SUPPORTING INFORMATION

The functional dissection of the plasma corona of SiO_2-NPs spots Histidine Rich Glycoprotein as a major player able to hamper nanoparticles capture by macrophages

Chiara Fedeli,[a,b] Daniela Segat,[c,†] Regina Tavano,[a,b] Luigi Bubacco,[c] Giorgia De Franceschi,[a] Patrizia Polverino de Laureto,[a] Elisa Lubian,[d] Francesco Selvestrel,[d] Fabrizio Mancin[d*] and Emanuele Papini[a,b *]

[a] Interdepartmental Research Center for Innovative Biotechnologies (CRIBI), Università di Padova, via U Bassi 58/B, I-35131, Padova, Italy

[b] Department of Biomedical Sciences, Università di Padova, via U Bassi 58/B, I-35131, Padova, Italy

[c] Department of Biology, Università di Padova, via U Bassi 58/B, I-35131, Padova, Italy

[d] Department of Chemistry, Università di Padova, via Marzolo 1, I-35131, Padova, Italy

[†] current address: Institute for Rare Diseases 'Mauro Baschirotto', via Bartolomeo Bizio 1, 36023 Costozza di Longare (Vicenza), Italy

*correspondence: emanuele.papini@unipd.it; fabrizio.mancin@unipd.it

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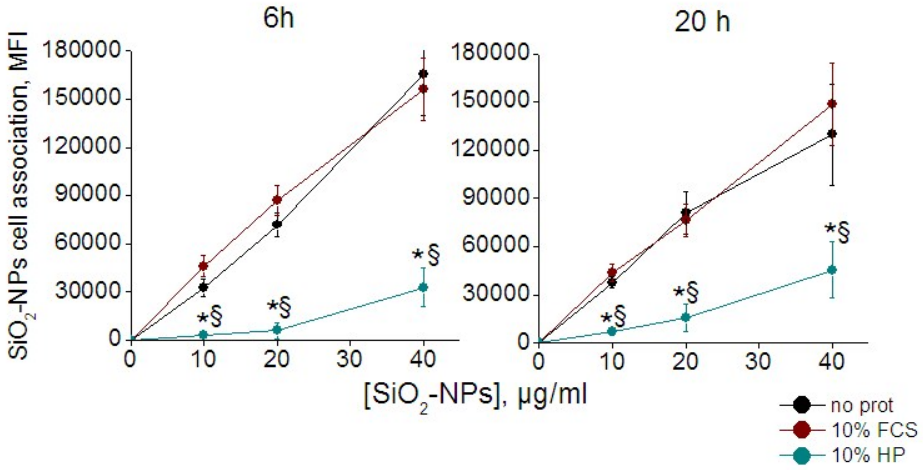
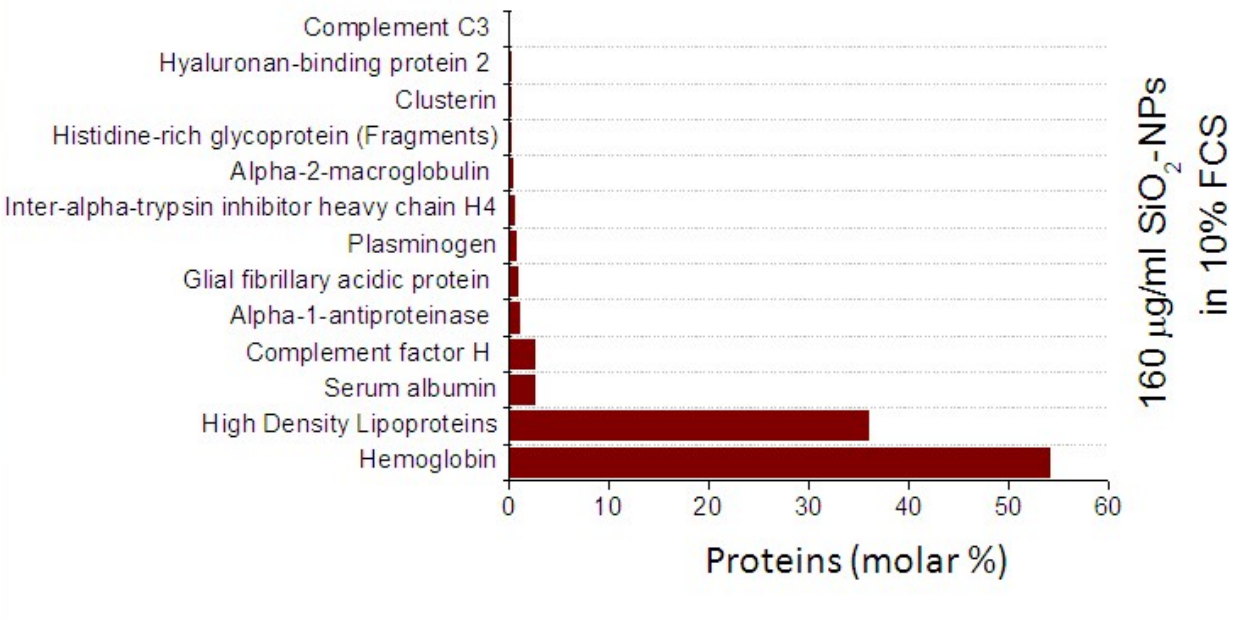
Figure S1. Time dependence of the association of SiO_2-NPs to macrophages.

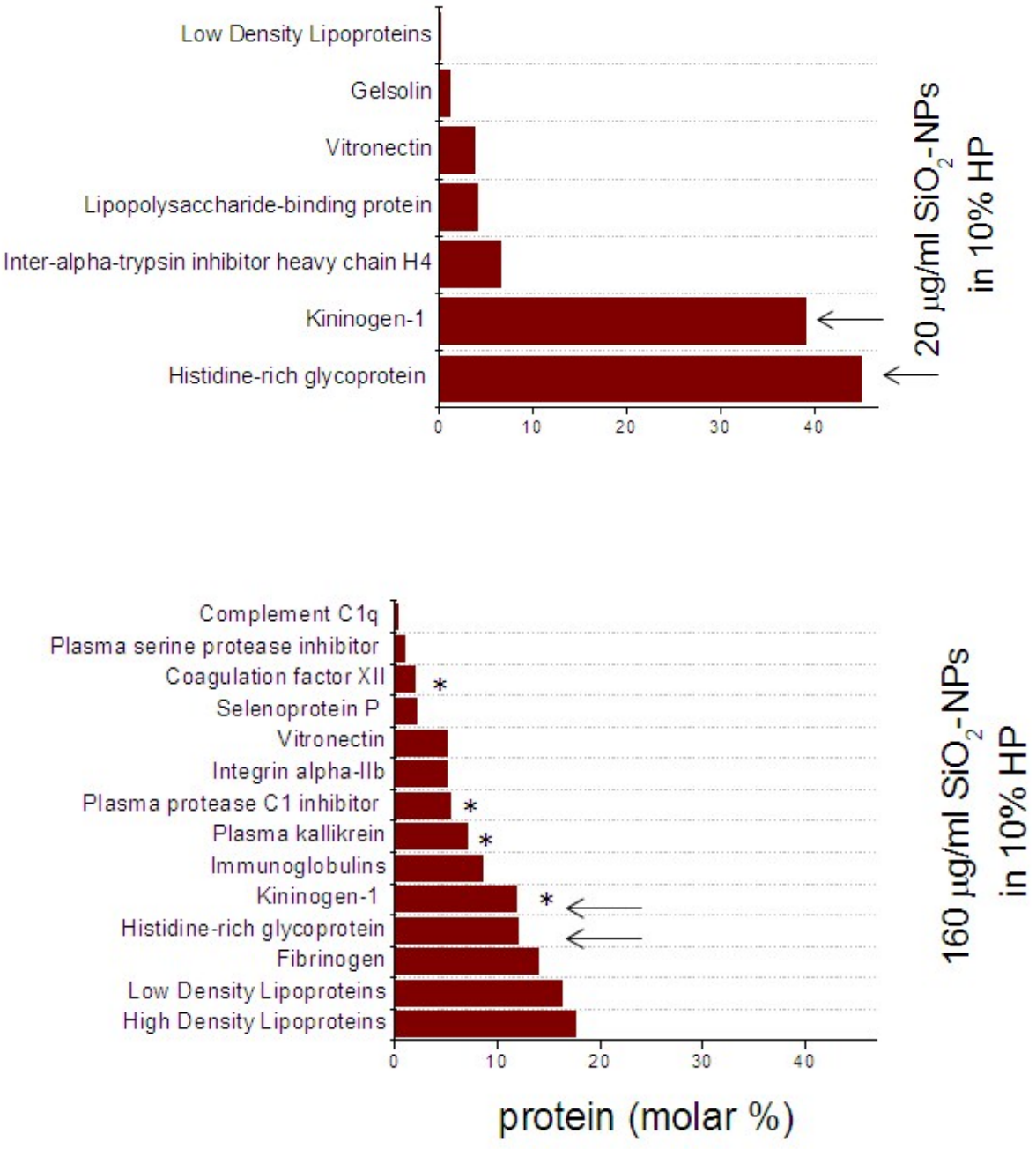


Macrophages were treated with SiO_2-NPs for 6h and 20h in RPMI 1640 without protein (no prot), plus 10% FCS or 10% HP, as indicated. After incubation, cells were washed and analyzed by flow-cytofluorimetry to quantify NPs-cell association (MFI). Data are the mean ± SE of four experiments run in duplicate. * significance ($p<0.05$) with respect to no protein; § significance ($p<0.05$) with respect to FCS.

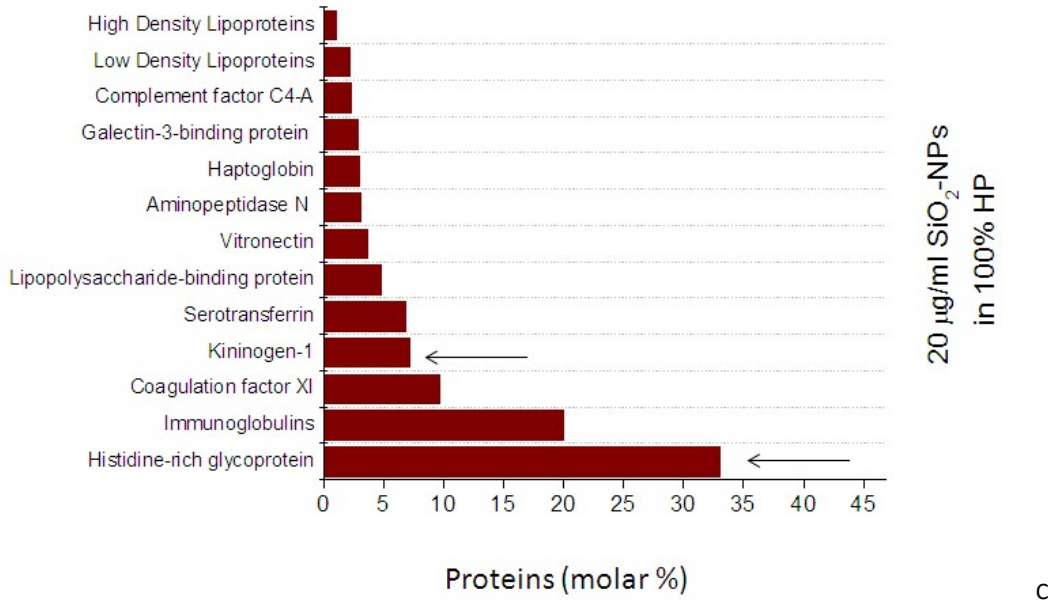
Figure S2. Estimation of the relative proportion of proteins of the corona in different media.



