A Peptide Aldehyde Microarray for High-Throughput Profiling of Cellular Events

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1. Materials and Methods:

1.1. General Information

All chemicals were purchased from commercial vendors and used without further purification. Aminomethyl (AM) polystyrene resin, Rink Amide resin, all Fmoc amino acids, O-benzotriazole-N,N,N',N'-tetramethyl-uronium-hexafluoro-phosphate (HBTU), N-hydroxy-benzotriazole (HOBt), triisopropylsilane (TIS) and N,N'-diisopropylcarbodiimide (DIC) used in peptide synthesis were purchased from GL Biochem (China). All reactions requiring anhydrous conditions were carried out under an argon or nitrogen atmosphere using oven-dried glassware. HPLC grade solvents were used. Unless otherwise stated, all reactions were carried out at room temperature. Reaction progress was monitored by TLC on pre-coated silica plates (Merck 60 F_{254}, 0.25 μm) and spots were visualized by UV, iodine or Cerium Ammonium Molybdate (CAM) stain. Peptide synthesis progress was monitored by ninhydrin test. Flash column chromatography was carried out using Merck 60 F_{254} 0.040-0.063 μm silica gel.

^{1}H and ^{13}C NMR spectra were recorded on Bruker Avance 300 MHz, or Bruker Avance 500 MHz spectrometer. Chemical shifts were reported in parts per million relative to internal standard Tetramethylsilane ($Si(CH_3)_4$ = 0.00 ppm) or residual solvent peaks ($CHCl_3$ = 7.26 ppm, DMSO-d_6 = 2.50 ppm). ^{1}H NMR data are reported as follows: chemical shift in ppm from the respective internal standard, multiplicity (s = singlet, d = doublet, t = triplet, m = multiplet, dd= doublet of doublets, ddd= doublet of doublets of doublets, dt = doublet of triplets or overlap of nonequivalent resonances), integration, coupling constant.

Mass spectra were recorded on a Finnigan LCQ mass spectrometer, a Shimadzu LC-IT-TOF spectrometer or a Shimadzu LC-ESI spectrometer. Analytical HPLC was carried out on Shimadzu LC-IT-TOF or Shimadzu LC-ESI system equipped with an autosampler, using reverse-phase Phenomenex Luna 5μm C_{18} 100 Å 50 × 3.0 mm columns. Preparative HPLC was carried out on Gilson preparative HPLC system using Trilution software and reverse-phase Phenomenex Luna 5 μm $C_{18(2)}$ 100 Å 50 × 30.00 mm column. 0.1% TFA/H_2O and 0.1% TFA/acetonitrile were used as eluents for all HPLC experiments. To purify selected compounds, semi-preparative HPLC was carried out on a Phenomenex Luna C_{18} column (250 × 21.2 mm). The flow rate was 0.6 mL/min for analytical HPLC and 8 mL/min for preparative HPLC.

Recombinant Caspases were expressed, purified and fluorescently labeled as previously described.[1,2] Cruzain and Rhodesain were kindly provided by the McKerrow lab (UCSF). The parasites (*P. Falciparum* and *T Brucei* (BSF)) were generously donated by the groups of Kevin Tan and Cynthia He (NUS), respectively.

1.2. Peptide Aldehyde Library Synthesis.

The peptide aldehyde library was synthesized in two different sublibraries (Positional Scanning Inhibitor Libraries (PSIL) and DEVX library) by using IRORI™ technology. The peptide aldehyde sequences are the followings (**Figure S1**):

Library	Component Structure	Number of sublibraries	Compounds per sublibraries
	P_4 P_3 P_2 P_1		
P_2	Biotin —G—G— Mix — Mix — □ — ▷	90	324
P_3	Biotin —G—G— Mix — □ — Mix — ▷	90	324
P_4	Biotin —G—G— □ — Mix — Mix — ▷	90	324
DEVX	Biotin —G—G— D — E — V — ▷	5	1

Mix = Isokinetic mixture of 18 amino acids omitting cysteine and methionine

□ = Individual amino acid of the 18 amino acids omitting cysteine and methionine

▷ = 5 different individual amino aldehydes

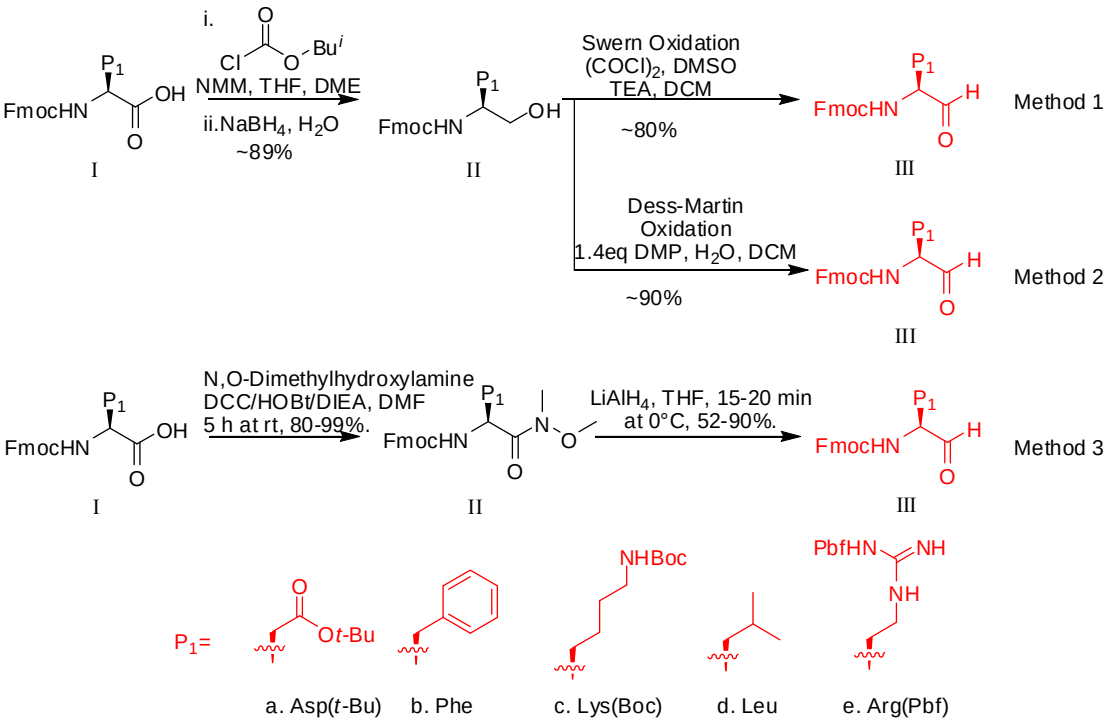
Figure S1. The diverse Peptide Aldehyde PSIL. The library was synthesized using five different amino aldehydes as P_1. *Mix* means an equimolar mixture of 18 amino acids (omitting cysteine and methionine). The library consists of P_2, P_3, and P_4 libraries, and each of them consists of 5 × 18=90 sublibraries. Each sub-library is composed of 18 × 18=324 species of tetra peptide-aldehyde inhibitors. The library totals 29,165 compound diversities.

1.2.1. General Procedure for Synthesis of Amino Aldehyde

All amino aldehyde Synthesis were based on previously published procedures (**Scheme S1**).[1,3] Three methods were used for synthesis of amino aldehydes. The Fmoc-protected amino acid **I** was initially activated using isobutylchloroformate, followed by reduction of the mixed anhydride to the amino alcohol **II**. Swern oxidation/ Dess-Martin oxidation gave the amino aldehyde **III (Method 1&2)**.

