Conference paper

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Competitive pseudo-ELISA based on molecularly imprinted nanoparticles for microcystin-LR detection in water

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Abstract: Microcystins (MCs) are dangerous cyanotoxins for the public health, and microcystin-LR (MC-LR) is one of most toxic, dangerous, and frequently found in water bodies. Typically, the detection of MCs is carried out by means of competitive ELISAs which, however, need special precautions for handling and storage, due to the stability of the antibodies used in this test. Molecularly imprinted nanoparticles (nanoMIPs) represents more robust and cost-effective alternative to antibodies. In this work, we developed a competitive pseudo-ELISA based on nanoMIPs (which are used in place of natural antibodies), for the detection of microcystin-LR (MC-LR). This pseudo-ELISA showed a linear response towards MC-LR, showing high affinity and low cross-reactivity against another analogue toxin (microcystin-YR). The analytical recovery of MC-LR in the analysis of water samples by the proposed pseudo-ELISA was 96 %–130 % and the limit of detection was 2.64×10^{-4} nM. The obtained results suggest that this competitive pseudo-ELISA could have high potential in the detection of toxins, due to its rapid, sensitive and accurate detection of toxin in water samples.

Keywords: Eurasia 2018; microcystin-LR; MIPs; pseudo-ELISA.

Introduction

In the last decade, harmful algal blooms (HABs) have increased its natural abundance in aquatic environments, due to eutrophication of water resources [1–3], producing numerous species of cyanotoxins such as anatoxins, cylindrospermopsins, saxitoxins and microcystins [4, 5]. Around 200 variants of microcystins are currently known [6, 7]. Within the microcystin family, the microcystin-leucine-arginine (MC-LR) is one of most toxic, dangerous, and frequently found in water bodies [6, 8, 9]. This toxin is a cyclic heptapeptides that contains three D-amino acids alanine (D-Ala), methylaspartic acid (MeAsp), and glutamic acid (D-Glu), two unusual amino acids *N*-methyldehydroalanine (Mdha) and 3-amino-9-methoxy-2,6,8-trimethyl-10-phe-

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nyldeca-4,6-dienoic acid (Adda), and two L-amino acids, leucine (L) and arginine (R) (Fig. 1) [10, 11]. The exposure to high concentrations of MC-LR due to recreational water or consumption of drinking water can affect the nervous system, the liver and the skin in both humans and animals, leading even to death [12–14]. Consequently, its levels are currently monitored in water to protect the public health.

Currently, numerous analytical techniques have been employed for the determination of MC-LR, such as chromatography (liquid and coupling with spectrometry mass) [15–17], capillary electrophoresis [18], protein phosphatase inhibition assays [19], and immunoassays [20]. The use of immunoassays has been raising in the past few years [7]. However, immunoassays rely on the use of antibodies, which need special precautions for their handling and storage, and they are relatively costly and time-consuming. Molecularly imprinted polymeric nanoparticles (nanoMIPs) represent a novel class of synthetic antibodies, which have the potential for replacing their natural counterparts in ELISAs [21–25].

Molecularly imprinted polymers (MIPs) are synthetic receptors that are produced in the presence of the target molecule. Following a self-assembling process and a polymerisation step, target-specific cavities (or imprints) are created within the polymers. After removal of the imprinted molecule, the polymer will contain target-shaped pockets which will be capable of rebinding the target molecule [26–28]. This specific recognition of the template is due to functional groups and weak complementary interactions (hydrogen bonds, Van der Waals forces, ionic bonds and hydrophobic interactions) occurring in the polymeric imprint [29, 30].

Given their small size, nanoMIPs are ideal for use in competitive assays. In this work, nanoMIPs specific for MC-LR are obtained by solid-phase synthesis, which [31] consists in immobilizing the template onto a support solid, allowing the orientation of the target molecule [32, 33]. Such orientation allows obtaining particles with a narrow distribution of affinities. The obtained nanoMIPs were evaluated in the pseudo-ELISA format, in both spiked and real samples. This assay may be a promising tool for a more robust and cost-effective detection of MC-LR in water.

