

# Metallo-responsive switching between hexadecameric and octameric supramolecular G-quadruplexes

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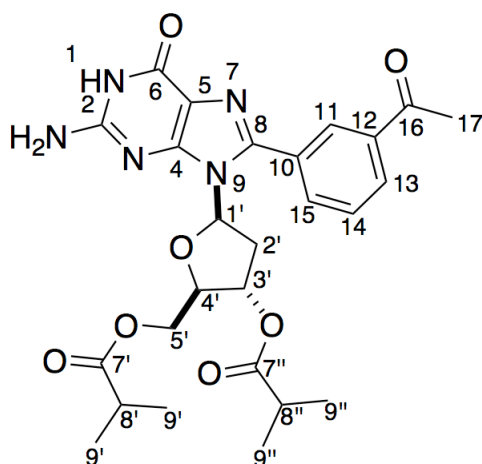
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## A. Characterization of the target compound

NMR spectra were recorded on Bruker DRX-500 or AV-500 spectrometer, equipped with either a 5 mm BBO or a TXI probe and with nominal frequencies of 500.13 MHz for proton and 125.77 MHz for carbon respectively. All NMR experiments were performed at 298.15 K unless otherwise stated. All the assemblies were characterized with  $^1\text{H}$  NMR and 2D-NMR techniques such as COSY and NOESY.

The synthesis and characterization for **1** was reported in Gubala, V.; Betancourt, J. E.; Rivera, J. M. *Org. Lett.* **2004**, 6, 4735-4738.



## B. $^1\text{H}$ NMR studies of **1** using potassium and strontium salts

A monovalent and a divalent cation were used to promote the cation-templated self-assembly of **1**. The addition of 0.5 equiv of KI and 0.125 equiv of  $\text{SrI}_2$ ,<sup>1</sup> into 30 mM solutions of the **1** in  $\text{CD}_3\text{CN}$  yield the formation of different GQs, different molecularities with high fidelity were observed (Table S1).<sup>2</sup>

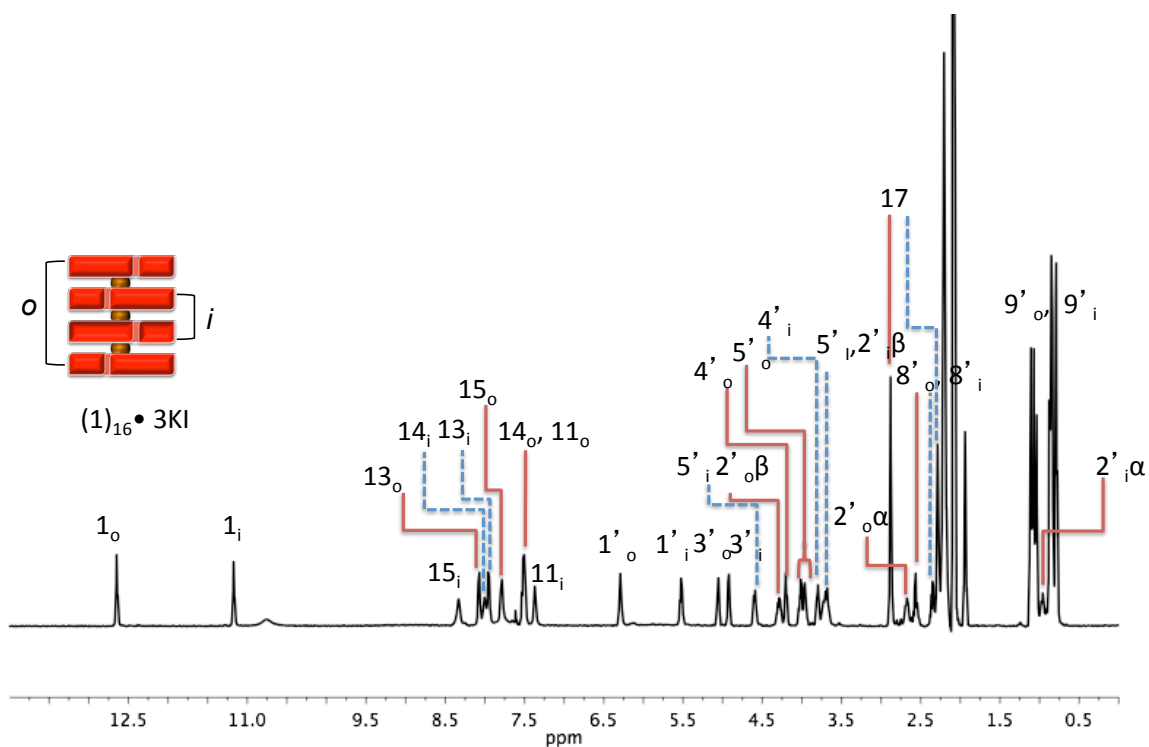
**Table S1** Type (molecularity) and relative amounts (fidelity) of quadruplexes formed by **1** (30 mM) determined by  $^1\text{H}$  NMR (0.5 equiv of KI, and 0.125 equiv of  $\text{SrI}_2$  at 298.2 K) in  $\text{CD}_3\text{CN}$ .

