

Supporting Information
Sequential and Phototriggered Supramolecular Self-Sorting Cascades
Using Hydrogen-Bonded Motifs

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General Considerations

All reagents were purchased from Aldrich or Alfa-Aesar and used without further purification unless otherwise stated. Where anhydrous solvents were required, THF was freshly distilled from sodium benzophenone ketyl radical, CH₂Cl₂ was freshly distilled from calcium hydride and CHCl₃ was freshly distilled from calcium chloride under a nitrogen atmosphere. Anhydrous DMF was obtained “sure-sealed” from Sigma-Aldrich. Triethylamine was distilled from calcium hydride and stored, under nitrogen, over potassium hydroxide pellets. All non-aqueous reactions were carried out under a nitrogen atmosphere. Analytical thin layer chromatography (TLC) was conducted using Merck Kiesegel 0.25 mm silica gel pre-coated aluminium plates with fluorescent indicator active at UV₂₅₄. Purification by column chromatography was carried out using Merck Kieselgel 60 silica gel. NMR spectra were obtained using Bruker DRX500 or Bruker DPX300 spectrometers operating at 500.13 MHz or 300.13 MHz for ¹H spectra and 125.76 MHz or 75.47 MHz for ¹³C spectra as stated. Proton spectra are referenced to TMS at 0.00 ppm, and carbon spectra to CDCl₃ at 77.4 ppm, unless otherwise stated. Melting points were determined using a Griffin D5 variable temperature apparatus and are uncorrected. IR spectra were obtained using Perkin-Elmer FTIR spectrometer. Microanalysis was carried out on a Carlo Erba Elemental Analyser MOD 1106 instrument. High Resolution Mass Spectra (HRMS) were recorded on a Micromass GCT Premier using electron impact ionisation (EI) or a Bruker Daltonics micrOTOF using electro spray ionisation (ESI).

Compounds **1**, **2** and **4** were synthesized as described previously.^{1,2} Compounds **3**, **5** and **6** were synthesized as described below:

2-Amino-6-tridecylpyrimidin-4-ol

This compound was synthesized following established literature procedures.³ Triethylamine (50.0 mL, 360 mmol) was added to a suspension of potassium ethyl malonate (40.8 g, 240 mmol) in MeCN (250 mL) that was stirring at 0°C. The reaction mixture was stirred for 15 mins before magnesium chloride (28.5 g, 300 mmol) in MeCN (80 mL) was added and allowed to warm to 10°C. After stirring for 2 hrs the reaction mixture was cooled to 0°C before myristoyl chloride (32.6 mL, 120 mmol) was added dropwise. The reaction mixture was warmed to room temperature and stirred for 16 hrs before the solvents were evaporated in vacuo. The resultant solid was dissolved in Et₂O (350 mL) and washed with 30% aqueous hydrochloric acid (250 mL) and saturated aqueous sodium bicarbonate (100 mL) before the

organics were dried (MgSO₄) and evaporated *in vacuo*. The resultant solid was purified by column chromatography (CHCl₃) to give ethyl 3-oxohexadecanoate (26.9 g, 75%) as a colourless powder and used without further purification. Guanidinium carbonate (12.8 g, 142.6 mmol) was added to a solution of ethyl 3-oxohexadecanoate (25.0 g, 83.9 mmol) and potassium tert-butoxide (9.4 g, 83.9 mmol) in EtOH (300 mL) and the reaction mixture was heated to reflux for 3 days. After cooling the reaction mixture was filtered and the filtrate was evaporated *in vacuo*. The resultant solid was dissolved in H₂O (500 mL) and the solution was acidified to pH 6 (acetic acid). The resultant suspension was filtered and the solid washed with acetone and Et₂O before being crystallised (propan-2-ol) to give *2-amino-6-tridecylpyrimidin-4-ol* as a cream coloured powder; m.p. 162-165°C; R_f 0.19 (1:9 MeOH:CH₂Cl₂); δ_H (300 MHz, DMSO-d₆); 6.58 (2H, s, NH₂), 5.33 (1H, s, ArCH), 2.20 (2H, t, *J* = 6.0 Hz, CH₂), 1.51 (2H, m, CH₂), 1.23 (20H, m, 10 x CH₂), 0.85 (3H, t, *J* = 6.0 Hz, CH₃); molecule was insufficiently soluble to obtain ¹³C NMR; ν_{max}/cm⁻¹ (neat); 3360, 3144, 2919, 2850, 2679 (br), 1637, 1468, 1401; ESI-HRMS found *m/z* 294.2529 [M+H]⁺, C₁₇H₃₂N₃O requires 294.2540.

Ethyl 4-(3-(4-oxo-6-tridecyl-1,4-dihydropyrimidin-2-yl)ureido)benzoate 3

Ethyl-4-isocyanatobenzoate (0.71 g, 3.75 mmol) was added to a refluxing solution of 2-Amino-6-tridecylpyrimidin-4-ol (1.00 g, 3.41 mmol) and triethylamine (0.48 mL, 3.75 mmol) in THF (50 mL). The reaction mixture was then stirred at reflux for 18 hrs before being allowed to cool and the volatiles evaporated *in vacuo*. The resultant solid was suspended in EtOAc (50 mL) and H₂O (50 mL) was added. The suspension was filtered and sonicated in EtOAc (50 mL) before being filtered and washed (Et₂O) to give *ethyl 4-(3-(4-oxo-6-tridecyl-1,4-dihydropyrimidin-2-yl)ureido)benzoate* (1.22 g, 74%) as a colourless powder; m.p. 218-220°C; R_f 0.38 (1:99 MeOH:CH₂Cl₂); δ_H (300 MHz, CDCl₃); 12.95 (1H, s, NH), 12.52 (1H, s, NH), 12.32 (1H, s, NH), 8.07 (2H, d, *J* = 8.7 Hz, ArCH), 7.86 (2H, d, *J* = 8.7 Hz, ArCH), 5.94 (1H, s, ArCH), 4.41 (2H, q, *J* = 7.2 Hz, CH₂), 2.47 (2H, t, *J* = 7.7 Hz, CH₂), 1.65 (4H, m, 2 x CH₂), 1.44 (3H, t, *J* = 7.2 Hz, CH₃), 1.32 (18H, m, 9 x CH₂), 0.92 (3H, t, *J* = 6.6 Hz, CH₃); δ_C (75 MHz, CDCl₃); 172.8, 166.2, 154.4, 154.3, 152.9, 142.8, 130.5, 125.3, 119.3, 105.9, 60.8, 32.4, 31.9, 29.6-29.2 (x7), 28.9, 26.3, 22.7, 14.4, 14.1; ν_{max}/cm⁻¹ (neat); 3466-2850, 1698, 1650; ESI-HRMS found *m/z* 485.3139 [M+H]⁺, C₂₇H₄₁N₄O₄ requires 485.3122.

