Time evolution and dynamic cellular uptake of PEGYlated gold nanorods

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Materials

Double distilled water was used as common solvent in this investigation. Hydrogen tetrachloroaurate (III) trihydrate ($HAuCl_4.3H_2O$), silver nitrate ($AgNO_3$), L-ascorbic acid (AA), sulphuric acid (H_2SO_4), nitric acid (HNO_3), cetyltrimethyl ammonium bromide (CTAB) and sodium borohydride ($NaBH_4$) were purchased from Sangon Biotech. MβCD, CPZ and EIPA were purchased from Sigma. Pooled validated siRANs targeting clathrin heavy chain, caveolin-1 and Ras-related C3 botulinum toxin substrate 1 were purchased from Dharmacom. The following antibodies were used: GAPDH (CST), clathrin heavy chain (BD transduction laboratories), caveolin-1 (Sigma), Ras-related C3 botulinum toxin substrate1 (Millipore), HRP-conjugated mouse and rabbit secondary antibodies (Invitrogen). Cell counting kit-8 and Fast Silver Stain Kit

were purchased from Beyotime Biotechnology. Cell culture plates were purchased from Corning Inc. and all other reagents were of analytical grade.

Preparation and characterization of GNRs

GNRs were synthesized using seed-mediated growth method according to previously described procedures with minor revision. First, we prepared CTAB-capped Au seeds through chemical reduction method: 7.5 mL 100 mM CTAB was mixed with 200 μL 10 mM $HAuCl_4$ and the volume was fixed to 9.4 mL by adding water followed by adding 0.6 mL 10mM ice-cold $NaBH_4$ aqueous solution. The seeds formed immediately and were incubated for 2.5 h at 29℃. The growth solution for GNRs consisted of a mixture of 100 mL 0.1 M CTAB, 5 mL 10 mM HAuCl4, 1 mL 10 mM AgNO3, 2 mL 0.92 M H2SO4 and 800 μL 100 mM Ascorbic acid. Then, 240 μL seeds were added into the solution and incubated for 12 h at 29℃. The reaction was stopped by centrifuging at 10000g for 15 min. The GNRs were washed once and dispersed in the water containing 20mg $mPEG_{5000}$-SH. After incubation for 8 h at 29℃, the reaction was stopped by centrifuging at 10000g. The PEGlated GNRs were washed three times and resuspended in water for subsequent use. The size and shape of GNRs and PEGlated GNRs were characterized under a transmission electron microscope (TEM, H-7650, Hitachi). The visible and near-infrared absorption spectrum was determined by Biotek synergy2 (Biotek). The nanorods zeta potential was determined by Malvern Instrument (Malbern Nano-ZS). The concentration of PEGlated GNRs were determined using inductively coupled plasma mass spectrometry (ICP-MS).

Cell culture

Human retinal endothelial cells were purchased from ScienCell (San Diego, USA) and cultured in Endothelial Cell Medium (ECM) supplemented with 5% FBS, 1% L-glutamine. The

cells were incubated at 37℃in a humidified atmosphere with 5% CO2. When growing to 80%-90% confluence, the cells were seeded into indicated plate for different assays.

Cell viability assay

The viabilities of cells were determined by Cell Counting Kit-8 assay using 96-well plate. 3×10^3cells were seeded into plates and cultured overnight. The grown medium was then replaced with medium containing various concentrations of PEGlated GNRs or chemical inhibitors and incubated for indicated times. We then dumped the medium, washed 2 times with phosphate buffered saline (PBS) and added 110μL fresh medium containing 10μL CCK-8. After incubation for about 2 hours, the optical density was measured at 450nm using Biotek synergy2 (Biotek).

Analysis of protein corona and PEGlated GNRs aggregation

After PEGlated GNRs were added into cell culture medium and incubated with HREC for 1 h, 24 h and 48 h, GNRs were collected by centrifuging at 8000g and washed twice with PBS. Total protein was extracted in a modified Buffer with 0.5% SDS in the presence of proteinase inhibitor cocktail. SDS-polyacrylamide gel electrophoresis (SDS-PAGE) was used to assess the proteins, while fetal bovine serum (FBS) solution was used as control sample. The proteins were stained with Fast Silver Kit according to the manufactory's instructions. Detailed proteins adsorbed by GNRs were analyzed using LC-MS by Shanghai Applied Protein Technology. For the TEM analysis, PEGlated GNRs cultured with HREC for 1h, 24 h and 48 h were drop on copper net and observed by TEM. For visible and near-infrared absorption spectrum detection, PEGlated GNRs collected from different time were washed once with PBS, resuspended in water and determined using Biotek synergy2 (Biotek).

Western blot analysis

After treatment with siRNAs for 48h, cells were washed with PBS twice and treated with RIPA Lysis Buffer. An equal amount of protein was loaded into sodium dodecyl sulfate–polyacrylamide electrophoresis (SDS–PAGE, 10%) and transferred to polyvinylidene fluoride (PVDF) membranes. After blocking with 5 % (w/v) skimmed milk for 2 h at room temperature, the membrane was incubated with specific primary antibodies overnight at 4℃ on the shaker. Then, membranes were incubated with indicated secondary antibodies for 2 hours at room temperature. Finally, membranes were visualized using the GeneGnome HR Image Capture System (Syngene, USA).

