Journal Name

ARTICLE

Probing Fibronectin Conformation on Protein Corona Layer around Nanoparticles

Mohammad Raoufi[1,2], Mohammad Javad Hajipour[3,4], Seyed Mehdi Kamali Shahri[5], Ingmar Schoen[6], Uwe Linne[7], Morteza Mahmoudi[8]*

[1]Nanotechnology Research Centre, Faculty of Pharmacy, Tehran University of Medical Sciences, Tehran 1417614411, Iran

[2]Department of New Materials and Biosystems, Max Planck Institute for Intelligent Systems, Heisenbergstraße 3, D-70569 Stuttgart, Germany

[3]Persian Gulf Marine Biotechnology Research Center, The Persian Gulf Biomedical Sciences Research Institute, Bushehr University of Medical Sciences, Bushehr 75147, Iran

[4]Non-Communicable Diseases Research Center, Endocrinology and Metabolism Population Sciences Institute, Tehran University of Medical Sciences, Tehran 13169-43551, Iran.

[5]Department of Chemical Engineering, The Pennsylvania State University, University Park, PA 16802, United States.

[6]Laboratory of Applied Mechanobiology, Department of Health Sciences and Technology, ETH Zurich, Vladimir-Prelog Weg 4, CH-8093 Zurich, Switzerland

[7]Fachbereich Physik/Chemie, Philipps-Universität Marburg, Marburg, Germany,

[8]Center for Nanomedicine and Department of Anesthesiology, Brigham and Women's Hospital, Harvard Medical School, Boston, Massachusetts 02115, United States

* Corresponding author: mmahmoudi@bwh.harvard.edu

EXPERIMENTAL SECTION

Citrate coated gold NPs were purchased from Sigma (diameter of 5 nm, Cas. Nr. 741949; diameter of 100 nm, Cas. Nr. 742031) and characterized by scanning electron microscopy (SEM) and transmission electron microscopy (TEM). Very dilute NP solutions were deposited on substrates. Superparamagnetic Iron Oxide (SPION) NPs were purchased from Micromode.

Labeled FN

FRET-labeled FN was prepared according to a previously described procedure [1, 2]. Briefly, FN was purified from human plasma by gel filtration and affinity chromatography, denatured to label the four internal cysteines using Alexa Fluor 546 maleimide, and transferred to sodium phosphate buffer for labeling surface-exposed lysines by Alexa Fluor 488 NHS ester [3, 4]. Absorption measurements showed an average degree of labeling with 8.5 donors and 4.0 acceptors [4]. For NP experiments, FRET-labeled FN was mixed with unlabeled FN at a ratio of 1:20 (to avoid intermolecular FRET) and suspended in phosphate-buffered saline (PBS; Life Technologies, Darmstadt, Germany) at a concentration of 100 µg ml^{-1}. For reference measurements with FRET-FN in solution, the mixing ratio of FRET-labeled:unlabeled FN was 2:3 in PBS or in PBS containing 1 M or 4 M guanidine hydrochloride (GdnHCl, Sigma Aldrich, Munich, Germany).

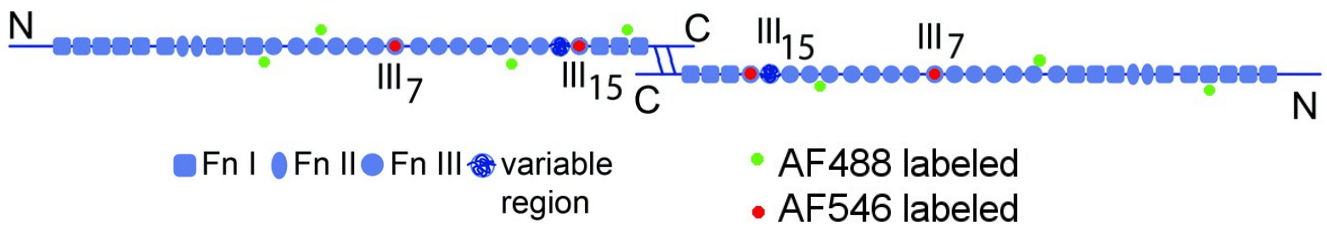

Figure S1. Schematic of Labeled Fn With Alexa Fluor 488 as donor and Alexa Fluor 546 as acceptor

FRET analysis

The emission spectra of FRET-labeled FN in solution with or w/o NPs was analyzed using a fluorospectrometer (FluoroMax, Horiba) with Casta 86 flat transparent well plates. Samples were excited at 470 nm, and emission was collected over the range 510-700 nm to capture the emission peaks of donor and acceptor. For each condition, measurements were repeated with three independently processed samples to assess sample-to-sample variability. The emission spectra of NPs at the two different concentrations was recorded as a blank and subtracted from the emission spectra of FRET-FN on the respective NPs before calculation of the FRET ratio. FRET ratios are presented as mean value ± standard error of the mean (SEM). All measured FRET ratios of FN on NPs fall in the range 0.5-0.8, which is consistent with the denaturing curve in solution for which we found FRET ratios of 0.93 (in PBS), 0.32 (1 M GdnHCl), and 0.17 (4 M GdnHCl).

