Electronic Supplementary Material (ESI) for Nanoscale.

Supplementary Information

High-resolution isolation of protein corona nanoparticles from complex physiological fluids

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Figure S1

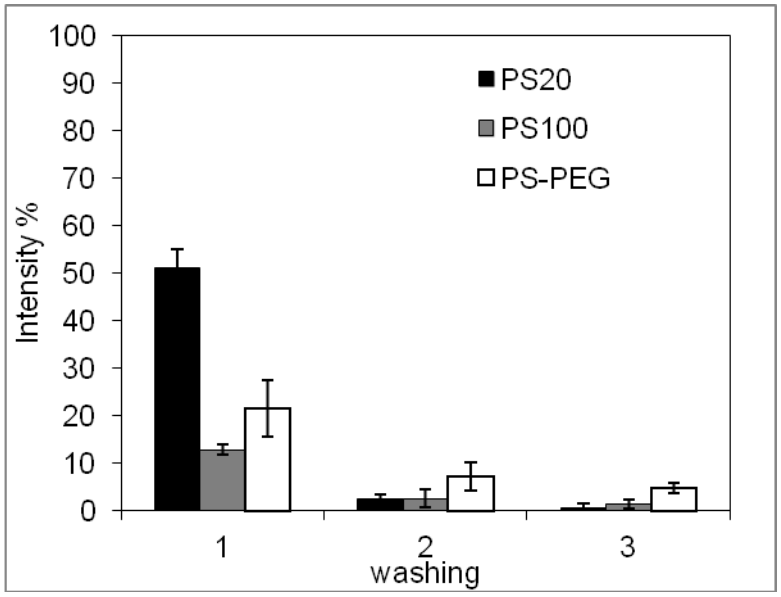


Figure S1. Fluorescence intensity emission of the surnatant solutions compared to that of the initial stock solutions when centrifugal washings are performed on *in situ* samples of polystyrene NPs prepared as described in the Material and Method section. The excitation and emission wavelengths weres 490 nm and 515 nm, respectively.

Figure S2

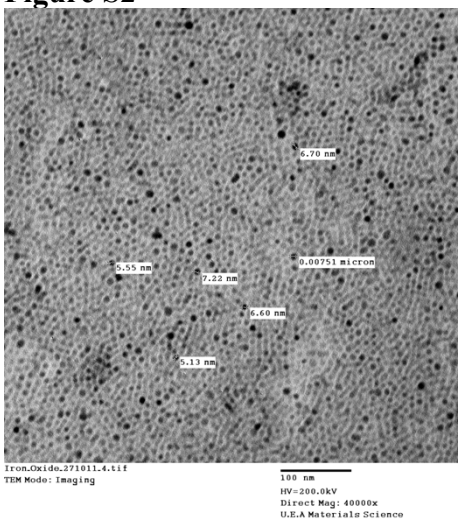


Figure S2. TEM image of Fe_3O_4 NPs stabilized by oleic acid and dispersed in toluene. 1 drop of the dispersionsì was placed on the carbon film 300 mesh Cu(50)(Agar Scientific). The instrument was Joel 200EX with tungsten filament and acceleration voltage of 180kV to 200kV.

Figure S3

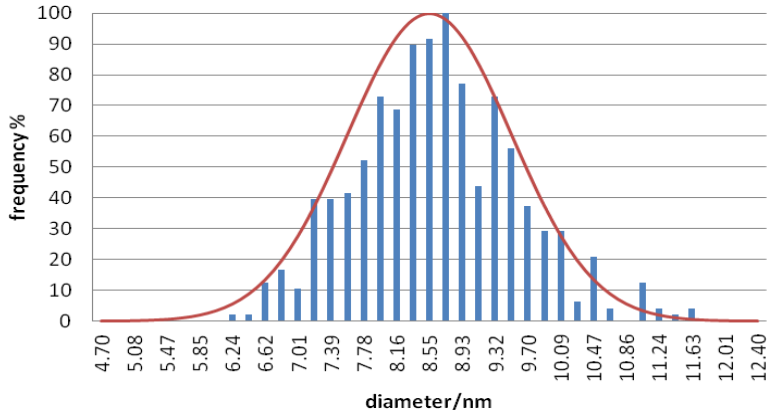


Figure S3. Statistical size distributions on 500 NPs imaged by TEM and analysed by ImageJ software.

