Supporting Information for

Photoactivatable Drug-Caged Fluorophore Conjugate Allows Direct Quantification of Intracellular Drug Transport

Sarit S. Agasti,[a,†] Ashley M. Laughney, [a,†] Rainer H. Kohler,[a] and Ralph Weissleder[a,b,*]

[a]Center for Systems Biology, Massachusetts General Hospital/Harvard Medical School, 185 Cambridge St., Boston, MA 02114 (USA)

[b]Department of Systems Biology, Harvard Medical School, 200 Longwood Ave., Alpert 536, Boston, MA 02115 (USA)

[†] These authors contributed equally to this work

*e-mail: rweissleder@mgh.harvard.edu

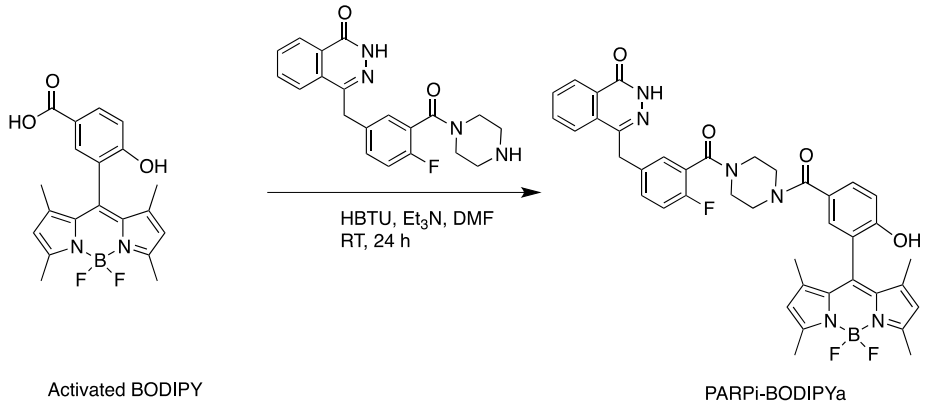
Reagents and instruments:

Unless otherwise stated, all the reagents for the synthesis of drug conjugates were obtained from Sigma-Aldrich (St. Louis, Missouri, USA) and used as received. ^{1}H NMR spectra were recorded using a Varian 400 MHz spectrometer. High performance liquid chromatography–mass spectrometry (HPLC-MS) analysis was performed using a Waters (Milford, MA) LC-MS system. A Waters XTerra C18 5 µm column was used for HPLC-MS analysis (eluents: 0.1% trifluoroacetic acid (v/v) in water and acetonitrile). Fluorescence spectra were recorded in a TECAN microplate reader. High-resolution electrospray ionization (ESI) mass spectra were recorded using a Bruker Daltonics APEXIV 4.7 Tesla Fourier Transform mass spectrometer (FT-ICR-MS) in the Department of Chemistry Instrumentation Facility at the Massachusetts Institute of Technology.

Synthesis of PARPi-BODIPYc:

The Carboxyl functionalized Caged BODIPY was synthesized by following a literature procedure.[1] PARPi was synthesized according to a previously described procedure.[2, 3] Carboxyl functionalized Caged BODIPY (2.8 mg, 4.96 µmol) and PARPi (1.8 mg, 4.96 µmol) were dissolved in dry DMF (500 µl). To this solution O-(Benzotriazol-1-yl)-N,N,N',N'-

tetramethyluronium Hexafluorophosphate (HBTU, 3.8 mg, 10.02 μmol) and triehylamine (Et_3N, 2.07 μl, 14.89 μmol) were added. The reaction mixture was stirred at room temperature (RT) for ~48 h. The reaction mixture was directly loaded onto a C18 reverse phase column for purification (eluents: 0.1% trifluoroacetic acid (v/v) in water and acetonitrile. gradient: 5% acetonitrile in water to 95% acetonitrile in water, v/v). The product isolated as brown solid. Yield: 2.95 mg, 65%. ^{1}H NMR (400 MHz, CD_3OD): 8.38 (m, 1H), 8.08 (d, 2H, J = 8 Hz), 7.95 (d, 1H, J = 7.2 Hz), 7.84 (m, 2H), 7.72 (t, 1H, J = 8.2 Hz), 7.67 (m, 1H), 7.50 (m, 1H), 7.40 (d, 1H, J = 8.4 Hz), 7.37 (m, 1H), 7.34 (m, 1H), 7.17 (t, 1H, J = 8.8 Hz), 6.10 (s, 2H), 5.50 (s, 2H), 4.39 (s, 2H), 3.80-3.34 (m, 8H), 2.45 (s, 6H), 1.50 (s, 6H). LC-ESI-MS: found m/z = 893.38 $[M - F]^+$, 911.35 $[M - H]^-$; calculated m/z = 893.30 $[M - F]^+$, 911.29 $[M - H]^-$. HRMS: $[M-F]^+$ m/z calculated 893.3030 for $C_{47}H_{40}BF_2N_8O_8$ found 893.3059.



