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Supplementary information

Preparation of near-infrared AIEgen-active fluorescent probes for mapping amyloid-β plaques in brain tissues and living mice

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Preparation of near-infrared AIEgen-active fluorescent probes for mapping amyloid-β plaques in brain tissues and living mice

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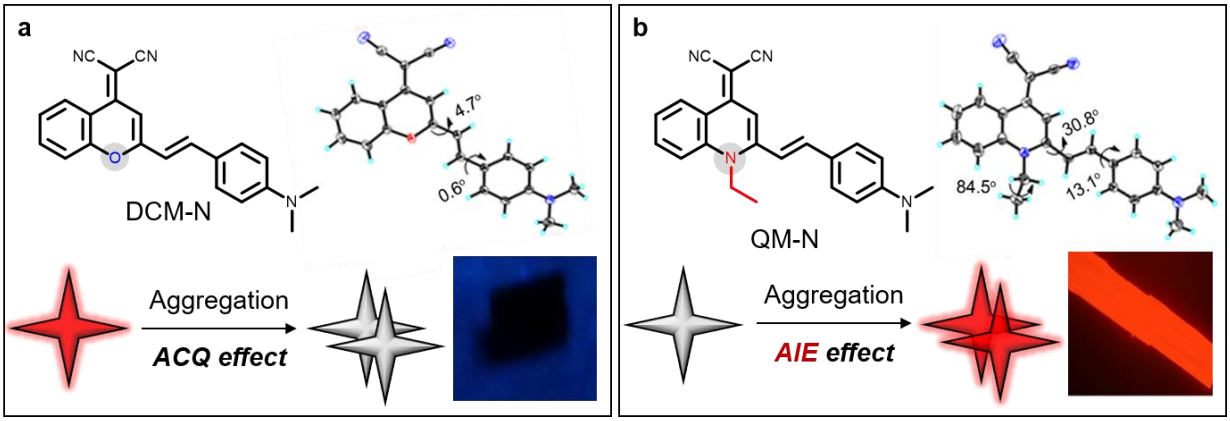
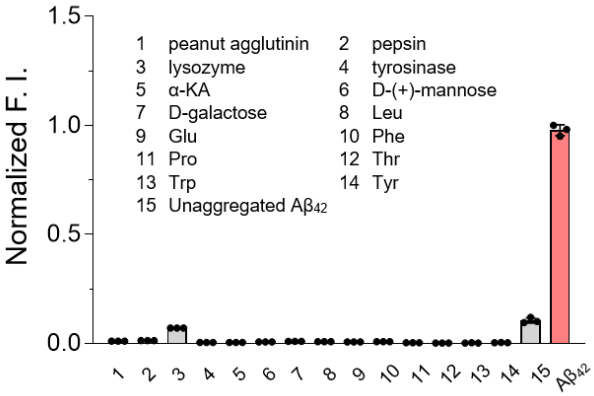
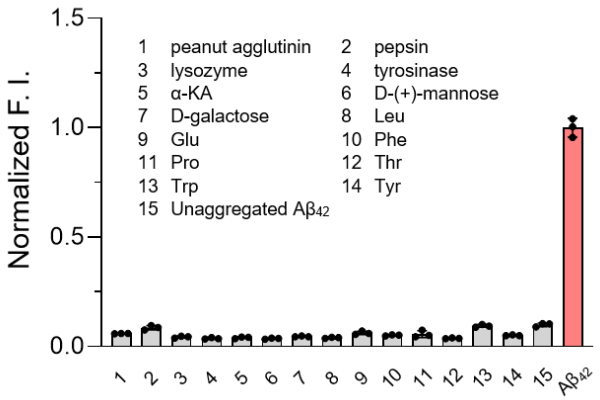
Supplementary Figures