Cellular localization of PEGlated GNRs

HREC cells were seeded in 10 cm dish and cultured for 24 hours. Then PEGlated GNRs were added into the medium and incubated for another 3 hours. In order to assess the uptake of PEGlated GNRs after culturing for 24 h and 48 h, we initially collected cell culture medium containing PEGlated GNRs after being cultured with HREC for 24 h and 48 h separately. Then cell culture medium containing nanorods were added into the dish and incubated for another 3 h. The cells treated with PEGlated GNRs were collected by scraping and fixed with a fixative mixture consisting of 2% paraformaldehyde and 2% glutaraldehyde overnight at 4℃. After three washes with PBS, the cells were coagulated with glutaraldehyde and a little plasma. The cell masses were cut into small pieces, washed with PBS, post-fixed with 1% osmium acid for 2 hours, dehydrated in a graded series of ethanol and acetone, and embedded in Epon812 epoxy resin. About 70 nm thick sections were cut, and stained with uranylacetate and lead nitrate and observed using TEM (H-7650, Hitachi).

Cellular uptake of PEGlated GNRs

For inductively coupled plasma mass spectrometry (ICP-MS) assay, HREC cells were seeded in 6-well plate and cultured for 24 hours. The culture medium was changed to fresh medium containing chemical inhibitors and the cells were incubated with these inhibitors for 1 h. Then PEGlated GNRs were added into the medium and incubated for another 3 hours. In order to assess the uptake of PEGlated GNRs after culturing for 24 h and 48 h, we initially collected cell culture medium containing PEGlated GNRs after being cultured with HREC for 24 h and 48 h separately. After the cells were incubated with chemical inhibitors for 1h, cell culture medium containing nanorods mixed with chemical inhibitors were added into the plate and incubated for another 3 h. Measurements for each group were repeated three times. After this process, the samples were collected and digested with 250 μL HNO_3 for 24 h. Once the samples were digested, ultrapure water was used to bring the final volume to 5 mL. Finally, the samples were measured by NexION 300X (GE).

Statistical analysis

All data were presented as mean ± standard deviation (SD). One-way ANOVA and t test were used to evaluate the data. A significance level of $p<0.05$ was supposed to be statistically significant.

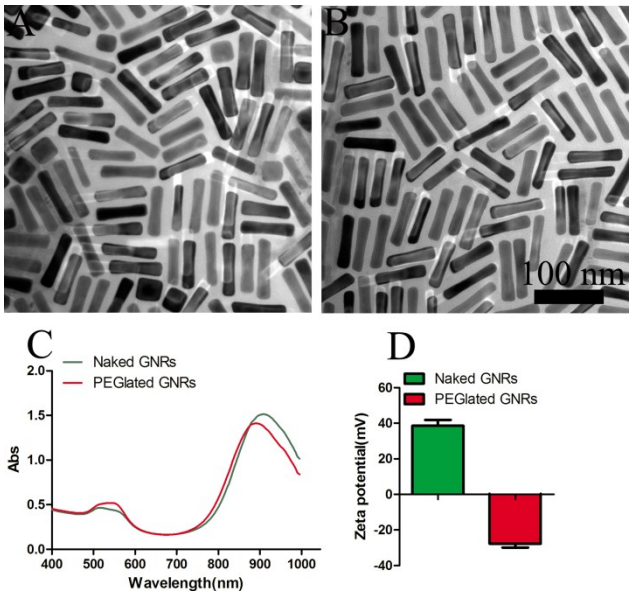

Fig. S1 Characterization of PEGlated GNRs. (A) TEM image of naked GNRs. (B) TEM image of PEGlated GNRs. (C) UV-Vis-NIR absorption spectra of GNRs. (D) Zeta potential of GNRs.

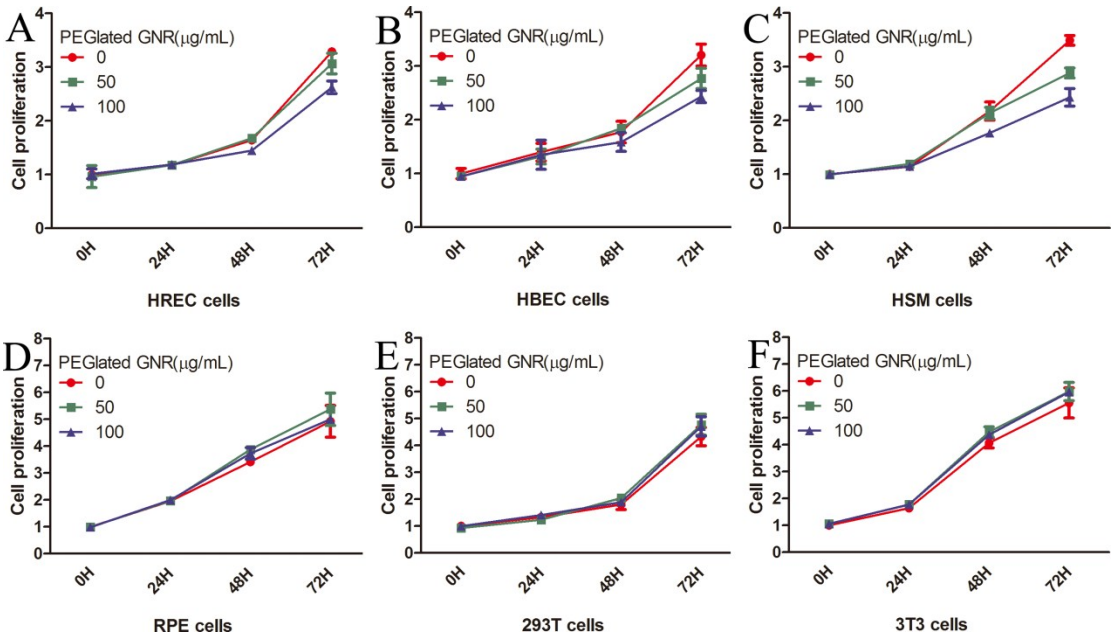

Fig. S2 Cell cytotoxicity assessment of PEGlated GNRs on different cells. A: human retinal endothelial cells (HREC), B: Human brain endothelial cells (HBEC), C: Human smooth muscular cells (HSMC), D: Human retinal pigment epithelium cells (RPE), E: Human embryonic kidney 293T cells (293T) and F: NIH 3T3 cells were exposed to PEGlated GNRs at different

