Electronic Supplementary Information

Heparin length in the coating of extremely small iron oxide nanoparticles regulates *in vivo* theranostic applications

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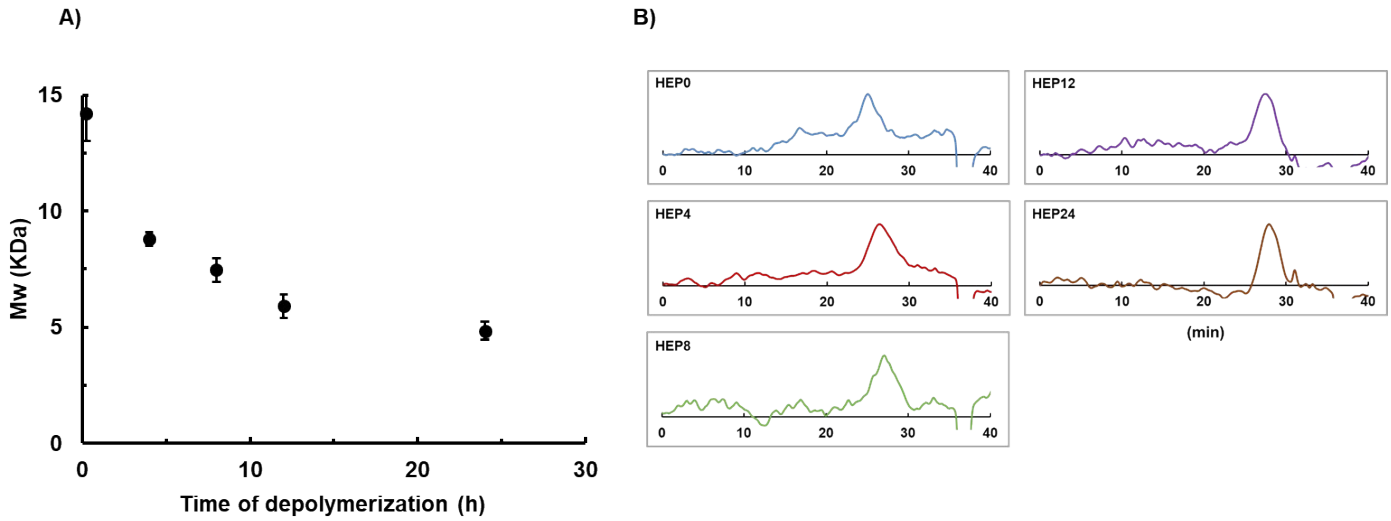


Figure S1: Preparation of the HO, depolymerisation of the native heparin. A) Effect of the H_2O_2-assisted radical depolymerisation method along time on the HO weight average molecular weight (Mw). B) SEC-HPLC analysis, x-axis is time in min and y-axis is normalized RID signal.

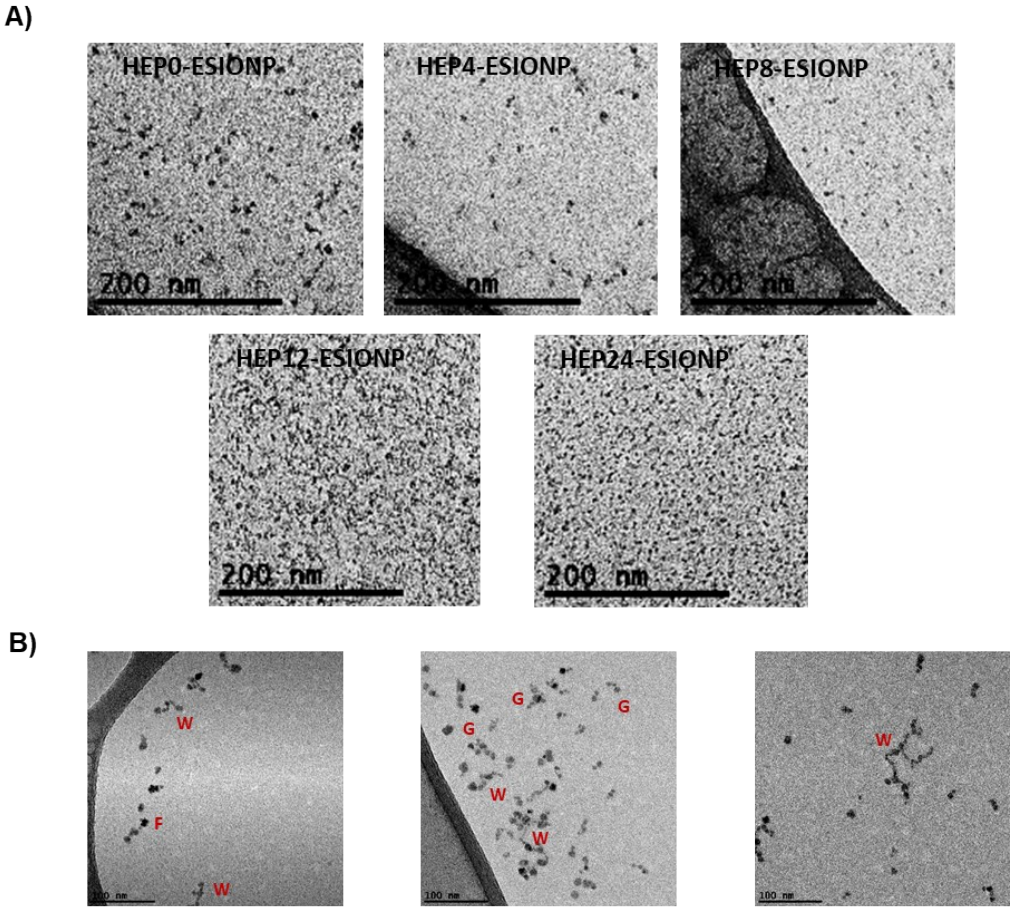


Figure S2: TEM images. A) TEM picture of the HEP-ESIONP, scale bar is 100 nm. B) Assemblies observed in the TEM images of the HEP0-ESIONP (W, worm; F, flower; G, grappe).