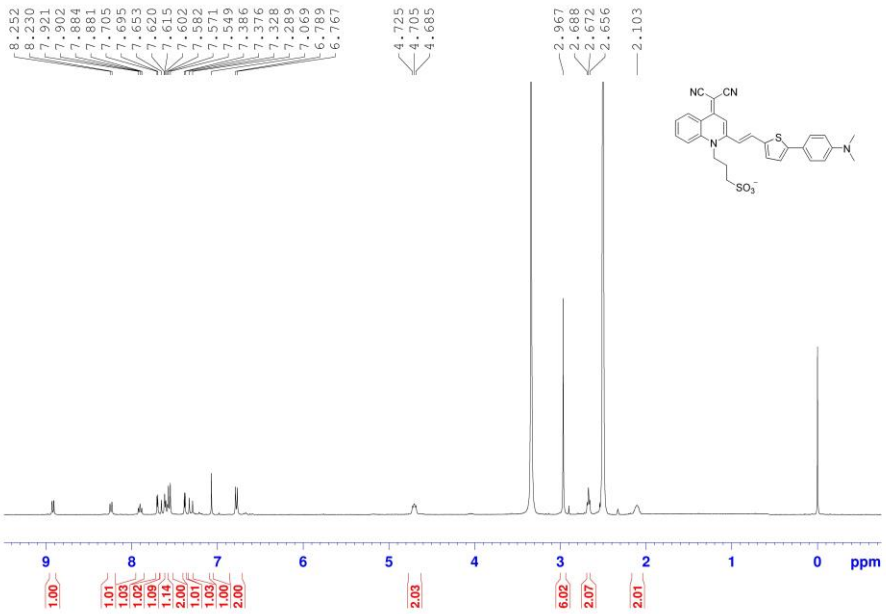
Supplementary Fig. 1. Molecular design strategy for AIEgens from ACQ fluorophores. Molecular structure, single crystals, and fluorescent photoimages (in the solid state) of DCM-N (**a**) and QM-N (**b**). **a-b** adapted from ref. [1].



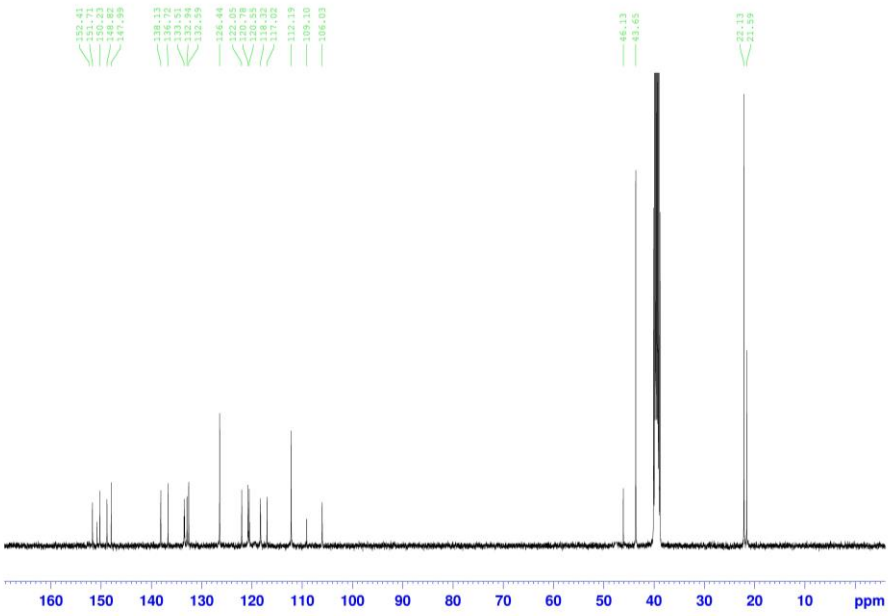
Supplementary Fig. 2. High selectivity toward potential competitive species and $A\beta_{42}$ aggregates in PBS. Normalized fluorescence at 660 nm (λ_{ex} = 500 nm) of QM-FN-SO_3 (10 μM) toward potential competitive species and $A\beta_{42}$ aggregates (10 μM) in PBS (pH 7.4, 1 % DMSO) for 0.5 h (Supplementary Method 1, Steps S1-S5). Legend of various amino compounds: (1) peanut agglutinin (0.25 μg mL^{-1}), (2) pepsin (10^{-2} mg mL^{-1}), (3) lysozyme (10 U/mL), (4) tyrosinase (10 U/mL), (5) α-KA (100 μM), (6) D-(+)-mannose (100 μM), (7) D-galactose (100 μM), (8) Leu (100 μM), (9) Glu (100 μM), (10) Phe (100 μM), (11) Pro (100 μM), (12) Thr (100 μM), (13) Trp (100 μM), (14) Tyr (100 μM), (15) unaggregated $A\beta_{42}$ (10 μM). Data with error bars are expressed as mean ± s.d., n = 3. Source data are provided (Supplementary Data 1). Adapted from ref. [2].



