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Electronic Supporting Information

Fluorogenic Response from DNA Templated Micrometer Range Self-Assembled Gold Nanorod

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1. Experimental details:

General Experimental Procedures. All the amine-modified and fluorophore tagged DNA samples and the synthetic DNA version of miR-21 were purchased from GeneX India Bioscience Pvt. Ltd. Hydrogen tetrachloroaurate trihydrate ($HAuCl_4.3H_2O$, ≥99.9%), CTAB (99%), silver nitrate ($AgNO_3$, ≥99.0%), L-ascorbic acid (≥99%), sodium borohydride ($NaBH_4$, 99%), were purchased from Himedia and were used without further purification. DI water was used in all experiments.

Extinction spectra were measured using an Agilent Cary 3500 UV−vis spectrophotometer. Emission measurements were performed using an Agilent Cary Eclipse fluorescence spectrophotometer with Xe flashlamp. Excitation and emission slit width was kept at 5 nm. All the measurements were performed at 495 nm excitation wavelength and emission spectra was recorded at 505 nm to 700 nm wavelength range. Transmission electron microscopy (TEM) images were collected on a FEI, Technai G2 20 S-TWIN transmission electron microscope, with an acceleration voltage of 200 kV. TEM samples were prepared by adding a single drop of nanorod assembly solution onto a carbon-coated copper grid placed on a filter paper. The grid was left overnight to dry at ambient temperature before the TEM analysis. ImageJ software was used to calculate the dimensions of the nanorods from TEM images. ζ-potential measurements were performed using a Malvern Nano-ZetaSizer. The values were reported by taking average of three consecutive measurements for each sample.

AuNR synthesis following the seed mediated method. The synthesis of AuNR were performed using a previously reported seed-mediated growth method comprising of two steps, i.e., (i) preparation of seed solution and (ii) the growth phase.

Preparation of seeds for gold nanorod. The seed solution was prepared by adding 1 mg of hydrogen tetrachloroaurate trihydrate ($HAuCl_4.3H_2O$, 0.25 mM) to a 10 ml aqueous solution 0.1 M CTAB (0.364 g). Then 600 µL of an aqueous solution of $NaBH_4$ was added in ice-cold condition with vigorous stirring for 10 min. A brownish yellow solution was obtained. This resultant seed solution was stored at 25 °C and left for 2 h to decompose of the excess $NaBH_4$.

Growth of AuNR. 3.46 g of CTAB was added to 95 ml water followed by the addition of an aqueous solution of 1 ml of $AgNO_3$ (10 mM) and an aqueous solution of 5 ml of $HAuCl_4$ (10 mM) with gentle mixing. Thereafter, an aqueous solution of a mild reducing agent ascorbic acid (0.55 ml, 0.1 M) was added to the growth solution resulting the solution to become colorless. In the final step, 0.12 mL of the seed was added to the growth solution at 25 °C. The color of the solution gradually changed within 10−20 min, confirming the formation of AuNRs. The entire solution was left undisturbed overnight and the colorless solution was transformed into deep brown. The solution was purified by two times centrifugation at 8000 rpm for 20 min to remove excess CTAB and other reagents present in the original solution. The supernatant was decanted and the pellet obtained was redispersed in fresh deionized water. The as prepared nanorods exhibit the LSPR peak at 700 nm and TSPR peak at 515 nm. As calculated from the TEM images, the length of the nanorod is 50 ± 3.13 nm and the width is 15 ± 1.08 nm (A.R 3.4).

Synthesis of AuNR': The synthesis was performed using a previously reported method[S1]. Briefly, growth solution was prepared at 25-30 ºC by adding 5 mL of 1 mM $HAuCl_4$ to 10 mL 0.2 M CTAB solution. Following this, 250 µL 4 mM $AgNO_3$ was added and the solution was gently shaken. 8 µL of 37% HCl was introduced to obtain a pH around 1-1.15. Then 70 µL of ascorbic acid (78.8 mM) was added and the solution was shaken gently until it was clear. Immediately afterward, ice-cold $NaBH_4$ (15 µL, 0.01 M) was injected to the unstirred growth solution and

allowed to react for 6 h. Nanorods were purified by centrifugation at 14000g for 1h. The as prepared nanorods exhibit the LSPR peak at 640 nm and TSPR peak at 515 nm. As calculated from the TEM images, the length of the nanorod is 24 ± 3.33 nm and the width is 8 ± 2.01 nm (A.R 3.0).

Self-Assembly of AuNRs. The OD value of the LSPR peak of the as synthesized AuNR solution was maintained at ~0.9 for all the experiments. As observed from the TEM images (Fig. S1a) the AuNRs have a length of (50 ± 3.13 nm) and a width of (15 ± 1.08 nm) and were well dispersed. To obtain side-by-side assembly, two microcentrifuge tubes (MCT) of 0.5 mL were labeled as A and B. In A, 150 μL of AuNR solution was incubated with 0.5 μM of **PMR** and in B the same was incubated with 0.5 μM of PML for 30 min at ambient temperature. These two solutions were labeled as **PMR** seed and **PML** seed. Now in another MCT 100 μL of each seed was mixed together to obtain a final 0.25 μM concentration of each probe DNA. Required concentration of targeted DNA (**tm-DNA**) was introduced to the mixture and incubated for another 75 min for the side-by-side assembly formation. In order to obtain end-to-end assembly, similar procedure was followed but with different DNA combination i.e., **PMR'** and **PML**. **PMR'** and **PML** were injected into two separate MCTs and incubated for 30 min. Then the two of them were mixed together in another MCT and target DNA was introduced for the end-to-end assembly formation.

Control experiments. To establish the selectivity of the DNA combination in forming the particular end-to-end and side-by-side assembly upon introduction of **tm-DNA**, we have performed control experiments using single DNA with **tm-DNA**. i.e., PMR and **tm-DNA**, **PML** and **tm-DNA** and **PMR'** and **tm-DNA**.

UV-vis absorbance study. Similar self-assembly formation procedure was followed for the UV-vis absorbance studies. Absorbance was measured for all the four cases of self-assembled nanorod solutions as well as for single probe DNA and mixture of probe DNA combinations as control. As prepared nanorod solution (8 nM) was diluted to 5, 3 and 1 nM and the self-assembly formation was performed as discussed previously keeping the stoichiometric ratio of AuNR to probe DNA at 1:63 and the absorbance spectra were recorded from 200-900 nm for all the cases with AuNR. In case of fluorophore tagged DNAs absorbance spectra were recorded from 350-750 nm in absence of AuNR.

Fluorescence enhancement experiments. Similar self-assembly formation procedure was followed for the fluorescence enhancement studies. For this purpose, exactly same DNA sequences with fluorophore tagging at the opposite of the amine-modified end was used (table-S4). For the fluorophore tagging of DNA, 6-carboxyfluorocein (6-FAM), tagged to **PMR** and **PMR'**, was used as a donor whereas rhodamine-6G, attached to **PML**, acted as an acceptor in the donor to acceptor energy transfer. After 75 min of incubation for the particular assembly formation, the emission was recorded. Extent of emission enhancement was calculated from 0.0625 μM to 1.25 μM concentration of tm-DNA at 0.25 μM of probe DNA in case of end-to-end assembly and 0.375 to 0.5 μM concentration of probe DNA at 0.5 μM **tm-DNA** concentration in case of side-by-side assembly.

