Supporting Information to:

A simple supramolecular assay for drug detection in urine

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Materials and methods

Neutral Red was purchased from Sigma-Aldrich as the other dyes tested initially. Cucurbit[7]uril (CB[7]) was synthesised following reported procedures.[1] Sodium phosphate buffers (from pH 6 to 7.5) were prepared using sodium phosphate monobasic monohydrate and sodium phosphate dibasic heptahydrate (J. T. Baker). Tris (base) and Tris hydrochloride (J. T. Baker) were used to prepare basic pH buffered solutions (from pH 8 to 9). Octreotide and synthetic urine (Surine ™) were also purchased from Sigma-Aldrich.

UV-Vis spectroscopy

UV-Vis spectroscopy was performed on a Cary 400 (Varian, Agilent) spectrophotometer, all the dyes were used at 20 μM concentration. For the titration experiments, the data points were collected at 520 nm and fit against the concentration using a standard one site competitive binding model with GraphPad Prism 7.02. The unknown samples were prepared at specific concentrations of Octreotide in synthetic urine and then a concentrated solutions of NR·CB[7] (1 and 2 mM, respectively, in 10 mM PB pH 7.4) was added to obtain a 20 μM solution of the dye.

Fluorescence spectroscopy

Fluorescence of NR and all of the dyes was evaluated using an F-7000 spectrophotometer with monochromator accessory (Hitachi High Technologies). The excitation wavelength varied depending on the dye evaluated, in specific: 485 nm for Acridine Orange; 520 nm for NR; 540 nm for Evans Blues; 600 nm for Brilliant green, Azure C and Nile Red; 633 Azure A and Nile Blue. The titrations were run in PB 10 mM pH 7.4 and all of the dyes were used at 10 μM, whereas CB[7] was added up to a 20 μM concentration. In the displacement experiments with Octreotide, NR was used at 10 μM and CB[7] at either 10 or 20 μM (buffer and synthetic urine, respectively).

Isothermal Titration Calorimetry (ITC)

ITC was performed using an Auto-ITC 200 (Malvern), with a standard temperature of 298 K, 750 rpm stirring, first injection of 0.4 μL, followed by 19 injections of 2 μL each. The CB[7] (concentration varying from 2.5 to 0.9 mM) was titrated from the syringe into the guest solution (either NR or Octreotide, 50 μM). All the solutions were prepared in 10 mM PB, pH 7.4. The data analyses was performed using Microcal Origin 7.

Nuclear Magnetic Resonance (NMR)

1D and 2D ^{1}H-NMR experiments were performed on a Bruker Avance Cryoprobe ATM TCI DRX 500 (Bruker BioSpin GmbH). Chemical shift (δ) are quoted in ppm with the internal reference tetramethylsilane.

