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(54) Title: METHODS OF DETERMINING ALZHEIMER'S DISEASE

(57) Abstract: The invention relates to molecular biomarkers and particular methods for assessing the likelihood of developing Alzheimer's disease, determining Alzheimer's disease and/or monitoring the progression of Alzheimer's disease in a subject.



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## METHODS OF DETERMINING ALZHEIMER'S DISEASE

### FIELD OF THE INVENTION

[0001] The invention relates to molecular biomarkers and particular methods for assessing the likelihood of developing Alzheimer's disease, determining Alzheimer's disease and/or monitoring the progression of Alzheimer's disease in a subject.

### INTRODUCTION

[0002] A biomarker, or biological marker, generally refers to a qualitative and/or quantitative measurable indicator of some biological state or condition. Biomarkers are typically molecules, biological species or biological events that can be used for the detection, diagnosis, prognosis and prediction of therapeutic response of diseases.

[0003] Proteins are the biological endpoints that govern most pathophysiological processes and they and the nucleic acid that encode them have therefore attracted most interest so far as biomarkers for diagnosis of diseases. Blood is the most valuable repertoire of biomarkers; however, the analysis of biomarkers directly from blood is hindered by the wide concentration range of blood proteins, in addition to the preponderance of highly abundant proteins. One particular problem is the extremely low concentration of particular protein biomarkers in biofluids. In addition, the 'swamping' effect, caused by other "non-specific" high abundant molecules, causes significant difficulties with detection.

[0004] Despite ongoing efforts and investment, there has been very little progress in the development of platforms for the discovery of biomarkers and the utilization of particular biomarkers as diagnostic tools for certain diseases, such as Alzheimer's disease.

[0005] Alzheimer's disease (AD) is a neurodegenerative disorder that results in a progressive and irreversible loss of memory and cognition. As life expectancy increases, the global economic and social burden of AD is expected to accelerate. However, currently there is no effective disease-modifying therapy for AD and existing pharmacological treatments are solely used to ameliorate symptoms.[1]

[0006] The implementation of effective treatments that can directly target the underlying mechanism of AD has largely failed so far, mainly due to the lack of early diagnostic tools. By the time symptoms emerge, the pathology is already well-established in the brain with the accumulation of amyloid- $\beta$  plaques preceding cognitive symptoms by 10-15 years. Stratification biomarkers that can detect the asymptomatic onset of AD could dramatically improve the outcomes of clinical trials for disease-modifying therapies, which are expected to be more efficacious at the earlier stages of the AD continuum. Interest is also increasing rapidly in the development of surrogate biomarkers –

indicators of AD progression that can be used as clinical endpoints – however, this currently remains a major challenge.[1]

[0007] Amyloid- $\beta$  deposition in the brain is the most clinically established diagnostic and disease monitoring marker and is currently assessed by positron-emission tomography (PET). In addition, measurements of amyloid- $\beta$  and tau protein levels in the cerebrospinal fluid (CSF) are clinically used to aid AD diagnosis, alongside standard cognitive assessments. However, CSF collection is relatively invasive and imaging modalities are expensive. Therefore, both techniques are impractical for early diagnosis at the asymptomatic phase of AD. The discovery of minimally invasive AD-specific signatures in blood, which may potentially track AD from the preclinical phase, to the prodromal phase of Mild Cognitive Impairment (MCI) and the onset of dementia are being investigated.[2]

[0008] Although conventionally considered as a Central Nervous System (CNS) disorder, there is now increasing evidence that AD coexists with systemic abnormalities directly associated with underlying disease processes. In addition to the systemic manifestations observed, blood-brain barrier breakdown has been shown to be an early indicator of cognitive dysfunction reinvigorating the discovery of peripheral biomarkers for CNS disorders.[3]

[0009] Despite recent progress in the analysis of amyloid- $\beta$ , tau and neurofilament light chain in blood [4, 5, 6], there is no AD-specific blood biomarker that has gone beyond the discovery phase to validation. In addition to the obstacles associated with blood biomarker discovery, the extremely low concentration of neurodegeneration-associated proteins in blood, together with the large dynamic range of proteins and the masking effect of albumin, makes the discovery of AD-specific biomarkers extremely challenging. While a few studies have previously attempted to analyse the blood proteome of AD patients,[7] the limited access to clinical samples at the asymptomatic stages has hampered the identification of early diagnostic biomarkers. Despite the conceptualization of AD as a biological and clinical continuum, most studies have so far attempted to discover molecular biomarkers at a single stage of the disease.[8]

[0010] It is therefore an aim of embodiments of the invention to overcome or mitigate at least one problem of the prior art, whether described herein or not.

## SUMMARY OF THE INVENTION

[0011] Surprisingly, the inventors have found biomarkers and methods of assessing the likelihood of developing Alzheimer's disease or of determining Alzheimer's disease or of monitoring Alzheimer's disease in a subject or in a biofluid taken from a subject. The inventors also apply a novel method which takes advantage of the interaction of nanoparticles with proteins to form biomolecule coronas,

which can be analysed to detect and monitor Alzheimer's disease and discover previously unknown Alzheimer's disease specific biomolecules. Advantageously, the methods may detect, identify, discover and/or quantify protein biomarkers which are in low abundance in the subject or the biofluid taken from the subject. Furthermore, the biomarkers and methods employed for detecting these may be used to identify and track longitudinal alternations of the blood proteome in subjects with Alzheimer's disease, and have the potential to track Alzheimer's disease from the preclinical phase, to the prodromal phase of MCI and the onset of dementia.

**[0012]** According to a first aspect of the invention there is provided a method of assessing the likelihood of developing Alzheimer's disease or of determining Alzheimer's disease or of monitoring Alzheimer's disease progression in a subject, comprising the step of determining the presence and/or amount of one or more protein biomarkers in the subject or biofluid taken from the subject, the one or more protein biomarkers being independently selected from those listed in Table 6a, Table 1 or Table 1a. Suitably, the protein biomarker is identified by protein name in Table 6a, Table 1 and Table 1a. Table 1 provides a SwissProt accession code for the murine version of the identified protein. The person skilled in the art can easily use this to identify the equivalent protein for the particular species of subject, e.g. human, for use in the invention. Table 1a and Table 6a provides a SwissProt accession code for the homo sapiens version of the identified protein.

**[0013] Table 1**

| Protein Name                                               | Accession  |
|------------------------------------------------------------|------------|
| 14-3-3 protein zeta/delta                                  | 1433Z      |
| 26S proteasome non-ATPase regulatory subunit 1             | PSMD1      |
| 26S proteasome non-ATPase regulatory subunit 14            | PSDE       |
| 26S proteasome non-ATPase regulatory subunit 2             | PSMD2      |
| 26S proteasome non-ATPase regulatory subunit 3             | PSMD3      |
| 26S proteasome non-ATPase regulatory subunit 6 (Fragment)  | A0A286YDW8 |
| 26S proteasome regulatory subunit 10B                      | PRS10      |
| 26S proteasome regulatory subunit 6A (Fragment)            | A0A087WPH7 |
| 40S ribosomal protein S9                                   | D3YWH9     |
| 55 kDa erythrocyte membrane protein                        | B7ZCL8     |
| 60S ribosomal protein L18                                  | A0A1B0GQU8 |
| 60S ribosomal protein L6                                   | Q3UCH0     |
| Aberrantly recombined kappa chain Vk8/J1 region (Fragment) | A2N494     |

|                                                                         |            |
|-------------------------------------------------------------------------|------------|
| Acylamino-acid-releasing enzyme (Fragment)                              | A0A0R4J107 |
| Adenylyl cyclase-associated protein 1                                   | CAP1       |
| Adiponectin                                                             | ADIPO      |
| ADP/ATP translocase 2                                                   | ADT2       |
| Alpha actinin 1a                                                        | A1BN54     |
| Aminopeptidase N (Fragment)                                             | A0A0U1RNS3 |
| Angiopietin-1                                                           | ANGP1      |
| Ankyrin-1                                                               | A0A0R4J1N7 |
| Annexin                                                                 | A0A2C9F2D2 |
| Annexin A2                                                              | ANXA2      |
| Annexin A5                                                              | ANXA5      |
| Annexin A6                                                              | ANXA6      |
| Anti-colorectal carcinoma light chain                                   | Q7TS98     |
| Anti-H5N1 hemagglutinin monoclonal antibody H5M9 heavy chain (Fragment) | U5LP42     |
| Anti-myosin immunoglobulin light chain variable region (Fragment)       | Q9JL74     |
| Anti-myosin immunoglobulin light chain variable region (Fragment)       | Q9JL78     |
| Anti-norfloxacin MAb light chain variable region (Fragment)             | A0A0M3RM39 |
| Anti-VIPase light chain variable region (Fragment)                      | Q8K1F1     |
| Aquaporin-1                                                             | AQP1       |
| Argininosuccinate lyase                                                 | ARLY       |
| Argininosuccinate synthase                                              | ASSY       |
| ATP synthase subunit alpha, mitochondrial                               | ATPA       |
| ATP synthase subunit beta, mitochondrial                                | ATPB       |
| Basigin                                                                 | BASI       |
| Bifunctional purine biosynthesis protein PURH                           | PUR9       |
| Bile acyl-CoA synthetase                                                | E9PXV4     |
| Bisphosphoglycerate mutase                                              | PMGE       |
| Calnexin                                                                | CALX       |
| Calreticulin                                                            | CALR       |
| Carboxypeptidase N catalytic chain                                      | CBPN       |
| Cartilage oligomeric matrix protein                                     | COMP       |

|                                                                                                                  |            |
|------------------------------------------------------------------------------------------------------------------|------------|
| Casein kinase II subunit alpha' (Fragment)                                                                       | A0A1D5RLQ2 |
| Caveolae-associated protein 1                                                                                    | CAVN1      |
| CD5 antigen-like                                                                                                 | CD5L       |
| CD9 antigen                                                                                                      | CD9        |
| Clathrin heavy chain 1                                                                                           | CLH1       |
| Clusterin                                                                                                        | CLUS       |
| Coagulation factor V                                                                                             | FA5        |
| Collagen alpha-1(VI) chain                                                                                       | CO6A1      |
| Collagen, type VI, alpha 3                                                                                       | A0A087WS16 |
| Colony stimulating factor 1 receptor                                                                             | Q0P0S6     |
| Complement C1q tumor necrosis factor-related protein 3                                                           | C1QT3      |
| Complement component 7                                                                                           | D3YXF5     |
| Complement component C8 alpha chain                                                                              | CO8A       |
| Coronin                                                                                                          | Q3T9L1     |
| C-type lectin domain family 11 member A                                                                          | CLC11      |
| Cytochrome b5                                                                                                    | CYB5       |
| Cytochrome P450 1A2                                                                                              | CP1A2      |
| Cytosolic 10-formyltetrahydrofolate dehydrogenase                                                                | AL1L1      |
| Decorin                                                                                                          | PGS2       |
| Dihydrolipoyllysine-residue acetyltransferase component of pyruvate dehydrogenase complex, mitochondrial         | ODP2       |
| Dihydrolipoyllysine-residue succinyltransferase component of 2-oxoglutarate dehydrogenase complex, mitochondrial | ODO2       |
| Dimethylaniline monooxygenase [N-oxide-forming] 5                                                                | FMO5       |
| Dipeptidyl peptidase 4                                                                                           | DPP4       |
| Disintegrin and metalloproteinase domain-containing protein 10                                                   | ADA10      |
| DNA helicase B                                                                                                   | HELB       |
| E3 ubiquitin-protein ligase UBR4                                                                                 | UBR4       |
| Early endosome antigen 1                                                                                         | EEA1       |
| EG214403 protein                                                                                                 | B2RUG5     |
| Elongation factor 1-alpha 1                                                                                      | EF1A1      |
| EMILIN-1                                                                                                         | EMIL1      |

|                                                                  |            |
|------------------------------------------------------------------|------------|
| Endoplasmin                                                      | ENPL       |
| Epidermal growth factor receptor                                 | EGFR       |
| Erythrocyte band 7 integral membrane protein                     | STOM       |
| Ezrin                                                            | EZRI       |
| Fermitin family homolog 3                                        | URP2       |
| Fibrinogen alpha chain                                           | FIBA       |
| Fibrinogen beta chain                                            | FIBB       |
| Fibrinogen gamma chain                                           | FIBG       |
| Fibronectin                                                      | A0A087WR50 |
| Filamin-A                                                        | FLNA       |
| Filamin-B                                                        | FLNB       |
| G protein-coupled receptor 179                                   | E9PY61     |
| Galectin-3-binding protein                                       | LG3BP      |
| GDH/6PGL endoplasmic bifunctional protein                        | A2A7A7     |
| Glucosidase 2 subunit beta                                       | GLU2B      |
| Glyceraldehyde-3-phosphate dehydrogenase                         | A0A0A0MQF6 |
| Glycogen phosphorylase, muscle form                              | PYGM       |
| Guanine nucleotide-binding protein G(I)/G(S)/G(T) subunit beta-1 | GBB1       |
| Haptoglobin                                                      | HPT        |
| HC protein                                                       | A0A0C6E3V3 |
| Heat shock 70 kDa protein 4                                      | Q3U2G2     |
| Hsp90aa1 protein (Fragment)                                      | A0PJ91     |
| Ig heavy chain V region 3-6                                      | HVM60      |
| Ig heavy chain V region 6.96                                     | HVM57      |
| Ig kappa chain V-III region 50S10.1                              | KV3A4      |
| Ig kappa chain V-III region PC 3741/TEPC 111                     | KV3A8      |
| Ig kappa chain V-V region MOPC 149                               | KV5A4      |
| IgA heavy chain variable region (Fragment)                       | K7THF9     |
| IgG1 heavy chain VDJ region (Fragment)                           | X5J5A8     |
| IgG1 heavy chain VDJ region (Fragment)                           | X5J4T6     |
| Igh protein                                                      | Q4VAB6     |
| IgM heavy chain VDJ region (Fragment)                            | X5J4G2     |

|                                                         |            |
|---------------------------------------------------------|------------|
| Immunoglobulin heavy variable 10-1 (Fragment)           | A0A0B4J1J6 |
| Immunoglobulin heavy variable 11-1 (Fragment)           | A0A075B5R6 |
| Immunoglobulin heavy variable 1-39 (Fragment)           | A0A075B5V5 |
| Immunoglobulin heavy variable 1-52                      | A0A075B5W2 |
| Immunoglobulin heavy variable 1-69                      | A0A075B5X7 |
| Immunoglobulin heavy variable 7-1 (Fragment)            | A0A075B5S2 |
| Immunoglobulin heavy variable V1-11 (Fragment)          | A0A0A6YWI9 |
| Immunoglobulin heavy variable V12-3 (Fragment)          | A0A075B5T1 |
| Immunoglobulin heavy variable V1-43                     | A0A075B5V7 |
| Immunoglobulin heavy variable V1-59                     | A0A075B5X0 |
| Immunoglobulin heavy variable V3-4 (Fragment)           | A0A0A6YXF1 |
| Immunoglobulin heavy variable V9-3 (Fragment)           | A0A0B4J1J5 |
| Immunoglobulin kappa chain variable 1-122 (Fragment)    | A0A140T8M9 |
| Immunoglobulin kappa chain variable 8-27                | A0A075B5N4 |
| Immunoglobulin kappa chain variable 9-124               | A0A075B5K2 |
| Immunoglobulin kappa variable 12-44 (Fragment)          | A0A140T8M2 |
| Immunoglobulin kappa variable 2-109 (Fragment)          | A0A075B5K6 |
| Immunoglobulin kappa variable 4-53                      | A0A075B677 |
| Immunoglobulin kappa variable 4-80 (Fragment)           | A0A075B5L7 |
| Immunoglobulin kappa variable 6-17 (Fragment)           | A0A140T8Q3 |
| Immunoglobulin kappa variable 6-23 (Fragment)           | A0A140T8N5 |
| Immunoglobulin kappa variable 8-28                      | A0A075B5N3 |
| Immunoglobulin kappa variable 8-34 (Fragment)           | A0A0G2JDU0 |
| Immunoglobulin lambda variable 3 (Fragment)             | A0A0B4J1K5 |
| Integrin alpha 2b                                       | B2RPR7     |
| Integrin beta                                           | M0QWA7     |
| Integrin beta-1                                         | ITB1       |
| Integrin beta-3                                         | ITB3       |
| Integrin-linked protein kinase                          | ILK        |
| Inter-alpha-trypsin inhibitor heavy chain H2            | ITIH2      |
| Intraflagellar transport protein 172 homolog (Fragment) | A0A0J9YU41 |
| Kell blood group glycoprotein homolog                   | KELL       |

|                                                        |            |
|--------------------------------------------------------|------------|
| Lactadherin                                            | MFGM       |
| Laminin subunit alpha-2                                | LAMA2      |
| Laminin subunit gamma-1                                | F8VQJ3     |
| Laminin subunit gamma-1                                | LAMC1      |
| Large neutral amino acids transporter small subunit 3  | A2ATS4     |
| Leucine-rich repeat neuronal protein 4                 | LRRN4      |
| Light chain variable region (Fragment)                 | A2NVX0     |
| L-lactate dehydrogenase                                | A0A1B0GSR9 |
| Long-chain-fatty-acid--CoA ligase 1                    | ACSL1      |
| Long-chain-fatty-acid--CoA ligase 5                    | ACSL5      |
| Low density lipoprotein receptor-related protein 1     | A0A0R4J0I9 |
| Lumican                                                | LUM        |
| Major urinary protein 10                               | A2BIN1     |
| Major vault protein                                    | MVP        |
| Maltase-glucoamylase                                   | B5THE2     |
| Maltase-glucoamylase                                   | A0A0J9YVE6 |
| Mannan-binding lectin serine protease 1                | MASP1      |
| Mannan-binding lectin serine protease 2                | MASP2      |
| MCG140437, isoform CRA_d                               | G3UW82     |
| MCG23377, isoform CRA_b                                | A0A0A6YW67 |
| MCG5400                                                | Q6ZWQ9     |
| Meprin A subunit                                       | A0A0R4J043 |
| Metalloproteinase inhibitor 3                          | TIMP3      |
| Microsomal triglyceride transfer protein large subunit | MTP        |
| Moesin                                                 | MOES       |
| Monocarboxylate transporter 1                          | MOT1       |
| MRP5 (Fragment)                                        | Q925S1     |
| Myosin-9                                               | MYH9       |
| NADPH--cytochrome P450 reductase (Fragment)            | E9PVT9     |
| Napsin-A                                               | NAPSA      |
| Neuronal membrane glycoprotein M6-a                    | GPM6A      |
| Oncoprotein-induced transcript 3 protein               | OIT3       |

|                                                                |            |
|----------------------------------------------------------------|------------|
| Pancreatic alpha-amylase                                       | AMYP       |
| Perilipin-4                                                    | PLIN4      |
| Peroxiredoxin-2                                                | PRDX2      |
| Phospholipid transfer protein                                  | PLTP       |
| Predicted gene 45713                                           | A0A1B0GS68 |
| Properdin                                                      | PROP       |
| Proteasome subunit alpha type                                  | Q3TN31     |
| Proteasome subunit alpha type                                  | Q3TUI9     |
| Proteasome subunit alpha type (Fragment)                       | Q3TL95     |
| Proteasome subunit alpha type-3                                | PSA3       |
| Proteasome subunit alpha type-6                                | PSA6       |
| Proteasome subunit beta type-3                                 | PSB3       |
| Proteasome subunit beta type-4                                 | PSB4       |
| Proteasome subunit beta type-5                                 | PSB5       |
| Proteasome subunit beta type-6                                 | PSB6       |
| Protein disulfide-isomerase                                    | PDIA1      |
| Protein disulfide-isomerase A3                                 | PDIA3      |
| Protein Igkv5-48 (Fragment)                                    | A0A140T8N2 |
| Protein KRI1 homolog                                           | KRI1       |
| Protein piccolo                                                | PCLO       |
| Protein-glutamine gamma-glutamyltransferase 2                  | TGM2       |
| Proteoglycan 4                                                 | A0A0R4J207 |
| Pyrethroid hydrolase Ces2e                                     | EST2E      |
| Rab GDP dissociation inhibitor (Fragment)                      | A0A1Y7VLG4 |
| RAS oncogene family protein                                    | A0A068BFR3 |
| RAS-related C3 botulinum substrate 3, isoform CRA_a (Fragment) | A2AC13     |
| Ras-related protein Rap-1b                                     | RAP1B      |
| Receptor expression-enhancing protein                          | D3Z6V2     |
| Retinal dehydrogenase 1                                        | AL1A1      |
| Retinol dehydrogenase 7                                        | RDH7       |
| Ribosomal protein L15                                          | Q3U7D2     |
| Ribosomal protein S2, pseudogene 6                             | A0A140T8L5 |

