# Real-Time Quantitative Monitoring of Specific Peptide Cleavage by a Proteinase for Cancer Diagnosis

**ARTICLE** *in* ANGEWANDTE CHEMIE INTERNATIONAL EDITION · JUNE 2012

Impact Factor: 11.26 · DOI: 10.1002/anie.201108830 · Source: PubMed

A Journal of the Gesellschaft Deutscher Chemiker

# Angewandte Chemie

International Edition

GDCh

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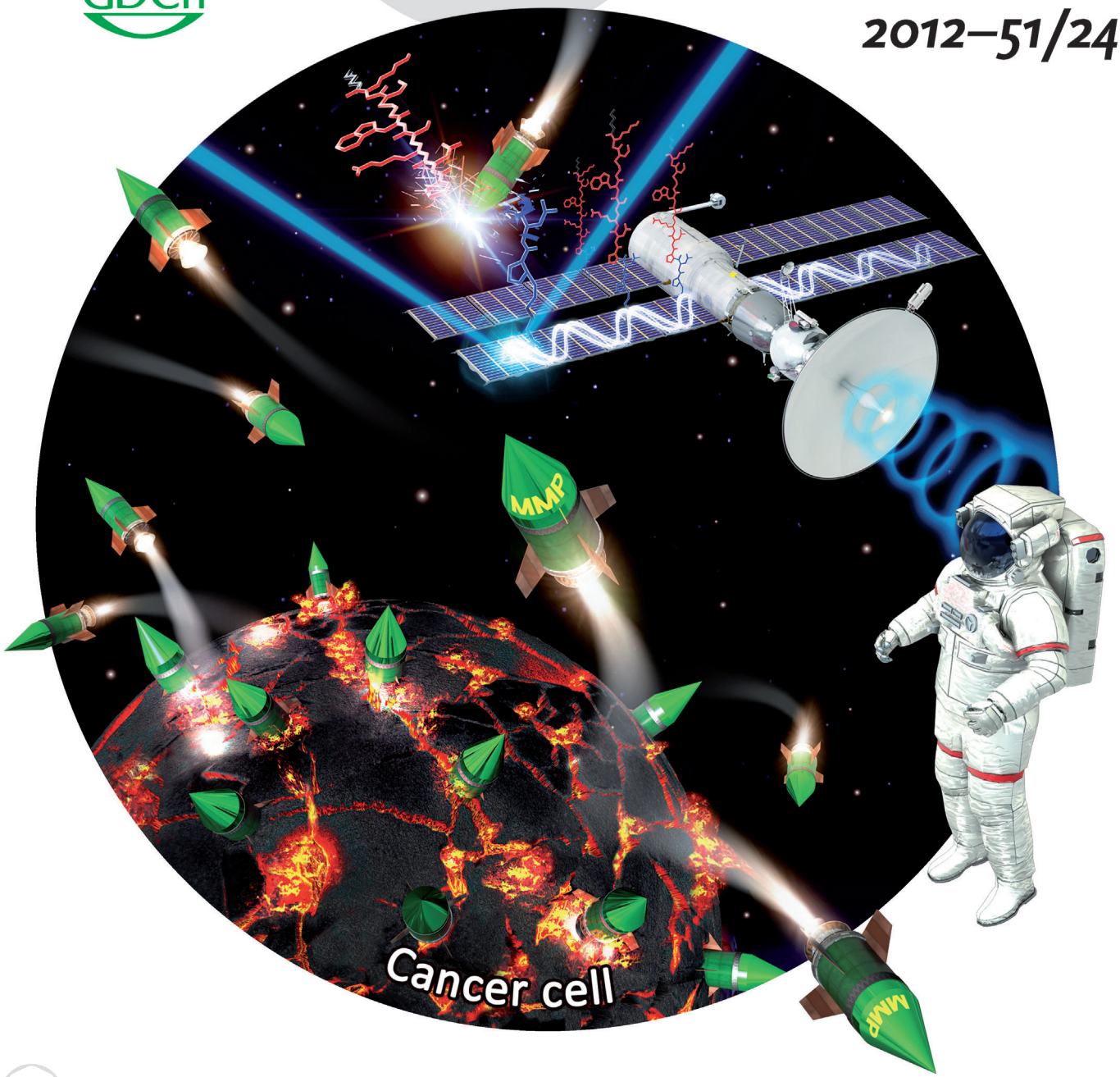
2012–51/24




## Active matrix metalloproteinases ...

… (MMPs; green missiles in the picture) expressed on a cancer cell surface can be sensed by a resonant cantilever device (satellite arm in the picture), as J. Yang, D. S. Yoon, T. Kwon et al. report in their Communication on page 5837 ff. Active MMPs attack the peptide sequence that is immobilized on the cantilever surface. The peptide cleavage leads to an increase in the resonant frequency of the cantilever, owing to a decrease in the mass of immobilized peptide.

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Biosensors

DOI: 10.1002/anie.201108830

# Real-Time Quantitative Monitoring of Specific Peptide Cleavage by a Proteinase for Cancer Diagnosis**

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The characterization of the proteolytic activity of proteases as a tumor biomarker is essential to the development of a prognostic model for cancer patients and to the detailed understanding of cancer cells in physiology and clinicopathology.[1] In particular, there have been recent efforts[2] to characterize the expression level of membrane type-1 matrix metalloproteinase (MT1-MMP) because of its functional role in cancer cell proliferation and metastasis.[2a,c,d] The mechanism of extracellular matrix (ECM) breakdown by MT1-MMP depends on the cell type; for instance, a tumor cell will experience a different ECM breakdown mechanism than a normal cell, and this difference is highly related to the metastasis[2d,3] and/or abnormal cell division in tumor cells.[4] This difference may be attributed not only to the expression level of MT1-MMP but also to the proteolytic activity of MT1-MMP that is expressed on the surface of an invasive cancer cell; in general, a cancerous cell exhibits overexpressed and highly activated MT1-MMPs on its surface.[5] This observation indicates that for an early diagnosis of cancers, it is essential to quantify the proteolytic activity as well as the acute expression level of MT1-MMP on the surfaces of invasive cancer cells.

