

# Single Molecule Analysis of Light-Regulated RNA-Spiropyran Interactions

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## Supplementary Information

### 1. Nanopore sensing control RNA

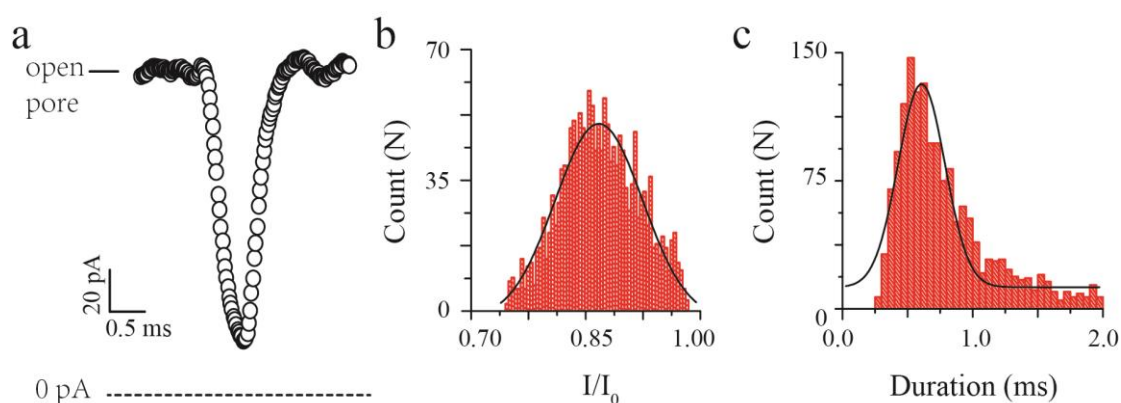


Fig. S1 (a) A translocation signal produced by control RNA. (b) Histogram of blockage current for control RNA. (c) The duration time histogram for control RNA.

### 2. Nanopore sensing spiropyran

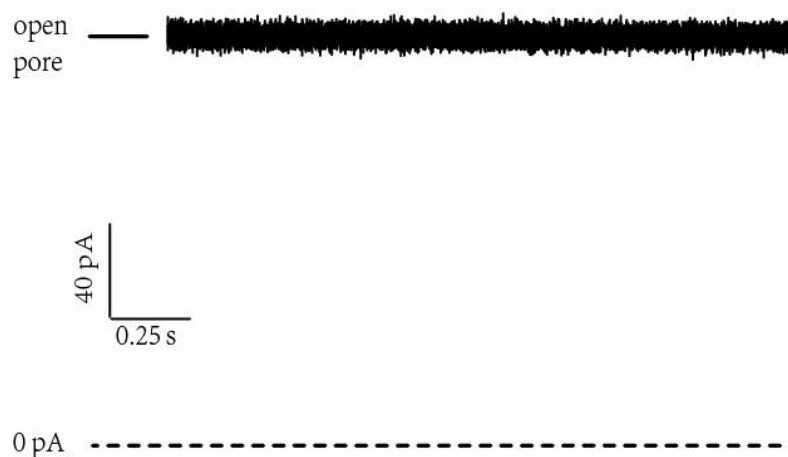


Fig. S2 The raw data for the open state of  $\alpha$ -HL in 1.0 M KCl at pH=7.98 Tris-HCl buffer by applying +150 mV

