Electronic Supplementary Information

A bimolecular i-motif mediated FRET strategy for imaging protein homodimerization on a living tumor cell surface

Ailing Kan,[a] Xiaoting Liu,[a, b] Xiaowen Xu,[a] Nan Zhang*[b] and Wei Jiang*[a]

[a] *School of Chemistry and Chemical Engineering, Shandong University, 250100 Jinan, P. R. China.*

[b] *Department of Oncology, Research Center of Basic Medicine, Jinan Central Hospital, Cheeloo College of Medicine, Shandong University, 250012 Jinan, P. R. China.*

Corresponding authors. Email: wjiang@sdu.edu.cn; zlkzn2016@126.com.

Experimental Details

Materials. All DNA oligonucleotides were synthesized and purified by Sangon Biological Engineering Technology and Services Co., Ltd (Shanghai, China). The DNA sequences and modifications are listed in Table S1. DMEM medium, RPMI 1640, certified foetal bovine serum (FBS), penicillin-streptomycin solution (PS, 100 U/ml), trypsin EDTA solution and phosphate buffered saline (PBS, pH 7.4) for cell culture were purchased from Biological Industries (Beit-Haemek, Israel). Wheat Germ Agglutinin, Alexa Fluor™ 488 conjugate (WGA-AF488) was purchased from Thermo Fisher Scientific (Waltham, USA). Recombinant human hepatocyte growth factor (HGF, #100-39) was purchased from PeproTech (USA). Anti-HGF antibody [ab83760] was purchased from Abcam (UK). RIPA lysis buffer (P0013D), protease and phosphatase inhibitor cocktail (P1050) were purchased from Beyotime (Shanghai, China). The primary antibody for β-Actin (AB0035) was purchased from Abways Technology (Shanghai, China). The primary antibodies for Met (#8198) and phospho-Met (Tyr1234/1235, #3077) were obtained from Cell Signaling Technology (USA). The secondary antibody Goat Anti-Rabbit lgG(H+L) HRP were obtained from Abways Technology (Shanghai, China). LumiQ HRP substrate solution kit was obtained from Share-bio Technology Co, Ltd (Shanghai, China). All aqueous solutions were prepared using ultrapure water (18.25 MΩ·cm).

Instruments. The native polyacrylamide gels for DNA analysis were imaged by GelDocTM XR$^+$ imaging system (Bio-RAD Laboratories Inc., USA). The SDS-

polyacrylamide gel images for western blot analysis were obtained by Amersham Imager 600 imaging system (GE Healthcare, USA). UV absorption spectra were recorded on a U-2910 spectrophotometer (Hitachi, Japan). Circular dichroism spectra were recorded using a Chirascan V100 circular dichroism spectrometer (Applied Photophysics, UK). Cytotoxicity assays were performed on a SPARK microplate reader (Tecan Austria GmbH, Austria). Flow cytometry assays were performed by a NovoCyte 3130 flow cytometer (ACEA Biosciences Inc., UAS). The confocal laser scanning microscopic (CLSM) imagings were carried through a Leica TCS SP8 STED confocal microscope (Leica, Germany) with an objective lens of 63×.

Cell lines and cell culture. All cell lines were cultured in a humidified incubator with 5% CO_2 at 37 ˚C. Human hepatocellular carcinoma cells (HepG2) were cultured in DMEM medium with 10% FBS and 1% PS. Human prostate adenocarcinoma cells (LNCaP) were cultured in RPMI 1640 medium with 10% FBS.

Preparation of the probe. The powders of DNA strands were first dissolved using TE buffer (10 mM Tris, 1 mM Na_2EDTA, pH 8.0) to result in concentrated stock solutions. The probe was assembled by mixing the stock solutions of Half-i@Apt strand and Blocker strand with a ratio of 1:1. Then the mixture was heated to 95 °C, slowly cooled down to room temperature and put at 4° C in the dark for 12 hours to allow adequate hybridization. The concentrated stock solution of probe was diluted using PBS buffer of different pH (10 mM NaH_2PO4, 10 mM Na_2HPO_4, 130 mM NaCl, 4.6 mM KCl, 5.0 mM $MgCl_2$) to appropriate concentrations for subsequent experiments.

Study of the pH-responsive ability of half-i. Native polyacrylamide gel electrophoresis (PAGE), circular dichroism (CD) spectroscopy and UV absorption spectroscopy were carried out to verify the ability of half-i's to combine into bimolecular i-motif in the environment of pH 6.5 (corresponding to the acidity of tumorous extracellular microenvironment).

Native PAGE assays. DNA oligonucleotides H14 (5'-ACTGTGACTACAGT-3', forming a 14-nt hairpin), PAL14 (5'-CTTGAGATCTCAAG-3', a palindromic sequence forming both 14-nt hairpin and 14-bp duplex), and PAL28 (5'-CTTGAGACTTGAGATCTCAAGTCTCAAG-3', a palindromic sequence forming both 28-nt hairpin and 28-bp duplex) were used as control size markers. The stock solution (100 μM) of size markers and half-i were diluted to the concentration of 2 μM using pH 7.4 and pH 6.5 PBS, and incubated for 30 minutes. The markers and half-i (0.012 nmol per lane) in pH 7.4 PBS were run on 15% native polyacrylamide gel in TAE buffer (40 mM Tris, 2 mM Na_2EDTA, 20 mM CH_3COOH, pH 7.4) for 80 minutes with a constant current of 26 mA at 14 °C. The markers and half-i in pH 6.5 PBS were

run in TAE buffer (40 mM Tris, 2 mM Na_2EDTA, 20 mM CH_3COOH, pH 6.5) for 120 minutes with a constant current of 26 mA at 14 °C.

