Small, Volume 10, Issue 6, March 2014, pp. 1086-1089
DOI: 10.1002/smll.201301996

Submitted to

Introducing MINA - The molecularly Imprinted Nanoparticle Assay

Roman V. Shutov, Antonio Guerreiro, Ewa Moczko, Isabel Perez de Vargas-Sansalvador, Iva Chianella, Michael J. Whitcombe, Sergey A. Piletsky*

Dr Antonio Guerreiro, Dr. Ewa Moczko, Dr. Isabel Perez de Vargas-Sansalvador, Dr. Iva Chianella, Dr. Michael J. Whitcombe, Prof. Sergey A. Piletsky
Cranfield Health, Cranfield University
Cranfield, Bedfordshire, MK43 0AL, UK
E-mail: a.guerreiro@cranfield.ac.uk
Dr. Roman V. Shutov, Saint-Petersburg State Chemical-Pharmaceutical Academy, Saint-Petersburg, Prof. Popov St. 14, 197376, Russian Federation

Keywords: molecular imprinting, core/shell materials, peroxidase, solid-phase synthesis

A totally abiotic ELISA-like assay, where no elements of biological origin are used, either for molecular recognition or for reporting purposes, is presented here. Using a novel synthetic approach, with immobilized template, surface-imprinted nanoparticles were prepared with a catalytic core capable of promoting a colorimetric reaction; these were used for combined target recognition and reporting. In microplate-competitive format the assay was capable of selectively determining vancomycin concentration from 10 nM to 1 mM, and with minimal modifications to standard assay protocols. Results point towards the future development of a new generation of assays based entirely on stable and potentially more affordable synthetic reagents.

Immunoassays have long been used in clinical, food, environmental and forensic industries to monitor a wide range of targets, ranging from proteins to DNA sequences and drugs.[1, 2] Of these, the enzyme-linked immuno-sorbent assay (ELISA) is probably the most common method, based on the competition between free and enzyme-labeled ligand to bind to immobilized targets or antibodies.[3] Although rapid, sensitive and cost effective for large sample loads, immunoassays have several disadvantages, mostly connected with limited

Published by Wiley. This is the Author Accepted Manuscript issued with: Creative Commons Attribution Non-Commercial License (CC:BY:NC 4.0).
The final published version (version of record) is available online at DOI:10.1002/smll.201301996. Please refer to any applicable publisher terms of use.

stability/batch variability and the expense of production and use of antibodies and reporter enzymes.

Molecularly Imprinted Nanoparticles (MIN) capable of recognition of a target molecule have been identified as promising alternatives to antibodies in a range of applications[4-6]. However, the added advantages (mainly storage/thermal stability and low cost) conferred by the use of imprinted materials as antibody replacements might not be effectively exploited if the assay system still requires the presence of a biological reporter enzyme. Accordingly, here we report the integration of catalytically active Fe_3O_4 with MIN as combined recognition and signaling functionalities in a core-shell nanoparticle format suitable for use in ELISA-like assays. The intrinsic peroxidase mimicking activity of γFe_3O_4 nanoparticles has been recently identified,[7] making them attractive substitutes for the enzyme in a variety of assays, with stable catalytic activity over a broad range of temperatures, low cost/long shelf life and ease of manufacture. In order to produce the composite core-shell Fe_3O_4-MIN, a variation of the solid-phase imprinting protocol[8] was used, **Scheme 1**. This method was devised taking into consideration its potential for scale-up and automation, both important factors to consider regarding potential practical applications of the technology. That synthesis and purification time < 1 h per batch of particles is also advantageous. Briefly, the protocol consisted of the addition of monomer mixture and UV initiator, containing a dispersion of 3-(trimethoxysilyl) propyl methacrylate modified γFe_3O_4 nanoparticles ($d \leq 50$ nm), to the solid phase (glass beads) bearing the immobilized target template (vancomycin). After a short period of UV irradiation (2.5 min), nanoparticles were formed,[9][8] and the solid phase containing the template and the bound MINs then functioned as an affinity support for the fractionation/purification of high-affinity MINs from the remaining polymer/unreacted monomers (see S. I. for full details), by washing with solvent at room temperature. High affinity materials were collected by elution at 60 °C. Subsequent magnetic separation of the high-affinity particles ensured that only those containing the magnetic Fe_3O_4 core were

