

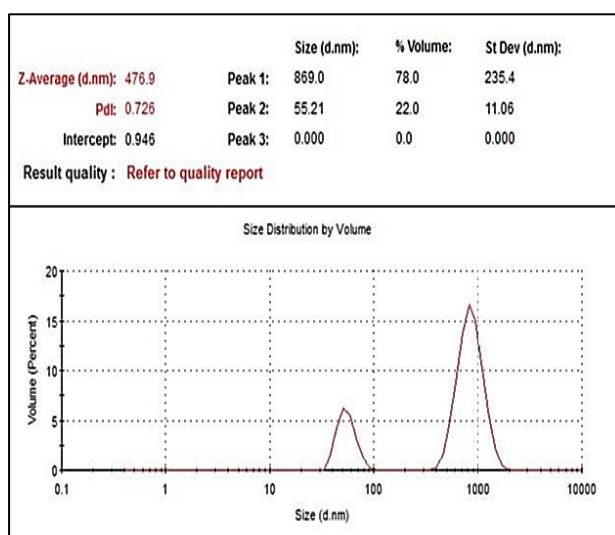
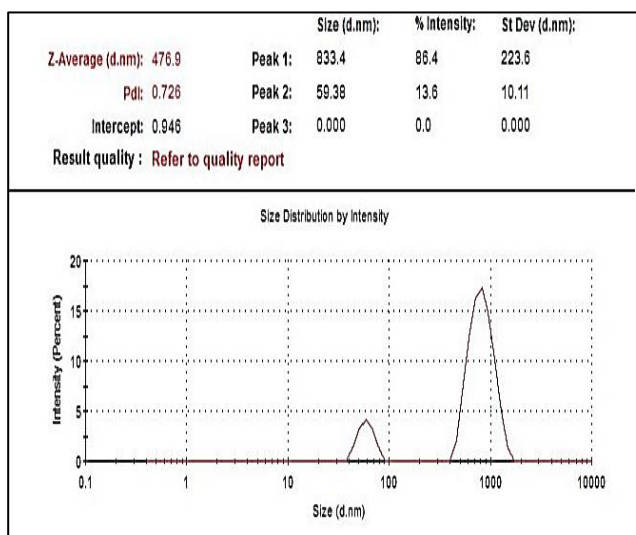


Fe/Mg-Modified Carbonate Apatite with Uniform Particle Size and Unique Transport Protein-Related Protein Corona Efficiently Delivers Doxorubicin into Breast Cancer Cells

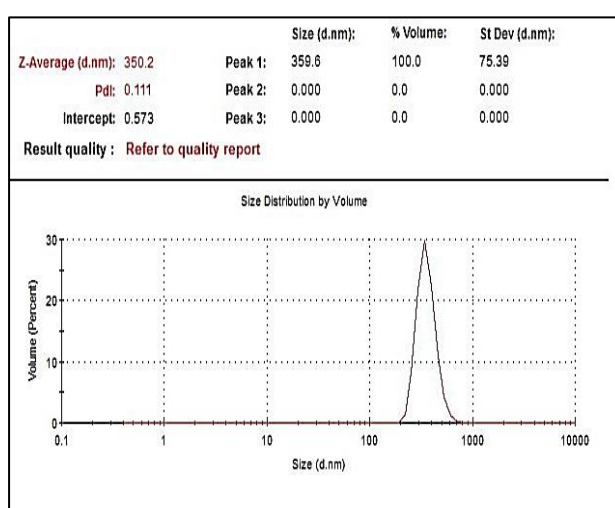
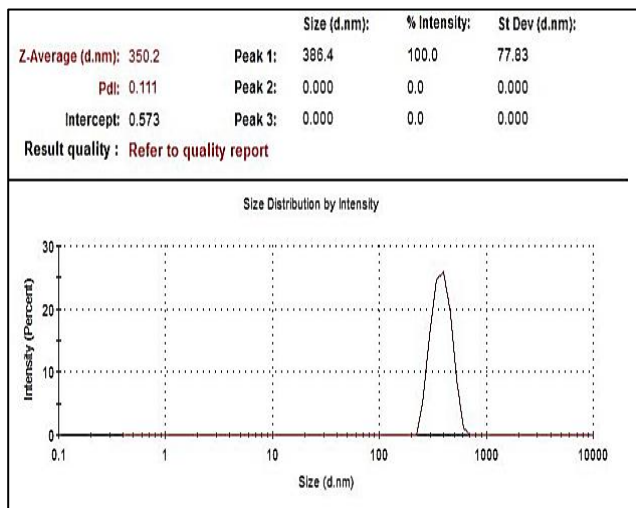
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Supplemental Materials:

A. CA



B. Low Fe/Mg-CA



C.

High Fe/Mg-CA

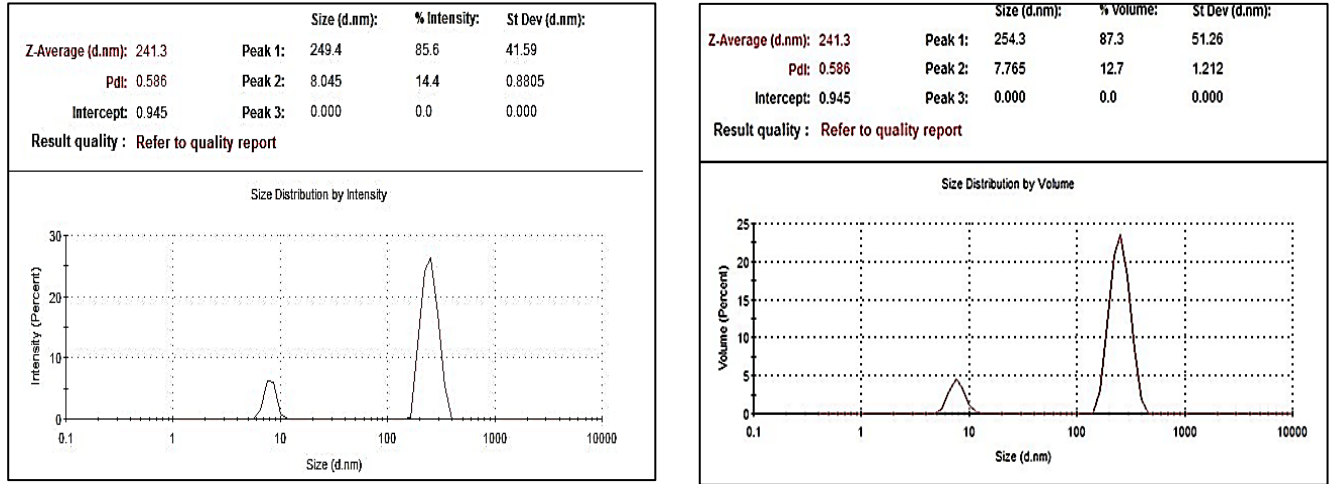


Figure S1: Representative data for dynamic light scattering technique used to demonstrate the particle size distribution by intensity and volume for CA and Fe/Mg-CA NPs. (A) Size distribution by intensity and volume for CA NPs. (B) Size distribution by intensity and volume for low Fe/Mg-CA NPs. (C) Size distribution by intensity and volume for high Fe/Mg-CA NPs.

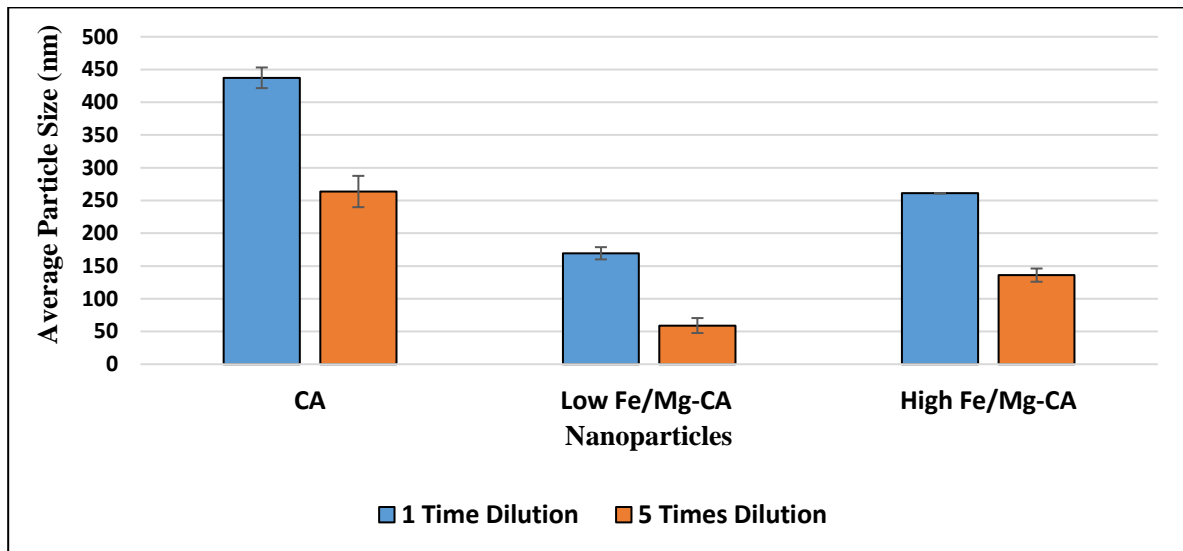
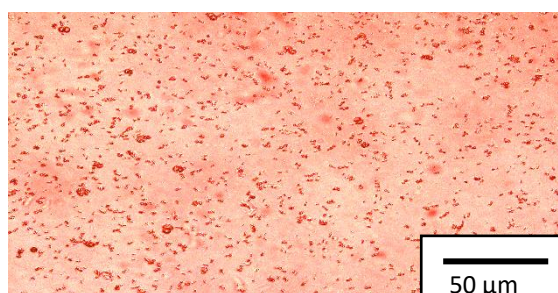
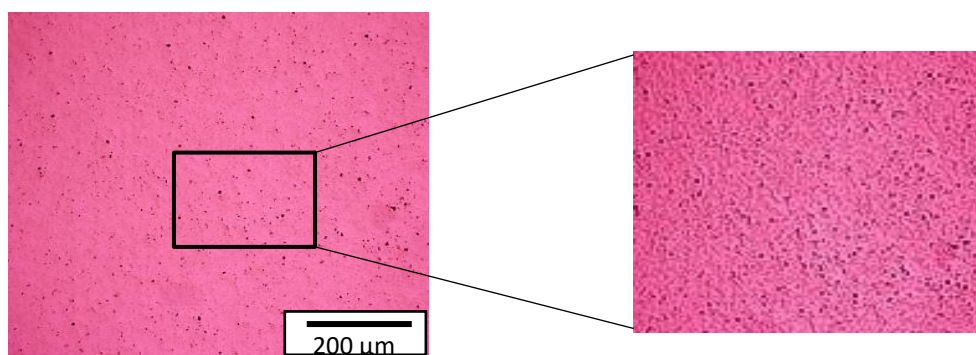
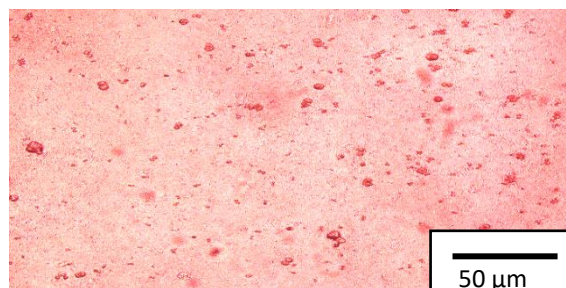
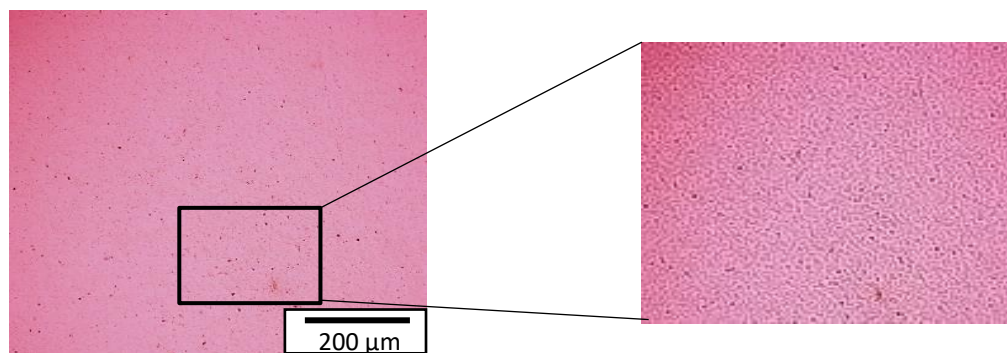