Scheme S1. Synthetic scheme for the preparation of PARPi-BODIPYa.

Synthesis of PARPi-BODIPYa:

The Carboxyl functionalized activated BODIPY was synthesized by following a literature procedure.[1] Carboxyl functionalized activated BODIPY (2.30 mg, 6 μmol) and PARPi (2.20 mg, 6 μmol) were dissolved in dry DMF (500 μl). To this solution O-(Benzotriazol-1-yl)-N,N,N',N'-tetramethyluronium Hexafluorophosphate (HBTU, 4.55 mg, 12 μmol) and triehylamine (Et_3N, 2.50 μl, 18 μmol) were added. The reaction mixture was stirred at room temperature (RT) for ~24 h. The reaction mixture was directly loaded onto a C18 reverse phase column for purification (eluents: 0.1% trifluoroacetic acid (v/v) in water and acetonitrile. gradient: 5% acetonitrile in water to 95% acetonitrile in water, v/v). The product isolated as orange solid. Yield: 0.80 mg, 18%. ^{1}H NMR (400 MHz, $CDCl_3$): 10.07 (s, 1H), 8.45 (m, 1H), 7.77 (m, 2H),

7.71 (m, 1H), 7.50 (m, 1H), 7.45 (m, 1H), 7.32 (m, 2H), 7.20 (m, 1H), 7.05 (t, 1H, J = 8.6 Hz), 5.99 (s, 2H), 4.27 (s, 2H), 3.80-3.04 (m, 8H), 2.53 (s, 6H), 1.50 (s, 6H). LC-ESI-MS: found m/z = 713.35 $[M - F]^+$ and 731.30 $[M - H]^-$; calculated m/z = 713.29 $[M - F]^+$ and 731.28 $[M - H]^-$. HRMS: $[M-F]^+$ m/z calculated 713.2859 for $C_{40}H_{36}BF_2N_6O_4$ found 713.2853.

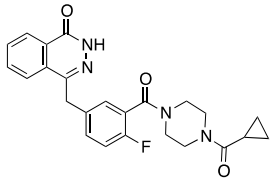
HPLC-MS characterization of the photocleavage of PARPi-BODIPYc:

A 3.0 mM solution of caged 4-OHC in 1:1 (v/v) water:acetonitrile was used for the HPLC-MS study. The solution of the PARPi-BODIPYc was placed in a glass vial and irradiated at ~365 nm using a hand held UV lamp. Aliquot was taken after 10 min time interval and injected to the HPLC-MS machine for analysis. Prism 5 (GraphPad, La Jolla, CA) for Mac was used to plot the data.

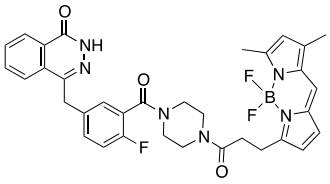
Fluorescence spectroscopy characterization of the photocleavage of PARPi-BODIPYc:

A 1 μM solution of PARPi-BODIPYc in 1:1 (v/v) water:acetonitrile was used for the fluorescence spectroscopic characterization of the photocleavage reaction. The solution of the PARPi-BODIPYc was placed in a 96 well black clear bottom microplate. The solution was then irradiated at ~365 nm using a hand held UV lamp. After light exposure, fluorescence spectra of the solution were recorded in a TECAN microplate reader. Time course of the photocleavage reaction was monitored by exposing the PARPi-BODIPYc solution to light for different durations, and subsequently recording the fluorescence spectra of the solution. Prism 5 (GraphPad, La Jolla, CA) for Mac was used to plot the data.

