## **Supporting Information**

# All-Organic Luminescent Nanodots from Corannulene and Cyclodextrin Nano-Assembly: Continuous-Flow Synthesis, Non-Linear Optical Properties, and Bio-Imaging Application

Sivaramapanicker Sreejith,<sup>a</sup> Nishanth Venugopal Menon,<sup>b</sup> Yue Wang,<sup>c</sup> Hrishikesh Joshi,<sup>a</sup> Shiying Liu,<sup>b</sup> Kok Chan Chong,<sup>a</sup> Yuejun Kang,<sup>d</sup> Handong Sun,<sup>a,e</sup> and Mihaiela C. Stuparu<sup>\*a,f</sup>

<sup>a</sup>Division of Chemistry and Biological Chemistry, School of Physical and Mathematical Sciences, Nanyang Technological University, 21 Nanyang Link, 637371, Singapore.

<sup>b</sup>School of Chemical and Biomedical Engineering, Nanyang Technological University, 62 Nanyang drive, 637459, Singapore.

<sup>c</sup>Division of Physics and Applied Physics, School of Physical and Mathematical Sciences, Nanyang Technological University, 21 Nanyang Link, 637371, Singapore.

<sup>d</sup>Faculty of Materials and Energy, Institute for Clean Energy and Advanced Materials, Southwest University, 2 Tiansheng Road, Beibei, Chongqing, 400715, China.

<sup>e</sup>Centre for Disruptive Photonic Technologies, School of Physical and Mathematical Sciences, Nanyang Technological University, 21-Nanyang Link, 637371, Singapore.

<sup>f</sup>School of Materials Science and Engineering, Nanyang Technological University, 639798, Singapore.

Email: mstuparu@ntu.edu.sg

### Experimental

**1. General:** All commercial reagents were used without further purification. Column chromatography for product purification was performed on DAVISIL® chromatographic silica gel LC60A (40–63 micron, GRACE Davison, Germany). NMR spectra were recorded at room temperature on Bruker AV-300 and Bruker 400-AV NMR instruments using CDCl<sub>3</sub> and DMSO-d<sub>6</sub>. Chemical shifts  $\delta$  were reported in ppm relative to CHCl<sub>3</sub> ( $\delta$  = 7.26 ppm) and coupling constants were given in Hz.

**2. Isothermal Titration Calorimetry (ITC):** OMNICAL SuperCRC Reaction micro calorimeter was used to study kinetic parameters in this study. The raw data from the ITC measurements were processed as follows. The raw data was integrated to obtain the heat added per addition and plotted with its corresponding mole ratio (Cor-DMA: $\gamma$ -CD). The scatter plot was fitted with a sigmoidal curve to obtain the enthalpy of the system and the association constant (*K*). Subsequently  $\Delta$ G and  $\Delta$ S were computed by the basic thermodynamic equations as the studies were conducted at constant temperature and pressure.

 $\Delta G = \Delta H - T \Delta S$ 

 $\Delta G = -RT \ln(K)$ 

### 3. Synthesis:

### 3.1. Trimethylsilylethynylcorannulene (1)



Bromocorannulene (1.00g, 3.04 mmol) was added into a mixture of triethylamine (80ml), bis(triphenylphosphine) palladium (II) dichloride (168mg, 0.24mmol), and copper(I) iodide (61.0 mg, 0.32mmol) at room temperature. The mixture was bubbled with N<sub>2</sub> gas for 15 minutes. Trimethylsilyl acetylene (1.3 mL, 9.12 mmol) was then added quickly all at once into the mixture, and resulting solution was stirred at 70 °C for 16 h. Subsequently, the solution was allowed to cool to room temperature and the solvent was evaporated to dryness. The crude product was purified by column chromatography on silica gel (hexane) to give the product as a white solid (0.735 g, 70%). <sup>1</sup>H NMR (400MHz, CDCl<sub>3</sub>)  $\delta$  8.03-7.71 (m, 9H), 0.35 (s, 9H).