Figure S4

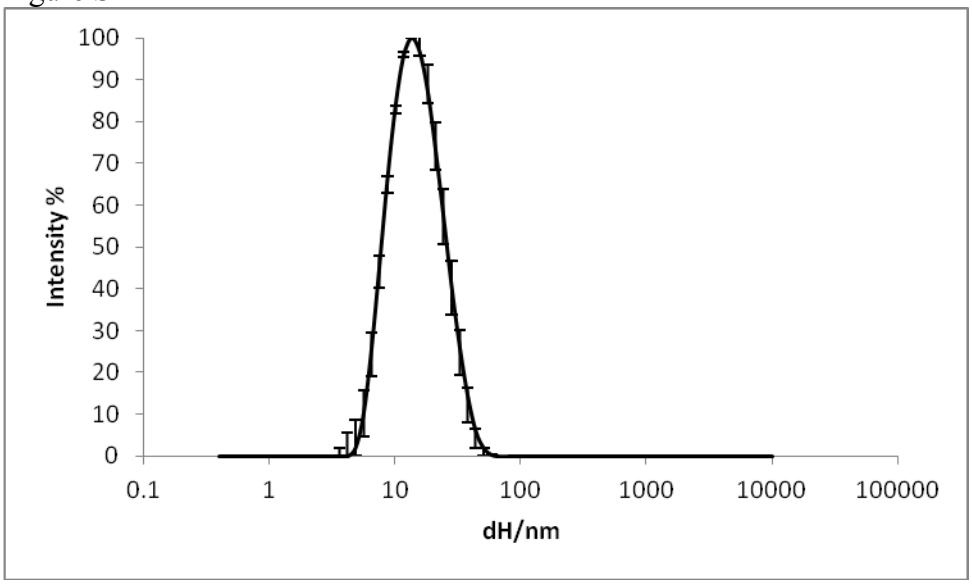


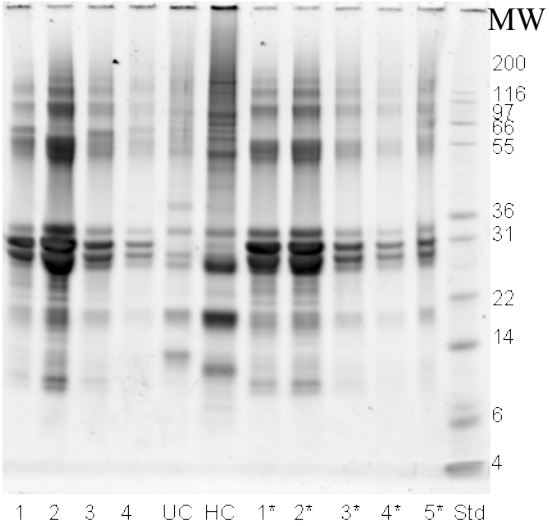
Figure S4. Intensity averaged hydrodynamic size distribution obtained fitting the DLS auto-correlation functions by cumulant analysis of Fe_3O_4 NPs stabilized by oleic acid in toluene.

Table S1. Physical parameters used to derive the concentration of the sample in NPs/ml from ICP- AES measures that give the total iron concentration in mg/l. The diameter value was extracted from the TEM images statistic.

NPs	Density [g/cm^3]	Diameter NP [nm]	Volume [cm^3]	Weight NP [g]
Fe_3O_4	5.2	8.5±1.0	$3.2*10^{-19}$	$1.7*10^{-12}$

Figure S5

a)



b)

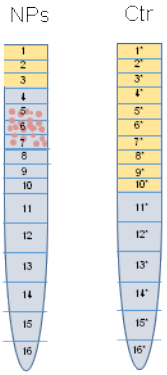
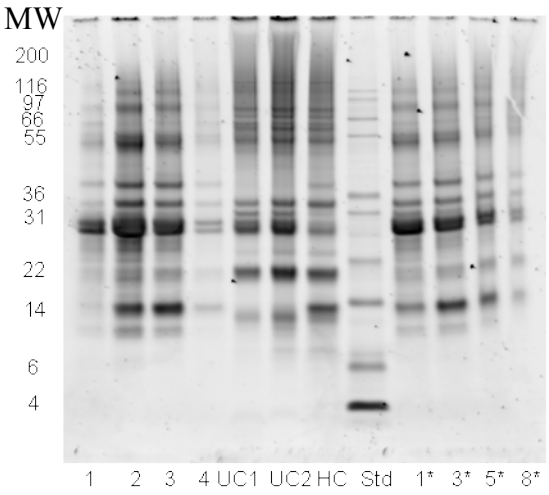


Figure S5. a) SDS-PAGE of Fe_3O_4 NPs in oral fluids. b) Scheme of the sucrose layer arrangement in the UC tube showing where NPs and proteins were located. A control sample was run on the fluid without NPs in the same experimental conditions. The first 5 ml of sucrose were collected in fractions of 0.5 ml, while from number 6th in 1ml aliquots. NPs were found in fractions 5 to 7 that were pooled together in one fraction (UC). HC was isolated centrifuging the same sample after incubation (HC). Samples decorated with * are the fractions from the control.