|                                                                                                            |            |
|------------------------------------------------------------------------------------------------------------|------------|
| RIKEN cDNA 1300017J02 gene                                                                                 | D3YY36     |
| Sarcoplasmic/endoplasmic reticulum calcium ATPase 2                                                        | AT2A2      |
| Serglycin                                                                                                  | SRGN       |
| Serine palmitoyltransferase 2                                                                              | SPTC2      |
| Serum amyloid A-4 protein                                                                                  | SAA4       |
| Serum paraoxonase/arylesterase 1                                                                           | PON1       |
| Single-chain variable                                                                                      | A0A1E1GJG6 |
| Solute carrier family 2, facilitated glucose transporter member 3                                          | GTR3       |
| Solute carrier family 29 (Nucleoside transporters), member 1, isoform CRA_a                                | Q3TCZ2     |
| Spectrin alpha chain, non-erythrocytic 1                                                                   | SPTN1      |
| Spectrin beta chain                                                                                        | B2RX08     |
| Spectrin beta chain, erythrocytic                                                                          | SPTB1      |
| Talin-1                                                                                                    | TLN1       |
| Tankyrase-2                                                                                                | TNKS2      |
| T-complex protein 1 subunit alpha                                                                          | TCPA       |
| T-complex protein 1 subunit gamma                                                                          | TCPG       |
| Tenascin                                                                                                   | TENA       |
| Thrombospondin-4                                                                                           | TSP4       |
| Thyrotropin-releasing hormone-degrading ectoenzyme                                                         | TRHDE      |
| Thyroxine-binding globulin                                                                                 | THBG       |
| Transferrin receptor protein 1                                                                             | TFR1       |
| Transketolase (Fragment)                                                                                   | A0A286YE28 |
| Translation initiation factor IF-3, mitochondrial (Fragment)                                               | D3YWL2     |
| Tripeptidyl-peptidase 2                                                                                    | TPP2       |
| Tubulin beta-4A chain                                                                                      | TBB4A      |
| Tyrosine 3-monooxygenase/tryptophan 5-monooxygenase activation protein, epsilon polypeptide, isoform CRA_c | Q5SS40     |
| UDP-glucuronosyltransferase 1-1                                                                            | UD11       |
| Uncharacterized protein                                                                                    | Q9CWK0     |
| Uncharacterized protein                                                                                    | Q3UBI6     |
| Uncharacterized protein                                                                                    | Q3T9M7     |

|                                              |            |
|----------------------------------------------|------------|
| Uncharacterized protein                      | A0A1S6GWH5 |
| Uncharacterized protein                      | I6L9E1     |
| Uncharacterized protein                      | Q9DBD9     |
| Uncharacterized protein                      | Q3TA14     |
| Uncharacterized protein                      | Q3TWZ3     |
| Uncharacterized protein                      | Q3TYT5     |
| Uncharacterized protein                      | A0A1S6GWH1 |
| Uncharacterized protein                      | Q3TR40     |
| Uncharacterized protein                      | Q3TI47     |
| Uncharacterized protein                      | Q2TAW9     |
| Uncharacterized protein (Fragment)           | Q3TB11     |
| Uncharacterized protein (Fragment)           | Q3TF08     |
| Uroplakin-3b                                 | A0A0R4J0S8 |
| UTP--glucose-1-phosphate uridylyltransferase | UGPA       |
| VH region (Fragment)                         | Q53VQ0     |
| Vimentin                                     | A0A0A6YWC8 |
| Vinculin                                     | VINC       |
| WD repeat-containing protein 90              | H7BX49     |
| Zinc finger protein 280D (Fragment)          | V9GXF6     |

**[0014]** In one embodiment of any aspect of the invention, the protein biomarker selected from Table 1 is not moesin.

**[0015] Table 1a**

| Protein name                                             | Gene name | Accession Number |
|----------------------------------------------------------|-----------|------------------|
| KIAA0100, isoform CRA_a                                  | KIAA0100  | K7EQ86           |
| Immunoglobulin kappa joining 3 (Fragment)                | IGKJ3     | A0A0A0MT96       |
| Anoctamin (Fragment)                                     | ANO7      | H7C220           |
| Apolipoprotein E (Fragment)                              | APOE      | Q8TCZ8           |
| Pancreas transcription factor 1 subunit alpha (Fragment) | PTF1A     | A0A1W2PRT5       |
| MS-D2 light chain variable region                        |           | A0A0X9USL5       |
| Proprotein convertase subtilisin/kexin type 9            | PCSK9     | PCSK9            |

|                                                                                                                   |                |            |
|-------------------------------------------------------------------------------------------------------------------|----------------|------------|
| Superoxide dismutase                                                                                              |                | A0A140VJU8 |
| Uncharacterized protein                                                                                           | DKFZp686K03196 | Q6N095     |
| Pyruvate kinase                                                                                                   | PKM            | B4DNK4     |
| Hepatocyte growth factor activator                                                                                | HGFAC          | HGFA       |
| Plasma kallikrein (Fragment)                                                                                      | KLKB1          | H0YAC1     |
| Latent-transforming growth factor beta-binding protein 1                                                          | LTBP1          | LTBP1      |
| Cathepsin D                                                                                                       | CTSD           | A0A1B0GV23 |
| Angiopoietin-like 6, isoform CRA_a                                                                                | ANGPTL6        | A0A024R7A9 |
| Histone H1.5                                                                                                      | HIST1H1B       | H15        |
| Catalase                                                                                                          | CAT            | CATA       |
| Uncharacterized                                                                                                   |                | Q7Z2U7     |
| V1-22 protein (Fragment)                                                                                          | V1-22          | Q5NV88     |
| cDNA FLJ45477 fis, clone BRSTN2018692, moderately similar to Complement C1q tumor necrosis factor-related protein |                | B3KXI7     |
| Proteoglycan 4, isoform CRA_a                                                                                     | PRG4           | A0A024R930 |
| cDNA FLJ52761, highly similar to Actin, aortic smooth                                                             |                | B4DUI8     |
| Adipocyte plasma membrane-associated protein                                                                      | APMAP          | APMAP      |
| Leukocyte elastase inhibitor                                                                                      | SERPINB1       | ILEU       |
| Anti-streptococcal/anti-myosin immunoglobulin kappa light chain variable region                                   |                | Q96SA9     |
| Transmembrane protein 163                                                                                         | TMEM163        | TM163      |
| Serpin peptidase inhibitor, clade C (Antithrombin), member 1, isoform CRA_a                                       | SERPINC1       | A0A024R944 |
| Neurogenic locus notch homolog protein 3                                                                          | NOTCH3         | NOTC3      |
| Leucine-rich alpha-2-glycoprotein                                                                                 | LRG1           | A2GL       |
| Myc target protein 1 (Fragment)                                                                                   | MYCT1          | H0YDV5     |
| IgG Fc-binding protein                                                                                            | FCGBP          | FCGBP      |
| Coagulation factor IX OS=Homo                                                                                     | F9 p22         | F2RM37     |
| IGL@ protein                                                                                                      | IGL@           | Q6IN99     |

|                                                                                |                |            |
|--------------------------------------------------------------------------------|----------------|------------|
| Heparan sulfate proteoglycan 2 (Perlecan), isoform CRA_b                       | HSPG2          | A0A024RAB6 |
| Transforming growth factor, beta 1 (Camurati-Engelmann disease), isoform CRA_a | TGFB1          | A0A024R0P8 |
| Uncharacterized protein DKFZp686K18196 (Fragment)                              | DKFZp686K18196 | Q6N092     |
| cDNA FLJ41552 fis, clone COLON2004478, highly similar to Protein Tro alpha1    |                | Q6ZW64     |
| Immunoglobulin lambda variable 10-54                                           | IGLV10-54      | A0A1W2PQ80 |
| Histone                                                                        |                | A2RUA4     |
| Podocalyxin                                                                    | PODXL          | PODXL      |
| Coagulation factor XI                                                          | F11            | FA11       |
| von Willebrand factor                                                          | VWF            | VWF        |
| von Willebrand factor                                                          | VWF            | L8E853     |
| Platelet factor 4 variant                                                      | PF4V1          | PF4V       |
| Annexin                                                                        | ANXA2          | A0A024R5Z7 |
| Immunoglobulin lambda variable 5-39                                            | IGLV5-39       | LV539      |
| Uncharacterized                                                                |                | Q6DHW4     |
| Protein tweety homolog                                                         | TTYH3          | A0A024R816 |
| Fc of IgG low affinity IIIa receptor isoform 2 (Fragment)                      | FCGR3A         | A0A0S2Z3S7 |
| Lysophospholipid acyltransferase 1                                             | MBOAT1         | MBOA1      |
| Anti-Mpl scFv                                                                  |                | A2J423     |
| Leukocyte surface antigen CD53                                                 | CD53           | CD53       |
| Platelet factor 4                                                              | PF4            | PLF4       |
| Cold agglutinin FS-1 L-chain                                                   |                | A2NB45     |
| Mucin-2                                                                        | MUC2           | A0A0G2JM87 |
| Extracellular glycoprotein lacritin                                            | LACRT          | LACRT      |
| Lysozyme                                                                       | LYZ            | A0A0B4J259 |
| Keratinocyte proline-rich protein                                              | KPRP           | KPRP       |
| GRB2-related adapter protein                                                   | GRAP           | I3L2P9     |
| Coiled-coil domain-containing protein 187                                      | CCDC187        | A0A075B733 |

|                                                                                |                |            |
|--------------------------------------------------------------------------------|----------------|------------|
| NADH dehydrogenase [ubiquinone] 1 alpha subcomplex subunit 3 (Fragment)        | NDUFA3         | H7C2R1     |
| Vimentin                                                                       | VIM            | VIME       |
| cDNA FLJ39539 fis, clone PUAEN2008228, highly similar to Platelet glycoprotein |                | B3KUB8     |
| ITIH4 protein                                                                  | ITIH4          | B7ZKJ8     |
| Inter-alpha (Globulin) inhibitor H4 (Plasma Kallikrein-sensitive glycoprotein) | ITIH4          | B2RMS9     |
| Fibrinogen alpha chain                                                         | FGA            | FIBA       |
| Transcobalamin-1                                                               | TCN1           | TCO1       |
| Immunoglobulin alpha-2 heavy                                                   |                | IGA2       |
| Alpha-2-macroglobulin                                                          | A2M            | A2MG       |
| Collagen alpha-1(II) chain                                                     | COL2A1         | CO2A1      |
| Protein HEG homolog 1                                                          | HEG1           | HEG1       |
| Uncharacterized protein DKFZp686O16217 (Fragment)                              | DKFZp686O16217 | Q6N041     |
| IgG L                                                                          |                | S6B2A1     |
| HRV Fab 027-VL                                                                 |                | A2IPI6     |
| Platelet endothelial cell adhesion molecule                                    | PECAM1         | A0A075B738 |
| Testicular secretory protein Li                                                |                | A0A140VK24 |
| Beta-2-glycoprotein I                                                          |                | D9IWP9     |
| Hyaluronan-binding protein 2                                                   | HABP2          | HABP2      |
| Apolipoprotein C-III                                                           | APOC3          | B0YIW2     |
| Apolipoprotein A-II                                                            | APOA2          | APOA2      |
| Apolipoprotein B variant                                                       |                | Q59HB3     |
| Serum paraoxonase/arylesterase 1                                               | PON1           | PON1       |
| Prenylcysteine oxidase 1                                                       | PCYOX1         | PCYOX      |
| Apolipoprotein A-IV                                                            | APOA4          | APOA4      |
| Hemopexin                                                                      | HPX            | HEMO       |
| Ankyrin repeat domain-containing protein 61                                    | ANKRD61        | ANR61      |
| Protein piccolo                                                                | PCLO           | PCLO       |
| Alpha-1-antitrypsin                                                            | SERPINA1       | A0A024R6I7 |

|                                                          |            |            |
|----------------------------------------------------------|------------|------------|
| Alpha-1-antitrypsin                                      | SERPINA1   | A1AT       |
| Glutathione peroxidase                                   | GPX3       | A0A087X1J7 |
| LINE-1 type transposase domain-containing protein 1      | L1TD1      | LITD1      |
| GCT-A3 heavy chain variable region                       |            | A0A0X9TD88 |
| Slit homolog 1 protein                                   | SLIT1      | SLIT1      |
| Anti-thyroglobulin heavy chain variable region           |            | Q8IZD7     |
| Apolipoprotein B (Including Ag(X) antigen)               | APOB       | C0JYY2     |
| Corticosteroid-binding globulin                          | SERPINA6   | CBG        |
| Immunoglobulin kappa variable 4-1                        | IGKV4-1    | KV401      |
| Vitamin D-binding protein                                | GC         | D6RF35     |
| Apolipoprotein A-V, isoform CRA_a                        | APOA5      | A0A0B4RUS7 |
| Apolipoprotein A-I, isoform CRA_a                        | APOA1      | A0A024R3E3 |
| Leucine-rich repeat-containing protein 7 (Fragment)      | LRRC7      | K7ELH5     |
| Apolipoprotein D                                         | APOD       | APOD       |
| Serum paraoxonase/lactonase 3                            | PON3       | PON3       |
| Immunoglobulin heavy variable 4-38-2                     | IGHV4-38-2 | HVD82      |
| Kelch-like protein 34                                    | KLHL34     | KLH34      |
| Immunoglobulin heavy variable 3-64D                      | IGHV3-64D  | HV64D      |
| Serum albumin                                            | ALB        | ALBU       |
| cDNA FLJ53691, highly similar to                         |            | B4E1B2     |
| Immunoglobulin heavy chain variable region               |            | Q0ZCI6     |
| Tissue factor pathway inhibitor (Fragment)               | TFPI       | C9JKV3     |
| Phosphatidylinositol-glycan-specific phospholipase D     | GPLD1      | PHLD       |
| Selenoprotein P (Fragment)                               | SELENOP    | A0A182DWH7 |
| Alpha-2-HS-glycoprotein                                  | AHSG       | FETUA      |
| Anthrax toxin receptor 2                                 | ANTXR2     | A0A0C4DG84 |
| GCT-A9 heavy chain variable region                       |            | A0A109PT03 |
| cDNA FLJ55673, highly similar to Complement factor B (EC |            | B4E1Z4     |

|                                                                                                    |             |            |
|----------------------------------------------------------------------------------------------------|-------------|------------|
| Transthyretin                                                                                      | TTR         | TTHY       |
| NADH-ubiquinone oxidoreductase chain 5                                                             | ND5         | A0A059RS62 |
| Epididymis luminal protein 180 (Fragment)                                                          | HEL180      | B6EDE2     |
| C-reactive protein                                                                                 | CRP         | CRP        |
| Transferrin variant                                                                                |             | Q53H26     |
| IgG H                                                                                              |             | S6C4S4     |
| Immunoglobulin lambda variable 3-1                                                                 | IGLV3-1     | LV301      |
| Single-chain Fv (Fragment)                                                                         | scFv        | Q65ZC9     |
| Serpin peptidase inhibitor, clade A (Alpha-1 antiproteinase, antitrypsin), member 4, isoform CRA_a | SERPINA4    | A0A024R6I9 |
| IGK@ protein                                                                                       | IGK@        | Q6P5S8     |
| SH3 domain-binding glutamic acid-rich-like protein 3                                               | SH3BGRL3    | Q5T123     |
| Immunoglobulin epsilon heavy                                                                       |             | IGE        |
| N-acetylmuramoyl-L-alanine amidase                                                                 | PGLYRP2     | PGRP2      |
| SAA2-SAA4 readthrough                                                                              | SAA2-SAA4   | A0A096LPE2 |
| Serum amyloid A                                                                                    |             | B2R5G8     |
| Coiled-coil domain-containing protein 40                                                           | CCDC40      | CCD40      |
| APOC4-APOC2 readthrough (NMD candidate)                                                            | APOC4-APOC2 | K7ER74     |
| Complement decay-accelerating factor                                                               | CD55        | A0A172WBW8 |
| IGHV5-51 protein (Fragment)                                                                        | IGHV5-51    | A0A0F7T891 |
| Histidine-rich glycoprotein                                                                        | HRG         | HRG        |
| cDNA, FLJ93914, highly similar to Homo sapiens histidine-rich glycoprotein (HRG),                  |             | B2R8I2     |
| APOB protein                                                                                       | APOB        | Q7Z7Q0     |
| L-lactate dehydrogenase A chain                                                                    | LDHA        | LDHA       |
| Lipoprotein B (Fragment)                                                                           | APOB        | S5FLF7     |
| MS-D1 heavy chain variable region                                                                  |             | A0A109PS45 |
| Immunoglobulin kappa variable 1D-16 (Fragment)                                                     | IGKV1D-16   | KVD16      |
| Immunoglobulin heavy variable 3-9                                                                  | IGHV3-9     | HV309      |
| Alpha-actinin-4                                                                                    | ACTN4       | ACTN4      |

|                                                                        |                |            |
|------------------------------------------------------------------------|----------------|------------|
| Apolipoprotein C-I, isoform CRA_a                                      | APOC1          | A0A024R0T8 |
| cDNA, FLJ93383, highly similar to Homo sapiens semenogelin II (SEMG2), |                | B2R7C8     |
| Complement component C8 gamma chain                                    | C8G            | CO8G       |
| IgG H                                                                  |                | S6AWF0     |
| Hemoglobin subunit beta                                                | HBB            |            |
| Ig heavy chain variable region                                         |                | A0A068LKQ2 |
| Ig heavy chain variable region                                         |                | A0A068LN03 |
| Alpha-1B-glycoprotein                                                  | A1BG           | A1BG       |
| Rheumatoid factor RF-ET9                                               |                | A2J1N6     |
| V2-17 protein (Fragment)                                               | V2-17          | Q5NV90     |
| Complement factor H-related protein 4                                  | CFHR4          | FHR4       |
| Alpha-1-antitrypsin (Fragment)                                         | SERPINA1       | G3V2B9     |
| MS-A3 light chain variable region                                      |                | A0A120HF56 |
| Biotinidase                                                            | BTB            | BTB        |
| Anti-staphylococcal enterotoxin D heavy chain variable region          |                | A0A1L2BU33 |
| Polymeric immunoglobulin receptor                                      | PIGR           | PIGR       |
| Histone PARylation factor 1                                            | HPF1           | HPF1       |
| IBM-A3 heavy chain variable region                                     |                | A0A0X9UWM4 |
| Rheumatoid factor RF-IP14                                              |                | A2J1N0     |
| Pescadillo homolog                                                     | PES1           | PESC       |
| Mannan-binding lectin serine protease 2                                | MASP2          | MASP2      |
| MS-F1 light chain variable region                                      |                | A0A0X9V9B3 |
| Uncharacterized protein DKFZp686M08189                                 | DKFZp686M08189 | Q6MZX9     |
| Immunoglobulin lambda variable 3-9                                     | IGLV3-9        | LV39       |
| Tenascin-X                                                             | TNXB           | A0A087X0I0 |
| Insulin-like growth factor-binding protein complex acid labile subunit | IGFALS         | ALS        |
| Immunoglobulin heavy variable 3-38 (non-functional) (Fragment)         | IGHV3-38       | A0A0C4DH36 |
| Apolipoprotein C-IV                                                    | APOC4          | APOC4      |