Cation	Assembly (%)		
	$\text{O}_{\text{D4}}$	H	UA
$\text{K}^+$	-	90	10
$\text{Sr}^{2+}$	100	-	-

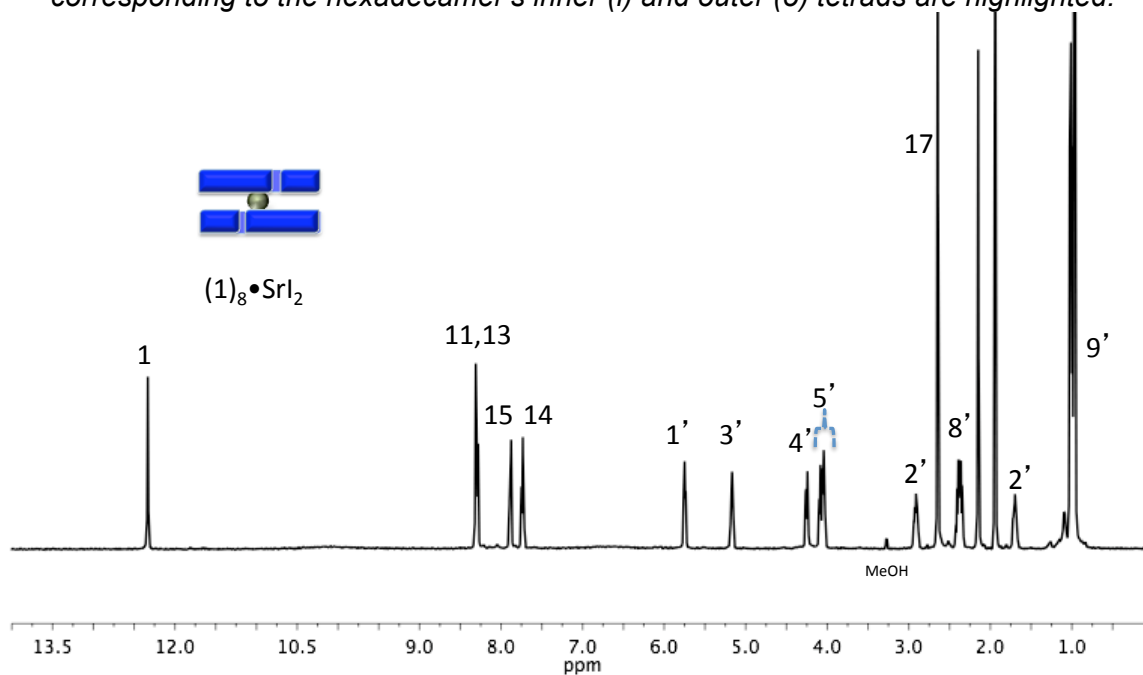
Note:  $\text{O}_{\text{D4}}$  = Octamer (D4 symmetry); H = Hexadecamer; UA = Unidentified Assembly  
All measurements have an estimated 5% error

<sup>1</sup> The equivalents of salt reported are calculated relative to the monomeric subunits. For example, 0.5 equiv of KI relative to **1** represents 8 equiv (or an excess) of  $\text{K}^+$  relative to  $\mathbf{1}_{16}$ .

<sup>2</sup> Fidelity refers to the percentage of the desired supramolecule when there are two or more potential outcomes. For more information see: Todd, E. M.; Quinn, J. R.; Park, T.; Zimmerman, S. C., Fidelity in the supramolecular assembly of triply and quadruply hydrogen-bonded complexes. *I. J. Chem.* **2005**, *45*, 381-389.



**Fig. S1**  $^1\text{H}$  NMR (500 MHz,  $\text{CD}_3\text{CN}$ , 298.2 K) of **1** (30 mM) with 0.5 equiv of KI. The signals corresponding to the hexadecamer's inner (i) and outer (o) tetrads are highlighted.



**Fig. S2**  $^1\text{H}$  NMR (500 MHz,  $\text{CD}_3\text{CN}$ , 298.2 K) of **1** (30 mM) with 0.125 equiv of  $\text{Srl}_2$ .

### C. Vapor Pressure Osmometry Studies

Vapor Pressure Osmometer (VPO) model Knauer K-7000 with the corresponding software EuroOsmo® version 1.3 was used. The calibration of the instrument was performed, using a standard of formula  $C_{54}H_{57}N_9O_{12}$  and a molecular weight of 1024.08 g/mol. Solutions with concentrations ranging from (0.02 - 0.05) molal were prepared, after the calibration procedure, a calibration curve and calibration constant ( $K_{\text{calib}}$ ) value were obtained. For each solution at different concentrations, 3-5 measured values were obtained with good to excellent standard deviation. Validation of the calibration method showed a molecular weight for the standard of 1010 g/mol, which compared to the theoretical value of 1024.08 g/mol, represents a discrepancy of 1.4%. Solutions of **1** (45 - 25 mM) in dry acetonitrile containing 0.5 equiv of the potassium and 0.125 equiv of the strontium salt. Again for each of these solutions 3-5 measurements were obtained with good to excellent standard deviation. The start gain was set to 128, the chamber temperature to 40 °C and the head temperature to 45 °C. To obtain a stable baseline, drops of solvent were added to the thermistors and the system was auto-zeroed several times together with a stabilization time of about an hour after obtaining the best possible baseline. Input of the concentrations in g/kg prior sample injection lead to computer calculated average molecular weight for a given sample. VPO experiments were performed for assemblies that show 90% of fidelity or higher.

