5 MHz center frequency ultrasound transducer to collect the PA signals emitted from the samples. The transducer was spherically focused, with a 2.54 cm focal length, a 1.91 cm diameter active element, and 72% nominal bandwidth. The scan head, consisting of the optical condenser and ultrasound transducer, was immersed in a water tank with a 5 cm x 5 cm opening sealed with thin clear membrane. Ultrasound gel provided acoustic coupling between the thin membrane and the sample. The PA and the photodiode signals were digitized using National Instruments data acquisition system with LabVIEW (National Instruments, USA) control software. The collected data was then analyzed offline using MATLAB (Mathworks, USA).

Sample preparation for photoacoustic experiments. We used chicken breast tissue (≈ 6 and 14 mm thickness) to simulate an optically turbid medium. Along with, Silastic tubes

containing bovine blood with oxygenated hemoglobin ($sO_2$: 99.3 %) (QuadFive) and the various OVGs samples, described above, were either placed on the chicken breast tissue, or embedded below the surface with the most superficial positions being at depths of 6 or 14 mm. As control sample, the Silastic tube contained 1 ml of the bovine blood with oxygenated hemoglobin ($sO_2$: 99.3 %), but without any OVG samples.

The samples were imaged with the PA system using excitation wavelengths in the range of 760-820 nm with a step size of 10 nm. A graphite sample was scanned at each tissue depth over the 760-820 nm wavelength range to compensate for wavelength- dependent fluence attenuation caused by the overlaid tissue. Since the absorption of graphite in this wavelength region remains spectrally flat, any changes in the PA signal are assumed to be due to differences in absorption and scattering of light by the tissue sample. The scanning distance for all experiments was 36 mm, with a step size of 100 μm (360 A-lines per B-scan). At each wavelength, 5, 20, or 30 B-scans were averaged for the experiments corresponding to the no tissue overlay, 6 mm, and 14 mm embedment depths, respectively.

***4. Photostability of OVGs***

To compare the photostability of the OVG constructs (20S sample) in response to increasing number of laser pulses with that of non-encapsulated (free) ICG, we embedded two samples at depth of 6 mm below the surface of the chicken breast phantom. One tube contained the 20S OVG constructs in BMV suspension buffer (pH 4.2). The second tube contained 20 μl $ml^{-1}$of ICG dissolved in BMV suspension buffer. The two samples were irradiated at 800 nm light using 10, 30, 50, 80, or100 pulses. The collected PA signals from both samples were normalized by their respective PA signal amplitudes in response to 10 laser pulses.

***5. In vivo immunogenicity assessment***

We used a multiplex bead-based assay to detect the presence of cytokines IFN-γ, IL-6, and TNF-α in response to BMV capsid protein (CP) administration through tail vein injection, under a protocol approved by the University of California, Riverside Animal Use and Care

Committee (A-20110042). Specifically, we injected 100 μg of purified BMV CP subunits into healthy female Swiss Webster mice (25-30 grams, 10-12 week old). As positive control, we administered ≈ 80 μg of lipopolysaccharide (LPS) dissolved in dimethyl sulfoxide (80 μl injection volume). Animals receiving the purified BMV CP subunits were euthanized at one and six hours post injection using $CO_2$ inhalation. LPS-injected animals were euthanized at six hours post-injection. Immediately, following euthanasia, we collected the blood by puncturing the heart using a 28-gauge needle syringe. The blood was allowed to clot overnight at 2-8 °C before centrifugation for 15 minutes at 1000×g. After centrifugation, we collected the serum, and immediately performed the assay using Milliplex® MAP mouse cytokine/chemokine magnetic bead panel kit (Catalogue # MCYTOMAG-70K or MCYTOMAG-70K-PX). All the samples were run in triplicate and assayed for IFN-γ, IL-6 and TNF-α according to manufacturer's recommended procedures. Briefly, all the reagents were first brought to room temperature. The serum samples were diluted 1:2 in the assay buffer provided with the kit. Mouse cytokine standards for the three analytes ranging between 3.2 pg $ml^{-1}$-10,000 pg $ml^{-1}$ were prepared in duplicates. We minimized the exposure of the beads to light by wrapping the tubes and/or plates with aluminum foil, and using appropriate agitation on a microplate shaker (initial agitation at 500-800 rpm for 10 min and then for 2 hours). After completing steps, the plate was read by Luminex 200 system.

**References**

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