Supporting Information

Stronger Host-guest Binding Does Not Necessarily Give Brighter Particles: A Case Study on a Polymeric AIEE-tunable and Size-tunable Suprasphere

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Experimental Procedures

General. All solvents were obtained from commercial resources or dried according to the standard procedure. Other materials were purchased from Aladdin, Adamas and J&K and were used for synthesis without further purification. $^1$H NMR and $^{13}$C NMR spectra were collected on a Bruker DRX-400 spectrometer with tetramethylsilane used as reference. High resolution mass spectrometry (HRMS) was performed with a Bruker maXis impact instrument. Gel-permeation chromatography (GPC) analysis in THF was measured with a Waters ACQUITY advanced polymer chromatography system. Scanning electron microscope (SEM) was carried out on a Zeiss LEO1530VP field-emission scanning electron microscope system. Transmission electron microscopy (TEM) was operated on a JEOL JEM-2100F field emission transmission electron microscopy. Fluorescence spectra were recorded on a Hitachi F-4500 fluorescence spectrophotometer. Dynamic light scattering experiments were performed with a Malvern Zetasizer Nano ZS system. The dependence of particle concentration on the particle size for each PASS system was determined by the multispectral nanoparticle tracking analysis (m-NTA) technique enabled by the ViewSizer™ 3000 (MANTA Instruments, USA) and was then used to calculate the relative particle density with the equations described below (see the section of Supplementary Equations).

Electron microscopic sample preparation. The supraspheres were prepared by adding the guests into P1 acetone solution with specific concentrations; the samples were then prepared on the carbon support films (TEM) or glass sheets (SEM) by drop method and dried under the infrared lamp.

Numerical analysis. The binding constants ($K$) and the relative fluorescence quantum yields ($\Phi$) were calculated by non-linear fitting of the fluorescent titration experiments with software Scientist 3 (Micromath, USA). The corresponding models used in the numerical analysis are listed in Supplementary Equations.

Materials

1,4-Dimethoxypillar[5]arene and 1,2-bis(4-ethynylphenyl)-1,2-diphenylethene (6) were prepared according to the reported procedures.

Synthesis of monomer 5. Dihydroxyl pillar[5]arene 3 (1445.6 mg, 2 mmol), 1,6-dibromhexan (1.5 mL, 10 mmol) and NaH (110.0 mg, 4.4 mmol) were dispersed in 30 mL DMF. The mixture was degassed with nitrogen and stirred under 80 °C overnight. The mixture was poured into water and extracted with dichloromethane. The crude product was concentrated under reduced pressure and then purified by column chromatography to get 4 as white powder (1783.2 mg, 1.7 mmol) in ~85% yield. Then, compound 4 (1048.9 mg, 1 mmol) was treated with NaN$_3$ (195.0 mg, 3 mmol) in DMF under 80 °C for 12 h. The mixture was extracted by water and dichloromethane for 3 times. The organic phase was concentrated under vacuum and subjected to silica gel chromatography (petroleum ether / dichloromethane / ethyl acetate: 10 / 5/ 1) to give 5 as a white powder (895.2 mg, 92% yield). M.p. 179.5–180.0 °C. $^1$H NMR (400 MHz, acetone-$_d$6, 298 K), δ (ppm): 6.90 (s, 2H), 6.89 (s, 2H), 6.88 (s, 2H), 6.87 (s, 2H), 3.90 (t, J = 6.6, 4H), 3.76 (s, 6H), 3.76 (s, 6H), 3.75 (s, 6H), 3.74 (s, 6H), 1.86–1.79 (m,
4H), 1.49–1.41 (m, 4H), 1.33–1.23 (m, 8H). $^{13}$C NMR (100 MHz, CDCl$_3$, 298 K), δ (ppm): 150.8, 150.7, 150.5, 150.4, 149.8, 128.8, 128.3, 128.0, 114.5, 114.0, 113.9, 113.4, 113.2, 68.5, 56.1, 55.9, 55.7, 55.4, 55.3, 50.7, 30.1, 29.6, 29.5, 29.2, 27.5, 26.0, 25.1. HRMS: m/z calcd. for [M+Na]$^+$ C$_55$H$_68$N$_6$NaO$_{10}$: 995.4895; found 995.4889.

