## 1 Porous Organic Polymer Nanosphere-Based Fluorescent Biosensing Platform for 2 Simultaneous Detection of Multiplexed DNA via Electrostatic Attraction and $\pi$ - $\pi$ Stacking Interactions 4 Yujie Sun<sup>#</sup>, Zhenzhong Lu<sup>#</sup>, Wenlin Ma, Rui Wang, Chengwu Zhang, Jinhua Liu<sup>\*</sup> 5,



24 Fig. S1. (a) The picture of POP powder; (b) TGA of POP.





Fig. S2. (a) Fluorescence emission spectra of P1, P1+POP and P1+ (POP/Mg<sup>2+</sup>); (b) 

The zeta potentials of POP+H<sub>2</sub>O and POP/Mg<sup>2+</sup>+H<sub>2</sub>O; (C) Normalization intensity of 

P1, P1+POP, P1+(POP/Metal ion) for Mg<sup>2+</sup>, Cd<sup>2+</sup>, Fe<sup>2+</sup> and Fe<sup>3+</sup>, respectively. 



61 Fig. S3. (a) Fluorescence emission spectra and quenching efficiency (b) of P1 (25 nM)

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<sup>62</sup> with different concentrations of POP (0-50 mg/mL).



75 Fig. S4. Fluorescence emission spectra of P1 (a), P2 (b), and corresponding quenching

- <sup>76</sup> efficiency (c) with different concentrations of POP (0-60 mg/mL).



93 Fig. S5. Fluorescence intensity of P1 (a), P2 (b) and P3 (c) change with time in the

94 presence of POP and Target DNA successively.



113 Fig. S6. Fluorescence intensity of P1 change with time upon the introduction of 114 different quenchers (GO and POP,  $30 \mu g/mL$ ) and T1 (200 nM).



Fig. S7. (a) Fluorescence spectra of probes (P1, P2, and P3) under different conditions
(P, P+POP, P+POP+T), respectively. (b) Quenching efficiency of P (P1, P2 and P3)
caused by POP with different concentrations (0, 10, 20, 25, 30, 50, 70 and 90 μg/mL).



146 Fig. S8. Fluorescence emission spectra of P1 (a), P2 (b) and P3(c) change with different

- 147 concentrations of POP (0-90 mg/mL).



Fig. S9. Fluorescence spectra of probes (P1, P2 and P3) in one system toward T1 (a),
T2 (b) and T3 (c); (d) Specificity of probes (P1, P2, P3) for targets (T1, T2, T3). B (13), blank; R (1-3), random sequence for P (1-3); M (1-3), base mismatched sequence
for P (1-3), respectively.

	Oligonucleotide	Sequence (5-3)*				
	P1	CAGTTACATTCTCCCAGTTGATT-FAM				
	P2	ACCTGGGGGAGTATTGCGGAGGAAGGT-Cy3				
	P3	AGACTCTTGAGTTCTCAGTATG-Texas Red				
	T1	AATCAACTGGGAGAATGTAACTG				
	T2	ACCTTCCTCCGCAATACTCCCCCAGGT				
	Т3	CATACTGAGAACTCAAGAGTCT				
	M1	AATCAACTG <u>T</u> GAGAATGTAACTG				
	M2	ACCTTCCTCCGCATTACTCCCCCAGGT				
	M3	CATA <u>T</u> TGAGAACTCAAGAGTCT				
100	R	ACCTGGGGGAGTATTGCGGAGGAAGGT				
180	* The mutation base	is indicated by underline				
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**Table S1.** DNA sequences used in this work.

	Materials	Sensitivity	Detection time	Fluorescent reporter	Multiplexed detection	Reference
	SWNT	4 nM	several hours	FAM	Not reported	[39]
	AuNP	nM	minutes	Rhodamine 6G	Yes	[40]
	Graphene oxide (GO)	100 pM	$\approx 1 \min$	FAM, Cy3, ROX	Yes	[41]
	GO (premixing)	$\approx \! 10 \ nM$	30 min	FAM	Not reported	[42]
	GO	2 nM	30 min	FAM	Not reported	[43]
	GO	0.5 nM	60-90 min	Silver	Yes	[44]
	GO	75 nM	30 min	Graphene quantum dots	Not reported	[45]
	g-C <sub>3</sub> N <sub>4</sub>	81 pM	Not reported	FAM, ROX	No	[46]
	MOFs(UiO-66-NH <sub>2</sub> )	10 nM	20 min	FAM	Not reported	[47]
	MOFs(Cu(H <sub>2</sub> dtoa))	3 nM	4 h	FAM	Not reported	[48]
194	РОР	50 pM	25 min	FAM, Cy3, Texas Red	Yes	This work
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## 193 Table S2. Comparison of different fluorescent DNA sensors.

System	Target DNA	QE (%)	Recovery (%)
Single chain	T1	94.43	38.37
	T2	83.12	55.90
	T3	85.80	53.04
		87.61	28.70
	T1&T2	68.18	83.09
Double chains		70.85	78.29
	T2&T3	48.92	70.34
		93.25	24.55
	T1&T3	86.92	29.68
		92.75	31.18
Three chains	T1&T2&T3	87.62	28.64
		89.63	25.05

209 Table S3. The quenching efficiency and recovery of different detected system.