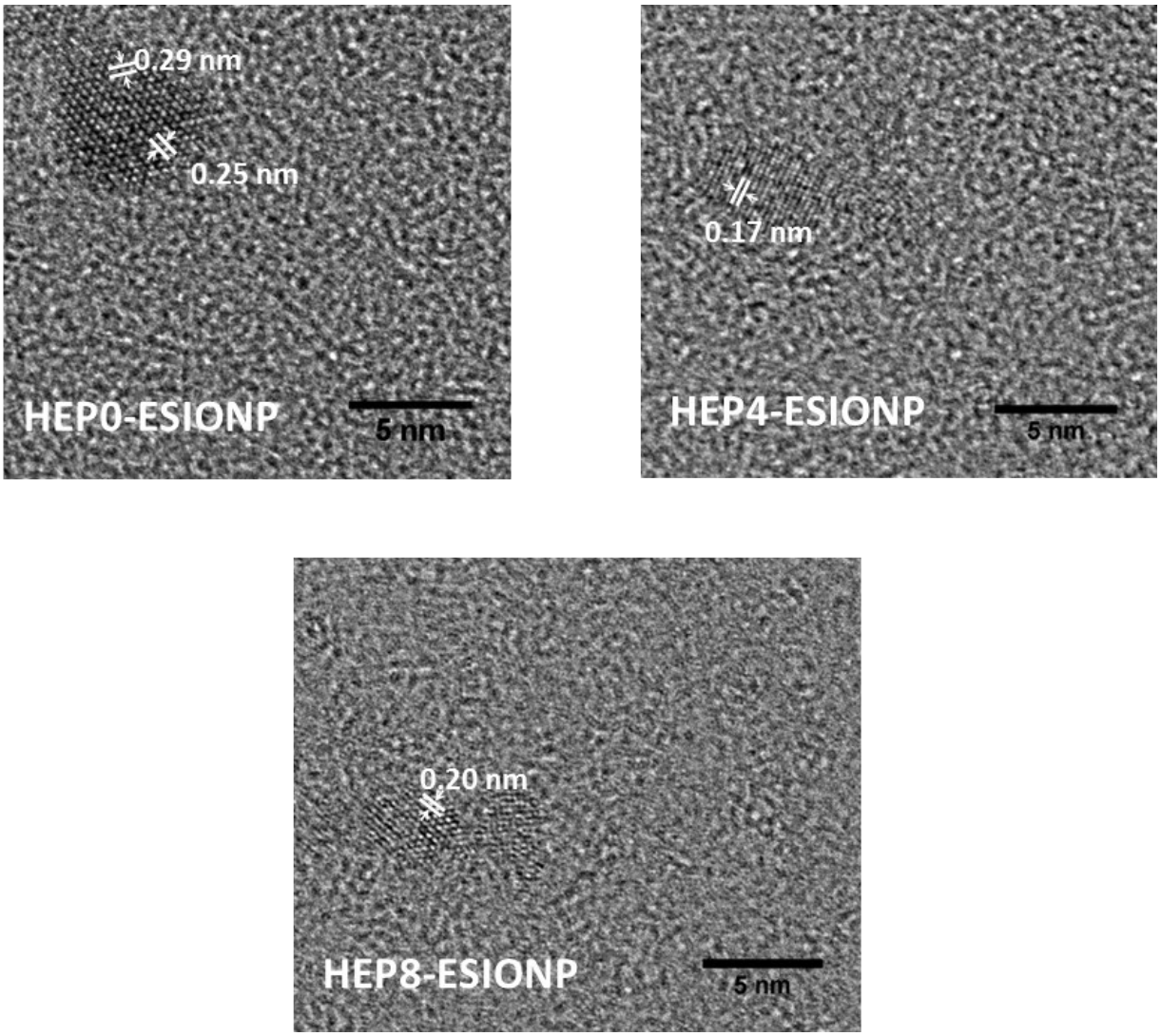


Figure S3: High-resolution TEM images. Examples of lattice fringes observed on the HEP-ESIONP cores, scale bar is 5 nm.

Table S1. The d-spacing values (nm) calculated from HRTEM images as compared with the standard atomic spacing for Fe_3O_4 and the respective *hkl* indexes from JCPS card (19-0629).

HEP-ESIONP	**Calculated d-spacing (nm)**	Assigned **JCPDS data for Fe_3O_4 (nm)**	***hkl***
HEP0-ESIONP	0.251 ± 0.006	0.2967	311
HEP0-ESIONP	0.203 ± 0.003	0.2099	400
HEP8-ESIONP	0.167 ± 0.001	0.1615	511
HEP8-ESIONP	0.199 ± 0.003	0.2099	400
HEP24-ESIONP	0.172 ± 0.001	0.1714	422
HEP24-ESIONP	0.197 ± 0.004	0.2099	400

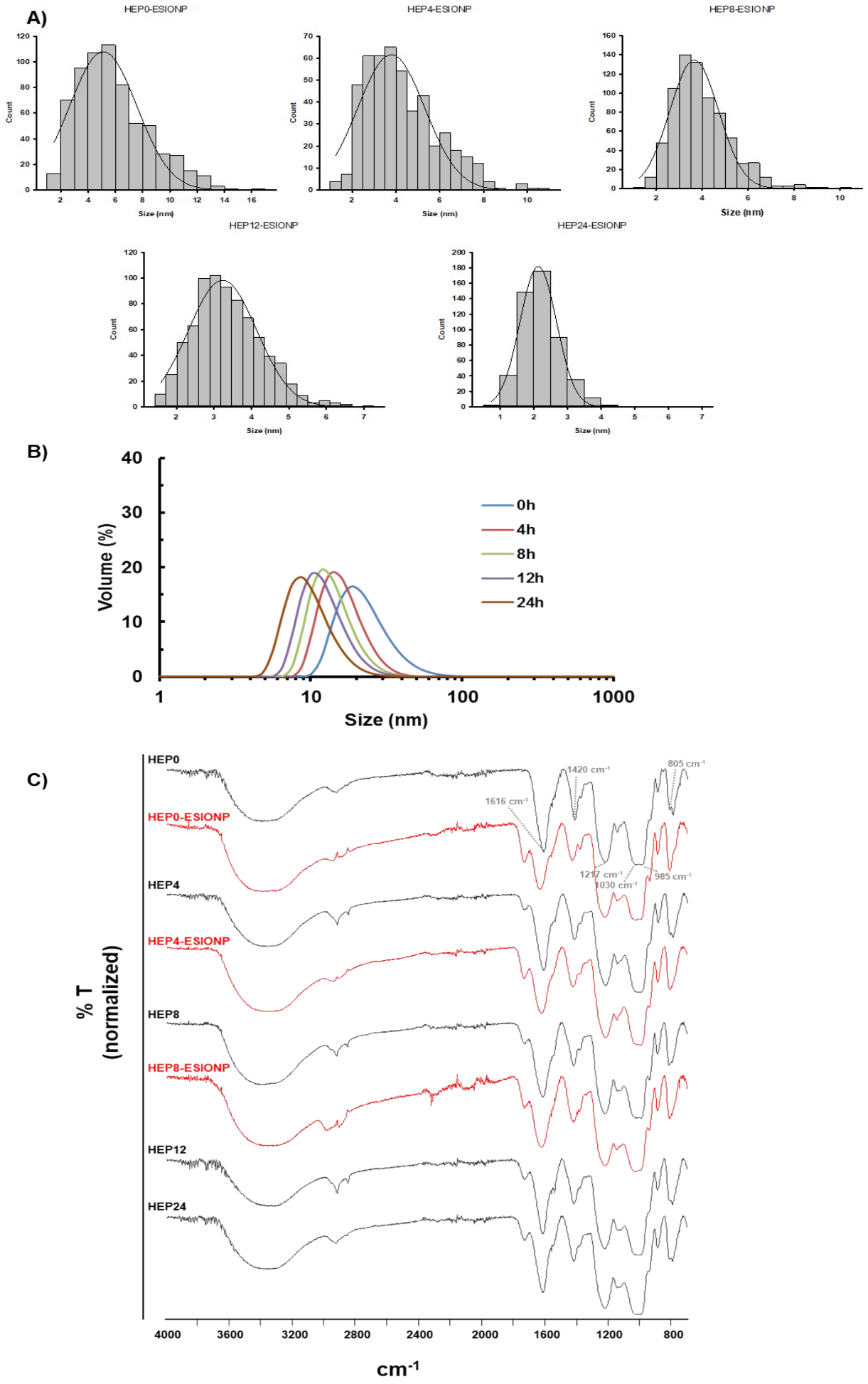


Figure S4: Physicochemical characterization of the HEP-ESIONP. A) Core size distribution of the HEP-ESIONP core measure on more than 300NP. B) Hydrodynamic size of the HEP-ESIONP. C) FTIR spectra of free HO and HEP-ESIONP

