Supporting Information

Reversible Control of Protein Corona Formation on Gold Nanoparticles Using Host-Guest Interactions

Jesús Mosquera[†±*], Isabel García[†], Malou Henriksen-Lacey[†], Miguel Martínez-Calvo[#], Mónica Dhanjani[†], José L. Mascareñas[#], Luis M. Liz-Marzán[†‡*]

[†]CIC biomaGUNE and CIBER-BBN, Basque Research and Technology Alliance (BRTA), Paseo de Miramón 182, 20014 Donostia-San Sebastián, Spain.

[±]Present address: Department of Chemical Engineering and Chemistry, Eindhoven University of Technology, The Netherlands

[#]Departamento de Química Orgánica and Centro Singular de Investigación en Química Biolóxica e Materiais Moleculares (CIQUS). Universidade de Santiago de Compostela. 15782 Santiago de Compostela, Spain

[‡]Ikerbasque, Basque Foundation for Science, 48013 Bilbao, Spain

*e-mail: j.mosquera@tue.nl; llizmarzan@cicbiomagune.es

Contents

General Experimental Procedures

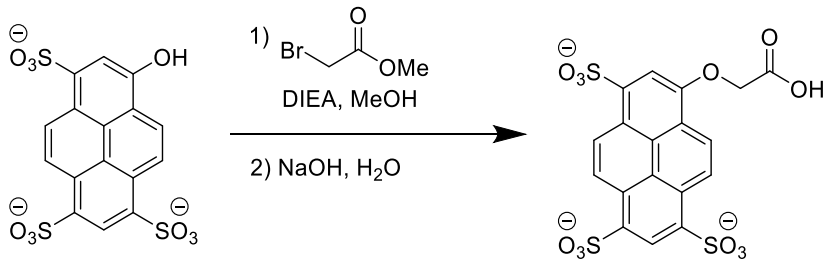
Cetyltrimethylammonium bromide (CTAB, 99%), hydrogen tetrachloroaurate trihydrate (HAuCl4·3H2O, ≥ 99.9%), L-ascorbic acid (≥ 99%), sodium borohydride ($NaBH_4$, 99%), silver nitrate (AgNO3, ≥ 99.0%), O-[2-(3-mercaptopropionylamino)ethyl]-O′-methylpolyethylene glycol 5 KDa (PEG-SH) and the rest of organic molecules were purchased from Sigma-Aldrich and were used without further purification. Alpha-Amino-omega-mercapto poly(ethylene glycol) hydrochloride 5 KDa (HS-PEG-NH2) was purchased from Iris Biotech. UV-Vis spectra were measured with an Agilent 8453 UV-Vis diode-array spectrophotometer. Transmission electron microscopy (TEM) images were obtained using a JEOL JEM-2100F electron microscope, at an acceleration voltage of 200 kV. Samples for TEM analysis were prepared by adding a single drop (2 μL) of the aqueous dispersion (*ca.* 0.1 mg/mL in milli-Q water) of gold nanoparticles onto a copper grid coated with a carbon film (Electron Microscopy Sciences) placed on parafilm. The grid was dried in air for several hours at room temperature. Zeta potential measurements were performed in a Malvern Zetasizer 3000 HS particle size analyzer (Malvern Instruments, UK). Fluorescence measurements were made with a PerkinElmer LS55 Fluorescence Spectrometer. Milli-Q water (resistivity 18.2 MΩ·cm at 25 °C) was used in all experiments. ICP-MS measurements were performed on a Thermo iCAP Q ICP-MS (Thermo Fisher Scientific GmbH, Bremen, Germany). An ASX-560 autosampler was coupled to the ICP-MS (CETAC Tech, Omaha, NE, USA).

The Image J software with the algorithm "Measure_ROI.class" was employed to measure the dimensions of gold nanoparticles from TEM images. About 100–300 nanoparticles per sample were measured.

HeLa and J774 cells were grown in DMEM supplemented with 10% FBS and 1% PBS (both Invitrogen). Cells were routinely checked for mycoplasma using MycoAlert Assay (Lonza).

Synthesis of organic molecules

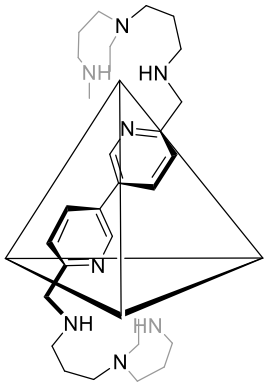
1. Synthesis of **Pyr-CO_2H**



Pyr **Pyr-CO_2H**

Pyr-CO_2H was synthesized as previously described in the literature.[1]

2. Synthesis of **A**



A

Cages **A** was synthesized according to the procedure reported in the literature.[2]

NS2 characterization

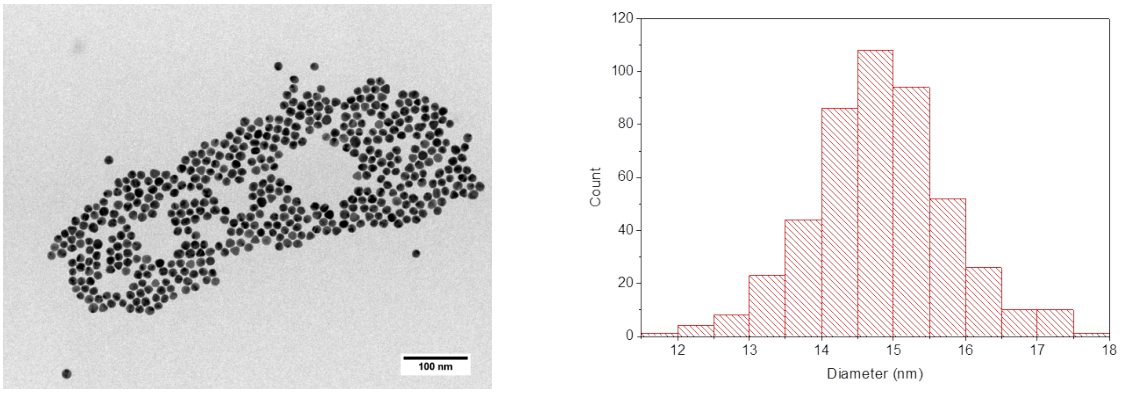


Figure S1. (Left) Representative transmission electron microscopy (TEM) image of **NS2**. (Right) Histogram size distribution from TEM images, the average diameter is 14.8±0.9 nm.

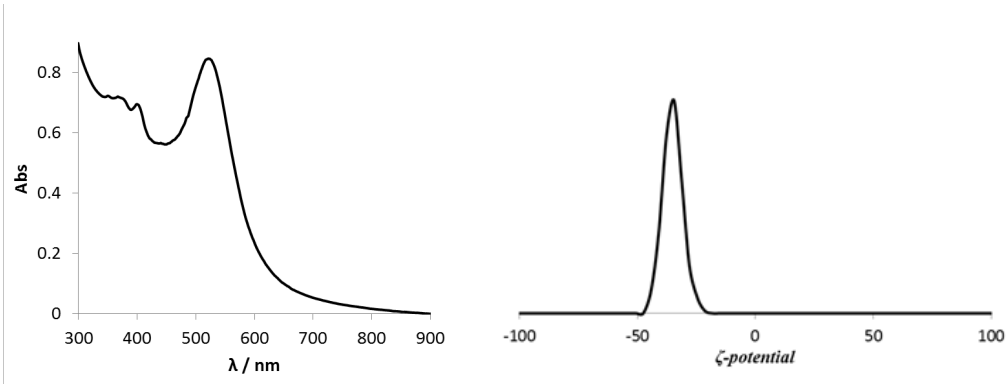


Figure S2. (Left) UV-visible-NIR spectra of **NS2**. (Right) Zeta potential distribution for **NS2** (-37±2 mV). Both measurements were performed in Milli-Q water at room temperature.

