Support Information

Label-free imaging, detection and mass measurement of single viruses by Surface Plasmon Resonance

Shaopeng Wang[1], Xiaonan Shan[1,2], Urmez Patel[1], Xinping Huang[1,3], Jin Lu[1,4], Jinghong Li[4], and Nongjian Tao[*1,2]

[1]Center for Bioelectronics and Biosensors, Biodesign Institute, Arizona State University, Tempe, AZ 85287; [2]Department of Electrical Engineering, Arizona State University, Tempe, AZ 85287;
[3]Department of Chemistry, Lanzhou University, Lanzhou, China
[4]Department of Chemistry, Tsinghua University, Beijing, China

Corresponding author, njtao@asu.edu, Fax: (480) 965-9457

10.1073/pnas.1005264107

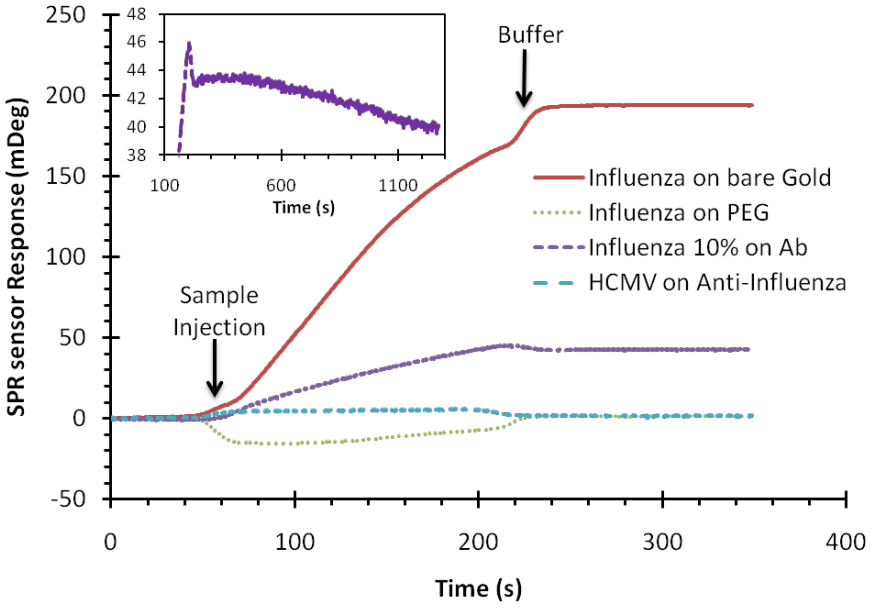

Figure S1. Control experiment results on a conventional SPR setup. SPR response of Influenza A injected into the flow cell of BI-2000 SPR instrument with different sensing surfaces: (a) bare gold, (b) PEG6 functionalized, and (c) PEG surface with antibody specific to Influenza A. HCMV virus on anti-influenza was also measured as a control. Flow rate: 30ul/min, sample volume: 100ul, flow buffer: 1x PBS, sample concentration: Influenza A virus ($3x10^{10}$ particle/ml, 0.05mg/ml), HCMV virus ($1.15x10^{10}$ particle/ml, 0.25 mg/ml). Anti-influenza was conjugated to PEG-COOH (1:20 mixed with PEG6) with standard EDC conjugation chemistry. The small kinks near the beginning and end of sample injection (marked with arrow) are due to bulk refractive index changes between the sample and buffer. The relative low binding of Influenza A to antibody surface is likely due to the purity of antibody. **Inset:** Zoom-in view of the slow

Influenza A dissociation process on anti-influenza surface. The slow dissociation and distorted dissociation curve could be due to multi-valence bindings of each viral particle with multiple antibodies on the sensor surface.

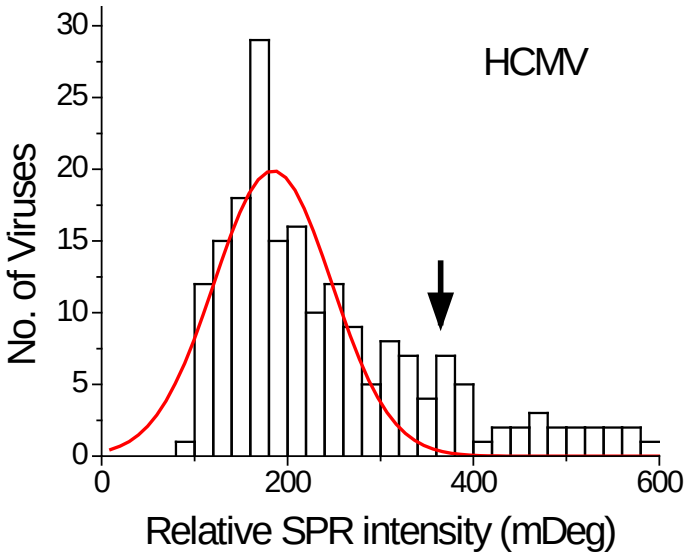

Figure S2. Histogram of relative SPR intensity distribution of HCMV viral particles. The center of the Gaussian fitting is at 184±20 mDeg at 95% confidence level. Arrow indicates the possible dimer peak. Some aggregated viral particles present in the sample, as indicated by the small number of high intensity counts.

