

A Sensitive Peptide-Nanotube Based Pathogen Assay

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ABSTRACT

Attomolar detection of viruses utilizing a multifunctional peptide nanotube-based assay has been developed. Details of the mechanism of detection have been elucidated by TEM. Proof of principle experiments have demonstrated the multiplex ability of this assay, and its limit of detection for several viruses is reported.

Keywords: biosensor, peptide nanotube, pathogen assay, flow cytometry, multiplex assay

1. INTRODUCTION

Rapid detection of pathogens is of crucial importance to ensuring the safety of regulated products, for example vaccines and blood supplies, as well as in the fight against bioterrorism. Many techniques such as electron microscopy[1], flow cytometry[2], carbon nanotubes[3] and fluorescent polymers[4] have been applied to virus detection problems, each with strengths and weakness unique to the particular method. However, one shortcoming of all methods is the ability to detect in-tact whole virus directly in low ($\sim 10^2$ pfu/mL) concentrations in small sample volumes. Here, a peptide-nanotube based pathogen assay was developed that addressed this shortcoming. Further, this is a multiplex assay capable of detecting intact whole virus at as low as attomolar concentrations. The assay exploits the unique chemical properties of peptide nanotube scaffolds to create multifunctional biosensors that drive both the detection and signaling on one platform.

2. DISCUSSION

This assay is based on the nanotubes binding a virus via the antibody attached selectively at only the ends of the tubes. As multiple nanotubes bind the same virus, they radiate outward like spokes around an axle or like the spikes of a sea urchin. At higher virus concentrations (typically greater than 10^4 pfu/mL for nanotube concentrations of 1mg/mL) these sea urchin structures can form networks of larger three dimensional aggregates that contain multiple virus vertices. Each nanotube has the same fluorescent intensity, creating a directly proportional relationship between virus concentration, network size, and fluorescence intensity. Using flow cytometry to observe the

size and fluorescence intensity of each network allows the determination of the virus concentration.

In the multiplex case, each antibody is assigned its own unique fluorophore. A cartoon of the mechanism is shown in figure 1. The multiplex mechanism is simply several single virus mechanisms running in parallel.

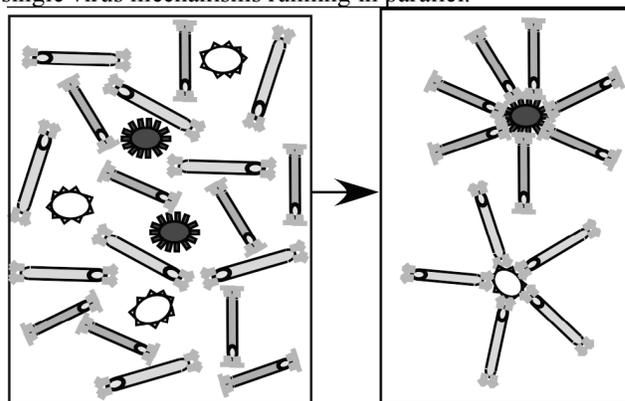


Figure 1. Cartoon of multiplex mechanism of detection.

The multifunctional peptide nanotube biosensors bind viruses via antibodies at the ends, while fluorescent dyes along the length provide tremendous signal to noise ratio signaling of binding into these networks.

Analysis of the networks was performed by flow cytometry. Each sample contained 0.5mL total volume for analysis. Data for the forward scattering intensity (FSC), a measure of relative size, the side scattering intensity (SSC), a measure of relative surface roughness, and fluorescence intensity for green (FL-1) and red (FL-2) were collected on each aggregate as the suspension was flowed through the instrument dropwise such that no more than one aggregate per drop was analyzed. Using the software to create a dot plot of FL-1 vs FSC and FL-2 vs FSC, selection of a gated region to observe only the region where sea urchin structures or network aggregates will occur and not where unbound nanotubes or unbound viruses would interfere with the detection was possible. This helps to minimize the amount of background noise. An example of the gate selections used for the flow cytometry data is shown in Figure 2. Analyzing the average fluorescence intensity of each network in the gated region at various known virus concentrations in terms of plaque forming units per mL (pfu/mL) allowed the creation of a standard curve. Standard curves were created using this process for all virus and antibody combinations shown in Table 1.

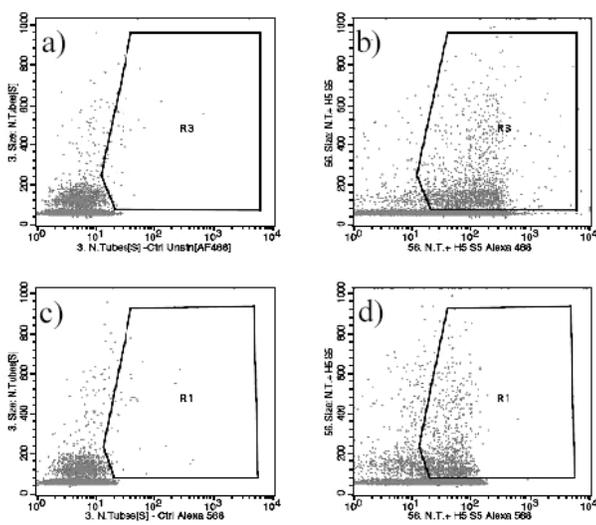


Figure 2. a) FL-1 vs. FSC for nanotube only negative control; b) FL-1 vs. FSC for spiked sample SV40= 10^6 pfu/mL & HSV2= 10^6 pfu/mL; c) FL-2 vs. FSC for nanotube only negative control; d) FL-2 vs. FSC for spiked sample SV40= 10^6 pfu/mL & HSV2= 10^6 pfu/mL.