One of the conventional assay tools that are able to characterize the expression level of proteinases is an immunoblot assay; however this assay does not give any insight of the kinetics of the proteolytic activity of MT1-MMP. To overcome this limitation, for a couple of decades, there have been attempts to characterize the proteolytic activity of matrix metalloproteinases (MMPs) by using fluorogenic nanosensors.[2e,6] However, because of photobleaching (or photoquenching) and/or light interference, a fluorogenic bioassay is not effective in deciphering the kinetics of MT1-MMP-driven proteolysis. This limitation indicates that the fluorogenic method is inappropriate for the quantification of proteolytic activities of MT1-MMPs on invasive cancer cells. Herein, we report a cantilever assay to decipher the kinetics of MT1-MMP activity for various invasive cancer cells. A cantilever bioassay enables not only highly sensitive detection but also quantitative depiction of biomolecular interactions based on mechanical signal transduction.[7] Cantilever-based label-free, real-time detection of the proteolytic activity of the MT1-MMP of invasive cancer cells can be a useful addition to conventional assays (e.g. immunoassay) and may lead to an early diagnosis of cancer at a molecular level. For specific measurements of the proteolytic activity, we functionalized a cantilever surface with a specific peptide sequence[8] that is able to selectively interact with MT1-MMP (Figure 1 a). Here, the peptide chains attached on a cantilever surface can be specifically snipped by MT1-MMP (Figure 1 b). The functionalization of a cantilever decreases its resonant frequency by $(9.4 \pm 2.8)$ kHz, because the attachment of molecules to a cantilever increases its mass (see Tables S1 and S2 in the Supporting Information). After functionalization of the cantilever, it was mounted on a liquid cell, into which the solution containing MT1-MMP was injected to observe the MT1-MMP-driven proteolysis. Our detection principle is based on the direct transduction of MT1-MMP-induced proteolysis into a shift in the resonant frequency of a cantilever owing to a decrease in the effective mass of a cantilever during proteolysis (Figure 1 c).

We have considered the dependence of MT1-MMP-driven peptide cleavage on MT1-MMP concentrations to validate our conjecture that MT1-MMP concentration (responsible for the invasion and metastasis for cancerous cells) may significantly affect the proteolysis efficacy. To confirm the specific interaction between MT1-MMP and peptide chains attached on a cantilever surface, we have

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[**] This work was supported by the National Research Foundation of Korea (NRF) (under Grant No. NRF-2010-0009428, and NRF-2011-0009885).

Supporting information for this article is available on the WWW under http://dx.doi.org/10.1002/anie.201108830.

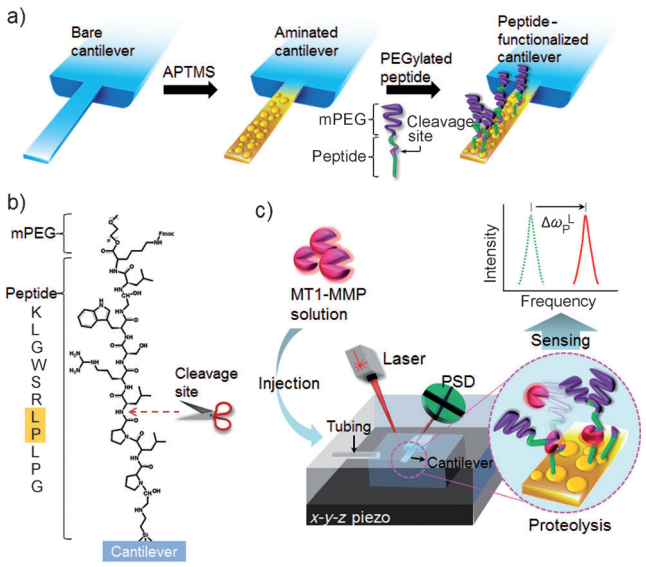


***Figure 1.*** a) Preparation of a peptide-functionalized cantilever for a bioassay. b) Chemical structure of peptide sequence, with cleavage site highlighted in yellow. c) Experimental setup and sensing mechanism. APTMS = 3-aminopropyltrimethoxysilane, mPEG = monomethyl poly(ethylene glycol), PSD = position-sensitive detector.

