Supporting Information

Cancer Cells-Targeted Two-Photon Fluorescence Probe for the Real-time Ratiometric Imaging of DNA Damage

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Contents

1. Procedures section

A.R. grade of solvents and reagents were used in this work. Column chromatographic was used to purify compounds and silica gel (200-300 mesh) was used as fillers. Commercial fluorescent dye, DAPI was purchased from Life Technologies Co. (USA). γ-Glutamyltranspeptidase (**GGT**, Enzyme Commission Number: 2.3.2.2) and N-acetyltransferase (**NAT**, Enzyme Commission Number: 2.3.1.5) were obtained from Sigma Chemical Co. (USA). **GGT** Activity Colorimetric Assay Kit and **NAT** Like Protein Assay Kit were obtained from Sigma Chemical Co. (USA). Doubly purified water was used in all experiments, which was prepared using by a Milli-Q system. **ANF-Glu** as stock was used in spectrographic determination and cell experiments. HPLC (Agilent 1290 Infinity)-HRMS (Bruker microToF II, Bruker Co., Switzerland) with an auto sampler operated in-line with a quantum triple quadrupole instrument was carried out in mass spectral studies on ESI positive or negative ion mode. NMR spectra were obtained from Avance 400 and 600 MHz spectrometer (Bruker Co., Switzerland).

Quantum Calculations. Gaussian 09 was used in the entire quantum chemical. The density functional theory (DFT) with B3-LYP functional was used in the geometry optimizations of the dyes. 6-31G (d, p) basis set was used. The electronic transition energies and corresponding oscillator strengths were calculated by time-dependent density functional theory (TD-DFT) at the B3LYP/6-31G (d, p) level.

Cell Culture. HeLa, MCF-7, HepG2, DU145, A549, V79, CHO and NIH3T3 cells were obtained from the Chinese Academy of Medical Sciences. The red-free Dulbecco's Modified Eagle's Medium (DMEM, WelGene) and eagle's minimum essential medium (MEM, WelGene) supplemented with penicillin/streptomycin and 10 % fetal bovine serum (FBS; Gibco) were used for culture cells in a CO_2 incubator at 37 ℃. One day before imaging, the cells mentioned above were seeded into confocal dishes with well glass bottom (MatTek, 1# glass, 0.13-0.16 mm). They were incubated at 37 ℃ in 5.0 wt %/vol CO_2 for 24 h. And then, the cells were incubated with ANF-Glu at a certain concentration.

Fluorescence Imaging in Cells and in Vivo. Olympus spectral confocal multiphoton microscope (FV1200) with MaiTai femtosecond laser source (Spectra-Physics) was used in image experiment. Inverted microscope was used in cell imaging, and uprighted microscope was used in animal imaging. The imaging parameters are as follow. Internal PMTs = 16 bit, pixels = 1600 × 1600. Lasers: 800 nm and 405 nm. The scan ranges were ascertained according to fluorescence of every dye.

Colocalization Imaging. DAPI was used as standard dye for DNA. The cells were incubated with DAPI and **ANF-Glu** for 30 min under 5.0 wt % /vol CO_2 at 37 ℃. After then, they were washed with PBS three times. And the cells were imaged in 1600 × 1600 pixels. The fluorescence imaging was analyzed by colocalization coefficient.

GGT Activity Assay. All samples and standards should be run in duplicate. pNA Standards for Colorimetric Detection Add 0, 4.0, 8.0, 12, 16, and 20 mL of the 2.0 mM standard solution into a 96 well plate, generating 0 (blank), 8.0, 16, 24, 32, and 40 nmole/well standards. Add **GGT** Assay Buffer to each well to bring the volume to 100 mL.

(a) Sample Preparation: Tissue (10 mg) or cells (1×10^6) can be homogenized in 200 mL of ice-cold **GGT** Assay Buffer. Centrifuge the samples at 13,000 $\times$ *g* for 10 minutes to remove insoluble material. For the positive control, add 10 mL of the **GGT** positive control solution to wells. Bring samples to a final volume of 10 mL with **GGT** Assay Buffer.

(b) Assay Reaction: (1) Add 90 mL of **GGT** Substrate Solution to each well containing test samples. Do not add to pNA Standards. (2) Incubate the plate at 37 ℃. After 3.0 min, take the initial measurement (Tinitial). Measure the absorbance at 418 nm at the initial time $(A418)_{initial}$. (3) Continue to incubate the plate at 37 ℃ taking measurements (A418) every 5.0 min. Protect the plate from light during the incubation. (4) Continue taking measurements until the value of the most active sample is greater than the value of the highest standard (40 nmole/well). At this time the most active sample is near or exceeds the end of the linear range of the standard curve. (5) The final measurement $[(A418)_{final}]$ for calculating the enzyme activity would be penultimate reading or the value before the most active sample is near or exceeds the end of the linear range of the standard curve. The time of the penultimate reading is T_{final}.

(c) Results Calculations: Plot the pNA standard curve from the initial measurement ($T_{initial}$). Calculate the change in measurement from $T_{initial}$ to T_{final} for the samples. DA418 = $(A418)_{final}$ - $(A418)_{initial}$. Compare the DA418 of each sample to the standard curve to determine the amount of pNA generated between $T_{initial}$ and T_{final}.

NAT Activity Assay. The test principle applied in this kit is Sandwich enzyme immunoassay. The microtiter plate provided in this kit has been pre-coated with an antibody specific to N-Acetyltransferase Like Protein. Standards or samples are then added to the appropriate microtiter plate wells with a biotin-conjugated antibody specific to N-Acetyltransferase Like Protein. Next, Avidin conjugated to Horseradish Peroxidase (HRP) is added to each microplate well and incubated. After TMB substrate solution is added, only those wells that contain N-Acetyltransferase Like Protein, biotin-conjugated antibodyand enzyme-conjugated Avidin will exhibit a change in color. The enzyme-substrate reaction is terminated by the addition of sulphuric acid solution and the color change is measured spectrophotometric wavelength of 450 nm ± 10 nm. The concentration of N-Acetyltransferase Like Protein in the samples is then determined by comparing the O.D. of the samples to the standard curve.

(a) Prepare all reagents, samples and standards; (b) Add 100 µL standard or sample to each well. Incubate 2.0 h at 37 ^oC; (c) Aspirate and add 100 µL prepared Detection Reagent A. Incubate 1.0 h at 37 ^oC; (d) Aspirate and wash 3 times; (e) Add 100 µL prepared Detection Reagent B. Incubate 30 min at 37 ^oC; (f) Aspirate and wash 5 times; (g) Add 90 µL Substrate Solution. Incubate 15-25 min at 37 ^oC; (h) Add 50 µL Stop Solution. Read at 450 nm immediately.

Comet Assay for DNA Damage. CometAssay®, that is, single cell gel electrophoresis assay provides a simple and effective method for evaluating DNA damage in cells. The principle of the assay is based upon the ability of denatured, cleaved DNA fragments to migrate out of the nucleoid under the influence of an electric field, whereas undamaged DNA migrates slower and remains within the confines of the nucleoid when a current is applied. Evaluation of the DNA comet tail shape and migration pattern allows for assessment of DNA damage. The Neutral CometAssay® is typically used to detect double-stranded breaks, whereas the Alkaline CometAssay® is more sensitive, and is used to detect smaller amounts of damage including single and double-stranded breaks.

Partition Coefficients. The logarithms of the water/octanol partition coefficients (log *P* values) of **ANF-Glu**, **ANF** and **N-acetyl ANF** were estimated using the computational procedure of Hansch and Leo.[29] This procedure was used as it permits calculation of the log *P* values, as was required to discuss permeability, and also for use with the QSAR models. Log *P* estimations using the software available at this time do not provide values for ions, but only for the related nonionic bases.

Tumor Model in Mice. In this part work, Guide for the Care and Use of Laboratory Animal Resources and the National Research Council were performed. The human cancer cell lines (HeLa) were used for in vivo studies. 1×10^6 to 2×10^6 cells in 200 to 300 μL of PBS were injected of in nude mice by subskin. After 20 days (tumor grew to about 0.5 mm in size), mice bearing tumor were performed in experimentse. Imaging tumor (HeLa cells) with **ANF-Glu** (30 μM) by injected intravenously in vivo, incubating time: 30 s, 10 min and 3 days.

Spectrographic determination in vitro

A Lambd 950 spectrophotometer from PerkinElmer (USA) and a LS-55 spectrophotometer from PerkinElmer (USA) were used to measure absorption spectra and fluorescence spectra, respectively. In all spectral experiments, the final solutions contained < 5 ‰ DMSO. Each experiment was carried out in five replicates (n = 5). The relative fluorescence quantum yields were determined using Rhodamine B (Φ_F = 0.97 in methanol) by the following equation:

$$\Phi_x = \Phi_s(F_x/F_s)(A_s/A_x)(\lambda_{exs}/\lambda_{exx})(n_x/n_s)^2 \quad (1)$$

where Φ represents quantum yield; F is the integrated area under the corrected emission spectrum; A is absorbance at the excitation wavelength; λex is the excitation wavelength; n is the refractive index of the solution (because of the low concentrations of the solutions, 10^{-7}-10^{-8} mol/L, the refractive indices of the solutions were replaced with those of the solvents); and the subscripts x and s refer to the unknown and the standard, respectively. The detection limit was calculated by three times the standard deviation divided by the slope of the blank. The data were obtained from replicate experiments (n=5)

Cytotoxicity.

