

Supporting Information

Mesoscopic structural aspects of Ca^{2+} -triggered polymer chain folding of a tetraphenylethene-appended poly(acrylic acid) in relation to its aggregation-induced emission behavior

Ken Morishima, Fumitaka Ishiwari, Satoko Matsumura, Takanori Fukushima, and

Mitsuhiro Shibayama*

*To whom correspondence should be addressed.

E-mail: sibayama@issp.u-tokyo.ac.jp

Table of Contents

Materials and Methods	S2
1. Materials	S2
2. General.....	S2
3. Evaluation of the apparent dissociation constant (K_d).....	S3
4. DLS and SLS measurements	S3
5. Synthesis	S4
Supporting Figures (Figures S1–S7)	S5
References	S10

Materials and Methods

1. Materials. Unless otherwise noted, all the commercial reagents were used as received. 4-(1,2,2-triphenylvinyl)phenyl acrylate (**1**) was prepared according to previously reported procedures¹ and unambiguously characterized by nuclear magnetic resonance (NMR) spectroscopy. Prior to use, *t*-butyl acrylate (**2**) was purified by passage through Al₂O₃ column to remove polymerization inhibitors. Azobisisobutyronitrile (AIBN) was purified by recrystallization from methanol.

2. General. Unless otherwise noted, all the experiments were conducted at 25 °C. Analytical GPC was performed at 40 °C regulated by column oven on a TOSOH GPC-8020 system equipped with a refraction index (RI) detector and a UV detector (UV-8020) using chloroform (CHCl₃) as an eluent at a flow rate of 0.40 mL/min on a column (Shodex LF-804). The molecular weight calibration curve was obtained by using standard polystyrenes (TSKstandard polystyrene, TOSOH). NMR spectroscopy measurements were carried out at 25 °C on a Bruker AVANCE-400 spectrometer (400 MHz for ¹H). Chemical shifts (δ) are expressed relative to the resonances of the residual non-deuterated solvent for ¹H (CDCl₃: ¹H(δ) = 7.26 ppm, CD₃OD ¹H(δ) = 3.31 ppm). Absolute values of the coupling constants are given in Hertz (Hz), regardless of their sign. Multiplicities are abbreviated as singlet (s), doublet (d), doublet of doublets (dd), triplet (t), quartet (q), multiplet (m), and broad (br). Infrared (IR) spectra were recorded at 25 °C on a JASCO FT/IR-660_{plus} Fourier-transform infrared spectrometer. Electronic absorption spectra were recorded using a quartz cell on a JASCO model V-670 UV/VIS spectrophotometer. Fluorescence spectra were recorded using a quartz cell on a JASCO model FP-6500 spectrophotometer. All the fluorescence spectra were measured at 25 °C by excitation at 307 nm (λ_{ex} = 307 nm).

3. Evaluation of the apparent dissociation constant (K_d). Ca^{2+} titration curves were obtained by measuring the fluorescence intensities of PAA-TPE_{0.01} under various Ca^{2+} concentrations. Because the number of effective Ca^{2+} -binding sites in PAA-TPE_{0.01} cannot be determined, a general stoichiometric analysis for determining the dissociation constant (K_d) is not applicable to these systems. Instead, we used the apparent K_d , which was obtained by fitting the Ca^{2+} titration curves with the following Hill's equation (1) using the least square method in R software (<http://www.R-project.org/>).

$$F = F_{\min} + \frac{(F_{\max} - F_{\min}) \times ([\text{Ca}^{2+}])^n}{([\text{Ca}^{2+}]) + (K_d)^n} \quad (1)$$