Figure S2: Comparison of z-average diameter of 1× and 5× diluted samples of CA, low Fe/Mg-CA and high Fe/Mg-CA NPs.

A. CA



B. Low Fe/Mg-CA



C. High Fe/Mg-CA

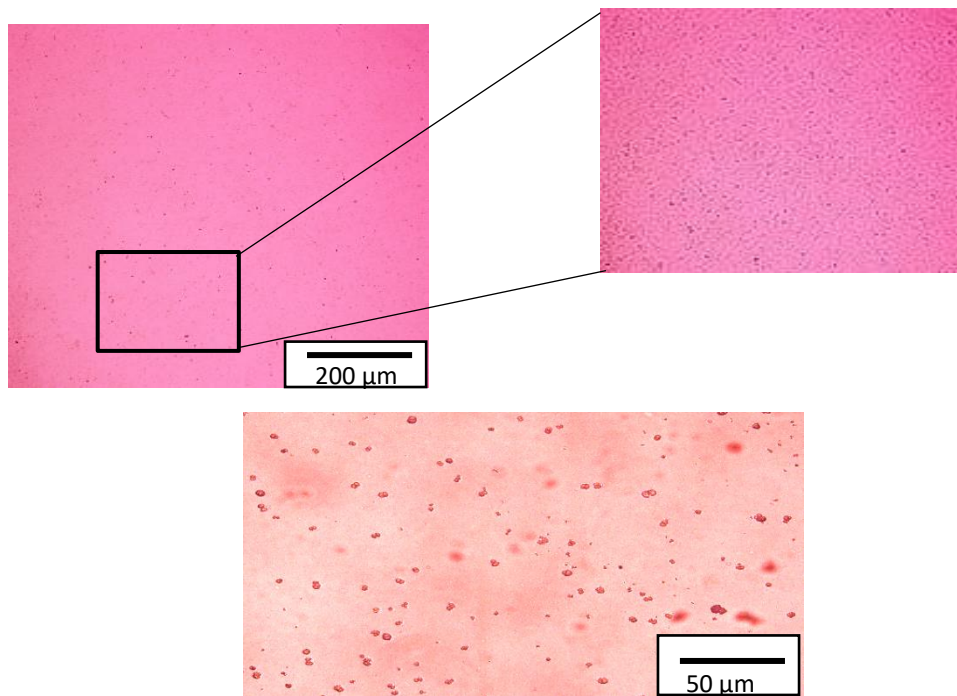


Figure S3: Observation of particle aggregates by optical microscopy with different magnifications, i.e. 40× (scale bar of 200 μm), 10× (scale bar of 50 μm) in presence of 10% FBS after 1 hour incubation. (A) CA, (B) Low Fe/Mg-CA and (C) High Fe/Mg-CA.

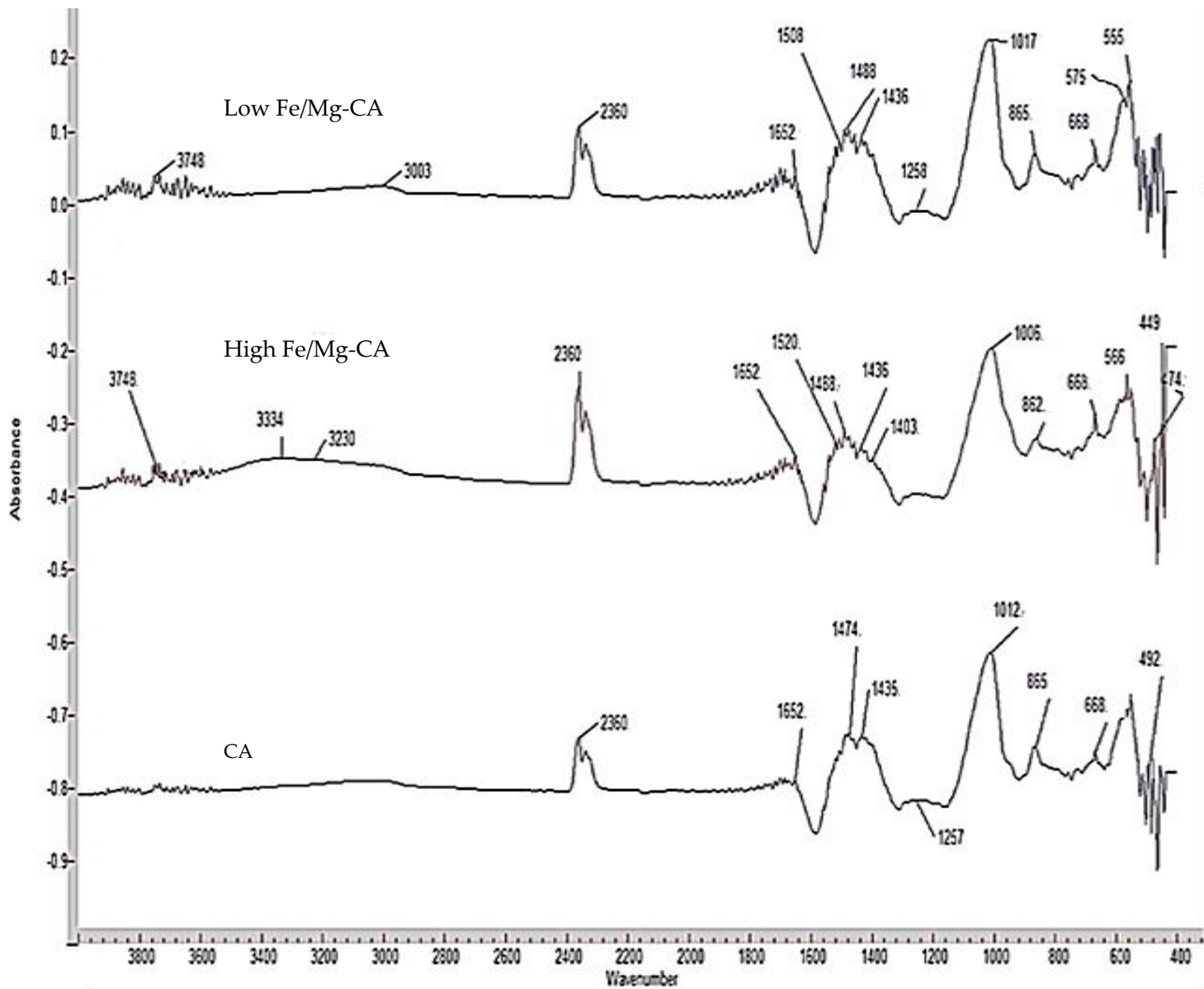


Figure S4: FTIR spectra for CA formulated with 40 mM Ca^{2+} , low Fe/Mg-CA generated with 40 mM Ca^{2+} , 10 μM Fe^{3+} and 40 mM Mg^{2+} , and high Fe/Mg-CA synthesized with 40 mM Ca^{2+} , 50 μM Fe^{3+} and 120 mM Mg^{2+} . The change in formulation was done in order to obtain significant amount of lyophilized powder for the analysis.

CA

0 H

4 H

A.