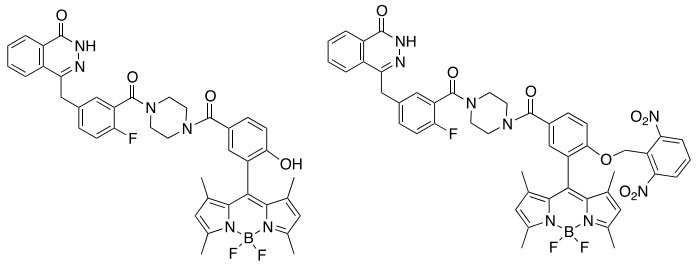
PARPi derivatives used for activity assay:



PARPi



PARPi-BODIPY Fl



Supporting figure 1. Chemical structures of the unlabeled and labeled variants of PARPi used for the activity assay.

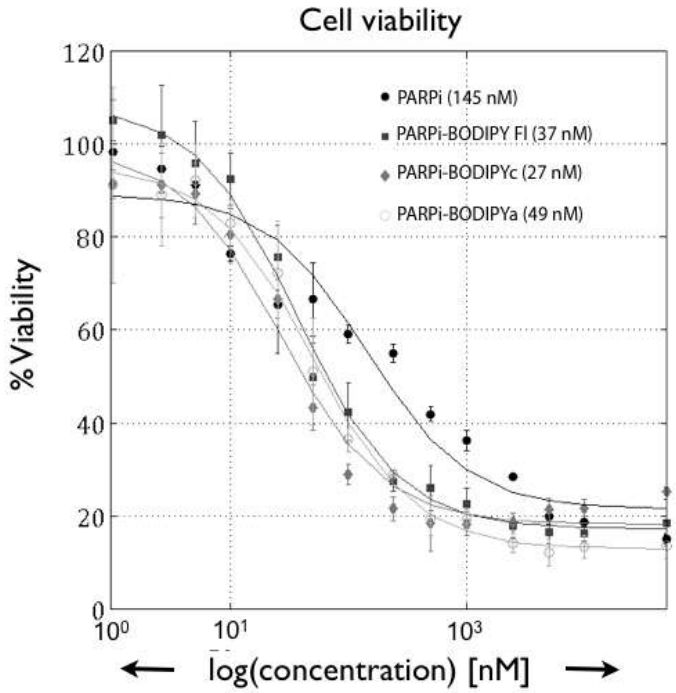
Inhibitory potential and cell viability assays:

The inhibitor potential of the PARPi derivatives was quantified using the commercially available HT Universal Colorimetric PARP Assay Kit (Trevigen, Gaithersburg, MD) in triplicate according to the manufacturers specifications. Two-fold dilutions of PARPi derivatives were incubated with 0.5U of PARP high specific activity (HSA) enzyme for 10 minutes in histone-coated 96-well plates; final concentrations of PARPi derivates ranged from 10 μM to 1 nM and 0 nM PARPi was included in the assay as an activity control. All reaction mixtures were adjusted to a final volume of 50 μL and a final concentration of 0.5% dimethyl sulfoxide (DMSO) in assay buffer. PARP-1 activity was measured by absorbance at 450nm in each well using a Tecan Safire2 microplate reader (Tecan Group, Mannedorf, Switzerland). The half maximal inhibitor concentration values and associated 95% confidence intervals were computed in MATLAB by minimizing the sum of squared residuals between the measured absorbance and a three-parameter sigmoidal dose response function. To achieve this, the nonlinear regression function, *nlpredci*, was applied to the normalized and log-transformed absorbance data. Toxicity profiles of the derivatives were assayed using 1X PrestoBlue Cell Viability Reagent (Life Technologies, Catalog #A-13261) after 120 h exposure to the PARPi derivatives; the viability assay was repeated for each derivate in triplicate. The BRCA1-mutant breast cancer cell line, MDA-MB-436, was cultured in RPMI 1640 medium supplemented with 10% Fetal Bovine Serum, L-glutamine, 100 IU penicillin, and 100 μg/mL streptomycin at 37°C and 5% CO_2. Cell viability assays were performed in a 96-well format and 2,000 cells were seeded per well 24 h prior to inhibitor administration. The concentration of inhibitor was serially diluted from 10 μM to 1 nM and similarly, half maximal inhibitor concentration values were computed using MATLAB

