Vancomycin architecture dependence on the capture efficiency of antibody-modified microbeads by magnetic nanoparticles†

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We show that the ability to control the architecture/orientation of vancomycin on the surface of magnetic nanoparticles has a drastic effect on the ability of the nanoparticles to magnetically confine vancomycin-antibody modified polystyrene microbeads.

Nanoparticles are well documented to have unique, material dependent properties and there is currently great interest in exploiting these properties in order to develop nanoparticle-based probes capable of specific cell or biomarker identification.[1] Recently superparamagnetic nanoparticles have shown utility as MRI contrast reagents[2–4] and magnetic capture probes[5–8] which can aid in the detection and identification of a variety of cells, pathogens and biomarkers from biological fluids. In order for a specific interaction to occur, nanoparticles must be modified with antibodies or small molecule probes which allow for biomolecular recognition and targeting. Though antibody–nanoparticle conjugates are extremely useful substrates that provide the opportunity to specifically target biomolecules and cells, antibodies are quite large and multifunctional, which can both limit the number that can bind to a surface and allow the antibody to adopt surface orientations not favourable for specific biomolecular interactions.[9] As such, Weissleder,[10,11] Zhang,[3,12] Xu[5] and Chen[8] have recently shown that small molecule probes can be extremely useful for targeted binding or internalization of superparamagnetic nanoparticles onto or into cells. Because of the emerging importance of small molecule probes for targeting biological substrates, it is important to investigate how the architecture/orientation of a substrate on a nanoparticle surface can affect its activity or general ability to participate in specific interactions with biological molecules. As an example, we report that vancomycin can be selectively anchored to a nanoparticle surface in two distinct orientations and demonstrate that one of the orientations results in a more expedient magnetic capture of vancomycin-antibody modified microbeads. These results highlight the importance of controlling the molecular architecture of substrates anchored to surfaces for use in assays dependent on specific molecular interactions.

The nanoparticles to which vancomycin is anchored are comprised of an iron-oxide core surrounded by a silicon dioxide shell (**SNP**).[13,14] These nanoparticles are superparamagnetic and can be easily terminated with amine groups (**SNP-1**) or carboxylate groups (**SNP-2**) following literature protocols.[15,16] As shown in Fig. 1, vancomycin has two individually addressable functional groups. When **SNP-1** is modified with vancomycin, the resulting nanoparticle has vancomycin anchored to the surface through the carboxylic acid group (**SNP-3**). When **SNP-2** is first converted to an NHS ester (**SNP-2(NHS)**) and subsequently modified with vancomycin, the resulting nanoparticle has vancomycin appended to the surface through the vancosamine nitrogen (**SNP-4**). This means that the vancomycin moiety is essentially flipped on the surface by 90°. Model reactions with fluorescent derivatives of vancomycin make it possible to estimate that there are 9 and 12 vancomycin molecules on **SNP-3** and **SNP-4**, respectively.[16] This corresponds to ~3 and 4% surface coverage for **SNP-3** and **SNP-4**, respectively.[16] These vancomycin-modified nanoparticles were then incorporated into an assay designed to magnetically capture vancomycin-antibody modified fluorescent (365/415 nm) 1 μm polystyrene beads (**vAb-PS**).[16]

In separate experiments, 25 μL aliquots (3×10^7 microspheres) of **vAb-PS** were added to 1 mL (5×10^{12} nanoparticles) of **SNP-3** and **SNP-4** in 30 mM MES buffered water (pH = 6) in a fluorescence cuvette. The solution was thoroughly mixed, then incubated for 30 minutes at room temperature allowing **SNP-3** and **SNP-4** to specifically interact with **vAb-PS**. This serves to magnetize the entire microbead and it can then be magnetically confined by a rare earth magnet. The relative efficiencies of magnetic capture of **vAb-PS** by **SNP-3** (Fig. 2A) and **SNP-4** (Fig. 2D) over time were elucidated by monitoring how quickly the fluorescence intensity of **vAb-PS** (λ_{max} = 416 nm) decreased as it