Figure S6

a)



b)

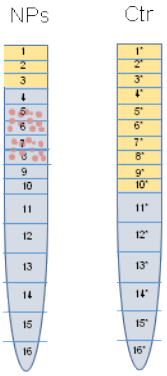


Figure S6. a) SDS-PAGE of Fe_3O_4 NPs in fluid before gastric phase. B) Scheme of the sucrose layer arrangement in the ultracentrifuge tubes showing where NPs and proteins were located. A control was performed on fluid in the same condition of NPs replacing them with water. The first 5 ml of sucrose were collected in fractions of 0.5 ml, while from number 6th in 1ml aliquots. NPs were found in fractions 5 to 7 that were pooled together in one fraction (UC1) and in 8 (UC2). HC was isolated centrifuging the same sample after incubation (HC). Samples decorated with * are the fractions from the control.

Figure S7

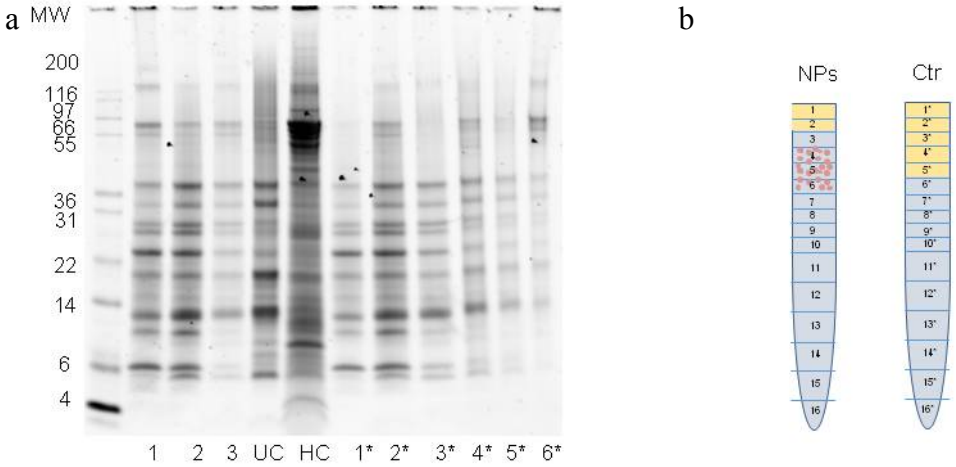


Figure S7 a) SDS-PAGE of Fe_3O_4 NPs in gastric fluid after 120 min. b) Scheme of the sucrose layer arrangement in the ultracentrifuge tubes showing where NPs and proteins were located. A control was performed on fluid in the same condition of NPs replacing them with water. The first 5 ml of sucrose were collected in fractions of 0.5 ml, while from number 6 in 1ml aliquots. NPs were found in fractions 4 to 6 that were pooled together in one fraction (UC). HC was isolated centrifuging the same sample after incubation (HC). Samples decorated with * are the fractions from the control.

