Supporting Information

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

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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 CO2 incubator at 37 °C. 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 °C in 5.0 wt %/vol CO2 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 CO2 at 37 °C. 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 \((1 \times 10^6)\) 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 °C. After 3.0 min, take the initial measurement \((T_{\text{initial}})\). Measure the absorbance at 418 nm at the initial time \((A418)_{\text{initial}}\). (3) Continue to incubate the plate at 37 °C 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 \((40 \text{nmole/well})\). 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 \([(A418)_{\text{final}}]\) 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 \(T_{\text{final}}\). (c) Results Calculations: Plot the pNA standard curve from the initial measurement \((T_{\text{initial}})\). Calculate the change in measurement from \(T_{\text{initial}}\) to \(T_{\text{final}}\) for the samples. \(DA418 = (A418)_{\text{final}} - (A418)_{\text{initial}}\). Compare the DA418 of each sample to the standard curve to determine the amount of pNA generated between \(T_{\text{initial}}\) and \(T_{\text{final}}\). **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 antibody and 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 °C; (c) Aspirate and add 100 µL prepared Detection Reagent A. Incubate 1.0 h at 37 °C; (d) Aspirate and wash 3 times; (e) Add 100 µL prepared Detection Reagent B. Incubate 30 min at 37 °C; (f) Aspirate and wash 5 times; (g) Add 90 µL Substrate Solution. Incubate 15-25 min at 37 °C; (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.\textsuperscript{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. $1 \times 10^6$ to $2 \times 10^6$ cells in 200 to 300 μL of PBS were injected in 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 Lambda 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 ($\Phi_F = 0.97$ in methanol) by the following equation:

$$\Phi = \Phi(F/F_{r})(A/A_{r})(\lambda_{ex}/\lambda_{ex})^{(n/n_0)^2}$$

where $\Phi$ represents quantum yield; $F$ is the integrated area under the corrected emission spectrum; $A$ is absorbance at the excitation wavelength; $\lambda_{ex}$ 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 °C. The DMEM or MEM was removed 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:

\[
\text{cell viability} (\%) = \frac{\text{OD}_{\text{dye}} - \text{OD}_{\text{K dye}}}{\text{OD}_{\text{control}} - \text{OD}_{\text{K control}}} \times 100
\]

(2)

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

**Flow Cytometry.**

Cells were cultured in different kinds of media supplemented with 10 % FBS under an atmosphere of 5.0 % CO₂ and 95 % air at 37 °C. After incubation at 37 °C with 5.0 % CO₂ 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 media 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. Yield 93%. Mp 247 °C. ¹H 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). ¹³C 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 C₁₂H₁₂NO₅: 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 CH₂OH/CH₂Cl₂=1:10 as eluent, affording B as yellow solid. Yield 87%. Mp > 300°C. ¹H 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). ¹³C NMR (600 MHz, DMSO-d6) δ: 149.49, 142.65, 134.27, 133.73, 128.54, 126.92, 125.24, 123.93. C₁₂H₁₂NO₅: 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 °C. ¹H 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). ¹³C NMR (600 MHz, DMSO-d6) δ:
The synthesis of ANF-Glu

To ANF (1.0 mmol) in N, N-dimethylformamide (DMF, 20 mL), 1-(3-dimethylaminopropyl)-3-ethylcarbodiimide hydrochloride (EDC) (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 (CH₂Cl₂) 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 H₂O) and eluent B (80:20 CH₃CN:H₂O) (A/B = 80:20 to 0:100, 40 min) to yield ANF-Glu which appears as a yellow powder. Yield 23 %. Mp > 300 °C. 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). ¹³C 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. C₂₁H₂₄N₄O₅: 412.1747, found: 412.1739.
4. Reaction rate and influence of ANF-Glu with GGT and NAT

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

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

<table>
<thead>
<tr>
<th></th>
<th>ANF-Glu</th>
<th>ANF</th>
<th>N-acetyl ANF</th>
</tr>
</thead>
<tbody>
<tr>
<td><strong>Formula</strong></td>
<td>C_{21}H_{24}N_{4}O_{5}</td>
<td>C_{16}H_{17}N_{2}O_{2}</td>
<td>C_{18}H_{19}N_{3}O_{3}</td>
</tr>
<tr>
<td><strong>λ_{ex}/nm</strong></td>
<td>417</td>
<td>423</td>
<td>428</td>
</tr>
<tr>
<td><strong>ε / M^{-1} cm^{-1}</strong></td>
<td>9678</td>
<td>18400</td>
<td>10850</td>
</tr>
<tr>
<td><strong>λ_{em}/nm</strong></td>
<td>441</td>
<td>530</td>
<td>455</td>
</tr>
<tr>
<td><strong>Φ</strong></td>
<td>0.135</td>
<td>0.39</td>
<td>0.134</td>
</tr>
<tr>
<td><strong>Lowest detection limit for GGT/ U/L</strong></td>
<td>182</td>
<td>-</td>
<td>-</td>
</tr>
<tr>
<td><strong>Octanol-water partition coefficient (log P)</strong></td>
<td>-2.9</td>
<td>1.18</td>
<td>-0.5</td>
</tr>
</tbody>
</table>

* QSAR model: entering into cells log P > 0

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

![Figure S2](image-url)

**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

![Graph showing two-photon action spectra 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 °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} \text{cm}^4 \text{s/photon}) at 800 nm.

8. DNA damage assay and NAT activity in living cells

<table>
<thead>
<tr>
<th></th>
<th>DNA damage</th>
<th>Normal DNA</th>
</tr>
</thead>
<tbody>
<tr>
<td>Olive Tail Moment(^1)</td>
<td>57.65 (DNA damage degree &gt; 70%)</td>
<td>1.23 (DNA damage degree &lt; 5%)</td>
</tr>
<tr>
<td>NAT activity(^2) (U/L)</td>
<td>483.1</td>
<td>27.12</td>
</tr>
</tbody>
</table>

\(^1\) The comet assay; \(^2\) The enzyme-linked immunosorbent assay
9. Living cells stained with ANF-Glu

Fluorescence channel
Incubating time: 250 s

Incubating time: 2.0 h

Incubating time: 5.0 h
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

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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 3.000000
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-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
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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
**************************************
**
* L I G A N D F I T - S C O R E *
**
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* 2009 Accelrys Software Inc. *
* www.accelrys.com *
**
* 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: lligfit_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
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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*                                 *
* Version 2.4 *                   *
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*                                 *
* 2009 Accelrys Software Inc. *   *
* www.accelrys.com *             *
*                                 *
* Build date: Jun 13 2009 22:12:30 *
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Obtained docking license for ligfit_ext...
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Run started at: Thu Dec 04 19:00:52 2014 on: PC0420YIU win32
Read docking and energy parameter file: LF_param.txt

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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
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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
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NONBOND_CUTOFF_DIS 10.000000
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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
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Molecule no. : 1
Rotatable bonds: 3
MC termination values: 3600 / 900
Flexible Docking ... done 22.3[s]
Molecule Pose Score dPMI

<table>
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<th>Pose</th>
<th>Score dPMI</th>
<th>Score dPMI</th>
<th>Score dPMI</th>
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Run completed at: Thu Dec 04 19:01:16 2014
Released license for ligfit_ext

**Parameters**

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**Reference:**
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.