**Table S2** Molecular weight (MW) as determined by VPO in  $CH_3CN$ .

Sample	Solution composition	MW (Da)		
		Calculated*	Experimental	Discrepancy
1	$1_{16} \cdot 3(KI)$	8907	8661	246
	$1_8 \cdot SrI_2$	4546	4566	20

\* Calculated assuming 100% fidelity in  $CH_3CN$ .

For VPO standard synthesis and preparation see: Rivera-Sánchez, M. d. C.; Andújar-de-Sanctis, I.; García-Arriaga, M.; Gubala, V.; Hopley, G.; Rivera, J. M. *J. Am. Chem. Soc.* **2009**, *131*, 10403–10405

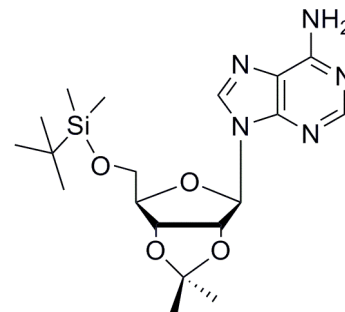
## D. Diffusion NMR studies

Diffusion NMR experiments were carried out in a Bruker DRX-500 spectrometer equipped with a 5 mm BBO probe, using the Stimulated Echo Pulse Gradient sequence (stebpgp1s) in FT mode. To improve homogeneity a “13 interval pulse sequence” was used with two pairs of bipolar gradients, all samples were prepared in Shigemi tubes (Shigemi, Inc., Allison Park, PA) and the temperature was actively controlled at  $(25.0 \pm 0.5)$  °C. Diffusion coefficients ( $D$ ) were derived using integration of the desired peaks to a single exponential decay, using the Bruker software package T1/T2 Relaxation (TopSpin v 2.0). The supramolecules were assumed to be spherical<sup>3</sup> and their

$$D = \frac{k_B T}{6 \pi \eta r};$$

hydrodynamic radii were calculated with the Stokes-Einstein equation:

where  $T$  denotes the temperature,  $\eta$  is the viscosity of the solvent at the given temperature,  $k_B$  is the Boltzmann-Constant,  $D$  is the measured diffusion constant and  $r$  is the hydrodynamic radii. For the calculations of the hydrodynamic radii in  $CD_3CN$  the value ( $\eta = 0.341 \times 10^{-3} \text{ Kg}\cdot\text{m}^{-1}\cdot\text{s}^{-1}$ , 298.15 K) reported elsewhere was used. All the measurements were performed in triplicate and the uncertainty is given as the standard deviation. An internal adenosine standard was used to validate the experiments,  $D = (12.7 \pm 1) \times 10^{-10} \text{ m}^2 \text{ s}^{-1}$  and  $r = (5.0 \pm 0.5) \text{ \AA}$  at 298.15 K.