Control experiments. To establish the role of plasmonic hotspot in the fluorescence enhancement over FRET, we have measured the fluorescence from the solutions obtained in case 1 and case 2. In the specific fluorophore tagged DNA combinations used for both the cases, we exchanged only the **PMR-F** and **PMR'-F** with **PMR** and **PMR'** respectively. Fluorescence spectra were recorded for rhodamine at λ_{ex}=495 nm.

Concentration calculation of naorod: The volume of the nanorod was calculated as per the previously reported equation given below:[S2]

$$\boldsymbol{V = \frac{4}{3}\pi\left(\frac{w}{2}\right)^3 + \left(\frac{w}{2}\right)^2 \pi(l - w)}$$

Where, '*l*' is the length and '*w*' is the width of nanorod.

The volume of the experimentally synthesized nanorod calculated using the formula was found to be 7952.1565 nm^3.

Assuming each gold atom constituting the nanorod to be spherical, volume of gold atom is:

$$\boldsymbol{V' = \frac{4}{3}\pi\,(0.144)^3}$$

$$\boldsymbol{= 0.0125\ nm^3}$$

Atomic radius of gold atom is 1.44 Å.

Concentration of nanorod was further calculated using a method reported previously.[S3]

Calculated concentration of nanorod is 4.734×10^{12} particles / mL or 7.86 nM or ~8 nM.

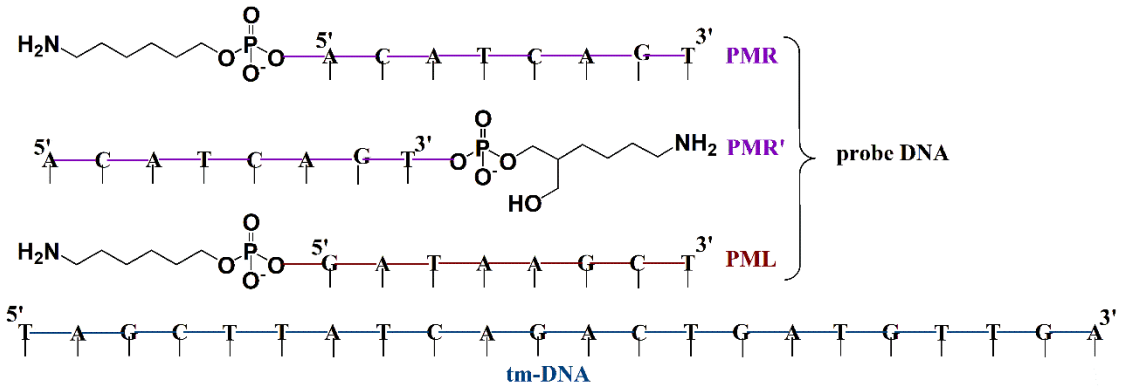


Figure S1: Pictorial representation of amine modified probe DNAs and unmodified template DNA.

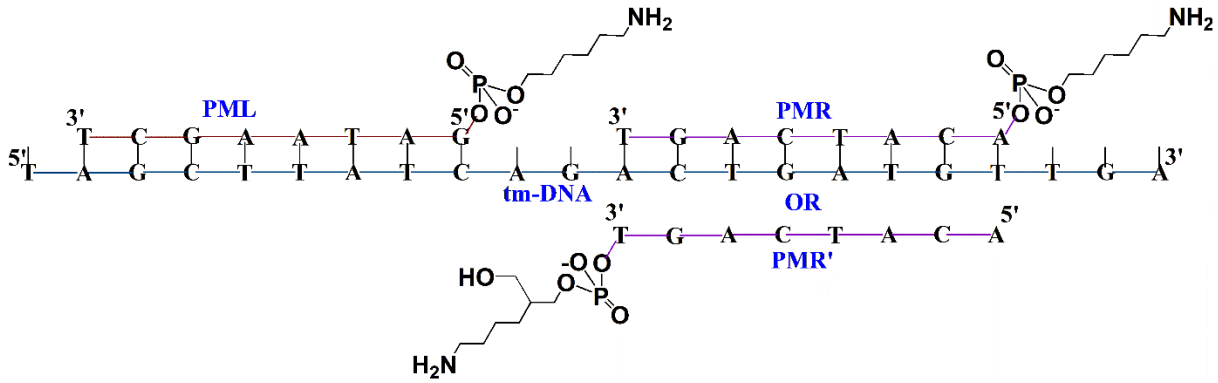


Figure S2: Pictorial representation of the sequence specific hybridisation between amine modified probe DNAs and unmodified template DNA.

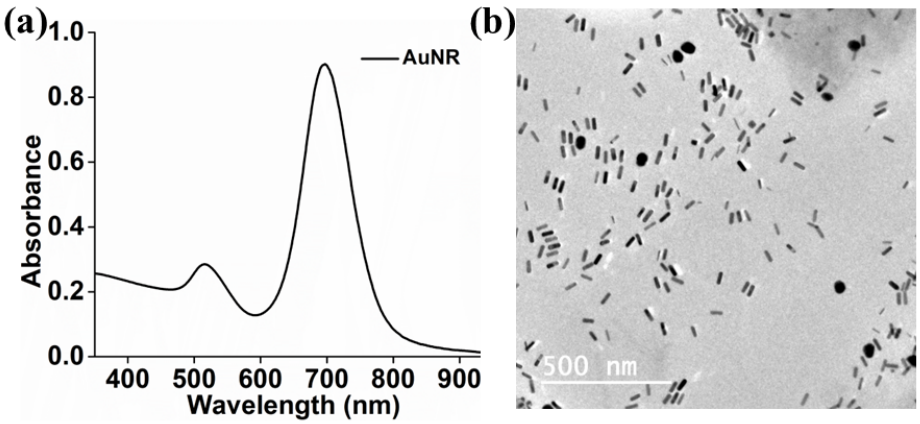


Figure S3: (a) UV-vis absorbance and (b) TEM image of gold Nanorod. (Scale bar 500 nm).

Table S1: Zeta potential of the experimental solution resulting from different combination of probe DNA with target DNA used in the nanorod assembly formation.

Experimental solution	Probe DNA combination and concentration (μM)	Presence of target DNA	Observed nanorod assembly	Zeta potential (mV)
NR	–	–	–	37.8
Probe DNA + AuNR	**PMR**/**PMR'**/**PML** (0.25)	No	No assembly	22.6
Probe DNA + AuNR	**PMR**/**PMR'**/**PML** (0.25)	Yes	No assembly	22.4
Mixture of Probe DNA	(**PMR** + **PML**)/ (**PMR'** + **PML**) (0.25 each)	No	No assembly	21.8
Case-1	**PMR'** + **PML** (0.25 each)	Yes	1D chain ETE	21.4
Case-2	**PMR** + **PML** (0.25 each)	Yes	No assembly	22.6
Case-3	**PMR'** + **PML** (0.45 each)	Yes	Thick chain ETE	20.7
Case-4	**PMR** + **PML** (0.45 each)	Yes	SBS	20.1

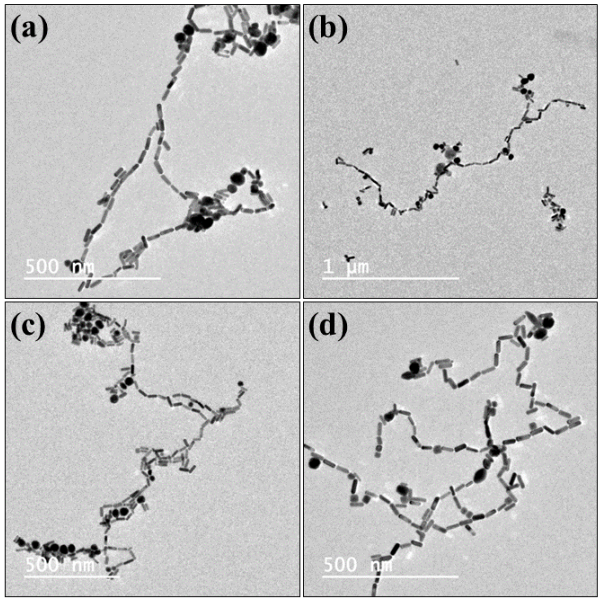


Figure S4: TEM images 1D ETE self-assembly of AuNR. (Scale bar: 500 nm for a, c, d and 1μm for b).