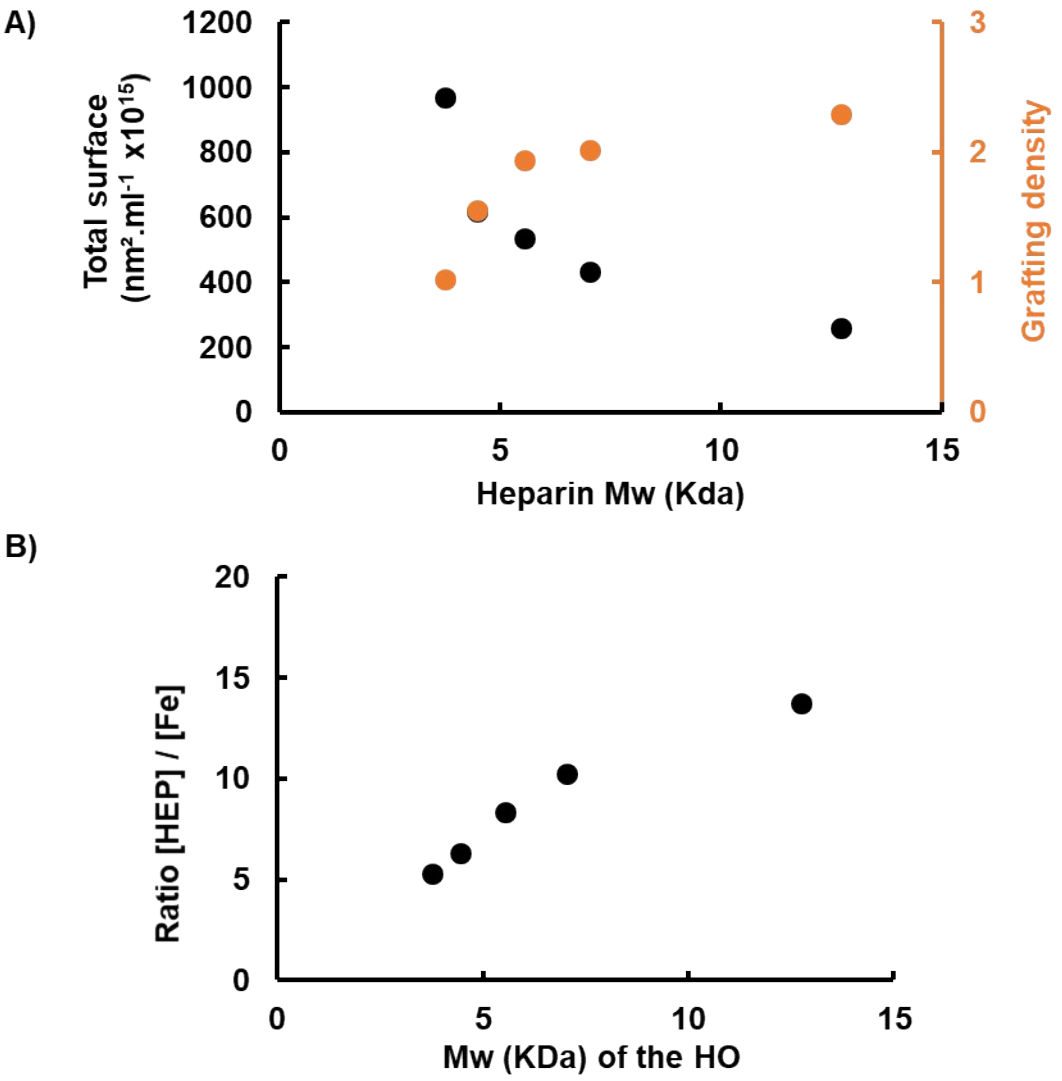


Figure S5: Characterization of the HO coating on the ESIONP. A) Total nanoparticle surface area per ml of HEP-ESIONP core (black) and grafting density of the HO on the HEP-ESIONP according to the MW of the HO coating (grey). B) Ratio [HEP]/ [Fe] for each HEP-ESIONP according to the MW of the HO coating.

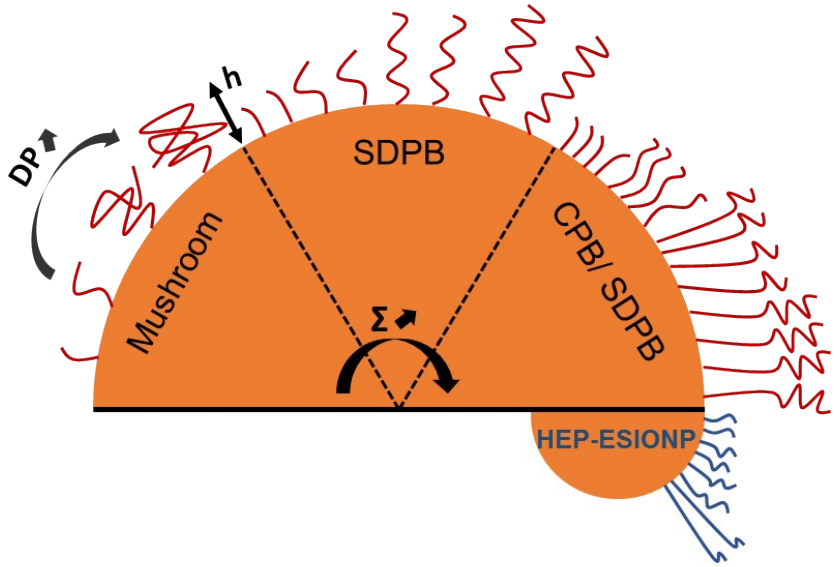


Figure S6: Schematic illustration of the coating configuration of the HEP-ESIONP. Usual configuration on polymers inserted at the surface of the NP according to the grafting density **σ** and the length (DP).

