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An amphiphilic asymmetric diblock copolymer with pH responsive fluorescent properties

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Abstract

Stimuli-responsive polymers with changeable fluorescent properties have numerous applications in sensing, bioimaging, and detection. Here we describe a facile synthesis of a pH-responsive amphiphilic asymmetric diblock copolymer of acrylic acid and butyl acrylate that incorporates a polarity-sensitive fluorophore. The asymmetric structure enhances the stimuli-responsive behavior, as the fluorescent intensity of the asymmetric diblock copolymer gradually increases as the environmental pH decreases whereas its symmetric block counterpart shows limited and stepwise change. Besides, this remarkable difference was demonstrated to be concentration independent as similar emission behavior was found for both polymers at lower concentrations. These results

indicate that the fluorescence properties of the copolymer can be adjusted by rationally designing the copolymer structure. This work provides a novel strategy for the design and synthesis of polymeric materials with stimuli-responsive fluorescent properties, to be applied as fluorescent probes.

Introduction

Fluorescent detecting technology has been widely applied in biological, material, and environmental sciences such as biological imaging,¹ substance detection,² and fluorescent switch³ due to its advantages including non-destructive detection,⁴ selective identification,⁵ high sensitivity,⁶ and fast response.⁷ Fluorescent probes, such as fluorescent proteins,⁸ nanoparticles,^{9, 10} organic small molecules,¹¹ and copolymers¹² can selectively convert microscopic changes into fluorescent signals that can be analyzed and detected. Among them, fluorescent copolymers have attracted significant attention due to their high stability, signal amplification, designable structure, and high water solubility.¹³

Stimuli-responsive copolymers which can respond to external stimuli or changes in the surrounding environment,^{14, 15} for example, pH,^{16, 17} temperature,^{18, 19} light,²⁰⁻²² magnetism,²³ and other types to make adaptable changes have aroused considerable interest.²⁴ Copolymers containing pH-responsive ionizable groups²⁵ such as carboxylic acids and amines have been extensively investigated. Their degree of ionization changes with the variation of pH, leading to changes in the properties of the copolymer solution.²⁶ By incorporating a polarity-sensitive fluorophore into the pH-responsive polymer structure, the fluorescence intensity of the polymer can be used as an output signal to sense the local environmental changes²⁷ and therefore used as a fluorescent probe²⁸ for realizing smart applications.²⁹

Controlled radical polymerization, including atom transfer radical polymerization

(ATRP)³⁰ and reversible addition-fragmentation chain transfer (RAFT) polymerization,³¹ is a powerful strategy to make polymers with specific monomer composition profile, topologies, molar masses, and properties, which enables researchers to synthesize polymers on demand.³²⁻³⁴ Through rational design of the stimuli-responsive copolymer structure, dynamic response to their environmental changes can be realized.³⁵⁻³⁷ Asymmetric copolymers, such as gradient copolymers and block copolymers having mixed segments in one or both of the blocks, have intriguing properties in terms of microphase separation and self-assembly.³⁸⁻⁴⁰ For example, a styrene/methyl methacrylate gradient copolymer prepared by He et al. exhibited dynamic self-assembly behavior responding to changing temperature, while the symmetric block copolymer displayed kinetically trapped self-assemblies.⁴¹ In our previous study, an asymmetric diblock copolymer of acrylic acid (AA) and butyl acrylate (BA) displayed dynamic self-assembly behavior in responding to the pH variation. Reversible changes in size were observed and the morphologies of the self-assemblies transitioned from spherical micelles to wormlike micelles to vesicles.⁴² In this work, a polarity-sensitive fluorophore (DBD-AE)⁴³ was incorporated into an asymmetric diblock copolymer with the molar mass of 10 kg mol⁻¹ (FD10), which consists of 84 mol% AA and 16 mol% BA as the first block followed by 16 mol% AA and 84 mol% BA as the second block with 0.05 mol% DBD-AE evenly distributed in each block.⁴² A fluorescent symmetric block copolymer with the same molar mass consisting of two equal segments of polyAA and polyBA (FB10) incorporated with 0.05 mol% DBD-AE in each block was also synthesized as a control polymer. Two other copolymers with the same AA and BA distributions as FD10 and FB10 but containing no DBD-AE units, D10 and B10, were prepared to verify that the fluorescence was caused by DBD-AE units rather than other groups.