Supplementary figures

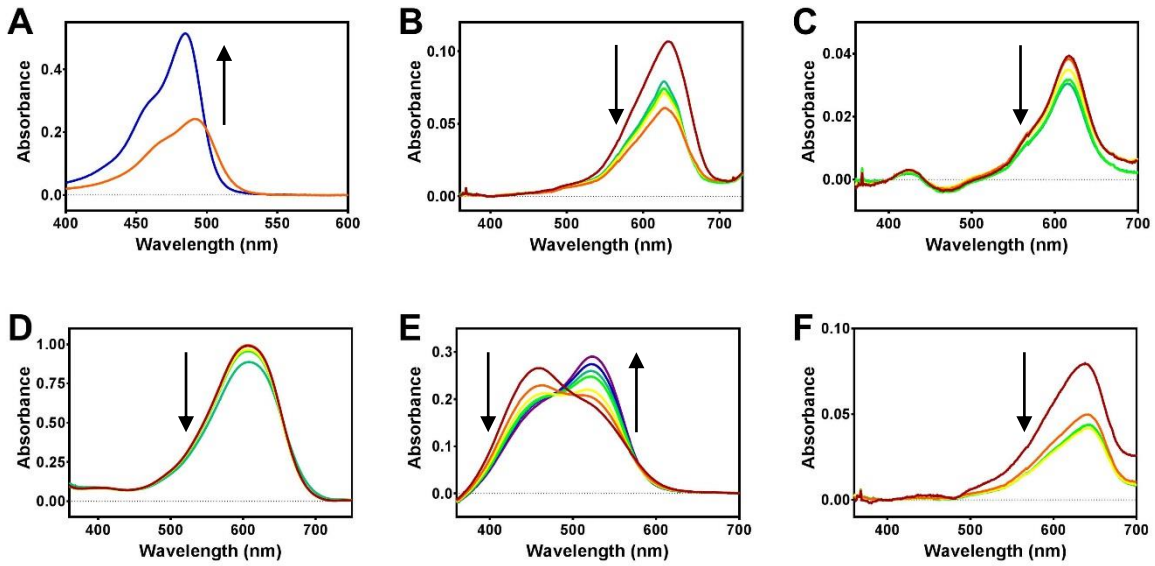

Figure S1 Titration of CB[7] from 0 to 30 µM into solutions of several dyes (20 µM) in 10 mM PB, pH 7.4. The dyes are, in order: **A**. Acridine Orange, **B**. Azure A, **C**. Brilliant Green, **D**. Evans Blue, **E**. Neutral Red, and **F**. Nile blue. The arrows show the change in absorbance intensity depending upon CB[7] addition. Acridine Orange is reported as dye solution only (orange line) and in the presence of 1:1 ratio of CB[7] (blue line).

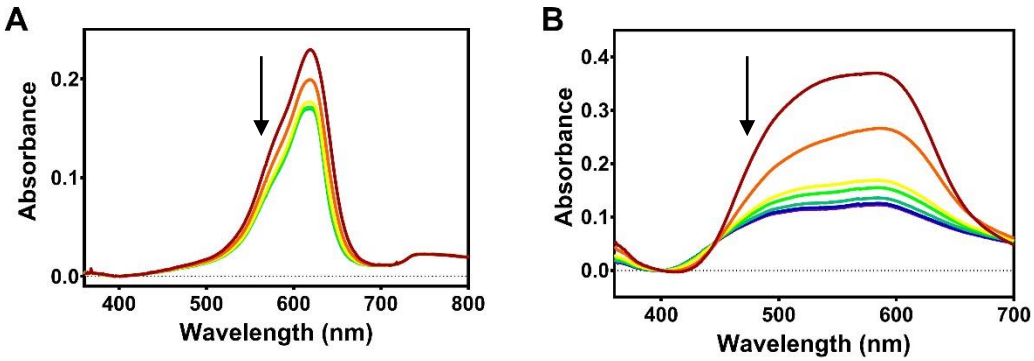

Figure S2 Titration of CB[7] from 0 to 30 µM into solutions of **A**. Azure C and **B**. Nile Red, both 20 µM in 10 mM PB, pH 7.4. The arrows show the change in absorbance depending upon CB[7] addition. These dyes do not show a shift in maximum absorption wavelength.