recovered. Consequently the collected MINs possessed both high-affinity (affinity separation step on the solid phase) and a Fe_3O_4 core (magnetic separation). Control (non-imprinted) particles were prepared in absence of immobilized template. Fe_3O_4-MINs had diameters of 113 ± 23 nm and Fe_3O_4-C (control) particles 81 ± 2 nm (by DLS). Particles possessed a core-shell structure, with a polymer shell surrounding the Fe_3O_4 core, Scheme 1. To increase polymer contrast during imaging, particles were stained with OsO_4. A TEM image of the core Fe_3O_4 is presented as S. I (**Figure S1**) confirming particles have a diameter below 50 nm. To demonstrate that specific interactions between polymer and template were responsible for retention of the particles by the solid phase (during the cold column washing), control experiments were performed by loading the polymerisation mixture containing Fe_3O_4 nanoparticles onto the solid phase. In the absence of a polymerization step, 99.59 % of the Fe_3O_4 was eluted during the cold wash. Following a polymerization step, only 82.75 % is eluted cold, with 17.25 % recovered during the hot wash (high affinity fraction) and included in the core-shell MINs. When the surface of the Fe_3O_4 core was not modified with methacrylate groups, a decrease in yield was observed (15.95 % recovered during hot wash) so modified Fe_3O_4 nanoparticles ware used on subsequent experiments. The composition of the polymerization mixture was based on a published protocol[8, 10] and included *N*-isopropylacrylamide, *N-tert*-butyl acrylamide, acrylic acid and the cross-linker N, N'-methylenebis(acrylamide). The full composition is given in S. I. Target recognition with this type of polymer can be attributed[4] to a combination of multiple weak electrostatic and hydrophobic interactions and is especially suited to the imprinting of large molecules.

The kinetics of the catalytic oxidation of TMB by Fe_3O_4 nanoparticles were studied by measuring the absorbance of the solution at 650 nm in 300 sec intervals, and compared for both free Fe_3O_4 and Fe_3O_4-MIN nanoparticles. Michaelis-Menten kinetics parameters (v_{max} and K_m) were calculated using the Lineweaver–Burk double reciprocal plot (see S. I, **Figure S2**). The catalytic constant (k_{cat}) was calculated according to Equation (1):

$$k_{cat} = v_{max}/[E] \quad (1)$$

where [E] is the Fe_3O_4 concentration (2.15×10^{-10} M). Catalytic parameters are summarized on **Table 1**. Results indicate that presence of the polymer shell does not significantly interfere with the catalytic activity of the nanoparticles. This can be attributed to the fact that the hydrophilic porous polymer shell, with low percentage of cross-linker (2%) is swollen in aqueous media, and so causes little resistance to the diffusion of the small-molecule reactants. Also, this is in accordance with Woo[11] who observed small increases in V_{max} values for Fe_3O_4 nanoparticles after surface derivatization/coating with proteins.

Although imprinted polymers have the potential to directly replace natural receptors in assays such as ELISA, the materials produced using classical protocols have generally proved unsuitable for such applications due to a number of factors. Firstly, traditional MIPs are highly "polyclonal" in relation to their binding behavior (i.e. binding sites distributed throughout the polymer bulk and surface which have a wide range of affinities towards the target molecule) and suffer from high levels of non-specific binding. Secondly, the protocols for integration of imprinted polymers with assays are complicated and non-generic, requiring significant modifications to the procedure used for their manufacture or performance of the assay. Aiming to solve some of these problems we have developed the solid-phase imprinting method for synthesis of MINs with pseudo-monoclonal properties.[8] As detailed in Scheme 1, by combining the affinity separation (for removal of low-affinity MINs) and the fact that particles have a limited number of binding sites (only at the point of contact with the solid-phase), the final product will have more uniform binding characteristics resembling those of monoclonal antibodies when compared with imprinted polymers prepared using traditional methods with free template in solution. MIN as produced here can be employed as direct replacements of antibodies/enzyme in standard ELISA-like assays with minimal modifications to the protocol. To demonstrate this, we selected the glycopeptide antibiotic vancomycin as analyte which also can point the way to the use of synthetic peptide epitopes

