Electronic Supplementary Material (ESI) for Materials Chemistry Frontiers.

Supporting Information

Structural Evolution from Disordered to Fibrous Assembly *via* a Dual Visual Dissipative Pathway

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Experimental Section

Materials. Indigo carmine (IC) was purchased from Macklin. Proline (Pro) was purchased from Innochem. Asparagine (Asn) and glutamine (Gln) were purchased from Aladdin. 1-Methylpyridinium chloride, aspartic acid (Asp) and glutamic acid (Glu) were purchased from TCI. Histidine (His) and phenylalanine (Phe) were purchased from Alfa. Alanine (Ala), arginine (Arg), glycine (Gly), methionine (Met) and valine (Val) were purchased from Ark. Cysteine (Cys), isoleucine (Ile), lysine (Lys), serine (Ser), leucine (Leu), threonine (Thr), tryptophan (Trp) and tyrosine (Tyr) were purchased from Adamas. All of these compounds were used without further purification. Calixpyridinium was synthesized and purified according to a previously reported procedure.[1] It was identified by ^{1}H and ^{13}C NMR spectroscopy in D_2O (Fig. S23), performed on a Bruker AV400 spectrometer, and by X-ray crystallographic analysis (Fig. S24), performed on a Bruker APEX-II CCD diffractometer.

The aqueous solution was adjusted to different pH values by HCl or NaOH.

Preparation of the static isothermal calixpyridinium−IC assembly. The static isothermal calixpyridinium−IC assembly was prepared by a simple mixture of calixpyridinium and IC in water. Calixpyridinium and IC were dissolved in double-distilled water. Then 1000 μL IC solution (100 μM) and 500 μL calixpyridinium solution (100 μM) were added to a 500 μL double-distilled water to obtain a 25 μM calixpyridinium–50 μM IC assembly solution. The static isothermal calixpyridinium−IC assembly achieved balance immediately.

Detection of *α*-amino acid. A 80 μL *α*-amino acid solution (20 mM) was added to a 2 mL calixpyridinium or calixpyridinium−IC assembly solution. Then the UV-Vis absorption spectra were recorded immediately.

A 25 μL Arg or Lys solution (20 mM) was added to a 2 mL calixpyridinium or calixpyridinium−IC assembly solution to obtain the response times.

Different volumes of Arg or Lys solutions (20 mM) were added to a 2 mL calixpyridinium or calixpyridinium−IC assembly solution. Then the UV-Vis absorption spectra were measured when the response time was reached to get the dynamic range.

A 15 μL Arg or Lys solution (20 mM) was added to three 2 mL calixpyridinium or calixpyridinium−IC assembly solutions, respectively, and the resulting concentration of Arg or Lys was 150 μM. The UV-Vis absorption spectra were measured when the response time was reached. The UV-Vis absorption spectra were obtained in the same way when the resulting concentrations of Arg or Lys were 200 μM and 300 μM, respectively. A total of 36 sets of data were measured to acquire the reproducibility.

Preparation of urine samples. Raw urine samples were provided by healthy volunteers from Tianjin Normal University, Tianjin, China. The test samples were prepared by adding a 20 μL of raw urine into a 2 mL calixpyridinium or calixpyridinium−IC assembly solution. The resulting urine samples for tests were diluted 100 times.

Measurements.

Electrical Conductivity Measurements. Electrical conductivity was measured by a DDJ-A automatic electrical conductivity instrument.

UV-Vis Absorption Spectra. UV-Vis absorption spectra were measured in a quartz cell (light path 10 mm) on a Persee TU-1810 spectrophotometer.

Fluorescence Emission Spectra. Fluorescence emission spectra were measured using a conventional quartz cell (light path 10 mm) on a FS5 fluorescence spectrometer from Edinburgh Instruments.

Transmission Electron Microscopy (TEM) Experiments. TEM images were acquired using a Talos F200X high-resolution TEM operating at an accelerating voltage of 200 kV.

Irradiation. The solution was irradiated using a Handhold UV Lamps (ZF-5) at 254 nm for taking fluorescent photos.

pH Measurements. The pH values were verified on a Sartorius pp-20 pH meter calibrated with two standard buffer solutions.

Dynamic Light Scattering (DLS) Measurements. DLS experiments were measured by NanoBrook 173 Plus at a scattering angle of 90°.

NMR Spectroscopy. ^{1}H and ^{13}C NMR spectra were recorded with a Bruker AV400 spectrometer.

Crystal X-ray Diffraction. X-ray crystallographic analysis was performed on a Bruker APEX-II CCD diffractometer.

Results and Discussion

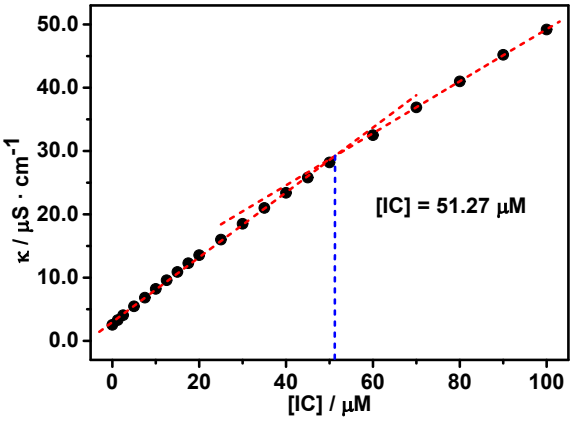


Figure S1. Dependence of the electrical conductivity on the concentration of IC.

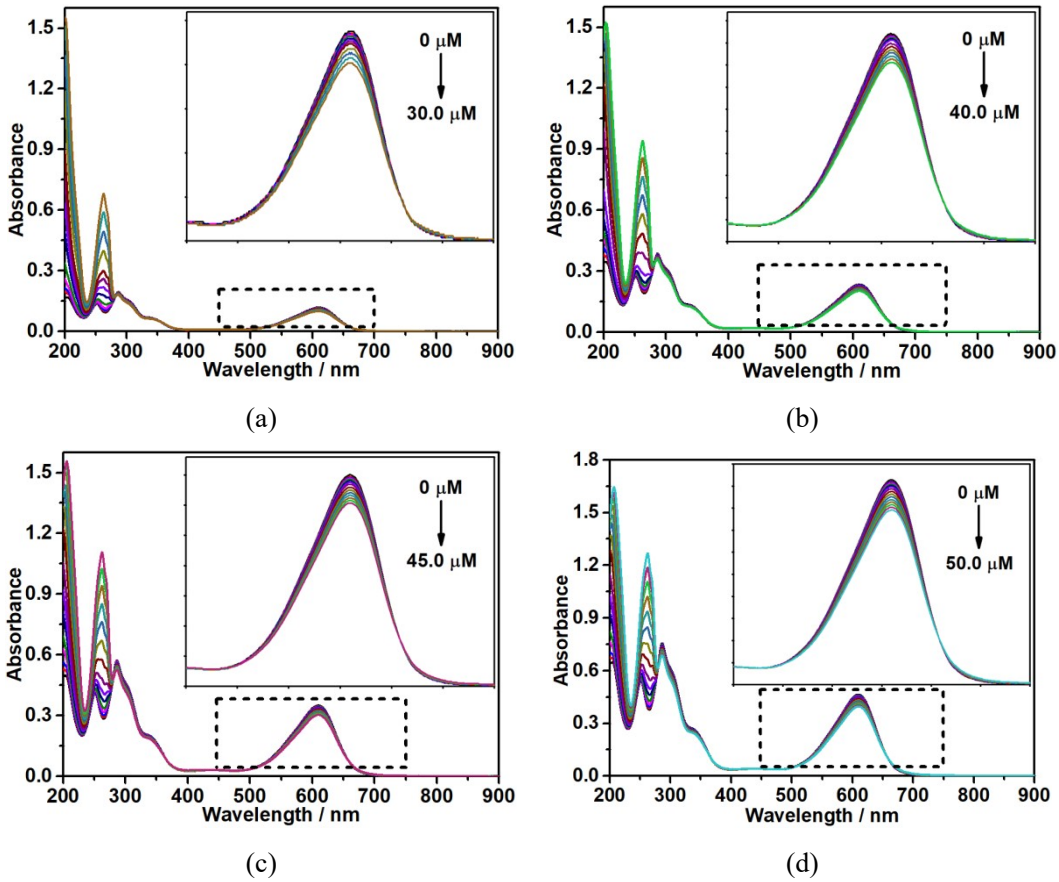


Figure S2. UV-Vis absorption spectra of 10 μM (a), 20 μM (b), 30 μM (c) and 40 μM (d) IC with the gradual addition of calixpyridinium.