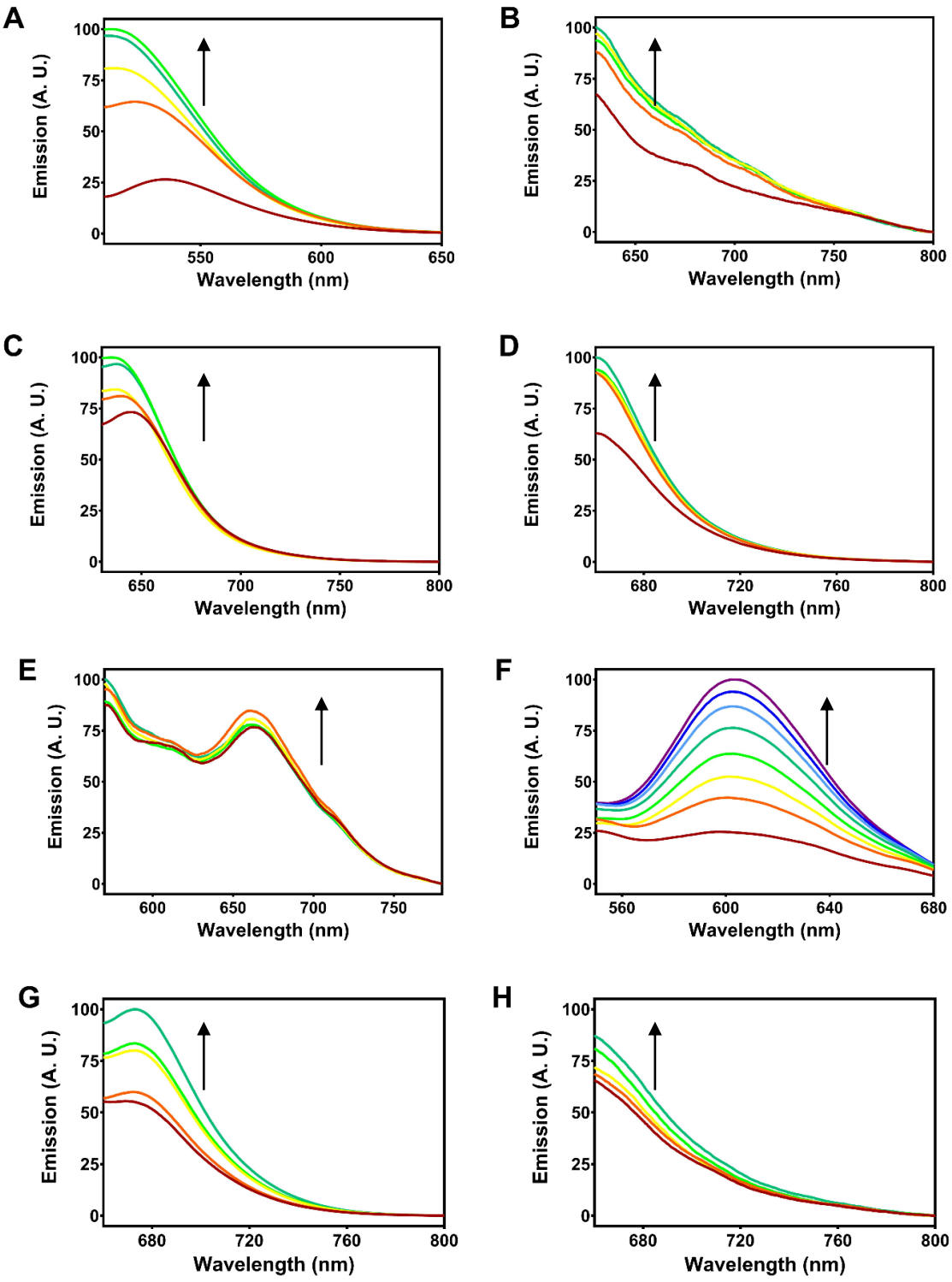

Figure S3 Titration of CB[7] from 0 to 20 µM into solutions of several dyes (10 µM) in 10 mM PB, pH 7.4. The dyes are, in order: **A**. Acridine Orange, **B**. Brilliant Green **C**. Azure C, **D**. Azure A, **E**. Evans Blue, **F**. Neutral Red, **G**. Nile blue, and **H**. Nile Red. The arrows show the change in emission intensity depending upon CB[7] addition.

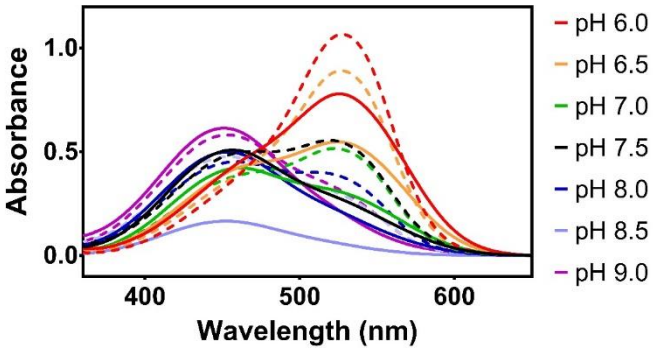

Figure S4 Shift in NR (20 µM) absorbance in different buffers (10mM) across a range of pH. The solid lines and the dashed lines represent samples in the absence and in the presence of CB[7] (20 µM), respectively.