instead of whole proteins in this type/format of assay,[12] again, to avoid using potentially unstable or expensive biomaterials. To develop the MIN-based assay, vancomycin was initially conjugated onto the well surface of a 96 well micro plate (via amine coupling, polystyrene plate surface derivatized with maleic anhydride). Then, in order to assess if imprinted nanoparticles cold bind preferentially to this surface, as opposed to surfaces without derivatization, particles were loaded onto the wells which were subsequently washed. Random/irreproducible peroxidase activity values were detected on wells without immobilized vancomycin, ranging from no activity to values similar to those of Fe_3O_4-C on vancomycin-derivatized surface (see text below). High peroxidase activity was only detected on surfaces containing immobilized vancomycin and attached nanoparticles were then visualized by Atomic Force Microscopy (AFM), **Figure 1**. The incomplete coverage of the surface with particles might indicate that the performance of the assay can be improved by optimization of the surface characteristics. This can be related with incomplete surface coverage with reactive groups, or some of these are too close to the surface to allow for MIN binding. The use of spacer groups between target molecule and surface, or the use of different coupling techniques will be investigated in the future order to improve the amount of nanoparticles which can be bound to the surface, and so increase the amount of catalytic sites, with consequent increases in measured absorbances. The peroxidase activity was determined by addition of standard colorimetric substrate 3,3′,5,5′-tetramethylbenzidine (TMB) for ELISA and hydrogen peroxide. TMB produces a blue color when oxidized by hydroxyl radicals produced in the presence of a catalyst (such as Fe_3O_4 or peroxidases) and H_2O_2.[7] By measuring the rate of color development in the reaction system with TMB, H_2O_2 and Fe_3O_4-MINs at different concentrations it was found that the quantity of nanoparticles remaining in the wells after incubation and washing (in the absence of free vancomycin) is equivalent to ca. 20 μg mL^{-1}. The MIN-based assay was then performed in competitive mode. Upon addition of MINs and vancomycin solutions, the extent of nanoparticles bound to the well surface was

found to be dependent on the amount of free vancomycin in solution (by measuring the peroxidase activity) after incubation and washing to remove unbound MINs. A good correlation was observed between the absorbance of the solution and the concentration of vancomycin over the range from 10 nM to 1 mM (**Figure 2**). When control Fe_3O_4-C particles were used, in the absence of free vancomycin, a response of 89 ± 11 mAU was recorded, as compared to 834 ± 47 mAU for the Fe_3O_4-MINs (both values obtained after 1 hour incubation with 100 μL nanoparticle solution, 50 μg/mL, loaded in each well and following washing). The absorbance values obtained with Fe_3O_4-C were not affected by the presence of vancomycin. While vancomycin was selected primarily as a model analyte, the obtained linear range is well within the recommended serum monitoring levels of 7 to 14 μM,[13, 14] demonstrating potential for the development of vancomycin assays based on this methodology. The assay was found to be specific, with minimal response to other antibiotics, each present at 0.1 μM Figure 1 (inset). While there is no vancomycin analogue available, bleomycin and gentamycin share chemical similarities with vancomycin, hence their selection. Whilst this demonstration study has been performed in buffer solution, and could possibly be translated with minor modifications for quantification of a wide range of analytes in relatively simple matrixes (such as urine or environmental samples), analysis in serum/blood will probably require optimization work related with type/length of linker between target and plate surface, washing/blocking agents and protocols. In conclusion, we have demonstrated the possibility to perform an ELISA-like assay where no biological material was used, either for recognition or reporting purposes, with minimal modifications to the standard protocol. Peroxidase-mimicking imprinted nanoparticles were produced by the solid-phase imprinting method in the presence of Fe3O4 and used for combined target recognition and reporting by catalyzing the oxidation of TMB. The results are a starting point for the development of a new generation of assays using stable synthetic reagents, which could find uses especially in

field/emergency conditions or in locations where a cold-chain for storage and transport is unavailable.

Supporting Information

Supporting Information is available online from the Wiley Online Library or from the author.

Acknowledgements

The authors thank The Wellcome Trust (UK) for the granting of a Translational Award, The Royal Society (UK) for a Brian Mercer Feasibility Award and the EU for the financial support through the project CHEBANA (FP7-PEOPLE-2010-ITN-204772). Financial support from the Zamaraev International Charitable Research Fund (Donation # DP-02/13) is gratefully acknowledged by R. S.

Received: ((will be filled in by the editorial staff))
Revised: ((will be filled in by the editorial staff))
Published online: ((will be filled in by the editorial staff))

[1] L. Anfossi, C. Baggiani, C. Giovannoli, G. D'Arco, G. Giraudi, *Anal. Bioanal. Chem.* **2013***, 405*, 467.