### 3.2. Corannulene acetylene (2)



To a stirred solution of methanol (30mL) and potassium carbonate (0.87 g, 6.36 mmol), was added **1** (0.735 g, 2.12 mmol) and CH<sub>2</sub>Cl<sub>2</sub> (20 mL). The yellow solution was then allowed to stir for a further 30 minutes at room temperature. Water (50 mL) was added to the solution and the resultant mixture was then extracted with CH<sub>2</sub>Cl<sub>2</sub> (15 mL  $\times$  3). The combined organic layers were dried over anhydrous Na<sub>2</sub>SO<sub>4</sub> and the solvent was evaporated under reduced pressure. The product was purified with flash column chromatography (hexane) to produce compound **2** as an orange-brown solid (0.570 g, 98%). <sup>1</sup>H NMR (400 MHz, DMSO)  $\delta$  8.07 – 7.75 (m, 9H), 3.43 (s, 1H).

### 3.3. (4-N, N-dimethylanilinoethynyl)corannulene (Cor-DMA)



Corannulene acetylene **2** (60.0 mg, 0.218 mmol) was added into a mixture of piperidine (5 mL), trans-bis(triphenylphosphine) palladium (II) dichloride (15.4 mg, 0.022 mmol), and copper(I) iodide (4.2 mg, 0.022 mmol) at room temperature. The mixture was bubbled with N<sub>2</sub> gas for 15 minutes. 4-bromo-N,N'-dimethylaniline (1.3 mL, 9.12 mmol) was then added quickly all at once into the mixture, and the resulting solution was stirred at 70°C for 18h. Subsequently, the solution was allowed to cool to room temperature and the solvent was evaporated to dryness. The crude product was purified by column chromatography (hexane: dichloromethane 1:1) to give Cor-DMA<sup>S1</sup> as an orange solid (10 mg, 60 %). <sup>1</sup>H NMR (300MHz, CDCl<sub>3</sub>)  $\delta$  8.14 (d, 1H, J = 8.79 Hz), 7.99 (s, 1H), 7.90-7.72 (m, 7H), 7.55 (d, 2H, J = 8.91 Hz), 6.72 (d, 2H, J = 8.88 Hz), 3.04 (s, 6H). <sup>13</sup>C NMR (75 MHz, CDCl<sub>3</sub>):  $\delta$  150.4, 136.2, 135.8, 135.7, 135.3, 134.9, 133.1, 131.1, 131.0, 130.7, 130.1, 127.5, 127.4, 127.3, 127.2, 127.16, 126.7, 126.4, 122.7, 112.0, 110.1, 95.1, 86.1, 40.3

### 4. Photophysical Study and Titration Experiments:



**Figure S1**. Photophysical properties of the electronically active assembly component. (a) Absorption (black curve) and emission (red curve,  $\lambda_{ex}$  380 nm) spectra of Cor-DMA in acetonitrile (1 × 10<sup>-5</sup> M). (b) Emission spectra of Cor-DMA in various solvents (1 × 10<sup>-5</sup> M). (c) Digital photograph showing corresponding solutions (from left) hexane, toluene, dichloromethane, THF, methanol, acetonitrile and DMF under 365 nm UV illumination.

### 5. <sup>1</sup>H NMR Experiments:



Figure S2. <sup>1</sup>H NMR (500 MHz) of  $\gamma$ -CD in DMSO- $d_6$ .



**Figure S3**. Comparison of <sup>1</sup>H NMR (500 MHz). (a)  $\gamma$ -CD; (b) Cor-DMA and (c) a 1:1 mixture in DMSO-*d*<sub>6</sub>.