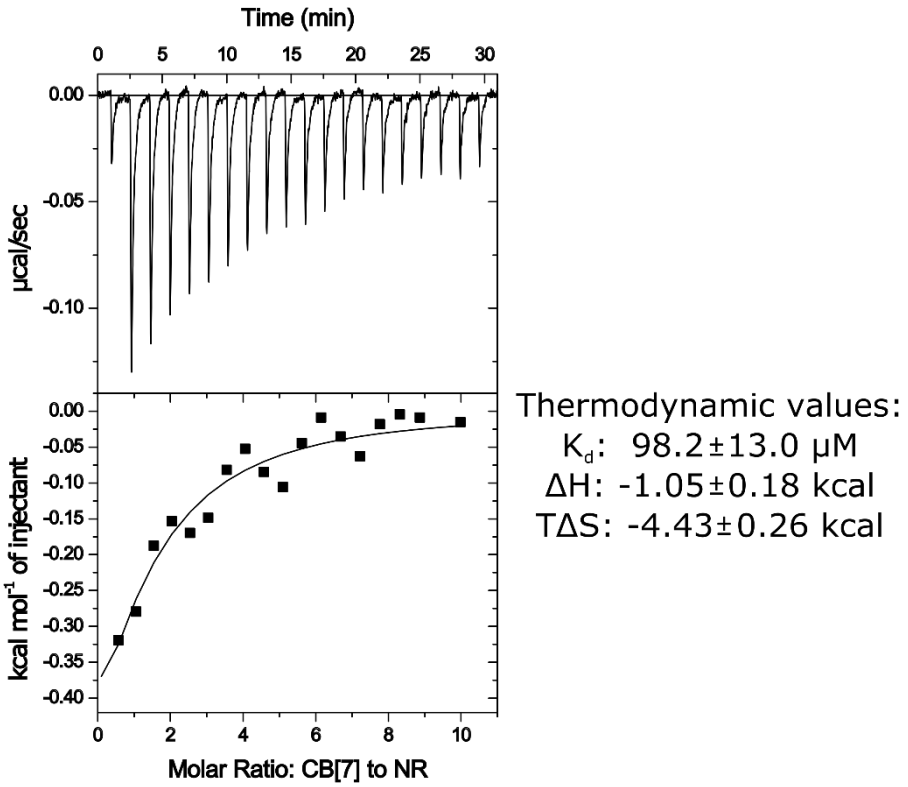

Figure S5 ITC of CB[7] (2.5 mM) titrated into NR (50 µM), both host and guest were in a 10 mM PB, pH 7.4. The obtained thermodynamic values are reported as average and STD of three independent measurements.