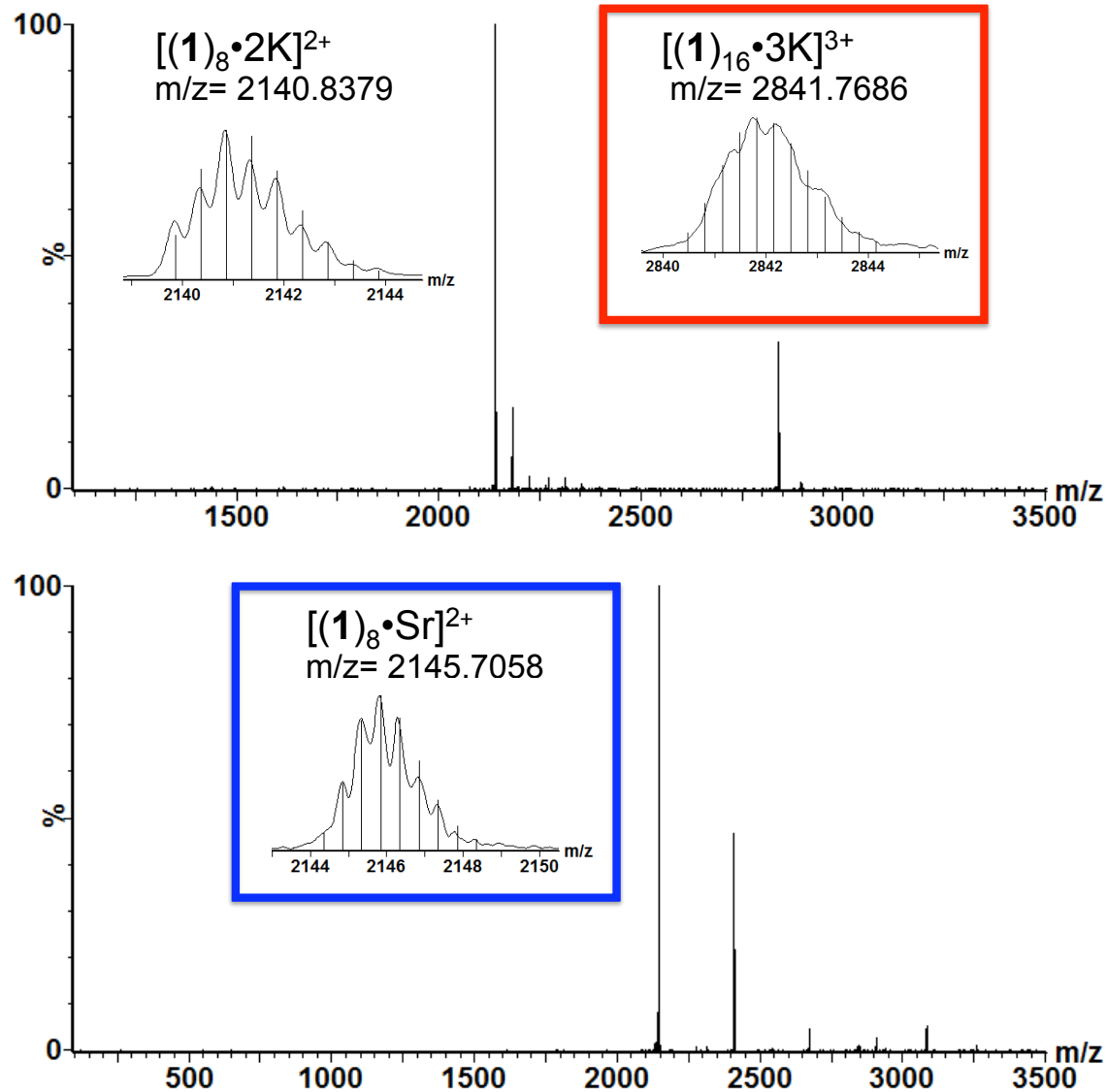
**Adenosine Standard**

**Table S3** Diffusion coefficients ( $D_s$ ) and hydrodynamic radii ( $R_H$ ) of the assemblies formed by **1**, determined by PFG–NMR in  $CD_3CN$ .

Assembly	$D_s$ ( $10^{-10} \text{ m}^2/\text{s}$ ) $\pm$ SD	( $R_H$ ) ( $\text{\AA}$ )
<b>1</b> <sub>16</sub> • <b>3</b> (Kl)	$6.4 \pm 0.1$	$10.0 \pm 0.2$
<b>1</b> <sub>8</sub> •Srl <sub>2</sub>	$7.0 \pm 0.2$	$9.2 \pm 0.3$

## E. Mass Spectrometry

High-resolution electrospray ionization mass spectrometry (ESI MS) was recorded on a Q-ToF Ultima Global mass spectrometer (Micromass) equipped with a Z-spray source. Electrospray ionization was achieved in the positive mode by 3 kV on the needle. 10mM solutions of monomer **1** in acetonitrile with 0.5 equiv of KI and 0.125 equiv of  $\text{SrI}_2$  were prepared. At room temperature these samples were directly and continuously infused at a flow rate of 5  $\mu\text{L}/\text{min}$  with a syringe pump. The source block temperature was maintained at 60° C and the desolvation gas was heated to 80° C. Argon was used as the collision gas and the cone voltage was set to 35 V. The mass spectrometer was operated in the mass range 0-4000 amu.



**Fig. S3** MS spectra of **1** (10 mM) solutions with 0.5 equiv of KI (top) and 0.125 equiv of  $SrI_2$  (bottom) in  $CH_3CN$ .