Cells were prepared for cell viability studies in 96-well plates (1×10^5 cells per well that were incubated in 100 μL). The cells were incubated for an additional 24 h with dye molecules **ANF-Glu**, **ANF** and **N-acetyl ANF** in different concentrations. Subsequently, 100 μL of 3-(4,5-dimethylthiazol-2-yl)-2,5-diphenyltetrazolium bromide (MTT, Sigma Chemical Co. USA) was added into each well, followed by further incubation for 4.0 h at 37 ℃. The DMEM or MEM was remove and DMSO (200 μL/well) added to dissolve the reddish-blue crystals. Optical density (OD) was determined by a microplate reader (Spectra Max M5, Molecular Devices) at 570 nm with subtraction of the absorbance of the cell-free blank volume at 630 nm.

The results from the six individual experiments were averaged. The relative cell viability (100%) was calculated using the following equation:

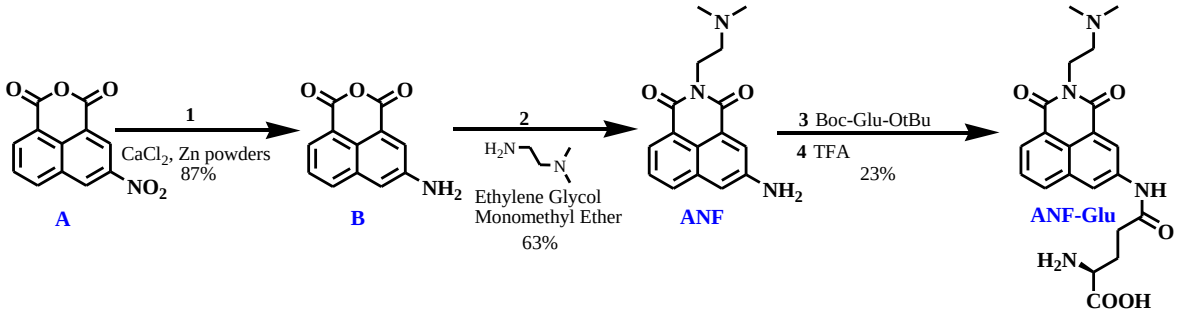
$$\text{cell viability (\%)} = (OD_{dye} - OD_{K\ dye})/(OD_{control} - OD_{K\ control}) \times 100 \quad (2)$$

where dye stands for the sample containing **ANF-Glu**, **ANF** and **N-acetyl ANF**.

Flow Cytometry.

Cells were cultured in ifferent kinds of mediums supplemented with 10 % FBS under an atmosphere of 5.0 % CO_2 and 95 % air at 37 ℃. After incubation at 37 ℃ with 5.0 % CO_2 for 1-2 days to reach 70-90% confluency, the medium was removed. Then the cells were washed with 2.0 mL of PBS buffer, and 2.0 mL of fresh different kinds of mediums was added along. Cells were incubated for 12 h prior to FCM analysis. Samples were illuminated with a sapphire laser at 488 nm on a FACScan flowcytometer (BD Biosciences Pharmingen, U.S.A.). The fluorescence of the forward- and side-scattered light from 10000 cells was detected at rate of 150 events/second. FCM data were analyzed with FACSDiva software.

2. Synthetic route of ANF-Glu



Scheme S1. The synthetic route of **ANF-Glu**.

3. Synthetic procedures of ANF-Glu and intermediates

The synthesis of A

Synthesis of **A** was synthesized by the previously reported method [S1]. Yield 93 %. Mp 247 ^{o}C. ^{1}H NMR (400 MHz, DMSO-d6), δ: 8.95 (d, *J* = 8.30 Hz, 1H), 8.48 (d, *J* = 6.80 Hz, 1H), 8.32 (d, *J* = 8.50 Hz, 1H), 7.74 (m, 1H), 6.95 (d, *J* = 8.70 Hz, 1H). ^{13}C NMR (600 MHz, DMSO-d6) *δ*: 149.51, 142.63, 134.21, 133.70, 128.50, 126.89, 125.20, 123.90. ESI-MS: m/z calcd for $C_{12}H_5NO_3$: 243.0168, found: 243.0175.

The synthesis of B

To **A** (1.0 mmol) in ethanol (20 mL), excessive Zn powders and crystallization of calcium chloride were added and the mixture was refluxed for 1.5 h. After then, the solvent was removed. The solid was purified by silica gel column chromatography by using CH_3OH/CH_2Cl_2=1:10 as eluent, affording **B** as yellow solid. Yield 87 %. Mp > 300^{o}C. ^{1}H NMR (400 MHz, DMSO-d6), δ: 8.95 (d, *J* = 8.30 Hz, 1H), 8.48 (d, *J* = 6.80 Hz, 1H), 8.32 (d, *J* = 8.50 Hz, 1H), 7.74 (m, 1H), 6.95 (d, J = 8.70 Hz, 1H), 3.42(d, J = 6.60 Hz, 2H). ^{13}C NMR (600 MHz, DMSO-d6) *δ*: 149.49, 142.65, 134.27, 133.73, 128.54, 126.92, 125.24, 123.93. $C_{12}H_7NO_3$: 213.0426, found: 213.0415.

The synthesis of ANF

To **B** (1.0 mmol) in hot ethyl alcohol (20 mL), N, N-dimethyl ethylene diamine (3.0 mmol) was added and the mixture refluxed for 7.0 h and then allowed to cool. A yellow solid precipitated and was filtered off and crystallized from chlorobenzene to yield the desired compound **ANF**. Yield 63 %. Mp: 170 ^{o}C. ^{1}H NMR (400 MHz, DMSO-d6), δ: 8.95 (d, *J* = 8.30 Hz, 1H), 8.48 (d, *J* = 6.80 Hz, 1H), 8.32 (d, *J* = 8.50 Hz, 1H), 7.74 (m, 1H), 6.95 (d, *J* = 8.70 Hz, 1H), 3.82 (t, *J* = 5.90 Hz, 2H), 3.62 (d, *J* = 5.60 Hz, 2H), 3.42(d, *J* = 6.60 Hz, 2H) 3.14-2.97 (m, 6H). ^{13}C NMR (600 MHz, DMSO-d6) *δ*:

159.49, 144.65, 134.27, 132.73, 130.61, 127.54, 125.41, 123.92, 123.74, 116.63, 115.97, 55.34, 47.12, 45.61. $C_{16}H_{17}N_3O_2$: 283.1321, found: 283.1325.

The synthesis of ANF-Glu

To **ANF** (1.0 mmol) in N, N-dimethylformamide (DMF, 20 mL), 1-(3-dimethylaminopropyl)-3-ethylcarbodiimide hydrochloride (EDCl) (0.70 mmol), and 4-dimethylaminopyridine (DMAP) (0.07 mmol) were added. The solution was stirred at room temperature under nitrogen for 24 h and then concentrated. After evaporation of the solvent, the residue was dissolved in 2 mL dichloromethane (CH_2Cl_2) and 2 mL trifluoroacetic acid (TFA). The reaction mixture was stirred at room temperature for 1.0 h. After evaporation of the solvent, the residue was purified by semi-preparative HPLC using eluent A (0.1 % TFA in H_2O) and eluent B (80:20 CH_3CN:H_2O) (A/B = 80:20 to 0:100, 40 min) to yield **ANF-Glu** which appears as a yellow powder. Yield 23 %. Mp > 300 ^oC. ^1H NMR (400 MHz, DMSO-d6), δ: 9.97 (s, 1H), 8.95 (d, *J* = 8.30 Hz, 1H), 8.48 (d, *J* = 6.80 Hz, 1H), 8.32 (d, *J* = 8.50 Hz, 1H), 8.17(s, 1H), 7.74 (m, 1H), 6.95 (d, *J* = 8.70 Hz, 1H), 4.36 (t, *J* = 5.90 Hz, 2H), 3.82 (d, *J* = 5.60 Hz, 2H), 3.62 (m, 3H), 3.41 (m, 3H), 3.14-2.97 (m, 6H), 2.85 (m, 4H). ^{13}C NMR (600 MHz, DMSO-d6) *δ*: 174.90, 173.01, 159.49, 140.65, 134.27, 132.73, 130.61 125.54, 123.92, 123.64, 116.93, 116.87, 55.30, 54.91, 47.20, 45.63, 30.43, 29.63. $C_{21}H_{24}N_4O_5$: 412.1747, found: 412.1739.