DLS evaluation of the protein corona size on NS2

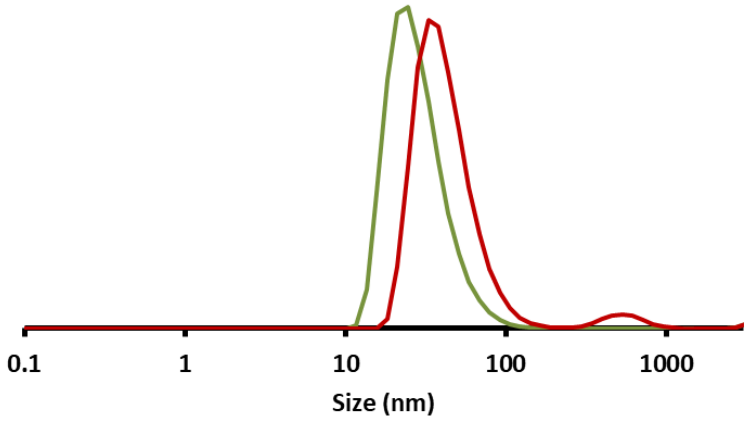


Figure S3. The effect of PC formation on the DLS size distribution (by volume) of **NS2**. Size distribution before (green line) and after (red lines) interaction with 5% of FBS. The average hydrodynamic diameter (three replicates) in the absence of FBS is 26±2 nm and in the presence of FBS is 43±1 nm.

Protein corona on NS2 and NS-PEG by SDS-PAGE

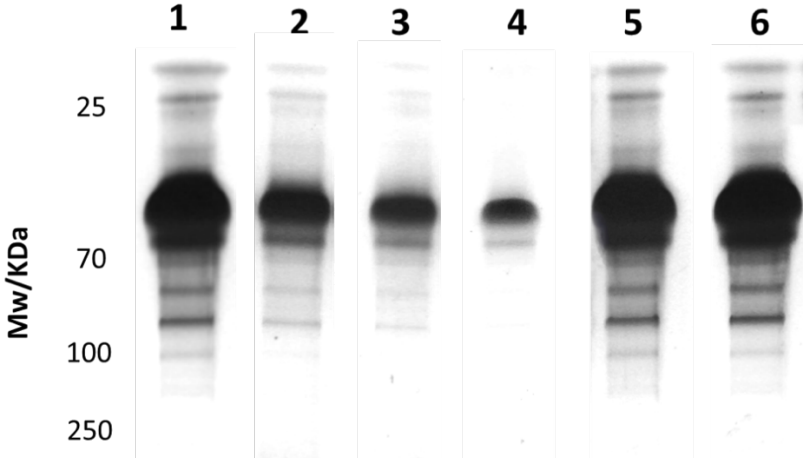


Figure S4: Comparison of the amount of FBS immobilized on **NS2** and **NS-PEG** particles, upon addition of cage **A**. Lane 1: 200 µL of **NS2** ($7.5x10^{11}$ particles/mL) diluted in FBS (5% in PBS) after 30 min of incubation. Lane 2: Same conditions as in Lane 1, but using 5 µM of cage **A** during incubation with FBS. Lane 3: Same conditions as in Lane 1, but using 10 µM of cage **A** during incubation with FBS. Lane 4: Same conditions as in Lane 1, but using 20 µM of cage **A** during incubation with FBS. Lane 5: 200 µL of **NS-PEG** ($7.5x10^{11}$ particles/mL) diluted in FBS (5% in PBS) after 30 min of incubation. Lane 6: Same conditions as in Lane 5 in the presence of 10 µM of cage **A**. The amount of protein was quantified using ImageJ (for the entire lane) and normalized against the result for lane 1: lane 1 (100%); lane 2 (42%); lane 3 (31%); lane 4 (18%); lane 5 (109%); lane 4 (117%).

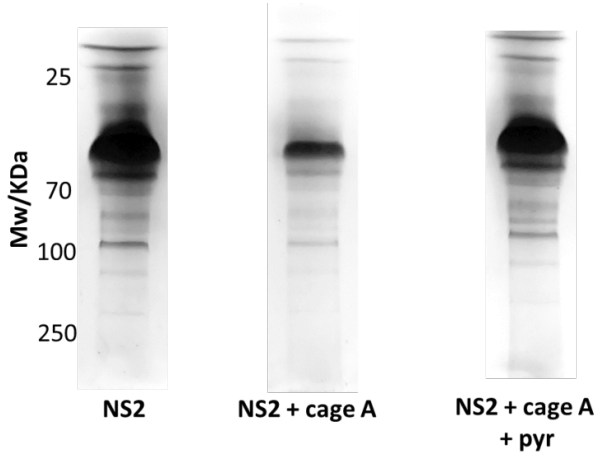


Figure S5. Comparison of the amount and type of FBS proteins immobilized onto **NS2** particles, upon addition of cage **A** and **pyr**. SDS-PAGE gels run for 40 min and stained with Coomassie Blue. Lane 1: **NS2** (200µL, $7.5x10^{11}$ particles/mL) diluted in FBS (5% in PBS) after 30 min of incubation. Lane 2: Same conditions as in lane 1, followed by addition of 20 µM of cage **A** and 10 min of incubation. Lane 3: Same conditions as in lane 2, followed by addition of 50 µM of free pyr and 10 min of incubation. The amount of protein was quantified using ImageJ and normalized against the result for lane 1: lane 1 (100%); lane 2 (21%); lane 3 (81%).

NS-PEG characterization

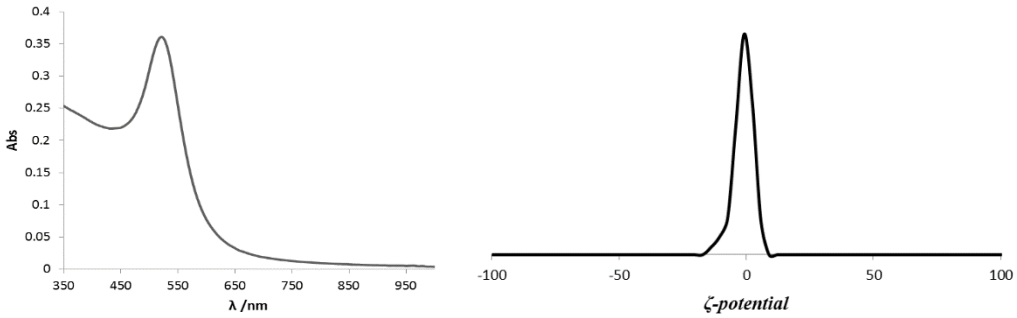
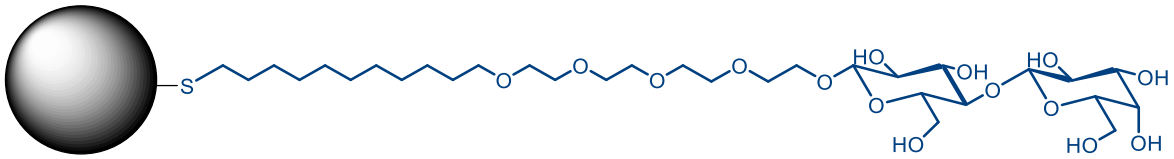


Figure S6. (Left) UV-visible-NIR spectra of **NS-PEG**. (Right) Zeta potential distribution for **NS-PEG** (-3.1±0.4 mV). Both measurements were performed in Milli-Q water at room temperature.