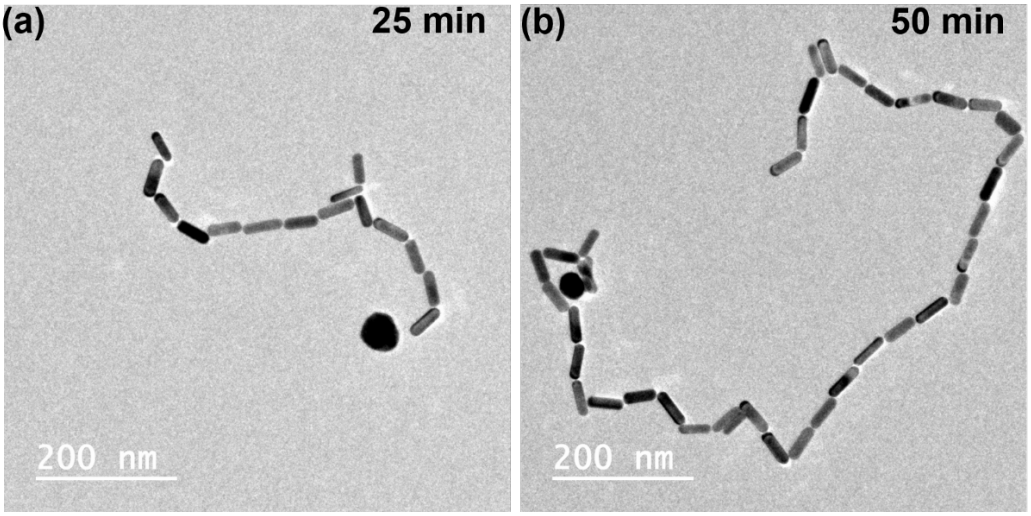


Figure S5: Time dependent TEM images of 1D ETE self-assembly of AuNR at (a) 25 min and (b) 50 min. (Scale bar: 200 nm).

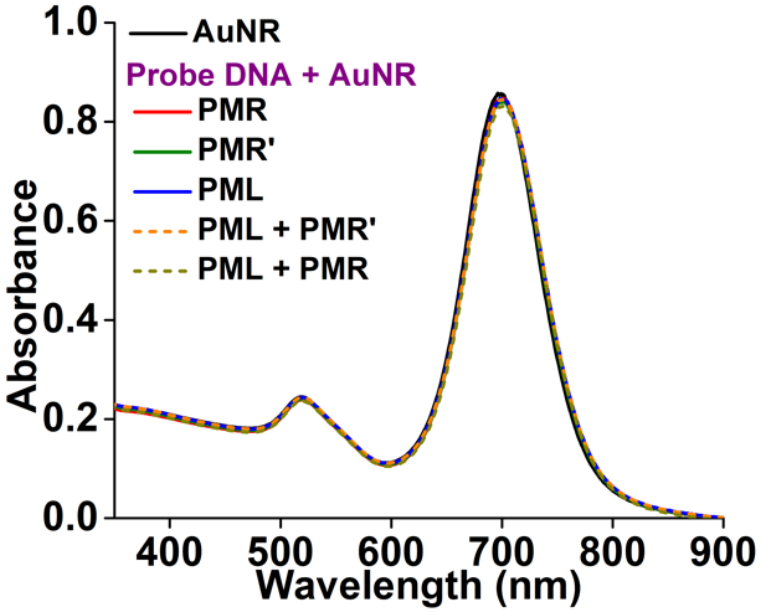


Figure S6: Absorbance spectra of control experiments for AuNR with probe DNAs only.

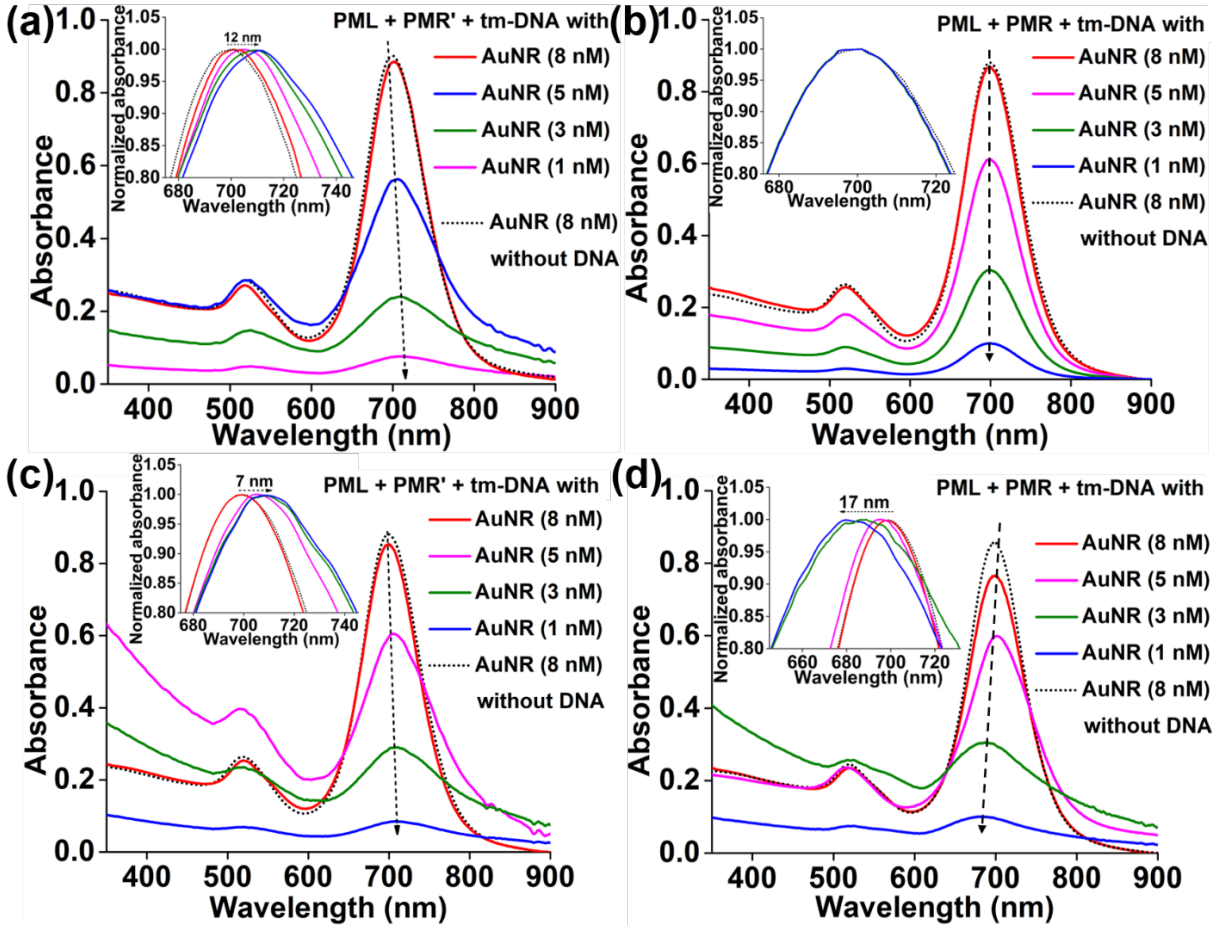


Figure S7: Absorbance of a) case-1, b) case-2, (c) case-3 and (d) case-4 at various concentration of AuNR (8 nM to 1 nM), inset: normalized absorbance and peak shift with varying concentration of AuNR.