Method 3: To a dried flask flushed with N_2 was added Fmoc-AA-OH (3 mmol), N-hydroxy-benzotriazole (0.51 g, 3.3 mmol), dicyclohexylcarbodiimide (0.68 g, 3.3 mmol) and 6mL DMF. After stirring for 30 min at room temperature, a solid formed and was removed by filtration. N,O-dimethylhydroxylamine (0.35 g, 3.6 mmol), N,N'-diisopropylethylamine (0.46 g, 3.6 mmol) and another 4 mL DMF were added. After 3 hr, the mixture was concentrated. The crude oil was dissolved in ethyl acetate and washed with saturated sodium bicarbonate,

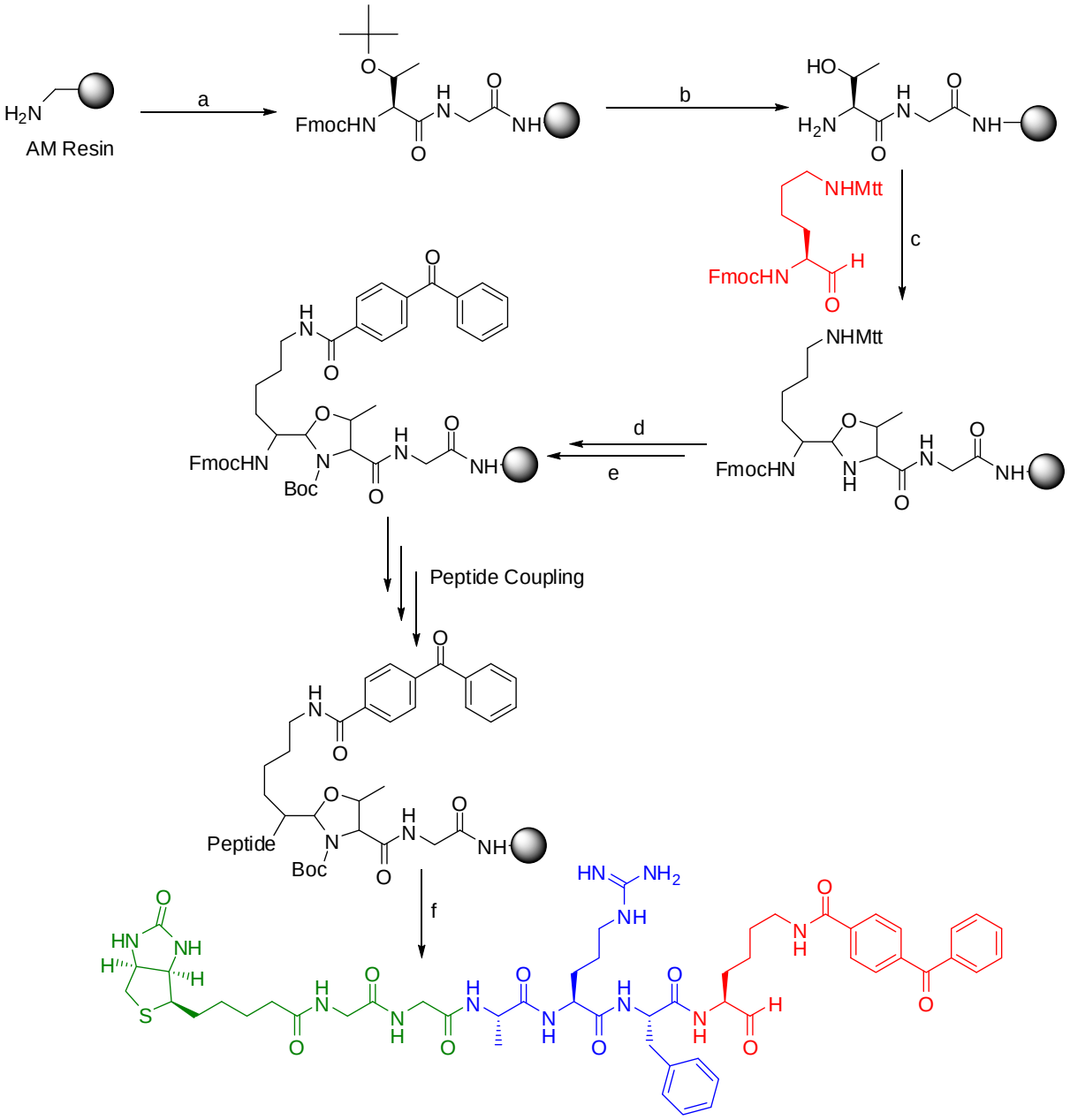
0.1M hydrochloric acid and brine. The organic layer was dried ($MgSO_4$), concentrated and further purified yield dimethylhydroxyl amide **II** intermediate**.** After then, $LiAlH_4$ (137 mg, 3.6 mmol) was added to Fmoc-AA-dimethylhydroxyl amide **II** (2.55 mmol) in THF (10 mL) at 0 °C. The reaction was stirred for 20 min and quenched by addition of 10 mL 5% $KHSO_4$. After aqueous work-up, the pure product was obtained by purification with flash chromatography to give amino aldehyde **III**.



Scheme S1. Synthesis of amino aldehyde

1.2.2. Synthesis of Photo-crosslinking Probe

The synthesis was followed by section in main text, except the peptide sequence was changed to Biotin-Gly-Gly-Ala-Arg-Phe-Lys(BP)-CHO, where BP stand for benzophenone (**Scheme S2**). LC-MS was performed to ensure the peptide aldehydes were of correct mass and sufficient purity (**Section 2.2**). All further work was carried out with these peptide aldehydes without purifications.



Scheme S2. Reagents and conditions for synthesis of the peptide aldehyde probe. (a) i: Fmoc-Gly-OH, HBTU, HOBt, DIEA, DMF, 4 hrs; ii: 20% piperidine/DMF, 30 min; iii Fmoc-Thr(O^{t}Bu)-OH, HBTU, HOBt, DIEA, DMF, 4 hrs; iv: Ac_2O, DIEA, DCM, 2 hr. (b) i: 20% piperidine/DMF, 30 min; ii: TFA/DCM (1:1) 1 hr; iii: 10% DIEA/DCM. (c) i:1%DIEA/MeOH , 2hr, 60°C. (d) Boc_2O, 1%DIEA/DCM, 2hrs. (e) i: 1% TFA/DCM, 30 min; ii: BP, HBTU, HOBt, DIEA, DMF, 4hrs. (f) i. TFA/TIS/EDT (95:5:0.1); ii. ACN:H_2O:TFA (60:40:0.1), 30 min, 60°C.

1.3. Microplate-based Screen of Sub-library against Caspase-3/-7/Cruzain/Rhodesain.

We performed screening on microplates to evaluate the relative potencies of Caspase-3/-7Cruzain/Rhodesain against the library. Assays were performed under optimized conditions as previous reported.[1,3] The relative potencies of each inhibitor were calculated from the normalized data through the following relationship:

$$\text{Inhibition Potency of } x = \left(1 - \frac{\text{Measured Intensity}, x}{\text{Uninhibited Intensity}}\right) \times 100\%$$

The data was presented as colored heatmaps using the treeview (http://rana.lbl.gov/ EisenSoftware.htm) software.

1.4. Microarray Work

1.4.1 Preparation of Avidin Slides.

25 × 75 mm glass slides (Sigma-Aldrich) were cleaned in piranha solution (sulfuric acid/ hydrogen peroxide, 7:3). An amine functionality was incorporated onto the slides by silanization using a solution of 3% (aminopropyl)triethoxysilane in 2% water and 95% ethanol. After 1-2 hr incubation, the slides were washed with ethanol and cured at 150 °C for at least 2 hr. The resulting amine slides were incubated in a solution of 180 mM succinic anhydride in DMF for 30 min before being transferred to a boiling water bath for 2 min. The slides were rinsed with ethanol and dried under a stream of nitrogen. The carboxylic acid-derivatized slide surface was activated with a solution of 100 mM HBTU, 200 mM DIPEA, and 100 mM N-hydroxysuccinimide in DMF, thus generating the NHS-derivatized slides. These surfaces were reacted with a solution of 1 mg/mL avidin in 10 mM $NaHCO_3$ (*p*H 9.0) for 40 min, washed with water and air dried. The unreacted NHS group was quenched with a solution of 2 mM aspartic acid in a 0.5 M $NaHCO_3$ buffer (*p*H 9.0). These slides were washed with water, dried and stored at 4 °C.

1.4.2 Microarray Preparation

Stock solutions of the peptide aldehydes were prepared to ~1.0 mM in 50% DMSO with 50% PBS, and were distributed in 384-well plates. All peptide aldehydes were shown to be completely soluble in this spotting solution. Slides were spotted on an ESI SMA arrayer (Ontario, Canada) with the printhead installed with four Stealth SMP8B Micro-spotting pins (Telechem, U.S.A.). Spots generated were of approximately 350 µm diameter and were printed with a spot-spot spacing of 375 µm. The pins were rinsed in between samples using two cycles of wash (for 10s) and sonication (for 10s) in reservoirs containing 70% ethanol followed by drying under reduced pressure (for 10s). The slides were allowed to stand for 2 hr on the printer platform and stored at 4ºC until use. Before incubation with the labeled protein, the slides were rinsed with PBS (*p*H 7.4) for 20 min and blocked with PBS containing 1.0% BSA for 1 hr.

1.4.3 Data extraction and Analysis

Microarray data was extracted using the ArrayWoRx software. Values from duplicated points were background subtracted and averaged (Duplicated spots with a standard deviation > 0.2 were rejected). The dataset was presented as colored heatmaps, using the Treeview software (http://rana.lbl.gov/EisenSoftware.htm).