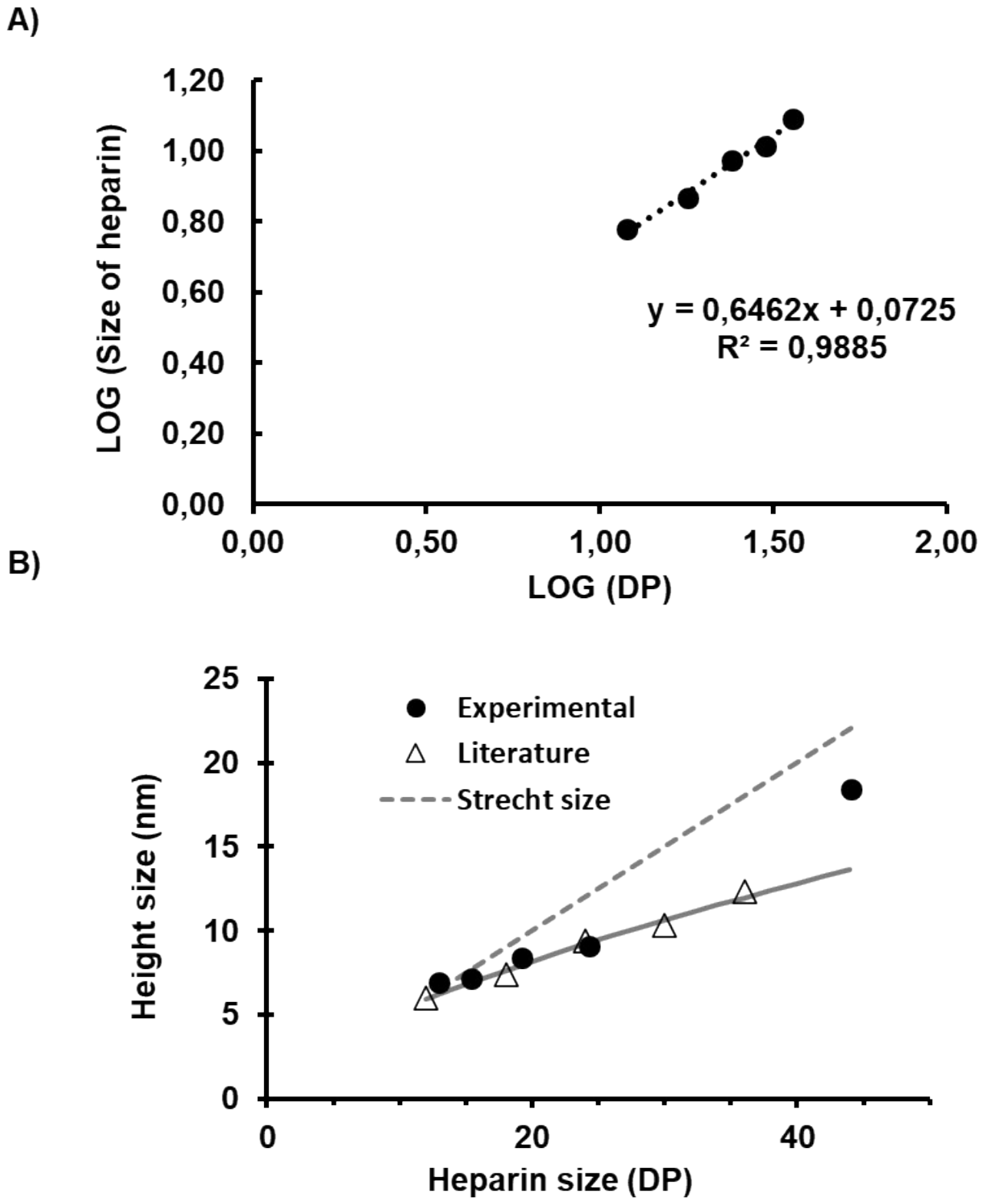


Figure S7: Size of the coating of the HEP-ESIONP. A) Extrapolation of the DP^n relationship of the free heparin length according to the size values of several HO by S. Khan et *al.* to extrapolate the size of our own HO produced in the DP range [10-50]. B) Height size of the different HO coating in the ESIONP, according to the length in DP of the HO. Triangles and grey line represent the extrapolated height size if the HO coating were under their free configuration without steric constraint. Dashed line represents the extrapolated height size, if the HO coating were fully stretch under steric constraint.

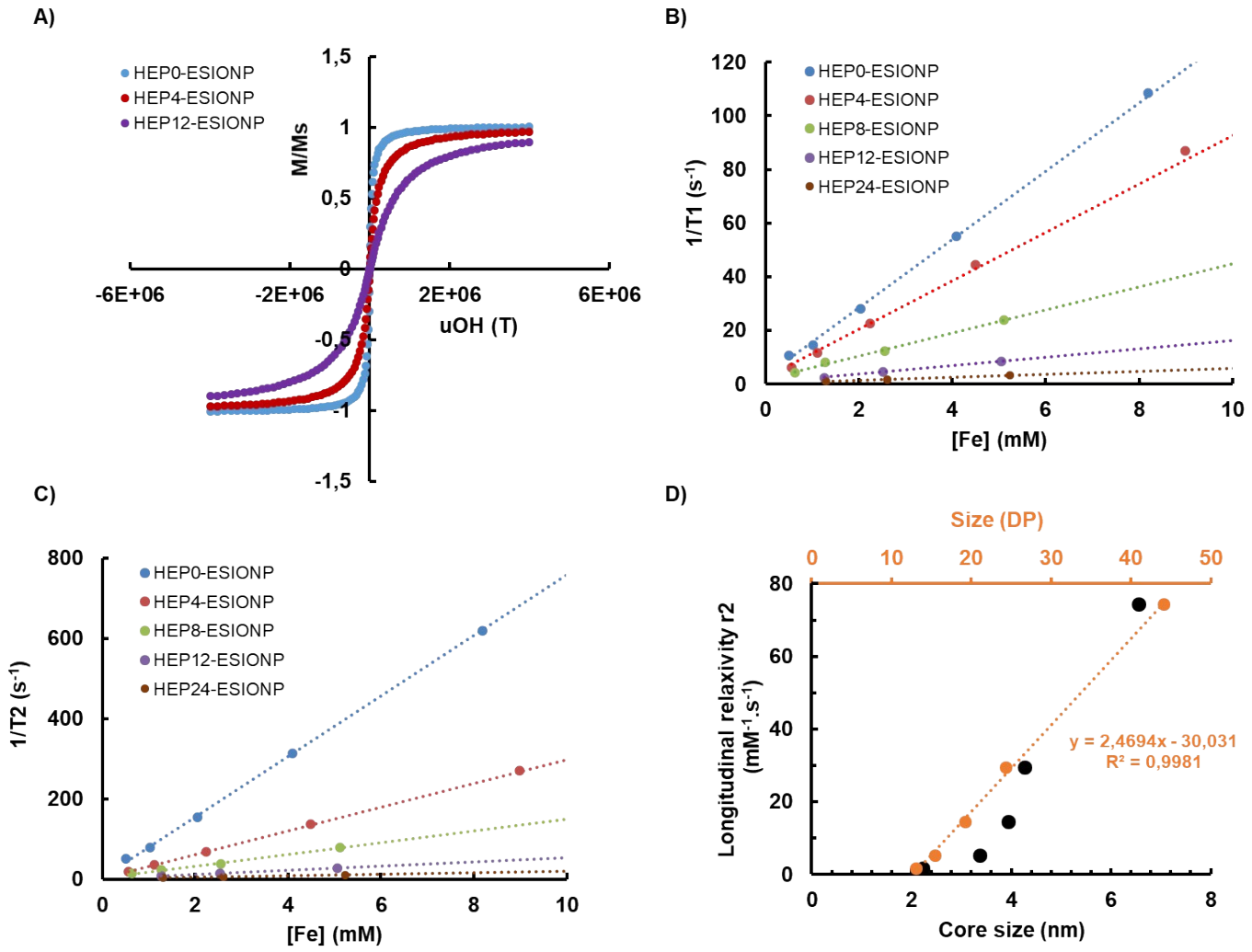


Figure S8: magnetic and relaxometric characterization of the HEP-ESIONP. A) Magnetization curves at 300 K of selected HEP-ESIONP ; B) Plot of the longitudinal (T1) and C) transversal (T2) relaxation rates measured at 1.5T of HEP-ESIONP at different iron concentration ; D) Transversal relaxivity *r2* according to the core size (black dot) and the length in DP of the HO coating (blue dot) of the HEP-ESIONP.

