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**Molecularly Imprinted Protein Recognition Thin Films Constructed by
Controlled/Living Radical Polymerization**

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Running title: Protein imprinting by ATRP

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Abstract

We demonstrated the synthesis of molecularly imprinted polymers (MIPs) with binding affinity toward a target protein, ribonuclease A (RNase) by atom transfer radical polymerization (ATRP) of acrylic acid, acrylamide, and *N,N'*-methylenebisacrylamide in the presence of RNase. The binding activity of the MIPs was evaluated by surface plasmon resonance (SPR) of the MIP thin layers prepared on the gold-coated sensor chips. The MIPs prepared by ATRP (MIP-ATRP) had a binding affinity toward RNase with larger binding amount compared to MIPs prepared by conventional free radical polymerization methods (MIP-RP). Moreover, protein selectivity was evaluated using reference proteins (cytochrome c, myoglobin, and α -lactalbumin) and was confirmed in MIP-ATRP of optimum film thickness determined experimentally to be 15-30 nm; however, protein selectivity was not achieved in all MIP-RP. We have shown that ATRP is powerful technique for preparing protein recognition materials by molecular imprinting.

1   **Introduction**

2   Molecular recognition materials for proteins are of great importance in the field of life science,
3   especially for use in protein separation, proteomics and diagnosis of cancers and other intractable
4   diseases (1). Currently, biomolecules such as enzymes, antibodies, and **aptamers** are used as
5   molecular recognition materials for proteins. Although highly selective binding activity can be
6   achieved, such biomolecules are difficult to control in production as well as being unstable in harsh
7   conditions and easily denatured. Polymer-based artificial molecular receptors serve as a more stable
8   and versatile alternative for application to explore protein-ligand interaction in biological systems.

9   Molecular imprinting, a template polymerization technique, is a powerful method for preparing
10   artificial receptors bearing predetermined binding cavities capable of recognizing target molecules.
11   Molecularly imprinted polymers (MIPs) are obtained by the co-polymerization of functional
12   monomers and crosslinkers in the presence of a target molecule or its derivative (template
13   molecule) (2-7). When proteins are selected as the template molecules for MIPs, the
14   complementary binding cavities left in the polymer matrices after removal of the templates are
15   capable of selective protein binding according to size and chemical properties, depending upon the
16   functional monomers used (6). In other words, the conformation of complexes formed between
17   the template proteins and functional monomer(s) during the polymerization step of preparing MIPs
18   is critical for the development of highly selective binding activity with strong affinity for the target
19   protein. **Previously, conventional free radical polymerization (RP) was employed for the**

1 preparation of MIPs for protein recognition; however, this method had several problems i.e.
2 formation of heterogeneous cross-linking matrix, difficulty of the film thickness control. (8-9)

3 Controlled/living radical polymerization (CLRP) is a powerful technique for the synthesis of
4 well-defined polymers with narrow molecular weight distribution or complex molecular
5 architectures such as block or star polymers (10-13). Many kinds of CLRP techniques have been
6 discovered and developed, but atom transfer radical polymerization (ATRP) is one of most studied
7 and highly developed of these techniques, involving radical species that are reversibly generated by
8 the redox reaction of copper ions. It is known that CLRP can be used to synthesize more
9 homogeneous cross-linking materials compared to those synthesized via RP (8, 14). Accordingly,
10 CLRP was applied to synthesize MIPs in order to improve the uniformity of molecular recognition
11 sites as well as recognition ability.

12 To date, several methods for the preparation of MIPs by CLRP have been reported. For
13 example, Ye et al. reported that MIPs prepared by nitroxide-mediated living radical polymerization
14 displayed more highly selective cholesterol binding compared to those prepared by RP (15). In
15 our previous work, we also tried using reverse ATRP with CuCl_2 and a thermal initiator in organic
16 solvents to synthesize the MIPs containing molecular recognition site for bisphenol A (BPA), which
17 is believed to be an endocrine disruptor that binds to estrogen receptors (16). It was discovered
18 that the MIPs obtained by ATRP had high recognition ability toward BPA compared to those
19 obtained by conventional free radical polymerization. Moreover, very recently Byrne et al.

1 investigated the role of CLRP in the formation of imprinted polymers in detail as well as elucidated that
2 binding activity was clearly increased when using CLRP in place of RP methods (17). MIPs for
3 protein recognition were also prepared by CLRP. Gai and coworkers prepared protein-imprinted
4 MIP by ATRP for protein separation (18). However, the effectiveness of the CLRP on the protein
5 recognition ability has not been discussed compared to RP.