1.4.4 K_D analysis of Selected High Binders

All peptide aldehydes were spotted onto slide where up to 4 identical sub-arrays were generated on the same microarray, so as to allow consistent/uniform screening and comparison of different protein samples, e.g. Caspases, Rhodesain, and cell lysates, to be carried out concurrently on the same platform. Using dose-dependent experiments, we extracted binding data of Caspase-3/-7 against 26 potential peptide aldehyde binders. The corresponding K_D was generated by fitting the data to the following equation, under the assumption that equilibrium is achieved during the incubation period:

$$\text{Observed Fluorescence of } x = \frac{(\text{Maximum Fluorescence}, x) \times [\text{Protein Concentration}]}{\text{K}_\text{D} + [\text{Protein Concentration}]}$$

Saturation dynamics observed when plotting *Observed Fluorescence* against the applied *Protein Concentration* were then fitted to the above equation using the Graph pad Prism software *ver* 4.03 (GraphPad, San Diego, USA) revealing the binding dissociation constant, K_D.[4]

1.5. Cell Culture and Western Blot

The human carcinoma epithelial carcinoma cell line Hela was cultured in growth media (DMEM supplemented with 10% fetal bovine serum, 100.0 mg/L streptomycin, and 100 IU/mL penicillin). Cells were maintained in a humidified atmosphere of 5% CO2 at 37 °C. To induce apoptosis, cells were incubated with 200 nM of staurosporine (STS), for overnight. Cells were detached by cell scraper, washed by cold PBS for three times, centrifuged, and keep in -80°C for further usage. Caspase-3, Calpain-1 and Cathepsin L antibodies were purchased from Abcam®. Briefly, equal amount of proteins were loaded and separated by SDS-PAGE and transferred onto PVDF membrane, blocked at room temperature for 1 hr using primary antibody blocking buffer (containing 0.1% Tween® 20) and incubated with HRP-conjugated secondary antibody for 1 hr at room temperature. At the end of this period, blots were washed thrice for 10 min each time in TBST (TBS containing 0.1% Tween® 20) and detected by chemiluminescence using ECL Plus Western Kit (Amersham, GE Healthcare, USA).

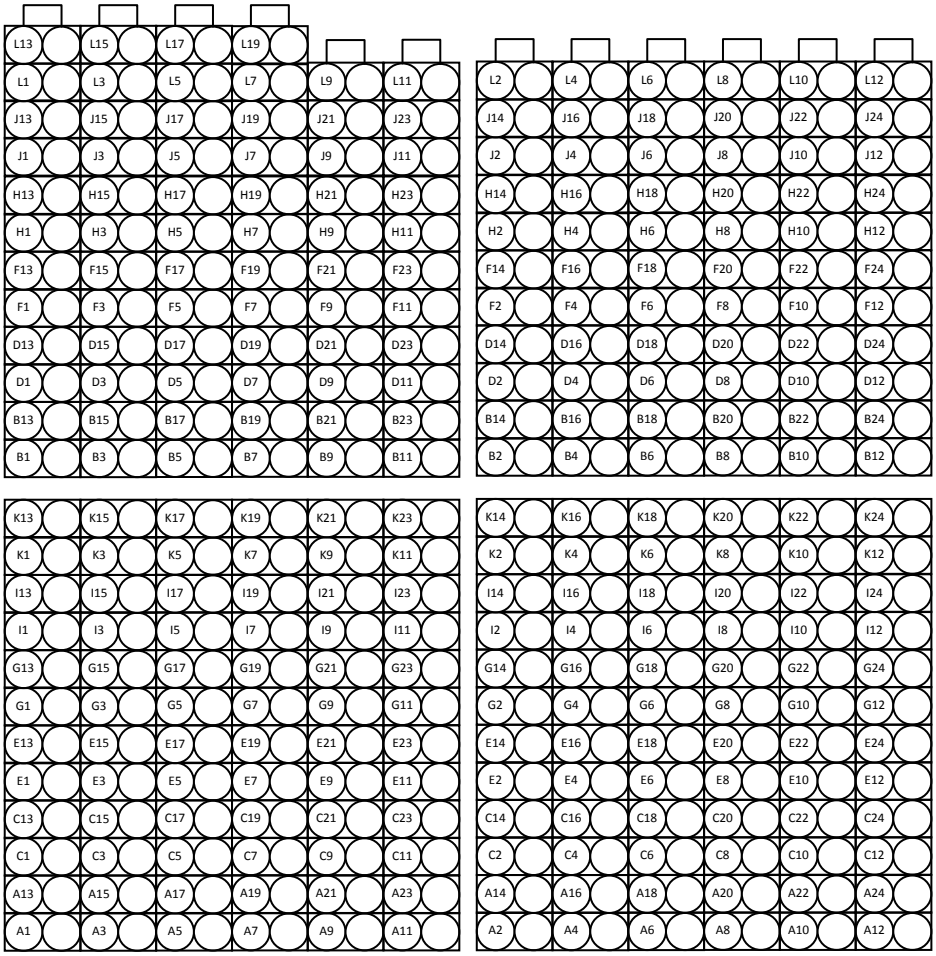


Figure S2. The spotting format of all the microarrays used in this study. Compounds were printed duplicate in horizontal direction. Decoding table see **Section 3**.

2. Results and Discussion:

2.1. ^{1}H and/or ^{13}C-NMR data of Amino Aldehyde

Fmoc-Asp(*tBu*)-H (IIIa): yield 86%. ^{1}H NMR (300 MHz, $CDCl_3$) 9.64 (s, 1H), 7.20-7.90 (m, 8H), 4.10-4.50 (m, 4H), 2.86 (m, 2H), 1.31 (s, 9H). ^{13}C NMR (60 MHz, $CDCl_3$) 198.7, 170.1, 156.1, 143.6, 141.2, 127.7, 127.0, 125.0, 120.0, 82.1, 67.2, 56.5, 47.0, 35.6, 27.9. (Method 1)

Fmoc-Phe-H (IIIb): yield 90%. ^{1}H NMR (300 MHz, $CDCl_3$) 9.64 (s, 1H), 7.10-7.90 (m, 12H), 4.10-4.60 (m, 4H), 3.15 (d, J = 6.4 Hz, 2H). ^{13}C NMR (60 MHz, $CDCl_3$) 198.6, 155.8, 143.6, 141.3, 135.4, 129.2, 128.7, 127.7, 127.1, 127.0, 124.9, 119.9, 66.9, 61.0, 47.1, 35.3. (Method 3)

Fmoc-Lys(Boc)-H (IIIc): yield 87%. ^{1}H NMR (300 MHz, $CDCl_3$) 9.57 (s, 1H) 7.20-7.90 (m, 8H), 4.10-4.60 (m, 4H), 3.11 (b, 2H), 1.2-1.7 (m, 15H). ^{13}C NMR (60 MHz, $CDCl_3$) 199.2, 156.1, 143.6, 141.2, 127.6, 127.0, 124.9, 119.9, 79.2, 66.9, 60.0, 47.1, 39.7, 33.8, 29.7, 28.4, 22.0. (Method 2)

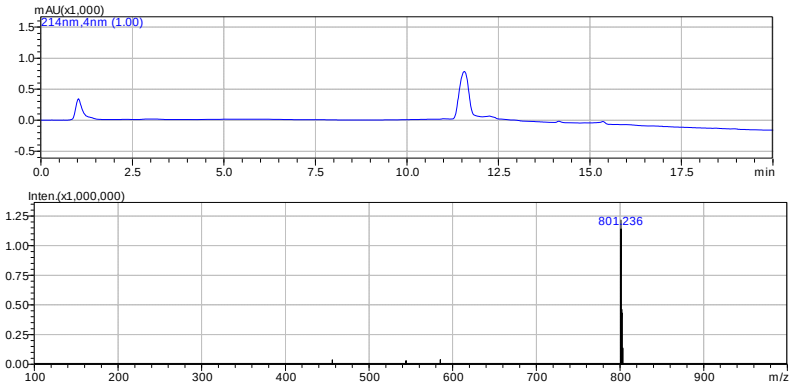
Fmoc-Leu-H (IIId): yield: 81.0%. ^{1}H NMR (300 MHz, $CDCl_3$) 9.58 (s, 1H), 7.20-7.90 (m, 8H), 4.10-4.60 (m, 4H), 1.71 (b, 2H), 1.30 (b, 1H), 0.97 (d, J = 4.4 Hz, 6H). ^{13}C NMR (60 MHz, CDCl3) 199.6, 156.1, 143.7, 141.2, 127.6, 127.0, 124.9, 119.9, 66.8, 58.7, 47.1, 38.0, 24.5, 23.0, 21.8. (Method 3)

Fmoc-Arg(Pbf)-H (IIIe): yield: 85%. 1H NMR (300 MHz, $CDCl_3$): δ 9.57 (s, 1H), 7.73 (d, J = 4.2 Hz, 2H), 7.56 (d, J = 4.2 Hz, 2H), 7.36 (t, J = 4.6 Hz, 2H), 7.28 (t, J = 3.4 Hz, 2H), 6.59 (s, 1H), 5.66 (s, 1H), 5.27 (d, J = 5.6 Hz, 1H), 4.76 (br s, 1H), 4.35 (d, J = 4.2 Hz, 2H), 4.17 (t, J = 4.2 Hz, 1H), 2.92 (s, 2H), 2.07-2.00 (m, 6H), 3.61, (m, 1H), 3.15 (t, J = 6.8 Hz, 1H), 1.77-1.52 (m, 4H), 1.44 (s, 6H). (Method 2)