Synthesis of host polymer P1. Compound 5 (486.6 mg, 0.5 mmol), 1,2-bis(4-ethynylphenyl)-1,2-diphenylethene (6, 190.2 mg, 0.5 mmol), CuBr (7.2 mg, 0.05 mmol) and 1,1,4,7,7-pentamethyldiethylenetriamine (PMDETA, 17.3 mg, 0.1 mmol) were dissolved in 20 mL dry THF. The mixture was degassed and stirred under room temperature for 48 hours. The mixture was concentrated under reduced pressure, and then precipitated in methanol. The precipitate was purified by flash silicone gel column chromatography with THF as eluent. The target polymer P1 was obtained by precipitation in methanol for 3 times and dried in vacuum under 55 °C as yellow-green powder (483.3 mg) in 71.4% yield.

$^1$H NMR (400 MHz, CDCl$_3$, 298 K), δ (ppm): 7.72–7.63 (m, 1H), 7.61–7.56 (m, 2H), 7.52–7.29 (m, 4H), 7.23–6.95 (m, 12H), 6.93–6.59 (m, 10H), 4.13–3.18 (m, 42H), 1.54–1.48 (m, 2H), 1.47–1.21 (m, 4H), 1.24–0.93 (m, 6H), 0.85–0.54 (m, 4H). As evaluated by gel-permeation chromatography (GPC), number-average molecular weight (Mn) of P1 is about 1.0 × 10$^4$ g/mol, with the polydispersity index (PDI) of 2.19.

Synthesis of guest G2. Compound 11 (609.8 mg, 1.5 mmol) and 5-azido-pentanenitrile (8, 744.6 mg, 6 mmol) were added to 20 mL dry THF. CuBr (21.5 mg, 0.15 mmol) was introduced into the mixture and the mixture was degassed with N$_2$, then stirred at room temperature for 12 h. The crude product was concentrated under reduced pressure, then extracted with brine and CH$_2$Cl$_2$ to get rid of CuBr. The organic phase was concentrated under reduced pressure and recrystallized in ethanol to produce the product G2 as white needle-like crystal (884.0 mg, 1.35 mmol, 90% yield). M.p. 120.2–121.8 °C. $^1$H NMR (400 MHz, CDCl$_3$, 298 K), δ (ppm): 7.60 (s, 2H), 6.93–6.88 (m, 4H), 6.84–6.80 (m, 4H), 5.16 (s, 4H), 4.42 (t, J = 6.8 Hz, 4H), 3.90 (t, J = 6.6 Hz, 4H), 2.13–2.06 (m, 4H), 1.79–1.65 (m, 8H), 1.48–1.37 (m, 8H). $^{13}$C NMR (100 MHz, CDCl$_3$, 298 K), δ (ppm): 153.8, 152.2, 144.8, 122.6, 118.9, 115.9, 115.4, 68.6, 62.7, 49.2, 29.3, 29.0, 26.0, 22.3, 16.7. HRMS: m/z calcd. for [M+Na]$^+$ C$_{36}$H$_{46}$N$_8$NaO$_4$: 677.3540; found 677.3534.
Synthesis of guest G3-1. Compound 14 (1153.2 mg, 1.3 mmol), 5-azido-pentanenitrile (8, 968.0 mg, 7.8 mmol) and CuBr (57.0 mg, 0.4 mmol) were added into 20 mL of degassed THF and protected in nitrogen atmosphere. The mixture was stirred under room temperature for 12 h, then poured into brine (100 mL) and extracted by dichloromethane. The organic layer was concentrated under vacuum, and recrystallized in ethanol to afford product G3-1 (1424.6 mg, 1.13 mmol, 87% yield) as white powder.\[2\] M.p. 101.4–102.1 °C. 1H NMR (400 MHz, CDCl₃, 298 K), δ (ppm): 7.59 (s, 3H), 6.92–6.88 (m, 6H), 6.84–6.80 (m, 6H), 6.06 (s, 3H), 5.15 (s, 6H), 4.41 (t, J = 6.6 Hz, 6H), 3.92–3.88 (m, 12H), 2.39 (t, J = 6.8 Hz, 6H), 2.12–2.05 (m, 6H), 1.79–1.64 (m, 18H), 1.47–1.35 (m, 24H). 13C NMR (100 MHz, CDCl₃, 298 K), δ (ppm): 161.0, 153.8, 152.2, 144.9, 122.5, 118.9, 115.9, 115.5, 93.9, 68.58, 68.0, 62.8, 49.2, 29.4, 29.3, 29.2, 29.0, 26.0, 26.0, 22.3, 16.7. HRMS: m/z calcd. for [M+Na]⁺ C₇₂H₆₀N₁₂NaO₉: 1295.7321; found 1295.7315.