6 In this study, we synthesized MIPs toward ribonuclease A (RNase) by activators generated by
7 electron transfer (AGET) ATRP (19, 20) (henceforth referred to as MIP-ATRP) in an aqueous
8 solution. In order to prepare MIPs bearing selective binding cavities, acrylic acid, which is often
9 used as a functional monomer for basic compounds, was introduced into a solution containing
10 RNase to form complexes with the protein molecules via electrostatic interactions and/or hydrogen
11 bonding. After the addition of a crosslinker, *N,N'*-methylenebisacrylamide (MBAA), AGET ATRP
12 of these monomers were carried out from 2-bromo-2-methylpropionic acid (ATRP
13 initiator)-immobilized gold surfaces of either a quartz crystal microbalance (QCM) or surface
14 plasmon resonance (SPR) sensor chips. QCM measurements were performed to estimate the
15 thickness of the MIP films, and the binding activity of the MIPs was evaluated by SPR
16 measurements. In order to compare the ATRP-based method with conventional radical
17 polymerization, RP-based MIPs (MIP-RP) were prepared on 2,2'-azobis(2-methylpropionamidine)
18 (V-50)-immobilized substrates, and the comparative effectiveness of AGET ATRP for the
19 formation of selective binding cavities in MIP thin films was discussed.

1   **Material and Methods**

2   *Materials*

3   RNase, ascorbic acid, CuBr₂, dichloromethane (CH₂Cl₂), 4,4'-dimethylaminopyridine (DMAP),
4   tetra-sodium ethylenediamine tetraacetate (EDTA-4Na), sodium chloride (NaCl), sodium hydroxide
5   (NaOH), ethanol, methanol, citric acid, 4-(2-hydroxyethyl)-1-piperazineethanesulfonic acid
6   (HEPES) were purchased from Nacalai Tesque Co. Ltd (Kyoto, Japan). Acrylic acid, V-50, MBAA,
7   acryl amide were purchased from Wako Pure Chemical Industries, Ltd (Osaka, Japan).
8   *N,N,N',N'',N'''*-Pentamethyldiethylenetriamine (PMDETA), N-hydroxy succinimide (NHS),
9   11-mercapto-1-undecanol, 11-mercapto-1-undecanoic acid, cytochrome c, myoglobin,
10   α-lactalbumin were purchased from Sigma-Aldrich (MO, USA).
11   1-Ethyl-3-(3-dimethylaminopropyl)carbodiimide hydrochloride (EDC) was purchased from Peptide
12   Institute Inc. (Osaka, Japan) and 2-bromo-2-methylpropionic acid was purchased from Tokyo
13   Chemical Industry (Tokyo, Japan). CH₂Cl₂ was purified by distillation prior to use in order to
14   remove water. V-50 was used without further purification.

15

16   *Preparation of MIP thin films by AGET ATRP (MIP-ATRP)*

17   The immobilization of ATRP initiator on QCM chips (QCA-A9M-AU (P); SEIKO EG&G Co.
18   Ltd) were carried out as follows: After the QCM chips were cleaned by a UV-O₃ cleaner
19   (Bioforce nanosciences) for 20 min, the QCM chips were dipped in 11-mercapto-1-undecanol (1.05

1 mg, 5 μ mol) in ethanol (1mL) for 1 h. The QCM chips were washed by ethanol and dried in
2 vacuo. The QCM chips were then dipped in 2-bromo-2-methylpropionicacid (3.26 mg, 20 μ mol),
3 DMAP (3.66 mg, 35 μ mol) and EDC (5.66 mg, 35 μ mol) in CH_2Cl_2 (1 mL) for 2 h, and the QCM
4 chips were washed by CH_2Cl_2 and dried in vacuo. Frequency of the QCM chips was measured by
5 using Quartz Crystal Analyzer QCA 917 (SEIKO EG&G Co. Ltd., Tokyo, Japan). The
6 concentration of 2-bromo-2-methylpropionicacid was changed from 0.2 μ mol to 20 μ mol.