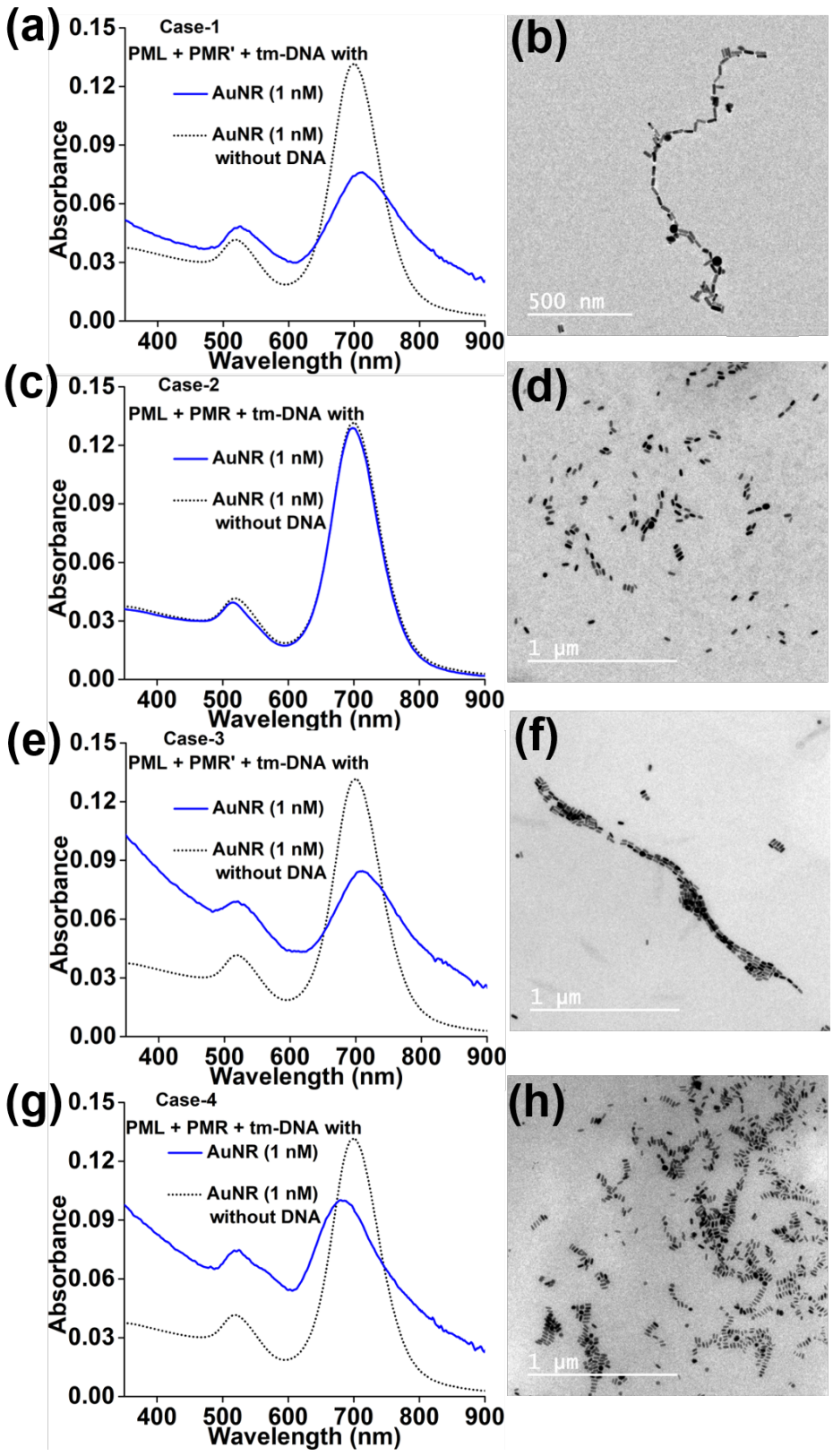


Figure S8: Absorbance (left column) and TEM images (right column) of case-1, case-2, case-3 and case-4 at 1 nM concentration of AuNR.

Table S2: Variation in the concentration of tm-DNA at constant probe DNA concentration in the 1D ETE assembly.

Set No.	Concentration (μM) of probe DNA and tm-DNA		
	PMR'	**PML**	**tm-DNA**
Set-1	0.25	0.25	0.0625
Set-2	0.25	0.25	0.125
Set-3	0.25	0.25	0.25
Set-4	0.25	0.25	0.5
Set-5	0.25	0.25	1.25

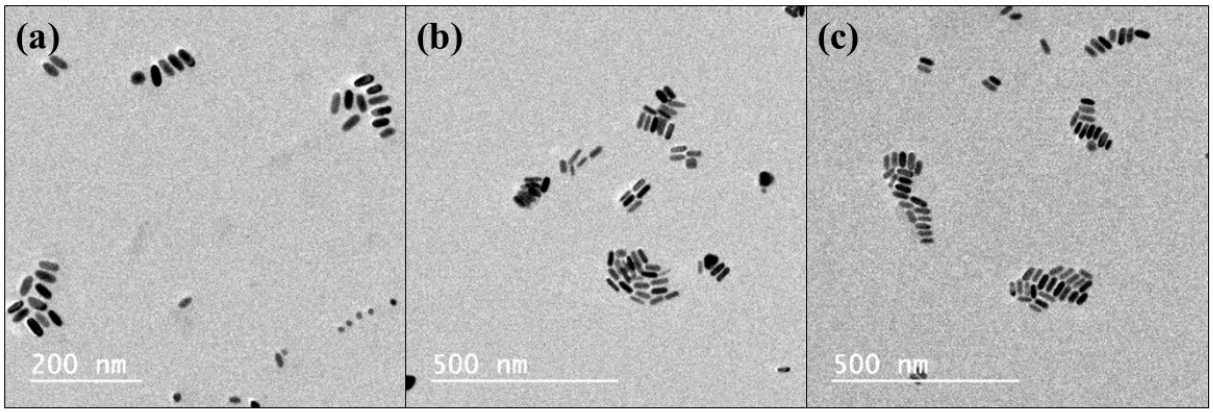


Figure S9. TEM images of control experiments with (a) **PMR** + **tm-DNA** (b) **PML** + **tm-DNA** and (c) **PMR' + tm-DNA** combination. Scale bar: (a) 200 nm; (b), (c) 500 nm.

Table S3: Variation in the concentration of probe DNA at constant target DNA concentration.

