# Supporting Information

**"A proteomic strategy for the identification of caspase-associating proteins"**

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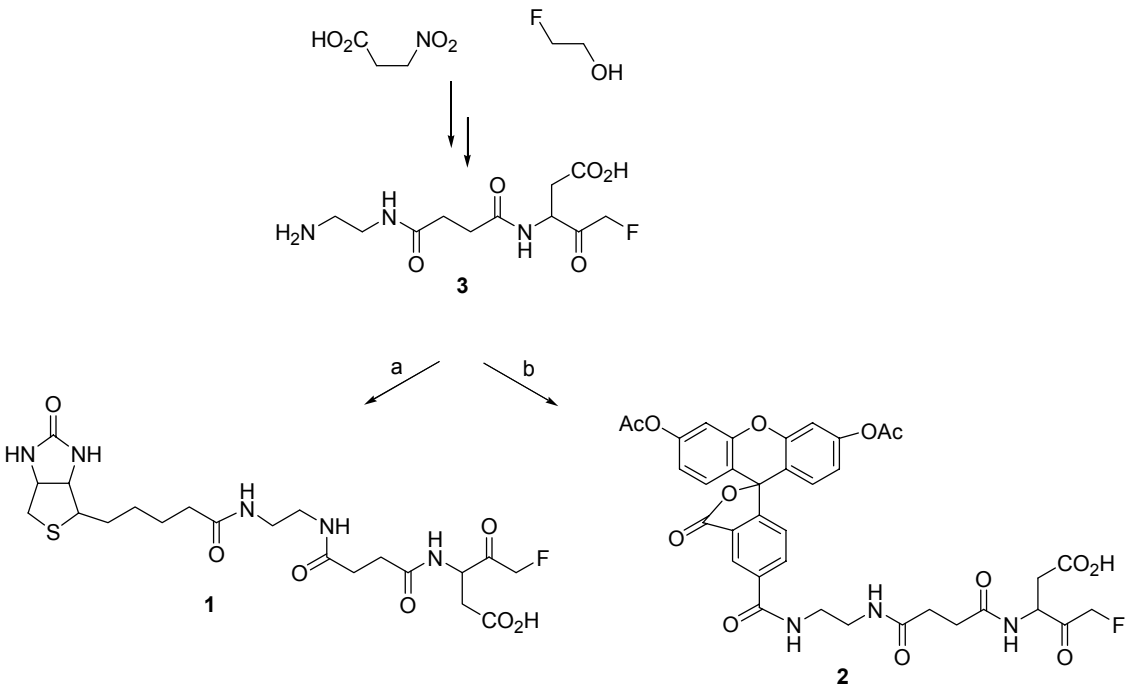
## 1. General:

All chemicals were purchased from commercial vendors and used without further purification, unless otherwise noted. $^1$H NMR spectra were taken on a Bruker 300 MHz NMR spectrometer. Chemical shifts are reported in parts per million referenced with respect to residual solvent ($CHCl_3$ = 7.26 ppm). HPLC was performed on a Waters Delta 600 HPLC equipped with a Phenomenex RP-18 (4.6 x 250 mm) column for analytical and Phenomenex RP-18 (21.2 x 250 mm) column for preparative, using an acetontrile–water gradient (with 0.1% TFA). MS was run on an electrospray mass spectrometer (Finnigan, USA).

## 2. Chemical synthesis




Reaction conditions : (a) Biotin-NHS, DIEA,DMF,12h, rt, 50 % yield.(b) Fluorescein diacetate-NHS, Pyridine,DMF,12h, rt, 30 % yield.

3-[3-(2-Boc-amino-ethylcarbomyl)-propionylamino]-5-fluoro-4-oxo-2-pentanoic acid (**3**):

The synthesis **3** was reported previously.[3] Briefly, it was made in 7 steps from 3-nitropropionic acid and 2-fluoroethanol. $^1$H-NMR (300 MHz, $CDCl_3$) δ 5.34-5.00 (m, 1H), 4.75(br, s, 1H), 4.56-4.30(m, 1H), 3.46(br, t, 2H), 3.06(br, t, 2H), 2.86-2.73(m, 2H), 2.58-2.50(m, 4H). m/z 292.1$[(M-H)]^-$.

Probe (**1**):

To **3** (0.21 g, 1 mmol) was added Biotin-NHS (0.341 g, 1 mmol) in DMF, and the solution was stirred for 12 hrs in the presence of 0.1 ml of DIEA at room temperature. The reaction mixture was concentrated under reduced pressure and dissolved in 50 ml of ethyl acetate layer, followed by washing with $NaHCO_3$ ( 2 X 25 ml), water ( 2 X 25 ml) and brine (2 X 25 ml) to give a crude solid, which upon further purification by Reverse phase HPLC afforded the probe **1** (yield: 50%)**.** $^1$H-NMR (300 MHz, $CDCl_3$) δ 4.91 (m, 2H), 4.82 (m, 1H), 4.37-4.32 (m, 2H), 3.58 – 3.27 (broad, 6 H), 2.99 (m, 2H), 2.74 (m, 2H), 2.57 (m, 4H), 2.05 (t, 2H), 1.59-1.29 (m, 6H). m/z 517.1$[(M+H)]^+$.