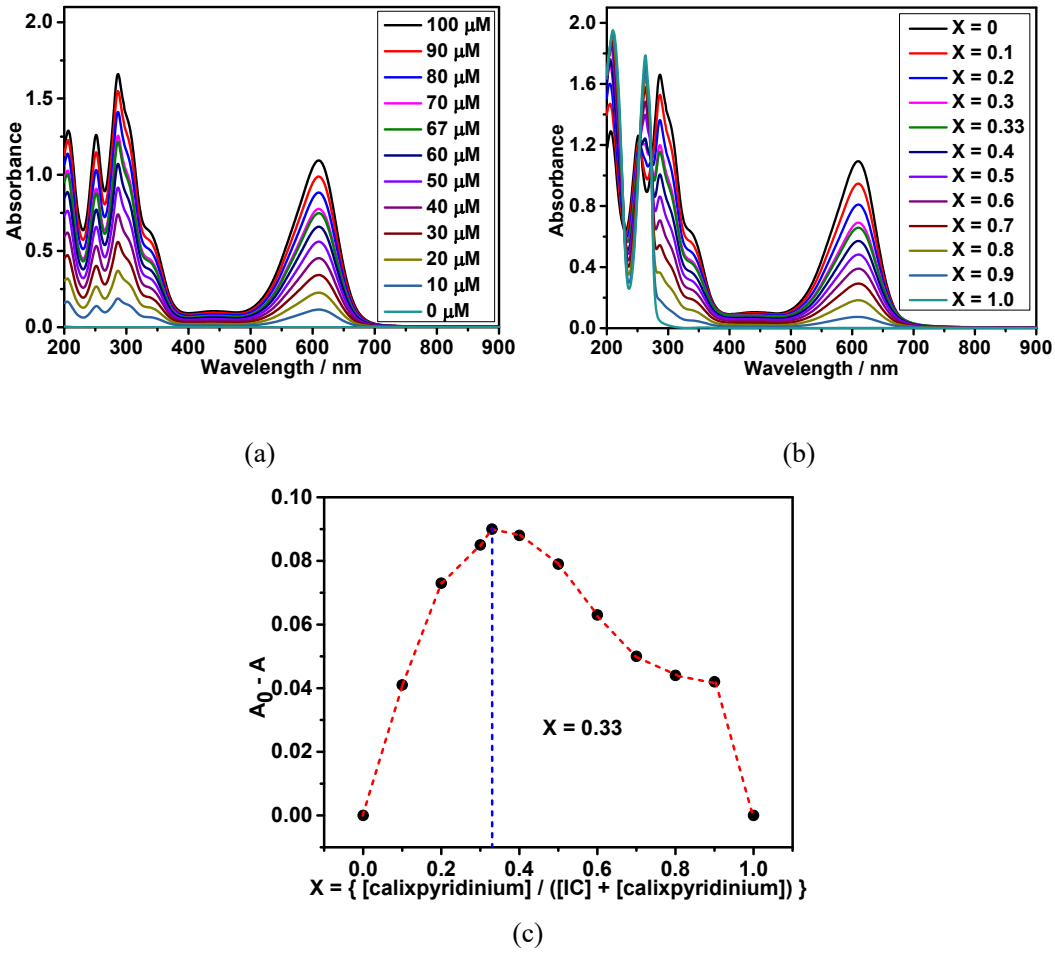


Figure S3. (a) UV-Vis absorption spectra of the IC solutions at different concentrations. (b) UV-Vis absorption spectra of the aqueous solutions of calixpyridinium and IC with different mixing molar ratios. X = [calixpyridinium] / ([IC] + [calixpyridinium]), [calixpyridinium] + [IC] = 100 μM. (c) Job's plot of calixpyridinium and IC at 610 nm (A_0: IC; A: IC + calixpyridinium).

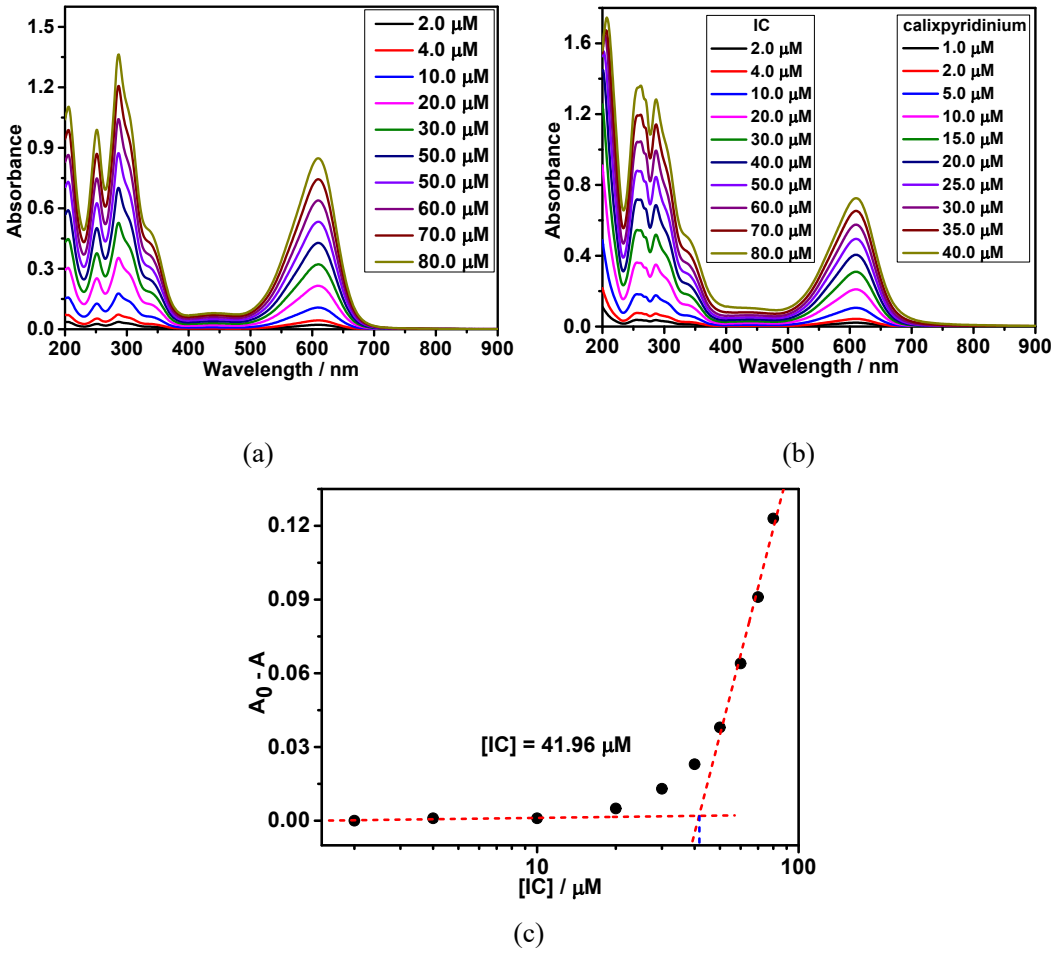


Figure S4. (a) UV-Vis absorption spectra of the IC solutions at different concentrations. (b) UV-Vis absorption spectra of the aqueous solutions of calixpyridinium−IC at different concentrations with a fixed stoichiometry of 1:2. The concentration of calixpyridinium changed from 1.0 μM to 40.0 μM. The concentration of IC changed from 2.0 μM to 80.0 μM. (c) Dependence of the variation of the absorbance at 610 nm on the IC concentration (A_0: IC; A: IC + calixpyridinium).

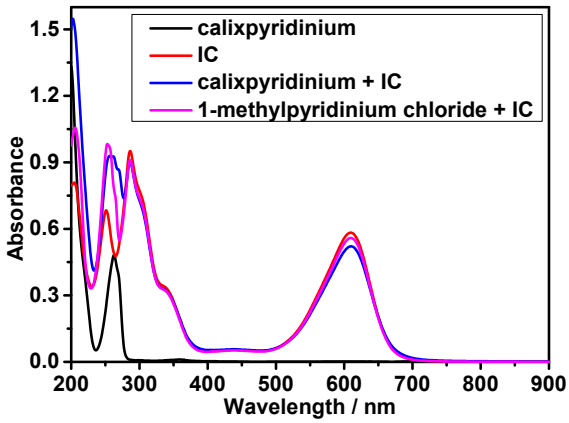


Figure S5. UV-Vis absorption spectra of calixpyridinium, IC, calixpyridinium + IC, and 1-methylpyridinium chloride + IC in water. [calixpyridinium] = 25 μM, [IC] = 50 μM and [1-methylpyridinium chloride] = 100 μM.

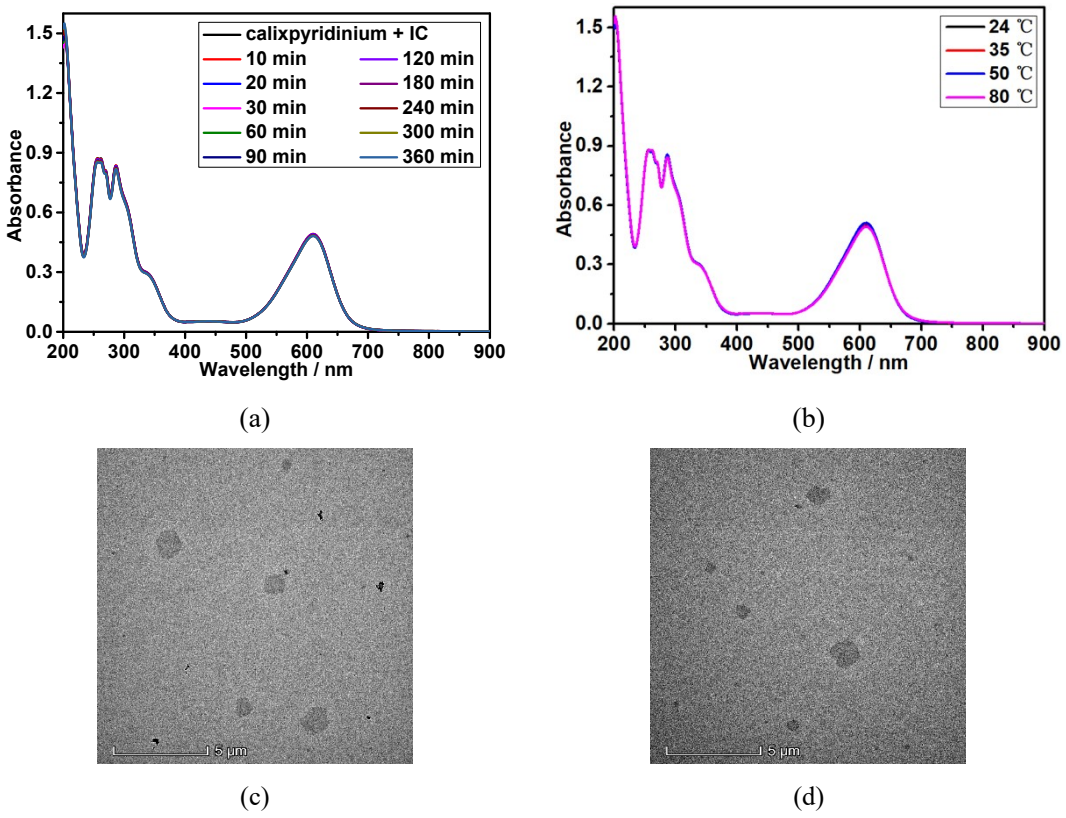


Figure S6. (a) UV-Vis absorption spectra of the calixpyridinium−IC solution at different time after preparation at room temperature. (b) UV-Vis absorption spectra of the calixpyridinium−IC solution at different temperatures. (c) TEM image of the calixpyridinium−IC aggregates after preparation for 6 h. (d) TEM image of the calixpyridinium−IC aggregates upon thermal annealing. [calixpyridinium] = 25 μM and [IC] = 50 μM.

