Supplementary Information

Metronidazole Non-Enzymatic Colorimetric Sensors Strip Based on Melamine-Functionalized Gold Nanoparticles Assembled Polyamide Nanofibers Membranes

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1. Supplementary Figures

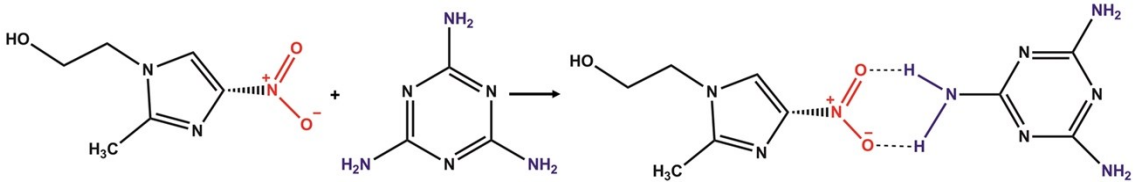

Fig. S1. The hydrogen bond between MTZ and MA.

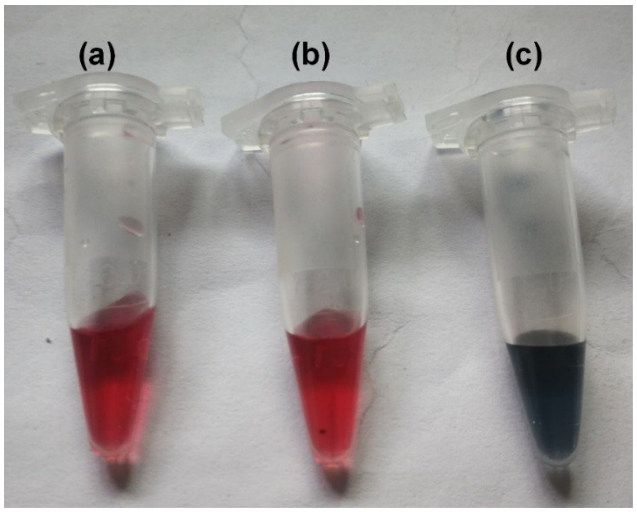

Fig. S2. Detection of MTZ (500μg/mL) based MA@GNPs solution (a) MA@GNPs, (b) MTZ-GNPs and (c) MTZ-MA@GNPs.

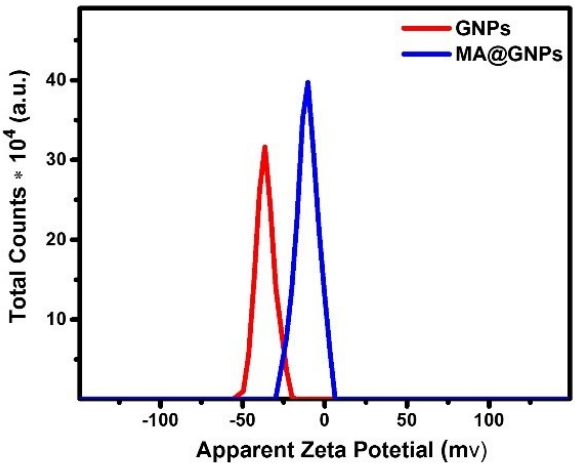

Fig. S3. The zeta potential result of GNPs and Mel@GNPs.

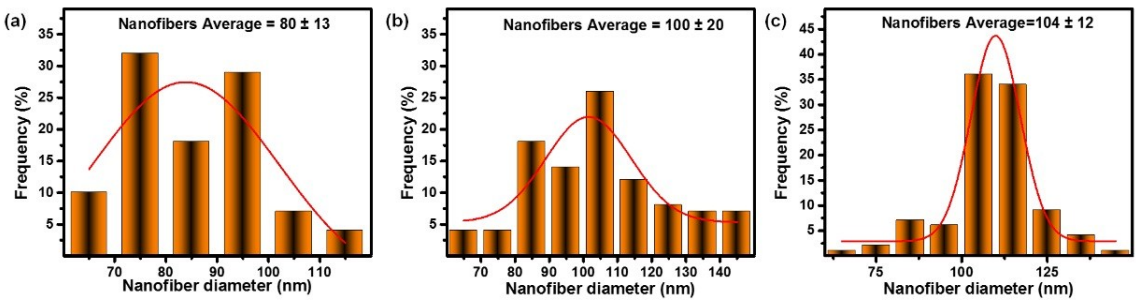

Fig. S4. Nanofiber diameter distribution of (a) electrospun PA6 NFMs (b) MA@GNPs NFMs and (c) MTZ-MA@GNPs NFMs.

