Supplementary information

Nanoparticle-Proteome In Vitro and In Vivo

Miaoyi Wang,[†] Ove J. R. Gustafsson,[§] Emily H. Pilkington,^{†‡} Aleksandr Kakinen,[†] Ibrahim Javed,[†] Ava Faridi,[†]

Thomas P. Davis^{\dagger *} and Pu Chun Ke^{\dagger *}

 [†]ARC Centre of Excellence in Convergent Bio-Nano Science and Technology, Monash Institute of Pharmaceutical Sciences, Monash University, 381 Royal Parade, Parkville, VIC 3052, Australia
[§]ARC Centre of Excellence in Convergent Bio-Nano Science and Technology, Future Industries Institute, University of South Australia, University Boulevard, Mawson Lakes, SA 5095, Australia
[‡]Department of Chemistry, University of Warwick, Gibbet Hill, Coventry, CV4 7AL, United Kingdom Table S1. In vitro protein corona, extracted from relevant literature as indicated.

Note: Due to space constraint, DOIs were indicated when there were too many types of nanoparticles or conditions used in the studies. NA indicates no relevant information available.

In vitro (1)

Literature	Dobrovolskaia et al (2009)	Tenzer et al (2011)	Zhang et	al (2011)
Nanoparticl e core	Colloidal gold particles (AuNP)	Silica nanoparticles (SiNP)	Polystyrene i	nanoparticles
Protein source	Human plasma containing sodium citrate as anticoagulant	Human plasma	Human	plasma
Incubation time	30 min	ıh	5 min, 15 mi	n, 1 h and 5 h
Techniques for identifying protein corona	Centrifugation followed by 2D-PAGE and in-gel trypsin digest (analysed by IT-MS)	Centrifugation followed by 1D and 2D- PAGE and in-gel trypsin digest (analysed by LC-MS)	SDS buffer elution, acetone precipitation followed by trypsin digest (analysed by LC-MS)	On particle digestion followed by centrifugation (analysed by LC- MS)
Surface chemistry	30 and 50 nm citrate stabilized AuNP	8, 20 and 125 nm amorphous SiNP	50 and 100 nm amir unmodified polysty	ie, carboxylated and vrene nanoparticles
Cell uptake	NA	NA	N	A

Cell toxicity	Ν	A	NA	NA
No. of	30 nm AuNP:	50 nm AuNP:	NA	NA
unique proteins	34	7		

Top 10 most abundant proteins (1)

Literature	Dobrovolska	ia et al (2009)	Те	enzer et al (20:	11)	Zhang et al (2011)
Nanoparticl	30 nm citrate	50 nm citrate	8 nm SiNP	20 nm	125 nm	See DOI: 10.1002/pmic.201100037
е	AuNP	AUNP		SiNP	SiNP	
1	Fibrinogen	Fibrinogen	Compleme	Serum	Serum	
	Fibrinogen α/αE	Fibrinogen α/αE	nt factor H	albumin	albumin	
	precursor	precursor				
2	Fibrinogen β-	Fibrinogen β-	Serum	Compleme	Alpha-2-	
	chain precursor chain precursor		albumin	nt factor H	macroglob	
					ulin	
3	Fibrinogen γ-	Fibrinogen γ-	Apolipopro	Compleme	Compleme	
	chain precursor	chain precursor	tein A-I	nt C3	nt C3	
4	Inter-α-trypsin	Inter-α-trypsin	Compleme	Apolipopro	lg gamma-	
	inhibitor heavy-	inhibitor heavy-	nt C3	tein A-I	1 chain C	
	chain H4	chain H4			region	
	precursor	precursor				
5	Plasma serine	Kininogen-	Fibronectin	Serotransf		
	protease inhibitor	1		errin		
	precursor					
6	Gelsolin precursor	Kininogen	Plasma	Apolipopro	Alpha-1-	
		precursor	protease	tein B-100	antitrypsin	

			C1 inhibitor		
7	Kininogen precursor	α-antitrypsin precursor	Nesprin-1	Nesprin-1	Haptoglobi n
8	α1- antichemotrypsin precursor	Protocadherin 17	Apolipopro tein B-100	Kininogen- 1	Apolipopro tein A-I
9	Protocadherin 17	Crumbs protein homologue precursor	Clusterin	C4b- binding protein alpha chain	lg kappa chain C region
10	Crumbs protein homologue precursor	Tropomyosin 4 chain	C4b- binding protein alpha chain	Plasma protease C1 inhibitor	lg gamma- 2 chain C region

In vitro (2)

Literature	Shannahan et al (2013)	Cai et al (2013)	Shannahan et al (2013)
Nanoparticl	Single and multi-walled carbon	Multi-walled carbon nanotubes	Silver nanoparticles (AgNP)
e core	nanotubes (SWCNT and MWCNT)	(MWCNTs) and carbon black (CB)	
Protein	Fetal bovine serum	Cell lysate prepared from the human	Dulbecco's Modified Eagle's Medium
source		cell line Hela	with glutamax and 10% heat inactivated
			fetal bovine serum
Incubation	ıh	3 h	ıh
time			
Techniques	Centrifugation followed by	Proteins pulled down by MWCNTs and	Centrifugation followed by solubilization
for	solubilization in situ using a lysis buffer	CB followed by SDS-PAGE and	in situ using a lysis buffer (8 M urea, 10

identifying	(8 N	/l urea	, 10 M	m DT	T) and	diges	sted	digested by trypsir	n (analysed by LC-	analysed by LC- Mm DTT) and digested by t		rypsin	
protein	by trypsin (analysed by LC-MS/MS)							M	S)	(a	(analysed by LC-MS/MS)		S)
corona											-	-	-
Surface	Na	М	М	SW	Μ	М	SW	Pristine MWCNTs	Colloidal CB	20 nm	110 nm	20 nm	110 nm
chemistry	noc	WC	WC	CN	WC	WC	CN	with mean		AgNP-	AgNP-	AgNP-	AgNP-
	lay	NT	NT	Т-	NT	NT	T-	diameter of 20-40		citrate	citrate	PVP	PVP
		-	-	ra	-	-	CO	nm					
		pur ra w PV CO OH				CO	ОН						
		e	w		P	ОН							
Cell uptake				NA				NANA			A		
Cell toxicity				NA				N	A		N	A	
No. of	1	6	7	3	6	16	34	N	A	10	1	15	10
unique													
proteins													

Top 10 most abundant proteins (2)

Literature	Shannahan et al (2013)							Cai et al (2013)	9	Shannahan	et al (2013	;)
Nanoparticl	Na	М	М	SW	М	М	SW	NA	20 nm	110 nm	20 nm	110 nm
e	noc	WC	WC	CN	WC	WC	CN		AgNP-	AgNP-	AgNP-	AgNP-
	lay	NT	NT	Т-	NT	NT	Т-		citrate	citrate	PVP	PVP
		-	-	ra	-	-	CO					
		pur	ra	w	PV	CO	ОН					
		е	w		Р	ОН						
1	TT	XIR	TT	TT	XIR	TT	XIR		Histone	Serum	Alpha-	Serum
	N	P2	N	Ν	P2	N	P2		-lysine	albumin	1-	albumin
									N-		antiprot	