7 MIP thin films were prepared by AGET ATRP as follows: RNase (13.4 mg, (1 μ mol)), acryl
8 amide (0.27 mg, 3.75 μ mol), acrylic acid (0.26 mg, 3.75 μ mol), MBAA (7.7 mg, 50 μ mol), CuBr_2
9 (0.94 mg, 4 μ mol), PMDETA (1.38 mg, 8 μ mol) were dissolved in a HEPES-NaOH buffer (pH 7.4)
10 in a polystyrene (PS) container. The QCM chips were set in a teflon cell (dip type) and dipped in
11 the PS container. As soon as ascorbic acid (3.2 mg, 12 μ mol) was added in the PS container, it
12 was sealed by a septum. After degassing the solution by vacuum/nitrogen cycles, the PS container
13 was set in thermostat bath at 40 $^\circ\text{C}$. After quenching polymerization at appropriate polymerization
14 time by air, the QCM chips were dipped in 1 M NaCl aqueous solution (NaCl aq.) for 1 h to remove
15 RNase. In order to remove the copper ions, the QCM chips were subsequently dipped in 1 M
16 EDTA-4Na aqueous solution (EDTA-4Na aq.) for 24 h. The frequencies of QCM chips were
17 measured both after polymerization and washing with NaCl aq.. The film thickness was calculated
18 by the following formula:

19

$$\text{Film thickness (nm)} = \frac{\text{deposits of PMBAA (ng)}}{\text{density of PMBAA (} 1.23 \times 10^{-12} \text{ ng/nm}^3 \text{)} \times \text{surface area of quartz (} 0.196 \times 10^{14} \text{ nm}^2 \text{)}}$$

$$\text{※deposits of PMBAA (ng)} = \text{change of frequency (s}^{-1}\text{)} \times \text{surface area of quartz (0.196 cm}^2\text{)} \times \left(\frac{\sqrt{u \times \rho}}{2 \times F_0^2} \times \text{surface area of quartz} \right)$$

(F_0 ; fundamental frequency (9003304 s⁻¹), surface area; 0.196 cm²,
 u ; shear stress of quartz (2.947×10²⁰ ng/cm · s²), ρ ; density of quartz

Preparation of MIP thin films by RP (MIP-RPs)

The immobilization of initiator for conventional radical polymerization on the QCM chips were carried out as follow. The QCM chips were cleaned by UV-O₃ treatment for 20 min and dipped in ethanol solution (1 mL) containing 11-mercapto-1-undecanoic acid (1.05 mg, 5 μmol) for 1 h. After washing the QCM chips by ethanol and drying in vacuo, the QCM chips were dipped in ethanol solution (1 mL) containing NHS (1.98 mg, 20 μmol) and EDC (3.26 mg, 10 μmol) for 1 h. The QCM chips were washed by ethanol and dried in vacuo, and the QCM chips were dipped in methanol solution (1 mL) of V-50 (2.7 mg, 10 μmol).(21) The QCM chips were washed by methanol and dried in vacuo. The frequency of the QCM chips was measured at each stage of modification.

The MIP-RPs were prepared by conventional radical polymerization as follows: RNase (13.4 mg, (1 μmol)), acryl amide (0.27 mg, 3.75 μmol), acrylic acid (0.26 mg, 3.75 μmol), MBAA (7.7 mg, 50 μmol) were dissolved in a HEPES-NaOH buffer (pH 7.4) in the PS container. Then, the QCM chips were set in Teflon cell (dip type) and dipped in the PS container. After sealing the PS container by septum and degassing the solution by vacuum/nitrogen cycles, the PS container was set in thermostat bath at 50 °C. After quenching the polymerization at appropriate polymerization

1 times by washing with pure water, the QCM chips were dipped in 1 M NaCl aq. for 1 h to remove
2 RNase. The frequency of the QCM chips was measured after the polymerization and the NaCl aq.
3 treatment, respectively, and the film thickness was calculated. Surface morphology of the QCM
4 chips after washing treatments was examined by Scanning Electron Microscopy (VE-9800,
5 KEYENCE Co. Ltd).

6

7 *Preparation of MIP thin films by AGET ATRP on SPR chips*

8 Immobilization of the ATRP initiator on SPR chips (SIA Kit Au (Biacore 3000, Biacore Co. Ltd))
9 were carried out under the same conditions as the QCM chips. A non-imprinted polymer film
10 (NIP-ATRP) was also prepared by the above-mentioned procedure without RNase.