concentrations for 0h, 24h, 48h and 72h separately. Cell viability of the nanorods treated cells was detected by cell counting-8 kit (CCK-8 kit). Data represent the means ± SD of three separate samples (**, p<0.01).

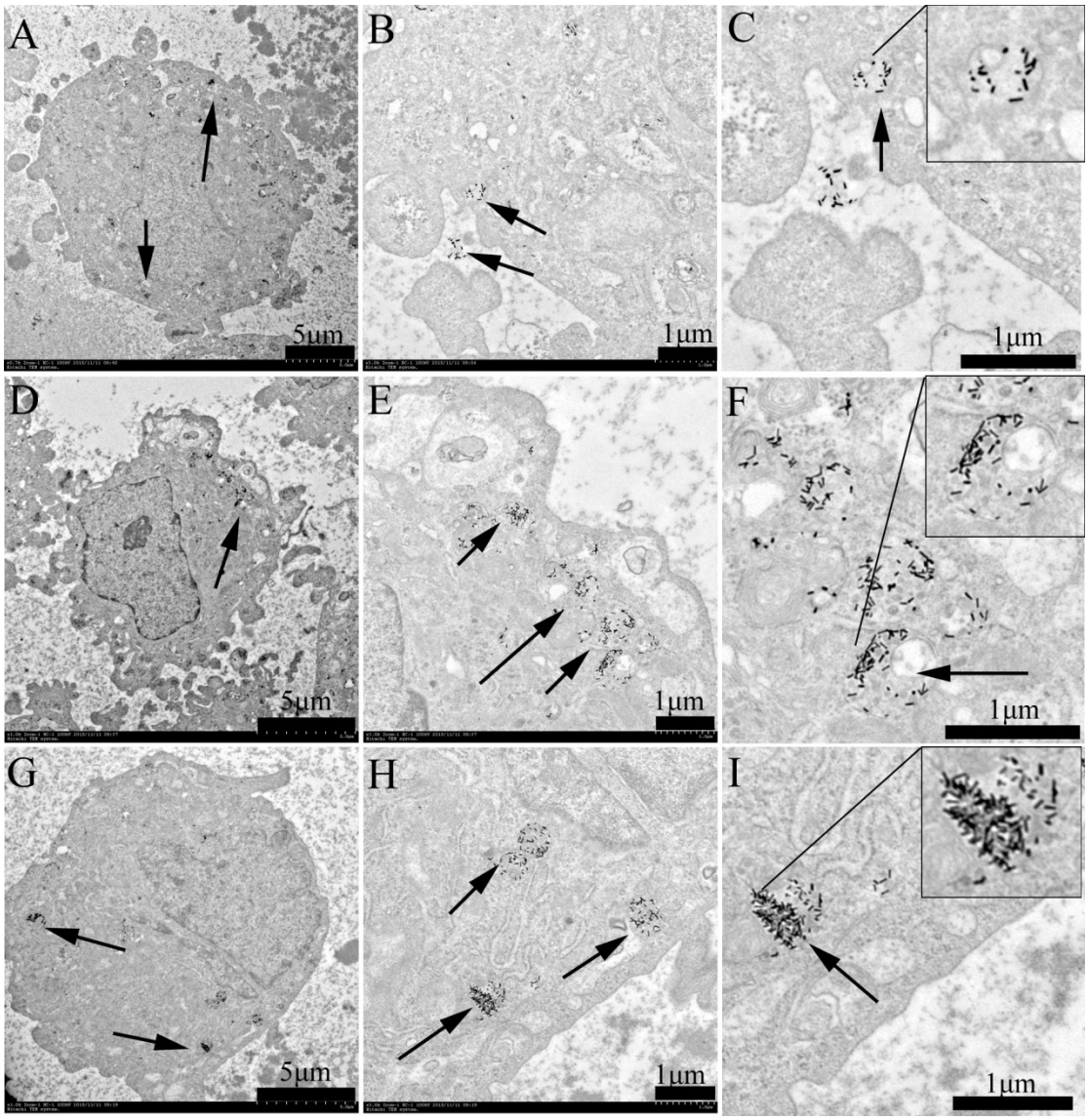

Fig. S3 Cellular location of internalized PEGlated GNRs at different time using TEM. Representative images of cellular location of internalized nanorods at 0h (A,B,C), 24h (D,E,F) and 48h (G,H,I). The arrows indicated the internalized nanorods.