CD spectroscopy. The concentrated stock solution of half-i (200 μM) was respectively diluted by PBS of pH 7.4 and pH 6.5 to a concentration of 15 μM for CD measurements. The spectra range from 220 nm to 320 nm were recorded. The background spectra of corresponding buffers were subtracted from the samples.

The melting curves of 15 μM half-i in pH 6.5 PBS were determined by collecting the ellipticities at 287 nm as the temperature gradually rises with a rate of 1 °C/min on a CD spectrometer equipped with a temperature-controlled water bath. Plot the ellipticities versus temperatures, and the temperature at which 50% of the bimolecular i-motif structures were dissociated was adopted as the $T_{m\text{-}CD}$ value.

To analyze the kinetics of the bimolecular i-motif formed from half-i's, the concentrated solution of half-i (200 μM) was directly added into pH 6.5 PBS and mixed quickly to result in a 15 μM concentration. The ellipticities at 287 nm were collected with a 90-second interval on the CD spectrometer at 25 °C. The ellipticity of the half-i at pH 7.4 PBS was measured as the initial point.

UV spectroscopy. The concentrated stock solution of half-i (200 μM) was respectively diluted by PBS of pH 7.4 and pH 6.5 to a concentration of 8 μM for UV measurements. The spectra range from 220 nm to 340 nm were recorded.

For UV-thermal denaturation experiment, the UV absorption spectra of the half-i (8 μM) in pH 6.5 buffer at different temperatures (10 ℃, 20 ℃, 30 ℃, 40 ℃, 50 ℃, 60 ℃, 70 ℃, 80 ℃) were measured. Melting curve was obtained by plotting the absorbances at 295 nm versus temperatures. The temperature at which 50% of the bimolecular i-motif structures were dissociated was adopted as the $T_{m\text{-}UV}$ value. The thermal difference spectrum (TDS) of the formed structure was obtained by subtracting the 30 ℃ spectrum from the 60 ℃ spectrum.

Fluorescence measurement. To make sure that the structural change of half-i's at pH 6.5 does cause FRET effect between the donor (Cy3) and acceptor (Cy5) on the probe, we used a simulated DNA sequence (5'-Cy5-TCCCCCCTCCCCCCATTGATGATCTATTTTTTTTTTTTT-Cy3-TAGATCATCAATGTGG-3') obtained by removing the aptamer part in the Half-

i@Apt strand to simulate the state of the probe on cytomembrane, and measured its fluorescence under 525 nm laser excitation at pH 7.4 and pH 6.5.

Cytotoxicity study of the probe. MTT assays were carried out to estimate the cytotoxicity of the probe. HepG2 and LNCaP cells were respectively seeded on 96-well plates at a concentration of 5000 cells per well and cultured at 37 ºC for 24 hours. Old medium was gently removed and replaced by 100 μl new medium containing different concentrations (0 nM, 10 nM, 50 nM, 100 nM, 250 nM, 500 nM) of probe. After incubating the cells with the probe for another 24 hours at 37 ºC, added 10 μl MTT (5 mg/ml) to each well and then maintained the cells at 37 °C for 4 hours, removed the medium and dissolved the formazan crystals generating from the reaction of MTT and succinodehydrogenase in living cells with 150 μl DMSO for each well. After shaking for 5 minutes, measured the absorbances at 490 nm to calculate cell viabilities. Cells without probe treatment were set as the control groups. Cell viabilities were calculated as the percentage of viable cells in total population. Each experiment was performed six replicates.

Western blot analysis.[1] Cells were cultured to 80% confluence in 6-well plates and treated with different conditions for different assays. After the treatments, the cells were washed with warm physiological saline for 3 times to remove the medium, then lysed in RIPA lysis buffer containing protease and phosphatase inhibitor cocktail for 15 minutes. Detached the cells from the plates by cell scrapers, then centrifuged the detached cells at 12000 rpm at 4 °C for 15 minutes and collected their supernatants. Added protein sample loading buffer and denatured the supernatants at 100 °C for 5 minutes. The total protein extracts were analyzed on 8% SDS-polyacrylamide gels (30 μg per lane). Proteins were transferred onto PVDF membranes (Millipore Immobilon-P, 0.45 μm), and nonspecific binding sites were blocked with 5% skim milk dissolved in TBST (10 mM Tris, 150 mM NaCl, 0.05%(v/v) Tween-20, pH 7.2-7.5) at room temperature for 1 hour, then washed the membranes 3 times with TBST. The primary antibody for Met, phospho-Met and β-Actin were incubated with the proteins on PVDF membranes overnight at 4 °C. After 3-time washes with TBST, the membranes were incubated with the secondary antibody on shaker at room temperature for 2 hours. After 3-time washes with TBST, the membranes were analyzed by ECL imaging.

Study on the specificity of the probe to Met. Met-positive HepG2 cells and Met-negative LNCaP cells were respectively used as experimental and control group to examine the specificity of the probe to target Met. HepG2 and LNCaP cells were separately seeded into confocal dishes and cultured for 24 hours to adhere. Then treated the cells with 100 nM probe containing 2 μg/ml WGA-AF488 in pH 6.5 PBS for 15 minutes. Cells were washed 2-3 times by pH 6.5 PBS and imaged by confocal laser

scanning microscope. The probe was excited by 525nm laser and the fluorescence range from 550 nm to 600 nm was collected.