Protein corona

Unlabeled FN, fibrinogen (FG, from human plasma; Calbiochem, San Diego, CA), or human serum albumin (HSA; 70029-90-7, Calbiochem,

Germany) were dissolved in PBS at a concentration of 1 mg ml^{-1}. Plasma protein solution was used as-is (100 %) or diluted to 0.1% with PBS. The pH of solutions was adjusted to 6.5 or 7.4 using 0.1 M NaOH or HCl solutions. Bare NPs (with particle concentrations of 100 and 500 µg ml^{-1}) were incubated with these protein solutions (FN, FG, HSA, or plasma) for 20 min at room temperature and then centrifuged (at 18,000 g for 20 min at room temperature) to collect the corona-coated NPs. The corona-coated NPs were re-suspended in PBS and centrifuged at 18,000 g for 20 min (washing step), repeated three times to get rid of loosely attached proteins on NPs [5]. Bare or corona-coated NPs were incubated with FRET-labeled FN solution and then purified from free FRET-FN by the same procedure as for the preparation of protein coronas.

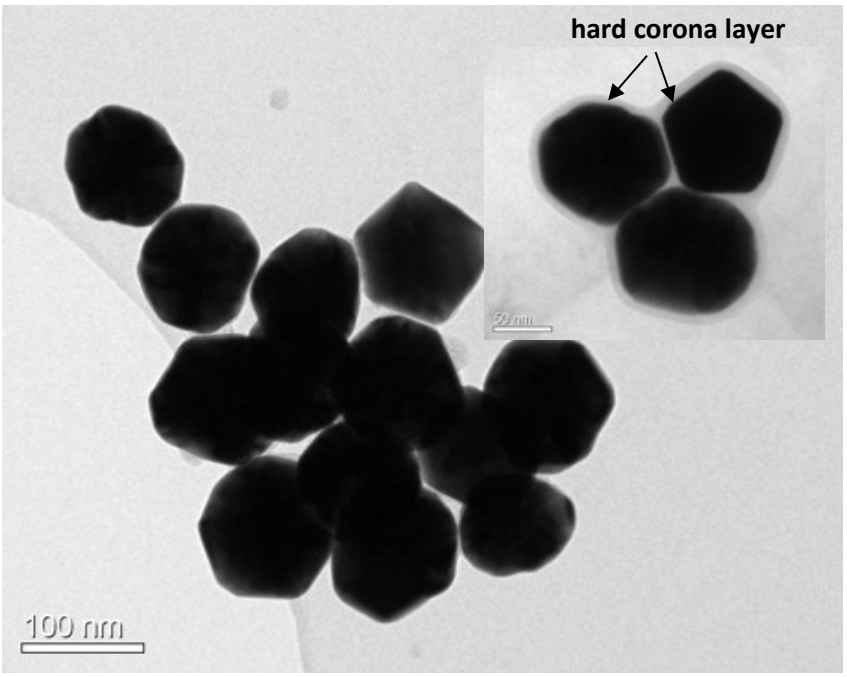

Figure S2. TEM image of gold NPs with diameter of 100 nm (Inset protein corona-coated gold