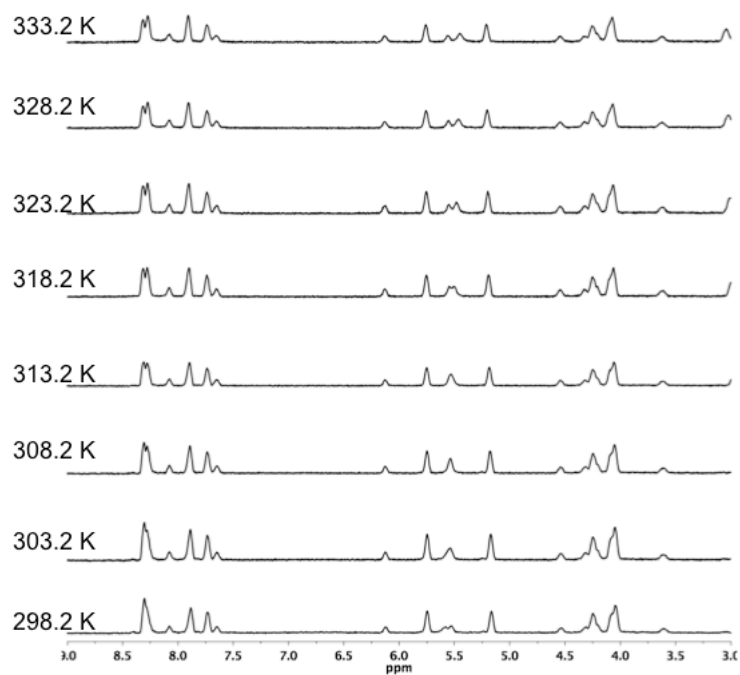


## F. Thermal stability studies

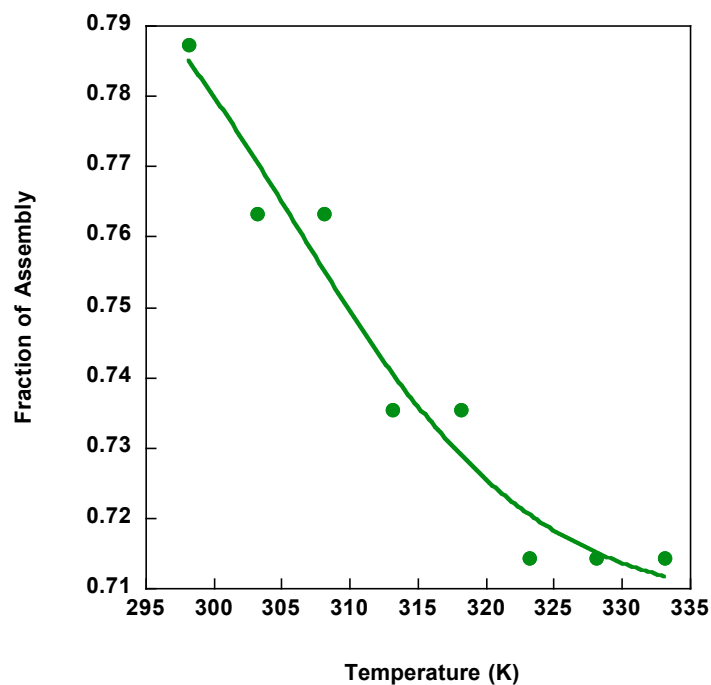
Variable temperature experiments were performed at 5 mM of **1**. At this particular concentration there is enough monomer and self-assembled species to enable the construction of a melting profile. Integration of the area under the H1' peaks and the first derivative calculations allows a good approximation of the melting temperature for the assemblies. The fraction ( $f$ ) of assembly (fidelity)  $f = X_{SA}/X_T$ , is reported as the ratio between the total self-assembled species ( $X_{SA}$ ) and the total concentration of **1** ( $X_T$ ), see Fig.S4 for more information. Value for the thermal stability of the hexadecamer form by **1** in KI was taken from Betancourt, J. E.; Martín-Hidalgo, M.; Gubala, V.; Rivera, J. M., *J. Am. Chem. Soc.* **2009**, *131*, 3186-3188.

**Table S4** Melting temperatures for the assemblies of **1** (5mM) as a function of different metal salts.

Metal salt	$T_m$ (K)
KI	329.2
SrI <sub>2</sub>	>333.2



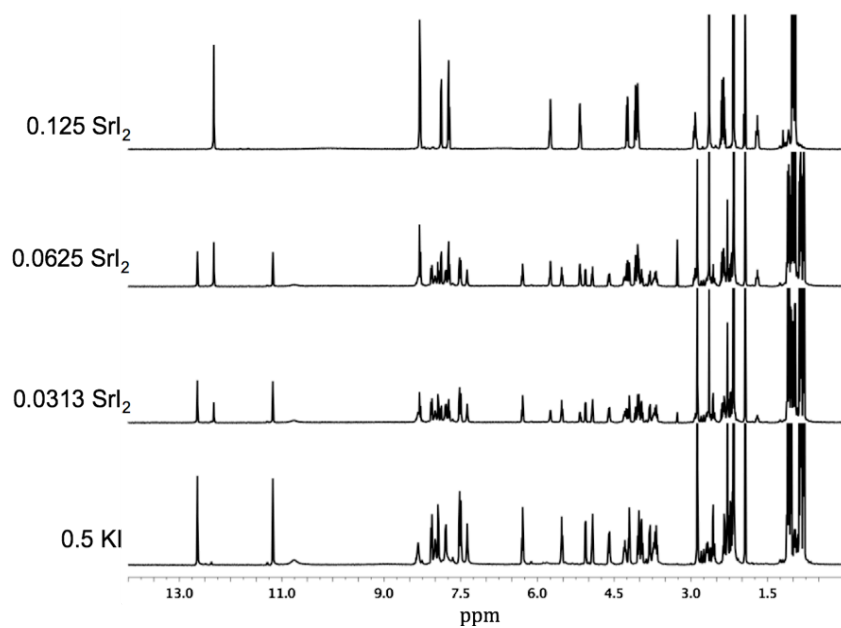
**Fig. S4** Variable temperature <sup>1</sup>H NMR (500 MHz, CD<sub>3</sub>CN) spectra **18**•Sr<sup>2+</sup> (5 mM in **1**, with 0.125 equiv of Srl<sub>2</sub>).