Interference of the probe on Met expression and homodimerization. To ensure the probe can reflect the natural status of the target protein, we explored the perturbations on Met expression and homodimerization resulting from the probe applications. HepG2 cells in six-well plates were grown to 80% confluence. For investigating the interference of probe on Met expression, 100 nM probe in pH 6.5 PBS was applied to the cells for 15-minute incubation. For investigating the interference of probe on Met homodimerization, the cells were first treated with 100 ng/ml HGF for 30 minutes, then 100 nM probe in pH 6.5 PBS was applied to the cells for 15-minute incubation. Then the cells were lysed and total protein was extracted for western blot analysis. All HGF and probe were applied in a volume of 1 ml. The influence of the probe on Met expression and dimerization were evaluated by comparing the content of Met and p-Met on cells with and without probe treatment.

Location analysis of the probe. The cellular location of the probe is quite essential for the aims of the probe. In order to investigate the location of the probe during the assays, we first stained the cytomembrane by 2 μg/ml WGA-AF488 for 15 minutes. Then washed off the excess WGA-AF488 with pH 6.5 PBS and added 100 nM probe to the cells. The cells were washed by pH 6.5 PBS to remove the excess probe after 15-min incubation and implemented confocal laser scanning microscopic (CLSM) imaging. Then the cells were incubated continuously, and images were captured when the incubation time reached 25 minutes, 35 minutes, 45 minutes and 55 minutes. By co-localizing the fluorescence of the probe and cytomembrane staining, we explored whether the probe remained stable on the cell membrane during the experiments. The WGA-AF488 was excited by 495nm laser, the emission range from 500 nm to 540 nm was recorded. The probe was excited by 525 nm laser and the emission range from 550 nm to 600 nm and 640 nm to 700 nm were collected for Cy3 and Cy5 respectively. These two excitations were set as two different sequences to avoid cross-color interference.

Imaging the Met homodimerization at different levels. HepG2 cells were treated with HGF of different concentrations (0 ng/ml, 10 ng/ml, 50 ng/ml and 100 ng/ml) to induce Met to homodimerize with different levels. After incubating the cells with HGF for 30 minutes, the mediums were removed. 100 nM probe containing 2 μg/ml WGA-AF488 in pH 6.5 PBS were added to the cells for 15-minute incubation in the dark. Then, the cells were washed 2-3 times with PBS of pH 6.5 for CLSM imaging.

CLSM imaging for relative quantitative analysis. Since we found that the introduction of membrane staining can reduce the fluorescence intensity of the probe, which may be due to the binding of WGA to the sugar complex on the plasma membrane interferes with the probe-protein, protein-protein or probe-probe interactions to some extent, the cells for relative quantitative analysis are exempt from cytomembrane staining to ensure the probe was interfered as little as possible. HepG2 cells were treated with HGF of different concentrations (0 ng/ml, 10 ng/ml, 50 ng/ml and 100 ng/ml) to induce Met to homodimerize with different levels. After incubating the cells with HGF for 30 minutes, the mediums were removed. 100 nM probe in pH 6.5 PBS were added to the cells for 15-minute incubation in the dark. Then, the cells were washed 2-3 times with PBS of pH 6.5 for CLSM imaging. The images were captured under the same parameters: excitation of the probe was 525 nm laser with 10% intensity, emission of Cy3 was collected in the range of 550 nm to 600 nm using a HyD detector with a gain value of 200%, Cy5 emission was recorded in the range from 640 nm to 700 nm via a PMT detector with a gain value of 700.

Inhibitor experiments. The inhibitor experiment was performed to further validate the specificity of the probe. 100 ng/ml HGF was first incubated with 2 μg/ml of anti-HGF antibody for 1 hour to occupy its binding site and then applied to HepG2 cells for 30-minute incubation. Then removed the medium and added 100 nM probe containing 2 μg/ml WGA-AF488 in pH 6.5 PBS to the cells for 15-minute incubation in the dark. The cells were washed 2-3 times with PBS of pH 6.5 for CLSM imaging.

Control probe assays. To explore the imaging mechanism of the probe, a control probe was constructed by replacing the half-i sequence with a dyad strand (GTTTTTTGTTTTTT) which is shaped like half-i but has no acid-responsive capacity. HepG2 cells were incubated with the medium containing 100 ng/ml of HGF for 30 minutes. 100 nM probe and control probe containing 2 μg/ml WGA-AF488 in pH 6.5 PBS were respectively added to cells for 15-minute incubation in the dark. Cells were washed 2-3 times with PBS of pH 6.5 for CLSM imaging.

Real-time imaging of Met homodimerization. HepG2 cells were incubated with 100 nM probe in pH 6.5 PBS for 15 minutes in the dark. Removed excess probe using pH 6.5 PBS. Found a clear imaging plane under confocal laser scanning microscope. 100 ng/ml HGF was added to the cells and a time-lapse fluorescence imaging was immediately captured under xyt scanning mode with an interval of 1 minute. The cells for real-time imaging are exempt from cytomembrane staining to avoid the interference of WGA binding to membrane.