***N*-(2-Nitrobenzyl)-*N*-(4-oxo-6-tridecyl-1,4-dihydropyrimidin-2-yl)benzylamide 5**

2-Nitrobenzylbromide (369 mg, 1.71 mmol) was added to a solution of 2-Amino-6-tridecylpyrimidin-4-ol (500 mg, 1.71 mmol) and K₂CO₃ (471 mg, 3.41 mmol) in DMF (20 mL) and the reaction mixture was heated to 50°C for 18 hrs. Once cooled the reaction mixture was filtered before the DMF was removed by vacuum distillation. The resultant solid was purified by column chromatography (gradient elution: 0:1-1:9 MeOH-CH₂Cl₂) to give a mixture of the *O*- and *N*-alkylation products (506 mg, 77%). These could not be separated so were both taken onto the next step. Benzoyl chloride (140 mL, 1.17 mmol) was added to a solution of the *O*- and *N*-alkylation products (500 mg, 1.17 mmol) and DMAP (14 mg, 0.12 mmol) in CHCl₃ (50 mL) and the reaction mixture was heated to reflux for 20 hrs. It was then allowed to cool before the solvent was removed *in vacuo* and the resultant solid was purified by column chromatography (gradient elution: hexane to CH₂Cl₂) to give *N*-(2-nitrobenzyl)-*N*-(4-oxo-6-tridecyl-1,4-dihydropyrimidin-2-yl)benzamide (170 mg, 27%) as a colourless sticky solid; R_f 0.42 (CH₂Cl₂); δ_H (300 MHz, CDCl₃); 13.94 (1H, s, NH), 8.04 (1H, d, *J* = 9.0 Hz, ArCH), 7.92 (2H, d, *J* = 9.0 Hz, 2 x ArCH), 7.44 (2H, m, 2 x ArCH), 7.32 (3H, m, 3 x ArCH), 7.12 (1H, d, *J* = 9.0 Hz, ArCH), 5.82 (1H, s, ArCH), 5.77 (2H, s, CH₂), 2.48 (2H, t, *J* = 7.5 Hz, CH₂), 1.65 (2H, m, CH₂), 1.25 (20H, m, 10 x CH₂), 0.81 (3H, t, *J* = 6.0 Hz, CH₃); δ_C (75 MHz, CDCl₃); 179.2, 161.5, 155.0, 153.4, 148.5, 136.4, 133.7, 132.6, 132.4, 129.4, 128.2, 128.0, 127.3, 125.1, 103.1, 42.3, 33.2, 31.9, 29.7 x 2, 29.6 x 2, 29.4 x 2, 29.2, 29.0, 26.9, 22.7, 14.2; ν_{max}/cm⁻¹ (neat); 3054, 2940, 2856, 1751, 1699, 1448, 1176, 917; ESI-HRMS found *m/z* 555.2936 [M+Na]⁺, C₃₁H₄₀N₄NaO₄ requires 555.2942.

***N*-(4-Oxo-6-tridecyl-1,4-dihydropyrimidin-2-yl)benzamide**

Benzoyl chloride (0.2 mL, 1.6 mmol) was added dropwise to a stirring solution of 2-amino-6-tridecylpyrimidin-4(1*H*)-one (0.4 g, 1.7 mmol) and 4-dimethylaminopyridine (2 mg, 0.2 mmol) in CHCl₃ (50 mL). The reaction mixture was heated to reflux for 16 hr. The reaction was cooled to room temperature and the solvent was removed *in vacuo*. The recovered solid was purified by column chromatography (60:40 hexane-ethyl acetate) to give *N*-(4-Oxo-6-tridecyl-1,4-dihydropyrimidin-2-yl)benzamide (0.21 g, 33 %) as a colourless powder; m.p: decomposition 176–178 °C; δ_H (300 MHz, CDCl₃); 7.88 (2H, d, *J* = 7.7 Hz, Ar-H), 7.59 (1H, t, *J* = 7.7 Hz, Ar-H), 7.45 (2H, t, *J* = 7.7 Hz, Ar-H), 5.95 (1H, s, pyrimidyl- H), 2.38 (2H, t, *J* = 7.7 Hz, pyrimidyl-CH₂CH₂-), 1.57 (2H, m, pyrimidyl-CH₂CH₂-), 1.18 (20H, m, alkyl- CH₂) and 0.81 (3H, t, *J* = 7.1 Hz, alkyl-CH₃); δ_C (75 MHz, CDCl₃); 163.4, 150.8, 145.5, 133.7,

132.1, 129.0, 127.9, 94.9, 36.7, 31.9, 29.7, 29.6, 29.5, 29.4, 29.1, 27.7, 22.7 and 14.1; $\nu_{\max}/\text{cm}^{-1}$ (solid state) = 3193, 1751 and 1644; ESI-HRMS found mass 398.2809 $[\text{M} + \text{H}]^+$ $\text{C}_{24}\text{H}_{36}\text{N}_3\text{O}_2$ requires 398.2729.