Figure S8

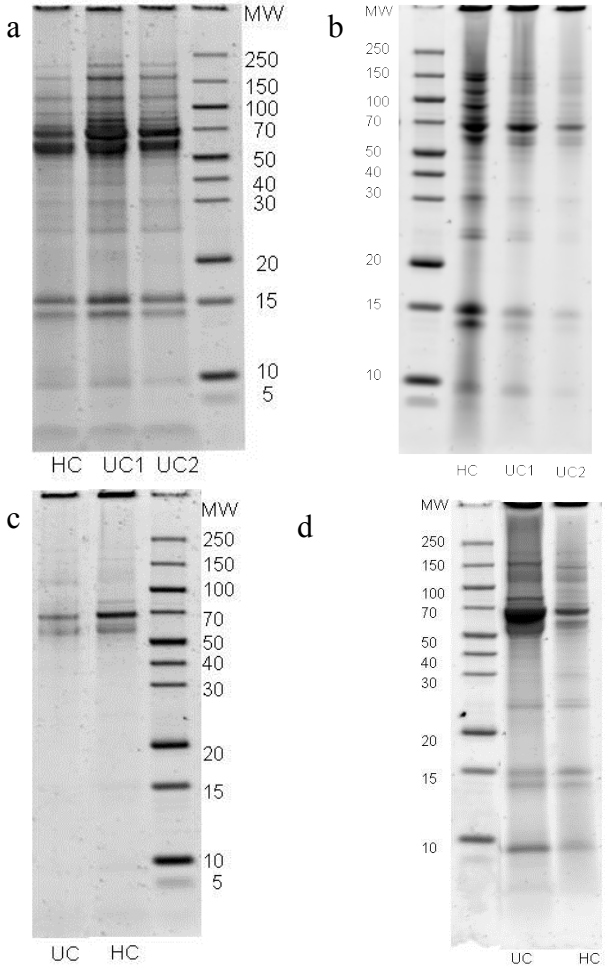


Figure S8. SDS PAGE of a) Fe_3O_4 NPs in 90% FBS, b) PS-COOH100 in 55% FBS, c) PS-PEG in 55% FBS, d) PS-COOH20 in 55% FBS. Lanes labeled as UCs and HCs represent samples isolated through ultracentrifugation and standard centrifugal washings, respectively.

Figure S9. LC-MS

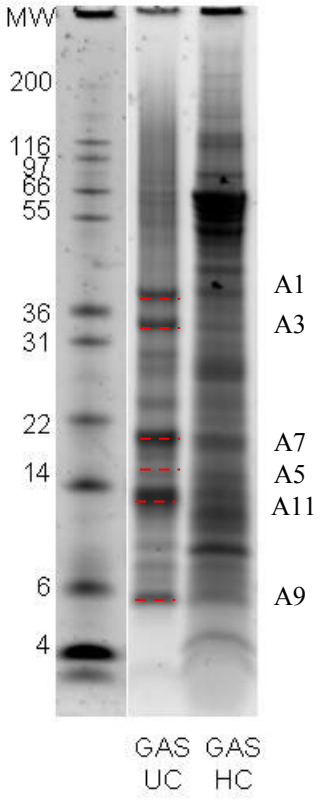


Figure S9 Gastric digestion gel in which are highlighted with a red dashed line all bands analysed by LC-MS. The band code is reported on the side of the gel in the correspondent order. Below tables listing major peptides found in bands analyzed.

Table S1. Mass Spectrometry results (from gel reported in Figure 7b)

Lane A1

Name	alpha-s2-casein	Major allergen beta-lactoglobulin	Butyrophilin	Vicilin 47k
coverage %	20	28	6	35
score	46	36	45	166
nominal mass	26173	20355	59912	49542
signal sequence length	15	16	26	24
total peptide length	222	178	562	437

Lane A3

name	Vicilin 47k	Major allergen beta-lactoglobulin	Butyrophilin	alpha-s1-casein	alpha-s2-casein	Convicilin	pepsine a
coverage %	39	34	26	32	31	5	4
score	299	178	134	99	69	62	48
nominal mass	49542	20355	59912	24498	26173	60139	41635
signal sequence length	24	16	26	15	15	-	15
total peptide length	437	178	562	214	222	511	385

Lane A5

name	alpha-s2-casein	Beta-lactoglobulin	Butyrophilin	Vicilin 47k	Galectin	Lactoferrin	alpha-s1-casein
coverage %	52	44	11	25	8	7	32
score	166	153	98	81	81	72	69
nominal mass	26173	20176	59912	49542	15095	79998	24498
signal sequence length	15	16	26	24	-	-	15
total peptide length	222	178	526	437	136	708	214

Lane A7

name	Major allergen beta-lactoglobulin	alpha-s2-casein	alpha-s1-casein	Alpha-lactalbumin	Vicilin 47k
coverage %	64	40	26	16	18
score	178	173	75	48	46
nominal mass	20355	26173	24498	16750	49542
signal sequence length	16	15	15	19	24
total peptide length	178	222	214	142	437

Lane A9

name	ALB protein	Alpha-lactalbumin	Beta-lactoglobulin;	Lactoferrin	Vicilin 47k	Beta casein	Butyrophilin	Albumin-1 A OS= Pisum sativum	Alpha-S2-casein
coverage %	16	38	17	6	8	37	4	6	29
score	140	115	68	67	42	49	46	34	23
nominal mass	71244	16750	20176	79998	49542	5164	59912	14473	26173
signal sequence length	25	19	16	19	24		26	26	22
total peptide length	607	142	178	708	437	45	526	130	222

Lane A11

name	Serum albumin	alpha-s2-casein	Butyrophilin
coverage %	19	38	4
score	169	128	47
nominal mass	55487	26173	59912
signal sequence length	-	15	26
total peptide length	476	222	526