Experimental section

Materials

Microcystin-LR (MC-LR or MCLR) was purchased from Biorbyt, UK. Microcystin-YR (MC-YR), acrylamide (ACA), *N*-*tert*-butylacrylamide (TBAm), acrylic acid (AAc), 2-hydroxyethyl methacrylate (HEMA), *N*-(3-ami-

Fig. 1: Microcystin-LR structure; formed by the following amino acids, alanine (D-Ala), methylaspartic acid (MeAsp), glutamic acid (D-Glu), *N*-methyldehydroalanine (Mdha), 3-amino-9-methoxy-2,6,8-trimethyl-10-phenyldeca-4,6-dienoic acid (Adda), leucine (L) and arginine (R).

nopropyl) methacrylamide hydrochloride (AMH), N,N'-methylene-bis-acrylamide (BIS), ammonium persulfate (APS), tetramethylethylenediamine (TEMED), sodium hydroxide (NaOH), glutaraldehyde (GA), 1,2-Bis(triethoxysilyl) ethane, (3-aminopropyl) triethoxysilane (APTES), toluene, acetone, methanol, sulfuric acid, 1-ethyl-3-(3-dimethylaminopropyl)-carbodiimide hydrochloride (EDC), N-hydroxysuccinimide (NHS), bovine serum albumin (BSA), non-fat dry milk (NFDM), horseradish peroxidase (HRP), 3,3',5,5'-tetramethylbenzidine (TMB), Tween 20 and 2-[morpholino]ethanesulfonic acid (MES) were obtained from Sigma-Aldrich, Chile. Phosphate buffered saline (PBS) was prepared from PBS buffer tablets, and glass beads (diameter of around 75 μm) were purchased from Sigma-Aldrich, Chile. 96-well flat-bottom microtiter plates (Nunc) were purchased from Thermo Scientific, Chile. The solvents and chemicals were analytical or HPLC grade and used without further purification if not otherwise specified.

Methods

Preparation of microcystin-LR (MC-LR) imprinted nanoMIPs by solid phase synthesis

The protocol for the solid-phase synthesis was adopted from Canfarotta *et al.* [31] with some modifications, as is detailed below.

Activation and immobilisation of microcystin-LR on the solid phase

The silica or glass microspheres (30 g) were activated by boiling them in a sodium hydroxide solution (4 M) for 15 min. After that, the glass microspheres ($\text{SiO}_2\text{-OH}$) were washed with deionised water (3 times) and placed in a sulfuric acid: water solution (50 %:50 %) for 1 h. Afterwards, the glass microspheres were washed several times with water, the pH neutralised with PBS, and finally washed with acetone (2 times). Following a drying process at 150 $^{\circ}\text{C}$, the solid-phase was placed in a bottle with APTES (3 %) and 1, 2-Bis (triethoxysilyl) ethane (0.12 %) in dry toluene, overnight at 70 $^{\circ}\text{C}$. Afterwards, the beads were washed with acetone (6 times), and dried at 150 $^{\circ}\text{C}$. The solid phase modification was confirmed by FTIR using a Nicolet Magna 550 spectrophotometer. For the infrared measurements, silica (SiO_2), silica treated with NaOH ($\text{SiO}_2\text{-OH}$) and silica functionalised with APTES ($\text{SiO}_2\text{-OH-APTES}$) were milled and mixed with potassium bromide.

The immobilisation of the MC-LR was carried out by mixing the silanised beads (bearing NH_2 groups) and the MC-LR previously activated with EDC/NHS. Briefly, the solid phase (30 g) was placed in a sealable bottle with 10 mL of phosphate buffer (100 mM) pH 7.4 at the same time, the activation of the carboxylic groups of the MC-LR was made with EDC/NHS. For this purpose, 1 mg of MC-LR was solubilized in 2 mL of MES (pH 5–6); next 1.90 mg of EDC and 1.70 mg of NHS were added. The system was incubated for 10 min at room temperature. The MC-LR activated was added to the glass bead solution and incubated for 4 h at room temperature. After the incubation, the beads were washed with distilled water 10 times.

Synthesis of MC-LR imprinted nanoMIPs

The polymerisation was carried out by mixing the MC-LR derivatized glass beads with the polymerisation mixture according to Table 1.

Preparation of the polymerisation mixture

ACA (24 mg), HEMA (15.6 mg) and BIS (2 mg) were mixed with 98 mL of water in a bottle (250 mL); next, 33 mg of TBAm were dissolved in 0.5 mL of ethanol and added to the bottle. In a separate vial, 22 μL of AAc were diluted to 1 mL with water, and then 400 μL of this solution was added to the main bottle. Afterwards, 5.8 mg of AMH were added to the previous solution. Next, the glass beads with immobilised MC-LR were added to the

Table 1: Molar amounts used in the polymerisation mixture for MC-LR imprinting.