Probe (**2**):

To **3** (0.21 g, 1 mmol) was added the NHS ester of 5-carboxyfluorescein diacetate (0.56 g, 1 mmol) in DMF, and the solution was stirred for 12 hrs in the presence of 0.2 ml of pyridine. Since the fluorescein diacetate is labile to bases. DIEA should be avoided. The reaction mixture was concentrated under reduced pressure and dissolved in 50 ml of ethyl acetate layer, followed by washing with $NaHCO_3$ ( 2 X 25 ml), water ( 2 X 25 ml) and brine ( 2 X 25 ml) to give the crude product, which upon further flash column purification afforded **2** (yield: 30%). $^1$H-NMR (300 MHz, $CDCl_3$) δ 8.47 (m, 1H), 8.20 (m, 1H), 7.32 (m, 1H), 7.17 (s, 2H), 6.87 (m, 4 H), 4.82 (b, 3H), 3.56 (2H, m), 3.29 (2H, m), 2.73 (m, 1H), 2.56 (m, 4H), 2.27 (s, 6H). m/z 735.1$[(M+H)]^+$.

**3. Biochemical experiments:**

Cell cultures:

The Hela cells were cultured in Dulbecco's modified Eagle's medium (DMEM; Sigma) containing 10% fetal calf serum (FCS, Gibco BRL), 100 units/ml Penicillin G and 100 μg/ml Streptomycin Sulfate( Irvine Scientific, Santa Ana, CA) at 37 $^oC$ in a 5% $CO_2$ atmosphere.

To induce apoptosis, HeLa cells were washed twice with phosphate-buffered saline (PBS), which was also added to the cell cultures during the time of UV irradiation. The cells were placed on a Hoefer™ MacroVue™ UV/Visible Transilluminator (Amersham, USA) at λ = 302 nm for 10 minutes. The average UV surface intensity is 7000 μW/cm$^2$ (in the low intensity mode). After UVB exposure, fresh culture medium was added to the dishes. Uninduced, control cells were subjected to identical procedures but without UV exposure. Apoptosis of cells was examined by their morphology changes using fluorescence microscopy. Cells with characteristic shrinking and membrane blebbing were scored as apoptotic. Phase contrast and fluorescence images were

recorded with a AxioSkop 40 fluorescence microscope (Carl Zeiss, Germany) equipped with a cooled CCD camera (AxioCam, Zeiss) using a 63 X objective and suitable filters.

To label the cells, probe **1** was added to culture media (to a final concentration of 25 μM) 12 h after UVB irradiation, unless otherwise stated. Labeling was carried for one and a half hour, and after which the cells washed extensively with PBS buffer before imaging, followed by harvesting where applicable. For pull-down experiments with probe **2**, cells were treated the same way.

To harvest the labeled cells, 0.2 mL of an ice-cold lysis buffer (PBS) was added to each of 150 mm dish of adherent HeLa cells. Cells were scraped off the dishes with a plastic cell scraper that has been cooled in ice-cold distilled water. The cell suspension was transferred to a centrifuge tube. An equal volume of acid-washed glass beads (Sigma, 425-600 microns) was added, and the tube was placed in a mixer mill (Retsch, Haan, Germany) and lysed for 3 x 6 min at 30 beat/sec at 4 $^{o}$C. The supernatant (containing soluble proteins) was collected by ultracentrifugation at 75 000 rpm for 1 h at 4$^{o}$C. For lysates labeled with probe **1**, SDS-PAGE or 2D-GE were carried out as described below. For lysates labeled with probe **2**, affinity pull-down experiments with Streptavidin beads were carried out, followed by SDS-PAGE or 2D-GE before MS analysis for protein identification.

Streptavidin pull-down experiments:

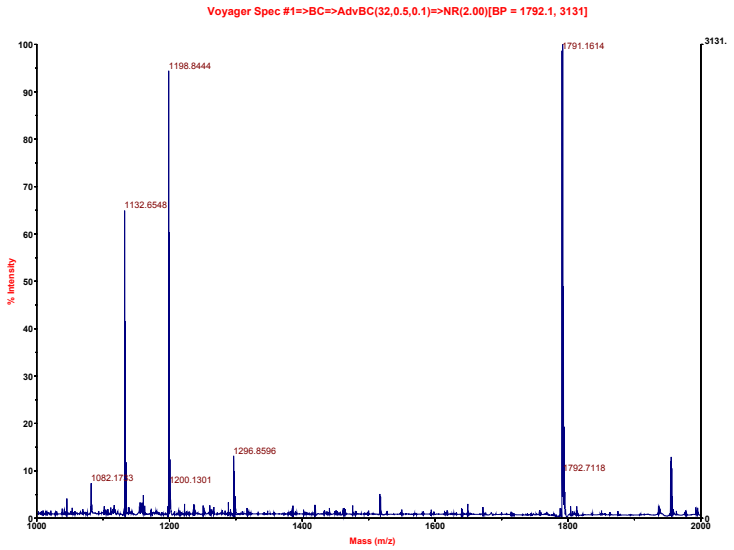
Upon being labeled with probe **2** as described above, the cell lysates were incubated with Streptavidin MagneSphere Paramagnetic Particles (Promega, MA) for 1 h. The resin was washed with 1% SDS (5 times) and bound proteins were eluted by boiling for 30 min in the same buffer. The supernatant was kept for SDS-PAGE or 2D-GE. For SDS-PAGE, the reaction was quenched with one volume of standard 2 X SDS-PAGE loading buffer, followed by heating the samples at 90$^{o}$C for 5 min before analysis on 12 % or 15% SDS-PAGE.