Results and Discussion

FD10 and FB10 were synthesized through one-pot RAFT polymerization of tert-butyl acrylate (tBA), BA, and DBD-AE, followed by the acidolysis of tBA with trifluoroacetic acid. The detailed synthetic process of all polymers can be found in the **Supporting Information (Scheme S1 and S2, Figure S1, Table S1)**. Size exclusion chromatography (SEC) of FD10 precursor (**Figure 1a**) displayed a monomodal shape and narrow polydispersity (\mathcal{D}) for each block and a clear shift to higher molar mass after chain extension, indicating that the polymerization was well-controlled. The ^1H NMR spectra of FD10 precursor (**Figure 1b**) after acidolysis revealed the complete disappearance of tert-butyl group indicating the formation of carboxylic group. In addition, the peak at 2.84 ppm corresponds to the protons of methyl groups connected to the sulfonyl group of DBD-AE, suggesting the fluorescent unit DBD-AE has been successfully incorporated into the copolymer structure. For the characterization of FB10, D10, and B10, see the **Supporting Information (Figures S2 and S3)**.

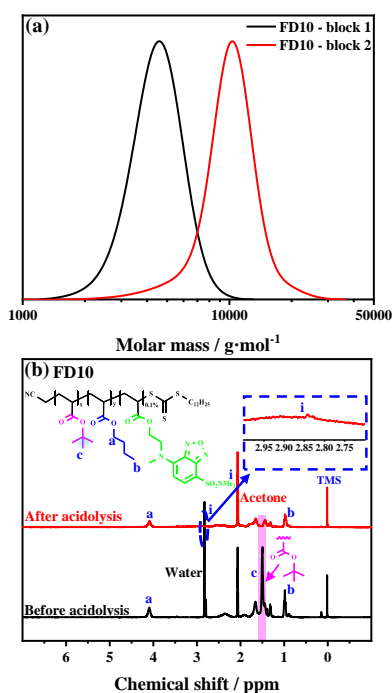


Figure 1. SEC curves of FD10 precursor (a) and ^1H NMR spectra of FD10 precursor before and after acidolysis (b).

The ultraviolet and visible (UV-Vis) spectra and digital photos under natural and UV light of FD10, FB10, D10, and B10 in aqueous solution (2 mg/mL) at pH 7 are displayed in **Figures 2a** and **2b**, respectively. FD10 and FB10 showed a maximum absorption at around 440 nm whereas D10 and B10 showed no UV absorbance. These results confirmed that the fluorescence of FD10 and FB10 was caused by the DBD-AE fluorophore. This was due to the polar benzoxadiazole group of DBD-AE, which absorbs strongly through its conjugated π - π bonds.⁴³ In addition, it is worth to note that simply mixing the fluorophore DBD-AE with either D10 or B10 in solution showed neither absorption nor fluorescence.

