Electronic Supplementary Information

In-Situ and Timed Extraction of Cellular Peptides from Live HeLa Cells by Photo-Switchable Mesoporous Silica Nanocarriers

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Experimental Procedures

Materials

Mbelliferone, allyl bromide, hexadecyltrimethylammonium bromide (CTAB), tetraethyl orthosilicate (TEOS), (3-aminopropyl)triethoxysilane (APTES), 2-(N-morpholino)ethanesulfonic acid (MES), N-hydroxysulfosuccinimide sodium salt (sulfo-NHS), 2,5-Dihydroxybenzoic acid (DHB), tetraethylammonium borohydride (TEAB), CH_2O, CD_2O, $NaBH_3CN$, formic acid (FA), trifluoroacetic acid (TFA), bovine serum albumin (BSA), dithiothreitol (DTT), iodoracetamide (IAA), ethylenediaminetetraacetic acid (EDTA), ethylene glycol tetraacetic acid (EGTA), trypsin, protease inhibitor Cocktail, NaCl, K_2CO_3, Pt (dvs) [platinum(0)-1,3-divinyl-1,1,3,3-tetramethyldisiloxane complex] were purchased from Sigma Aldrich (St. Louis, MO). Triethoxysilane, N-(3-dimethylaminopropyl)-N`-ethylcarbodiimide hydrochloride (EDC) were obtained from J&K Scientific Ltd (Beijing, China). ACN was HPLC grade and obtained from Merck (Darmstadt, Germany). RPMI 1640 cell culture medium and penicillin/streptomycin solution (100×) were obtained from Gibco Invitrogen Corporation (Carlsbad, CA). Reagents for SILAC labeling, including L-Lysine-2HCl, D4 L-Lysine-2HCl, lysine-depleted RPMI 1640 medium and dialyzed fetal bovine serum (FBS) were obtained from Thermo Scientific (USA). TAT peptides (NH_2-YGRKKRRQRRR-COOH) were purchased from Chinese Peptide Company (Hangzhou, China) with the purity at 95%. Other reagents, including toluene, NH_4NO_3, ethanol, isopropanol, HCl were of analytical grade. Deionized water was purified using a Milli-Q water system (Millipore, USA).

The construction of controllable nanocarrier based on MSN

The MSN nanoparticles were synthesized based on a reported strategy.[1] Basically, 350 μL NaOH (2 M) was added in 50 mL CTAB (5 mM), then 0.5 mL TEOS was added into the solution under vigorous stirring; the MSN was formed at 60 ℃ for 2 h. The products were collected by centrifugation, washed with ethanol and H_2O for 3 times, and dried in a vacuum oven at 60 ℃ overnight.

The synthesis of 7-[(3-triethoxysilyl)propoxy]coumarin and construction of MSN-coumarin was followed the previous report with a few modifications.[2] As a general procedure, 600 mg umbelliferone (3.0 mmol) was dissolved in 30 mL acetone, followed by the addition of 500 mg K_2CO_3 (3.6 mmol). Later, 435 mg allyl bromide (3.6 mmol) was dropwise added in the solution, and

the reaction was kept under reflux for 5 h. After filtration, 7-allyloxycoumarin was collected and purified by recrystallization using ethanol, and dried in a vacuum oven at 60 ℃ overnight. 324 mg 7-allyloxycoumarin (1.6 mmol) and 332 mg triethoxysilane (1.76 mmol) was dissolved in 20 mL toluene under N_2 atmosphere, after which 80 μL Pt (dvs) [platinum(0)-1,3-divinyl-1,1,3,3-tetramethyldisiloxane complex] (2 mM in toluene) was further added; the reaction would be finished at room temperature for 20 h.

To link 7-[(3-triethoxysilyl)propoxy]coumarin on the MSN, 200 mg MSN was first dispersed in 20 mL n-hexane, and the 40 mg 7-[(3-triethoxysilyl)propoxy]coumarin (in 1 mL n-hexane) was added, where the mixture was stirred at room temperature for 10 min. The n-hexane was then removed by vacuum distillation. The products of MSN-coumarin were re-dispersed in ethanol containing NH_4NO_3 (0.5 mg/mL), and CTAB templates were removed by ion exchange at 70 ℃ for 2 hours. Finally, the MSN-coumarin was collected by centrifugation, washed with ethanol until no 7-[(3-triethoxysilyl)propoxy]coumarin left, dried in a vacuum oven and stored under dry condition.

Before further functionalization, the MSN-coumarin was treated with APTES to form the amino groups on the nanoprobes. TAT peptides were linked on the MSN-coumarin through the EDC/sulfo-NHS strategy. 2 μmol of TAT peptides were first dissolved in 1 mL 2-morpholinoethanesulfonic acid (MES) buffer (0.1 M MES, 0.5 M NaCl, pH=6.0), followed by the addition of 20 μmol EDC and 50 μmol sulfo-NHS, and the activation of TAT peptides was achieved at room temperature for 15 min. 100 mg aminated MSN-coumarin (in 10 mL PBS buffer, pH=7.4) was mixed with the activated TAT peptides, and the mixture was kept in room temperature for 2 h. Finally, the products of MSNcg were collected by centrifugation, washed with ethanol and H_2O, dried in a vacuum oven and stored under dry condition before further application.