Synthesis of guest G3-2. 1,3,5-Triethynylbenzene (18, 211.0 mg, 1.4 mmol) and 5-azido-pentanenitrile (632.9 mg, 5.1 mmol) were added into 30 mL dry THF, and then CuBr (57.4 mg, 0.4 mmol) was introduced into the reaction mixture. The mixture was stirred at room temperature for 12 h under the protection of nitrogen. The crude product was concentrated under reduced pressure. The residue was washed by water, and then recrystallized in ethanol to yield G3-2 as white powder.
extracted by CHCl₃. The CHCl₃ phase was concentrated under vacuum and then recrystallized in ethanol to get G3-2 (607.6 mg, 1.16 mmol, 83% yield) as white powder. M.p. 177.4–178.4 °C. 

**1H** NMR (400 MHz, CDCl₃, 298 K), δ (ppm): 8.29 (s, 3H), 7.98 (s, 3H), 4.51 (t, J = 6.8 Hz, 6H), 2.45 (t, J = 7.0 Hz, 6H), 2.21–2.13 (m, 6H), 1.79–1.61 (m, 6H). **13C** NMR (100 MHz, CDCl₃, 298 K), δ (ppm): 147.4, 131.7, 122.5, 120.2, 118.9, 49.4, 29.1, 22.4, 16.7. HRMS: m/z calcd. for [M+Na]+ C₂₇H₃₀N₁₂Na: 545.2614; found 545.2609.

**Synthesis of guest G1.** Ethynyl-benzene (19, 204.3 mg, 2 mmol), 5-azido-pentanenitrile (298.0 mg, 2.4 mmol), and CuBr (28.7 mg, 0.2 mmol) were added into 20 mL degassed THF. The mixture was stirred under room temperature for 12 h, and then concentrated under vacuum. The crude product was obtained by extraction with CH₂Cl₂ and concentration under vacuum. Then the crude product was further purified by recrystallization in ethanol to afford G1 (209.0 mg, 0.92 mmol) as white fibrous. Yield: 46%. M.p. 59.6–60.2 °C. 

**1H** NMR (400 MHz, CDCl₃, 298 K), δ (ppm): 7.83–7.80 (m, 2H), 7.79 (s, 1H), 7.43–7.40 (m, 2H), 7.35–7.31 (m, 1H), 4.42 (t, J = 7.0 Hz, 2H), 2.37 (t, J = 7.0 Hz, 2H), 2.12–2.04 (m, 2H), 1.71–1.63 (m, 2H). 

**13C** NMR (100 MHz, CDCl₃, 298 K), δ (ppm): 147.9, 130.5, 128.9, 128.3, 125.7, 119.7, 119.0, 49.2, 29.1, 22.3, 16.7. HRMS: m/z calcd. for [M+Na]+ C₁₃H₁₄N₄Na: 249.1116; found 249.1111.

**Synthesis of control polymer PC.** 1,4-Bis-(6-bromo-hexyloxy)-benzene (21, 872.4 mg, 2 mmol) and 5-azido-pentanenitrile (260 mg, 4 mmol) were dispersed in 30 mL DMF, and then degassed with nitrogen and stirred under 80 °C for 12 h. Mixture was poured into water and then extracted with dichloromethane. The crude product was concentrated under reduced pressure, then purified by column chromatography (petroleum ether / ethyl acetate: 5 / 1) to afford compound 22 as white powder (648.8 mg, 1.8 mmol) with 90% yield. 

