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Aggregation–Deaggregation-Triggered, Tunable Fluorescence of an Assay Ensemble Composed of Anionic Conjugated Polymer and Polypeptides by Enzymatic Catalysis of Trypsin

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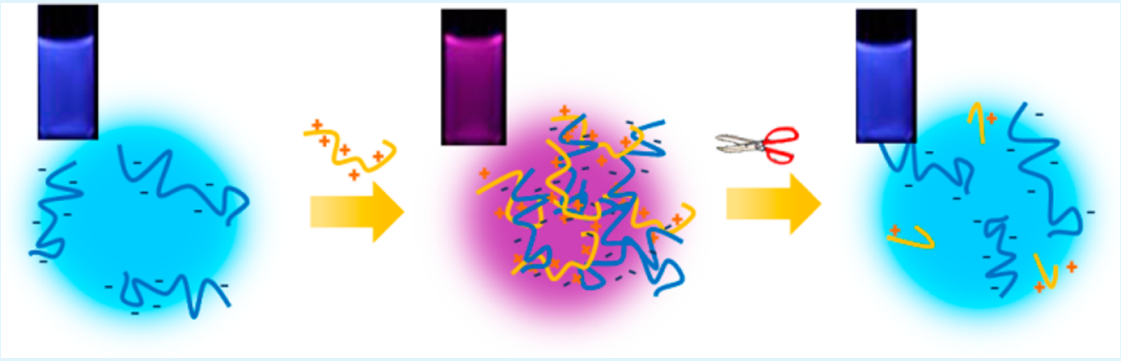
Supporting Information



ABSTRACT: We prepared a water-soluble conjugated polymer composed of electron-donating units and electron-accepting groups in the backbone. The polymer exhibits a short wavelength (blue) emission in aqueous solution and long wavelength (red) emission in the solid state, because of intermolecular energy transfer. Considering this, we develop a new approach for the sensitive detection of trypsin, which is known to control pancreatic exocrine function, using an ensemble system composed of the anionically charged conjugated polymer and cationically charged polypeptides (such as polylysine and polyarginine). The blue-emitting, water-soluble conjugated polymer becomes aggregated upon exposure to the polypeptides, leading to a red-emitting assay ensemble. The red-emitting assay ensemble becomes dissociated in the conjugated polymer and polypeptide fragments by selective degradation of trypsin, which then exhibits recovery of blue emission. This emission-tuning assay ensemble allows for detection of trypsin at nanomolar concentrations, which enables naked-eye detection. Importantly, this strategy can be employed for label-free, continuous assay for trypsin.

KEYWORDS: *sensors, water-soluble conjugated polymers, fluorescence, trypsin, polypeptides*

INTRODUCTION

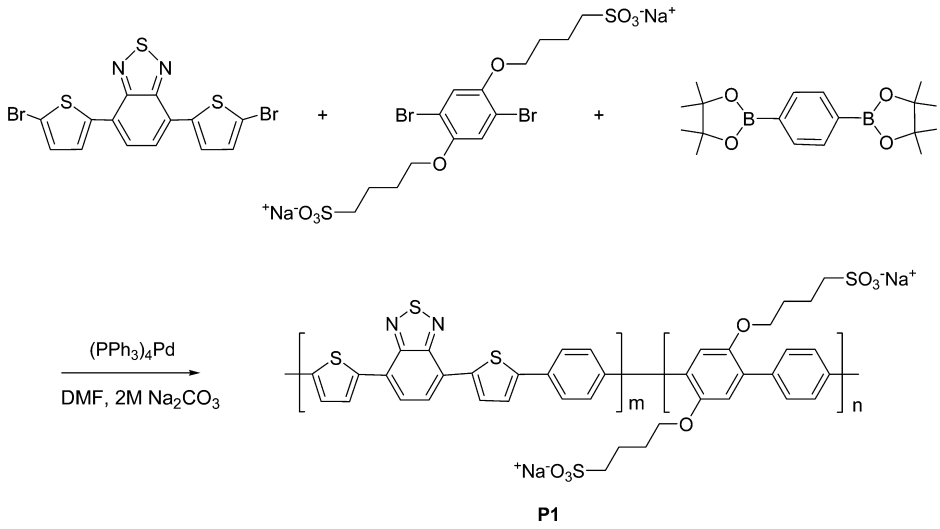
In the past decade, fluorescent conjugated polymers have attracted considerable interest for their widespread applications in the areas of optoelectronics, microelectronics, and sensors.[1−3] However, because of the hydrophobic nature of the backbone, conjugated polymers are limited in application to biologically relevant fields. Thus, water-soluble conjugated polymers have been developed by the introduction of functional groups including phosphonates, sulfonates, and quaternary ammonium salts to the polymer chain.

Their optoelectronic properties can be modulated facilely through structural alterations in the conjugated backbones of the polymers and the ionic functional group provides them with versatile features including electrostatic interaction with oppositely charged chemical or biological species, which supports a new basis for optoelectronic devices and biological and chemical detection such as DNA, proteins, and other biological agents.[4−17] Moreover, in contrast to polymeric materials, one of the shortcomings of conventional small molecular fluorescent assays without ionic groups is the relatively poor solubility in the aqueous phase, which significantly hinders their practical use in biomedical investigations.