performed a few negative-control experiments (that validate specific interaction between MT1-MMP and the peptide sequence) as described in Figure S1 in the Supporting Information. Based on the frequency shift that is due to proteolysis, we have found that the proteolysis efficacy depends on the MT1-MMP concentration (Figure S2a in the Supporting Information); as the concentration of MT1-MMP increases, so does the proteolysis efficacy. Moreover, we have taken into account the in situ frequency shift measured in buffer solution that is due to MT1-MMP-driven proteolysis for gaining insights into the kinetics of proteolysis. As described in our previous study,[9] the in situ frequency shift ($\Delta\omega_p^L$) can be theoretically fitted to a Langmuir kinetic model that describes the frequency shift in the form $\Delta\omega_p^L(t) = \Delta\omega_0\ [1-\exp(-k_p t)]$ and allows extraction of the rate constant for proteolysis, $k_p$ (Figure 2a). For instance, the kinetic rate of proteolysis at an MT1-MMP concentration of 4.8 nM is estimated to be $k_p \approx 0.088\ \text{min}^{-1}$ (Figure 2b). We found that the kinetic rate strongly depends on the MT1-MMP concentration, and that it increases with the MT1-MMP concentration. The increase rate ($R$) of the kinetic rate with respect to MT1-MMP concentration is estimated to be $R = 0.16 \times 10^{-9}\ \text{min}^{-1}\text{M}^{-1}$ (Figure 2b). This increase rate clearly elucidates that the evaluation of the kinetic rate of proteolysis as a function of MT1-MMP concentration leads to quantitative predictions on the amount of expressed MT1-MMPs.

For validation of our strategy toward cancer diagnostics, we have measured small-molecule-mediated inhibition of MT1-MMP-driven proteolysis, since such a characterization is a first requisite for understanding the regulation of MT1-MMP expression. We have utilized the MMP inhibitor GM6001 as a small-molecule reagent that interrupts MT1-MMP-induced proteolysis. In an inhibition assay, we have fixed the MT1-MMP concentration at 48 nM, while the GM6001 concentration ranges from 48 nM to 4.8 μM. It is shown in Figure S2b in the Supporting Information that the proteolysis efficacy is significantly affected by the GM6001 concentration in such a way that when the GM6001 concentration is increased, the proteolysis efficacy is remarkably decreased.[2e,10] Moreover, we have estimated the kinetic rate of MT1-MMP-driven proteolysis as a function of GM6001 concentration based on in situ frequency shifts (Figure 2c). It is shown that an increase of GM6001 concentration significantly decreases the kinetic rate of MT1-MMP-induced proteolysis in such a way that a decrease in kinetic rate is proportional to GM6001 concentration (Figure 2d). This result implies that our cantilever biosensor exhibits a high detection sensitivity, which may result in effective cancer diagnosis.

For verifying interaction between MT1-MMP and target peptides as well as small-molecule-mediated inhibition, we have utilized tapping-mode AFM (tmAFM),[11] which can visualize the cantilever surface on which MT1-MMP-driven

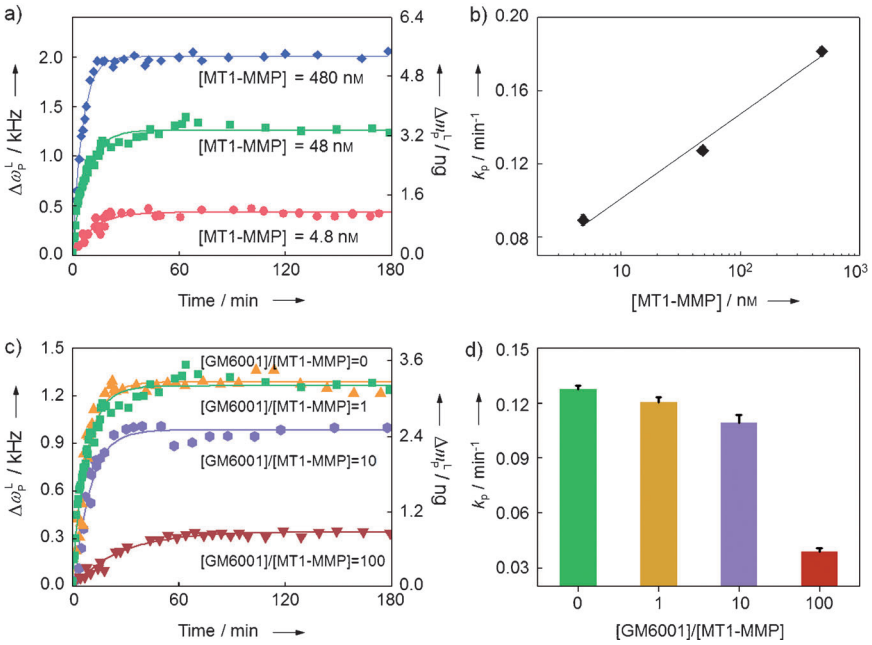


***Figure 2.*** In situ monitoring of MT1-MMP activities and small-molecule-mediated inhibition. a) Resonant frequency shifts ($\Delta\omega_p^L$) and mass of cleft peptide ($\Delta m_p^L$) owing to proteolysis are measured in buffer solution with different MT1-MMP concentrations. b) Kinetic rate, $k_p$, for proteolysis with respect to MT1-MMP concentration. c) Resonant frequency shift owing to proteolysis by MT1-MMP in the presence of the small-molecule inhibitor GM6001. d) Rate constant of proteolysis measured in the presence of the small-molecule inhibitor.

proteolysis occurs (Figure 3a–d). The immobilization of peptides onto a cantilever surface increases both the AFM average height and the roughness of a cantilever surface (Figure 3b), whereas MT1-MMP-driven proteolysis