Concentration (μM) of probe DNA in the AuNR assembly		**Concentration (μM) of target DNA**
PMR/PMR'	**PML**	**tm-DMA**
0.25	0.25	0.5
0.375	0.375	0.5
0.5	0.5	0.5

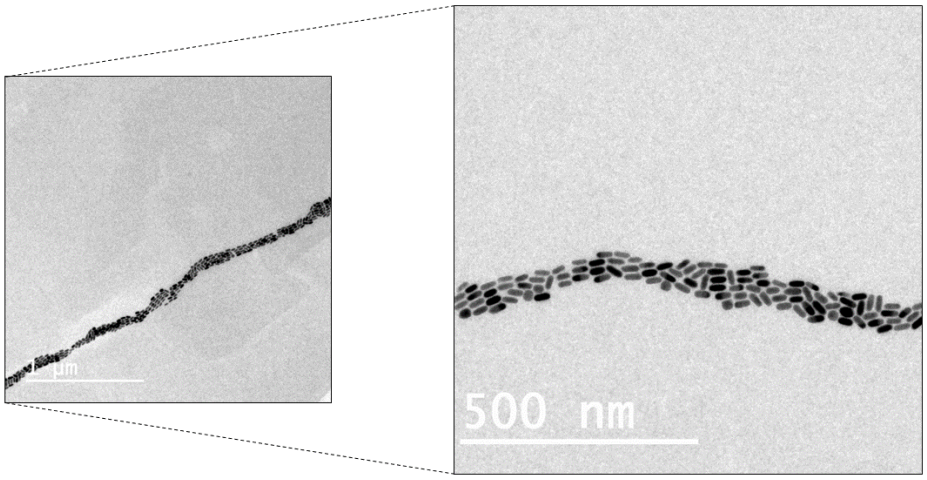
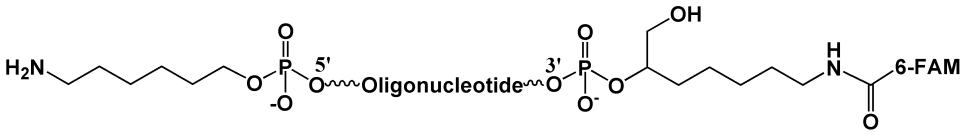
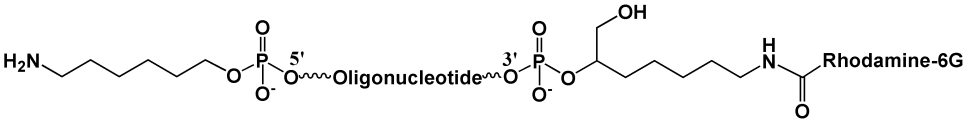
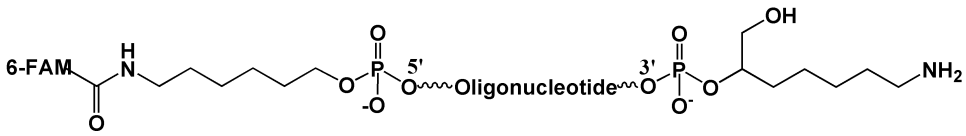


Figure S10. Magnified TEM image of paralleled ETE self-assembly of AuNR (Case-3, scheme-2).

Table S4: Fluorophore modification and sequence of the DNA used in emission experiment.

Probe DNA name	Sequence
PMR-F	NH_2-$(CH_2)_6$-5'-ACATCAGT-3'-CH(CH_2OH)$(CH_2)_5$-6-FAM 
PML-R	NH_2-$(CH_2)_6$-5'-GATAAGCT-3'-CH(CH_2OH)$(CH_2)_5$-Rhodamine-6G 
PMR'-F	6-FAM-$(CH_2)_6$-5'-ACATCAGT-3'-CH(CH_2OH)$(CH_2)_5$-NH_2 

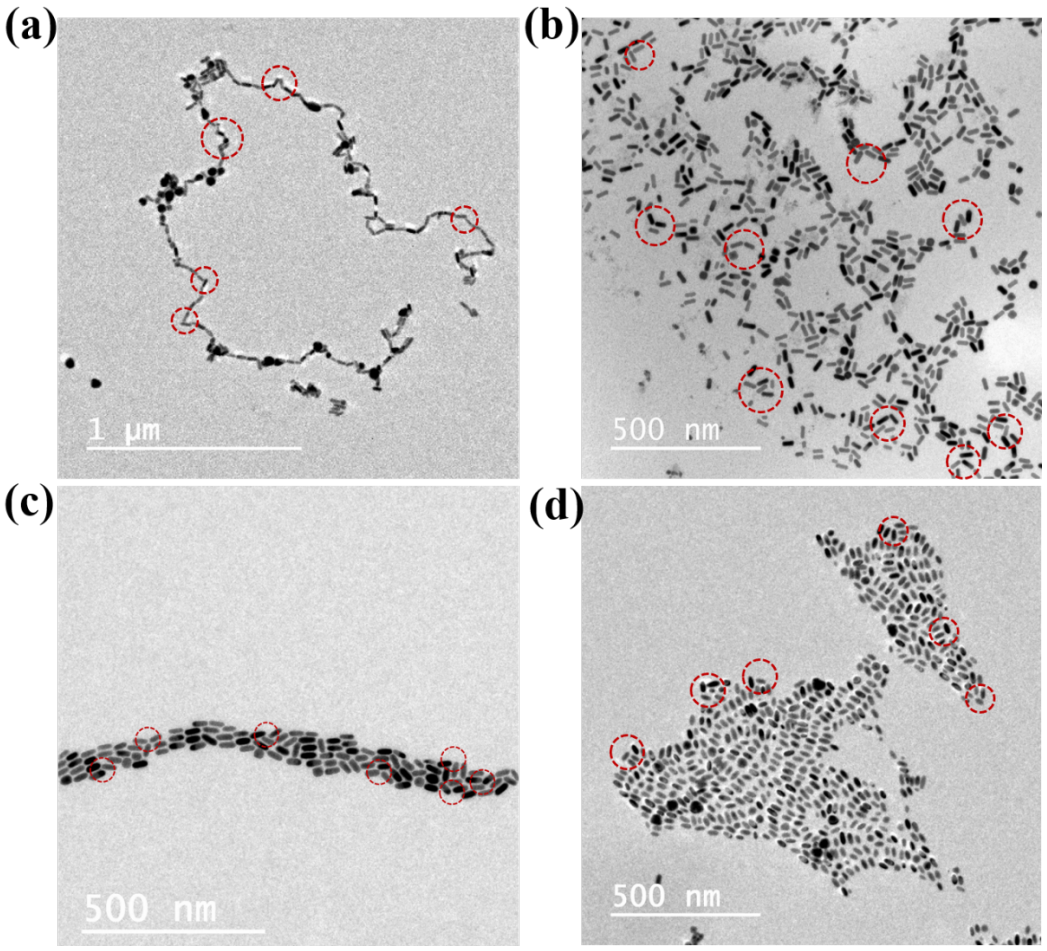


Figure S11. TEM images of AuNR assembly in (a) case-1, (b) case-2, (c) case-3 and (d) case-4 showing presence of hinged orientation (red dotted circle) in all the cases due to flexible nature of DNA.