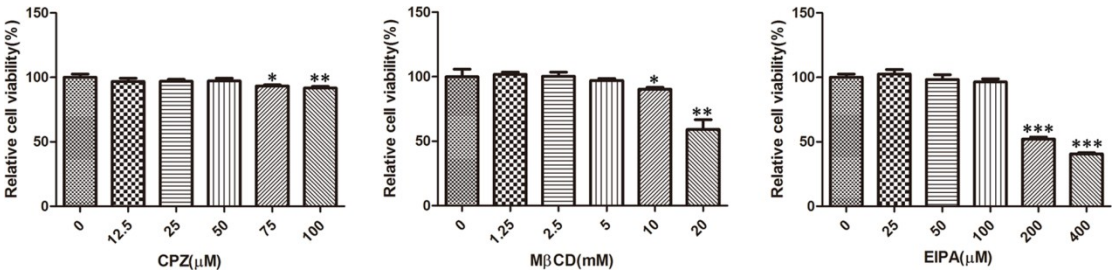

Fig. S4 Determination of concentrations of chemical inhibitors. Cell viability assay of CPZ, MβCD and EIPA were detected using CCK-8. Data represent the means ± SD of three separate samples (*, $p<0.05$, **, $p<0.01$, ***, $p<0.001$).

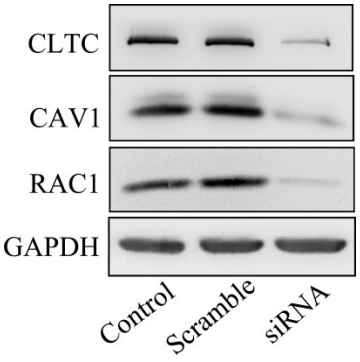

Fig. S5 Silence efficiency of siRNAs targeting CLTC, CAV1 and RAC1.

Table S1. Proteins identified by LC-MS on PEGlated GNRs cultured with HREC for 1 h.

Access [a)]	Protein Name	SpC[b)]	MV[c)]	Species[d)]
P02769	Serum albumin	8	69292.69	B
P12763	Alpha-2-HS-glycoprotein	3	38418.34	B
Q03247	Apolipoprotein E	3	35979.44	B
P63267	Actin, gamma-enteric smooth muscle	3	41876.43	H
E1BGN3	Histone H3	2	15417.87	B
P01030	Complement C4 (Fragments)	2	101550.02	B
P01045	Kininogen-2	2	68709.21	B
P01966	Hemoglobin subunit alpha	2	15184.18	B
P13384	Insulin-like growth factor-binding protein 2	2	34014.62	B
P63258	Actin, cytoplasmic 2	2	41792.4	B
Q05718	Insulin-like growth factor-binding protein 6	2	24966.7	B
Q28107	Coagulation factor V	2	248980.17	B
Q29443	Serotransferrin	2	77752.38	B
Q3MHN5	Vitamin D-binding protein	2	53341.5	B
Q3SZ57	Alpha-fetoprotein	2	68586.95	B
Q5J801	Endopin 2B	2	47001.11	B
P18065	Insulin-like growth factor-binding protein 2	2	34813.78	H

Q6NXT2	Histone H3.3C	2	15213.56	H
E2DRY6	Enolase	2	36554.25	H
P02765	Alpha-2-HS-glycoprotein	2	39324.24	H
A0A075B6Z2	Protein TRAJ56 (Fragment)	2	2110.41	H
A0A077SA06	Lysozyme	1	17898.63	B
A5PKK5	SMARCA2 protein	1	176920.88	B
B0JYN3	L-lactate dehydrogenase	1	36724.17	B
E1B9F6	Elongation factor 1-alpha	1	50169.26	B
F1MB08	Alpha-enolase	1	47283.5	B
F1MCF8	Uncharacterized protein	1	24501.16	B
F1MD79	Uncharacterized protein (Fragment)	1	130051.7	B
F2Z4E8	Histone H2B	1	13921.97	B
G3N000	Uncharacterized protein (Fragment)	1	155793.72	B
P07195	L-lactate dehydrogenase B chain	1	36638.07	H
P07910	Heterogeneous nuclear ribonucleoproteins C1/C2	1	33669.62	H
P08670	Vimentin	1	53651.05	H
P17936	Insulin-like growth factor-binding protein 3	1	31673.9	H
P31146	Coronin-1A	1	51025.7	H
P49913	Cathelicidin antimicrobial peptide	1	19301.17	H
P51531	Probable global transcription activator SNF2L2	1	181277.31	H
Q05639	Elongation factor 1-alpha 2	1	50469.57	H
Q14004	Cyclin-dependent kinase 13	1	164921.24	H
Q6ZMW3	Echinoderm microtubule-associated protein-like 6	1	217896.93	H
Q8IYT2	Cap-specific mRNA (nucleoside-2'-O-)-methyltransferase 2	1	88118.8	H
Q8IZ21	Phosphatase and actin regulator 4	1	78210.25	H
Q8NGT2	Olfactory receptor 13J1	1	34688.41	H

Table S2. Proteins identified by LC-MS on PEGlated GNRs cultured with HREC for 24 h.