|                                                                    |                       |            |
|--------------------------------------------------------------------|-----------------------|------------|
| Apolipoprotein C-IV                                                | APOC4                 | A5YAK2     |
| Variable immunoglobulin anti-estradiol heavy chain                 |                       | A2NZ55     |
| IgG H                                                              |                       | S6BGD4     |
| Plexin domain-containing protein 2                                 | PLXDC2                | PXDC2      |
| Fermitin family homolog 3                                          | FERMT3                | URP2       |
| IGL@ protein                                                       | IGL@                  | Q6PIK1     |
| Cathelicidin antimicrobial peptide                                 | CAMP                  | CAMP       |
| Probable G-protein-coupled receptor 179                            | GPR179                | A0A087WW83 |
| Apolipoprotein M                                                   | APOM                  | APOM       |
| Fab Hu 15C1 Heavy                                                  |                       | A0A0M3KKW6 |
| Phospholipid transfer protein                                      | PLTP                  | PLTP       |
| Semenogelin-1                                                      | SEMG1                 | SEMG1      |
| Immunoglobulin kappa variable 3-11                                 | IGKV3-11              | KV311      |
| Thyroxine-binding globulin                                         | SERPINA7              | THBG       |
| Immunoglobulin heavy variable 4-59                                 | IGHV4-59              | HV459      |
| Neutrophil defensin 1                                              | DEFA1                 | DEF1       |
| Complement C1r subcomponent-like protein                           | C1RL                  | C1RL       |
| Beta-Ala-His dipeptidase                                           | CNDP1                 | CNDP1      |
| Vitamin D binding protein (Fragment)                               | Gc                    | A0A1B1CYC8 |
| MS-A2 heavy chain variable region                                  |                       | A0A125U0U3 |
| Anti-folate binding protein (Fragment) OS=Homo                     | HuC4lambda<br>Vlambda | A2NYQ7     |
| Uncharacterized protein DKFZp686C02218<br>(Fragment)               | DKFZp686C02218        | Q7Z374     |
| Ceruloplasmin                                                      | CP                    | CERU       |
| Immunoglobulin heavy variable 3-74                                 | IGHV3-74              | HV374      |
| Angiotensinogen                                                    | AGT                   | ANGT       |
| Cholesteryl ester transfer protein plasma isoform 1<br>(Fragment)  | CETP                  | A0A0S2Z3F6 |
| Immunoglobulin heavy variable 3-43                                 | IGHV3-43              | HV343      |
| Immunoglobulin heavy variable 3-35 (non-<br>functional) (Fragment) | IGHV3-35              | A0A0C4DH35 |

|                              |      |            |
|------------------------------|------|------------|
| Alpha-1-acid glycoprotein 2  | ORM2 | A1AG2      |
| Testicular tissue protein Li |      | A0A140VK00 |

**[0016]** In one embodiment of any aspect of the invention, the protein biomarker selected from Table 1a is not apolipoprotein E or vitamin D binding protein.

**[0017]** According to a second aspect of the invention there is provided a method of testing a patient's biofluid sample for one or more biomarkers of Alzheimer's disease, comprising the steps of:

- a) contacting a plurality of nanoparticles in a biofluid sample taken from a subject to allow a biomolecule corona to form on the surface of said nanoparticles;
- b) optionally isolating the nanoparticles and surface-bound biomolecule corona;
- c) determining the abundance of one or more protein biomarkers in the corona, the one or more protein biomarkers being independently selected from those listed in Table 1 or Table 1a; and
- d) comparing the abundance of the one or more protein biomarkers in the corona to the abundance of the same one or more protein biomarkers in a non-diseased control reference.

**[0018]** According to a third aspect of the invention there is provided a method for monitoring AD progression in a subject, comprising the steps of:

- a) contacting a plurality of nanoparticles with a biofluid sample from a subject with AD to allow a biomolecule corona to form on the surface of said nanoparticles;
- b) optionally isolating the nanoparticles and surface-bound biomolecule corona;
- c) determining the abundance of one or more protein biomarkers in the corona, the one or more protein biomarkers being independently selected from those listed in Table 2, Table 1a or Table 6a; and
- d) comparing the abundance of the one or more protein biomarkers in the corona to the abundance of the same one or more protein biomarkers in a non-diseased control reference;

wherein down regulation of one or more of the proteins in Table 2, Table 1a, or Table 6a relative to a previous measurement in the same subject indicates AD progression.

**[0019] Table 2**

| Protein Name                                 | Accession Number |
|----------------------------------------------|------------------|
| Immunoglobulin heavy variable 7-1 (Fragment) | A0A075B5S2       |
| Transferrin receptor protein 1               | TFR1             |

|                                                    |            |
|----------------------------------------------------|------------|
| Light chain variable region (Fragment)             | A2NVX0     |
| Proteasome subunit alpha type-6                    | PSA6       |
| 26S proteasome non-ATPase regulatory subunit 3     | PSMD3      |
| Glyceraldehyde-3-phosphate dehydrogenase           | A0A0A0MQF6 |
| Proteasome subunit beta type-6                     | PSB6       |
| Myosin-9                                           | MYH9       |
| Immunoglobulin heavy variable V1-11 (Fragment)     | A0A0A6YWI9 |
| Uncharacterized protein                            | A0A1S6GWH1 |
| Proteasome subunit alpha type-3                    | PSA3       |
| Acylamino-acid-releasing enzyme (Fragment)         | A0A0R4J107 |
| Aminopeptidase N (Fragment)                        | A0A0U1RNS3 |
| Uroplakin-3b                                       | A0A0R4J0S8 |
| T-complex protein 1 subunit gamma                  | TCPG       |
| Pregnancy zone protein                             | PZP        |
| WD repeat-containing protein 90                    | H7BX49     |
| Proteasome subunit beta type-3                     | PSB3       |
| Cartilage oligomeric matrix protein                | COMP       |
| Moesin                                             | MOES       |
| Kell blood group glycoprotein homolog              | KELL       |
| Immunoglobulin heavy variable 1-34 (Fragment)      | A0A075B5V2 |
| Bifunctional purine biosynthesis protein PURH      | PUR9       |
| Laminin subunit alpha-2                            | LAMA2      |
| Peroxiredoxin-2                                    | PRDX2      |
| Laminin subunit gamma-1                            | F8VQJ3     |
| Endoplasmin                                        | ENPL       |
| Filamin-A                                          | FLNA       |
| Thrombospondin-4                                   | TSP4       |
| Tankyrase-2                                        | TNKS2      |
| Glucosidase 2 subunit beta                         | GLU2B      |
| Thyrotropin-releasing hormone-degrading ectoenzyme | TRHDE      |
| Laminin subunit beta-1                             | LAMB1      |
| Clusterin                                          | CLUS       |

|                                                           |            |
|-----------------------------------------------------------|------------|
| Proteasome subunit alpha type                             | Q3TN31     |
| Heat shock 70 kDa protein 4                               | Q3U2G2     |
| 26S proteasome non-ATPase regulatory subunit 6 (Fragment) | A0A286YDW8 |
| Ezrin                                                     | EZRI       |
| Clathrin heavy chain 1                                    | CLH1       |
| Proteasome subunit beta type-1                            | PSB1       |
| Erythrocyte band 7 integral membrane protein              | STOM       |
| Fructose-bisphosphate aldolase A                          | ALDOA      |
| Laminin subunit gamma-1                                   | LAMC1      |
| Protein disulfide-isomerase                               | PDIA1      |
| T-complex protein 1 subunit alpha                         | TCPA       |
| 26S proteasome non-ATPase regulatory subunit 14           | PSDE       |
| Tripeptidyl-peptidase 2                                   | TPP2       |
| Nidogen-1                                                 | NID1       |
| 26S proteasome regulatory subunit 10B                     | PRS10      |
| Serglycin                                                 | SRGN       |
| Proteasome subunit beta type-5                            | PSB5       |
| 26S proteasome regulatory subunit 6A (Fragment)           | A0A087WPH7 |
| 26S proteasome non-ATPase regulatory subunit 2            | PSMD2      |
| Alpha-2-macroglobulin-P                                   | A2MG       |
| Immunoglobulin kappa variable 2-109 (Fragment)            | A0A075B5K6 |
| Carboxylesterase 3A                                       | EST3A      |
| Early endosome antigen 1                                  | EEA1       |
| Complement C4-B                                           | CO4B       |
| Sarcoplasmic/endoplasmic reticulum calcium ATPase 2       | AT2A2      |
| Uncharacterized protein                                   | Q3TYT5     |
| Proteasome subunit beta type-4                            | PSB4       |
| Aldehyde dehydrogenase family 16 member A1                | A0A1B0GSU0 |
| MCG23377, isoform CRA_b                                   | A0A0A6YW67 |
| Ras-related protein Rap-1b                                | RAP1B      |
| Collectin-11                                              | A0A0R4J0M6 |
| Large neutral amino acids transporter small subunit 3     | A2ATS4     |

|                                                                             |            |
|-----------------------------------------------------------------------------|------------|
| Adiponectin                                                                 | ADIPO      |
| Cytochrome P450 2D9                                                         | CP2D9      |
| Uncharacterized protein                                                     | Q3TR40     |
| Retinol dehydrogenase 7                                                     | RDH7       |
| Single-chain variable                                                       | A0A1E1GJG6 |
| Carbonic anhydrase 1                                                        | CAH1       |
| 26S proteasome non-ATPase regulatory subunit 12                             | B1AT36     |
| LOC207685 protein (Fragment)                                                | A4FU62     |
| Collagen alpha-1(I) chain                                                   | CO1A1      |
| MCG5400                                                                     | Q6ZWQ9     |
| Cytoplasmic dynein 1 heavy chain 1                                          | DYHC1      |
| Spectrin alpha chain, non-erythrocytic 1                                    | SPTN1      |
| Proteasome subunit alpha type                                               | Q3TUI9     |
| Proteasome subunit alpha type (Fragment)                                    | Q3TL95     |
| Purine nucleoside phosphorylase                                             | PNPH       |
| Cytochrome P450 2F2                                                         | CP2F2      |
| Cytochrome P450 2D10                                                        | CP2DA      |
| Uncharacterized protein                                                     | Q3TI47     |
| Integrin alpha 2b                                                           | B2RPR7     |
| Cytosolic 10-formyltetrahydrofolate dehydrogenase                           | AL1L1      |
| Metalloproteinase inhibitor 3                                               | TIMP3      |
| Laminin subunit alpha-1                                                     | LAMA1      |
| Uncharacterized protein                                                     | Q3U5V3     |
| Solute carrier family 29 (Nucleoside transporters), member 1, isoform CRA_a | Q3TCZ2     |
| Annexin A5                                                                  | ANXA5      |
| Immunoglobulin kappa variable 8-34 (Fragment)                               | A0A0G2JDU0 |
| Microsomal triglyceride transfer protein large subunit                      | MTP        |
| Caveolae-associated protein 1                                               | CAVN1      |
| Immunoglobulin kappa variable 1-135 (Fragment)                              | A0A0B4J1H7 |
| Ig heavy chain V region 3-6                                                 | HVM60      |
| Solute carrier family 2, facilitated glucose transporter member 3           | GTR3       |

|                                                                                                                  |            |
|------------------------------------------------------------------------------------------------------------------|------------|
| Cytochrome P450 2D26                                                                                             | CP2DQ      |
| NADPH--cytochrome P450 reductase (Fragment)                                                                      | E9PVT9     |
| Immunoglobulin heavy variable V1-59                                                                              | A0A075B5X0 |
| Integrin beta-1                                                                                                  | ITB1       |
| Dimethylaniline monooxygenase [N-oxide-forming] 5                                                                | FMO5       |
| Galectin-3-binding protein                                                                                       | LG3BP      |
| ATP synthase subunit alpha, mitochondrial                                                                        | ATPA       |
| Uncharacterized protein                                                                                          | Q3U8F5     |
| Peroxisomal protein 1 (Fragment)                                                                                 | B1AXW5     |
| Uncharacterized protein (Fragment)                                                                               | Q3TB11     |
| Protein KRI1 homolog                                                                                             | KRI1       |
| Perilipin-4                                                                                                      | PLIN4      |
| Spectrin beta chain, erythrocytic                                                                                | SPTB1      |
| 55 kDa erythrocyte membrane protein                                                                              | B7ZCL8     |
| Immunoglobulin kappa variable 8-16                                                                               | A0A075B5N6 |
| Mannan-binding lectin serine protease 1                                                                          | MASP1      |
| Vimentin                                                                                                         | A0A0A6YWC8 |
| 26S proteasome non-ATPase regulatory subunit 1                                                                   | PSMD1      |
| UDP-glucuronosyltransferase 2B17                                                                                 | UDB17      |
| Ig lambda-2 chain C region                                                                                       | LAC2       |
| Dihydrolipoyllysine-residue succinyltransferase component of 2-oxoglutarate dehydrogenase complex, mitochondrial | ODO2       |
| Carbonic anhydrase 2                                                                                             | CAH2       |
| Complement component 6                                                                                           | E9Q6D8     |
| UDP-glucuronosyltransferase 1-1                                                                                  | UD11       |
| L-gulonolactone oxidase                                                                                          | GGLO       |
| Bile acyl-CoA synthetase                                                                                         | E9P XV4    |
| DNA helicase B                                                                                                   | HELB       |
| Ig heavy chain V region 6.96                                                                                     | HVM57      |
| NADH-cytochrome b5 reductase 3 (Fragment)                                                                        | A0A0A0MQM3 |
| Heat shock protein 84b                                                                                           | Q71LX8     |
| Ig heavy chain V region 3                                                                                        | HVM05      |

|                                                                  |            |
|------------------------------------------------------------------|------------|
| Disintegrin and metalloproteinase domain-containing protein 10   | ADA10      |
| Basigin                                                          | BASI       |
| Uncharacterized protein                                          | Q3THG2     |
| Heat shock protein HSP 90-alpha                                  | HS90A      |
| Collagen, type VI, alpha 3                                       | A0A087WS16 |
| EMILIN-1                                                         | EMIL1      |
| Retinal dehydrogenase 1                                          | AL1A1      |
| Immunoglobulin heavy variable 1-39 (Fragment)                    | A0A075B5V5 |
| Igh protein                                                      | I6L985     |
| Spectrin beta chain                                              | B2RX08     |
| Alpha actinin 1a                                                 | A1BN54     |
| Glutathione peroxidase 3                                         | GPX3       |
| Integrin beta-3                                                  | ITB3       |
| Spectrin alpha chain, erythrocytic 1                             | SPTA1      |
| Protein Igkv5-48 (Fragment)                                      | A0A140T8N2 |
| Immunoglobulin heavy variable 10-1 (Fragment)                    | A0A0B4J1J6 |
| Talin-1                                                          | TLN1       |
| Immunoglobulin kappa variable 4-53                               | A0A075B677 |
| RAS oncogene family protein                                      | A0A068BFR3 |
| Guanine nucleotide-binding protein G(I)/G(S)/G(T) subunit beta-1 | GBB1       |

**[0020]** In one embodiment of any aspect of the invention, the protein biomarker selected from Table 2 is not peroxiredoxin-1 (Fragment) or moesin,

**[0021]** Suitably, the protein biomarker is identified by protein name in Table 2. Table 2 provides a SwissProt accession code for the murine version of the identified protein. The person skilled in the art can easily use this to identify the equivalent protein for the particular species of subject, e.g. human, for use in this aspect of the invention.

**[0022]** According to a fourth aspect of the invention there is provided a method for assessing the stage of AD in a subject, comprising the steps of:

- a) contacting a plurality of nanoparticles with a biofluid sample from the subject to allow a biomolecule corona to form on the surface of said nanoparticles;
- b) optionally isolating the nanoparticles and surface-bound biomolecule corona;

- c) determining the abundance of one or more protein biomarkers in the corona, the one or more protein biomarkers being independently selected from those listed in Table 1; and
- d) comparing the abundance of the one or more protein biomarkers in the corona to the abundance of the same one or more protein biomarkers in a non-diseased control reference;

wherein a change in abundance relative to a non-diseased control reference of one or more proteins in Table 3 indicates that the patient has early stage asymptomatic AD, or the presence of one or more proteins in Table 4 indicates that the patient has intermediate stage AD, or the presence of one or more proteins in Table 5 indicates that the patient has advanced stage AD.

**[0023] Table 3**

| Protein Name                                    | Accession Number |
|-------------------------------------------------|------------------|
| Pancreatic alpha-amylase                        | AMYP             |
| Early endosome antigen 1                        | EEA1             |
| Cytochrome b5                                   | CYB5             |
| T-complex protein 1 subunit alpha               | TCPA             |
| IgG1 heavy chain VDJ region (Fragment)          | X5J4T6           |
| 60S ribosomal protein L18                       | A0A1B0GQU8       |
| Receptor expression-enhancing protein           | D3Z6V2           |
| E3 ubiquitin-protein ligase UBR4                | UBR4             |
| Uncharacterized protein                         | A0A1S6GWH1       |
| 26S proteasome non-ATPase regulatory subunit 1  | PSMD1            |
| Integrin beta                                   | M0QWA7           |
| Predicted gene 45713                            | A0A1B0GS68       |
| Ribosomal protein L15                           | Q3U7D2           |
| 26S proteasome regulatory subunit 6A (Fragment) | A0A087WPH7       |
| Uncharacterized protein                         | Q9CWK0           |
| Long-chain-fatty-acid--CoA ligase 5             | ACSL5            |
| Ribosomal protein S2, pseudogene 6              | A0A140T8L5       |
| Calreticulin                                    | CALR             |
| 60S ribosomal protein L6                        | Q3UCH0           |

|                                                      |            |
|------------------------------------------------------|------------|
| 26S proteasome regulatory subunit 10B                | PRS10      |
| Talin-1                                              | TLN1       |
| Laminin subunit alpha-2                              | LAMA2      |
| IgM heavy chain VDJ region (Fragment)                | X5J5A8     |
| Uncharacterized protein                              | Q3UBI6     |
| VH region (Fragment)                                 | Q53VQ0     |
| Colony stimulating factor 1 receptor                 | Q0P635     |
| Proteoglycan 4                                       | A0A0R4J207 |
| 26S proteasome non-ATPase regulatory subunit 14      | PSDE       |
| Immunoglobulin heavy variable 10-1 (Fragment)        | A0A0B4J1J6 |
| GDH/6PGL endoplasmic bifunctional protein            | A2A7A7     |
| Endoplasmin                                          | ENPL       |
| WD repeat-containing protein 90                      | H7BX49     |
| Proteasome subunit beta type-5                       | PSB5       |
| Proteasome subunit beta type-6                       | PSB6       |
| 40S ribosomal protein S9                             | D3YWH9     |
| Elongation factor 1-alpha 1                          | EF1A1      |
| Myosin-9                                             | MYH9       |
| Proteasome subunit beta type-4                       | PSB4       |
| Immunoglobulin kappa chain variable 1-122 (Fragment) | A0A140T8M9 |
| Immunoglobulin kappa chain variable 9-124            | A0A075B5K2 |
| Ig kappa chain V-V region MOPC 149                   | KV5A4      |
| Immunoglobulin heavy variable V1-11 (Fragment)       | A0A0A6YWI9 |
| Immunoglobulin kappa variable 12-44 (Fragment)       | A0A140T8M2 |
| Immunoglobulin kappa variable 8-28                   | A0A075B5N3 |
| Cartilage oligomeric matrix protein                  | COMP       |
| Immunoglobulin kappa chain variable 8-27             | A0A075B5N4 |
| Proteasome subunit alpha type-6                      | PSA6       |
| Proteasome subunit alpha type (Fragment)             | Q3TL95     |
| Immunoglobulin kappa variable 6-17 (Fragment)        | A0A140T8Q3 |
| Immunoglobulin kappa variable 2-109 (Fragment)       | A0A075B5K6 |
| Immunoglobulin heavy variable V1-59                  | A0A075B5X0 |

