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# Viral-Induced Self-Assembly of Magnetic Nanoparticles Allows the Detection of Viral Particles in Biological Media

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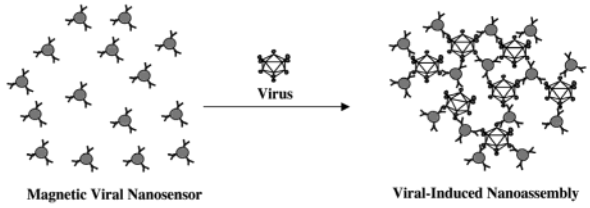
Biologic (e.g., viruses) and synthetic (e.g., metallic) nanoparticles can be used as unique building blocks in the design of supramolecular architectures with multivalent and three-dimensional interactions.[1] Of particular value would be supramolecular assemblies that take advantage of changes in the nanoparticle's optical or magnetic properties upon viral-induced assembly. Such structures could potentially be used to devise nanosensors for rapid and sensitive detection of clinically relevant viruses. We have previously described a unique magnetic phenomenon during the self-assembly of magnetic nanoparticles.[2] During this process, the nanoparticles act as magnetic relaxation switches by causing extensive spin−spin relaxation time changes ($\delta$T2) of surrounding water molecules. This observation has allowed us to design magnetic nanosensors capable of detecting a variety of targets such as nucleic acids (DNA, RNA) and proteins via the target-induced self-assembly of magnetic nanoparticles.

Here we report the application of this technique to the detection of low levels of viral particles in serum by MRI. Both herpes simplex virus (HSV) and adenovirus (ADV) were used in the current study because of their relevance to human pathology and their use as viral vectors in gene therapy. We hypothesized that, due to the multivalent interactions between nanoparticle and virus, a highly sensitive assay could be developed. We show that these readily detectable magnetic changes can be used to directly detect viral particles at low concentrations (5 viral particles in 10 $\mu$L) in biological samples. The developed magnetic viral nanosensors are composed of a superparamagnetic iron oxide core caged with a dextran coating[3] onto which virus-surface-specific antibodies were attached (Scheme 1). Attachment of anti-adenovirus 5 (ADV-5) or anti-herpes simplex virus 1 (HSV-1) antibodies was accomplished via Protein G coupling, attached to the caged dextran via *N*-succinimidyl-3-(2-pyridyldithio)propionate (SPDP). The overall particle size of the magnetic nanoparticles was 46 ± 0.6 nm, R1 was 33.4 $s^{-1}$ $mM^{-1}$, and R2 was 79.5 $s^{-1}$ $mM^{-1}$.

We first tested whether incubation of the ADV-5 viral particles with the anti-ADV-5 magnetic nanosensor would result in the formation of nanoassemblies in solution. Immediately after addition of $10^4$ viral particles to the magnetic nanosensors (20 $\mu$g Fe/mL), two distinct populations of particles were detected by light scattering, corresponding to magnetic nanoparticles alone (46 ± 0.6 nm**)** and viral particles (100 ± 18 nm**)**. Within 30 min of incubation, the viral particle population became undetectable by light scaterring and a larger-size population (494 ± 23 nm) appeared, corresponding to the viral-induced nanoassembly (Figure 1A). The size of the nanoassembly continued to increase slightly, reaching a plateau within 2 h (550 ± 30 nm**)**. Atomic force microscopy (AFM) studies confirmed the presence of viral-induced nanoassemblies in solution (Figure 1B). Next, we investigated the effects of nanoassembly formation on the T2 relaxation times of water at 0.47 T (20 MHz) with a tabletop relaxometer. For these

***Scheme 1.*** Diagram of Viral-Induced Nanoassembly of Magnetic Nanoparticles[a]




[a] Virus-surface-specific antibodies are immobilized on the magnetic nanoparticles to create magnetic viral nanosensors. When exposed to viral particles in solution, clustering of the nanoparticles occurs with a corresponding change in the MR signal ($\delta$T2).

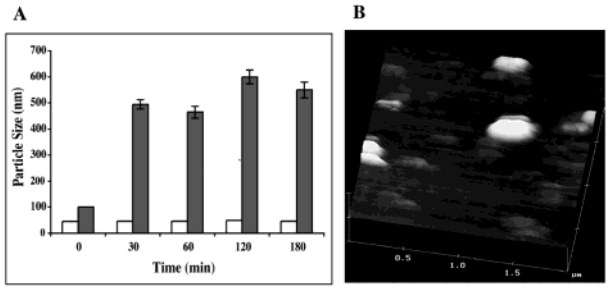



***Figure 1.*** (A) Particle size analysis by light scattering of a mixture of anti-ADV-5 magnetic nanosensors with ADV-5 virus at various time points. Empty bars represent the average size for the disperse magnetic nanoparticle population, while filled bars correspond to the average size for the viral-induced nanoassembly population. (B) AFM image (2 $\mu$m × 2 $\mu$m) taken on tapping mode in liquid of the viral-induced nanoassembly showing the formation of a 522 ± 11 nm viral-induced assembly. Control sample of ADV-5 magnetic nanosensors with no viral particle shows monodisperse magnetic particles of 50 nm.

experiments, we incubated anti-ADV-5 magnetic nanosensors (10 $\mu$g Fe/mL) with viral particles at two different concentrations ($10^4$ and $10^2$ viral particles/250 $\mu$L) in PBS. $\delta$T2 was measured over time, and Figure 2 summarizes these changes. Within 30 min, $\delta$T2 was half-maximum and reached a plateau in less than 2 h. A control sample consisting of heat-denatured virus only showed minimal T2 changes, indicating that sensing occurs only with complete viral particles.