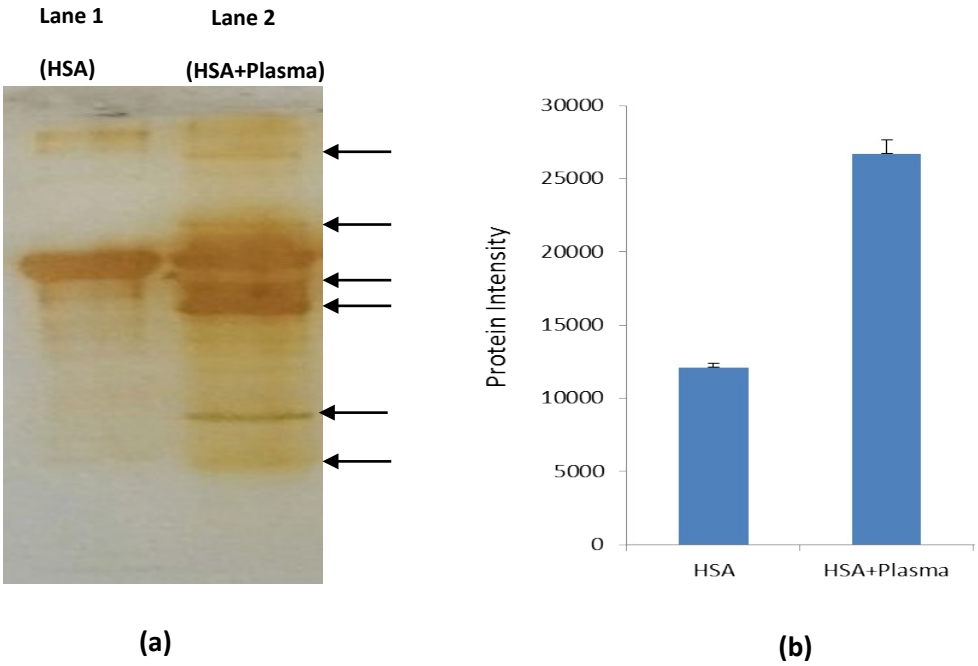

Figure S3: a) SDS-PAGE gel (15%) of corona-coated gold NPs after incubation with HSA or HSA and human plasma (two times). The black arrows show the new proteins adsorbed onto the HSA-coated NPs after incubation with human plasma. b) The intensity of HSA and other proteins involved in the corona of gold NPs were measured using Image J software. (HSA: the intensity of HSA protein in lanes 1, HSA + plasma: the intensity of all proteins in lanes 2). Error of intensities are determined by several analyses.

Gravity effect on protein corona

To clear the gravity effect, after incubation of FRET-FN with pre-coated NPs, the sample centrifuged with higher relative centrifugal force (RCF) (1.5 and 2 time RCF) resulting not significant changes on FRET ratio. This indicate that demonstrating of gravity force could not press the protein for more attached to NPs. The only important for replacing the FRET-FN by protein coated on NPs is affinity of FN. This indicates that the higher RCF leads to more stable attachment of protein to NPs.

Uv-visible spectra of gold NPs coated with protein corona

The Uv-visible spectra of untreated and corona-coated NPs were measured at 400-780 nm wavelength range. The surface plasmon resonances (SPRs) of corona-coated NPs showed no significant shift in peak position. This means NPs do not aggregate after protein corona formation.

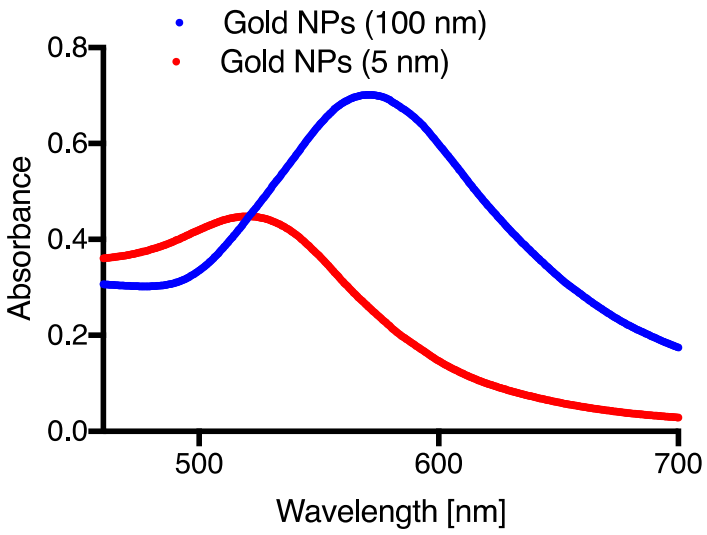

Figure S4. Uv-visible spectra of coated NPs.

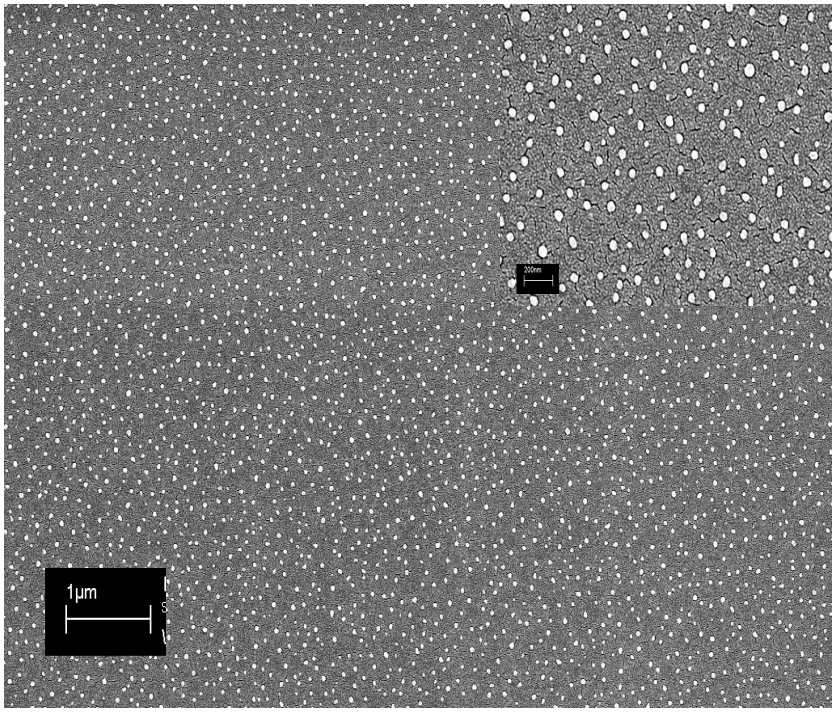

Figure S5. SEM image of protein corona-coated gold NPs at pH 6.5. No aggregation of the 100-nm gold NPs was observed.