								methylt		einase	
								ransfera			
								se			
2	AL	TT	AL	AL	TT	AL	TT	Serum	Alpha-	Serum	Alpha-
	В	N	В	В	N	В	N	albumin	1-	albumin	2-HS-
									antiprot		glycopr
									einase		otein
3	AP	AL	Bt.	то	AL	NC	NC	Alpha-	Alpha-	Apolipo	Alpha-1-
	OA	B	105	P2	В	OA	OA	1-	2-HS-	protein	antiprot
	1		991	В		6	6	antiprot	glycopr	B-100	einase
								einase	otein		
4		KIF	AP	AA	AB	Bt.	AL	PDZ	Apolipo	Apolipo	Apolipo
	L6	7	0A	RS	CA	105	В	domain	protein	protein	protein
	A6		1	2	1	991		contain	A-I	A-I	A-I
								ng			
								protein			
		<u> </u>		СГ			De		Caratra	Alaba	Caratra
5								Apolipo	Serotra		Serotra
		160	56		52			protein	nsiemin	2-II-S-	nsiemin
	2	100					5	A-1		otein	
6	кс	Δн	SE	K B	Δн		ΔΤ	Alpha	Alpha	Pentidyl	Alpha
U U		SG		T ₁	SG	Т		2-HS-		nrolvl	2-
	2		NA	0		'	2B	alvcopr	macroal	isomera	
	5		1	Ŭ			20	otein	obulin	se	obulin
			_							domain	
										and WD	
										repeat-	
										containi	
										ng	

										protein	
										1	
7	CS	P2	AT	AT	SE	AH	AL	Comple	Alpha-	40S	A
	N1	RX	AD	AT	RPI	SG	S2	ment C	fetoprot	ribosom	fet
	Sı	5	2B	1	NA				ein	al	
					1					protein	
										S12	
8	AT	SE	AL	AP	CO	SE	LC	Throm	Apolipo	Kalirin	Ap
	AD	RPI	S2	OA	L3	RPI	Т	ospond	protein		pro
	2B	NA		1	Aı	NA		n-1	B-100		B-
		1				1					
9	AH	AP	AP	CP	HB	AA	KIF	Titin	Alpha-	Keratin,	Co
	SG	OA	OA	Sı	A	RS	7		2-	type ll	me
		1	2			2			antiplas	cytoskel	
									min	etal 1	
10	SE	HB	CO	CS	KR	AT	AB	Tight	Comple	Titin	Alı
	RPI	Α	L6	N1	T1	AD	CA	junctior	ment C3		
	NA		A6	Sı	0	2B	1	protein			ant
	1							ZO-1			n

In vitro (3)

Literature	Tenzer et al (2013)	Walkey et al (2014)	Sakulkhu et al (2014)
Nanoparticl e core	Commercial and lab-synthesized silica nanoparticles and polystyrene nanoparticles (AmSil, SiNP and PsNP)	Gold nanoparticles (GNP) and silver nanoparticles (SNP)	Superparamagnetic iron oxide nanoparticles (SPION)
Protein source	Human blood plasma	Human serum	Rat blood
Incubation	o.5 min at the earliest, o.5-480 min for	ıh	15 min

time	pro	longed expos	ure						
Techniques	Centrifu	gation follow	ed by 1D	Centrifugation followed by	Ma	agnetic separat	or		
identifying		F and tryps	in digest	(PAGE) and trypsin digest (analysed					
protein	(an	alvsed by I C-I	MS)	hy I C-MS/MS)					
corona	(un			<i>xy</i> <u>-</u> <i>c msymsy</i>					
Surface	Silica	Negatively	Positively	Various surface modifications (67	Positively	Negatively	Neutral		
chemistry	nanoparticl	charged	charged	molecules or combinations of	charged	charged	PVA		
	es	polystyren	polystyren	molecules used for surface	PVA (amino	PVA			
		е	е	modification) and sizes (15, 30 or 60	group)	(carboxyl			
		nanoparticl	nanoparticl	nm)		group)			
		es (nPsNP)	es (pPsNP)						
Cell uptake	Protein co	rona formatio	n increased	Inductively coupled plasma-atomic		NA			
	cell uptake	for fluorescer	nt silica and	emission spectroscopy (ICP-AES) was					
	pPsNP in er	ndothelial cell	s. Uptake of	used to measure nanoparticle					
	nPsNP wa	s less affected	l by corona	association with A549 human lung					
		formation		epithelial carcinoma cells. Cationic					
				gold nanoparticles associated with					
				cells greater than anionic or neutral					
				ones					
Cell toxicity	Protein coro	ona prevented	l haemolysis	NA	No toxicity o	n RAW 264.7 c	ells up to o.8		
	and rec	luced primary	human			mg Fe per mL			
	endothe	elial and micro	vascular						
	endothelial	cell death mo	stly notably						
	for	the early cord	ona						
No. of	Rising: 11	19	19	NA		NA			
unique	Falling: 8	27	15						
proteins	Peak: 12	16	20						

Top 10 most abundant proteins (3)

Literature	Те	nzer et al (20	13)	Walkey et al (2014)	Sak	ulkhu et al (20	014)
Nanoparticl	AmSil30	nPsNP (0.5	pPsNP (o.5	NA	Positively	Negatively	Neutral
e	(o.5 min	min	min		charged	charged	SPION
	exposure)	exposure)	exposure)		SPION	SPION	
1	Serum	Serum	Serum		Apolipoprot	Haptoglobi	Apolipoprot
	albumin	albumin	albumin		ein A-I	n	ein A-I
2	Apolipopro	Compleme	Apolipopro		Apolipoprot	Serotransfe	Apolipoprot
	tein A-I	nt C3	tein A-I		ein E	rrin	ein E
3	Compleme	Compleme	lg γ-1 chain		Hemoglobi	Serum	Serum
	nt C3	nt factor H	C region		n subunit	albumin	amyloid P-
					alpha-1/2		component
4	lg γ-1 chain	β2-	Inter-α-		Hemoglobi	Apolipoprot	Alpha-2-
	C region	glycoprotei	trypsin		n	ein A-ll	HS-
		n 1	inhibitor		subunit		glycoprotei
			heavy		beta-1		n
			chain H4				
5	Compleme	Kininogen-	lg μ chain		Serum	Apolipoprot	Hemoglobi
	nt factor H	1	C region		amyloid P-	ein E	n subunit
					component		alpha-1/2
6	Kininogen-	Inter-α-	lg γ-3		Coagulatio	Coagulatio	Hemoglobi
	1	trypsin	chain C		n factor X	n factor X	n subunit
		inhibitor	region				beta-1
		heavy					
		chain H4					

7	Compleme	lg γ-1 chain	lg к chain C	Alpha-2	Ficolin-1	Coagulatio
	nt C4-A	C region	region	HS-		n factor X
				glycopro	ei	
				n		
8	Ig к chain C	Vitronectin	Vitronectin	Compler	e Secreted	T-
	region			nt	phosphopro	Kininogen 2
				compone	nt tein 24	
				Cg		
9	Serotransf	Compleme	Compleme	Myosir	Fibrinogen	Serum
	errin	nt C1r	nt C3	light	alpha chain	albumin
		subcompo		polypept	de	
		nent		6		
10	Gelsolin	lg γ-3	Compleme	Prothron	bi Glyceraldeh	T-
		chain C	nt C1r	n	yde-3-	Kininogen 1
		region	subcompo		phosphate	
			nent		dehydrogen	
					ase	

In vitro (4)

