From Rational Design to Probe Optimization: The Role of Nanoparticle Valency in Single Cancer Cell Detection in Blood via Magnetic Relaxation

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ABSTRACT

The conjugation of targeting ligands to iron oxide nanoparticles has achieved the development of specific assays for the detection of biomedical targets through magnetic relaxation. However, limited studies investigated how the nanoparticle valency modulates the detection response and the assays’ sensitivity. Through nanoparticle valency grafting, we were able to achieve single cancer cell detection in blood, with a small-molecule-carrying multivalent nanoparticle in just 15 minutes. Although exhibiting a similar diagnostic trend, a corresponding high-valency antibody-carrying nanosensor did not achieve the same detection threshold, indicating that small molecules might be attractive probes for cancer diagnostics. Overall, these studies indicate that nanoparticle multivalency is a critical parameter in the design of robust and sensitive nanoprobes for diverse clinical and field-based applications.

Keywords: iron oxide nanoparticles, small molecule probes, mammalian cells, bacteria.

Multivalent interactions are abundant in nature, providing specificity, affinity and fidelity. Considering this, the need for sensitive cancer diagnostics and the utility of magnetic nanosensors in diverse clinical diagnostics, we investigated how the nanoparticle’s valency – the amount of targeting ligands on the nanoparticle – can direct the magnetic relaxation detection pattern and sensitivity threshold. We hypothesized that high-valency nanoparticles may demonstrate prominent changes in the magnetic relaxation response (ΔT2) at high cell concentrations, due to the formation of elaborate assemblies (Fig. 1). On the other hand, low-valency nanosensors may exhibit high ΔT2 at low cell concentrations, due to the multiple interactions between a population of nanosensors and a target (Fig. 1). Hence, we anticipated that a multivalent nanosensor may be faster and more sensitive than its low valency counterpart.

In order to test our hypothesis, we prepared polyacrylic-acid-coated iron oxide nanoparticles with different levels of folic acid (MW: 441 Da) and anti-folate-receptor antibody (MW: 150 kDa). Valency grafting was achieved by varying the conjugation reactions’ stoichiometry, resulting in high- and low-valency folate (120 Vs 1 moieties) or antibody (4 Vs 1) nanosensors. Interestingly, the high-valency folate nanosensor was able to detect a single cancer cell in blood within 15 minutes, exhibiting a gradual increase in the ΔT2 as the cell population increased (Fig. 2). Alternatively, the low-folate nanosensors had slower detection kinetics and quantified a narrower region, with the ΔT2 decreasing as the cancer cells’ numbers increased (Fig. 2). Similar valency-dependent findings were observed with the antibody-carrying nanoparticles, yet these sensors could not achieve single cell detection (Fig. 2).

Concluding, in this study we delineate the pivotal role of valency in the sensitivity optimization of magnetic nanosensors, as well as the use of small molecules to perform detection, isolation and confirmation of cancer biomarkers in clinical samples.
Figure 1. Discrete associations between magnetic nanosensors and cancer cells are directed by the nanoparticles’ valency. Multiple low-valency nanosensors interact with a single target at low concentrations, inducing large shifts in the $\Delta T_2$ response. Alternatively, multivalent nanosensors can associate with multiple target moieties on a single cancer cell at low concentrations, yielding a lower signal. As the cell concentration increases, these nanosensors can form elaborate complexes, through their interaction with targets on neighboring cells and causing the $\Delta T_2$ to increase.

Figure 2. Single-cancer-cell detection by multivalent small-molecule-carrying nanosensors. The signal response is directed by the nanoparticle valency, regardless of the nature of the targeting probe (small molecule and antibody).

REFERENCES