Supplementary Fig. 3. High selectivity toward potential competitive species and $A\beta_{42}$ aggregates in DMEM. Normalized fluorescence at 710 nm (λ_{ex} = 500 nm) of QM-FN-SO_3 (10 μM) toward potential competitive species and $A\beta_{42}$ aggregates (10 μM) in mixture of DMEM and PBS (1:1, vol/vol) for 0.5 h (Supplementary Method 1, Steps S6-S9). Legend of various amino compounds: (1) peanut agglutinin (0.25 μg mL^{-1}), (2) pepsin (10^{-2} mg mL^{-1}), (3) lysozyme (10 U/mL), (4) tyrosinase (10 U/mL), (5) α-KA (100 μM), (6) D-(+)-mannose (100 μM), (7) D-galactose (100 μM), (8) Leu (100 μM), (9) Glu (100 μM), (10) Phe (100 μM), (11) Pro (100 μM), (12) Thr (100 μM), (13) Trp (100 μM), (14) Tyr (100 μM), (15) unaggregated $A\beta_{42}$ (10 μM). Data with error bars are expressed as mean ± s.d., n = 3. Source data are provided (Supplementary Data 1).



Supplementary Fig. 4. **^{1}H NMR spectrum of QM-FN-SO$_3$ in DMSO-d_6.** Reproduced from ref. [2].



Supplementary Fig. 5. **^{13}C NMR spectrum of QM-FN-SO$_3$ in DMSO-d_6.** Reproduced from ref. [2].

Monoisotopic Mass, Even Electron Ions
51 formula(e) evaluated with 1 results within limits (up to 1 best isotopic matches for each mass)
Elements Used:
C: 0-29 H: 0-45 N: 0-4 O: 0-3 S: 0-2

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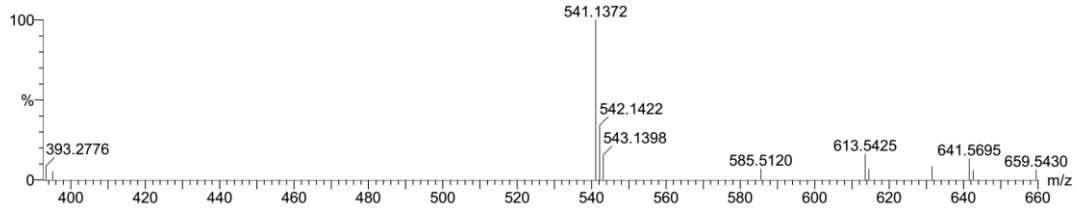
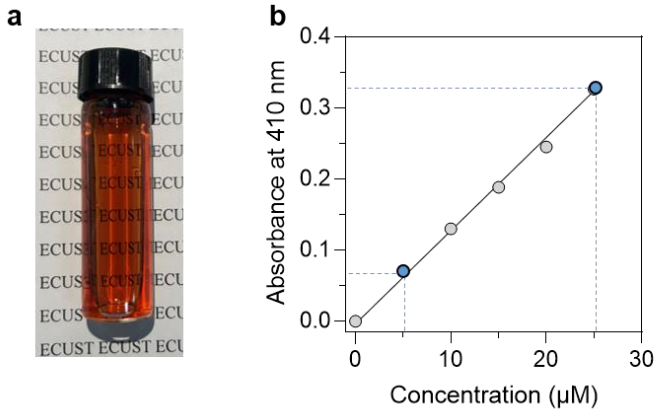
ZW-FW-SO3 142 (3.235) Cm (141:144) 1: TOF MS ES- 5.38e+004