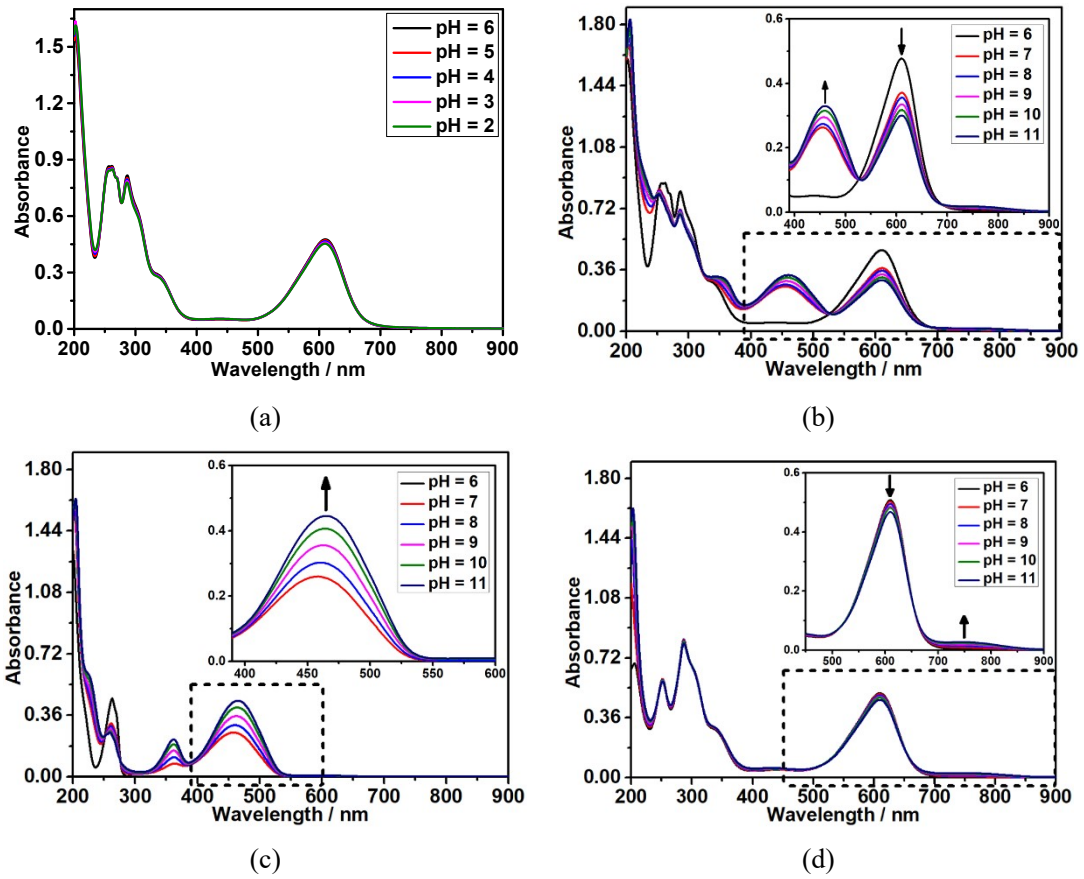


Figure S7. (a and b) UV-Vis absorption spectra of the calixpyridinium−IC solution at different pH values. UV-Vis absorption spectra of the calixpyridinium (c) and the IC (d) solutions at different pH values. [calixpyridinium] = 25 μM and [IC] = 50 μM.

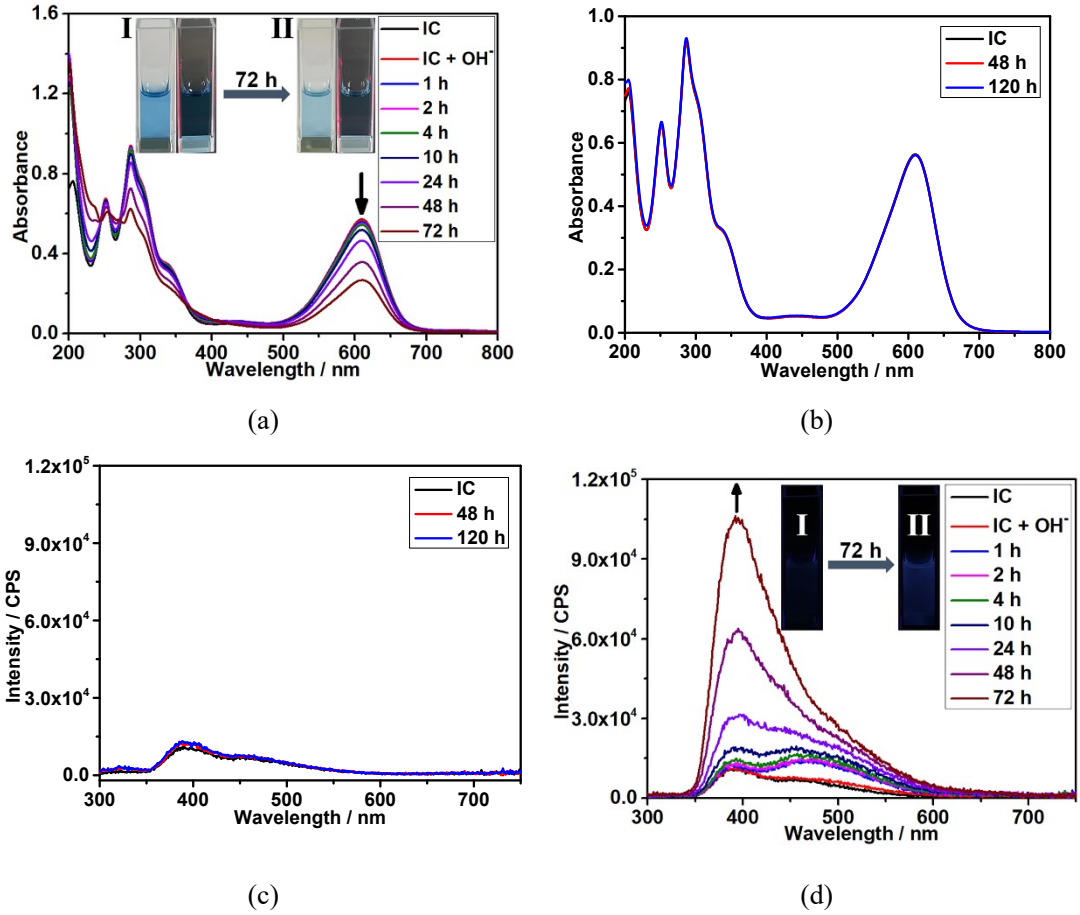
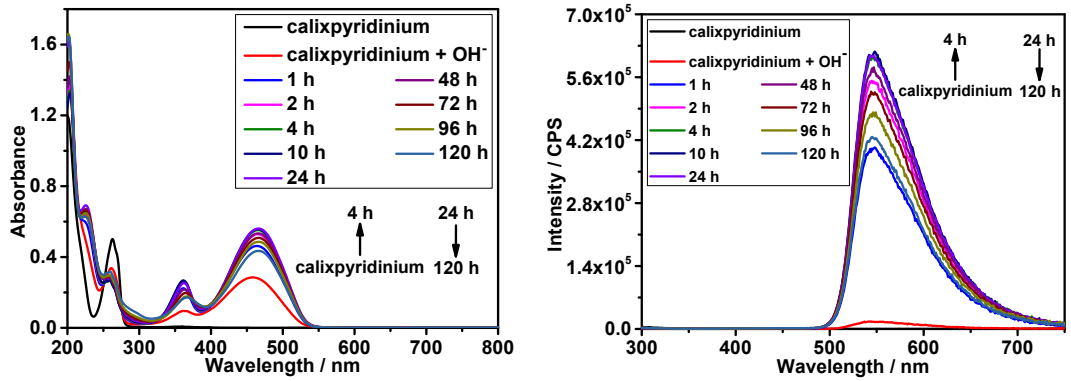
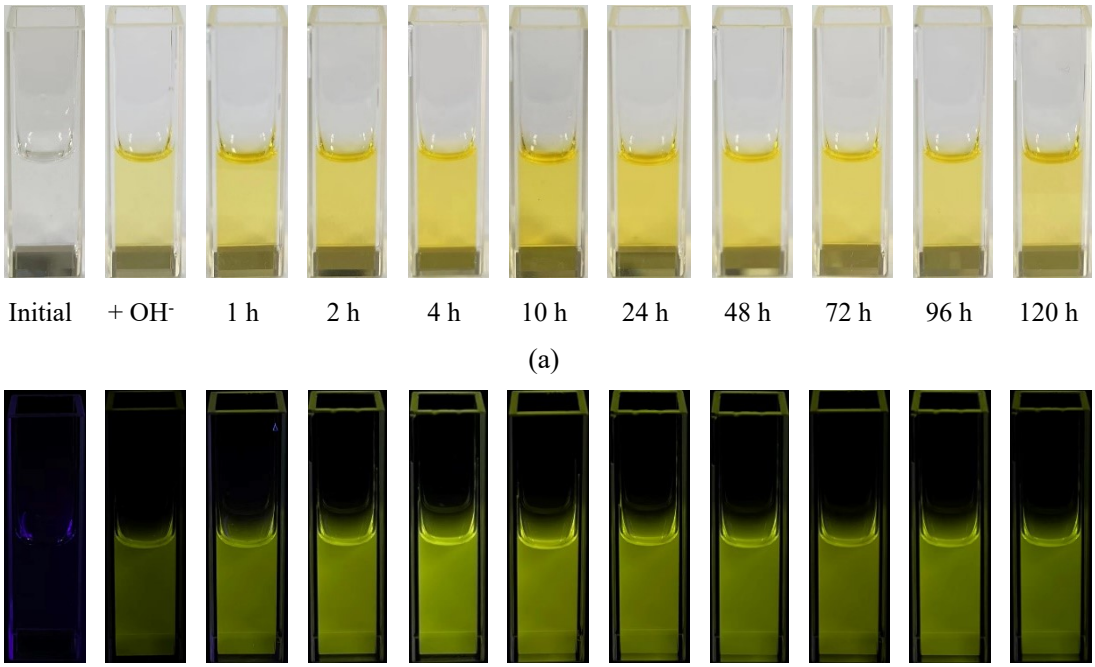


Figure S8. (a) UV-Vis absorption spectra of the IC solution before addition of alkali (pH = 6) and at different time after adding an alkali to adjust its pH to 8. Inset: photos showing the color and the Tyndall effect of the IC solution immediately (I) and 72 h later (II) after adding an alkali to adjust its pH to 8. UV-Vis absorption (b) and fluorescence emission (c) spectra of the IC solution at different time after preparation (pH = 6). (d) Fluorescence emission spectra of the IC solution before addition of alkali (pH = 6) and at different time after adding an alkali to adjust its pH to 8. Inset: photos showing the fluorescence of the IC solution under a UV lamp at 254 nm immediately (I) and 72 h later (II) after adding an alkali to adjust its pH to 8. [IC] = 50 μM, λ_{ex} = 263 nm, slit width: ex 2 nm, em 2 nm.