Extraction of proteins associated within the protein corona and LCMS/MS analysis

The contribution of FRET-FN to the corona in response to the diameter and concentration of NPs as well as variations in the type of proteins on pre-coated NP was investigated using LC-MS/MS. The obtained hard corona-coated NPs were incubated with trypsin, with final

concentration of 2ng ml^{-1}, at 37 ºC for 24 hours. Then, this solution was centrifuged at 18000 g for 60 min and the supernatant containing the peptides was collected (First peptide solution). The pelleted NPs were resuspended in 2% formic acid and incubated at 37 ºC for 45 min. In the next step, this solution was centrifuged at 18000 g for 60 min and the supernatant containing the peptides was added to the first peptide solution. Finally the extracted proteins was incubated with 1% dithiothreitol and injected into LC-MS/MS and two or three replicates were measured by LC-MS/MS to ensure high accuracy in the analysis [6]. This analytical approach determined the intensity and type of proteins bound to the NPs. The proteins adsorbed onto the corona-coated NPs are listed in table 1-16. The FN protein, which was subjected for dual FRET labeling, extracted from human plasma using gel filtration and affinity chromatography[3, 4]. Undoubtedly, low concentrations of other plasma proteins were also extracted through FN extraction. The additional proteins detected in the hard corona of HSA/FG/FN- pre-coated NPs incubated with FRET-FN are those previously extracted from serum human plasma. These additional proteins may limit FRET-FN accessibility to NP surface. Therefore, they can reduce the discrepancy between FRET ratio of FN adsorbed on the untreated and corona-coated NPs

Calculation of the intensity of proteins bound to the NPs

The contribution of each protein to the protein corona was calculated and normalized using the following equation.

$$N_p S_p C_k = \left(\frac{(S_p C / M_w)_K}{(\sum_{i=1}^{n} S_p C / M_w)_i} \right) \times 100$$

SpC is the spectral count identified for each protein in LC-MS/MS analysis and M_w is molecular weight (kDa) of protein [7].

SDS-PAGE

The corona-coated NPs were mixed with sample buffer and boiled for 10 min at 100 ºC. In the next step, the identical amounts of boiled samples were loaded on a 15% polyacrylamide gel and run at 100 V, 80 mA for 120 min. The obtained gel was stained using highly sensitive silver nitrate. The semi-quantitative intensity of proteins adsorbed on the NP surface was measured using Image J software [8].

SEM/TEM analysis

For SEM, samples were coated with approximately 7 nm carbon. SEM was performed using a Zeiss Ultra 55cv device (Oberkochen, Germany, operation voltage of 5 kV), and TEM was carried out with JEOLJEM1010.3 by placing a 20-µl drop of the sample onto a 200-nm mesh formvar/carbon-coated copper grid.

ITC experimental procedure

NanoITC calorimeters (TA instruments) were used for ITC experiments equipped with gold and hastelloy materials for sample and reference cells (cell volume=1.0 ml). All the experiments were performed at 37.5 ^{o}C using a 250 µl syringe at a stirring rate of 250 rpm. The sample cell contains with different concentrations of 5 nm Au NPs (NPs) solution (0-0.353 mM). The reference cell contains the same aqueous solution as the sample cell has for each experiment. The cells were initially flushed with the Au NPs solutions for three times to eliminate any possible interactions of Au NPs with the cell walls during all experiments. In a typical experiment, a desired volume of plasma and/or FN solution was incrementally injected in series into the preferred concentrations of Au NPs, and the power compensation was simultaneously measured as a function of time. The experiments were conducted under identical conditions in ITCs with Au and hastelloy cells, and the data reported in here is from ITC with hastelloy material of cells. A control experiment was run to eliminate the heat of mixing because the area under the peak contains heat of mixing and adsorption at the same time. Data analysis was performed using the NanoAnalyze software from TA instruments.

