Supporting Information

An Adenoviral Platform for Selective Self-Assembly and Targeted Delivery of Nanoparticles

Vaibhav Saini, Dmitri V. Martyshkin, Sergei B. Mirov, Alex Perez, Guy Perkins, Mark H. Ellisman, Victoria D. Towner, Hongju Wu, Larisa Pereboeva, Anton Borovjagin, David T. Curiel, Maaike Everts*

Supporting Information

Atomic absorption spectroscopy of AuNP-labeled Ad vectors

The gold (Au) atomic absorption standard solution (1 mg/mL in 0.5N HCl) was obtained from Acros Organics (Belgium). It was further diluted in ultra pure HPLC grade water (Chromasolv® Plus, for HPLC, Sigma-Aldrich) containing 0.5N HCl to 10 ppb, 20 ppb, 50 ppb, and 100 ppb concentration respectively. The obtained Au atomic absorption standard solutions were used for instrument calibration as well as for a quality control measurement.

The atomic absorption measurements were performed at Atomspec DF Workstation (Thermo Jarrell Ash Corporation). Atomic absorption of Au was measured at 242.8 nm using the Smith-Hieftje background correction method. The atomic absorption signal corresponds to an integral of the absorbance integrated over time. The instrument was calibrated using Au atomic absorption standard solution prior to virus sample measurements.

The Ad vector samples collected from the CsCl gradients were dialyzed to remove CsCl and replace it with water. Following this, the viral particle number was determined by measuring absorbance at 260 nm using a conversion factor of 1.1 x 10^{12} viral particles per absorbance unit[1]

Ad vectors containing a 6-His tag in hexon coupled to AuNPs were ultrasonicated for 5 min and diluted 20 times in ultra pure HPLC grade water before measurements due to high initial concentration of Au. The dilution factor (20) and the final concentration of Au in virus sample were carefully chosen in order to fall into a linear response of the instrument.

The typical measurement procedure consists of 5 stages; sample drying, pyrolysis, another pyrolysis, atomization, and cuvette cleaning. The parameters of each section should be experimentally optimized for each individual analyte. The following analytical protocol has been found to be optimal for measurements of the amount of Au atoms in samples containing viral particles and 1.8 nm AuNPs (Table 2).

For repeatability, five consecutive measurements of the same virus sample have been done followed by the quality control measurement of the atomic absorption standard solution, followed by a control virus sample (Ad5) measurement containing no AuNPs. The Au concentration was determined by using arithmetic average of the results of five measurements (Table 3).

Figure 5 shows the atomic absorption calibration curve obtained using standard solutions and concentration of Au for Ad vector sample containing a 6-His tag in hexon coupled to AuNPs.

The results of atomic absorption measurements of Au concentration in control Ad5 samples are summarized in Table 4. The Ad5 samples contain no Au and the observed signal corresponds to a noise level. Figure 6a shows signal obtained from control Ad5 sample without nanoparticles. As one can see there is no signal associated with gold atoms absorption. The atomic absorption of Au in Ad vector samples with a 6-His tag in hexon coupled to AuNPs and the atomization furnace temperature profile are depicted in Figure 6b and Figure 6c respectively.

The number of AuNPs per virion was calculated by comparing the atomic absorption readings for the viral samples with the Au standard, assuming 180 atoms of Au per AuNP calculated as follows:

Volume of 1.8 nm AuNPs is:

$$V_{nano} = \frac{\pi D^3}{6} = \frac{\pi \left(1.8 \cdot 10^{-9}\right)^3}{6} = 3.052 \cdot 10^{-27} m^3 = 3.052 \cdot 10^{-21} cm^3 , \tag{1}$$

where D=1.8 nm is diameter of nanoparticle.

Mass of one 1.8 nm AuNPs is:

$$m_{nano} = V_{nano} \cdot \rho = 58.9 \cdot 10^{-21} g , \tag{2}$$

where ρ=19.3 g/cm^3 is Au density.

Number of Au atoms in one AuNP is:

$$N_{Au} = \frac{m_{nano} \cdot N_A}{M.W.} = 180 \ atoms , \tag{3}$$

where M.W=197 g mol^{-1} is Au molecular weight and N_A=6.022·10^{23} is Avogadro's number.