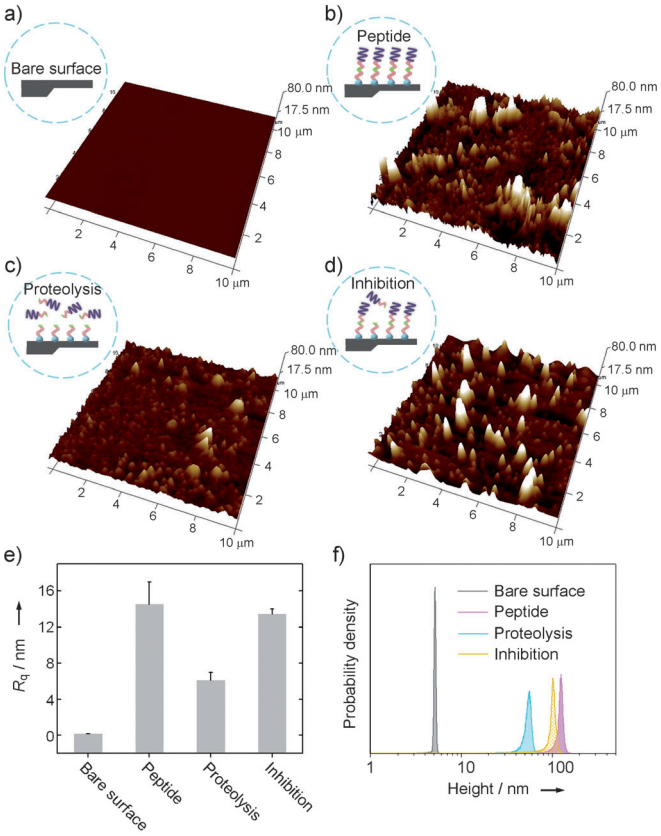


***Figure 3.*** AFM tapping-mode images ($10\times10\ \mu m^2$) of cantilever surfaces in different states. a) A bare cantilever surface is very flat (root-mean-squared height $R_q = (0.19 \pm 0.01)$ nm), b) a cantilever surface functionalized with peptides ($R_q = (14.5 \pm 2.5)$ nm), c) a functionalized surface exposed to MT1-MMP ($R_q = (6.1 \pm 0.9)$ nm), and d) a functionalized surface exposed to both MT1-MMP and inhibitor GM6001 ($R_q = (13.4 \pm 0.6)$ nm). e) Root-mean-squared height $R_q$ for cantilever surfaces in different states and f) Gaussian distribution of cantilever surface roughness in each state.

decreases the AFM average height as well as the roughness of a cantilever surface (Figure 3c). An increase in surface roughness owing to peptide immobilization is attributed to peptide aggregation. A decrease in surface roughness induced by proteolysis may be ascribed to the hypothesis that during proteolysis, most of the peptide chain is cleaved off. The surface profile clearly validates the interaction between MT1-MMP and peptides immobilized on a cantilever surface. Moreover, we have corroborated the small-molecule-mediated inhibition of MT1-MMP-driven proteolysis based on surface morphology. The decrease in AFM average height for the cantilever surface in the presence of both inhibitor GM6001 and MT1-MMP is smaller than in the presence of only MT1-MMP (Figure 3d–f). This finding suggests that inhibitor GM6001 effectively prevents MT1-MMP from cleaving the peptide chains on a cantilever surface.

To evaluate the potential of our sensor for cancer diagnostics, we have studied the cantilever-based detection of the proteolysis activity of MT1-MMPs that were extracted from membranes of live cancer cells. Specifically, in a cantilever assay a buffer solution containing various proteins, which were obtained from whole cell lysate of fibrosarcoma HT1080 cells,[10] was injected into a liquid cell, in which a cantilever vibrated (see the Supporting Information). The expression of MT1-MMP on HT1080 cells (and on HT1080 cells treated with inhibitor GM6001) was validated by western blotting analysis (Figure 4a), which enables the understanding of small interfering RNA (siRNA)-based regulation of MT1-MMP expression,[2d,12] while it cannot provide an insight into small-molecule-mediated regulation of MT1-MMP activity. This limitation of western blotting analysis for studying proteolytic activity of MT1-MMP can be overcome by using our cantilever sensor. We have observed a resonant frequency shift owing to MT1-MMP-driven proteolysis of peptides as well as GM6001-mediated regulation of such a proteolysis (Figure 4c). The frequency shift induced by peptide cleavage by MT1-MMP (extracted from the invasive cancerous cell line HT1080) is estimated to be 0.3 kHz, which is equivalent to the mass of cleft peptides of approximately 0.9 ng, whereas the frequency shift of a cantilever exposed to both MT1-MMP (obtained from whole cell lysate) and inhibitor GM6001 is below 0.1 kHz (equivalent to a mass of cleft peptide of approximately 0.12 ng). Moreover, based on the Langmuir kinetic model, the kinetic rate for proteolysis driven by MT1-MMP expressed on HT1080 cells is found to be $k_p \approx 0.066\ min^{-1}$, whereas GM6001-mediated inhibition significantly decreases the kinetic rate of proteolysis by MT1-MMP (i.e. $k_p \approx 0.023\ min^{-1}$). This result clearly suggests that a cantilever sensor is able to effectively detect the small-molecule-mediated regulation of enzymatic activity,[2d,12] thus implying that our cantilever assay may be useful for drug screening with high sensitivity.