11

12 *Preparation of MIP thin films by RP on SPR chips*

13 Immobilization of the initiator for conventional radical polymerization on SPR chips were carried
14 out under the same conditions as the QCM chips. A non-imprinted polymer film (NIP-RP) was
15 also prepared by the above-mentioned procedure without RNase.

16

17 *SPR measurements*

18 Binding experiments for MIP-ATRP, MIP-RP, NIP-ATRP and NIP-RP were performed by
19 using a surface plasmon resonance sensing system (BIACORE 3000, Biacore AB, Sweden). Four

1 kinds of different proteins (RNase, cytochrome c, myoglobin, and α -lactalbumin) were dissolved in
2 a HEPES–NaOH buffer (pH 7.4) at 25 °C. The conditions of SPR measurements were as follows:
3 running buffer; 10 mM HEPES–NaOH buffer (pH 7.4, 20 μ L/min), injection volume; 20 μ L,
4 regeneration solution; 10 mM HEPES–NaOH buffer containing 1M NaCl and 5 mM NaCl aq..
5 The amounts of bound proteins were calculated by measuring the signal intensity at 120 s after the
6 end of each sample introduction period. Protein concentrations were varied from 0.25 to 5.0 μ M.
7 Binding isotherms were drawn by using the Δ RU at each protein concentration. The binding
8 constants and the number of binding sites were calculated by Scatchard plots from the obtained
9 binding isotherms.

10

11 *Surface characterization using Scanning Electron Microscopy (SEM)*

12 Surface morphology of the obtained QCM chips was examined by Scanning Electron Microscopy
13 (VE-9800, KEYENCE Co. Ltd).

14

15 **Results and Discussion**

16 Fig. 1(a) shows MIP-ATRP film thicknesses at different AGET ATRP polymerization times on
17 QCM chips, where a self-assembled monolayer (SAM) of 11-mercapto-1-undecanol was formed on
18 QCM chips via strong chemical binding between the gold surface and thiol groups, and the hydroxy
19 end groups were coupled with various concentrations (0.02, 2.0, and 20 mM) of

1 2-bromo-2-methylpropionic acid in the presence of EDC and **DMAP**. Film thickness increased
2 linearly with polymerization time on all QCM chips for each different concentration of
3 2-bromo-2-methylpropionic acid, indicating that the “living” nature was maintained throughout
4 polymerization. In addition, somewhat greater film thickness was obtained overall at higher
5 concentrations of 2-bromo-2-methylpropionic acid. According to these results, the film thickness
6 could be controlled not only by polymerization time but also by changing the concentration of
7 2-bromo-2-methylpropionic acid used for immobilization on the chip.

8 We also investigated the polymer film thickness at different polymerization times for RP, where
9 a SAM of 11-mercapto-1-undecanoic acid was formed onto QCM chips, followed by amine
10 coupling with 10 mM of V-50 in the presence of EDC and NHS **(21)**. The MIP-RP film thickness
11 increased up to approximately 50 nm after only 2 h and then gradually increased to 84 nm over 24 h
12 (Fig. 1b). SEM images of the MIP-RP films at each polymerization time supported the rapid
13 increase of the film thickness compared to the MIP-ATRP films. **(Supplementary data Fig. S1 and**
14 **Fig. S2)**.

15 (Fig. 1)

16 The differing behavior of polymer growth between MIP-ATRP and MIP-RP is the result of the
17 different polymerization mechanisms that take place in each method. In AGET ATRP, the polymer
18 chain length increases gradually with increase of conversion; on the other hand, in RP the polymer
19 chains propagate dramatically in just a few seconds. From these results, we therefore surmised

1 that the film thickness could be controlled precisely by applying AGET ATRP for different
2 polymerization times and the initiator density on the substrates, whereas this level of control would
3 be difficult in RP for film thicknesses under 50 nm.

4 In this study, RNase was noncovalently imprinted by AGET ATRP-based copolymerization of
5 acrylic acid, acrylamide, and MBAA as the functional monomer, the co-monomer, and the crosslinker,
6 respectively. Polymerization was followed by the removal of RNase by washing with 1M NaCl aq.
7 to form selective binding cavities, the successful formation of which was confirmed by the reduced
8 mass on the sensor chip (Fig. 2). Thus obtained MIP-ATRP thin films were subsequently washed
9 with EDTA-4Na aq. to remove copper ions; the red color associated with the presence of copper
10 complexes completely disappeared after the EDTA-4Na aq. treatment. Moreover, the mass on the
11 chip after washing with EDTA-4Na aq. clearly decreased (Fig. 2), indicating that the template RNase
12 and copper ions were successfully removed from the MIP-ATRP thin films.