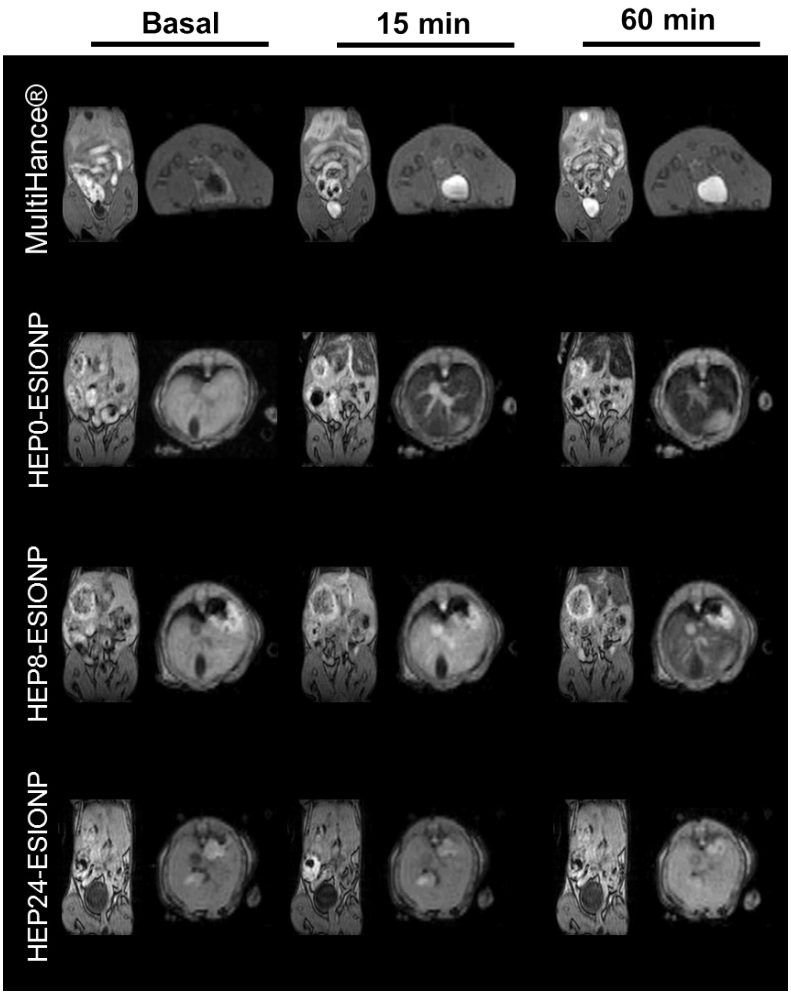


Figure S9: *In vivo* positive contrast MRI of the HEP0, HEP8 and HEP24-ESIONP Coronal and axial images of mice liver performed on a 1T MRI (ICON 1T-MRI; Bruker BioSpin GmbH) after i.v.a of HEP-ESIONP (50µl, [Fe]=1mg.ml^{-1}) and of mice bladder after i.v.a of Gadobenate dimeglumine (Multihance®; 529 mg.mL^{-1}). A T1-weighted gradient echo sequence was used with a repetition time (TR) = 21 ms, an echo time (TE) = 3 ms and a flip angle of 20º.

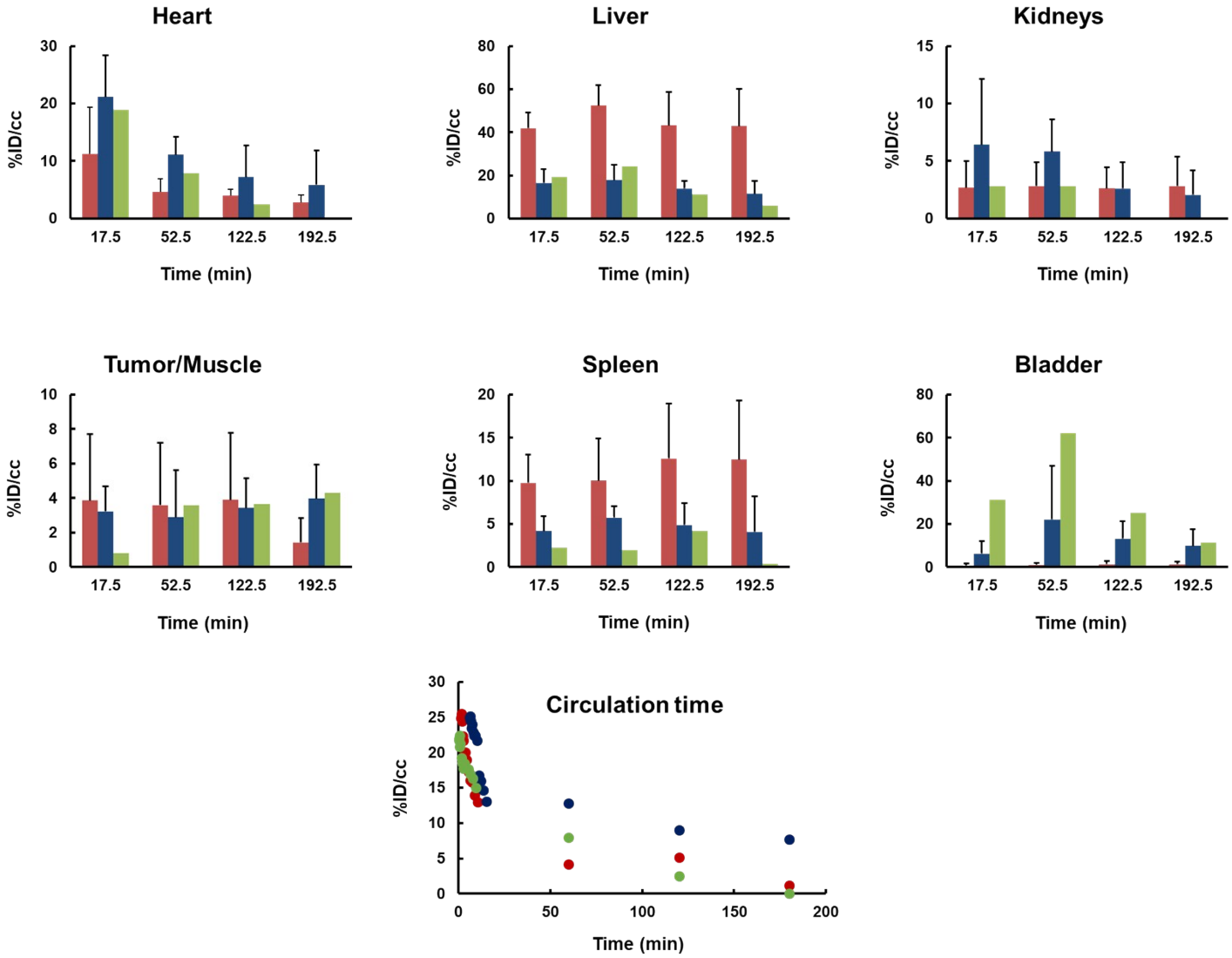
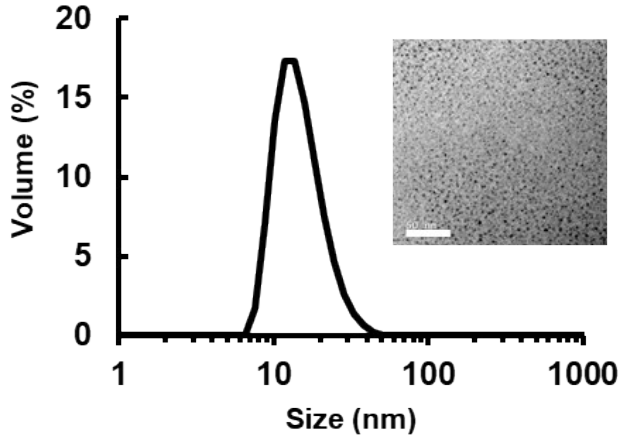
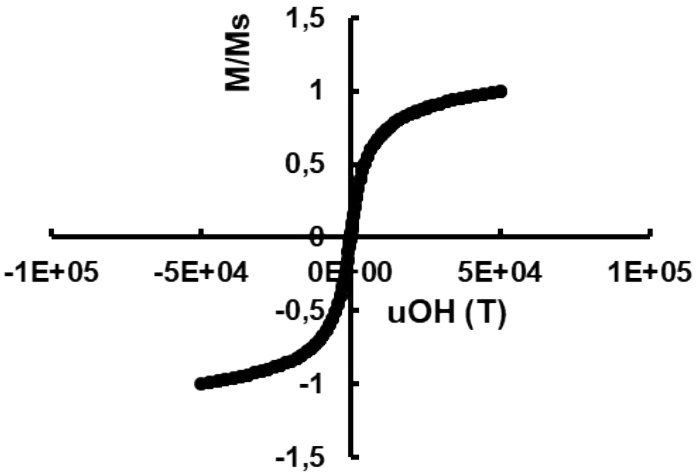