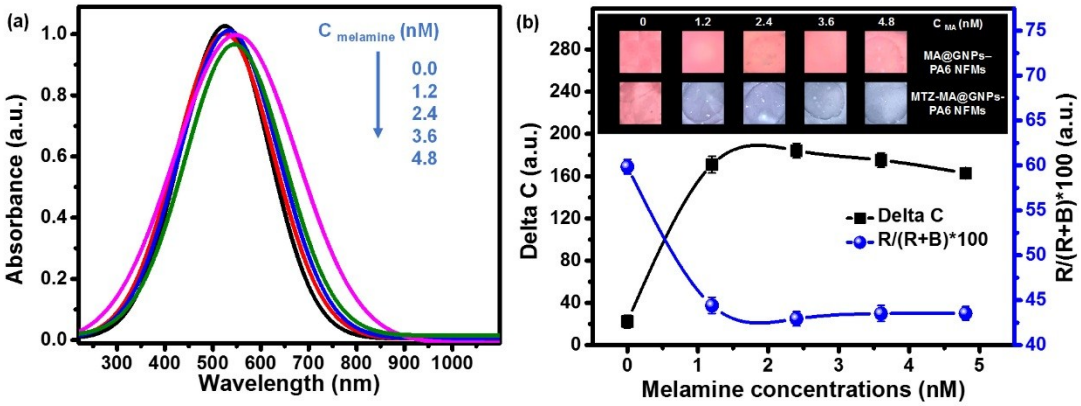

Fig. S5. (a) UV-Vis absorption spectra of colorimetric strips MA_x@GNPs-PA6 with MA concentrations (0, 1.2, 2.4, 3.6, and 4.8 nM), and (b) the red color ratio and the color difference values of colorimetric strips before and after incubation for 20 min with 100 µg/mL of MTZ as a function of MA concentrations; the corresponding optical images (inset)

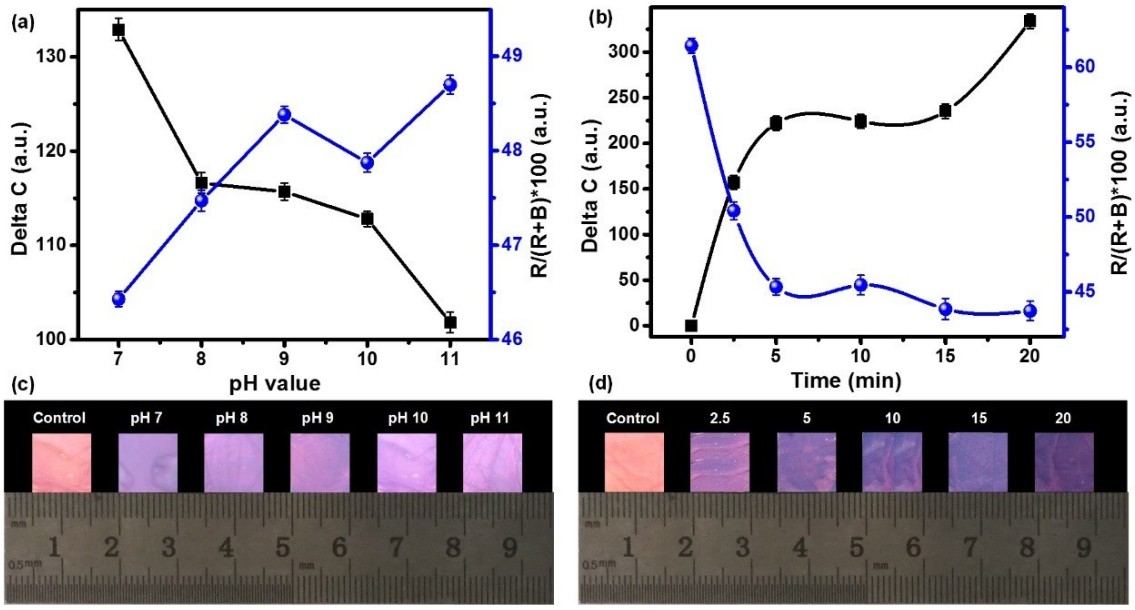

Fig. S6. Optimization of metronidazole sensing conditions: the color difference values and the red color ratio as a function of (a) pH and (b) time; (c) and (d) the corresponding optical images are displayed