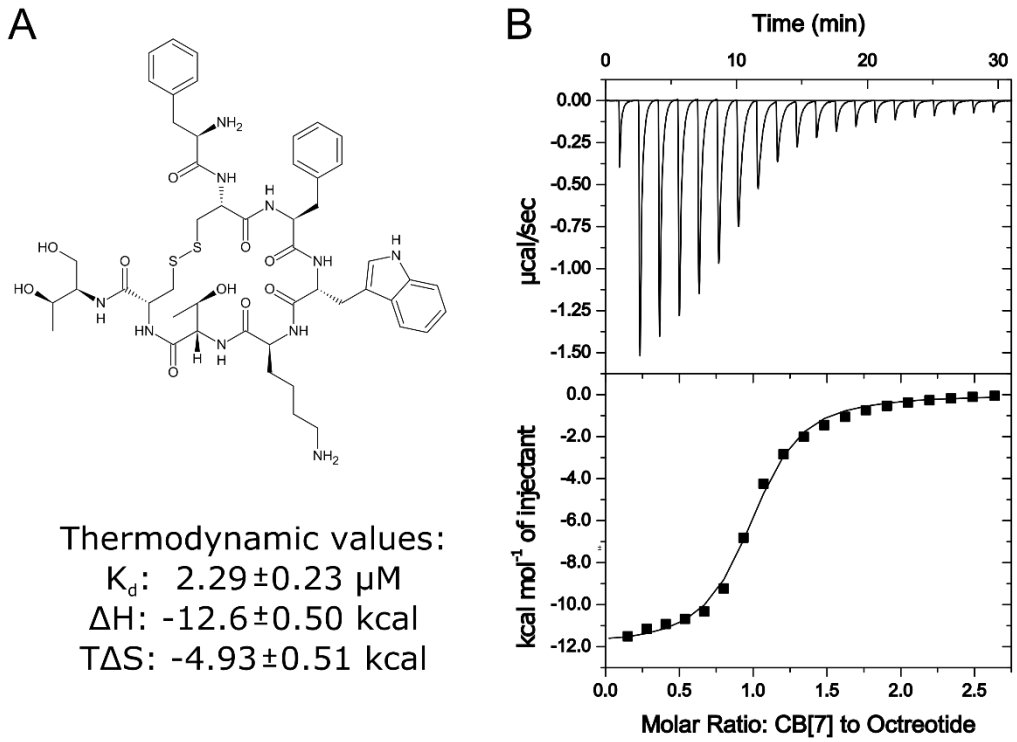

Figure S6 A. Structural chemical formula of the synthetic peptide drug Octreotide. **B**. ITC of CB[7] (0.9 mM) titrated into Octreotide (70 µM), both host and guest were in a 10 mM PB, pH 7.4. The obtained thermodynamic values are reported as average and STD of three independent measurements.