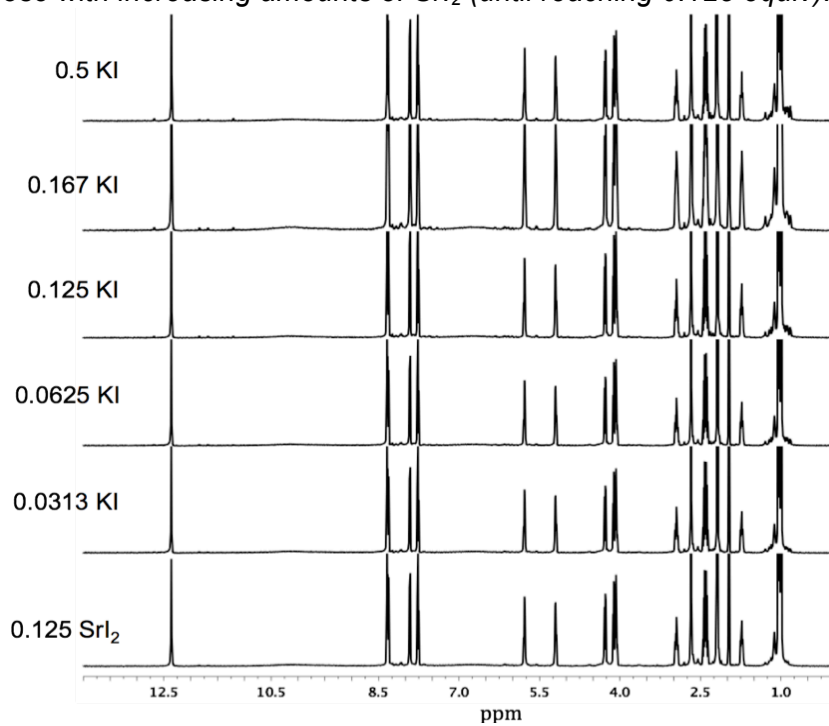
**Fig. S5** Melting profile as determined by VT-NMR measurements for  $1_8 \cdot \text{Sr}^{2+}$  (5 mM in **1**, 0.125 equiv.  $\text{SrI}_2$ ).

### G. $^1\text{H}$ NMR titration experiments of **1** with KI and $\text{SrI}_2$

**1** (30 mM) was assembled into the hexadecamer  $1_{16} \cdot 3\text{K}^+$  by adding 0.5 equiv of KI in  $\text{CD}_3\text{CN}$ . Upon incremental addition of  $\text{SrI}_2$  the signals corresponding to  $1_{16} \cdot 3\text{K}^+$  decreased with the concomitant increase of those corresponding to  $1_8 \cdot \text{Sr}^{2+}$  reaching ~100% of fidelity at 0.125 equiv of  $\text{SrI}_2$  (Fig. S6). Similarly, **1** was assembled with 0.125 equiv of  $\text{SrI}_2$  and upon incremental addition of KI the supramolecule remains as an octamer (Fig. S7). Only small peaks corresponding to a putative hexadecamer are detected (ca. 5 %).



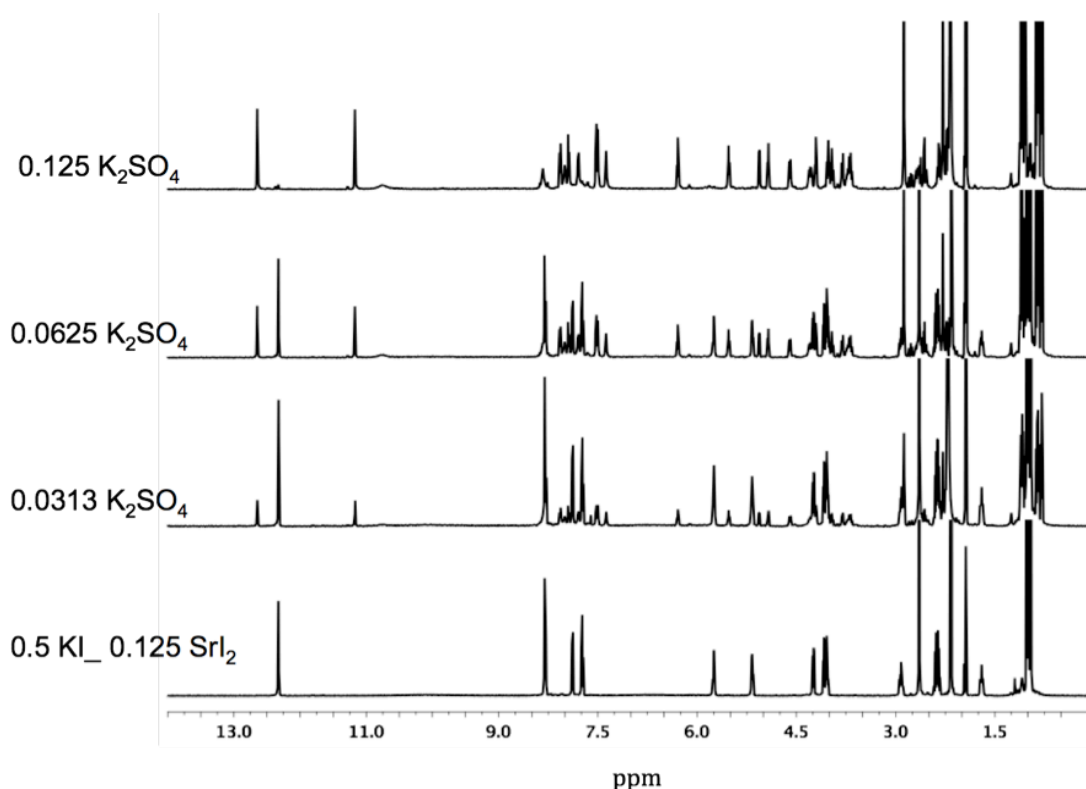
**Fig. S6**  $^1\text{H}$  NMR (500 MHz,  $\text{CD}_3\text{CN}$ , 298.2 K) spectra of **1** (30 mM, with 0.5 equiv of KI) followed by those with increasing amounts of  $\text{Srl}_2$  (until reaching 0.125 equiv).



