Supporting Information

Design, synthesis and cell imaging of a simple peptide-based probe for selective detection of RNA

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Experimental Procedures

1. Materials and reagents

Bis (2-hydroxyethyl) amino-tris (hydroxymethyl) methane (Bis-Tris) was purchased from Aladdin Biochemical Technology Co., Ltd (Shanghai, China). Deoxyribonucleic acid sodium salt from calf thymus (etDNA) was supplied by Sigma-Aldrich (Sigma-Aldrich Company, USA). p(dA•dT), p(dG•dC); and p(A+U) were purchased from InvivoGen (San Diego, CA, USA) and prepared as 1mg/mL sterile stock solution with physiological water which was supplied by InvivoGen. Deionized water was used throughout this work. Fmoc-based amino acids used in the solid phase synthesis of P1-P5 such as Fmoc-Lys(Fmoc)-OH, Fmoc-Dpr(Boc)-OH, Fmoc-Thr(Bu)-OH and Fmoc-Trp(Boc)-OH and so on were all supplied by Toppeptide Co., Ltd. (Shanghai, China). Other chemical reagents such as dansyl chloride, Rink Amide resin, DMSO, N,N-dimethylformamide(DMF), dichloromethane (DCM), ethanol, Trifluoroacetic acid(TFA), triisopropylsilane(TIS) were acquired from commercial suppliers. All the reagents used were analytically pure grade and further purification is not required. ER tracker Red and Lyso Tracker were used in this text. The sequence of Forward single-stranded RNA was: AUAACUUAAUGUUUUUAUAUGGUUGCAUUUACGCCAAUAAU.

2. Instruments

1.1 Materials and reagents

1.2 Fluorescence intensity measurements

The stock solution of P1 was dissolved in deionized water at a concentration of 10 mM and aliquoted into tubes and stored at -20 °C. The stock solutions of four different kinds of nucleic acids (etDNA, p(dA•dT), p(dG•dC) and p(A+U)) and all kinds of single strand RNA (A-rich, CG-rich, AU-rich, L=41, 34, 27, 20, 13, 5) were prepared in physiological water at 1 mg/mL and upon suspension, they were aliquoted and stored at -20 °C for long term storage or at 4°C for short term storage to keep their natural conformation. All the fluorescence measurements were performed in 10 mM Bis-Tris buffer at pH 7.4. The fluorescence emission spectra of P1 in the presence of four different kinds of nucleic acids were taken by a 10 nm path length quartz cuvette. All the fluorescence measurements were performed under the excitation wavelength set at 330 nm and the recording emission range from 400 to 650 nm. The slit sizes for excitation was 5 nm and for emission was 20 nm. The following formula was used to calculate the fold of emission enhancement of P1: (F2/F1)/F0, where F0 was the initial fluorescence intensity of P1 alone and F2 represented the fluorescence intensity of P1 in presence of different kinds of nucleic acids. All the fluorescence intensity at 535 nm was used.

1.5 Circular Dichroism measurements

The Circular Dichroism spectra were taken on a JASCO-810-Circular Dichroism (CD) Spectrometer using a 10 mm path length quartz cuvette. The CD spectrum of 2 mL Bis-Tris buffer was first examined and used as a baseline corrected for all the other results. 10 μM P1 in Bis-Tris buffer was then measured and followed by titration of p(A+U) with incubation for 5 min. Finally, the CD spectrum of 50 μg/mL pure p(A+U) was also measured. All the CD spectra were recorded as a scan from 200-500 nm (only 200-300 nm data were shown) at scanning speed 100 nm/min and the data pitch was 1.0 nm. All measurements were performed at room temperature.
1.6 Molecular modelling and analysis

3D structures of DNA and RNA double helices were modeled with sybyl 8.1 software expect for ctDNA. Modeled p(dA•dT) and p(dG•dC) were set as B-form and added AMBER7 FF99 charges. Modeled p(A•U) was set as A-form and added AMBER7 FF99 charges. Crystal structure of ctDNA was extracted from complex obtained from RCSB Protein Data Bank (PDB ID: 1BNA). The hydrogen atoms in polar orientation and AMBER7 FF99 charges were added before the docking. 3D structures of five synthesized compound P1-P3 were generated with sybyl 8.1 software. All structures were added Gasteiger-Marsili charges and minimized for 1000 cycles. The molecular docking and result analysis were conducted by surflex_dock program. The additional starting conformations per molecular was set as 6. The angstroms to expand search grid was 10. The max conformations per fragment were set as 20. The ring softness was considered during the dock for all compounds. The binding site was defined using automatic method. The threshold for the protomol generation was set with default value (0.5 Ang). Complexes of docking results were generated and analyzed and mapped using VMD software.