***N*-(4,5-Dimethoxy-2-nitrobenzyl)-*N*-(4-oxo-6-tridecyl-1,4-dihydropyrimidin-2-yl)benzamide 6**

2-Amino-6-tridecylpyrimidin-4(1*H*)-one (0.5 g, 1.7 mmol) and potassium carbonate (0.47 g, 3.4 mmol) were suspended in dimethylformamide. With stirring, 4,5-dimethoxy-2-nitrobenzyl bromide (0.47 g, 1.71 mmol) was added and the reaction mixture subsequently protected from light with aluminium foil and heated to 50 °C for 18 hr. After cooling, the reaction mixture was filtered and the solvent removed under reduced pressure. The recovered solid was purified by column chromatography (60:40 hexane–ethyl acetate) to give a mixture of the *O*- and *N*- alkylation products (0.59 g, 71 %) as a yellow powder (ESI-HRMS found mass 488.2999 $[\text{M} + \text{H}]^+$ $\text{C}_{27}\text{H}_{41}\text{N}_4\text{O}_5$ requires 489.3084). These could not be separated so were both taken on to the next step; 2-((4,5-Dimethoxy-2-nitrobenzyl)amino)-6-tridecylpyrimidin-4(1*H*)-one (0.5 g, 1.0 mmol) and 4-dimethylaminopyridine (0.01 g, 0.10 mmol) were dissolved in CHCl_3 (50 mL). Benzoyl chloride (0.12 mL, 1.0 mmol) was added dropwise with stirring and the reaction mixture subsequently heated to reflux for 20 hr. After cooling to room temperature, the solvent was removed *in vacuo* and the recovered solid was purified twice by column chromatography (60:40 hexane–ethyl acetate) then CH_2Cl_2 to provide *N*-(4,5-Dimethoxy-2-nitrobenzyl)-*N*-(4-oxo-6-tridecyl-1,4-dihydropyrimidin-2-yl)benzamide (50 mg, 9 %) as a white powder. δ_{H} (500 MHz, CDCl_3): 7.97 (2H, d, $J = 8.2$, benzoyl-Ar-H), 7.57 (1H, s, nitrobenzyl-Ar-H), 7.43 (1H, t, $J = 7.3$, benzoyl-Ar-H), 7.33 (2H, t, $J = 7.3$, benzoyl-Ar-H), 6.63 (1H, s, nitrobenzyl-Ar-H), 5.81 (1H, s, pyrimidyl-H), 5.79 (2H, s, nitrobenzyl-CH₂), 3.85 (3H, s, nitrobenzyl-O-CH₃), 3.67 (3H, s, nitrobenzyl-O-CH₃), 2.47 (2H, t, $J = 7.8$, pyrimidyl-CH₂CH₂-), 1.66 (2H, quin., $J = 7.3$, pyrimidyl-CH₂CH₂-), 1.19 (20H, m, alkyl-CH₂) and 0.81 (3H, t, $J = 7.3$ Hz, alkyl-CH₃); δ_{C} (75 MHz, CDCl_3): 193.2, 187.5, 184.9, 172.9, 153.3, 147.8, 142.4, 137.3, 136.4, 132.6, 129.4, 128.3, 127.0, 126.6, 109.1, 108.1, 103.0, 56.2, 42.1, 33.1, 29.6, 33.1, 31.9, 29.6, 29.5, 29.4, 28.9, 22.7 and 14.1; ESI-HRMS found mass 615.3146 $[\text{M} + \text{Na}]^+$ $\text{C}_{33}\text{H}_{44}\text{N}_4\text{O}_6\text{Na}$ requires 615.3158. Melting points and IR spectra were not obtained due to the sensitive nature of this compound.

NMR complexation and titration experiments

^1H NMR complexation spectra are given in the following section for previously unpublished interactions so as to further facilitate comparison with the self-sorting ^1H NMR spectra given in the main manuscript. NMR complexation and titration data for the following complexes was taken from the literature: UIM:UIM **1:1**⁴ AIC:UIM **1:2**² UIM:UPy **1:3** UIM:DAN **1:4**¹ AIC:AIC **2:2**⁴ UPy:UPy **3:3**³ UPy:DAN **3:4**⁵ DAN:DAN **4:4**⁶. For those binding constants reported by other groups the methods used to determine the binding constants are sufficiently similar to justify their use in speciation calculations. Titration experiments for uncharacterized pairings were performed as previously described; representative isotherms are given where applicable.²

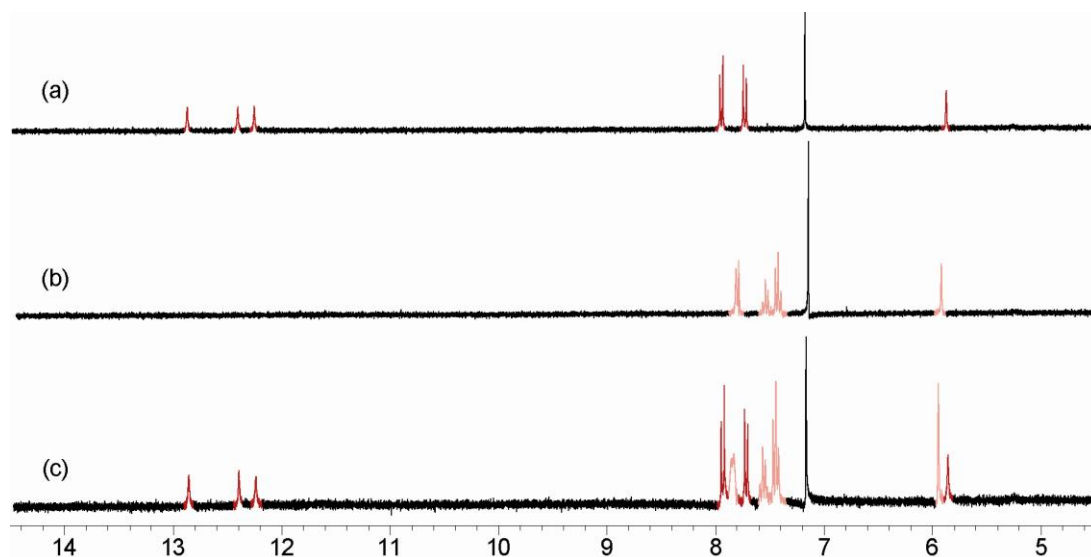


Figure S2. ^1H NMR spectra (10mM, 300 MHz, CDCl_3) of the UPy **3** and AIC **2** (a) UPy **3**, (b) AIC **2** and (c) a 1:1 mixture of UPy **3** and AIC **2**. The data indicate minimal interaction between UPy **3** and AIC **2**.