**Figure S4**. Snap shots showing formation of Cor-DMA- $\gamma$ -CD particle precipitate in a quartz cuvette under stirring at 350 rpm. (a) Solution immediately after addition of  $\gamma$ -CD (1 mg/mL) in water into Cor-DMA (1 × 10<sup>-5</sup> M), in acetonitrile in a 1:16 ratio. (b)-(h), images obtained after time intervals of (b) 120s; (c) 240s; (d) 300s; (e) 360s; (f) 420s; (g) 540s, and (h) 720s.



**Figure S5**. Transmission Electron Microscope (TEM) images of sedimented Cor-DMA- $\gamma$ -CD complex. TEM image of Cor-DMA- $\gamma$ -CD aggregate obtained after (a) 240s (b) 740s post-addition of  $\gamma$ -CD (1 mg/ml) in water into Cor-DMA (1 × 10<sup>-5</sup> M), in acetonitrile in a 1:16 (Cor-DMA: $\gamma$ -CD) ratio.



**Figure S6**. DLS profiles of Cor-DMA-γ-CD aggregates formed directly.

### 6. Microfluidic chip fabrication

Microfluidic chips for this study were fabricated by a combination of photolithography and soft lithography. Photolithography comprises the use of a negative photoresist SU8 2050 to create a mold with a thickness of 180 µm. Briefly, the photoresist is spin coated on a silicon wafer following which it is soft baked at 95 °C, exposed to UV radiation (365 nm), baked again at 95 °C, and finally developed using the SU8 developer. Following mold preparation, soft lithography is performed by replica molding where a polymer, polydimethylsiloxane (PDMS), is used to prepare chips using the SU8 mold. PDMS is prepared by mixing the elastomer and the curing agent in the ratio of 10:1 by weight, followed by which it is cured at 70 °C for 2 h. A punch is used to define the inlets and the outlets. Plasma bonding, using air plasma is used to bond the chip onto a glass cover slip.



**Figure S7**. Microfluidic chip design and chip performance. (a) Microscope snapshot of mixing performance of "Y" junction fluidic device using blue and green food colors (Flow rate 5mL per hour). (b) Full microscopic image of "T" junction appended 'zigzag' fluidic chip selected for size-controlled synthesis of Cor-DMA and  $\gamma$ -CD inclusion mediated soft-aggregates. (i) Higher magnification microscopic image of 'T' junction. (ii) Microscopic image of "T" junction showing flow of red and green food color (in water) mixing (channel dimensions: inlet channel - 5  $\mu$ m and mixing channel - 2  $\mu$ m). (iii) Microscopic image showing food color mixing in the first turn in the channel after expansion from 2 to 5  $\mu$ m with a flow rate of 2 mL/hour.



**Figure S8**. TEM image and particle size distribution. (a) TEM image of Cor-DMA- $\gamma$ -CD dots and (b) DLS profile of Cor-DMA- $\gamma$ -CD dots obtained by fluidic method.

### 7.1. Isothermal Titration Calorimetry (ITC) of Cor-DMA with γ-CD

First experiment was conducted using a 3 mL solution of  $1 \times 10^{-5}$  M Cor-DMA, in which 25 µl of 1 mg/mL  $\gamma$ -CD was added at regular intervals at 298 K. The corresponding changes in heat were recorded *via* the ITC instrument. Subsequently, the corresponding thermodynamic parameters of this process were computed by fitting the plot of heat *vs* mole ratio by a sigmoidal curve (R<sup>2</sup> = 0.9965).



**Figure S9**. Isothermal titration calorimetry and kinetics of interaction of Cor-DMA with  $\gamma$ -CD. (a) Titration of  $\gamma$ -CD (1 mg/mL) into Cor-DMA (1 × 10<sup>-5</sup> M), in acetonitrile following different ratios. The cell volume is 2.0 ml and the injection volume if 25 µL. (b) Titration isotherm obtained from data Fig. S8a after automated thermogram processing. Analysis of the data sets yields the association constant, binding enthalpy, and binding entropy.