Literature	Groult et al (2014)	Wan et al (2015)	Hadjidemetrious et al (2015)
Nanoparticl	Superparamagnetic iron oxide	Silica nanoparticles (SiNP)	Liposome
e core	nanoparticles (SPION)		
Protein	Rat serum	Human plasma	CD-1 mouse plasma
source			
Incubation	15, 90 or 180 min	ıh	10 min

time						
Techniques	Centrifugation follo	owed by SDS-PAGE	Centrifugation followed by SDS-PAGE	Size exclusio	n chromatogra	phy followed
for	and trypsin digest (analysed by LC-		and trypsin digest (analysed by LC-	by membrane ultrafiltration		
identifying	MS/	MS)	MS/MS)			
protein						
corona						
Surface	Micellar	Micellar	FITC	Bare	PEG	Anti-MUC-1
chemistry	phosphatidylcholi	polysorbate 8o-				antibody
	ne-coated	coated				conjugated
	superparamagnet	superparamagnet				PEG
	ic iron oxide	ic iron oxide				
	nanoparticles (PC nanoparticles					
	SPION)	(P8o SPION)				
Cell uptake	Effective intern	alization of the	The deglycosylated (removal of	Protein	Poor cell	Protein
	nanomicelles inte	o C57BL/6 mouse	glycan) protein corona coated	corona	uptake with	corona
	embryonic fibrob	lasts in a time and	nanoparticles showed higher cell	significantly	and without	significantly
	concentration-de	ependent manner	uptake in M1 and M2 macrophages	reduced cell	protein	reduced cell
				uptake in	corona in	uptake in
				MCF7 cells	MCF7 cells	MCF7 cells
				(More		(More
				pronounced		pronounced
				than in		than in
				vivo)		vivo)
Cell toxicity	Low toxicity u	p to 4o µg/mL	NA		NA	
No. of	N	A	NA	26	24	41
unique						
proteins						

Top 10 most abundant proteins (4)

Literature	Groult et al (2014)	Wan et al (2015)	Hadjid	emetrious et a	l (2015)
Nanoparticl	See DOI: 10.1002/chem.201404221	SiNP (Deglycosylated proteins after	Bare	PEGylated	Targeted
e		enzymatic deglycosylation reaction)	liposome	liposome	liposome
1		Fibrinogen alpha chain	Apolipoprot ein E	Apolipoprot ein E	Apolipoprot ein E
2		Plasminogen	Alpha-2- macroglobu lin	Fibrinogen beta chain	Fibrinogen beta chain
3		Serotransferrin	Fibrinogen beta chain	Alpha-2- macroglobu lin	Alpha-2- macroglobu lin
4		Kininogen-1	Fibrinogen gamma chain	Fibrinogen gamma chain	Fibrinogen gamma chain
5		Cartilage acidic protein 1	lg mu chain C region	Protein Fga	Protein Fga
6		Coagulation factor XII	Protein Fga	lg kappa chain C region	lg mu chain C region
7		Histidine-rich glycoprotein	Apolipoprot ein C-III	Apolipoprot ein B-100	Apolipoprot ein C-III
8		Plasma protease C1 inhibitor	Apolipoprot ein C-I	Apolipoprot ein C-III	Apolipoprot ein B-100
9		Vitronectin	Apolipoprot ein B-100	lg mu chain C region	Serum albumin

10	Vimentin	Serum	Serum	lg heavy
		albumin	albumin	chain V
				region

In vitro (5)

Literature	Pozzi et al	Sopotnik et al (2015)			Vogt et	al (2015)		
Nanoparticl e core	Liposome		Carbon black (CB), multi-walled carbon nanotubes (MWCNT) and graphene oxide sheets (GO)		Superparamagnetic iron oxide nanoparticles (SPION)			
Protein source	Fetal bov	ine serum	Hur	nan serum pro	otein	Human	plasma	
Incubation time	90 min static incubation	90 min dynamic incubation	ıh			1	ıh	
Techniques for identifying protein corona	Centrifugation followed by in-solution trypsin digestion and desalting (analysed by NanoLC-MS/MS)		Centrifugati and in-gel t	ion followed b rypsin digest (LC-ESI-MS)	y SDS-PAGE analysed by	Centrifugation followed by in-solution trypsin digestion and desalting (analysed by mass spec)		
Surface chemistry	None		СВ	MWCNT	GO	Silica	Dextran	
Cell uptake	N	IA	NA		Protein corona increased primary	Protein corona didn't affect		

				human monocyte- derived macrophage (HMDM) cell uptake	HMDM cell uptake
Cell toxicity	Ν	IA	NA	Low HMDM cell viability was observed at 100 µg/mL but completely restored with pre- formed hard protein corona	Non-toxic with or without a protein corona
No. of unique proteins	44	48	NA	N	İA

Top 10 most abundant proteins (5)

Literature	Pozzi et al (2015)		Sop	ootnik et al (20	015)	Vogt et al (2015)	
Nanoparticl e	Liposome under static conditions	Liposome under static conditions conditions		MWCNT	GO	Silica	Dextran
1	Apolipoprotein A- II	Apolipoprotein A- II	Compleme nt component C3	Transferrin	Compleme nt 9	Fibrinogen beta	Kininogen 1 microtubule associated ser/thr

2	Serum albumin	Serum albumin	Serum	Apolipopro	Inter-	Fibrinogen gamma	Kinase-like
			albumin	tein A-I	alpha-		
				precursor	trypsin		
					inhibitor		
					heavy		
					chain H4		
					precursor		
3	Alpha-2-HS-	Antitrypsin	Compleme	Proapolipo	Compleme	Fibrinogen alpha	Platelet factor 4
	glycoprotein		nt C4-A	protein	nt C3		
			protein	6			
4	Hemoglobin fetal	Alpha-2-HS-	Transferrin	Serum	Iransferrin	Vitronectin	Actin, beta
	subunit beta	giycoprotein					
5	Antitrypsin	Hemoglobin fetal	Ig G1 H Nie	Vitamin K-	Compleme	Histidine-rich	Integrin, alpha 2b
		subunit beta		dependent	nt	giycoprotein	
				protein S	Component		
					Co alpina		
6	Inter alpha	Apolipoprotoin C	Compleme		Cindin	Thrombosponding	Bro platalat basis
0	trypsin inhibitor		compleme nt	iy yanina-	complettie		protoin
	heavy chain Ha	111	component	region	component		protein
			Component	region	C8 heta		
			C3		chain		
7	Apolipoprotein C-	Apolipoprotein C-	Trypsin	Clusterin	Compleme	Otoferlin	Kallikrein B.
,			precursor	Closterin	nt C1s		plasma 1
			P		subcompo		P
					nent		
8	Apolipoprotein C-	Apolipoprotein A-	Compleme	Inter-	Compleme	Coagulation factor	Lactotransferrin
			nt C1q	alpha-	, nt	XII	
			subcompo	trypsin	component		
			nent	inhibitor	C7		

			subunit B	heavy			
				chain H2			
9	Alpha-2-	Hemoglobin	Keratin	Trypsin	Apolipopro	Complement	Glycoprotein lb
	macroglobulin	subunit beta		inhibitor	tein E	4A/4B	(platelet)
10	Protein AMBP	Hemoglobin	Clusterin	Apolipopro	Compleme	Complement	Integrin, beta 3
		subunit alpha		tein J	nt	factor H-related 1	
					component		
					C3		

In vitro (6)

Literature	Raesch et al (2015)	Schöttler et al (2016)	Saha et al (2016)
Nanoparticl	Oleic acid-coated primary magnetite	Polystyrene nanoparticle (PS)	Gold nanoparticles (AuNP)
e core	nanoparticles		
Protein	Native porcine surfactant	Human plasma	Human serum (10 % and 50 %)
source			
Incubation	ıh	ıh	ıh
time			
Techniques	Repeated magnetic separation and	Centrifugation followed by SDS-PAGE	Centrifugation followed by LC-MS/MS
for	centrifugation followed by 1D	and trypsin digest (analysed by LC-	procedure
identifying	polyacrylamide gel electrophoresis	MS)	
protein	(1D-PAGE), and trypsin digest		
corona	(analysed by LC-MS)		