4. Reaction rate and influence of ANF-Glu with GGT and NAT

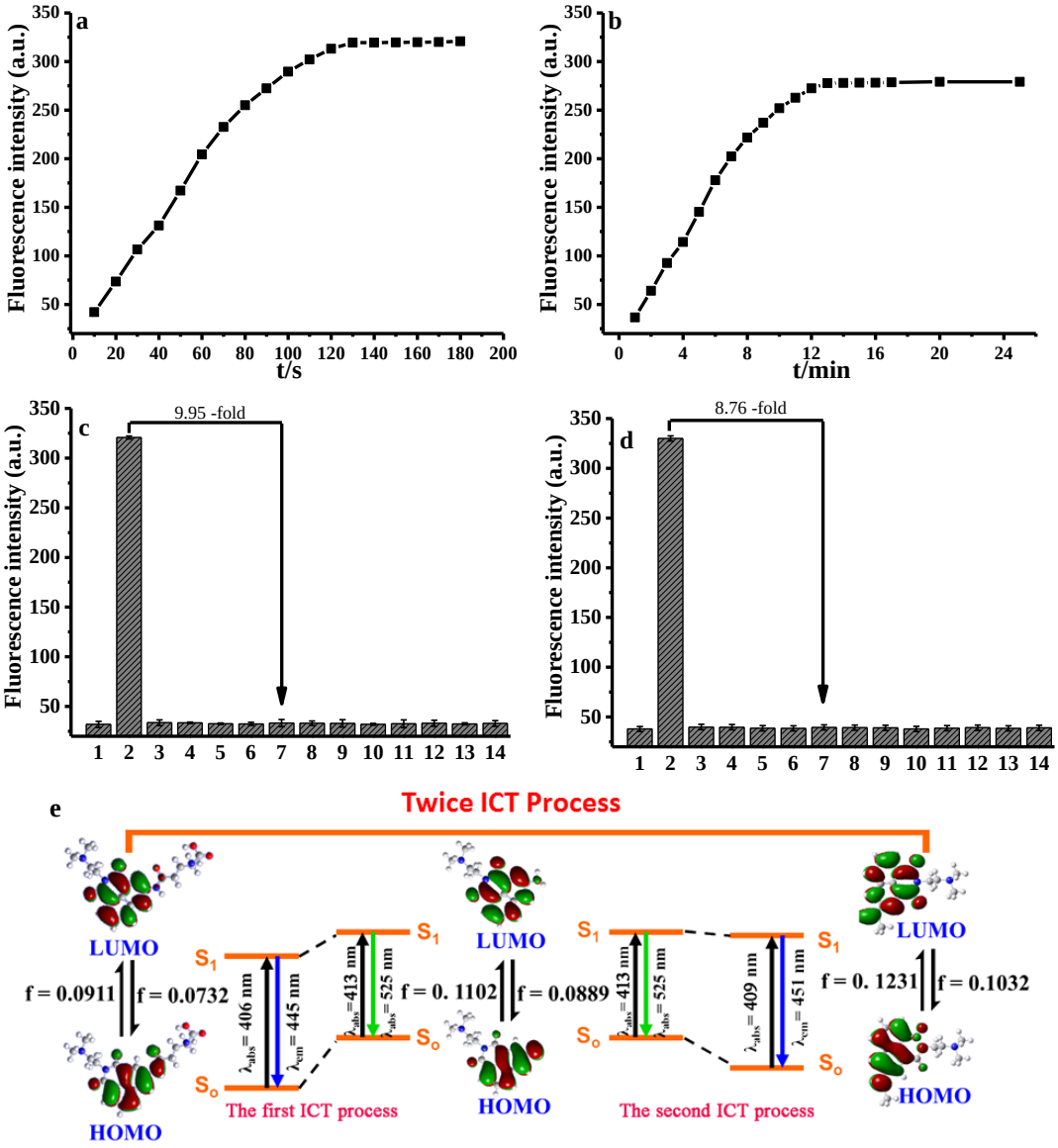


Figure S1. (a) The reaction rate of **ANF-Glu** (3.0 μM) with **GGT** (1000 U/L). (b) The reaction rate of probe with **NAT** (200 U/L) (c) The influence of transferases for the recognition of **ANF-Glu** for **GGT**. The influence of transferases in b: 1. Control, 2. **GGT** (1000 U/L), 3. P-Beta Aminopropionaldehyde, 4. amino transferase, 5. NAT, 6. Cyclodextrin glucanotransferase, 7. Alpha-Cyclodextringlucosyltransferase, 8. Dnmt3a(cytosine-5)-methyltransferase 3A), 9. α-Glucosyltransferasetreated stevia, 10. Kinase (phosphorylating), 11. Glutamic oxalacetic transaminase, 12. Acyltransferase, 13. lactose synthetase, 14. Phosphotransacetylase; (d) The influence of transferases for probe in the the presence of acetyl-CoA: 1. Control + **GGT**, 2. **NAT** (200 U/L) + **GGT**, 3. P-Beta Aminopropionaldehyde + **GGT**, 4. amino transferase + **GGT**, 5. **GGT**, 6. Cyclodextrin glucanotransferase + **GGT**, 7. Alpha-Cyclodextringlucosyltransferase + **GGT**, 8. Dnmt3a(cytosine-5)-methyltransferase 3A) + **GGT**, 9. α-Glucosyltransferasetreated stevia + **GGT**, 10. Kinase (phosphorylating) + **GGT**, 11. Glutamic oxalacetic transaminase + **GGT**, 12. Acyltransferase + **GGT**, 13. lactose synthetase + **GGT**, 14. Phosphotransacetylase + **GGT**. Condition: Fluorescence intensity at 531 nm; phosphate buffer (pH 7.4, 25^{o}C).d, pH (3-11) influence on the fluorescent signals. Data were obtained from replicate experiments (n = 5). (e) Frontier molecular orbital of **ANF-Glu**, **ANF** and **N-acetyl ANF** calculated by Gaussian 09 software (DFT/TDDFT in B3LYP/ 6-31G (d, p) level)

5. The basic optical data of ANF-Glu with GGT and NAT

Table S1. The data of ANF-Glu, ANF and N-acetyl ANF

	ANF-Glu	**ANF**	**N-acetyl ANF**
Fomula	$C_{21}H_{24}N_4O_5$	$C_{16}H_{17}N_3O_2$	$C_{18}H_{19}N_3O_3$
λ_{ex}/nm	417	423	428
ε / M^{-1} cm^{-1}	9678	18400	10850
λ_{em}/nm	441	530	455
Φ	0.135	0.39	0.134
Lowest detection limit for **GGT**/ U/L	182	-	-
Octanol-water partition coefficient (log *P*)	-2.9	1.18	-0.5

* QSAR model: entering into cells log *P* > 0

6. Photostability and cytotoxicity of ANF-Glu, ANF and N-acetyl ANF

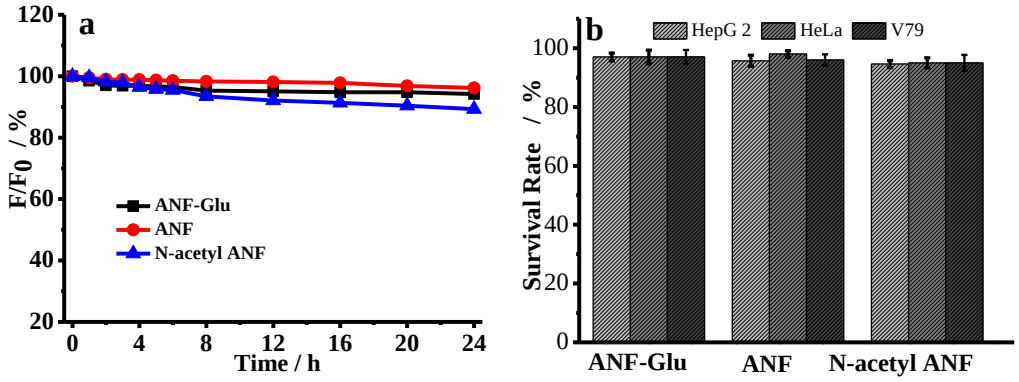


Figure S2. (a) The photostability of **ANF-Glu** (3.0 μM), **ANF** (3.0 μM) and **N-acetyl ANF** (3.0 μM). (b) Cytotoxicity toxicity of **ANF-Glu** (3.0 μM) and **ANF** (3.0 μM) and **N-acetyl ANF** (3.0 μM).