A


Low Density Lipoproteins
Gelsolin
Vitronectin
Lipopolysaccharide-binding protein
Inter-alpha-trypsin inhibitor heavy chain H4
Kininogen-1
Histidine-rich glycoprotein
0
10
20
30
40
20 µg/ml SiO2-NPs
in 10% HP
Complement C1q
Plasma serine protease inhibitor
Coagulation factor XII
Selenoprotein P
Vitronectin
Integrin alpha-IIb
Plasma protease C1 inhibitor
Plasma kallikrein
Immunoglobulins
Kininogen-1
Histidine-rich glycoprotein
Fibrinogen
Low Density Lipoproteins
High Density Lipoproteins
*
0
10
20
30
40
protein (molar %)
160 µg/ml SiO2-NPs
in 10% HP

B



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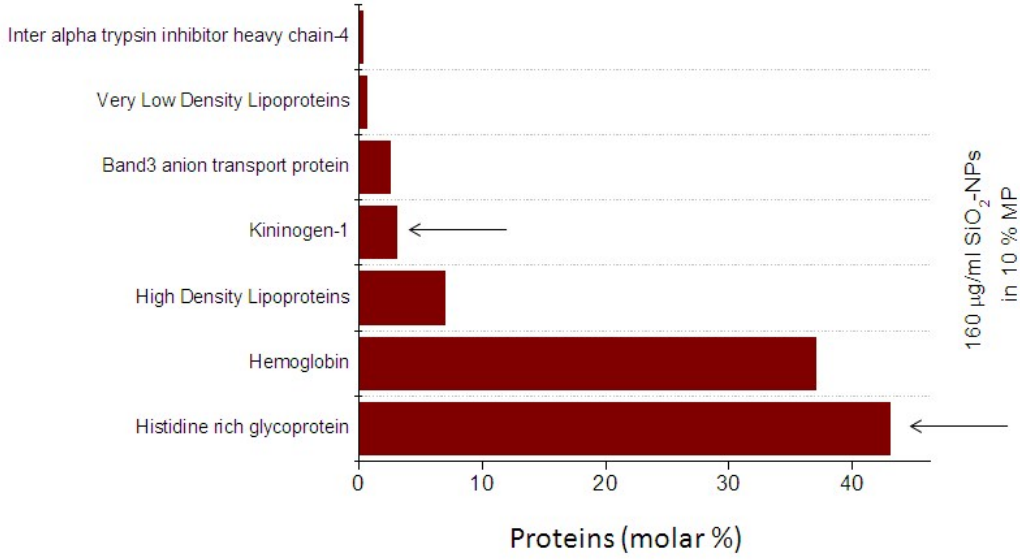
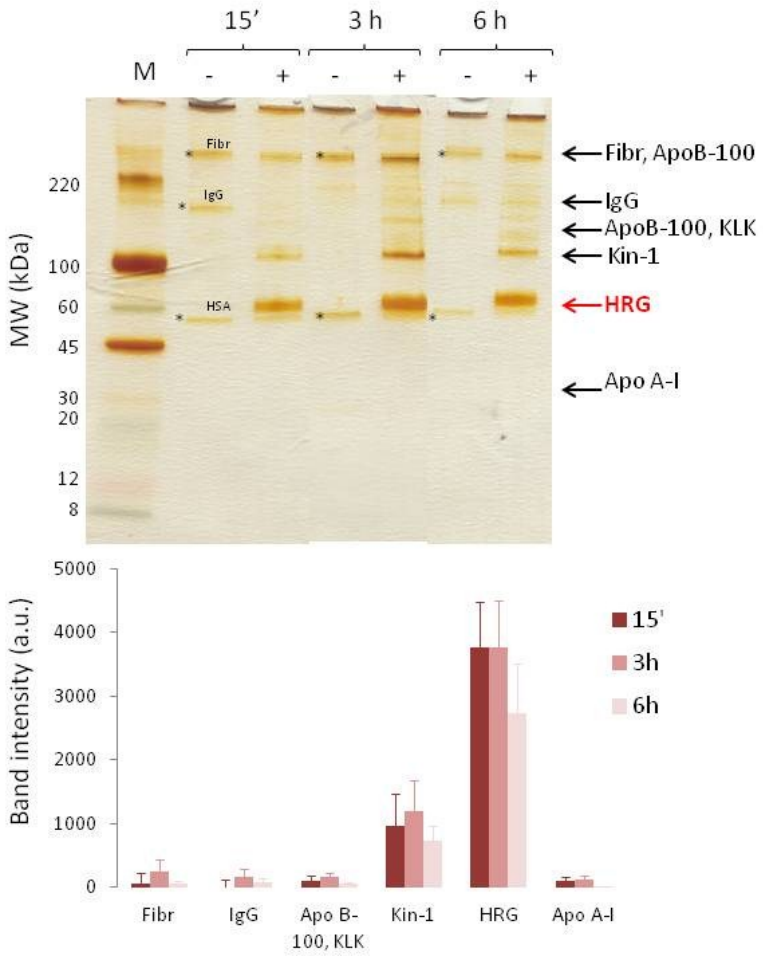


D

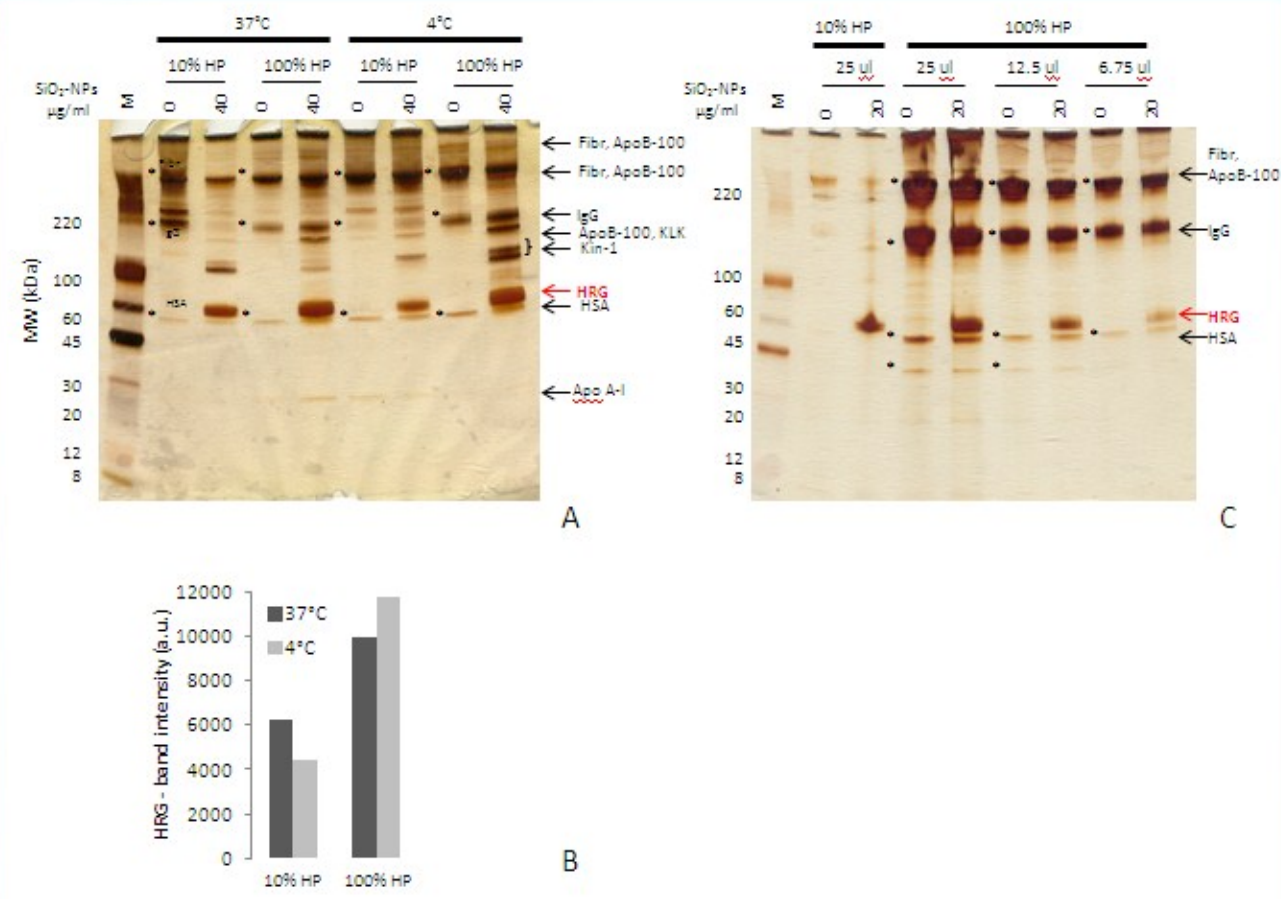
Label free emPAI values corresponding to the same polypeptides found in the SDS-PAGE major bands of the same lane, analyzed by LC MS/MS (see Figures 3A-B, S5A-B and tables S1,S2,S3,S4), were grouped after normalization for different sample loading in the chromatographic system, to obtain the % values of the indicated main proteins in FCS (A), HP (B and C) and MS (D) in the specified conditions. Arrows point to HRG and Kin-1. The coagulation *contact system* components (Kin-1, Kallikrein, F-XII) and its main regulator (plasma protease C1 inhibitor) are indicated by asterisks in the 10% HP samples (SiO_2-NPs 160 mg/ml). Data are adjusted considering the quaternary protein structures (ex= 3 Apo A-I per HDL molecule, 2 H chains and 2 L chains per Ig molecules, CF-XI is a homodimer, Fibrinogen is made of 2 α, 2 β and 2 γ subunits, C1q is made by 6 sub A, 6 sub B and 6 sub C, hemoglobin is made by 2 α sub and 2 β sub, α 2-macroglobulin is a homotetramer). HDL and LDL amounts are best estimated by using Apo A-I and Apo B-100, while minor components of the two lipoproteins classes, like Apo A-IV or Apo E were not considered since substoichiometric. Ig types (IgG, IgA) or sub-types (IgG_1, IgG_2, IgG_3 IgG_4 in HP) were grouped under the notation *immunoglobulins.*

Figure S3. Time-evolution of the HP-derived SiO_2-NPs corona.



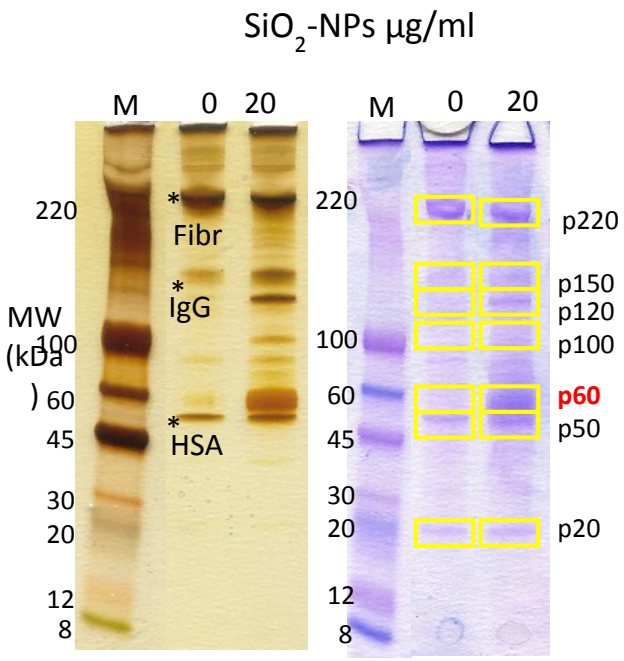
RPMI 1640 + 10% HP was incubated with (+) or without (-) SiO_2-NPs (20 µg/ml) at 37°C for 15', 3h and 6h. After ultracentrifugation, NPs were subjected to non-reducing gradient SDS-PAGE (25 µl loaded) followed by silver staining and bands corresponding to the main proteins, indicated by arrows, were quantified by densitometry (the semi quantitative estimation of the amount of the indicated proteins is represented in lower histogram). * indicate the major background proteins (Fibr, IgG and HSA) present in the samples treated without NPs (mock samples). Data are the mean ± SE (N=5).

Figure S4. Effect of temperature, HP amount and NPs recovering method on SiO_2-NPs protein corona.



A) SiO_2-NPs were incubated for 15' at 37°C or 4°C in 10% HP in RPMI 1640 or in 100% HP (final volume 2 ml), as indicated, ultracentrifuged with a fixed angle rotor, washed twice with PBS pH 7.4 and subjected to non-reducing SDS-PAGE (4-20%) loading the same sample volume (25 µl of NPs). After silver staining, the major corona proteins (indicated by arrows) were identified by LC MS/MS. B) Histogram shows the semi quantitative estimation of the amount of HRG (the main protein of SiO_2-NPs corona) in different experimental conditions. C) SiO_2-NPs were incubated for 15' at 37°C or 4°C in 10% HP in RPMI 1640 or in 100% HP (2 ml), layered on a sucrose cushion (0.7 M in PBS), ultracentrifuged with a swing-out rotor and washed twice with PBS. After NPs loading on a non-reducing SDS-PAGE (decreasing volumes were loaded to reduce background signal), gel was silver stained and the main corona proteins were identified (indicated by arrows). Mock samples (HP with no NPs) were always run in parallel to evaluate non-specific recovery of protein plasma precipitates, indicated with * in the lanes. These are Fibr, IgGs, and HAS plus another non-identified polypeptide with a mass < 45 KDa.

