*Electronic Supplementary Information*

# A label-free and fluorescence turn-on assay for sensitive detection of hyaluronidase based on hyaluronan-induced perylene self-assembly

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## Experimental section

### Synthesis of PDI

The cationic perylene diimide derivative (PDI) was synthesized following the literature procedure.[1] Briefly, under nitrogen atmosphere, a mixture of perylene tetracarboxylic dianhydride (1.0 g, 2.42 mmol) and N, N-Dimethyl-1, 3-propanediamine (5 mL, 39.3 mmol) in 50 mL of isobutanol was stirred at 90 °C overnight. After filtering, the solid was washed twice with water and ethanol. The unreacted perylene tetracarboxylic dianhydride was then removed from the crude product by treating with 5% aqueous NaOH solution at 90 °C for 1 h. After filtering, washing with triply distilled water and ethanol, and drying under vacuum, 1.1 g of N, N-bis (propylenedimethylamine)-3, 4, 9, 10-perylenediimide was obtained as a red solid. It was added together with 1.5 mL of methyl iodide (24.1 mmol) to 50 mL of toluene, and heat to reflux for 3 h under nitrogen. The mixture was slowly brought to room temperature, and then filtered and washed with cool ether to give an iodide of compound 1 as a brown-red solid. The nitrate of compound 1 was further prepared by treating it with silver nitrate. It was characterized using $^1$H NMR and MS analytical spectroscopic techniques, and the results agree well with the previously reported data.[1] $^1$H NMR (DMSO-$d_6$, 400 MHz, 80 °C), δ (ppm) = 2.18-2.25 (m, 4H), 3.09 (s, 18H), 3.47-3.52 (m, 4H), 4.20 (t, 4H, $J$ = 6.6 Hz), 8.56 (d, 4H, $J$ = 7.8 Hz), 8.84 (d, 4H, $J$ =7.9 Hz). MS(TOF) m/e: 295.2 [$M^{2+}$/2].

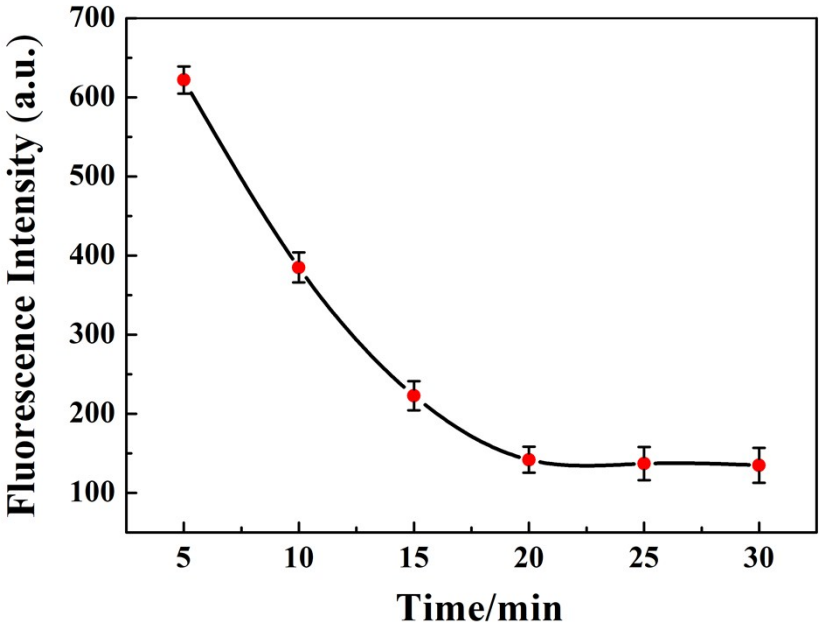


**Fig. S1** Effect of the self-assembly time on the fluorescence emission of HA-PDI nanoaggregates at 550 nm; [PDI] = 0.2 μM, [HA] = 20 μg/mL. The error bars were estimated from three replicate measurements.

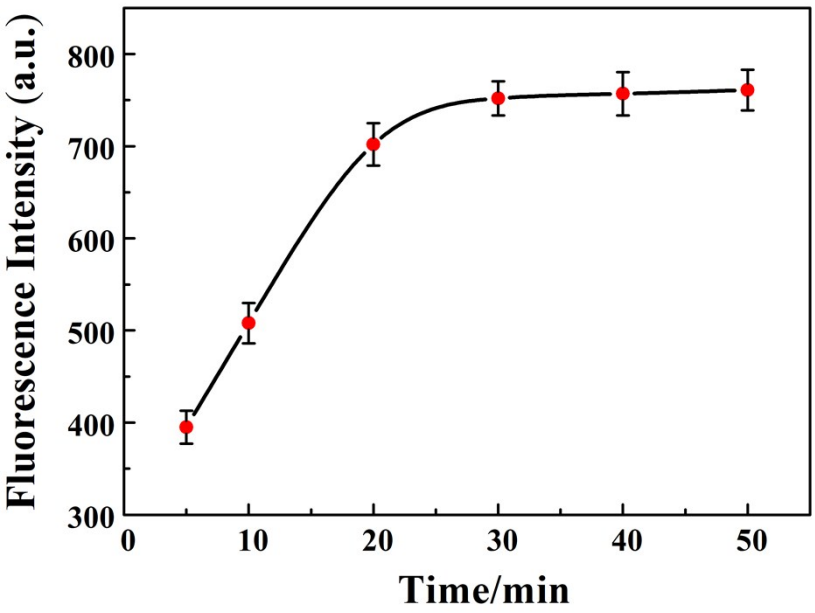


**Fig. S2** Effect of the enzymatic reaction time of HAase on the fluorescence emission of HA-PDI based assay system at 550 nm; [PDI] = 0.2 μM, [HA] = 20 μg/mL, [HAase] = 10 U/mL. The error bars were estimated from three replicate measurements.

**Table S1.** Comparison of various fluorescence methods for the detection of HAase.

| Probe | Linear range | Detection limit | Ref. |
|---|---|---|---|
| FITC-HA- AuNPs | 1.25−50 U/mL | 0.63 U/mL | 2 |
| $MoS_2$ QDs/HA- AuNPs | 1-50 U/mL | 0.7 U/mL | 3 |
| Cationic conjugated polymer PFEP/HA-Dox complex | 0-1.30 U/mL | 0.075 U/mL | 4 |
| Polyethylenimine-modified carbon dots (P-CDs)/HA-Doxs | 0−400 U/mL | 0.65 U/mL | 5 |
| Positively-charged pyrene analog (N-Py)/HA | —— | 0.007 U/mL | 6 |
| Carbon dots (CDs)/HA-AuNPs | 0.1−80 U/mL | 0.06 U/mL | 7 |
| Carbon dots (CDs)/HA | 0.2-10000 U/mL | 0.1 U/mL | 8 |
| Graphitic carbon nitride (g-CN) nanosheets/HA-AuNPs | 0-0.015 U/mL | 0.6 mU/mL | 9 |
| HA-PDI | 0.1-10 U/mL | 0.03 U/mL | this work |

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