Proteases are important biocatalysts that are involved in the cleavage of specific peptide bonds in analyte proteins and thus cause proteins to dissociate into small fragments. Therefore, these enzymatic proteins control various essential physiological processes.[18] As a type of protease, trypsin is one of the important digestive enzymes, which is formed in the pancreas as an inactive proenzyme trypsinogen,[19−22] which plays an

Received: September 26, 2013
Accepted: December 20, 2013
Published: December 20, 2013

Scheme 1. Synthetic Methods for Polymer



important role in controlling the pancreatic exocrine function (also, the level of trypsin increases with certain types of pancreatic-related diseases).[20−22] Thus, this protease is known to be overexpressed in pathological conditions of inflammation[23] and cancer.[24] It has been known that trypsin is one of the enzymatic proteins that can selectively degrade peptide at the carboxylic side of amino acids such as lysine and arginine.[25]

Considering its biological importance, new and convenient assays for trypsin are in high demand for developing effective diagnostic and therapeutic techniques toward the treatment of pancreatic diseases and application in the proteomics field. A variety of techniques for trypsin detection have been developed on the basis of mass spectroscopy,[26,27] gel electrophoresis,[28] enzyme-linked immunosorbent assay (ELISA),[29] colorimetric,[30,31] and fluorometric methods.[32−39] In general, the colorimetric and fluorometric methods are favorable to the application, because other techniques require specialized instruments or are time-consuming.

Fluorescence detection techniques for trypsin have also been reported based on labeled substrate peptides,[40] conjugated polymers,[37,41−43] gold nanoclusters,[44] photonic nanoparticles,[45] and natural fluorophore epicocconone.[46] However, the development of a new assay for trypsin is still challenging, because several obstacles including tedious synthetic chemistry of conjugated fluorophores, a need for labeling, and the toxicity of quantum dots limit their applications.

Compared to the emission turn-on or turn-off switchable polymeric assays, considerable interest has been paid to fluorescent conjugated polymers with tunable emission colors upon aggregation. The features of tunable fluorescence emissions are mainly attributed to the intrinsic properties of the aggregation-induced exciton migration in conjugated polymer backbones. Typical applications of such emission color-tunable conjugated polymers include platforms for proteins,[47−49] heparin,[50,51] multicationic amines,[52] mercury ion,[53] cysteine,[54] and bacteria sensing.[55]

Herein, we present an ensemble system as an assay for trypsin based on the aggregation of positively charged polypeptides such as polylysine and polyarginine and initially blue-emitting, negatively charged conjugated polymer, of which emission can be tuned blue-to-red upon aggregation. The conjugated polymer is functionalized with ionic side-chains of sulfonate groups to provide water solubility and to ensure ionic interactions with cationically charged polypeptides. The red-emitting, aggregated ensemble recovers its blue emission upon exposure to a protease, trypsin, which degrades a specific sequence of amino acids. Therefore, our ensemble system may have potential for a wide range of clinical applications. As far as we know, this ensemble system, based on the distinctive red-to-blue emission color change, caused by the aggregation−deaggregation protocol, has been rarely exploited for trypsin detection and, thus, it is advantageous over other detection system, in terms of possible detection by the naked eye.

EXPERIMENTAL SECTION

Materials. Monomers for Suzuki cross-coupling polymerization, such as 4,7-dibromo-2,1,3-benzothiadiazole, 4,7-bis(5-bromothiophen-2-yl)benzo-2,1,3-thiadiazole, and 1,4-dibromo-2,5-bis(4-sulfonatobutoxy)benzene sodium salt were synthesized according to the previously published methods.[56−58] All polypeptides including poly(L-lysine hydrobromide) (pLys; mol wt = 4000−15000), poly(L-arginine hydrochloride) (pArg; mol wt = 15 000−70 000), poly(L-histidine hydrochloride) (pHis; mol wt ≥ 5000), and trypsin (mol wt = 23 300) were purchased from Aldrich and used as received. Other reagents were purchased from Aldrich and Acros and used without further purification.

Characterization. The ^{1}H NMR and ^{13}C NMR spectra were recorded on a Bruker Model DRX-300 spectrometer with tetramethylsilane as an internal standard (Korea Basic Science Institute). UV−vis absorption spectra were collected on a Perkin−Elmer Model Lambda 35 spectrometer. Photoluminescence spectra were obtained from a Varian Cary Model Eclipse fluorescence spectrometer. Elemental analysis was carried out on an elemental analyzer (Fisons Instruments, Model EA 1108).

Polymerization. 4,7-Bis(5-bromothiophen-2-yl)benzo-2,1,3-thiadiazole (26.1 mg, 0.057 mmol), 1,4-dibromo-2,5-bis(4-sulfonatobutoxy)benzene sodium salt (300 mg, 0.514 mmol), and 1,4-benzenediboronic acid bis(pinacol)ester (188 mg, 0.571 mmol) were dissolved in a mixture of dimethylformamide (DMF) (8 mL) and 2 M Na_2CO_3 aqueous solution (12 mL) in a flask. The detailed procedures for synthesis and workup of **P1** are described elsewhere.[49,54] After purification,

dx.doi.org/10.1021/am405120y | ACS Appl. Mater. Interfaces 2014, 6, 918−924

the final yellow solid was obtained (yield: 162 mg, 53%). ^{1}H NMR (300 MHz, D_2O): δ_{ppm} 8.1−7.3 (br, 3.1H), 7.2−6.7 (br, 2H), 4.0 (br, 2.8H), 3.0 (br, 3H), 2.0−1.5 (br, 5H). ^{13}C NMR (D_2O): δ_{ppm} 150.17, 130.56, 128.85, 128.26, 120.40, 117.63, 114.98, 68.97, 65.76, 51.21, 49.86, 43.04, 27.93, 23.14, 21.30, 20.87. Anal. Calcd (%) for $C_{20}H_{20.8}N_{0.2}S_{2.1}O_{7.2}Na_{1.8}$: C, 49.24; H, 4.27; N, 0.57; S, 13.79. Found: C, 48.48; H, 4.34; N, 0.50; S, 13.39.

Emission Color Change of P1 upon Exposure to Polypeptides. Changes in fluorescence color (blue-to-red) could be observed upon successive addition of pLys, pArg, or pHis (0, 2, 5, 10, 15, and 20 μg/mL) to an aqueous solution of **P1** (1×10^{-4} M, based on repeat units) at ambient temperature. The fluorescence spectra were recorded at various concentrations of polypeptides.