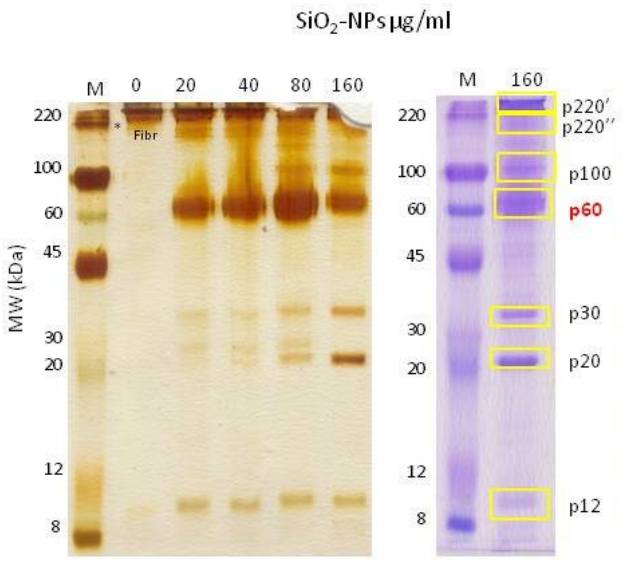
Figure S5. SiO2-NPs corona characterization in 100% HP and 10% MP



A

Band (20 µg/ml)	Polypeptide (molar % within the band)
p220	**Fibr (45) [β (28), α (17)]**, α- 2MG (34), ApoB-100 (21)
p150	**Ig (72) [Igκ-C (28), Igλ2,3-C (24), Igγ1,2,3,4-C (22), IgκVIII (4), Igα1-C (2)]**, NG9 (SIE,WOL,BRO (5), IgLL5 (3), Hp (2), Cf-XI (2), Fibr-β (2), Kin-1 (1)
p120	**Ig (48) [Igκ-C (31), Igα1-C (7), Igγ2-C (6), Igγ3-C (4)], Cf-XI (25)**, ANPEP (5), HRG (5), C4-A (4), HSA (3), Kin-1 (2), KLK (2), C3 (1), ITGα-2 (1)
p100	**Kin-1 (50), Cf-XI (50)**
p60	**HRG (42)**, HSA (19), Tf (10), LBP (7),Kin-1 (6), ITIH4 (6), VTN (5), LGALS3BP (5)
p50	**HSA (79)**, HRG (21)
p20	**ApoA-I (100)**

Band (0 µg/ml)	Polypeptide (molar % within the band)
p220	**Fibr (55)[β (35), α (20)]**, α- 2MG (20), ApoB-100 (13), FN (11)
p150	**Ig (100)**
p120	**ITGα6 (100)**
p100	**XXYLT1 (67)**
p60	**HSA (65)**
p50	**HSA (87)**
p20	**ApoA-I (95)**

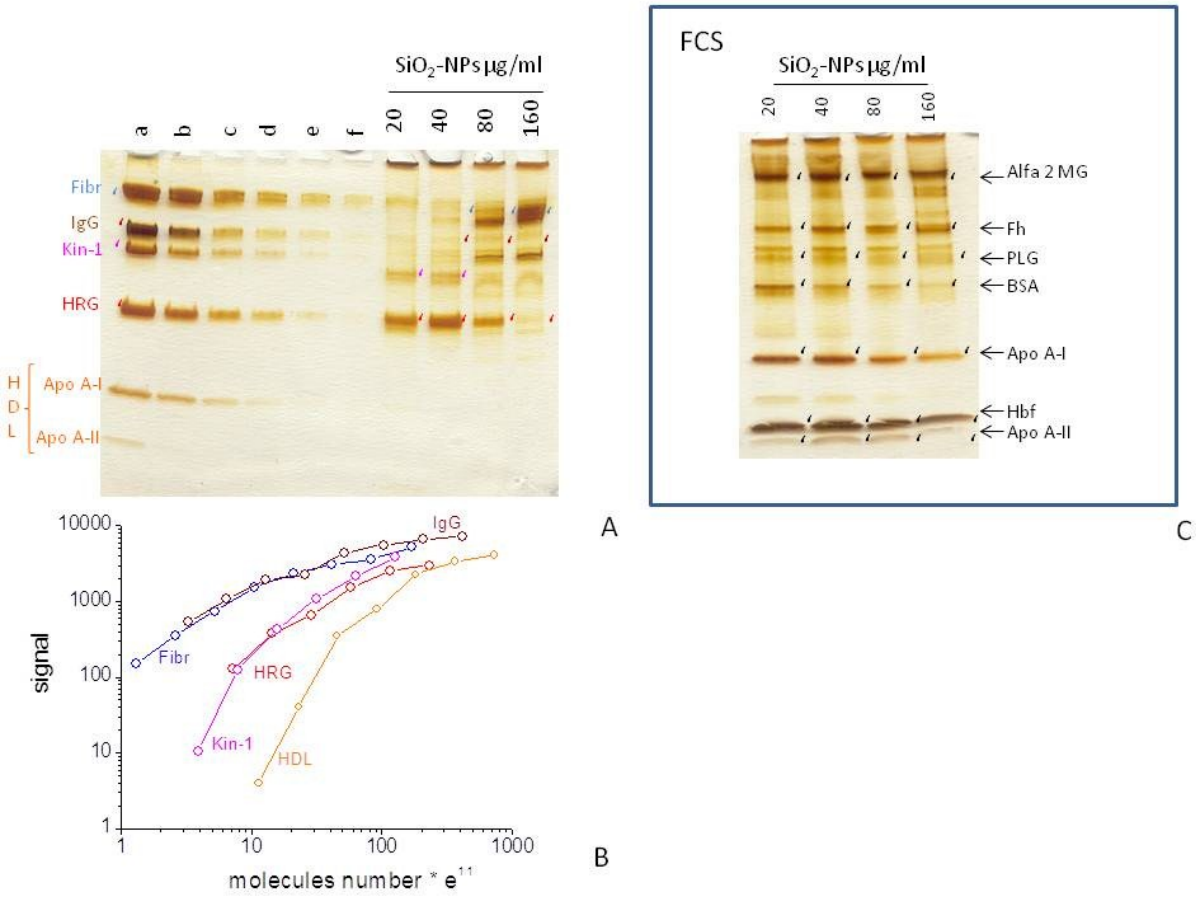


band	Polypeptide (molar % within the band)
p220′	**Fibr (82) [β (55), γ (27)]**, α- 2MG (12), IgM-C (5), FN (1)
p220″	**Fibr (27) [β (21), γ (6)], Igγ2A-C (11), Igγ2A-C memb bound (11)**, HRG (10), SERPING 1 (9), KLK (8), FN (6), C3 (6), α- 2MG (5), Cf-XI (3), Mug-1 (2), ApoB-100 (1)
p100	**Hbf-β1 (38), HRG (30)**, Kin-1 (17), Band3 (13), ITIH4 (2)
p60	**HRG (100)**
p30	**ApoE (100)**
p20	**ApoA-I (100)**
p12	**Hbf (89) [β1 (50), β2 (28), α (11)]**, ApoC-III (5), SAA-4 (5), ApoA-I (1)

B

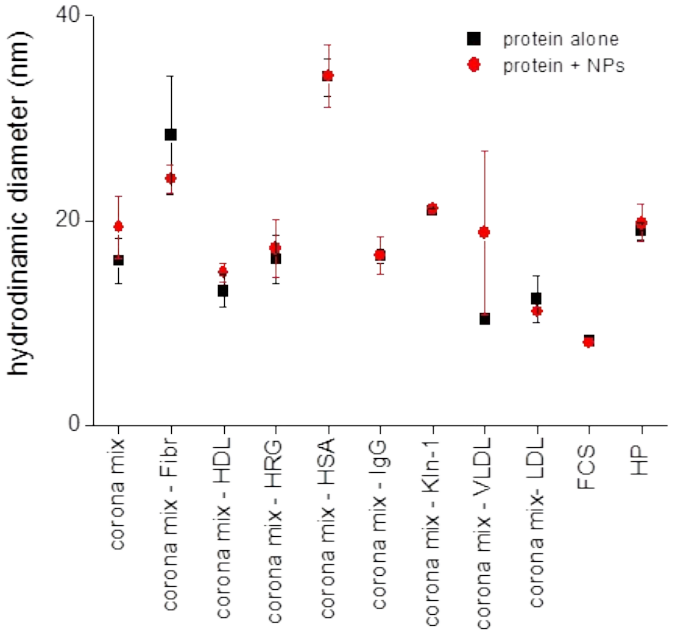
SDS-PAGE (silver or Coomassie G-250 staining) of the protein corona isolated from SiO_2-NPs after incubation for 15' at 37°C with RPMI plus 100% HP (A) and 10% MP (B). Yellow boxed indicated bands were excised from Coomassie stained gel and analyzed with LC MS/MS to identify their protein composition (see Materials and Methods for details). Positive hits of each spot (proteins with at least 2 significant matches, $p<0.05$) are listed in the tables and molar % within the band, based on emPAI, are reported in brackets. Main proteins are indicated in bold; asterisks indicate proteins present in the background. M: standard molecular weight; □- 2MG: alpha-2 macroglobulin; ANPEP: aminopeptidase N; Apo A-I/B-100/C-III/E: apolipoprotein A-I/B-100/C-III/E; Band-3: band 3 anion transport protein; Cf-XI, XII: coagulation factor XI, XII; C3,C4-A: complement factor C3,Ca-A; Fh: complement factor H; Fibr: fibrinogen; FN: fibronectin GSN: gelsolin; Hbf □/□: fetal haemoglobin a/□□□Hp: haptoglobin□□HRG: histidine rich glycoprotein; HSA: human serum albumin□ Ig: immunoglobulins; ITG-□2,6: integrin alpha 2,6; ITIH4: Inter-alpha-trypsin inhibitor heavy chain H4; Kin-1: kininogen-1; KLK: kallikrein; LGALS3BP: galectin 3 binding protein; LBP: lipopolysaccharide-binding protein; Mug-1: murinoglobulin-1; SAA-4: serum amyloid A-4 protein; SERPING1: plasma serine protease C1 inhibitor; Tf: serotransferrin; VTN: vitronectin; XXYLT1: xyloside-xylosìyl trasferase-1.

Figure S6. Calibration curves for the absolute quantification of the protein composition of the HP-derived hard corona of SiO_2-NPs as a function of the nanoparticles concentration.

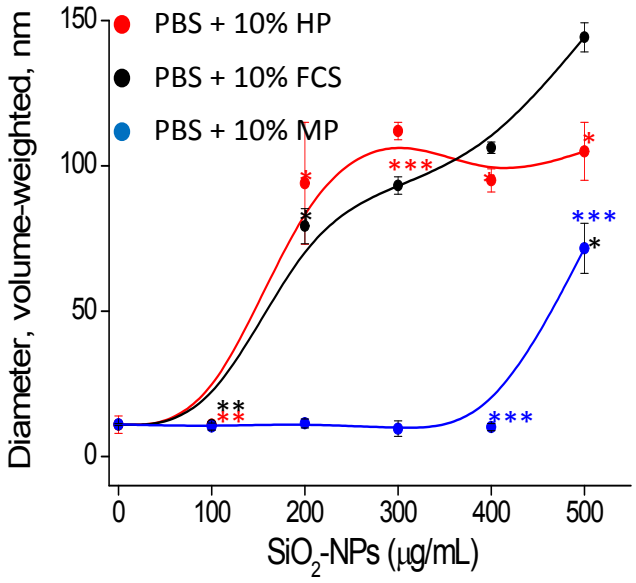


A) The picture reports a typical SDS-PAGE used for this analysis. The intensity of the bands corresponding to the proteins bound to SiO_2-NPs after incubation at different nanoparticle concentrations as indicated (same quantity of NPs/lane, 13 µg) were compared with those of known quantities of purified human standard proteins (lanes a-f) (loading scheme = a: 10 µg of Fibr, 2.5 µg of IgG, Kin-1, HRG and HDL; b: 5 µg of Fibr, 1.25 µg of IgG, Kin-1, HRG and HDL; c: 2.5 µg of Fibr, 0.625 µg of IgG, Kin-1, HRG and HDL; d: 1.25 µg of Fibr, 0.3125 µg of IgG, Kin-1, HRG and HDL, e: 0.625 µg of Fibr, 0.15625 of IgG, Kin-1, HRG and HDL; f: 0.3125 µg of Fibr, 0.0078 µg of IgG, Kin-1, HRG and HDL. B) Representative calibration curves run after densitometry of gels as above is shown. The number of molecules per particle was calculated based on the known MW of each protein, assuming that each HDL contains three Apo-AI units. The molecular weight of a single SiO_2-NPs of 26 nm diameter was calculated based on its density 2 g/ml and found to be ~ 10^7 Da. C) The same experiment was performed in FCS to show the much less marked evolution of the FCS-derived corona as a function of NPs concentration, for comparison.