Monomers	Acronyms	Amount (mmol)
Acrylamide	ACA	0.34
<i>N</i> - <i>tert</i> -butylacrylamide	TBA _m	0.26
Acrylic acid	AAc	0.13
2-hydroxyethyl methacrylate	HEMA	0.062
<i>N</i> -(3-aminopropyl) methacrylamide hydrochloride	AMH	0.033
<i>N,N'</i> -Methylenebisacrylamide	BIS	0.026
Ammonium persulfate	APS	0.13
<i>N,N,N',N'</i> -Tetramethylethylenediamine	TEMED	0.33

polymerisation mixture. After that, 30 mg of APS were diluted in 500 μ L of water and 24 μ L of TEMED were added. Subsequently, the polymerisation was initiated by adding the initiator to the polymerisation mixture. The mixture was allowed to proceed for 1.5 h at 20 °C.

Collection of the nanoMIPs

After the polymerisation, all the content from the polymerisation mixture was transferred into a SPE cartridge, and then washed with 30 mL of cold water (4 °C, 8 times). Next, the high-affinity MIPs were eluted with hot water (60 °C), until a final volume of 100 mL was collected.

Characterisation of MC-LR imprinted nanoMIPs

The concentration of the collected high-affinity nanoMIPs was determined spectrophotometrically. The average size of the nanoMIPs, polydispersity and morphology were examined using Dynamic Light Scattering (DLS, 90Plus particle size analyser) and transmission electron microscopy (TEM, JEOL/JEM 1200 EX II).

Immobilisation of nanoMIPs in microtiter plate

The nanoparticles were deposited via physical absorption. For this purpose, 40 μ L of the nanoMIPs solution (0.06 mg mL⁻¹) were placed to each well in microtiter plate. Finally, the microtiter plate was left overnight at room temperature until complete evaporation of the water.

Preparation of the MC-LR labeled with horseradish peroxidase (HRP-MCLR) and blocking conditions

The conjugate (HRP-MCLR) was prepared by mixing the activated MC-LR in excess (3.013×10^{17} molecules) with HRP (2.665×10^{16} molecules), to ensure that each molecule of HRP react with at least one MC-LR molecule. In particular, the activated MC-LR was obtained by mixing a solution of 0.5 mg mL⁻¹ of MC-LR in 0.1 M MES buffer (pH 6), EDC (1.9 mg) and NHS (1.7 mg), and this reaction was carried out for 10 min at room temperature. After that, the activated MC-LR was mixed with a solution of HRP (1.5 mg mL⁻¹) in PBS (pH 7.4), and incubated for 2 h at 4 °C. To remove unreacted reagents, the solution was washed 5 times using 10 mL of PBS by using an Amicon Ultracentrifugal filter unit (30 kDa MWCO). Afterwards, the content was diluted with 2 mL of deionised water and stored at -18 °C until further use.

After immobilisation of the nanoMIPs and preparation of the conjugate (HRP-MCLR), blocking of the free surface in the well was performed. For this purpose, four blocking solutions were tested, BS₁: 0.2 % NFDM, 1 % Tween 20; BS₂: 2 % NFDM, 1 % Tween 20; BS₃: 0.1 % BSA, 1 % Tween 20 and BS₄: 0.3 % BSA, 1 % Tween 20.

In addition, in this assay the optimum dilution of the conjugate was investigated. For that reason, four dilutions of the conjugate were prepared (from 1:50 to 1:400) and their absorbance evaluated. The highest relation of absorbance between the signal of the wells with nanoMIPs and empty wells was selected as optimum dilution and blocking conditions to perform the competitive pseudo-ELISA.