|                                                                             |            |
|-----------------------------------------------------------------------------|------------|
| Immunoglobulin kappa variable 4-53                                          | A0A075B677 |
| Coagulation factor V                                                        | FA5        |
| Single-chain variable                                                       | A0A1E1GJG6 |
| DNA helicase B                                                              | HELB       |
| Aberrantly recombined kappa chain Vk8/J1 region (Fragment)                  | A2N494     |
| Adiponectin                                                                 | ADIPO      |
| Long-chain-fatty-acid--CoA ligase 1                                         | ACSL1      |
| Transferrin receptor protein 1                                              | TFR1       |
| Anti-VIPase light chain variable region (Fragment)                          | Q8K1F1     |
| Maltase-glucoamylase                                                        | B5THE2     |
| Proteasome subunit alpha type-3                                             | PSA3       |
| Serum amyloid A-4 protein                                                   | SAA4       |
| Anti-myosin immunoglobulin light chain variable region (Fragment)           | Q9JL74     |
| Argininosuccinate lyase                                                     | ARLY       |
| Clusterin                                                                   | CLUS       |
| Rab GDP dissociation inhibitor (Fragment)                                   | A0A1Y7VLG4 |
| Monocarboxylate transporter 1                                               | MOT1       |
| Protein piccolo                                                             | PCLO       |
| Solute carrier family 29 (Nucleoside transporters), member 1, isoform CRA_a | Q3TCZ2     |
| Protein KRI1 homolog                                                        | KRI1       |
| Basigin                                                                     | BASI       |
| Uncharacterized protein                                                     | Q3TA14     |
| Sarcoplasmic/endoplasmic reticulum calcium ATPase 2                         | AT2A2      |
| Disintegrin and metalloproteinase domain-containing protein 10              | ADA10      |
| Filamin-A                                                                   | FLNA       |
| ATP synthase subunit beta, mitochondrial                                    | ATPB       |
| Kell blood group glycoprotein homolog                                       | KELL       |
| Erythrocyte band 7 integral membrane protein                                | STOM       |

|                                                                |            |
|----------------------------------------------------------------|------------|
| Moesin                                                         | MOES       |
| RAS-related C3 botulinum substrate 3, isoform CRA_a (Fragment) | A2AC13     |
| Aquaporin-1                                                    | AQP1       |
| Annexin A5                                                     | ANXA5      |
| Glucosidase 2 subunit beta                                     | GLU2B      |
| Clathrin heavy chain 1                                         | CLH1       |
| Complement C1q tumor necrosis factor-related protein 3         | C1QT3      |
| Ezrin                                                          | EZRI       |
| Ig kappa chain V-III region 50S10.1                            | KV3A4      |
| Ras-related protein Rap-1b                                     | RAP1B      |
| Ig kappa chain V-III region PC 3741/TEPC 111                   | KV3A8      |
| Protein Igkv5-48 (Fragment)                                    | A0A140T8N2 |
| Immunoglobulin lambda variable 3 (Fragment)                    | A0A0B4J1K5 |
| Spectrin alpha chain, non-erythrocytic 1                       | SPTN1      |
| MCG23377, isoform CRA_b                                        | A0A0A6YW67 |
| Aminopeptidase N (Fragment)                                    | A0A0U1RNS3 |
| Retinol dehydrogenase 7                                        | RDH7       |
| Uncharacterized protein                                        | Q3TI47     |
| Uncharacterized protein                                        | Q3TR40     |
| Fibronectin                                                    | A0A087WR50 |
| Uroplakin-3b                                                   | A0A0R4J0S8 |
| Tankyrase-2                                                    | TNKS2      |
| Integrin beta-1                                                | ITB1       |
| Immunoglobulin kappa variable 6-23 (Fragment)                  | A0A140T8N5 |
| Napsin-A                                                       | NAPSA      |
| Light chain variable region (Fragment)                         | A2NVX0     |

**[0024]** In one embodiment of any aspect of the invention, the protein biomarker selected from Table 3 is not moesin,

**[0025]** Table 4

| Protein Name                                           | Accession Number |
|--------------------------------------------------------|------------------|
| T-complex protein 1 subunit gamma                      | A0A075B5S2       |
| Meprin A subunit                                       | A0A075B5T1       |
| Protein piccolo                                        | A0A075B5V5       |
| G protein-coupled receptor 179                         | A0A075B5V7       |
| C-type lectin domain family 11 member A                | A0A0R4J043       |
| Immunoglobulin heavy variable 7-1 (Fragment)           | A2BIN1           |
| Complement C1q tumor necrosis factor-related protein 3 | AL1L1            |
| Cytosolic 10-formyltetrahydrofolate dehydrogenase      | ASSY             |
| Tankyrase-2                                            | CD5L             |
| Immunoglobulin heavy variable V12-3 (Fragment)         | CLC11            |
| Anti-colorectal carcinoma light chain                  | E9PY61           |
| Retinol dehydrogenase 7                                | EST2E            |
| Uroplakin-3b                                           | OIT3             |
| Bisphosphoglycerate mutase                             | PMGE             |
| Argininosuccinate synthase                             | PROP             |
| Immunoglobulin heavy variable V1-43                    | Q6ZWQ9           |
| Oncoprotein-induced transcript 3 protein               | Q7TS98           |
| Properdin                                              | TCPG             |
| Thrombospondin-4                                       | TRHDE            |
| MCG5400                                                | TSP4             |
| Immunoglobulin heavy variable 1-39 (Fragment)          | X5J4G2           |

[0026] Table 5

| Protein Name                                  | Accession Number |
|-----------------------------------------------|------------------|
| Caveolae-associated protein 1                 | CAVN1            |
| Bile acyl-CoA synthetase                      | E9PXV4           |
| MRP5 (Fragment)                               | Q925S1           |
| Tripeptidyl-peptidase 2                       | TPP2             |
| Uncharacterized protein                       | A0A1S6GWH5       |
| Peroxiredoxin-2                               | PRDX2            |
| Immunoglobulin heavy variable 11-1 (Fragment) | A0A075B5R6       |

|                                                                  |            |
|------------------------------------------------------------------|------------|
| Heat shock 70 kDa protein 4                                      | Q3U2G2     |
| Integrin beta-3                                                  | ITB3       |
| Alpha actinin 1a                                                 | A1BN54     |
| EMILIN-1                                                         | EMIL1      |
| Large neutral amino acids transporter small subunit 3            | A2ATS4     |
| Integrin alpha 2b                                                | B2RPR7     |
| Angiopoietin-1                                                   | ANGP1      |
| Glyceraldehyde-3-phosphate dehydrogenase                         | A0A0A0MQF6 |
| Uncharacterized protein                                          | I6L9E1     |
| Uncharacterized protein (Fragment)                               | Q3TB11     |
| Microsomal triglyceride transfer protein large subunit           | MTP        |
| Bifunctional purine biosynthesis protein PURH                    | PUR9       |
| Neuronal membrane glycoprotein M6-a                              | GPM6A      |
| Guanine nucleotide-binding protein G(I)/G(S)/G(T) subunit beta-1 | GBB1       |
| ATP synthase subunit alpha, mitochondrial                        | ATPA       |
| Dimethylaniline monooxygenase [N-oxide-forming] 5                | FMO5       |
| Integrin-linked protein kinase                                   | ILK        |
| Galectin-3-binding protein                                       | LG3BP      |
| RAS oncogene family protein                                      | A0A068BFR3 |
| Adenylyl cyclase-associated protein 1                            | CAP1       |
| Annexin A2                                                       | ANXA2      |
| CD9 antigen                                                      | CD9        |
| Tubulin beta-4A chain                                            | TBB4A      |
| Spectrin beta chain                                              | B2RX08     |
| Lactadherin                                                      | MFGM       |
| Decorin                                                          | PGS2       |
| Leucine-rich repeat neuronal protein 4                           | LRRN4      |
| Mannan-binding lectin serine protease 2                          | MASP2      |
| Immunoglobulin heavy variable V9-3 (Fragment)                    | A0A0B4J1J5 |
| Igh protein                                                      | Q4VAB6     |
| Immunoglobulin kappa variable 8-34 (Fragment)                    | A0A0G2JDU0 |

|                                                                                                                  |            |
|------------------------------------------------------------------------------------------------------------------|------------|
| Dihydrolipoyllysine-residue succinyltransferase component of 2-oxoglutarate dehydrogenase complex, mitochondrial | ODO2       |
| Transketolase (Fragment)                                                                                         | A0A286YE28 |
| Serglycin                                                                                                        | SRGN       |
| Laminin subunit gamma-1                                                                                          | F8VQJ3     |
| Mannan-binding lectin serine protease 1                                                                          | MASP1      |
| Vinculin                                                                                                         | VINC       |
| Ankyrin-1                                                                                                        | A0A0R4J1N7 |
| Retinal dehydrogenase 1                                                                                          | AL1A1      |
| Serum paraoxonase/arylesterase 1                                                                                 | PON1       |
| L-lactate dehydrogenase                                                                                          | A0A1B0GSR9 |
| ADP/ATP translocase 2                                                                                            | ADT2       |
| Annexin                                                                                                          | A0A2C9F2D2 |
| Solute carrier family 2, facilitated glucose transporter member 3                                                | GTR3       |
| Dihydrolipoyllysine-residue acetyltransferase component of pyruvate dehydrogenase complex, mitochondrial         | ODP2       |
| NADPH--cytochrome P450 reductase (Fragment)                                                                      | E9PVT9     |
| Uncharacterized protein                                                                                          | Q3T9M7     |
| Phospholipid transfer protein                                                                                    | PLTP       |
| Maltase-glucoamylase                                                                                             | A0A0J9YVE6 |
| Perilipin-4                                                                                                      | PLIN4      |
| Hsp90aa1 protein (Fragment)                                                                                      | A0PJ91     |
| Coronin                                                                                                          | Q3T9L1     |
| Spectrin beta chain, erythrocytic                                                                                | SPTB1      |
| Dipeptidyl peptidase 4                                                                                           | DPP4       |
| Calnexin                                                                                                         | CALX       |
| 26S proteasome non-ATPase regulatory subunit 2                                                                   | PSMD2      |
| UDP-glucuronosyltransferase 1-1                                                                                  | UD11       |
| Annexin A6                                                                                                       | ANXA6      |
| Glycogen phosphorylase, muscle form                                                                              | PYGM       |
| 55 kDa erythrocyte membrane protein                                                                              | B7ZCL8     |

|                                                                   |            |
|-------------------------------------------------------------------|------------|
| Anti-myosin immunoglobulin light chain variable region (Fragment) | Q9JL78     |
| Uncharacterized protein                                           | Q9DBD9     |
| UTP--glucose-1-phosphate uridylyltransferase                      | UGPA       |
| Fermitin family homolog 3                                         | URP2       |
| Laminin subunit gamma-1                                           | LAMC1      |
| Immunoglobulin kappa variable 4-80 (Fragment)                     | A0A075B5L7 |

**[0027]** Suitably, the protein biomarker is identified by protein name in Tables 3, 4 or 5. Tables 3, 4 and 5 provides a SwissProt accession code for the murine version of the identified protein. The person skilled in the art can easily use this to identify the equivalent protein for the particular species of subject, e.g. human, for use in the invention.

**[0028]** According to a fifth aspect of the invention there is provided a method for assessing the stage of AD in a subject, comprising the steps of:

- a) contacting a plurality of nanoparticles with a biofluid sample from the subject to allow a biomolecule corona to form on the surface of said nanoparticles;
- b) optionally isolating the nanoparticles and surface-bound biomolecule corona;
- c) determining the abundance of one or more protein biomarkers in the corona, the one or more protein biomarkers being independently selected from those listed in Table 1a; and
- d) comparing the abundance of the one or more protein biomarkers in the corona to the abundance of the same one or more protein biomarkers in a non-diseased control reference;

wherein a change in abundance relative to a non-diseased control reference of one or more proteins in Table 3a indicates that the patient has early stage asymptomatic AD, or the presence of one or more proteins in Table 4a indicates that the patient has intermediate stage AD, or the presence of one or more proteins in Table 5a indicates that the patient has advanced stage AD.

**[0029] Table 3a**

| Protein Name | Gene Name | Accession Number |
|--------------|-----------|------------------|
|              |           |                  |

|                               |      |            |
|-------------------------------|------|------------|
| Proteoglycan 4, isoform CRA_a | PRG4 | A0A024R930 |
| Protein piccolo               | PCLO | PCLO       |

**[0030] Table 4a**

| Protein Name    | Gene Name | Accession Number |
|-----------------|-----------|------------------|
| Protein piccolo | PCLO      | PCLO             |

**[0031] Table 5a**

| Protein Name                            | Gene Name | Accession Number |
|-----------------------------------------|-----------|------------------|
| Phospholipid transfer protein           | PLTP      | PLTP             |
| Serum paraoxonase/arylesterase 1        | PON1      | PON1             |
| Mannan-binding lectin serine protease 2 | MASP2     | MASP2            |
| Fermitin family homolog 3               | FERMT3    | URP2             |

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**[0032]** Suitably, the protein biomarker is identified by the protein name in Tables 3a, 4a, or 5a. Tables 3a, 4a and 5a provides a SwissProt accession code for the homo sapiens version of the identified protein. The person skilled in the art can easily use this to identify the equivalent protein for the particular species of subject for use in the invention.

- 10 **[0033]** According to a sixth aspect there is provided a method for identifying neurodegeneration-associated protein biomarkers in the blood of a subject, comprising (a) (i) contacting a plurality of nanoparticles with a blood-derived fluid sample from one or more healthy subjects; and (ii) contacting a plurality of nanoparticles with a blood-derived fluid sample from one or more subjects with neurodegeneration, under conditions to allow a biomolecule corona to form on the surface of said
- 15 nanoparticles; (b) optionally isolating the nanoparticles and surface-bound biomolecule corona; (c) determining the abundance of one or more protein biomarkers in the corona from each subject; (d) comparing the abundance of the one or more protein biomarkers in the corona of subjects in (i) to the abundance of the same one or more protein biomarkers in the corona of subjects in (ii); wherein protein biomarkers which display statistically significant differential expression between (i) and (ii) are
- 20 identified as neurodegeneration-associated protein biomarkers.

**[0034]** Suitably, in any of the aspects of the invention, the biofluid is a blood or blood fraction sample, such as serum or plasma. Suitably the blood or blood fraction sample is from circulating blood.

**[0035]** Biomolecule corona formation has become an active area of research and the potential exploitation of protein corona as a proteomic biomarker discovery platform that enables a higher-definition, in-depth analysis of the blood proteome and the enrichment of low abundant disease-specific molecules has previously been evaluated and reported (see WO2018/046542 and [9, 10, 11, 12]).

**[0036]** In certain embodiments, the methods of the invention result in an interaction between the nanoparticles and a greater number of different types of protein biomarkers than can be detected by direct analysis of biofluids taken from a subject, such as one in a diseased state. It is to be understood that the methods involve identification of a biomarker or biomarkers that provides a measurable indicator of some biological state or condition. This includes, but is not limited to, the discovery of unique disease-specific biomolecules (those biomolecules that are only present in a diseased state) but also includes detection of changes (for example, a statistically significant change) in the amount and/or abundance of biomolecule(s) that are present in both healthy and diseased states, for example upregulation or down regulation of biomolecules in a diseased state when compared to the healthy state or at a different time point. It will also be understood that in order to identify a potential disease-specific biomarker, comparison against a suitable non-diseased control reference can be required.

**[0037]** By up-regulation or down-regulation of a particular biomarker we mean an increase or decrease, respectively, in the amount and/or abundance of the biomarker.

**[0038]** In particular embodiments, the biomarker level is reduced or down-regulated to less than 90%, such as less than 80% such as less than 70% for example less than 60%, for example less than 50%, such as less than 40%, such as less than 30% such as less than 20% for example less than 10%, for example less than 5%, such as completely inhibited (0%) compared to the control level.

**[0039]** In particular embodiments, the biomarker level is increased or up-regulated to more than 110%, such as more than 120% such as more than 130% for example more than 150%, for example more than 175%, such as more than 200%, such as more than 250% such as more than 300% for example more than 350% of the control amount.

**[0040]** In particular embodiments, the methods facilitate the detection (which may include identification or discovery) of previously unknown disease-specific biomolecules.

**[0041]** In one particular embodiment, the methods involve identifying panels of biomarkers, which can lead to increased sensitivity and specificity of detection.

[0042] In a particular embodiment of any aspect of the invention, the methods allow identification or detection of a biomarker without the need for invasive tissue sampling, e.g. a biopsy.

[0043] It is to be understood that the methods are applicable to a wide range of nanoparticles and allow the benefit of removal of unbound and highly abundant biomolecules to allow identification of low abundant protein biomarkers that would otherwise be undetected. In addition to identification of potential biomarkers, the methods can also be employed to monitor changes in biomarkers, for example in response to therapy and/or to assist in diagnosis.

[0044] Suitably, the methods can be used to detect or monitor a disease in a subject. The methods disclosed herein are applicable to any stage of Alzheimer's disease, in which detection and/or monitoring of biomarkers would be beneficial. In particular, the methods of the invention can be used to assess the likelihood of developing Alzheimer's disease or determine Alzheimer's disease or monitor Alzheimer's disease progression in a subject or in biofluid taken from a subject. The methods of the invention can also be employed to discover novel biomarkers and biomarker fingerprints.

[0045] In a seventh aspect of the invention, there is provided a diagnostic kit comprising nanoparticles and reagents capable of detecting one or more protein biomarkers listed in Table 1 or Table 1a.

[0046] In an eighth aspect of the invention, there is provided any one or more of the protein biomarkers listed in Table 1, Table 1a, or any combinations thereof, for use as a biomarker for Alzheimer's disease.

[0047] Any embodiment described herein can be applied to any aspect of the invention unless indicated otherwise or it is apparent to the person of skill in the art that such embodiment cannot apply.

[0048] Accession numbers herein detailed in Tables 1, 2, 3, 4, 5, 6, 7, 8, 9, 10, 11 and 12 are based on the SwissProt\_2018\_01 database.

[0049] Accession numbers herein detailed in Tables 1a, 3a, 4a, 5a, 6a, 6b, and 13 are based on the SwissProt\_2019\_10 fasta; Trembl\_2019\_10 databases.

#### DESCRIPTION OF THE DRAWINGS

[0050] **Figure 1: Blood-circulating nanoparticle scavengers in APP/PS1 and WT mice.**

(a) Quantification of A $\beta$  plaque burden (percentage cortical area) in APP/PS1 and wild-type (WT mice (C57/B6j, male) at 2, 6 and 12 months of age with example images; cortical plaque deposition visualised with 6e10 antibody for A $\beta$  3-8. (Scale bars: 1mm; Sidak corrected posthoc test; \*\*\*p.value<0.001 APP/PS1 vs WT at the same time point; ### p.value <0.001 APP/PS1 vs APP/PS1 at the previous time point; ns - not significant, n=4-5). (b-d) Reference memory performance of APP/PS1 and WT (C57/B6j) mice at 2, 6 and 12 months of age in the Morris water maze as measured

by time (s) in correct quadrant during the 30s probe trial. Error bars indicate mean  $\pm$  SEM (n=19-25 mice, Welch's test). 25% line represents expected performance from random chance. **(e)** Physicochemical characteristics of liposomes employed in this study. **(f)** Schematic description of the experimental design. PEGylated liposomes were intravenously injected and subsequently recovered from the blood circulation of 2-, 6- and 12-month-old APP/PS1 and wild-type C57 male mice. 'Healthy' and 'diseased' *in vivo* formed protein coronas were comprehensively characterized and compared by label-free mass spectrometry (LC-MS/MS) to identify differentially abundant proteins. **(g)** The total amount of protein adsorbed onto the surface of the blood-recovered liposomes, expressed as Pb values ( $\mu\text{g}$  of protein/ $\mu\text{M}$  lipid). Error bars indicate mean  $\pm$  SEM of n=3 biological replicates; n=3 mice/replicate. (Mann-Whitney t-test).