Characterization of the MSN and MSNcg

The TEM images of MSN were obtained using a JEM-2000 EX (JEOL, Japan) electronic microscope using the accelerating voltage at 120 keV. The nitrogen adsorption curve of MSN was measured with the static-volumetric method at 77 K using an ASAP 2010 instrument (Micromeritics), and the specific area, pore size and pore volume were calculated by the nitrogen adsorption curve using the BET equation. The FTIR analysis of MSNcg was carried out using the Spectrum GX (PE, USA).

Adsorption capacity of peptides in MSN

100 μg MSN was mixed with 100 μg BSA digested peptides (in PBS buffer) for required incubation times (0, 5, 10, 15, 30, 60, 120 min) at room temperature, after which MSN was removed by centrifugation. The amounts of peptides in the solution before and after extraction were measured by HPLC, and the extraction amounts were calculated by comparing the changed amounts of peptides in the supernatants during the extraction procedure.

To investigate the mechanism of peptide adsorption in the MSN based materials, different concentration (0, 5, 10, 15, 20, 30%, v/v) of methanol was utilized to elute the peptides adsorbed in the pores, and the recovery of eluted peptides was measured with UV spectrometry.

Cell viability assay

Viability of HeLa cells under the treatment of MSNcg was measured by Cell Counting Kit-8 (CCK-8, DOJINDO, Japan) according to the protocol. At first, 5 000 HeLa cells were pre-planted in a 96-well plate, and then MSNcg was added and incubated with cells as required. 1 000, 2 000, 3 000, 4 000, 5 000 HeLa cells were also planted in the plate to serve as the standard data of cell viability. After the treatments, the nanoprobes were removed and cells were washed with PBS buffer for 3 times. The eluted CCK-8 solution was added and incubated with cells for 30 min, the absorbance value was obtained by a microplate reader (BioRek, USA) at 450 nm. All the measurements were repeated for six times.

To test the influence of UV irradiation (at 254 nm) during the pore opening procedure, the apoptotic ratio of cells after the UV treatment was evaluated by flow cytometry. The treated cells were stained with FITC Annexin V Apoptosis Detection Kit I (BD, Franklin Lakes, NJ) following the instructions, and all the measurements were performed with a FACS Vantage SE flow cytometer from BD (Franklin Lakes, NJ). All the data were technically repeated for 3 times.

Cell culture and stable isotope labeling by amino acids in cell culture (SILAC)

HeLa cells were cultured using RPMI 1640 culture medium containing 10% bovine serum (BS) and penicillin/streptomycin (1×) at 37 ℃ under 5% CO_2 atmosphere, and the cells were split with a 90% confluency. SILAC treated HeLa cells were cultured in lysine-depleted 1640 medium containing 0.1 mg/mL D4 L-lysine (K4).

Tryptic digestion of BSA and dimethylation isotope labeling of digested peptides

Protein digestion was followed a protocol developed in our lab.[3] Briefly, 1 mg BSA was dissolved and denatured in 150 μL solution of 8 M urea and 100 mM TEAB (pH=8.0), reduced by

DTT (2 μmol), and alkylated by IAA (4 μmol). Then, 1 mL 100 mM TEAB buffer (pH=8.0) was added into the protein solution, followed by the addition of 4 μg trypsin. The digestion was kept at 37 ℃ for 20 h. The digested peptides were then went through dimethylation isotope labeling by adding the light labeling reagent (0.2% CH_2O and 30 mM $NaBH_3CN$) and heavy labeling reagent (0.2% CD_2O and 30 mM $NaBH_3CN$) as required.[4] Finally, the isotope labeled peptides were desalted with reverse phase C18 SPE columns, lyophilized, and stored at −80 ℃ before further usage.

Intracellular localization of MSNcg by TEM imaging

After HeLa cells were treated by MSNcg and MSN-coumarin (200 μg/mL) for 1 h, the cells were washed with PBS buffer for three times, fixed by PBS buffer containing 2.5% glutaraldehyde overnight at 4℃ and further fixed with PBS buffer containing 1% OsO_4. The cell pellets were dehydrated using ethanol, treated using propylene oxide and embedded in Epon. Then, the cell slices (ca. 80 nm) were obtained, stained with uranyl acetate and lead citrate. The observation of the intracellular localization of MSNcg was carried out using a JEM-2000 EX (JEOL, Japan) electronic microscope with an accelerating voltage at 120 keV.

Controlling the coumarin gates inside live HeLa cells

8 mg MSNcg was mixed with 4 mL DOX solution (0.5 mg/mL) and incubated overnight under gentle vibration. After that, the mixture was irradiated at 310 nm for 5 min to close the gates. DOX@MSNcg was washed completely with PBS buffer. The DOX@MSNcg (25 μg/mL in cell culture medium) was ingested by HeLa cells during incubation for 12 h. Then, the cells were treated with UV irradiation at 254 nm for 2 min, where the cells in the control group were not irradiated for gate opening, and the release of DOX in cells was observed by a fluorescence microscope (Olympus) after 10 min.

Controllable extraction of peptides in vitro and in live HeLa cells

BSA digested peptides were utilized as standard peptides for in vitro extraction. As a general procedure, coumarin gates on the MSNcg were first closed by irradiation >310 nm for 10 min using a high pressure mercury lamp coupled with a long-wave-pass filter. 1 mg BSA digested peptides (heavy or light isotope labeled) were mixed with 4 mg MSNcg, and the coumarin gates were opened by irradiated at 256 nm for 2 min. The adsorption equilibrium could be reached within 15 min (by optimization using the MSN), after which the gates on MSNcg were closed again and the MSNcg

was collected by centrifugation. Non-specific adsorbed peptides outside MSNcg were removing with the washing solution (15% methanol, 85% H_2O containing 0.1% TFA) until no peptides could be detected by MALDI-TOF analysis. Finally, the gates of MSNcg were opened again and the adsorbed peptides were eluted with the washing solution, lyophilized and stored at -80 ℃ before analysis.