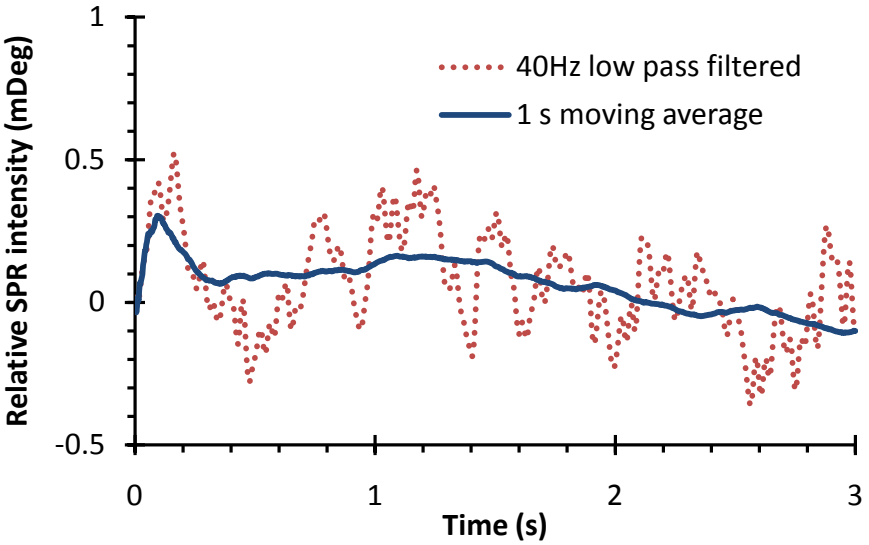

Figure S3. Noise level of current SPRM setup: noise level of averaged intensity of a small region (3x5µm) of SPRM images of PBS buffer over time. Both FFT low pass filtered data (to remove 60 Hz noise from laser), and 1 second moving average smoothed data are shown. The data was recorded with the Pike camera at 380 fps. Standard deviation for 3 second data is 0.3 mDeg, and 1s moving average reduced the noise level to 0.04mDeg.

Table 1. Size, volume and mass of silica nanoparticles and viruses.

Type of particles	Diameter (nm)	Size CV	Volume (μm^3)	Mass (fg)	Mass CV	Density (g/ml)
Silica NP 98 nm[1]	98	<14%	0.00049	0.99	<48%	2.0
Silica NP 150nm	150	<15%	0.00177	3.53	<52%	2.0
Silica NP 205 nm	205	<5%	0.00451	9.02	<15%	2.0
Influenza A (H1N1)[2]	80-120	20%	0.00027-0.00090	0.42-1.2	3 fold	1.19
HCMV (AD 169)[3]	230	N/A	0.00637	7.8	N/A	1.219

Notes for Table 1:

1. The diameter and size variations of nanoparticles are provided by the manufacturers, and the volume, mass and mass variations of the nanoparticles are calculated from the size and size variations. The mass density of silica nanoparticles is 2.0 g/ml.
2. The reported size and mass of influenza virus are from different sources: 90-110 nm and 0.52 fg in (1), 80-120 nm and $2.5x10^8$ Da in (2), and 120nm and 0.6 fg dry weight or 1.2 fg hydrated weight in (3). The variations could be due to that influenza A is an enveloped virus, containing a lipid membrane from its host cell, which may vary.
3. Human cytomegalovirus (HCMV) has a reported diameter of 230 nm (4) and density of 1.219 g/ml (5), and volumes and mass are calculated from these data. No size variation information could be found for HCMV in the literature, but HCMV is also an enveloped virus, so some size and mass variations are also expected.

References:

1. Vollmer F, Arnold S, & Keng D (2008) Single virus detection from the reactive shift of a whispering-gallery mode. *Proc Natl Acad Sci U S A* 105(52):20701-20704.
2. International Committee on Taxonomy of Viruses., Van Regenmortel MHV, & International Union of Microbiological Societies. Virology Division. (2000) *Virus taxonomy : classification and nomenclature of viruses : seventh report of the International Committee on Taxonomy of Viruses* (Academic Press, San Diego) pp xii, 1162 p.
3. Karl Maramorosch & Lauffer MA eds (1974) *Advances in Virus Research* (Academic Press), Vol 18.
4. Shenk T & Stinski M (2008) *Human cytomegalovirus* (Springer, Berlin) pp xiii, 475 p.
5. Hart H & Norval M (1981) Association of human cytomegalovirus (HCMV) with mink and rabbit lung cells. *Arch Virol* 67(3):203-215.