And after 24 h incubation with ANF-Glu, there was still greater than 93% living cells

7. Two-photon action cross section of ANF-Glu, ANF and N-acetyl ANF

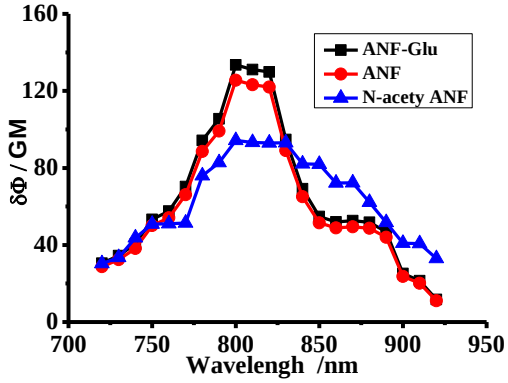


Figure S3. Two-photon action spectra of **ANF-Glu**, **ANF** and **N-acetyl ANF** in PBS buffer (pH 7.4) at 25 ^{o}C. Data are from replicate experiments (n = 5).

The maximal two-photon action cross section (Φδ) of probe is 133 GM (1 GM = 10^{-50} cm^4 s/photon) at 800 nm.

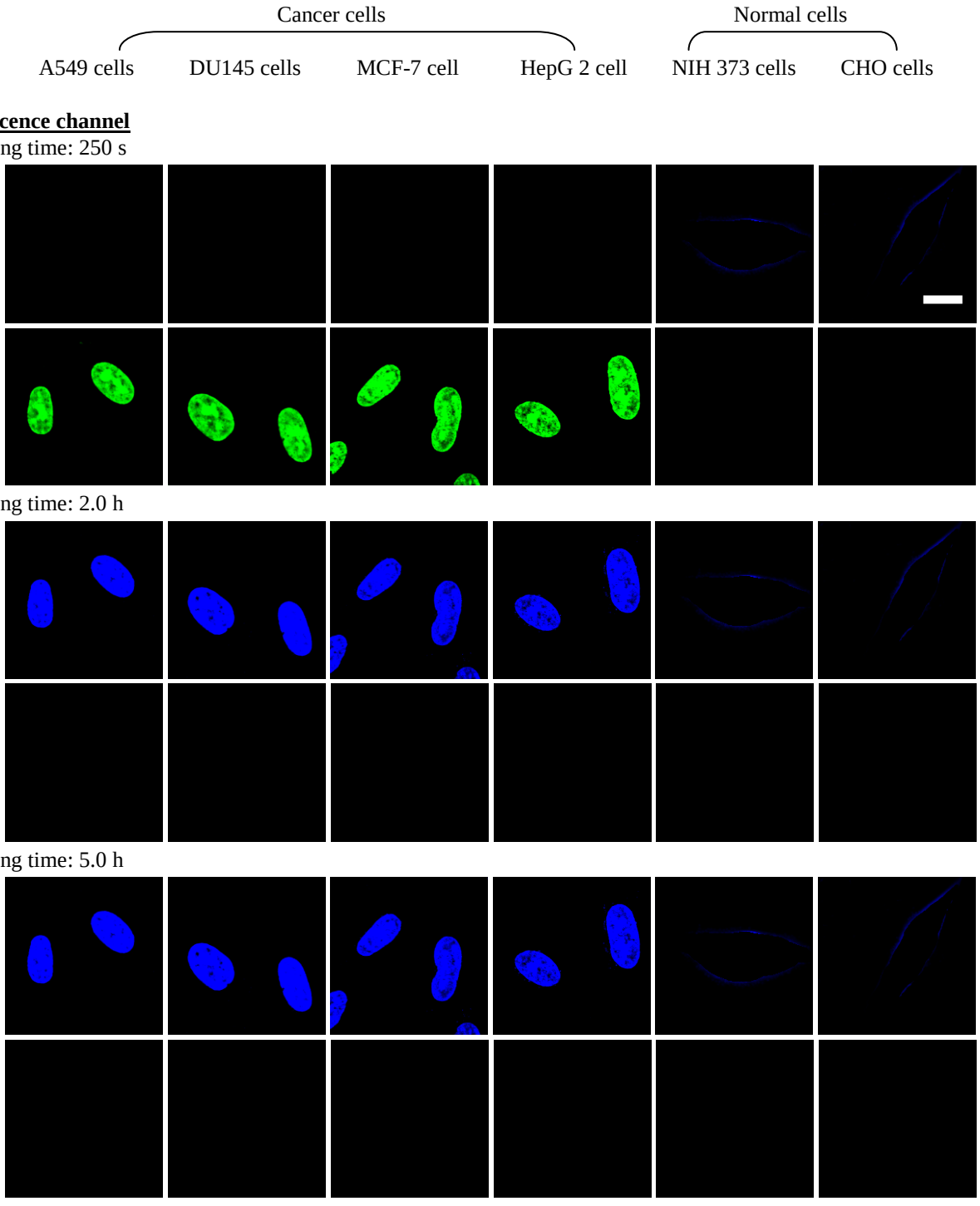
8. DNA damage assay and NAT activity in living cells

Table S2. DNA damage assay[1] and NAT activity[2] in living cells

	DNA damage	Normal DNA
Olive Tail Moment[1]	57.65 (DNA damage degree > 70%)	1.23 (DNA damage degree < 5%)
NAT activity[2] (U/L)	483.1	27.12

[1] The comet assay; [2] The enzyme-linked immunosorbent assay

9. Living cells stained with ANF-Glu



Light channel

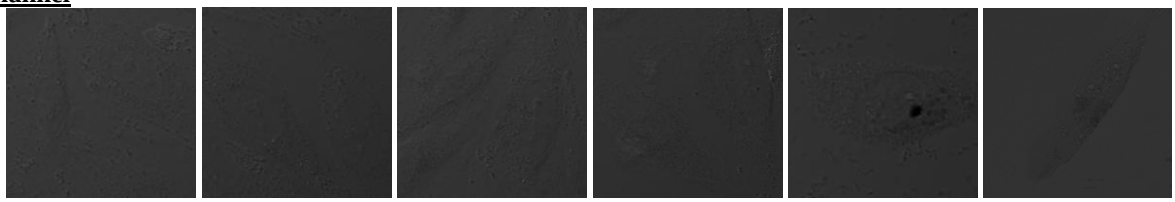


Figure S4. Living cells stained with **ANF-Glu** (3.0 μM). Excitation wavelength = 800 nm; incubating time: 250 s and 2.0 h; scale bar, 10.0 μm. Images are representative of replicate experiments (n = 5).

10. The assay of GGT in cells by enzyme-linked immunosorbent assay and flow cytometry

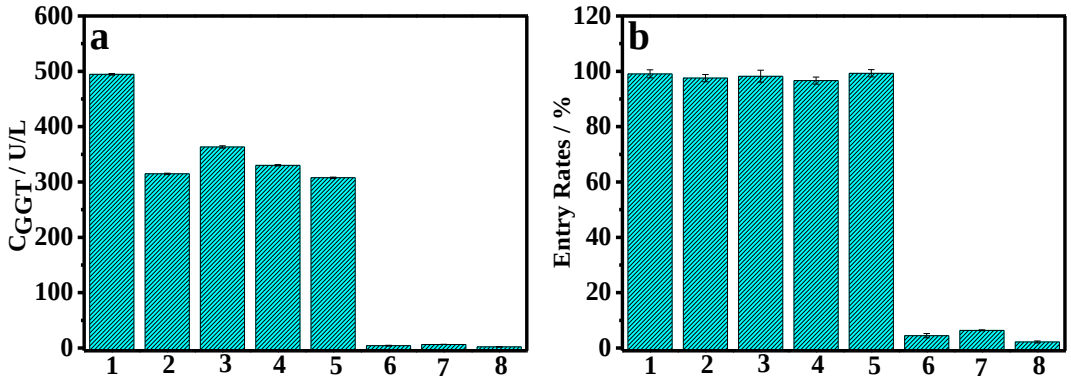


Figure S5. (a) The **GGT** activity in cancer and normal cells detected by **GGT** Activity Colorimetric Assay Kit. (b) The entry rates of **ANF-Glu** into cells detected by flow cytometry. 1. Hela cell line, 2. 549 cell line, 3. DU145 cell line, 4. MCF-7 cell line, 5. HepG 2 cell line, 6. V79 cell line, 7. NIH-3T3 cell line, 8. CHO cell line. Data are representative of replicate experiments (n = 5).