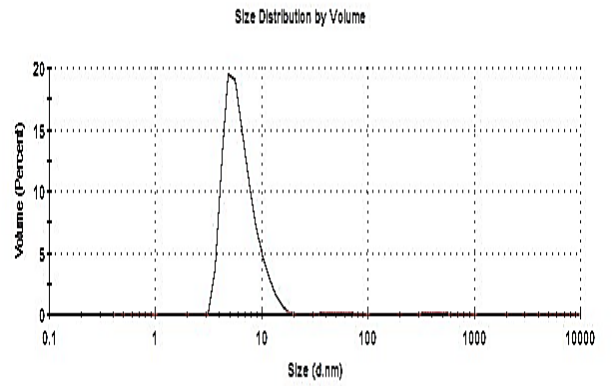
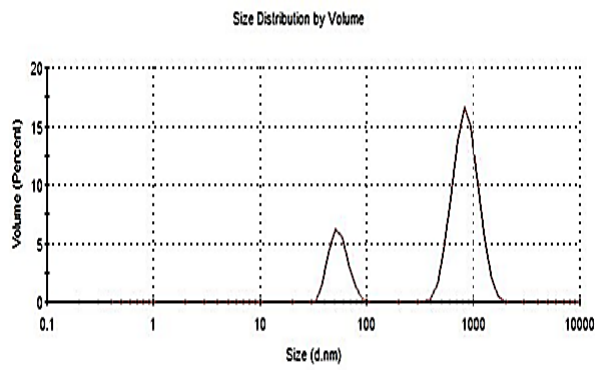
B.

	Size (d.nm):	% Volume:	St Dev (d.nm):
Z-Average (d.nm): 476.9	Peak 1: 869.0	78.0	235.4
Pdl: 0.726	Peak 2: 55.21	22.0	11.06
Intercept: 0.946	Peak 3: 0.000	0.0	0.000

	Size (d.nm):	% Volume:	St Dev (d.nm):
Z-Average (d.nm): 275.1	Peak 1: 451.4	0.5	127.0
Pdl: 0.459	Peak 2: 54.35	0.9	16.72
Intercept: 0.975	Peak 3: 6.403	98.6	2.279

Result quality : Refer to quality report

Result quality : Refer to quality report



24 H

72 H

C.

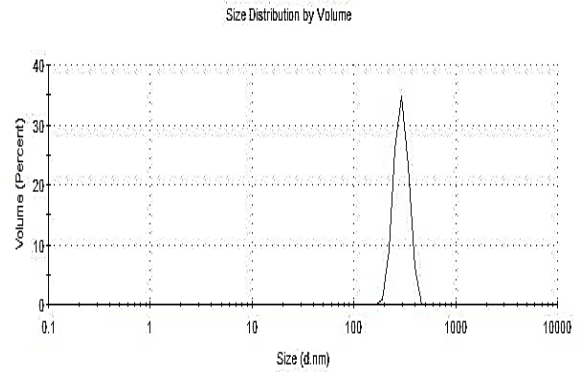
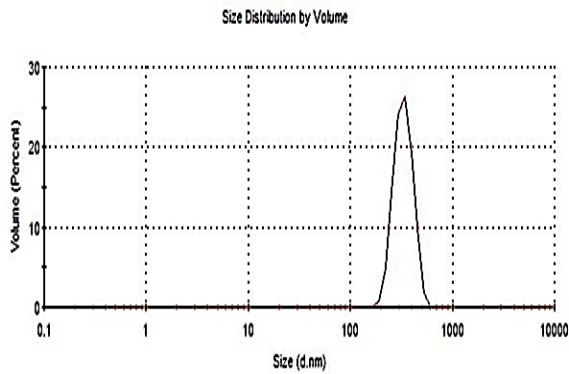
D.

	Size (d.nm):	% Volume:	St Dev (d.nm):
Z-Average (d.nm): 334.8	Peak 1: 334.7	100.0	69.19
Pdl: 0.343	Peak 2: 0.000	0.0	0.000
Intercept: 0.939	Peak 3: 0.000	0.0	0.000

	Size (d.nm):	% Volume:	St Dev (d.nm):
Z-Average (d.nm): 361.9	Peak 1: 294.1	100.0	46.41
Pdl: 0.652	Peak 2: 0.000	0.0	0.000
Intercept: 0.949	Peak 3: 0.000	0.0	0.000

Result quality : Refer to quality report

Result quality : Refer to quality report



Low Fe/Mg-CA

0 H

4 H

E.

F.

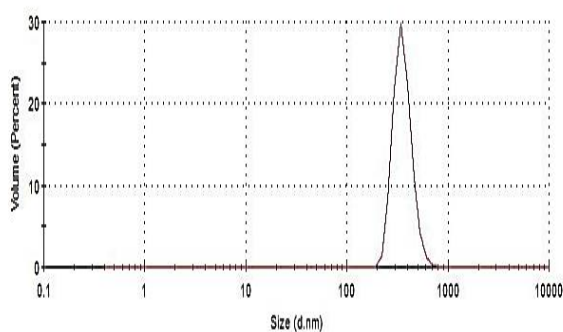
	Size (d.nm):	% Volume:	St Dev (d.nm):
Z-Average (d.nm): 350.2	Peak 1: 359.6	100.0	75.39
Pdl: 0.111	Peak 2: 0.000	0.0	0.000
Intercept: 0.573	Peak 3: 0.000	0.0	0.000

	Size (d.nm):	% Volume:	St Dev (d.nm):
Z-Average (d.nm): 223.5	Peak 1: 273.0	0.1	88.54
Pdl: 0.412	Peak 2: 17.98	3.9	3.904
Intercept: 0.979	Peak 3: 5.639	96.0	1.188

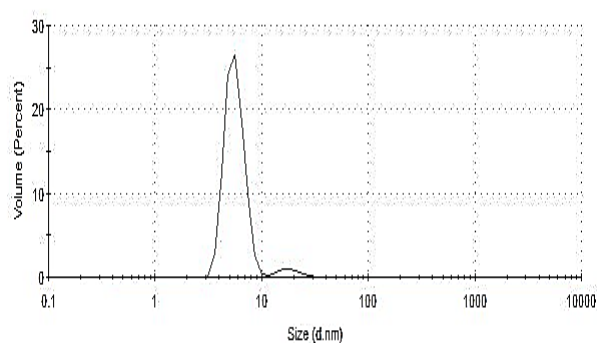
Result quality : Refer to quality report

Result quality : Refer to quality report

Size Distribution by Volume



Size Distribution by Volume



24 H

72 H

G.

H.

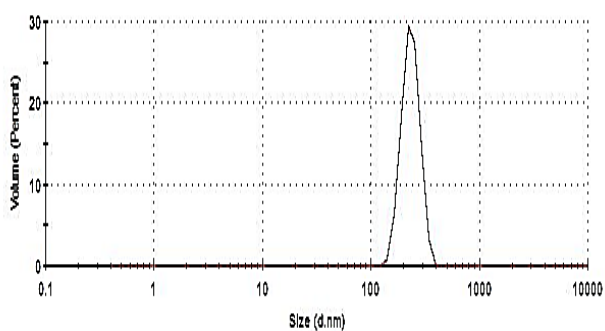
	Size (d.nm):	% Volume:	St Dev (d.nm):
Z-Average (d.nm): 296.4	Peak 1: 234.9	100.0	42.79
Pdl: 0.031	Peak 2: 0.000	0.0	0.000
Intercept: 0.971	Peak 3: 0.000	0.0	0.000

	Size (d.nm):	% Volume:	St Dev (d.nm):
Z-Average (d.nm): 374.9	Peak 1: 440.0	100.0	76.14
Pdl: 0.264	Peak 2: 0.000	0.0	0.000
Intercept: 0.947	Peak 3: 0.000	0.0	0.000

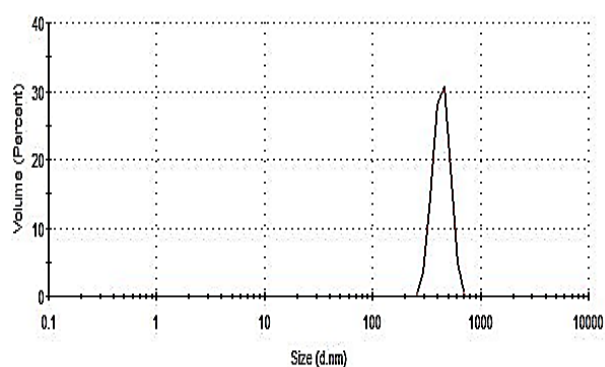
Result quality : Refer to quality report

Result quality : Refer to quality report

Size Distribution by Volume



Size Distribution by Volume



High Fe/Mg-CA

0 Hour

4 H

I.

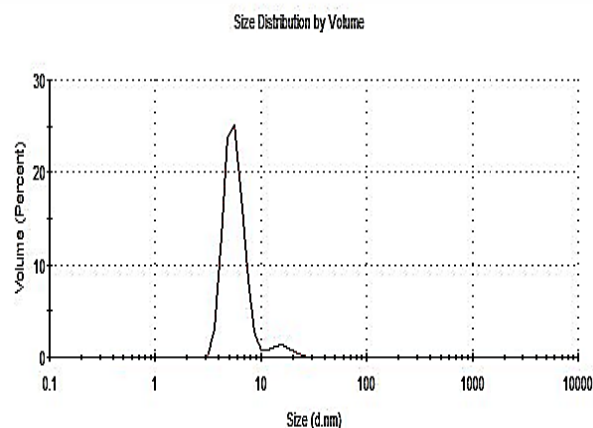
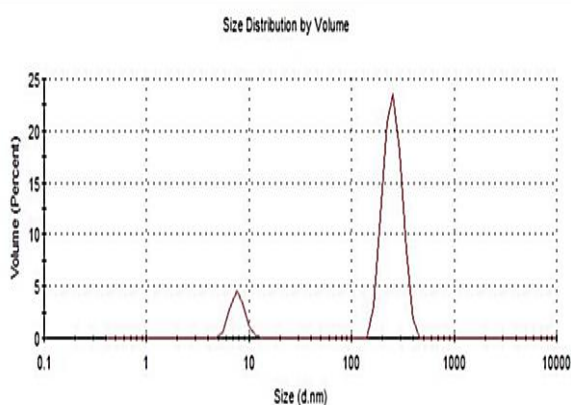
	Size (d.nm):	% Volume:	St Dev (d.nm):
Z-Average (d.nm): 241.3	Peak 1: 254.3	87.3	51.26
Pdl: 0.586	Peak 2: 7.765	12.7	1.212
Intercept: 0.945	Peak 3: 0.000	0.0	0.000

Result quality : Refer to quality report

J.