The in vivo extraction went through a similar procedure with the in vitro extraction. Briefly, MSNcg was treated with 10 min irradiation (> 310 nm) for gate closing, and then the MSNcg were dispersed in the culture medium (200 μg/mL) and incubated with HeLa cells for 1 h. After internalization, the treated cells were washed with PBS buffer for several times to fully remove the endogenous peptides in bovine serum. Then, the cells were collected, followed by the initiation (open the coumarin gate) and termination (close the coumarin gate) of the adsorption. The cells were later lysed using the lysing buffer (20 mM Hepes, 25 mM SDS, 0.5 mM EGTA, 1 mM EDTA, 1 mM PMSF, 2% Cocktail, pH=7.4), and the ingested MSNcg with the adsorbed peptides were gathered using centrifugation and washed using the washing buffer. Finally, the coumarin gate of MSNcg was opened and captured peptides were eluted as described above.

MALDI-TOF analysis of extracted peptides

MALDI-TOF analysis was carried out with AB Sciex 5800 MALDI-TOF/TOF mass spectrometer (AB, SCIEX, USA) using linear positive ion mode with a pulsed laser at 355 nm. 2,5-Dihydroxybenzoic acid was sissolved in ACN/H_2O/H_3PO_4 (70:29:1, v/v/v) as matrix. Before analysis, peptides were disoved in 0.1% FA solution, and 0.5 μL of peptides and 0.5 μL DHB matrix were added on the sample plate stepwised.

LC-MS analysis of the intracellular peptides from HeLa cells

The bottom up analysis of extracted peptides was carried out using Q Exactive (Thermo Scientific, Waltham, MA). The peptides was re-dissolved in 0.1% FA (Buffer A), and loaded to a 3 cm ×200 μm trap column packed with C18 AQ beads (Michrom Bio Resources, 5 μm, 120 Å, USA) using Buffer A at 5 μL/min, and separated by a 15 cm ×75 μm C18 AQ column (5 μm, 120 Å, USA) at 300 nL/min with a gradient of 10%~43% Buffer B (80% ACN, 0.1% FA) in 90 min. The full scan was obtained at a resolution of 70000 (m/z=200) with an AGC of 1×10^6, and the Top 15 ions (intensity above $1e^4$) was subjected to HCD fragmentation at a normalized collision energy of 28 with an constant injection time of 60 ms. The product ion was analysis with a resolution of 17500 (m/z=200). The dynamic exclusion was enabled with a value of 30 s.

Data processing

The data LC-MS/MS analysis were searched against human database (Uniprot_human, version 201307) by Thermo Proteome Discoverer^{TM} (Thermo Scientific, Waltham, MA; version 1.4.1.14) using Sequest HT strategy. No enzyme was selected for digestion with unspecific cleavage sites. Oxidation of methionine was selected as the dynamic modification. Precursor and fragment mass tolerance were set at 20 ppm and 0.05 Da, respectively. The false positive rates for identification of both proteins and peptides were less than 1%.

References:

[1] C. T. Kresge, M. E. Leonowicz, W. J. Roth, J. C. Vartuli, J. S. Beck, *Nature* **1992**, *359*, 710-712.
[2] N. K. Mal, M. Fujiwara, Y. Tanaka, *Nature* **2003**, *421*, 350-353.
[3] F. Wang, J. Dong, X. Jiang, M. Ye, H. Zou, *Anal. Chem.* **2007**, *79*, 6599-6606.
[4] P. J. Boersema, R. Raijmakers, S. Lemeer, S. Mohammed, A. J. R. Heck, *Nat. Protoc.* **2009**, *4*, 484-494.