F : Fluorescence intensity

F_{\max} : Maximum fluorescence intensity

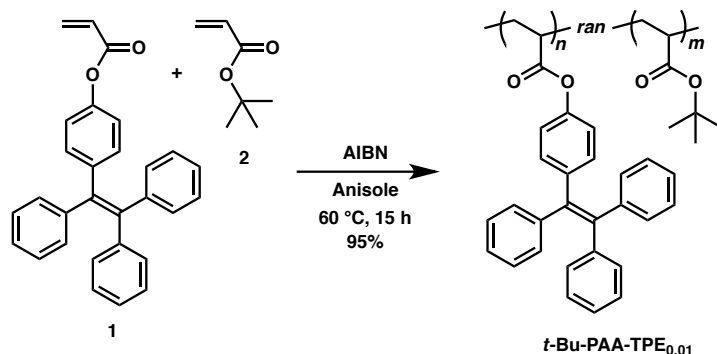
F_{\min} : Minimum fluorescence intensity

K_d : Apparent dissociation constant

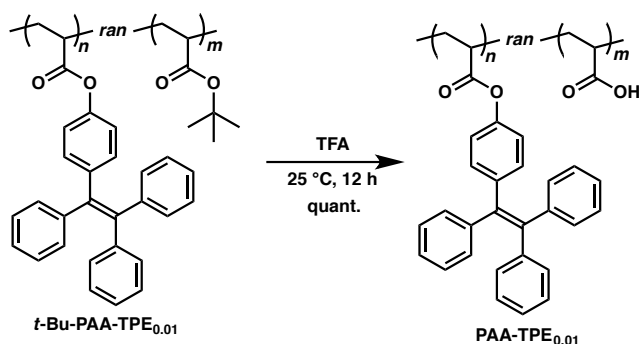
n : Apparent Hill coefficient

4. DLS and SLS measurements. DLS and SLS measurements were simultaneously conducted by using ALV/DLS-5000 light scattering system equipped with an ALV photon correlator (ALV, Germany) and a He-Ne laser (wavelength, $\lambda = 633$ nm) as an incident beam. The scattering intensity was detected at $\theta = 30^\circ$ – 120° of scattering angles. The measurement temperature was at 25°C . All the test solutions for light scattering measurement were optically cleaned by filtration through a $0.45\ \mu\text{m}$ pore-size membrane filter before adding salts. The specific refractive index increment $\partial n/\partial c$ of PAA-TPE_{0.01} in acetic acid (1.0 M) / methanol and HEPES buffer solution was determined to be 0.164 and $0.177\ \text{cm}^3/\text{g}$, respectively, using DRM-1021 (Otsuka Electronics, Japan). The time-autocorrelation function of the scattering intensity $g^{(2)}(\tau)$ measured by DLS was analyzed with CONTIN method to obtain the relaxation spectrum $G(\Gamma^{-1})$ of the relaxation time Γ^{-1} . The average-relaxation time was calculated as $\Sigma[\Gamma^{-1}G(\Gamma^{-1})]/\Sigma[G(\Gamma^{-1})]$. The scattering from toluene was referred to standard intensity to obtain the excess Rayleigh ratio R_θ .

5. Synthesis.



***t*-Bu-PAA-TPE_{0.01}:** An anisole solution (100 μL) of a mixture of monomer **1** (13.9 mg, 35 μmol), *t*-butyl acrylate (**2**, 500 μL , 3.4 mmol) and AIBN (2.8 mg, 17 μmol) was degassed by freeze-pump-thaw cycles (three times) and purged with argon. The mixture was stirred at 60 $^\circ\text{C}$ for 12 h, allowed to cool to 25 $^\circ\text{C}$, and then evaporated to dryness under a reduced pressure. The residue was freeze-dried from toluene to afford *t*-Bu-PAA-TPE_{0.01} as white solid (429 mg, 95%): ^1H NMR (400 MHz, CDCl_3) δ (ppm) 6.79–7.11 (br), 2.05–2.39 (br), 1.71–1.86 (br), 1.20–1.63 (br). FT-IR (KBr) ν (cm^{-1}) 2979, 2935, 1731, 1481, 1457, 1393, 1368, 1257, 1149, 1034, 909, 846, 751, 701, 471, 430. $M_n = 4.62 \times 10^5$ g/mol, $M_w = 5.64 \times 10^5$ g/mol, PDI=1.22 (SEC; based on polystyrene standards).