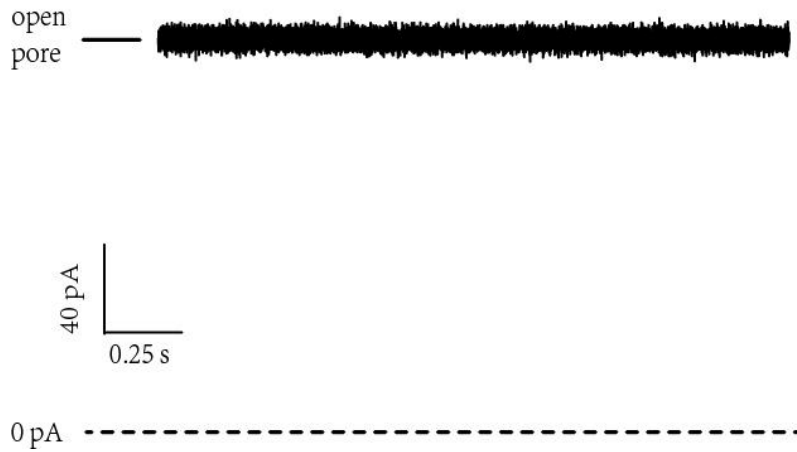


Fig. S3 The raw data of 0.1  $\mu\text{M}$  spiropyran in 1.0 M KCl at pH=7.98 Tris-HCl buffer by applying +150 mV.

### 3. RNA aptamers irradiate with visible light

Table S1. Blockage currents and durations of RNA aptamers under different irradiation conditions. <sup>a</sup>

	Irradiation	$I_1/I_0$	$t_1$ (ms)	$I_2/I_0$	$t_2$ (ms)	$I_3/I_0$	$t_3$ (ms)
RNA aptamer	/	$0.56 \pm 0.01$	$50.02 \pm 1.95$	$0.51 \pm 0.01$	$5.96 \pm 0.25$	$0.87 \pm 0.01$	$0.60 \pm 0.01$
	Visible light	$0.56 \pm 0.01$	$49.16 \pm 1.13$	$0.51 \pm 0.01$	$5.99 \pm 0.37$	$0.88 \pm 0.01$	$0.61 \pm 0.01$

<sup>a</sup> Data of the values were based on three separate experiments.

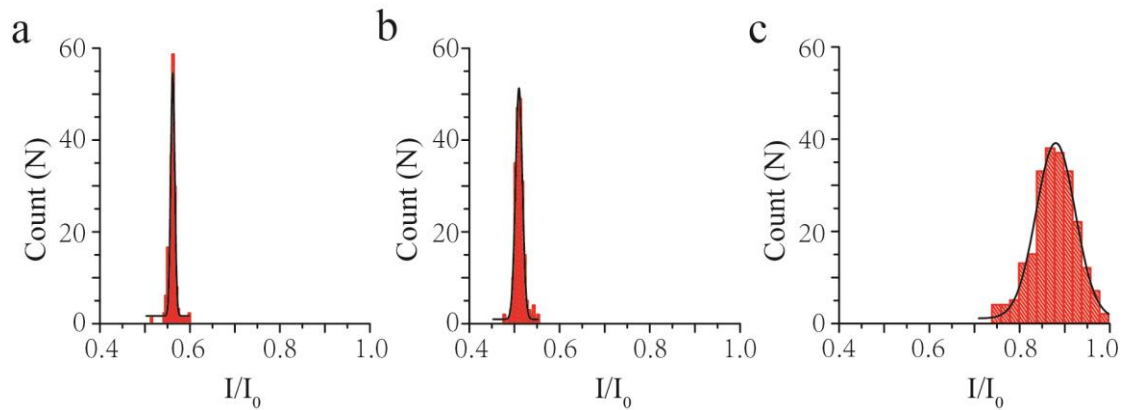


Fig. S4 Histograms of blockage currents for Level 1 (a), Level 2 (b) and Level 3 (c), which were fitted by a Gaussian function, respectively.