**[0051] Figure 1h:** Negative stain TEM of liposomes before and after intravenous administration and recovery from the blood circulation of APP/PS1 and WT (C57/B6j) mice at 2, 6 and 12 months of age. All scale bars are 100nm.

**[0052] Figure 2: AD-specific longitudinal proteomic alterations in blood.**

Proteomic comparison between 'healthy' and 'diseased' protein coronas at the 2-month (2M), 6-month (6M) and 12-month (12M) time points (n=3 biological replicates; n=3 mice/replicate). MS peak intensities were analysed using Progenesis LC-MS software (version 3.0; Nonlinear Dynamics). Only proteins that differed between APP/PS1 and WT mice with a *p* value < 0.05 are shown. Venn diagrams report the number of unique and common differentially abundant proteins discovered for the three time points (2M, 6M & 12M) by proteomic analysis of **(a)** plasma control samples and **(b)** corona samples. **(c-e)** Volcano plots display the relationship between fold change and significance for the differentially abundant corona proteins at 2M, 6M and 12M. The full list of differentially abundant proteins is shown in **Tables 7, 8 and 9**. **(f)** Comparison of the differentially abundant proteins discovered at 2M and 12M. Filled dots represent the n=31 common proteins between the two time points. **(g)** Longitudinal fluctuation in the fold change values of the n=105 proteins identified to be differentially abundant between APP/PS1 and WT mice at the 2M time point.

**[0053] Figure 2h:** Longitudinal fluctuation in the fold change values of the n=31 common proteins that were found to be differentially abundant between APP/PS1 and WT mice in both 2M and 12M time points.

**[0054] Figure 3: Systemic monitoring of AD progression.**

Time evolution of the liposomal coronas in APP/PS1 mice. Proteomic comparisons between **(a)** 2M vs 6M and **(b)** 6M vs 12M time points (n=3 biological replicates; n=3 mice/replicate). MS peak intensities were analysed using Progenesis LC-MS software (version 3.0; Nonlinear Dynamics). Only proteins that differed by a *p* value < 0.05 are shown. Proteins that were differentially abundant

between 2M vs 6M and 6M vs 12M WT mice as a result of ageing were excluded and only AD-specific monitoring proteins are shown. Volcano plot displays the relationship between fold change and significance between the two groups. The full list of potential biomarker proteins are shown in **Tables 10 and 11**. Longitudinal kinetics in the abundance of **(c)** all the disease-monitoring proteins identified and **(d)** the n=18 common proteins that displayed differential abundance between 2M and 6M and between 6M and 12M time points. Proteins are classified in three groups: proteins with increased abundance with AD progression (labelled “up” and shown in blue), proteins with decreased abundance with AD progression (labelled “down” and show in red) and proteins characterized by more complex kinetics (labelled “complex” and shown in green).

**[0055] Figure 4: Molecular pathway enrichment analysis.**

**(a)** Clustegram illustrating the 10 most enriched pathways (columns) from Kyoto Encyclopedia of Genes and Genomes (KEGG) and the 56 differentially abundant blood proteins involved (rows). Proteins are ranked from high to low frequency and pathways are rank according to the significance-level of enrichment. The proteins identified to be involved in the KEGG Alzheimer’s disease pathway are also shown. Proteins with increased abundance with AD progression are shown in blue, proteins with decreased abundance with AD progression are shown in red and proteins characterized by more complex kinetics are shown in green. **(b)** Protein interaction network. Nodes, representing the 10 most enriched pathways are sized according to the number of constituent proteins. Connections between nodes are sized according to the number of shared proteins between the pathways.

**[0056] Figure 5: Proteomic comparison of the ex vivo liposomal coronas formed in plasma samples obtained from AD patients (n=20 females; n=20 males) and healthy controls (n=20 females; n=20 males).** Proteomic comparison of ‘healthy’ and ‘diseased’ protein coronas. MS peak intensities were analysed using Progenesis LC-MS software (version 3.0; Nonlinear Dynamics). Data was filtered to a 1% false discovery rate (FDR). The peptide intensities were compared between groups by one way analysis of variance (ANOVA). Only proteins with p value < 0.05 are shown. Volcano plots displays the relationship between fold change and significance between the two groups. The full list of proteins are shown in Table 13.

**[0057] Figure 6: Proteomic comparison of the ex vivo liposomal coronas formed in plasma samples obtained from AD patients (n=20 females; n=20 males) and healthy controls (n=20 females; n=20 males).** Proteomic comparison of ‘healthy’ and ‘diseased’ protein coronas. MS peak intensities were analysed using Progenesis LC-MS software (version 3.0; Nonlinear Dynamics). Data was filtered to a 1% false discovery rate (FDR). The peptide intensities were compared between groups by one way analysis of variance (ANOVA). Only proteins with p value < 0.05 and which have

been shortlisted (n=27) are shown and Volcano plots displays the relationship between fold change and significance between the two groups. The shortlisted proteins are show in Table 6a.

## DETAILED DESCRIPTION OF THE INVENTION

5 **[0058]** The practice of particular embodiments of the invention will employ, unless indicated specifically to the contrary, conventional methods of chemistry, biochemistry, organic chemistry, molecular biology, microbiology, recombinant DNA techniques, genetics, immunology, and cell biology that are within the skill of the art, many of which are described below for the purpose of illustration. Such techniques are explained fully in the literature. See, e.g., Sambrook, et  
10 al., Molecular Cloning: A Laboratory Manual (3rd Edition, 2001); Ausubel et al., Current Protocols in Molecular Biology (John Wiley and Sons, updated July 2008); Short Protocols in Molecular Biology: A Compendium of Methods from Current Protocols in Molecular Biology, Greene Pub. Associates and Wiley-Interscience.

**[0059]** Unless defined otherwise, all technical and scientific terms used herein have the same  
15 meaning as commonly understood by those of ordinary skill in the art to which the invention belongs. Although any methods and materials similar or equivalent to those described herein can be used in the practice or testing of the invention, preferred embodiments of compositions, methods and materials are described herein.

### Definitions

20 **[0060]** The articles “a,” “an,” and “the” are used herein to refer to one or to more than one (i.e. to at least one) of the grammatical object of the article.

**[0061]** The use of the alternative (e.g., “or”) should be understood to mean either one, both, or any combination thereof of the alternatives.

**[0062]** The term “and/or” should be understood to mean either one, or both of the alternatives.

25 **[0063]** As used herein, the term “about” or “approximately” refers to a quantity, level, value, number, frequency, percentage, dimension, size, amount, weight or length that varies by as much as 15%, 10%, 9%, 8%, 7%, 6%, 5%, 4%, 3%, 2% or 1% to a reference quantity, level, value, number, frequency, percentage, dimension, size, amount, weight or length. In one embodiment, the term “about” or “approximately” refers a range of quantity, level, value, number, frequency, percentage,  
30  $\pm 15\%$ ,  $\pm 10\%$ ,  $\pm 9\%$ ,  $\pm 8\%$ ,  $\pm 7\%$ ,  $\pm 6\%$ ,  $\pm 5\%$ ,  $\pm 4\%$ ,  $\pm 3\%$ ,  $\pm 2\%$ , or  $\pm 1\%$  about a reference quantity, level, value, number, frequency, percentage, dimension, size, amount, weight or length.

**[0064]** The term "biomarker" as used herein includes proteins, fragments of proteins such as polypeptides or peptides. As used herein, "peptide" means peptides of any length and includes polypeptides and proteins.

**[0065]** As used herein, the term "in vitro" means performed or taking place in a test tube, culture dish, or elsewhere outside a living organism. The term also includes ex vivo because the analysis takes place outside an organism.

**[0066]** As used herein, the term "isolated" means material that is substantially or essentially free from components that normally accompany it in its native state. In particular embodiments, the term "obtained" or "derived" is used synonymously with isolated.

**[0067]** As used herein, "proteomics" is the analysis of proteins and elements of protein (referred to herein as a protein element or protein derivative) such as peptides (short chains of amino acids, e.g. 2-10 amino acids) and polypeptides (longer chains of amino acids).

**[0068]** A "subject," "individual," or "patient" as used herein, includes any animal that exhibits a symptom of a condition that can be detected or identified with compositions contemplated herein.

Suitable subjects include laboratory animals (such as mouse, rat, rabbit, or guinea pig), farm animals (such as horses, cows, sheep, pigs), and domestic animals or pets (such as a cat or dog). In particular embodiments, the subject is a mammal. In certain embodiments, the subject is a non-human primate and, in a particular embodiment, the subject is a human.

**[0069]** As used herein, the term "disease-specific biomarker" refers to a biomarker which is associated with or indicative of a disease.

**[0070]** The term "control reference" as used herein refers to a biofluid sample from a human or non-human subject not diagnosed or presenting symptoms of Alzheimer's disease.

#### Methods of the invention

**[0071]** According to a first aspect of the invention there is provided a method of assessing the likelihood of developing Alzheimer's disease or of determining Alzheimer's disease or of monitoring Alzheimer's disease progression in a subject, comprising the step of determining the presence and/or amount of one or more protein biomarkers in the subject or biofluid taken from the subject, the one or more protein biomarkers being independently selected from those listed in Table 6a, Table 1 or Table 1a.

**[0072]** In one embodiment, there is provided a method of assessing the likelihood of developing Alzheimer's disease in a subject or in a biofluid taken from a subject, comprising the step of determining the presence and/or amount of one or more protein biomarkers in the subject or biofluid

taken from the subject, the one or more protein biomarkers being independently selected from those listed in Table 6a, Table 1 or Table 1a.

**[0073]** In another embodiment, there is provided a method of determining Alzheimer's disease in a subject or in a biofluid taken from a subject, comprising the step of determining the presence and/or amount of one or more protein biomarkers in the subject or biofluid taken from the subject, the one or more protein biomarkers being independently selected from those listed in Table 6a, Table 1 or Table 1a.

**[0074]** In a further embodiment, there is provided a method of monitoring Alzheimer's disease progression in a subject or in a biofluid taken from a subject, comprising the step of determining the presence and/or amount of one or more protein biomarkers in the subject or biofluid taken from the subject, the one or more protein biomarkers being independently selected from those listed in Table 6a, Table 1 or Table 1a.

**[0075]** In a preferred embodiment, the one or more protein biomarkers are independently selected from those listed in Table 6a, or Table 1a, suitably Table 6a.

**[0076]** Detection of the presence or absence of a protein biomarker, or increases or decreases in protein biomarker levels, can be used according to the invention.

**[0077]** The step of determining the presence and/or amount of one or more protein biomarkers in the subject or biofluid taken from the subject may comprise any methods that determine the quantity or the presence of the biomarkers at the protein level. Such methods are well known in the art and include but are not limited to western blots, northern blots, southern blots, enzyme linked immunosorbent assay (ELISA), immunoprecipitation, immunofluorescence, flow cytometry, bead-based immunochemistry, immunochemistry, molecular imprinting, nucleic acid aptamers, nucleic acid hybridization techniques, nucleic acid reverse transcription methods, and nucleic acid amplification methods.

**[0078]** Immunohistochemistry, ELISA, bar-coded antibody or mass spectroscopy methods, such as liquid-chromatography mass spectroscopy (LC-MS) are particularly suitable methods. A digital barcoded antibody is an antibody whereby DNA molecules are attached to the antibody as a barcode. The antibody can be specific for a particular protein biomarker. To detect distinct protein biomarkers, multiple barcoded antibodies can be assayed in parallel and subsequently analysed by DNA sequencing (e.g. see Agasti et al. J Am Chem Soc. 134(45):18499–18502, 2012).

**[0079]** Suitably, detection of a biomarker of the present invention is performed using an antibody molecule that specifically binds to the biomarker. These antibodies can be used in various methods such as Western blot, ELISA, or immunoprecipitation techniques.

**[0080]** Antibodies that can be used herein are polyclonal or monoclonal antibodies, preferably monoclonal antibodies. Antibodies can be commonly used in the art, such as fusion methods (Kohler and Milstein, European Journal of Immunology, 6: 511-519 (1976)), recombinant DNA methods (US Pat. No. 4,816,56) or phage antibody library methods (Clackson et al, Nature, 352: 624-628 (1991) and Marks et al, J. Mol. Biol., 222: 58, 1-597 (1991)). General procedures for antibody preparation are described in Harlow, E. and Lane, D., Using Antibodies: A Laboratory Manual, Cold Spring Harbor Press, New York, 1999; Zola, H., Monoclonal Antibodies: A Manual of Techniques, CRC Press, Inc., Boca Raton, Florida, 1984; And Coligan, CURRENT PROTOCOLS IN IMMUNOLOGY, Wiley / Greene, NY, 1991, which are incorporated herein by reference.

**[0081]** An antibody is optionally conjugated with a detectable label. Such antibodies can be polyclonal or monoclonal. An intact antibody, a fragment thereof (e.g., Fab or F(ab')<sub>2</sub>), or an engineered variant thereof (e.g., sFv) can also be used. Such antibodies can be of any immunoglobulin class including IgG, IgM, IgE, IgA, IgD and any subclass thereof.

**[0082]** Techniques for detecting antibody binding through the use of a detectable label are well known in the art. For example, antibody binding may be detected through the use of chemical reagents that generate a detectable signal that corresponds to the level of antibody binding and, accordingly, to the level of biomarker protein expression. In some embodiments, the detection antibody is coupled to an enzyme, particularly an enzyme that catalyses the deposition of a chromogen at the antigen-antibody binding site. Suitable enzymes include but are not limited to horseradish peroxidase (HRP) and alkaline phosphatase (AP). Commercial antibody detection systems may also be used to practice the invention.

**[0083]** Although antibodies are illustrated herein for use in the invention because of their extensive characterization, any other suitable agent (e.g., a peptide, an aptamer, or a small organic molecule) that specifically binds a biomarker is optionally used in place of the antibody. For example, an aptamer that specifically binds a selected biomarker may be used. Aptamers are nucleic acid-based molecules that bind specific ligands. Methods for making aptamers with a particular binding specificity are known in the art.

**[0084]** Alternative methods of detecting a protein biomarker in a sample include high performance liquid chromatography (HPLC) and other high-throughput techniques.

**[0085]** Additionally, identification and quantification of one or more biomarkers can be performed using mass spectrometry. One specific example of mass spectrometry that may be useful is tandem mass spectrometry; another example is high mass accuracy/high mass resolution mass spectrometry (e.g. Orbitrap™, Thermo Scientific).

**[0086]** Tandem mass spectrometry, for example, can be used for quantitative analysis of peptides in biological samples due to high sensitivity and specificity. High mass accuracy/high mass resolution mass spectrometers (e.g. Orbitrap™, Thermo Scientific) also can be utilized for analysis. Generally, a product of digestion can be purified using separation techniques and ionized to generate ions detectable by mass spectrometry, where the concentration of peptides is determined by mass spectrometry, and amount detected is related to the amount of biomarker in the test sample. The ions can be single charged or multiple charged. In one aspect, ions selected in the first stage of mass analysis can be monoisotopic or isotopic. In another aspect, ions selected in the second stage of mass analysis can be monoisotopic or isotopic. Additionally, it is contemplated that in some cases ions selected in all following stages of mass analysis can be monoisotopic or isotopic.

**[0087]** Measurements can be obtained separately for individual parameters or can be obtained simultaneously for a plurality of parameters. Any suitable platform can be used to obtain parameter measurements.

**[0088]** Useful platforms for simultaneously quantifying multiple protein parameters include, for example, those described in PCT Publication No. WO2007/067819. An example of a useful platform utilizes MIMS label-free assay technology developed by Precision Human Biolaboratories, Inc. (now Ridge Diagnostics, Inc., Research Triangle Park, N.C.).

**[0089]** Another example of a platform useful for quantifying multiple parameters is the FDA-approved, flow-based LUMINEX® assay system (xMAP®; Luminex Corporation, Austin, TX). This multiplex technology uses flow cytometry to detect antibody/peptide/ oligonucleotide or receptor tagged and labeled microspheres. In addition, LUMINEX® technology permits multiplexing of up to 100 unique assays within a single sample. Since the system is open in architecture, LUMINEX® can be readily configured to host particular disease panels.

**[0090]** The methods of the first aspect of the invention may comprise determining the presence and/or amount of at least 1, 2, 3, 5, 10, 20, 30, 40, 50, 75, 100, 150 or at least 200 of the proteins selected from Table 1 or Table 1a. The methods may comprise determining the presence and/or amount of substantially all of the proteins listed in Table 1 or Table 1a.

**[0091]** The methods may comprise determining the presence and/or amount and/or abundance of at least 1, 2, 3, 5, 10, 15, 20, 25 or at least 30 of the proteins selected from Table 6. The methods may comprise determining the presence and/or amount and/or abundance of substantially all of the proteins listed in Table 6.

**[0092]** Suitably, the protein biomarker is identified by protein name in Table 6. Table 6 provides a SwissProt accession code for the murine version of the identified protein. The person skilled in the

art can easily use this to identify the equivalent protein for the particular species of subject, e.g. human, for use in the invention

**[0093] Table 6**

| <b>Protein Name</b>                             | <b>Gene Name</b> |
|-------------------------------------------------|------------------|
| Ankyrin-1                                       | Ank1             |
| Adenylyl cyclase-associated protein 1           | Cap1             |
| Clathrin heavy chain 1                          | Cltc             |
| Ezrin                                           | Ezr              |
| Hsp90aa1 protein (Fragment)                     | Hsp90aa1         |
| Integrin alpha 2b                               | Itga2b           |
| Integrin beta-1                                 | Itgb1            |
| Integrin beta-3                                 | Itgb3            |
| Laminin subunit gamma-1                         | Lamc1            |
| Moesin                                          | Msn              |
| Myosin-9                                        | Myh9             |
| MCG5400                                         | MyI12a           |
| Proteasome subunit alpha type-3                 | Psm3             |
| Proteasome subunit alpha type (Fragment)        | Psm4             |
| Proteasome subunit alpha type-6                 | Psm6             |
| Proteasome subunit beta type-4                  | Psm4             |
| Proteasome subunit beta type-5                  | Psm5             |
| Proteasome subunit beta type-6                  | Psm6             |
| 26S proteasome regulatory subunit 6A (Fragment) | Psm3             |
| Uncharacterized protein                         | Psm5             |
| 26S proteasome regulatory subunit 10B           | Psm6             |
| 26S proteasome non-ATPase regulatory subunit 1  | Psm1             |
| 26S proteasome non-ATPase regulatory subunit 14 | Psm14            |
| 26S proteasome non-ATPase regulatory subunit 2  | Psm2             |
| Uncharacterized protein                         | Rpl14            |
| Ribosomal protein L15                           | Rpl15            |
| 60S ribosomal protein L18                       | Rpl18            |
| 60S ribosomal protein L6                        | Rpl6             |

|                                          |        |
|------------------------------------------|--------|
| Uncharacterized protein                  | Rpl7   |
| 40S ribosomal protein S9                 | Rps9   |
| Spectrin alpha chain, non-erythrocytic 1 | Sptan1 |
| Spectrin beta chain, erythrocytic        | Sptb   |
| MKIAA1027 protein (Fragment)             | Tln1   |
| Tubulin beta-4A chain                    | Tubb4a |

**[0094]** In one embodiment of any aspect of the invention, the protein biomarker selected from Table 6 is not apolipoprotein E (Fragment).