# 7.2. Isothermal Titration Calorimetry (ITC) of Cor-DMA with $\gamma\text{-}\text{CD}$ in a lower concentration

For dilute conditions, experiment was conducted using a 3 mL solution of  $1 \times 10^{-6}$  M Cor-DMA, in which 25 µL of 0.12 mg/ml  $\gamma$ -CD was added at regular intervals at 298 K. The corresponding changes in heat were recorded *via* the ITC instrument. It is evident from the plot that this system reached equilibrium after 10 additions and additionally no precipitation was observed in the solution. Therefore, this was considered as the equilibrium between complex and reactants (Step 1). On the contrary, if it were a single step process, a similar result to Fig. S7 would have been observed in this study, along with some minor dilution effects. As similar results were not observed, the two-step theory was proposed for the formation of Cor-DMA- $\gamma$ -CD aggregates. The data was fitted with 1:1 binding model and the thermodynamic properties for this step were computed.



**Figure S10**. Isothermal titration calorimetry and kinetics of interaction of Cor-DMA with  $\gamma$ -CD in dilute conditions. (a) Titration of  $\gamma$ -CD (0.1 mg/mL) into Cor-DMA (1 × 10<sup>-6</sup> M), in acetonitrile following different ratios. The cell volume is 2.0 mL and the injection volume if 25  $\mu$ L. (b) Titration isotherm obtained from data Fig. S9a after automated thermogram processing. Analysis of the data sets yields the association constant, binding enthalpy, and binding entropy.

The step was exothermic with ( $\Delta H$ ) of *c. a.* -47.32 kJ mol<sup>-1</sup>, which was *c.a.* 6 times lower than the overall step. Interestingly, as the thermodynamic properties are state properties, the thermodynamic nature of step 2 can be qualitatively established by subtracting the results of second study from first, which is rather difficult to estimate experimentally. The entropy change in second step was suspected to be higher than the first step, due to the spontaneous selfassembly assisted process that resulted in spherical nanoparticles. Therefore, the step 2 was found to be exothermic process with  $\Delta H = -252.53$  kJ mol<sup>-1</sup>. Moreover, both the steps were spontaneous and supporting the forward direction of reaction. The exothermic nature of the steps can be attributed to the release of energy during complexation as well as the self-arrangement step. Moreover, negative enthalpy also indicates the stable nature of products (complex and nanoparticles) compared to their respective reactants. Overall, the thermodynamic results from both these studies clearly reflect the feasibility of the entire process and also highlight the feasibility of the constituent steps.



Scheme S1. Proposed multistep interaction of Cor-DMA with  $\gamma$ -CD. (a) Reaction scheme and thermodynamic parameters estimated for titration of  $\gamma$ -CD (1 mg/mL) into Cor-DMA (1 × 10<sup>-5</sup> M), in acetonitrile leading to Cor-DMA- $\gamma$ -CD aggregates. (b) Diluted conditions and thermodynamic parameters estimated for titration of  $\gamma$ -CD (0.1 mg/mL) into Cor-DMA (1 × 10<sup>-6</sup> M), in acetonitrile. (c) Over all multistep host-guest interaction pathway for the formation of Cor-DMA- $\gamma$ -CD aggregates.



Scheme S2. Proposed multistep interaction mechanism of Cor-DMA with  $\gamma$ -CD towards formation of Cor-DMA- $\gamma$ -CD dots.

### 8. Control experiment



Figure S11. TEM image of Cor-DMA- $\gamma$ -CD dots post-treated with TFA in water.