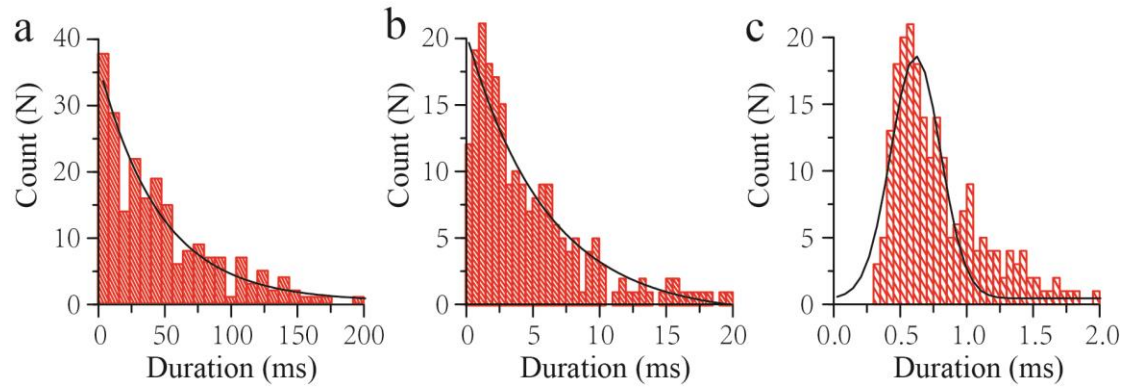


Fig. S5 Histograms of duration time for Level 1 (a), Level 2 (b) and Level 3 (c). The histogram of Level 1 and Level 2 was fitted by the exponential function, respectively. A Gaussian function was used for Level 3.

#### 4. $\alpha$ -HL irradiate with UV light and visible light

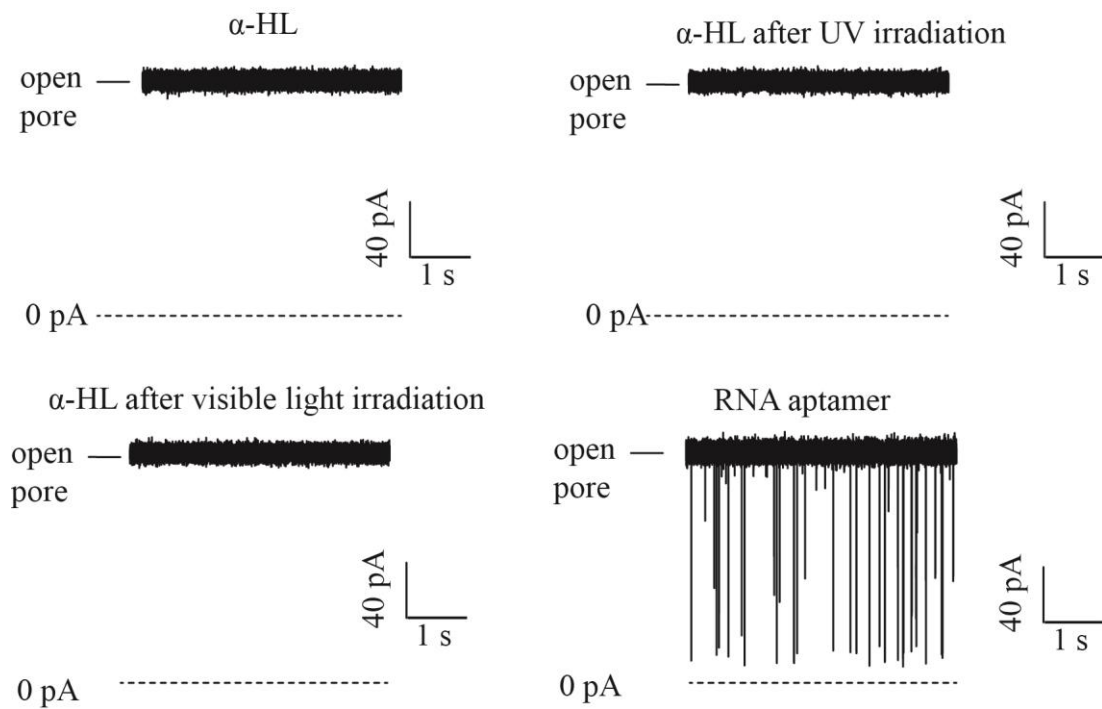


Fig. S6 The current traces recording at the holding potential of +150 mV. (a)  $\alpha$ -HL, (b)  $\alpha$ -HL after 30 min UV irradiation, (c)  $\alpha$ -HL after 30 min visible light irradiation, (d)  $\alpha$ -HL after add RNA aptamer.