**[0095]** The methods may comprise determining the presence and/or amount and/or abundance of at least 1, 2, 3, 5, 10, 15, 20 or at least 25 of the proteins selected from Table 6a. The methods may comprise determining the presence and/or amount and/or abundance of substantially all of the proteins listed in Table 6a.

**[0096]** Suitably, the protein biomarker is identified by protein name in Table 6a. Table 6a provides a SwissProt accession code for the homo sapiens version of the identified protein. The person skilled in the art can easily use this to identify the equivalent protein for the particular species of subject for use in the invention

**[0097] Table 6a**

| Protein Name                                | Gene Name | Accession Number |
|---------------------------------------------|-----------|------------------|
| Angiopoietin-like 6, isoform CRA_a          | ANGPTL6   | A0A024R7A9       |
| Anoctamin (Fragment)                        | ANO7      | H7C220           |
| Apolipoprotein E (Fragment)                 | APOE      | Q8TCZ8           |
| Leukocyte surface antigen CD53              | CD53      | CD53             |
| IGL@ protein                                | IGL       | Q6IN99           |
| Proteoglycan 4, isoform CRA_a               | PRG4      | A0A024R930       |
| Transmembrane protein 163                   | TMEM163   | TM163            |
| Ankyrin repeat domain-containing protein 61 | ANKRD61   | ANR61            |
| Apolipoprotein A-I, isoform CRA_a           | APOA1     | A0A024R3E3       |
| Apolipoprotein A-II                         | APOA2     | APOA2            |
| Apolipoprotein A-IV                         | APOA4     | APOA4            |

|                                                                |             |            |
|----------------------------------------------------------------|-------------|------------|
| Apolipoprotein C-III                                           | APOC3       | B0YIW2     |
| APOC4-APOC2 readthrough (NMD candidate)                        | APOC4-APOC2 | K7ER74     |
| Apolipoprotein D                                               | APOD        | APOD       |
| Fermitin family homolog 3                                      | FERMT3      | URP2       |
| Glutathione peroxidase                                         | GPX3        | A0A087X1J7 |
| Hyaluronan-binding protein 2                                   | HABP2       | HABP2      |
| Epididymis luminal protein 180 (Fragment)                      | HEL180      | B6EDE2     |
| Immunoglobulin heavy variable 3-38 (non-functional) (Fragment) | IGHV3-38    | A0A0C4DH36 |
| Prenylcysteine oxidase 1                                       | PCYOX1      | PCYOX      |
| Pescadillo homolog                                             | PES1        | PESC       |
| Phospholipid transfer protein                                  | PLTP        | PLTP       |
| Serum paraoxonase/arylesterase 1                               | PON1        | PON1       |
| Serum paraoxonase/lactonase 3                                  | PON3        | PON3       |
| Selenoprotein P (Fragment)                                     | SELENOP     | A0A182DWH7 |
| Alpha-1-antitrypsin                                            | SERPINA1    | A0A024R6I7 |
| Tissue factor pathway inhibitor (Fragment)                     | TFPI        | C9JKV3     |

**[0098]** Suitably, the methods may comprise determining the presence and/or amount and/or abundance of ANGPTL6, ANO7, APOE, CD53, IGL, PRG4, TMEM163, ANKRD61, APOA1, APOA2, APOA4, APOC3, APOC4-APOC2, APOD, FERMT3, GPX3, HABP2, HEL180, IGHV3-38, PCYOX1, PES1, PLTP, PON1, PON3, SELENOP, SERPINA1, or TFPI.

**[0099]** In one embodiment of any aspect of the invention, the protein biomarker selected from Table 6a is not apolipoprotein E (Fragment), fermitin family homolog 3 and/or serum paraoxonase/lactonase 3.

**[0100]** The methods may comprise determining the presence and/or amount and/or abundance of at least 1, 2, or 3 of the proteins selected from Table 6b. The methods may comprise determining the presence and/or amount and/or abundance of substantially all of the proteins listed in Table 6b.

**[0101]** Suitably, the protein biomarker is identified by protein name in Table 6b. Table 6b provides a SwissProt accession code for the homo sapiens version of the identified protein. The person skilled

in the art can easily use this to identify the equivalent protein for the particular species of subject for use in the invention.

**[0102] Table 6b**

| Protein Name                     | Gene Name | Accession Number |
|----------------------------------|-----------|------------------|
| Proteoglycan 4, isoform CRA_a    | PRG4      | A0A024R930       |
| Fermitin family homolog 3        | FERMT3    | URP2             |
| Phospholipid transfer protein    | PLTP      | PLTP             |
| Serum paraoxonase/arylesterase 1 | PON1      | PON1             |

5 **[0103]** Suitably, the methods may comprise determining the presence and/or amount and/or abundance of PRG4, FERMT3, PLTP, or PON1. Suitably, the methods may comprise determining the presence and/or amount and/or abundance of PRG4 and FERMT3. Suitably, the methods may comprise determining the presence and/or amount and/or abundance of PRG4 and PLTP. Suitably, the methods may comprise determining the presence and/or amount and/or abundance of PRG4 and  
10 PON1. Suitably, the methods may comprise determining the presence and/or amount and/or abundance of FERMT3 and PLTP. Suitably, the methods may comprise determining the presence and/or amount and/or abundance of FERMT3 and PON1. Suitably, the methods may comprise determining the presence and/or amount and/or abundance of PLTP and PON1. Suitably, the methods may comprise determining the presence and/or amount and/or abundance of PLTP and  
15 PON1. Suitably, the methods may comprise determining the presence and/or amount and/or abundance of PRG4, FERMT3 and PLTP. Suitably, the methods may comprise determining the presence and/or amount and/or abundance of PRG4, FERMT3 and PON1. Suitably, the methods may comprise determining the presence and/or amount and/or abundance of PRG4, PLTP and PON1. Suitably, the methods may comprise determining the presence and/or amount and/or  
20 abundance of FERMT3, PLTP and PON1. Suitably, the methods may comprise determining the presence and/or amount and/or abundance of PRG4, FERMT3, PLTP and PON1.

**[0104]** In one embodiment of any aspect of the invention, the protein biomarker selected from Table 6b is not serum paraoxonase/lactonase 3.

25 **[0105]** In one embodiment, the presence and/or amount of the one or more protein biomarkers in the subject or biofluid taken from a subject is compared to the presence and/or amount of the one or more protein biomarkers in a non-diseased control reference.

[0106] The methods may further comprise a step of determining the abundance (such as normalised abundance, mean normalised abundance, % abundance, for example) of the or each protein biomarker.

[0107] The methods of the first aspect of the invention may comprise any appropriate method step described in relation to any one of, or any combination of, steps a) to d) of the methods of the second, third, fourth, fifth or sixth aspects of the invention. Any of the methods of determining the abundance described in relation to step c) of the second, third, fourth, fifth or sixth aspects of the invention may be applicable to the first aspect of the invention, but may be used to determine the presence and/or amount of the one or more biomarkers and not necessarily the abundance *per se* (although in some embodiments, the method may comprise determining abundance).

[0108] According to a second aspect of the invention there is provided a method of testing a patient's biofluid sample for one or more biomarkers of Alzheimer's disease, comprising the steps of:

- a) contacting a plurality of nanoparticles in a biofluid sample taken from a subject to allow a biomolecule corona to form on the surface of said nanoparticles;
- b) optionally isolating the nanoparticles and surface-bound biomolecule corona;
- c) determining the abundance of one or more protein biomarkers in the corona, the one or more protein biomarkers being independently selected from those listed in Table 1 or Table 1a; and
- d) comparing the abundance of the one or more protein biomarkers in the corona to the abundance of the same one or more protein biomarkers in a non-diseased control reference.

[0109] In one embodiment, the method comprises determining the abundance of at least 1, 2, 3, 5, 10, 20, 30, 40, 50, 75, 100, 150 or at least 200 of the proteins selected from Table 1 or Table 1a. The method may comprise determining the abundance of substantially all of the proteins listed in Table 1 or Table 1a.

[0110] The method may comprise determining the abundance of at least 1, 2, 3, 5, 10, 15, 20, 25 or at least 30 of the proteins selected from Table 6. The methods may comprise determining the abundance of substantially all of the proteins listed in Table 6.

[0111] The method may comprise determining the abundance of at least 1, 2, 3, 5, 10, 15, 20 or at least 25 of the proteins selected from Table 6a. The methods may comprise determining the abundance of substantially all of the proteins listed in Table 6a.

[0112] The method may comprise determining the abundance of at least 1, 2 or 3 of the proteins selected from Table 6b. The methods may comprise determining the abundance of substantially all of the proteins listed in Table 6b.

[0113] Suitably, the methods may comprise determining the abundance of PRG4, FERMT3, PLTP, or PON1. Suitably, the methods may comprise determining the abundance of PRG4 and FERMT3. Suitably, the methods may comprise determining the abundance of PRG4 and PLTP. Suitably, the methods may comprise determining the abundance of PRG4 and PON1. Suitably, the methods may comprise determining the abundance of FERMT3 and PLTP. Suitably, the methods may comprise determining the abundance of FERMT3 and PON1. Suitably, the methods may comprise determining the abundance of PLTP and PON1. Suitably, the methods may comprise determining the abundance of PLTP and PON1. Suitably, the methods may comprise determining the abundance of PRG4, FERMT3 and PLTP. Suitably, the methods may comprise determining the abundance of PRG4, FERMT3 and PON1. Suitably, the methods may comprise determining the abundance of PRG4, PLTP and PON1. Suitably, the methods may comprise determining the abundance of FERMT3, PLTP and PON1. Suitably, the methods may comprise determining the abundance of PRG4, FERMT3, PLTP and PON1.

[0114] In a particular embodiment, step a) comprises contacting a plurality of nanoparticles in a biofluid sample taken from a subject under conditions to allow a biomolecule corona to form on the surface of said nanoparticles. Suitably, such incubation can be carried out *ex vivo* or *in vitro* (herein the term *in vitro* includes *ex vivo*). In this approach, the biomolecule corona is formed *in vitro* by incubating the plurality of nanoparticles in a biofluid sample to be analysed. Conveniently, this involves incubating at a suitable temperature, such as at about 37°C, for a suitable length of time. The biomolecule corona can form almost immediately, but typically the incubation is carried out for a period of 5 to 60 minutes, or more; such as 5, 10, 15, 20, 30, 40, 50, 60 or more minutes. Conveniently, the mixture can be subject to agitation, for example by way of an orbital shaker set at approximately 250rpm to mimic *in vivo* conditions. Suitably, the biofluid sample from the subject to be analysed has been previously taken and the sample extraction step is not part of the method.

[0115] In a particular embodiment, step a) is performed *in-vivo* and comprises administering a plurality of nanoparticles to a subject, such as by intravenous, oral, intracerebral, spinal, intraperitoneal or intra-ocular administration, or any combination thereof in order to allow a biomolecule corona to form on the surface of said nanoparticles. Conveniently, the route of administration is by intravenous injection. The biomolecule corona typically forms within less than 10 minutes from administration.

[0116] In a particular embodiment, the method comprises isolating the nanoparticles with surface-bound biomolecule corona from the biofluid and purifying them to remove unbound and highly abundant biomolecules, for example albumin and/or immunoglobins.

[0117] Step b) may comprise any isolation technique that is capable of preserving the surface-bound biomolecule corona. In some embodiments, the method comprises isolating the nanoparticles with surface-bound biomolecule corona from the biofluid and purifying them to remove unbound and highly abundant biomolecules (for example albumin and/or immunoglobulins, which can constitute 90% of the plasma proteome), which may allow detection and/or quantification of the biomarkers when they are in lower abundance, as masking by highly abundant proteins is minimised.

[0118] In a particular embodiment, step b) comprises performing size exclusion chromatography and/or ultrafiltration. In a particular embodiment, step b) comprises performing size exclusion chromatography followed by ultrafiltration.

[0119] In a particular embodiment, the step c) and/or step d) comprises a step of identifying the one or more Alzheimer's disease biomarkers in the corona and a step of quantifying the one or more Alzheimer's disease biomarkers in the corona.

[0120] In a particular embodiment, step c) and/or step d) comprises performing gel electrophoresis, mass spectrometry, an immunoassay, UV-Vis. absorption, fluorescence spectroscopy, chromatography, liquid chromatography or NMR methodology, or any combination thereof. In a particular embodiment, step c) and/or step d) comprises performing mass spectrometry or LC/MS.

[0121] In a particular embodiment, the non-diseased control reference comprises a biomolecule corona or protein corona obtained from a healthy subject or a sample of biofluid obtained therefrom.

[0122] In a particular embodiment, the corona obtained from the healthy subject is obtained by a method substantially the same as or identical to steps a) and b) and/or the protein biomarker abundance thereof is analysed by a method substantially the same as or identical to step c).

[0123] The method may comprise determining the abundance of at least 1, 2, 3, 5, 10, 20, 30, 40, 50, 75, 100, 150 or at least 200 of the proteins selected from Table 1 or Table 1a, in step c) and comparing the results with the abundance of the same proteins in a non-diseased control reference in step d). The method may comprise determining and/or comparing the abundance of substantially all of the proteins listed in Table 1 or Table 1a, in step c) and comparing the results with the abundance of the same proteins in a non-diseased control reference in step d). The method may comprise determining the abundance of at least 1, 2, 3, 5, 10, 15, 20, 25 or at least 30 of the proteins selected from Table 6, in step c) and comparing the results with the abundance of the same proteins in a non-diseased control reference in step d). The method may comprise determining the abundance of at least 1, 2, 3, 5, 10, 15, 20 or at least 25 of the proteins selected from Table 6a in step c) and comparing the results with the abundance of the same proteins in a non-diseased control reference in step d). The method may comprise determining the abundance of at least 1, 2 or 3 of the proteins selected from Table 6b, in step c) and comparing the results with the abundance of the same proteins

in a non-diseased control reference in step d). The method may comprise determining and/or comparing the abundance of substantially all of the proteins listed in Table 6, Table 6a or Table 6b, in step c) and comparing the results with the abundance of the same proteins in a non-diseased control reference in step d).

5 **[0124]** The use of multiple biomarkers may provide increased sensitivity and specificity of detection.

**[0125]** In a particular embodiment, step d) comprises determining and/or calculating relative or differential abundance between the corona and the non-diseased control reference with respect to the or each of the one or more protein biomarkers.

**[0126]** According to a third aspect of the invention there is provided a method for monitoring  
10 Alzheimer's disease (AD) progression, comprising the steps of:

- a) contacting a plurality of nanoparticles with a biofluid sample from a subject with AD to allow a biomolecule corona to form on the surface of said nanoparticles;
- b) optionally isolating the nanoparticles and surface-bound biomolecule corona;
- c) determining the abundance of one or more protein biomarkers in the corona, the one  
15 or more protein biomarkers being independently selected from those listed in Table 2, Table 1a or Table 6a; and
- d) comparing the abundance of the one or more protein biomarkers in the corona to the abundance of the same one or more protein biomarkers in a non-diseased control reference;

20 wherein down regulation of one or more of the proteins in Table 2, Table 1a or Table 6a relative to a previous measurement in the same subject indicates AD progression.

**[0127]** The applicants have surprisingly found that certain proteins are upregulated at early time points and gradually became downregulated as pathophysiological changes culminate in cognitive impairment. Interestingly, this downregulation effect has been observed for the majority of the  
25 upregulated proteins identified at the 2-month time point in the studies described in the Examples.

**[0128]** By down-regulation of a particular biomarker we mean a decrease in the amount and/or abundance of the biomarker.

**[0129]** In particular embodiments, the biomarker level is reduced or down-regulated to less than 90%, such as less than 80% such as less than 70% for example less than 60%, for example less  
30 than 50%, such as less than 40%, such as less than 30% such as less than 20% for example less than 10%, for example less than 5%, such as completely inhibited (0%) compared to the control level.

**[0130]** In one embodiment, the method comprises determining the abundance of at least 1, 2, 3, 5, 10, 20, 30, 40, 50, 75 or at least 100 of the proteins selected from Table 2. The method may comprise

determining the abundance of substantially all of the proteins listed in Table 2. Alternatively, the method may comprise determining the abundance of at least 1, 2, 3, 5, 10, 15 or at least 20 of the proteins listed in Table 6. The method may comprise determining the abundance of substantially all of the proteins listed in Table 6.

5 **[0131]** The method may comprise determining the abundance of at least 1, 2, 3, 5, 10, 20, 30, 40, 50, 75 or at least 100 of the proteins selected from Table 2, in step c) and comparing the results with the abundance of the same proteins in a non-diseased control reference in step d).

**[0132]** In one embodiment, the method comprises determining the abundance of at least 1, 2, 3, 5, 10, 20, 30, 40, 50, 75 or at least 100 of the proteins selected from Table 1a. The method may  
10 comprise determining the abundance of substantially all of the proteins listed in Table 1a.

**[0133]** The method may comprise determining the abundance of at least 1, 2, 3, 5, 10, 20, 30, 40, 50, 75 or at least 100 of the proteins selected from Table 1a, in step c) and comparing the results with the abundance of the same proteins in a non-diseased control reference in step d).

**[0134]** In one embodiment, the method comprises determining the abundance of at least 1, 2, 3, 5, 10, 15, 20 or at least 25 of the proteins selected from Table 6a. The method may comprise  
15 determining the abundance of substantially all of the proteins listed in Table 6a.

**[0135]** The method may comprise determining the abundance of at least 1, 2, 3, 5, 10, 15, 20 or at least 25 of the proteins selected from Table 6a, in step c) and comparing the results with the abundance of the same proteins in a non-diseased control reference in step d).

20 **[0136]** In one embodiment, the previous measurement was acquired at least 1, 3, 6, 9, 12, 18, 24, or 36 months previously. In one embodiment, the previous measurement was obtained by following the steps a), b), and c) and/or d) of the method.

**[0137]** In a further aspect of the invention, the method according to the third aspect can be used to compare the abundance of the one or more protein biomarkers in the corona to the abundance of  
25 the same one or more protein biomarkers in a non-diseased control reference; wherein up regulation of one more proteins from Tables 7, 8, or 9 relative to a previous measurement indicates AD progression.

**[0138]** Suitably, the protein biomarker is identified by protein name in Table 7, 8 or 9. Tables 7, 8 and 9 provide a SwissProt accession code for the murine version of the identified protein. The person  
30 skilled in the art can easily use this to identify the equivalent protein for the particular species of subject, e.g. human, for use in the invention.

**[0139]** According to a fourth aspect of the invention there is provided a method for assessing the stage of Alzheimer's disease (AD) in a subject, comprising the steps of:

- a) contacting a plurality of nanoparticles with a biofluid sample from the subject to allow a biomolecule corona to form on the surface of said nanoparticles;
- b) optionally isolating the nanoparticles and surface-bound biomolecule corona;
- c) determining the abundance of one or more protein biomarkers in the corona, the one or more protein biomarkers being independently selected from those listed in Table 1; and
- d) comparing the abundance of the one or more protein biomarkers in the corona to the abundance of the same one or more protein biomarkers in a non-diseased control reference;

wherein a change in abundance relative to a non-diseased control reference of one or more proteins in Table 3 indicates that the patient has early stage asymptomatic AD, or the presence of one or more proteins in Table 4 indicates that the patient has intermediate stage AD, or the presence of one or more proteins in Table 5 indicates that the patient has advanced stage AD.

**[0140]** In one embodiment, the method comprises determining the abundance of at least 1, 2, 3, 5, 10, 20, 30, 40, 50 or at least 60 of the proteins selected from Table 3, 4 or 5. In one embodiment, the method comprises determining the abundance of substantially all of the proteins listed from Table 3, 4 or 5.

**[0141]** The method may comprise determining the abundance of at least 1, 2, 3, 5, 10, 20, 30, 40, 50 or at least 60 of the proteins selected from Table 3, 4 or 5, in step c) and comparing the results with the abundance of the same proteins in a non-diseased control reference in step d). The method may comprise determining and/or comparing the abundance of substantially all of the proteins listed in Table 3, 4 or 5.