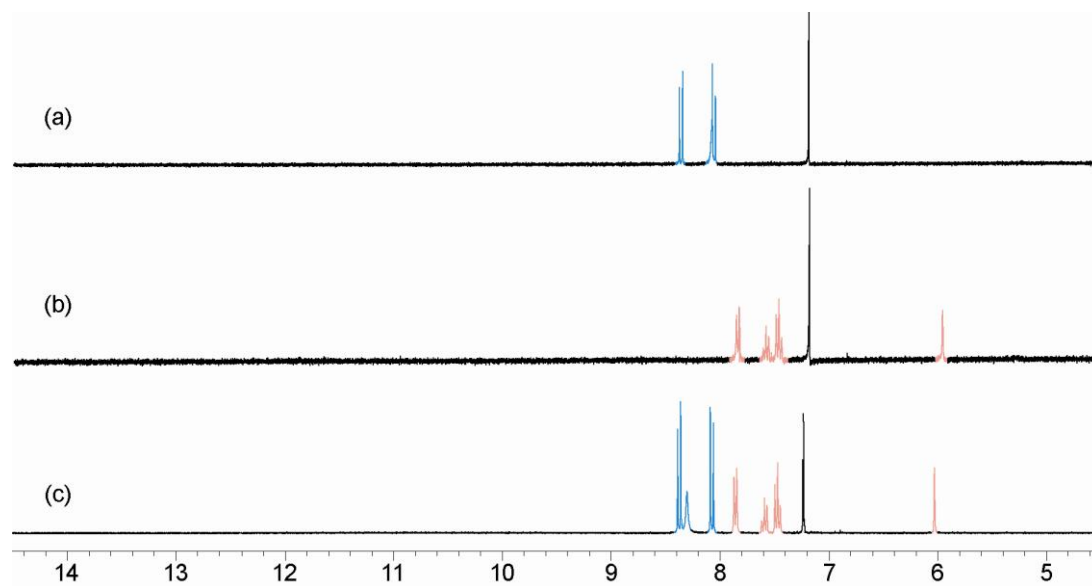


Figure S2. ¹H NMR spectra (10mM, 300 MHz, CDCl₃) of the DAN **4** and AIC **2** (a) DAN **4**, (b) AIC **2** and (c) a 1:1 mixture of DAN **4** and AIC **2**. The data indicate minimal interaction between DAN **4** and AIC **2**.

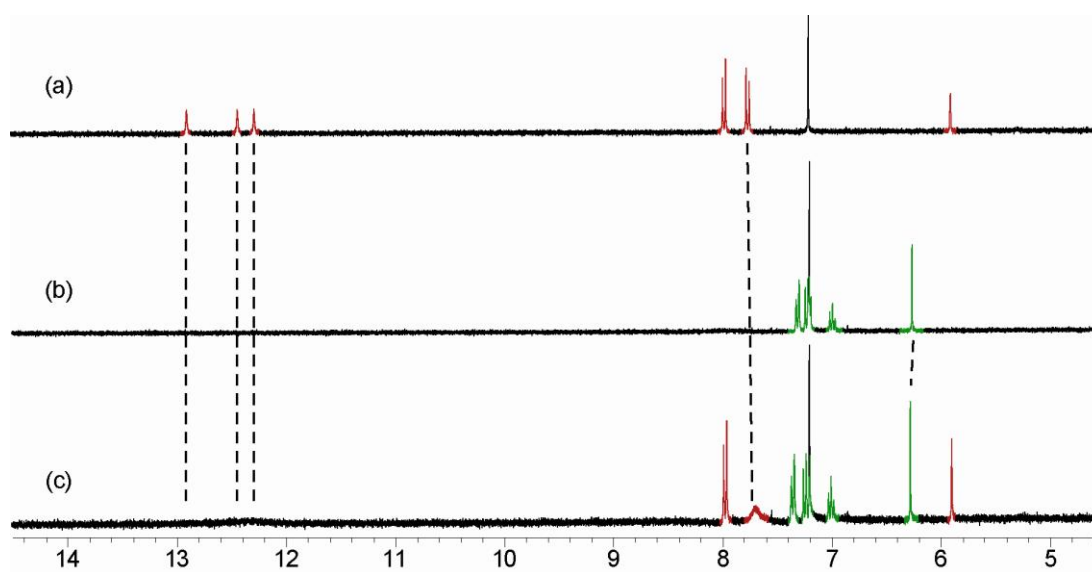


Figure S3 ¹H NMR spectra (10 mM, 300 MHz, CDCl₃) of the UPy **3** and UIM **1** (a) UPy **3**, (b) UIM **1** and (c) a 1:1 mixture of UPy **3** and UIM **1**.

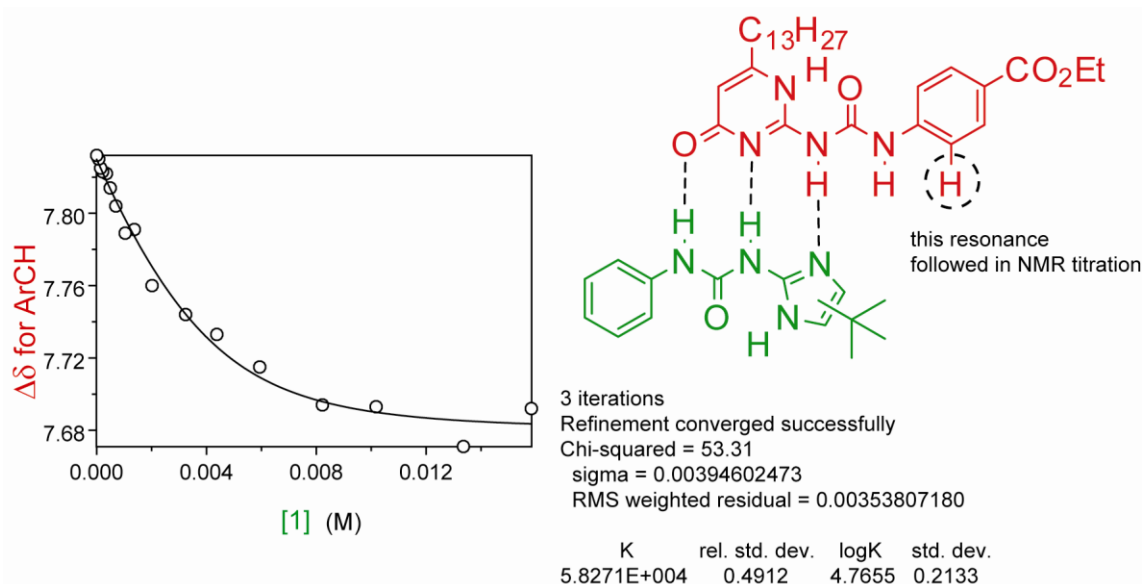


Figure S4 Titration isotherm and fitting data for the binding of UIM **1** to UPy **3**.