Figure S10: ***In vivo*** **quantification of the biodistribution of the ^{68}Ga-HEP0-ESIONP and ^{68}Ga-HEP24-ESIONP in the major organs and circulation time (^{68}Ga-HEP8-ESIONP)**

A)



B)



C)

Cit-ESIONP	
Hydrodynamic size	21.2 nm
TEM core size	3.1 ± 0.6
Potential Zeta	- 28.5 mV
r1	10.2 $mM^{-1} \cdot s^{-1}$
r2	69.3 $mM^{-1} \cdot s^{-1}$

D)

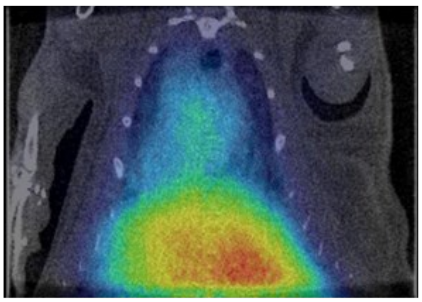


Figure S11: Physicochemical characterization of Cit-ESIONP. A) Hydrodynamic size, B) Magnetization curves at 300 K, C) physicochemical characterizations and D) typical PET images showing the accumulation of 68Ga-Cit-ESIONP in liver after 30 min

Table S2. List of the unique proteins found in the coronas, comparing the different HEP-ESIONP with Cit-ESIONP (Venn diagrams). The proteins with % abundance below 0.15 % in all the four ESIONP coronas were excluded)

Cit-ESIONP	%	vs	HEP0-ESIONP
Ig kappa chain V-III region PC 7940	1,17		Ig kappa chain V-V region HP 124E1
Ig kappa chain V-III region PC 7175	0,97		Ig kappa chain V-III region PC 7210
Ig kappa chain V-VI region NQ5-61.1.2	0,86		Ig heavy chain V region 6.96
Ig heavy chain V region 5-84	0,65		Ig kappa chain V-III region ABPC 22/PC 9245
Platelet factor 4	0,44		Ig kappa chain V-II region 2S1.3
Ig kappa chain V-III region PC 3741/TEPC 111	0,35		Ig kappa chain V-II region MOPC 511
Ig heavy chain V region M603	0,30		Ig kappa chain V-II region 7S34.1
Lysozyme C-2	0,25		Ig heavy chain V region RF
Fructose-bisphosphate aldolase A	0,21		Ig kappa chain V-V region L7 (Fragment)
Tubulin beta-5 chain	0,18		Ig kappa chain V-III region 50S10.1
Ribonuclease 4	0,14		Hemoglobin subunit beta-2
Ig kappa chain V-V region T1	0,11		Serum amyloid P-component
Beta-2-glycoprotein 1	0,11		Ig heavy chain Mem5 (Fragment)
Isocitrate dehydrogenase [NADP]	0,07		Ferritin light chain 1
Alpha-2-HS-glycoprotein	0,07		Ig kappa chain V-VI region NQ2-17.4.1
			Ig lambda-1 chain V region
			Tubulin alpha-4A chain
			Keratin, type II cytoskeletal 8
			C-reactive protein
			Apolipoprotein A-IV
			Moesin
			CD9 antigen
			Hemopexin
			Tubulin alpha-1C chain

Cit-ESIONP		vs	HEP8-ESIONP
Ig kappa chain V-III region PC 7940	1,17		Ig kappa chain V-III region PC 7210
Ig kappa chain V-III region PC 7175	0,97		Ig heavy chain V-III region A4
Ig kappa chain V-VI region NQ5-61.1.2	0,86		Ig heavy chain Mem5 (Fragment)
Ig heavy chain V region 5-84	0,65		Ig kappa chain V-V region L7 (Fragment)
Alpha-1-antitrypsin 1-2	0,49		Ig heavy chain V region RF
Platelet factor 4	0,44		Ig heavy chain V region 6.96
Ig kappa chain V-III region PC 3741/TEPC 111	0,35		Ig kappa chain V-II region MOPC 511
Ig heavy chain V region M603	0,30		Hemoglobin subunit beta-2
Fructose-bisphosphate aldolase A	0,21		Ig kappa chain V-V region HP 124E1
Tubulin beta-5 chain	0,18		Ig kappa chain V-III region 50S10.1
Proteasome subunit alpha type-3	0,15		Protein S100-A9
C4b-binding protein	0,14		Serum amyloid P-component
Ribonuclease 4	0,14		Ig kappa chain V-III region ABPC 22/PC 9245
Ig kappa chain V-V region T1	0,11		Ig kappa chain V-II region 7S34.1
Proteasome subunit alpha type-2	0,06		Ig kappa chain V-II region 2S1.3
			Tubulin alpha-4A chain
			Hemopexin
			Tubulin alpha-1C chain
			Moesin
			Ezrin
			Apolipoprotein A-IV
			C-reactive protein
			Keratin, type II cytoskeletal 8

Cit-ESIONP	%	vs	HEP24-ESIONP
Ig kappa chain V-III region PC 7940	1,17		Ig kappa chain V-V region HP 124E1
Ig kappa chain V-III region PC 7175	0,97		Ig kappa chain V-III region PC 7210
Ig kappa chain V-VI region NQ5-61.1.2	0,86		Ig heavy chain Mem5 (Fragment)
Immunoglobulin J chain	0,83		Ig heavy chain V region 6.96
Ig heavy chain V region 5-84	0,65		Ig kappa chain V-III region PC 6684

Platelet factor 4	0,44		Ig kappa chain V-III region 50S10.1
Ig kappa chain V-III region PC 3741/TEPC 111	0,35		Ig kappa chain V-V region L7 (Fragment)
Ig heavy chain V region M603	0,30		Serum amyloid P-component
Lysozyme C-2	0,25		Ig heavy chain V-III region A4
Fructose-bisphosphate aldolase A	0,21		Ig kappa chain V-II region MOPC 511
Tubulin beta-5 chain	0,18		Ig kappa chain V-II region 7S34.1
C4b-binding protein	0,14		Ig heavy chain V region RF
Ribonuclease 4	0,14		Tubulin alpha-4A chain
Ig kappa chain V-V region T1	0,11		Moesin
Beta-2-glycoprotein 1	0,11		Hemoglobin subunit beta-2
Heat shock cognate 71 kDa protein	0,08		Ig kappa chain V-III region ABPC 22/PC 9245
			Tubulin alpha-1C chain
			Ig kappa chain V-II region 2S1.3
			Keratin, type II cytoskeletal 8
			Hemopexin
			C-reactive protein
			Ezrin

Cit-ESIONP	**%**	**vs**	**HEP-ESIONP**
Ig kappa chain V-III region PC 7940	1,17		Ig kappa chain V-V region HP 124E1
Ig kappa chain V-III region PC 7175	0,97		Ig kappa chain V-III region PC 7210
Ig kappa chain V-VI region NQ5-61.1.2	0,86		Ig heavy chain Mem5 (Fragment)
Ig heavy chain V region 5-84	0,65		Ig heavy chain V region 6.96
Platelet factor 4	0,44		Ig kappa chain V-III region 50S10.1
Ig kappa chain V-III region PC 3741/TEPC 111	0,35		Ig kappa chain V-V region L7 (Fragment)
Ig heavy chain V region M603	0,30		Serum amyloid P-component
Fructose-bisphosphate aldolase A	0,21		Ig kappa chain V-II region MOPC 511
Tubulin beta-5 chain	0,18		Ig kappa chain V-II region 7S34.1
Ribonuclease 4	0,14		Ig heavy chain V region RF
Ig kappa chain V-V region T1	0,11		Tubulin alpha-4A chain
			Moesin
			Hemoglobin subunit beta-2
			Ig kappa chain V-III region ABPC 22/PC 9245
			Tubulin alpha-1C chain
			Ig kappa chain V-II region 2S1.3
			Keratin, type II cytoskeletal 8
			Hemopexin
			C-reactive protein

Table S3. List of the proteins with significant changes (p-values < 0.05), assessing HEP-ESIONP vs Cit-ESIONP. In brown, proteins related with acute phase and in yellow proteins related with pro-coagulant activities.