Figure S7. DLS analysis of SiO_2-NPs in the presence of FCS, HP, MP and corona mix.



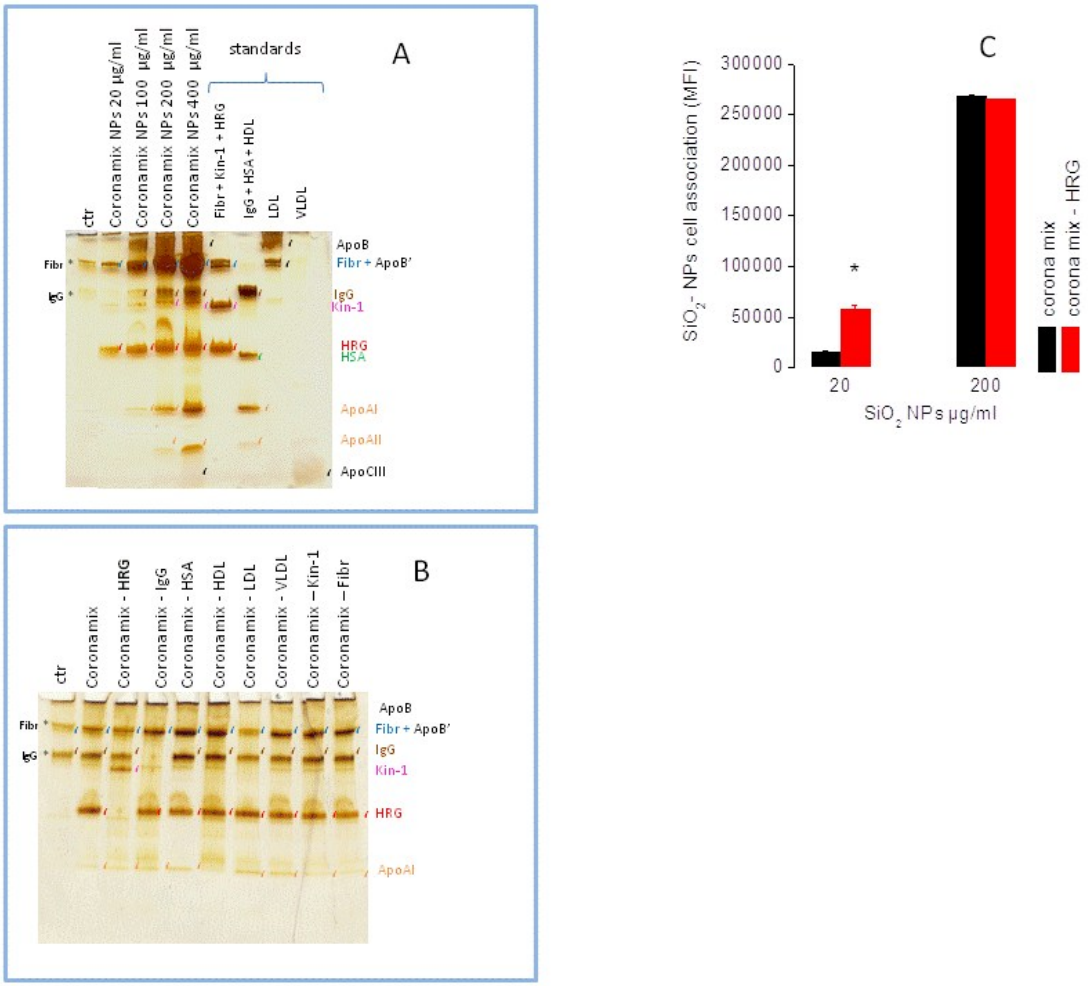
A



B

A) DLS distribution (PBS buffer pH 7.4, 37°C) of 10% FCS, 10% HP, the protein mixture together (corona mix) or selectively deprived of each of them (- proteins) alone (black points) and after incubation (10 minutes, 37°C) with 20 µg/ml SiO_2-NPs (red points). Data are means ± SE (N=3). B) SiO_2-NPs (0-500 µg/ml) were incubated for 10 minutes at 37°C with 10% HP, FCS or MP diluted in PBS pH 7.4, and volume weighted distribution analysis were performed. * a second, minor, population with 8-11 nm size. ** a second, minor, population with 70-75 nm size. *** a second, minor population with 4500-4900 nm size. Data are means ± SE (N=3).

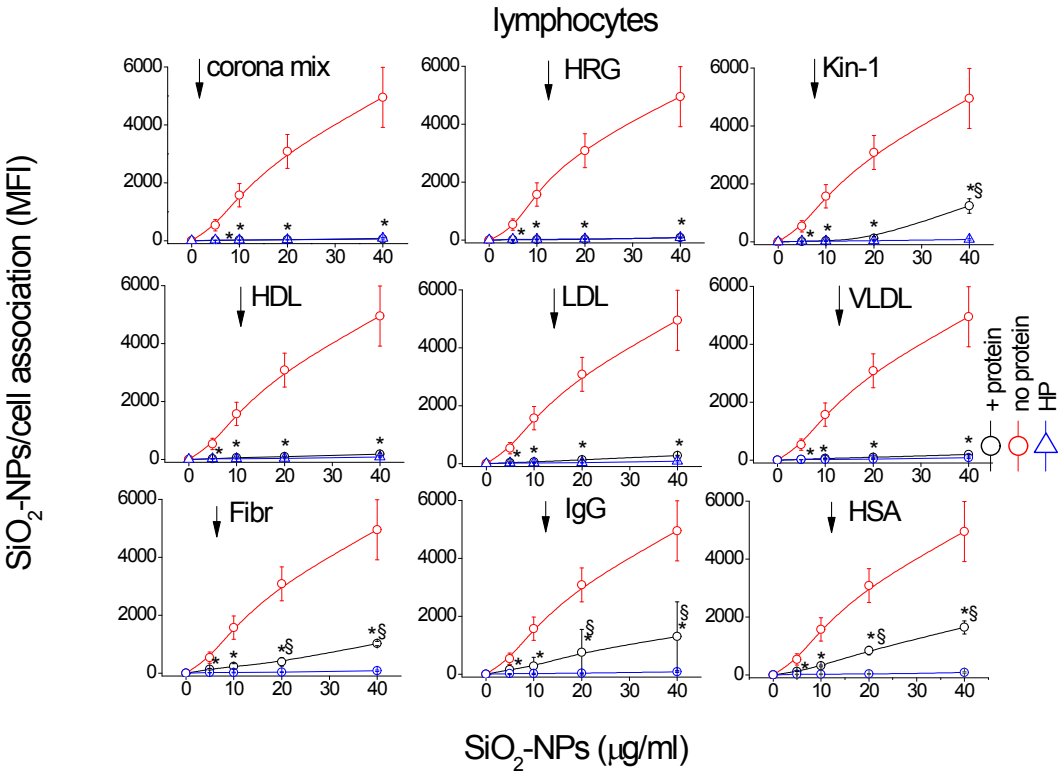
Figure S8. Analysis of the protein corona composition of SiO_2-NPs formed in the presence of plasma proteins combinations.



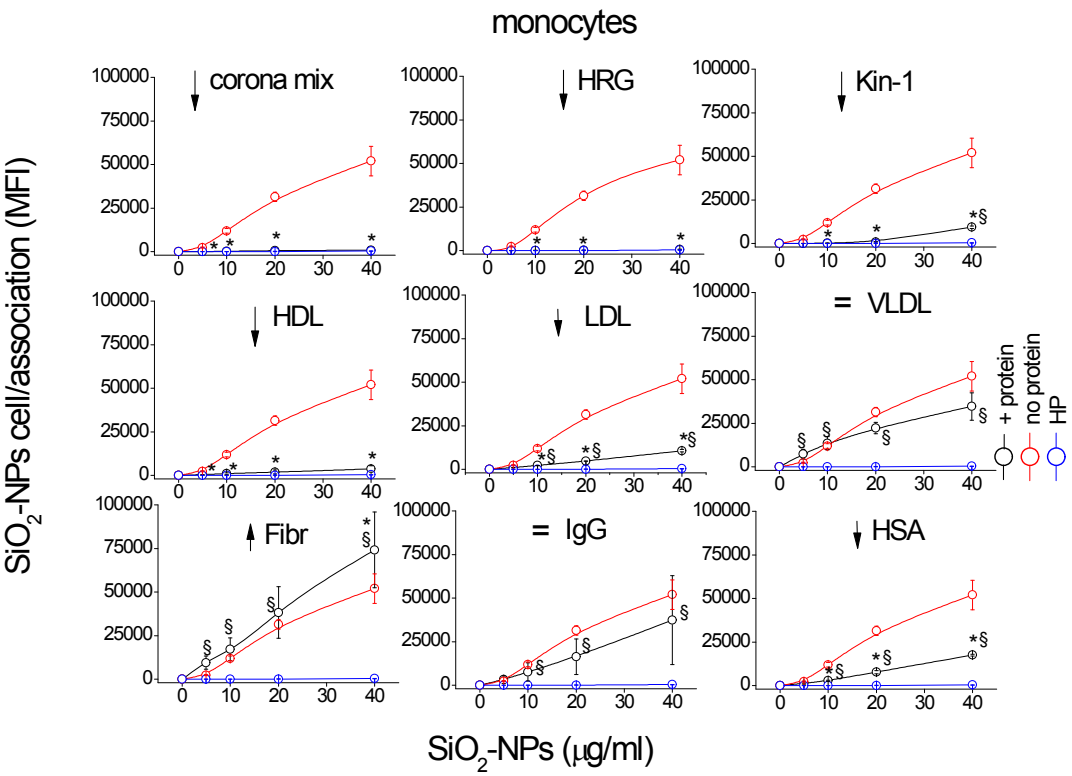
A) SiO_2-NPs (0-400 µg/ml) were incubated in RPMI at 37°C for 15 minutes in the presence of the complete mixture formed by 10% human plasma-like concentrations of HRG, Kin-1, HDL, LDL, VLDL, HSA, IgG and Fibr. After ultracentrifugation, an equal volume (25 µl) of loading buffer-dissolved NPs was loaded on a non-reducing gradient SDS-PAGE and silver stained. Single protein bands were identified by comparison with parallely run purified standard proteins. All proteins could be distinguished based on their electrophoretic mobility, while a fragment of ApoB (ApoB') co-migrated with Fibr. B) SiO_2-NPs (20 µg/ml) were incubated in RPMI at 37°C in the presence of different proteins, as indicated, and analyzed by SDS-PAGE and silver staining, as above. Gel is representative of four experiments. C) Macrophages were treated with SiO_2-NPs (20, 200 µg/ml) at 37°C for 3h in RPMI 1640 plus the complete corona mix as in panel A and the corona mix devoid of HRG, and analyzed by FACS to measure NPs cell capture (MFI). Data are the mean ± SE (N=2). * significance $p < 0.05$ with respect to corona mix sample.

Figure S9. Effect of the main proteins of the HP-derived corona on the association of SiO_2-NPs to lymphocytes, monocytes and macrophages: nanoparticle dose-response.