	Size (d.nm):	% Volume:	St Dev (d.nm):
Z-Average (d.nm): 197.1	Peak 1: 332.7	0.1	106.9
Pdl: 0.380	Peak 2: 124.2	0.0	26.58
Intercept: 0.974	Peak 3: 16.37	5.3	3.770

Result quality : Refer to quality report



24 H

72 H

K.

	Size (d.nm):	% Volume:	St Dev (d.nm):
Z-Average (d.nm): 347.9	Peak 1: 335.4	100.0	66.78
Pdl: 0.353	Peak 2: 0.000	0.0	0.000
Intercept: 0.964	Peak 3: 0.000	0.0	0.000

Result quality : Refer to quality report

L.

	Size (d.nm):	% Volume:	St Dev (d.nm):
Z-Average (d.nm): 380.7	Peak 1: 454.8	100.0	84.55
Pdl: 0.312	Peak 2: 0.000	0.0	0.000
Intercept: 0.950	Peak 3: 0.000	0.0	0.000

Result quality : Refer to quality report

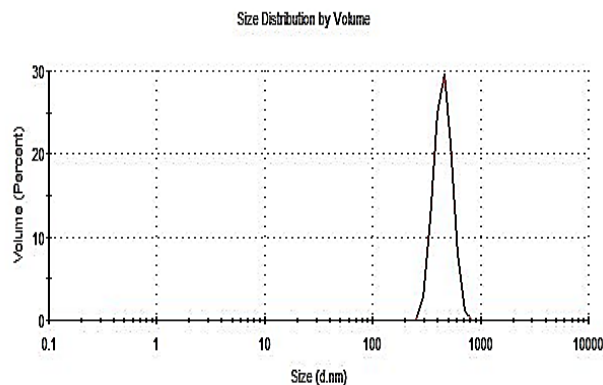
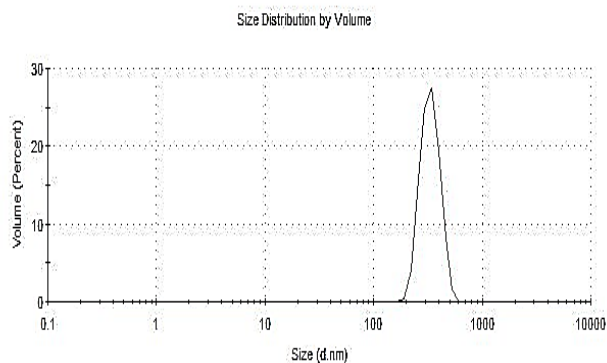


Figure S5: Representative data for dynamic light scattering technique used to demonstrate the PDI value and particle size distribution by volume for CA and Fe/Mg-CA NPs. (A) CA at 0 h. (B) CA at 4 h. (C) CA at 24 h. (D) CA at 72 h. (E) Low Fe/Mg-CA at 0 h. (F) Low Fe/Mg-CA 4 h. (G) Low Fe/Mg-CA at 24 h. (H) Low Fe/Mg-CA at 72 h. (I) High Fe/Mg-CA at 0 h. (J) High Fe/Mg-CA at 4 h. (K) High Fe/Mg-CA at 24 h. (L) High Fe/Mg-CA at 72 h.

The representative data for the elemental analysis of CA, low Fe/Mg-CA and high Fe/Mg-CA were presented below-

A. CA

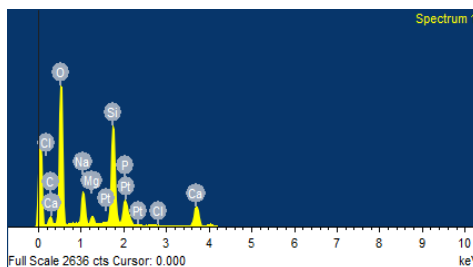
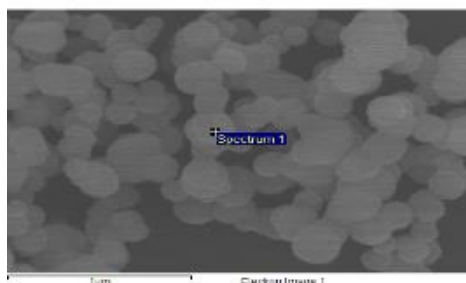


Table S1: Elemental analysis for CA.

Element	Weight %	Atomic %
Ca	11.67	12.76
Mg	1.09	0.94
C	5.04	8.86
P	4.24	2.89
O	47.38	62.55
Si	16.97	12.76
Pt	8.08	0.87
Na	5.16	4.74
Cl	0.34	0.22
Total	100	

B. Low Fe/Mg-CA

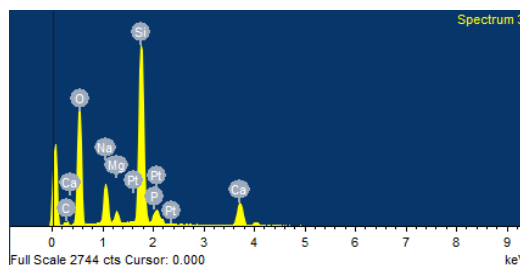
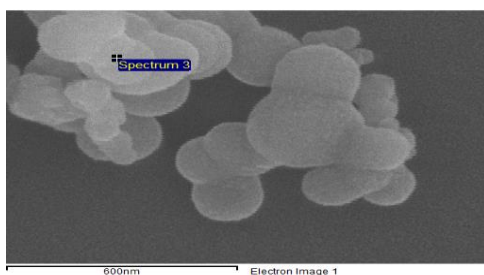


Table S2: Elemental analysis for low Fe/Mg-CA.

Element	Weight %	Atomic %
Ca	6.71	3.39
Mg	1.55	1.29
C	4.57	7.70
P	1.46	0.95
O	49.49	62.58
Si	24.48	17.63
Pt	4.98	0.52
Na	6.75	5.94
Total	100	

C. High Fe/Mg-CA

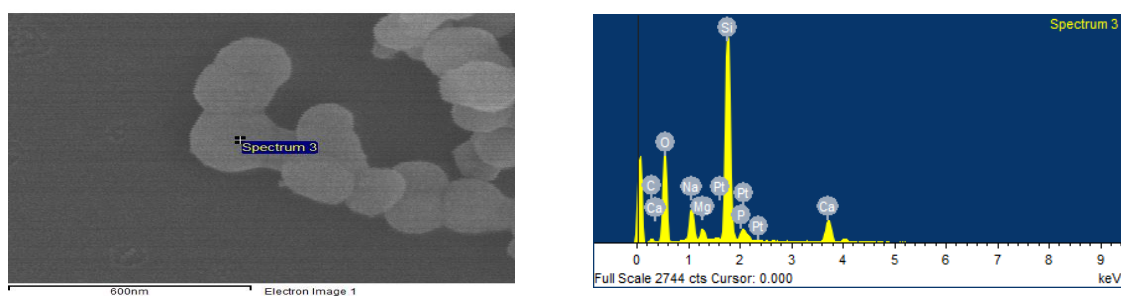


Table S3: Elemental analysis for high Fe/Mg-CA.

Element	Weight %	Atomic %
Ca	7.1	3.69
Mg	1.74	1.49
C	4.44	7.69
P	1.24	0.83
O	44.6	58.03
Si	30.47	22.58
Pt	4.69	0.5
Na	5.73	5.19
Total	100	

Figure S6: Representative data for the elemental analysis for (A) CA (B) Low Fe/Mg-CA (C) High Fe/Mg-CA.

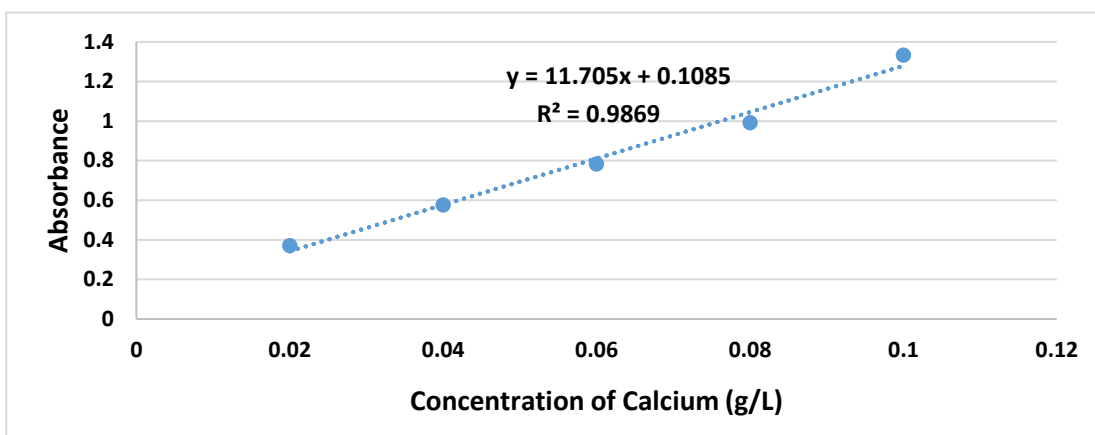


Figure S7: Standard curve for Calcium.

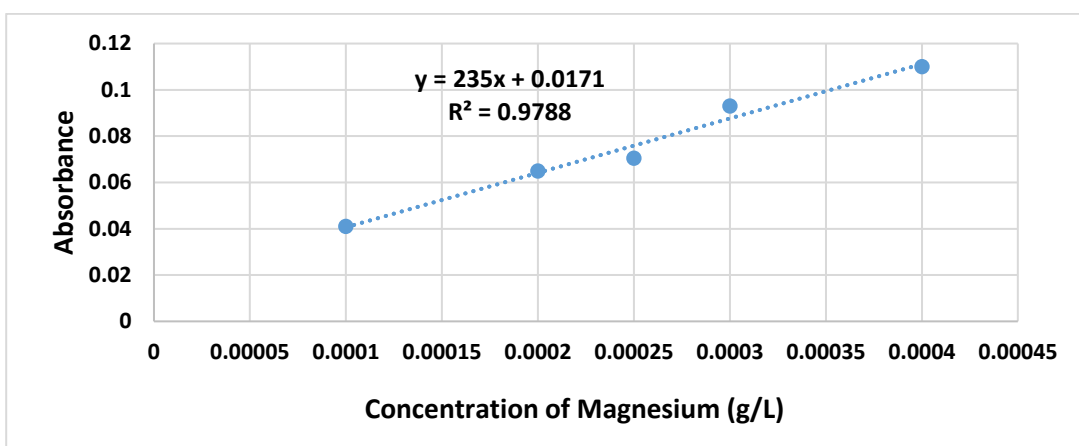


Figure S8: Standard curve for Magnesium.