PAA-TPE_{0.01}: A trifluoroacetic acid (6 mL) was added to *t*-Bu-PAA-TPE_{0.01} (300 mg, 0.38 μmol). The mixture was stirred at 25 $^\circ\text{C}$ for 12 h and evaporated to dryness under a reduced pressure. The residual volatile compounds were azeotropically removed with methanol (10 mL, five times) to afford PAA-TPE_{0.01} quantitatively as white solid (298 mg): ^1H NMR (400 MHz, CD_3OD) δ (ppm) 6.79–7.21 (br), 2.28–2.65 (br), 1.40–2.22 (br). FT-IR (KBr) ν (cm^{-1}) 2961, 2361, 1716, 1503, 1454, 1417, 1249, 1168, 802, 701, 614, 503, 414.

Supporting Figures.

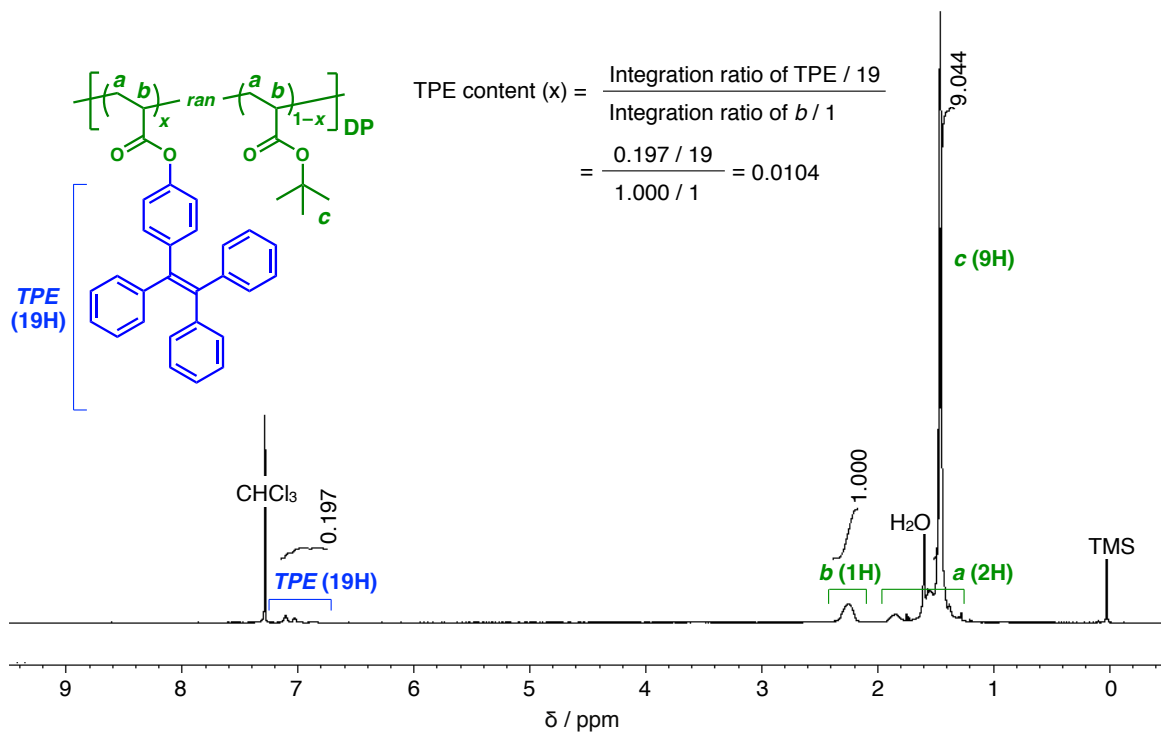


Figure S1. ^1H NMR spectrum (400 MHz) of $t\text{-Bu-PAA-TPE}_{0.01}$ in CDCl_3 at 25 °C.