Ensemble Assays for Trypsin Activity. A cuvette containing **P1** (1×10^{-4} M, based on repeat units) and pLys or pArg (20 μg/mL) was exposed to trypsin (30 μg/mL) in deionized water (Millipore Nanopure). The mixture was incubated at 37 °C for a desired time interval. The fluorescence intensities of the mixtures at 417 and 628 nm were recorded at the excitation wavelength of 350 nm.

RESULTS AND DISCUSSION

The synthetic procedure for the preparation of polymer is shown in Scheme 1. Bromination of 2,1,3-benzothiadiazole enabled us to obtain 4,7-dibromo-2,1,3-benzothiadiazole in good yield using published methods.[55] 4,7-Bis(5-bromothiophen-2-yl)benzo-2,1,3-thiadiazole was obtained from the bromination of 4,7-bis(thiophen-2-yl)benzo-2,1,3-thiadiazole with *N*-bromosuccinimide (NBS) in a mixture of tetrahydrofuran (THF) and acetic acid.[57] A reaction between 2,5-dibromo-1,4-hydroquinone and 1,4-butanesultone was carried out in the presence of sodium hydroxide to prepare 1,4-dibromo-2,5-bis(4-sulfonatobutoxy) benzene sodium salt, according to the literature method.[58]

The water-soluble conjugated polymer (**P1**) was synthesized via Suzuki cross-coupling reaction of compounds 4,7-bis(5-bromothiophen-2-yl)benzo-2,1,3-thiadiazole, 1,4-dibromo-2,5-bis(4-sulfonatobutoxy)benzene sodium salt, and 1,4-benzenediboronic acid bis(pinacol)ester using palladium catalyst (Scheme 1). The yield of polymer was found to be 53%. The chemical structure of the polymer was confirmed by ^{1}H NMR, ^{13}C NMR, and elemental analysis. The chemical composition (mole fraction) in **P1** was determined by elemental analysis and measured to be 0.07:0.93 (m:n). The polymer showed good solubility in water, but it was not soluble in THF, chloroform, methanol, or ethanol.

A reliable molecular weight of **P1** was barely obtainable. It was not easy to obtain a feasible molecular weight of **P1** by gel permeation chromatography (GPC) using an aqueous eluent, because the polymer was adsorbed in the polystyrene gel in the GPC column. Moreover, determination of molecular weight was attempted using matrix-assisted laser desorption/ionization time-of-flight (MALDI-TOF), feasible data could not be obtainable.[52,59,60] However, it is likely that the molecular weight of **P1** was more than 12 000, because **P1** was dialyzed with a membrane of 12400 cutoff.

Absorption and fluorescence emission spectra of **P1** in aqueous solution are shown in Figure S1 in the Supporting Information. **P1** showed absorption at 326 nm in the aqueous solution and at 345 nm in solid state. The absorption resulted from the $\pi-\pi^*$ transition of the phenylene groups in the polymer chain. The spectrum of polymer has broad absorption band at ∼500−600 nm, which is assigned to bis-thienylbenzothiadiazole (TBT) units. A red shift in absorption in the solid state, compared to the solution, was typically observed because of a conformational change in the solid state, resulting in π-electron delocalization along the chain.[61,62] In aqueous solution, **P1** emits blue fluorescence with the main band at 417 nm. In contrast, **P1** begins to emit red fluorescence as it aggregates by interchain aggregation-induced exciton migration, exhibiting a much smaller intensity of blue emission at ∼417 nm and intensified red emission, indicative of intermolecular energy transfer.

It has been reported that the mechanism of aggregation-enhanced energy transfer (exciton migration) can be employed in the sensing applications, which are involved in biorelated, multicationic targets.[47−55] Using the fact that the negatively charged **P1** showed visually distinctive blue (solution) and red (solid or aggregate) emission colors, an assay ensemble could be obtained by simply mixing suitable concentrations of negatively charged **P1** and positively charged polypeptides, such as pLys, pArg, and pHis, to induce aggregation via electrostatic interaction, leading to red emission. The emission intensity at 417 nm (short wavelength) of the sensor ensemble (**P1**-polypeptides) decreased and a new emission at 636 nm (long wavelength) was simultaneously formed with an increase in intensity as the amounts of polypeptides increased (see Figure 1 and Figure S2 in the Supporting Information). This was generated by the self-aggregation of the mixture, resulted from the intermolecular interaction between **P1** and polypeptides.

Figure 1a shows the fluorescence spectra of **P1** with the addition of pLys, ranging from 0 to 20 μg/mL. Initially, the blue emission band at 417 nm is dominant in the free **P1** solution with a comparably weak red emission at ∼600 nm. As the concentration of pLys increased, the blue emission band at 417 nm decreased significantly, in conjunction with the aggregation-induced exciton migration between the polymer chains. Concomitantly, the fluorescence spectra of the polymer showed an intensified band at 636 nm resulting from the aggregation-induced enhancement of red emission as the concentration of pLys increased. The result clearly indicates the aggregation-induced blue-to-red emission color tuning of **P1** in the presence of pLys, as shown in the inset photograph in Figure 1a. The positively charged pLys interacted with **P1** via an electrostatic charge interaction, resulting in aggregation, which was accompanied by enhanced exciton migration, finally leading to a visually observable blue-to-red emission color change. Similar aggregation-enhanced emission color change in the anionic **P1** was observed in the presence of a cationically charged pArg or pHis, respectively (see Figure 1b and Figure S2 in the Supporting Information). The degree of aggregation (increment in the red emission) depends on the polypeptides used.