Surface chemistry	PLGA	PEG	Lipid	Amine	PEG (Degree of polymeriza tion: 44 and 110)	PEEP (Degree of polymeriza tion: 49 and 92)	Thiolated ligand with increasing hydrophobicity
Cell uptake		NA		High RAW and PEEP fu without pro with protein RAV	264.7 cell upta nctionalized n tein corona bu corona. Clust V264.7 cell up	ake of PEG anoparticles ut no uptake erin reduces take	Lower cell (RAW264.7) uptake for hydrophobic NPs compared to hydrophilic NPs with protein corona. High nonspecific uptake for all NPs without protein corona. Specifically, complement proteins in the corona increased cell uptake while immunoglobulins showed the opposite effect
Cell toxicity	/ NA				NA		Non-toxic at the concentration of 50 nM for all AuNPs
No. of unique proteins		NA			NA		NA

Top 10 most abundant proteins (6)

Literature	Raesch et al (2015)	Schöttler et al (2016)	Saha et al (2016)

Nanoparticl e	PLGA-NP	PEG-NP	Lipid-NP	Amine- functionali zed PS	PEG ₄₄ -PS	PEEP ₄₉ -PS	See DOI: 10.1021/acsnano.6booo53
1	Tubulin alpha-4A chain	Tubulin alpha-4A chain	Tubulin alpha-4A chain	Serum albumin	Clusterin	Clusterin	
2	Actin, cytoplasmi c 1	Actin, cytoplasmi c 1	Actin, cytoplasmi c 1	Fibrinogen beta chain	Apolipopro tein A-I	Fibronectin	
3	Hemoglobi n subunit beta	Hemoglobi n subunit beta	Hemoglobi n subunit beta	Fibrinogen gamma chain	Vitronectin	Apolipopro tein A-IV	
4	L-xylulose reductase	L-xylulose reductase	L-xylulose reductase	Vitronectin	Apolipopro tein C-III	Serum amyloid P- component	
5	Tubulin beta-4B chain	Tubulin beta-4B chain	Myosin-9	Fibrinogen alpha chain	Fibrinogen beta chain	Apolipopro tein C-III	
6	Tubulin alpha-1A chain	Tubulin alpha-1A chain	Tubulin beta-4B chain	Clusterin	Fibronectin	Vitronectin	
7	Deleted in malignant brain tumors 1 protein	Tubulin beta chain	Pulmonary surfactant- associated protein A	lg gamma- 1 chain C region	Fibrinogen gamma chain	Apolipopro tein D	
8	Tubulin beta chain	Myosin-9	Deleted in malignant brain tumors 1	lg kappa chain C region	Serum albumin	Compleme nt C1q subcompo nent	

			protein			subunit B
9	Pulmonary	Fibronectin	Tubulin	Apolipopro	Ig kappa	lg mu chain
	surfactant-		alpha-1A	tein C-III	chain C	C region
	associated		chain		region	
	protein A					
10	Myosin-9	Glyceralde	Tubulin	Apolipopro	Compleme	lg kappa
		hyde-3-	beta chain	tein A-I	nt C1q	chain C
		phosphate			subcompo	region
		dehydroge			nent	
		nase			subunit C	

In vitro (7)

Literature	Koshkina et al (2016)	Corbo et al (2016)	Wang et al (2016)
Nanoparticl	Poly(organosiloxane) nanoparticles	Liposome	CdSe/ZnS qantum dots (QDs)
e core	(POS)		
Protein	Fetal calf serum	BALB/C mice blood plasma	PBS solution of human serum
source			
Incubation	ıh	1 h	10 min
time			
Techniques	Centrifugation followed by 1D SDS-	Centrifugation followed by 1D SDS-	Centrifugation followed by SDS-PAGE
for	PAGE and in-solution trypsin digest	PAGE and in-gel trypsin digest	and in-gel trypsin digest (analysed by
identifying	(analysed by LC-MS)	(analysed by LC-MS)	MALDI-ToF/MS)
protein			
corona			

Surface	Negativ	Positive	Poly(et	Poly(2-	None	Bidentate	Zwitterioni	Poly(ethyle
chemistry	ely	ly	hylene	ethyl-2-		anionic	c D-	ne glycol)
	charge	charge	glycol)	oxazoli		dihydrolipoi	penicillamin	with
	d POS	d POS	(PEG)	ne)		c acid	e (DPA)	cationic
	(СООН	(NEt ₃)		(PEtOx)		(DHLA)		amino
)							group
								(PEG)
Cell uptake	Cell uptake of POS-PEG and POS- PEtOx was much lower than that of POS-COOH and POS-NEt ₃ . Protein corona decreased cell uptake for all four NPs, and POS-PEtOx was the most efficient to avoid protein		d POS- o that of Protein re for all was the otein	Protein corona facilitated liposome cell uptake by both J774 mouse macrophage and 4T1 mouse mammary tumor cells		NA		
Coll toxicity			ings A		ΝΙΔ		ΝΙΔ	
	NA			INA		INA		
No. of		N	A		NA		NA	
unique								
proteins								

Top 10 most abundant proteins (7)

Literature	Koshkina et al (2016))	Corbo et al (2016)	Wang et al (2016)
Nanoparticl e	POS- COOH	POS- NEt ₃	POS- PEG	POS- PEtOx	Liposome	NA

1	Serum	Apolipo	Serum	Serum	Serum albumin	
	albumi	protein	albumi	albumi		
	n	A-II	n	n		
2	Hemogl	Hemogl	Hemogl	Hemogl	Apolipoprotein-E	
	obin	obin	obin	obin		
	subunit	subunit	subunit	subunit		
	alpha	alpha	alpha	alpha		
3	Alpha-	Apolipo	Alpha-	Alpha-	lg mu chain C region	
	2-HS-	protein	2-HS-	2-HS-		
	glycopr	A-I	glycopr	glycopr		
	otein		otein	otein		
4	Apolipo	Serum	Alpha-	Alpha-	Apolipoprotein A-IV	
	protein	albumi	1-	1-		
	A-I	n	antipro	antipro		
			teinase	teinase		
5	Alpha-	Alpha-	Hemogl	Hemogl	Myosin	
	1-	1-	obin	obin		
	antipro	antipro	fetal	fetal		
	teinase	teinase	subunit	subunit		
			beta	beta		
6	Apolipo	Hemogl	Actin,	Apolipo	Alpha-2 macroglobulin	
	protein	obin	cytopla	protein		
	A-II	fetal	smic 2	A-II		
		subunit				
		beta				
7	Hemogl	Apolipo	Serotra	Serotra	Serotransferrin	
	obin	protein	nsferrin	nsferrin		
	fetal	C-III				
	subunit					
	beta					

8	Beta-2-	Apha-	Apolipo	Actin,	Apolipoprotein A-I
	glycopr	2-HS-	protein	cytopla	
	otein-1	glycopr	A-II	smic 2	
		otein			
9	Vitrone	Apolipo	Alpha-	Alpha-	Phospholipid transfer protein
	ctin	protein	2-	2-	
		D	macrog	macrog	
			lobulin	lobulin	
10	Serotra	Coagul	Comple	Apolipo	Hemoglobin sub beta-1
	nsferrin	ation	ment	protein	
		factor	C3	C-III	
		XIII, B			
		polype			
		ptide			