It was observed that the limit of detection for each virus in the multiplex system was the same as when the virus was quantified independently. Results from several example viruses characterized individually are summarized in Table 1. By careful selection of antibodies, the differentiation between strains of the same virus is possible, as demonstrated on Adenovirus Types 3 and 5, with antibodies specific to each strain.

Antibody	Virus	Detection Limit (atto molar)	Detection Limit (log pfu/mL)
Herpes Simplex Virus 2	HSV-2	120aM	2.5
Influenza Type A	Influenza A	60 aM	2.0
Influenza Type B	Influenza B	60 aM	2.0
SV40 Lg T antigen	SV40	120aM	2.5
Adenovirus Type 3	AV3	600aM	3.0
Adenovirus Type 5	AV3	n.d.	n.d.
Adenovirus Type 5	AV5	60 aM	2.0
Adenovirus Type 3	AV5	n.d.	n.d.
Vaccinia(MVA) marker 1	MVA	6000aM	4.0
Vaccinia(MVA) marker 2	MVA	6000aM	4.0

Table 1. Detection limits for several example antibody and virus combinations.

The sensitivity of this assay allows detection of concentrations as little as 60 attomolar in a sample volume of 0.5mL. Achieving the maximum sensitivity depends on the ability of the antibody to bind the antigen. The stronger

these interactions are and the more antibodies that can bind per antigen will increase the sensitivity of the assay. Further research is required to optimize specific antibodies with specific receptor targets on given analytes to find what is the best combination for any given system, however these results do show that femtomolar concentration detection is always attainable.

Transmission Electron Microscope (TEM) images were obtained of the aggregates. These images are shown in figure 3. In frame a, low virus concentrations such as 10^2 pfu/mL produce a sea urchin-like aggregate, with multiple nanotube spokes radiating outward around the virus hub. At slightly higher virus concentrations, such as 10^3 pfu/mL in frame b, these hub-and-spoke shaped structures begin to merge together and form larger structures. Eventually, at the highest virus concentrations, such as 10^5 pfu/mL in frame c, very large aggregations form a network structure, with each additional virus that joins the network adding a proportional number of nanotubes to the network and in turn increasing the fluorescent intensity and size of the network proportionally.

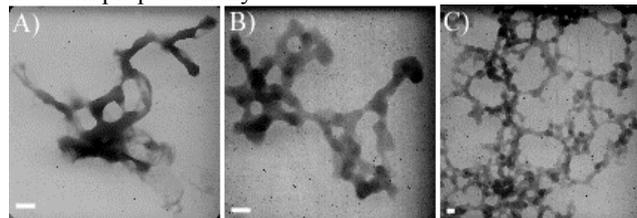


Figure 3. TEM images of networks structures used in flow cytometry detection. a) 10^2 pfu/mL nanotube-virus structure; b) 10^3 pfu/mL nanotube-virus structure; c) 10^5 pfu/mL nanotube-virus structure.

Additionally, this system can provide rapid qualitative visualization of the presence of target viruses by light microscopy. As shown in figure 4, one can qualitatively identify the fluorescent network structures using a benchtop fluorescent light microscope.

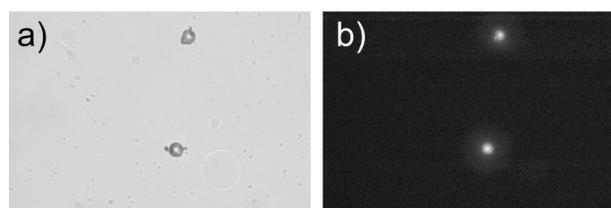


Figure 4. Light microscope (a) and fluorescence microscope (b) images of the nanotube-virus network structures.

3. CONCLUSION

Here we present a sensitive pathogen assay based on peptide nanotube biosensors. The ability to detect attomolar concentrations of in-tact whole virus rapidly solves an important need in the monitoring of regulated substances

such as blood supplies and vaccines. TEM images confirm the structures quantified by flow cytometry follow the proposed hypothesis for the mechanism of detection. The proof-of-principle of this system's multiplex ability was demonstrated using Herpes Simplex Type 2 Virus and Simian Virus 40 spiked samples to create standard curves for various potential analyte conditions, with no impact on detection limits compared to the single pathogen detection system.

4. ACKNOWLEDGEMENTS

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