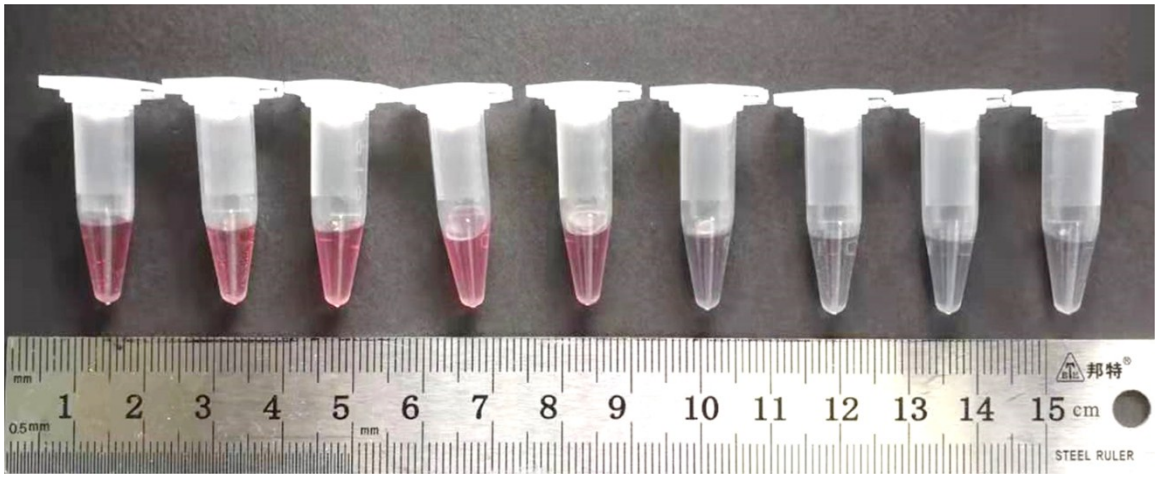

Fig. S7. Visual color changes of the MA@GNPs upon addition of different concentrations of MTZ (0, 2, 5, 10, 20, 40, 60, 80, and 100 nM)

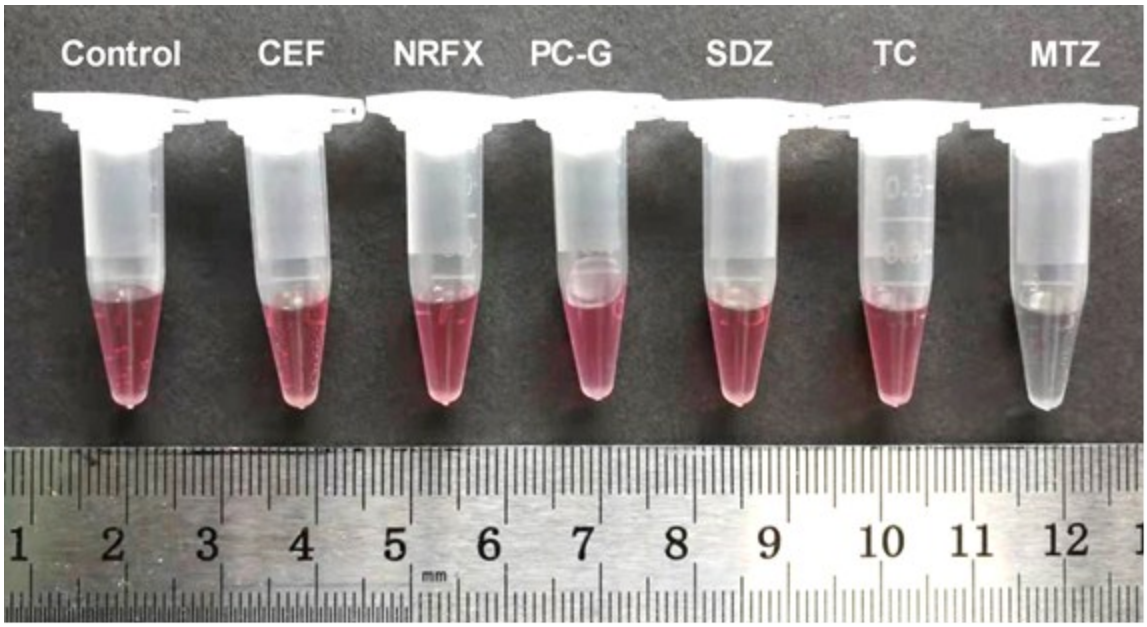

Fig. S8. Visual color changes of the MA@GNPs upon addition of different antibiotics (100 nM)