To further confirm the diagnostic potential of our sensor, we have considered HEK293 cells that are genetically transfected with MT1-MMP or mutant MT1-MMP. Here, three different kinds of cells—original HEK293 cells (N/C), HEK293 cells transfected with MT1-MMP (WT), and HEK293 cells transfected with mutant MT1-MMP (E/A)[2b]—were used for the cantilever bioassay, and the correct protein expression in the different cells was confirmed by western blotting analysis as shown in Figure 4b. When a solution obtained from whole cell lysate of cells transfected with MT1-MMP (WT) was injected into a liquid cell, a shift in the frequency of a cantilever was observed, which is attributed to peptide cleavage by MT1-MMP. However, when a cantilever is exposed to solutions obtained from cell lysates of original HEK293 cells (N/C) and from cells transfected with the mutant MT1-MMP (E/A), the shift in the frequency is smaller than 0.1 kHz (Figure 4d), thus indicating that peptide cleavage is unlikely to occur. Interestingly, in this experiment, we could validate the proteolytic ability of mutant MT1-MMP (E/A). Although an expression of MT1-MMP in the cells transfected with mutant MT1-MMP (E/A) was shown in western blot analysis, the cantilever bioassay reveals that the proteolytic activity of mutant MT1-

  

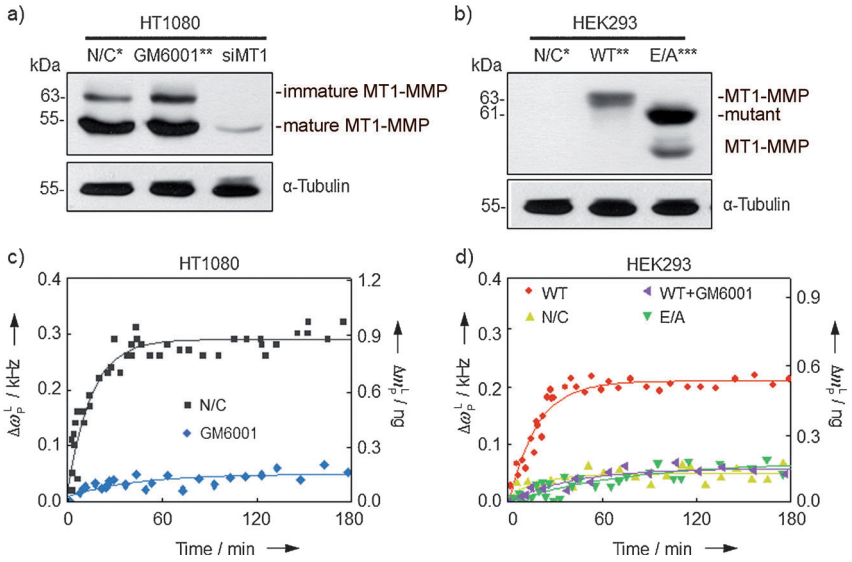


***Figure 4.*** Quantifying MT1-MMP concentration in whole cell lysate of invasive cancer cells. a) Western blot analysis of MT1-MMP from whole cell lysates of HT1080 cells using α-tubulin as a loading control (*N/C: negative control—normal HT1080 cells; **GM6001 concentration is 20 μM; siMT1: siRNA directed against MT1-MMP). The treatment with siMT1 inhibits the expression of MT1-MMP in a cell, whereas treatment with inhibitor GM6001 does not. b) Expression of MT1-MMP from HEK293 cells (*N/C: negative control—normal HEK293 cells; **WT: up-regulation of MT1-MMP expression; ***E/A: mutant MT1-MMP expression). The expected molecular weights for immature MT1-MMP and mutant MT1-MMP are 63 and 61 kDa, respectively. c, d) Resonant frequency shift owing to peptide cleavage by MT1-MMP acquired from HT1080 (c) and HEK293 (d) whole cell lysates (ca. $1\times10^5$ cells $mL^{-1}$). Injection volume of lysate into the liquid cell is 50 μL and the concentration of inhibtor GM6001 (20 μM) is identical in all bioassays where GM6001 is added.

MMP was forfeited, thereby suggesting that the mutation of MT1-MMP affects its enzymatic activity.

In conclusion, we have quantitatively described the proteolytic activities of MT1-MMP (acquired from invasive cancer cells and also genetically engineered cells) by using a resonant microcantilever. The label-free detection of the activity of MT1-MMP extracted from invasive cancer cells is based on the measurement of both the mass of peptides cleaved by MT1-MMP and the kinetic rate of MT1-MMP-driven proteolysis. It has been shown that our cantilever bioassay exhibits a detection sensitivity below 1 nM for sensing MT1-MMP-driven proteolysis (see the Supporting Information), which is mostly higher than the sensitivity of other methods, such as methods using fluorogenic probes,[2e,6a,b] except a method using nanoporous films[13] for detecting MMP activity. Our study sheds light on a cantilever assay for quantifying enzymatic activity (for cancerous cells) as well as the regulation of such an activity. For future applications in cancer diagnosis our cantilever sensor will be used with more realistic samples, such as various cancerous cells with different metastasis status or in different stages of cancer development.[13,14]

Received: December 15, 2011
Revised: April 24, 2012
Published online: May 15, 2012

**Keywords:** atomic force microscopy · biosensors · cancer · peptides · proteases

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Angewandte Chemie
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Supporting Information



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# Real-Time Quantitative Monitoring of Specific Peptide Cleavage by a Proteinase for Cancer Diagnosis**

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# Supporting Information:

**S1. Preparation of PEGylated peptide**

A peptide (Peptide 2.0 Inc., VA, USA), whose sequence is given by Gly-Pro-Leu-Pro-Leu-Arg-Ser-Trp-Gly-Leu-Lys-Fmoc[1], was chemically linked to PEG chain. This PEGylation was performed to improve water-solubility and drastic mass change due to peptide cleavage[2]. In particular, the N-terminus of the peptide was conjugated with the carboxyl group of methoxy PEG succinimidyl carboxymethyl ester (M-SCM-5000, JenKem Technology, TX, USA) by esterification reaction. The mPEG-NHS (2.0 μmol) and 1-ethyl-3-(3-dimethylaminopropyl)-carbodiimide (EDC, 2.1 μmol) were dissolved in phosphate buffered saline (PBS; 1.0 ml, pH 7.4, 10 mM). After incubation of the mixture for 1 hour at room temperature, the peptide (2.0 μmol) was additionally dissolved in the solution. The PEGylated peptide solution was incubated for 24 hours.