1.7 Molecular dynamic simulations.

Complexes obtained from the docking results were used as the starting structures for molecular dynamic simulations. The Amber ff14SB force field for the protein (1, 2) and solvation in a rectangular box of TIP3P water (3), with a minimum distance between the nucleic acid and the box edge of 8 Å. The solvated protein was subsequently neutralized with Na ion or Cl ion. Optimization and equilibration protocols were applied to all systems before running production MD simulation. Initial optimization of the solvent consisted of 1000 cycles of energy minimization followed by 50 ps of MD simulation at 298K (applying a positional restraint of 10 kcal mol$^{-1}$ Å$^{-2}$ on all solute atoms). The whole system was then optimized by 1000 cycles of energy minimization with a mild positional restraint on the nucleic acid atoms (2.0 mol$^{-1}$ Å$^{-2}$). To prepare for production simulations, first, the temperature was increased from 50 to 298 K over a period of 10 ps (maintaining the mild restraint on nucleic acid atoms). Second, 50 ps simulation in the NPT ensemble at 298 K and 1 bar was performed, again maintaining the mild restraint on nucleic acid atoms. Thereafter, the whole system was briefly further equilibrated by 100 ps of NPT MD simulation (298 K, 1 bar). After this equilibration procedure, 2 ns MD simulation in the NPT ensemble at 298 K and 1 bar was carried out. Throughout, periodic boundary conditions were applied and the SHAKE algorithm was applied to fix all bond lengths involving hydrogen atoms. A time step of 2 fs was used, with a direct-space cut off radius of 8.0 Å for non-bonded interactions and particle mesh Ewald for long-range electrostatic interactions. The trajectory was sampled every 1 ps (500 steps intervals) for analysis. All simulations were performed using AMBER18 (1). The AmberTools programs ptraj, cpptraj (4) and MMGBSA (5) were used for analysis. Simulations were visualized using VMD (http://www.ks.uiuc.edu/Research/vmd/) (6).

1.8 Cell culture and imaging

HeLa cells were used for imaging experiments. Cells were all cultured in DMEM growth medium (Biological Industries) containing 10% FBS and 1% penicillin-streptomycin at carbon dioxide incubator with 5% CO$_2$. The temperature was maintained at 37°C. Firstly, cells were plated on 18 × 18 mm glass coverslips and allowed to adhere for more than 12 h. And then the cells were transfected with H2B-mCherry to indicate the location of chromosome. The next day, cells were washed with phosphate buffer saline (PBS) buffer and fixed with 4% paraformaldehyde for 10 min at room temperature and further incubated with P1, P4 or P5 for another 30 min after washing with PBS buffer. Finally, the coverslips were mounted with fluorescent mounting media and sealed on glass slides. Fluorescence imaging was performed with a fully automatic inverted confocal fluorescence microscope (Zeiss LSM880) with a 60× oil immersion or 20× objective lens. Cells were excited with 405 nm laser and the emission was collected at 489-589 nm. Meanwhile mCherry were excited with 543 nm laser and the emission was collected at 547-703 nm.

1.9 Cytotoxicity assays

The cell cytotoxicity of P1 was measured on HeLa cells using cell counting kit-8 (CCK-8). HeLa cells were plated on 96-well cell culture plate at a concentration of 5×10^3/mL with 100 μL cell suspension per well and then incubated for approximately 12 h at 37°C under 5% CO$_2$. And then these cells were treated with different concentrations of P1 and incubated for another 24 h. Cells were then treated with 10 μL CCK-8 for 0.5 h and finally the absorbance was measured using microplate reader at 450nm.