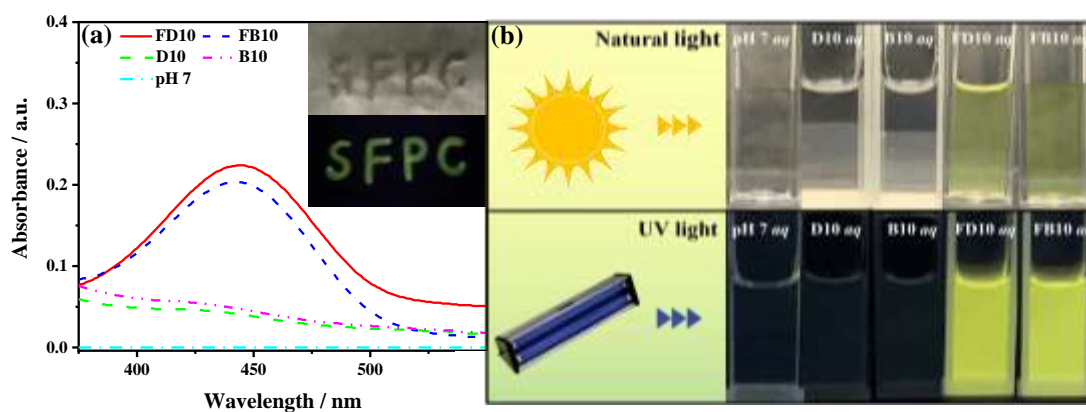


Figure 2. UV-vis spectra of different copolymers of 2 mg/mL in pH 7 buffer (inset is the digital photos FD10 aqueous solution writing pattern under natural light (top) and UV light (bottom, $\lambda = 365$ nm)) (a) and digital photos under natural light and UV light ($\lambda = 365$ nm) (b).

The UV-Vis spectra of FD10 (**Figure 3a**) and FB10 (**Figure 3b**) in aqueous solution of 2 mg/mL at different pH showed maximum absorptions at about 440 nm for both copolymers. As the pH decreased from 10 to 5, the absorbance increased while the maximum absorption wavelength exhibited a blue shift. Interestingly, FD10 demonstrated a more gradual increase in maximum absorbance, whereas the maximum absorbance of FB10 stayed nearly constant as the pH decreased from 10 to 6 before abruptly increasing when the pH decreased to 5.

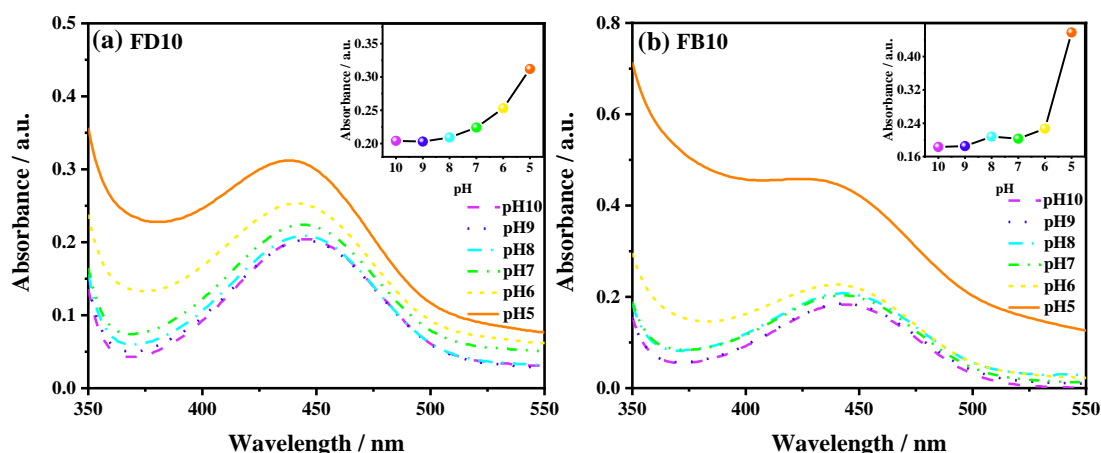


Figure 3. UV-vis spectra of FD10 (a) and FB10 (b) in 2 mg/mL aqueous solution at different pH.

Aqueous solutions of FD10 (**Figure S4a**) and FB10 (**Figure S4b**) (2 mg/mL at pH 7) showed maximum emissions at excitation wavelengths of 444 nm (FD10) and 442 nm (FB10). In addition, FD10 showed generally stronger emission in comparison with FB10.

The fluorescent properties of FD10 (**Figure 4a**) and FB10 (**Figure 4b**) in 2 mg/mL aqueous solution were further analyzed by varying the pH. The asymmetric copolymer, FD10, exhibited a dynamic pH response as the fluorescence intensity gradually increased while decreasing the pH. In contrast, the block copolymer, FB10, showed nearly constant fluorescence intensity as the pH decreased from 10 to 6 and only a slight increase when the pH further decreased to 5. Notably, FD10 showed a much stronger fluorescence intensity than FB10 at pH 5. In addition, a blue shift was observed for the emission maxima of FD10: decreasing the solution pH from 10 to 5 caused a shift in the emission peak from 545.6 nm to 541.4 nm. In contrast, FB10 presented a red shift, with a shift in the emission peak from 540.6 nm to 543 nm over the same pH range.