Flow cytometry. Cells were seeded in six-well plates and cultured to 80% confluence. For homodimerization levels analysis, different degrees of Met dimerization were induced by applying 1 ml of medium containing 0 ng/ml, 10 ng/ml, 50 ng/ml and 100 ng/ml HGF respectively to the cells for 30-minute incubation. For inhibitor experiments, 1 ml of 100 ng/ml HGF was first incubated with 2 μg/ml inhibitor for 1 hour and then applied to the cells for 30-minute incubation. After the treatments, cells were gently washed by pH 6.5 PBS to remove the medium. 1 ml of 100 nM probe in pH 6.5 PBS was added to the cells and maintained for 15 minutes in the dark. Then softly washed the cells 2-3 times with pH 6.5 PBS. The cells were scraped off with cell scrapers for flow cytometry analysis. The probe was excited by 488 nm laser. The emission from 544 nm to 600 nm (BL2, 572/28) were recorded for Cy3. The emission from 645 nm to 705 nm (BL4, 675/30) were recorded for Cy5.

Relative quantitative analysis. The fluorescence intensities of the CLSM images were quantified by measuring the mean fluorescence intensities of 10-15 cells using the stack profile tools equipped on the microsystem. The intensity of Cy3 and Cy5 were converted into relative values expressed in percentages. The conversion formulas are: $I_{Cy3}\% = [\ I_{Cy3} / (I_{Cy3} + I_{Cy5})\ ]* 100\%$, $I_{Cy5}\% = [\ I_{Cy5} / (I_{Cy3} + I_{Cy5})\ ]* 100\%$. The gray analysis of the western blot bands were performed by ImageJ software. Convert the mean gray values of Met and p-Met strips into relative values expressed in percentages by the following formulas: $I_{Met}\%= [I_{Met} / (I_{Met} + I_{p\text{-}Met})]*100\%$; $I_{p\text{-}Met}\%= [I_{p\text{-}Met} / (\ I_{Met} + I_{p\text{-}Met}\ )]*100\%$. The I_{Met} and $I_{p\text{-}Met}$ were obtained by dividing the raw gray values of Met and p-Met by the gray values of the corresponding internal reference protein Actin, respectively. For flowcytometry, the average fluorescence intensities of the predominant cell species in Cy3 and Cy5 channels were converted into relative values expressed in percentages. The conversion formulas are: ICy3% = [ICy3 / (ICy3 + ICy5)]* 100%; ICy5% = [ICy5 / (ICy3 + ICy5)]* 100%. Since the intensities of fluorescence or brightness of bands are related to the test parameters (laser, gain value, exposure time and voltage, etc.), the absolute values obtained by different methods cannot be directly compared. Therefore, we compared the increments in the proportion of Cy5 (p-Met in western blot) among different treated groups.

Table S1 The sequences of DNA employed in this work.

Name	Sequence (5'→3')
Half-i@Apt [a]	Cy5-TCCCCCCTCCCCCCATTGATGATCTATTTTTTTTGGATGGTAGCTCGGTCGGGGTGGGTGGGTTGGCAAGTCTTTTTTTTT-Cy3-TAGATCATCAATGTGG
Blocker	AACCCACCCACCCCGACCGAGCTACCATCCAAAAAAAATAGATTATCAATGGGG
Half-i	TCCCCCCTCCCCCC
H14	ACTGTGACTACAGT
PAL14	CTTGAGATCTCAAG
PAL28	CTTGAGACTTGAGATCTCAAGTCTCAAG
Simulated sequence [b]	Cy5-TCCCCCCTCCCCCCATTGATGATCTATTTTTTTTTTTTTTT-Cy3-TAGATCATCAATGTGG
Scramble sequence [c]	GTTTTTTGTTTTTT
Scr@Apt [d]	Cy5-GTTTTTTGTTTTTTATTGATGATCTATTTTTTTTGGATGGTAGCTCGGTCGGGGTGGGTGGGTTGGCAAGTCTTTTTTTTT-Cy3-TAGATCATCAATATAA
Control blocker [d]	AACCCACCCACCCCGACCGAGCTACCATCCAAAAAAAATAGATTATCAATAAAA

[a] In the Half-i@Apt, underlined sequence indicate the aptamer of Met. [b] Simulated sequence obtained by removing the aptamer part in the Half-i@Apt was used for in vitro fluorescence measurement. [c] Scramble sequence was used to replace the half-i for control probe construction. [d] Scr@Apt and Control blocker were used for control probe assembling. The Cy3 and Cy5 labels are in red.

Supplementary Figures

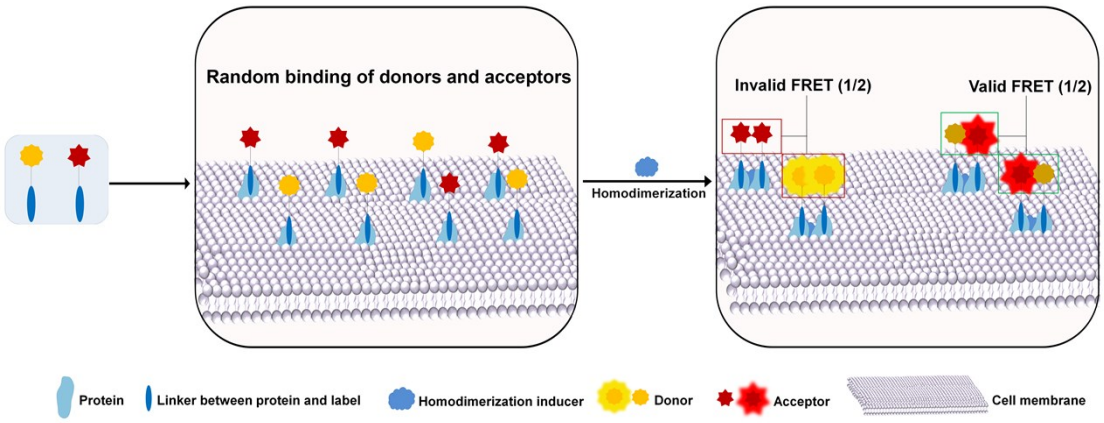


Fig. S1 Schematic illustration of the classical FRET-based methods for protein homodimerization detection in which half homodimeric proteins lead to FRET-invalid label combinations.