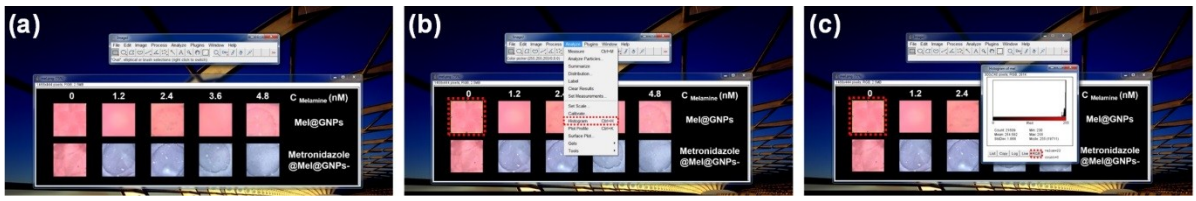

Fig. S9. Using ImageJ software to calculate the Red Green Blue (RGB) values from the photographs of strips

2. Supplementary Tables

Table S1. The R, G, and B values of Ma_x@GNPs-PA6 before and after MTZ detection (100 nM)

$C_{[MA]}$ nM	Samples					
	Control Samples			Detected samples		
	R	G	B	R	G	B
0	254.6±1.8	144.1±7.3	160.9±7.4	243.0±13.1	147.1±15.3	162.6±15.3
1.2	254.9±0.1	154.2±8.8	172.0±6.8	167.9±10.3	122.6±11.3	209.1±11.0
2.4	254.3 ±0.7	151.7±6.2	153.9±4.4	161.5±14.1	170.0±16.6	214.3±14.0
3.6	254.2±0.8	155.4±4.7	165.8±5.3	160.7±15.0	175.0±14.8	208.7±15.0
4.8	254.8±0.2	163.1±7.3	177.2±6.6	165.8±16.1	180.4±16.0	214.4±15.5

Table S2. The R, G, and B values of $Ma_{2.4}$@GNPs-PA6 before and after MTZ detection (100 nM) under different pH conditions.

pH value	Samples		
	R	G	B
Control	251.2±3.5	164±4.3	173.3±5.2
7	197.2±6.3	149±5.3	218±5.4
8	182.3±4.2	147±4.6	210±7.2
9	194.5±8.2	140±5.6	207±4.6
10	225.4±6.3	165±6.3	245±4.2
11	224.0±5.8	163±5.8	236±5.3

Table S3. The R, G, and B values of $Ma_{2.4}$@GNPs-PA6 before and after MTZ detection (100 nM) for different time.

$C_{[MTZ]}$ nM	Samples		
	R	G	B
Control	254.2±0.6	165±4.3	160.3±5.2
2.5	180±5.3	120±5.2	177±5.2
5	141±6.2	108±4.5	170±6.3
10	140±3.6	107±8.2	168±4.8
15	132±7.2	107±4.2	169±3.9
20	94±9.2	67±4.3	121±7.5

Table S4. The R, G, and B values of $Ma_{2.4}$@GNPs-PA6 before and after the detection of MTZ with different concentrations under optimal condition.

Time (min)	Samples		
	R	G	B
Control	254.1±0.8	183.5±4.3	177.3±5.2
2	253.2±0.9	188±6.3	193±5.2
5	226±6.5	170±5.89.2	247±6.3
10	219±5.2	172±4.2	254±7.5
20	213±4.9	185±7.2	254±5.3
40	207±8.2	170±4.3	252±3.6
60	181±4.8	1663.7±7.2	234±6.5
80	155±5.2	138±4.2	202±5.7
100	144±5.8	116±8.2	186±7.3

Table S5. The R, G, and B values of $Ma_{2.4}$@GNPs-PA6 before and after the detection of different antibiotics (100 nM).