13 (Fig. 2)

14 In order to investigate the effect of ATRP on binding activity, binding experiments on MIP-ATRP
15 and MIP-RP were performed by SPR sensors, where MIP-ATRP and MIP-RP were prepared on
16 SPR sensor chips in the same manner with that on QCM chips. When RNase was injected on each
17 chip in a concentration dependent manner, Δ RU values on the MIP-ATRP chip were found to be
18 larger than those on the MIP-RP (Fig. 3), indicating that the binding activity of the MIP thin films
19 toward RNase was improved when AGET ATRP was used as the polymerization method (61

1 fmol/g) instead of RP (45 fmol/g), however the binding constants were similar in those cases ($6.9 \times$
2 10^5 M^{-1} for MIP-ATRP and $7.0 \times 10^5 \text{ M}^{-1}$ for MIP-RP).

3 (Fig. 3)

4 Selectivity of MIP-ATRP was examined by measuring SPR response for RNase (14 kDa, $pI =$
5 8.6), cytochrome c (Cyt; 12 kDa, $pI = 10.1$), myoglobin (Myo; 18 kDa, $pI = 8.2$), and α -lactalbumin
6 (Lac; 14 kDa, $pI = 4.5$). When thin films were prepared on the SPR chips with polymerization times
7 of 3 h, 4.5 h, and 6 h, and a 2-bromo-2methylpropionic acid concentration of 0.02 mM
8 (MIP-ATRP-002), the binding activities of Myo and Lac were small and negligible compared with
9 those of Cyt and RNase (Fig. 4). Because acrylic acid was used as the functional monomer, Lac,
10 an acidic protein, could not interact with MIP-ATRP due to electrostatic repulsion. Myo is a weak
11 basic protein, therefore weak interactions may have occurred, but they were not strong enough to
12 bind Myo tightly. MIP-ATRP prepared with the polymerization time of 3 h showed weak binding
13 to Cyt and RNase (Fig. 4a), suggesting that the binding cavities may not have been completely
14 formed. However, the binding activity of Cyt and RNase significantly increased with
15 polymerization time, *i.e.* the number of active binding cavities increased with film thickness. The
16 binding activity of RNase was larger than that of Cyt when MIP-ATRP was prepared with the
17 polymerization time of 4.5 h (Fig. 4b). In the case of 6 h polymerization, the binding activity of
18 RNase was smaller than that of Cyt (Fig. 4c). This may be due to an increase in non-specific
19 binding to the polymer matrix. These results suggest that under the conditions of 0.02 mM

1 2-bromo-2methylpropionic acid and an AGET ATRP polymerization time of 4.5 h (resulting in a
2 film thickness of approximately 20 nm), larger binding amounts toward RNase among the proteins
3 was observed.

4 (Fig. 4)

5 We also investigated the protein selectivity of MIP-ATRP toward RNase using SPR chips
6 prepared with a higher concentration of 2-bromo-2-methyl propionic acid (20 mM) (MIP-ATRP-20).
7 The protein selectivity of MIP-ATRP prepared with 3 h, 6 h and 12 h polymerization times were
8 shown in Fig. 5. As was the case with MIP-ATRP-0.02, the binding selectivity for both Myo and
9 Lac was small and negligible. Interestingly, binding selectivity for RNase was only observed
10 when the polymerization time was 3 h, where the film thickness was estimated to be approximately
11 30 nm (Fig. 5a). Because polymer chains propagate gradually in AGET ATRP with conversion,
12 these results imply that selective binding cavities toward RNase were formed in the early stages of
13 polymerization whereas non-specific binding sites seem to form in the later stages. Ergo, it is clear
14 that MIP-ATRP films must be the appropriate thickness in order to display the selectivity toward
15 RNase, and the present study indicates that this optimal thickness is 15 - 30 nm.

16 (Fig. 5)

17 The imprinting effect of MIP-ATRP was confirmed by the comparison with non-imprinted
18 polymer (NIP) thin film prepared by AGET ATRP in a similar manner in the absence of RNase.
19 The selective binding property toward RNase was not confirmed in NIP-ATRP, which was prepared

1 for 3 h polymerization on the SPR chip treated with 20 mM 2-bromo-2-methylpropionic acid
2 (Supplementary data Fig. S3). Similarly, when NIP-ATRP was prepared for 4.5 h polymerization
3 on SPR chip treated with 0.02 mM 2-bromo-2-methylpropionic acid, the selectivity toward RNase
4 was not observed (Supplementary data Fig. S4). These results indicated that the imprint cavities
5 were successfully formed during the imprinting process in MIP-ATRPs, and RNase was recognized
6 by the imprint cavities of the resultant MIP-ATRPs.