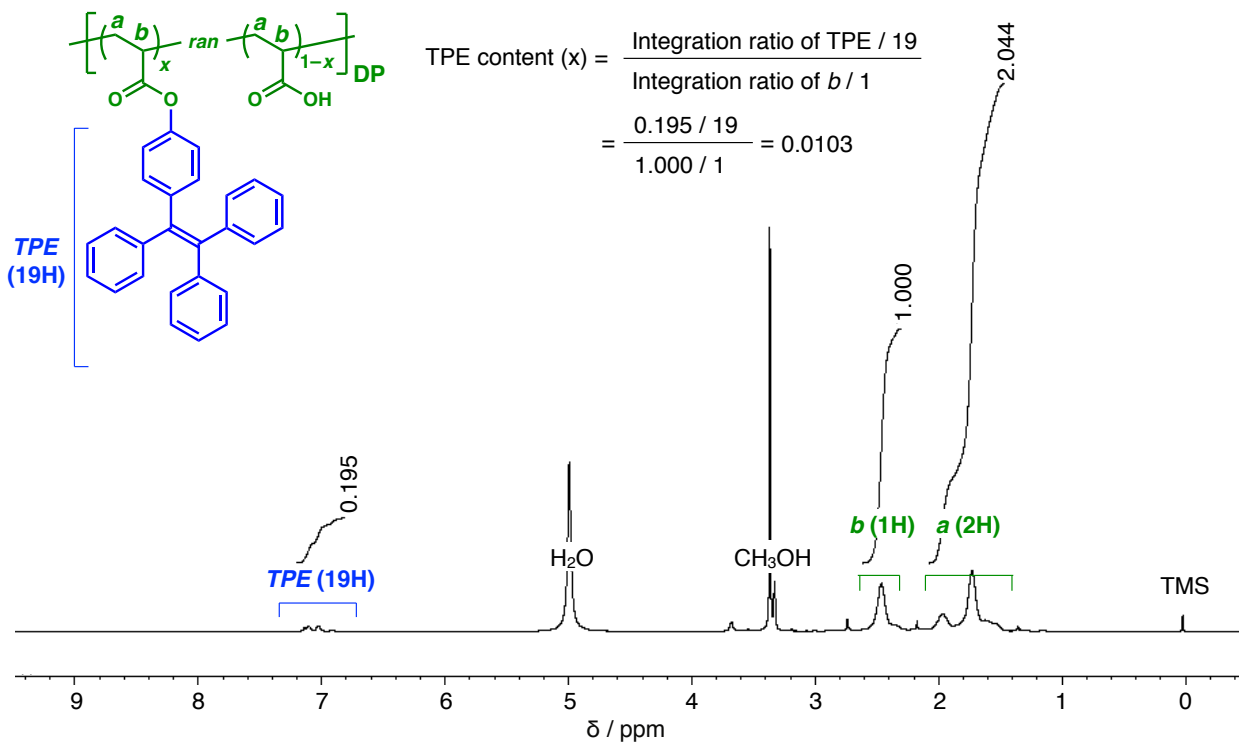


Figure S2. ^1H NMR spectrum (400 MHz) of $\text{PAA-TPE}_{0.01}$ in CD_3OD at 25 °C.