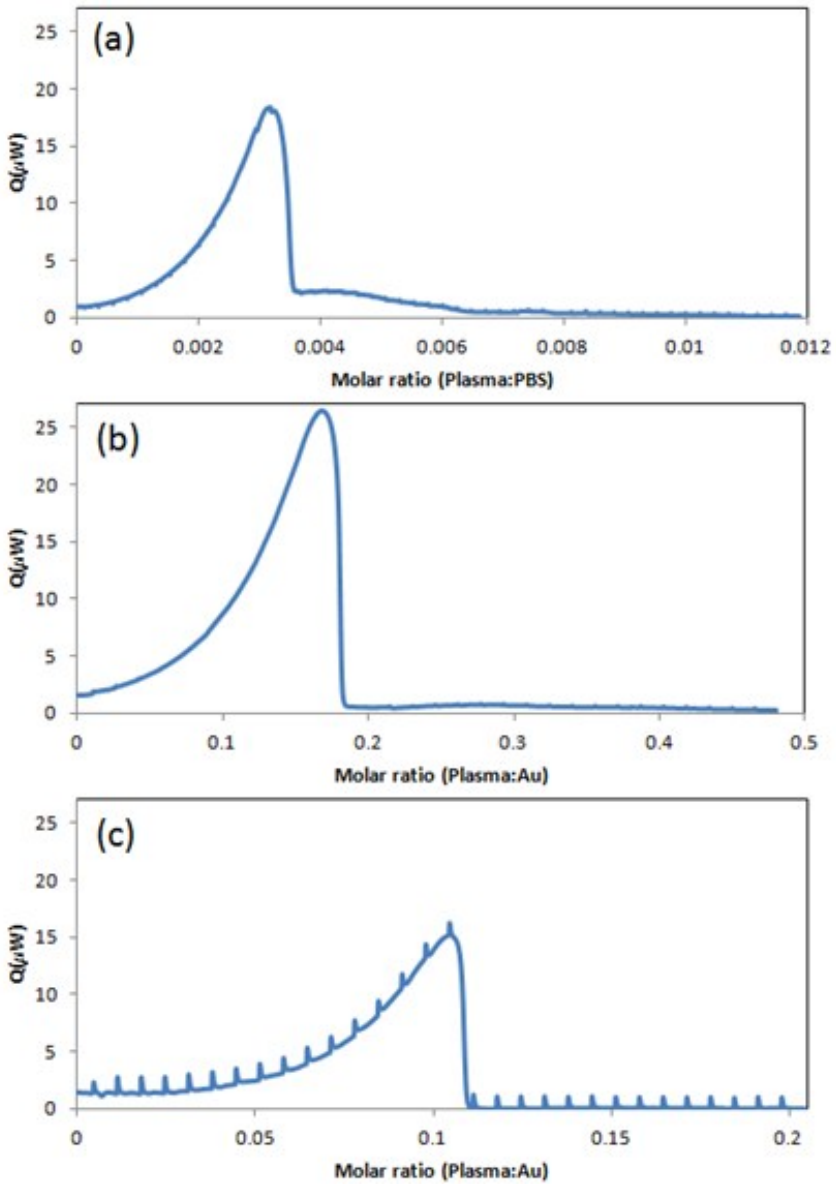

Figure S6. Real-time ITC thermograms for binding the plasma (0.47 mM) to, a) PBS (10 mM), b) Au NPs (0.12 mM), and c) Au NPs (0.353 mM) at 37.5 °C. Plasma in syringe was injected into PBS and Au NPs.

Table S1. The FRET ratio of FRET-labeled Streptavidin on untreated and pre-coated Au and Iron oxide NPs. As Streptavidin is not expected to undergo major conformational changes upon physisorption, these experiments served as a control to determine the contribution of surface plasmons to the measured changes in FRET between untreated and pre-coated particles.

Table S2. Proteins associated within the unlabeled FN-coated NPs (5 nm, 100 μg/ml) incubated with labeled FN, as identified by LC-MS/MS analysis

Table S3. Proteins associated within the Fg-coated NPs (5 nm, 100 μg/ml) incubated with labeled FN, as identified by LC-MS/MS analysis

Table S4. Proteins associated within the HSA-coated NPs (5 nm, 100 μg/ml) incubated with labeled FN, as identified by LC-MS/MS analysis

Table S5. Proteins associated within the hard corona (0.1% plasma)-coated NPs (5 nm, 100 μg/ml) incubated with labeled FN, as identified by LC-MS/MS analysis

Table S6. Proteins associated within the unlabeled FN-coated NPs (5 nm, 500 μg/ml) incubated with labeled FN, as identified by LC-MS/MS analysis

Table S7. Proteins associated within the Fg-coated NPs (5 nm, 500 μg/ml) incubated with labeled FN, as identified by LC-MS/MS analysis

Table S8. Proteins associated within the HSA-coated NPs (5 nm, 500 μg/ml) incubated with labeled FN, as identified by LC-MS/MS analysis

Table S9. Proteins associated within the hard corona (0.1% plasma)-coated NPs (5 nm, 500 μg/ml) incubated with labeled FN, as identified by LC-MS/MS analysis

Table S10. Proteins associated within the unlabeled FN-coated NPs (100 nm, 100 μg/ml) incubated with labeled FN, as identified by LC-MS/MS analysis

Table S11. Proteins associated within the Fg-coated NPs (100 nm, 100 μg/ml) incubated with labeled FN, as identified by LC-MS/MS analysis

Table S12. Proteins associated within the HSA-coated NPs (100 nm, 100 μg/ml) incubated with labeled FN, as identified by LC-MS/MS analysis

Table S13. Proteins associated within the hard corona (0.1% plasma)-coated NPs (100 nm, 100 μg/ml) incubated with labeled FN, as identified by LC-MS/MS analysis

Table S14. Proteins associated within the unlabeled FN-coated NPs (100 nm, 500 μg/ml) incubated with labeled FN, as identified by LC-MS/MS analysis

Table S15. Proteins associated within the Fg-coated NPs (100 nm, 500 μg/ml) incubated with labeled FN, as identified by LC-MS/MS analysis

Table S16. Proteins associated within the HSA-coated NPs (100 nm, 500 μg/ml) incubated with labeled FN, as identified by LC-MS/MS analysis