11. Moleclaur docking

a. the moleclaur docking result of ANF-Glu with DNA

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* L I G A N D F I T - S C O R E *
* *
* Version 2.4 *
* Copyright (C) 2009 *
* *
* 2009 Accelrys Software Inc. *
* www.accelrys.com *
* *
* Build date: Jun 13 2009 22:12:30 *
****************************************
Obtained docking license for ligfit_ext...
Checked out license feature: DS_LigandFit <v2009.03> [for for DS 2.0] (1 copy)
Run started at: Thu Dec 04 19:01:53 2014 on: PC0420YIU win32
Read docking and energy parameter file: LF_param.txt
FLEX_DOCKING 1
NUM_POSE_SAVED 10
SAMPLE_ALL_ORIENTATION 1
RBM_ITER_SD 10
RBM_ITER_BFGS 20
RBM_ITER_FINAL 0
RBM_LINE_CONVG 0.000100
RBM_ENG_CONVG 0.010000
RBM_ITER_FINAL 0
SITE_PARTITION_NUMBER 1
SITE_PARTITION_SEED 1
SITE_MATCH_RMS 2.000000
SITE_FUSING_FLAG 0
SITE_MERGE_RMS 2.000000
SITE_PARTITION_OVERRIDE 0
DIVERSE_SAVE_FLAG 0
DIVERSE_SAVE_RMS 1.500000
DIVERSE_SAVE_ENG 20.000000
CLUSTER_FLAG 0
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CLUSTER_NUMBER 5

CLUSTER_RMS 1.500000

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ENG_GRID_SPACING 0.500000

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ENG_PROT_SIZEC 2.500000

ENG_MODE dreiding

ENG_CFF_LIB

NONBOND_CUTOFF_DIS 10.000000

ENG_DIELE 1.000000

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ENG_USE_DDE 1

ENG_ALPHA 1.000000

ENG_BETA 0.050000

ENG_KAPPA 2.000000

ENG_GRID_PENALTY 0.000000

Read site data file: BindingSite.txt

Site partition: 1 site partition(s) produced

Site partition(s) from site data file: 1

Prepare dreiding grid, size: 29 23 34

Grid calculation finished in 1.0[s]

Monte Carlo Docking Parameters:

TORSION ITERATIONS STOP_STEPS

(1) 2 500 120

(2) 4 1200 300

(3) 6 1500 350

(4) 10 2000 500

(5) 25 3000 750

(6) 100 0 0

Molecule: **ANF-Glu**

Molecule no. : 1

Rotatable bonds: 10

MC termination values: 20000 / 5000

Flexible Docking ... done 48.1[s]

MC termination condition encounted. Stopped at 18027 iterations.

Poses rejected by DockScore filtering: 10

Poses saved: 0

Total Poses rejected by DockScore filtering: 10

Total elapsed time: 0:49 [min:sec]

Run completed at: Thu Dec 04 19:02:42 2014

Released license for ligfit_ext...

b. the moleclaur docking result of ANF-Glu with DNA under transferance of GGT

Obtained Scoring license for ligfit_ext...

Checked out license feature: DS_LigScore <v2009.03> [for for DS 2.0] (1 copy)

C:\Program Files\Accelrys\PipelinePilot\apps\scitegic\dscore\server\share/SBD/\pmf.par

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* L I G A N D F I T - S C O R E *

* *

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* *

* Build date: Jun 13 2009 22:12:30 *

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Running LigandScore on: PC0420YIU win32

Run started at: Thu Dec 04 19:01:17 2014

10 molecules in sd file: ligfit_in.sd

Reading Receptor File: 1bna.pdb

Receptor atoms: 998

Molecule: Molecule-1

Molecule: Molecule-1

Molecule: Molecule-1

Molecule: Molecule-1

Molecule: Molecule-1

Molecule: Molecule-1

Molecule: Molecule-1

Molecule: Molecule-1

Molecule: Molecule-1

Molecule: Molecule-1

Scored 10 molecules in: 0.1 [sec]

Molecules written: 10

Run ended at: Thu Dec 04 19:01:18 2014

Released license for ligfit_ext...

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* L I G A N D F I T - S C O R E *

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* Build date: Jun 13 2009 22:12:30 *

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Obtained docking license for ligfit_ext...

Checked out license feature: DS_LigandFit <v2009.03> [for for DS 2.0] (1 copy)

Run started at: Thu Dec 04 19:00:52 2014 on: PC0420YIU win32

Read docking and energy parameter file: LF_param.txt

FLEX_DOCKING 1

NUM_POSE_SAVED 10

SAMPLE_ALL_ORIENTATION 1

RBM_ITER_SD 10

RBM_ITER_BFGS 20

RBM_ITER_FINAL 0

RBM_LINE_CONVG 0.000100

RBM_ENG_CONVG 0.010000

RBM_ITER_FINAL 0

SITE_PARTITION_NUMBER 1

SITE_PARTITION_SEED 1

SITE_MATCH_RMS 2.000000

SITE_FUSING_FLAG 0

SITE_MERGE_RMS 2.000000

SITE_PARTITION_OVERRIDE 0

DIVERSE_SAVE_FLAG 0
DIVERSE_SAVE_RMS 1.500000
DIVERSE_SAVE_ENG 20.000000
CLUSTER_FLAG 0
CLUSTER_NUMBER 5
CLUSTER_RMS 1.500000
LIG_ES_FLAG 0
LIG_REJECT_ENG 10000.000000
POLAR_H_STEP 30.000000
DOCK_SCORE_FILTER 0.000000
ENG_GRID_EXT 3.000000
ENG_GRID_SPACING 0.500000
ENG_PROT_SIZEH 2.000000
ENG_PROT_SIZEC 2.500000
ENG_MODE dreiding
ENG_CFF_LIB
NONBOND_CUTOFF_DIS 10.000000
ENG_DIELE 1.000000
ENG_USE_SOFT 1
ENG_USE_DDE 1
ENG_ALPHA 1.000000
ENG_BETA 0.050000
ENG_KAPPA 2.000000
ENG_GRID_PENALTY 0.000000
Read site data file: BindingSite.txt
Site partition: 1 site partition(s) produced
Site partition(s) from site data file: 1
Prepare dreiding grid, size: 29 23 34
Grid calculation finished in 1.0[s]
Monte Carlo Docking Parameters:
TORSION ITERATIONS STOP_STEPS
(1) 2 500 120
(2) 4 1200 300
(3) 6 1500 350
(4) 10 2000 500
(5) 25 3000 750
(6) 100 0 0

Molecule: **ANF**

Molecule no. : 1

Rotatable bonds: 3

MC termination values: 3600 / 900

Flexible Docking ... done 22.3[s]

Molecule Pose Score dPMI

1 1 9.78 1.18

1 2 9.01 1.38

1 3 8.51 1.40

1 4 8.34 1.48

1 5 8.31 1.43

1 6 7.99 1.34

1 7 7.88 1.49

1 8 7.75 1.29

1 9 7.63 1.47

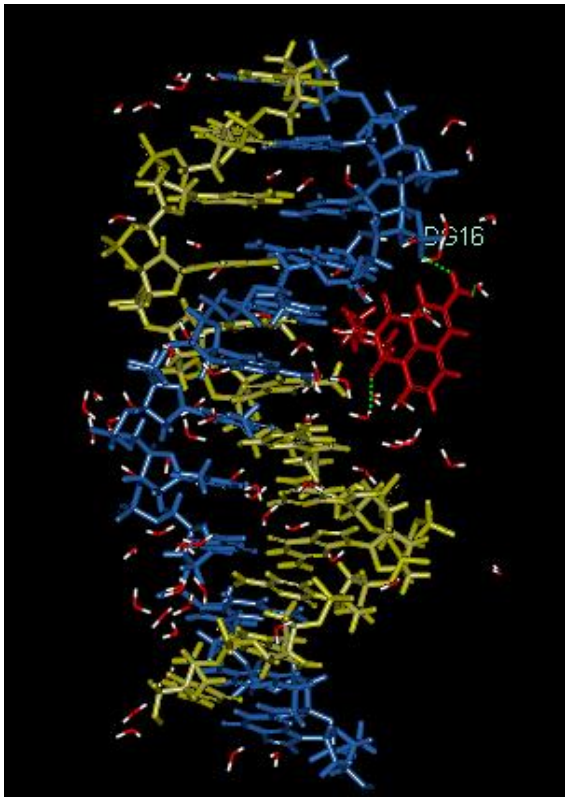
1 10 7.53 1.28

Poses saved: 10

Total elapsed time: 0:24 [min:sec]

Run completed at: Thu Dec 04 19:01:16 2014

Released license for ligfit_ext



Parameters

Input Receptor	../Input/1bna.dsv
Input Binding Site	../Input/BindingSite.txt
Input Ligands	../Input/untitled.sd
Input Control Ligands	
Input Preserve Controls In Input	True
Input Control Ligand Property	Control
Energy Grid	Dreiding
Energy Grid Extension from Site	3.0
Energy Grid Outside Binding Site Penalty	0.0
Energy Grid Softened Potential Energy	True
Energy Grid Dielectric Constant	1.0
Energy Grid Nonbonded Cutoff Distance	10.0