Supplementary Figures

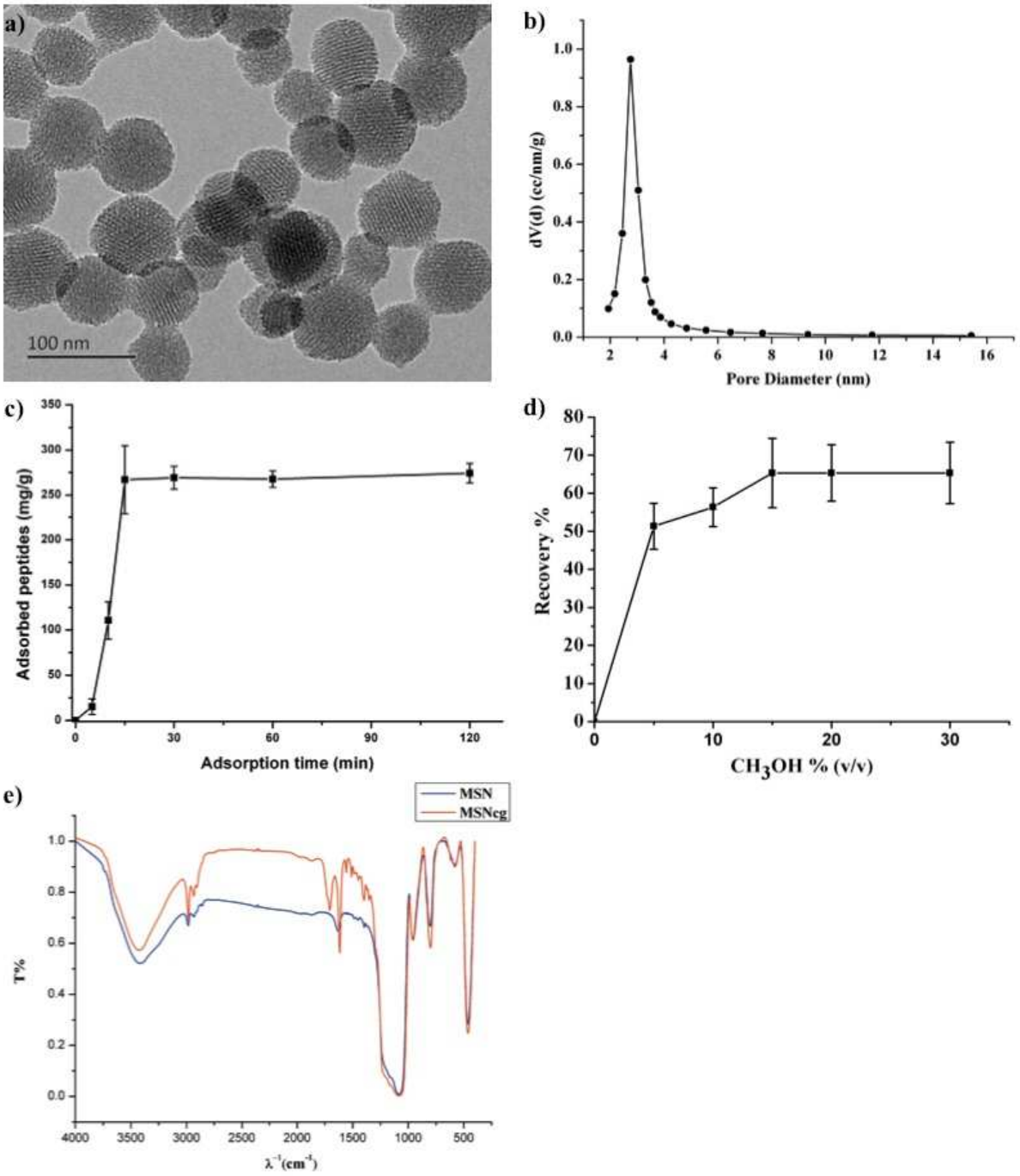
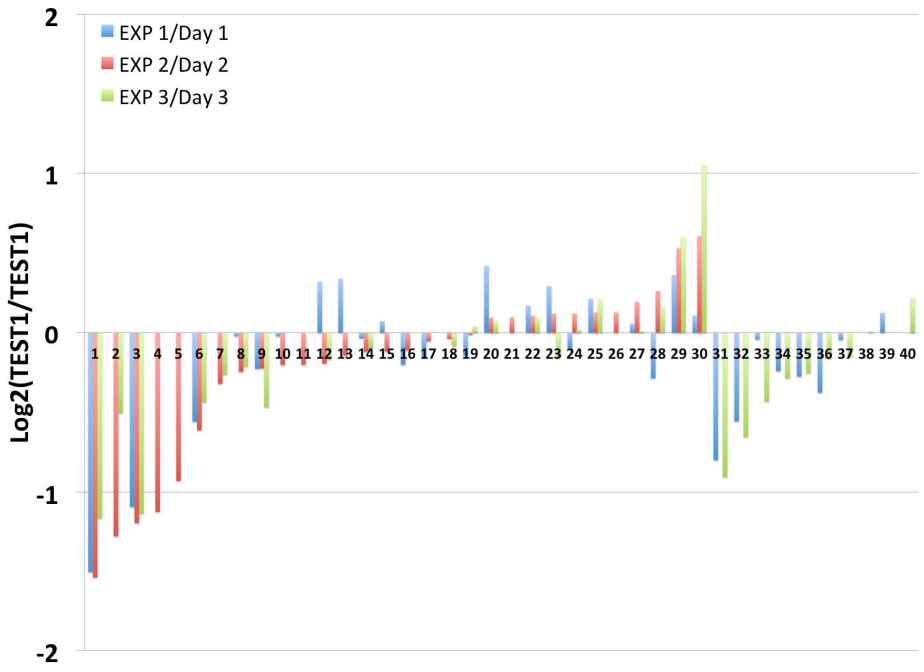


Figure S-1. Characterization of MSN and MSNcg. a) TEM image and b) pore size distribution of MSN, c) the adsorption of BSA digested peptides by MSN, d) the elution of loaded peptides from the pores of MSN, where more peptides could be obtained with higher concentrations of methanol, and e) FT-IR analysis of MSN (blue) and MSNcg (red). Peaks at 1710 cm^{-1}, 1450-1550 cm^{-1} at FT-IR spectrum represented the existence of lactone and benzene from coumarin.