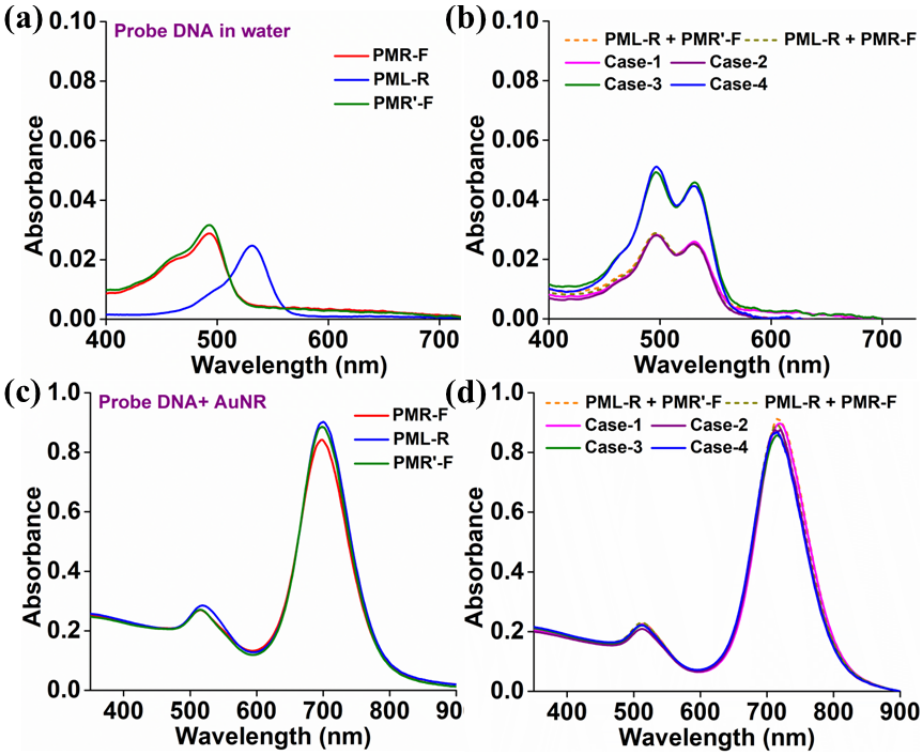


Figure S12: Absorbance of a) single fluorophore labeled DNA and b) their combination in absence of AuNR; c) single fluorophore labeled DNA and d) their combination in presence of AuNR. Dashed and solid lines represent DNA combination in absence and presence of **tm-DNA** respectively.

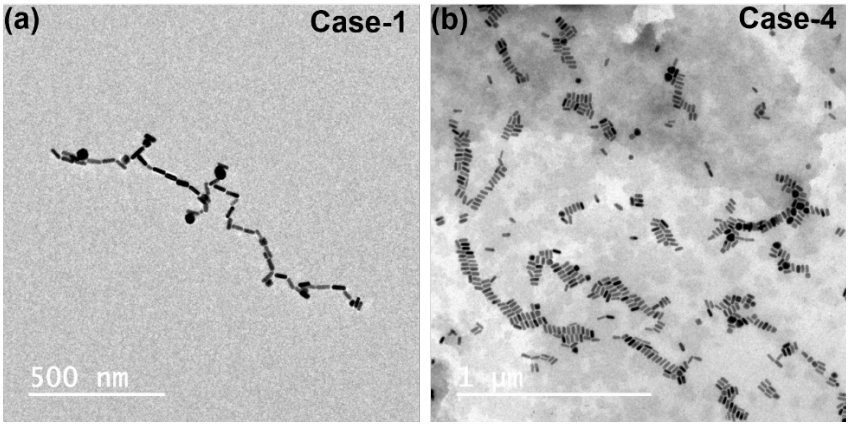


Figure S13: TEM images of a) ETE (case-1) and b) SBS (case-4) self-assembly of AuNR in presence of fluorophore tagged probe DNA.

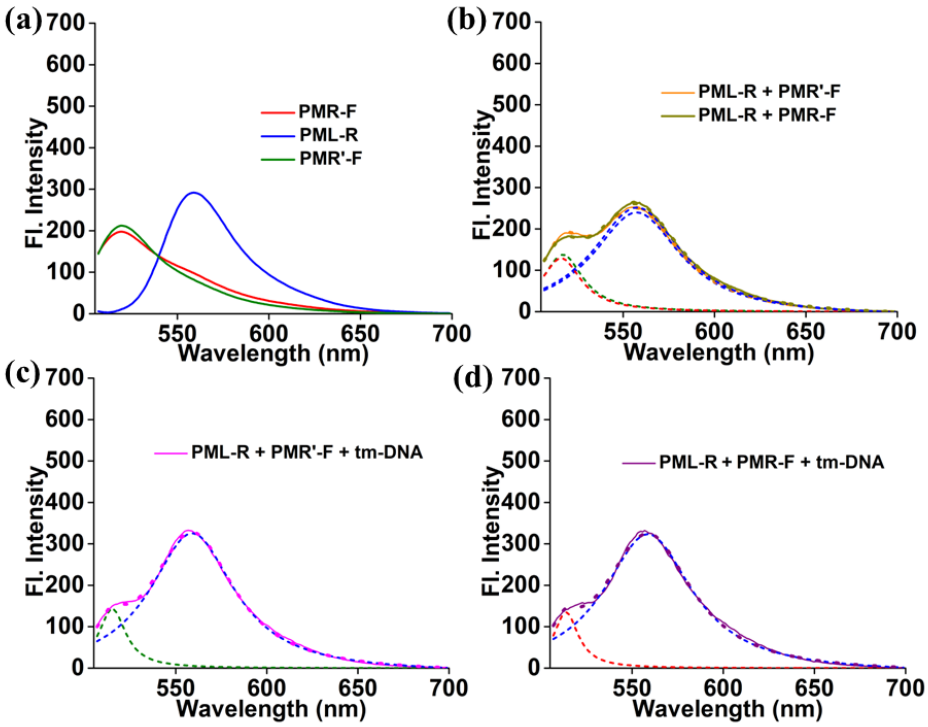


Figure S14: Fluorescence from a) single fluorophore labeled DNA and b) their combination in absence of **tm-DNA**; (c), (d) in presence of **tm-DNA** in water. dashed lines represent the fit peaks (R^2>0.99 for all the fittings); color codes for **PMR-F**, **PMR'-F** and **PML-R** are red, green and blue respectively. The emission spectra are in water. The concentration of probe DNA: 0.25 μM and tm-DNA: 0.5 μM respectively, λ_{ex}=495 nm.

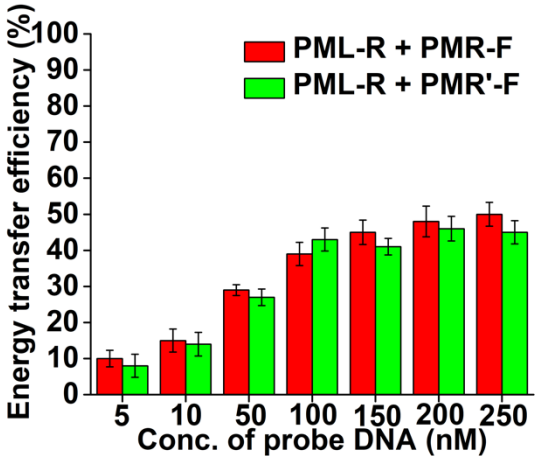


Figure S15: FRET efficiency variation with dilution of probe DNAs in absence of tm-DNA.

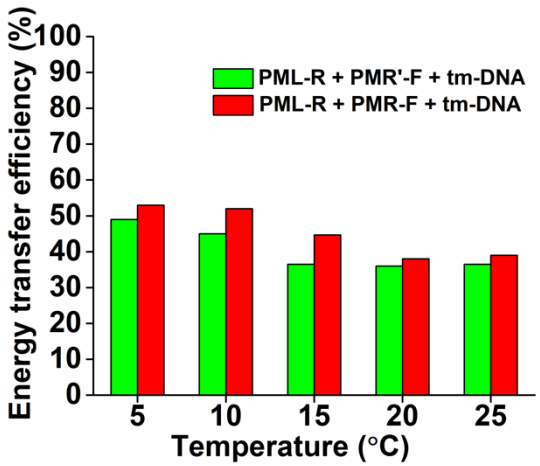


Figure S16: FRET efficiency of free strand (probe DNA + tm-DNA in water) in absence of AuNR and variation with temperature.