Energy Grid Distance Dependent Dielectric	True
Number of Monte Carlo Trials	"2 500 120, 4 1200 300, 6 1500 350, 10 2000 500, 25 3000 750"
Conformation Search	
Conformation Search Include Electrostatic Energy	False
Conformation Search Torsional Step Size for Polar H	30.0
Conformation Search Maximum Internal Energy	10000.00
Docking	Docking
Docking RMS Threshold for Ligand/Site Match	2.0
Docking Site Fusing RMS Cutoff	
Docking Rigid Body SD Iterations	10
Docking Rigid Body BFGS Iterations	20
Maximum Poses Retained	10
Pose Saving	
Pose Saving DockScore Threshold	0.0
Pose Saving Rigid Body BFGS Iterations	0
Pose Saving RMS Threshold for Diversity	1.50
Pose Saving Score Threshold for Diversity	20.0
Pose Saving Perform Clustering	False
Pose Saving Maximum Clusters per Molecule	5
Pose Saving RMS Threshold for Clustering	1.5
Interaction Filters	
Interaction Filters Minimum Number of Features	0
Interaction Filters Donor Include SH	True
Interaction Filters Acceptor Include Aromatic F	True
Interaction Filters Metal include S	True
Interaction Filters Save Only Poses with Minimum Features	False
Minimization Algorithm	Do not minimize
Minimization Sphere of Flexible Atoms	

Minimization Forcefield	CHARMm
Minimization Iterations	1000
Minimization Dielectric Constant	1.0
Minimization Distant-Dependent Dielectrics	True
Minimization Energy Tolerance	0.0
Minimization Gradient Tolerance	0.001
Minimization Nonbond Cutoff	13.0
Scoring Functions	LigScore1, LigScore2, PLP1, PLP2, Jain, PMF
LigScore	
Scoring LigScore Forcefield	Dreiding
Scoring LigScore Energy Grid Extension	5.0
Scoring LigScore1 Label	LigScore1
Scoring LigScore1 Minimum Score	
Scoring LigScore1 Report Individual Contributions	False
Scoring LigScore2 Label	LigScore2
Scoring LigScore2 Minimum Score	
Scoring LigScore2 Report Individual Contributions	False
PLP	
Scoring PLP1 Label	-PLP1
Scoring PLP1 Minimum Negative Score	
Scoring PLP2 Label	-PLP2
Scoring PLP2 Minimum Negative Score	
Jain	
Scoring Jain Label	Jain
Scoring Jain Minimum Score	
Scoring Jain Ignore Non-Polar Hydrogens	False
Scoring Jain Ignore Water	False
PMF	
Scoring PMF Label	-PMF
Scoring PMF Minimum Negative Score	

Scoring PMF Ignore Hydrogens	True
Scoring PMF Carbon-Carbon Cutoff	12.0
Scoring PMF Other Interactions Cutoff	12.0
PMF04	
Scoring PMF04 Label	-PMF04
Scoring PMF04 Minimum Negative Score	
Scoring PMF04 Ignore Hydrogens	True
Scoring PMF04 Carbon-Carbon Cutoff	6.0
Scoring PMF04 Other Interactions Cutoff	9.0
Ludi	
Scoring Ludi Score Label	Ludi
Scoring Ludi 1 Minimum Score	
Scoring Ludi 2 Minimum Score	
Scoring Ludi 3 Minimum Score	
Scoring Ludi Report Individual Contributions	False
Rotated Atoms Property	Receptor.Positions, Gold.Protein.RotatedAtoms
Parallel Processing	False
Parallel Processing Batch Size	25
Parallel Processing Server	localhost
Parallel Processing Server Processes	2
Parallel Processing Preserve Order	True

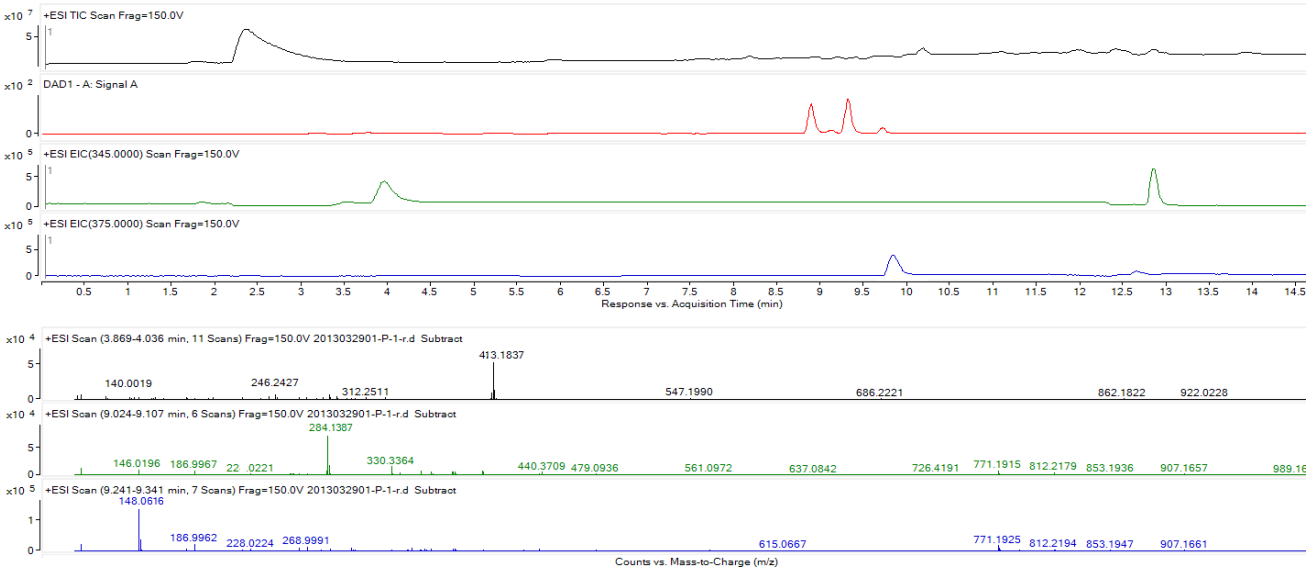
Reference:

S1. Cao, H.; Diaz, D. I.; DiCesare, N.s; Lakowicz, J. R.; Heagy, M. D. Organic Letters, 4, 1503-1505 (2002).

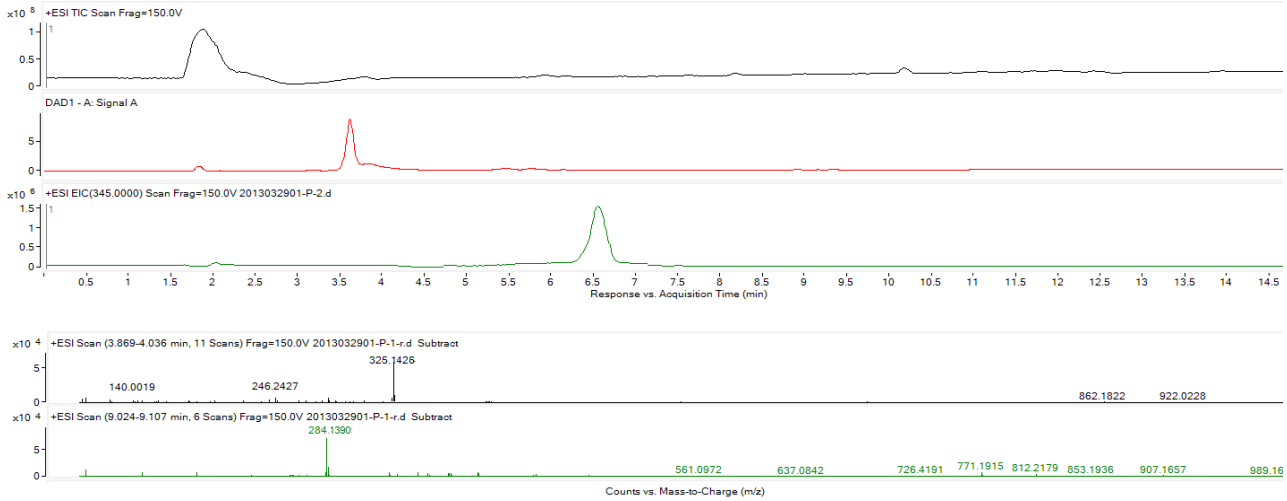
Attached Spectra

1. HPLC-HRMS

(1) AFN-Glu with GGT



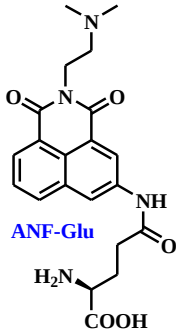
(2) AFN with NAT in the presence of acetyl-CoA