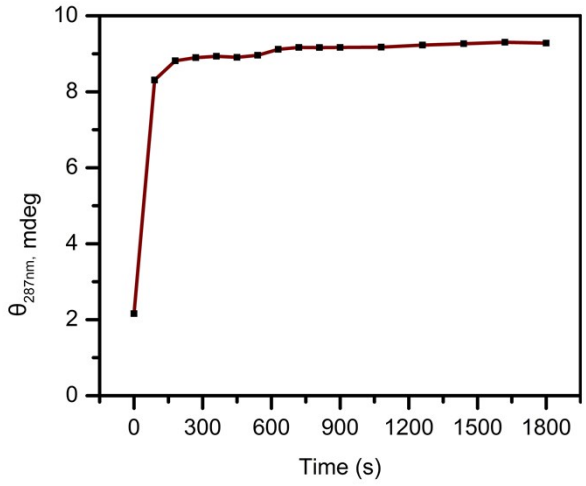


Fig. S2 Time dependence of ellipticities at 287 nm of half-i in pH 6.5 PBS. The pattern

indicates the bimolecular i-motif is formed rapidly in 180 seconds at pH 6.5.

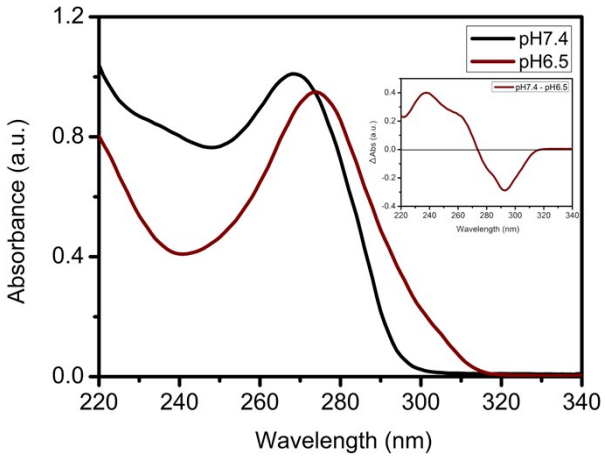


Fig. S3 UV absorption spectra of half-i in pH 7.4 and pH 6.5 buffers. Inset shows the difference spectrum obtained by subtracting the spectrum at pH 6.5 from the one at pH 7.4. The absorbances at 265 nm and 295 nm are respectively characterized by hypochromism and hyperchromic effect as the pH dropped from 7.4 to 6.5, which is considered to be the signature of the presence of C•C+ base pairs.[2] The difference spectrum is similar in shape to the thermal difference spectrum at pH 6.5 (Fig. 1d), which further proves that the half-i's form some structure containing C•C+ base pairs[3] when the pH dropped from 7.4 to 6.5.

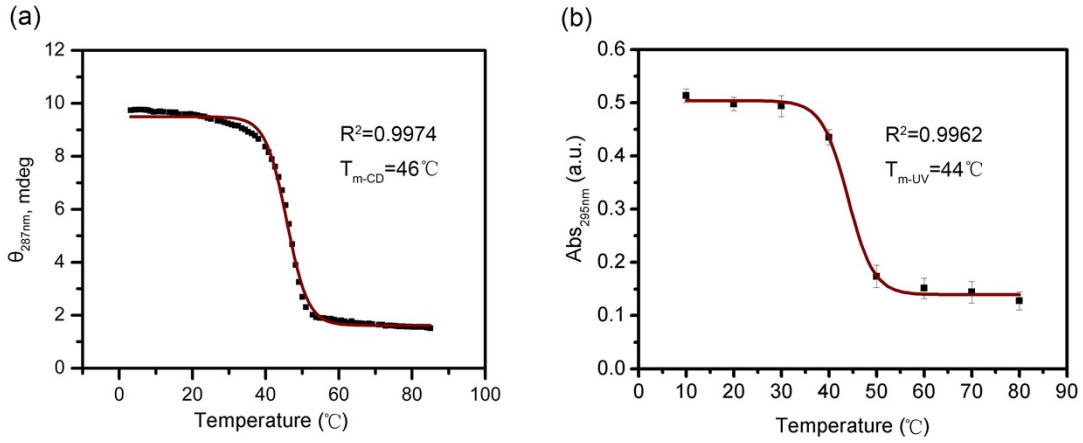


Fig. S4 (a) The ellipticities at 287 nm of half-i versus temperature in pH 6.5 PBS. (b) The absorbances at 295 nm of half-i versus temperature in pH 6.5 PBS. All points were fitted by a boltzmann function. The $T_{m\text{-}CD}$ and $T_{m\text{-}UV}$ of the bimolecular i-motif were

determined as 46℃ and 44℃ respectively, suggesting the bimolecular i-motif can function stably at room temperature or 37 ℃ for imaging on living cells.