(a) (b)

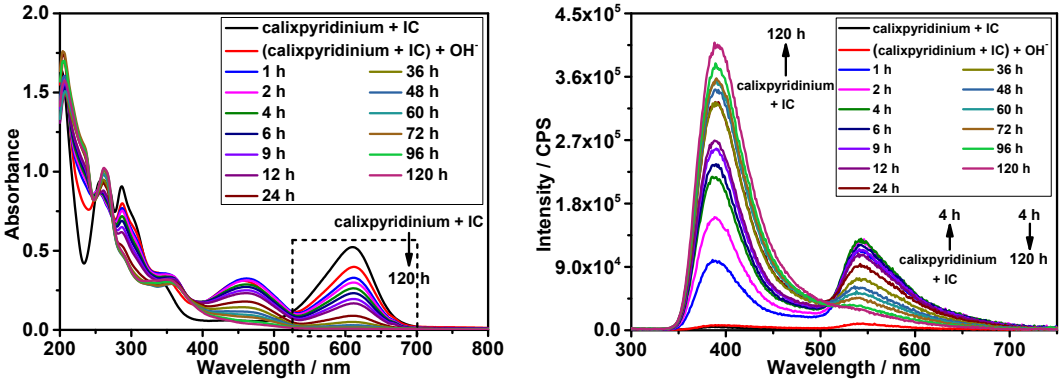
Figure S9. UV-Vis absorption (a) and fluorescence emission (b) spectra of the calixpyridinium solution before and at different time after adding an alkali to adjust its pH to 8. [calixpyridinium] = 25 μM, λ_{ex} = 263 nm, slit width: ex 2 nm, em 2 nm.



(a)

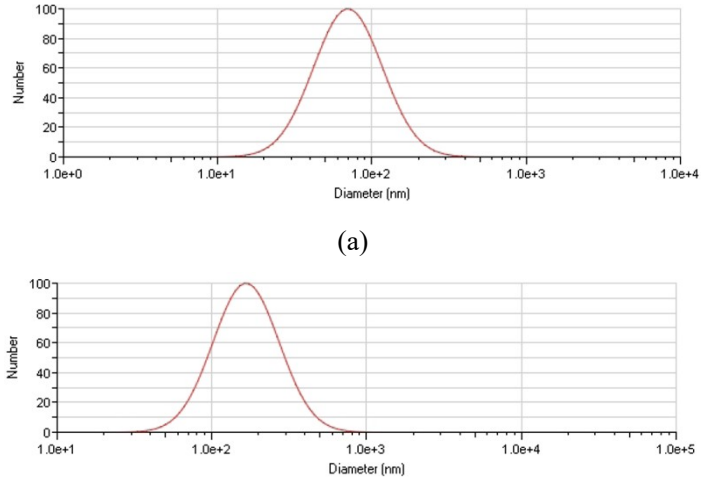
(b)

Figure S10. (a) Photos showing the color of the calixpyridinium solution before and at different time after adding an alkali to adjust its pH to 8. (b) Photos showing the fluorescence of the calixpyridinium solution before and at different time after adding an alkali to adjust its pH to 8. The solution was irradiated under a UV lamp at 254 nm for taking the fluorescent photos. [calixpyridinium] = 25 μM.



(a) (b)

Figure S11. UV-Vis absorption (a) and fluorescence emission (b) spectra of the calixpyridinium−IC solution before and at different time after adding an alkali to adjust its pH to 8. [calixpyridinium] = 25 μM, [IC] = 50 μM, λ_{ex} = 263 nm, slit width: ex 2 nm, em 2 nm.



(a)

(b)

Figure S12. DLS data of the calixpyridinium−IC aggregates after adding an alkali to adjust its pH to 8 for 4 h (a) and 120 h (b). [calixpyridinium] = 25 μM, [IC] = 50 μM.

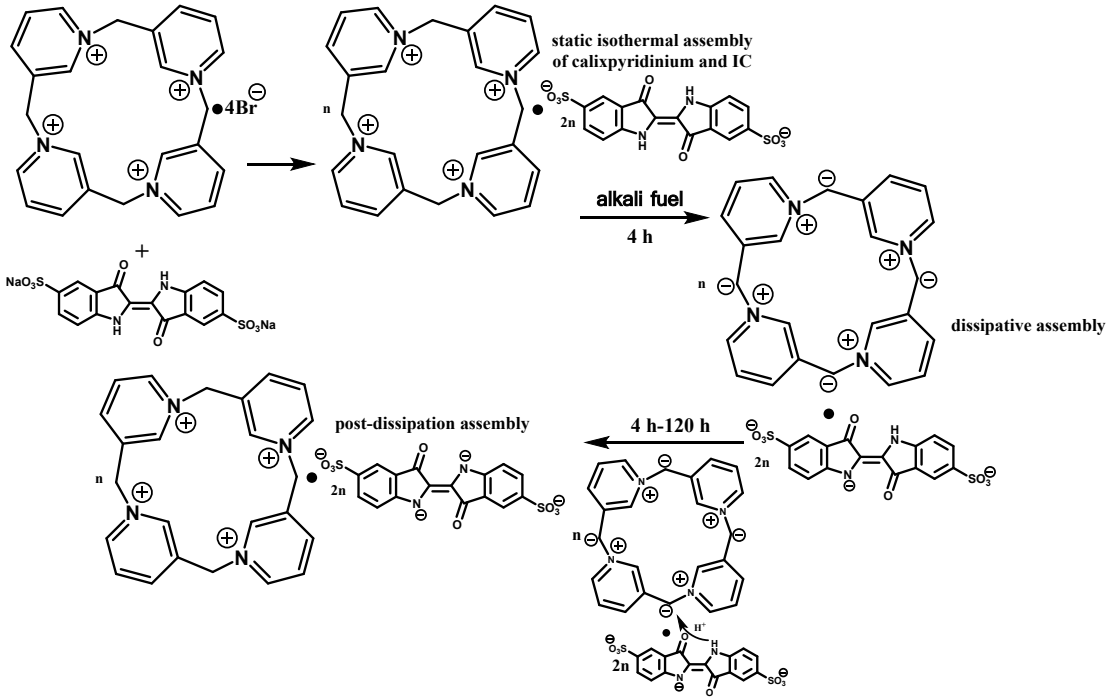
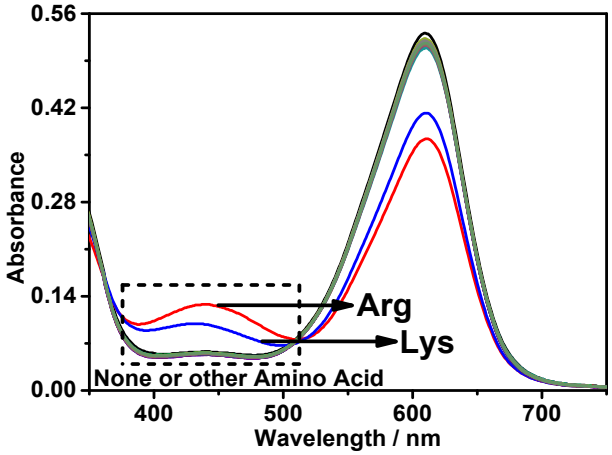
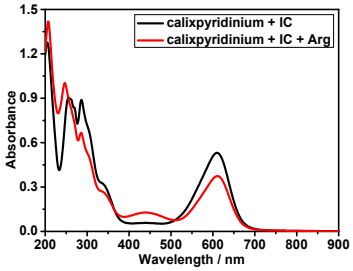
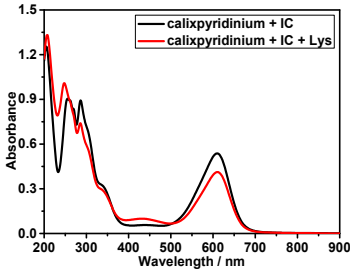
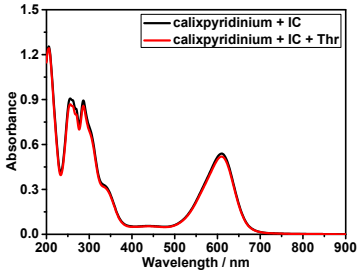


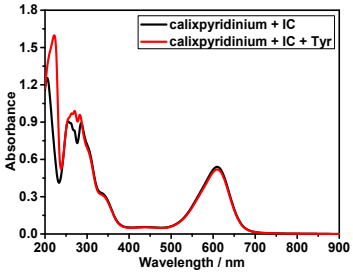
Figure S13. Chemical structures of calixpyridinium and IC showing their deprotonation with base and proton transfer from IC to calixpyridinium.