2.0 Isothermal titration calorimetry

Isothermal titration calorimetry (ITC) performing on a MicroCal PEAQ-ITC instrument was used to measure binding affinity between P1 and p(A•U)$_2$. Before titration, P1 and p(A•U)$_2$ were both diluted with 10mM HEPES buffer (pH 7.0) and centrifuged at 1,2000 rpm for 15 min to remove bubbles. And then 300 μL P1 (50 μM) and 40 μL p(A•U)$_2$ (400 μM) were loaded into the sample cell and syringe respectively. 2 μL of p(A•U)$_2$ was injected every 180 s until P1 was saturated (a total of 20 injections) at 25°C , except for the first injection point being 0.4 μL. Many thermodynamic parameters including the stoichiometric ratio (N), equilibrium dissociation constant (Kd), enthalpy (ΔH), Gibbs free energy (ΔG) and binding entropy (ΔS) were calculated from the titration curve.
Supplementary Figures

Fig. S1 Chemical structure and synthesized route of fluorescent peptide P1.
HPLC Chromatogram of P1

Sample: P1
Sequence: (Dansyl-Trp-Thr-Dpr)₂-Lys-NH₂
Column: 4.6×150 mm, kromasil C18-5
Solvent A: 0.1% Trifluoroacetic in 100% Acetonitrile
Solvent B: 0.1% Trifluoroacetic in 100% Water

Gradient:

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<tr>
<td>25.0 min</td>
<td>70%</td>
<td>30%</td>
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Flow rate: 1.0 ml/min
Wavelength: 214 nm
Volume: 10 μL

Fig. S2 HPLC Chromatogram of P1.

Table S1 HPLC Chromatogram data of P1.

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<th>Conc.</th>
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<td>Total</td>
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MS Analysis data

Sample: P1

Expected MS: 1359

Flow rate: 0.2ml/min  Run time: 1min

Buffer A: 0.1% HCOOH in water  Buffer B: 0.1% HCOOH in Acetonitrile

**Fig. S3** MS spectrum of P1.
Fig. S4 Fluorescence emission spectra for the titration of P1 (10 μM) with p(dA・dT)$_2$ (a), p(dG・dC)$_2$ (b) and ctDNA (c) with their concentrations ranged from 0-8 μg/ml in 10 mM Bis-Tris buffer at pH 7.4. The direction of black arrow indicates an increase in the concentrations of three typical double-stranded DNAs. The scatter plot at upper right corner of each graph shows the fluorescence intensity changes at 535 nm emission wavelength along with the titration of the indicated DNAs.

Fig. S5 (a) Normalized fluorescence intensity of P1 with concentrations of 0, 1, 2, 3, 4 μg/mL of AU-rich RNA and CG-rich RNA. (b-c) Fluorescence emission spectra of P1 (10μM) with the titration of AU-rich RNA (b) and CG-rich RNA (c).

Fig. S6 (a) Normalized fluorescence intensity of P1 with different length of RNA (4 μg/mL). (b-g) Fluorescence emission spectra of P1 with the titration of different length of RNA.
Molecular dynamics simulations

For P1 bound with p(A·U)$_2$, ctDNA, p(dG·dC)$_2$ and p(dA·dT)$_2$, binding free energies were calculated as -20.37, -16.56, -12.16 and -15.16 kcals/mol, respectively. Lower binding free energy indicates higher binding affinity. It is obvious that P1 binds with p(A·U)$_2$ best with the highest binding affinity. ctDNA would be the second preferred nucleic acid with the lower binding free energy. Much weaker binding with p(dG·dC)$_2$ and p(dA·dT)$_2$ are predicted. All these results are correlated with the molecular docking and fluorescent measurements.

Besides the strong binding with p(A·U)$_2$, the binding free energy of P1 with p(dG·dC)$_2$ is a little higher than that of p(dA·dT)$_2$, which is different from the results obtained by molecular docking score. The difference might be caused by the sensitivity of the modelled solvent environment and random ions due to the weak binding for two systems.

Table S3 Calculated binding free energy (kcals/mol) of P1 with p(A·U)$_2$, p(dA·dT)$_2$, p(dG·dC)$_2$ and ctDNA.