Protein corona on a NS2 analogue with a short organic ligand



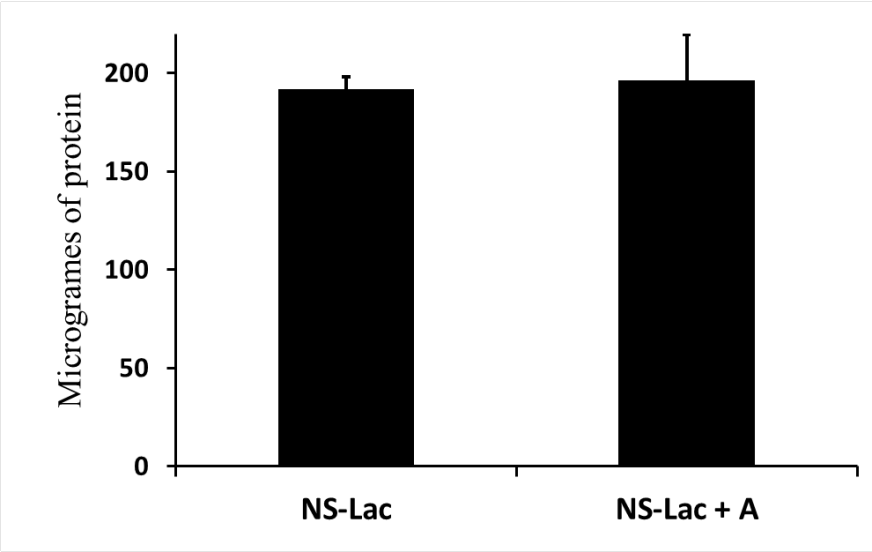


Figure S7. Comparison of the amount of FBS immobilized on **NS-Lac** particles in the presence and absence of cage **A**. (Top) Chemical structure of the ligand bound to the surface for **NS-Lac**. The metallic core of this particle is the same as **NS1**. The organic ligand was synthesized following a reported protocol.[3] (Bottom) Comparison of the amount of FBS protein immobilized on **NS-Lac** particles. **NS-Lac**: only **NS-Lac** (200µL, 7.5×10^{11} particles/mL) diluted in 5% of FBS. **NS-Lac + A**: same conditions as the previous case, followed by addition of 20 µM of cage **A** and 10 min of incubation.

Fluorescence evaluation of the interaction between NS2 and cage A

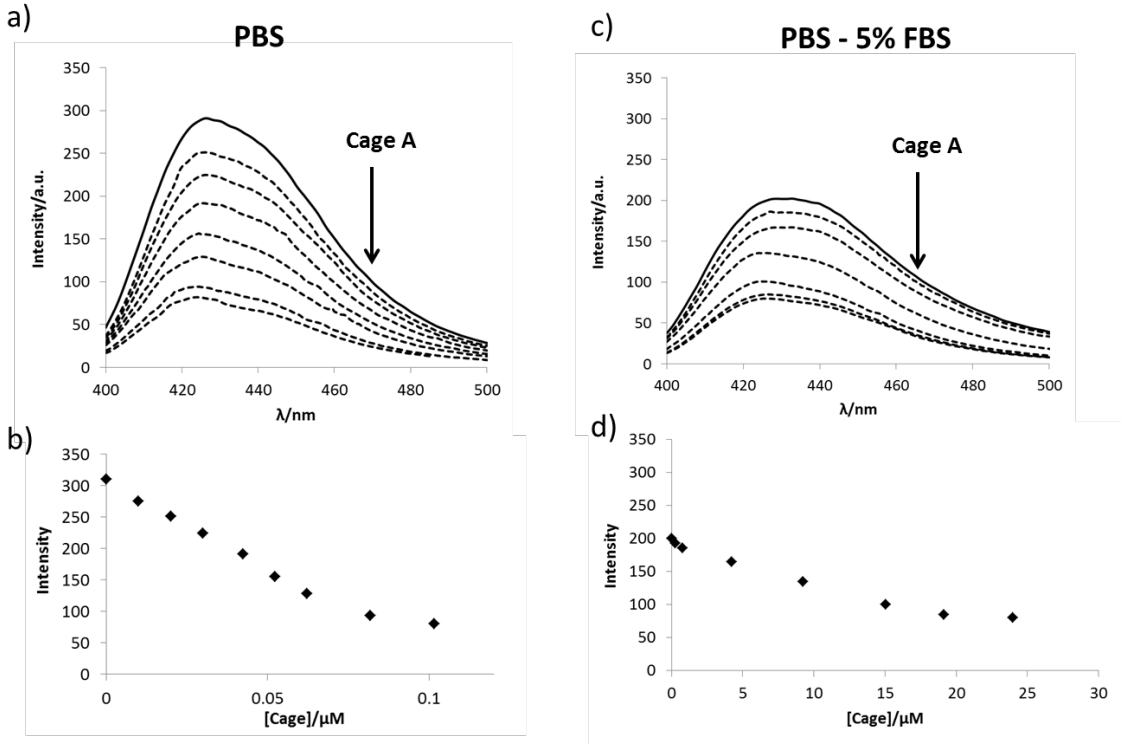


Figure S8. (a) Solid line: fluorescence emission spectrum from a suspension of **NS2** ($1.25x10^{11}$ NP/mL) in PBS. Dashed lines: fluorescence emission spectra from **NS2** after addition of increasing amounts of cage **A**. (b) Variation of pyranine fluorescence as a function of concentration of **A**, monitored at 425 nm. (c) Solid line: fluorescence emission spectrum from **NS2** ($1.25x10^{11}$ NP/mL) in PBS with 5% FBS. Dashed lines: fluorescence emission spectra of **NS2** after addition of increasing amounts of cage **A**. (d) Variation of the pyranine fluorescence as a function of concentration of **A**, monitored at 425 nm.

Bicinchoninic acid (BCA) quantification

The Bicinchoninic acid (BCA) assay was performed to quantify the amount of protein recovered in the washing steps and the amount of protein remaining on the particle. A calibration curve of 5 points was first generated by serial dilutions of BSA in Milli-Q water, from 1500 to 0 µg/mL. BCA reagents A and B were mixed at a ratio 50 : 1 and 200 µL of the BCA mixture was dispensed into 96 well-plates. Then, 10 µL of each standard or each sample of adsorbed proteins was added to each well. The plate was incubated for 1 hour at 37 °C and then the absorbance at 574 nm was recorded on a plate reader (Fluostar Omega).

Proteins adsorbed onto AuNPs were detached from Au NPs upon treatment with sodium dodecyl sulfate (SDS) (10%) and dithiothreitol (DTT; 0.5 mM), at 90 ºC for 10 min, purified by dialysis (Micro DispoDialyzer, 10K MWCO) and quantified by a BCA Protein assay kit.