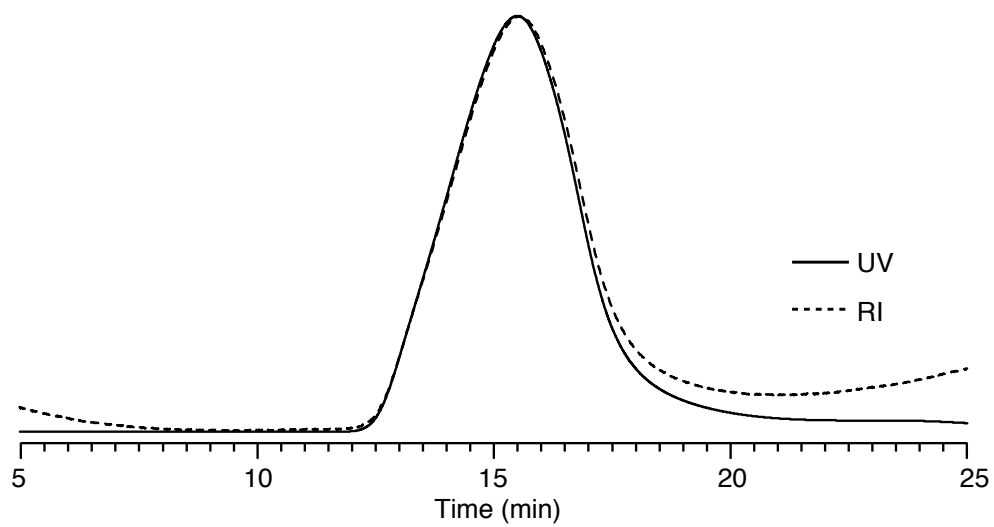


Figure S3. SEC profile of *t*-Bu-PAA-TPE_{0.01} (PDI = M_w/M_n = 1.22).

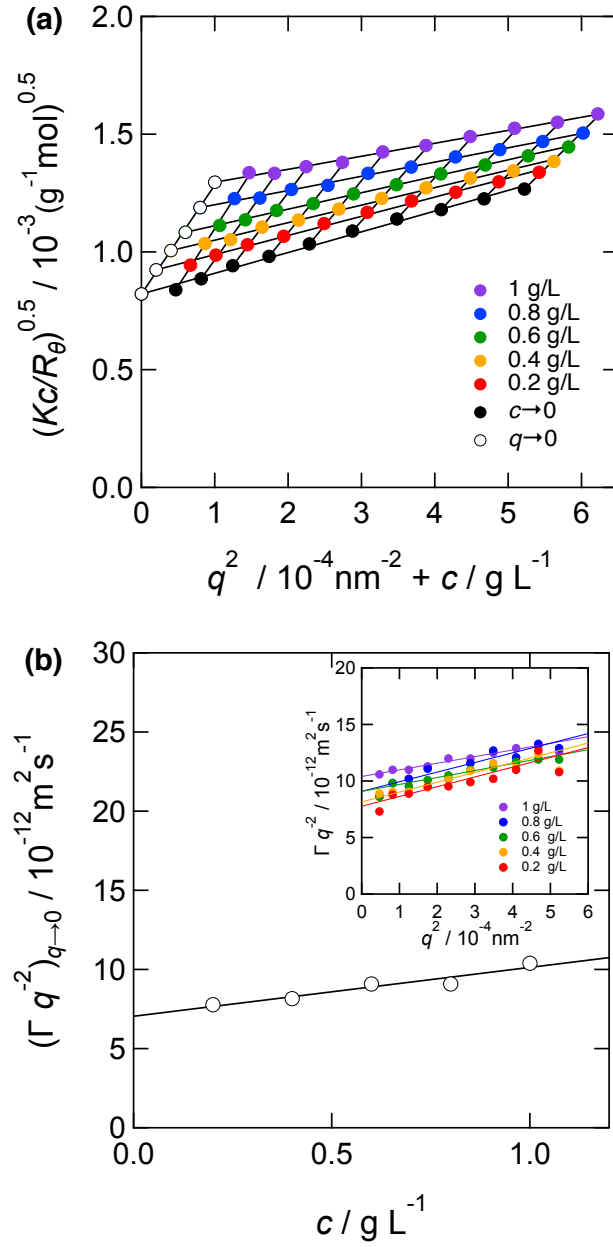


Figure S4. Light scattering results for molecularly dispersed PAA-TPE_{0.01} in organic solvent [acetic acid (1.0 M) / methanol solution]. (a) Zimm plot obtained from SLS measurement. The weight-average molecular weight M_w and radius of gyration R_g of the polymer were determined to be 1.48×10^6 g/mol and 80.0 nm, respectively. (b) Concentration and angular dependences of apparent diffusion constant (Γq^{-2}) obtained from DLS measurement. The hydrodynamic radius R_H was determined to be 55.8 nm.