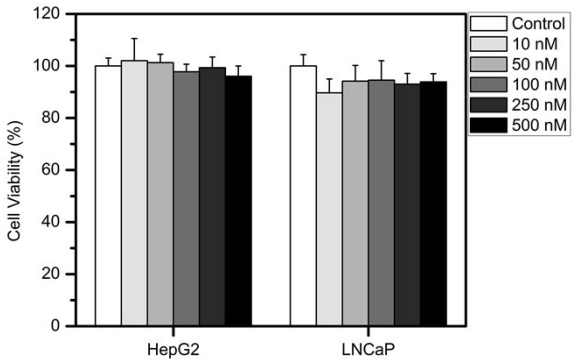
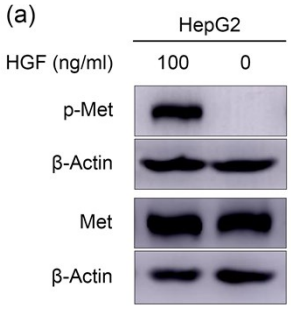


Fig. S5 Cell viability of HepG2 and LNCaP cells treated with different concentrations of probe. The viabilities of cells treated with different concentrations of probe are all above 85%, indicating that the probe has no significant cytotoxicity to cells, paving the way for living cell imaging.

(a)



(b)

Treatment	0 HGF+Probe	100 HGF+Probe
IntDen of Met/Actin	1.51	1.21
IntDen of p-Met/Actin	0.0148	1.02
p-Met (%)	1	46

Fig. S6 (a) Western blot of the Met (monomeric Met) and p-Met (homodimeric Met) in probe-treated HepG2 cells. (b) The relative gray analysis of Met and p-Met in western blot. The cells treated with probe present consistent content with that without probe treatment (Fig. 2b) on the Met monomer and homodimer expressions. The results verify that the probe has little interference in target protein expression and homodimerization, thus the images obtained from the probe can trustworthily reveal the natural level of protein monomer and homodimer.

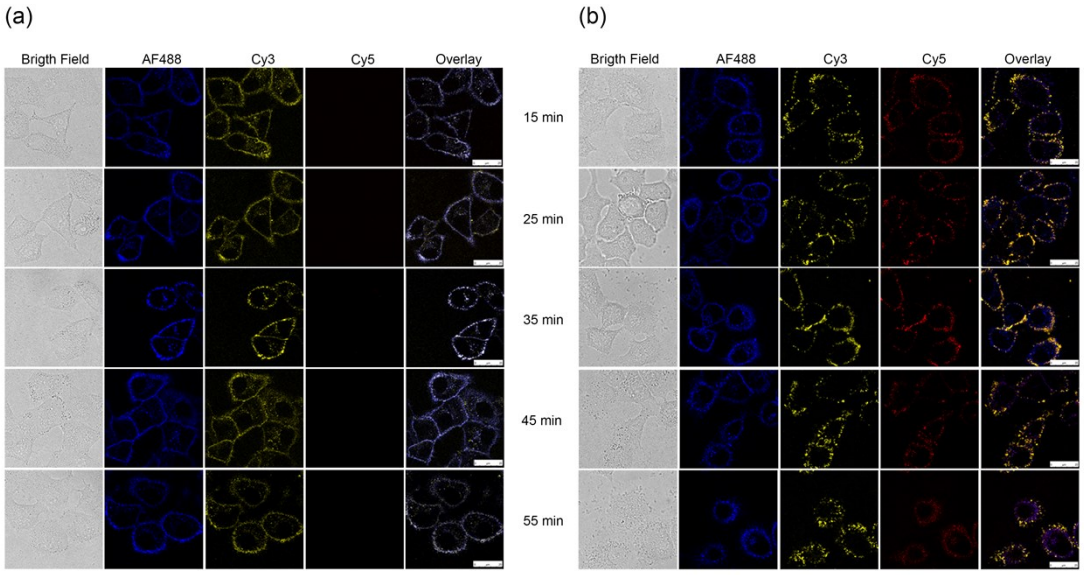


Fig. S7 Co-localization of the probe and cytomembrane at different incubation times. (a) Cells without HGF treatment. (b) Cells with HGF treatment. The fluorescence of the probe coincides well with that of AF488 on the cell membrane during 15 to 55 minutes, indicating that the probe can remain stable on cell membrane within 55 minutes. WGA-AF488 and the probe were respectively excited by 495nm and 525nm laser in different sequences. The scale bars are 25 μm.

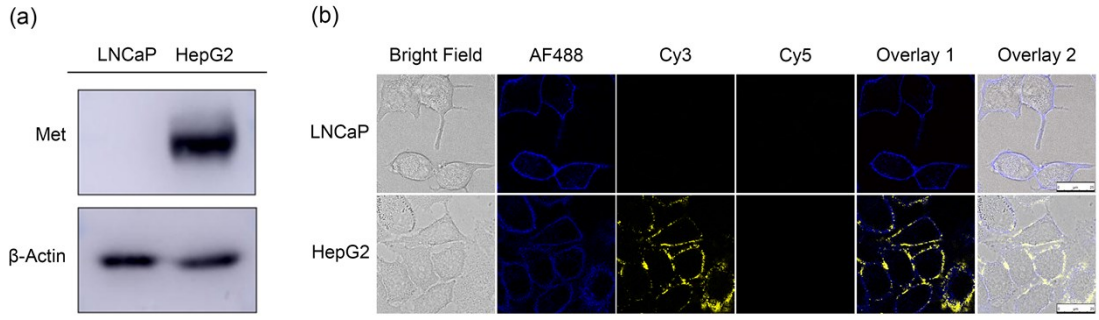


Fig. S8 (a) Western blot analysis of Met in LNCaP and HepG2 cells. (b) CLSM images of LNCaP and HepG2 cells. WGA-AF488 and the probe were respectively excited by 495nm and 525nm laser in different sequences. The scale bars are 25 μm.