**[0142]** In one embodiment, the change in abundance relative to a non-diseased control reference of one or more proteins in Table 3 indicates that the patient has early stage asymptomatic AD is a statistically significant change in abundance.

**[0143]** In one embodiment, the change in abundance relative to a non-diseased control reference of one or more proteins in Table 4 indicates that the patient has immediate stage AD is a statistically significant change in abundance.

**[0144]** In one embodiment, the change in abundance relative to a non-diseased control reference of one or more proteins in Table 5 indicates that the patient has advanced stage AD is a statistically significant change in abundance.

**[0145]** According to a fifth aspect, there is provided a method for assessing the stage of Alzheimer's disease (AD) in a subject, comprising the steps of:

- a) contacting a plurality of nanoparticles with a biofluid sample from the subject to allow a biomolecule corona to form on the surface of said nanoparticles;
- b) optionally isolating the nanoparticles and surface-bound biomolecule corona;
- c) determining the abundance of one or more protein biomarkers in the corona, the one or more protein biomarkers being independently selected from those listed in Table 1a; and
- d) comparing the abundance of the one or more protein biomarkers in the corona to the abundance of the same one or more protein biomarkers in a non-diseased control reference;

wherein a change in abundance relative to a non-diseased control reference of one or more proteins in Table 3a indicate that the patient has early stage asymptomatic AD, or the presence of one or more proteins in Table 4a indicate that the patient has intermediate stage AD, or the presence of one or more proteins in Table 5a indicate that the patient has advanced stage AD.

**[0146]** In one embodiment, the method comprises determining the abundance of at least 1, 2 or 3 of the proteins selected from Table 3a, 4a or 5a. In one embodiment, the method comprises determining the abundance of substantially all of the proteins listed from Table 3a, 4a or 5a.

**[0147]** The method may comprise determining the abundance of at least 1, 2 or 3 of the proteins selected from Table 3a, 4a or 5a, in step c) and comparing the results with the abundance of the same proteins in a non-diseased control reference in step d). The method may comprise determining and/or comparing the abundance of substantially all of the proteins listed in Table 3a, 4a or 5a.

**[0148]** In one embodiment, the change in abundance relative to a non-diseased control reference of one or more proteins in Table 3a indicates that the patient has early stage asymptomatic AD is a statistically significant change in abundance.

**[0149]** In one embodiment, the change in abundance relative to a non-diseased control reference of one or more proteins in Table 4a indicates that the patient has immediate stage AD is a statistically significant change in abundance.

**[0150]** In one embodiment, the change in abundance relative to a non-diseased control reference of one or more proteins in Table 5a indicates that the patient has advanced stage AD is a statistically significant change in abundance.

**[0151]** According to a sixth aspect there is provided a method for identifying neurodegeneration-associated protein biomarkers in the blood of a subject, comprising (a) (i) contacting a plurality of nanoparticles with a blood-derived fluid sample from one or more healthy subjects; and (ii) contacting a plurality of nanoparticles with a blood-derived fluid sample from one or more subjects with neurodegeneration, under conditions to allow a biomolecule corona to form on the surface of said nanoparticles; (b) optionally isolating the nanoparticles and surface-bound biomolecule corona; (c) determining the abundance of one or more protein biomarkers in the corona from each subject; (d) comparing the abundance of the one or more protein biomarkers in the corona of subjects in (i) to the abundance of the same one or more protein biomarkers in the corona of subjects in (ii); wherein protein biomarkers which display statistically significant differential expression between (i) and (ii) are identified as neurodegeneration-associated protein biomarkers.

**[0152]** In one embodiment, the neurodegeneration-associated protein biomarker is an Alzheimer's disease-associated protein biomarker and the subjects with neurodegeneration are subjects with Alzheimer's disease.

**[0153]** The method may be used to identify new previously unknown neurodegeneration-associated protein biomarker, e.g. Alzheimer's disease-associated protein biomarkers. In a particular embodiment, the unknown biomarker is a unique biomolecule, meaning that it is a biomolecule that would not have been detected if analysis was carried out directly on biofluid, such as plasma, isolated from the subject.

**[0154]** In particular embodiments of any aspect of the invention, the presence and/or amount and/or abundance of the protein biomarker(s) in the biomolecule corona can be used to detect a disease state. Protein biomarker detection in the biomolecule corona can therefore be used to indicate the presence of Alzheimer's disease and/or the stage of Alzheimer's disease in a subject.

**[0155]** In particular embodiments of any aspect of the invention, the method may be useful in the early detection of AD in a subject. The method can also be useful for monitoring disease progression and/or response to a therapeutic intervention. Suitably the method involves detecting one or more AD-specific biomarkers over time.

**[0156]** In particular embodiments of any aspect of the invention, the Alzheimer's disease is early stage asymptomatic Alzheimer's disease, intermediate stage Alzheimer's disease, or advanced stage Alzheimer's disease.

**[0157]** The stage of disease, e.g. early stage, intermediate stage and advanced stage Alzheimer's disease can be identified by the physician using clinically approved standards. One well established clinical standard is the seven stage Reisberg scale [13]. Suitably, stages 1-3 of the Reisberg scale

can be classed as early stage asymptomatic AD, stages 4-5 as intermediate stage AD and stages 6-7 as advanced stage AD.

**[0158]** In particular embodiments of any aspect of the invention, monitoring Alzheimer's disease progression can be progression according to the Reisberg scale [13].

5 **[0159]** In particular embodiments of any aspect of the invention, the Alzheimer's disease is early stage asymptomatic Alzheimer's disease (stages 1-3 of Reisberg scale), intermediate stage Alzheimer's disease (stages 4-5 of Reisberg scale), or advanced stage Alzheimer's disease (stages 6-7 of Reisberg scale).

10 **[0160]** In particular embodiments of any aspect of the invention, the method is performed at two or more different time points, and the method comprises comparing the presence and/or amount and/or abundance of the protein biomarker(s) in the subject or biofluid taken from the subject determined at the different time points. In one embodiment, the different time points are at least 1 month apart, such as at least 3, 6, 9, 12, 18, 24, or 36 months apart.

15 **[0161]** In a particular embodiment, the up-regulation of one or more protein biomarkers relative to the previous measurement indicates that the patient's Alzheimer's disease has progressed.

**[0162]** In a particular embodiment, the down-regulation of one or more protein biomarkers relative to the previous measurement indicates that the patient's Alzheimer's disease has progressed.

20 **[0163]** In a particular embodiment, the method comprises determining the abundance of one or more protein biomarkers in the corona, the one or more protein biomarkers being independently selected from those listed in Table 2, wherein down regulation of one or more of the protein biomarker in Table 2 relative to the previous measurement indicates AD progression.

25 **[0164]** In a particular embodiment, the method comprises determining the abundance of one or more protein biomarkers in the corona, the one or more protein biomarkers being independently selected from those listed in Table 1a or Table 6a, wherein down regulation of one or more of the protein biomarker in Table 1a or Table 6a relative to the previous measurement indicates AD progression.

30 **[0165]** The methods of any of the aspects of the invention may offer high sensitivity and a high level of precision which allows for the identification, detection and/or quantification of neurodegeneration disease markers, e.g. Alzheimer's disease biomarkers and/or the abundance thereof, even when present in low abundance, which otherwise may be very difficult to identify.

### *Biomarkers*

**[0166]** In a particular embodiment of any aspect of the invention, the relative amount of a protein biomarker in the sample is determined by reference to a control protein in the sample.

**[0167]** A control protein may be a protein that is representative of a wild-type/healthy level.

5 **[0168]** In one embodiment of any aspect of the invention, the protein biomarker has a molecular weight of less than 80 kDa, such as less than 40 kDa or less than 20 kDa.

**[0169]** In particular embodiments of any aspect of the invention, the method may comprise determining the abundance of at least 1, 2, 3, 4, 5, 6, 7, 8, 9, 10, 15, 20, 25, 30, 35, 40, 45, 50, 75, 100, 150, 200 or at least 250 biomarkers, and optionally, comparing the results with the abundance of the same proteins in a non-diseased control reference.

10 **[0170]** In particular embodiments, the methods described herein comprise determining the presence and/or amount and/or abundance of at least 1, 2, 3, 4, 5, 6, 7, 8, 9, 10, 15, 20, 25, 30, 35, 40, 45, 50, 60, 75, 100, 150, 200 of the proteins selected from Table 1, Table 1a, Table 2, Table 3, Table 3a, Table 4, Table 4a, Table 5, Table 5a, Table 6, Table 6a or Table 6b, or substantially all of the proteins listed in Tables 1, 1a 2, 3, 3a, 4, 4a, 5, 5a, 6, 6a or 6b. In particular embodiments, the  
15 method comprises additionally determining the presence and/or amount and/or abundance of other disease-specific proteins along with proteins listed in Table 1, Table 1a, Table 2, Table 3, Table 3a, Table 4, Table 4a, Table 5, Table 5a, Table 6, Table 6a or Table 6b.

**[0171]** The methods may comprise determining the presence and/or amount and/or abundance of at least 1, 2, 3, 5, 10, 15, 20, 25 or at least 30 of the proteins selected from Table 6. The methods  
20 may comprise determining the presence and/or amount and/or abundance of substantially all of the proteins listed in Table 6.

**[0172]** The methods may comprise determining the presence and/or amount and/or abundance of at least 1, 2, 3, 5, 10, 15, 20 or at least 25 of the proteins selected from Table 6a. The methods may  
25 comprise determining the presence and/or amount and/or abundance of substantially all of the proteins listed in Table 6a.

**[0173]** The methods may comprise determining the presence and/or amount and/or abundance of at least 1, 2 or 3 of the proteins selected from Table 6b. The methods may comprise determining the presence and/or amount and/or abundance of substantially all of the proteins listed in Table 6b.

30 **[0174]** In particular embodiments, the method comprises determining the presence and/or amount and/or abundance of at least 1, 2, 3, 4, 5, 6, 7, 8, 9, 10, 15, 20, 25, 30, 35, 40, 45, 50, 60, 75, 100, 150, 200 of the proteins selected from Table 1, Table 1a, Table 2, Table 3, Table 3a, Table 4, Table 4a, Table 5, Table 5a, Table 6, Table 6a or Table 6b and comparing the results with the presence and/or amount and/or abundance of the same proteins in a non-diseased control reference. In particular embodiments, the method comprises determining the presence and/or amount and/or

abundance of substantially all the proteins listed in Table 1, Table 1a, Table 2, Table 3, Table 3a, Table 4, Table 4a, Table 5, Table 5a, Table 6, Table 6a or Table 6b and comparing the results with the presence and/or amount and/or abundance of the same proteins in a non-diseased control reference.

5 **[0175]** In one embodiment of any aspect of the invention, the protein biomarker is clusterin.

**[0176]** Tables 1, 2, 3, 4, 5, 6, 7, 8, 9, 10 and 11 herein recite the protein biomarkers by name and provide a Swissprot accession code for the murine version of this protein. The person skilled in the art can easily use this to identify the equivalent protein biomarker for the particular species of subject, e.g. human, for use in the invention.

10 **[0177]** Tables 1a, 3a, 4a, 5a, 6a, 6b, and 13 herein recite the protein biomarkers by name and provide a Swissprot accession code for the homo sapiens version of this protein.

#### *Biofluid*

**[0178]** The biofluid can be any fluid obtained or obtainable from a subject. The subject can be an animal. In a particular embodiment of any aspect of the invention, the subject is a human.

**[0179]** In particular embodiments of any aspect of the invention, the biofluid is selected from blood, plasma, serum, saliva, sputum, ascites, lacrimal, cerebrospinal and ocular fluids. Suitably the biofluid is a blood or blood fraction sample, such as serum or plasma. In a particular embodiment, the biofluid is plasma. In a particular embodiment of any aspect of the invention, the analysis is conducted on a single biofluid sample. Suitably, the sample is a plasma sample.

#### *Nanoparticles*

**[0180]** A plurality of nanoparticles can be a population of the same type of nanoparticle (a population of nanoparticles) or more than one population of nanoparticles, wherein each population is of a different type of nanoparticle; and so when combined can be termed a heterogeneous population of nanoparticles (i.e. a plurality of distinct nanoparticle populations).

**[0181]** Certain classes of nanoparticle are more effective at adsorbing different biomolecules, therefore by utilizing a mixture of distinct nanoparticles (i.e. two or more distinct nanoparticle populations) it will be possible to create a corona that comprises a particular complement of biomolecules and/or as many biomolecule species as needed.

**[0182]** Thus, in a particular embodiment the plurality of nanoparticles used is a heterogeneous population of nanoparticles.

**[0183]** In a particular embodiment, all the nanoparticles used in the method are of the same type of nanoparticle, and so can be termed a homogeneous population of nanoparticles.

[0184] The methods are applicable to any types of nanoparticles capable of forming a biomolecule corona. In particular embodiments of any aspect of the invention, the nanoparticles are selected from liposomes, metallic nanoparticles (such as gold or silver), polymeric nanoparticles, fibre-shaped nanoparticles (such as carbon nanotubes and two dimensional nanoparticles such as graphene oxide nanoparticles), or any combination thereof.

[0185] Conveniently, the nanoparticles comprise liposomes. Liposomes are generally spherical vesicles comprising at least one lipid bilayer. Liposomes are often composed of phospholipids. In a particular embodiment, the liposomes are composed of phospholipid molecules and functionalised amphiphilic molecules (e.g. PEGylated DSPE). In a particular embodiment, the liposomes are composed of phospholipid molecules and functionalised amphiphilic molecules (e.g. PEGylated DSPE) that are able to self-assemble into unilamellar vesicles. In a particular embodiment, the liposomes are PEGylated DSPE. In a particular embodiment, the nanoparticles are PEGylated liposomes.

#### *Biomolecule corona*

[0186] The corona formed on the nanoparticles is a biomolecule corona. Conveniently, the biomolecule corona comprises a protein corona. Conveniently the biomolecule corona comprises one or more protein biomarkers. Conveniently, the biomolecule corona comprises one or more protein biomarkers selected from Table 1, Table 1a, Table 2, Table 3, Table 3a, Table 4, Table 4a, Table 5, Table 5a, Table 6, Table 6a or Table 6b, such as at least 1, 2, 3, 4, 5, 6, 7, 8, 9, 10, 15, 20, 25, 30, 35, 40, 45, 50, 60, 75, 100, 150, 200, or substantially all of the proteins listed in Table 1, Table 1a, Table 2, Table 3, Table 3a, Table 4, Table 4a, Table 5, Table 5a, Table 6, Table 6a or Table 6b.

[0187] In particular embodiments, the method comprises contacting a plurality of nanoparticles with a biofluid sample from a subject with or suspected of having Alzheimer's disease to allow a biomolecule corona to form on the surface of said nanoparticles. Conveniently, the method comprises administering a plurality of nanoparticles to a subject to allow a biomolecule corona to form on the surface of said nanoparticles or incubating a plurality of nanoparticles in a biofluid sample taken (such as previously taken) from a subject to allow a biomolecule corona to form on the surface of said nanoparticles.

[0188] In certain embodiments of the invention, step a) is performed in-vivo and comprises administering a plurality of nanoparticles to a subject by intravenous, oral, intracerebral, spinal, intraperitoneal or intra-ocular administration, or any combination thereof in order to allow a biomolecule corona to form on the surface of said nanoparticles. Conveniently, the route of administration is by intravenous injection. The biomolecule typically forms within less than 10 minutes

from administration.

**[0189]** In the methods of the invention that involve administration of the nanoparticles to a subject, a biofluid sample comprising some of the introduced nanoparticles is then extracted from the subject; for example, by taking a blood sample, after a period of time has elapsed from administration to allow the corona to form. In particular embodiments, the biofluid sample comprising nanoparticles is extracted/removed from the subject at least 5 minutes after administration of the nanoparticles to the subject, such as at least 6, 7, 8, 9, 10, 12, 15, 20, 30, 40, 60, 90, 120 minutes or more. The volume of the biofluid sample comprising nanoparticles extracted can be determined by the physician and will depend on the source of the biofluid sample. For example, if it is a blood sample, it may be in a volume of 2-50 mL, such as 5-20 mL. In a particular embodiment, the nanoparticles are isolated from the biofluid sample prior to analysis

**[0190]** In particular embodiments, the method comprises administering a plurality of nanoparticles to a subject, a biofluid sample is then taken from the subject and analysed. Prior to analysis, the particles are isolated from the biofluid and purified to remove unbound and highly abundant biomolecules. In one embodiment the plurality of nanoparticles are administered to the subject by intravenous injection.

**[0191]** In particular embodiments, the method comprises incubating a plurality of nanoparticles in a biofluid sample taken from a subject under conditions to allow a biomolecule corona to form on the surface of said nanoparticles.

**[0192]** Suitably, such incubation can be carried out *ex vivo* or *in vitro* (herein the term *in vitro* includes *ex vivo*). In this approach, the biomolecule corona is formed *in vitro* by incubating the plurality of nanoparticles in a biofluid sample to be analysed. Conveniently, this involves incubating at a suitable temperature, such as at about 37°C, for a suitable length of time. The biomolecule corona can form almost immediately, but typically the incubation is carried out for a period of 5 – 60 minutes, or more; such as 5, 10, 15, 20, 30, 40, 50, 60 or more minutes. Conveniently, the mixture can be subject to agitation, for example by way of an orbital shaker set at approximately 250 rpm to mimic *in vivo* conditions. Suitably, the biofluid sample from the subject to be analysed has been previously taken and the sample extraction step is not part of the method.

**[0193]** Thus, according to a particular embodiment, the plurality of nanoparticles are incubated in the test biofluid sample *in vitro* under conditions to allow a biomolecule corona to form on the surface of said nanoparticles.

**[0194]** Suitably, the subject is suffering from a disease (is in a diseased state) or suspected of having the disease.

[0195] In particular embodiments of any aspect of the invention, the corona may be digested prior to step c) in order to facilitate analysis. In embodiments where the non-diseased control reference comprises a biomolecule corona obtained from a healthy or non-Alzheimer's disease control subject, said corona may be digested prior to the equivalent steps of its own analysis.

5

*Isolating the nanoparticles and surface-bound corona*

[0196] In a particular embodiment of any aspect of the invention, the method comprises isolating the nanoparticles and surface-bound biomolecule corona. Any isolation technique that is capable of preserving the surface-bound biomolecule corona is suitable. In a particular embodiment, the method

10 comprises isolating the nanoparticles and surface-bound biomolecule corona from the biofluid and purifying them to remove unbound and highly abundant biomolecules, (for example albumin and/or immunoglobulins, which can constitute 90% of the plasma proteome).

[0197] The method therefore allows minimization of any masking caused by the highly abundant proteins and allows identification of lower abundant biomarkers. Conveniently, the method comprises

15 performing size exclusion chromatography and/or ultrafiltration. In a particular embodiment, the method comprises performing size exclusion chromatography followed by ultrafiltration.

[0198] In a particular embodiment, the nanoparticles are isolated from the biofluid sample prior to analysis.

20 *Proteomic analysis*

[0199] The various aspects of the invention are directed to the detection/identification of one or more protein biomarkers. In a particular embodiment of any aspect of the invention, at least one of the protein biomarker(s) is a protein or protein derivative.

[0200] The results obtained in the step of determining the presence and/or amount and/or

25 abundance of one or more protein biomarkers in the subject or biofluid taken from the subject can be compared to a non-diseased control reference which may comprise the results obtained from a healthy subject. The results obtained from a healthy subject may be obtained by the same or similar method steps of the method and may be analysed by the same or similar method steps of the method. The healthy subject may be a subject who does not have the type of disease (e.g. Alzheimer's

30 disease) for which the likelihood thereof is being assessed, who does not have the particular stage of Alzheimer's disease and/or who does not have any serious illnesses or diseases (e.g. a subject who is generally considered, for example by doctors or other medical practitioners, to be healthy and/or substantially free from disease or illness or serious disease or illness).