Based on the new formation of a long wavelength (red) emission upon aggregation with two oppositely charged species, we proposed a new assay ensemble to evaluate trypsin activity via restoration of the short wavelength (blue) emission. The trypsin detection assay is demonstrated in Scheme 2. To prepare the assay ensemble, the blue-emitting solution of anionic polyelectrolyte **P1** was exposed to polypeptides such as pLys and pArg in the aqueous phase. At this stage, a strong red emission induced by aggregation could be observed, as mentioned above. Upon the addition of trypsin to the assay,

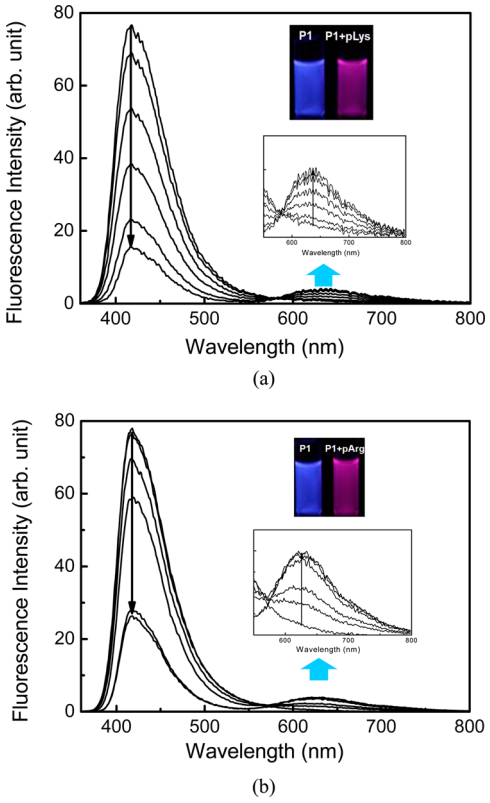


Figure 1. Changes in fluorescence spectra of **P1** after addition of different concentrations of (a) pLys and (b) pArg. [**P1**] = 1×10^{-4} M (based on repeat units), [pLys] and [pArg] = 0, 2, 5, 10, 15, and 20 μg/mL. Inset shows photographs of the aqueous solutions of **P1** before and after addition of polypeptides (20 μg/mL) under UV (365 nm) illumination. Excitation wavelength: λ_{ex} = 350 nm.

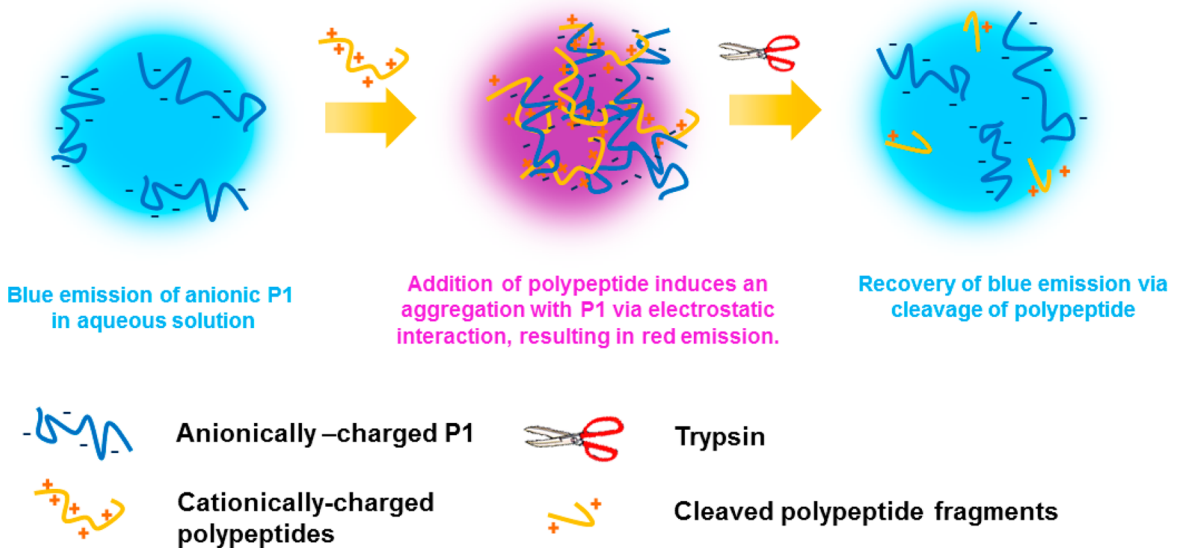
the aggregates disappeared and the original blue emission was recovered. Introduction of trypsin to the ensemble of **P1**−polypeptide induces a hydrolysis of lysine and arginine residues in pLys and pArg, respectively, resulting in peptide fragments along with weakened electrostatic interaction with **P1**.[38] As a result, the **P1** could restore its initial blue fluorescence after dissociation of the assay complex into **P1** and lysine and arginine fragments, which allows qualitative and quantitative detection of trypsin via a naked-eye-detectable emission color change.

As illustrated in Figure 1, the solution of an assay ensemble composed of **P1** (1.0×10^{-4} M) and pLys or pArg (20 μg/mL) showed weakening of blue emission with intensifying red emission. Upon the addition of trypsin (30 μg/mL) to the ensemble solutions (**P1**−pLys and **P1**−pArg), the blue fluorescence of the ensemble increased considerably and, at the same time, the red emission decreased gradually with incubation time (Figure 2). After the further addition of trypsin to this ensemble solution and subsequent incubation for 40 min, the blue emission was recovered up to 90% (for **P1**−pLys) and 95% (for **P1**−pArg) of original blue intensity. These results clearly demonstrate that an emission-shift assay for trypsin can be established using the ensembles of **P1**−pLys and **P1**−pArg.

The intensity of blue emission of the ensemble with trypsin increased and saturated after the solution was incubated at 37 °C for 40 min. Although similar recovery behaviors of blue emission were observed for both the ensembles of **P1**−pLys and **P1**−pArg, the rates of trypsin-mediated hydrolysis were found to be different in both cases. In detail, in the case of **P1**−pLys, the initial rate of hydrolysis was observed to be higher, while the final rate was higher for the case of **P1**−pArg. These results demonstrate the effect of trypsin activity on the hydrolysis of different polypeptides and the activity can be regarded as the fluorescence change of the ensemble of **P1** and polypeptides.