Table S17. Proteins associated within the hard corona (0.1% plasma)-coated NPs (100 nm, 500 μg/ml) incubated with labeled FN, as identified by LC-MS/MS analysis

Diameter	5 nm	100 nm
Untreated Au NPs	0,391	0,401
Pre-coated Au NPs	0,426	0,434

Table S1

Diameter	150 nm
Untreated Iron oxide NPs	0,662
Pre-coated Iron oxide NPs	0,701

Table S2

Protein identity	NSpC
Fibronectin	10.5
Serum albumin	6.6
Vinculin	1.8
Ubiquitin-40S ribosomal protein S27a	12.8
Ig gamma-1 chain C region	6.3
Elongation factor 1-alpha 2	4.5
Ig gamma-3 chain C region	5.5
Dihydrolipoyllysine-residue succinyltransferase component of 2-oxoglutarate dehydrogenase complex, mitochondrial	4.7
78 kDa glucose-regulated protein	3.1
40S ribosomal protein S14	14.1
Prolyl 4-hydroxylase subunit alpha-2	3.7
Probable G-protein coupled receptor 141	6.4
Kinectin	1.47

Table S3

Protein identity	NSpC
Fibronectin	5.9
Serum albumin	15.6
Fibrinogen alpha chain	6.5
Fibrinogen beta chain	5.5
Ig gamma-1 chain C region	8.6
Elongation factor 1-alpha 2	3.0
40S ribosomal protein S14	9.5
Vinculin	1.2
Ubiquitin-40S ribosomal protein S27a	8.6
Isoform 11 of Sorbin and SH3 domain-containing protein 2	1.1
Fibrinogen gamma chain	3.0
Ig gamma-3 chain C region	3.7
Kinectin	0.9
POTE ankyrin domain family member E	1.2
LINE-1 type transposase domain-containing protein 1	1.5
Lactotransferrin	1.9
78 kDa glucose-regulated protein	2.1
Isoform 13 of Dysferlin	0.6
Dihydrolipoyllysine-residue succinyltransferase component of 2-oxoglutarate dehydrogenase complex, mitochondrial	3.1
Isoform 5 of Bromodomain-containing protein 9	2.7
Isoform 5 of Membrane-associated guanylate kinase, WW and PDZ domain-containing protein 1	1.1
Stromal interaction molecule 1	2.0

Table S4

Protein identity	NSpC
Serum albumin	36.2
fibronectin	2.6
Actin, aortic smooth muscle	4.2
Fibrinogen alpha chain	2.6
Elongation factor 1-alpha 3	3.5
Ubiquitin-40S ribosomal protein S27a	9.9
Dihydrolipoyllysine-residue succinyltransferase component of 2-oxoglutarate dehydrogenase complex, mitochondrial	3.6
Rho GTPase-activating protein 30	1.2
E3 ubiquitin-protein ligase TRIM18	3.2
Transmembrane 9 superfamily member 2	2.5
79 kDa glucose-regulated protein	2.4
Integrator complex subunit 8	1.6
40S RIBOSOMAL PROTEIN S7	6.2
Syntaxin-6	4.5
Acetylcholine receptor subunit alpha	3.4
Protein phosphatase 1 regulatory subunit 3B	5.5

Table S5

Protein identity	NSpC
Serum albumin	13.1
Fibronectin	3.4
Ig gamma-1 chain C region	4.6
Alpha-1-antitrypsin	7.1
Complement C3	1.3
Ig gamma-3 chain C region	2.0
POTE ankyrin domain family member E	0.6
Serotransferrin	2.1
Vinculin	0.6
Ig heavy chain V-III region BRO	6.2
Fibrinogen beta chain	1.4
Ig heavy chain V-III region TRO	6.1
Ubiquitin-40S ribosomal protein S27a	4.6
Alpha-2-HS-glycoprotein	2.1
Elongation factor 1-alpha 2	1.6
78 kDa glucose-regulated protein	1.1
40S ribosomal protein S14	5.1
RAS guanyl-releasing protein 1	0.9
Ig lambda-2 chain C regions	7.3
Isoform 13 of Dysferlin	0.3
Tumor suppressor candidate 2	6.8
LINE-1 type transposase domain-containing protein 1	0.8
Isoform 4 of Probable helicase senataxin	0.2
Cullin-1	0.9
Lactotransferrin	1.0
Potassium/sodium hyperpolarization-activated cyclic nucleotide-gated channel 4	0.6
Dynein heavy chain 10, axonemal	0.1
Kinectin	0.5

Table S6

Protein identity	NSpC
Fibronectin	9.9
Serum albumin	15.6
Ig gamma-1 chain C region	6.0
Fibrinogen beta chain	3.8
Vinculin	1.7
Ig gamma-3 chain C region	5.2
Isoform 11 of Sorbin and SH3 domain-containing protein 2	1.6
Ubiquitin-40S ribosomal protein S27a	12.0
Kinectin	1.3
Elongation factor 1-alpha 2	4.2
78 kDa glucose-regulated protein	2.9
40S ribosomal protein S14	13.3
LINE-1 type transposase domain-containing protein 1	2.1
Lactotransferrin	2.7