**1H** NMR (400 MHz, CDCl₃, 298 K), δ (ppm): 6.81 (s, 4H), 3.91 (t, J = 6.4 Hz, 4H), 3.28 (t, J = 7.0 Hz, 4H), 1.80–1.73 (m, 4H), 1.65–1.60 (m, 4H), 1.56–1.42 (m, 8H).

**13C** NMR (100 MHz, CDCl₃, 298 K), δ (ppm): 153.2, 115.4, 68.4, 51.4, 29.3, 28.8, 26.5, 25.7. Compound 22 (180.2 mg, 0.5 mmol), 1,2-bis(4-ethynylphenyl)-1,2-diphenylethene (6, 190.2 mg, 0.5 mmol), CuBr (7.2 mg, 0.05 mmol) and 1,1,4,7,7-pentamethyl-diethylenetriamine (PMDETA, 17.3 mg, 0.1 mmol) were dispersed in 20 mL dry THF, then degassed and stirred under room temperature for 48 hours. The mixture was concentrated under reduced pressure, and then precipitated in methanol. The precipitate was purified by flash silicone gel column chromatography with THF as eluent. The target polymer PC was obtained by precipitation in methanol as yellow-green powder (190.4 mg). Yield: 51.4%. 

**1H** NMR (400 MHz, CDCl₃, 298 K), δ (ppm): 7.64–7.64 (m, 2H), 7.59–7.53 (m, 3H), 7.17–6.95 (m, 15H), 6.81–6.75 (m, 4H), 4.36 (s, broad, 4H), 3.89–3.86 (m, 4H), 1.93 (s, broad, 4H), 1.73 (s, broad, 4H), 1.50 (s, broad, 4H), 1.39 (s, broad, 4H).
Supplementary Figures

**Figure S1.** $^1$H NMR spectrum of compound 5 (400 MHz, acetone-$d_6$, 298 K).

**Figure S2.** $^{13}$C NMR spectrum of compound 5 (100 MHz, CDCl$_3$, 298 K).
Figure S3. HRMS (ESI) spectrum of compound 5.

Figure S4. $^1$H NMR spectrum of P1 (400 MHz, CDCl$_3$, 298 K).
Figure S5. $^1$H NMR spectrum of guest G2 (400 MHz, CDCl$_3$, 298 K).

Figure S6. $^{13}$C NMR spectrum of guest G2 (100 MHz, CDCl$_3$, 298 K).
Figure S7. HRMS (ESI) spectrum of G2.

Figure S8. $^1$H NMR spectrum of guest G3-1 (400 MHz, CDCl$_3$, 298 K).
Figure S9. $^{13}$C NMR spectrum of guest G3-1 (100 MHz, CDCl$_3$, 298 K).

Figure S10. HRMS (ESI) spectrum of G3-1.
**Figure S11.** $^1$H NMR spectrum of guest G3-2 (400 MHz, CDCl$_3$, 298 K).

**Figure S12.** $^{13}$C NMR spectrum of guest G3-2 (100 MHz, CDCl$_3$, 298 K).
Figure S13. HRMS (ESI) spectrum of G3-2.

Figure S14. $^1$H NMR spectrum of guest G1 (400 MHz, CDCl$_3$, 298 K).
Figure S15. $^{13}$C NMR spectrum of guest G1 (100 MHz, CDCl$_3$, 298 K).

Figure S16. HRMS (ESI) spectrum of G1.
Figure S17. $^1$H NMR spectrum of compound 22 (400 MHz, CDCl$_3$, 298 K).

Figure S18. $^{13}$C NMR spectrum of compound 22 (100 MHz, CDCl$_3$, 298 K).
Figure S19. $^1$H NMR spectrum of polymer PC (400 MHz, CDCl₃, 298 K).