[2] A. Valanne, S. Huopalahti, T. Soukka, R. Vainionpää, T. Lövgren, H. Härmä, *J. Clin. Virol.* **2005***, 33*, 217.

[3] A. Vieira, *Appl. Biochem. Biotech.* **1998***, 10*, 247.

[4] Y. Hoshino, T. Kodama, Y. Okahata, K. J. Shea, *J. Am. Chem. Soc.* **2008***, 130*, 15242.

[5] Y. Ge, A. P. F. Turner, *Chem. Eur. J.* **2009***, 15*, 8100.

[6] A. Poma, A. P. F. Turner, S. A. Piletsky, *Trends Biotechnol.* **2010***, 28*, 629.

[7] L. Gao, J. Zhuang, L. Nie, J. Zhang, Y. Zhang, N. Gu, T. Wang, J. Feng, D. Yang, S. Perrett, X. Yan, *Nat. Nanotechnol.* **2007***, 2*, 577.

[8] A. Poma, A. Guerreiro, M. J. Whitcombe, E. V. Piletska, A. P. F. Turner, S. A. Piletsky, *Adv. Funct. Mater.***2013**, *23*, 2821.

[9] A. R. Guerreiro, I. Chianella, E. Piletska, M. J. Whitcombe, S. A. Piletsky, *Biosens. Bioelectron.* **2009***, 24*, 2740.

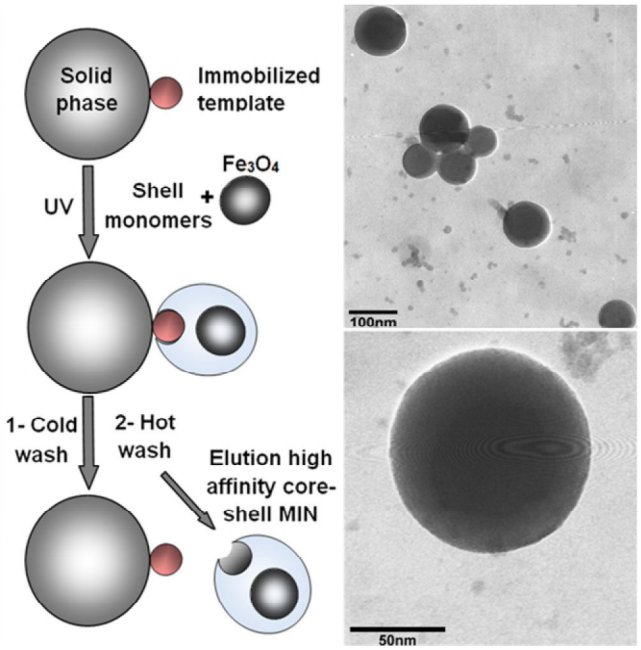
[10] Y. Hoshino, H. Koide, T. Urakami, H. Kanazawa, T. Kodama, N. Oku, K. J. Shea, *, J. Am. Chem. Soc.* **2010***, 132*, 6644.

[11] M. Woo, I. Kim, J. H. Jung, K. S. Park, T. S. Seo, H. G. Park, *Int. J. Mol. Sci.* **2013**, *14*, 9999.

[12] H. Nishino, C. Huang, K. J. Shea, *Angew. Chem. Int. Ed.* **2006**, *45*, 2393.

[13] A. H. Thomson, C. E. Staatz, C. M. Tobin, M. Gall, A. M. Lovering, , *J. Antimicrob. Chemother.* **2009**, *63*, 1050.

[14] M. Rybak, B. Lomaestro, J. C. Rotschafer, R. Moellering Jr., W. Craig, M. Billeter, J. R. Dalovisio, D. P. Levine, C. Reilly, *Am. J. Health-Syst. Ph.* **2009**, *66*, 82.



Scheme 1. Representation of the solid-phase synthesis protocol with addition of Fe_3O_4 for preparation of peroxidase-mimicking core-shell MIN (left) and TEM image of the obtained Fe_3O_4-MIN particles (right).