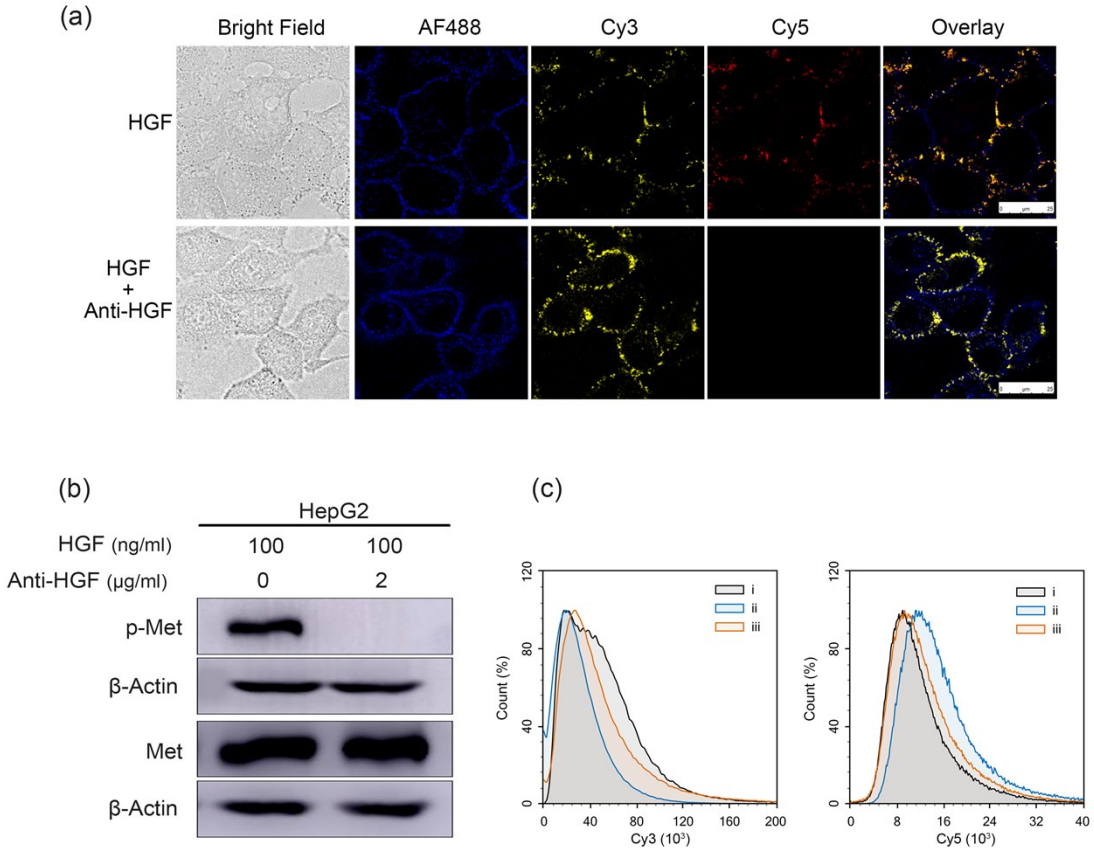
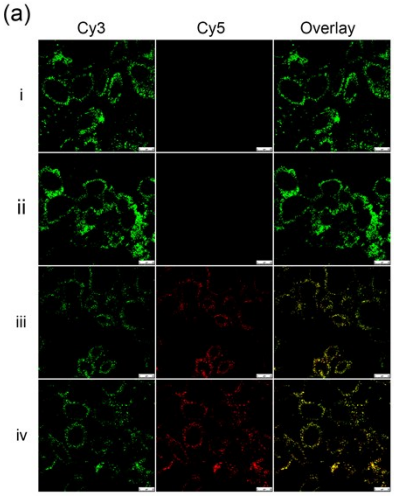


Fig. S9 (a) CLSM images of Met monomer and homodimer in HepG2 cells with different treatments. WGA-AF488 and the probe were respectively excited by 495nm and 525nm laser in different sequences. The scale bars are 25 μm. (b) Western blot of the Met (monomeric Met) and p-Met (homodimeric Met) in HepG2 cells with different treatments. (c) Flow cytometry analysis of HepG2 cells: i. addition of 0 ng/ml HGF, ii. addition of 100 ng/ml HGF, iii. addition of 100 ng/ml HGF that was pretreated with anti-HGF antibody.