Figure S20. (a) Fluorescence spectra of P1 in THF–water mixtures with different solvent ratios ($\lambda_{ex}$=340 nm); (b) The corresponding fluorescence under a UV lamp (365 nm). [RU] = 10 μM.
Figure S21. Limit of detection (LOD) for the binding of P1 towards GMs. GMs = G2 (a1, a2), G3-1 (b1, b2) and G3-2 (c1, c2).

LOD for the binding P1 towards G2, G3-1 or G3-2 was evaluated by the following equation (3σ criterion), where ‘σ’ was the standard deviation of the blank test (N=20).

\[ \text{LOD} = \frac{3\sigma}{\text{Slope}} \]
Figure S22. Fluorescence response of P1 or PC toward the guests: (a) Fluorescence response of P1 toward the monotopic guest G1 in acetone; (b) Fluorescence response of PC toward the multitopic guests G2, G3-1 and G3-2 in acetone. The spectra of P1 in the presence of G3-2 were used for comparison. [RU] = 10 μM. [GM] = 16.7 μM. λex = 340 nm.

Figure S23. 1H NMR spectra for the binding of compound 5 with guest G1. 1H NMR spectra of G1 (a), compound 5 (c) and the mixture of G1 and compound 5 at 1:1 (b) were determined using CD3OD as solvent (400 MHz, 298 K). [Compound 5] = 0.05 M. [G1] = 0.05 M.

The alkyl protons a, b, c, d and triazole protons e of the guest G1 shifted upfield upon the binding to the compound 5. Meanwhile, the signal peaks of compound 5 were widened during the complexation process. Due to the slow chemical exchange between the guests free in solvent and bound as the complex, the signals for both guest species can be simultaneously observed.
Figure S24. Fluorescence response of the aggregated P1 towards guests in the mixture of THF and water. [RU] = 10 μM, [G] = 16.7 μM, λ_ex = 340 nm.

Figure S25. Dependence of Fluorescence of P1 on the concentration of GMs in acetone. GMs = G2 (a1, a2), G3-1 (b1, b2) and G3-2 (c1, c2), λ_ex = 340 nm, [RU] = 1 μM (a1, b1 and c1) or 10 μM (a2, b2 and c2).
Figure S26. Job plots for the binding of RU to G2 (a), G3-1 (b) and G3-2 (c).
Figure S27. Non-linear fitting of the binding isotherm with different binding models. The data in panels a1 and a2 were for guest G2 and fitted with a RU:G2 2:1 model; figures b1 and b2 were for guest G3-1 and fitted with a RU:G3 3:1 model; figures c1 and c2 were for guest G3-2 and fitted with a RU:G3 3:1 model. The fluorescence intensities were integrated from 450 nm to 550 nm in the fluorescence spectra.
Figure S28. TEM images of the PASS for G2⊂P1 (a, b), G3-1⊂P1 (c, d) and G3-2⊂P1 (e, f). [RU] = 10 μM. [GM] = 16.7 μM.

Figure S29. TEM images of the PASS at very low concentration of guest. [RU] = 10 μM. [G3-2] = 1 × 10^{-4} μM.
Figure S30. SEM images of P1 (a), G2 (b), G3-1 (c) and G3-2 (d).

Figure S31. DLS experiments for PASS at different concentrations of P1 with different guests used. [GM] = 10 μM.
Figure S32. Results determined by the multispectral nanoparticle tracking analysis (m-NTA): Particle concentration and size distribution of PASS in acetone. GM = G2 (a1, a2), G3-1 (b1, b2) and G3-2 (c1, c2). [GMs] = 10 μM. [RU] = 1 μM (a1, b1 and c1) or 10 μM (a2, b2 and c2). The data were fitted with the Gauss distribution (red curves) to determine the average diameter of the nanoparticles.

Fig. S33. Fluorescent titration of G3-2⊂P1 with adiponitrile in acetone. Inset: fit of the binding isotherm. [RU] = 10 μM, [G3-2] = 16.7 μM.
Figure S34. TEM images of disaggregated polymeric host-guest suprasphere (G3-2⊂P1) with the addition of adiponitrile. Shapeless stains with size from hundreds to dozens of nanometers in diameter were observed instead of regular nano-spheres (Figure 3).
Supplementary Equations

Equations for the binding isotherm

We sequentially choose RU:GM 1:1, 2:1 and 3:1 models to fit the binding isotherm determined for the PASS systems until the residues between the data and the fit are random around 0. The overall binding constant for RU:GM 1:1 complex, RU:GM 2:1 complex and RU:GM 3:1 complex were represented as $K_{HG}, \beta_{HG}, \beta_{HG}$. The value of constant for the binding of RU to a single arm of GM (i.e. $K$ reported in the paper) was calculated by the following equations under the assumption that the sequential binding of RU to the first, the second and the third arm (if available) of GM are equal to each other in each PASS system.

$K = K_{HG}$

$K = \sqrt{\beta_{HG}}$

$K = \sqrt[3]{\beta_{HG}}$

Model 1: RU:Guest 1:1 binding model:

IndVars: [GT]

// [GT]: total guest concentration

DepVars: I, [G], [H], [HG]

Params: $K_{HG}, \Phi_{HG}, [H_0]$ //

$$[HG] = K_{HG} \times [H] \times [G]$$  

$$[H] = [H_0] - [HG]$$  

$$[G] = [GT] - [HG]$$  

$$I = (\Phi_{HG} -1) \times [HG] / [H_0]$$

// The following section is for the guessing values of parameters.

$K_{HG} = 9.88 \times 10^5 //$

$\Phi_{HG} = 8.14 //$

// The following section is for the concentration of RU.

$[H_0] = 1.0 \times 10^{-6} //$

// The following section is for the range of the variables.

$0 < [G] < 1.4 \times 10^{-5}$

$0 < [H] < 1.3 \times 10^{-5}$
Model 2: RU:GM 2:1 binding model:

IndVars: [GT],
// [GT]: total guest concentration
DepVars: I, [H₂G], [H], [G]
Params: Φ, [H₀], β₃G

\[ [H₂G] = β₃G \times [H]^2 \times [G] \]

\[ [H] = [H₀] − 2 \times [H₂G] \]

\[ [G] = [GT] − [H₂G] \]

\[ I = \frac{( 2 \times Φ \times [H₂G] + [H] − [H₀] )}{[H₀]} \]

// The following section is for the guessing values of parameters.
\[ β₃G = 4.88 \times 10^{18} // \]
\[ Φ = 8.14 // \]

// The following section is for the concentration of RU.
\[ [H₀] = 1 \times 10^{-6} \]

// The following section is for the range of variables.
\[ 0 < [G] < 1 \times 10^5 \]
\[ 0 < [H] < 1 \times 10^5 \]
\[ 0 < [H₂G] < 1 \times 10^5 \]

Model 3: RU:GM 3:1 binding model:

IndVars: [GT], // [GT]: total guest concentration
DepVars: I, [H₃G], [H], [G]
Params: Φ, [H₀], β₃G

\[ [H₃G] = β₃G \times [H]^3 \times [G] \]
\[ [H] = [H_0] - 3 \times [H_3G] \]  
\[ [G] = [GT] - [H_3G] \]  
\[ I = (3 \times \Phi \times [H_3G] + [H] - [H_0]) / [H_0] \]  
\[ \beta_{HDG} = 4.88 \times 10^{18} \]  
\[ \Phi = 8.14 \]  

// The following section is for the concentration of RU.  
\[ [H_0] = 1 \times 10^6 \]  

// The following section is for the range of the variables.  
\[ 0 < [G] < 1 \times 10^4 \]  
\[ 0 < [H] < 1 \times 10^5 \]  
\[ 0 < [H_3G] < 1 \times 10^{-5} \]  

Model 4: Model for the competition experiment:  
IndVars: [GcT]  
// [GcT]: competitive guest concentration  
DepVars: I, [H_3G], [H], [G], [HGc]  
Params: \( \Phi_{HG} \), [HT], [GT], \( \beta_{H3G} \), \( K_{HGc} \)  
// [HT]: total concentration of RU; [GT]: total guest concentration.  