Table S7

Protein identity	NSpC
Fibronectin	5.7
Serum albumin	14.4
Ig gamma-1 chain C region	4.6
Fibrinogen beta chain	5.9
Fibrinogen gamma chain	3.2
Fibrinogen alpha chain	5.2
Isoform 11 of Sorbin and SH3 domain-containing protein 2	1.2
Vinculin	1.3
Ubiquitin-40S ribosomal protein S27a	9.3
Alpha-1-antitrypsin	7.1
POTE ankyrin domain family member E	1.3
Elongation factor 1-alpha 2	3.3
Kinectin	1.0
LINE-1 type transposase domain-containing protein 1	1.6
Isoform 4 of Probable helicase senataxin	0.5
Isoform 13 of Dysferlin	0.6
Suppression of tumorigenicity 18 protein	1.4
Zinc finger protein 48	2.4
Phosphoinositide 3-kinase adapter protein 1	1.8
Transcription factor SOX-30	2.0
Cullin-1	1.8
X-ray radiation resistance-associated protein 1	1.8
Protein bassoon	0.4

Table S8

Protein Identity	NSpC
Fibronectin	5.7
Serum albumin	14.4
Ig gamma-1 chain C region	4.6
Fibrinogen beta chain	5.9
Fibrinogen gamma chain	3.2
Fibrinogen alpha chain	5.2
Isoform 11 of Sorbin and SH3 domain-containing protein 2	1.2
Vinculin	1.3
Ubiquitin-40S ribosomal protein S27a	9.3
Alpha-1-antitrypsin	7.1
POTE ankyrin domain family member E	1.3
Elongation factor 1-alpha 2	3.3
Kinectin	1.0
LINE-1 type transposase domain-containing protein 1	1.6
Isoform 4 of Probable helicase senataxin	0.5
Isoform 13 of Dysferlin	0.6
Suppression of tumorigenicity 18 protein	1.4
Zinc finger protein 48	2.4
Phosphoinositide 3-kinase adapter protein 1	1.8
Transcription factor SOX-30	2.0
Cullin-1	1.8
X-ray radiation resistance-associated protein 1	1.8
Protein bassoon	0.4

Table S9

Protein identity	NSpC
Serum albumin	12.8
Fibronectin	4.8
Ig gamma-1 chain C region	7.0
Vinculin	1.0
Alpha-1-antitrypsin	8.1
Ig gamma-3 chain C region	3.0
POTE ankyrin domain family member E	1.0
Fibrinogen beta chain	2.2
Complement C3	0.6
Isoform 11 of Sorbin and SH3 domain-containing protein 2	0.9
40S ribosomal protein S14	7.8
Ubiquitin-40S ribosomal protein S27a	7.0
Elongation factor 1-alpha 2	2.5
78 kDa glucose-regulated protein	1.7
Dihydrolipoyllysine-residue succinyltransferase component of 2-oxoglutarate dehydrogenase complex, mitochondrial	2.6
Isoform 13 of Dysferlin	0.5
Tumor suppressor candidate 2	10.5
LINE-1 type transposase domain-containing protein 1	1.2
Isoform 4 of Probable helicase senataxin	0.4
Isoform 2 of Cell division cycle protein 27 homolog	1.3
Serotransferrin	1.6
Fibrinogen alpha chain	1.3
Oxysterol-binding protein-related protein 2	2.3
Lactotransferrin	1.6
Laminin subunit gamma-1	0.7
Kinectin	0.8

Table S10

Protein identity	NSpC
Fibronectin	10.9
Serum albumin	4.5
Elongation factor 1-alpha 2	6.3
Isoform 11 of Sorbin and SH3 domain-containing protein 2	2.3
Vinculin	2.5
Ubiquitin-40S ribosomal protein S27a	17.7
Dihydrolipoyllysine-residue succinyltransferase component of 2-oxoglutarate dehydrogenase complex, mitochondrial	6.5
Ig gamma-1 chain C region	8.8
40S ribosomal protein S14	19.5
78 kDa glucose-regulated protein	4.4
Phosphatidylinositol 4,5-bisphosphate 3-kinase catalytic subunit alpha isoform	2.5
Isoform 13 of Dysferlin	1.3
E3 ubiquitin-protein ligase HECTD1	1.1
Integrin beta-8	3.7
Laminin subunit gamma-1	1.7
Centromere protein F	0.8

Table S11

Protein identity	NSpC
Fibronectin	5.2
Fibrinogen alpha chain	11.5
Fibrinogen gamma chain	5.3
Fibrinogen beta chain	9.7
Isoform 11 of Sorbin and SH3 domain-containing protein 2	2.0
Vinculin	2.2
Ubiquitin-40S ribosomal protein S27a	15.2
Ig gamma-1 chain C region	7.5
Elongation factor 1-alpha 2	5.4
Dihydrolipoyllysine-residue succinyltransferase component of 2-oxoglutarate dehydrogenase complex, mitochondrial	5.6
Integrin beta-8	3.1
Nuclear pore complex protein Nup98-Nup96	1.3
DnaJ homolog subfamily B member 9	10.7
N-acetylglucosamine-1-phosphotransferase subunits alpha/beta	1.9