PEP No.	PEP Sequence
1	ECCHGDIIECADDR
2	DDSPDIPK
3	EYEATIEECCAK
4	ETYGDMADCCEK
5	DAIPENIPPITADFAEDK
6	TCVADESHAGCEK
7	QNCDQFEK
8	IVNEITEFAK
9	ECCDKPIIEK
10	IKECCDKPIIEK
11	ATEEQIK
12	QTAIVEIIK
13	GACIIPK
14	CCTESIVNR
15	IVTDITK
16	DIGEEHFK
17	QEPERNECFISHK
18	NECFISHKDDSPDIPK
19	SIHTIFGDEICK
20	VPQVSTPTIVEVSR
21	NECFISHK
22	YIYEIAR
23	SHCIAEVEK
24	IKPDPNTICDEFK
25	KVPQVSTPTIVEVSR
26	ICVIHEK
27	FKDIGEEHFK
28	RPCFSAITPDETYVPK
29	IGEYGFQNAIIVR
30	TVMENFVAFVDK
31	DAIPENIPPITADFAEDKDV CK
32	YNGVFQECCQAEDK
33	HIVDEPQNIIK
34	AEFVEVTK
35	YICDNQDTISSK
36	RHPEYAVSVIIR
37	EACFAVEGPK
38	IFTFHADICTIPDTEK
39	CCAADDKEACFAVEGPK
40	KQTAIVEIIK

Figure S-2 Repeatability of the controllable extraction with MSNcg during 3 days.

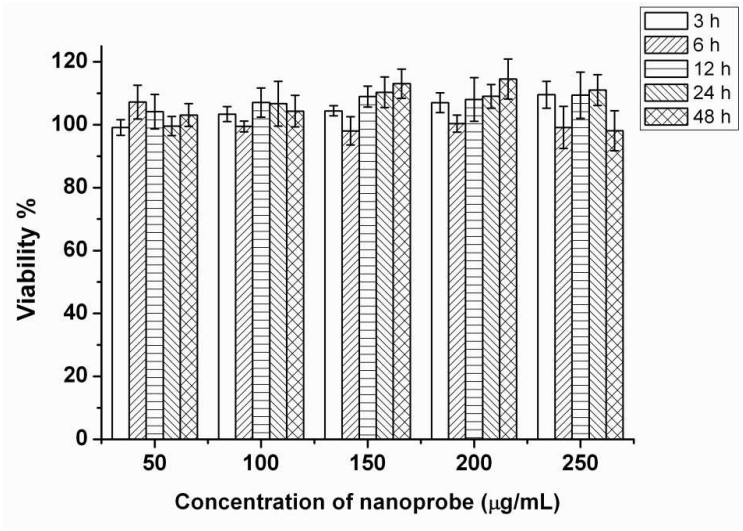


Figure S-3 Viability of HeLa cells treated with MSNcg.

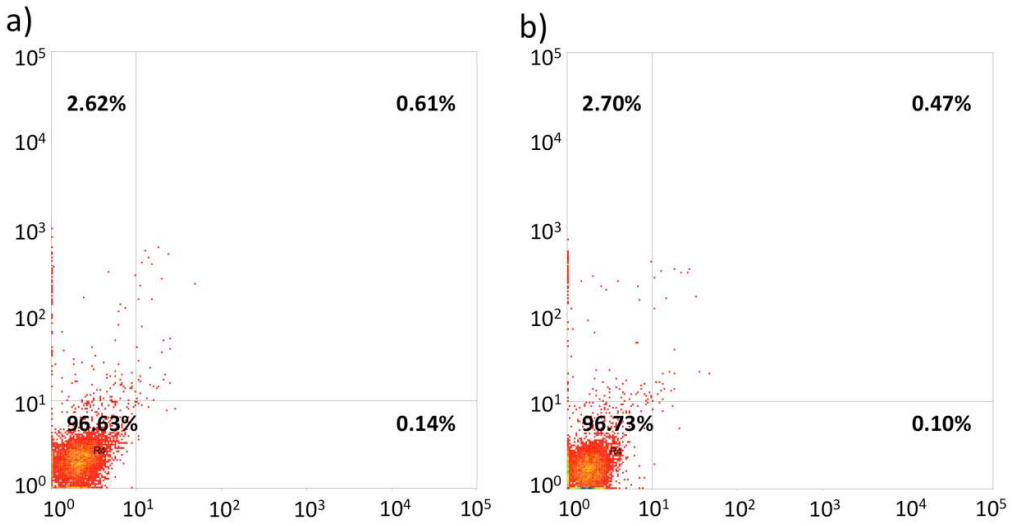


Figure S-4 Ratio of apoptotic HeLa cells a) before and b) after UV irradiation for opening the coumarin gates of MSNcg.

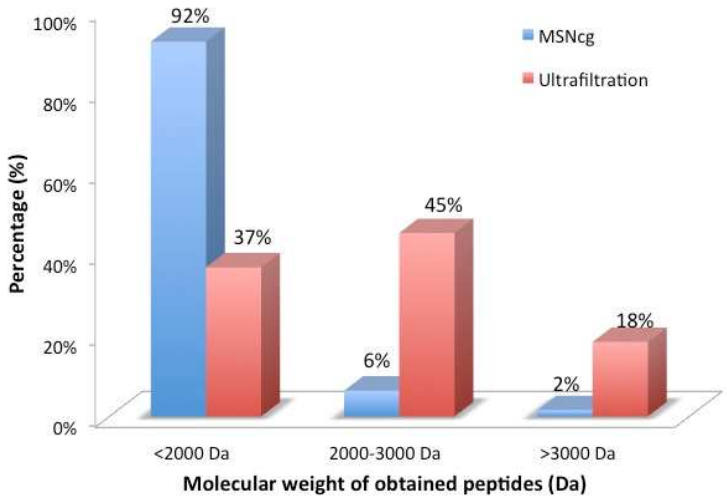


Figure S-5 Molecular weight of peptides obtained by both MSNcg and ultrafiltration from HeLa cells.