Meanwhile, a control experiment was carried out to determine the trypsin activity using the **P1**−pHis ensemble, in which trypsin could not hydrolyze a histidine linkage. As expected, trypsin cannot recover the blue emission of the ensemble composed of **P1**−pHis, as the emission of the ensemble maintained almost unchanged for the long wavelength emission and only slightly changed for the short wavelength emission (Figure S3 in the Supporting Information).

Scheme 2. Schematic Illustration of the Design Rationale for the Emission Shift Assay for Trypsin



dx.doi.org/10.1021/am405120y | ACS Appl. Mater. Interfaces 2014, 6, 918−924

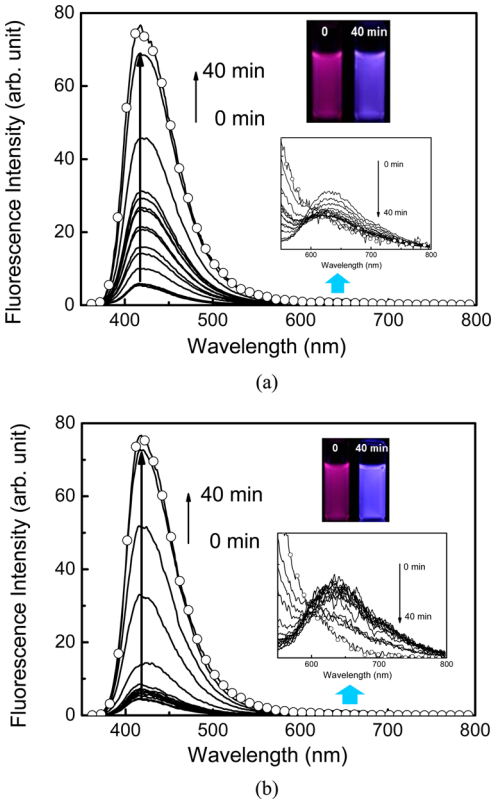


Figure 2. Changes in fluorescence spectra of (a) **P1**−pLys and (b) **P1**−pArg ensembles in the presence of trypsin (30 μg/mL) after incubation at 37 °C during different incubation times (0, 5, 10, 20, 40 s; 1, 1.5, 2, 4, 8, 10, 20, 30, 40 min). [**P1**] = 1×10^{-4} M (based on repeat units); [pLys] and [pArg] = 20 μg/mL. Inset shows photographs of the aqueous solutions of (a) **P1**−pLys and (b) **P1**−pArg in the presence of trypsin (30 μg/mL) for incubation times of 0 and 40 min under UV illumination (365 nm). Excitation wavelength: λ_{ex} = 350 nm. Curves with empty circles represent the spectra of **P1** without polypeptides.

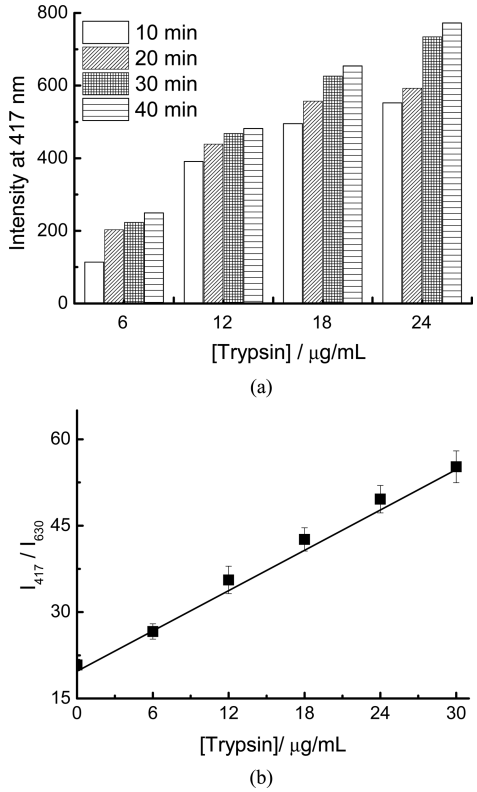


Figure 3. (a) Effects of incubation time and the concentration of trypsin on degradation efficiency of trypsin and (b) changes in the fluorescence intensity of **P1**−pArg, with respect to the concentrations of trypsin for 40 min of incubation time. Excitation wavelength: λ_{ex} = 350 nm. [**P1**] = 1×10^{-4} M; [pArg] = 20 μg/mL. I_0 and I correspond to the emission intensities of **P1**−pArg ensemble at 417 nm in the absence and presence of trypsin, respectively.

These observations clearly indicate the high specificity of trypsin toward enzymatic degradation of lysine and arginine. The enzymatic degradation at the lysine and arginine linkages resulted in specific dissociation of pLys and pArg in the ensemble of **P1**−pLys and **P1**−pArg, respectively, and the fluorescence of **P1** was then recovered to the original blue emission. For a further assay test, the ensemble of **P1**−pArg was employed, because this ensemble was found to be beneficial for a prolonged incubation time, compared to that of **P1**−pLys.

The changes in the emission spectra of the ensemble of **P1**−pArg were also investigated with variable concentrations of trypsin. The ensemble of **P1** (1×10^{-4} M) and pArg (20 μg/mL) was employed in the presence of different amounts of trypsin (6, 12, 18, and 24 μg/mL). Each solution was incubated at 37 °C for various time intervals, and the emission spectra of the solutions were then investigated. Figure 3a exhibits the emission intensity at 417 nm versus the reaction time for the **P1**−pArg in the presence of trypsin with different concentrations during different incubation times.

Changes in the size of **P1** before and after the addition of pArg were investigated via a dynamic light scattering (DLS) technique, and an increase in size was measured (from 91 nm to 230 nm). After treatment of trypsin, the size of **P1**−pArg was decreased to 151 nm, which is in good agreement with quantum yields (QYs) of such systems, as shown in Table S1 in the Supporting Information.