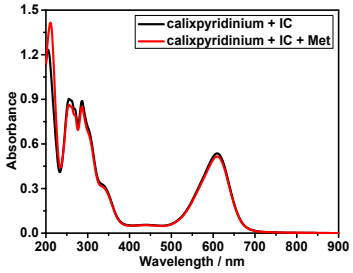

Absorbance
Wavelength / nm
Arg
Lys
None or other Amino Acid

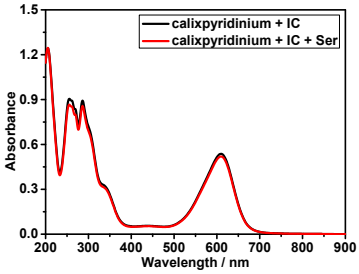

calixpyridinium + IC
calixpyridinium + IC + Arg
Absorbance
Wavelength / nm

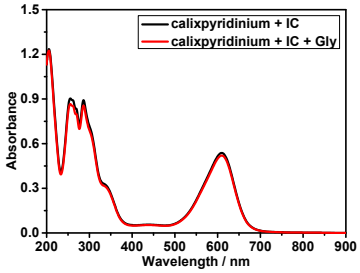

calixpyridinium + IC
calixpyridinium + IC + Lys
Absorbance
Wavelength / nm

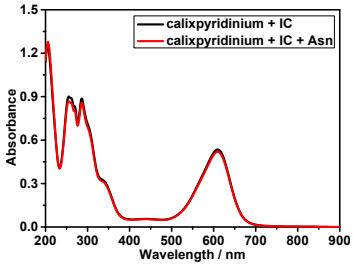

calixpyridinium + IC
calixpyridinium + IC + Thr
Absorbance
Wavelength / nm

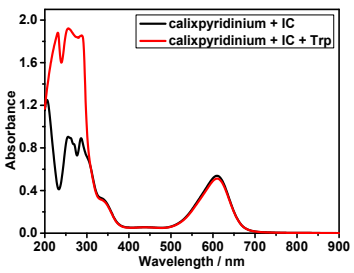

calixpyridinium + IC
calixpyridinium + IC + Tyr
Absorbance
Wavelength / nm

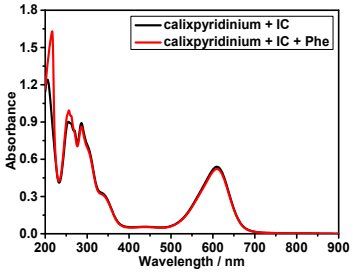

calixpyridinium + IC
calixpyridinium + IC + Met
Absorbance
Wavelength / nm

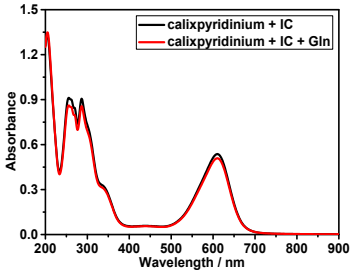

calixpyridinium + IC
calixpyridinium + IC + Ser
Absorbance
Wavelength / nm

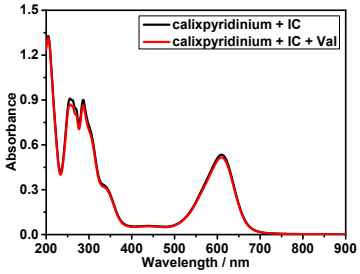

calixpyridinium + IC
calixpyridinium + IC + Gly
Absorbance
Wavelength / nm


calixpyridinium + IC
calixpyridinium + IC + Asn
Absorbance
Wavelength / nm


calixpyridinium + IC
calixpyridinium + IC + Trp
Absorbance
Wavelength / nm


calixpyridinium + IC
calixpyridinium + IC + Phe
Absorbance
Wavelength / nm


calixpyridinium + IC
calixpyridinium + IC + Gln
Absorbance
Wavelength / nm


calixpyridinium + IC
calixpyridinium + IC + Val
Absorbance
Wavelength / nm

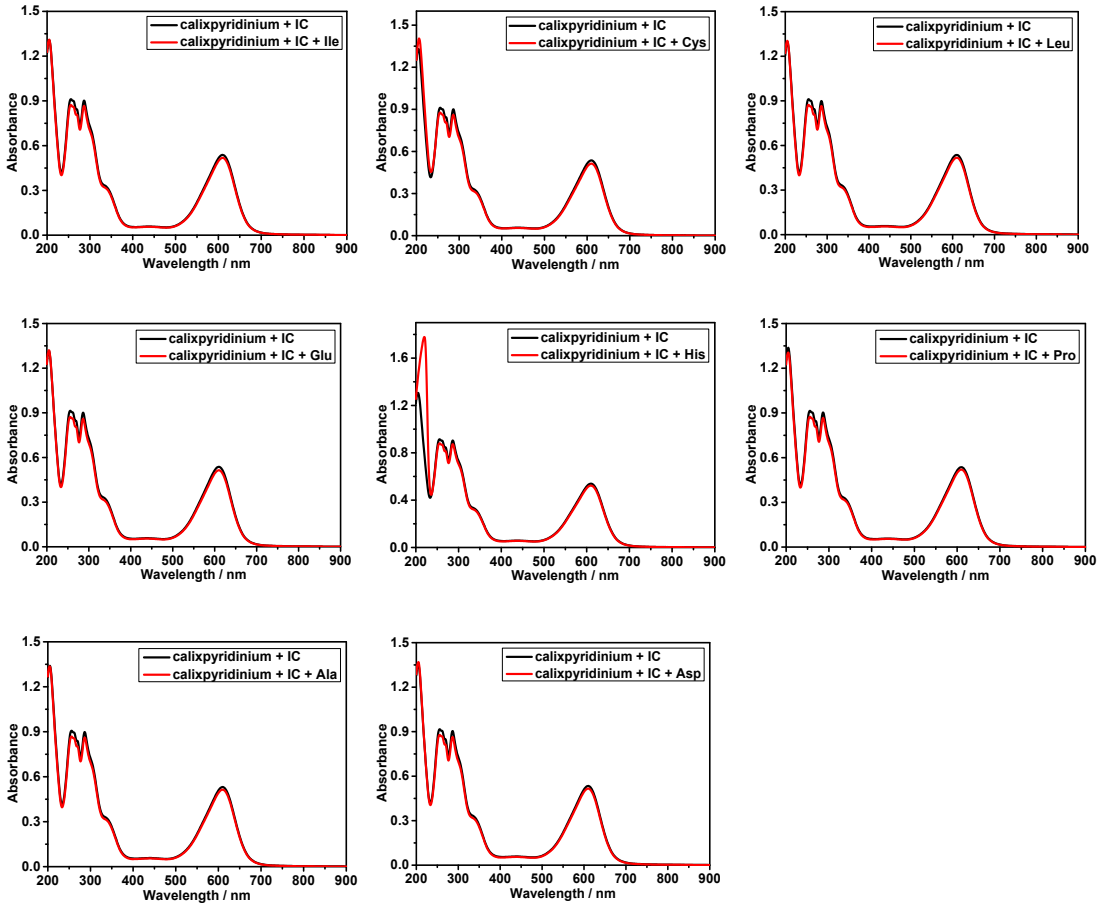
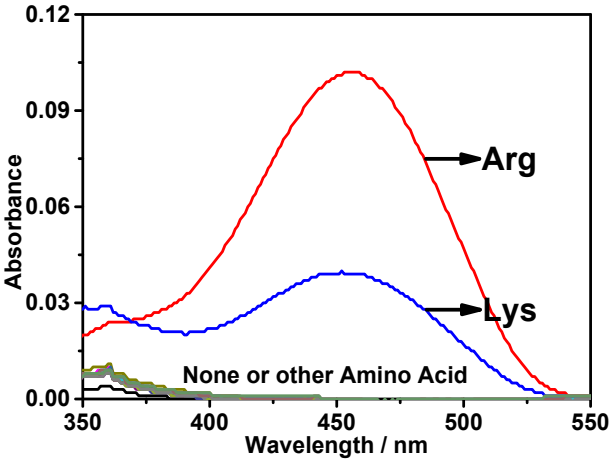
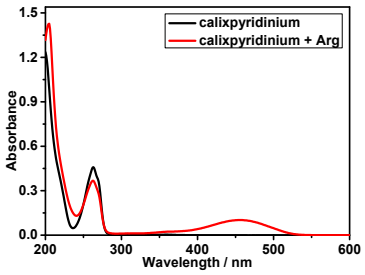
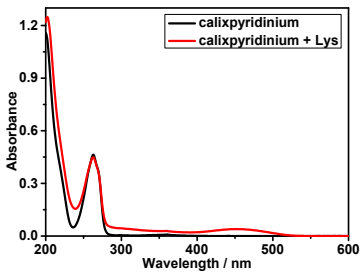
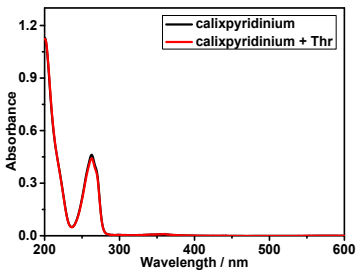


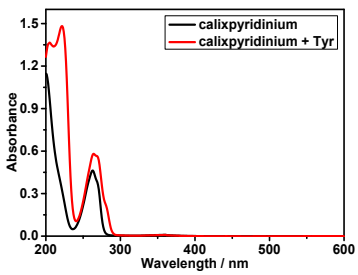
Figure S14. UV-Vis absorption spectra of the calixpyridinium−IC solution in the absence and presence of 20 native α-amino acids in water. [calixpyridinium] = 25 μM, [IC] = 50 μM and [α-amino acid] = 800 μM.