**S2. Preparation of MT1-MMP/GM6001-dissolved solution**

To obtain MT1-MMP-dissolved solution (with MMP concentrations of 4.8 nM, 48 nM, and 480 nM, respectively, the recombinant catalytic domain of human MT1-MMP (MT1-CAT, Calbiochem), which is initially dissolved in stock solution with concentration of 0.2 mg/ml, was diluted in a buffer solution (150 mM NaCl, 50 mM Tris-HCl, 5 mM $CaCl_2$, and 0.025 % Brij-35, pH 7.5) at room temperature. GM6001 (1 mg/ml, galardin or ilomastat, Calbiochem) was diluted in phosphate buffered saline buffer solution (pH 7.4) to acquire GM6001-dissolved solution with GM6001 concentrations of 48 nM, 480 nM, and 4.8 μM, respectively. In addition, we have prepared a buffer solution that contains MT1-MMP obtained from whole cell lysate (1 ml). The details of cell culture and western blot analysis are specified in S6 section in this material.

**S3. Preparation of Functionalized Cantilevers**

We have used a commercially available microcantilever (TESP, Veeco, Santa Barbara, CA, USA), whose dimension was given as 40 × 4 × 125 $\mu m^3$ (width × thickness × length) with a force constant of ~42 N/m. The fundamental resonant frequency of a cantilever was measured as $f$ = 341.0 kHz, which is consistent with a continuum beam theory such as Euler-Bernoulli beam theory. To functionalize a cantilever surface, PEGylated peptide chains were immobilized to an aminated cantilever[2a]. In detail, the cantilever surface was chemically customized by 3-aminopropyltrimethoxysilane (200 μl) in deionized water (40 ml) at 85 ℃ for 24 hours. After chemical reaction, aminated surface was purified by excessive deionized water and ethanol. The amine-functionalized cantilever was immersed in the PEGylated peptide solution for 24 hours at room temperature after adding EDC (3 μmol). After PEGylated peptide immobilization, the cantilever was rinsed by excessive buffer solution and dried out for 24 hours in desiccator.

**S4. Cantilever Bioassay**

We have utilized Nanoscope V controller (PicoForce, Veeco, Santa Barbara, CA, USA) to estimate the resonant frequency of a cantilever and frequency shift induced by MT1-MMP-driven proteolysis. In particular, a software Nanoscope v7.0 (Veeco, Santa Barbara, CA, USA) provides the frequency-amplitude curve, in which the sharp

peak indicates the fundamental resonance (Figure S3). For *in situ* monitoring of proteolysis, a microcantilever functionalized with peptide chains was mounted on a liquid cell with its volume of ~50 μl. At room temperature, MT1-MMP solution with three different concentrations (4.8 nM, 48 nM, and 480 nM) was injected into the liquid cell in order to evaluate the proteolysis-induced frequency shift. Subsequently, the resonant frequency of a cantilever immersed in a buffer solution was monitored for every 1 min after injection of MT1-MMP-dissolved buffer solution. Moreover, we have measured the frequency shift due to MT1-MMP for a cantilever in both air and liquid (see Tables S1 and S2) in order to quantify the mass of cleft peptides and the change of hydrodynamic loading effect due to hydrophilicity change induced by proteolysis [2a]. The kinetic rate of proteolysis is measured from Langmuir kinetic model that can be fitted to a measured frequency shift due to proteolysis (for details, see Ref. [2a]). For inhibition bioassay, a buffer solution containing MT1-MMP (48 nM) and GM6001 (48 nM, 480 nM, or 4.8 μM) was injected to a liquid cell, where a functionalized cantilever was mounted. In the similar manner, the frequency shift for a cantilever due to small-molecule-mediated proteolysis is measured for every 1 min.

**S5. Analysis of AFM images**

The AFM height images for specific peptide hydrolysis due to MT1-MMP-driven proteolysis or inhibition test is quantified by comparing surface roughness defined as root mean squared value $R_q = \sqrt{(1/n)\sum_{i=1}^{n} y_i^2}$ .

**S6. Cell culture and western blot analysis**

HT1080, human colon cancer cell line, and HEK293, human embryonic kidney cell line, were cultured in Dulbecco's modified eagle medium (DMEM) supplemented with 10% fetal bovine serum (FBS), streptomycin (100 μg/ml) and penicillin (100 units/ml) at 37 ℃ with 5.0% $CO_2$. Western blot analysis of MT1-MMP is employed from whole-cell lysates ($1 \times 10^5$ cells/ml) using tubulin as a loading control. Cells were lysed in a buffer composed of 50 mM Tris, pH 7.4, 150 mM NaCl, 1% Triton X-100, 1 mM EDTA, 1 mM sodium orthovanadate, 1 mM PMSF, and protease inhibitors. Equal amount of protein extracts was subjected to electrophoresis on SDS-polyacrylamide gels and then transferred to polyvinylidene fluoride (PVDF) membranes. The following antibodies were used: MMP14 (ab3644, Abcam, Cambridge, UK), α-tubulin (Oncogene Science, Cambridge, MA, USA). Secondary antibodies were anti-rabbit IgG peroxidase (Cell signaling). The signals were developed by Lumi-Light Western Blotting Substrate (Roche, Indianapolis, IN, USA) according to the manufacturer's protocol.