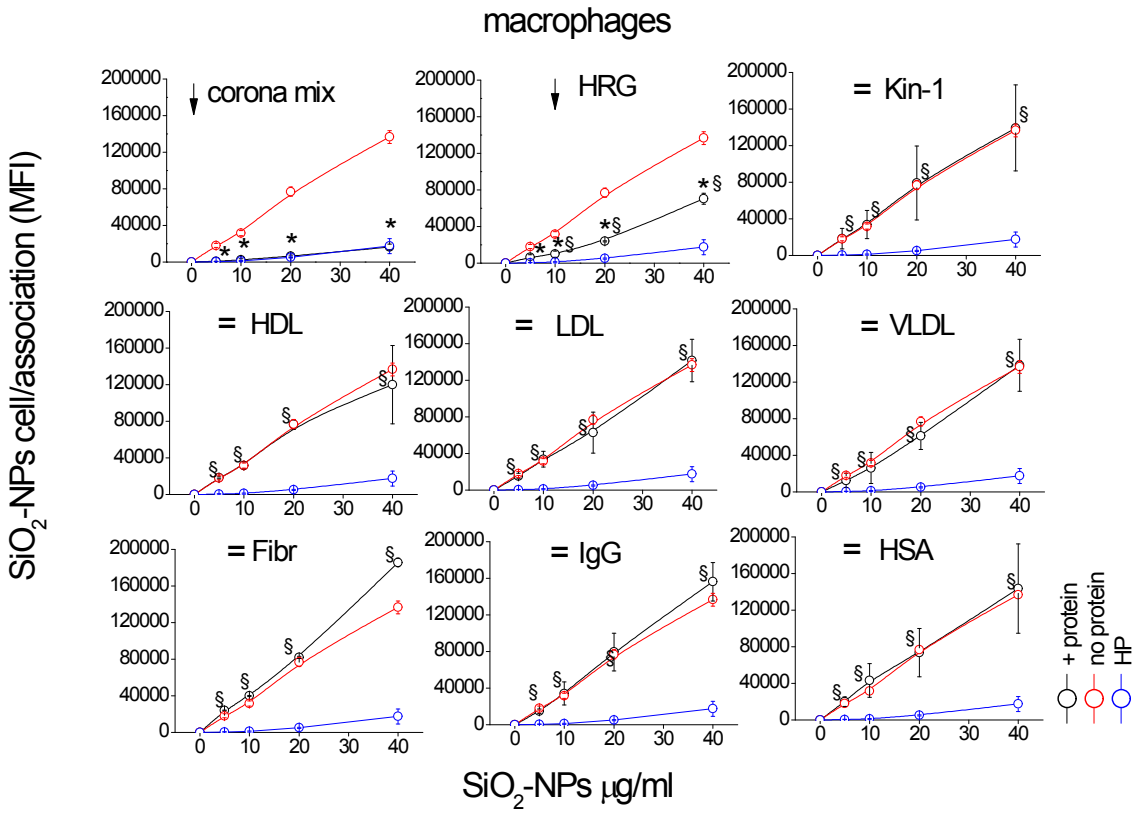
A



B

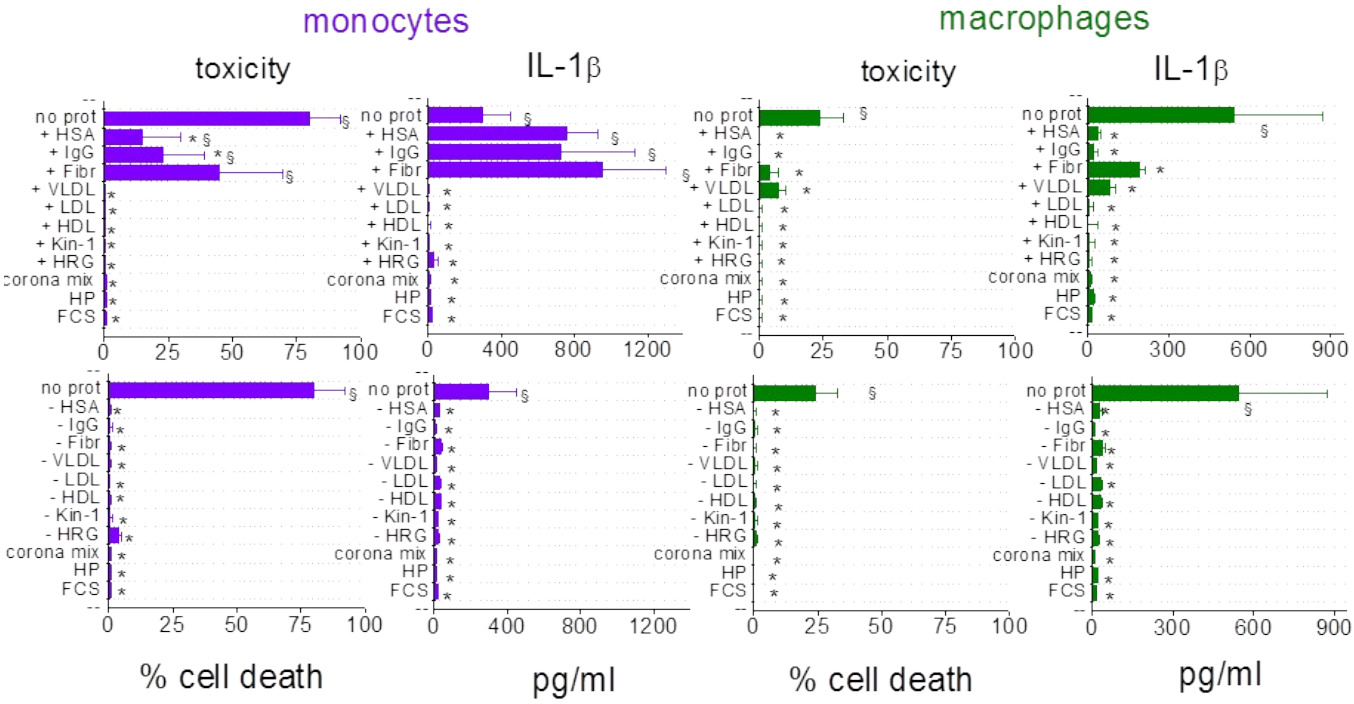


C



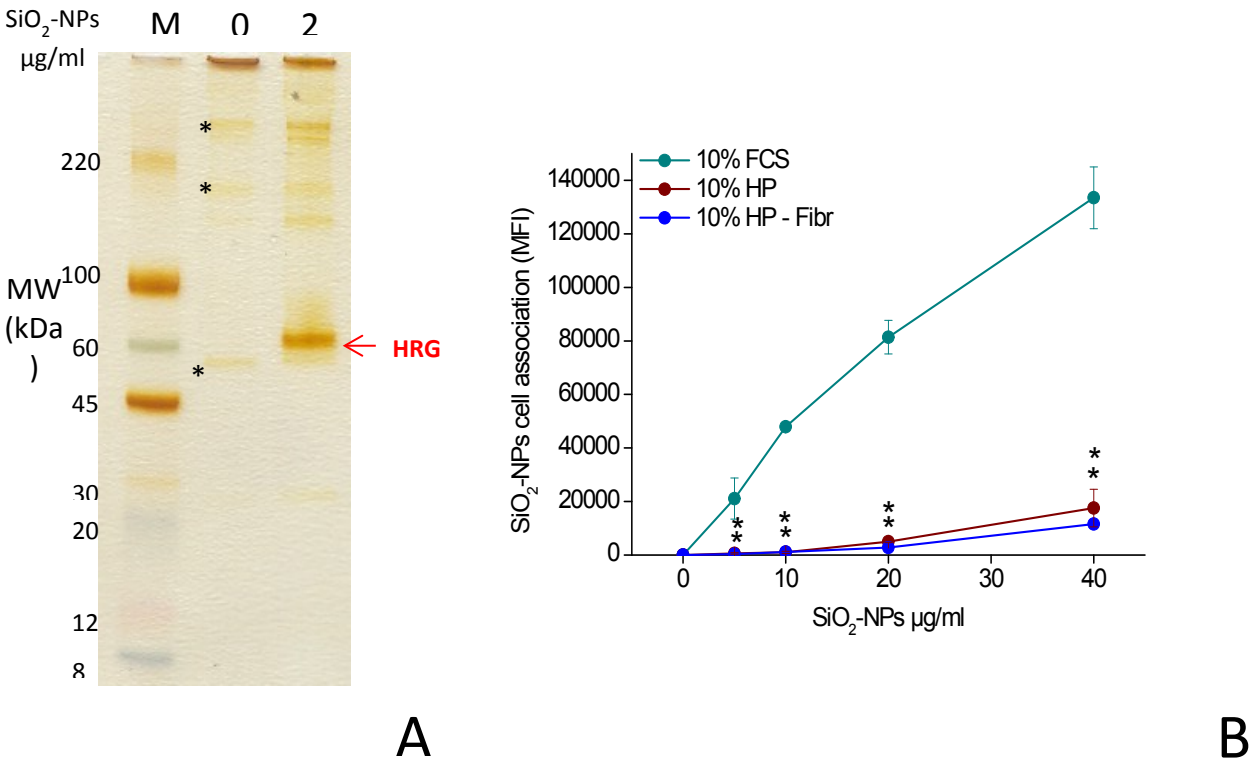
Lymphocytes (A), monocytes (B) and macrophages (C), were incubated with SiO_2-NPs (0-40 µg/ml) for 3 h in RPMI 1640 at 37°C in the presence of each purified indicated protein at the concentration typically found in 10% HP (HRG=15 µg/ml; Kin-1 = 8 µg/ml; HDL= 150 µg/ml ; LDL= 78 µg/ml ; VLDL= 12 µg/ml; Fibr= 300 µg/ml; IgG= 700 µg/ml; HSA= 5 mg/ml) or in the presence of a mixture of all of them at the same concentrations (corona-mix). MFI intensity was measured after cell washings by FACS. Data are the mean of duplicate experiments ± SE (N=3). Black symbols refer to the effect of plasma proteins, red and blue curves refer to the cell-NPs association in protein free-medium or in 10% HP for comparison. * significance ($p<0.05$) with respect to no protein; § significance ($p<0.05$) with respect to HP. Downward ↓ or upward ↑ arrows indicate an inhibitory or a stimulatory effect, respectively, of a given protein on NPs cell binding compared to that of bare NPs, while = indicates no effect.

Figure S10. Effect of the main proteins of the HP-derived corona and of their selective depletion from corona mix on SiO_2-NPs cytotoxicity and pro-inflammatory effects in monocytes and macrophages.



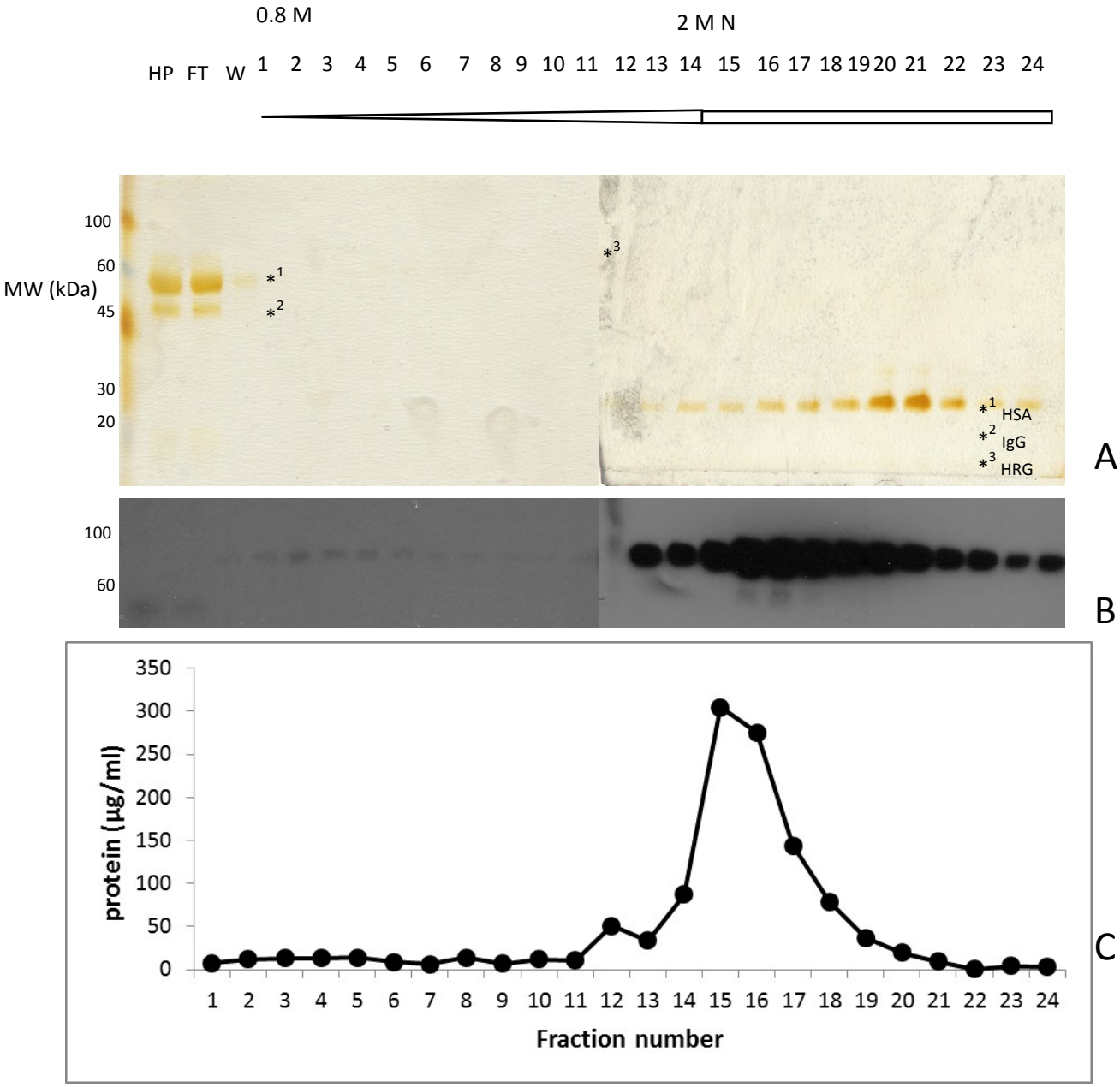
Monocytes and macrophages were incubated at 37°C with 20 µg/ml SiO_2-NPs in RPMI 1640 without proteins (no prot), plus 10% HP (HP), plus 10% FCS (FCS) and with the indicated purified human plasma proteins alone (top panels), all together (corona mix) or all-together with the selective lack of each of them (– protein) (bottom panels) as indicated. After 20 h cell mortality and IL-1 β production were measured with MTS and ELISA assays, respectively. Data are the mean ± SE of three experiments run in duplicate. * significance ($p<0.05$) with respect to no protein; § significance ($p<0.05$) with respect to HP.