#### 5. Nanopore sensing RNA:SP

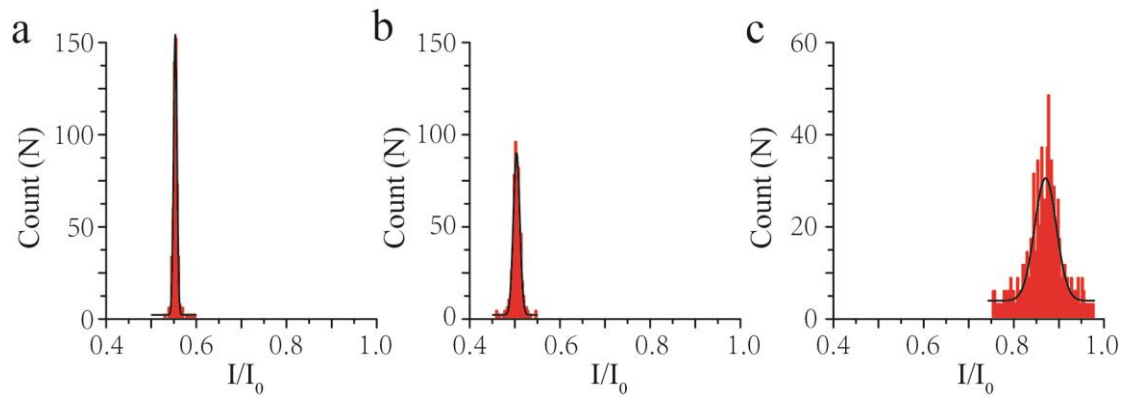


Fig.S7 Histograms of blockage currents for Level 1 (a), Level 2 (b) and Level 3 (c), which were fitted by a Gaussian function, respectively.

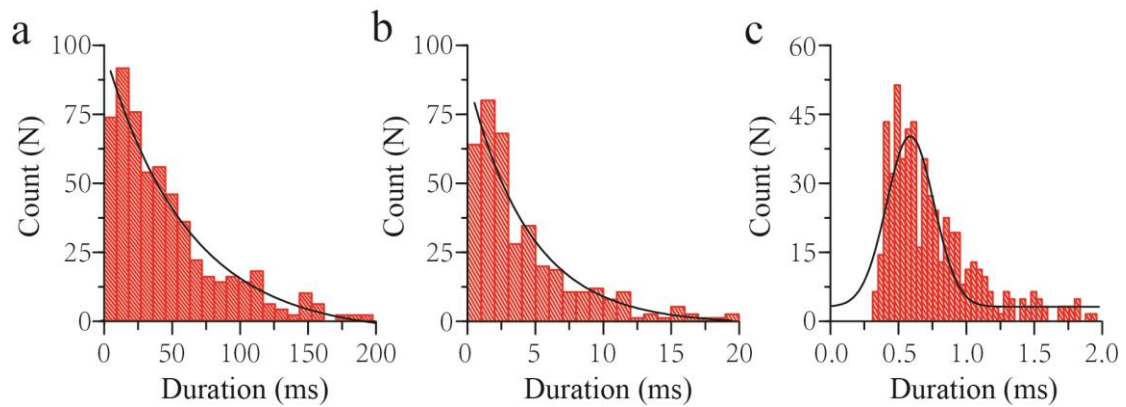


Fig. S8 Histograms of duration time for Level 1 (a), Level 2 (b) and Level 3 (c). The histogram of Level 1 and Level 2 was fitted by the exponential function, respectively. A Gaussian function was used for Level 3.