Comment on the difference in binding affinity for UIM 1:UPy 3 and UIM 1:DAN 4. We attribute the 1 order of magnitude difference in binding affinity between these two complexes to differences in secondary interactions which for the former are attractive whilst for the later are repulsive in the proposed structures of the complexes as illustrated below.

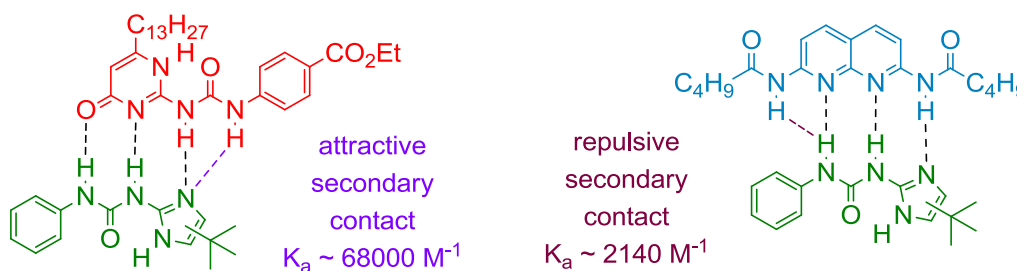


Figure S5 Proposed structures for UIM **1**:UPy **3** and UIM **1**:DAN **4**, together with an explanation for the differences in binding affinity.

Additional Self-Sorting Cascades

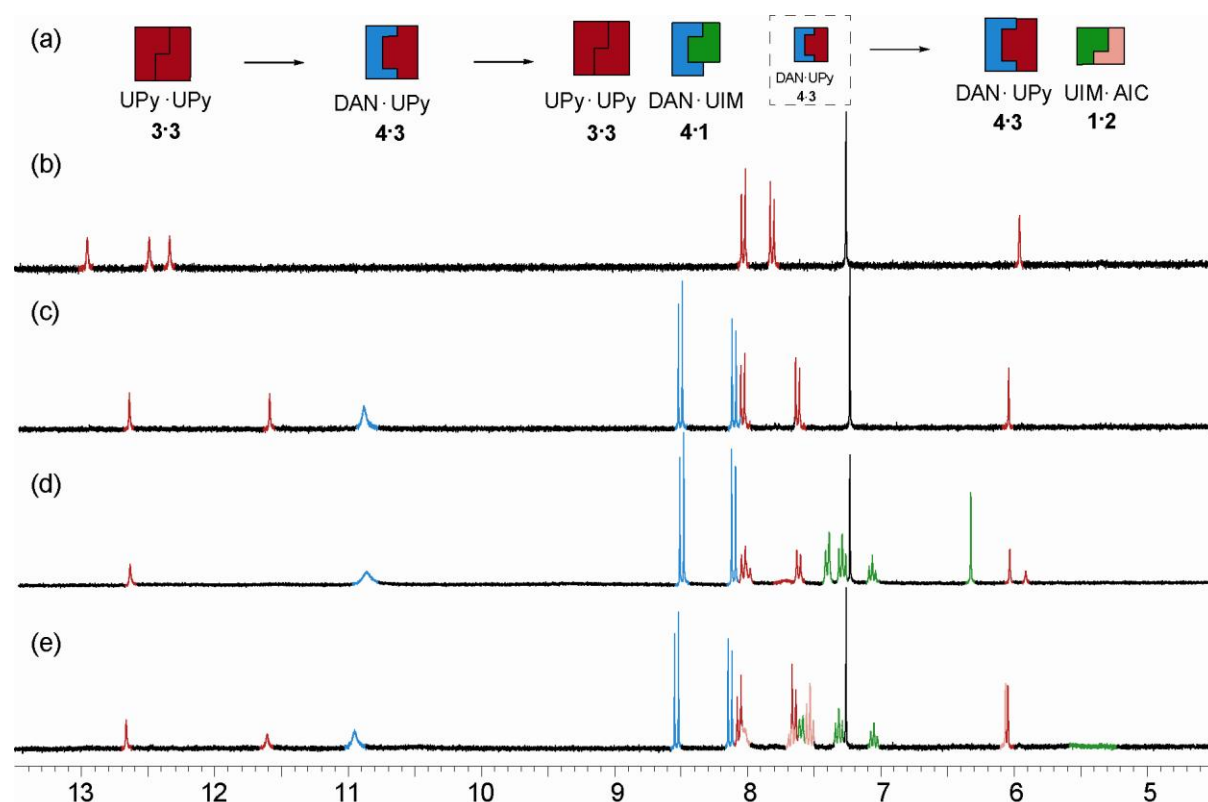


Figure S6. Signalling cascade using hydrogen-bonding motifs **1-4** (a) a schematic depicting complexes that form upon addition of different components to the supramolecular ensemble (b-e) 300 MHz NMR spectra (10 mM, CDCl_3 , 293K) of the signalling cascade following *path II* (b) UPy **3** (c) UPy **3** and DAN **4**, (d) UPy **3**, UIM **1** and DAN **4**, (e) UPy **3**, UIM **1**, AIC **2** and DAN **4**.