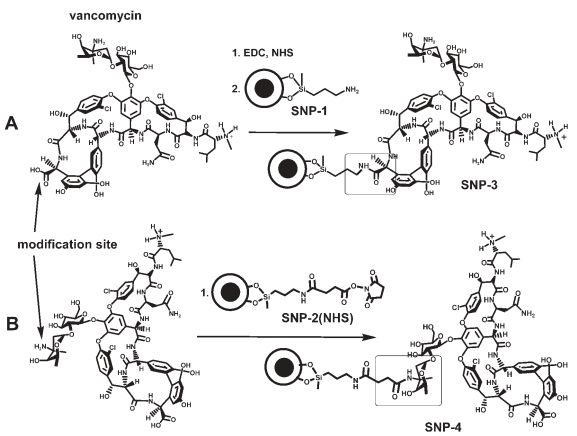

Fig. 1 The modification site on vancomycin and the resulting vancomycin-modified nanoparticles.

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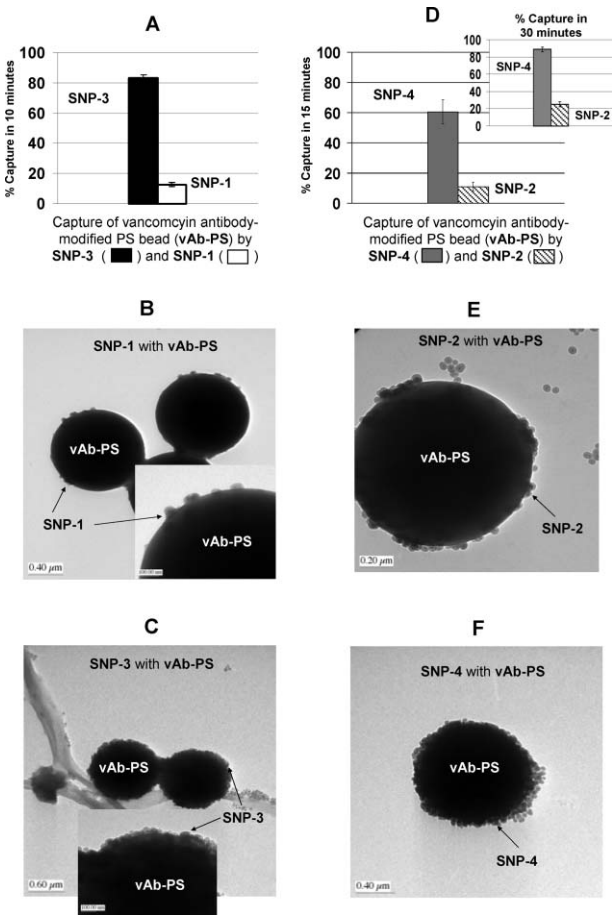

Fig. 2 Bar graph representations of the percentage loss of fluorescence intensity from **vAb-PS** as it is captured by **SNP-3** and **SNP-1** following 10 minutes of magnetic confinement (A) and by **SNP-4** and **SNP-2** following 15 minutes and 30 minutes (inset) of magnetic capture (D). TEM images of **SNP-1** (B), **SNP-3** (C), **SNP-2** (E) and **SNP-4** (F) mixed with **vAb-PS**. Note that **SNP-3** (C) and **SNP-4** (F) densely cover **vAb-PS** and **SNP-1** (B) and **SNP-2** (E) sparsely cover **vAb-PS**.