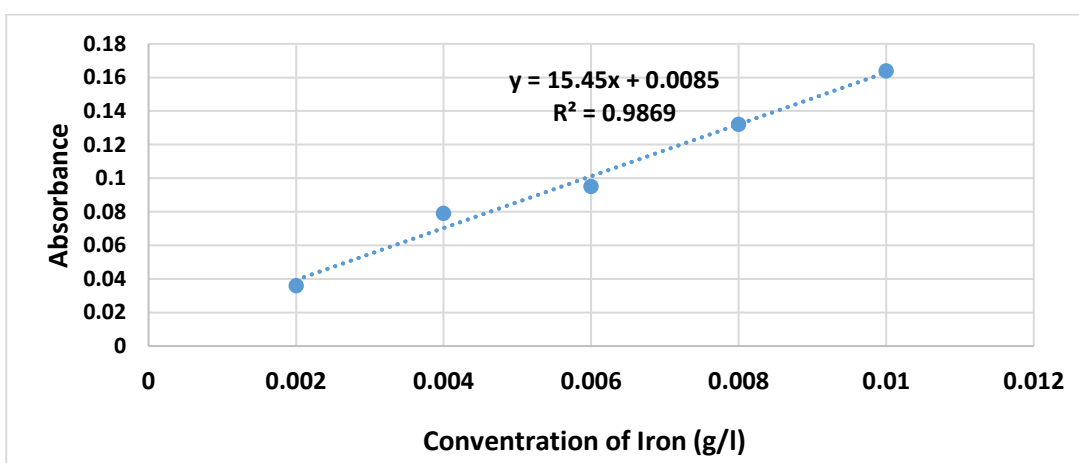


Figure S9: Standard curve for Iron.

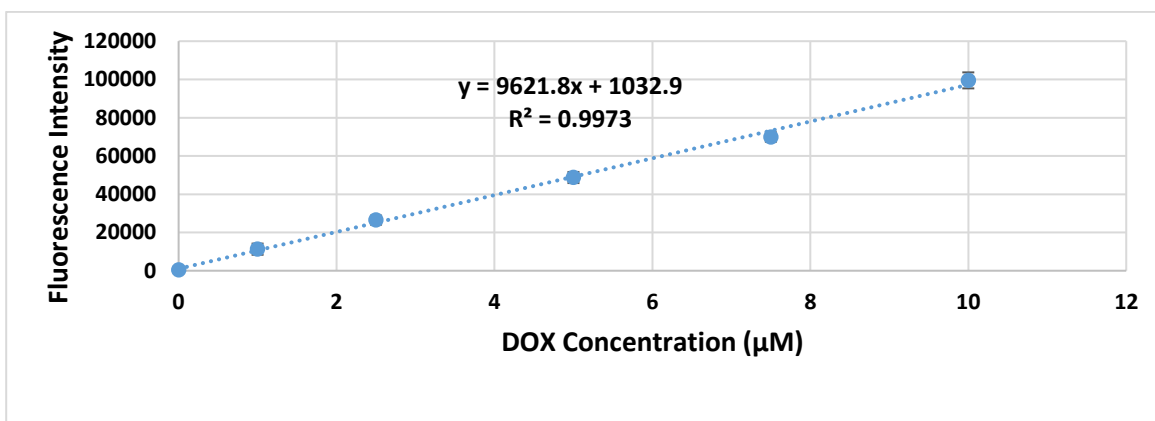


Figure S10: Standard Curve for free DOX at different concentrations.

Table S4: Cytotoxicity enhancement (%) of DOX-CA and DOX-Fe/Mg-CA NPs by thiazolyl blue tetrazolium bromide (MTT) assay in Michigan Cancer Foundation-7 (MCF-7) cells after 48 h of treatment.

NP's with different concentrations of DOX	Cytotoxicity enhancement (%) in MCF-7 cells
CA+DOX 1 nM	1± 0.45
CA+DOX 10 nM	3 ± 0.38
CA+DOX 100 nM	2 ± 0.29
CA+DOX 1µM	11± 0.81
Low Fe/Mg-CA+DOX 1 nM	3± 0.48
Low Fe/Mg-CA+DOX 10 nM	5± 0.79
Low Fe/Mg-CA+DOX 100 nM	15± 0.29
Low Fe/Mg-CA+DOX 1µM	11± 0.82
High Fe/Mg-CA+DOX 1 nM	2± 0.48
High Fe/Mg-CA+DOX 10 nM	6± 0.38
High Fe/Mg-CA+DOX 100 nM	12± 0.28
High Fe/Mg-CA+DOX 1µM	13± 0.44

Table S5. List of identified proteins with CA in 10% FBS along with their isoelectric point (pI) and major biological functions.

Functional classification of protein	Identified Protein	pI	Detailed Function
Transport Protein	ALB protein	5.7	1. Zn ²⁺ binding
	Serum Albumin	5.53	1. Transport 2. Lipid binding 3. Metal binding 4. Bilirubin binding 5. Helps maintain osmolarity of blood 6. Dysopsonin
	Alpha-2-HS glycoprotein	5.94	1. Enhance endocytosis 2. Ca ²⁺ binding 3. Post-translational protein modification 4. Protein metabolism
	cDNA FLJ51509	7.42	1. Behaves similar to Alpha-fetoprotein, i.e- it takes part in metal, fatty acids and bilirubin binding, involved in cellular protein and progesterone metabolism
	Transthyretin	5.6	1. Thyroid hormone binding 2. Identical protein binding 3. Transport iron 4. Transport thyroxine to the brain 5. Cellular protein metabolism 6. Neutrophil degranulation 7. Retinol metabolism
	Serotransferrin	6.81	1. Fe ³⁺ binding 2. Bicarbonate binding 3. Transport iron
	Transferrin variant	6.68	1. Fe ³⁺ binding 2. Transport Fe ³⁺ within transmembrane 3. Involved in iron ion homeostasis in cells
	Transferrin	6.97	1. Fe ³⁺ binding 2. Transport Fe ³⁺ within transmembrane 3. Involved in iron ion homeostasis in cells
	LMAN1 protein	6.3	1. Mannose (monosaccharide hexose) binding. 2. Endoplasmic reticulum (ER) to golgi body facilitated transport
	Globin C1	9.79	1. Metal binding (binding with Ca ²⁺ , Mg ²⁺ and Fe ³⁺) 2. Heme-binding 3. Transport oxygen
	Globin B1	6.75	1. Fe ²⁺ , Fe ³⁺ binding 2. Heme binding 3. Organic acid binding 4. Oxygen binding 5. Hemoglobin alpha binding 6. Transports oxygen
	Globin A1	7.02	1. Fe ²⁺ , Fe ³⁺ binding 2. Heme binding 3. Oxygen binding 4. Transports oxygen
	Alpha-1-acid glycoprotein (positive acute phase protein)	4.93	1. Behave as transport protein in blood stream. 2. Involved in regulation of immune system. 3. Inhibits aggregation of platelets and neutrophils.

	Mutant hemoglobin alpha 1 globin chain	8.08	<ol style="list-style-type: none"> 1. Fe²⁺, Fe³⁺ binding 2. Heme binding 3. Oxygen binding 4. Transport oxygen 5. Transport bicarbonate
	Hemoglobin alpha-1 globin chain	8.08	<ol style="list-style-type: none"> 1. Fe²⁺, Fe³⁺ binding 2. Heme binding 3. Oxygen binding 4. Transport oxygen 5. Transport bicarbonate
	Hemoglobin subunit alpha	8.72	<ol style="list-style-type: none"> 1. Fe²⁺, Fe³⁺ binding 2. Heme binding 3. Oxygen binding 4. Transport oxygen
	Alpha-2 globin chain	8.72	<ol style="list-style-type: none"> 1. Fe²⁺, Fe³⁺ binding 2. Heme binding 3. Oxygen binding 4. Transport oxygen 5. Transport bicarbonate
	Mutant hemoglobin alpha 2 globin chain	8.72	<ol style="list-style-type: none"> 1. Fe²⁺, Fe³⁺ binding 2. Heme binding 3. Oxygen binding 4. Transport oxygen
	Lactoferrin	8.70	<ol style="list-style-type: none"> 1. Fe²⁺, Fe³⁺ binding 2. Involved in serine-type endopeptidase activity 3. Engender antimicrobial humoral response 4. Regulates the production of cytokine
	Beta-globin protein	6.64	<ol style="list-style-type: none"> 1. Heme binding 2. Metal ion binding 3. Oxygen binding 4. Transports oxygen 5. Makes haemoglobin (HbA) along with alpha-globin in adults
	Hemoglobin fetal subunit beta	6.75	<ol style="list-style-type: none"> 1. Fe²⁺, Fe³⁺ binding 2. Heme binding 3. Organic acid binding 4. Oxygen binding 5. Hemoglobin alpha binding 6. Transports oxygen
	Truncated beta-globin	7.96	<ol style="list-style-type: none"> 1. Heme binding 2. Oxygen binding
	Mutant beta-globin	7.92	<ol style="list-style-type: none"> 1. Heme binding 2. Oxygen binding 3. Transports oxygen
	Beta-globin chain	5.57	<ol style="list-style-type: none"> 1. Fe²⁺, Fe³⁺ binding 2. Heme binding 3. Oxygen binding 4. Transport oxygen
	Hemoglobin subunit beta	6.25	<ol style="list-style-type: none"> 1. Heme binding 2. Oxygen binding 3. Transports oxygen
	Hemoglobin beta chain	6.74	<ol style="list-style-type: none"> 1. Fe²⁺, Fe³⁺ binding 2. Heme binding 3. Oxygen binding 4. Transport oxygen
	Hemoglobin beta chain variant Hb.Sinai-Bel Air	5.41	<ol style="list-style-type: none"> 1. Fe²⁺, Fe³⁺ binding 2. Heme binding 3. Oxygen binding