The NMR spectrum shown in Fig S6d does not show the diagnostic NH resonances associated with the UPy **3** homodimer or those associated with the UPy·DAN **3·4** heterodimer. Two distinct resonances for UPy **3** are observed consistent with the presence of at least two different complexes (in all likelihood in slow exchange). Although the possibility of higher order (e.g. ternary) complexes cannot be discounted, a more likely interpretation is that the presence of several complexes involving UPy **3** results in signal broadening of those resonances directly involved in H-bonding. In support of this, the speciation calculations shown in Fig S9 suggest that based on association constants, the dominant complex at 10 mM is **1·4** with **3·3** and **3·4** also present in significant quantities.

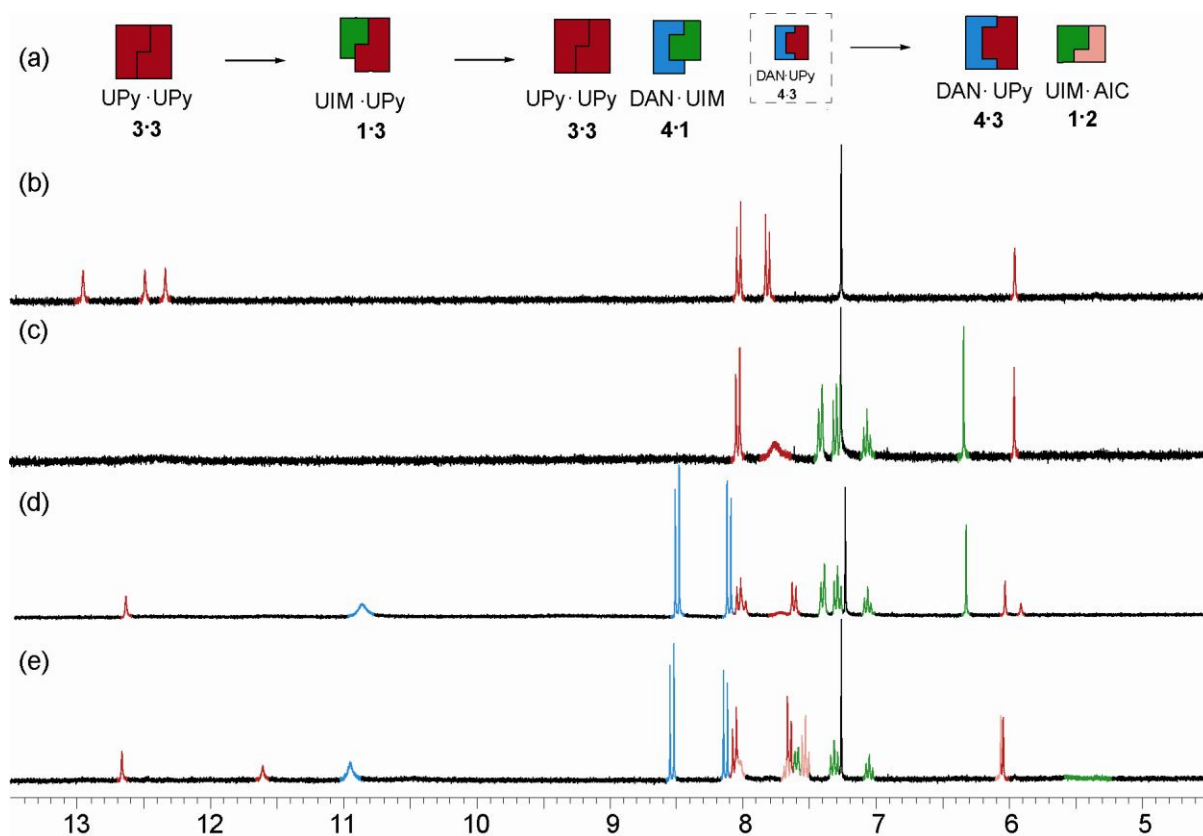


Figure S7. Signalling cascade using hydrogen-bonding motifs **1-4** (a) a schematic depicting complexes that form upon addition of different components to the supramolecular ensemble (b-e) 300 MHz NMR spectra (10 mM, CDCl_3 , 293K) of the signalling cascade following *path III* (b) UPy **3** (c) UPy **3** and UIM **1**, (d) UPy **3**, UIM **1** and DAN **4**, (e) UPy **3**, UIM **1**, AIC **2** and DAN **4**.

Similarly to Path II, the NMR spectrum shown in Fig S6d suggests the probability of several complexes. The rationale is the same as this phase of Path III is identical to the corresponding phase of Path II. Similarly, the speciation calculations shown in Fig S10 suggest that based on association constants, the dominant complex at 10 mM is **1·4** with **3·3** and **3·4** also present in significant quantities.

Determination of Fidelity

Fidelity was calculated as described by Zimmerman.⁷ Briefly, the composition of the mixture at each stage of the signalling cascade was calculated using HySS and the measured binding constants.⁸ The concentration of all molecules already present was set to 10mM and the concentration of the molecule being added was increased from 0-50mM. The fidelity of each step of the signalling cascade during addition of the new component was calculated using equation 1. As a representative example for the 4 component system, equation 2 is given.

$$Fidelity = \frac{\text{total concentration of desired complexes}}{\text{total concentration of all complexes}}$$

(Equation 1)

$$Fidelity = \frac{[DAN. UPy] + [AIC. UIM]}{[DAN] + [UPy] + [AIC] + [UIM] + [UPy. UPy] + [DAN. DAN] + [AIC. AIC] + [UIM. UIM] + [DAN. UPy] + [DAN. AIC] + [DAN. UIM] + [UPy. UIM] + [UPy. AIC] + [UIM. AIC]}$$

(Equation 2)