Access[a)]	Protein Name	SpC[b)]	MV[c)]	Species[d)]
B0JYQ0	ALB protein	37	69292.74	B
P02769	Serum albumin	37	69292.69	B
P98160	Basement membrane-specific heparan sulfate proteoglycan core protein	29	468825.05	H
Q28085	Complement factor H	27	140372.52	B
F1MQ37	Uncharacterized protein	26	227100.21	B
A0A024R1	Myosin, heavy polypeptide 9, non-muscle,	23	226529.66	H

N1	isoform CRA_a			
Q7SIH1	Alpha-2-macroglobulin	22	167574.07	B
P07996	Thrombospondin-1	19	129381.43	H
Q03247	Apolipoprotein E	18	35979.44	B
F1N3A1	Thrombospondin-1	17	129390.46	B
Q5M8T4	Connective tissue growth factor	17	38069.16	H
G3X6N3	Serotransferrin	16	77665.31	B
F1MKS5	Histidine-rich glycoprotein	16	60742.97	B
Q2UVX4	Complement C3	15	187250.98	B
P01044	Kininogen-1	15	68889.4	B
P34955	Alpha-1-antiproteinase	15	46103.42	B
Q2TBQ1	Coagulation factor XIII, B polypeptide	15	75166.21	B
Q3ZBS7	Uncharacterized protein	15	53574.53	B
P13384	Insulin-like growth factor-binding protein 2	14	34014.62	B
Q27975	Heat shock 70 kDa protein 1A	14	70257.65	B
V9HWB8	Pyruvate kinase	14	57936.25	H
F1MER7	Uncharacterized protein (Fragment)	13	466023.94	B
K4JDT2	Alpha-2-macroglobulin variant 20	13	98342.31	B
K4JF16	Alpha-2-macroglobulin variant 23	13	101358.71	B
P01045	Kininogen-2	13	68709.21	B
P19120	Heat shock cognate 71 kDa protein	13	71239.66	B
P334331	Histidine-rich glycoprotein (Fragments)	13	44470.06	B
P08107	Heat shock 70 kDa protein 1A/1B	13	70051.38	H
P63261	Actin, cytoplasmic 2	13	41792.4	H
E9PKE3	Heat shock cognate 71 kDa protein	13	68804.98	H
F1MSZ6	Antithrombin-III	12	52439.59	B
O18739	Connective tissue growth factor	12	37923.87	B
Q28107	Coagulation factor V	12	248980.17	B
P60712	Actin, cytoplasmic 1	12	41736.29	B
P15497	Apolipoprotein A-I	12	30275.95	B
E1BH06	Uncharacterized protein	11	192762.75	B
F1MDH3	Uncharacterized protein (Fragment)	11	270812.35	B
P02081	Hemoglobin fetal subunit beta	11	15859.03	B
E1BNR0	Uncharacterized protein	10	515752.39	B
Q28065	C4b-binding protein alpha chain	10	68885.75	B
Q76LV1	Heat shock protein HSP 90-beta	10	83252.2	B
P02751	Fibronectin	10	262621.56	H
Q9Y490	Talin-1	10	269764.06	H
A0A024RD80	Heat shock protein 90kDa alpha (Cytosolic), class B member 1, isoform CRA_a	10	83263.22	H
A5D984	Pyruvate kinase 3	9	57948.27	B
G3X7F3	Uncharacterized protein (Fragment)	9	74144.22	B
G5E5A9	Fibronectin	9	272119.4	B
G3MXL3	Uncharacterized protein (Fragment)	9	62891.54	B

A5PJE3	Fibrinogen alpha chain	9	66997.31	B
O02717	Non-muscle myosin heavy chain (Fragment)	9	72370.21	B
P00735	Prothrombin	9	70504.75	B
Q14767	Latent-transforming growth factor beta-binding protein 2	9	195049.92	H
P06733	Alpha-enolase	9	47168.41	H
Q86YZ3	Hornerin	9	282386.64	H
Q92176	Coronin-1A	8	50978.63	B
F1MB08	Alpha-enolase	8	47283.5	B
P62935	Peptidyl-prolyl cis-trans isomerase A	8	17869.16	B
P07355	Annexin A2	8	38603.6	H
B8ZX62	Tissue plasminogen activator	8	62964.55	H
P18065	Insulin-like growth factor-binding protein 2	8	34813.78	H
A0A024R611	Coronin	8	51025.7	H
A2I7N1	Serpin A3-5	7	46396.82	B
F1N169	Uncharacterized protein	7	281051.32	B
G3N0V2	Uncharacterized protein	7	63150.56	B
M0QVY0	Uncharacterized protein	7	60803.12	B
P04272	Annexin A2	7	38611.63	B
P28800	Alpha-2-antiplasmin	7	54710.2	B
P12763	Alpha-2-HS-glycoprotein	7	38418.34	B
Q2KIG3	Carboxypeptidase B2	7	48821.13	B
Q76LV2	Heat shock protein HSP 90-alpha	7	84729.76	B
P01966	Hemoglobin subunit alpha	7	15184.18	B
A0A087WWY3	Filamin-A	7	245848.1	H
Q2VPJ6	HSP90AA1 protein (Fragment)	7	68370.64	H
P62937	Peptidyl-prolyl cis-trans isomerase A	7	18012.3	H
A4IFP2	KRT4 protein	6	58045.64	B
O18977	Tenascin-X	6	447378.84	B
P68103	Elongation factor 1-alpha 1	6	50140.28	B
Q3SZ57	Alpha-fetoprotein	6	68586.95	B
Q2KJD0	Tubulin beta-5 chain	6	49670.28	B
Q92626	Peroxidasin homolog	6	165272.78	H
B4DE59	cDNA FLJ60424, highly similar to Junction plakoglobin	6	62615.29	H
Q9NZS6	Elongation factor 1-alpha (Fragment)	6	46268.72	H
Q5SU16	Beta 5-tubulin	6	49670.28	H
I1VZV6	Hemoglobin alpha 1	6	15279.41	H
F1MVS9	Uncharacterized protein	5	81297.58	B
P49907	Selenoprotein P	5	43686.82	B
Q2KJ63	Plasma kallikrein	5	70993.3	B
Q3SX14	Gelsolin	5	80729.71	B