Competitive pseudo-ELISA for microcystin-LR detection

After immobilising the nanoMIPs in microtiter plates, selection of blocking conditions and dilution of the conjugate, the wells were conditioned with 250 μL of PBS twice. After that, the wells were blocked with 300 μL of a selected blocking solution for 2 h. Afterward, the wells were washed with PBS ($3 \times 250 \mu\text{L}$). Then 100 μL of sample (concentrations of conjugate or problem sample) were added and incubated for 1 h at room temperature in the dark. Next, the microtiter plate was washed with the blocking solution, three times (300 μL). After that, 50 μL of TMB reagent were added and incubated for 10 min. The enzymatic reaction was stopped by adding 50 μL of sulfuric acid 0.5 M. Finally, the absorbance was measured at 450 nm using an Epoch 2 microplate spectrophotometer. In addition, to confirm the specificity of the nanoMIPs, cross-reactivity was assessed using microcystin-YR (MC-YR) as free template in the competitive assay.

Analysis of MC-LR in real samples

Real samples were spiked and analysed with the developed competitive pseudo-ELISA. For this purpose, water sample from a lake (Las Tres Pascualas, Concepción, Chile) was collected in amber glass bottle and filtered through 0.45- μm PVDF membrane. Moreover, drinking water was analysed. In all cases, the samples were stored at 8 °C and analysed within 24 h. The real samples were spiked with 1 nM and 10 nM of MC-LR, finally the concentration and recovery were determined using the calibration curve obtained from the standards.

Results and discussion

Synthesis and characterization of MC-LR imprinted nanoMIPs

The nanoMIPs were produced by solid phase synthesis, according to Canfarotta *et al.* [31]. Initially, the surface of the silica (solid phase) was modified using APTES, which converts the surface of the solid-phase such that terminal amino groups are exposed and available for the following immobilisation of the MC-LR. The FTIR spectra of silica (SiO_2), silica treated with NaOH ($\text{SiO}_2\text{-OH}$) and silica functionalised with APTES ($\text{SiO}_2\text{-APTES}$) are shown in Fig. 2. Among the main differences of the spectra is the widening of the band at 3449 cm^{-1} for $\text{SiO}_2\text{-OH}$ and $\text{SiO}_2\text{-APTES}$, which are attributed to O–H and N–H bond stretching vibrations, respectively. For $\text{SiO}_2\text{-APTES}$, two bands appear at 2919 and 2857 cm^{-1} , which are attributed to the asymmetric and symmetric stretching vibration of methylene groups. Finally, all the spectra show two wide bands around 1043 and 774 cm^{-1} , which are attributed to the asymmetrical and symmetrical stretching vibrations of the Si–O bond, respectively. In this way, the modification of the SiO_2 microspheres with APTES is demonstrated [34].

After functionalisation of solid phase with APTES, the MC-LR (template) was covalently immobilised onto the solid phase, allowing the coupling between carboxyl groups to primary amine groups on the glass beads.

Once the template is immobilised, the glass microspheres are placed in contact with the monomer mixture. After polymerization, nanoMIPs are produced around the template (Fig. 3). Then, the glass beads act as an affinity support to isolate the high-affinity nanoMIPs from the remaining monomers and low-affinity polymers, which are removed by washing the beads with cold distilled water at 4 °C. Subsequently, increasing

Fig. 2: The FTIR of (a) SiO_2 (solid phase), (b) solid phase treated with NaOH ($\text{SiO}_2\text{-OH}$) and (c) solid phase functionalised with APTES ($\text{SiO}_2\text{-OH-APTES}$).

the temperature of the solvent (60°C) breaks the interaction nanoMIPs-template allowing the elution of the high affinity nanoMIPs, suitable for its use as capture antibody in competitive pseudo-ELISA for the detection MC-LR.

The particle size distribution was determined by DLS, which revealed an average hydrodynamic size in water of 247 ± 6.1 nm, which was also confirmed by TEM (Fig. 4).

Optimisation of the conjugate concentration (HRP-MCLR) and blocking conditions

Pseudo-ELISA was performed according to Chianella *et al.* [22], and the steps of the assay are shown in Table 2. The values obtained from the absorbance ratio of the assay with nanoMIPs/empty well for conjugate dilutions using different blocking solutions are shown in Table 3. High values of this absorbance ratio indicate that the conjugate (HRP-MCLR) interacts specifically with the nanoMIPs. In this sense, in order to minimize non-specific interactions, the objective of this optimisation was to obtain the highest absorbance ratios in the assay with nanoMIPs/empty well. Therefore, the blocking buffer that allows obtaining highest values of absorbance ratio (nanoMIPs/empty well) is the BS_3 , for that reason, BS_3 was the optimum blocking solution for the following competitive assays. On the other hand, the optimum conjugate dilution was selected

Fig. 3: Solid phase synthesis of nanoMIPs for MC-LR detection. (a) Covalent immobilisation of MC-LR on the solid phase, (b) Formation of the nanoMIPs around of the template, (c) removal of low-affinity nanoMIPs and unreacted monomers, (d) Elution of high-affinity nanoMIPs suitable for pseudo-ELISA.