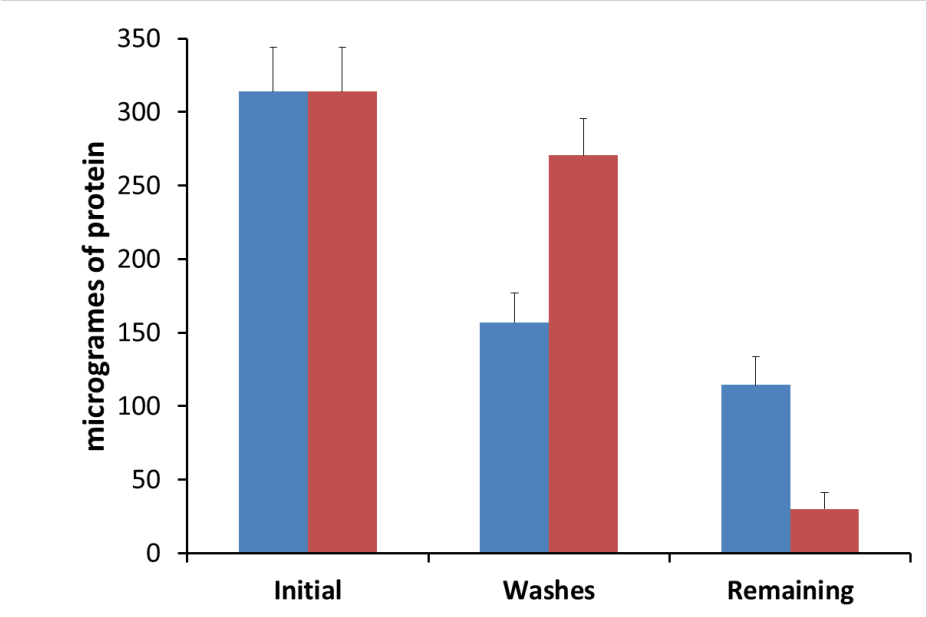


Figure S9. Bicinchoninic acid (BCA) quantification of the protein content in the absence (blue) and in the presence of cage **A** (20 µM, red) when **NS2** ($1.5x10^{11}$ particles) was incubated with dilute FBS (5% in PBS) for 30 min at 25 °C. *Initial* represents the amount of protein incubated with the NPs. *Washes* represents the amount of protein recovered in the washing steps. *Remaining* represents the amount of protein still bound to the NPs after the washing steps.

Protein corona on NS2 in 100% rat serum

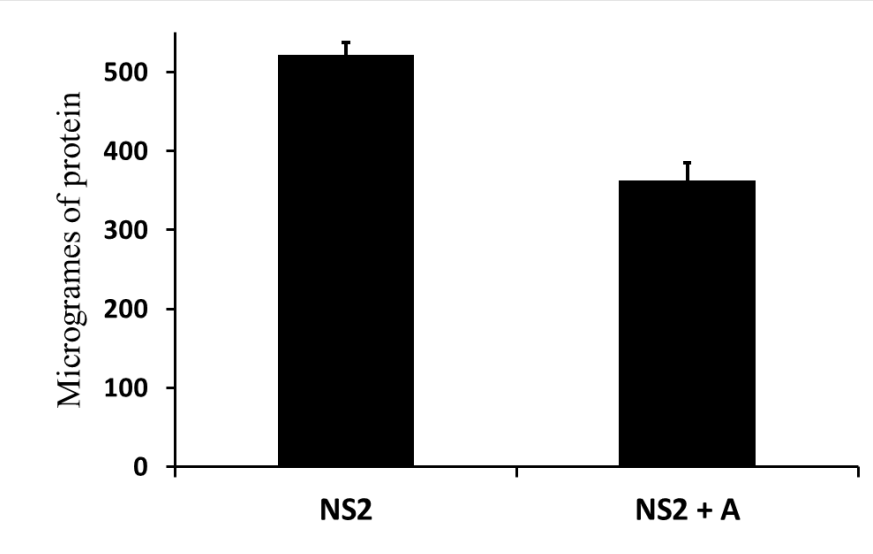


Figure S10. Comparison of the amount of rat serum protein immobilized on **NS2** particles. **NS2**: only **NS2** (200µL, $7.5x10^{11}$ particles/mL) diluted in 100% rat serum. **NS2 + A**: same conditions as the previous case, followed by addition of 100 µM of cage **A** and 10 min of incubation. Sprague Dawley Rat serum was recovered from whole blood donations from normal healthy rats.

Protein corona on NS2 in the presence of spermine

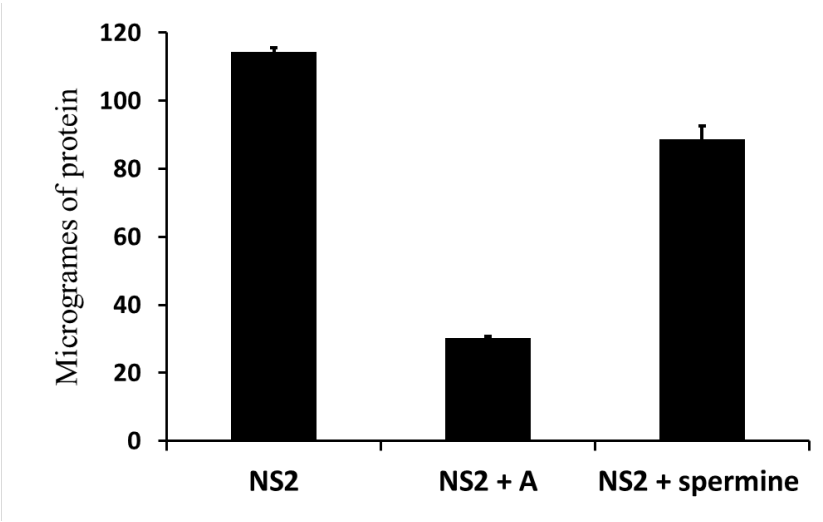


Figure S11. Comparison of the amount of FBS proteins immobilized onto **NS2** particles, upon addition of cage **A** or spermine by BCA analysis. **NS2**: only **NS2** (200µL, $7.5x10^{11}$ particles/mL) diluted in FBS (5% in PBS). **NS2 + A**: same conditions as the previous case, followed by addition of 20 µM of cage **A** and 10 min of incubation. **NS2**+ **spermine**: same conditions as **NS2**, followed by addition of 20 µM of spermine and 10 min of incubation.

Mass spectrometry analysis

Samples were analyzed in a hybrid trapped ion mobility spectrometry – quadrupole time of flight mass spectrometer (timsTOF Pro with PASEF, Bruker Daltonics) coupled online to a nanoElute liquid chromatograph (Bruker). This mass spectrometer takes advantage of a scan mode termed parallel accumulation – serial fragmentation (PASEF), which multiplies the sequencing speed with no sensitivity loss (https://www.ncbi.nlm.nih.gov/pubmed/26538118) and has been shown to provide outstanding analytical speed and sensibility for proteomics analyses (https://www.ncbi.nlm.nih.gov/pubmed/30385480). The sample (200 ng) was directly loaded in a 15 cm Bruker nanelute FIFTEEN C18 analytical column (Bruker) and resolved at 400 nL/min with a 30 min gradient. The column was heated to 50 ºC using an oven. PEAKS software (Bioinformatics Solutions) was used for protein identification and differential analysis. Only proteins identified with a FDR<5% and two different peptides were considered as reliable hits. Regarding the differential analysis, proteins with a PEAKSQ significance higher than 20 (corresponding to a $p<0.01$) and a ratio higher than 2 in either direction were considered as differentially present between samples. Significantly differential proteins ($p<0.01$) were analyzed using DAVID (https://david.ncifcrf.gov/) in order to characterize the biological processes they are involved in.