In vitro (8)

Literature	Chen et al (2016)		e Chen et al (2016) Pisani et al		Pisani et al (2017)	Zhu et al (2017)
Nanoparticl	Superparamagnetic iron oxide (SPIO)		Magnetic mesoporous silica NPs (M-	Hydroxyapatite (HA) and magnetic		
e core	nanoworms		MISINS)	light oxyapatite (MIRA) scallolus		
Protein source	Human plasma	Human serum	Human serum and fetal bovine serum	100 % fetal bovine serum (100% FBS), extracellular secretion (ES) of MC3T3-E1 cells and the combination of 10% FBS, and extracellular secretion (FBS+ES)		
Incubation time	1	h	From 0.5 min to 7 days	24 h		

Techniques for identifying protein corona	Ultracentrifugation followed by SDS- PAGE and trypsin digest (analysed by Nano LC-MS)	NP-protein corona complexes were magnetically separated and followed by NuPAGE gel electrophoresis and trypsin digest (analysed by Nano LC- MS/MS)	Hard corona was collected by SDT lysis buffer, boiled for 10 min at 99 °C. Protein in the supernatant was separated by 1D-SDS-PAGE and followed by trypsin digest (analysed by
Surface chemistry	Dextran	None	UC-MS/MS) With and without magnetic Fe ₃ O ₄ nanoparticles
Cell uptake	Higher cell (leukocyte) uptake of SPIO nanoworms in serum than in plasma. 10 mM EDTA significantly reduced cell uptake in both serum and plasma	NA	NA
Cell toxicity	NA	NA	NA
No. of	NA	NA	NA
unique proteins			

Top 10 most abundant proteins (8)

Literature	Chen et al (2016)		Chen et al (2016) Pisani et al (2017)		Zhu et a	Zhu et al (2017)	
Nanoparticl	SPIO nanoworms	SPIO nanoworms	See DOI: 10.1039/C6NR04765C	HA in 100% FBS	MHA in 100% FBS		
e	in plasma	in serum					
1	Complement C ₃	Apolipoprotein B-		Serum albumin	Serum albumin		
		100					

2	Fibrinogen beta chain	Serum albumin	Serotransferrin	Serotransferrin
3	Fibrinogen alpha chain	Complement C ₃	Alpha-2-HS- glycoprotein	Alpha-2-HS- glycoprotein
4	Fibronectin	Apolipoprotein A	Alpha-1- antiproteinase	Apolipoprotein A
5	Myosin-9	Serotransferrin	Alpha-fetoproteir	Alpha-1- antiproteinase
6	Filamin-A	Alpha-2- macroglobulin	Apolipoprotein A-	Alpha-fetoproteir
7	Talin-1	Fibronectin	Alpha-2- macroglobulin	Alpha-2- macroglobulin
8	Fibrinogen gamma chain	Complement C4- B	Hemoglobin subunit alpha	Vitamin D binding protein
9	Thrombospondin- 1	Plasma kallikrein	Vitamin D-binding protein	Transthyretin
10	Serum albumin	Complement factor B	Hemoglobin fetal subunit beta	Antithrombin-III

In vitro (9)

Literature	Gao et a	I	_undqvist	et al (2017	7)	Bonvin et al (2017)		
Nanoparticl e core	Silver nanopa		Silica nan	oparticles		Iron oxide nanoparticles		
Protein source	Smallmouth bass plasma (male)	Smallmouth bass plasma (female)	Whole blood	Whole blood with EDTA	Plasma	Serum	Human blood (flow rate: 1400 and 20 rpm)	Lymph serum (flow rate: 1400 and 20 rpm)

Incubation	1	h	24	h h	5 min for whole blood and 2 h for	24 h
time					other samples	
Techniques	Centrifug	jation follo	owed by SI	DS-PAGE	Centrifugation followed by 1D SDS-	Magnet to collect nanoparticle-protein
for	and in-gel trypsin digest (analysed by				PAGE and in-gel trypsin digest	corona complexes followed by multiple
identifying	LC-MS/MS)				(analysed by MALDI-TOF MS)	centrifugation and SDS-PAGE in-gel
protein						digest (analysed by LC-MS)
corona						
Surface	PVP				None	None
chemistry						
Cell uptake		NA			NA	NA
Cell toxicity	NA				NA	NA
No. of	Male 1 Female Male 24 Female		Female	NA	NA	
unique	h: 1 h: h: 64 24 h: 26		24 h: 26			
proteins	67	19				

Top 10 most abundant proteins (9)

Literature	Gao et al (2017)				Lundqvist et al (2017)	Lundqvist et al (2017) Bonvin et al (
Nanoparticl	AgNP	AgNP	AgNP	AgNP	NA	Human	Human	Lymph	Lymph
e	in	in	in	in		blood at	blood at	serum	serum
	male	female	male	female		1400	20 rpm	at 1400	at 20
	plasma	plasma	plasma	plasma		rpm		rpm	rpm
	for 1 h	for 1 h	for 24 h	for 24 h					

1	Immun	Immun	Immun	Immun	Ar	Apolipo	Apolipo	Apolipo	Fib
	oglobul	oglobul	oglobul	oglobul	pr	orotein	protein	protein	en
	in light	in light	in	in light	В	B-100	B-100	B-100	c ł
	chain	chain	heavy	chain					
			chain						
			variable						
			region						
2	Immun	Hemogl	Immun	Immun	Co	Comple	Comple	Comple	Fibr
	oglobul	obin	oglobul	oglobul	me	nent C3	ment C3	ment C ₃	en a
	in	embryo	in M	in					ch
	heavy	nic	heavy	heavy					
	chain	subunit	chain	chain					
	variable	alpha		variable					
	region			region					
3	Immun	Hemogl	Immun	Immun	S	Serum	Comple	Serum	Fibr
	oglobul	obin	oglobul	oglobul	alt	lbumin	ment	albumin	e e
	in M	subunit	in light	in M			C4-A		gan
	heavy	alpha-1	chain	heavy					ch
	chain			chain					
4	Immun	Immun	Immun	Immun	Co	Comple	Apolipo	Comple	Ser
	oglobul	oglobul	oglobul	oglobul	r	ment	protein	ment	albu
	in mu	in M	in	in mu	(C4-A	A-I	C4-A	
	heavy	heavy	mu/tau	heavy					
	chain	chain	heavy	chain					
			chain						
5	Fibrino	Immun	Comple	Hemogl	Co	Comple	Serum	Alpha-	Cor
	gen	oglobul	ment	obin	r	ment	albumin	2-	men

	beta chain	in mu heavy chain	C3	subunit alpha-1	C4-B		macrogl obulin	
6	Hemogl obin subunit beta	Comple ment C ₃	Hemogl obin subunit alpha-1	Apolipo protein E	Apolipo protein A-I	Prothro mbin	Apolipo protein A-I	Fibrone ctin
7	Beta- fibrinog en	Comple ment C8 beta	lmmun oglobul in mu heavy chain	Immun oglobul in mu/tau heavy chain	Apolipo protein E	Apolipo protein E	Apolipo protein E	Apolipo protein B-100
8	Comple ment C8 beta	Immun oglobul in heavy chain variable region	Comple ment C8 beta	Comple ment C ₃	Antithro mbin-III	Antithro mbin-III	Antithro mbin-III	Comple ment C4-A
9	Immun oglobul in kappa chain V region Mem5	Apolipo protein E	Fibrino gen beta chain	Comple ment C8 beta	Alpha- 2- macrogl obulin	Plasmin ogen	Histidin e-rich glycopr otein	Comple ment factor H
10	Hemogl obin embryo	Alpha- 2- macrog	Apolipo protein E	Hemogl obin subunit	Histidin e-rich glycopr	Vitronec tin	Plasmin ogen	Plasmin ogen

nic	lobulin	alpha-A	otein		
subunit					
alpha					

In vitro (10)