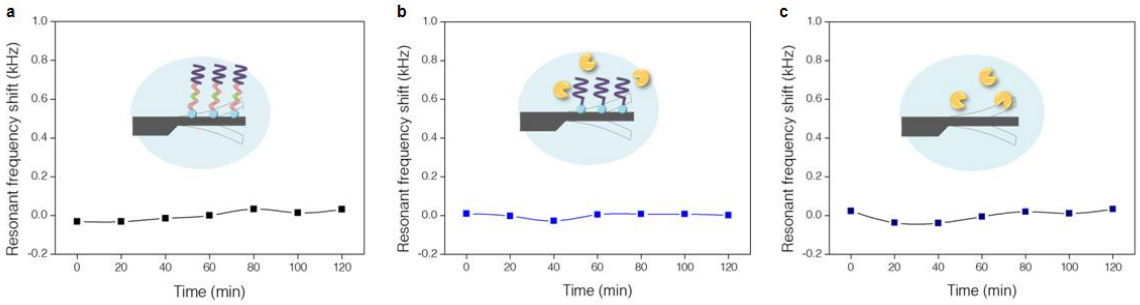


**Figure S1.** Negative control experiments. **a,** Resonance behaviors of cantilever, which is functionalized with PEGylated peptide chains, immersed in buffer solution that does not contain any proteases, **b,** Resonant frequency for a cantilever functionalized only with PEG chain in response to injection of MT1-MMP-dissolved buffer solution (with MT1-MMP concentration of 48 nM), and **c,** The frequency behavior of a cantilever when exposed to MT1-MMP. These negative control experiments have proved that the vibration of a cantilever immersed in buffer solution is stable, that MT1-MMP is unable to unspecifically cleave PEG chain, and that non-specific binding of MT1-MMP into a silicon cantilever surface is unlikely to occur.

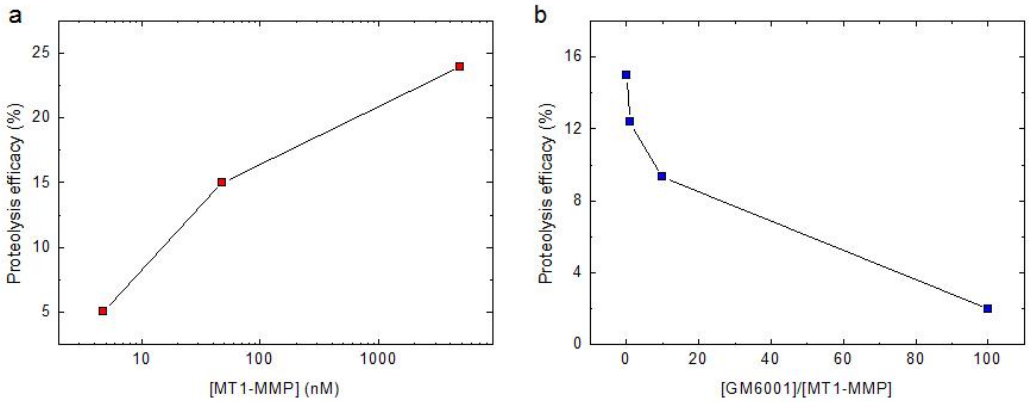


**Figure S2.** Relationship between proteolysis efficacy, $r$, and MT1-MMP concentration, [MT1-MMP]. Here, proteolysis efficacy, $r$, is defined as $r = \Delta m_P / \Delta m_I$, where $\Delta m_P$ and $\Delta m_I$ represent the total mass of cleft peptides and immobilized peptides on the cantilever surface, respectively.

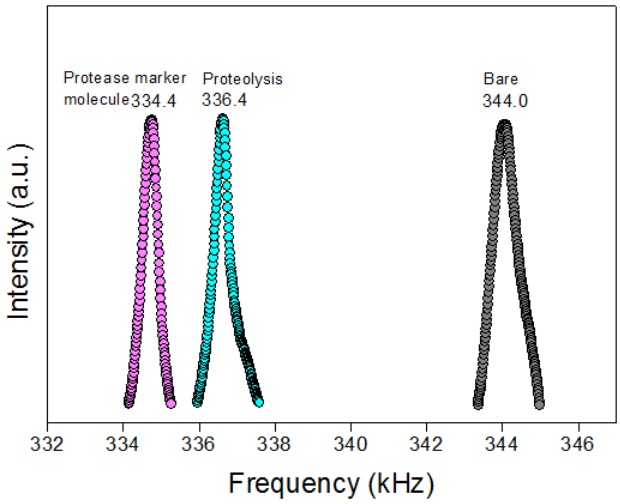


**Figure S3.** The resonant frequency of microcantilever measured in dry air in response to peptide immobilization and MT1-MMP-driven proteolysis. The frequency of a bare cantilever is shown as dark gray curve, and the frequency behavior of a cantilever in response to peptide immobilization is dictated by pink curve, while the frequency response to MT1-MMP proteolysis is depicted by cyan curve. It is shown that peptide immobilization reduces the resonance of cantilever (due to increase of overall mass) whereas MT1-MMP-driven proteolysis of peptides increases the resonance of a cantilever (arising from the decrease of the effective mass of a functionalized cantilever due to the mass of cleft peptides).