Figure S11. Effect of fibrinogen depletion from plasma on HP-derived SiO_2-NPs corona and SiO_2-NPs macrophages association.



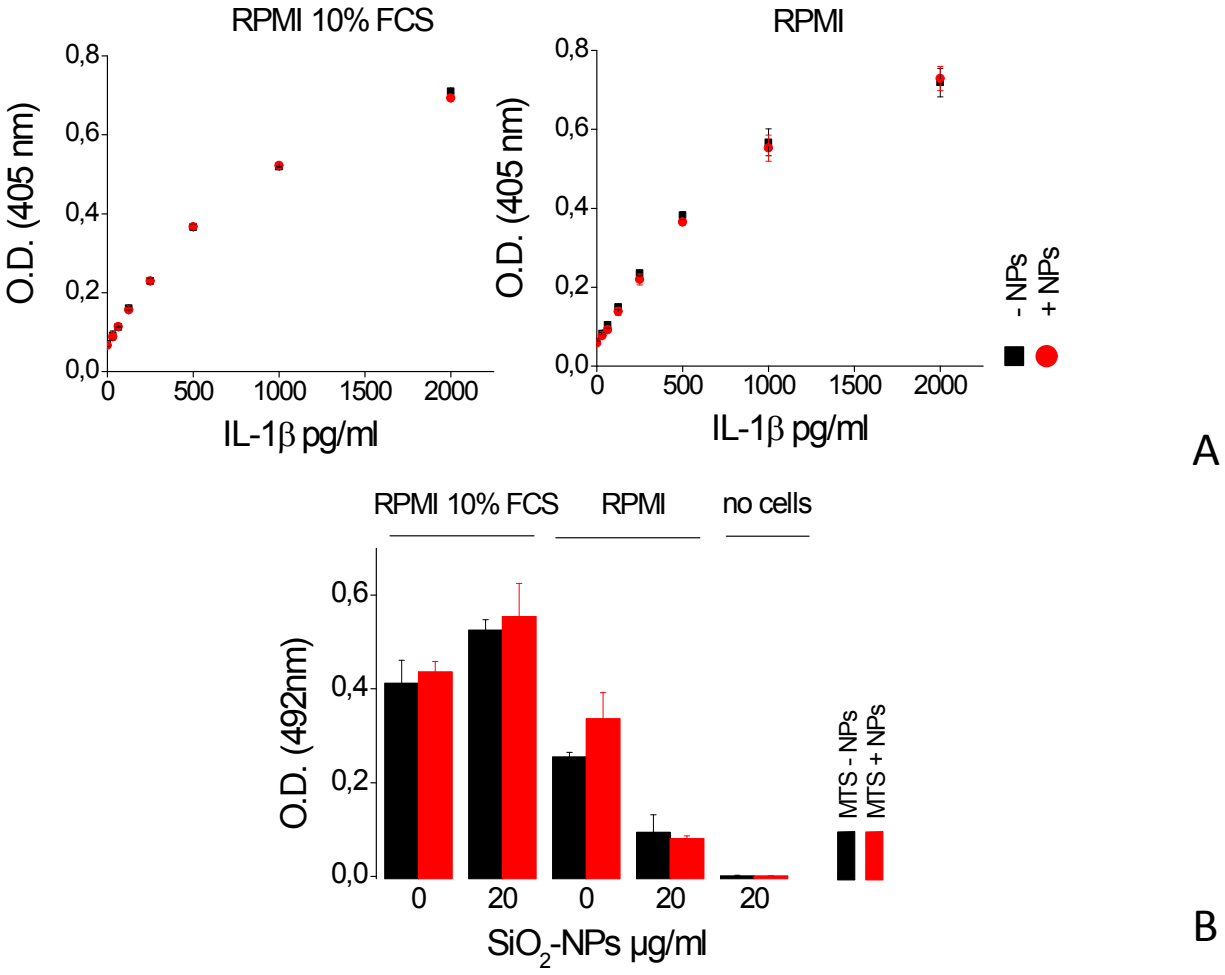
A) SiO_2-NPs (0-20 µg/ml) were incubated in RPMI + 10% HP-Fibrinogen (HS) for 15' at 37°C, ultracentrifuged, washed twice with PBS pH 7.4 and subjected to non-reducing SDS-PAGE (4-20%). The gel, representative of two, was silver stained. M: standard molecular weight marker. HRG is indicated by an arrow, while nonspecifically recovered proteins from HS were indicated by an asterisk. B) Macrophages were incubated with SiO_2-NPs at the indicated concentrations for 3h at 37°C in RPMI supplemented with 10% FCS, 10% HP or 10% HP-fibrinogen, washed and analyzed by flow-cytofluorimetry to quantify NPs-cell association (MFI). Data are the mean ± SE of two experiments run in duplicate. * significance ($p<0.05$) with respect to 10% FCS.

Figure S12. Elution chromatogram of HRG purification from human plasma.



A) Silver staining of eluted fractions from phospho-cellulose chromatographic column (see methods for details) for HRG purification from human plasma. 20 µl of diluted (1:200) human plasma (HP) and Flow Through (FT) (50 ml of initial HP), 20 µl of wash (W, 400 ml total volume), 20 µl of NaCl 0.8-2 M gradient fractions (1-14, 1.6 ml/fraction) and 20 µl of elution fractions (2M NaCl, 15-24, 3.2 ml/fraction) were loaded onto a 12% v/v reducing SDS-PAGE and subjected to silver staining. Bands corresponding to highly abundant plasma proteins HSA (*1) and IgG (*2) were visible in HP and FT lanes, as indicated, *3 are the peak of NaCl eluted ~ 60 KDa protein expected to be HRG B) Western Blot validation of HRG, using specific anti-HRG antibodies, on the same samples used for silver staining. C) Gradient and elution fractions protein profile using a Bradford assay.

Figure S13. Controls of the interference of SiO_2-NPs with the ELISA and MTS assays in both complete and serum free medium.



A) ELISA assay was performed, after overnight seeding of anti IL-1β capture antibody, by incubating standard IL-1β (0-2000 pg/ml) diluted in RPMI 10% FCS or in RPMI alone with or without SiO_2-NPs 20 µg/ml (2 hours). After extensive washing, IL-1β biotinylated detection antibody was added for 1 hour and washed. HRP-conjugated streptavidin was then added for 30 minutes. TMB substrate solution was added until blue color development, and sample absorbance was read at 405 nm with a spectrophotometer. B) Monocytes were incubated for 20 h with or without SiO_2-NPs 20 µg/ml in complete (RPMI 10% FCS) or serum free medium (RPMI). MTS assay was developed in the absence or in the presence of SiO_2-NPs 20 µg/ml in the substrate solution (10 µl MTS + 100 µl RPMI / well). "No cell" sample was added to check if SiO_2-NPs 20 µg/ml induced signal *per se* (either by scattering or by non-specific color development).

14. Calculations of the HRG and Kin-1 binding capabilities of SiO_2-NPs in the conditions of our experiments in 10% HP

The surface area of a 26 nm diameter nanoparticle is 2120 nm^2. The protein corona compositions obtained by quantitative gel electrophoresis are respectively 20 HRG, 6 Kin-1, and 3 Fibr at 40 µg/ml SiO_2-NP concentration, 8 HRG, 3 Kin-1 and 6 Fibr at 80 µg/ml NPs, and 1 HRG, 1 Kin-1 and 10 Fibr at 160 µg/ml NPs.

Footprints were numerically estimated by iteratively assigning incremental footprints values to the proteins and calculating the total surface coated. Since HRG and Kin-1 have a similar molecular weight we decided to assign the same footprint to both. Final numbers obtained for HRG/Kin-1 and Fibr footprints respectively of 60 and 200 nm^2 are reported in the table below:

SiO_2-NPs (µg/ml)	Protein	N	Surface coated (nm^2)	Total (nm^2)
40	HRG + Kin-1	26	1560	2160
	Fibr	3	600	
80	HRG + Kin-1	16	960	2160
	Fibr	6	1200	
160	HRG + Kin-1	2	120	2120
	Fibr	10	2000	

The molecular weight of a 26 nm diameter nanoparticle with a density of 2.0 g/ml is 1.1×10^7 Da. The molar surface area is 1.3×10^9 m^2/mol (which corresponds to 115 m^2/g). The molar concentration of HRG and Kin-1 in 10% HP are respectively 0.25 µM and 0.11 µM. The molecular footprint of 60 nm^2 can be determined by the protein quantification experiments. Based on this assumptions, it is possible to calculate that the molar coating ability of the two proteins is 3.6×10^7 m^2/mol. Consequently, at the concentration they are present in 10% HP, HRG and Kin-1 can coat a surface area corresponding to about 106 mg/mL nanoparticles in the hypothesis of full binding (HRG alone can coat a surface area corresponding to about 74 µg/mL nanoparticles).

Since the nanoparticle affinity of HRG and Kin-1 is in the nanomolar range, nanoparticle binding in the concentration range used in the experiments is expected to be strong. Indeed, the amount of NPs-bound HRG with respect to the total nanoparticles concentration, and the depletion of free HRG, can be independently calculated using the data obtained in the nanoparticles affinity experiments, taking into account the real affinity of the HRG-NPs interaction and the extrapolated maximal HRG binding (30 molecules/particle), in 10% HP and the following equation, , which is deduced by the on one-site binding equilibrium:

$$\mathbf{HRG^{NPbound} = \{(30xNP_0 + HRG_0 + Kd) - [(30xNP_0 + HRG_0 + Kd)^2 - 4x30xNP_0xHRG_0)]^{1/2}\}/2}$$

Where:

HRG_0 is the nM total concentration of 10% HP (~ 15 µg/ml; ~ 250 nM , being the MW 64 KDa)

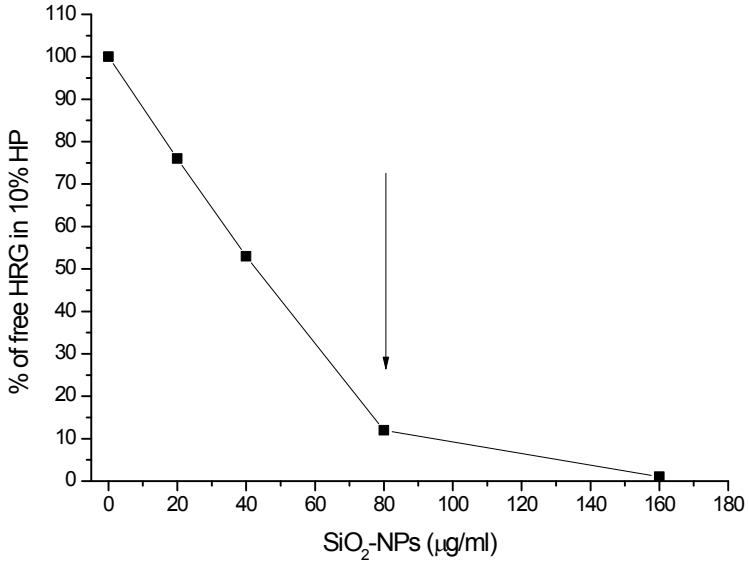
NP_0 is the nM total concentration of SiO_2-NPs (mw~ 10^7 Da; ex 20 µg/ml is 2 nM)

Kd is the dissociation constant of the NPs-HRG complex (2.4 nM)

The simulation of the HRG recruitment on NPs depending on the nanoparticle concentration (shown below) is in good agreement with the above described calculation of the same phenomenon. In fact, a SiO_2-NPs concentration of 80 µg/ml (highlighted in red in the table and by the arrow in the corresponding graph) is predicted to capture about 90% of the HRG present in a 10% HP solution.