Table S1. List of proteins comprising the PC on **NS2**, for which the relative abundance increases or decreases more than two times during the cage/pyranine cycle of the SDS-PAGE assay showed in Figure 3 (main manuscript) based on LC-MS/MS analysis.

Identified protein	Ratio	Step 1	Step 3	Step 5	Step 2	Step 4	Step 6
Beta-enolase	**0.15**	0.86	0.84	1.30	0.00	0.18	0.00
Plasminogen	**0.21**	0.90	0.74	1.37	0.16	0.28	0.20
Pantetheinase	**0.24**	1.09	0.91	1.00	0.29	0.19	0.25
Tetranectin	**0.25**	0.78	1.12	1.10	0.20	0.25	0.29
Plasma kallikrein	**0.29**	0.81	1.03	1.15	0.36	0.24	0.29
Carboxypeptidase B2	**0.30**	0.97	0.94	1.09	0.40	0.21	0.32
Inter-alpha-trypsin inhibitor heavy chain H4	**0.30**	1.18	0.75	1.06	0.31	0.32	0.26
Prothrombin	**0.31**	0.95	0.90	1.15	0.34	0.32	0.28
Complement component C6	**0.32**	1.14	1.00	0.86	0.35	0.37	0.24
Alpha-1B-glycoprotein	**0.33**	1.05	0.84	1.11	0.48	0.29	0.26
Plasma serine protease inhibitor	**0.35**	0.96	1.03	1.01	0.39	0.35	0.31
Complement factor H	**0.35**	0.85	0.96	1.19	0.44	0.36	0.27
C4b-binding protein alpha chain	**0.36**	0.79	1.08	1.13	0.34	0.36	0.35
Thrombospondin-1	**0.36**	0.92	0.91	1.17	0.35	0.39	0.33
Gelsolin	**0.37**	0.95	0.92	1.13	0.31	0.48	0.33
Keratin type I cytoskeletal 26	**0.37**	0.91	1.22	0.87	0.49	0.33	0.31
Kininogen-1	**0.40**	1.08	0.98	0.94	0.51	0.31	0.39
Inter-alpha-trypsin inhibitor heavy chain H3	**0.40**	1.13	0.82	1.05	0.53	0.33	0.36
Antithrombin-III	**0.41**	1.05	0.91	1.04	0.49	0.36	0.38
Factor XIIa inhibitor	**0.41**	0.92	0.93	1.16	0.44	0.38	0.40
Protein AMBP	**0.43**	1.11	0.76	1.13	0.46	0.43	0.39
Complement component C9	**0.47**	0.90	1.13	0.97	0.45	0.49	0.46
Pigment epithelium-derived factor	**0.47**	0.81	1.07	1.12	0.50	0.47	0.43
Beta-2-glycoprotein 1	**0.48**	1.20	0.82	0.98	0.58	0.40	0.46
Alpha-fetoprotein	**0.49**	1.00	0.87	1.13	0.61	0.43	0.46
Tudor domain-containing protein 7	**2.52**	0.76	1.08	1.16	2.50	2.39	2.54

Table S2. Proteins immobilized onto **NS2** during the cage **A**/**pyr** cycle.

Identified protein	p value	Step 1	Step 3	Step 5	Step 2	Step 4	Step 6
ALBU_BOVIN	0.253285	3889896	3739982	4360010	3840067	3320111	3240084
TRFE_BOVIN	0.004223	1319994	1330005	1740053	1019973	820020	928988
A2MG_BOVIN	0.006907	975985	688979	906973	506991	311010	303009
FETUA_BOVIN	0.336998	795003	768987	830028	767018	614009	676017
A1AT_BOVIN	0.441825	753005	797984	824980	795995	599999	622019
CO3_BOVIN	0.066283	573010	682988	619996	439012	496007	415014
APOA1_BOVIN	0.359742	340006	369010	371010	359991	379989	366995
FETA_BOVIN	0.001377	336000	294009	380992	205005	143999	154003
K1C10_BOVIN	1.05E-05	264004	267004	287002	413005	432998	428014
VTDB_BOVIN	0.486142	228004	183002	235000	170996	87999	127003
ANT3_BOVIN	0.001502	198999	172998	197994	93703	68400	71800
HBA_BOVIN	0.272589	194998	276995	219993	194998	219004	211002
ITIH4_BOVIN	0.000848	172998	109997	154999	45801	47400	37401
HBBF_BOVIN	0.025327	143999	147996	154003	167001	207997	214007
K2C5_BOVIN	0.234632	136998	120996	143004	165997	239008	161000
FETUB_BOVIN	0.785751	136998	154003	145999	119000	84602	104998
APOE_BOVIN	0.581172	130004	233005	165997	170003	175998	153003
CFAB_BOVIN	0.131515	120996	120996	99997	76200	70498	67402
PLMN_BOVIN	0.000914	116003	95598	177001	20300	36800	26101
ITIH3_BOVIN	0.018219	103998	75700	96397	48601	30201	32800
A2AP_BOVIN	0.007803	84602	66198	77898	51999	34699	43301
A1BG_BOVIN	0.003611	81200	64401	85599	37099	22000	20400
CFAH_BOVIN	0.022193	78201	87500	109002	40401	32600	24801
HEMO_BOVIN	0.174013	74803	57801	66299	58102	37401	43301
TPM3_BOVIN	0.080031	69701	89998	92901	96899	99100	134002
SPA37_BOVIN	0.106058	69499	51999	63899	36401	22000	31799
A1AG_BOVIN	0.097652	67201	68799	77898	58701	40100	51401
AMBP_BOVIN	0.000804	62100	42799	63002	25500	23999	21700
CO4_BOVIN	0.088221	55500	60700	75099	35900	31700	29200
PEDF_BOVIN	0.154082	54501	72099	75198	33701	31799	29099
CO7_BOVIN	0.017837	50500	65101	68699	41701	49999	42201
GELS_BOVIN	0.000607	45501	43899	53799	15000	22999	15800
FINC_BOVIN	0.947686	39801	34699	34800	35001	27999	29701
AFAM_BOVIN	0.003828	39101	32299	32400	24600	18900	16700
THRB_BOVIN	3.97E-05	33200	31700	40401	12000	11100	9760
TTHY_BOVIN	0.75199	32501	37200	44899	31901	27800	37900
K1C17_BOVIN	0.92662	31301	24901	30400	29600	88697	31599
APOH_BOVIN	0.496791	30101	20499	24400	14600	10100	11500