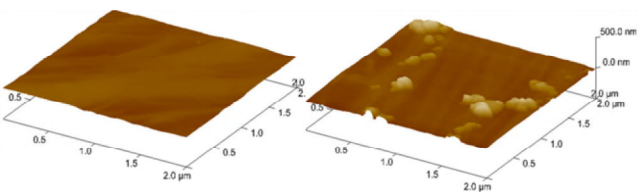


Figure 1. AFM images showing topography of the micro plate well surface before (left) and after 1 hour incubation with 100 µL Fe_3O_4-MINs (50 µg mL^{-1}) and washing (3 × 100 µL) with deionized water.

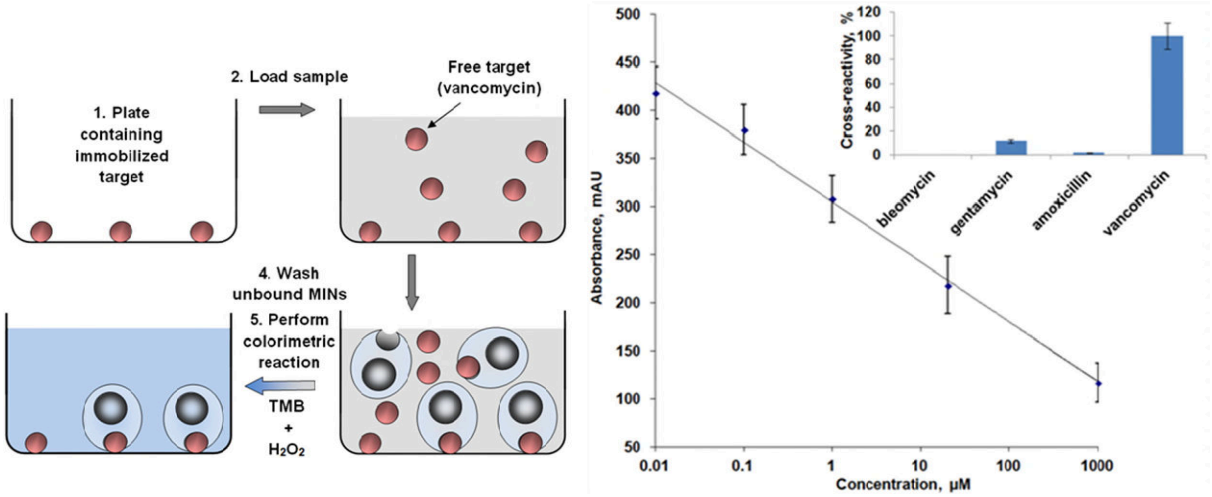


Figure2. On the left, scheme of the assay in competitive mode, and on the right, calibration curve for vancomycin obtained with core-shell catalytic MIN (main) and (inset) normalized assay response (cross-reactivity) to different antibiotics, all experiments performed under physiological conditions(PBS 1×, pH 7.2). Solutions of Fe_3O_4-MINs (100 µL, 50 µg mL^{-1}) were added to each of the vancomycin modified wells, followed by 50 µL vancomycin solution. After one hour incubation, wells were washed (3 × 100 µL PBS) and colorimetric TMB assay performed, full details in S. I. Error bars represent ± 1 standard deviation and are for experiments performed in triplicate.

Table 1. Table 1. Apparent kinetic parameters of Fe_3O_4 nanoparticles and Fe_3O_4-MIN as peroxidase mimics.

Particle type	K_m [M]	v_{max} [M s^{-1}]	k_{cat} [s^{-1}]	(k_{cat}/K_m) [(M s)$^{-1}$]
Fe_3O_4	0.49	16.8 × 10^{-9}	77.8	156.3
Fe_3O_4-MIN	0.48	19.8 × 10^{-9}	92.1	190.6

Submitted to small

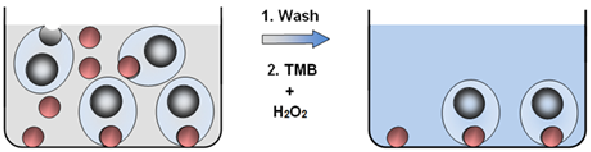
A new ELISA-like assay where no elements of biological origin are used for molecular recognition or signaling is demonstrated here. Synthesized using a solid-phase approach, composite imprinted nanoparticles containing a catalytic core can simultaneously act as recognition/signaling elements, and be used with minimal modifications to standard assay protocols. This provides a new route towards replacement of unstable biomolecules in immunoassays.