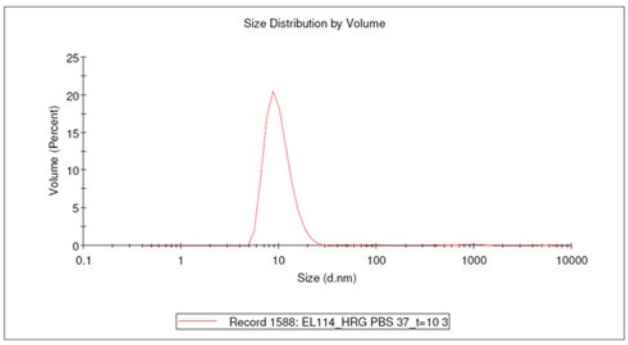
SiO_2-NPs (µg/ml)	NPs HRG-binding sites (nM) *	NPs bound HRG (nM) [a]	Free HRG 10% HP (nM)- [%]
0	0	0	250 [100%]
20	60	60	190 [76%]
40	120	118	132 [53%]
80	240	220	30 [12%]
160	480	247	3 [1%]

* assuming 30 as the maximal binding capacity of a single SiO_2-NP

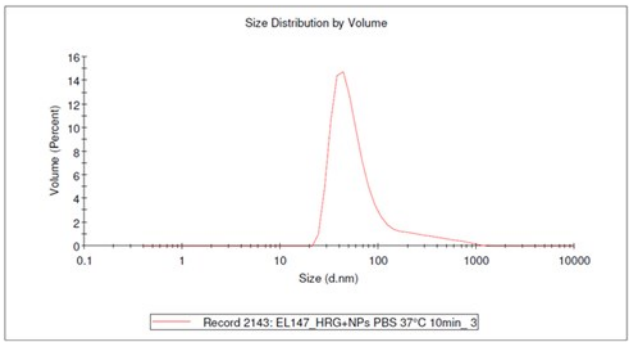


15. DLS profiles of proteins alone and after incubation with SiO_2-NPs 20 µg/ml in the absence or in the presence of HRG 15 µg/ml

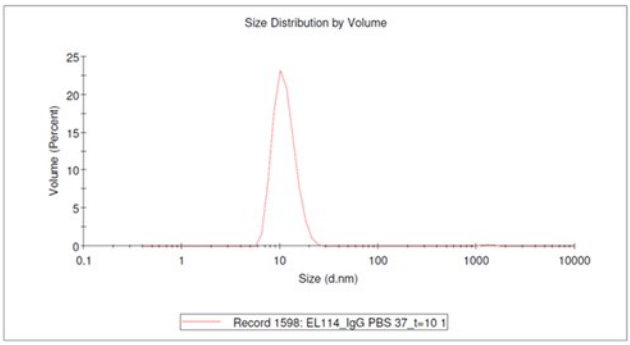
HRG 15µg/mL in PBS at 37°C
Size 9 nm



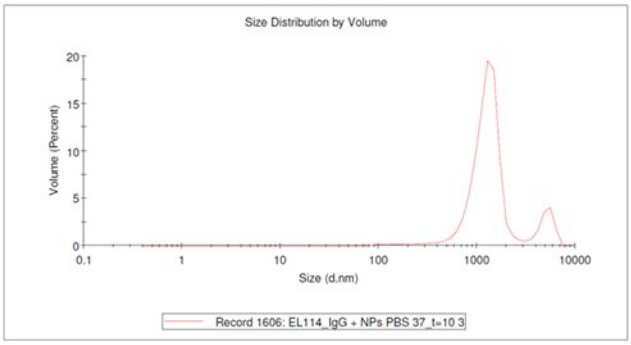
HRG 15µg/mL + SiO_2-NPs 20µg/mL in PBS at 37°C
Size 56 nm



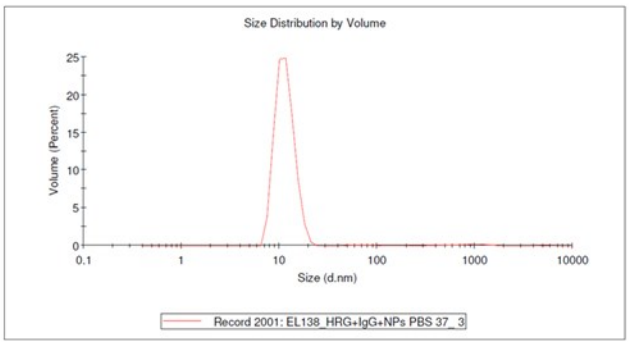
IgG 700µg/mL in PBS at 37°C
Size 11 nm



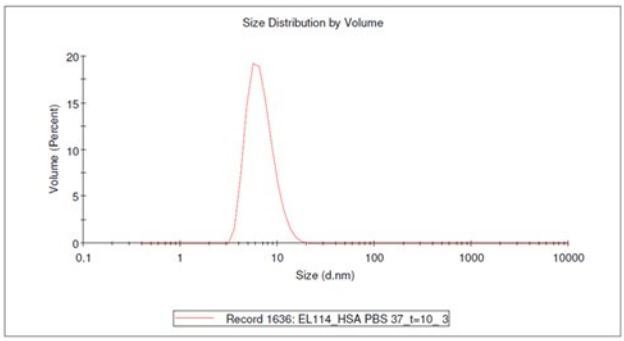
IgG 700µg/mL + SiO_2-NPs 20µg/mL in PBS at 37°C
Size 1288 nm



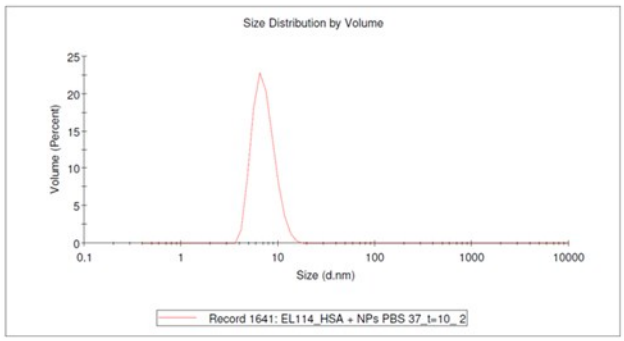
HRG 15µg/mL + IgG 700µg/mL + SiO_2-NPs 20µg/mL in PBS at 37°C
Size 11 nm



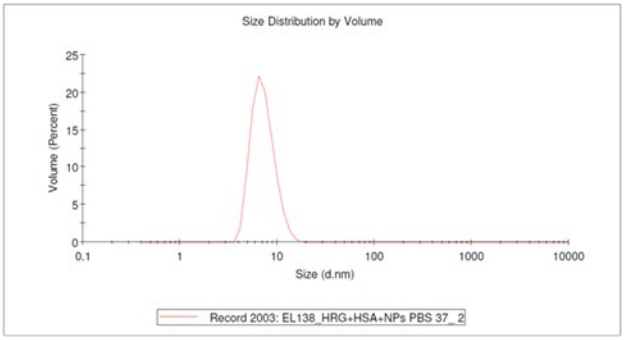
HSA 5000µg/mL in PBS at 37°C
Size 7 nm



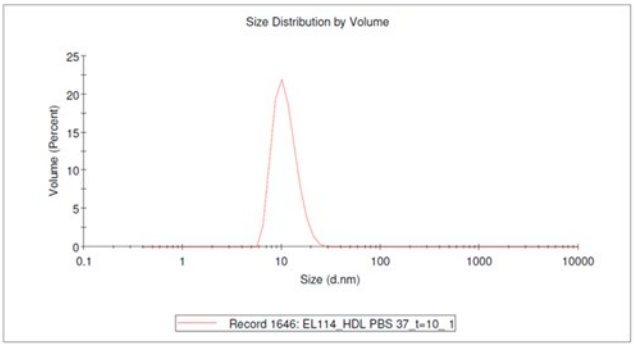
HSA 5000µg/mL + SiO_2-NPs 20µg/mL in PBS at 37°C
Size 7 nm



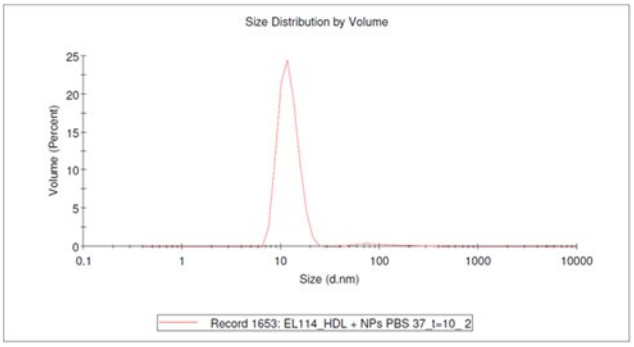
HRG 15µg/mL + HSA 5000µg/mL + SiO_2-NPs 20µg/mL in PBS at 37°C
Size 7 nm



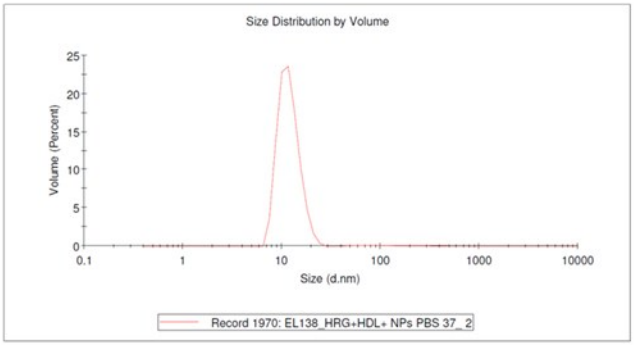
HDL 150µg/mL in PBS at 37°C
Size 11 nm



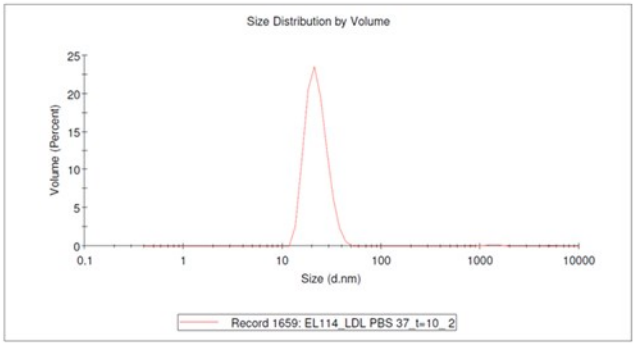
HDL150µg/mL + SiO_2-NPs 20µg/mL in PBS at 37°C
Size 12 nm



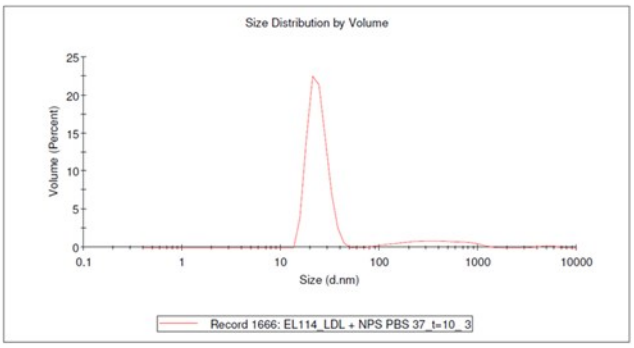
HRG 15µg/mL + HDL 150µg/mL + SiO_2-NPs 20µg/mL in PBS at 37°C
Size 11 nm



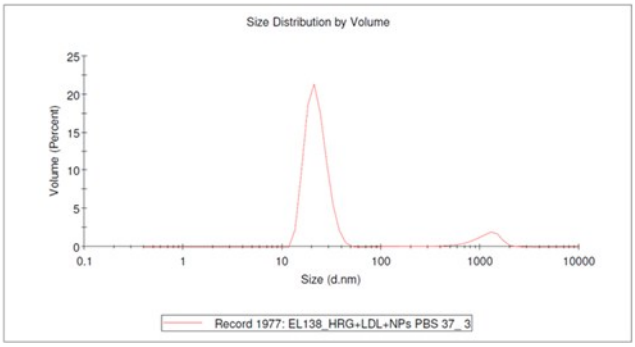
LDL 78µg/mL in PBS at 37°C
Size 22 nm



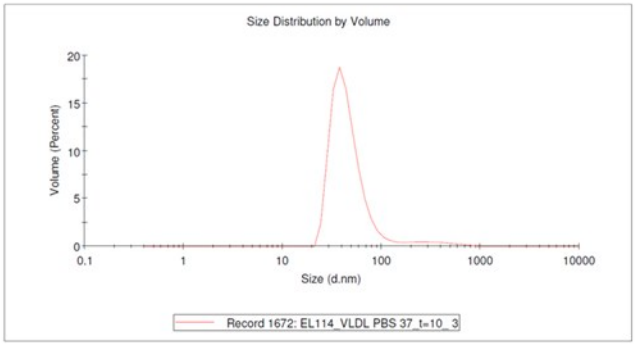
LDL 78µg/mL + SiO_2-NPs 20µg/mL in PBS at 37°C
Size 25 nm