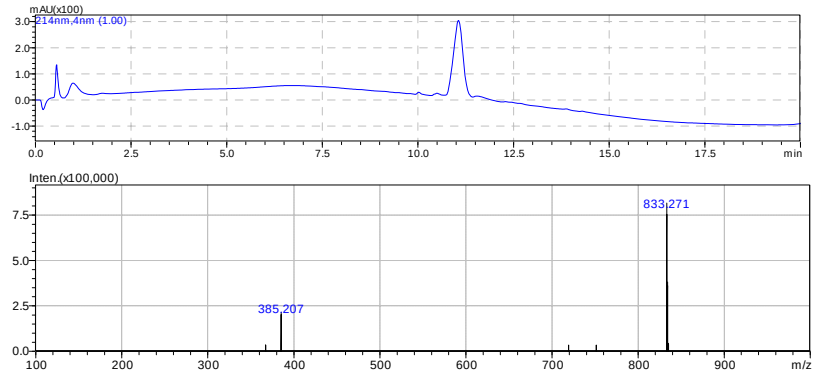
2.2. HPLC-MS Profiles of DEVX-CHO Sublibrary

ID	Inhibitors	M.W.	Obs Mass
H21	Biotin –GG- D-E-V-D-CHO	800.83	801.236
G22	Biotin –GG- D-E-V-F-CHO	832.92	833.271
D14	Biotin –GG- D-E-V-K-CHO	813.92	814.333
D16	Biotin –GG- D-E-V-L-CHO	798.90	799.297
D18	Biotin –GG- D-E-V-R-CHO	841.93	842.317