Supplementary Tables

Table S1. Sequences and detailed information of extracted cytosol peptides from live HeLa cells with a) MSNcg and b) MSN.

a) Peptides extracted by MSNcg

Sequence	PSMs	Proteins	Protein Groups	Protein Group Accessions	MH+ [Da]	q-Value	PEP	XCorr
IQAESGCK	1	2	1	Q96I24-2	839.4280871	0	0.1089	1.654422522
FAGDDAPR	4	29	2	P60709;P60712;P63261;P63258	848.3934802	0	0.1155	1.517790198
PGDSDIIR	1	1	1	Q3T0D5	872.4514636	0	0.1182	0.97250849
TEAPLNPK	1	23	2	P60709;P60712;P63261;P63258	873.5009021	0	0.04736	2.412272215
SGATAGAAGGR	1	2	1	P50991-2	875.4311999	0	0.00207	3.185145617
VAPEEHPV	1	11	2	P63261;P63258;P60709;P60712	877.4460315	0	0.05523	0.999101937
TPCGEGSKT	1	3	1	G3XAN0	883.4127673	0	0.01381	2.700140476
AASSSSLEK	1	13	2	P60709;P60712;P63261;P63258	883.4688586	0	0.09965	1.436336637
GGAGVGSMTK	1	1	1	P39019	884.4442615	0	0.05767	1.91283834
VEGDCDIH	1	1	1	P12763	887.3603992	0	0.04071	1.485003591
IQQESGCK	1	5	1	C9JSZ1	896.4440784	0	0.0363	1.724055052
GFAGDDAPR	2	29	2	P63261;P63258;P60709;P60712	905.4167956	0	0.09808	1.959263206
GGSDGYGSGR	1	2	1	P22626-2;Q2HJ60	912.3831042	0	0.08805	2.491816759
GSSGLGGGSSR	1	2	1	Q04695	921.441698	0	0.01749	2.539993286
SECEEQAK	1	1	1	Q7KZF4	927.4019641	0	0.05363	1.474758148
MDSGDGVTH	1	11	2	P63261;P63258;P60709;P60712	934.3574695	0	0.01466	2.756661415
IDNGSGMCK	1	11	1	P63261;P63258	944.4108752	0	0.007544	2.326663017
GQLFRPDN	4	5	1	P04350;Q3ZBU7	946.4785632	0	0.09171	1.425071716

ESSSGEAFH	1	1	1	P12763	950.3888415	0.004	0.1803	1.368982553
KLESTESR	1	7	1	P02545-2	953.5210437	0	0.04532	1.9061836
AVEGDCDIH	2	1	1	P12763	958.3979968	0	0.138	1.700157523
AVAATDCIAK	1	1	1	P12763	962.5028552	0	0.07074	1.756940484
NSELVQSGK	1	4	1	Q04695	965.525133	0	0.003706	2.051731348
AGFAGDDAPR	4	29	2	P60709;P60712;P63261;P63258	976.4534778	0	0.03693	2.352322578
QDGSVDFGR	5	3	1	P02675	980.4484729	0	0.00426	2.310837269
CFNKPEDK	1	1	1	P62979	988.5045642	0	0.03211	1.96306932
VDNGSGMCKA	1	7	1	P60709;P60712	1001.434435	0	0.06223	1.760012984
VVDNGSGMCK	1	7	1	P60709;P60712	1009.445482	0	0.08303	1.854108691
GGAGPVGGQGPR	1	1	1	P23246	1009.521227	0	0.09709	1.424232721
VVDNGSGMCK	1	7	1	P60709;P60712	1013.47368	0	0.004346	2.151490211
ALGGEDVRVT	3	1	1	P12763	1016.542345	0	0.07735	1.185128808
EAGGGGVGGPGAK	1	4	1	Q8NC51-4	1017.530077	0	0.004497	2.206112385
VCDNSPEVR	1	5	1	B4E0R6	1018.466112	0	0.05594	1.593203425
GTGSETESPR	1	2	1	P02671-2	1020.459154	0	0.1144	2.389992476
VIDNGSGMCK	1	11	1	P63261;P63258	1027.490404	0	0.0005259	2.406390429
VVDNGSGMCK	1	7	1	P60709;P60712	1029.464891	0	0.0009667	2.983365536
LNESGDPSSK	1	4	1	Q01105-3	1037.506029	0	0.07234	2.043890715
PDPDAAIEGR	2	1	1	P00735	1040.506273	0	0.01389	1.945333958
VIDNGSGMCK	2	11	1	P63261;P63258	1043.483568	0	0.01517	2.15725708
SGAGPGGSGGGGAR	1	4	1	H7C2X0	1044.480394	0	0.07078	2.293560028
SVSGSGSTAGSR	1	9	1	Q15149-7	1052.495409	0	0.01362	2.646725893
SSPDSAEDVR	2	2	1	P12763	1062.473192	0	0.03763	1.860526681
KTDASDVKPC	2	1	1	P17690	1063.512255	0	0.01352	2.352246761
GGGGNFGPGPGSN	4	2	1	P22626-2;Q2HJ60	1074.466112	0	0.0211	3.039511919
GMESCGIHET	1	6	2	P63261;P63258;P60709;P60712	1079.418627	0	0.09772	1.590104461
VSSSSTASASAK	1	4	1	J3KR89	1086.555834	0	0.0005671	2.226454735
GSGGGSSGGSIGGR	1	1	1	P04264	1092.505052	0	0.007527	2.140908957