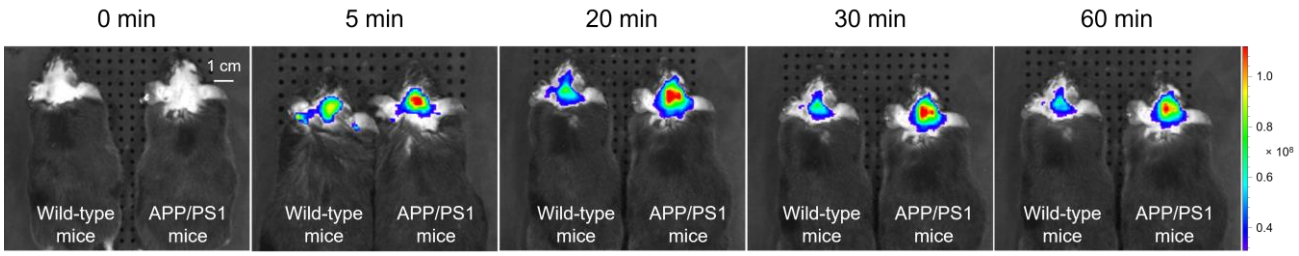


Minimum:				-1.5			
Maximum:		30.0	30.0	100.0			
Mass	Calc. Mass	mDa	PPM	DBE	i-FIT	i-FIT (Norm)	Formula
541.1372	541.1368	0.4	0.7	19.5	17.0	0.0	C29 H25 N4 O3 S2

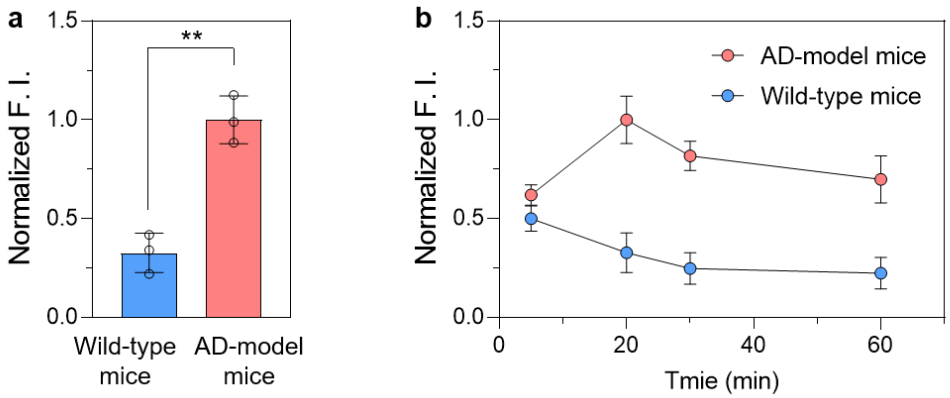
Supplementary Fig. 6. HRMS spectrum of QM-FN-SO$_3$. Reproduced from ref. [2].



Supplementary Fig. 7. Excellent water solubility of QM-FN-SO$_3$. a Potoimage of QM-FN-SO$_3$ stock solution (100 μM, mixture of DMSO and DI water 1:99, vol/vol). **b** The absorbance at 410 nm of QM-FN-SO$_3$ solution (0-25 μM, gray data points) and diluted supernatant of QM-FN-SO$_3$ stock solution (blue data points) (Supplementary Method 2, Steps S10-S19). Source data are provided (Supplementary Data 1). As shown in **a**, QM-FN-SO$_3$ stock solution (mixture of DMSO and DI water 1:99, vol/vol) is fully transparent, demonstrating good water solubility of QM-FN-SO$_3$. Then, we evaluate the solubility by using the shake-flask solubility method. As shown in **b**, the concentration-absorbance curve was established for quantification of sample concentrations. Then, QM-FN-SO$_3$ solutions (100 and 500 μM) were stirred for 24 h, and the supernatant was collected and diluted. These solutions were analyzed by using a UV-vis spectrophotometer. The concentration of these diluted solutions is calculated as 5.01 and 25.23 μM. Thus, the concentration of these supernatants is calculated as 100.20 and 504.60 μM. These results well fit their initial concentrations, and highlight the excellent water solubility of QM-FN-SO$_3$.



Supplementary Fig. 8. *In vivo* mapping of Aβ deposition. APP/PS1 transgenic mice (AD model mice, n = 3, C57BL6, APP/PS1, 22-month-old, male) and age-matched wild-type mice (n = 3, C57BL6, 22-months-old, male) were intravenously injected with QM-FN-SO_3 (2.0 mg kg^{-1}). *In vivo* fluorescent images of wild-type mice and APP/PS1 mice at different time points after intravenous injection (filter set: λ_{ex} = 500 nm, λ_{em} = 680 ± 20 nm). Adapted from ref. [2].



Supplementary Fig. 9. Fluorescent intensities of the ROI of wild-type mice and APP/PS1 mice with time. a Fluorescent intensities of wild-type mice and APP/PS1 mice at 20 min after intravenous injection. Data with error bars are expressed as mean ± s.d., n = 3. Source data are provided (Supplementary Data 1). The *p*-value was performed with one-way ANOVA, $^{**}p < 0.01$. **b** Fluorescent intensities of wild-type mice and APP/PS1 mice at different time points after intravenous injection. Data with error bars are expressed as mean ± s.d., n = 3. Source data are provided (Supplementary Data 1).

Supplementary Method 1

Reagents for selectivity test

- Peanut agglutinin (Sigma-Aldrich, cat. no. L7759-250UG)
- Pepsin (≥250 units/mg; Sigma-Aldrich, cat. no. P7000-25G)
- Lysozyme (>23000 U/mg; Sigma-Aldrich, cat. no. 10837059001)
- Tyrosinase (Sigma-Aldrich, cat. no. T3824-25KU)
- 2-ketoglutaric acid (α-KA, Adamas, cat. no. 48577A)
- D-(+)-mannose (Adamas, cat. no. 50198F)
- D-galactose (Adamas, cat. no. 68252D)
- L-Leu (Adamas, cat. no. 70880A)
- L-Glu (Greagent, cat. no. G66304A)
- L-Phe (Adamas, cat. no. 73340A)
- L-Pro (Adamas, cat. no. 24922B)
- L-Thr (Adamas, cat. no. 79193B)
- L-Trp (Adamas, cat. no. 79767B)
- L-Tyr (Adamas, cat. no. 69060A)
- DMEM (Bioagrio, cat. no. LD1111-500)

Reagent setup for selectivity test

Peanut agglutinin (25 μg mL^{-1})

Dissolve 25 μg of peanut agglutinin in 1 mL of DI water. This solution should be freshly prepared.

Pepsin (1 mg mL^{-1})

Dissolve 1 mg of pepsin in 1 mL of DI water. This solution should be freshly prepared.

Lysozyme (1 KU mL^{-1})

Dissolve 1 mg of lysozyme in 23 mL of DI water. This solution should be freshly prepared.

Tyrosinase (1 KU mL^{-1})

Dissolve 1 mg of tyrosinase in 25 mL of DI water. This solution should be freshly prepared.

2-ketoglutaric acid stock solution (10 mM)

Dissolve 14.6 mg of 2-ketoglutaric acid in 10 mL of DI water. This solution should be freshly prepared.

D-(+)-mannose stock solution (10 mM)

Dissolve 18.0 mg of D-(+)-mannose in 10 mL of DI water. This solution should be freshly prepared.

D-galactose stock solution (10 mM)

Dissolve 18.0 mg of D-galactose in 10 mL of DI water. This solution should be freshly prepared.

L-Leu stock solution (10 mM)

Dissolve 13.1 mg of L-Leu in 10 mL of DI water. This solution should be freshly prepared.

L-Glu stock solution (10 mM)

Dissolve 14.7 mg of L-Glu in 10 mL of 1 M HCl solution. This solution should be freshly prepared.

L-Phe stock solution (10 mM)

Dissolve 16.5 mg of L-Phe in 10 mL of DI water. This solution should be freshly prepared.

L-Pro stock solution (10 mM)

Dissolve 11.5 mg of L-Pro in 10 mL of DI water. This solution should be freshly prepared.

L-Thr stock solution (10 mM)

Dissolve 11.9 mg of L-Thr in 10 mL of DI water. This solution should be freshly prepared.

L-Trp stock solution (10 mM)

Dissolve 20.4 mg of L-Trp in 10 mL of 1 M HCl solution. This solution should be freshly prepared.

L-Tyr stock solution (10 mM)

Dissolve 18.1 mg of L-Tyr in 10 mL of 1 M HCl solution. This solution should be freshly prepared.

Procedure for selectivity test in PBS

S1 Add 1.5 mL of PBS to a 2-mL volumetric flask. And add 20 μL of the 1 mM QM-FN-SO_3 stock solution to the 2-mL volumetric flask.

S2 Repeat Step S1 to prepare a total of 16 samples. and add to them 20 μL of Peanut agglutinin (25 μg mL^{-1}), 20 μL of Pepsin (1 mg mL^{-1}), 20 μL of Lysozyme (1 KU mL^{-1}), 20 μL of Tyrosinase (1 KU mL^{-1}), 20 μL of 2-ketoglutaric acid stock solution (10 mM), 20 μL of D-(+)-mannose stock solution (10 mM), 20 μL of D-galactose stock solution (10 mM), 20 μL of L-Leu stock solution (10 mM), 20 μL of L-Glu stock solution (10 mM), 20 μL of L-Phe stock solution (10 mM), 20 μL of L-Pro stock solution (10 mM), 20 μL of L-Thr stock solution (10 mM), 20 μL of L-Trp stock solution (10 mM), 20 μL of L-Tyr stock solution (10 mM), 100 μL of 200 μM unaggregated $A\beta_{42}$ stock solution, and 100 μL of 200 μM $A\beta_{42}$ aggregates stock solution, respectively.

S3 Make up the final volume of each solution to 2.0 mL by using PBS.

S4 Transfer the solutions to 4-mL glass vials, and incubate the solution at 37 °C for 30 min.

S5 Transfer the solutions to 1-cm quartz sample cells, and record the fluorescence intensities at 660 nm by using a fluorescence spectrometer (excitation wavelength: 500 nm).

Procedure for selectivity test in mixture of DMEM and PBS

S6 Add 1.5 mL of mixture of DMEM and PBS (1:1, vol/vol) to a 2-mL volumetric flask. And add 20 μL of the 1 mM QM-FN-SO_3 stock solution to the 2-mL volumetric flask.

S7 Repeat Step S2 for the preparation of samples.

S8 Make up the final volume of each solution to 2.0 mL by using mixture of DMEM and PBS (1:1, vol/vol).

S9 Repeat Steps S4-S5 for incubation and spectroscopic assays.

Supplementary Method 2

Procedure for solubility determination

S10 Add 1.5 mL of DMSO to a 2-mL volumetric flask.

S11 S2 Repeat Step S10 to prepare a total of six samples. Add different volumes of 1 mM QM-FN-SO_3 stock solution (DMSO) in each volumetric flask, and quickly make up the final volume of each solution to 2 mL by using DMSO. The volume of 1 mM QM-FN-SO_3 stock solution added in each volumetric flask is 0, 10, 20, 30, 40, and 50 μL, respectively. The final concentration of QM-FN-SO_3 is 0, 5, 10, 15, 20, and 25 μM, respectively.

S12 Transfer the solutions to 1-cm quartz sample cells, and record the absorption at 410 nm of the solution in the quartz sample cell by using the UV-vis spectrophotometer.

S13 Establish the concentration-absorbance curve (for quantification of sample concentrations).

S14 Prepare the QM-FN-SO_3 stock solution (100 μM, mixture of DMSO and DI water 1:99, vol/vol) following the "Reagent setup" section. And keep stirring for 24 h.

S15 Transfer 100 μL of supernatant to a 2-mL volumetric flask, and quickly make up the final volume of each solution to 2 mL by using DMSO.

S16 Transfer the solution to 1-cm quartz sample cells, and record the absorption at 410 nm of the solution in the quartz sample cell by using the UV-vis spectrophotometer.

S17 Prepare the QM-FN-SO_3 stock solution (500 μM) following the "Reagent setup" section. And keep stirring for 24 h.

S18 Transfer 100 μL of supernatant to a 2-mL volumetric flask, and quickly make up the final volume of each solution to 2 mL by using DMSO.

S19 Transfer the solution to 1-cm quartz sample cells, and record the absorption at 410 nm of the solution in the quartz sample cell by using the UV-vis spectrophotometer.

References

1 Shi, C. *et al.* Self-assembly solid-state enhanced red emission of quinolinemalononitrile: optical waveguides and stimuli response. *ACS Appl. Mater. Interfaces* **5**, 192-198 (2013).

2 Fu, W. *et al.* Rational design of near-infrared aggregation-induced-emission-active probes: *in situ* mapping of amyloid-β plaques with ultrasensitivity and high-fidelity. *J. Am. Chem. Soc.* **141**, 3171-3177 (2019).