HEP-ESIONP « enrichment » proteins vs. Cit-ESIONP
COMMON TO THE THREE HEP-ESIONP
Ig kappa chain V-III region PC 7210
Ig kappa chain V-II region MOPC 511
Ig kappa chain V-II region 7S34,1
Ig kappa chain V-V region L7 (Fragment)
Ig heavy chain V region RF
Ig kappa chain V-III region PC 7210
Ig kappa chain V-II region MOPC 511
Ig kappa chain V-II region 7S34,1
Ig kappa chain V-V region L7 (Fragment)
Ig heavy chain V region RF
Ig kappa chain V-III region PC 7210
Ig kappa chain V-II region MOPC 511
Ig kappa chain V-II region 7S34,1
Serum amyloid P-component
Complement C5
Tubulin alpha-4A chain
Mannose-binding protein A
Coagulation factor XIII A chain
Talin-1
COMMON TO HEP0 and HEP8-ESIONP
Ig kappa chain V-III region ABPC 22/PC 9245
Ig kappa chain V-V region MOPC 41
Ig kappa chain V-III region ABPC 22/PC 9245
Ig kappa chain V-V region MOPC 41
Hemoglobin subunit beta-2
Apolipoprotein A-IV
Serine protease inhibitor A3K
Band 3 anion transport protein
Coagulation factor V
COMMON TO HEP0 and HEP24-ESIONP
Ig kappa chain V-V region HP 124E1
Inter alpha-trypsin inhibitor, heavy chain 4
COMMON TO HEP8 and HEP24-ESIONP
Ig heavy chain Mem5 (Fragment)
Fibronectin
Moesin
Fermitin family homolog 3
Hemoglobin subunit beta-1
OF HEP0-ESIONP
Ig kappa chain V-II region 2S1,3
Ig kappa chain V-V region K2
Ig kappa chain V-II region 2S1,3
Ferritin light chain 1

CD9 antigen
C-reactive protein
Proteasome subunit alpha type-5
Hemoglobin subunit alpha
C-type lectin domain family 4 member F
Complement C4-B
Complement C1q subcomponent subunit B
OF HEP8-ESIONP
Protein S100-A9
Tubulin alpha-1C chain
Hemopexin
Ezrin
Transthyretin
Isocitrate dehydrogenase [NADP] cytoplasmic
Kininogen-1
Hypoxia up-regulated protein 1
Apolipoprotein A-I
Vitronectin
OF HEP24-ESIONP
Ig heavy chain V region 6,96
Ig kappa chain V-III region PC 2880/PC 1229
Serine protease inhibitor A3N
Phosphatidylinositol-glycan-specific phospholipase D
Tubulin beta-1 chain
Heat shock-related 70 kDa protein 2
Matrix metalloproteinase-19
Heparin cofactor 2
HEP-ESIONP « depleted » proteins vs. Cit-ESIONP
COMMON TO THE THREE HEP-ESIONP
Ig heavy chain V region 5-84
Ig kappa chain V-III region PC 7940
Ig kappa chain V-VI region NQ5-61,1,2
Serum albumin
Thrombospondin-1
Properdin
Fructose-bisphosphate aldolase A
Platelet factor 4
Histidine-rich glycoprotein
COMMON TO HEP0 and HEP8-ESIONP
Polymeric immunoglobulin receptor
Tubulin beta-5 chain
COMMON TO HEP0 and HEP24-ESIONP
Ig gamma-2B chain C region
Coagulation factor XI
COMMON TO HEP8 and HEP24-ESIONP
Ig kappa chain V-III region PC 7175
Ig mu chain C region
Complement factor H
Complement C1r-A subcomponent

OF HEP0-ESIONP
Ig heavy chain V region PJ14
Ig gamma-2A chain C region, A allele
Complement C1r-A subcomponent
Carboxypeptidase N catalytic chain
Complement C1q subcomponent subunit A
Multimerin-1
Complement factor B
Prothrombin
OF HEP8-ESIONP
Pigment epithelium-derived factor
CD5 antigen-like
Proteasome subunit alpha type-3
Alpha-1-antitrypsin 1-2
Alpha-1-antitrypsin 1-3
Inter-alpha-trypsin inhibitor heavy chain H2
OF HEP24-ESIONP
Immunoglobulin J chain
Mannan-binding lectin serine protease 2

Table S4. List of the unique proteins found in the coronas, comparing the different HEP-ESIONP together (Venn diagrams). The proteins with % abundance below 0.15 % in all the four ESIONP coronas were excluded)

Unique to HEP0-ESIONP
Ferritin light chain 1 (0.25%)
Ig kappa chain V-VI region NQ2-17.4.1 (0.21%)
Ig lambda-1 chain V region (0.2%)
CD9 antigen (0.14%)
C4b-binding protein (0.05%)
Unique to HEP8-ESIONP
Protein S100-A9 (0.38%)
Beta-2-glycoprotein 1 (0.05%)
Lysozyme C-2 (0.36%)
Unique to HEP24-ESIONP
Ig kappa chain V-III region PC 6684 (0.79%)
Only shared by HEP0 and HEP8-ESIONP
Immunoglobulin J chain (0.13 & 0.20%)
Heat shock cognate 71 kDa protein (0.05 & 0.16%)
Apolipoprotein A-IV (0.16 & 0.15%)
Only shared by HEP8 and HEP24-ESIONP
Isocitrate dehydrogenase [NADP] cytoplasmic (0.17 an 0.19%)
Alpha-2-HS-glycoprotein (0.07 & 0.16%)
Ig heavy chain V-III region A4 (0.8 & 0.44 %)
Ezrin (0.19 & 0.06%)

Only shared by HEP0 and HEP24-ESIONP
Alpha-1-antitrypsin 1-2 (0.4 & 0.19%)
Proteasome subunit alpha type-3 (0.08 & 0.08%)
Proteasome subunit alpha type-2 (0.411 & 0.1%)

Table S5. List of the proteins with significant changes (p-values < 0.05), assessing HEP-ESIONP together

« ENRICHMENT » IN HEP0-ESIONP	%	vs	« ENRICHMENT » IN HEP8-ESIONP
Ig kappa chain V-III region ABPC 22/PC 9245	0,59		Complement factor B
Proteasome subunit alpha type-7	0,06		Histidine-rich glycoprotein
Keratin, type I cytoskeletal 10	0,10		Moesin
Complement C4-B	0,65		Myosin-9
Ig lambda-2 chain C region	1,60		
« ENRICHMENT » IN HEP0-ESIONP		**vs**	**« ENRICHMENT » IN HEP24-ESIONP**
CD9 antigen	0,14		Apolipoprotein E
Complement C5	0,21		Coagulation factor XII
Serum albumin	1,49		Glia-derived nexin
			Phosphatidylinositol-glycan-specific phospholipase D
			Inter-alpha-trypsin inhibitor heavy chain H2
« ENRICHMENT » IN HEP8-ESIONP	**%**	**vs**	**« ENRICHMENT » IN HEP 24-ESIONP**
Protein S100-A9	0,38		Clusterin
Transferrin receptor protein 1	0,35		Coagulation factor XII
Vitronectin	1,33		Inter-alpha-trypsin inhibitor heavy chain H1
			Ig heavy chain V region 102
			Actin, cytoplasmic 1
			Serine protease inhibitor A3N
			Lactotransferrin
			Thrombospondin-1
			Proteasome subunit alpha type-3