PEPAKSAPAPK	1	6	1	P62807;P62808	1092.606968	0	0.006064	3.043016434
HAVEGDCDIH	1	1	1	P12763	1095.453051	0	0.01236	1.567427635
VVDNGSGMCKA	1	7	1	P60709;P60712	1100.504808	0	0.0005798	2.974669933
PEPAKSAPAPK	2	6	1	P62807;P62808	1100.656101	0	0.0007528	3.96082449
VLSAADKGNVK	4	1	1	P01966	1101.62932	0	0.01106	2.609865427
KAGFAGDDAPR	4	29	2	P63261;P63258;P60709;P60712	1108.574388	0	0.04953	1.83305788
VIDNGSGMCKA	1	11	1	P63261;P63258	1114.521654	0	0.084	1.735392332
SAPGGGSKVPQK	1	4	1	P06748-3	1120.656908	0	0.0186	1.846408844
SSQTQGGGSVTK	1	7	1	P02545-2	1140.576219	0	0.002272	2.940981388
MTEDAIDGER	2	1	1	Q2UVX4	1152.482347	0	0.03306	2.319306374
VVDNGSGMCKAG	1	7	1	P60709;P60712	1157.524095	0	0.001669	2.8980124
TGSQGQCTQVR	4	1	1	P62857;Q56JX6	1164.54326	0	0.007118	2.042942286
SGVASVESSSGEA	1	1	1	P12763	1166.523363	0	0.04858	2.834527254
QAGQCGNQIGAK	2	24	1	P04350;Q3ZBU7	1178.595018	0	0.0003367	2.911138773
GQGGAGPVGGQGPR	2	1	1	P23246	1194.603685	0	0.005435	2.599571943
GGGGPGYGNQGGGY	4	4	1	P22626-2;Q2HJ60	1197.498705	0	0.003139	3.026599169
DSYVGDEAQSK	1	23	2	P60709;P60712;P63261;P63258	1202.550463	0	0.009084	2.124384642
HQGVMVGMGQK	3	23	2	P60709;P60712;P63261;P63258	1207.589769	0	0.004971	2.168776274
CKAGFAGDDAPR	3	24	2	P63261;P63258;P60709;P60712	1211.585253	0	0.03864	1.987827301
TEAPLNPKANR	1	20	2	P60709;P60712;P63261;P63258	1214.682543	0	0.06692	1.365045547
KEPACDDPDTE	2	1	1	P12763	1219.483934	0	0.07686	2.624964476
GGGGNFGPGPGSNF	2	2	1	P22626-2;Q2HJ60	1221.532762	0	0.09495	2.713013172
ICDNQDTISSK	1	1	1	P02769	1223.560228	0	0.003416	2.119036913
AVQETDDTSHQ	1	1	1	P34955	1230.523973	0	0.0452	1.22696805
GGGPGGGNFGGSPG	1	1	1	P22626-2;Q2HJ60	1237.529711	0	0.1192	2.165133238

Y								
QEMQEVQSSR	2	2	1	P22626-2;Q2HJ60	1237.551073	0	0.03724	1.901958704
GGTTMYPGIADR	3	10	2	P60709;P60712;P63261;P63258	1254.585741	0	0.07026	2.133411169
AAAEIDEEPVSK	2	7	1	F8VZ58	1262.649706	0	0.01057	2.068374395
CDSSPDSAEDVR	3	2	1	P12763	1280.511034	0	0.005919	1.899081707
VTEVENGGSLGSK	1	5	1	H3BQ34	1280.670092	0	0.0004955	2.444029331
QGGGGGGGSVPGIER	2	4	1	M0R019	1284.6364	0	0.001203	2.146699429
IQAGQCGNQIGAK	4	24	1	P04350;Q3ZBU7	1291.680345	0	0.03566	2.26593256
GGGNYGPGGSGGSGGY	4	2	1	P22626-2;Q2HJ60	1300.526171	0	0.0008483	3.726836443
NVTGPGGVPVQGSK	1	3	1	P67809;P67808	1300.722948	0	0.0005895	3.174743652
HPDYSVVLLLR	31	6	1	C9JKR2	1311.754442	0	0.0000421 7	3.076092005
GVQVETISPGDGR	2	2	1	P18203	1314.667284	0	0.03319	2.038385391
GNLGAGNGNLQGPR	2	12	1	D6RAM9	1324.67766	0	0.1164	2.236612558
SGGTTMYPGIADR	5	10	2	P60709;P60712;P63261;P63258	1325.622606	0	0.01263	2.747023582
AAAAEIDEEPVSK	1	7	1	F8VZ58	1333.687303	0	0.0432	2.085813046
SGGTTMYPGIADR	17	10	2	P60709;P60712;P63261;P63258	1341.616747	0	0.0000835 6	3.576370478
FGQGGAGPVGGQGPR	2	1	1	P23246	1341.675096	0	0.0594	2.121561289
KEPACDDPDTEQ	2	1	1	P12763	1347.543016	0	0.04596	2.386503696
SGAQASSTPLSPTR	1	4	1	P02545-2	1359.694139	0	0.09619	2.318849564
PVEEPDPEVMAK	1	6	1	E7ETM7	1360.666063	0	0.02742	2.157238483
SYPARVPPPPPIA	1	13	1	G3V555	1361.766405	0	0.009006	1.946058512
IPLDPVAGYKEPA	4	1	1	P12763	1369.742113	0	0.0002525	3.721466303
DIEIDTLETTCH	2	1	1	P12763	1389.626024	0	0.009855	2.252911568
PPPPEDFPAADEL	1	3	0		1394.645023	0	0.1046	2.357812881