Q3T0P6	Phosphoglycerate kinase 1	5	44537.11	B
G1K1X9	Vitamin K-dependent protein Z	5	47734.83	B
K4JDR8	Alpha-2-macroglobulin variant 5	5	45074.14	B
Q0VCX2	glucose-regulated protein	5	72399.13	B
P01008	Antithrombin-III	5	52601.83	H
Q16270	Insulin-like growth factor-binding protein 7	5	29130.05	H
Q2TSD0	Glyceraldehyde-3-phosphate dehydrogenase	5	36048.8	H
P11021	78 kDa glucose-regulated protein	5	72332.07	H
B4DPR2	cDNA FLJ50830, highly similar to Serum albumin	5	59573.13	H
G3V1N2	HCG1745306, isoform CRA_a	5	11947.65	H
A0A0A0MPA0	Uncharacterized protein	4	46882.16	B
A5PJT7	ECM1 protein	4	57636.3	B
A6QPD4	LOC790886 protein	4	45427.87	B
A7E3S8	Heat shock 70kD protein binding protein	4	41444.38	B
F1MMK9	Protein AMBP	4	39293.46	B
F1MMP5	Inter-alpha-trypsin inhibitor heavy chain H1	4	101236.44	B
F1MPP2	Uncharacterized protein	4	29049.99	B
F1MR86	Uncharacterized protein	4	33593.37	B
F1MY85	Complement C5a anaphylatoxin	4	189044.2	B
F1N5M2	Vitamin D-binding protein	4	53355.53	B
G5E505	Integrin-linked protein kinase	4	51493.77	B
P00432	Catalase	4	59914.57	B
P10096	Glyceraldehyde-3-phosphate dehydrogenase	4	35867.68	B
P20959	Insulin-like growth factor-binding protein 3	4	31569.81	B
P98140	Coagulation factor XII	4	67159.64	B
Q27967	Secreted phosphoprotein 24	4	23133.89	B
Q3SZV7	Hemopexin	4	52208.67	B
E1BEL8	Uncharacterized protein	4	16070.34	B
O46375	Transthyretin	4	15726.78	B
P17697	Clusterin	4	51113.36	B
Q9TS85	Histidine-rich GLYCOPROTEIN=FACTOR XIIIA substrate (Fragments)	4	23982.36	B
P00558	Phosphoglycerate kinase 1	4	44614.2	H
P01023	Alpha-2-macroglobulin	4	163289.06	H
P03956	Interstitial collagenase	4	54006.23	H
P10809	60 kDa heat shock protein, mitochondrial	4	61053.94	H
P39060	Collagen alpha-1(XVIII) chain	4	178185.48	H
Q13418	Integrin-linked protein kinase	4	51418.66	H
Q15582	Transforming growth factor-beta-induced protein ig-h3	4	74680.04	H
Q16658	Fascin	4	54529.35	H
B7Z9A0	FLJ56212, highly similar to Gelsolin	4	83102.45	H

E7EPG1	Multimerin-1	4	110751.27	H
P02765	Alpha-2-HS-glycoprotein	4	39324.24	H
P14625	Endoplasmin	4	92467.76	H
P04004	Vitronectin	4	54304.97	H
Q9BWU5	Mutant hemoglobin beta chain (Fragment)	4	11501.07	H
A2VDN8	Coronin, actin binding protein, 1C	3	53125.26	B
A5D792	DCK protein	3	17680.22	B
A5D7R6	ITIH2 protein	3	106185.12	B
A6QPP2	SERPIND1 protein	3	55206.55	B
A6QQA8	Sulfhydryl oxidase	3	62974.43	B
A7YWB6	LOC539596 protein	3	74406.72	B
A8E654	COL18A1 protein	3	135065.93	B
D4QBB4	Hemoglobin beta	3	15954.2	B
E1B726	Plasminogen	3	91242.33	B
E1B991	Uncharacterized protein	3	64333.02	B
F1MAV0	Fibrinogen beta chain	3	56440.15	B
F1MJK3	Uncharacterized protein	3	165608.65	B
F1MUZ9	60 kDa heat shock protein, mitochondrial	3	60976.85	B
O60687	Sushi repeat-containing protein SRPX2	3	52971.33	H
P00338	L-lactate dehydrogenase A chain	3	36688.3	H
P02649	Apolipoprotein E	3	36153.66	H
P05121	Plasminogen activator inhibitor 1	3	45059.47	H
P06732	Creatine kinase M-type	3	43100.62	H
P07814	Bifunctional glutamate/proline--tRNA ligase	3	170588.9	H
P27348	14-3-3 protein theta	3	27763.94	H
Q96QV1	Hedgehog-interacting protein	3	78850.52	H
Q9Y4G6	Talin-2	3	271610.07	H
B2R4R0	Histone H4	3	11367.2	H
O95084	Serine protease 23	3	43000.75	H

Table S3. Proteins identified by LC-MS on PEGlated GNRs cultured with HREC for 48 h.