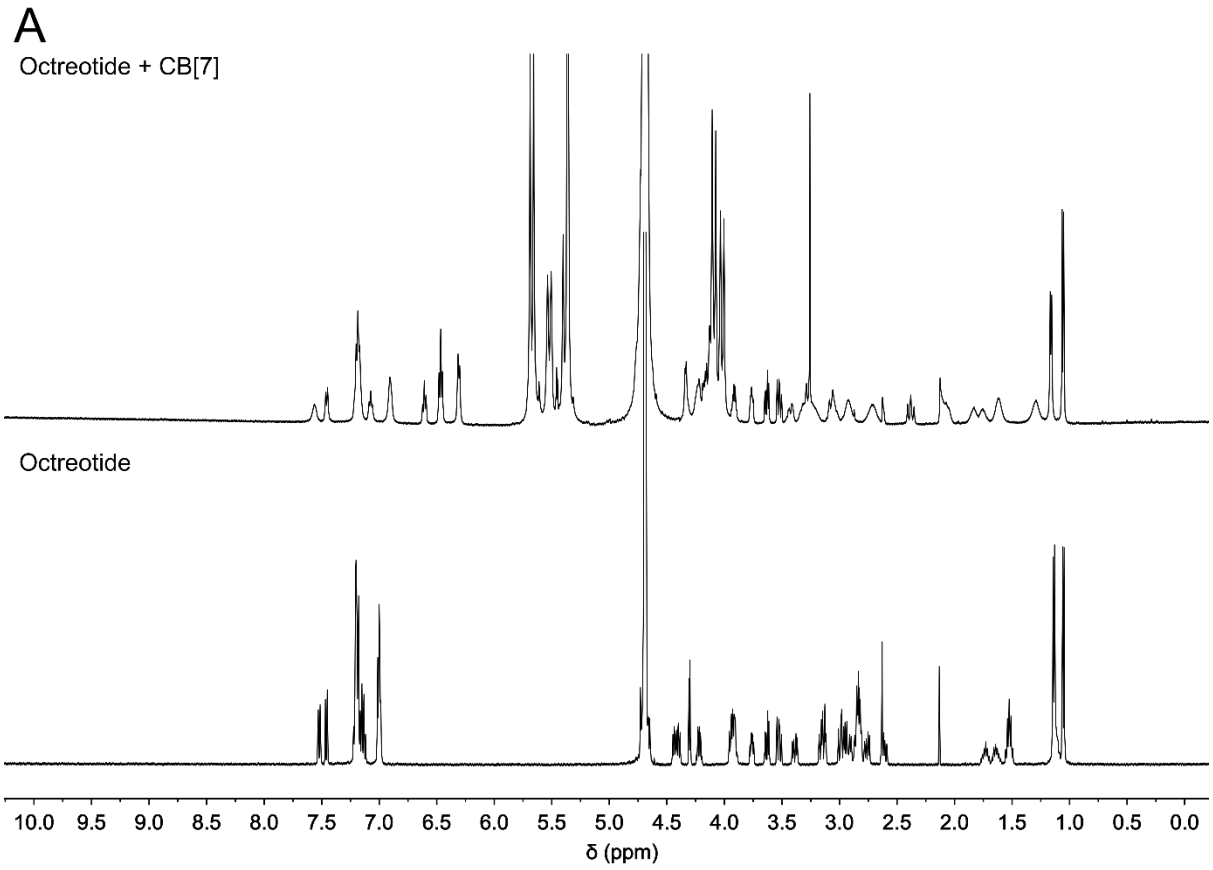

Figure S7 ^{1}H-NMR of Octreotide and CB[7], both 1 mM in D_2O. **A.** Comparison of full ^{1}H-NMR spectra of Octreotide in the presence and absence of CB[7]; **B.** Detail of the aromatic region of Octreotide in the presence and absence of CB[7], the upfield shift of the signals from one of the Phe residues was highlighted through proton assignments; **C.** Aromatic region of COSY-NMR of Octreotide in the presence of CB[7].

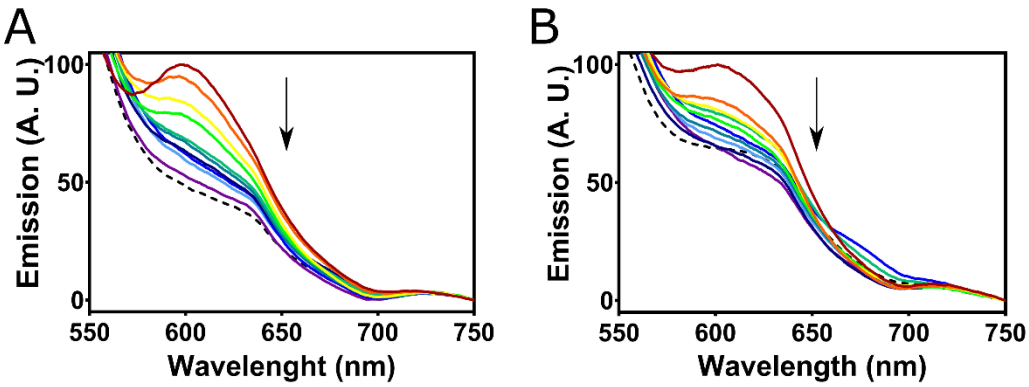

Figure S8 Fluorescence emission of a NR (10 µM) and CB[7] (**A, B**: 10 and 20 µM, respectively) solution upon addition of Octreotide (**A, B**: 0 to 33 and 0 to 40 µM, respectively). The arrows indicate the direction of the change in emission; the black dashed line in each graph represents NR emission in the same solvent conditions. The solvent system used was 10 mM sodium phosphate buffer, pH 7.4 in **A**, and synthetic urine in **B**.

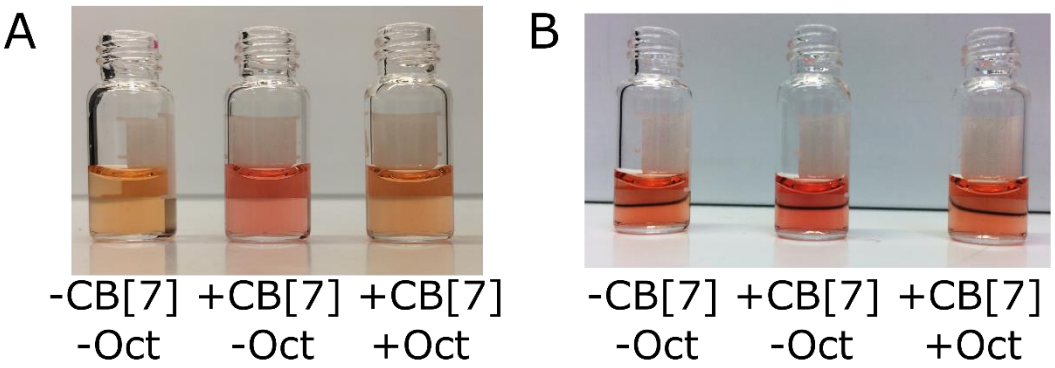

Figure S9 Colour change upon addition of CB[7] (**A, B**: 50 and 100 µM, respectively) to a NR solution (50 µM) and change back upon addition of Octreotide (**A, B**: 50 and 150 µM, respectively). The solvent system used was 10 mM sodium phosphate buffer, pH 7.4 in **A**, and synthetic urine in **B**.

References

1 K. I. Assaf and W. M. Nau, *Chem. Soc. Rev.*, **2015**, *44*, 394-418.