To gain further insight into the linear relationship between fluorescence intensity and trypsin concentration, the emission intensities of **P1**−pArg ensemble at 417 nm were monitored with various concentrations of trypsin (Figure 3b). It is noted that the emission intensity at 417 nm increases after the addition of trypsin with prolonged incubation time. Moreover, as the trypsin concentration in the solution increased, the fluorescence intensity of the ensemble increased more rapidly. From the fluorescence changes of the ensemble upon exposure to the trypsin, the limit of detection (3σ/slope) was determined to be 37.1 ng/mL (1.59 nM based on molecular weight), as estimated according to a previously reported method.[63−65]

It is known that trypsin is highly activated at pH 8.0 and in the presence of Ca^{2+}, because Ca^{2+} plays the role of activating trypsin and increasing its stability against self-digestion.[34,66,67] The dependence of the relative fluorescence intensity (see Figure S4 in the Supporting Information). The rate of fluorescence intensity increased dominantly in the presence of Ca^{2+} but decreased when further Ca^{2+} was added above the optimal concentration (in this case, 5×10^{-3} M). It is presumed that, instead of boosting the enzymatic activity of trypsin, the Ca^{2+} ions with excess concentration interacted with the anionic **P1**, because of the electrostatic interaction between the cationic Ca^{2+} ion and negatively charged polymer.

In order to clarify the selectivity of this assay ensemble, control experiments were carried out with trypsin analogues

such as pepsin and glucose oxidase (GOx). Aqueous solutions containing blue-emitting **P1** and a pArg ensemble were tested with 30 μg/mL of pepsin or GOx under the same experimental conditions used for the trypsin assay. In contrast to the case of trypsin, much fewer changes in emission intensity were observed in the cases of pepsin and Gox (see Figures 4a and 4b). Only the addition of trypsin could result in the maximum fluorescence change, which demonstrated the high specificity of the trypsin sensing platform, as shown in Figure 4c. We investigated trypsin in the mixture of these proteases and found that other proteases have no notable influences on trypsin detection.

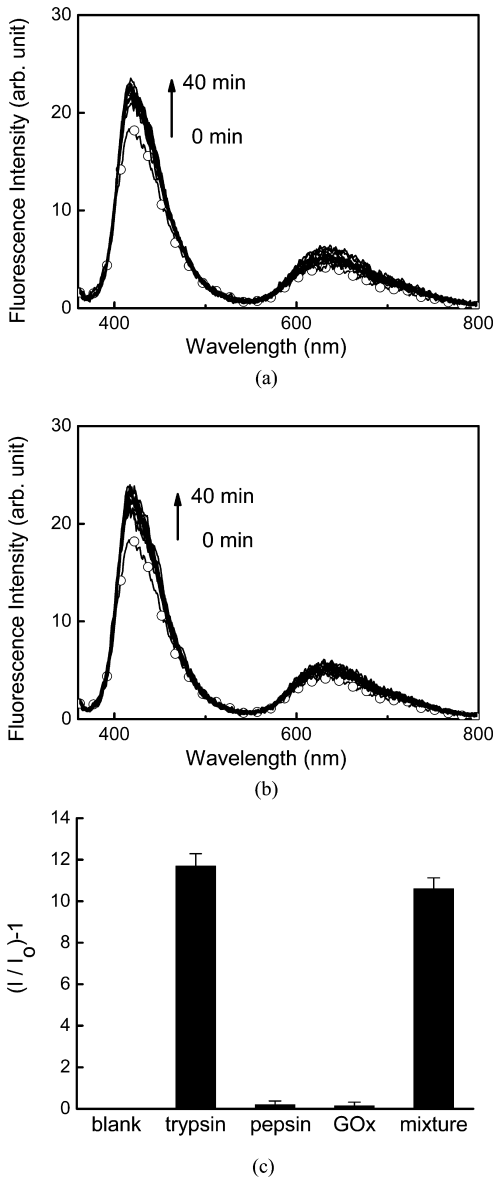


Figure 4. Selectivity for trypsin over other proteases according to incubation time: (a) pepsin and (b) glucose oxidase (GOx). (c) Relative efficiency of degradation upon addition of enzymes. Mixture includes trypsin, pepsin, and GOx. Excitation wavelength: λ_{ex} = 350 nm. [**P1**] = 1 × 10^{-4} M, [pArg] = 20 μg/mL, and [proteases] = 30 μg/mL. Curves with empty circles represent the spectra of **P1**−pArg without polypeptides.

These results clearly demonstrate that the enzymatic hydrolysis of pArg by trypsin specifically caused the emission color tuning. Therefore, the **P1**−pArg ensemble can be used as a fluorescent assay for the specific detection of trypsin with negligible interference from other nonspecific proteases. In terms of noticeable blue-to-red color change, this system has potential advantages over other turn-off and turn-on systems, which enables naked-eye detection.

CONCLUSIONS

We successfully developed a new fluorescent detection method with an ensemble of conjugated polymer and polypeptide for a trypsin assay. This new detection method is developed to exploit the manipulation of electrostatic interactions between the polymer and polypeptide in the presence of trypsin, resulting in the possible tuning of fluorescence emissions of the polymer. Blue-emitting, anionically charged conjugated polymer containing TBT units showed aggregation in the presence of positively charged molecules such as polypeptide, exhibiting blue-to-red emission color change. The ensemble composed of the polymer and pArg can be dissociated to each species, leading to the recovery of the short wavelength blue emission color, which can be utilized to detect trypsin with a concentration of 37.1 ng/mL; thus, acceptable sensitivity of this new assay for trypsin is obtainable. Both the polymer and pArg are facilely accessible, and detection can be accomplished in water-based solutions. Because of such simplicity, possible naked-eye detection, and high sensitivity, this fluorometric assay ensemble is potentially useful for high-throughput screening, which may be beneficial to analyze pancreatic disease-related fields, in which trypsin plays a key function.

ASSOCIATED CONTENT

Supporting Information

Absorption and fluorescence spectra of the polymer, with polyhistidine in the presence and absence of trypsin, and the effect of the Ca^{2+} ion. This material is available free of charge via the Internet at http://pubs.acs.org.