ADIPO_BOVIN	0.479786	29099	26599	25100	18900	11900	17200
CCD25_BOVIN	0.203046	28501	38100	32400	43400	50200	40900
VNN1_BOVIN	0.000102	27900	23400	25800	7520	4950	6320
PSD11_BOVIN	0.894666	27600	32800	32600	25800	8630	20200
KNG1_BOVIN	0.017206	26401	23800	22999	12500	7650	9500
ITIH1_BOVIN	0.003371	25700	17700	26500	11500	6000	7770
TETN_BOVIN	0.008667	21700	31301	30900	5670	6940	8060
IPSP_BOVIN	5.7E-05	21599	23299	22799	8750	7830	6950
FA5_BOVIN	0.071865	20900	27800	27800	12800	14000	13000
K1C19_BOVIN	0.569878	20601	3510	4440	4680	7560	3500
F12AI_BOVIN	0.001379	19700	19900	24801	9430	8110	8610
KLKB1_BOVIN	0.003558	19500	24801	27700	8670	5720	6880
CO9_BOVIN	0.018064	18500	23100	19900	9190	9950	9400
CASA1_BOVIN	0.518525	17900	17200	18701	19700	19200	17600
DBNL_BOVIN	0.096234	16999	21200	28501	29399	26000	29300
FA10_BOVIN	0.320193	15100	9650	15800	6460	5260	3660
G3P_BOVIN	0.088338	14900	14800	12600	9580	11800	11100
THBG_BOVIN	0.721845	14300	7900	10300	5840	2880	4450
SYWC_BOVIN	0.329755	13900	10400	11000	12700	14400	12300
APOA2_BOVIN	0.047567	13700	13900	18400	16800	21301	22499
ASPM_BOVIN	0.003159	13600	18701	13700	27400	28100	26401
RET4_BOVIN	0.782166	13300	11700	14900	8030	4840	8010
TSP1_BOVIN	0.000292	12600	12400	16000	4770	5300	4490
APOA4_BOVIN	0.827052	12600	12900	9170	10900	8990	8330
EF1A1_BOVIN	0.304077	12100	20800	15300	11200	19800	9630
K2C79_BOVIN	0.870414	12100	17500	20499	19400	24400	17600
PROS_BOVIN	0.013418	11900	8620	7140	4550	4420	3320
C4BPA_BOVIN	0.015976	11700	16100	16800	5080	5390	5190
BIP_BOVIN	0.04955	11200	16000	10600	11200	6660	6890
CO6_BOVIN	0.042938	11000	9630	8330	3410	3600	2310
CLUS_BOVIN	0.972088	11000	11300	9960	8520	8440	7000
CO1A1_BOVIN	0.445343	10800	14600	13500	13900	18701	13200
ANXA2_BOVIN	0.272467	10400	7680	4090	9760	25399	10700
MPRI_BOVIN	0.017105	9280	7960	15200	4830	5270	6370
KNG2_BOVIN	0.009189	8380	8870	10800	5160	1780	1690
FIBA_BOVIN	0.021695	8240	8190	7650	7110	5040	6040
HS71A_BOVIN	0.130627	7870	4330	6070	4800	4960	3810
HS71B_BOVIN	0.130627	7870	4330	6070	4800	4960	3810
PACN1_BOVIN	0.193331	7630	6860	10100	11100	45099	10300
AOC3_BOVIN	0.288954	7270	4360	6430	3530	2700	2080
AOCY_BOVIN	0.288954	7270	4360	6430	3530	2700	2080

PLAK_BOVIN	0.898926	7270	6600	6410	8160	10400	8930
ENOA_BOVIN	0.17626	7120	4970	6120	4370	4710	3580
TRFL_BOVIN	0.072367	7070	6390	33601	2630	3300	2010
CAND1_BOVIN	0.543758	6850	5970	10500	5500	5880	5860
SYCP3_BOVIN	0.83711	6680	5350	5790	4830	6220	3380
PI4KB_BOVIN	0.158597	6470	5510	8280	8880	9250	8130
PDIA1_BOVIN	0.113679	6440	6510	6920	4270	5180	6690
KC1G1_BOVIN	0.815685	6170	11000	10300	8950	5820	7030
UBP16_BOVIN	0.089803	5710	5000	6570	3180	3030	2440
H4_BOVIN	0.669273	5500	2210	3690	3700	6950	2850
RL40_BOVIN	0.007274	5380	5650	4640	7530	11900	8260
UBC_BOVIN	0.007274	5380	5650	4640	7530	11900	8260
RS27A_BOVIN	0.007274	5380	5650	4640	7530	11900	8260
UBB_BOVIN	0.007274	5380	5650	4640	7530	11900	8260
HS90A_BOVIN	0.152754	5330	3410	4560	3050	3790	2120
K2C71_BOVIN	0.55175	5000	5410	1640	4290	3770	3750
LUM_BOVIN	0.596661	4980	5620	4500	6470	6270	8260
DSG1_BOVIN	0.328925	4820	2670	3000	3940	2700	2230
FIBB_BOVIN	0.875387	4670	6750	5240	4310	3990	4010
CAP1_BOVIN	0.011109	4440	5460	6400	3550	2100	2680
MYH10_BOVIN	0.097618	4430	3940	6610	3440	3880	2400
PGK1_BOVIN	0.951098	4180	4230	3400	2700	4450	3190
ACTS_BOVIN	0.84594	4090	6200	3520	4600	6030	3220
ACTC_BOVIN	0.84594	4090	6200	3520	4600	6030	3220
STK38_BOVIN	0.248798	4040	3890	4760	4230	5330	5230
TDRD7_BOVIN	0.001367	4030	5730	6180	13300	12700	13500
APOD_BOVIN	0.393819	4000	6000	5260	4550	6050	5350
H2A2C_BOVIN	0.156429	3820	1240	727	1240	749	437
ARGI1_BOVIN	0.303055	3720	2320	2490	3140	3220	2890
K2C73_BOVIN	0.2134	3520	2310	3090	3840	3980	2870
HPT_BOVIN	0.078562	3500	4640	2450	2430	1880	2430
FA11_BOVIN	0.759451	3480	3850	2870	3370	2820	2900
SPA38_BOVIN	0.830371	3460	2360	1280	1040	1330	1410
HRG_BOVIN	0.011525	3330	2200	2490	1580	535	611
ACTB_BOVIN	0.162988	3260	2620	2650	2050	2730	1570
CE290_BOVIN	0.377515	3150	4150	5120	4620	4560	4040
CBPB2_BOVIN	0.003565	3060	2970	3440	1260	665	1020
PKP3_BOVIN	0.108398	3030	2070	1730	3640	2520	5390
1433Z_BOVIN	0.236352	3000	4860	5590	2260	5470	2290
F13A_BOVIN	0.018737	2830	2770	2130	1340	301	176
HS90B_BOVIN	0.961561	2750	378	1350	1230	2570	1110