## 6. Nanopore sensing RNA/MR

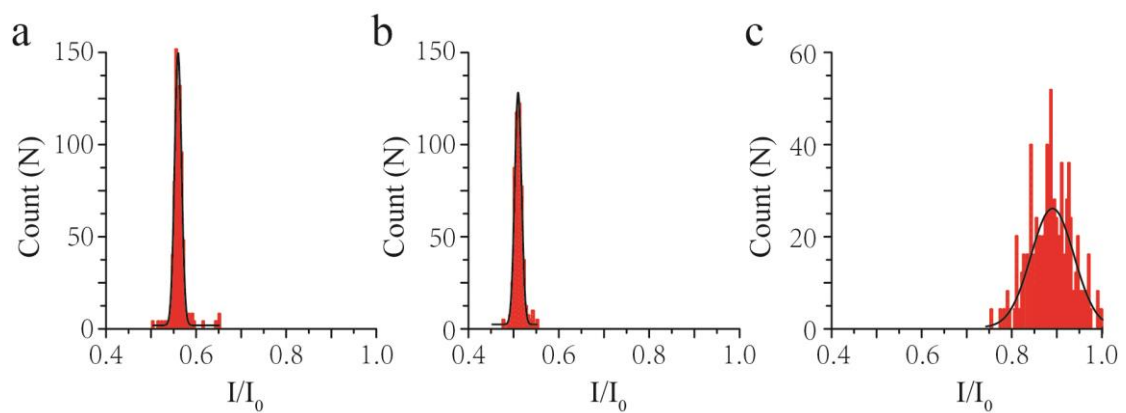


Fig. S9 Histograms of blockage currents for Level 1, Level 2 and Level 3, which were fitted by a Gaussian function, respectively.

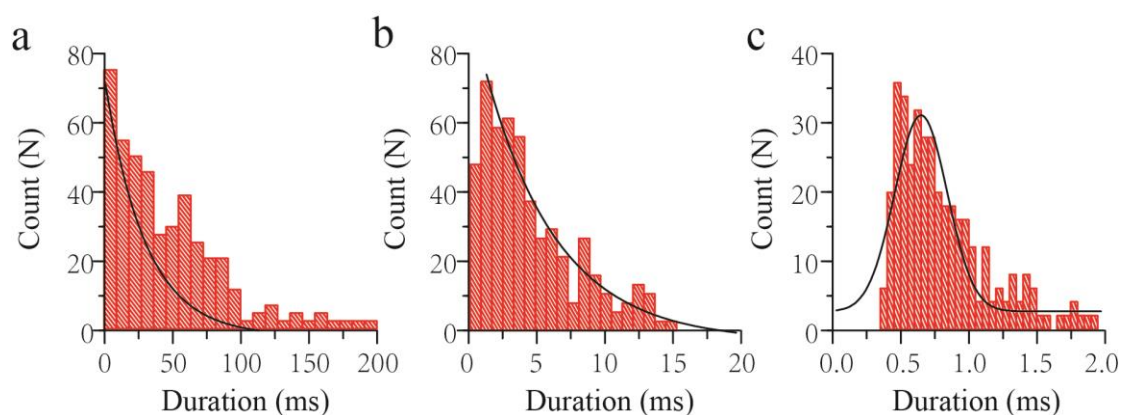
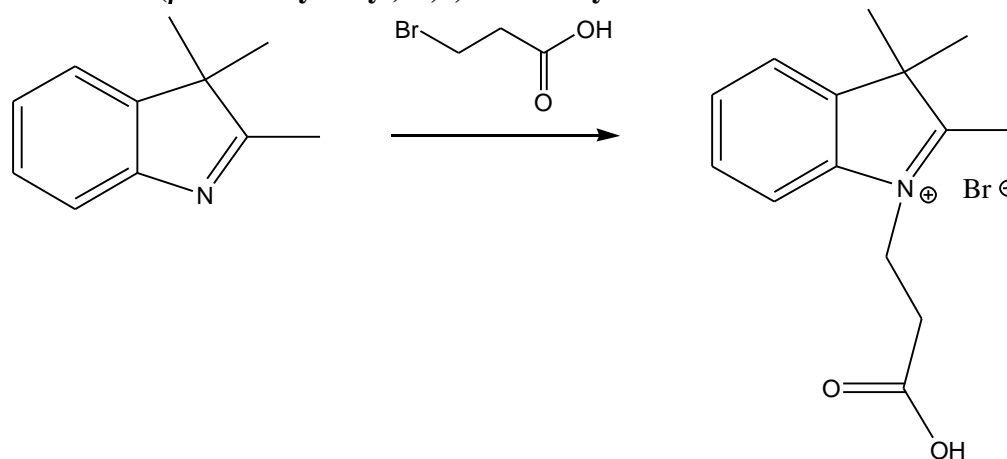


Fig. S10 Histograms of duration time for Level 1, Level 2 and Level 3 (from left to right). The histogram of Level 1 and Level 2 was fitted by the exponential function, respectively. A Gaussian function was used for Level 3.