Table S5: Emission intensity and FRET efficiency comparison chart of fluorophores under different conditions.

Conditions	Probe DNA combination	Emission intensity [a]		Em. enhancement from R (in fold) [c,d]	FRET efficiency (%) [f]	Emission intensity [b]		Em. enhancement from R (in fold) [c,d]	FRET efficiency (%) [f]
		Fluorescein (F)	Rhodamine (R)			Fluorescein (F)	Rhodamine (R)		
– tm-DNA – AuNR[c]	PMR-F	196	–	–	–	400	–	–	–
	PML-R	–	296	–	–	–	597	–	–
	PMR'-F	208	–	–	–	410	–	–	–
– tm-DNA – AuNR[c]	PMR-F + PML-R	128	238	0.8	35	–	–	–	–
	PMR'-F + PML-R	138	253	0.85	34	–	–	–	–
+ tm-DNA – AuNR[c]	PMR-F + PML-R	137	320	1.08	37	–	–	–	–
	PMR'-F + PML-R	150	322	1.08	39	–	–	–	–
+ AuNR[d]	PMR-F	120	–	–	–	238		–	–
	PML-R	–	8	–	–		15.2	–	–
	PMR'-F	90	–	–	–	175		–	–
+ AuNR – tm-DNA [d]	PMR'-F + PML-R	65	85	11	42	–	–	–	–
	PMR-F + PML-R	47	70	10	50	–	–	–	–
+ AuNR + tm-DNA [d,e]	PMR'-F + PML-R	59	672	84	76	162	621	43	88
	PMR-F + PML-R	63	136	17	69	216	567	39	77

[a] Conc. Of fluorophore tagged probe DNA: 0.25 µM.
[b] Conc. of fluorophore tagged probe DNA: 0.50 µM.
[c] Emission intensities of rhodamine has been compared among the free, unhybridized (– tm-DNA) and hybridized (+ tm-DNA) condition in absence of AuNR (– AuNR).
[d] Emission intensity of rhodamine has been compared among the free, unhybridized (– tm-DNA) and hybridized (+ tm-DNA) condition in presence of AuNR (+ AuNR).
[e] This condition refers to the experimental four cases of AuNR assembly (Case 1-4, refer to table 2 in the main text).
– Data is not applicable for the experimental conditions.

[f] FRET efficiency has been calculated using relative quantum yield of the FRET donor.

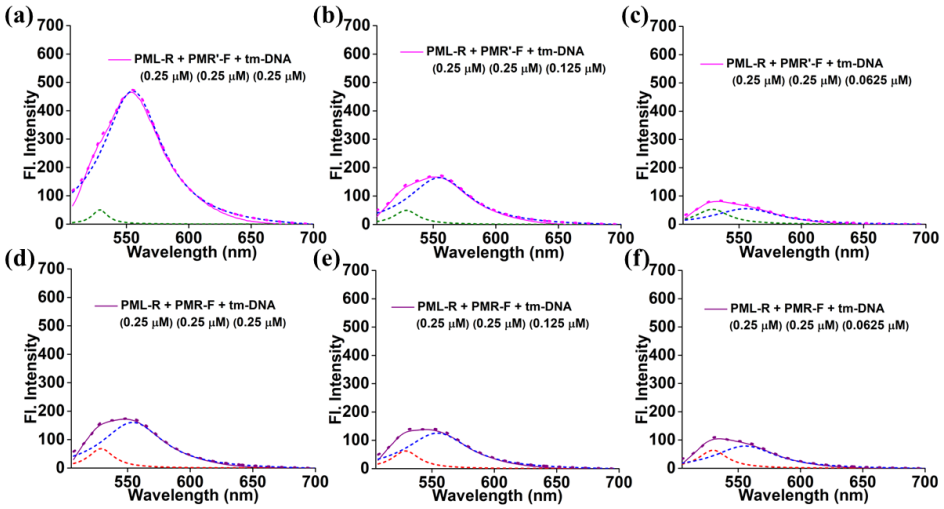


Figure S17. Variation in the fluorescence enhancement of rhodamine at variable **tm-DNA** concentration (a)-(c) in **PML-R** + **PMR'-F** and (d)-(f) in **PML-R** + **PMR-F** combination.

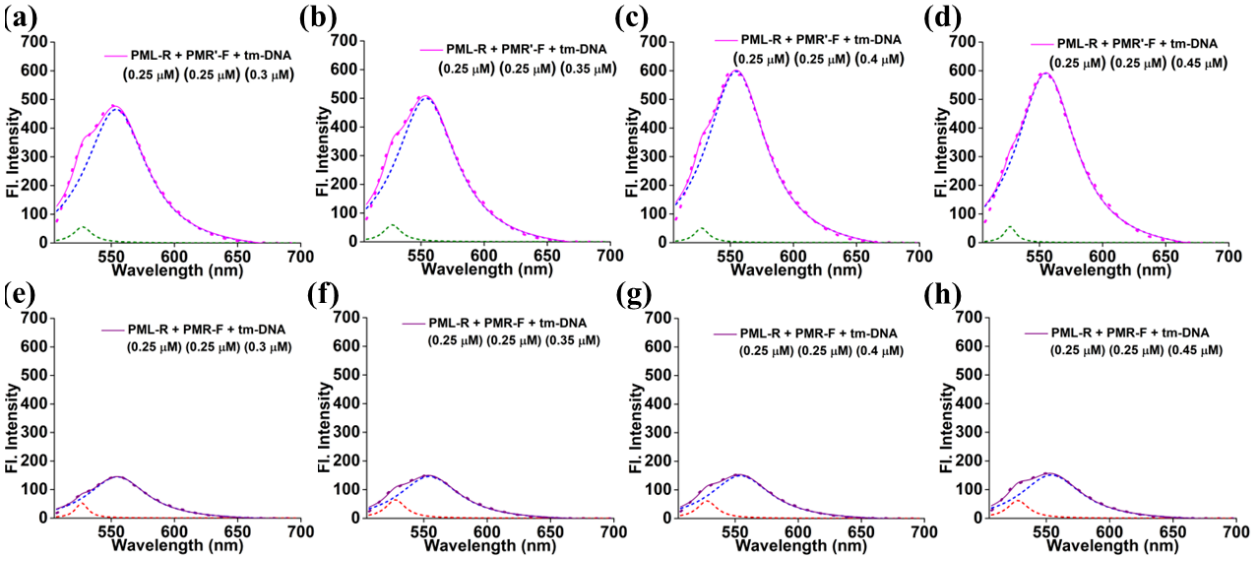


Figure S18. Variation in the fluorescence enhancement of rhodamine at variable **tm-DNA** concentration from 0.3 μM to 0.45 μM in (a)-(d) **PML-R** + **PMR'-F** and in (e)-(h) **PML-R** + **PMR-F** combination.