Number of Au atoms per mL in 1 ppb solution is:

$$N_{Au\ 1ppb} = \frac{10^{-9} g}{M.W.} \cdot N_A = 3.06 \cdot 10^{12}\, atoms \cdot mL^{-1}. \tag{4}$$

Number of AuNPs requared to produce 1 mL of 1ppb Au solution is:

$$N_{nano\ 1ppb} = \frac{N_{Au\ 1ppb}}{N_{Au}} = 1.7 \cdot 10^{10}\, nanoparticles\,. \tag{5}$$

Number of AuNPs coupled to hexon per virus is:

$$N = \frac{c_{Au} \cdot \eta \cdot N_{nano\ 1ppb}}{c_{vp}} = 56\ nanoparticles \cdot virus^{-1}, \tag{6}$$

where c_{Au}=42.55 ppb is Au concentration found from atomic absorption measurements, η=20 is dilution factor and c_{vp}=0.2607·10^{12} vp mL^{-1} is concentration of virus particles.

Error associated with atomic absorption spectroscopy measurements:

Atomic absorption spectroscopy is one of the most sensitive techniques designed for trace detection of metal ions in aqueous solutions. It has been used for over three decades as an analytical tool in environmental, industrial and scientific laboratories. Over this extended period of time the analytical methods and data treatment have substantially evolved in order to minimize the errors associated with the method features. We also made great efforts to optimize this method particularly for Au ions detection in an organic biological matrix and achieved signals of ideal shapes without any parasitic backgrounds, as one can see in Figure 6. Therefore we believe that it is safe to assume that noise associated with method imperfection can be neglected. However noise associated with random signal fluctuation and detection system noise due to electronics and the quantum nature of the photomultiplier tube (PMT) can not be avoided. The rough estimation of noise level was

made based on 5 measurements of 20 ppb gold standard solutions used as control measurements throughout the analytical protocol. The relative standard deviation

$$\%RSD = \frac{s}{x} \cdot 100\%,\ \text{where}\ \ s = \sqrt{\frac{1}{N-1}\sum_{i}^{N}(x_i - x)^2}\ \ \text{is the sample variance, N is the}$$

number of measurements, and x is the mean value. The RSD was estimated to be 6.5%. The same estimation can be done for the Ad vector sample containing a 6-His tag in hexon that was coupled to AuNPs, resulting in 2.3% relative standard deviation. Taking into account the above error estimations one can find that the number of Au nanoparticles attached to the viruses was 56±4.

Table 1: Positions of viral bands in centrifugation tubes after CsCl density gradient ultracentrifugation

Ad-AuNP combination	Distance from bottom to the viral band (cm)	Total height gradient (cm)
Ad5	4.6	7.2
Ad5 + AuNPs	4.6	7.2
Ad5 with 6-His in FF + AuNPs	4.6	7.2
Ad5 with 6-His in pIX + AuNPs	4.6	7.2
Ad5 with 6-His in hexon + AuNPs	3.8	7.2

Table 2: The procedure for measurement of atomic absorption

		Furnace information			
	Dry	Pyro1	Pyro2	Atom	Clean
Temp	150	600	600	2250	2300
Ramp	60	10	10	1	0
Hold	80	15	5	4	3
Purge	Low	Low	Med	Off	Med

Table 3: Atomic absorption measurement for Ad vector with AuNPs coupled in hexon

Ad vector with 6-His in hexon + AuNPs	
Absorption	Concentration (ppb)
0.5371	43.77048
0.5178	41.73367
0.517	41.65116
0.54	44.08451
0.5156	41.50713
Average	42.54939

Table 4: Atomic absorption measurement for control Ad5 sample

Control sample (Ad5) without AuNPs	
Absorption	Concentration (ppb)
0.0069	0.54 ppb

Figure Legends

Figure 5. Atomic absorption calibration curve. The calibration curve was obtained using standard solutions (open circles). The concentration of Au for the Ad vector sample containing a 6-His tag in hexon that was coupled to AuNPs is shown as blue filled circles.

Figure 6. Atomic absorption spectra of a) control sample (Ad5) without AuNPs b) signal obtained from Ad vector sample with a 6-His tag in hexon that was coupled to AuNPs, and c) temperature of the furnace.