Keyword molecular imprinting, core/shell materials, peroxidase, solid-phase synthesis

Roman V. Shutov, Antonio Guerreiro,* Ewa Moczko, Isabel Perez de Vargas-Sansalvador, Iva Chianella, Michael J. Whitcombe, Sergey A. Piletsky

Introducing MINA - The molecularly Imprinted Nanoparticle Assay

ToC figure



Copyright WILEY-VCH Verlag GmbH & Co. KGaA, 69469 Weinheim, Germany, 2013.

Submitted to small NANO MICRO

Supporting Information

for *Small*, DOI: 10.1002/smll.((please add manuscript number))

Introducing MINA - The molecularly Imprinted Nanoparticle Assay

Roman V. Shutov, Antonio Guerreiro, Ewa Moczko, Isabel Perez de Vargas-Sansalvador, Iva Chianella, Michael J. Whitcombe, Sergey A. Piletsky*

Experimental procedures

Materials

3-(trimethoxysilyl) propyl methacrylate, 3-aminopropyltrimethyloxysilane (APTMS), glutaraldehyde (GA), phosphate buffered saline (PBS), N-isopropylacrylamide (NIPAm), acrylic acid (AA), *N,N'*-methylene-bis-acrylamide (BIS), *N-tert*-butylacrylamide (TBAm), vancomycin, gentamycin, bleomycin, amoxicillin, ethanolamine, 3,3′,5,5′-tetramethylbenzidine (TMB) ready solution for ELISA, hydrogen peroxide (34%), Fe_3O_4 nanopowder (particle size ≤ 50 nm), osmium tetroxide were purchased from Sigma-Aldrich, UK. Acetonitrile (ACN) and sodium hydroxide were obtained from Fisher Scientific (UK). Diethyldithiocarbamic acid benzyl ester was obtained from TCI Europe (Belgium). RO ultrapure water (Millipore) was used throughout. All chemicals and solvents were analytical or HPLC grade and were used without further purification. Phosphate buffered saline (PBS) was prepared as directed from 1 × PBS buffer tablets (Sigma-Aldrich, UK), pH adjusted to 7.2, at 25 ° C. Amine-binding maleic anhydride 96-well micro plates were purchased from Pierce (Thermo-Fisher Scientific, USA), and used as directed by the manufacturer. Solid glass beads (40 μm ≤ diameter ≤ 106 μm, average diameter 90 μm, were purchased from Blagden, UK.

Preparation of vancomycin-derivatized glass beads

The protocol for the immobilization of vancomycin on glass beads has already been described elsewhere.[SI1] Briefly, glass beads were activated by boiling them in 1M NaOH for 10 min and washed with double-distilled water followed by acetone and then dried. The beads were then incubated overnight in a solution of APTMS in toluene (0.4 mL solution/g glass beads), washed with acetone and subsequently incubated overnight at 4 °C in a solution of 5 % GA in PBS pH 7.2 (0.4 mL solution/g glass beads) then washed with water. The surface immobilization of vancomycin was performed by incubating the beads with a solution of vancomycin (5 mg mL^{-1}) in PBS, pH 7.2, overnight at 4 °C, again with 0.4 mL solution per g glass beads. This method yields *ca*. 0.26 ligand molecules nm^{-2} of glass bead surface.[SI 1] Finally, the glass beads were washed with double-distilled water, dried under vacuum and stored at 4 °C until used.

Preparation of Fe_3O_4 derivatized with 3-(trimethoxysilyl) propyl methacrylate

One gram of Fe_3O_4 nanopowder was suspended in 45 mL of dry toluene to which 3-(trimethoxysilyl) propyl methacrylate (5 mL) was added under stirring. The suspension was incubated in an ultrasound bath for 4.5 hours. The derivatized Fe_3O_4 nanopowder was then separated from solution using a magnet, washed with five times 45 mL toluene and dried under vacuum overnight.