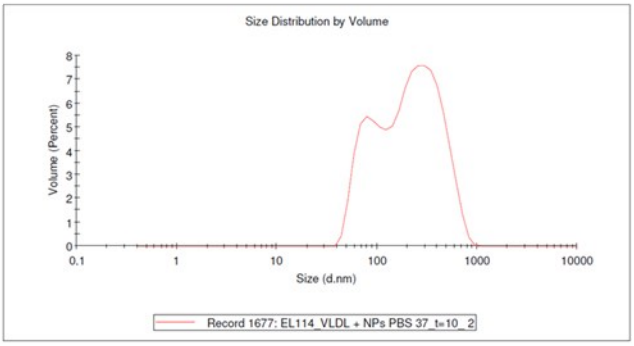
HRG 15µg/mL + LDL 78µg/mL + SiO_2-NPs 20µg/mL in PBS at 37°C
Size 23 nm



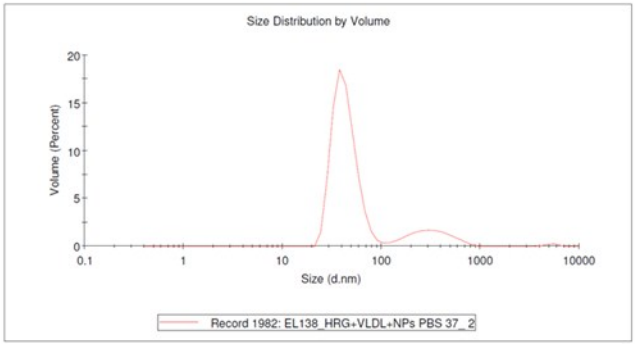
VLDL 12µg/mL in PBS at 37°C
Size 48 nm



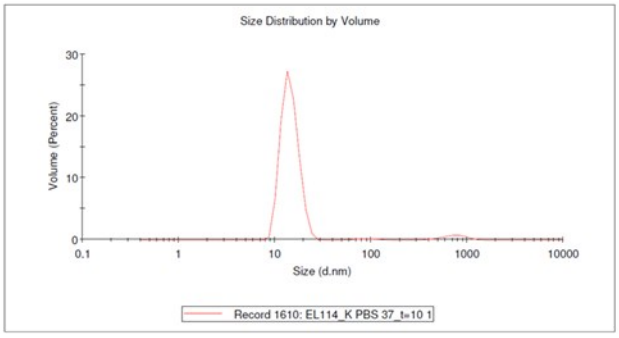
VLDL12µg/mL + SiO_2-NPs 20µg/mL in PBS at 37°C
Size 283 nm



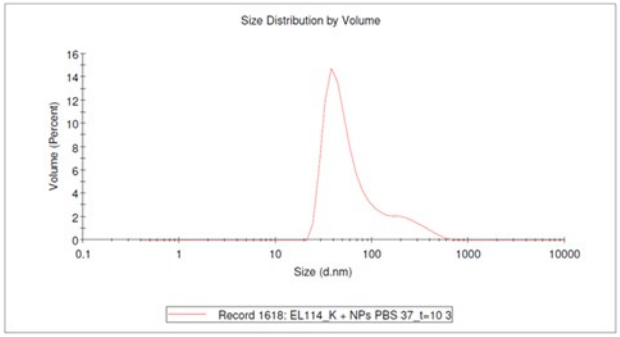
HRG 15µg/mL + VLDL 12µg/mL + SiO_2-NPs 20µg/mL in PBS at 37°C
Size 42 nm



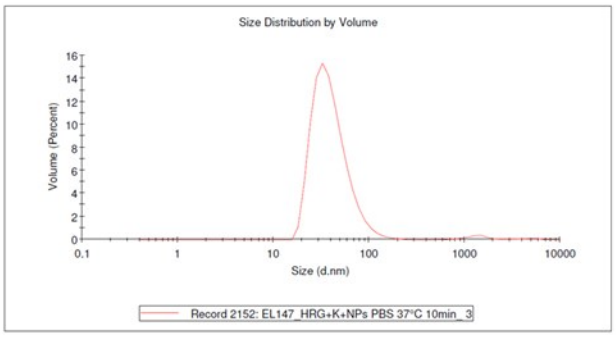
Kininogen 8µg/mL in PBS at 37°C
Size 16 nm



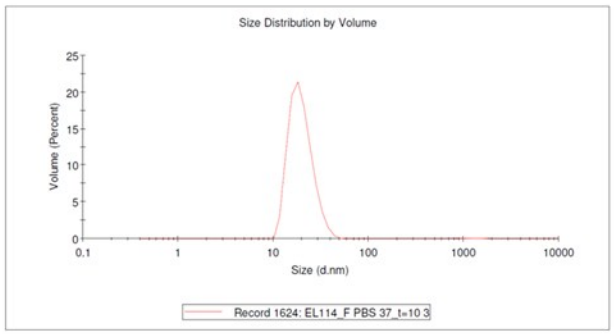
Kininogen 8µg/mL + SiO_2-NPs 20µg/mL in PBS at 37°C
Size 48 nm



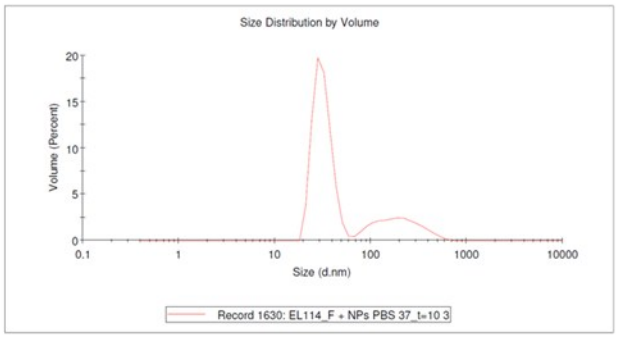
HRG 15µg/mL + Kininogen 8µg/mL + SiO_2-NPs 20µg/mL in PBS at 37°C
Size 41 nm



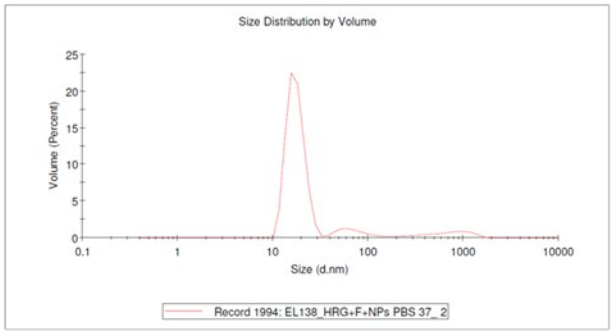
Fibrinogen 300µg/mL in PBS at 37°C
Size 11 nm



Fibrinogen 300µg/mL + SiO_2-NPs 20µg/mL in PBS at 37°C
Size 12 nm



HRG 15µg/mL + Fibrinogen 300µg/mL + SiO_2-NPs 20µg/mL in PBS at 37°C
Size 11 nm



16. Supplementary Tables legend. LC MS/MS analysis of the polypeptides found in SDS-PAGE bands from the corona of SiO_2-NPs formed in FCS 10% (S1), HP 10% (S2), HP 100% (S3) and MS 10% (S4). After bands excision from Coomassie G-250 stained gels (see Figures 3A,B and S5A,B), polypeptides were identified by LC MS/MS tandem analysis (see Materials and Methods for details). Polypeptides are ordered based on their score values from analysis of Q-TOF data using in-house MASCOT. All files were searched against the SwissProt database_2014_06 (545536 total sequences; 66370 sequences after taxonomy filter [Mammalia]). Prot_score: MASCOT protein score/ion score identity threshold ($p<0.05$). Protein matches/protein sequences: number of peptide matches/sequences matched, significant ($p< 0.05$) are reported in brackets. emPAI: Exponentially Modified Protein Abundance Index, automatically calculated by MASCOT. Polypeptide % molar abundance within the same band, are calculated based on emPAI parameter: % number in black consider any polypeptide identified in the band, while % number in red only those with more than 1 significant hit.

17. Supplementary Methods

RPMI-1640 composition (as from supplier datasheet)

Amino Acids	**mM**
Glycine	0.13333334
L-Arginine hydrochloride	1.1374408
L-Asparagine	0.37878788
L-Aspartic acid	0.15037593
L-Cystine	0.20833333
L-Glutamic Acid	0.13605443
L-Glutamine	2.0547945
L-Histidine	0.09677419
L-Hydroxyproline	0.15267175
L-Isoleucine	0.3816794
L-Leucine	0.3816794
L-Lysine hydrochloride	0.21857923
L-Methionine	0.10067114
L-Phenylalanine	0.09090909
L-Proline	0.17391305
L-Serine	0.2857143
L-Threonine	0.16806723
L-Tryptophan	0.024509804
L-Tyrosine	0.110497236
L-Valine	0.17094018

Vitamins	**mM**

Amino Acids	**mM**
Biotin	8.1967213E-4
Choline chloride	0.021428572
D-Calcium pantothenate	5.24109E-4
Folic Acid	0.0022675737
Niacinamide	0.008196721
Para-Aminobenzoic Acid	0.00729927
Pyridoxine hydrochloride	0.004854369
Riboflavin	5.319149E-4
Thiamine hydrochloride	0.002967359
Vitamin B12	3.690037E-6
i-Inositol	0.19444445

Inorganic Salts	**mM**
Calcium nitrate (Ca(NO3)2 4H2O)	0.42372882
Magnesium Sulfate (MgSO4-7H2O)	0.40650406
Potassium Chloride (KCl)	5.3333335
Sodium Bicarbonate (NaHCO3)	23.809525
Sodium Chloride (NaCl)	103.44827
Sodium Phosphate dibasic (Na2HPO4) anhydrous	5.633803

Other components	**mM**
D-Glucose (Dextrose)	11.111111
Glutathione (reduced)	0.0032573289
Phenol Red	0.013283741

CPDA-1 composition (as from supplier datasheet)

Adenine 2 mM, Citric acid 17 mM, Dextrose 175 mM, Sodium citrate 8.9 mM, Sodium phosphate monobasic 18 mM

Silver Staining

Gels were fixed for 30' in 50% methanol 10% acetic acid, incubated for 15' in 5% methanol 1% acetic acid, washed 3 times with water and exposed for 90'' to thiosulfate solution (200 µg/ml $Na_2S_2O_3$ pentahydrate). After extensive washing with water, gels were

incubated in the dark for 30' with 2 g/l $AgNO_3$, rinsed and developed for 5'-15' with a solution containing 60 mg/ml Na_2CO_3, 4 μg/ml $Na_2S_2O_3$ pentahydrate and 0.01875% formaldehyde. Reaction was stopped with 6% acetic acid.

Colloidal Coomassie G-250 Staining

Gels were fixed for 18 h in 50% methanol 2% H_3PO_4, rinsed 3 times for 30' with water, and incubated for 1 h in a solution containing 34% methanol, 2% H_3PO_4 and 17% $(NH_4)_2SO_4$. Staining was performed for 3 days in 34% methanol, 2% H_3PO_4, 17% $(NH_4)_2SO_4$ and 0.066% Coomassie G-250, and it was followed by de-staining in water for additional 3 days.

Western Blot.

Proteins were transferred on a PVDF membrane (Biorad) (80 V, 200 mA, 1.5 h for HRG and Apo A-I; 80 V 30 mA 18 h for Fibr). After membranes blocking for 1-2 h at RT with 5% nonfat dry milk in TBS 0.1% Tween, primary antibodies (diluted in 3% nonfat dry milk in TBS 0.1% Tween or 1% nonfat dry milk in TBS for Fibr) were added over night at 4°C (Apo A-I: Calbiochem, 178463; HRG: Abnova, B01P; Fibr: Dako, F0111). Then membranes were washed three times with TBS 0.1% Tween, incubated 1 h with secondary antibodies, washed four times and developed using enhanced chemo luminescence (Apo A-I and HRG) or fluorescence (Fibr).

Cytotoxicity and cytokine assay.

Monocytes and macrophages (3×10^5 cells/well; 0.92×10^6 cells /cm^2) seeded into 96-wells plates (Falcon) were incubated at 37°C for 20 h with 20 μg/ml Stöber NPs in RPMI 1640 alone or supplemented with 10% (v/v) FCS, 10% (v/v) HP, the mix of proteins (corona mix, total or selectively lacking one protein) or single human plasma proteins at a concentration corresponding to 10% human plasma (see "human proteins and plasma" section). Cellular viability was evaluated by CellTiter 96® AQueous One Solution Reagent (Promega), according to manufacturer's instructions; IL-1β levels were measured by ELISA assay (Bender MedSystems) in the extracellular media of monocytes and macrophages treated as for MTS assay. In control experiments, Stöber NPs (20 μg/ml) were added to MTS substrate solution or to IL-1β standard curve to assess any interference with the assays (either scattering or specific color development) (Figure S13).