We next investigated the linearity and detection threshold at 0.47 T (20 MHz). Using the same setup as described above, we were able to detect 2500 viral particles in the 250 $\mu$L used in our experiments. The assay was linear over at least 2 orders of magnitude. When lower-volume samples (100 $\mu$L) were used, we were able to detect as few as 100 viral particles. To determine the specificity of the assay, we performed experiments similar to those described above but using HSV-1 instead of ADV-5 as viral particles. In these experiments, no $\delta$T2 change was observed when anti-ADV-5 nanosensors were used; however, when an anti-HSV-1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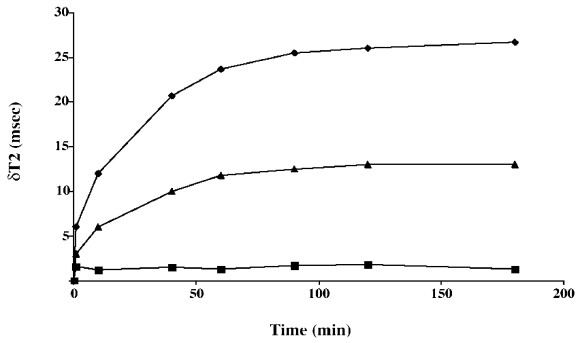


***Figure 2.*** Time course of water T2 change at 0.47 T (20 MHz) of anti-ADV-5 magnetic nanosensors in the presence of $10^4$ (◆) and $10^2$ (▲) ADV-5 viral particles. As control, denatured ADV-5 viral particles were used (■), showing no change in T2.

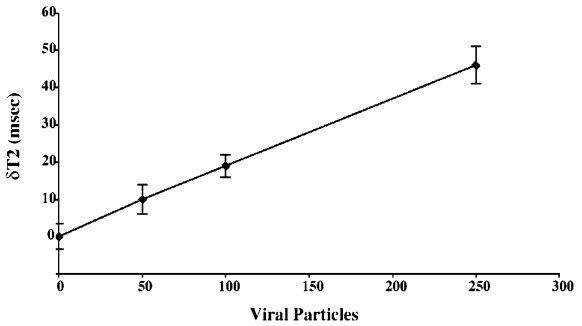


***Figure 3.*** Dose dependence of water T2 changes at 1.5 T (60 MHZ) of anti-HSV-1 magnetic nanosensors with decreasing amount of viral particles. Sample size = 100 $\mu$L.

antibody was attached to the magnetic nanoparticles, similar $\delta$T2 changes became detectable. These results confirm that the observed $\delta$T2 values are selective and that viral sensing depends primarily on the coat protein specificity of a given antibody incubated with the protein G-coupled magnetic sensors.

Finally, we investigated if the method can be used to directly detect low levels of viral particles in biological media such as cell lysates or serum, obviating any need for purification or amplification. For these experiments, an anti-HSV-1 polyclonal antibody was directly attached to the magnetic nanoparticle. A bifunctional linker, suberic acid bis(*N*-hydroxysuccinimide ester) (DSS) was utilized to cross-link the amino groups in the antibody with the amino groups in the nanoparticle. This approach was chosen rather than using protein G in order to minimize nonspecific interactions between serum/cell proteins and magnetic particles. Experiments were also performed at 1.5 T (60 MHz) to lower the detection limit at this higher magnetic field strength.[4] For these experiments, serial dilutions of a HSV-1 virus stock were made in either cell lysate or serum and incubated with the anti-HSV-1 magnetic nanosensor (3 $\mu$g/mL). Using this approach, it was possible to detect as few as 50 viral particles ($\delta$T2 = 10 ± 3 ms) in 100 $\mu$L of solution (25% serum in PBS) (Figure 3). When a lower sample volume (10 $\mu$L) is used, it is possible to detect as few as five viral particles. However, when the incubations were performed in 100% serum (100 $\mu$L), the detection limit for HSV-1 was 100 viral particles.

In conclusion, the reported viral-induced nanoassembly of magnetic nanoparticles allows for rapid, sensitive, and selective detection of a virus in solution by measurement of changes in water T2 relaxation times. The method was able to detect as few as five viral particles in 10 $\mu$L of 25% protein solution without the need of PCR amplification. This method represents an improvement over current PCR methods[5] for viral detection as it is fast, less prone to artifacts, and does not require removal of proteins or the use of amplifying enzymatic reactions. It is also independent of the optical properties of the solution, allowing detection of the virus in complex turbid media. The described assay is also more sensitive than current ELISA assays and does not require attachment of the virus to a solid surface. Our method utilizes common NMR and MRI instrumentation and allows for experiments to be performed in complex biological media like blood, cell suspensions, culture media, lipid emulsions, and even whole tissue. Recent advances in NMR technologies[4,6,7] would allow the analysis of multiple samples and smaller sample sizes. Furthermore, since the viruses used in this study are currently used in gene therapy studies, the technique could potentially be used to detect the distribution of HSV and ADV viruses by MRI in vivo.

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**Supporting Information Available:** Experimental section, describing the synthesis protocol for magnetic viral nanosensors and viral particles preparation, and a T2 map image at 1.5 T of viral nanosensors detecting low amount of virus (PDF). This material is available free of charge via the Internet at http://pubs.acs.org.

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