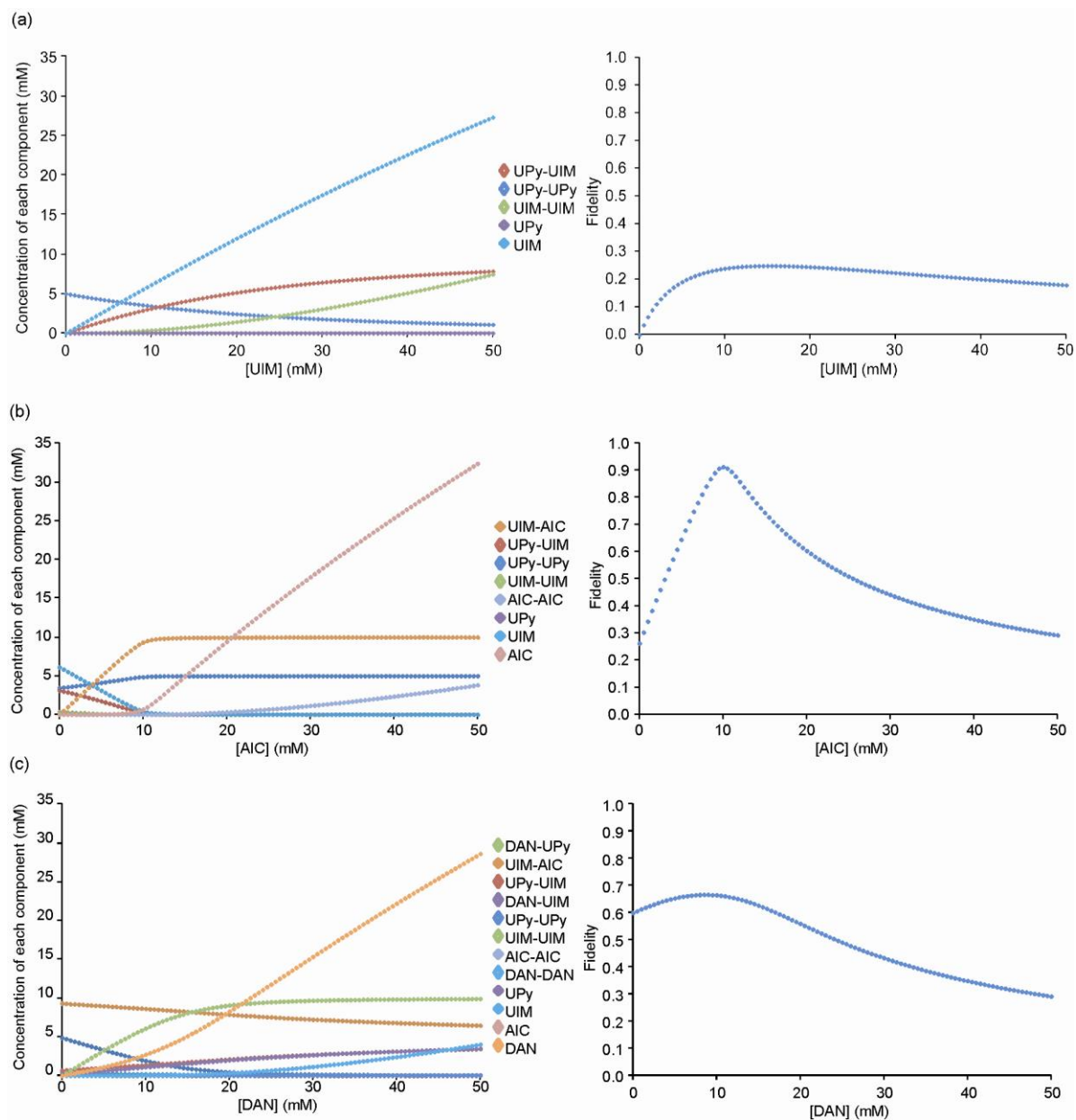


Figure S8. The calculated speciation and fidelity plots for all components in the *path I* cascade were determined as each molecule was added. (a) UPy 3:UPy 3 > UPy 3:DAN 2, (b) UPy 3:UIM 1 > UPy 3:UPy 3 + UIM 1:AIC 2. (c) UPy 3:UPy 3 + UIM 1:AIC 2 > UPy 3:DAN 4 + UIM 1:AIC 2. All experiments begin at 10mM.