SAHH_BOVIN	0.240367	2730	4920	2580	3810	4330	3770
K2C75_BOVIN	0.039436	2700	2530	1840	3820	5880	3100
RET3_BOVIN	0.582315	2670	2110	1580	2190	1380	1860
PGRP1_BOVIN	0.651339	2670	6940	6040	2780	3950	3350
K1C20_BOVIN	0.246624	2480	1060	2300	2320	2560	2080
KPCA_BOVIN	0.664558	2470	912	2910	3270	3160	3910
K2C78_BOVIN	0.120657	2450	2570	3720	3710	3130	3910
SPA36_BOVIN	0.728977	2360	1500	866	1140	244	1200
CASK_BOVIN	0.191161	2350	4190	3640	4700	5520	5520
VIME_BOVIN	0.450416	2300	3310	1850	2710	3510	831
PKP1_BOVIN	0.081407	2270	1960	2390	1190	1820	1580
TPIS_BOVIN	0.444407	2270	804	797	1610	1860	257
IBP2_BOVIN	0.429107	1850	1280	2190	144	907	963
K1C26_BOVIN	0.00156	1830	2460	1740	977	669	617
PHLD_BOVIN	0.090895	1810	1120	1280	152	570	218
KRT83_BOVIN	0.858105	1800	884	875	1010	867	936
COMP_BOVIN	0.106818	1750	1680	843	909	716	1120
K2C4_BOVIN	0.709511	1730	354	710	787	5540	912
ERO1A_BOVIN	0.745358	1720	799	2610	739	1950	634
DSC1_BOVIN	0.443629	1710	277	602	1390	705	605
TBB5_BOVIN	0.962849	1610	590	142	1490	2010	599
CSTF2_BOVIN	0.235607	1580	3600	3420	4340	3070	3630
K1C14_BOVIN	0.657092	1580	1470	1540	2220	4530	2320
ATPB_BOVIN	0.706788	1580	6050	1500	2620	1910	1390
KRT35_BOVIN	0.186557	1450	676	302	750	269	201
CHIA_BOVIN	0.588631	1430	2170	2430	1240	248	1230
K2C72_BOVIN	0.714161	1390	1940	1540	713	4980	2030
HABP2_BOVIN	0.56873	1350	2030	1350	2230	916	1670
ROA2_BOVIN	0.749706	1320	919	1120	992	1510	874
CO2A1_BOVIN	0.564622	1250	3910	2240	2500	1550	2580
EF2_BOVIN	0.215331	1240	1880	1670	1170	1740	1060
RGN_BOVIN	0.306067	1170	1270	837	19500	723	799
TGO1_BOVIN	0.108674	1150	1450	1770	2430	4700	2150
NFX1_BOVIN	0.208631	1090	1030	818	907	2100	2020
RS3A_BOVIN	0.026851	1060	1370	1370	899	408	367
GLNA_BOVIN	0.871401	1060	464	211	715	321	451
ENOB_BOVIN	0.003469	1030	1000	1550	262	218	91
ATPA_BOVIN	0.382422	1020	899	599	213	2260	392
CBG_BOVIN	0.610117	966	849	1180	1080	667	222
TSP4_BOVIN	0.192279	939	1530	1880	360	595	551
FA9_BOVIN	0.819305	929	1040	520	872	339	790

C1S_BOVIN	0.064577	895	923	1160	470	176	621
SPA31_BOVIN	0.392677	891	439	414	95	461	456
KPCB_BOVIN	0.315309	886	1020	999	780	257	79
LACB_BOVIN	0.823682	850	304	991	1750	740	532
ACTG_BOVIN	0.342581	827	182	621	515	865	471
K1C25_BOVIN	0.976582	823	4010	809	100	4490	3620
RUSD3_BOVIN	0.33511	809	1330	2100	531	1050	1130
MCM5_BOVIN	0.219528	785	1600	1120	655	511	979
ARF1_BOVIN	0.098994	770	1000	1420	1410	1280	1710
ARF3_BOVIN	0.098994	770	1000	1420	1410	1280	1710
ARF2_BOVIN	0.098994	770	1000	1420	1410	1280	1710
HSP7C_BOVIN	0.88357	753	841	215	376	1980	1030
PSB2_BOVIN	0.497658	730	305	935	1040	774	996
ACTN4_BOVIN	0.56297	730	1510	1500	1990	3070	2150
K2C8_BOVIN	0.569633	691	765	649	641	517	610
TERA_BOVIN	0.063508	668	920	1130	787	200	257
CATA_BOVIN	0.045268	649	734	712	637	389	323
CADH5_BOVIN	0.27584	621	1000	366	648	514	276
HGFL_BOVIN	0.168701	615	255	1430	550	293	142
KASH5_BOVIN	0.060853	603	564	630	875	1050	610
1433E_BOVIN	0.397559	603	956	732	668	1120	401
CLIC1_BOVIN	0.599268	582	240	124	376	814	460
TCPB_BOVIN	0.673209	576	1300	591	1530	1390	1310
1433S_BOVIN	0.60358	575	495	254	516	2610	374
K1C27_BOVIN	0.731166	564	121	414	325	588	382
OLFL3_BOVIN	0.872259	562	882	785	845	692	708
CO1A2_BOVIN	0.40795	539	2470	1830	1690	376	1140
CLH1_BOVIN	0.29566	504	145	183	349	181	56
PGAM1_BOVIN	0.552067	495	1380	737	782	864	364
TCPZ_BOVIN	0.107568	369	332	946	1320	841	739
TBB4B_BOVIN	0.454661	337	1610	355	896	1130	886
MOES_BOVIN	0.414187	335	601	3290	1840	957	1270
PABP1_BOVIN	0.507582	301	369	1120	382	4460	1910
K2C74_BOVIN	0.169045	229	1200	266	3150	7630	1570
CAZA2_BOVIN	0.997682	118	1030	426	681	909	402
HBB_BOVIN	0.509141	75	1350	640	391	219	3520
CTNB1_BOVIN	0.865931	55	498	122	731	102	239

Protein corona on NS1

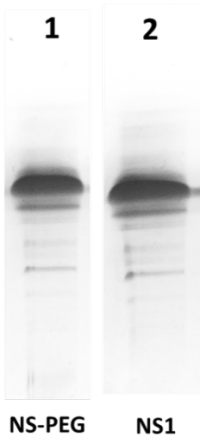


Figure S12. Comparison of the amount of FBS immobilized on **NS1** and **NS-PEG** particles. Lane 1: 200 µL of **NS-PEG** ($7.5x10^{11}$ particles/mL) diluted in FBS (5% in PBS) after 30 min of incubation. Lane 2: 200 µL of **NS1** ($7.5x10^{11}$ particles/mL) diluted in FBS (5% in PBS) after 30 min of incubation. The amount of protein was quantified using ImageJ and normalized against the result for lane 1: lane 1 (100%); lane 2 (168%).

Cell viability

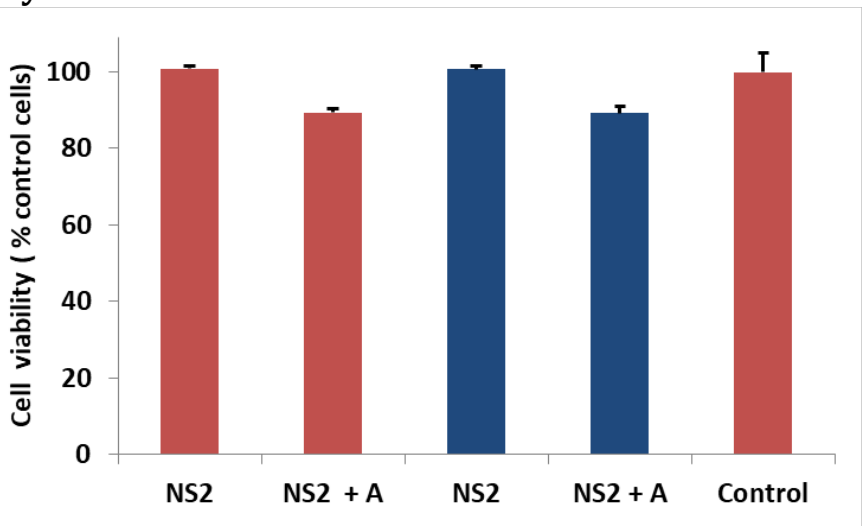


Figure S13. MTT assay (Lonza) of HeLa cells treated with **NS2** ($9x10^{10}$ particles/mL for red bars; $3x10^{10}$ particles/mL for blue bars), in the presence and absence of cage **A** (5 µM). HeLa cells were plated in 96-well plates and allowed to adhere overnight. The following day cell media was replaced with NPs, with and without cage **A**, diluted in DMEM containing 10% FBS. Cells were incubated at 37 °C for 24h. MTT reagent was added to the cells for 1h, followed by washing and addition of DMSO. Cell absorbance at 550 nm was measured and the mean cell viability is presented as a percentage of control cells, +/- SD. N=3.