Preparation of Fe_3O_4-MIN

The polymerization mixture was prepared by mixing NIPAm (0.936 g) as backbone monomer, BIS (0.048 g) as cross-linker, TBAm (0.8 g) as hydrophobic functional monomer, AA (56.8 µL) as negatively-charged functional monomer and N,N-diethyldithiocarbamic acid benzyl ester (0.152 g) as initiator (iniferter) in ACN (7.87 g) (total monomer concentration: 18%

w/v) and 0.05 g Fe_3O_4 nanopowder derivatized with 3-(trimethoxysilyl) propyl methacrylate. The mixture was placed in a 20 mL glass vial and purged with N2 for 20 min and sonicated in an ultrasound bath during 10 min. Vancomycin-derivatized glass beads (solid phase, 50 g), prepared as described above were placed in a 200 mL flat-bottomed glass beaker fitted with a flat glass lid and degassed under vacuum for 20 min, the air inside the beaker then replaced with N_2. The polymerization mixture was poured onto the solid phase and the vessel then placed between two UV light sources (both Philips model HB/171/A, each fitted with 4×15 W lamps) for 2.5 min under a nitrogen atmosphere. After polymerization, the contents of the beaker were transferred into an SPE cartridge fitted with a polyethylene frit (20 µm porosity) in order to perform the temperature-based affinity separation of Fe_3O_4- MIN. The temperature of ACN and the SPE cartridge was kept at 20 °C (same as polymerization step). Washing was performed with 8 bed volumes of ACN (relative to the volume of the solid phase, approx. 8 × 30 mL), using a vacuum manifold. This was done in order to remove non polymerized monomers and low affinity polymer. The effectiveness of the washing was verified by measuring the UV absorbance of washing aliquots, in order to ensure complete monomer removal. Afterwards the SPE cartridge containing the solid-phase with high-affinity Fe_3O_4-MIN attached was heated up to 60 °C and eluted with 3×30 mL ACN at 60 °C. The particles were then collected at the bottom of the collection vial by a magnet, the supernatant discarded, followed by re-suspension of material in 20 mL ACN. This procedure was repeated three times. Concentration of Fe_3O_4-MIN in the final solution was determined by freeze-drying. The working solution for the assay was prepared by transferring an aliquot of Fe_3O_4-MIN solution in ACN, followed by magnetic separation and re-suspension in PBS. Two magnetic separation/re-suspension cycles were performed; concentration of Fe_3O_4-MIN was adjusted to 50 µg/mL for the assay experiments. Molar concentration of Fe_3O_4 in stable suspensions was determined spectrophotometrically. Control non-imprinted particles were prepared in absence of template-derivatized solid phase. After polymerization, materials were removed from

solution by magnetic separation. The particles were then washed with ACN. For this, six cycles of separation/re-suspension with 20 mL ACN/cycle were performed. The working solution for the assay was prepared by transferring an aliquot of Fe_3O_4-MIN solution in ACN, followed by magnetic separation and re-suspension in PBS. Two magnetic separation/re-suspension cycles were performed.

Size analysis of nanoparticles

Solutions of nanoparticles at 50 μg/mL were sonicated for 5 minutes and then analyzed at 25 °C using a Zetasizer Nano (Nano-S) from Malvern Instruments Ltd (UK). For transmission electron microscope analyses, Fe_3O_4-MIN samples were stained with osmium tetroxide by adding 50 μL of 2 % solution to 1 mL nanoparticles solution in water. Solutions were held for 4 hours and then vented on a fume hood overnight to remove excess osmium tetroxide. Caution! Osmium tetroxide is highly poisonous, even at low exposure levels. After staining, particles were placed on a carbon-coated copper grid and dried on air. The microscope used was a Philips CM20 transmission electron microscope.

AFM analysis

The surface of the interior bottom section of a well from the 96 well micro plate was used for AFM analysis. The instrument was a Bruker Dimension 3100, and analysis performed in tapping mode.

Vancomycin conjugation to the micro plate surface

Micro plate wells were washed with PBS (3×100 μL) then vancomycin solution was added (20 μM, 100 μL, pH 8) and allowed to stand overnight at room temperature. Then the solution was poured out and ethanolamine solution (50 mM, 100 μL) in PBS was added as blocking

solution and allowed to stand for 1 h. Afterwards wells were washed twice (100 µL) and air-dried.

Assay procedure

To the vancomycin-modified micro plate wells containing nanoparticle suspension (100 µl) and vancomycin solution at various concentrations (50 µl) were incubated for 1 h with constant shaking. Then the solution was poured out, the wells were washed with PBS (3×100 µl) and afterwards 100 µL of TMB solution and 50 µL of hydrogen peroxide were added and incubated for 1 h in the dark to develop the color. Then 50 µl of 0,1 M sulfuric acid were added and absorbance was measured using UV/Visible micro plate reader (Dynex, UK) at 450 nm.