Antibiotics	*Samples*		
	R	*G*	*B*
Control	*254.1±0.8*	*183.5±4.3*	*177.3±5.2*
Cephalexin (CEF)	*230±5.6*	*181±3.5*	*166±2.8*
Norfloxacin (NRFX)	*232±6.4*	*175±5.7*	*165±8.5*
Penicillin G (PC-G)	*210±4.6*	*141±7.6*	*135±5.7*
Sulfadiazine (SDZ)	*230±6.3*	*175±6.3*	*165±7.6*
Tetracycline (OTC)	*216±3.7*	*141±3.8*	*140±6.7*
MTZ	*113±5.8*	*135±8.2*	*94±7.3*

Table S6. Determination of MTZ in the drinking water and milk

Sample	***Added (nM)***	***Found (nM)*** [a]	***ICP-AES (nM)***[a]	***Recovery (%)***	***RSD (%)***
Drinking water	*2.00*	*2.08±0.04*	*1.94±0.04*	*104.20*	*3.40*
	5.00	*4.99±0.14*	*5.01±0.08*	*99.84*	*2.76*
	10.0	*9.96±0.04*	*9.98±0.18*	*99.80*	*1.83*
	20.0	*20.04±0. 28*	*19.84±0.20*	*100.18*	*1.38*
	40.0	*40.05±0.94*	*40.06±0.96*	*100.14*	*2.33*
Milk	*2.00*	*1.92±0.09*	*1.90±0.04*	*95.90*	*4.66*
	5.00	*4.96±0.14*	*4.99±0.15*	*99.24*	*2.74*
	10.0	*9.92±0.18*	*10.08±0.12*	*99.22*	*1.76*
	20.0	*19.94±0.22*	*20.02±0.11*	*99.68*	*1.12*
	40.0	*39.69±1.17*	*40.16±1.15*	*99.22*	*2.96*

a Average value of five determination ± standard deviation

Table S7. An overview on recently reported methods for determination of MTZ

Method	***Assay time (min)***	***Linear range (μM)***	***Limit of detection (μM)***	***Ref.***
Luminescent	*N/A*	*10-60*	*3.00*	[1]
Luminescent	*<60*	*0.25-300*	*0.10*	[2]
UHPLC-MS/MS[a]	*1.3*	*0.05-0.2*	*0.03*	[3]
Electrochemical sensor	*20*	*0.05-1000*	*0.12*	[4]
Electrochemical sensor	*2*	*0.5-1600*	*58.0*	[5]
Surface-enhanced Raman spectroscopy	*N/A*	*30-300*	*6.43*	[6]
Electrochemistry	*N/A*	*0.05-1.00*	*0.02*	[7]
Fluorescence	*N/A*	*0.2-15*	*0.15*	[8]
Photoelectrochemical immunoassay	*30*	*0.01-500*	*0.005*	[9]
UPLC-MS/MS	*N/A*	*0.001-0.234*	*0.0005*	[10]
Colorimetric based nanofiber	*2.5*	*0.00-0.1*	*0.002*	*our work*

a. Ultra-High-Performance Liquid Chromatography-Tandem Mass Spectrometry

3. Extraction of RGB values and the calculation process

1. The ImageJ software was used to extract the Red Green Blue (RGB) data from the photographs of strips before (R_1, G_1, B_1) and after (R_2, G_2, B_2) MTZ detection.

- First open the photograph of strips using ImageJ software as shown in Fig. S9a
- Then select the Rectangular Selection tool in the ImageJ toolbar and drag a rectangle over the image of the specific strip (Fig. S9b).
- Choose Analyze > Histogram (Fig. S9b), then click RGB (Fig. S9c). This sets to measure R, G, and B for only the highlighted pixels within the rectangular selection.
- The data can be recorded or saved in CSV file.

2. ΔR, ΔG, and ΔB values were attained from differences between RGB values before (R_1, G_1, B_1) and after (R_2, G_2, B_2) MTZ detections.

- $\Delta R = R_1 - R_2$
- $\Delta G = G_1 - G_2$
- $\Delta B = B_1 - B_2$

3. The color difference (ΔC) was calculated using the Equation[11]

$$\Delta C = \sqrt{2(R_1 - R_2)^2 + 4(G_1 - G_2)^2 + 3(B_1 - B_2)^2 + \frac{r^2((R_1 - R_2) - (B_1 - B_2))}{256}}$$

Where r is

$$r = \frac{R_1 + R_2}{2}$$

4. The red color ratio was calculated using the Equation

$$The\ red\ color\ ratio = \frac{R}{R + B}$$

4. Supplementary references

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