TEM images of NS2 internalization in the absence of cage A

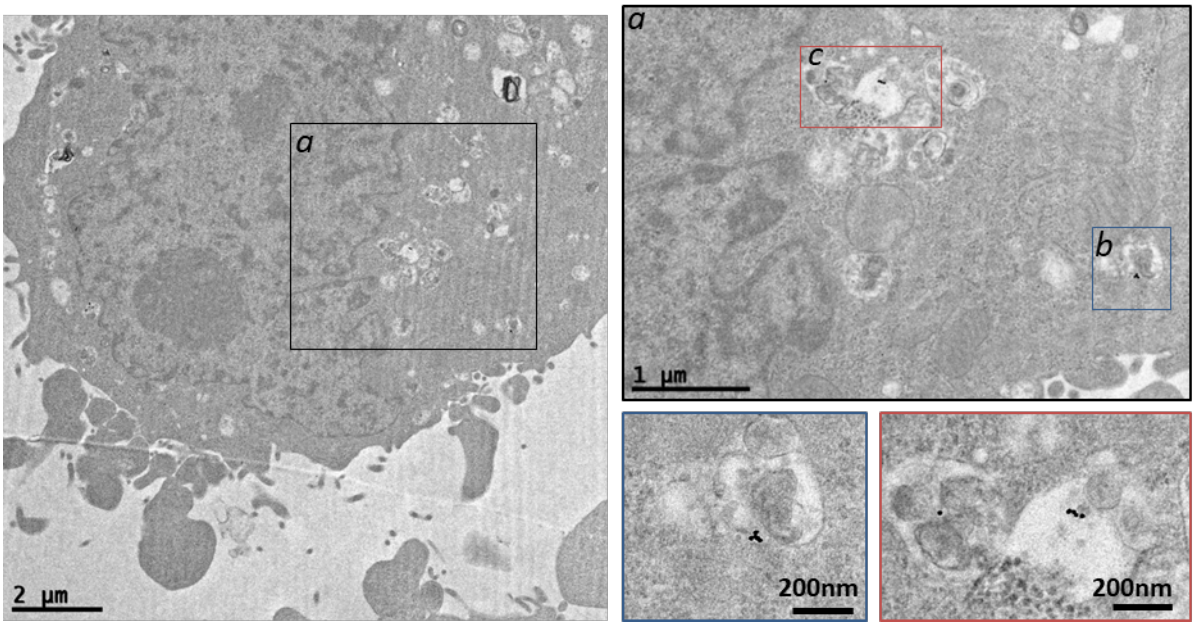


Figure S14. TEM images of one HeLa cell incubated with **NS2 (**$9x10^{10}$ NP/mL) in DMEM with 10% FBS, for 24 h, at a concentration of $9x10^{10}$ NP/mL in the absence of cage **A**.

NR3 characterization

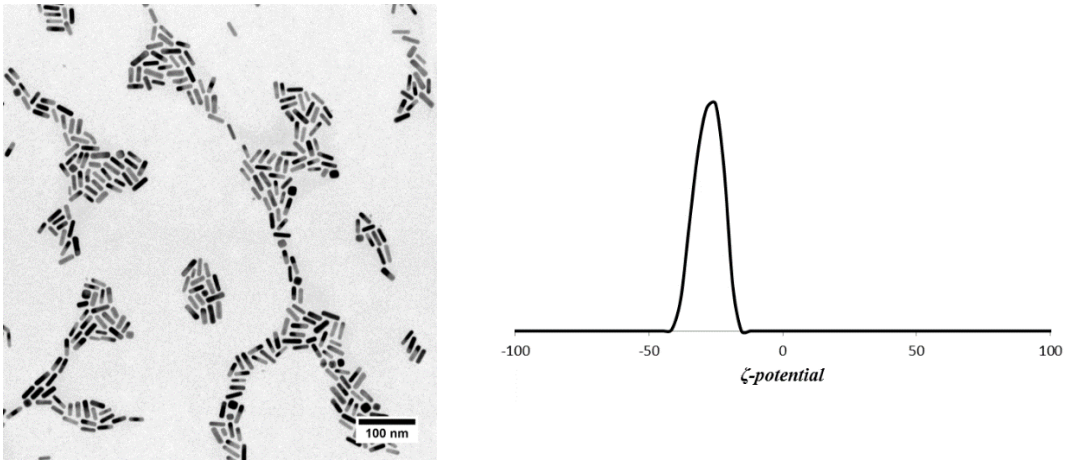


Figure S15. (Left) Representative TEM image of **NR3**. Based on TEM images the average dimensions are 29±4 nm × 9±1 nm. (Right) Zeta potential distribution for **NR3** (-27±2 mV). Both measurements were performed in Milli-Q water at room temperature.

Protein corona on NR3

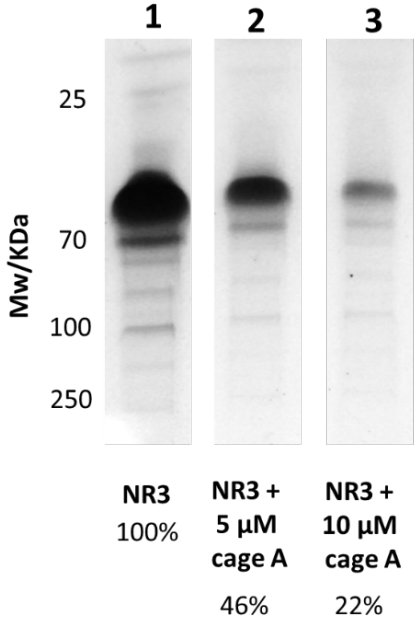


Figure S16. Comparison of the amount of FBS immobilized on **NR3**, upon addition of cage **A**. Lane 1: **NR3** ($7.5x10^{11}$ particles/mL, 200 µL) diluted in FBS (5% in PBS) after 30 min of incubation. Lane 2: Same conditions as in Lane 1, but using 5 µM of cage **A** during incubation with FBS. Lane 3: Same conditions as in Lane 1, but using 10 µM of cage **A** during incubation with FBS. The amount of protein was quantified using ImageJ and normalized against the result for lane 1: lane 1 (100%); lane 2 (46%); lane 3 (22%).

References

(1) Nyren-Erickson, E. K.; Haldar, M. K.; Gu, Y.; Qian, S. Y.; Friesner, D. L.; Mallik, S. Fluorescent Liposomes for Differential Interactions with Glycosaminoglycans. *Anal. Chem.* **2011**, *83*, 5989–5995.

(2) Mosquera, J.; Zarra, S.; Nitschke, J. R. Aqueous Anion Receptors through Reduction of Subcomponent Self-Assembled Structures. *Angew. Chem. Int. Ed.* **2014**, *53*, 1556–1559.

(3) García, I.; Sánchez-Iglesias, A.; Henriksen-Lacey, M.; Grzelczak, M.; Penadés, S.; Liz-Marzán, L. M. Glycans as Biofunctional Ligands for Gold Nanorods: Stability and Targeting in Protein-Rich Media. *J. Am. Chem. Soc.* **2015**, *137*, 3686-3692.