			4. Transport oxygen
	Hemoglobin beta chain variant Hb-I_Toulouse	5.41	1. Fe ²⁺ , Fe ³⁺ binding 2. Heme binding 3. Oxygen binding 4. Transport oxygen
	Vitamin D-binding protein	5.2	1. Vitamin D binding 2. Transport vitamin within transmembrane
Cell adhesive protein	cDNA FLJ54111	6.95	1. Ca ²⁺ binding 2. Collagen binding
Coagulation protein	Prothrombin	5.64	1. Non-covalently calcium ions (Ca ²⁺) binding. 2. Blood coagulation 3. Involved in serine-type endopeptidase activity
Metabolic Protein	Adiponectin	5.42	1. Involved in fat metabolism and hormonal activity 2. Identical protein binding 3. Sialic acid binding 4. Signaling receptor binding 5. Involved in glucose metabolism 6. Involved in the negative regulation of macrophage differentiation and phagocytosis
Protease inhibitor	Kininogen-1	6.02	1. Inhibits thiol proteases 2. Inhibits the thrombin- and plasmin-induced aggregation of thrombocytes in blood coagulation 3. Heparin binding 4. Zinc ion binding 5. Regulates cell adhesion
	KNG1 protein	6.4	1. Inhibits cysteine-type endopeptidase
	Fetuin-B	8.7	1. Inhibits metalloenzyme, metalloprotease and protease, i.e- inhibits cysteine-type endopeptidase and metallo-endopeptidase. 2. Involved in fertilization.
	Alpha-1-antitrypsin	6.05	1. Inhibits protease and serine protease. 2. Inhibits trypsin, chymotrypsin and plasminogen activator. 3. Identical protein and protease binding.
Structural Protein	Keratin, type I cytoskeletal 10	5.13	1. Cross linking of peptides 2. Protein heterodimerization and heterotetramerization activity 3. Keratinization
	Keratin 1	8.15	1. Involved in structural molecular activity
	KRT6A protein	8.09	1. Involved in the structural molecular activity
	Keratin, type I cytoskeletal 14	5.09	1. Responding to Zn ²⁺ 2. Keratin filament binding 3. Structural constituent of cytoskeleton 4. Keratinization
	Histone H2B	10.32	1. DNA binding 2. Involved in protein heterodimerization and nucleosome assembly
Regulatory Protein	Junction mediating and regulatory protein p53 cofactor	5.95	1. Actin binding. 2. Arp2/3 complex binding 3. Involved in transcription coactivator activity. 4. Involved in cell cycle arrest. 5. Involved in positive regulation of apoptosis.
Others	Uncharacterized protein	6.28	1. Fe ²⁺ , Fe ³⁺ binding 2. Heme binding 3. Oxygen binding 4. Transport oxygen

	Epididymis secretory protein Li 51	5.4	1. Vitamin D binding 2. Transports vitamin transmembrane
	Alpha-fetoprotein	4.57	1. Metal (copper, nickel) binding 2. Fatty acids binding 3. Bilirubin binding 4. Cellular protein metabolism 5. Progesterone metabolism
	cDNA FLJ95666	5.92	1. Involved in aspirin acetylated lysine 2. Bilirubin binding
	cDNA FLJ57154	6.29	1. Helps in heterologous expression
	cDNA FLJ55606	5.84	1. Cysteine-type endopeptidase inhibitor

Table S6. List of identified proteins with low Fe/Mg-CA in 10% FBS along with their isoelectric point (pI) and major biological functions.

Functional classification of protein	Identified Protein	pI	Detailed Function
Transport protein	Albumin	5.53	1. Transport 2. Systemic calcification inhibitor 3. Lipid binding 4. Metal binding 5. Maintaining intravascular colloid osmotic pressure (COP) 6. Dysopsonin
	Mutant hemoglobin alpha 1 globin chain	8.72	1. Fe ²⁺ , Fe ³⁺ binding 2. Heme binding 3. Oxygen binding 4. Transport oxygen 5. Transport bicarbonate
	Hemoglobin alpha-1 globin chain	8.72	1. Fe ²⁺ , Fe ³⁺ binding 2. Heme binding 3. Oxygen binding 4. Transport oxygen 5. Transport bicarbonate
	Alpha-2 globin chain	8.72	1. Fe ²⁺ , Fe ³⁺ binding 2. Heme binding 3. Oxygen binding 4. Transport oxygen 5. Transport bicarbonate
	Hemoglobin subunit alpha piens	8.72	1. Fe ²⁺ , Fe ³⁺ binding 2. Heme binding 3. Oxygen binding 4. Transport oxygen 5. Transport bicarbonate 6. Receptor mediated endocytosis 7. Regulation of cell death 8. Protein heterooligomerization
	Mutant hemoglobin alpha 2 globin chain	8.72	1. Fe ²⁺ , Fe ³⁺ binding 2. Heme binding 3. Oxygen binding 4. Transport oxygen
	Hemoglobin subunit alpha	8.72	1. Fe ²⁺ , Fe ³⁺ binding 2. Heme binding 3. Oxygen binding

			4. Transport oxygen
	Globin C1	9.79	1. Metal binding (binding with Ca ²⁺ , Mg ²⁺ and Fe ³⁺) 2. Heme-binding 3. Transport oxygen
	Beta-globin protein	6.64	1. Heme binding 2. Fe ²⁺ , Fe ³⁺ binding 3. Oxygen binding 4. Transports oxygen 5. Makes haemoglobin (HbA) along with alpha-globin in adults
	Beta-globin chain	5.57	1. Fe ²⁺ , Fe ³⁺ binding 2. Heme binding 3. Oxygen binding 4. Transport oxygen
	Truncated beta-globin	7.96	1. Heme binding 2. Oxygen binding
	Mutant beta-globin	7.92	1. Heme binding 2. Oxygen binding 3. Transports oxygen
	Hemoglobin subunit beta	6.25	1. Heme binding 2. Oxygen binding 3. Transports oxygen
	Hemoglobin beta	5.45	1. Fe ²⁺ , Fe ³⁺ binding 2. Heme binding 3. Oxygen binding 4. Transport oxygen
	Hemoglobin beta chain variant Hb-I_Toulouse	5.41	1. Fe ²⁺ , Fe ³⁺ binding 2. Heme binding 3. Oxygen binding 4. Transport oxygen
	Mutant hemoglobin beta chain (Fragment)	6.5	1. Fe ²⁺ , Fe ³⁺ binding 2. Heme binding 3. Oxygen binding 4. Transport oxygen
	Hemoglobin beta globin chain	6.74	1. Fe ²⁺ , Fe ³⁺ binding 2. Heme binding 3. Oxygen binding 4. Transport oxygen
	Hemoglobin beta	6.74	1. Fe ²⁺ , Fe ³⁺ binding 2. Heme binding 3. Oxygen binding 4. Transport oxygen
	Hemoglobin beta chain variant Hb.Sinai-Bel Air	5.41	1. Fe ²⁺ , Fe ³⁺ binding 2. Heme binding 3. Oxygen binding 4. Transport oxygen
	Alpha-2-HS glycoprotein	5.94	1. Enhance endocytosis 2. Ca ²⁺ binding 3. Post-translational protein modification 4. Protein metabolism
	Transthyretin	5.6	1. Thyroid hormone binding 2. Identical protein binding 3. Transport iron 4. Transport thyroxine to the brain 5. Cellular protein metabolism 6. Neutrophil degranulation 7. Retinol metabolism

	Serotransferrin	6.81	<ol style="list-style-type: none"> 1. Fe³⁺ binding 2. Bicarbonate binding 3. Transport iron
	Transferrin variant	6.68	<ol style="list-style-type: none"> 1. Fe³⁺ binding 2. Transport Fe³⁺ within transmembrane 3. Involved in iron ion homeostasis in cells
	Transferrin	6.97	<ol style="list-style-type: none"> 1. Fe³⁺ binding 2. Transport Fe³⁺ within transmembrane 3. Involved in iron ion homeostasis in cells
	Lactotransferrin	8.7	<ol style="list-style-type: none"> 1. Fe³⁺ binding 2. Cu²⁺ binding 3. Bicarbonate binding 4. Involved in serine-type endopeptidase activity 5. Engender antimicrobial humoral response 6. Regulates the production of cytokine
	Lactoferrin	8.7	<ol style="list-style-type: none"> 1. Fe²⁺, Fe³⁺ binding 2. Involved in serine-type endopeptidase activity 3. Engender antimicrobial humoral response 4. Regulates the production of cytokine
	Delta globin	7.85	<ol style="list-style-type: none"> 1. Fe²⁺, Fe³⁺ binding 2. Heme binding 3. Oxygen binding 4. Transport oxygen 5. Blood coagulation
	Hbbm fused globin protein	6.17	<ol style="list-style-type: none"> 1. Fe²⁺, Fe³⁺ binding 2. Heme binding 3. Oxygen binding 4. Transport oxygen
Coagulation protein	Prothrombin	5.64	<ol style="list-style-type: none"> 1. Non-covalently calcium ions (Ca²⁺) binding. 2. Blood coagulation 3. Involved in serine-type endopeptidase activity
Cell adhesive protein	cDNA FLJ54111	6.95	<ol style="list-style-type: none"> 1. Ca²⁺ binding 2. Collagen binding
	Proliferation-inducing protein 33	4.71	<ol style="list-style-type: none"> 1. Ca²⁺ binding 2. Collagen binding
	C-type lectin domain family 3 member B isoform CRA_a	5.21	<ol style="list-style-type: none"> 1. Carbohydrate binding 2. Ossification
Enzymes	N-acetyltransferase 5	5.84	<ol style="list-style-type: none"> 1. Involved in transferase activity 2. Catalyzes the transfer of acetyl groups from acetyl CoA to arylamines
Structural protein	cDNA FLJ56708	8.44	<ol style="list-style-type: none"> 1. Involved in structural molecular activity
	Keratin, type I cytoskeletal 10	5.13	<ol style="list-style-type: none"> 1. Cross linking of peptides 2. Protein heterodimerization and heterotetramerization activity 3. Keratinization
	Keratin, type I cytoskeletal 28	5.33	<ol style="list-style-type: none"> 1. Involved in structural molecular activity 2. Cornification 3. keratinization
	Keratin 1	8.15	<ol style="list-style-type: none"> 1. Involved in structural molecular activity
	Keratin, type II cytoskeletal 2	8.07	<ol style="list-style-type: none"> 1. Cytoskeletal protein binding 2. Cross linking of peptides 3. Structural constituent of cytoskeleton 4. Keratinization