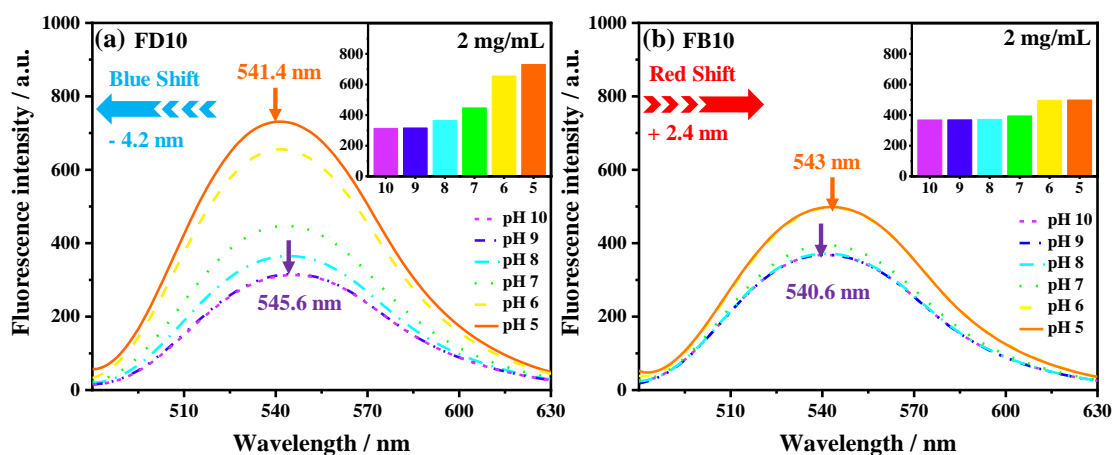


Figure 4. Fluorescence intensity of 2 mg/mL for FD10 (a) and FB10 (b) at different pH.

The small response of FB10 corresponded well to a frozen system which can be verified by the dynamic light scattering (DLS) analysis as the sizes of the self-assemblies were nearly constant with changing pH (**Figure 5a**), in agreement with our previous studies on non-fluorescent polymer.^{42, 44} We assumed that nearly all the fluorescence that was observed came from the core of the micelles, which were unaffected by changes in pH as mixtures of fluorophore DBD-AE with D10 or B10 showed no fluorescence. Once the pH decreased to 5, the degree of ionization of AA groups significantly decreased, and some fluorescence from the corona would be observed (especially the parts that were close to the core). This is where we previously⁴² saw aggregation of poly(BA-*b*-AA) block copolymer micelles. It also explains why the peak maxima shifted to higher wavelengths, as fluorescence from areas that contained mostly AA and were more polar than the core started to be observed, thus causing the red shift (**Figure 5c**).^{45, 46} However, for FD10, as AA groups existed in the core of the micelles, it was possible to change the environment in the core by changing the pH.⁴⁷ Under acidic conditions, the degree of ionization of the AA groups decreased which decreased the polarity, and *vice versa*.⁴⁴ As a result, the peak maximum shifted to higher wavelengths with increasing pH as the micelle core became more polar (unlike FB10, which did not change). Additionally, the dynamic self-assembly of FD10 caused the particles to increase in size as the pH

decreased (**Figure 5b**), thus the core volume increased, and the core became more isolated from the surrounding water (**Figure 5c**). This could explain both the increase in fluorescence intensity and the greater intensity compared to FB10.