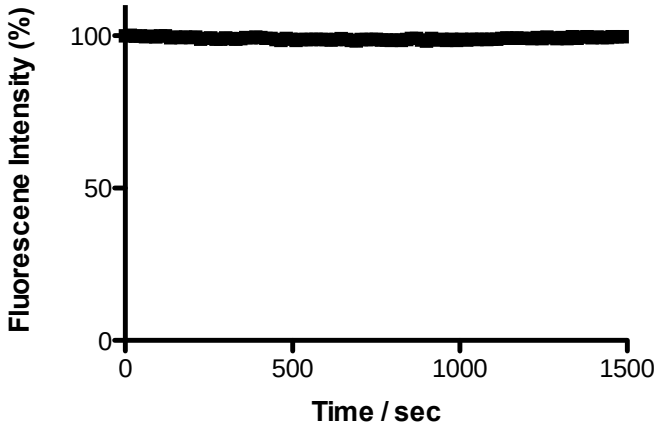
according to a three-parameter sigmoidal dose response function.

Photoactivation and imaging:

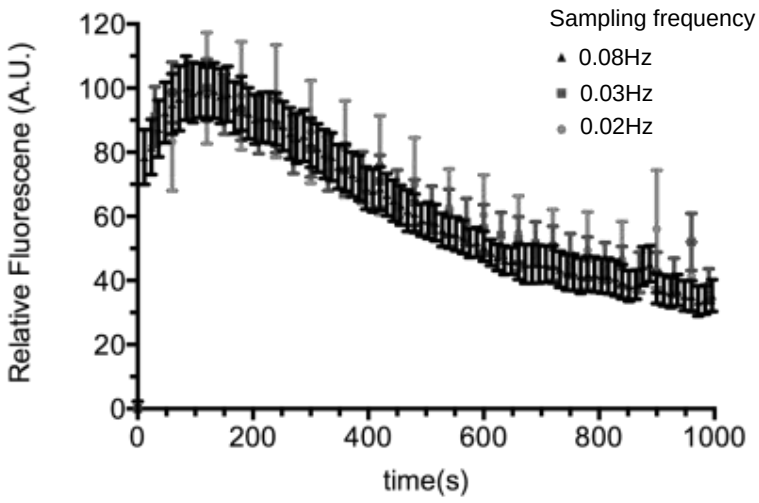
The construction of pTag-H2B-Apple has previously been reported;[3] this vector was transfected in HT1080 cells using the X-tremeGENE HP transfection reagent (Roche, Basel, Switzerland), followed by selection in 500 μg/mL G418. Single clones were screened for H2B-Appple expression by fluorescence microscopy and cells were maintained in minimum essential medium supplemented with 10% fetal bovine serum, 100 IU penicillin, 100 μg/mL streptomycin, 2 mM L-glutamine, nonessential amino acids and 100 μg/mL G418. 300,000 cells were plated per 35mm dish in phenol red-free medium and allowed to attach for 48 h. The cells were incubated at with PARPi-BODIPYc for 1 h at 37℃ prior to imaging; no washout steps were necessary. Imaging was performed on a DeltaVision microscope (Applied Precision Instruments, Issaquah, WA) with a 20x objective; pre- and post-activation images were acquired with 4x zoom; and single cells were activated at 10x zoom with just 6 scans from the 405 nm laser at full power. Time-lapse microscopy was performed to trace drug activation and loss in single cells. To validate nuclear localization of BODIPY fluorescence after substrate washout, cells were incubated with PARPi-BODIPYc conjugate for 1h and subsequently, were washed twice with phenol red-free media. Three hours after inhibitor washout, single cells were activated as previously described using the 405 nm laser. Nuclear co-localization was observed by overlaying nuclear Apple fluorescence with BODIPY fluorescence using ImageJ software.