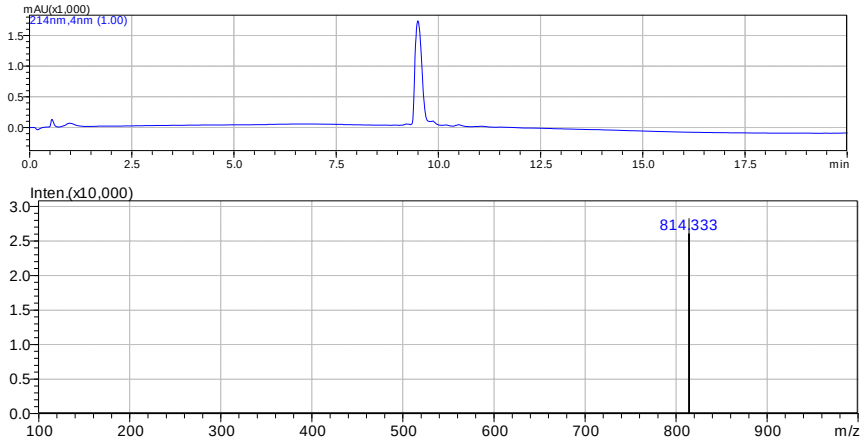
H21. Biotin-G-G-DEVD-CHO



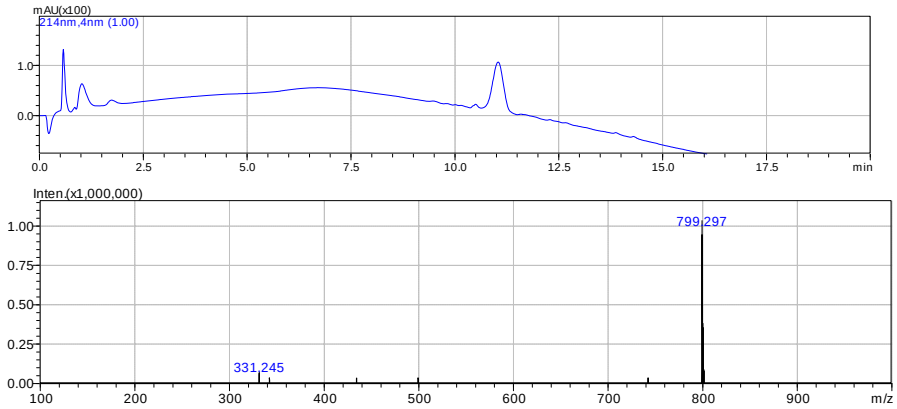
G22. Biotin-G-G-DEVF-CHO



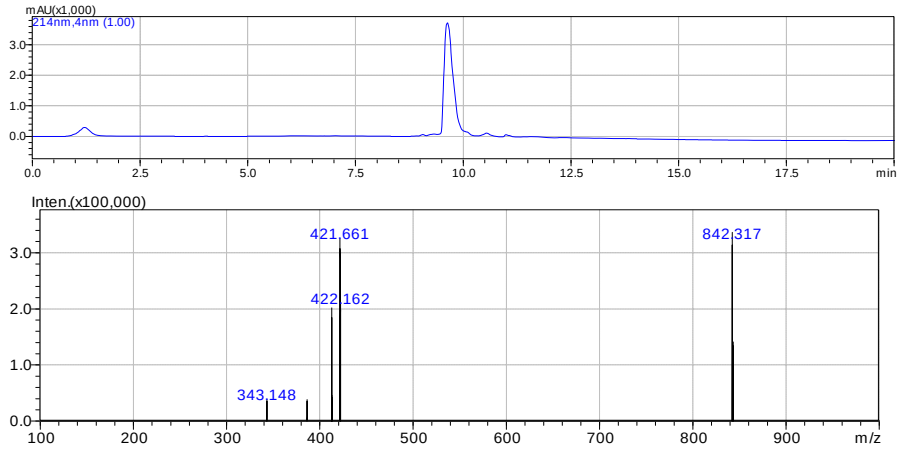
D14. Biotin-GG-DEVK-CHO



D16. Biotin-GG-DEVL-CHO



D18. Biotin-GG-DEVR-CHO



2.3. Microplate and Microarray Specificity Profiles of Pure Cysteine Proteases

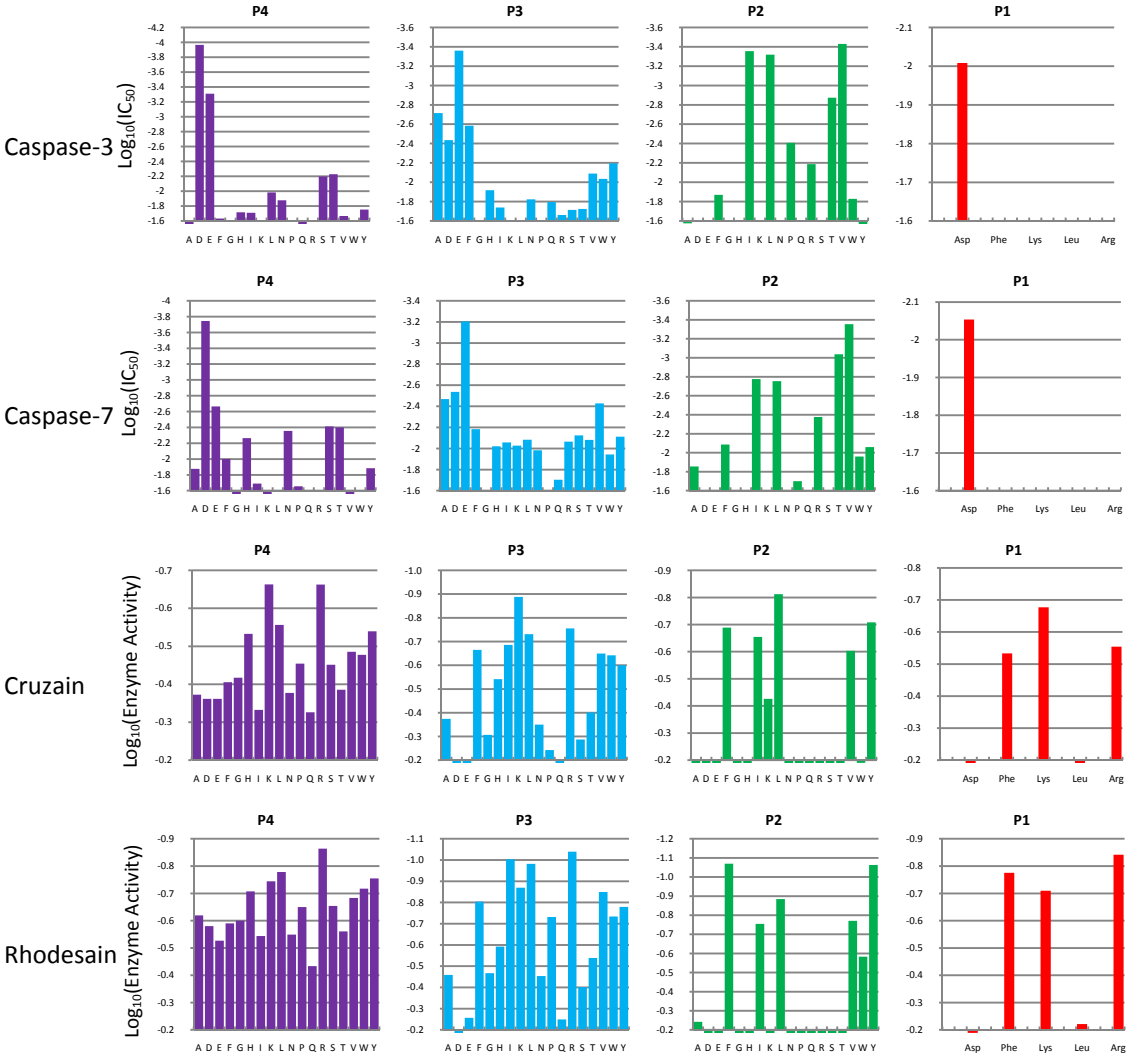


Figure S3. Microplate inhibitor specificity screening of cysteine proteases determined using the complete diverse inhibitor library. The results are presented in the order of amino acid sequence identity to each protease. All assays were performed in triplicate. The height of the bars denotes mean of **$Log_{10}(IC_{50})$** value for caspase-3/-7 and **Log_{10}(Enzyme Activity)** for Cruzain and Brucipain. The *x* axis in P2/P3/P4 indicates 18 amino acids held constant at each position, designated by the single-letter code. The *x* axis in P1 indicates five amino aldehydes.

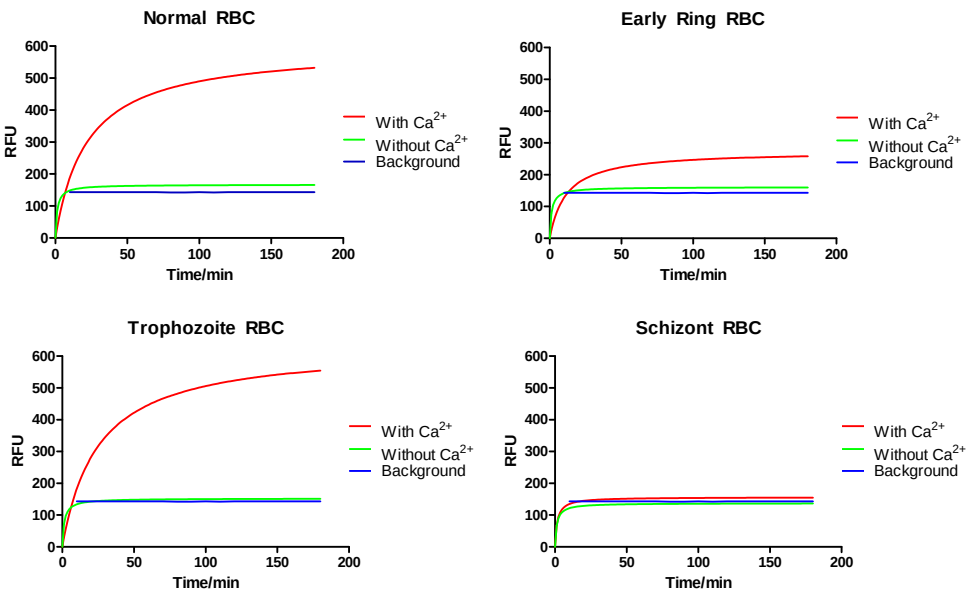


Figure S4 Kinetic reading of Ca^{2+} activated protease assay using non-/infected RBC lysates monitored by microplate reader. Curves shown in red are lysates pre-activated by $CaCl_2$ (20 μM). Curves shown in green are those without activation of Ca^{2+}. Curves shown in blue are background readings of the fluorogenic substrate (BODIPY FL casein).

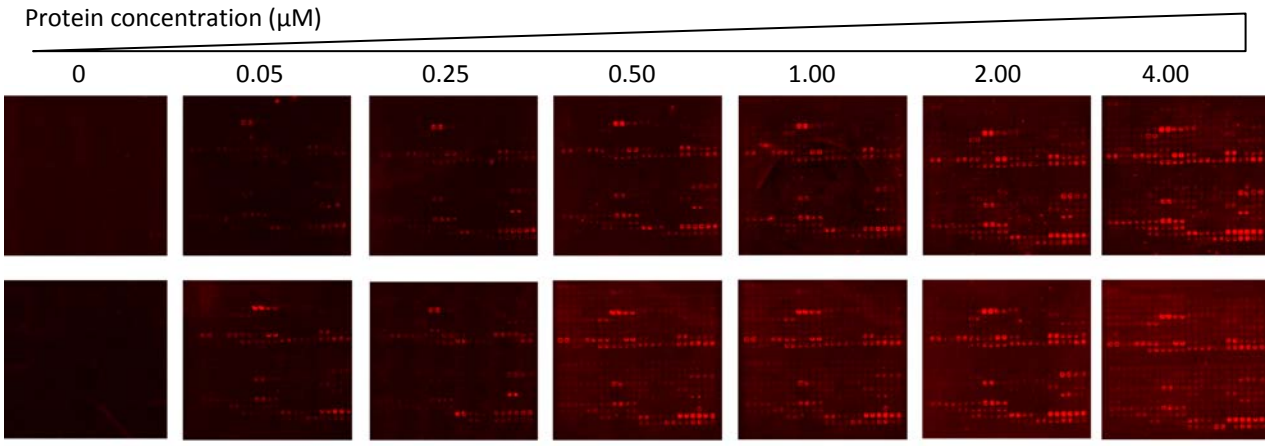


Figure S5. Microarray images of concentration-dependent experiments with Caspase-7 (top), and Caspase-3 (bottom). Data were extracted and fitted, assuming a saturation binding model, to derive the corresponding K_D.

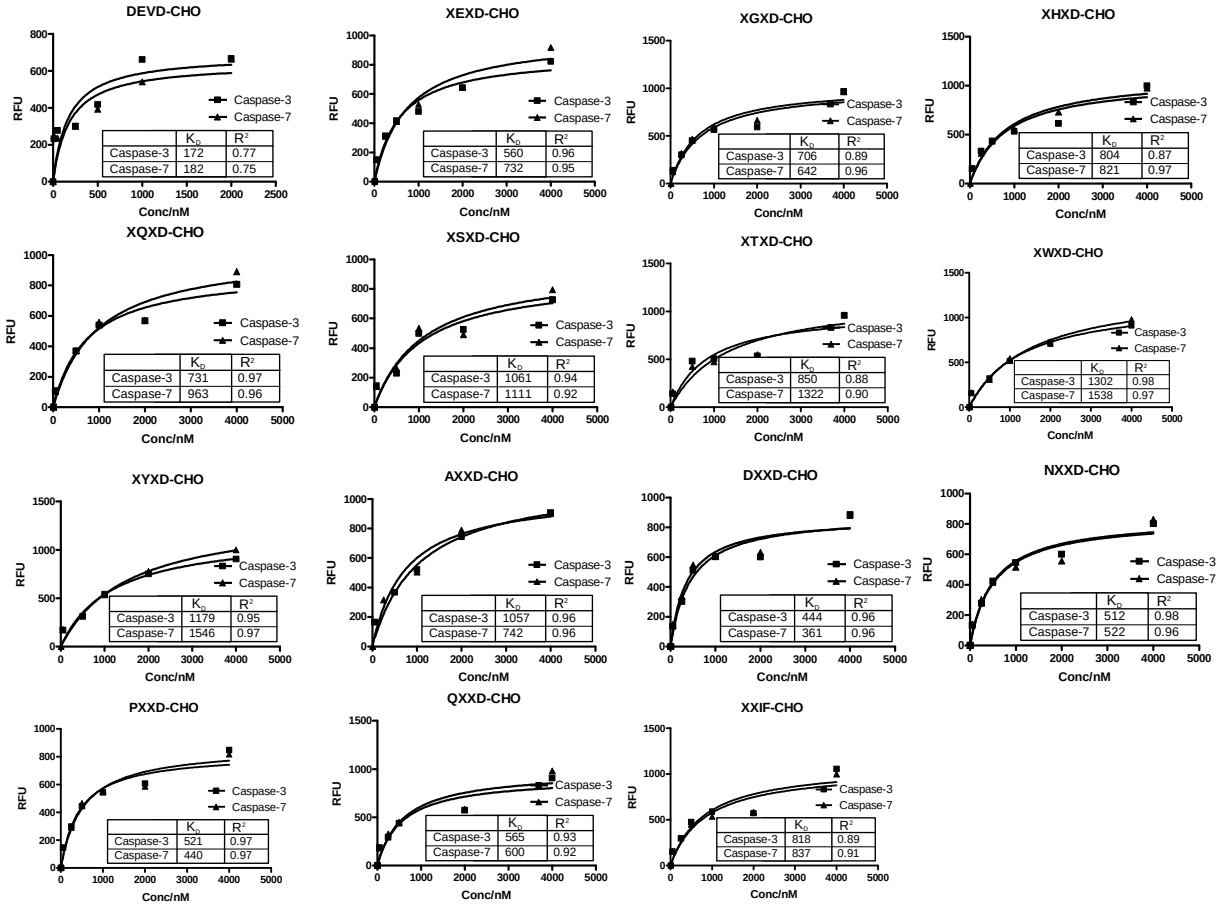


Figure S6. Inhibitory binding curves of K_D analysis for selected compounds.