was attracted to the rare earth magnet placed beside the cuvette. In Figs. 2A and 2D the loss of fluorescence intensity has been converted to a percentage and plotted as a bar graph. A significant portion of the nanoparticle surface in **SNP-3** and **SNP-4** is not covered by vancomycin ($\sim$ 95%), so a control experiment was carried out to ensure that the capture was mediated by antibody–antigen interactions between the vancomycin on the modified nanoparticles and the antibody on the PS bead. Solutions of **SNP-1** and **SNP-2** (1 mL, 5×10^{12} nanoparticles) in MES buffered water were spiked with 25 μL aliquots of **vAb-PS** (3×10^7 particles) and incubated for 30 minutes. As shown in Figs. 2A and 2D, respectively, there is significantly less capture of the **vAb-PS** by **SNP-1** (terminated by amine) and **SNP-2** (terminated by carboxylic acid) than **SNP-3** and **SNP-4**, respectively, over identical timescales. This suggests that the capture of **vAb-PS** by **SNP-3** and **SNP-4** is mediated by vancomycin–vancomycin antibody interactions and there is little non-specific absorption from the unmodified parts of the nanoparticles contributing to the magnetic confinement. All of these experiments were repeated at least three times. These data also show that anchoring the vancomycin to the nanoparticle surface through the carboxylic acid moiety (**SNP-3**) results in a much more efficient magnetic confinement of **vAb-PS** (85% capture in 10 minutes) than when it is anchored through the vancosamine nitrogen (**SNP-4**) (60% capture in 15 minutes). It is also worth noting that **SNP-4** requires 30 minutes to capture 88% of the **vAb-PS** (inset of Fig. 2D). The strong interaction of **SNP-3** and **SNP-4** with **vAb-PS** can also be observed by TEM. **SNP-3** (Fig. 2C) and **SNP-4** (Fig. 2F) interact with **vAb-PS** much more effectively (note the dense coverage of nanoparticle in the TEM images) than the precursor nanoparticles, **SNP-1** (Fig. 2B) and **SNP-2** (Fig. 2E), respectively (note the sparse surface coverage of nanoparticle on the PS bead in the TEM images). However, it is difficult to discern any differences in affinity between **SNP-3** and **SNP-4** for **vAb-PS** from the TEM images.

The differences in capture efficiency could be due to a combination of factors including architectural, orientational and binding constant effects. We do not feel that the differences are due to surface coverage differences because there is a maximum vancomycin surface coverage of 3 and 4% for **SNP-3** and **SNP-4**, respectively, which should not introduce any steric hindrance impeding the approach of **vAP-PS**. It is possible that the binding sites (epitopes) on vancomycin targeted by the vancomycin antibody are more exposed in the case of **SNP-3** and more hindered in the case of **SNP-4**. That is, if the majority of epitopes are near the disaccharide moiety on the vancomycin molecule, the binding of the vancomycin antibody to these epitopes may be hindered in **SNP-4**. However, because the antibody employed in this study is polyclonal to vancomycin (*i.e.* it consists of a variety of antibodies that target a variety of epitopes on vancomycin), it is possible that the variety of epitopes targeted in **SNP-3** and **SNP-4** would be equally affected by linking through the two sites on vancomycin. It is also possible that there is a dependence of the association (K_a) and dissociation constant (K_d) of the antigen–antibody interaction on the modification site of vancomycin. Adamczyk and coworkers have reported that the K_d for a vancomycin derivative modified through the vancosamine nitrogen (as in **SNP-3**) can be 0.6–2900 times greater than the K_d for the analogous derivative modified at the carboxylic acid (as in **SNP-4**).[17] However, because we employ a 1.7×10^5 times excess of nanoparticle over **vAb-PS** it could be possible that even very large differences in K_d could be overcome with the extremely large excess of nanoparticle. We are currently exploring if a nanoparticle based system such as this could find use as an assay to qualitatively assess the relative position of the epitopes on vancomycin by exploring the interaction between **SNP-3** and **SNP-4** and PS beads modified with a variety of monoclonal vancomycin antibodies. In addition, because vancomycin is a well known glycopeptide antibiotic capable of targeting gram-positive pathogens,[5,8,18] we are also expanding this investigation in order to elucidate if the architecture/orientation of vancomycin on the nanoparticle surface affects the magnetic capture efficiency of pathogens from solution.

We have shown that the ability to control the orientation/architecture of vancomycin on the surface of nanoparticles has a drastic effect on the ability of the nanoparticles to magnetically capture vancomycin-antibody modified polystyrene microbeads. This investigation nicely highlights the importance of being able to control the molecular architecture of substrates on nanoparticle surfaces and illustrates the power of utilizing small molecule probes for the capture of biomolecules.

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