### 9. Two-photon experiments

The measurements of TPA cross-sections. The measurements were performed by using femtosecond pulses from the Mai-Tai laser (80 MHz, 100 fs, Spectra-Physics, Inc.), with tunable wavelength range from 720 to 920 nm, as the excitation source. The laser beam was focused onto the sample that was contained in a quartz cuvette with a path length of 1 cm. The emission of the samples was collected from the edge and the signals were dispersed by a 750 mm monochromator combined with suitable filters, and detected by a photomultiplier tube (Hamamatsu R928) using a standard lock-in amplifier technique. A short pass filter with cut-off wavelength at 700 nm was placed before the spectrometer to minimize the scattering from the pump beam. TPA cross-section was determined from TPA induced fluorescence method using

rhodamine B as a reference with a known value. The TPA cross-section of samples can be calculated at each wavelength according to,

$$\delta = \delta_{ref} \frac{\mathbf{n}_{ref} \cdot \boldsymbol{\Phi}_{ref} \cdot \boldsymbol{C}_{ref} \cdot \boldsymbol{F}}{n \cdot \boldsymbol{\Phi} \cdot \boldsymbol{C} \cdot \boldsymbol{F}_{ref}} \tag{1}$$

In the equation, the subscript ref stands for the reference molecule.  $\delta$  is the TPA cross-section value, *n* is the refractive index of the solution,  $\phi$  is the fluorescence quantum yield, C is the concentration of solution and F is the two-photon emission intensity at each wavelength.

### 10. Cell culture, In vitro cytotoxicity assay and two-photon imaging

*Cell Culture:* MCF-7 cells were cultured in DMEM (Dulbecco's Modified Eagle's Medium) containing 10% FBS (fetal bovine serum) and nonessential aminoacids (0.1 mM). The culture was maintained at 37 °C in a humidified atmosphere containing 5% CO<sub>2</sub>.

*Cytotoxicity analysis*: Inherent cytotoxicity of Cor-DMA- $\gamma$ -CD dots was evaluated using the MTT (3-(4,5-dimethylthiozolyl-2)-2,5-diphenyltetrazolium bromide) viability assay with MCF-7 breast cancer cell lines (MCF-7 cell lines) incubated for 24 h. The cell viability was estimated by varying the concentrations of Cor-DMA- $\gamma$ -CD dots in microgram range. Results obtained from viability assay are shown in Fig. S11. The results clearly indicate that both Cor-DMA- $\gamma$ -CD dots and native  $\gamma$ -CD remain non-cytotoxic at low to moderate concentrations. The low cytotoxicity and good dispensability of Cor-DMA- $\gamma$ -CD dots in aqueous conditions with excellent luminescence properties further support its usage as a potential probe for *in vitro* two-photon bio-imaging.

*Imaging*: MCF-7 cells were seeded in 35 mm plastic-bottom  $\mu$ -dishes and grown in complete DMEM medium for 24 h before treated with Cor-DMA- $\gamma$ -CD dots (100 $\mu$ g mL<sup>-1</sup>). After 24 h

incubation, the medium was removed and cells were washed with PBS (Phosphate buffered saline) buffer (pH=7.4) for three times to remove any free dot residues in the culture dish. Formaldehyde (4%, 1mL) was used to fix the cells at room temperature for 15 min. After removing formaldehyde and washing with PBS buffer (pH=7.4) for three times before capturing images by using two-photon microscope (60x oil objective).



**Figure S12**. Cytotoxicity results of Cor-DMA- $\gamma$ -CD dots (control as  $\gamma$ -CD). The assay was carried out using MTT stain evaluated using MCF-7 cells lines for 24 h.



**Figure S13**. Flow cytometry profiles of Cor-DMA- $\gamma$ -CD dots conducted using MCF-7 cell lines. Green curve shows control without any stains. Red curve indicates results obtained with a batch of MCF-7 cells incubated and stained with Cor-DMA- $\gamma$ -CD dots.

### References

S1. Wu, Y.; Stuparu, M. C.; Boudon, C.; Gisselbrecht, J.; Schweizer, W. B.; Baldridge, K. K.; Seigel, J. S.; Diederich, F. Structural. Optical and Electrochemical Properties of Threedimensional Push-pull Corannulenes. *J. Org. Chem.*, **2012**, *77*, 11014.