SDS-PAGE/2D-GE and MS:

SDS-PAGE was carried using standard protocols. All 2D experiments were performed on an Ettan IPGphor. isoelectric focusing and Ettan DALT gel system (Amersham, Cleveland, OH, USA) using standard protocols recommended by the same vendor (Ettan DIGE User Manual, Amersham). Fluoresecently labeled sample (with probe **1**) or affinity-purified sample (labeled by probe **2**) was reconstituted in the rehydration buffer before being applied to the immobilized pH gradient strip (pH 3–10, 18 cm; Amersham, USA). Passive rehydration was performed for 1 h, followed by 12 h of active rehydration (20 $^{o}$C, 50 V). The total voltage-hour in isoelectric focusing (IEF) was 32 kVh. After IEF, the strips were equilibrated in equilibration buffer 1 (6 M urea, 2% SDS, 0.375 M Tris (pH 8.8), 20 % glycerol, 130 mM dithiothreitol; 15 min), then in equilibration buffer 2 (6 M urea, 2 % SDS, 0.375 M Tris (pH 8.8), 20% glycerol, 135 mM iodoacetamide; 15 min), before being applied directly to a 12% SDS-PAGE gel. The SDS electrophoresis was run first at 2.5 W/gel (for 30 min), then 17 W/gel until the dye front was 1 mm from the bottom of the gel. Each gel was repeated at least once. Similar results were obtained.

For experiments labeled with probe **2**, gels were visualized by western blotting with HRP-conjugated anti-biotin antibody (NEB, USA) and the Enhanced ChemiLuminescent (ECL) kit (Amersham). For experiments carried out with probe **1**, gel images were collected using a Typhoon™ 9200 fluorescence gel scanner (Amersham, USA).

MS experiments were carried out on a matrix-assisted laser desorption ionization-time of flight (MALDI-TOF) mass spectrometer (Voyager-DE STR™; Applied Biosystems, USA) following protein digestion/extraction (using standard protocols). The MALDI-TOF MS data was used for protein identification by searching the NCBI database using Mascot™ search engine at http://www.matrixscience.com.

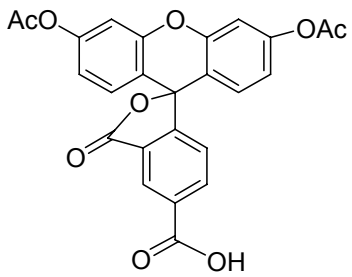



MDFSRNLYDIGEQLDSEDLASLKFLSLDYIPQRKQEPIKDALMLFQRLQEKRMLEESNLS
FLKELLFRINRLDLLITYLNTRKEEMERELQTPGRAQISAYRVMLYQISEEVSRSELRSF
KFLLQEEISKCKLDDDMNLLDIFIEMEKR**VILGEGKLDILK**RVCAQINKSLLKIINDYEE
FSK**DFGQSLPNEK**QTSGILSDHQQSQFCK**STGESAQTSQH**

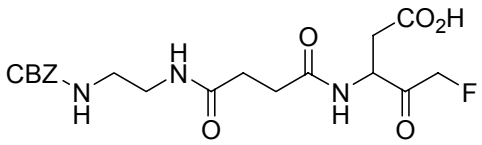
Figure S1. MALDI-TOF-MS spectrum of caspase-8 and its matched peptide alignment sequences (underlined and in bold), identified from the band isolated in Fig. 1B, panel *ii* (in maintext).

Control experiments and regents used:

The structure of the control dye used in fluorescence microscopic experiments (e.g. panel *i* in Figure 1A, maintext) is shown below:




The unlabeled fluoromethylketone (fmk)-containing probe used in the control experiment related to Figure 1B (both gels) was shown below.




The control experiment was done by first incubating the apoptotic cells with the above unlabeled fluoromethylketone probe, then treated with either **1** or **2** before cells were collected and analyzed by SDS-PAGE: all bands as shown in Fig. 1B (both gels, in maintext) were abolished, indicating they originally arose from specific labelling by the two probes, respectively.