\[ [H_3G] = \beta_{H3G} \times [H_3] \times [G] \]  
\[ [HGc] = K_{HGc} \times [H] \times [Gc] \]  
\[ [H] = [HT] - 3 \times [H_3G] - [HGc] \]  
\[ [G] = [GT] - [H_3G] \]  
\[ [Gc] = [GcT] - [HGc] \]  
\[ I = ( [H] + 3 \times \Phi_{HG} \times [H_3G] + [HGc] ) / ([H]_0 + 3 \times \Phi_{HG} \times [H_3G])_0 \]  

// The initial values of \([H]_0\) and \([H_3G]_0\) were calculated to be 1.1772 and 2.98418 µM, respectively, under the specific experimental condition using Scientist 3 with model 3 program.  

// The following section is for the guessing values of parameters.
\( \Phi_{HG} = 11 \)  
\( K_{HG} = 4.88 \times 10^{-3} \)

// The following section is for the variables with known values. 

\( \beta_{HG} = 1.31 \times 10^{13} / \mu M \)

\[ [HT] = 10 \ \mu M \]
\[ [GT] = 16.7 \ \mu M \]

// The following section is for the range of the variables.

\[ 0 < [G] < 1 \times 10^{-5} \]
\[ 0 < [Ge] < 1 \times 10^{-2} \]
\[ 0 < [H] < 1 \times 10^{-4} \]
\[ 0 < [H_3G] < 1 \times 10^{-5} \]

Equations for the relative particle density determined by using m-NTA technology

As shown in Fig. S32, the raw data determined by using m-NTA technology were given as the dependence of the particle numbers per sample volume (\( [P] \)) on the diameter of particle (\( d \)).

\[ [P] = f(d) \]

The dependence of \( [P] \) on the particle volume (\( V_p \)) was then determined by equations 46 and 47.

\[ V_p = \frac{4}{3} \pi \left( \frac{d}{2} \right)^3 \]

The total particle volume per sample volume (\( V_p^{total} \)) was determined as the integration of \( [P] \) over \( V_p \) (i.e. \( \sum ([P] \times V_p) \)).

The total number of GMcRU complex embedded in the PASS particles per sample volume (\( [GMcRU]_{PASS} \)) was related to the total number of GMcRU complex generated in the samples per sample volume (\( [GMcRU]_{sample} \)) by the following equation:

\[ [GM \subset RU]_{PASS} = [GM \subset RU]_{sample} \times a \]

where the parameter \( a \) was related to the experimental conditions such as the laser power, frame rate, exposure, video length, the number of videos, as well as other parameters selected. The parameter \( a \) was considered to be the same for the experiments conducted in the same day under the same experimental conditions. The variable of \( [GMcRU]_{sample} \) can be calculated according to the model 2 (equations 11-20) or 3 (equations 21-30) aforementioned.

The particle density of the PASS (\( \rho \)) was related to \( [GMcRU]_{PASS} \) and \( V_p^{total} \) (equation 49):

\[ \rho = \frac{MW_{GMcRU} \times [GMcRU]_{PASS}}{V_p^{total}} \]
where MW_{GM\subset RU} corresponds to the molecular weight of the specific GM\subset RU complex.

Equation 50 was derived from equations 48 and 49.

\[
\rho = \frac{MW_{GM\subset RU \times [GM\subset RU]_{sample \times a}}}{V_{p2}^{\text{total}}} \tag{49}
\]

As a result, the relative particle density for each PASS system (RD_i) can be determined by equation 50.

\[
RD_i = \frac{\rho_i}{\rho_2} = \frac{MW_i \times [GM\subset RU]_{i \times a}}{V_{p1}^{\text{total}}} \times \frac{V_{p2}^{\text{total}}}{MW_2 \times [GM\subset RU]_2 \times a} \tag{50}
\]

In this study, the PASS of G3-2\subset RU system prepared at 1 \mu M RU and 10 \mu M G3-2 was employed as the reference sample, i.e. sample 2 in equation 50. The values of RD_i were then determined and listed in Table 1 of the paper.
References