Fig. 4: Representative TEM images of MC-LR imprinted nanoMIPs.

Table 2: Steps involved in the optimum assay for MC-LR detection using nanoMIPs as synthetic antibodies.

Steps: pseudo-ELISA assay for MC-LR detection		
1	NanoMIPs immobilisation	2.4 μg per well
2	Washing	Wash buffer: 0.01 M PBS pH 7.4 (2 times × 250 μL)
3	Incubation with blocking solution	300 μL, 2 h: Blocking solution
4	Washing	Wash buffer: 0.01 M PBS pH 7.4 (3 times × 250 μL)
5	Conjugate and free target addition	Conjugate: 1:100, Free target molecule dilutions: from 10 ⁻⁵ to 100 nM (100 μL)
6	Washing	Blocking solution (3 times × 300 μL)
7	TMB (substrate)	50 μL by 10 min
8	Stop solution	50 μL of H ₂ SO ₄ 0.05 M
9	Measurement of the signal	Absorbance at 450 nm

Table 3: Selection of the concentration of the conjugate and blocking conditions.

Blocking solution		Conjugate concentration	nanoMIPs/Empty well ^a
BS ₁	0.2 % NFDM 1 % Tween 20	1:50	2.31
		1:100	1.32
		1:200	1.34
		1:400	1.25
BS ₂	2 % NFDM, 1 % Tween 20	1:50	1.42
		1:100	1.08
		1:200	1.12
		1:400	1.09
BS ₃	0.1 % BSA, 1 % Tween 20	1:50	3.16
		1:100	4.16
		1:200	2.81
		1:400	2.86
BS ₄	0.3 % BSA, 1 % Tween 20	1:50	2.26
		1:100	2.66
		1:200	2.34
		1:400	2.04

^aAbsorbance ratio: nanoMIPs/empty wells.

from the highest absorbance ratio obtained from the dilutions (Fig. 5). The optimum conjugate concentration was 1:100, due to the highest absorbance ratio was presented using this conjugate concentration generating the greatest colour difference developed between well with nanoMIPs and empty wells. For that reason, the conjugate dilution 1:100 was selected as optimum concentration of the HRP-MCLR for development the following competitive pseudo-ELISAs.

Competitive pseudo-ELISA for MC-LR detection

The competitive pseudo-ELISA was developed for the detection of MC-LR with optimal blocking conditions (0.1% BSA, 1% Tween 20 in PBS) and conjugate concentration (HRP-MCLR, 1:100). Initially, the nanoMIPs were deposited in the microtiter plate via physical adsorption through interactions hydrophobic, ionic, Van

Fig. 5: Comparison of the signal detected of the conjugate dilutions (HRP-MCLR) blocking the wells with 0.1 % BSA, 1 % Tween 20 in PBS.

der Waals forces and hydrogen bonds that occur between the wells of the polystyrene microplate and residual functional groups of the nanoMIPs and, after that, the wells were conditioned with PBS. Subsequently, to prevent the non-specific binding, the blocking solution was added and incubated for 2 h [21]. Then, the competitive binding assay between free MC-LR and the competitor HRP-MCLR (conjugate) was carried out by addition of the standards between 0.00001 nM and 100 nM and optimal dilution of the conjugate (1:100) to each well. Next, each well in microtiter plate was washed with PBS, and the substrate reagent was added producing a colour change. Finally, sulfuric acid was added and the absorbance was measured immediately at 450 nm. The competitive pseudo-ELISA is illustrated in Fig. 6.

The results obtained from competitive assay plotted in Fig. 7 showed a linear response for free MC-LR from 10^{-5} to 100 nM in the presence of the competitor (HRP-MCLR). These results show a competition between free MC-LR and HRP-MCLR towards the nanoMIPs cavity, detecting low concentrations of MC-LR in the assay. The competitive pseudo-ELISA presented a LOD of 2.6×10^{-4} nM, demonstrating high sensibility towards MC-LR. Besides, the MC-LR did not show any response in the presence of nanoNIPs (non-imprinted nanoparticles) and in empty wells, which confirms the specific binding of the nanoMIPs to the target molecule. The high signal at high concentration of MC-LR is due to the time the reaction and non-specific binding. For that reason, it is recommend doing a deeper study of the optimization of blocking conditions and reaction time in the last step of the ELISA.