## 7. Synthesis of spiropyran

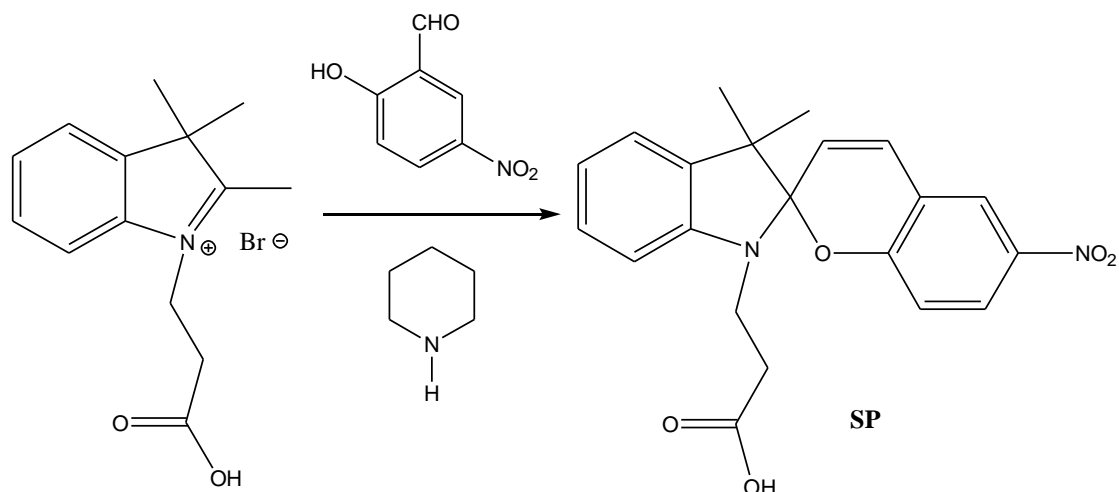
The materials was prepared by a slight modification of a previously reported procedure.<sup>1</sup>

### Synthesis of N-( $\beta$ -Carboxy-ethyl)-2,3,3-trimethyl-3H-indolium bromide



To a 50 mL flask, 2,3,3-trimethyl-3H-indole (6.4 mL, 40 mmol) and  $\beta$ -bromopropionic acid (7.8g, 40 mmol) were dissolved in 50 mL acetonitrile. The mixture was refluxed for 3 hours. After cooling to room temperature, the acetonitrile was condensed to 20 mL and cold acetone was added with the precipitation of red crystalline. After filtration, the red crystalline was washed with cold acetone to yield N-( $\beta$ -Carboxy-ethyl)-2,3,3-trimethyl-3H-indolium bromide (9 g, 69.2%).

### Synthesis of N-( $\beta$ -Carboxy-ethyl)-Spiropyran (SP)



N-(β-Carboxy-ethyl)-2,3,3-trimethyl-3H-indolium bromide (2 g, 5.7 mmol) was dissolved in boiling acetone (10 mL) in the presence of piperidine (1 mL). The solution was then reacted with a solution of 2-hydroxy-5-nitrobenzaldehyde (1 g, 6 mmol) in acetone (5 mL). The resulting mixture was refluxed for 30 min and allowed to cool to room temperature and react overnight. The resulting precipitate was filtered and washed with cold acetone to yield a yellow powder (1.7 g, 60.7%).

$^1\text{H NMR}$  ( $\text{CDCl}_3$ ):  $\delta$ =1.14 (s, 3H), 1.28 (s, 3H), 2.71 (t, 2H), 3.65 (t, 2H), 5.85 (d, 1H), 5.91 (d, 1 H), 6.62–7.18 (m, 5 H), 8.05 (m, 2H).

## References

1. J. Zhang, M. Riskin, R. Tel-Vered, H. Tian and I. Willner, *Chem. Eur. J.*, 2011, **17**, 11237-11242.