Literature	Kokkin	iopoulou et a	(2017)	W	ang et al (201	17)	Müller et al (2017)					
Nanoparticl	Polystyrer	ne nanoparticl	es (PS-NP)	Iron oxide nanoparticles (IONPs)			Po	lystyre	ne clos	Magr	Magnetite	
Protein	Human serum			Human plasma			Huma	in S	heep	Mouse	Rabbit	
source					I		plasm	na pl	lasma	plasma	plasma	
Incubation time	1 h,	multiple wasł	ning		24 h				1	h		
Techniques for	Multiple of followed by	entrifugation	and wash d in-solution	Centrifuga PAGE ar	tion followed	by 1D SDS- in digest	Centrifugation followed by SDS-PAGE				S-PAGE	
identifying	trypsin digest (analysed by LC-MS)			(analysed by LC-MS/MS)			LC-MS)					
corona												
Surface chemistry	Unfunction alizedNegatively chargedPositively chargedpolystyren epolystyrenpolystyreneeenanoparticl es (PS)nanoparticl es (PS- CCOH)nanoparticl NH2)			Bare	Bare Brushed Brushed polyethyle phosphoryl ne glycol choline (bPEG) (bPC)			Carb oxyl- end grou p (PS- COO H)	Ami no- end grou p (PS- NH ₂)	Dextran (M- DEX)	Hydroxy ethyl starch (HES)	
Cell uptake	Cellular (R corona coa	AW264.7) upt ated nanopart	ake of soft icles lower	NA			Coated with human plasma proteins, cell (murine macrophage cell line RAW264.7)					

	than hard corona and uncoated nanoparticles. Lower cellular uptake of PS and PS-NH2 than PS-COOH				uptake of polystyrene nanoparticles decreased. On the contrary, cell uptake increased when polystyrene nanoparticles were coated with mouse plasma proteins				
Cell toxicity	NA		NA			N	А		
No. of	NA	13	8	9	PS-NP	Sheep	Mouse	Rabbit	
unique					in	plasma:	plasma:	plasma:	
proteins					human	7	78	17	
					plasma:				
					48				
					M-DEX	Sheep	Mouse	Rabbit	
					in	plasma:	plasma:	plasma:	
					human	14	80	4	
					plasma:				
					57				

Top 10 most abundant proteins (10)

Literature	Kokkinopoulou et al (2017)	W	ang et al (201	.7)	Müller et al (2017)
Nanoparticl	See DOI: 10.1039/C7NR02977B	Bare IONPs	bPEG-	bPC-IONPs	See DOI: 10.1021/acs.biomac.7b01472
e			IONPs		
1		Serum	Serum	lg heavy	
		albumin	albumin	constant	
				mυ	
2		Compleme	Vitronectin	Apolipopro	
		nt C3		tein B-100	

3	Vitronectin	Compleme	Serum
		nt C3	albumin
4	lg heavy	Fibronectin	Vitronectin
	constant		
	gamma 1		
5	Coagulatio	lg heavy	Ig kappa
	n factor XI	constant	variable 3-1
		mu	1
6	Kininogen-	lg heavy	Apolipopro
	1	constant	tein(a)
		gamma 1	
7	Compleme	Kininogen-	Compleme
	nt C1q	1	nt C3
	subcompo		
	nent		
	subunit C		
8	Apolipopro	Coagulatio	lg heavy
	tein B-100	n factor XI	constant
			gamma 1
9	Compleme	Compleme	Fibronectin
	nt C4-A	nt C1q	
		subcompo	
		nent	
		subunit C	
10	Ig kappa	Histidine-	Alpha-2-
	constant	rich	HS-glyco
		glycoprotei	protein
		l n	

In vitro (11)

Literature		Simo	on et al (2018)		V	/ang et al (20:	18)	Castagnola	a et al (2018)	
Nanoparticl	Po	lystyren	e nanop	articles (PS)	Nano-	graphene oxid	e (nGO)	Graphene	nanoflakes	
e core										-	
Protein		Hυ	man pla	sma		Human plasma			Human serum (10	Fetal bovine serum	
source									% and 100 %)	(10 % and 100 %)	
Incubation		ıh					24 h		1-4 h under u	ultrasonication	
time											
Techniques	Cer	ntrifugat	ion follo	wed by S	SDS-	Centrifuga	ation followed	by 1D SDS-	A series of washin	ng by centrifugation	
for	P	AGE and	l in-solu [.]	tion dige	est	PAGE a	nd in-gel tryps	sin digest	followed by 1D SE	DS-PAGE and in-gel	
identifying		(analy	rsed by L	.C-MS)		(analysed by LC-MS/MS)			trypsin digest (ana	lysed by LC-MS/MS)	
protein											
corona											
Surface	Ami	PEGy	Hydr	Copol	Hydr	Bare	Polyethyle	Poly(2-	N	one	
chemistry	no-	lation	ophili	ymer	opho		ne glycol	ethyl-2-			
	funct	(PS-	С	s (PS-	bic		(PEG)	oxazoline)			
	ional	PEG)	poly	P(1 ₄₂ -	copol			(PEtOx)			
	ized		mer	co-2 ₄)	ymer						
	PS-		(PS-		s (PS-						
	NH₂)		P(1) ₄₅		P(1 ₃₁ -						
)		CO-						
		<u> </u>			2 ₁₅)						
Cell uptake	With	plasma p	protein c	oatings,	strong		NA		NA		
	cell	ular (RA	W 264.7) uptake	was						
	observed for positively charged			ged							
	nanocarriers and weaker cellular			lular							
	uptak	e observ	ed for P	S-PEG a	nd PS-						
	P(1	L) ₄₅ . With	i increas	ing polyi	mer						

	hydrophobicity, cellular uptake was strongly enhanced				
Cell toxicity	Non-toxic up to 75 µg/mL in RAW 264.7 cells	Lower toxic PEtOx thar Non-toxic f below 100 µ red blood ce toxicity for	ity of nGO-PE n bare nGO at for all three ty g/mL on HEK Ils. Protein con all three types red blood cells	G and nGO- 100 µg/mL. pes of nGO 293 cells and rona reduced s of nGO on	NA
No. of unique proteins	NA	11	2	4	NA

Top 10 most abundant proteins (11)

Literature	Simon et al (2018)	W	ang et al (201	.8)	Castagnola et al (2018)	
Nanoparticl	See DOI: 10.1002/anie.201800272	Bare nGO	nGO-PEG	nGO-	Graphene	Graphene
е				PEtOx	nanoflakes	nanoflakes
					exfoliated in 100 %	exfoliated in 100 %
					human serum	fetal bovine serum
1		Serum	Fibrinogen	Fibrinogen	Serum albumin	Serum albumin
		albumin	alpha chain	alpha chain		
2		Fibrinogen	Fibrinogen	Fibrinogen	Apolipoprotein A-I	Hemoglobin
		gamma	gamma	beta chain		subunit alpha
		chain	chain			
3		Compleme	Fibrinogen	Fibrinogen	Apolipoprotein E	Apolipoprotein A-I
		nt C4-B	beta chain	gamma		
				chain		