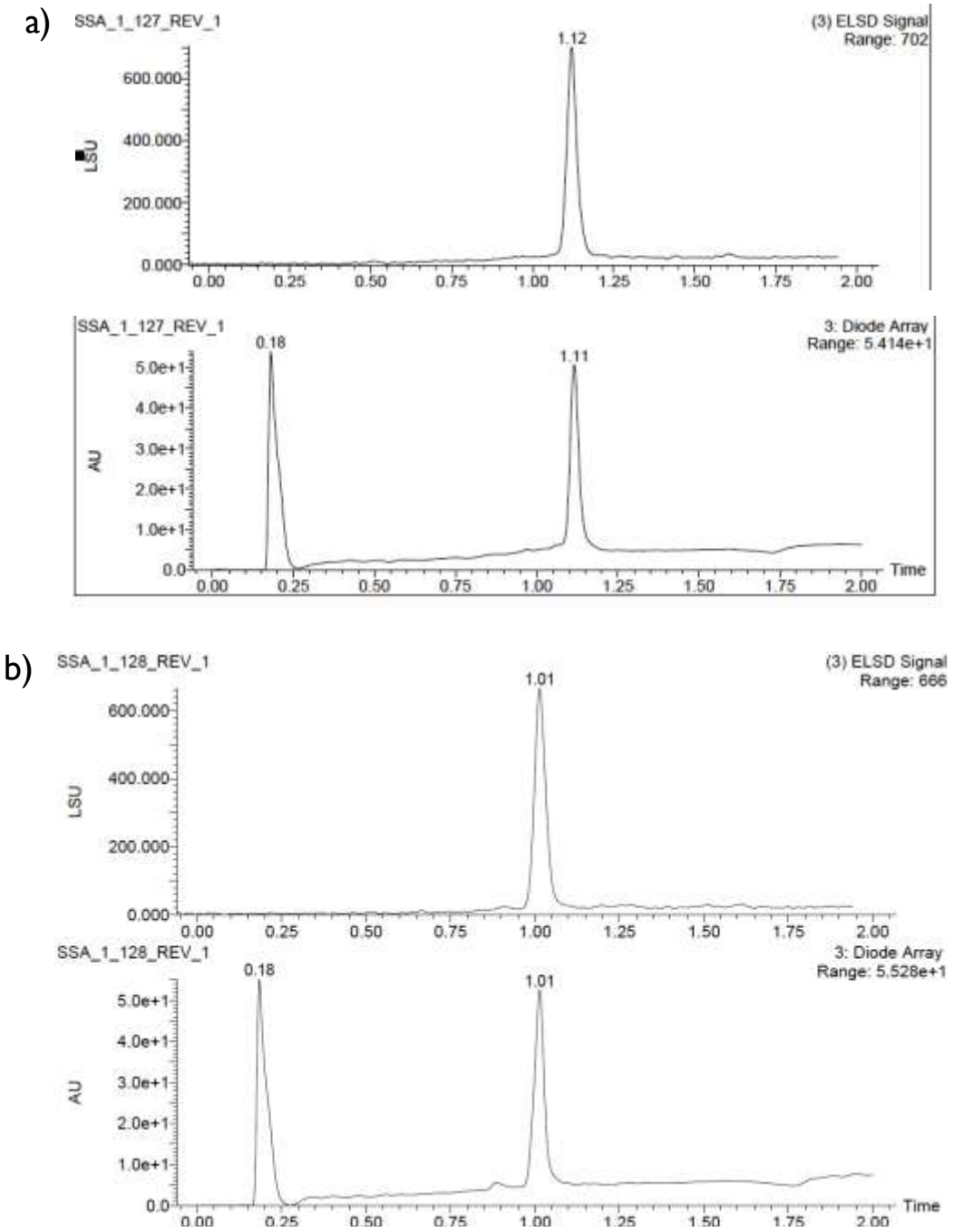
Supporting figure 2. Cell viability of MDA-MB-436 cells (breast cancer) after 120 h exposure to unlabeled and labeled variants of PARPi (● PARPi, ▪PARPi-BODIPY Fl, ◆PARPi-BODIPYc, ○ PARPi-BODIPYa). Corresponding IC50 values are included in the legend.



Supporting figure 3. Change in fluorescence intensity during repetitive signal acquisition from PARP-BODIPYa. A 1 μM solution of PARPi-BODIPYa in 1:1 (v/v) water:acetonitrile was excited at 470 nm for 100 times (every 15 sec interval) and the fluorescence intensity was measured at 519 nm. Fluorescence intensity was normalized against fluorescence at t=0 sec. A minimal decrease in fluorescence was observed over time, indicating good photostablity of the fluorescent derivatives.



Supporting figure 4. Population kinetics of activated drug efflux acquired at three sampling frequencies (reported as the mean +/-SEM of 9-11 cells per condition). No significant differences were observed between the population efflux slopes (p=0.26) for frames sampled every 12, 30 and 60s, indicating negligible photobleaching effects. Relative fluorescence values were computed as a fraction of the maximum signal achieved after photo activation. Single cells were segmented using an active contour algorithm developed in MATLAB.