<table>
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<th>Binding Free Energy</th>
<th>P1-p(A·U)$_2$</th>
<th>P1-ctDNA</th>
<th>P1-p(dG·dC)$_2$</th>
<th>P1-p(dA·dT)$_2$</th>
</tr>
</thead>
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<tr>
<td>$\Delta G_{\text{gas}}$</td>
<td>-100.80±0.95</td>
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<td>-44.81±1.36</td>
<td>-74.32±1.29</td>
</tr>
<tr>
<td>$\Delta G_{\text{solv}}$</td>
<td>80.43±0.87</td>
<td>115.06±0.82</td>
<td>32.65±1.25</td>
<td>59.16±1.25</td>
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<tr>
<td>$\Delta G_{\text{total}}$</td>
<td>-20.37±0.43</td>
<td>-16.56±0.34</td>
<td>-12.16±0.28</td>
<td>-15.16±0.23</td>
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</table>
**Fig. S7** Snapshots of P1 bound with p(A·U)$_2$, p(dA·dT)$_2$, p(dG·dC)$_2$ and ctDNA at 0ns, 1ns and 2ns. DNA/RNA skeletons were displayed in cartoon. P1 molecule was displayed in sticks. Bases interacting with P1 were displayed in lines and colored with atom type.
Docking and molecular dynamics simulation of A-form DNA

3D structures of DNA double strand p(dA·dT)₂ and p(dG·dC)₂ in A-form were modeled with sybyl 8.1 software. Using the exactly the same parameters with the previous studies, binding affinity of PI sensor and DNA were calculated through molecular docking and molecular dynamics simulation studies.

Docking scores of PI sensor with p(dA·dT)₂ and p(dG·dC)₂ in A-form were measured as 6.03 and 7.28 kcals/mol, respectively. By conducting MD simulations of 2ns, binding free energy were measured as -11.43 and -12.60 kcals/mol respectively.

Fig. S8 (a, b) Binding modes of PI bound with p(dA·dT)₂ and p(dG·dC)₂ in A-form. Dansyl group was displayed in purple spheres. Tryptophan was displayed in orange spheres. (c) Snapshots of PI bound with p(dA·dT)₂ and p(dG·dC)₂ in A-form at 0ns, 1ns and 2ns. DNA skeletons were displayed in cartoon. PI molecule was displayed in sticks. Bases interacting with PI were displayed in lines and colored with atom type.
HPLC Chromatogram of P2

Sample: P2

Sequence: (Dansyl-Trp-Thr-Gly)$_2$-Lys-NH$_2$

Column: 4.6-150 mm, kromasil C18-5

Solvent A: 0.1% Trifluoroacetic in 100% Acetonitrile

Solvent B: 0.1% Trifluoroacetic in 100% Water

Gradient:

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<tr>
<th>Time</th>
<th>A</th>
<th>B</th>
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<td>0.01 min</td>
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<tr>
<td>25.0 min</td>
<td>70%</td>
<td>30%</td>
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Flow rate: 1.0 ml/min

Wavelength: 214 nm

Volume: 10 μL

Fig. S9 HPLC Chromatogram of P2.

Table S4 HPLC Chromatogram data of P2.

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<td>Total</td>
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HPLC Chromatogram of P3

Sample: P3
Sequence: (Dansyl-Trp-Thr-Asp)₂-Lys-NH₂
Column: 4.6-150 mm, kromasil C18-5
Solvent A: 0.1% Trifluoroacetic in 100% Acetonitrile
Solvent B: 0.1% Trifluoroacetic in 100% Water

Gradient: | Time | A | B |
----------|------|---|---|
0.01 min  | 5%   | 95%|
25.0 min  | 70%  | 30%|

Flow rate: 1.0 ml/min
Wavelength: 214 nm
Volume: 10 μL

Fig. S10 HPLC Chromatogram of P3.

Table S5 HPLC Chromatogram data of P3.

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Sample: P2

Expected MS: 1299.48

Flow rate: 0.2ml/min                       Run time: 1min
Buffer A: 0.1% HCOOH in water              Buffer B: 0.1% HCOOH in Acetonitrile

Fig. S11 MS spectrum of P2.
Sample: P3

Expected MS: 1416.43

Flow rate: 0.2 ml/min

Run time: 1 min

Buffer A: 0.1% HCOOH in water

Buffer B: 0.1% HCOOH in Acetonitrile

Fig. S12 MS spectrum of P3.
Fig. S13 Characterization of P4 synthesis. (a) HPLC Chromatogram of P4. (b) MS spectrum of P4.
Fig. S14 Characterization of P5 synthesis. (a) HPLC Chromatogram of P5. (b) MS spectrum of P5.
Fig. S15 Binding mode of P4 (a) and P5 (b) with p(A·U)$_2$.

Fig. S16 (a) – (d) Fluorescence emission spectra for the titration of P2 – P5 (10 μM) with p(A·U)$_2$ in 10 mM Bis-Tris buffer at pH 7.4. Direction of the black arrow indicates an increase in the
concentrations of $p(A\cdot U)_2$. The scatter plot at upper right corner of each graph shows the fluorescence intensity changes at 535 nm emission wavelength along with the increase of $p(A\cdot U)_2$.