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Notes

The authors declare no competing financial interest.

ACKNOWLEDGMENTS

This research was supported by Basic Science Research Program (No. 2012R1A2A2A01004979) through the National Research Foundation of Korea (NRF), funded by Korean government.

REFERENCES

(1) *Handbook of Conducting Polymers*, 2nd Edition; Skotheim, T. A., Elsenbaumer, R. L., Reynolds, J. R., Eds.; Marcel Dekker: New York, 1997.
(2) *Semiconducting Polymers*; Hadziioannou, G., van Hutten, P. F., Eds.; Wiley−VCH: Weinheim, Germany, 2000.
(3) *Conducting Polymers, Fundamentals and Applications*; Chandrasekhar, P., Ed.; Kluwer Academic Publishers: Boston, 1999.
(4) Swager, T. M. *Acc. Chem. Res.* **1998**, *31*, 201−207.
(5) Thomas, S. W., III; Joly, G. D.; Swager, T. M. *Chem. Rev.* **2007**, *107*, 1339−1386.
(6) Bunz, U. H. F. *Chem. Rev.* **2000**, *100*, 1605−1644.
(7) Liu, B.; Bazan, G. C. *Chem. Mater.* **2004**, *16*, 4467−4476.

(8) Ho, H. A.; Najari, A.; Leclerc, M. *Acc. Chem. Res.* **2008**, *41*, 168−178.
(9) Gaylord, B. S.; Heeger, A. J.; Bazan, G. C. *Proc. Natl. Acad. Sci. U.S.A.* **2002**, *99*, 10954−10957.
(10) Wang, S.; Gaylord, B. S.; Bazan, G. C. *J. Am. Chem. Soc.* **2004**, *126*, 5446−5451.
(11) Liu, B.; Bazan, G. C. *J. Am. Chem. Soc.* **2006**, *128*, 1188−1196.
(12) Lee, K.; Povlich, L. K.; Kim, J. *Adv. Funct. Mater.* **2007**, *17*, 2580−2587.
(13) Kwak, C. K.; Kim, D. G.; Kim, T. H.; Lee, C.-S.; Lee, M.; Lee, T. S. *Adv. Funct. Mater.* **2010**, *20*, 3847−3855.
(14) Kim, J.; McQuade, D. T.; McHugh, S. K.; Swager, T. M. *Angew. Chem., Int. Ed.* **2000**, *39*, 3868−3872.
(15) Wang, Y.; Zhan, R.; Li, T.; Pu, K.-Y.; Wang, Y.; Tan, Y. C.; Liu, B. *Langmuir* **2012**, *28*, 889−895.
(16) An, L.; Tang, Y.; Feng, F.; He, F.; Wang, S. *J. Mater. Chem.* **2007**, *17*, 4147−4152.
(17) Feng, F.; Liu, L.; Yang, Q.; Wang, S. *Macromol. Rapid Commun.* **2010**, *31*, 1405−1420.
(18) Alfonta, L.; Blumenzweig, I.; Zayats, M.; Baraz, L.; Kotler, M.; Willner, I. *ChemBioChem* **2004**, *5*, 949−957.
(19) Hirota, M.; Ohmuraya, M.; Baba, H. *J. Gastroenterol.* **2006**, *41*, 832−836.
(20) Rinderknecht, H. *Dig. Dis. Sci.* **1986**, *31*, 314−321.
(21) Lankisch, P. G.; Burchard-Reckert, S.; Lehnick, D. *Gut* **1999**, *44*, 542−544.
(22) Yamashita, K.; Mimori, K.; Inoue, H. *Cancer Res.* **2003**, *63*, 6575−6578.
(23) Caughey, G. H. *Immunol. Rev.* **2007**, *217*, 141−145.
(24) Yamamoto, H.; Iku, S.; Adachi, Y.; Imsumran, A.; Taniguchi, H.; Nosho, K.; Min, Y.; Horiuchi, S.; Yoshida, M.; Itoh, F.; Imai, K. *J. Pathol.* **2003**, *199*, 176−184.
(25) Olsen, J. V.; Ong, S. E.; Mann, M. *Mol. Cell. Proteomics* **2004**, *3*, 608−614.
(26) Wang, Y.; Zagorevski, D. V.; Stenken, J. A. *Anal. Chem.* **2008**, *80*, 2050−2057.
(27) Kim, Y.; Lee, B. S.; Kim, E.; Choi, I. S.; Moon, D. W.; Lee, T. D.; Kim, H. *Anal. Chem.* **2008**, *80*, 5094−5102.
(28) Michaud, D. *Anal. Chim. Acta* **1998**, *372*, 173−185.
(29) Gutiérrez, O. A.; Salas, E.; Hernández, Y.; Lissi, E. A.; Castrillo, G.; Reyes, O.; Garay, H.; Aguilar, A.; García, B.; Otero, A.; Chavez, M. A.; Duarte, C. A. *Anal. Biochem.* **2002**, *307*, 18−24.
(30) Li, Y.; Bai, H.; Li, C.; Shi, G. *ACS Appl. Mater. Interfaces* **2011**, *3*, 1306−1310.
(31) Xue, W.; Zhang, G.; Zhang, D. *Analyst* **2011**, *136*, 3136−3141.
(32) Gu, X.; Yang, G.; Zhang, G.; Zhang, D.; Zhu, D. *ACS Appl. Mater. Interfaces* **2011**, *3*, 1175−1179.
(33) Zauner, T.; Berger-Hoffmann, R.; Muller, K.; Hoffmann, R.; Zuchner, T. *Anal. Chem.* **2011**, *83*, 7356−7363.
(34) Xue, W.; Zhang, G.; Zhang, D.; Zhu, D. *Org. Lett.* **2010**, *12*, 2274−2277.
(35) Lowe, S. B.; Dick, J. A. G.; Cohen, B. E.; Stevens, M. M. *ACS Nano* **2012**, *8*, 851−857.
(36) Feng, F.; Tang, Y.; Wang, S.; Li, Y.; Zhu, D. *Angew. Chem., Int. Ed.* **2007**, *46*, 7882−7886.
(37) Wang, Y.; Zhang, Y.; Liu, B. *Anal. Chem.* **2010**, *82*, 8604−8610.
(38) Xue, W.; Zhang, G.; Zhang, D.; Zhu, D. *Org. Lett.* **2010**, *12*, 2274−2277.
(39) Li, W.; Chen, J.; Jiao, H.; Zhang, Q.; Zhou, H.; Yu, C. *Chem. Commun.* **2012**, *48*, 10123−10125.
(40) Farmer, W. H.; Yuan, Z. *Anal. Biochem.* **1991**, *197*, 347−352.
(41) An, L.; Tang, Y.; Feng, F.; He, F.; Wang, S. *J. Mater. Chem.* **2007**, *17*, 4147−4152.
(42) Zhu, Q.; Zhan, R.; Liu, B. *Macromol. Rapid Commun.* **2010**, *31*, 1060−1064.
(43) Dwivedi, A. K.; Iyer, P. K. *J. Mater. Chem. B* **2013**, *1*, 4005−4010.
(44) Hu, L.; Han, S.; Parveen, S.; Yuan, Y.; Zhang, L.; Xu, G. *Biosens. Bioelectron.* **2012**, *32*, 297−299.
(45) Cordovilla, C.; Swager, T. M. *J. Am. Chem. Soc.* **2012**, *134*, 6932−6935.
(46) Cleemann, F.; Karuso, P. *Anal. Chem.* **2008**, *80*, 4170−4174.
(47) Yu, D.; Zhang, Y.; Liu, B. *Macromolecules* **2008**, *41*, 4003−4011.
(48) Pu, K.-Y.; Zhan, R.; Liu, B. *Chem. Commun.* **2010**, *46*, 1470−1472.
(49) Lee, J. H.; Kim, D. G.; Kwon, N. Y.; Jang, G. S.; Son, J. H.; Lee, M.; Cho, H.-J.; Kweon, H.-S.; Lee, T. S. *J. Polym. Sci., Part A: Polym. Chem.* **2011**, *49*, 138−146.
(50) Pu, K.-Y.; Liu, B. *Macromolecules* **2008**, *41*, 6636−6640.
(51) Shi, H.; Sun, H.; Yang, H.; Liu, S.; Jenkins, G.; Feng, W.; Li, F.; Zhao, Q.; Liu, B.; Huang, W. *Adv. Funct. Mater.* **2013**, *23*, 3268−3276.
(52) Satrijo, A.; Swager, T. M. *J. Am. Chem. Soc.* **2007**, *129*, 16020−16028.
(53) Kwon, N. Y.; Kim, D.; Son, J. H.; Jang, G. S.; Lee, J. H.; Lee, T. S. *Macromol. Rapid Commun.* **2011**, *32*, 1061−1065.
(54) Kwon, N. Y.; Kim, D.; Jang, G.; Lee, J. H.; So, J.-H.; Kim, C.-H.; Kim, T. H.; Lee, T. S. *ACS Appl. Mater. Interfaces* **2012**, *4*, 1429−1433.
(55) Disney, M. D.; Zheng, J.; Swager, T. M.; Seeberger, P. H. *J. Am. Chem. Soc.* **2004**, *126*, 13343−13346.
(56) Yang, R.; Tian, R.; Yan, J.; Zhang, Y.; Yang, J.; Hou, Q.; Yang, W.; Zhang, C.; Cao, Y. *Macromolecules* **2005**, *38*, 244−253.
(57) Hou, Q.; Xu, Y.; Yang, W.; Yuan, M.; Peng, J.; Cao, Y. *J. Mater. Chem.* **2002**, *12*, 2887−2892.
(58) Vetrichelvan, M.; Hairong, L.; Ravindranath, R.; Valiyaveettil, S. *J. Polym. Sci., Part A: Polym. Chem.* **2006**, *44*, 3763−3777.
(59) Bao, Y.; Li, Q.; Liu, B.; Du, F.; Tian, J.; Wang, H.; Wang, Y.; Bai, R. *Chem. Commun.* **2012**, *48*, 118−120.
(60) Kim, S.; Jackiw, J.; Robinson, E.; Schanze, K. S.; Reynolds, J. R.; Baur, J.; Rubner, M. F.; Boils, D. *Macromolecules* **1998**, *31*, 964−974.
(61) Schwartz, B. J. *Annu. Rev. Phys. Chem.* **2003**, *54*, 141−172.
(62) Pu, K.-Y.; Cai, L.; Liu, B. *Macromolecules* **2009**, *42*, 5933−5940.
(63) Thomsen, V.; Schatzlein, D.; Mercuro, D. *Spectroscopy* **2003**, *18*, 112−114.
(64) *Orange Book: IUPAC Compendium of Analytical Nomenclature*, 2nd Edition; Freiser, H., Nancollas, G. H., Eds.; Blackwell Scientific Publications: Oxford, U.K., 1987; p 5.
(65) Zhu, B.; Gao, C.; Zhao, Y.; Liu, C.; Li, Y.; Wei, Q.; Ma, Z.; Du, B.; Zhang, X. A. *Chem. Commun.* **2011**, *47*, 8656.
(66) Sipos, T.; Merkel, R. J. *Biochemistry* **1970**, *9*, 2766−2775.
(67) Hu, L.; Han, S.; Parveen, S.; Yuan, Y.; Zhang, L.; Xu, G. *Biosens. Bioelectron.* **2012**, *32*, 297−299.

NOTE ADDED AFTER ASAP PUBLICATION

This paper published to the Web on December 31, 2013, with an incorrect journal title for ref 43. It was changed to J. Mater. Chem. B, and the corrected version was republished on January 2, 2014.