Figure 7. NP-labeled Ad vectors demonstrate a change in density in CsCl gradients. To confirm the specificity of interaction between Ni-NTA-AuNPs and the Ad vector expressing a 6-His tag in hexon, the coupling reaction was performed in the presence and absence of 250 mM imidazole. The presence of imidazole competitively inhibited the AuNP binding as no change in Ad band density was observed in the CsCl density gradient (middle tube).
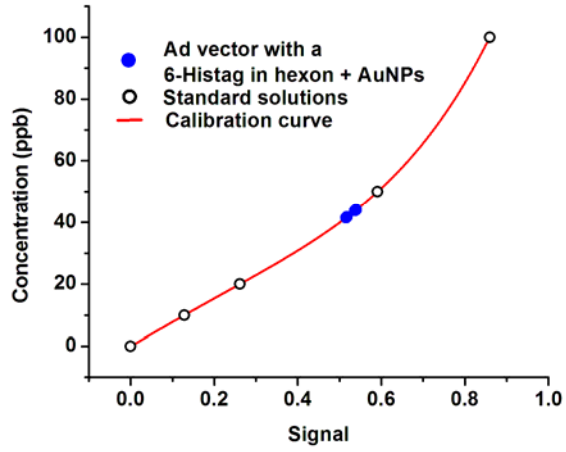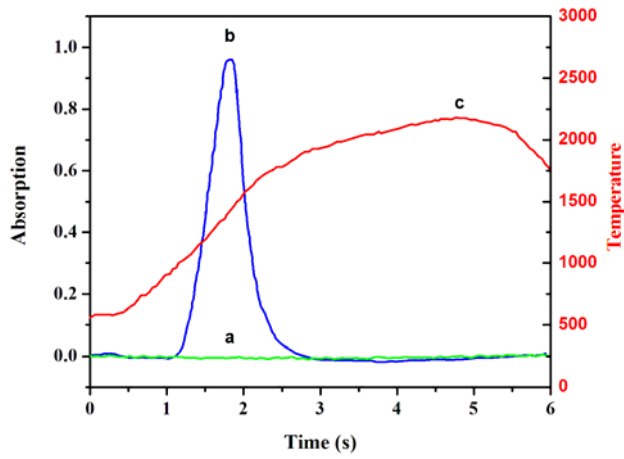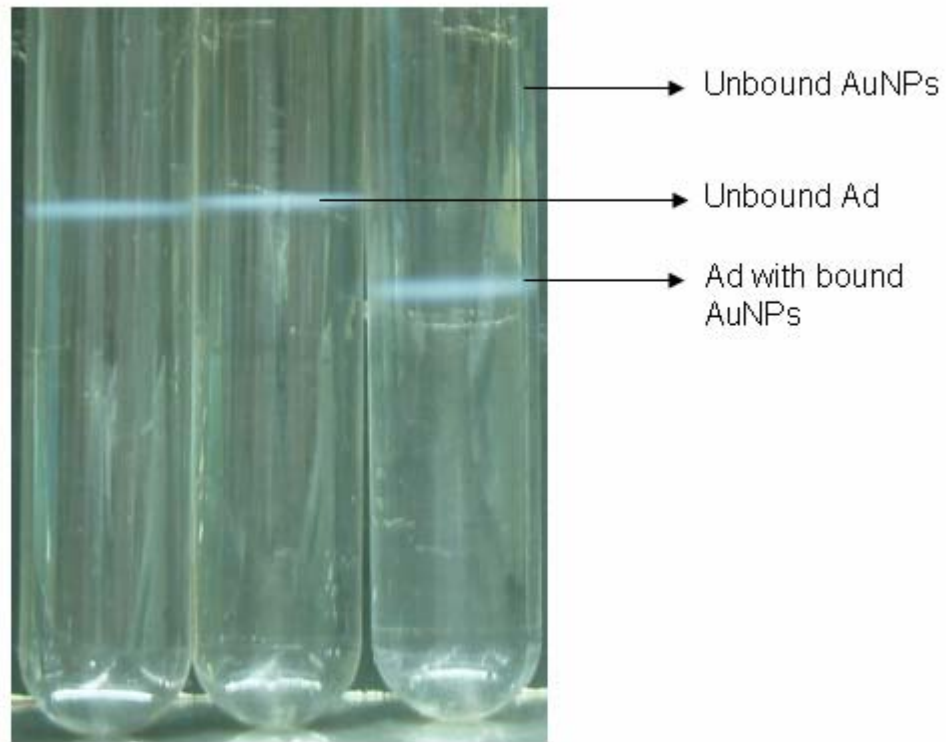
Figures

Figure 5

Figure 6

Figure 7

Reference

[1]   J. V. Maizel, Jr., D. O. White ,M. D. Scharff, Virology 1968, 36, 115-25.