7 The selectivity of MIP-RP was also examined to confirm the effectiveness of AGET ATRP as a
8 preparation method of protein-imprinted MIPs (Fig. 6). As seen with MIP-ATRP, the binding
9 activities of Myo and Lac were low, as expected. However, MIP-RPs prepared with
10 polymerization times of 3h (Fig. 6a), 6 h (Fig. 6b) and 12 h (Fig. 6c) all consistently showed no
11 binding specificity for RNase, indicating that effective binding cavities were not formed in MIP-RP.
12 According to these results, ATRP was proven to be an effective technique to prepare
13 protein-imprinted MIPs.

14 (Fig. 6)
15

16 In conclusion, we demonstrated the preparation of MIPs bearing binding property toward for
17 RNase on initiator-immobilized gold surfaces by AGET ATRP of acrylic acid, acryl amide, and
18 MBAA in the presence of RNase. Binding properties were evaluated by SPR measurements, and
19 it was found that MIP-ATRP has affinity toward RNase compared to MIP-RP. Moreover, the
20 binding amount of target protein of MIP-ATRP was the highest at the optimum film thickness of

1 around 30 nm, which may be the result of the density gradation of RNase binding cavities in the
2 MIP-ATRP thin films, *i.e.* because the binding cavities formed at the early stages of ATRP
3 polymerization due to the adsorption of RNase-acrylic acid conjugates onto the
4 initiator-immobilized surface of SPR sensor chips. The results from this study suggested that the
5 parameters affecting the binding activity of MIPs are not limited to the selection of reactant species
6 (functional monomers, co-monomers, and crosslinkers) but also include polymer film thickness, an
7 attribute controllable in AGET ATRP by varying polymerization time and the density of the initiator
8 on the substrates. We expect that CLRP techniques will be applied more frequently in future
9 studies in order to prepare MIPs with high affinity and selectivity towards target proteins, and we
10 believe that the present study will prove valuable in the pursuit of this exciting direction in the field
11 of molecularly imprinted polymers.

12

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15

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Figure legends

Fig. 1. Film thickness of MIPs on QCM sensor chips versus time plots of (a) MIP-ATRP and (b) MIP-RP. 2-Bromo-2-methylpropionic acid concentration used for immobilization: 0.02 mM (open circle), 2 mM (open diamond) and 20 mM (open square); V-50 concentration for the immobilization: 10 mM (closed triangle).

Fig. 2 Mass of MIP-ATRP thin films on QCM sensor chips before (black) and after successive washing with NaCl (gray) and EDTA-4Naq (white). 2-Bromo-2-methylpropionic acid concentration for the immobilization: 20 mM; AGET ATRP reaction time: 24 h.

Fig. 3 Binding of RNase to MIP-ATRP (open circle) and MIP-RP (closed circle) on SPR sensor chips. 2-Bromo-2-methylpropionic acid concentration for immobilization: 0.02 mM; V-50 concentration for the immobilization: 10 mM; AGET ATRP reaction time: 4.5 h; RP reaction time: 12 h.

Fig. 4 Binding of RNase, Cyt, Myo, and Lac to MIP-ATRP prepared on SPR chips (0.02 mM 2-bromo-2-methylpropionic acid concentration; (a) 3 h, (b) 4.5 h, and (c) 6 h polymerization times). Protein concentration: 1 μ M.

Fig. 5 Binding of RNase, Cyt, Myo, and Lac on MIP-ATRP prepared on SPR chips (20 mM 2-bromo-2-methylpropionic acid concentration; (a) 3 h, (b) 6 h, and (c) 12 h polymerization times). Protein concentration: 1 μ M.

Fig. 6 Binding of RNase, Cyt, Myo, and Lac on MIP-RP prepared on SPR chips (10 mM V-50 concentration; 3 h, 6 h, and 12 h polymerization times). Protein concentration: 1 μ M.

Figure 1

Figure 2

Figure 3

Figure 4

Figure 5

Figure 6