**Table S6** Binding mode of P2-P5 with double-stranded RNA $p(A\cdot U)_2$

<table>
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<td>9.19</td>
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<tr>
<td>P3</td>
<td>8.96</td>
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<tr>
<td>P4</td>
<td>12.27</td>
<td>$180^\circ/180^\circ$</td>
</tr>
<tr>
<td>P5</td>
<td>9.24</td>
<td>$180^\circ/120^\circ$</td>
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**Fig. S17** Fluorescence imaging of 293T, A549 and LM3 cells incubated with P1 (40 μM). Dansyl and mCherry were shown to indicate the relative location for P1 and chromosome DNA.
Cell imaging treated with P1 in living cells and fixed cells

In order to clarify the efficiency of P1 detecting RNA in living cells and fixed cells, Hela cells were treated with P1 under two different methods. Cells were plated on 18 × 18 mm glass coverslips and allowed to adhere for more than 12 h. All cells were all cultured in DMEM growth medium (Biological Industries) containing 10% FBS and 1% penicillin-streptomycin at carbon dioxide incubator with 5% CO2. The temperature was maintained at 37°C. Then, part of cells were incubated with P1 (40μM in DMEM) for 1h before washed with PBS buffer and fixed with pre-cold methanol. Part of cells were fixed with pre-cold methanol first and then treated with P1 (40μM in PBS buffer) for only 20 min. The coverslips were mounted with fluorescent antifade mounting medium and sealed with glass slides. Fluorescence imaging was performed with a fully automatic inverted confocal fluorescence microscope (Zeiss LSM880) with a 40× oil immersion lens. Cells were excited with 405 nm laser.

It is obviously that P1 could enter the living cells and detecting RNA. However, living cells with P1 incubating for 1h perform gloomier than fixed cells with P1 treated with only 20 min. Moreover, the efficiency of P1 entering nuclear might also be weaker since few fluorescence emission was observed in nucleus. Thus, we strongly recommend using P1 in fixed cells for better cell imaging. Longer incubating time will be taken for using P1 in living cells.

Fig. S18 Fluorescence imaging of HeLa cells incubated with P1 in living cells and fixed cells.
Measurement of fluorescence quantum yield of P1

The fluorescence quantum yield of P1 excited by RNA was determined using comparative method. Considering the emission range of P1, anthracene solvated in ethanol was used as the standard material [1]. The absorbance values of solution were record with spectrophotometer (Hitachi, U-3900H). Fluorescence emission spectra of all solutions were record at 25 °C with excitation wavelength of 405 nm (Hitachi, F-2700). The fluorescence quantum yield of P1 was calculated according to the following equation [2]:

\[
\Phi_x = \frac{n_x^2}{n_i^2} \frac{A_x \cdot D_x}{A_i \cdot D_i} \cdot \Phi_i
\]

Here, \( \Phi_x \) and \( \Phi_i \) represents the quantum yield of P1 in the presence of RNA and anthracene respectively. \( n_x \) and \( n_i \) are the refractive index of the solutions. \( A_x \) and \( A_i \) are the absorbance of P1 and anthracene respectively. \( D_x \) and \( D_i \) are the integral areas of fluorescent emission spectra of P1 and anthracene. Same solutions were used for measuring the absorbance and fluorescent emission spectra.

With exciting wavelength of 405nm, the absorbance of anthracene solution was measured as 0.002 and P1 with p(A·U)2 was measured as 0.024. Integral areas were measured as 500.3 and 6651.1 respectively. It is known that refractive indexes for water and ethanol were 1.33 and 1.10 at 25 °C respectively. Fluorescence quantum yield of anthracene solvated in ethanol was 0.27. According to the above equation, the quantum yield of P1 excited by RNA was determined as 0.44.
Co-staining experiment with SYTO™ RNA select™ dye

To fully examine the staining ability of P1 in fixed cells, SYTO™ RNA select™ dye (refers as SYTO dye) was used to co-stain with P1. Cell coverslips were prepared with the same method as previous. Then the cells were fixed with pre-cold methanol at -20°C for 10min. After washed by PBS buffer, the cells were incubated with PBS buffer containing P1 of 30 µM and SYTO dye of 500 nM for 0.5 h at room temperature. The coverslips were mounted with fluorescent antifade mounting medium after washing with buffer and sealed with glass slides. Fluorescence imaging was performed with a fully automatic inverted confocal fluorescence microscope (Zeiss LSM880) with a 40× oil immersion lens. Cells were excited with 405 nm laser for P1 imaging and 488 nm for SYTO dye imaging.