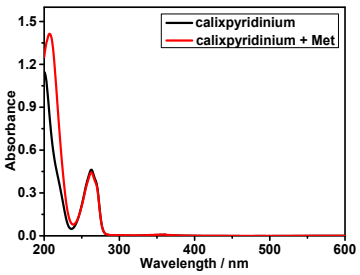

Absorbance
Wavelength / nm
Arg
Lys
None or other Amino Acid

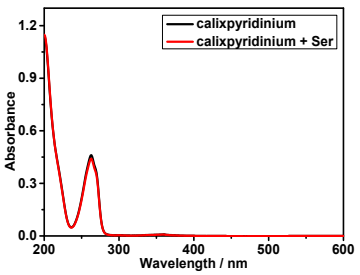

calixpyridinium
calixpyridinium + Arg
Absorbance
Wavelength / nm

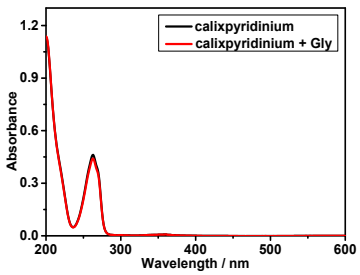

calixpyridinium
calixpyridinium + Lys
Absorbance
Wavelength / nm

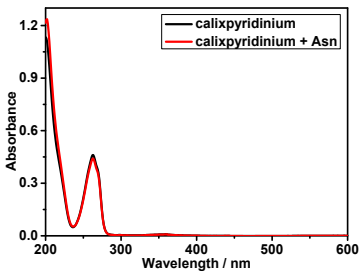

calixpyridinium
calixpyridinium + Thr
Absorbance
Wavelength / nm

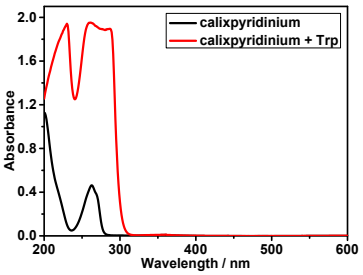

calixpyridinium
calixpyridinium + Tyr
Absorbance
Wavelength / nm

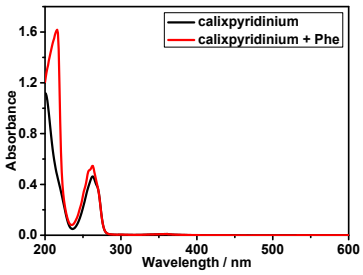

calixpyridinium
calixpyridinium + Met
Absorbance
Wavelength / nm

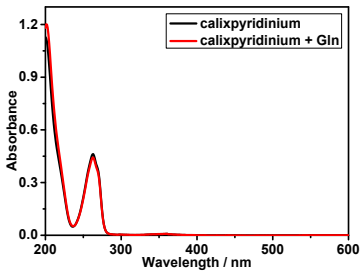

calixpyridinium
calixpyridinium + Ser
Absorbance
Wavelength / nm

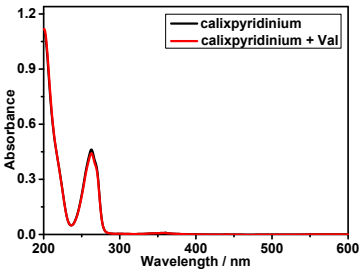

calixpyridinium
calixpyridinium + Gly
Absorbance
Wavelength / nm


calixpyridinium
calixpyridinium + Asn
Absorbance
Wavelength / nm


calixpyridinium
calixpyridinium + Trp
Absorbance
Wavelength / nm


calixpyridinium
calixpyridinium + Phe
Absorbance
Wavelength / nm


calixpyridinium
calixpyridinium + Gln
Absorbance
Wavelength / nm


calixpyridinium
calixpyridinium + Val
Absorbance
Wavelength / nm

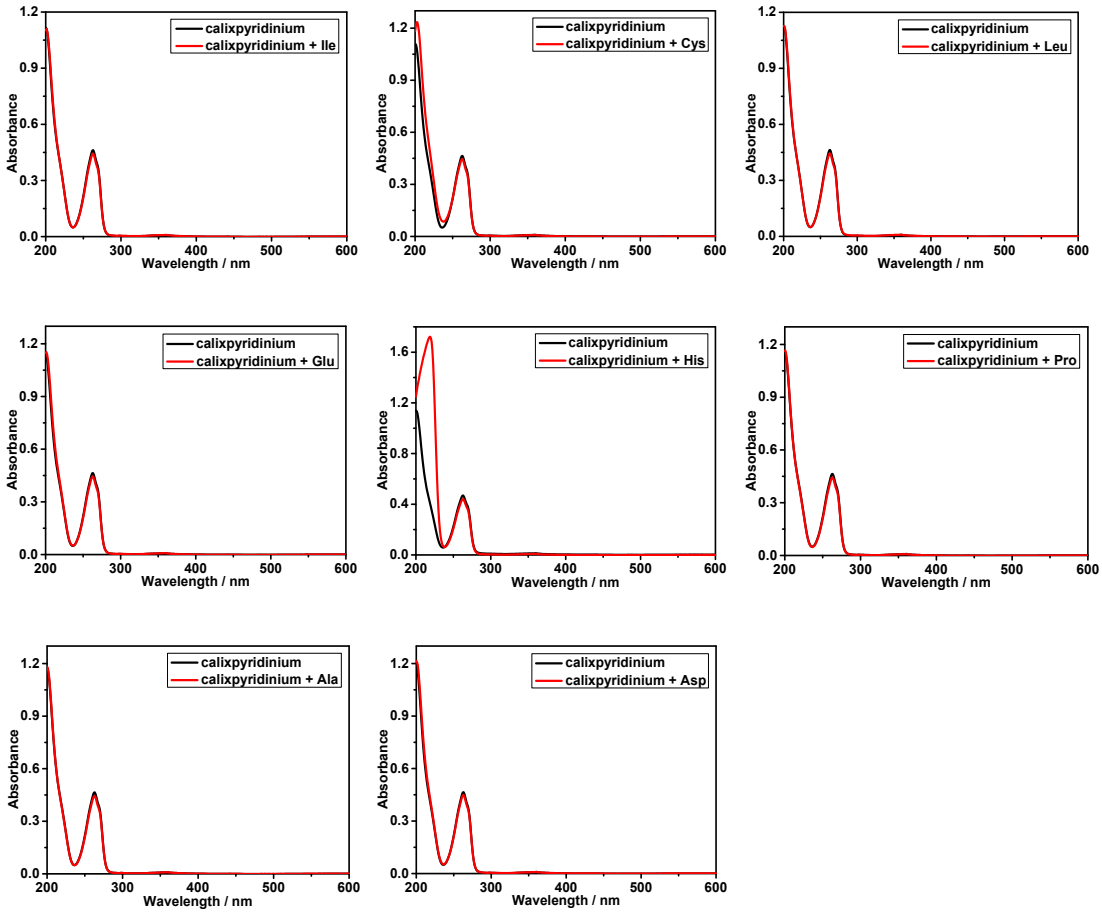


Figure S15. UV-Vis absorption spectra of the calixpyridinium solution in the absence and presence of 20 native α-amino acids in water. [calixpyridinium] = 25 μM and [α-amino acid] = 800 μM.

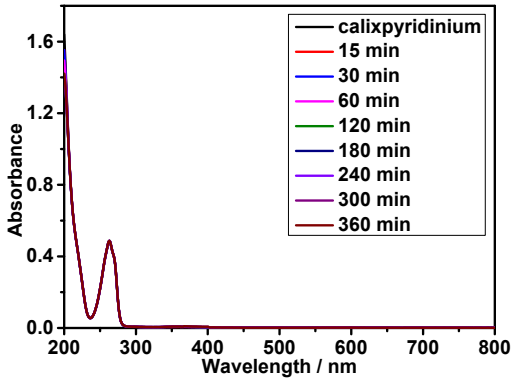


Figure S16. UV-Vis absorption spectra of the calixpyridinium solution at different time after preparation at room temperature. [calixpyridinium] = 25 μM.

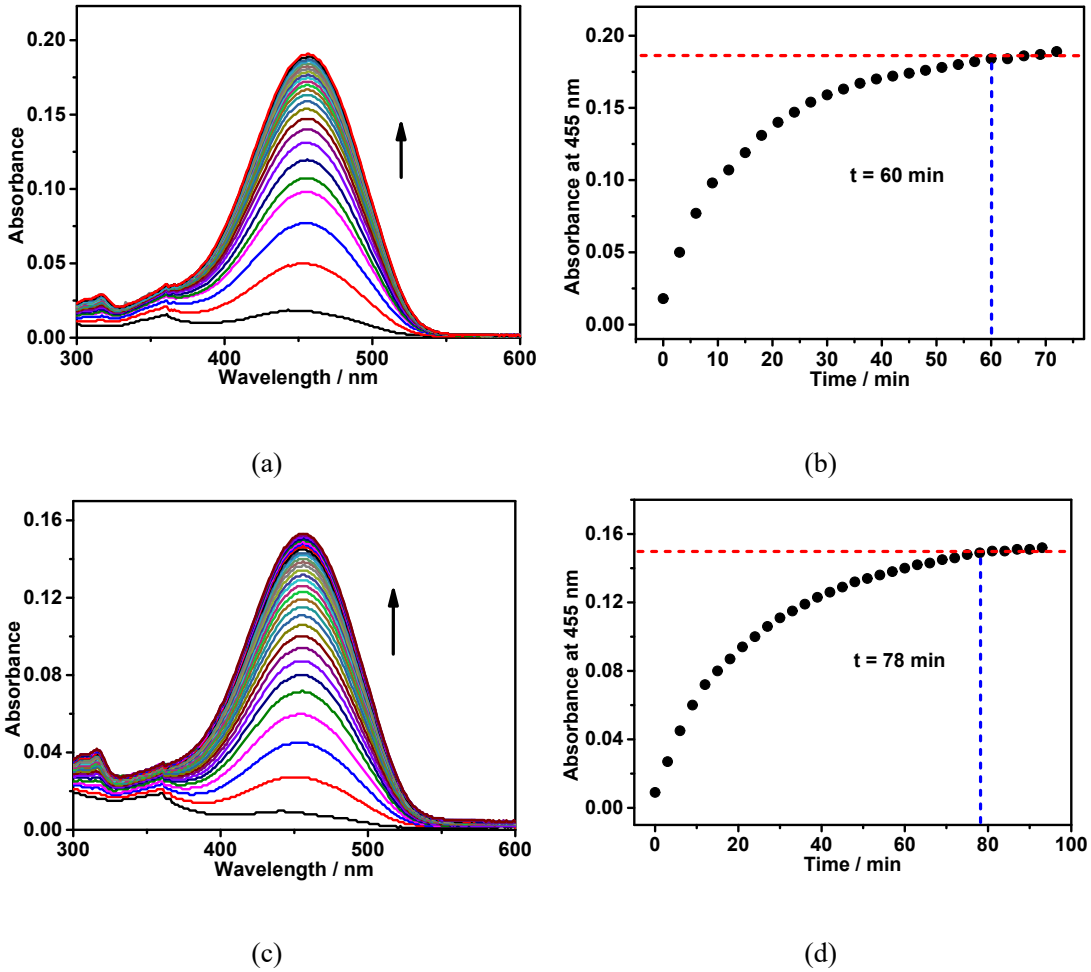


Figure S17. (a) UV-Vis absorption spectra of the calixpyridinium solution at different time after adding Arg. (b) Dependence of the absorbance at 455 nm on time. (c) UV-Vis absorption spectra of the calixpyridinium solution at different time after adding Lys. (d) Dependence of the absorbance at 455 nm on time. [calixpyridinium] = 25 μM, [Arg]

= 250 μM and [Lys] = 250 μM.