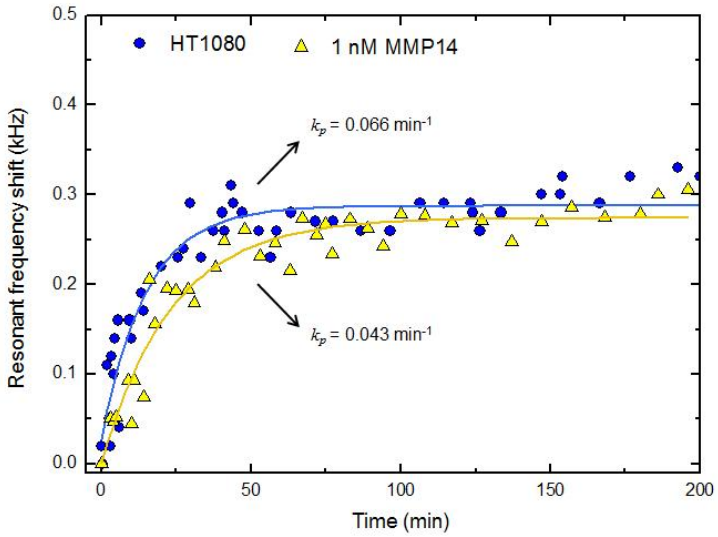


**Figure S4.** *In situ* measurement of resonant frequency shift of microcantilever in MT1-MMP 1 nM solution, in compare with HT1080 whole cell lysate (~$1\times10^5$ cells/ml).

| Cant. # | MT1-MMP (nM) | $\omega_0$ (kHz) | $\omega_I$ (kHz) | $\Delta\omega_I$ (kHz) | $\Delta m_I$ (ng) | $\omega_I^L$ (kHz) | $\omega_P^L$ (kHz) | $\Delta\omega_P^L$ (kHz) | $\Delta m_P^L$ (ng) |
|---|---|---|---|---|---|---|---|---|---|
| | | ***in air*** | | | | ***in liquid*** | | | |
| 1 | 4.8 | 349.6 | 334.9 | -14.7 | 3.25 | 143.4 | 143.8 | +0.45 | 1.20 |
| 2 | 48 | 326.9 | 318.4 | -8.5 | 1.90 | 144.2 | 145.5 | +1.31 | 3.44 |
| 3 | 480 | 344.0 | 334.4 | -9.6 | 2.16 | 158.9 | 161.0 | +2.00 | 4.83 |
| | *[MT1-MMP] : [GM6001] | | | | | | | | |
| 4 | 1 : 1 | 334.5 | 325.5 | -9.0 | 2.02 | 149.7 | 150.9 | +1.28 | 3.26 |
| 5 | 1 : 10 | 345.1 | 335.8 | -9.3 | 2.08 | 160.9 | 162.0 | +1.00 | 2.38 |
| 6 | 1 : 100 | 346.2 | 334.5 | -11.7 | 2.61 | 160.6 | 160.9 | +0.34 | 0.81 |

*[MT1-MMP] = 48 nM. $\Delta\omega_I = \omega_0 - \omega_I$, $\Delta\omega_P^L = \omega_P^L - \omega_I^L$

**Table S1.** The measurement of resonant frequency of microcantilevers in dry liquid. Resonance of bare cantilevers, cantilevers after peptide immobilization, and such peptide-immobilized cantilever after exposure to protease with MT1-MMP concentration of 4.8 nM, 48 nM, and 480 nM, and protease (48 nM) with small-molecule inhibitor (48 nM, 480 nM, and 4800 nM), respectively, were measured in dry air or liquid.

| Cant. # | MT1-MMP (nM) | $\omega_0$ (kHz) | $\omega_I$ (kHz) | $\omega_P$ (kHz) | $\Delta\omega_I$ (kHz) | $\Delta\omega_P$ (kHz) | $\Delta m_I$ (ng) | $\Delta m_P$ (ng) | $\Delta m_P/\Delta m_I$ (%) |
|---|---|---|---|---|---|---|---|---|---|
| 7 | 4.8 | 357.2 | 347.4 | 347.9 | -9.8 | +0.5 | 2.18 | 0.11 | 5.04 |
| 8 | 48 | 317.1 | 309.1 | 310.2 | -8.0 | +1.2 | 1.80 | 0.27 | 15.00 |
| 9 | 480 | 313.9 | 306.7 | 308.4 | -7.2 | +1.7 | 1.63 | 0.39 | 23.92 |
| | *[MT1-MMP] : [GM6001] | | | | | | | | |
| 10 | 1 : 1 | 336.5 | 330.0 | 330.8 | -6.5 | +0.8 | 1.37 | 0.17 | 12.40 |
| 11 | 1 : 10 | 318.7 | 305.3 | 306.5 | -13.4 | +1.2 | 3.00 | 0.28 | 9.33 |
| 12 | 1 : 100 | 313.1 | 308.6 | 308.7 | -4.5 | +0.1 | 1.02 | 0.02 | 1.96 |

*[MT1-MMP] = 48 nM.

**Table S2.** The measurement of resonant frequency of microcantilevers in dry air

## References


[1] S. Ohkubo, K. Miyadera, Y. Sugimoto, K.-i. Matsuo, K. Wierzba, Y. Yamada, *Biochem. Biophys. Res. Commun.* **1999**, *266*, 308-313.

[2] a) T. Kwon, J. Park, J. Yang, D. S. Yoon, S. Na, C.-W. Kim, J.-S. Suh, Y.-M. Huh, S. Haam, K. Eom, *PLoS ONE* **2009**, *4*, e6248; b) F. M. Veronese, *Biomaterials* **2001**, *22*, 405-417.