Access[a)]	Protein Name	SpC[b)]	MV[c)]	Species[d)]
P98160	Basement membrane-specific heparan sulfate proteoglycan core protein	76	468825.05	H
P02769	Serum albumin	65	69292.69	B
B0JYQ0	ALB protein	62	69292.74	B
P07996	Thrombospondin-1	46	129381.43	H
A0A024R9Q1	Thrombospondin 1, isoform CRA_a	46	129351.4	H
F1N3A1	Thrombospondin-1	40	129390.46	B
Q28178	Thrombospondin-1	39	129532.66	B
F1MQ37	Uncharacterized protein	37	227100.21	B

E1BNR0	Uncharacterized protein	36	515752.39	B
Q28085	Complement factor H	32	140372.52	B
F1MER7	Uncharacterized protein (Fragment)	30	466023.94	B
P35579	Myosin-9	30	226529.66	H
Q7SIH1	Alpha-2-macroglobulin	28	167574.07	B
B0JYN6	Alpha-2-HS-glycoprotein	25	38418.34	B
P02751	Fibronectin	24	262621.56	H
F1MC45	Complement factor H (Fragment)	22	96591.9	B
Q28107	Coagulation factor V	22	248980.17	B
E1BH06	Uncharacterized protein	21	192762.75	B
P01044	Kininogen-1	20	68889.4	B
Q2TBQ1	Coagulation factor XIII, B polypeptide	19	75166.21	B
Q5M8T4	Connective tissue growth factor	19	38069.16	H
Q2UVX4	Complement C3	18	187250.98	B
P01045	Kininogen-2	18	68709.21	B
P02081	Hemoglobin fetal subunit beta	18	15859.03	B
Q96QV1	Hedgehog-interacting protein	17	78850.52	H
F1N169	Uncharacterized protein	16	281051.32	B
O02717	Non-muscle myosin heavy chain (Fragment)	16	72370.21	B
Q27965	Heat shock 70 kDa protein 1B	16	70227.56	B
Q28065	C4b-binding protein alpha chain	16	68885.75	B
Q03247	Apolipoprotein E	16	35979.44	B
Q92626	Peroxidasin homolog	16	165272.78	H
P34955	Alpha-1-antiproteinase	15	46103.42	B
P08107	Heat shock 70 kDa protein 1A/1B	15	70051.38	H
F1MVK1	Uncharacterized protein (Fragment)	14	173971.74	B
P19120	Heat shock cognate 71 kDa protein	14	71239.66	B
F1MDH3	Uncharacterized protein (Fragment）	14	270812.35	B
F1MKS5	Histidine-rich glycoprotein	14	60742.97	B
Q9BGU1	Histidine-rich glycoprotein	14	61947.27	B
P11142	Heat shock cognate 71 kDa protein	14	70897.24	H
P07589	Fibronectin	13	272150.41	B
Q3ZBS7	Uncharacterized protein	13	53574.53	B
E7EPG1	Multimerin-1	13	110751.27	H
Q6MZF4	Putative uncharacterized protein DKFZp686F219 (Fragment)	13	122112.27	H
F1MNW4	Inter-alpha-trypsin inhibitor heavy chain H2	12	106155.03	B
Q76LV1	Heat shock protein HSP 90-beta	12	83252.2	B
O18739	Connective tissue growth factor	12	37923.87	B
P00735	Prothrombin	12	70504.75	B
Q99715	Collagen alpha-1(XII) chain	12	333142.67	H
Q60FE5	Filamin A	12	278223.09	H
P08238	Heat shock protein HSP 90-beta	12	83263.22	H

A5D984	Pyruvate kinase 3	11	57948.27	B
A5PJW9	HHIP protein	11	78536.16	B
F1N2P8	Insulin-like growth factor-binding protein 2	11	30784.95	B
G3X7F3	Uncharacterized protein (Fragment)	11	74144.22	B
P41361	Antithrombin-III	11	52346.55	B
V6F9A2	Apolipoprotein A-I preproprotein	11	30275.95	B
P60709	Actin, cytoplasmic 1	11	41736.29	H
B8ZX62	Tissue plasminogen activator	11	62964.55	H
V9HW11	Epididymis secretory sperm binding protein Li 83p	11	51146.95	H
V9HWB8	Pyruvate kinase	11	57936.25	H
Q9Y490	Talin-1	11	269764.06	H
A5PJE3	Fibrinogen alpha chain	10	66997.31	B
P63258	Actin, cytoplasmic 2	10	41792.4	B
Q2KIG3	Carboxypeptidase B2	10	48821.13	B
P01966	Hemoglobin subunit alpha	10	15184.18	B
P33433	Histidine-rich glycoprotein (Fragments)	10	44470.06	B
G3V511	Latent-transforming growth factor beta-binding protein 2	10	189239.69	H
P06733	Alpha-enolase	10	47168.41	H
P68133	Actin, alpha skeletal muscle	10	42050.59	H
F1MY85	Complement C5a anaphylatoxin	9	189044.2	B
A4IFM8	Actin, alpha 1, skeletal muscle	9	42022.58	B
Q9XSJ4	Alpha-enolase	9	47325.58	B
Q9Y4K0	Lysyl oxidase homolog 2	9	86723.9	H
Q53FK3	Matrix metalloproteinase 1 preproprotein variant (Fragment)	9	53976.21	H
P05121	Plasminogen activator inhibitor 1	9	45059.47	H
P02768	Serum albumin	9	69365.97	H
P28800	Alpha-2-antiplasmin	8	54710.2	B
Q3SZ57	Alpha-fetoprotein	8	68586.95	B
Q5EA25	Sushi repeat-containing protein SRPX2	8	53067.65	B
Q76LV2	Heat shock protein HSP 90-alpha	8	84729.76	B
G3N0V2	Uncharacterized protein	8	63150.56	B
P62935	Peptidyl-prolyl cis-trans isomerase A	8	17869.16	B
K9JA46	Epididymis luminal secretory protein 52	8	84658.73	H
M5FKF4	Insulin-like growth factor binding protein, acid labile subunit	7	65992.41	B
Q92176	Coronin-1A	7	50978.63	B
F1MJH1	Gelsolin	7	80701.66	B
G3MXL3	Uncharacterized protein (Fragment)	7	62891.54	B
G3N0S9	Uncharacterized protein	7	22335.74	B
Q28194	Thrombospondin-1 (Fragment)	7	25015.17	B