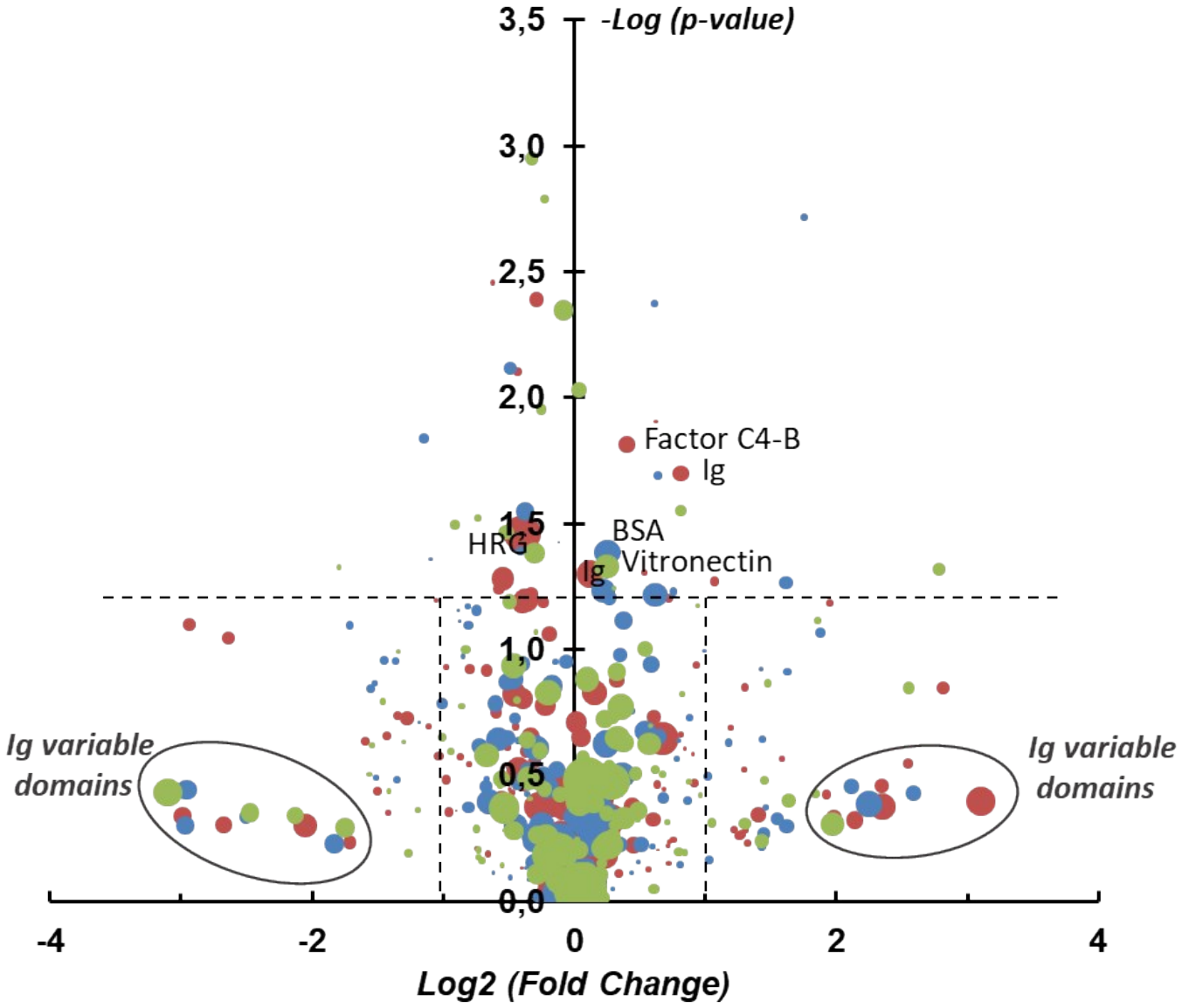


Figure S12: Comparison of the three HEP0, HEP8 and HEP24-ESIONP protein coronas. Plot of the fold-changes in abundance of each protein alongside their p-value significance, assessing the coronas of the three different HEP-ESIONP. Positive changes means a higher abundance of the protein in HEP0-ESIONP than in HEP8-ESIONP, HEP0-ESIONP than in HEP24-ESIONP and in HEP24-ESIONP than HEP8-ESIONP; opposite for the negative changes. Y-axis indicates significance of the change according to the p-value obtained with a Student's T-Test statistic. Surface of the dot represented are proportional to the abundance of the protein in the HEP-ESIONP depending whether the fold-change is positive or negative.

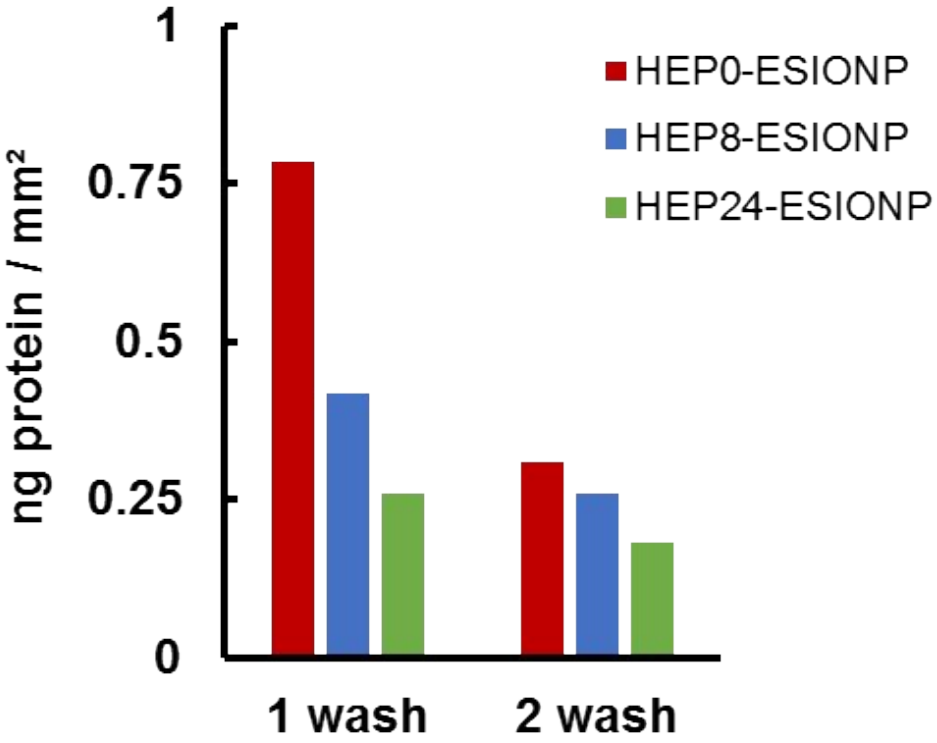


Figure S13: Amount of the protein in the HEP0, HEP8 and HEP24-ESIONP protein coronas. Plot of the amount of protein calculated by BCA assays and normalized to surface of each HEP-ESIONP. NP were incubated 15 min in mouse serum followed by isolation by centrifugation and one or two washing steps. Differences observed in the values measured between one and two washes reflected desorption of the low-binding proteins in the corona during the additional centrifugation step.