Supporting figure 5. HPLC analysis coupled with evaporative light scattering detection (ELSD) and absorbance detection of the a) caged derivative (PARPi-BODIPYc) and b) the fluorescent derivative (PARPi-BODIPYa) of PARPi. A single peak (both in ELSD and absorbance based detection) was observed in the HPLC chromatogram of the respective compound, indicating the high purity of the synthesized compounds.

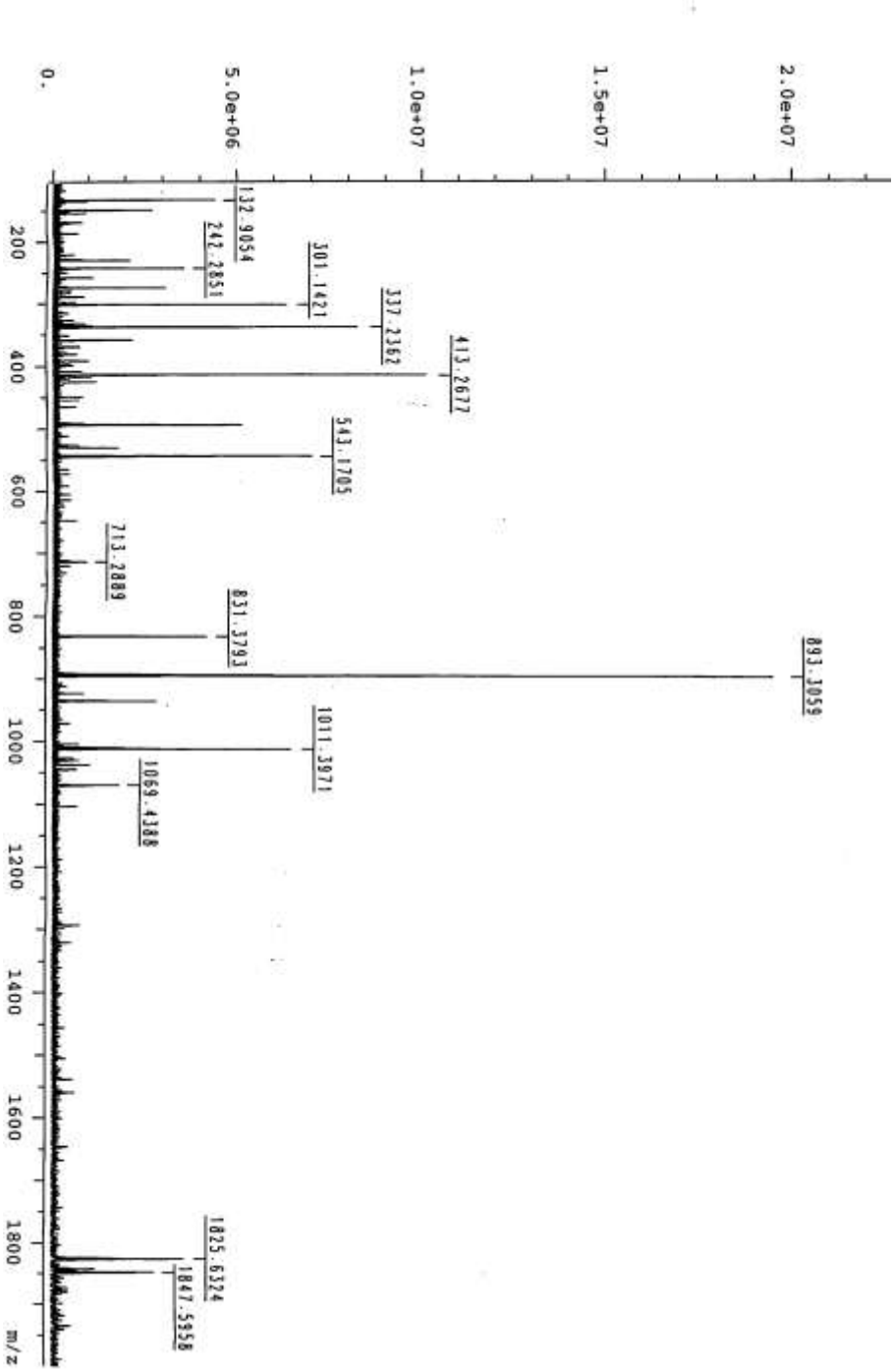


Figure 6. HRMS spectra of PARPi-BODIPYc. HRMS: $[M-F]^+$ m/z calculated 893.3030 for $C_{47}H_{40}BF_2N_8O_8$ found 893.3059.