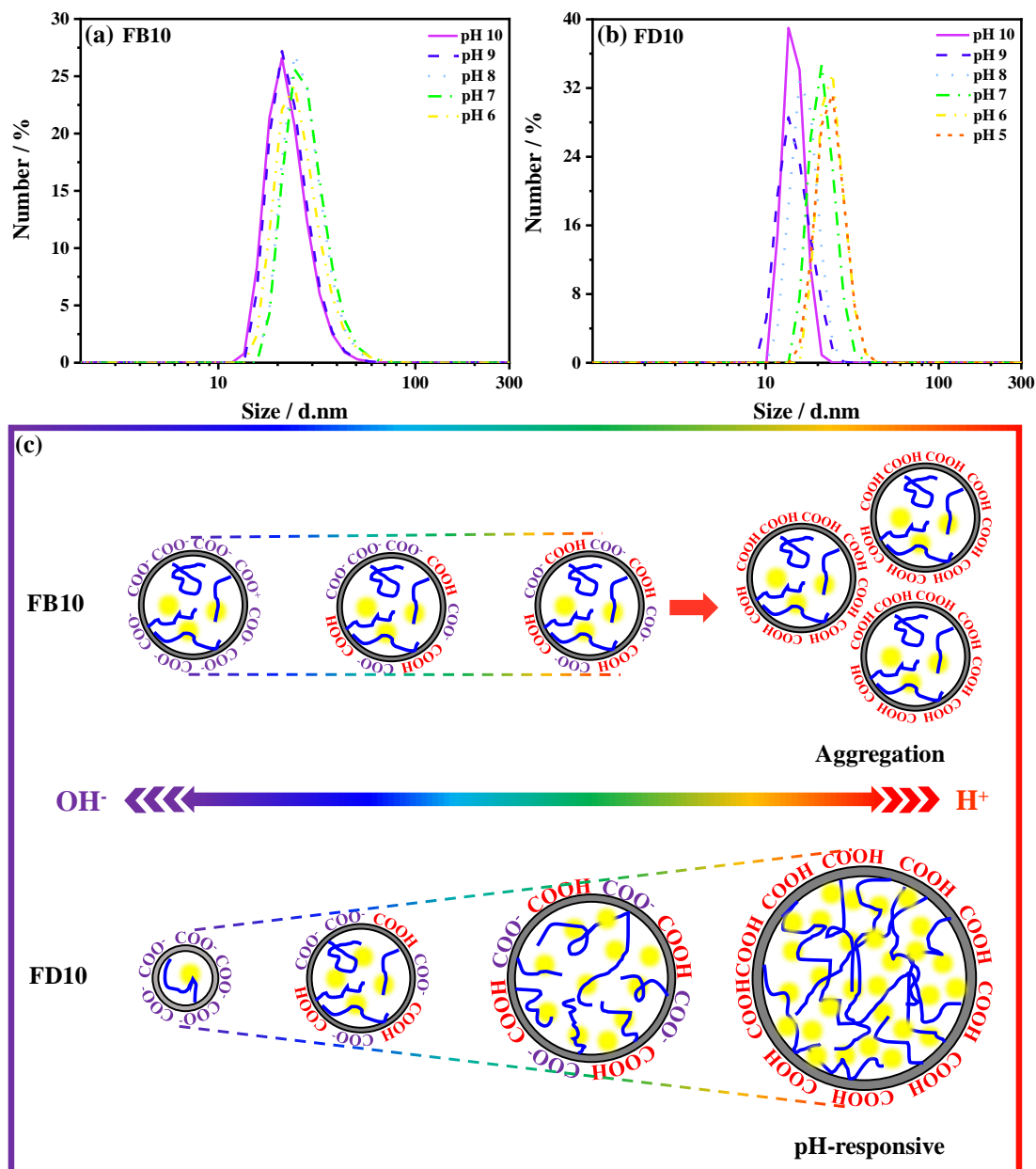


Figure 5. Size distributions of 2 mg/mL FB10 (a) and FD10 (b) at different pH analyzed by DLS; (c) Mechanism of the change in fluorescence intensity with changing pH for FB10 (top) and FD10 (bottom).

Analysis of the fluorescence emission at lower concentrations demonstrated that this remarkable difference of response between FD10 and FB10 was independent of

concentration. FD10 showed a similar dynamic response (**Figure S5 (a-a')**) while FB10 still showed limited and stepwise response (**Figure S5 (b-b')**) at lower concentrations of 0.1 and 0.5 mg/mL, respectively. However, stronger fluorescent intensity was observed for both FD10 and FB10 at higher concentrations.