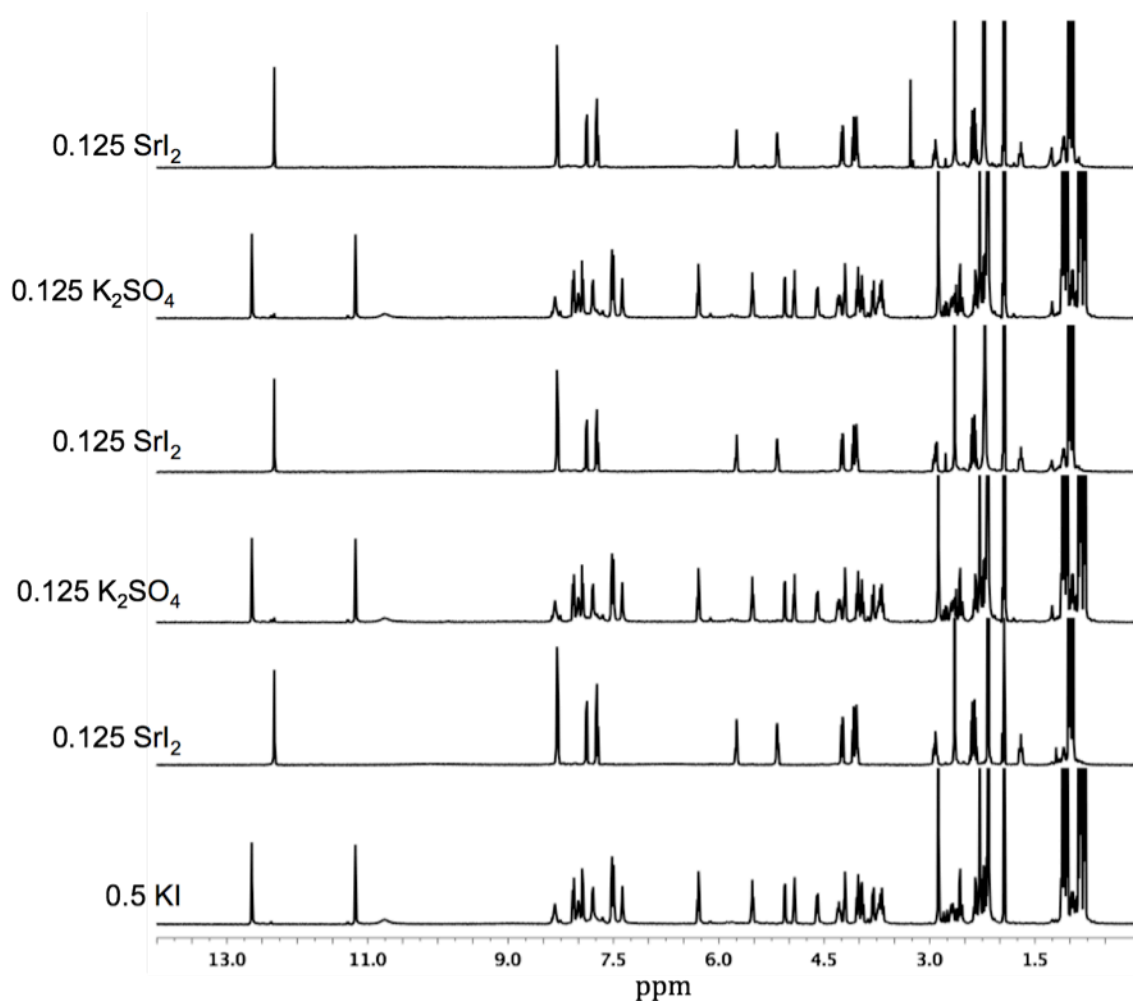
**Fig. S7**  $^1\text{H}$  NMR (500 MHz,  $\text{CD}_3\text{CN}$ , 298.2 K) spectra of **1** (30 mM) with  $\text{Srl}_2$  (0.125 equiv) followed by those with increasing amounts of KI (until reaching 0.5 equiv).