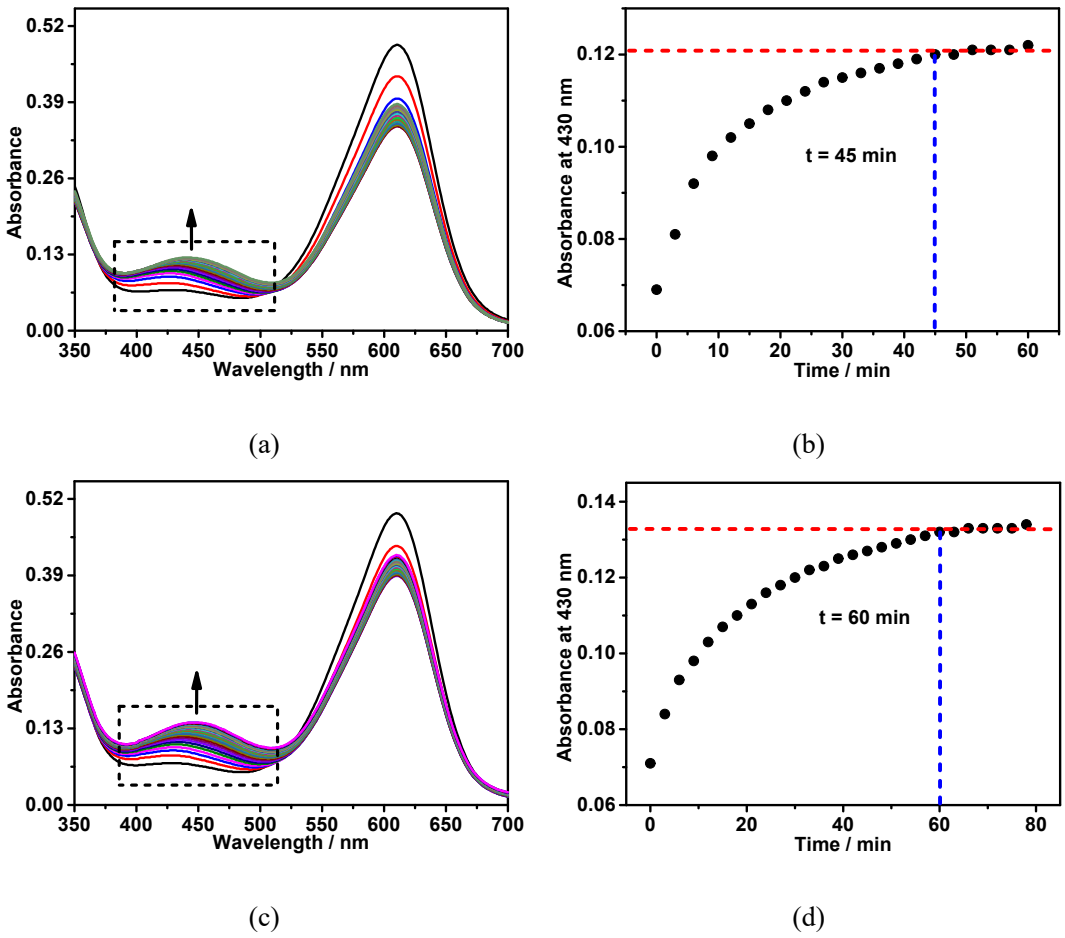


Figure S18. (a) UV-Vis absorption spectra of the calixpyridinium−IC solution at different time after adding Arg. (b) Dependence of the absorbance at 430 nm on time. (c) UV-Vis absorption spectra of the calixpyridinium−IC solution at different time after adding Lys. (d) Dependence of the absorbance at 430 nm on time. [calixpyridinium] = 25 μM, [IC] = 50 μM, [Arg] = 250 μM and [Lys] = 250 μM.

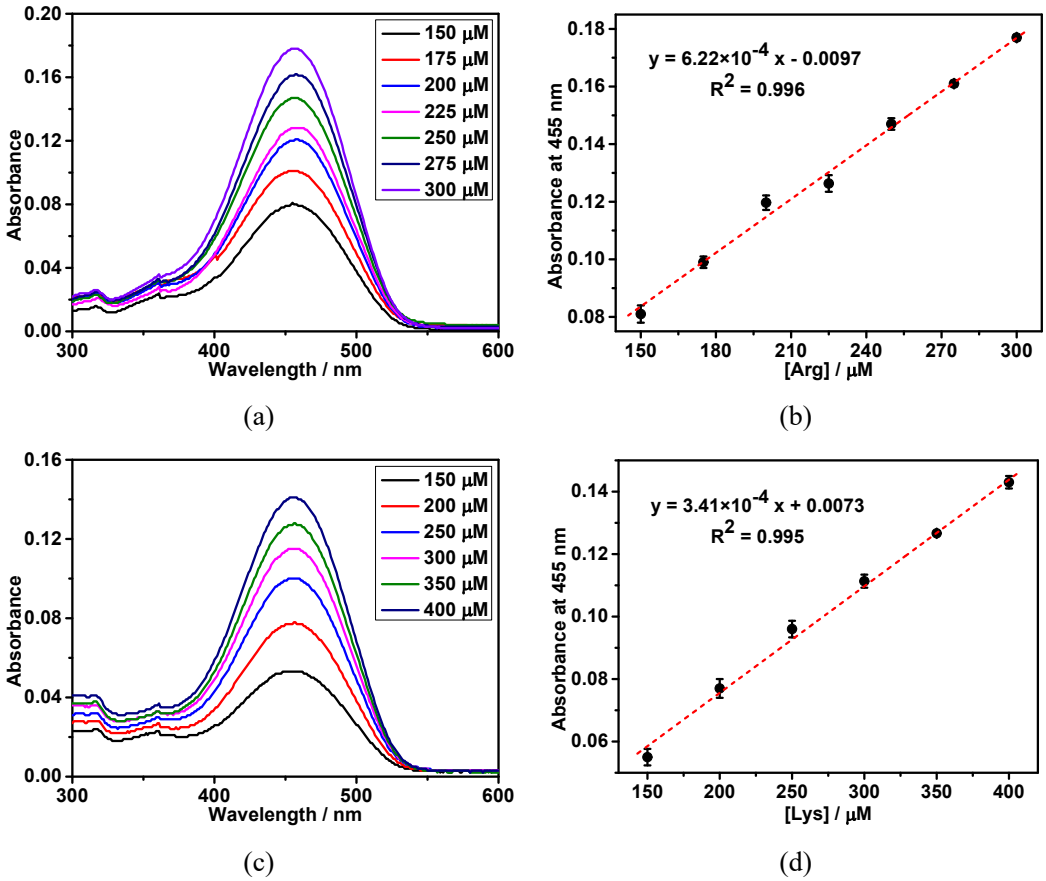


Figure S19. (a) UV-Vis absorption spectra of the calixpyridinium solution in the presence of different concentrations of Arg. (b) Dependence of the absorbance at 455 nm on the concentration of Arg. (c) UV-Vis absorption spectra of the calixpyridinium solution in the presence of different concentrations of Lys. (d) Dependence of the absorbance at 455 nm on the concentration of Lys. [calixpyridinium] = 25 μM.

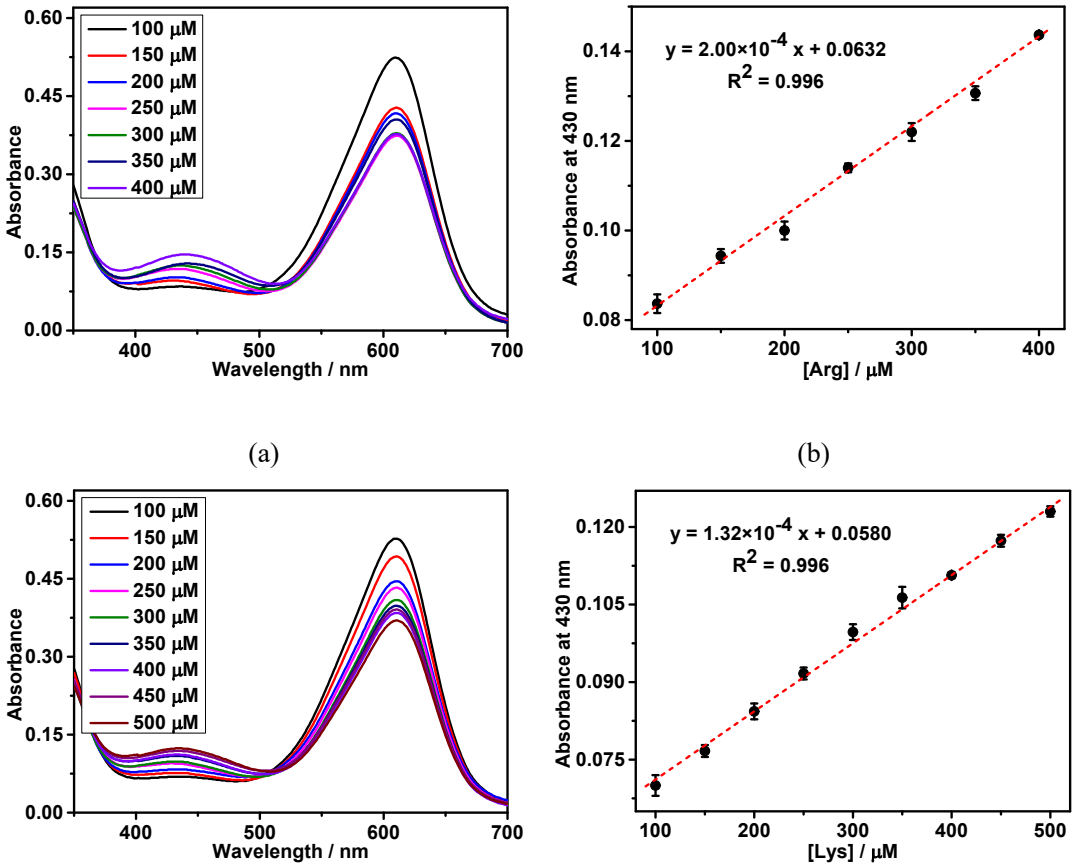


Figure S20. (a) UV-Vis absorption spectra of the calixpyridinium−IC solution in the presence of different concentrations of Arg. (b) Dependence of the absorbance at 430 nm on the concentration of Arg. (c) UV-Vis absorption spectra of the calixpyridinium−IC solution in the presence of different concentrations of Lys. (d) Dependence of the absorbance at 430 nm on the concentration of Lys. [calixpyridinium] = 25 μM and [IC] = 50 μM.

Table S1

Reproducibility of the detection of Arg by free calixpyridinium.

[Arg] / M	Absorbance at 455 nm	Average	RSD (n = 3)
1.5×10^{-4}	0.080; 0.092; 0.087	0.086	7.03 %
2.0×10^{-4}	0.131; 0.120; 0.128	0.126	4.52 %
3.0×10^{-4}	0.173; 0.177; 0.168	0.173	2.62 %

Reproducibility of the detection of Lys by free calixpyridinium.

[Lys] / M	Absorbance at 455 nm	Average	RSD (n = 3)
1.5×10^{-4}	0.057; 0.050; 0.055	0.054	6.68 %
2.0×10^{-4}	0.087; 0.080; 0.083	0.083	4.26 %
3.0×10^{-4}	0.116; 0.110; 0.112	0.113	2.73 %

Reproducibility of the detection of Arg by calixpyridinium−IC assembly.

[Arg] / M	Absorbance at 430 nm	Average	RSD (n = 3)
1.5×10^{-4}	0.098; 0.095; 0.096	0.096	1.65 %
2.0×10^{-4}	0.105; 0.103; 0.108	0.105	2.43 %
3.0×10^{-4}	0.122; 0.126; 0.126	0.125	1.88 %

Reproducibility of the detection of Lys by calixpyridinium−IC assembly.

[Lys] / M	Absorbance at 430 nm	Average	RSD (n = 3)
1.5×10^{-4}	0.082; 0.076; 0.083	0.080	4.76 %
2.0×10^{-4}	0.088; 0.086; 0.087	0.087	1.15 %
3.0×10^{-4}	0.103; 0.099; 0.101	0.101	1.98 %

Table S2

Detection of Arg in urine samples by free calixpyridinium.