							0.0000669	
PPPPEDDENKEK	1	6	1	P63208;Q3ZCF3	1394.645023	0	6	3.055523157
PPPPEDDENKEK	1	6	1	P63208;Q3ZCF3	1402.693058	0	0.001599	2.850319862
				P60709;P60712;P63261;P6				
TTGIVMDSGDGVTH	3	11	2	3258	1405.633226	0	0.003022	2.517249107
CDSSPDSAEDVRK	2	1	1	P12763	1408.606005	0	0.0328	1.938174486
KCDSSPDSAEDVR	1	1	1	P12763	1408.606127	0	0.06212	1.420137048
KEPACDDPDTEQA	14	1	1	P12763	1418.579027	0	0.0006362	3.337440014
PNETNEIANANSR	2	4	1	P84098;Q3T0W9	1429.67705	0	0.003704	1.894564867
AAGGGAGSSEDDAQ							0.0000165	
SR	1	2	1	D6REM1	1435.603075	0	4	2.256657839
AGQSAAGAAPGGGV								
DTR	1	3	1	Q5HY54	1442.703905	0	0.01437	2.763138056
SGVASVESSSGEAFH	1	1	1	P12763	1450.642504	0	0.03132	2.124135017
CVESFSDYPPLGR	2	2	1	P68104;P68103	1469.674486	0	0.003151	2.296670437
TPIVGQPSIPGGPVR	17	1	1	P12763	1474.841234	0	0.0002156	3.709882736
KEPACDDPDTEQAA	5	1	1	P12763	1489.615892	0	0.05574	2.845000029
AVQETDDTSHQEAA	1	1	1	P34955	1501.644213	0	0.01686	2.545996428
TKCDSSPDSAEDVR	13	1	1	P12763	1509.65311	0	0.1041	3.030259609
GNVAGDSKNDPPME								
A	2	2	1	P68104;P68103	1521.683641	0	0.008779	2.671720266
QGVNDNEEGFFSAR	3	1	1	P02675	1569.700975	0	0.004426	1.864581943
GGSTSYGTGSETESP								
R	2	2	1	P02671-2	1572.684008	0	0.007303	2.404025078
YKEPACDDPDTEQA	4	1	1	P12763	1581.641161	0	0.02063	2.274344683
TPIVGQPSIPGGPVRL	1	1	1	P12763	1587.934862	0	0.003088	3.63397789
EGTGSTATSSSSTAG								
AAGK	1	7	1	E5RHC7	1631.772264	0	0.02077	1.773420453
TKCDSSPDSAEDVRK	1	1	1	P12763	1637.757358	0	0.0004107	2.231179237
PPAENSSAPEAEQGG								
AE	1	1	1	P67809;P67808	1640.709154	0	0.01062	2.507757425

AGYKEPACDDPDTE								
QA	34	1	1	P12763	1709.705126	0	0.0000014 41	4.43173027
AGYKEPACDDPDTE								
QAA	3	1	1	P12763	1780.742845	0	0.0006752	3.056024551
TPIVGQPSIPGGPVRL								
CPG	1	1	1	P12763	1845.007982	0	0.02441	2.468435764
HVGKTPIVGQPSIPG								
GPVR	2	1	1	P12763	1896.097202	0	4.748E-08	6.637650967
KTFRIKRFLAKKQKQ								
N	1	1	1	Q3T051	2034.254307	0.004	0.1607	1.511747599
SSGNSSSSGSGSGSTS								
AGSSSPGAR	1	6	1	E5RHJ2	2102.880663	0	8.38937E-06	3.070529461
QLQLILLKVALILGIE								
IHV	1	1	1	G3MWR8	2126.371332	0	0.1438	0.24153395
LGEDNINVVEGNEQF								
ISASK	17	1	1	P00760	2163.067919	0	3.311E-08	4.748443127
IPLDPVAGYKEPACD								
DPDTEQA	12	1	1	P12763	2344.069384	0	2.97169E-10	6.136271477
IPLDPVAGYKEPACD								
DPDTEQAA	3	1	1	P12763	2415.118456	0	0.001077	2.713061094
IPLDPVAGYKEPACD								
DPDTEQAAL	4	1	1	P12763	2528.201633	0	0.0008835	4.139038086
IPLDPVAGYKEPACD								
DPDTEQAALA	2	1	1	P12763	2599.23662	0	0.1542	2.005493164
KAPAASAPPRKAPA								
VPAPSQKAPAVPAPS								
QKAPAIPA	1	2	1	Q8ND99	3523.128561	0	0.0881	2.442393064
AADEDDDDDDEEDD								
DEDDDDDDFDDEEA								
EEKAPVKK	1	3	1	P06748-3	4145.555807	0	0.01306	3.799960852

b) Peptides extracted by MSN

Sequence	PSMs	Proteins	Protein Groups	Protein Group Accessions	MH+ [Da]	q-Value	PEP	XCorr
AQKAVDEVFESCFNDH EPFGR	1	1	1	IPI00840292.2	2425.09838	0	1	0.28
THSTTSL	1	2	1	Q86YS3-2	746.36846	0	1	0.75
VLSAADKG	1	1	1	IPI00710783.2	760.41973	0	1	1.22
VPPASSTPYKPPYGKLL LPPGTDGGKEGPRVLN G	1	8	1	Q8WXH0-6	3459.79506	0	1	2.30