## H. Switching experiments

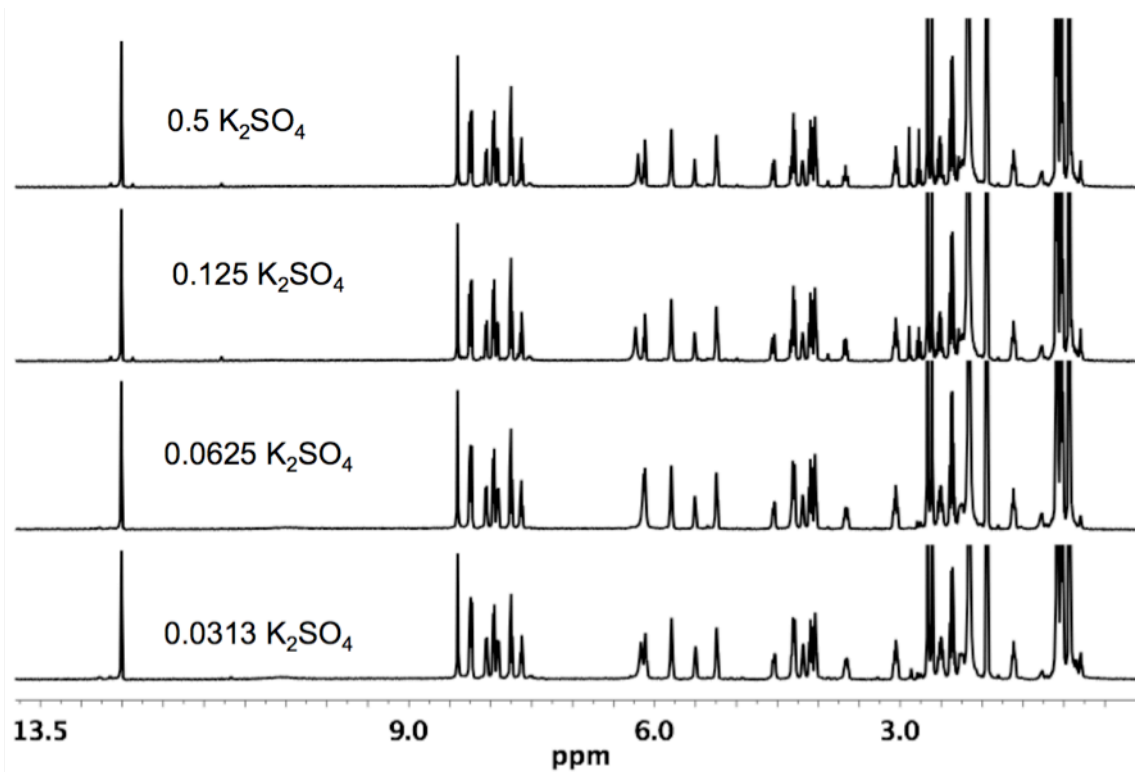
The switching studies were performed using a solution of the hexadecamer of **1** (30 mM, 0.5 equiv KI) in deuterated acetonitrile, after adding 0.0625 equiv of  $\text{SrI}_2$  an interconversion from  $\mathbf{1}_{16}\cdot\mathbf{3K}^+$  to  $\mathbf{1}_8\cdot\text{Sr}^{2+}$  was observed (Fig. S8). The controlled addition of  $\text{K}_2\text{SO}_4$  caused the precipitation of  $\text{SrSO}_4$  with the simultaneous switching of  $\mathbf{1}_8\cdot\text{Sr}^{2+}$  back to  $\mathbf{1}_{16}\cdot\mathbf{3K}^+$  (Fig. S9). The subsequent filtration of the precipitate and further addition of  $\text{SrI}_2$  regenerated the octamer  $\mathbf{1}_8\cdot\text{Sr}^{2+}$ . The process was repeated three times with no significant loss in fidelity. The cycle can be started from the octamer of  $\text{SrI}_2$  followed by the addition of 0.5 equiv of  $\text{K}_2\text{SO}_4$ . On the other hand **1** in  $\text{K}_2\text{SO}_4$  only promotes the formation of an octamer ( $\mathbf{1}_8\cdot\text{K}^+$ ) with a moderate fidelity of 66% (Fig. S10).



**Fig. S8**  $^1\text{H}$  NMR (500 MHz,  $\text{CD}_3\text{CN}$ , 298.2 K) spectra of **1** (30 mM) with 0.5 equiv of KI and 0.125 equiv of  $\text{SrI}_2$ , followed by the addition of increasing amounts of  $\text{K}_2\text{SO}_4$  until reaching 0.125 equiv of  $\text{K}_2\text{SO}_4$ .



**Fig. S9**  $^1\text{H}$  NMR (500 MHz,  $\text{CD}_3\text{CN}$ , 298.2 K) spectra of **1** (30 mM) with 0.5 equiv of KI followed by the addition of 0.125 equiv of  $\text{SrI}_2$ . The eventual addition of 0.125 equiv of  $\text{K}_2\text{SO}_4$  followed by the further addition of 0.125 equiv of  $\text{SrI}_2$  induces the switching between the octameric and hexadecameric states.



**Fig. S10.** Titration of **1** (30 mM) solution with  $K_2SO_4$  as measured by  $^1H$  NMR (500 MHz,  $CD_3CN$ , 298.2 K).

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