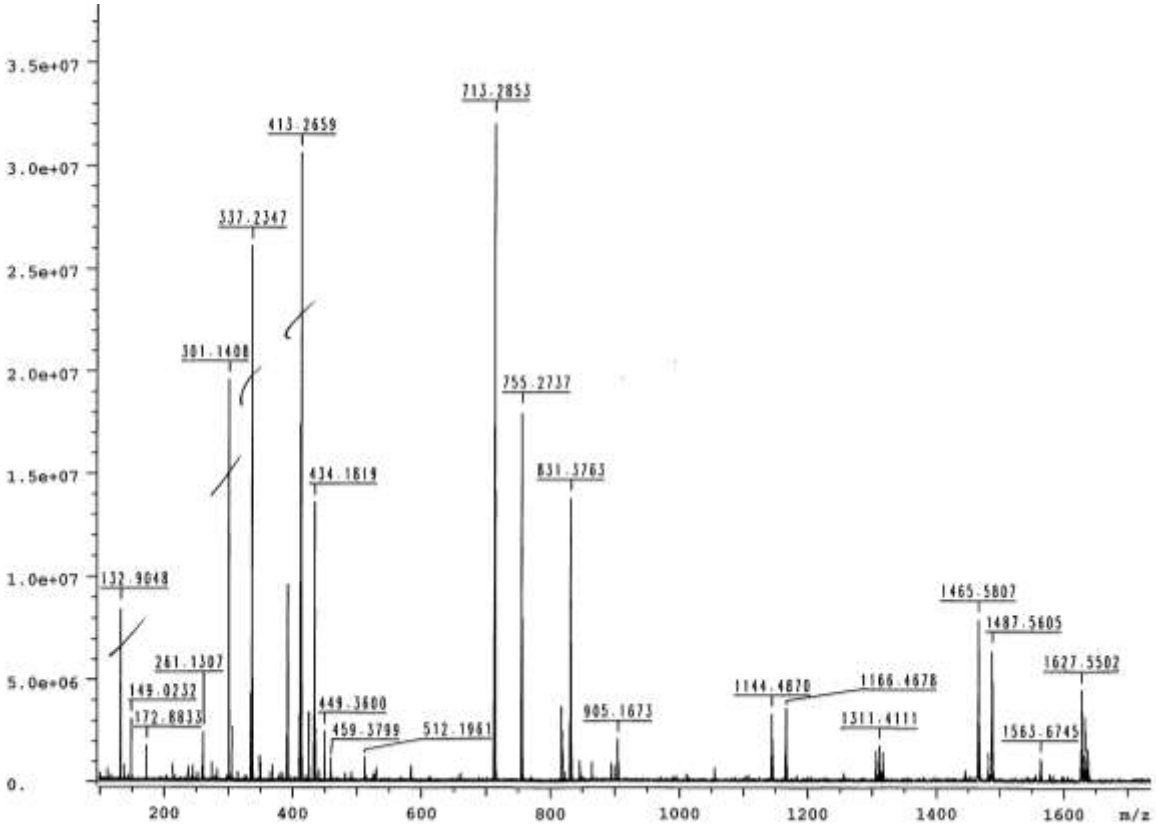


Figure 7. HRMS spectra of PARPi-BODIPYa. HRMS: $[M-F]^+$ m/z calculated 713.2859 for $C_{40}H_{36}BF_2N_6O_4$ found 713.2853.

Supporting References:

1. T. Kobayashi, T. Komatsu, M. Kamiya, C. Campos, M. Gonzalez-Gaitan, T. Terai, K. Hanaoka, T. Nagano, Y. Urano, *J. Am. Chem. Soc.* 2012, **134**, 11153.
2. K. A. Menear, C. Adcock, R. Boulter, X.-l. Cockcroft, L. Copsey, A. Cranston, K. J. Dillon, J. Drzewiecki, S. Garman, S. Gomez, H. Javaid, F. Kerrigan, C. Knights, A. Lau, V. M. Loh, Jr., I. T. W. Matthews, S. Moore, M. J. O'Connor, G. C. M. Smith, N. M. B. Martin, *J. Med. Chem.* 2008, **51**, 6581.
3. T. Reiner, J. Lacy, E. J. Keliher, K. S. Yang, A. Ullal, R. H. Kohler, C. Vinegoni, R. Weissleder, *Neoplasia* 2012, **14**, 169.