Furthermore, the cross-reactivity or specify of the MC-LR imprinted nanoMIPs in the presence of another cyanobacterial toxin, microcystin-YR, was evaluated. The results plotted in Fig. 8 demonstrate that MC-LR imprinted nanoMIPs do not present any affinity to MC-YR in the same range of concentrations. According to the results shown, the pseudo-ELISA did not present a cross reactivity in presence of this analogous toxin. This allows to conclude that the assay has a high selectivity towards the MC-LR.

Analysis of MC-LR in water samples

The global climate change is leading to an increase the production of harmful algae, especially cyanobacteria blooms [35]. Hence, the present assay can be very useful in the detection of toxins in lakes. For this reason,

Fig. 6: Scheme of the competitive pseudo-ELISA base on nanoMIPs for the detection of MC-LR. (a) NanoMIPs immobilised, (b) solution of free and conjugate analyte, (c) addition of free (MC-LR) and conjugate target (HRP-MCLR, 1:100), (d) addition of 3, 3', 5, 5'-tetramethylbenzidine, (e) addition of stop solution (sulfuric acid).

Fig. 7: Calibration plot determined for competitive pseudo-ELISA for the detection of MC-LR in presence of nanoMIPs (0.06 mg mL^{-1}) and nanoNIPs and in empty wells (without polymeric nanoparticles). Measurements were performed in triplicates.

Fig. 8: Calibration plot of competitive pseudo-ELISA base on nanoMIPs for microcystin-YR (MC-YR). Assay conditions: concentration of nanoMIPs was 0.06 mg mL^{-1} , HRP-MCLR conjugate dilution was 1:100, standard solution concentrations of MC-YR from 10^{-5} nM to 100 nM . Measurements were performed in triplicates.

samples of water (drinking and lake water) were spiked with different concentrations of MC-LR and then analysed with the proposed assay. The lake water was obtained from Las Tres Pascualas Lake in Concepción, Chile ($36^{\circ}48'50.9''\text{S}$ $73^{\circ}02'46.6''\text{W}$). The sample from the lake was filtered through a $0.45 \mu\text{m}$ membrane, spiked and analysed within 24 h. The drinking water sample was analysed directly. Table 4 shows the results of the added and found concentrations of MC-LR by the competitive pseudo-ELISA at two levels of concentrations, showing an excellent recovery of the spiked samples. These results demonstrate the potential of this ELISA in the detection of MC-LR in real water samples.

Table 4: MC-LR recovery values from spiked water sample by competitive pseudo-ELISA.

Water sample	MC-LR spiked, nM	MC-LR found by pseudo-ELISA, nM	Recovery (%) ^a
Drinking water	1.0	1.05 ± 0.2	105
	10	9.60 ± 0.2	96
Lake water	1.0	1.18 ± 0.05	118
	10	13.0 ± 0.1	130

^aNumber of replicates ($n=6$).

Conclusions

In this study, we developed a new assay for microcystin-LR detection in water, based on molecularly imprinted nanoparticles (nanoMIPs), which are used as detection tools in place of the typical natural antibodies. The nanoMIPs were obtained via solid phase synthesis, which has the potential for being automated and hence suitable for large-scale production. The nanoMIPs herein developed exhibited very low cross-reactivity towards another toxin (MC-YR), and excellent results were obtained also in real samples, thus confirming that these “plastic antibodies” hold great potential for diagnostic uses. It is worth mentioning that, being made of synthetic polymers, nanoMIPs are capable of withstanding exposure to high temperature for prolonged periods without affecting their recognition properties [22, 36]. This feature allows the nanoMIPs (and related MIP-based assays) to be stored at room temperature, without the need of any refrigeration. This can be very cost-efficient, as it makes nanoMIPs viable for use in more remote geographical areas where healthcare infrastructure or cold chain availability might be limited. Moreover, the generic nature of the synthetic approach employed in the preparation of nanoMIPs allows for the manufacture of many other nanoMIP types.

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