Others	Protein unc-13 homolog B	5.67	<ol style="list-style-type: none"> 1. Ca²⁺binding 2. Calmodulin binding 3. Phospholipid binding 4. Diacylglycerol binding 5. Intracellular signal transduction 6. Presynaptic and synaptic vesicle Exocytosis
	MHC class I antigen	6.64	<ol style="list-style-type: none"> 1. Peptide binding engendered by cytosolic protein degradation via the proteasome 2. Exhibit intracellular proteins to cytotoxic T cells 3. Function as an inhibitory ligand for natural killer cells
	Alpha-fetoprotein	4.57	<ol style="list-style-type: none"> 1. Metal (copper, nickel) binding 2. Fatty acids binding 3. Bilirubin binding 4. Cellular protein metabolism 5. Progesterone metabolism
	cDNA FLJ57154	6.29	<ol style="list-style-type: none"> 1. Helps in heterologous expression
	cDNA FLJ79229	8.10	<ol style="list-style-type: none"> 1. Fe²⁺, Fe³⁺ binding 2. Controls cytokine production 3. Involved in antimicrobial humoral response
	Uncharacterized protein	6.28	<ol style="list-style-type: none"> 1. Fe²⁺, Fe³⁺ binding 2. Heme binding 3. Oxygen binding 4. Transport oxygen
	A-kinase anchor protein	8.84	<ol style="list-style-type: none"> 1. Protein kinase A (PKA) binding 2. Specific substrate targeting by means of phosphorylation (through PKA) and dephosphorylation (through phosphatases)
	Zinc finger SWIM domain-containing protein 4	6.47	<ol style="list-style-type: none"> 1. Zn²⁺ binding 2. DNA binding
	Capsid scaffolding protein	8.10	<ol style="list-style-type: none"> 1. Identical protein binding 2. Involved in nuclear capsid macromolecular assembly 3. Involved in serine-type endopeptidase activity
	cDNA FLJ51509	7.42	<ol style="list-style-type: none"> 1. Behaves similar to Alpha-fetoprotein, i.e- takes part in metal, fatty acids and bilirubin binding, involved in cellular protein and progesterone metabolism
cDNA FLJ55606	5.84	<ol style="list-style-type: none"> 1. Cysteine-type endopeptidase inhibitor 	

Table S7. List of identified proteins with high Fe/Mg-CA in 10% FBS along with their isoelectric point (pI) and major biological functions.

Functional classification of protein	Identified Protein	pI	Detailed Function
Transport protein	Albumin	5.53	<ol style="list-style-type: none"> 1. Transport 2. Systemic calcification inhibitor 3. Lipid binding 4. Metal binding 5. Maintaining intravascular colloid osmotic pressure (COP)

	Globin C1	9.79	<ol style="list-style-type: none"> 1. Metal binding (binding with Ca^{2+}, Mg^{2+} and Fe^{3+}). 2. Heme-binding 3. Transport oxygen.
	Alpha-2 globin chain	8.72	<ol style="list-style-type: none"> 1. Fe^{2+}, Fe^{3+} binding 2. Heme binding 3. Oxygen binding 4. Transport oxygen 5. Transport bicarbonate
	Hemoglobin subunit alpha	8.72	<ol style="list-style-type: none"> 1. Fe^{2+}, Fe^{3+} binding 2. Heme binding 3. Oxygen binding 4. Transport oxygen
	Mutant hemoglobin alpha 1 globin chain	8.72	<ol style="list-style-type: none"> 1. Fe^{2+}, Fe^{3+} binding 2. Heme binding 3. Oxygen binding 4. Transport oxygen 5. Transport bicarbonate
	Hemoglobin alpha-1 globin chain	8.72	<ol style="list-style-type: none"> 1. Fe^{2+}, Fe^{3+} binding 2. Heme binding 3. Oxygen binding 4. Transport oxygen 5. Transport bicarbonate
	Mutant hemoglobin alpha 2 globin chain	8.72	<ol style="list-style-type: none"> 1. Fe^{2+}, Fe^{3+} binding 2. Heme binding 3. Oxygen binding 4. Transport oxygen
	Beta-globin protein	6.64	<ol style="list-style-type: none"> 1. Heme binding 2. Makes haemoglobin (HbA) along with alpha-globin in adults
	Alpha-2-HS glycoprotein	5.94	<ol style="list-style-type: none"> 1. Enhance endocytosis 2. Ca^{2+} binding 3. Post-translational protein modification 4. Protein metabolism
	Transthyretin	5.6	<ol style="list-style-type: none"> 1. Thyroid hormone binding 2. Identical protein binding 3. Transport iron 4. Transports thyroxine to the brain 5. Cellular protein metabolism 6. Neutrophil degranulation 7. Retinol metabolism
	Serotransferrin	6.81	<ol style="list-style-type: none"> 1. Fe^{3+} binding 2. Bicarbonate binding 3. Transport iron
	Transferrin variant	6.68	<ol style="list-style-type: none"> 1. Fe^{3+} binding 2. Transport Fe^{3+} within transmembrane 3. Involved in iron ion homeostasis in cells
	Transferrin	6.97	<ol style="list-style-type: none"> 1. Fe^{3+} binding 2. Transport Fe^{3+} within transmembrane 3. Involved in iron ion homeostasis in cells
	Tetranectin	5.8	<ol style="list-style-type: none"> 1. Ca^{2+} binding 2. Heparin binding 3. Carbohydrate binding 4. Cellular metabolism 5. Bone mineralization 6. Ossification 7. Platelet degranulation
	cDNA FLJ54839	7.42	1. Behaves similar to Lactotransferrin precursor
	cDNA FLJ53691	6.81	1. Fe^{3+} binding

			2. Transport Fe ³⁺ within transmembrane
	Lactotransferrin	8.7	1. Fe ³⁺ binding 2. Cu ²⁺ binding 3. Bicarbonate binding 4. Involved in serine type endopeptidase activity 5. Engender antimicrobial humoral response 6. Regulates the production of cytokine
	Lactoferrin	8.7	1. Fe ²⁺ , Fe ³⁺ binding 2. Involved in serine type endopeptidase activity 3. Engender antimicrobial humoral response 4. Regulates the production of cytokine
	Delta globin	7.85	1. Fe ²⁺ , Fe ³⁺ binding 2. Heme binding 3. Oxygen binding 4. Transport oxygen 5. Blood coagulation
	Hemoglobin subunit gamma-2	6.64	1. Fe ²⁺ , Fe ³⁺ binding 2. Heme binding 3. Oxygen binding 4. Transport oxygen 5. Blood coagulation
	Globin B3	8.67	1. Fe ²⁺ , Fe ³⁺ binding 2. Heme binding 3. Hemoglobin alpha binding 4. Oxygen binding 5. Transport oxygen
	Gamma-G globin	7.2	1. Fe ²⁺ , Fe ³⁺ binding 2. Heme binding 3. Oxygen binding 4. Transport oxygen
	Hbbm fused globin protein	6.17	1. Fe ²⁺ , Fe ³⁺ binding 2. Heme binding 3. Oxygen binding 4. Transport oxygen
Cell adhesive protein	SPARC-like protein 1	4.73	1. Ca ²⁺ binding 2. Collagen binding 3. Extracellular matrix binding 4. Adhesion of synaptic membrane 5. Cellular protein metabolism
	Proliferation-inducing protein 33	4.71	1. Ca ²⁺ binding 2. Collagen binding
	C-type lectin domain family 3 member B isoform CRA_a	5.21	1. Carbohydrate binding 2. Ossification
	cDNA FLJ54111	6.95	1. Ca ²⁺ binding 2. Collagen binding
	cDNA FLJ54278	4.83	1. Ca ²⁺ binding 2. Collagen binding
	cDNA FLJ54387	4.71	1. Ca ²⁺ binding 2. Collagen binding
	cDNA FLJ55140	4.73	1. Ca ²⁺ binding 2. Collagen binding

Coagulation protein	Prothrombin	5.64	1. Non-covalently calcium ions (Ca ²⁺) binding. 2. Blood coagulation 3. Involved in serine-type endopeptidase activity
Structural protein	Keratin, type II cytoskeletal 2 epidermal	8.07	1. Cytoskeletal protein binding 2. Cross linking of peptides 3. Structural constituent of cytoskeleton 4. Keratinization
	Keratin, type II cytoskeletal 5	7.58	1. Scaffold protein binding 2. Structural constituent of cytoskeleton 3. Keratinization
	Keratin, type I cytoskeletal 14	5.09	1. Responding to Zn ²⁺ 2. Keratin filament binding 3. Structural constituent of cytoskeleton 4. Keratinization
	Keratin 77	5.8	1. Involved in structural integrity of epithelial cells 2. Involved in structural molecular activity
	Keratin 1	8.15	1. Significant for structural integrity of a complex or its assembly within or outside a cell.
	Keratin, type I cytoskeletal 9	5.14	1. Keratin filament binding 2. Structural constituent of cytoskeleton 3. Keratinization 4. Epidermis development 5. Intermediate filament organization
	Keratin, type I cytoskeletal 10	5.13	1. Cross linking of peptides 2. Protein heterodimerization and heterotetramerization activity 3. Structural constituent of epidermis 4. Keratinization
Others	cDNA FLJ57154	6.29	1. Helps in heterologous expression
	cDNA FLJ58606	7.82	1. Helps in heterologous expression
	Protein S100-A7A	6.89	1. Ca ²⁺ binding 2. Transition metal binding 3. Involved in protein self-association
	A-kinase anchor protein	8.84	1. Protein kinase A (PKA) binding 2. Specific substrate targeting by means of phosphorylation (through PKA) and dephosphorylation (through phosphatases)
	Epididymis luminal protein 110	7.8	1. Fe ²⁺ , Fe ³⁺ binding 2. Involved in serine type endopeptidase action 3. Engender antimicrobial humoral response 4. Regulates the production of cytokine
	Zinc finger SWIM domain-containing protein 4	6.47	1. Zn ²⁺ binding 2. DNA binding
	Alpha-fetoprotein	4.57	1. Metal (copper, nickel) binding 2. Fatty acids binding 3. Bilirubin binding 4. Cellular protein metabolism 5. Progesterone metabolism
	Protein unc-13 homolog B	5.67	1. Ca ²⁺ binding 2. Calmodulin binding 3. Phospholipid binding 4. Diacylglycerol binding 5. Intracellular signal transduction 6. Presynaptic and synaptic vesicle exocytosis

Table S8: Protein corona profile of CA in 10% FBS in terms of their molecular weight (Da), -10lgP and coverage (%).