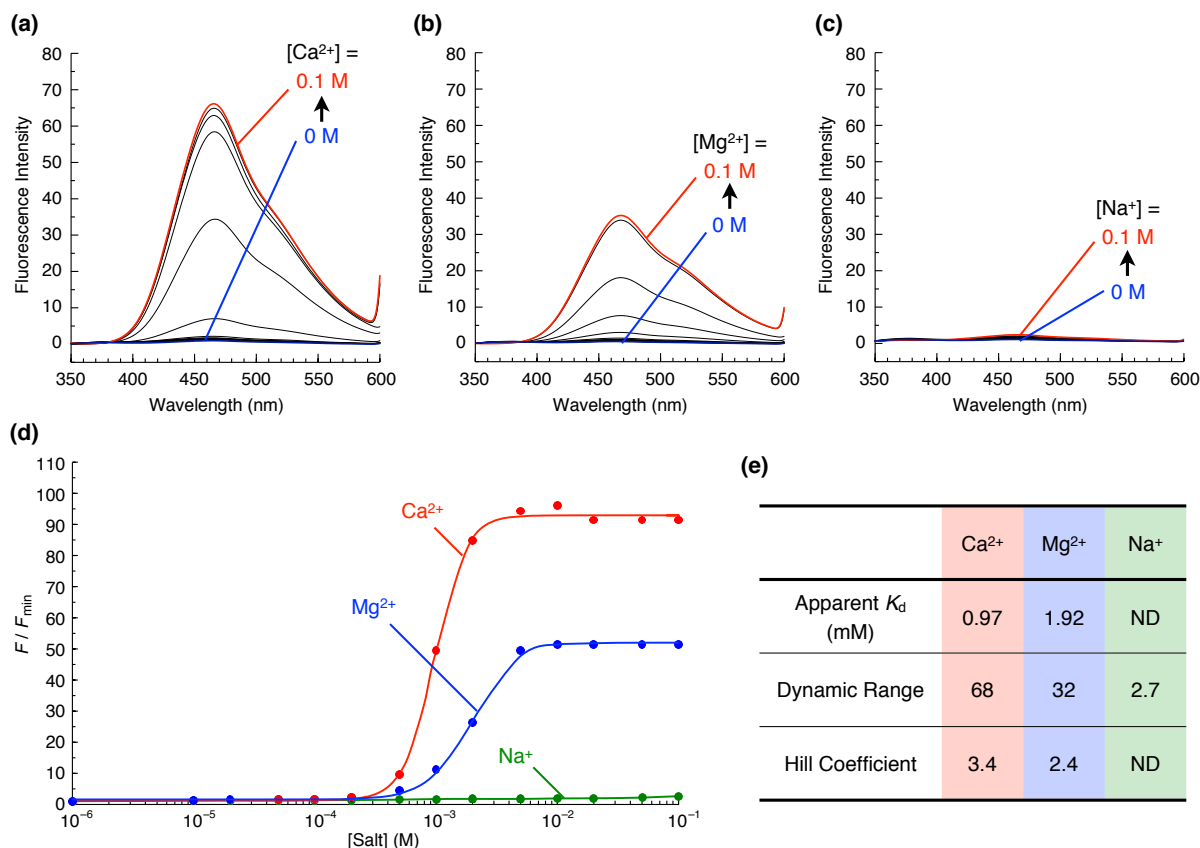


Figure S5. (a) Fluorescence spectral changes of PAA-TPE_{0.01} (0.1 g/L) in a HEPES buffer solution (70 mM, pH = 7.4) at 25 °C upon addition of CaCl₂ (blue: 0 M → red: 0.1 M). (b) Fluorescence spectral changes of PAA-TPE_{0.01} (0.1 g/L) in a HEPES buffer solution (70 mM, pH = 7.4) at 25 °C upon addition of MgCl₂ (blue: 0 M → red: 0.1 M). (c) Fluorescence spectral changes of PAA-TPE_{0.01} (0.1 g/L) in a HEPES buffer solution (70 mM, pH = 7.4) at 25 °C upon addition of NaCl (blue: 0 M → red: 0.1 M). (d) Titration curves of PAA-TPE_{0.01} (0.1 g/L) with CaCl₂, MgCl₂ and NaCl in a HEPES buffer solution (70 mM, pH = 7.4) at 25 °C. F and F_{\min} represent the observed and minimum fluorescence intensities at 465 nm, respectively. (e) Ion-sensing properties of PAA-TPE_{0.01} (0.1 g/L) in a HEPES buffer solution (70 mM, pH = 7.4).