Table S12

Protein identity	NSpC
Serum albumin	66.9
fibronectin	4.4
Fibrinogen alpha chain	3.6
Elongation factor 1-alpha 3	4.8
Ubiquitin-40S ribosomal protein S27a	13.5
Endoplasmin	2.6

Table S13

Protein identity	NSpC
Serum albumin	21.2
Ig gamma-1 chain C region	8.6
Fibronectin	1.1
Ig gamma-4 chain C region	2.1
Alpha-1-antitrypsin	3.3
POTE ankyrin domain family member E	0.6
Vinculin	0.62
Elongation factor 1-alpha 2	1.5
Ig heavy chain V-III region BRO	5.8
Ig heavy chain V-III region POM	5.9
Isoform 2 of Ig mu chain C region	1.5
Serotransferrin	4.0
Isoform 3 of Vitamin D-binding protein	1.4
Fibrinogen alpha chain	0.8
Ig heavy chain V-III region TRO	5.7
Ig kappa chain V-III region SIE	6.5
78 kDa glucose-regulated protein	1.0
Fibrinogen beta chain	1.3
Ig lambda-2 chain C regions	6.8
Tumor suppressor candidate 2	6.4
Isoform 4 of Probable helicase senataxin	0.2
Kinectin	0.4
LINE-1 type transposase domain-containing protein 1	0.7
Isoform 11 of Sorbin and SH3 domain-containing protein 2	0.5
Inactive caspase-12	1.9
Mucin-5B	0.1
Aldehyde oxidase	0.5
Syntaxin-5	1.9

Table S14

Protein identity	NSpC
Fibronectin	10.5
Fibronectin	3.9
Vinculin	2.2
Ubiquitin-40S ribosomal protein S27a	15.4
Ig gamma-1 chain C region	7.6
Isoform 13 of Dysferlin	1.1
Isoform 11 of Sorbin and SH3 domain-containing protein 2	2.0
78 kDa glucose-regulated protein	3.8
Elongation factor 1-alpha 2	5.4
Uncharacterized protein C1orf198	7.6
Isoform 2 of Testis-expressed sequence 101 protein	9.6
Cullin-1	3.0
Isoform 4 of NUT family member 1	2.2
NMDA receptor synaptonuclear signaling and neuronal migration factor	4.6
Probable G-protein coupled receptor 141	7.8

Table S15

Protein indentity	NSpC
Fibronectin	6.0
Fibrinogen beta chain	3.5
Fibrinogen alpha chain	8.3
Fibrinogen gamma chain	3.8
Serum albumin	5.7
Ig gamma-1 chain C region	5.5
Vinculin	1.6
Ubiquitin-40S ribosomal protein S27a	11.0
Cytoplasmic dynein 1 heavy chain 1	0.3
Elongation factor 1-alpha 2	3.9
78 kDa glucose-regulated protein	2.7
Prostate stem cell antigen	15.4
Salivary acidic proline-rich phosphoprotein 1/2	11.6

Table S16

Protein identity	NSpC
Serum albumin	50.1
fibronectin	3.5
Ubiquitin-40S ribosomal protein S27a	10.7
Elongation factor 1-alpha 3	3.8
Fibrinogen alpha chain	2.8
79 kDa glucose-regulated protein	2.6
cleavage stimulation factor subunit 3	3.1
alpha-2-macroglobulin	1.1
Synaptosomal-associated protein 26	8.2
Protein VAC14 homolog	2.1
Syntaxin-6	4.8

Table S17

Protein identity	NSpC
Serum albumin	22.9
Fibronectin	3.4
Ig gamma-1 chain C region	3.1
Vinculin	0.9
POTE ankyrin domain family member E	0.9
POTE ankyrin domain family member E	3.6
Alpha-1-antitrypsin	7.2
Fibrinogen beta chain	2.0
Ig gamma-3 chain C region	2.7
Serotransferrin	4.4
Isoform 11 of Sorbin and SH3 domain-containing protein 2	0.8
Fibrinogen alpha chain	1.1
Ig heavy chain V-III region BRO	8.5
Complement C3	1.2
LINE-1 type transposase domain-containing protein 1	1.1
78 kDa glucose-regulated protein	1.5
Kinectin	0.7
Elongation factor 1-alpha 2	2.2
Ubiquitin-40S ribosomal protein S27a	6.3
Fibrinogen gamma chain	2.2
Isoform 13 of Dysferlin	0.4
Laminin subunit gamma-1	0.6
Integral membrane protein GPR155	1.1
Lymphocyte function-associated antigen 3	4.0
Oxysterol-binding protein-related protein 2	2.0
Testis-specific Y-encoded protein 4	3.1
Isoform 2 of Ig mu chain C region	2.1

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