4	Inter-	Inter-	Inter-	Vitronectin	Hemoglobin
	alpha-	alpha-	alpha-		subunit beta
	trypsin	trypsin	trypsin		
	inhibitor	inhibitor	inhibitor		
	heavy	heavy	heavy		
	chain H4	chain H4	chain H4		
5	lg heavy	lg heavy	lg heavy	Alpha-1-	Alpha-2-HS-
	constant	constant	constant	antitrypsin	glycoprotein
	gamma 1	gamma 1	gamma 1		
6	Fibrinogen	Apolipopro	Kininogen-	Apolipoprotein A-	Histone H4
	alpha chain	tein A-I	1	IV	
7	Fibrinogen	Gelsolin	Gelsolin	Hemoglobin	Apolipoprotein E
	beta chain			subunit beta	
8	Compleme	Serum	Compleme	Apolipoprotein A-II	Alpha-1-
	nt C3	albumin	nt C3		antiproteinase
9	Gelsolin	Kininogen-	Vitronectin	Complement C3	Complement C ₃
		1			
10	Apolipopro	Apolipopro	Apolipopro	L-lactate	Alpha-2-
	tein B-100	tein B-100	tein B-100	dehydrogenase A	macroglobulin
				chain	

In vitro (12)

Literature	Tavano et al (2018)				
Nanoparticle	Organically modified silica				
core	nanoparticles				
Protein	Human sera	Mouse sera			

source								
Incubation	15 min							
time								
Techniques	Sho	t-gun	proteo	mics:				
for identifying	Centrifu	gation	follow	ved by in-				
protein	solutio	n diges	st and c	lesalting				
corona	(an	alyzed	by LC-	-MS)				
	MS sp	ectron	netry a	nalysis:				
	Centrifug	gation f	followe	ed by SDS-				
	PAGE and	in-gel o	digest (analyzed by				
			-IVIS)					
Surface	Uncoate	Poly(ethyl	Poly(2-				
chemistry	d	ene g	Iycol)	methyl-2-				
	(PEG) oxazoli							
				(PMOXA)				
Cell uptake	PMOX/	PMOXA and PEG-coated NPs						
I I	exhibited	l highe	r uptak	ke in blood				
	phao	gocytes	, s and h	uman				
	macropha	ages th	an unc	oated NPs.				
	Wherea	as PMO	XA-co	ated NPs				
	showed	lower	uptake	in mouse				
	monocyte-derived macrophages							
	than uncoated NPs.							
	Human sera from 8 individual							
	donors pro	moted	efficie	nt uptake of				
	PMOXA	A-coate	ed NPs.	Variable				
	macropha	age upt	ake wa	as observed				

	for PEGylated NPs
Cell toxicity	Very low percentage of hemolysis of human erythrocytes and low toxicity on monocyte-derived human macrophages for all three types of NPs
No. of unique proteins	NA

Top 10 most abundant proteins (12)

Literature	Tavano et al (2018)
Nanoparticl	See DOI: 10.1021/acsnano.8bo1806
е	
1	
2	
3	
4	
5	
6	
7	
8	

10	9
	10

Table S2. In vivo protein corona, extracted from relevant literature as indicated.

In vivo (1)

Literature	Sakulkhu et al (2014) (in vitro vs in vivo)	Hadjidemetriou et al (2015) (in vitro vs in vivo)	Hadjidemetriou et al (2016) (in v only)		
Nanoparticl e core	Superparamagnetic iron oxide nanoparticles (SPION)	Liposome	Liposome		
Protein source	Rat sera	CD-1 mouse plasma	CD-1 mouse plasma		
Incubation time	15 min	10 min	10 min	ıh	3 h
Technique of separating	Magnetic separator	Size exclusion chromatography followed by membrane ultrafiltration	Size exclusion chromatograph followed by membrane ultrafiltra		ography trafiltration

nanoparticl e-PC complex from free proteins									
Surface chemistry	Positively charged PVA (amino group)	Negatively charged PVA (carboxyl group)	Neutral PVA	Bare	PEG	Anti- MUC-1 antibody conjugate d PEG	Doxorul	picin-encapsula	ated PEG
Cell uptake		NA			Poor cell uptake with and without protein corona in MCF7 cells	Protein corona reduced cell uptake in MCF7 cells	NA		
Cell toxicity	No toxicity on RAW 264.7 cells up to 0.8 mg Fe per mL			NA			NA		
No. of unique proteins	32	51	55	12	18	34	90	25	35

Top 10 most abundant proteins (1)

Literature	Sakulkhu et al (2014) (in vitro vs in	Hadjidemetriou et al (2015) (in vitro	Hadjidemetriou et al (2016) (in vivo
	vivo)	vs in vivo)	only)

Nanoparticl	Positively	Negatively	Neutral	Bare	PEGylated	Targeted	10 min	ıh	3 h
e	charged	charged	PVA	liposome	liposome	liposome	Doxorubici	Doxorubici	Doxorubici
	PVA	PVA					n-	n-	n-
							encapsulate	encapsulate	encapsulate
							d	d	d
							PEGyalted	PEGyalted	PEGyalted
							liposome	liposome	liposome
1	Hemoglobi	Hemoglobi	Fibrinogen	Apolipoprot	Apolipoprot	Apolipopr	Alpha-2-	Apolipoprot	Hemoglobi
	n	n subunit	alpha chain	ein C-III	ein C-III	otein E	macroglobu	ein E (PE=2	n subunit
	subunit	beta-2					lin	SV=1)	beta-1
	beta-2								
2	Hemoglobi	Hemoglobi	Fibrinogen	Apolipoprot	Apolipoprot	Apolipopr	Apolipoprot	Alpha-2-	Apolipoprot
	n subunit	n subunit	beta chain	ein E	ein E	otein C-III	ein C-III	macroglobu	ein E (PE=2
	alpha-1/2	alpha-1/2						lin	SV=1)
3	Hemoglobi	Hemoglobi	Hemoglobi	Haemoglob	Hemoglobi	Alpha-2-	Hemoglobi	Apolipoprot	Apolipoprot
	n subunit	n subunit	n subunit	in subunit	n subunit	macroglo	n subunit	ein C-III	ein C-III
	beta-1	beta-1	alpha-1/2	beta-1	beta-1	bulin	beta-1		
4	Apolipopro	Apolipopro	Fibrinogen	Beta-globin	Alphaglobi	Haemoglo	Apolipoprot	Serum	Alpha-2-
	tein E	tein A-ll	gamma		n 1	bin	ein E (PE=1	albumin	macroglobu
			chain			subunit	SV=2)		lin
						beta-1			
5	Fibrinogen	Apolipopro	Hemoglobi	Alpha-2-	Alpha-2-	Apolipopr	Beta-	Apolipoprot	Beta-
	alpha chain	tein E	n subunit	macroglobu	macroglobu	otein C-IV	globin,	ein E (PE=1	globin,
			beta-1	lin	lin		Hbbt1	SV=2)	Hbbt1
							(A8DUK2)		(A8DUK ₂)
6	Secreted	Coagulatio	Secreted	Alphaglobi	Haemoglob	lg mu	Apolipoprot	Hemoglobi	Hemoglobi
	phosphopr	n factor X	phosphopr	n 1	in subunit	chain C	ein A-I	n subunit	n subunit
	otein 24		otein 24		beta-2	region		beta-1	beta-2