2.4. Cellular lysates screening on microarray

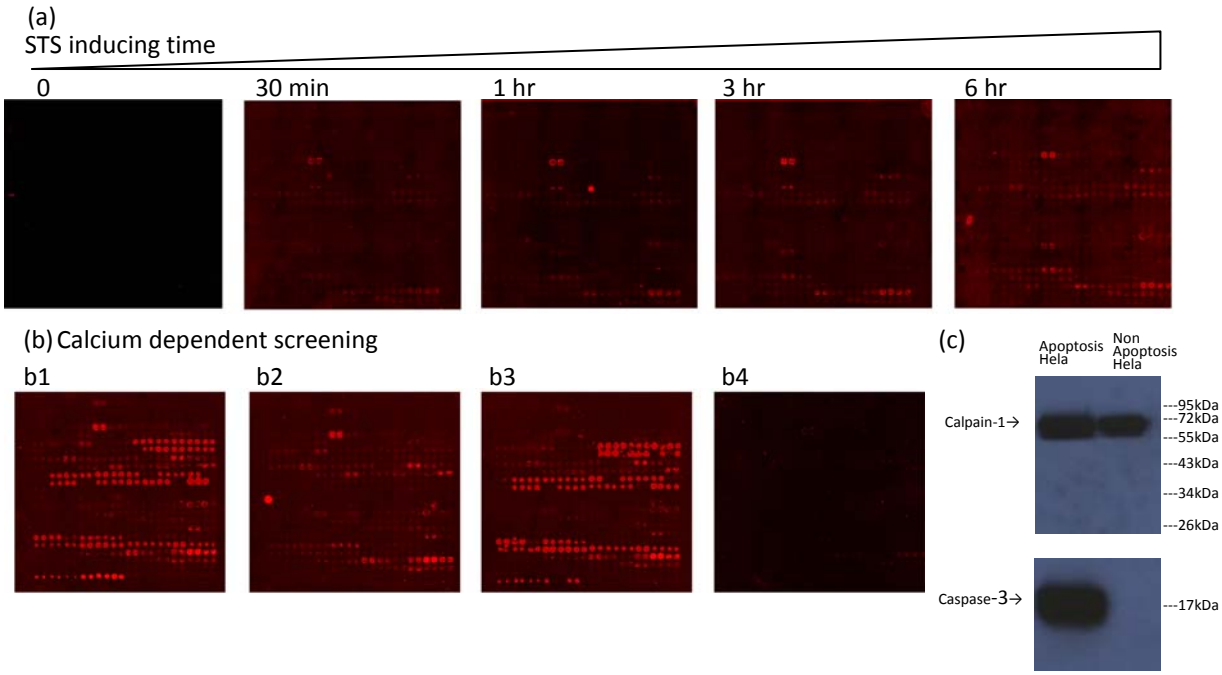


Figure S7. Cellular lysates screening on microarray including STS induced and Calcium dependent activity. (a) Hela cell was induced by STS for different lengths of time (as indicated), after which the cell lysates was collected and labeled. Subsequently, 0.16 μg/μL of the lysate was applied onto each grid; (b) Calcium-dependent microarray images (0.16 μg/μL of lysate on each gride); b1, Cy5-labeled apoptotic Hela cells (ApHela) induced with 20 μM Ca^{2+}; b2, Cy5-labeled apoptotic Hela cells only; b3, Cy5-labeled Hela cells (Non-apoptotic) induced with 20 μM Ca^{2+}; b4, Cy5-labeled Hela cells (Non-apoptotic) only. (c) Western blots of non-apoptosis/apoptosis Hela lysates, indicating the successful activation of the corresponding cysteine proteases (e.g. caspases and calpains).

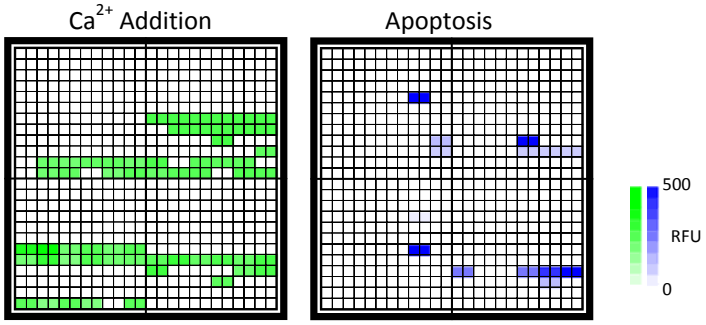


Figure S8. 2D barcode representation from different cellular events based on the microarray binding profiles obtained (left) Ca^{2+}-activated Hela Cells; (right) STS-induced ApHela Cells. The RFU values were normalized from scale 0 to 500.

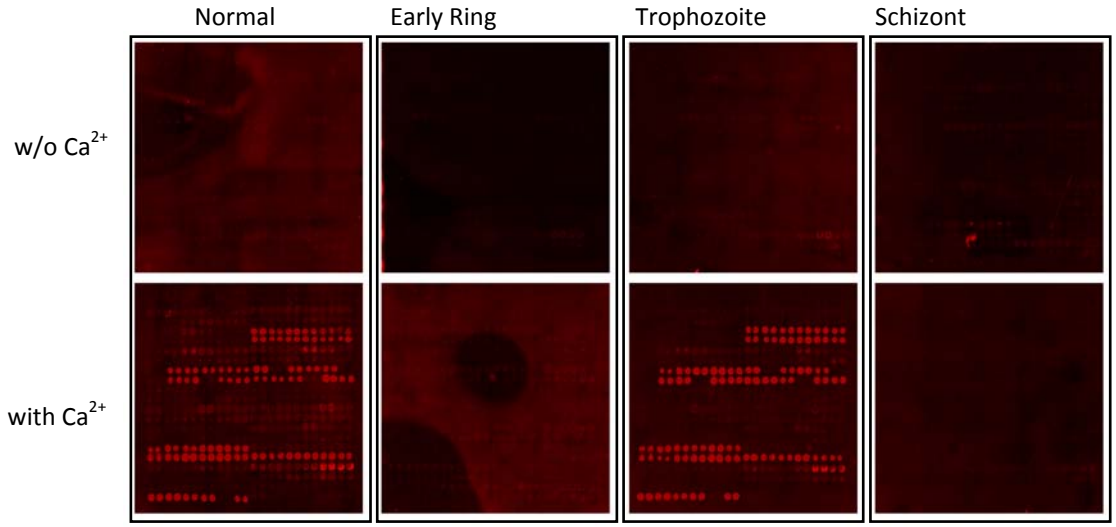
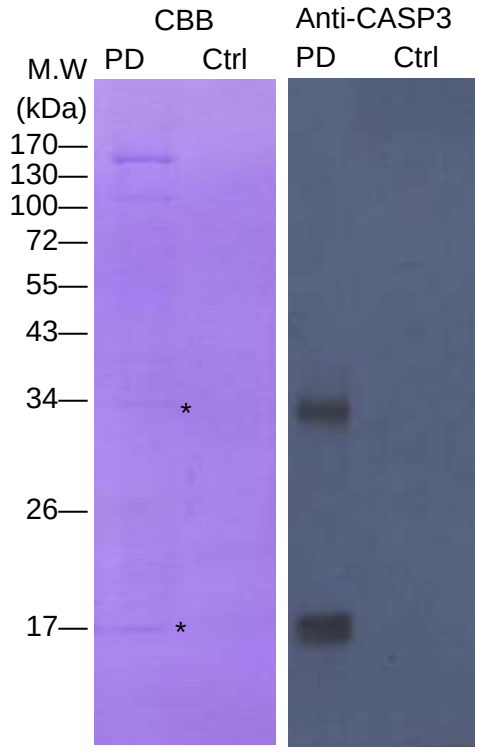


Figure S9. Ca^{2+} induced RBC lysate of different malaria parasite stages (Normal erythrocyte, without infection; Early-ring stage of *P. Falciparum* infected erythrocyte; Trophozoite staged erythrocyte; and Schizont staged erythrocyte). Parts of above figure were reproduced as Figure 4b in the maintext.