Determination of kinetic parameters

To microplate wells containing nanoparticles solution (final concentration Fe_3O_4 0.21 nM), 100 µL of TMB solution and 50 µL of H_2O_2 were added. H_2O_2 was added at different concentrations in order to obtain the following final concentration of 1.47 M, 0.98 M, 0.73 M, 0.49 M, 0.49 M, 0.36 M, 0.18 M. Absorbance was then measured every 300 seconds for 45 minutes at 650 nm. Absorbance data were back-calculated to concentration using a molar absorption coefficient of 39000 $M^{-1}cm^{-1}$ for TMB-derived oxidation products.[SI2] Michaelis-Menten kinetics parameters were calculated using the Lineweaver–Burk (double reciprocal) plot.

Supporting data

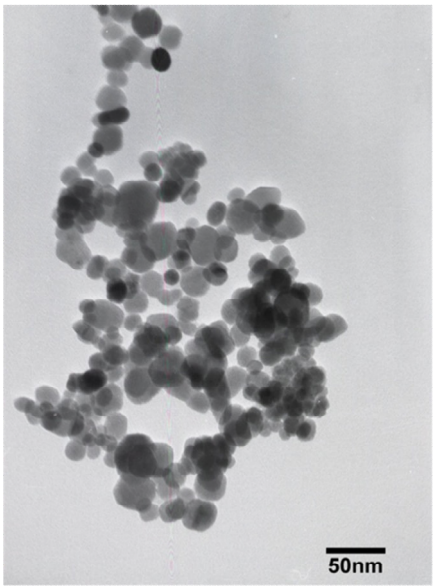


Figure S1. TEM image of core Fe_3O_4 nanoparticles.

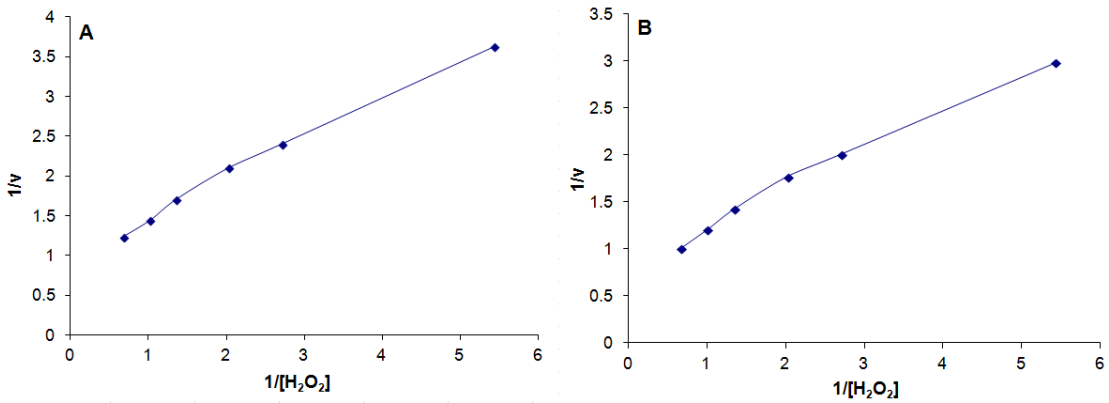


Figure S2. Lineweaver–Burk plots for catalysis of H_2O_2 by core Fe_3O_4 nanoparticles (A) and Fe_3O_4 –MIN nanoparticles (B).

References for S.I.

S1- A. Poma, A. Guerreiro, M. J. Whitcombe, E. Piletska, A. P. F. Turner, S. Piletsky, *Adv. Funct. Mat.***2013**, *23*, 2821.
S2- Q. Zhang, W. Gong, Y. Zhang, T. Yang, Y. Wang, N. Gu, *J. Mater. Chem.* **2010**, *20*, 5110.

https://dspace.org/

Introducing MINA-The Molecularly Imprinted Nanoparticles Assay

Shutov, Roman V.; Guerreiro, Antonio R.; Moczko, Ewa; Perez De Vargas Sansalvador, Isabel M.; Chianella, Iva ...

2014-02-06
Wiley
https://doi.org/10.1002/smll.201301996
Shutov RV, Guerreiro A, Moczko E, et al., (2014) Introducing MINA - The Molecularly Imprinted Nanoparticles Assay. Small, Volume 10, Issue 6, March 2014, pp. 1086-1089

Downloaded from a DSpace repository. https://dspace.org/