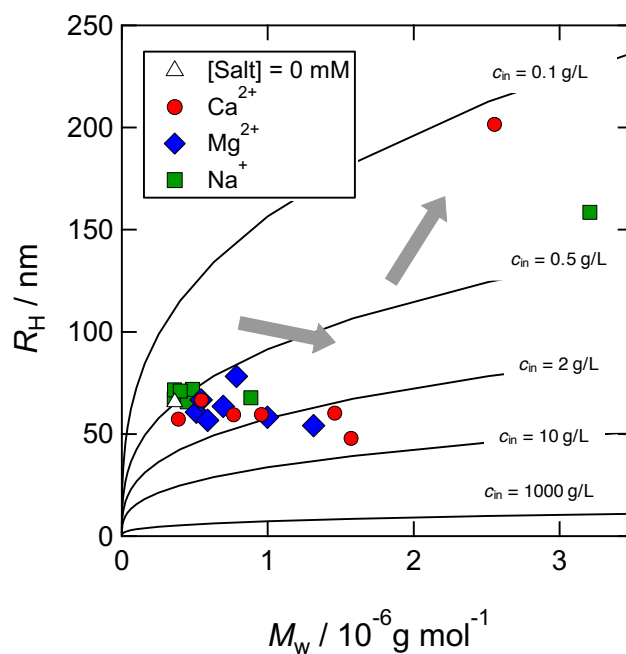


Figure S6. Conformational plot (hydrodynamic radius R_H vs. weight average molar mass M_w) of PAA-TPE_{0.01}. The thick arrow represents the conformational change with increasing salt concentrations. The black solid lines represent the iso-inner density lines.

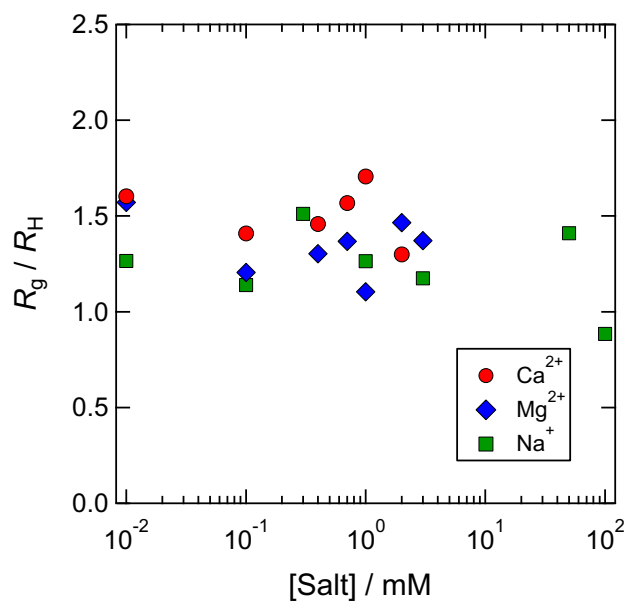


Figure S7. Plots of the value of R_g/R_H of PAA-TPE_{0.01} (0.1 g/L) in a HEPES buffer solution (70 mM, pH = 7.4) in the presence of various concentrations of CaCl₂, MgCl₂, or NaCl versus salt concentrations.

References

1. Ishiwari, F.; Hasebe, H.; Matsumura, S.; Hajjaj, F.; Horii-Hayashi, N.; Nishi, M.; Someya, T.; Fukushima, T., Bioinspired design of a polymer gel sensor for the realization of extracellular Ca^{2+} imaging. *Scientific reports* **2016**, *6*, 24275.
2. Berne, B.; Pecora, R. *Dynamic Light Scattering*. Wiley: New York, 1976.
3. Yamakawa, H. *Modern theory of Polymer Solutions*. Harper and Row: New York, 1971.