The fluorescent signals of P1 and SYTO RNA select dye show highly consistency with each other. It is obvious that P1 shows clear fluorescent response for nucleus RNA with weaker signals than in cytoplasm. Compared to the SYTO RNA selective dye, P1 performs better fluorescent response to cytoplasm RNA than nucleus RNA, which might be caused by the lower efficiency of P1 entering the nuclear.
Fluorescence imaging of HeLa cells incubated with P1 sensor (30 µM) and SYTO™ RNA select™ dye (500 nM) in fixed cells.

Co-staining of living cells with P1 and MitoTracker™ Red CMXRos, ER tracker RED and Lyso Tracker™ Red DND-99

To reveal where P1 is accumulated in the cytoplasm, MitoTracker™ Red CMXRos, ER tracker RED and Lyso Tracker™ Red DND-99 (refers as Mito-tracker, ER-tracker, Lyso-tracker) were used to co-stain with P1. Cell coverslips were prepared with the same method as previous. Then the cells were incubated with DMEM containing P1 of 40 µM and Mito-tracker, ER-tracker and Lyso-tracker of 500 nM for 1 h at 37 °C, respectively. After washed with PBS buffer, the cells were fixed with pre-cold methanol at -20°C for 10min. The coverslips were then mounted with fluorescent antifade mounting medium after washing with buffer and sealed with glass slides. Fluorescence imaging was performed with a fully automatic inverted confocal fluorescence microscope (Zeiss LSM880) with a 40× oil immersion lens. Cells were excited with 405 nm laser for P1 imaging and 543, 561, 561 nm for Mito-tracker, ER-tracker and Lyso-tracker imaging, respectively.

Merged view shows obvious overlay of the signal of Mito-tracker with fluorescent signal of P1 sensor, which indicates accumulation of P1 sensor in the mitochondria. Moreover, there are still uncovered fluorescence signal of P1 beside the Mito-tracker signal. Thus, P1 sensor exists around the whole cytoplasm with obvious accumulation in mitochondria. Similar accumulation could also be observed for ER, which reveals the existing of attached ribosomes on ER. No overlap signals were observed with
lysosome.

**Fig. S21** Fluorescence imaging of Hela cells incubated with P1 sensor (40 µM) and MitoTracker™ Red CMXRos (500 nM) in living cells.

**Fig. S22** Fluorescence imaging of HeLa cells incubated with P1 sensor (40 µM) and ER Tracker™ Red (500 nM) in living cells.

**Fig. S23** Fluorescence imaging of HeLa cells incubated with P1 sensor (40 µM) and LysoTracker™ Red (500 nM) in living cells.
RNase digestion experiments in fix-cells

To reveal the RNA selectivity of $\textbf{P1}$ in cells, fluorescent imaging of cells was recorded before and after treated with RNase. Cell coverslips were prepared with the same method as previous. Then the cells were fixed with pre-cold methanol at -20°C for 10 min. All cells were treated with 10% Triton-20 to make cell membrane penetrable for DNase and RNase. After being washed with PBS buffer, cells in control group were incubated with PBS buffer for 30 min at 37°C. Cells in RNase were incubated with RNase working solution (1U/µL, 10×Reaction buffer) for 30 min at 37°C. All cells were then incubated with $\textbf{P1}$ sensor (40 µM) after washing with PBS buffer for 20 min at room temperature. Finally, the cells were fixed with pre-cold methanol at -20°C for 10 min after washing. The coverslips were then mounted with fluorescent antifade mounting medium after washing with buffer and sealed with glass slides. Fluorescence imaging was performed with a fully automatic inverted confocal fluorescence microscope (Zeiss LSM880) with a 40× oil immersion lens. Cells were excited with 405 nm laser for $\textbf{P1}$ imaging.

![Image](image.png)

**Fig. S24** Fluorescence imaging of Hela cells incubated with $\textbf{P1}$ sensor (40 µM) without (Control) and with the treatment of RNase. Overall fluorescent signal intensity was measured.
References:


