Supporting Information

From aggregation-induced to solution emission: A new strategy for designing ratiometric fluorescent probes and its application for in vivo HClO detection

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1. Fluorescence and UV-vis spectra affected by AIE

Fig. S1 (a) Fluorescence spectra of PDAM-Me in the MeCN-water mixtures with different water contents ($\lambda_{ex} = 410$ nm). (b) Fluorescence peak intensity of PDAM-Me versus water content of the solvent mixture.

Fig. S2 UV-vis spectra of PDAM-Lyso (20 $\mu$M) in CH$_3$CN, upon increasing volume percentages of water from 0% to 98%. 

S3
2. Mechanism research

Fig.S3 (a) LCMS spectra of PDAM-Lyso. (b) LCMS spectra of PDAM-Lyso + NaClO.
3. Detection limit calculation

Fluorescence titration was carried out in CH$_3$CN/PBS buffer (1/9, v/v, 10 mM, pH = 7.4) to determine the detection limit, which was then calculated with the equation:

Detection of limit = $3 \times \sigma_i / m$

where $\sigma_i$ is the standard deviation of blank measurements and is calculated with twenty ($n = 20$) experiments, and $m$ is the slope between intensity ration ($I_{470}/I_{620}$) and sample concentration. It was calculated that the detection limit was 440 nm.

![Dependence curve of fluorescence intensity ratios](image)

**Fig. S4** The dependence curve of fluorescence intensity ratios ($I_{470}/I_{620}$) in the presence of ClO$^-$ in CH$_3$CN/PBS buffer (1/9, v/v, 10 mM, pH = 7.4), ($\lambda_{ex} = 410$ nm).
Fig. S5 Reaction-time profiles of PDAM-Lyso (20 μM) in the absence and presence of NaClO in CH$_3$CN/PBS buffer (1/9, v/v, 10 mM, pH = 7.4) ($\lambda_{ex}$=410 nm).
5. pH-dependent research

Fig. S6 pH-dependent profiles of PDAM-Lyso (20 μM) in the absence and presence of NaClO (2.5 eq.) with pH range of 4.5 – 8.5 in CH₃CN/PBS buffer (1/9, v/v, 10 mM) ($\lambda_{ex}$=410 nm).
6. Cytotoxicity study

![Cytotoxicity data of PDAM-Lyso for Raw264.7 cells (blue) and Hela cells (red).](image1)

Fig. S7 Cytotoxicity data of PDAM-Lyso for Raw264.7 cells (blue) and Hela cells (red).

![Cytotoxicity data of PDAM-Me for Raw264.7 cells (blue) and Hela cells (red).](image2)

Fig. S8 Cytotoxicity data of PDAM-Me for Raw264.7 cells (blue) and Hela cells (red).
7. Cell imaging

Fig. S9 Confocal fluorescent images of Hela cells of PDAM-Lyso and Lyso-Tracker red ($\lambda_{\text{ex}} = 405$ nm, $\lambda_{\text{em}} = 560 – 690$ nm).

Fig. S10 Confocal fluorescent images of Hela cells of PDAM-Lyso and Lyso-Tracker red ($\lambda_{\text{ex}} = 405$ nm, $\lambda_{\text{em}} = 430 – 560$ nm).
Fig. S11 Confocal fluorescent images of Hela cells of PDAM-Me and Lyso-Tracker red ($\lambda_{ex} = 405$ nm, $\lambda_{em} = 560 – 690$ nm).

Fig. S12 Confocal fluorescent images of Hela cells of PDAM-Me and Lyso-Tracker red ($\lambda_{ex} = 405$ nm, $\lambda_{em} = 430 – 560$ nm).
Fig. S13 Confocal fluorescence images of PDAM-Me in cells (λ_\text{ex} = 410 nm). (a) Hela cells, PDAM-Me (3.0 μM), 420 – 550 nm; (b) Hela cells, PDAM-Me (3.0 μM), 570 – 700 nm; (c) The overlay of (a) and (b); (d) The scatter distributing plot of (c). (e) Hela cells, PDAM-Me (3.0 μM), NaClO (60 μM), 420 – 550 nm; (f) Hela cells, PDAM-Me (3.0 μM), NaClO (60 μM), 570 – 700 nm; (g) The overlay of (e) and (f); (h) The scatter distributing plot of (g). (i) Raw 264.7 cells, PDAM-Me (3.0 μM), LPS (1.0 μg/mL), PMA (1.0 μg/mL), 420 – 550 nm; (j) Raw 264.7 cells, PDAM-Me (3.0 μM), LPS (1.0 μg/mL), PMA (1.0 μg/mL), 570 – 700 nm; (k) The overlay of (i) and (j); (l) The scatter distributing plot of (k).
Fig. S14 Confocal fluorescent images of PDAM-Lyso (3.0 μM) in Hela cells ($\lambda_{\text{ex}} = 410$ nm, $\lambda_{\text{em}} = 420 – 550$ nm).

Fig. S15 Confocal fluorescent images of PDAM-Lyso (3.0 μM) in Hela cells ($\lambda_{\text{ex}} = 410$ nm, $\lambda_{\text{em}} = 570 – 700$ nm).
Fig. S16 Confocal fluorescent images of PDAM-Lyso (3.0 μM) in Hela cells with the existence of NaClO (60 μM) ($\lambda_{ex} = 410$ nm, $\lambda_{em} = 420 – 550$ nm).

Fig. S17 Confocal fluorescent images of PDAM-Lyso (3.0 μM) in Hela cells with the existence of NaClO (60 μM) ($\lambda_{ex} = 410$ nm, $\lambda_{em} = 570 – 700$ nm).
Fig. S18 Confocal fluorescent images of PDAM-Lyso (3.0 μM) in Raw 264.7 cells with LPS (1.0 μg/mL) and PMA (1.0 μg/mL) cells ($\lambda_{ex} = 410$ nm, $\lambda_{em} = 420 – 550$ nm).

Fig. S19 Confocal fluorescent images of PDAM-Lyso (3.0 μM) in Raw 264.7 cells with LPS (1.0 μg/mL) and PMA (1.0 μg/mL) cells ($\lambda_{ex} = 410$ nm, $\lambda_{em} = 570 – 700$ nm).
8. Crystal data of PDAM-Me

Table S1 Crystal data of compound PDAM-Me

<table>
<thead>
<tr>
<th>Compound</th>
<th>PDAM-Me</th>
</tr>
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<tbody>
<tr>
<td>Empirical formula</td>
<td>C\textsubscript{18}H\textsubscript{13}N\textsubscript{5}S</td>
</tr>
<tr>
<td>Formula weight</td>
<td>331.39</td>
</tr>
<tr>
<td>Crystal system</td>
<td>monoclinic</td>
</tr>
<tr>
<td>Space group</td>
<td>\textit{P}2\textsubscript{1}/c</td>
</tr>
<tr>
<td>(a[\text{Å}])</td>
<td>18.09(2)</td>
</tr>
<tr>
<td>(b[\text{Å}])</td>
<td>6.977(9)</td>
</tr>
<tr>
<td>(c[\text{Å}])</td>
<td>13.863(19)</td>
</tr>
<tr>
<td>(\alpha[^\circ])</td>
<td>90</td>
</tr>
<tr>
<td>(\beta[^\circ])</td>
<td>109.407(12)</td>
</tr>
<tr>
<td>(\gamma[^\circ])</td>
<td>90</td>
</tr>
<tr>
<td>(V[\text{Å}^3])</td>
<td>1650(4)</td>
</tr>
<tr>
<td>(Z)</td>
<td>4</td>
</tr>
<tr>
<td>(T[\text{K}])</td>
<td>291(2)</td>
</tr>
<tr>
<td>(D_{\text{calc}}[\text{g} \cdot \text{cm}^{-1}])</td>
<td>1.334</td>
</tr>
<tr>
<td>(F(000))</td>
<td>688</td>
</tr>
<tr>
<td>(\mu[\text{mm}^{-1}])</td>
<td>0.205</td>
</tr>
<tr>
<td>(\theta \text{ range}[^\circ])</td>
<td>2.388 - 24.999</td>
</tr>
<tr>
<td>(R_1)</td>
<td>0.1562</td>
</tr>
<tr>
<td>(wR_2)</td>
<td>0.4408</td>
</tr>
<tr>
<td>(S)</td>
<td>1.124</td>
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Table S2 Intermolecular hydrogen bonding parameters (Å, °) in PDAM-Me

<table>
<thead>
<tr>
<th>D–H···A</th>
<th>D–H</th>
<th>H···A</th>
<th>D···A ((d))</th>
<th>(\angle \text{DHA})</th>
<th>Symmetry code</th>
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<tr>
<td>N5–H5A···N3</td>
<td>0.86</td>
<td>2.22</td>
<td>3.031(15)</td>
<td>157</td>
<td>(x, -1+y, z)</td>
</tr>
<tr>
<td>N5–H5B···N4</td>
<td>0.86</td>
<td>2.19</td>
<td>3.028(14)</td>
<td>166</td>
<td>(-x, -1/2+y, 1/2-z)</td>
</tr>
</tbody>
</table>
9. Supplemental spectra

$^1$H NMR spectrum of PDAM-Me.

$^{13}$C NMR spectrum of PDAM-Me.
-MS spectrum of the compound 4.

$^1$H NMR spectrum of compound 4.
$^{13}$C NMR spectrum of compound 4.

$^1$H NMR spectrum of compound 5.
\(^{13}\)C NMR spectrum of compound 5.

-MS spectrum (positive) of PDMA-Lyso.
$^1$H NMR spectrum of PDMA-Lyso.

$^{13}$C NMR spectrum of PDMA-Lyso.