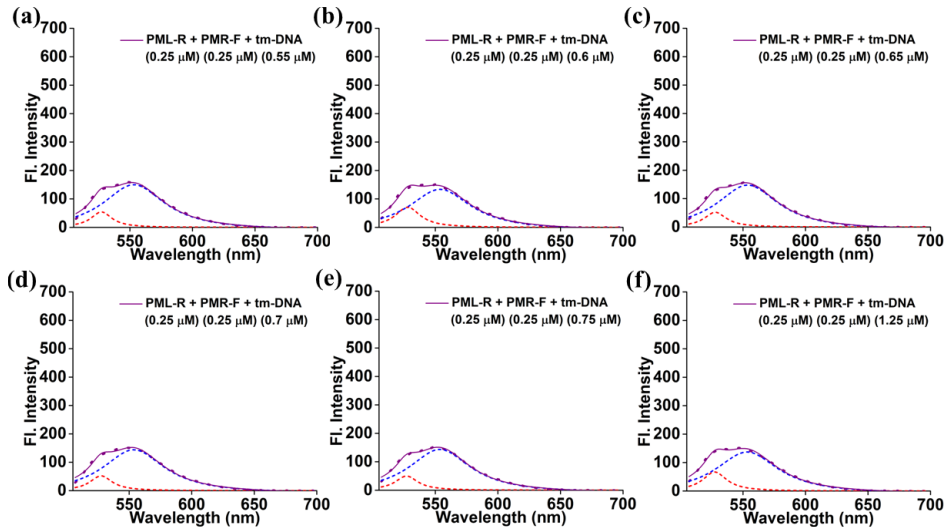


Figure S19. Variation in the fluorescence enhancement of rhodamine at variable **tm-DNA** concentration from (a) 0.55, (b) 0.6, (c) 0.65, (d) 0.7, (e) 0.75 and (f) 1.25 μM in **PML-R** + **PMR-F** combination.

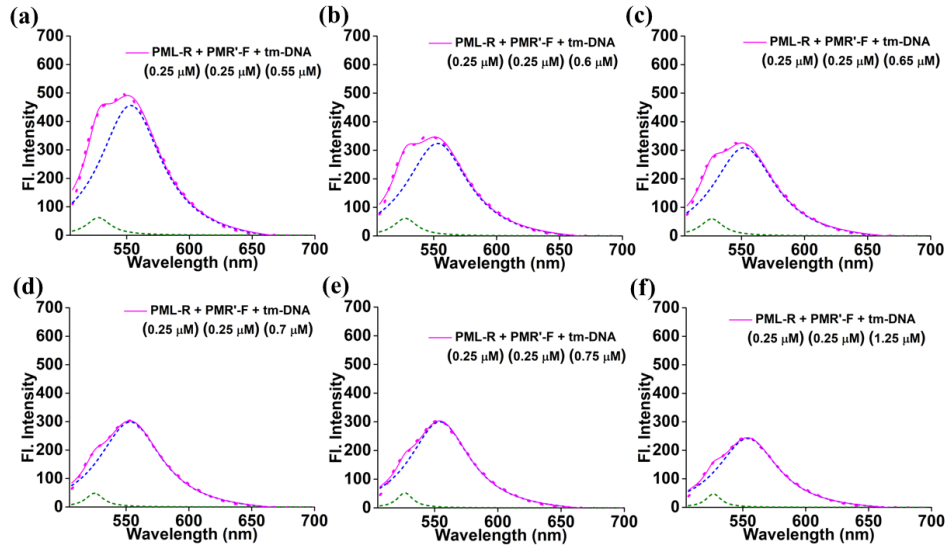


Figure S20. Variation in the fluorescence enhancement of rhodamine at variable **tm-DNA** concentration from (a) 0.55, (b) 0.6, (c) 0.65, (d) 0.7, (e) 0.75 and (f) 1.25 μM in **PML-R** + **PMR'-F** combination.

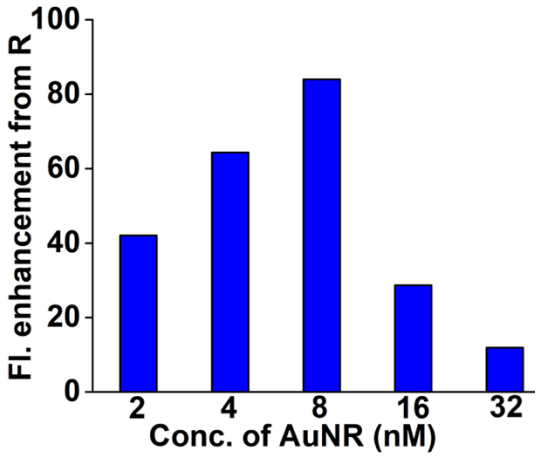


Figure S21. Fluorescence enhancement of rhodamine with varying AuNR concentration.

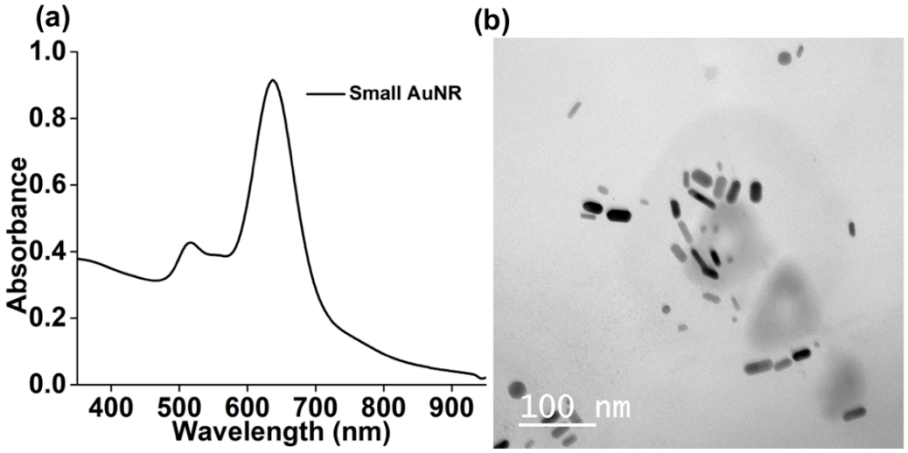


Figure S22. (a) UV-vis absorbance and (b) TEM image of AuNR'. Length and width of nanorod is 24 ± 3.33 and 8.0 ± 2.01 nm respectively. (Scale bar 100 nm).

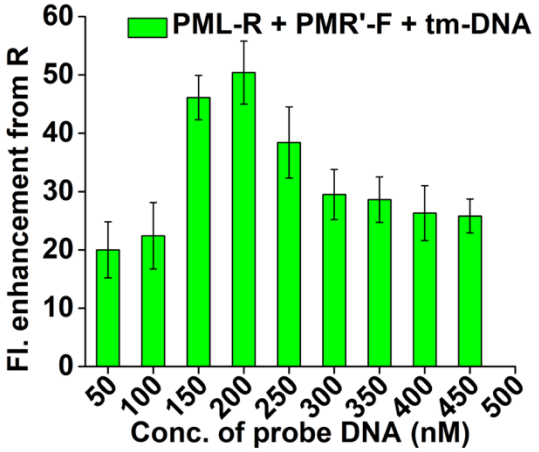


Figure S23. Fluorescence enhancement of rhodamine with varying probe DNA concentration in case of small AuNR'.

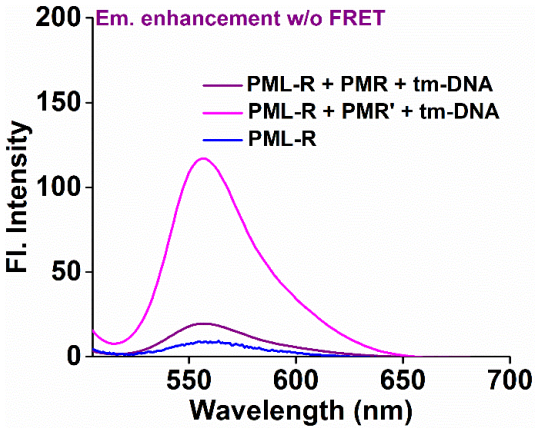


Figure S24. Fluorescence enhancement of rhodamine from case 1 and case 2 in absence of FRET from fluorescein.

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