O60687	Sushi repeat-containing protein SRPX2	7	52971.33	H
C9JMY1	Insulin-like growth factor-binding protein 2	7	20280.87	H
Q6IPN6	Elongation factor 1-alpha	7	50122.24	H
B2RE56	Peptidyl-prolyl cis-trans isomerase	7	18008.31	H
A5PJT7	ECM1 protein	6	57636.3	B
F1N4K8	Fibrillin-1	6	312247.58	B
G8JKW7	Uncharacterized protein	6	46343.7	B
Q9TS85	Histidine-rich GLYCOPROTEIN=FACTOR XIIIA substrate (Fragments)	6	23982.36	B
A6QQA8	Sulfhydryl oxidase	6	62974.43	B
P68103	Elongation factor 1-alpha 1	6	50140.28	B
P02070	Hemoglobin subunit beta	6	15954.2	B
E1BEL8	Uncharacterized protein	6	16070.34	B
P07355	Annexin A2	6	38603.6	H
P35555	Fibrillin-1	6	312235.65	H
A0A0A0MT01	Gelsolin	6	84744.29	H
P02765	Alpha-2-HS-glycoprotein	6	39324.24	H
Q9BWU5	Mutant hemoglobin beta chain (Fragment)	6	11501.07	H
P69905	Hemoglobin subunit alpha	6	15257.36	H
F1N401	Collagen alpha-1(XII) chain	5	340074.21	B
G3X6N3	Serotransferrin	5	77665.31	B
P04272	Annexin A2	5	38611.63	B
Q27967	Secreted phosphoprotein 24	5	23133.89	B
Q2KJ63	Plasma kallikrein	5	70993.3	B
Q3ZCJ7	Tubulin alpha-1C chain	5	49856.71	B
G1K1X9	Vitamin K-dependent protein Z	5	47734.83	B
P68363	Tubulin alpha-1B chain	5	50151.07	H
Q16270	Insulin-like growth factor-binding protein 7	5	29130.05	H
Q99988	Growth/differentiation factor 15	5	34139.85	H
D3DSM4	Collagen, type XVIII, alpha 1, isoform CRA_d	5	135507.96	H
E5RGA1	Tissue-type plasminogen activator (Fragment)	5	20078.57	H
P00734	Prothrombin	5	70036.12	H
G3V1N2	HCG1745306, isoform CRA_a	5	11947.65	H
Q96T46	Hemoglobin alpha 2 (Fragment)	5	8391.38	H
A2VDN8	Coronin, actin binding protein, 1C	4	53125.26	B
A5D7S8	Fibulin-1	4	77529.65	B
A6QLY8	IGFBP7 protein	4	29078	B
A6QPD4	LOC790886 protein	4	45427.87	B
A6QPP2	SERPIND1 protein	4	55206.55	B
F1MAV0	Fibrinogen beta chain	4	56440.15	B
F1MJ12	Complement C1s subcomponent	4	77381.29	B
F1MTT3	Coagulation factor XII	4	67234.67	B

F1MVS9	Uncharacterized protein	4	81297.58	B
G3MZ95	Uncharacterized protein	4	31909.53	B
K4JDR8	Alpha-2-macroglobulin variant 5	4	45074.14	B
P52898	Dihydrodiol dehydrogenase 3	4	36783.88	B
Q1RMN9	C4b-binding protein alpha-like	4	21996.34	B
Q2KIV9	Complement C1q subcomponent subunit B	4	26398.91	B
Q3MHL4	Adenosylhomocysteinase	4	47637.45	B
Q95M18	Endoplasmin	4	92425.6	B
A6QLC4	CDH1 protein	4	97973.51	B
P17697	Clusterin	4	51113.36	B
P52556	Flavin reductase (NADPH)	4	22132.09	B
Q0VCX2	78 kDa glucose-regulated protein	4	72399.13	B
Q1RMH5	C1QC protein (Fragment)	4	29022.83	B
Q2HJ49	Moesin	4	67974.39	B
Q15582	Transforming growth factor-beta-induced protein ig-h3	4	74680.04	H
A0A024R611	Coronin	4	51025.7	H
A0A024R8E5	Collagen, type V, alpha 1, isoform CRA_a	4	183557.64	H
A0A024RBI5	Coronin	4	53248.46	H
A0A0A0MRJ7	Coagulation factor V	4	252233.03	H
O95084	Serine protease 23	4	43000.75	H
P14625	Endoplasmin	4	92467.76	H
P26038	Moesin	4	67819.26	H
J7M2B1	Tyrosine-protein kinase receptor	4	98947.39	H
V9HWB4	Epididymis secretory sperm binding protein Li 89n	4	72332.07	H
A4FV50	MGC142792 protein	3	38421.35	B
A5D9E9	Complement component 1, r subcomponent	3	80212.02	B
P01024	Complement C3	3	187145.88	H
P07737	Profilin-1	3	15054.07	H

a)UniProt accession number; b)Total spectral counts; c)Protein molecular weight, calculated from the theoretical amino acid sequence; d) Species of origin: ‘B’ is bovine protein from fetal bovine serum; ‘H’ is human protein from HREC cells.