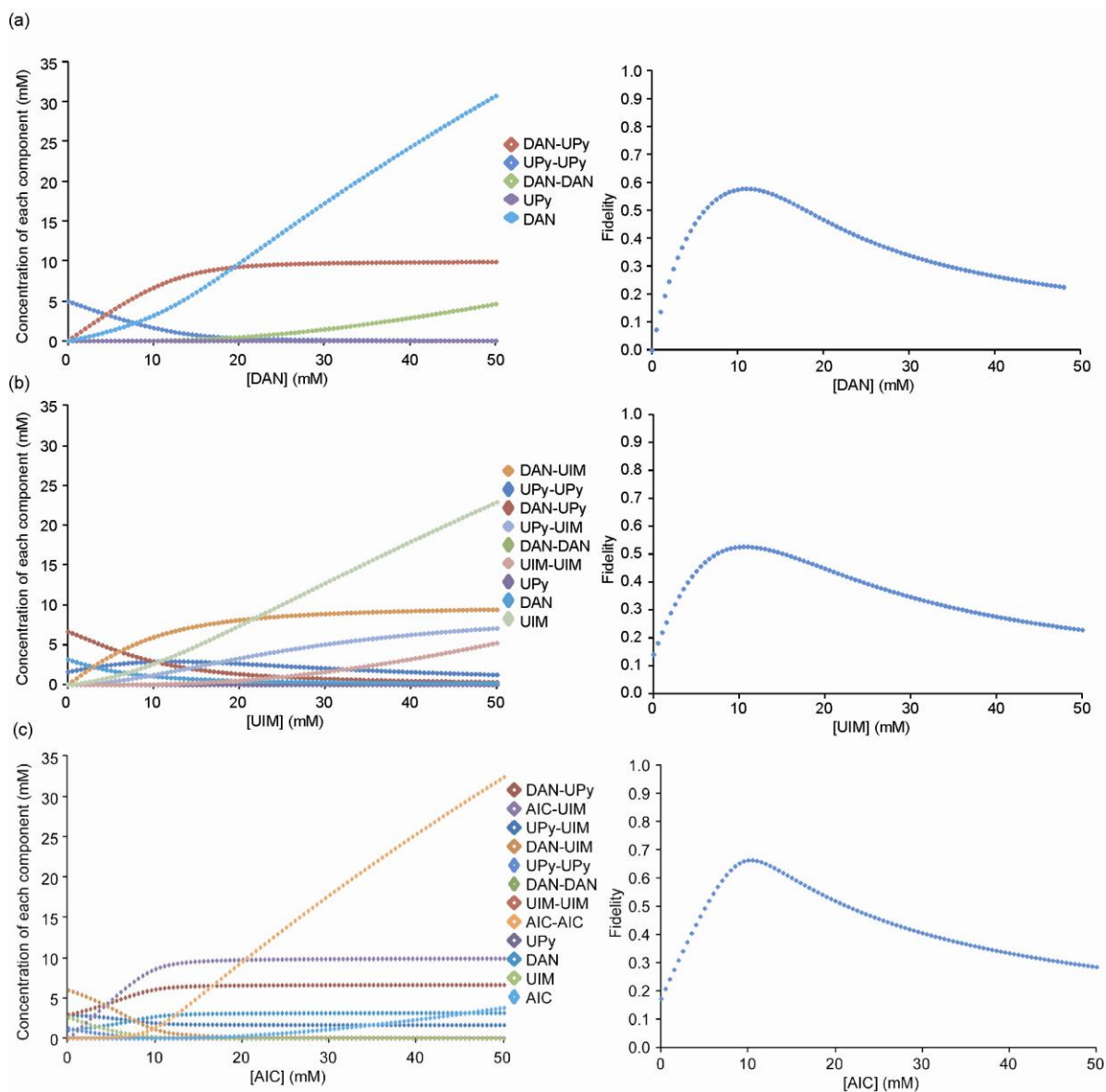


Figure S9. The calculated speciation and fidelity plots for all components in the *path II* cascade were determined as each molecule was added. (a) UPy 3:UPy 3 > UPy 3:DAN 4, (b) UPy 3:DAN 4 > UPy 3:UPy 3 + UIM 1:DAN 4. (c) UPy 3:UPy 3 + UIM 1:DAN 4 > UPy 3: DAN 4 + UIM 1:AIC 2. All experiments begin at 10mM.

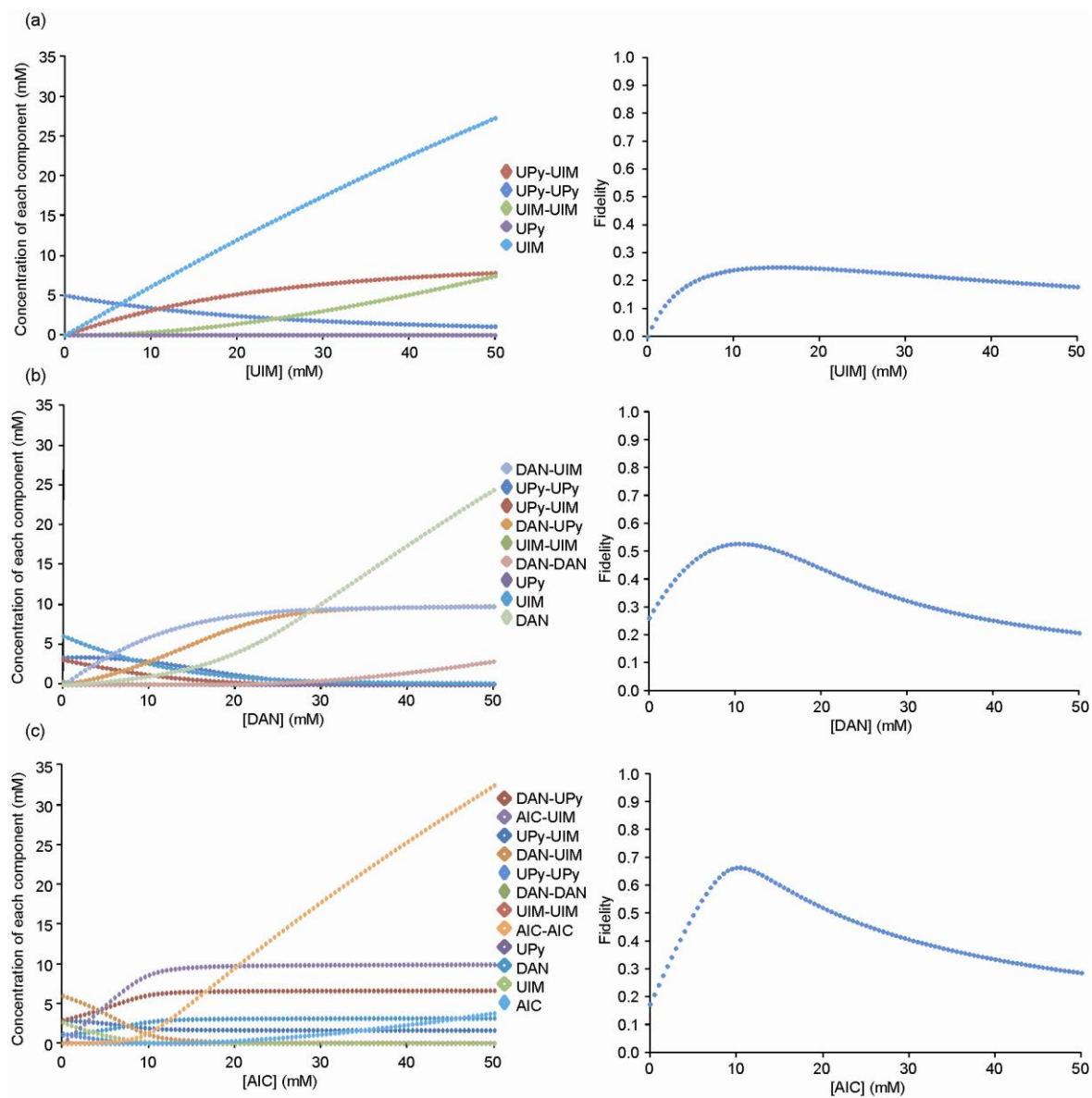


Figure S10. The calculated speciation and fidelity plots for all components in the *path III* cascade were determined as each molecule was added. (a) UPy 3:UPy 3 > UPy 3:UIM 1, (b) UPy 3:UIM 1 > UPy 3:UPy 3 + UIM 1:DAN 4. (c) UPy 3:UPy 3 + UIM 1:DAN 4 > UPy 3:DAN 4 + UIM 1:AIC 2. All experiments begin at 10mM.

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