2.5 Pull-down results



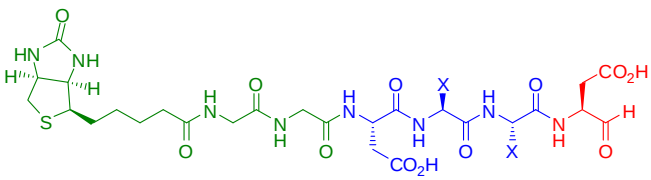


Figure S10. Proteomic validation of protein targets by pull-down and western blotting experiments. Pull-down of Hela lysate with non-covalent compound D10 (structure shown on the right) as described in **maintext**. Pull-down proteins positively identified by immunoblotting with the corresponding antibody. Asterisks show the expected locations of pro-Caspase-3 and active Caspase-3 bands. CBB: Coomassie Brilliant Blue; PD: Pull-Down assay; Ctrl: negative pull-down (with avidin beads only); CASP3: Caspase-3.

No	ID	Description	Mass	Score	emPAI	Peptides Matched	Related References
1	IPI00295741	Cathepsin B	30781	119	0.36	11	
2	IPI00002745	Cathepsin Z	34530	87	0.20	3	
3	IPI00219018	GAPDH	36201	508	0.69	12	6a
4	IPI00645452	β-Tubulin	48135	493	1.21	21	6b
5	IPI00027493	SLC3A2	58023	119	0.12	6	6c
6	IPI00022774	VCP	89950	113	0.11	3	6d
7	IPI00013508	ACTN1	103563	105	0.06	2	6e
8	IPI00011062	CPS1	165975	95	0.04	2	6f
9	IPI00784154	HSPD1	61187	395	0.37	15	
10	IPI00021439	Actin	42052	350	0.83	25	
11	IPI00414676	HSP90AB1	83554	317	0.31	10	
12	IPI00302592	FLNA	282581	185	0.07	8	
13	IPI00550731	IGKC	26503	146	0.27	2	
14	IPI00643920	Transketolase	69382	141	0.15	4	
15	IPI00001639	KPNB1	98420	140	0.10	4	
16	IPI00793443	IPO5	125032	118	0.08	3	
17	IPI00169383	PGK1	44985	92	0.24	3	
18	IPI00339226	FN1	243861	90	0.03	3	
19	IPI00219217	LDHB	36900	83	0.29	6	
20	IPI00302925	CCT8	60011	78	0.05	2	

Table S1. Proteins identified by pull-down and mass spectrometry. (Score is greater than 70)

3. References:

1. Ng, S.L.; Yang, P.-Y.; Chen, K.Y.-T.; Srinivasan, R.; Yao, S.Q. " "Click" Synthesis of Small Molecule Inhibitors Targeting Caspases", *Org. Biomol. Chem.*, **2008**, *6*, 844 – 847.
2. Uttamchandani, M.; Lee, W.L.;Wang, J.; Yao, S. Q. "Inhibitor Fingerprinting of Matrix Metalloproteases Using a Combinatorial Peptide Hydroxamate Library", *J. Am. Chem. Soc.*, **2007**, *129*, 13110 – 13117.
3. Yang, P-Y.; Wu, H.; Lee, M.Y.; Xu, A.; Srinivasan, R.; Yao, S.Q. "Solid-Phase Synthesis of Azidomethylene Inhibitors Targeting Cysteine Proteases", *Org. Lett.*, **2008**, *10*, 1881 – 1884.
4. Lu, C.H.S.; Sun, H.; Bakar, F.B.A.; Uttamchandani, M.; Zhou, W.; Liou, Y.-C.; Yao, S.Q. "Rapid Affinity-Based Fingerprinting of 14-3-3 Isoforms Using A Combinatorial Peptide Microarray", *Angew. Chem. Intl. Ed.*, **2008**, *47*, 7438 – 7441.
5. (a) Denault, J.B.; Salvesen, G.S. "Caspases: Keys in the Ignition of Cell Death", *Chem. Rev.*, **2002**, *102*, 4489 – 4499, (b) www.merops.sanger.ac.uk, (c) Choe, Y.; Leonetti, F.; Greenbaum, D.C.; Lecaille F.; Bogyo, M.; Bromme, D.; Ellman, J.A.; Craik, C.S. "Substrate Profiling of Cysteine Proteases Using a Combinatorial Peptide Library Identifies Functionally Unique Specificities", *J. Biol. Chem.*, **2006**, *281*, 12824 – 12832.
6. (a) Nakajima, H.; Amano, W.; Fujita, A.; Fukuhara, A.; Azuma, Y.-T.; Hata, F.; Inui, T.; Takeuchi, T. *J. Biol. Chem.* **2007**, *282*, 26562–26574. (b) Ludueña, R. F.; Banerjee, A.; Khan, I. A. *Curr. Opin. Cell Biol.* **1992**, *4*, 53–57. (c) Fort, J.; de la Ballina, L. R.; Burghardt, H. E.; Ferrer-Costa, C.; Turnay, J.; Ferrer-Orta, C.; Uson, I.; Zorzano, A.; Fernandez-Recio, J.; Orozco, M.; Lizarbe, M. A.; Fita, I.; Palacín, M. *J. Biol. Chem.* **2007**, *282*, 31444–31452. (d) Noguchi, M.; Takata, T.; Kimura, Y.; Manno, A.; Murakami, K.; Koike, M.; Ohizumi, H.; Hori, S.; Kakizuka, A. *J. Biol. Chem.* **2005**, *280*, 41332–41341. (e) Harper, B. D.; Beckerle, M. C.; Pomies, P. *Biochem. J.* **2000**, *350*, 269–274. (f) Corvi, M. M.; Soltys, C.-L. M.; Berthiaume, L. G. *J. Biol. Chem.* **2001**, 276, 45704–45712.

4. Microarray Decoding Table:

ID	Sequence
A01	Biotin-GG-IXXF-CHO
A02	Biotin-GG-XXAD-CHO
A03	Biotin-GG-KXXF-CHO
A04	Biotin-GG-XXDD-CHO
A05	Biotin-GG-LXXF-CHO
A06	Biotin-GG-XXED-CHO
A07	Biotin-GG-XRXK-CHO
A08	Biotin-GG-XXFD-CHO
A09	Biotin-GG-PXXF-CHO
A10	Biotin-GG-XXGD-CHO
A11	Biotin-GG-XXFK-CHO
A12	Biotin-GG-XXHD-CHO
A13	Biotin-GG-XXAK-CHO
A14	Biotin-GG-XXRD-CHO
A15	Biotin-GG-XXDK-CHO
A16	Biotin-GG-XXSD-CHO
A17	Biotin-GG-XXEK-CHO
A18	Biotin-GG-XXTD-CHO
A19	Biotin-GG-QXXF-CHO
A20	Biotin-GG-XXDR-CHO
A21	Biotin-GG-XXGK-CHO
A22	Biotin-GG-XXWD-CHO
A23	Biotin-GG-XXHK-CHO
A24	Biotin-GG-XXYD-CHO
B01	Biotin-GG-AXXK-CHO
B02	Biotin-GG-IXXK-CHO
B03	Biotin-GG-DXXK-CHO
B04	Biotin-GG-KXXK-CHO
B05	Biotin-GG-EXXK-CHO
B06	Biotin-GG-LXXK-CHO
B07	Biotin-GG-FXXK-CHO
B08	Biotin-GG-NXXK-CHO

ID	Sequence
B09	Biotin-GG-GXXK-CHO
B10	Biotin-GG-PXXK-CHO
B11	Biotin-GG-HXXK-CHO
B12	Biotin-GG-QXXK-CHO
B13	Biotin-GG-RXXK-CHO
B14	Biotin-GG-XXAL-CHO
B15	Biotin-GG-SXXK-CHO
B16	Biotin-GG-XXDL-CHO
B17	Biotin-GG-TXXK-CHO
B18	Biotin-GG-XXEL-CHO
B19	Biotin-GG-VXXK-CHO
B20	Biotin-GG-XXFL-CHO
B21	Biotin-GG-WXXK-CHO
B22	Biotin-GG-XXGL-CHO
B23	Biotin-GG-YXXK-CHO
B24	Biotin-GG-XXHL-CHO
C01	Biotin-GG-XAXF-CHO
C02	Biotin-GG-XIXF-CHO
C03	Biotin-GG-XDXF-CHO
C04	Biotin-GG-XKXF-CHO
C05	Biotin-GG-XEXF-CHO
C06	Biotin-GG-XXIF-CHO
C07	Biotin-GG-XFXF-CHO
C08	Biotin-GG-XNXF-CHO
C09	Biotin-GG-XGXF-CHO
C10	Biotin-GG-XXKF-CHO
C11	Biotin-GG-XHXF-CHO
C12	Biotin-GG-XQXF-CHO
C13	Biotin-GG-XRXF-CHO
C14	Biotin-GG-AXXF-CHO
C15	Biotin-GG-XSXF-CHO
C16	Biotin-GG-DXXF-CHO
C17	Biotin-GG-XTXF-CHO
C18	Biotin-GG-EXXF-CHO