Volunteer 1

	Arg added (M)	Arg recovered ± SD (M)	Recovery	RSD (n = 3)
Sample 1	1.5×10^{-4}	$5.42\times10^{-5} \pm 4.23\times10^{-6}$	36.13 %	7.81 %
Sample 2	2.0×10^{-4}	$8.10\times10^{-5} \pm 8.87\times10^{-6}$	40.50 %	10.95 %
Sample 3	3.0×10^{-4}	$1.25\times10^{-4} \pm 1.06\times10^{-5}$	41.67 %	8.49 %

Volunteer 2

	Arg added (M)	Arg recovered ± SD (M)	Recovery	RSD (n = 3)
Sample 1	1.5×10^{-4}	$4.34\times10^{-5} \pm 3.33\times10^{-6}$	28.93 %	7.67 %
Sample 2	2.0×10^{-4}	$7.51\times10^{-5} \pm 4.80\times10^{-6}$	37.55 %	6.39 %
Sample 3	3.0×10^{-4}	$1.16\times10^{-4} \pm 4.06\times10^{-6}$	38.67 %	3.50 %

Detection of Lys in urine samples by free calixpyridinium.

Volunteer 1

	Lys added (M)	Lys recovered ± SD (M)	Recovery	RSD (n = 3)
Sample 1	1.5×10^{-4}	$6.94\times10^{-6} \pm 1.69\times10^{-6}$	4.63 %	24.38 %
Sample 2	2.0×10^{-4}	$3.24\times10^{-5} \pm 8.96\times10^{-6}$	16.20 %	27.65 %
Sample 3	3.0×10^{-4}	$8.12\times10^{-5} \pm 8.80\times10^{-6}$	27.07 %	10.84 %

Volunteer 2

	Lys added (M)	Lys recovered ± SD (M)	Recovery	RSD (n = 3)
Sample 1	1.5×10^{-4}	$2.16\times10^{-5} \pm 1.73\times10^{-6}$	14.40 %	8.02 %
Sample 2	2.0×10^{-4}	$5.68\times10^{-5} \pm 6.09\times10^{-6}$	28.40 %	10.73 %

Sample 3	3.0×10^{-4}	$1.03\times10^{-4} \pm 9.17\times10^{-6}$	34.33 %	8.90 %

Detection of Arg in urine samples by calixpyridinium−IC assembly.

Volunteer 1

	Arg added (M)	Arg recovered ± SD (M)	Recovery	RSD (n = 3)
Sample 1	1.5×10^{-4}	$1.49\times10^{-4} \pm 8.66\times10^{-6}$	99.33 %	5.81 %
Sample 2	2.0×10^{-4}	$2.19\times10^{-4} \pm 1.32\times10^{-5}$	109.50 %	6.04 %
Sample 3	3.0×10^{-4}	$3.24\times10^{-4} \pm 1.00\times10^{-5}$	108.00 %	3.09 %

Volunteer 2

	Arg added (M)	Arg recovered ± SD (M)	Recovery	RSD (n = 3)
Sample 1	1.5×10^{-4}	$1.36\times10^{-4} \pm 7.65\times10^{-6}$	90.67 %	5.62 %
Sample 2	2.0×10^{-4}	$1.87\times10^{-4} \pm 1.16\times10^{-5}$	93.50 %	6.18 %
Sample 3	3.0×10^{-4}	$2.86\times10^{-4} \pm 7.65\times10^{-6}$	95.33 %	2.67 %

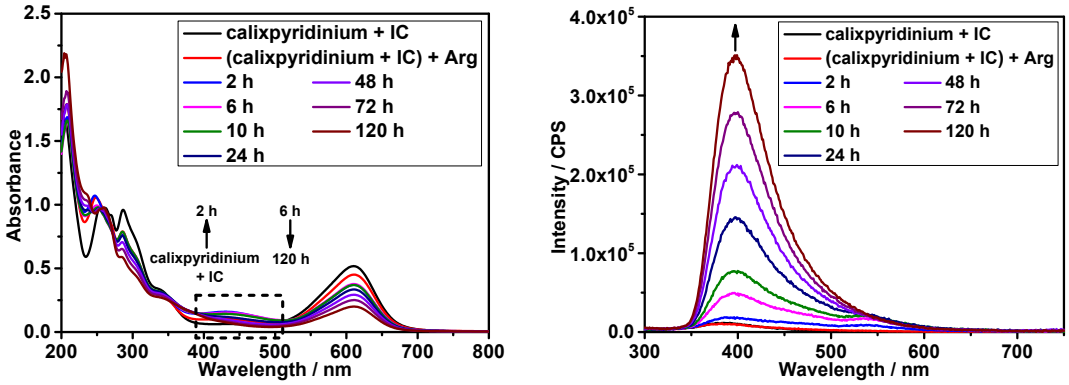
Detection of Lys in urine samples by calixpyridinium−IC assembly.

Volunteer 1

	Lys added (M)	Arg recovered ± SD (M)	Recovery	RSD (n = 3)
Sample 1	1.5×10^{-4}	$1.57\times10^{-4} \pm 1.17\times10^{-5}$	104.67 %	7.44 %
Sample 2	2.0×10^{-4}	$2.02\times10^{-4} \pm 1.18\times10^{-5}$	101.00 %	5.84 %
Sample 3	3.0×10^{-4}	$3.08\times10^{-4} \pm 1.61\times10^{-5}$	102.67 %	5.23 %

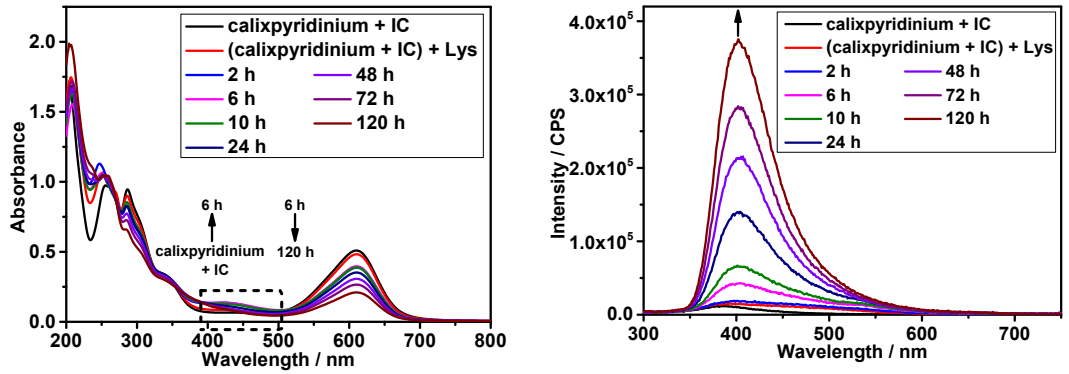
Volunteer 2

	Lys added (M)	Arg recovered ± SD (M)	Recovery	RSD (n = 3)
Sample 1	1.5×10^{-4}	$1.54\times10^{-4} \pm 8.66\times10^{-6}$	102.67 %	5.62 %
Sample 2	2.0×10^{-4}	$2.17\times10^{-4} \pm 1.12\times10^{-5}$	108.50 %	5.18 %
Sample 3	3.0×10^{-4}	$3.08\times10^{-4} \pm 1.91\times10^{-5}$	102.67 %	6.22 %



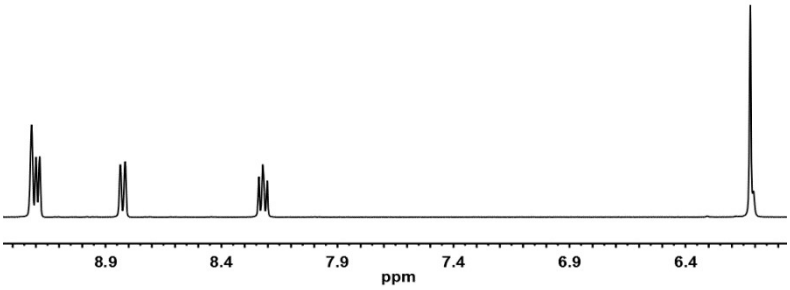
(a) (b)

Figure S21. UV-Vis absorption (a) and fluorescence emission (b) spectra of the calixpyridinium−IC solution before and at different time after adding Arg in urine samples. [calixpyridinium] = 25 μM, [IC] = 50 μM, [Arg] = 1 mM, λ_{ex} = 263 nm, slit width: ex 2 nm, em 2 nm.

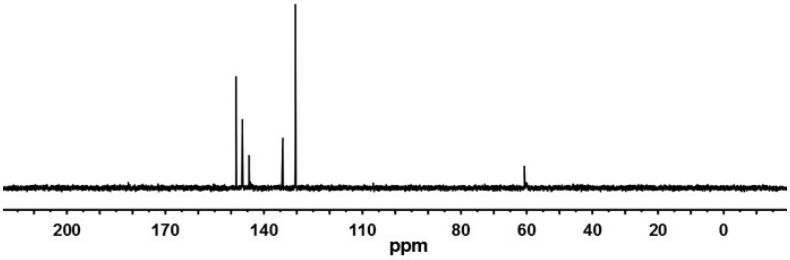


(a) (b)

Figure S22. UV-Vis absorption (a) and fluorescence emission (b) spectra of the calixpyridinium−IC solution before and at different time after adding Lys in urine samples. [calixpyridinium] = 25 μM, [IC] = 50 μM, [Lys] = 1 mM, λ_{ex} = 263 nm, slit width: ex 2 nm, em 2 nm.



(a)



(b)

Figure S23. ^{1}H NMR spectrum (a) and ^{13}C NMR spectrum (b) of calixpyridinium in D_2O.

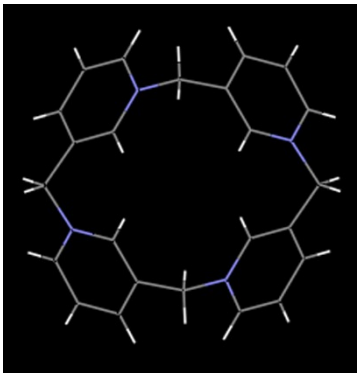


Figure S24. Crystal structure of calixpyridinium.

References

1 S. Shinoda, M. Tadokoro, H. Tsukube and R. Arakawa, One-step synthesis of a quaternary tetrapyridinium macrocycle as a new specific receptor of tricarboxylate anions, *Chem. Commun.*, 1998, 181−182.