Emission intensity of fluorescent polymer has been found to be highly concentration dependent. The fluorescence emission of FD10 and FB10 was measured for a wide range of concentrations from 0.01 to 50 mg/mL at pH 7 (**Figure 6** and **Figure S6**). The emission intensity of both FD10 and FB10 increase with increasing concentration from 0.01 to 10 mg/mL. Meanwhile, the emission wavelength showed a pronounced red shift. It is noteworthy that the emission wavelength of FD10 was relatively constant for > 1 mg/mL, while FB10 varied significantly with concentration. A linear relationship between the concentration and fluorescent intensity was observed in the range of 0.01 to 2 mg/mL (**Figure S7**). Above 2 mg/ml, the emission intensity started to deviate from the linear relationship, reaching a maximum at 10 mg/mL before decreasing at even higher concentrations. Digital photos of FD10 and FB10 solution at various concentrations under natural light and UV light clearly show the change of color and brightness (**Figure S8**), which was consistent with the evolution of fluorescent emission. The fluorescence quenching and red shift of wavelength emission maxima at concentrations over 10 mg/mL was mainly ascribed to non-radiative energy transfer between ground and excited states caused by the intermolecular aggregation when individual polymer chains were close enough. Furthermore, the inner-filter effect at high concentration of fluorophore resulted from the partial overlap of emission band and absorption band also contributed to the significant fluorescence extinction.^{48, 49}

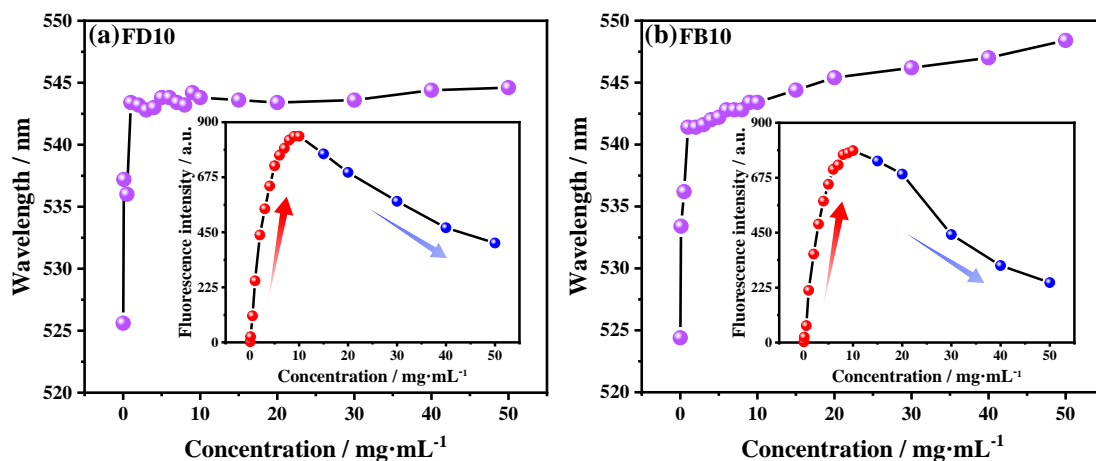


Figure 6. Fluorescence emission behavior of FD10 (a) and FB10 (b) solution at different concentrations at pH 7.

Conclusions

In summary, a pH responsive amphiphilic asymmetric diblock copolymer FD10 incorporated with a microenvironmental polarity sensitive fluorophore DBD-AE was prepared through a facile one-pot RAFT polymerization of tBA, BA, and DBD-AE, followed by the acidolysis of tert-butyl group. The fluorescent emission behavior of FD10 was remarkably different to its symmetric block counterpart FB10. The fluorescent intensity of FD10 showed a dynamic pH response, displaying a gradual increase as pH decreased, whereas FB10 demonstrated nearly identical fluorescent intensity with the change of pH. Notably, this difference in response was independent of concentration. FD10 and FB10 demonstrated a concentration dependent fluorescent behavior as the emission intensity increased with increasing the concentration when the concentration was lower than 10 mg/mL while fluorescence quenching was observed at higher concentrations. Our finding reveals the importance of the polymer structure to its properties, particularly when dynamically responsive systems are desired. This work provides a novel strategy for the design and synthesis of dynamic polymeric materials with stimuli-responsive fluorescent properties for imaging, detecting, and sensing.

Acknowledgements

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Conflict of interest

The authors declare no conflict of interest.

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