ID	Sequence
C19	Biotin-GG-XVXF-CHO
C20	Biotin-GG-XXNF-CHO
C21	Biotin-GG-XWXF-CHO
C22	Biotin-GG-XXPF-CHO
C23	Biotin-GG-XYXF-CHO
C24	Biotin-GG-XXQF-CHO
D01	Biotin-GG-XXIL-CHO
D02	Biotin-GG-XQXD-CHO
D03	Biotin-GG-XXKL-CHO
D04	Biotin-GG-XSXD-CHO
D05	Biotin-GG-XXLL-CHO
D06	Biotin-GG-XTXD-CHO
D07	Biotin-GG-XWXD-CHO
D08	Biotin-GG-XYXD-CHO
D09	Biotin-GG-AXXD-CHO
D10	Biotin-GG-DXXD-CHO
D11	Biotin-GG-NXXD-CHO
D12	Biotin-GG-PXXD-CHO
D13	Biotin-GG-QXXD-CHO
D14	Biotin-GG-DEVK-CHO
D15	Biotin-GG-XDXL-CHO
D16	Biotin-GG-DEVL-CHO
D17	Biotin-GG-XEXD-CHO
D18	Biotin-GG-DEVR-CHO
D19	Biotin-GG-XFXL-CHO
D20	Biotin-GG-XEXL-CHO
D21	Biotin-GG-XGXL-CHO
D22	Biotin-GG-DEVY-CHO
D23	Biotin-GG-XHXL-CHO
D24	Biotin-GG-DEVH-CHO
E01	Biotin-GG-XXID-CHO
E02	Biotin-GG-RXXD-CHO
E03	Biotin-GG-XXKD-CHO
E04	Biotin-GG-SXXD-CHO

ID	Sequence
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E06	Biotin-GG-TXXD-CHO
E07	Biotin-GG-XXND-CHO
E08	Biotin-GG-VXXD-CHO
E09	Biotin-GG-XXPD-CHO
E10	Biotin-GG-WXXD-CHO
E11	Biotin-GG-XXQD-CHO
E12	Biotin-GG-YXXD-CHO
E13	Biotin-GG-XAXD-CHO
E14	Biotin-GG-XLXF-CHO
E15	Biotin-GG-XDXD-CHO
E16	Biotin-GG-XPXF-CHO
E17	Biotin-GG-DEVQ-CHO
E18	Biotin-GG-XXLF-CHO
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E23	Biotin-GG-XHXD-CHO
E24	Biotin-GG-HXXF-CHO
F01	Biotin-GG-XRXL-CHO
F02	Biotin-GG-AXXL-CHO
F03	Biotin-GG-XSXL-CHO
F04	Biotin-GG-DXXL-CHO
F05	Biotin-GG-XTXL-CHO
F06	Biotin-GG-EXXL-CHO
F07	Biotin-GG-XVXL-CHO
F08	Biotin-GG-FXXL-CHO
F09	Biotin-GG-XWXL-CHO
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F12	Biotin-GG-HXXL-CHO
F13	Biotin-GG-IXXL-CHO
F14	Biotin-GG-RXXL-CHO

ID	Sequence
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F16	Biotin-GG-SXXL-CHO
F17	Biotin-GG-LXXL-CHO
F18	Biotin-GG-TXXL-CHO
F19	Biotin-GG-NXXL-CHO
F20	Biotin-GG-VXXL-CHO
F21	Biotin-GG-PXXL-CHO
F22	Biotin-GG-WXXL-CHO
F23	Biotin-GG-QXXL-CHO
F24	Biotin-GG-YXXL-CHO
G01	Biotin-GG-XIXD-CHO
G02	Biotin-GG-XRXD-CHO
G03	Biotin-GG-XKXD-CHO
G04	Biotin-GG-XXSL-CHO
G05	Biotin-GG-XLXD-CHO
G06	Biotin-GG-XXTL-CHO
G07	Biotin-GG-XNXD-CHO
G08	Biotin-GG-XVXD-CHO
G09	Biotin-GG-XPXD-CHO
G10	Biotin-GG-XXNL-CHO
G11	Biotin-GG-XXRL-CHO
G12	Biotin-GG-XXVL-CHO
G13	Biotin-GG-XXPL-CHO
G14	Biotin-GG-IXXD-CHO
G15	Biotin-GG-HXXD-CHO
G16	Biotin-GG-KXXD-CHO
G17	Biotin-GG-EXXD-CHO
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G22	Biotin-GG-DEVF-CHO
G23	Biotin-GG-XXAR-CHO
G24	Biotin-GG-XAXL-CHO

ID	Sequence
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H03	Biotin-GG-DEVW-CHO
H04	Biotin-GG-XXFR-CHO
H05	Biotin-GG-XXWL-CHO
H06	Biotin-GG-XXGR-CHO
H07	Biotin-GG-XXYL-CHO
H08	Biotin-GG-XXHR-CHO
H09	Biotin-GG-XIXL-CHO
H10	Biotin-GG-XXRR-CHO
H11	Biotin-GG-XKXL-CHO
H12	Biotin-GG-XXSR-CHO
H13	Biotin-GG-XLXL-CHO
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H17	Biotin-GG-XPXL-CHO
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H19	Biotin-GG-XQXL-CHO
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H22	Biotin-GG-XXIR-CHO
H23	Biotin-GG-XXVD-CHO
H24	Biotin-GG-XXKR-CHO
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I03	Biotin-GG-XXDF-CHO
I04	Biotin-GG-SXXF-CHO
I05	Biotin-GG-XXEF-CHO
I06	Biotin-GG-TXXF-CHO
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I08	Biotin-GG-VXXF-CHO
I09	Biotin-GG-XXGF-CHO
I10	Biotin-GG-WXXF-CHO

ID	Sequence
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I14	Biotin-GG-XXIK-CHO
I15	Biotin-GG-XXSF-CHO
I16	Biotin-GG-XXKK-CHO
I17	Biotin-GG-XXTF-CHO
I18	Biotin-GG-XXLK-CHO
I19	Biotin-GG-XXVF-CHO
I20	Biotin-GG-XXNK-CHO
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I23	Biotin-GG-XXYF-CHO
I24	Biotin-GG-XXQD-CHO
J01	Biotin-GG-XIXR-CHO
J02	Biotin-GG-XRXR-CHO
J03	Biotin-GG-XKXR-CHO
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J10	Biotin-GG-XWXR-CHO
J11	Biotin-GG-XQXR-CHO
J12	Biotin-GG-XYXR-CHO
J13	Biotin-GG-AXXR-CHO
J14	Biotin-GG-IXXR-CHO
J15	Biotin-GG-DXXR-CHO
J16	Biotin-GG-KXXR-CHO
J17	Biotin-GG-EXXR-CHO
J18	Biotin-GG-LXXR-CHO
J19	Biotin-GG-FXXR-CHO
J20	Biotin-GG-NXXR-CHO

ID	Sequence
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J22	Biotin-GG-PXXR-CHO
J23	Biotin-GG-HXXR-CHO
J24	Biotin-GG-QXXR-CHO
K01	Biotin-GG-XXRK-CHO
K02	Biotin-GG-XAXK-CHO
K03	Biotin-GG-XXSK-CHO
K04	Biotin-GG-XDXK-CHO
K05	Biotin-GG-XXTK-CHO
K06	Biotin-GG-XEXK-CHO
K07	Biotin-GG-XXVK-CHO
K08	Biotin-GG-XFXK-CHO
K09	Biotin-GG-XXWK-CHO
K10	Biotin-GG-XGXK-CHO
K11	Biotin-GG-XXYK-CHO
K12	Biotin-GG-XHXK-CHO
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L03	Biotin-GG-XXNR-CHO
L04	Biotin-GG-VXXR-CHO
L05	Biotin-GG-XXPR-CHO
L06	Biotin-GG-WXXR-CHO

ID	Sequence
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L08	Biotin-GG-YXXR-CHO
L09	Biotin-GG-XAXR-CHO
L10	Biotin-GG-XEXR-CHO
L11	Biotin-GG-XDXR-CHO

ID	Sequence
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L13	Biotin-GG-XGXR-CHO
L15	Biotin-GG-XHXR-CHO
L17	Biotin-GG-RXXR-CHO
L19	Biotin-GG-SXXR-CHO