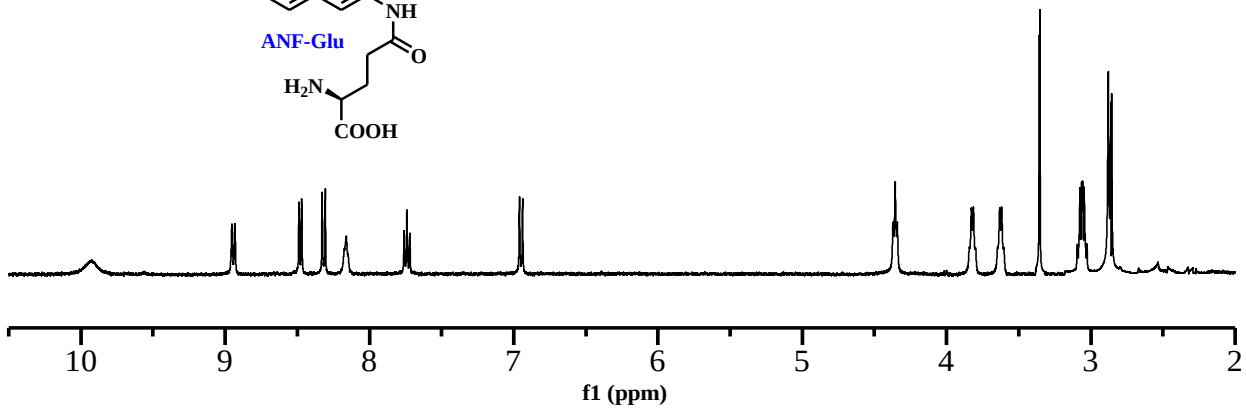


2. NMR

^{1}H NMR

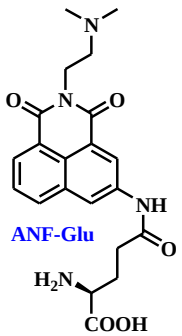
ANF-Glu

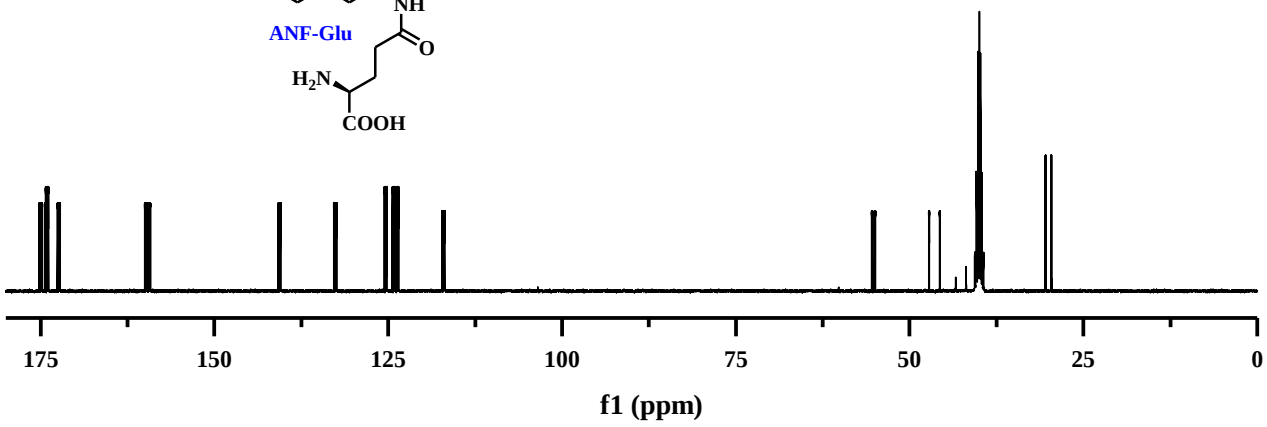




^{13}C NMR

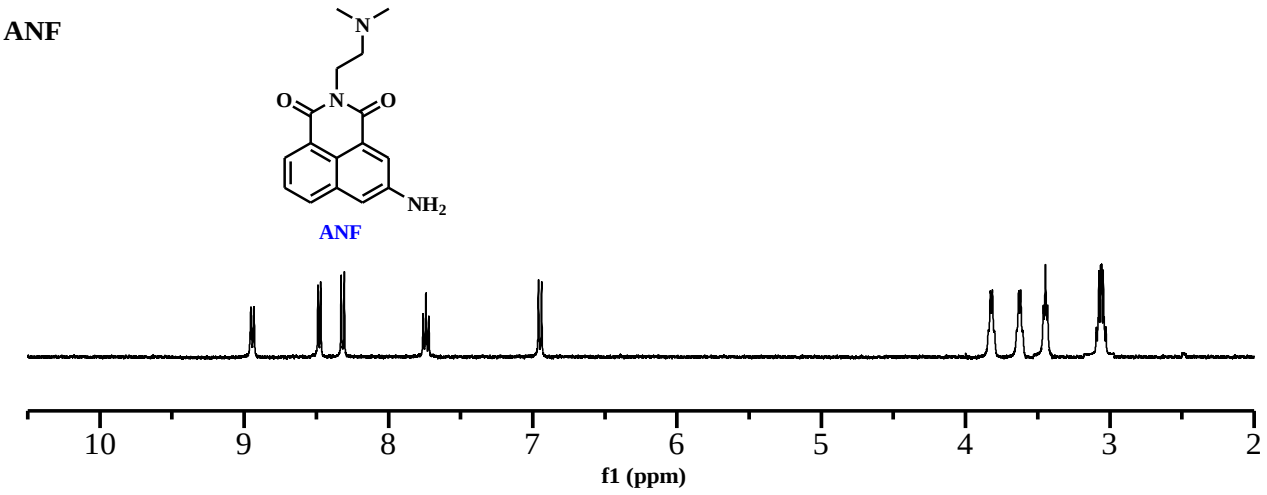
ANF-Glu





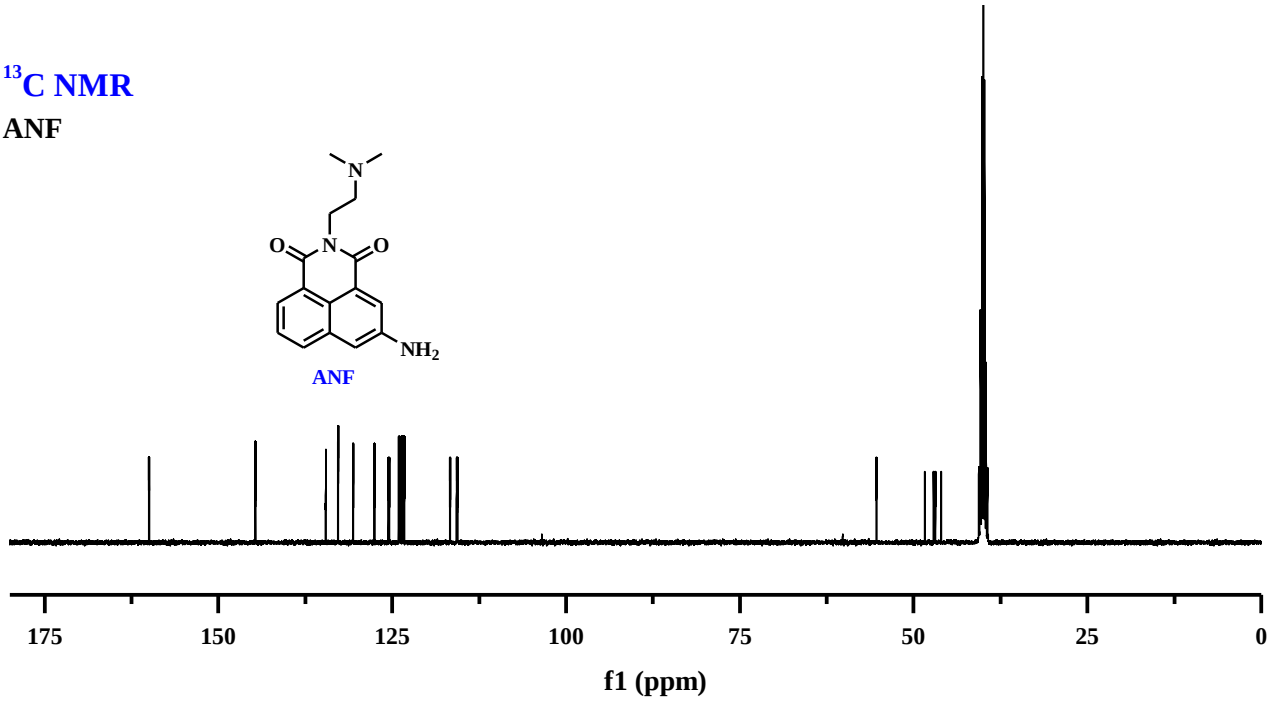
^{1}H NMR

ANF



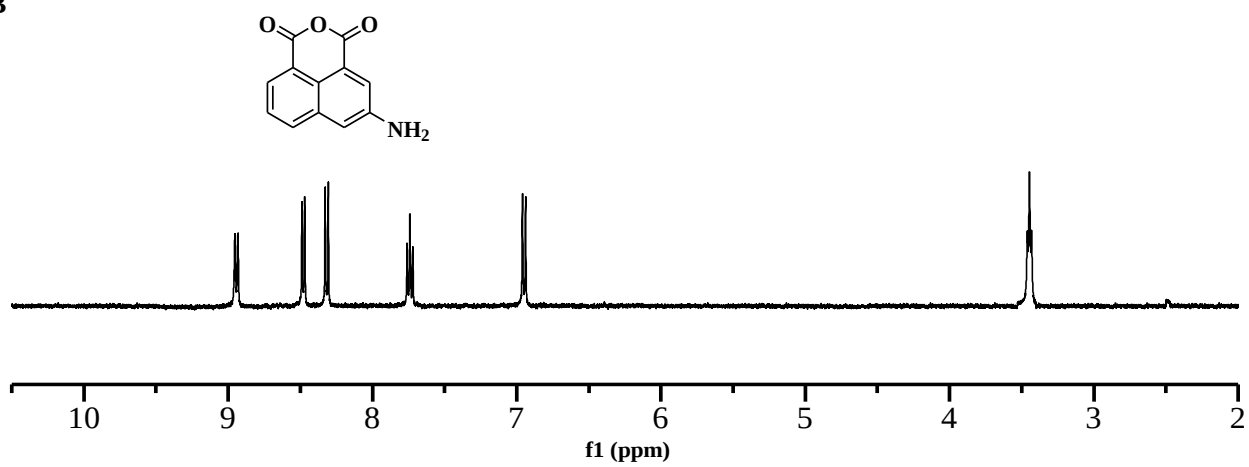
^{13}C NMR

ANF



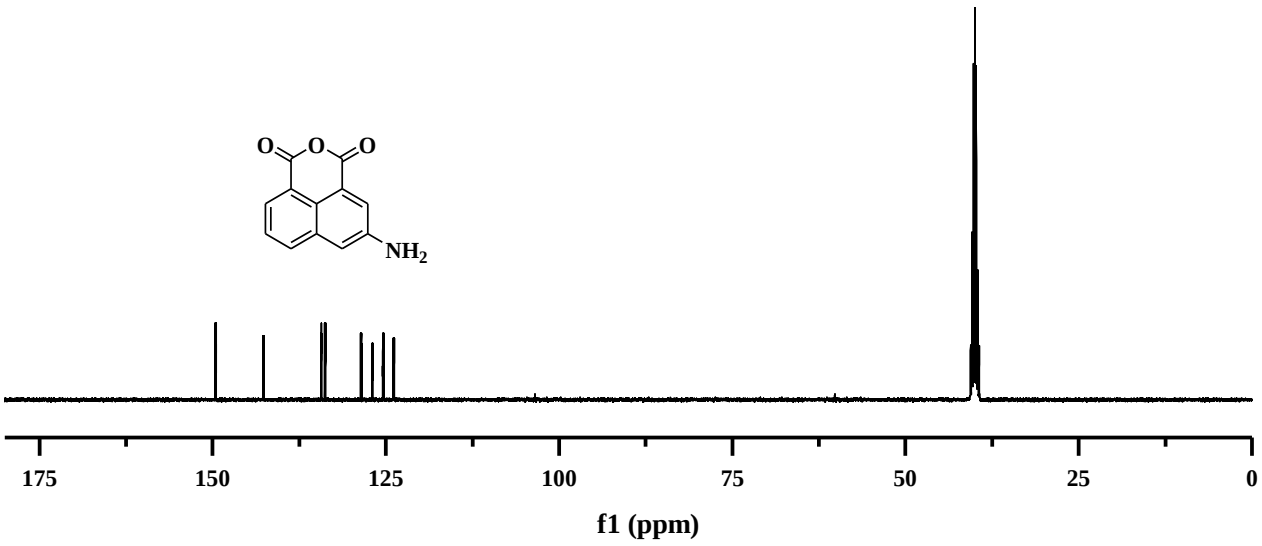
^{1}H NMR

B



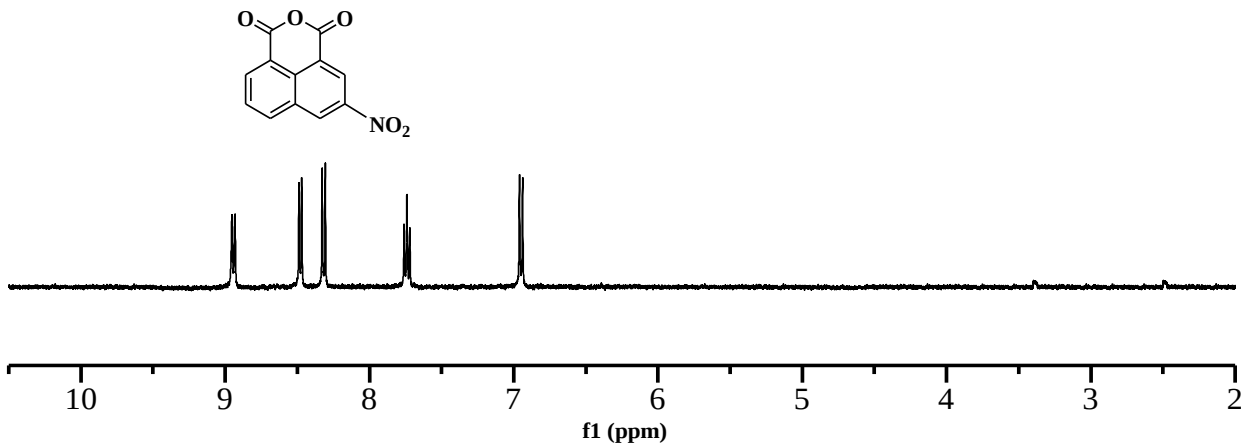
^{13}C NMR

B



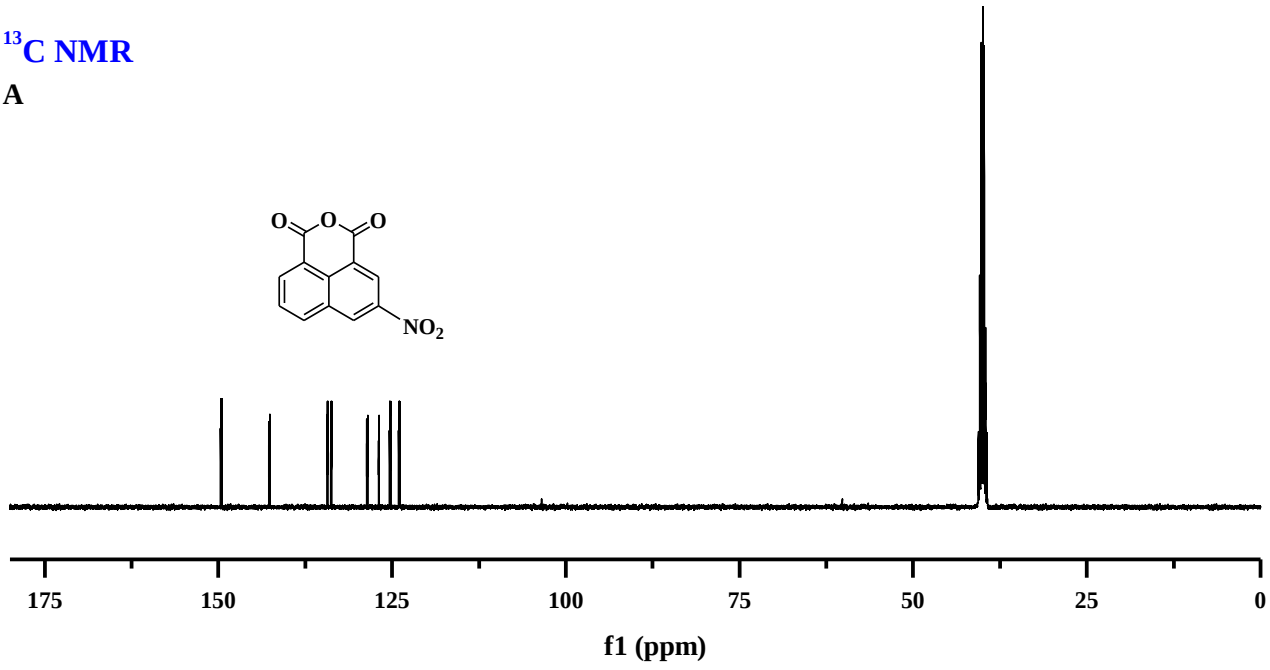
^{1}H NMR

A



^{13}C NMR

A



Supporting Information-Video S1

The dynamic change of DNA damage in cancer cells was observed on time-scan mode by Olympus spectral confocal multiphoton microscope (FV1200) with MaiTai femtosecond laser source (Spectra-Physics). Inverted microscope was used in cell imaging. The imaging parameters are as follow. Internal PMTs = 16 bit pixels = 1600×1600. Lasers: 405 nm. Time interval = 10 s. Imaging number = 50.

Supporting Information-Video S2

The dynamic change of DNA damage in cancer cells was observed on time-scan mode by Olympus spectral confocal multiphoton microscope (FV1200) with MaiTai femtosecond laser source (Spectra-Physics). Inverted microscope was used in cell imaging. The imaging parameters are as follow. Internal PMTs = 16 bit pixels = 1600×1600. Lasers: 800 nm. Time interval = 25 s. Imaging number = 50.