Functional classification of protein	Identified Protein	Molecular Weight (Da)	-10lgP	Coverage (%)
Transport Protein	ALB protein	69294	260.46	80
	Serum Albumin	69227	207.46	40
	Alpha-2-HS glycoprotein	39325	121.73	11
	cDNA FLJ51509	54207	107.1	15
	Transthyretin	15872	80.92	28
	Serotransferrin	77064	92.84	7
	Transferrin variant	77080	92.84	7
	Transferrin	76960	92.84	7
	LMAN1 protein	57549	21.54	1
	Globin C1	19232	78.40	23
	Globin B1	16071	33.31	7
	Globin A1	15954	33.31	7
	Alpha-1-acid glycoprotein (positive acute phase protein)	23512	37.40	6
	Mutant hemoglobin alpha 1 globin chain	10753	78.40	40
	Hemoglobin alpha-1 globin chain	10783	78.40	40
	Hemoglobin subunit alpha	20146	78.40	28
	Alpha-2 globin chain	15258	78.40	28
	Mutant hemoglobin alpha 2 globin chain	15258	78.40	28
	Lactoferrin	78357	69.91	4
	Beta-globin protein	4550	59.21	56
	Hemoglobin fetal subunit beta	15859	33.31	7
	Truncated beta-globin	6998	59.21	37
	Mutant beta-globin	9689	59.21	26
	Beta-globin chain	11476	59.21	26
	Hemoglobin subunit beta	9670	59.21	26
	Hemoglobin beta chain	11023	59.21	23

	Hemoglobin beta chain variant Hb.Sinai-Bel Air	11548	59.21	22
	Hemoglobin beta chain variant Hb-I_Toulouse	11505	59.21	22
	Vitamin D-binding protein	53021	47.50	1
Cell adhesive protein	cDNA FLJ54111	63437	92.84	8
Coagulation protein	Prothrombin	35932	39.89	3
Metabolic Protein	Adiponectin	26133	39.64	4
Protease inhibitor	Kininogen-1	71957	43.16	2
	KNG1 protein	33055	43.16	3
	Fetuin-B	42663	49.43	11
	Alpha-1-antiproteinase	46104	118.31	18
Structural Protein	Keratin, type I cytoskeletal 10	54848	86.65	14
	Keratin 1	66126	101.91	11
	KRT6A protein	60045	62.63	4
	Keratin, type I cytoskeletal 14	51561	72.04	8
	Histone H2B	13926	20.26	7
Regulatory Protein	Junction mediating and regulatory protein p53 cofactor	109383	21.54	1
Others	Uncharacterized protein	18931	59.21	13
	Epididymis secretory protein Li 51	52964	47.50	1
	Alpha-fetoprotein	68678	107.1	12
	cDNA FLJ95666	69393	206.8	39
	cDNA FLJ57154	50756	107.10	16
	cDNA FLJ55606	46627	121.73	9

Table S9: Protein corona profile of low Fe/Mg-CA in 10% FBS in terms of their molecular weight (Da), -10lgP and coverage (%).

Functional classification of protein	Identified Protein	Molecular Weight (Da)	-10lgP	Coverage (%)
Transport protein	Albumin	66531	180.69	36
	Mutant hemoglobin alpha 1 globin chain	10753	95.34	47
	Hemoglobin alpha-1 globin chain	10783	95.34	47
	Alpha-2 globin chain	15258	95.34	33
	Hemoglobin subunit alpha piens	15258	95.34	33
	Mutant hemoglobin alpha 2 globin chain	15258	95.34	33
	Hemoglobin subunit alpha	20146	95.34	33
	Globin C1	19232	95.34	27
	Beta-globin protein	4550	57.88	56
	Beta-globin chain	11476	62.94	22
	Truncated beta-globin	6998	57.88	37
	Mutant beta-globin	9689	62.94	26
	Hemoglobin subunit beta	9670	57.88	26
	Hemoglobin beta chain	11023	57.88	23
	Hemoglobin beta chain variant Hb-I_Toulouse	11505	62.94	22
	Mutant hemoglobin beta chain (Fragment)	11501	57.88	22
	Hemoglobin beta globin chain	11494	57.88	22
	Hemoglobin beta	11482	62.94	22
	Hemoglobin beta chain variant Hb.Sinai-Bel Air	11548	57.88	22
	Alpha-2-HS glycoprotein	39325	62.15	13
	Transthyretin	15872	88.27	44
	Serotransferrin	77064	77.82	5
	Transferrin variant	77080	77.82	5
	Transferrin	76960	77.82	5
	Lactotransferrin	76626	55.45	3
	Lactoferrin	77981	55.45	3
	Delta globin	16055	48.92	7
	Hbbm fused globin protein	10928	40.72	10
Coagulation protein	Prothrombin	35932	37.17	3
Cell adhesive protein	cDNA FLJ54111	63437	71.87	6
	Proliferation-inducing protein 33	75230	72.24	5
	C-type lectin domain family 3 member B isoform CRA_a	22537	48.09	10
Enzymes	N-acetyltransferase 5	18857	24.33	4
Structural protein	cDNA FLJ56708	18049	62.03	15
	Keratin, type I cytoskeletal 10	58827	154.11	34
	Keratin, type I cytoskeletal 28	50567	59.40	5

	Keratin 1	66126	145.46	22
	Keratin, type II cytoskeletal 2	65433	103.32	13
Others	Protein unc-13 homolog B	180679	21.64	1
	MHC class I antigen	12392	23.42	4
	Alpha-fetoprotein	68678	70.56	6
	cDNA FLJ57154	50756	54.72	7
	cDNA FLJ79229	73170	68.49	3
	Uncharacterized protein	18931	57.88	13
	A-kinase anchor protein	161184	21.18	1
	Zinc finger SWIM domain-containing protein 4	99649	29.84	1
	Capsid scaffolding protein	66941	21.94	1
	cDNA FLJ51509	54207	70.56	8
	cDNA FLJ55606	46627	62.15	11

Table S10: Protein corona profile of high Fe/Mg-CA in 10% FBS in terms of their molecular weight (Da), -10lgP and coverage (%).

Functional classification of protein	Identified Protein	Molecular Weight (Da)	-10lgP	Coverage (%)
Transport protein	Albumin	66531	158.89	27
	Globin C1	19232	90.79	23
	Alpha-2 globin chain	15258	86.99	28
	Hemoglobin subunit alpha	20146	86.99	28
	Mutant hemoglobin alpha 1 globin chain	10753	86.99	40
	Hemoglobin alpha-1 globin chain	10783	86.99	40
	Mutant hemoglobin alpha 2 globin chain	15258	86.99	28
	Beta-globin protein	2104	40.72	59
	Alpha-2-HS glycoprotein	39325	55.71	9
	Transthyretin	15872	47.21	24
	Serotransferrin	77064	71.87	5
	Transferrin variant	77080	71.87	5
	Transferrin	76960	71.87	5
	Tetranectin	17794	48.09	13
	cDNA FLJ54839	54207	55.45	7
	cDNA FLJ53691	74832	71.87	5
	Lactotransferrin	76626	55.45	3
	Lactoferrin	77981	55.45	3
	Delta globin	16055	48.92	7
	Hemoglobin subunit gamma-2	15319	40.72	7
	Globin B3	16203	40.72	7

	Gamma-G globin	16969	40.72	6
	Hbbm fused globin protein	10928	40.72	10
Cell adhesive protein	SPARC-like protein 1	75208	72.24	5
	Proliferation-inducing protein 33	75230	72.24	5
	C-type lectin domain family 3 member B isoform CRA_a	22537	48.09	10
	cDNA FLJ54111	63437	71.87	6
	cDNA FLJ54278	58507	72.24	7
	cDNA FLJ54387	64141	72.24	6
	cDNA FLJ55140	55519	72.24	7
Coagulation protein	Prothrombin	35932	35.09	3
Structural protein	Keratin, type II cytoskeletal 2 epidermal	65433	131.06	13
	Keratin, type II cytoskeletal 5	62378	97.24	11
	Keratin, type I cytoskeletal 14	51562	95.71	13
	Keratin 77	61802	81.98	5
	Keratin 1	66126	232.15	40
	Keratin, type I cytoskeletal 9	62064	183.04	33
	Keratin, type I cytoskeletal 10	58827	172.78	37
Others	cDNA FLJ57154	50756	54.72	7
	cDNA FLJ58606	91670	29.84	1
	Protein S100-A7A	11305	36.53	11
	A-kinase anchor protein	161184	21.18	1
	Epididymis luminal protein 110	78182	55.45	3
	Zinc finger SWIM domain-containing protein 4	99649	29.84	1
	Alpha-fetoprotein	68678	54.72	5
	Protein unc-13 homolog B	180679	21.64	1