(a)



(b)

	HGF (ng/ml)	0	10	50	100
CLSM	Fluo. of Cy3	27.4	30.04	13.6	13.27
	Fluo. of Cy5	2.66	2.56	10.47	13.44
	Cy5 (%)	9	8	43	50
	Increment of Cy5 (%)	—	**-1**	**35**	**7**
WB	IntDen of Met/Actin	1.10	1.05	1.35	1.14
	IntDen of p-Met/Actin	0.0187	0.0385	0.924	1.09
	p-Met (%)	2	4	41	49
	Increment of p-Met (%)	—	**2**	**37**	**8**
FCM	Mean intensity of Cy3	73806	72843	49337	48011
	Mean intensity of Cy5	14481	14452	16886	17975
	Cy5 (%)	16	17	25	27
	Increment of Cy5 (%)	–	**1**	**8**	**2**

Fig. S10 (a) CLSM images of Met monomers and homodimers on HepG2 cells treated with different concentrations of HGF (scale bars are 25 μm). (b) Relative quantitative analysis of fluorescent images (Fig. S10a), western blot bands (Fig. 2b) and flow cytometry (Fig. 2c).

For cells treated with 0 ng/ml, 10 ng/ml, 50 ng/ml and 100 ng/ml HGF, the percentage of Cy5 in CLSM increased by -1%, 35% and 7% in turn. The percentage of p-Met in western blot increased by 2%, 37% and 8% in turn. The percentage of Cy5 in flow cytometry increased by 1%, 8% and 2%, in turn. The relative fluorescence intensities of Cy5 and Cy3 in the CLSM images correspond well with the relative contents of p-Met and Met in western blot, indicating that the probe can in situ image target protein homodimerization on tumor cell surface with high sensitivity and accuracy. The trend of fluorescence change in flow cytometry is consistent with that of CLSM imaging and western blot, but it is not in good agreement with them quantitatively. It may be because that the cells need to be scraped and suspended for flow cytometry, which may disturb the protein-protein or probe-probe interactions.

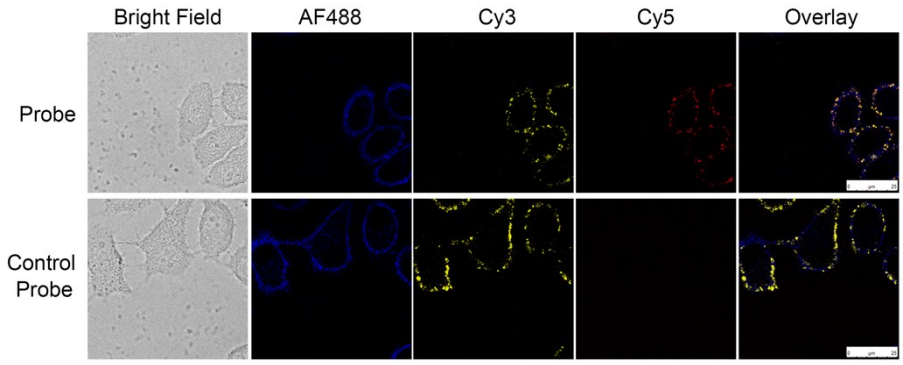


Fig. S11 CLSM images of HGF-treated HepG2 cells probed by the proposed probe and control probe. The control probe was constructed by replacing the half-i sequence in the proposed probe with a dyad strand (GTTTTTTGTTTTTT) which is shaped like half-i but has no acid-responsive capacity. WGA-AF488 and the probe were respectively excited by 495nm and 525nm laser in different sequences. The scale bars are 25 μm.

The cells imaged by the proposed probe show apparent fluorescence in both Cy3 and Cy5 channels to indicate the homodimerization of Met monomers. Cells imaged by the control probe show strong Cy3 signals and negligible Cy5 fluorescence even though the Met on HGF-treated cells has been proven to be dimerized. The results confirm that

the acid-responsive allostery of the half-i in the probe is the key to homodimerization imaging.

Supplementary Videos

Video S1 Visualization of Met receptor in HepG2 cells without HGF treatment (related to Fig. 3a, channel Cy3).

Video S2 Visualization of Met receptor in HepG2 cells without HGF treatment (related to Fig. 3a, channel Cy5).

Video S3 Visualization of Met receptor in HepG2 cells without HGF treatment (related to Fig. 3a, channel Overlay).

Video S4 Visualization of Met receptor in HepG2 cells with HGF treatment (related to Fig. 3b, channel Cy3).

Video S5 Visualization of Met receptor in HepG2 cells with HGF treatment (related to Fig. 3b, channel Cy5).

Video S6 Visualization of Met receptor in HepG2 cells with HGF treatment (related to Fig. 3b, channel Overlay).

References

[1] M. Z. Noman, S. Buart, J. V. Pelt, C. Richon, M. Hasmim, N. Leleu, W. M. Suchorska, A. Jalil, Y. Lecluse, F. E. Hage, M. Giuliani, C. Pichon, B. Azzarone, N. Mazure, P. Romero, F. M. Chouaib and S. Chouaib, *J Immunol.*, 2009, **182,** 3510-3521.

[2] (a) A. Singh and S. Kukreti, RSC Adv., 2018, 8, 34202-34214; (b) J. L. Leroy, M. Guéron, J. L. Mergny and C. Hélène, Nucleic Acids Res., 1994, 22, 1600-1606; (c) J. L. Mergny, L. Lacroix, X. Han, J. L. Leroy and C. Helene, J. Am. Chem. Soc., 1995, 117, 8887-8898.

[3] J. L. Mergny, J. Li, L. Lacroix, S. Amrane and J. B. Chaires, Nucleic Acids Res., 2005, 33, e138.