7	Compleme	Ficolin-1	Apolipopro	lg mu chain	lg mu chain	Harmoglo	Hemoglobi	Apolipoprot	Alpha-
	nt C3		tein A-II	C region	C region	bin	n subunit	ein A-I	globin
						subunit	beta-2		
						beta-2			
8	Fibrinogen	Secreted	Apolipopro	Serum	Beta-globin	Serum	Alpha-	Serine	Apolipoprot
	beta chain	phosphopr	tein A-I	albumin		albumin	globin	protease	ein A-I
		otein 24						inhibitor	
								A3K	
9	Matrix Gla	Fibrinogen	Coagulatio	Hemoglobi	Apolipoprot	Alphaglob	lg mu chain	lg mu chain	Fibrinogen
	protein	alpha chain	n factor X	n subunit	ein C-IV	in 1	C region	C region	beta chain
				beta-2					
10	Serum	Glyceralde	Apolipopro	Apolipoprot	Ig kappa	lg kappa	Putative	Hemoglobi	Fibrinogen
	albumin	hyde-3-	tein E	ein A-I	chain C	chain C	uncharacter	n subunit	gamma
		phosphate					ized protein	beta-2	chain
		dehydroge							
		nase							

In vivo (2)

Literature	Corbo et al (2017) (in vivo only)		Zhu et al (2017) (in vitro vs in vivo)	Bertrand et al (2017) (in vivo only)
Nanoparticl	Liposome	Leukosome	Hydroxyapatite (HA) and magnetic	PEG-PLGA nanoparticles
e core			hydroxyapatite (MHA) scaffolds	
Protein	Healthy BALB/c mice blood		Female SD rats blood	Male mice or Sprague-Dawley rats
source				blood
Incubation	10 min and 1 h		24 h	15 min
time				
Technique	Centrifugation		Boiled in SDT lysis buffer and	Size exclusion chromatography
of	_		supernatants collected	followed by membrane ultrafiltration
separating				

nanoparticl							
e-PC							
complex							
from free							
proteins							
Surface		No	one		Without magnetic	With magnetic	PEG chains with different density (15,
chemistry			Fe ₃ O ₄	Fe ₃ O ₄	18, 25 and 45 chains per 100 nm ²)		
_			nanoparticles	nanoparticles			
Cell uptake	Protein	corona	Protein	corona	N	A	NA
	increas	ed cell	decrea	sed cell			
	uptake uptake						
Cell toxicity	NA		NA		NA		
No. of	10 min:	1 h:	10 min:	1 h:	99	138	NA
unique	14	27	11	11			
proteins							

Top 10 most abundant proteins (2)

Literature	Corbo et al (2017) (in vivo only)			only)	Zhu et al (2017) (in vitro vs in vivo)		Bertrand et al (2017) (in vivo only)
Nanoparticl	10 min	ıh	10 min	10 min	HA	MHA	NA
	Liposo me	Liposo me	Leukos ome	Leukos ome			

		I .	1	I .		
1	Vitrone	Fibrino	Vitrone	Fibrino	Hemoglobin	Hemoglobin
	ctin	gen	ctin	gen	subunit beta-1	subunit beta-1
		gamma		gamma		
		chain		chain		
2	Serum	Fibrino	lg mu	Fibrino	Protein Hbb-b1	Protein Hbb-b1
	amyloi	gen	chain C	gen,		
	d P-	beta	region	alpha		
	compo	chain		polype		
	nent			ptide		
3	lg mu	lg mu	Fibrone	Fibrino	Protein Hba-a2	Protein Hba-a2
	chain C	chain C	ctin	gen		
	region	region		beta		
				chain		
4	Actin,	Actin,	Serum	Serum	Hemoglobin	Hemoglobin
	cytopla	cytopla	amyloi	albumi	subunit beta-2	subunit beta-2
	smic 1	smic 1	d P-	n		
			compo			
			nent			
5	Fibrone	Fibrino	Hemogl	lg mu	Hemoglobin	Serum albumin
-	ctin	gen,	obin	chain C	subunit alpha-1/2	
		alpha	subunit	region		
		polype	beta-1	5		
		ptide				
6	Serum	Serum	Plasmin	Actin,	Serum albumin	Hemoglobin
	albumi	albumi	ogen	cytopla		subunit alpha-1/2
	n	n		smic 1		
7	Hemogl	Apolipo	Serum	Vitrone	Zero beta-globin	Beta-glo
	obin	protein	albumi	ctin	(Fragment)	
	subunit	A-I	n			
	alpha					

8	Hemogl	Myosin	Fibrino	Apolipo	Epsilon 1 globin	Epsilon 1 globin
	obin	-9	gen	protein		
	subunit		gamma	A-I		
	beta-1		chain			
9	Plasmin	Hemogl	Hemogl	Serum	Rat haemoglobin	Protein Myh1
	ogen	obin	obin	amyloi	beta-chain	
		subunit	subunit	d P-		
		beta-1	beta-2	compo		
				nent		
10	lg	Plasmin	lg	Plasmin	Histone H4	Histone H ₂ A
	kappa	ogen	heavy	ogen		
	chain C		chain V			
	region		regions			

In vivo (3)

Literature	Garcia-Alvarez et al (2018) (in vivo						
	only)						
Nanoparticl	Gold Gold Gold Go						
e core	nanoro	nanost	nanoro	nanost			
	d 40	ar 40	d 70 nm	ar 70			
	nm	nm	(NR 70)	nm			
	(NR40) (NS40) (NS7						
Protein	CD-1 female mice						
source							
Incubation		10	min				

time								
Technique	Size exclusion chromatography							
of	followed by membrane ultrafiltration							
separating								
nanoparticl								
e-PC								
complex								
from free								
proteins								
Surface	Polyethylene glycol							
chemistry								
Cell uptake	NA							
Cell toxicity		N	IA					
No. of	(The	NS40:	NR70:	NS70:				
unique	effect	224	43	33				
proteins	of size)							
	NR40:							
	95							
	(The NS40: NR 70: NS70:							
	effect 143 69 38							
	of 'S S S							
	shape)							
	NR40:							
	35							

Top 10 most abundant proteins (3)

Literature	Garcia-Alvarez et al (2018) (in vivo only)						
Nanoparticl	NR40	NS40	NR70	NS70			
e							
1	Serum	Serum	Serum	Serum			
	albumi	albumi	albumi	albumi			
	n	n	n	n			
2	Alpha-	Alpha-	Alpha-	Alpha-			
	2-	2-	2-	2-			
	macrog	macrog	macrog	macrog			
	lobulin	lobulin	lobulin	lobulin			
3	Fibrino	Serine	Serine	Serine			
	gen	proteas	proteas	proteas			
	beta	е	е	е			
	chain	inhibito	inhibito	inhibito			
		r A3K	r A3K	r A3K			
4	Apolipo	Fibrino	Apolipo	Fibrino			
	protein	gen	protein	gen			
	A-I	beta	A-I	beta			
		chain		chain			
5	Comple	Apolipo	Fibrino	Alpha-			
	ment	protein	gen	1B-			
	factor	E	beta	glycopr			
	Н		chain	otein			
6	Serine	Fibrino	Fibrino	Fibrino			
	proteas	gen	gen	gen			
	е	gamma	gamma	gamma			
	inhibito	chain	chain	chain			

	r A ₃ K			
7	lg mu chain C region (fragme nt)	Apolipo protein A-I	Comple ment C ₃	Apolipo protein A-I
8	Fibrino gen gamma chain	lg mu chain C region (fragme nt)	Apolipo protein E	Apolipo protein E
9	Arginin osuccin ate synthas e	Comple ment factor H	Murino globuli n-1	Fibrino gen alpha chain
10	Plasmin ogen	lg kappa light chain (fragme nt)	Comple ment factor H	Murino globuli n-1