**[0201]** When the biofluid sample is from a subject with or suspected of having AD, the presence and/or amount and/or abundance of one or more biomarkers in the corona can be compared to the abundance of the same one or more biomarkers in a non-diseased control reference.

**[0202]** The methods of the invention may comprise determination and/or calculation of relative or differential abundance between the corona and a non-diseased control reference (such as analysis results of a corona obtained by the same or similar method, but wherein the subject is a healthy subject from a healthy subject) with respect to the or each of the one or more protein biomarkers. The method may comprise the use of a computer program or software tool. The method may comprise analysis (such as computer or software analysis) of raw data obtained from analyses and/or measurements, for example raw data obtained from LC/MS of the or each corona. The method may comprise a statistical comparison between the protein abundance of the one or more protein biomarkers in the corona and in the non-diseased control reference.

**[0203]** Abundance and comparison of abundance (such as differential or relative abundance) may be calculated and/or represented in any appropriate way or format, for example as a normalised abundance, mean normalised abundance, % abundance, etc.

**[0204]** In a particular embodiment of any aspect of the invention, the protein biomarker is analysed directly without prior extraction or purification from the biomolecule corona.

**[0205]** In some embodiments of any aspect of the invention, albumin and/or immunoglobins may not be depleted from corona samples (which may include for example a corona from a healthy subject) prior to analysis.

**[0206]** In a particular embodiment of the invention, the biomolecule corona is analysed by any suitable technique capable of identifying and/or quantifying the protein biomarkers and/or determining and/or calculating the abundance thereof. In a particular embodiment, analysis comprises performing gel electrophoresis, mass spectrometry, an immunoassay, UV-Vis. absorption, fluorescence spectroscopy, chromatography, liquid chromatography or NMR methodology, or any combination thereof. In a particular embodiment, analysis comprises performing mass spectrometry or LC/MS.

**[0207]** In particular embodiments of any aspect of the invention, the biomolecule corona is subjected to proteomic analysis, such as via LC-MS/MS or a bicinchoninic acid assay (BCA assay), such as further described herein. The total protein biomolecule content of the biomolecule corona can be determined by any method capable of quantifying the level of said biomolecules in the surface-bound corona. In one embodiment, the total protein content is determined by bicinchoninic acid (BCA) assay.

[0208] Conveniently, the biomolecule corona is analysed by mass spectrometry, such as LC-MS, which can allow qualitative and quantitative analysis of the biomolecule corona present on the nanoparticles. Advantageously, the methods of the invention may detect and quantify protein biomarkers even in low abundance. In a particular embodiment, the methods allow identification of unique biomolecules without the need for highly specialized and ultra-sensitive analytical mass spectrometry instrumentation such as using an UltiMate® 3000 Rapid Separation LC (RSLC, Dionex Corporation, Sunnyvale, CA) coupled to a Q Exactive™ Hybrid Quadrupole Orbitrap™ (Thermo Fisher Scientific, Waltham, MA) mass spectrometer.

[0209] In certain embodiments, analysis of the biomolecule corona is carried out after administering a plurality of nanoparticles to a subject in a diseased state to allow a biomolecule corona to form on the surface of said nanoparticles and isolating the nanoparticles and surface-bound biomolecule corona. When compared to other methods, such methods can yield high levels of unique low abundant biomolecules and allow identification of such unique biomolecules without the need for highly specialized and ultra-sensitive analytical mass spectrometry instrumentation such as using an UltiMate® 3000 Rapid Separation LC (RSLC, Dionex Corporation, Sunnyvale, CA) coupled to a Q Exactive™ Hybrid Quadrupole Orbitrap™ (Thermo Fisher Scientific, Waltham, MA) mass spectrometer.

[0210] In a particular embodiment of the invention, the beneficial sensitivity and high level of precision provided by the method allows the identification of intracellular protein disease related biomarkers that are present in low abundance and would otherwise be very difficult to identify.

[0211] Conveniently, the method allows identification of protein biomarkers with molecular weight of less than 80kDa. More conveniently, the method allows identification of protein biomarkers with molecular weight of less than 40kDa or less than 20kDa.

#### *Monitoring effects of therapy*

[0212] The methods of the invention may be useful for monitoring changes in the amount of the biomarkers in response to a therapeutic treatment. For example, a determination of one or more protein biomarkers in a patient's biofluid can be conducted prior to a therapeutic intervention (such as administration of any therapeutic drug) and then at one or more time points during or after treatment. A change in the amount of the protein biomarker(s) detected can then be used to determine the effectiveness of the treatment.

[0213] Therefore, in some embodiments, the method may comprise an extra step, during or (preferably before step a)) of administering a therapy to the subject, for example administering a drug molecule to the subject diagnosed with AD or whose AD has progressed. In a particular embodiment,

the therapy comprises administration of a drug molecule to the subject. In a separate embodiment, there is provided a method for monitoring the changes in biomarkers in a subject in response to therapy, comprising the step of a) contacting a plurality of nanoparticles with a biofluid from a therapeutically treated subject with AD to allow a biomolecule corona to form on the surface of said nanoparticles.

**[0214]** In a particular embodiment of any aspect of the invention, the methods also provide the ability to monitor changes in biomarkers in response to therapy.

**[0215]** In a particular embodiment, a change in total protein content in a biofluid from a subject in response to therapy is monitored. In a particular embodiment, a change in at least one biomarker (such as a biomarker) in response to therapy is monitored. Conveniently, the therapy administered to the subject prior to testing is a drug molecule.

#### *Panels of biomarkers*

**[0216]** In addition to the identification of a single biomarker, the methods of the invention also provide the ability to identify and use panels of biomarkers. This approach can lead to increased sensitivity and specificity of detection. In a particular embodiment of any aspect of the invention, the protein biomarker is part of a panel of AD-specific biomolecule biomarkers. In a further embodiment, the panel comprises a combination of unknown and known AD-specific biomolecule biomarkers.

#### *Kits*

**[0217]** In a seventh aspect of the invention, there is provided a diagnostic kit comprising nanoparticles and reagents capable of detecting one or more of the protein biomarkers listed in Table 1, Table 1a, Table 2, Table 3, Table 3a, Table 4, Table 4a, Table 5, Table 5a, Table 6, Table 6a or Table 6b.

#### *Use of protein biomarkers*

**[0218]** In an eighth aspect of the invention, there is provided any one or more of the protein biomarkers listed in Table 1, Table 1a or Table 2, or any combinations thereof, for use as a biomarker for Alzheimer's disease.

**[0219]** In one embodiment, there is provided any one or more of the protein biomarkers listed in Table 3 or Table 3a, or any combinations thereof, for use as a biomarker for early stage Alzheimer's disease; one or more of the protein biomarkers listed in Table 4 or Table 4a, or any combinations thereof, for use as a biomarker for immediate stage Alzheimer's disease; or one or more of the protein

biomarkers listed in Table 5 or Table 5a, or any combinations thereof for use as a biomarker for advanced stage Alzheimer's disease.

**[0220]** In some embodiments, there is provided at least one biomarker selected from Table 6, Table 6a or Table 6b, or any combinations thereof, for use as a biomarker for Alzheimer's disease.

5 **[0221]** The use may comprise the use of any combination of at least biomarker as described in relation to the first, second, third, fourth, fifth or sixth aspects of the invention.

**[0222]** In one embodiment, the use comprises the use of at least 1, 2, 3, 5, 10, 20, 30, 40, 50, 60, 75, 100, 150, 200, or substantially all of the proteins identified or listed in Table 1, Table 1a, Table 2, Table 3, Table 3a, Table 4, Table 4a, Table 5, Table 5a, Table 6, Table 6a or Table 6b.

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## EXAMPLES

### Materials and Methods for Examples 1 to 3

**[0223] Animals:** APP<sup>swe</sup> PSEN1 $\Delta$ E9 (APP/PS1) transgenic mice [14] on a C57BL/6j background were obtained from the Jackson Laboratory<sup>TM</sup> (#005864) and bred to produce hemizygous APP/PS1 male mice and C57BL/6j (wild-type; WT) littermates. All mice were housed under standard conditions at 22 $\pm$ 2°C and a standard 12 hour light/dark cycle with free access to food and water. All animal experiments were carried out in accordance with the United Kingdom Animals (Scientific Procedures) Act 1986 and approved by ethical committees under licence #P1D200E0B. Animals were coded in order to blind the individual evaluating the behavioural tasks and immunohistological sections.

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**[0224] Immunohistochemistry:** Mouse brains were split into hemispheres and the right hemisphere was immerse-fixed in 4% paraformaldehyde for 24 hours. The hemispheres were then dehydrated, paraffin embedded and 5  $\mu$ m sections were taken sagittally every 1 mm from the central sulcus and mounted onto Superfrost<sup>TM</sup> Plus slides (VWR). Antigen retrieval was performed by 30 minutes incubation in 0.2 mM citrate buffer pH 6 at 96°C followed by 10 minute immersion in 90% formic acid. Sections were then washed (3x 5min) with 0.1% tween in phosphate buffered saline (PBST) and blocked for 1 hour in a 1% bovine serum albumin (BSA, A9647 Sigma-Aldrich), 0.2 M phosphate buffer (PB) solution. The slides were then incubated overnight at 4°C in 1:200 biotinylated 6e10 antibody (#SIG-39340-200, Covance) in a 1% BSA, 0.2 M PB solution. The sections were washed (3x 5min, PBST), exposed to 1:20 Strep-Avidin (P188503, RnD) 1% BSA, 0.2 M PB solution for 2 hour at room temperature, washed and then visualised with a DAB-nickel solution (D0426-50SET, Sigma-Aldrich). The slides were then dehydrated in ethanol and xylene, and then covered slipped using DPX (DI5319/05, Fisher Scientific). The sections were then scanned and the

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percentage stained area calculated through threshold-particle analyses performed on Image J. The 3 sagittal sections 1mm apart per mouse were processed and analysed in this way.

**[0225] Morris water maze:** Morris water maze (MWM) was used to evaluate memory deficits at the 2, 6 and 12 month time points in the APP/PS1 and WT mice. The tank was 1 m in diameter with a 10 cm platform and large visual cues in all directions. Water temperature was maintained at  $22\pm 2^{\circ}\text{C}$  and white noise (40 db) was on during habituation to the room and the MWM task. The MWM was performed as previously described [15] with some modifications. A 6-day protocol was used consisting of habituation, a cued trial day, 4 trial days and a probe trial day. Mice were placed in the behaviour room during the entire period of the study.

**[0226]** Tracking analysis was performed on ANY-maze software. To ensure equal motivation and ability for the task, a cued trial was performed. A platform with a large black and white flag was placed in a random quadrant (NE, SE, NW, SW). Mice were placed in the water maze at a random starting location. The trial was stopped when the mouse found the platform or 60 seconds had lapsed, in which case the mice were guided to the platform. The mice were then dried and placed in warmed cages. This was repeated four times with new platform and starting locations and a minimum of 60 minutes between trials. During the trial days the hidden platform was placed in the NE quadrant of the maze. As above, mice were placed in the maze at a random starting location, given 60 seconds to find the platform, dried and placed in warmed cages and four repeats were performed per day. The mice were tasked with learning the location of the hidden platform over four trial days. During the probe trial the hidden platform was removed. The mice were placed in the maze facing the wall and allowed to swim for 30 seconds. Swim speed, total distance travelled, time in each quadrant, number of entries in the platform zone and a number of other factors were measured. Time (%) in the correct quadrant (NE) was our primary measure of memory performance.

**[0227] Preparation of liposomes:** HSPC:Chol:DSPE-PEG2000 (56.3:38.2:5.5) liposomes were prepared, by thin lipid film hydration method followed by extrusion, as previously described [16, 17]. The physicochemical properties of the liposomes employed, were measured using Zetasizer Nano ZS (Malvern, Instruments, UK) and are shown in **Figure 1e**.

**[0228] *In vivo* administration of liposomes:** Liposomes were intravenously injected via the lateral tail vein (at a lipid dose of 0.125 mM/g body weight) and subsequently recovered by cardiac puncture (10 min post-injection) from the blood circulation of 2-, 6- and 12-month-old APP/PS1 and WT mice. For each time point three biological replicates were performed. For each of these biological replicates 3 mice were used ( $n = 3$  biological replicates,  $n = 3$  mice/replicate). The plasma samples obtained from three mice were pooled together for each biological replicate. Blood, containing corona-coated liposomes, was collected in K2EDTA coated tubes. Approximately 0.5 mL of blood sample was

collected from each mouse. Plasma was then prepared by centrifugation for 12 min at 1200 RCF at 4°C after inverting the collection tubes to ensure mixing of blood with EDTA. Plasma was collected into Protein LoBind Eppendorf Tubes. The plasma samples obtained from three mice were pooled together for a final plasma volume of 1 mL.

5 **[0229]** Corona-coated liposomes were separated from unbound and weakly bound plasma proteins by size exclusion chromatography followed by membrane ultrafiltration as previously described.[18, 19]

**[0230] Transmission Electron Microscopy (TEM).** Bare and corona-coated liposomes were stained with uranyl acetate (UA) solution 1% and visualized with transmission electron microscopy (FEI Tecnai 12 BioTwin) before and after their *in vivo* interaction with plasma proteins. Samples were  
10 diluted to 0.5 mM lipid concentration and carbon Film Mesh Copper Grids (CF400-Cu, Electron Microscopy Science) were used.

**[0231] Quantification of adsorbed proteins.** Proteins associated with recovered liposomes were quantified by BCA Protein assay kit according to the manufacturer's instructions. To make sure that  
15 liposomes in solution do not interfere with the absorbance at 562 nm, the absorbance of corona-coated liposomes in HEPES buffer was measured and subtracted from the total absorbance, measured when corona-coated liposomes were mixed with the BCA reagent. Lipid concentration was quantified by Stewart assay and Pb values ( $\mu\text{g}$  of protein/ $\mu\text{mol}$  lipid) were then calculated.

**[0232] Mass Spectrometry.** In-gel digestion of corona proteins (40  $\mu\text{g}$ ) was performed prior to LC-  
20 MS/MS analysis, as we have previously described.[18, 19] Digested samples were analysed by LC-MS/MS using an UltiMate® 3000 Rapid Separation LC (RSLC, Dionex Corporation, Sunnyvale, CA) coupled to a Q Exactive™ Hybrid Quadrupole-Orbitrap™ (Thermo Fisher Scientific, Waltham, MA) mass spectrometer.

**[0233] Mass Spectrometry data analysis.** To statistically compare the abundance of proteins  
25 identified in the liposomal coronas formed in APP/PS1 and WT mice, MS peak intensities were analysed using Progenesis LC-MS software (version 3.0; Nonlinear Dynamics). RAW files were imported into Progenesis LC-MS software (version 3.0; Nonlinear Dynamics) with automatic feature detection enabled. A representative reference run was selected automatically, to which all other runs were aligned in a pair-wise manner. Automatic processing was selected to run with applied filters for  
30 peaks charge state (maximum charge 5) and protein quantitation method the relative quantitation using Hi-N with N=3 peptides to measure per protein. The resulting MS/MS peak lists were exported as a single Mascot generic file and loaded onto a local Mascot Server (version 2.3.0; Matrix Science, UK). The spectra were searched against the UniProt database using the following parameters: tryptic enzyme digestion with one missed cleavage allowed, peptide charge of +2 and +3, precursor mass

tolerance of 15 mmu, fragment mass tolerance of 8 ppm, oxidation of methionines as variable modifications and carbamidomethyl as fixed modifications, with decoy database search disabled and ESI-QUAD-TOF the selected instrument. Each search produced an XML file from Mascot and the resulted peptides (XML files) were imported back into Progenesis LC-MS to assign peptides to features. Data were filtered to present a 1 % false discovery rate (FDR) and a score above 21 through the 'refine identification' tab of Progenesis QI toolbox. The resulting peptides were exported as an XML file from Mascot and imported back into Progenesis LC-MS to assign peptides to features.

**[0234] Pathway enrichment analysis:** Proteins were classified by Kyoto Encyclopaedia of Genes & Genomes (KEGG) database using the Enrichr analysis tool. The pathway overlap network was constructed using the Gephi visualization platform. For each enriched pathway (network nodes) we calculated the proportion of constituent proteins that are shared with all other pathways (network edges). We then visualised all connections between pathways sharing more than a third of their constituent proteins. Pathway node size was determined by the number of constituent proteins in each pathway and network edge thickness was determined by the proportion of shared proteins between the two connected pathways.

**[0235] Statistical analysis.** The effects of genotype on memory performance and protein abundance extracted were evaluated with Welch corrected t-tests at each time point. Homoscedasticity and normality were evaluated graphically using predicted vs (pearson) residuals and Q-Q plots. Plaque burden was analysed by two-way ANOVA followed by Sidak corrected post-hoc analyses. Statistical analyses of the data were performed using GraphPad Prism software. Accepted levels of significance were \*P < 0.05, \*\*P < 0.01, and \*\*\*P < 0.001.

### Example 1 – Blood circulating nanoparticle scavengers

**[0236]** The double transgenic mouse model of AD, APP<sup>swe</sup>/PS1<sup>dE9</sup>,<sup>[14]</sup> was employed and plaque deposition and memory deficits were assessed in APP/PS1 and wild-type (WT) control mice at 2-, 6- and 12- months of age utilizing the amyloid- $\beta$  (6e10 antibody) staining and the Morris Water Maze (MWM) test, respectively (**Fig. 1a-d**). In agreement with previous studies,<sup>[20]</sup> nominal plaque deposition was observed at the 2-month time point and no significant effects on memory were detected, which corresponds to a pre-diseased state (**Fig. 1a and b**). The 6-month time point revealed statistically significant but mild plaque burden and no memory deficits, modelling the period between the pathophysiological manifestations of AD-related amyloidopathy and cognitive symptoms of the disease (**Fig. 1a and c**), as described by the Jack *et al* 2013 model of AD progression.<sup>[21]</sup> As expected, the 12-month time point corresponded to symptomatic AD with significant plaque burden and substantial memory deficits (**Fig. 1a and d**).

[0237] PEGylated liposomes (HSPC:Chol:DSPE-PEG2000) were intravenously injected (via the tail vein) and subsequently recovered by cardiac puncture (10 min post-injection) from the blood circulation of 2-, 6- and 12- months old APP/PS1 and WT C57BL/6 male mice (n = 3 mice/replicate; 3 independent biological replicates). For each biological replicate plasma samples obtained from three mice were pooled together for a final volume of 1 mL. This not only ensures adequate concentration of recovered corona-coated liposomes but also minimizes any mouse-to-mouse variation of the plasma proteome. The physicochemical characteristics of the liposomes employed are summarized in **Figure 1e**. Corona-coated liposomes were purified from any unbound plasma components by a second-step purification protocol which is based on size exclusion chromatography followed by membrane ultrafiltration, as previously described.[9, 18, 19] This protocol has been previously shown to completely eliminate unbound proteins and to result in a reproducible composition of protein corona.[12] The resultant purified *in vivo* protein coronas at the three different time points were comprehensively characterised and compared (**Fig. 1f**).

[0238] Negative stain transmission electron microscopy (TEM) revealed intact blood-recovered, protein-coated liposomes (**Fig. 1h**) To quantitatively compare the total amount of protein adsorbed onto the surface of liposomes at the three different time points of investigation, Bicinchoninic Acid (BCA) protein assay was performed and Protein binding (Pb) values were calculated (expressed as  $\mu\text{g}$  of protein/ $\mu\text{mol}$  of lipid). As shown in **Fig. 1g**, the average Pb value increased with age only in the APP/PS1 mice, while significant changes were observed between APP/PS and WT mice only at the 12-month time point.

### *Discussion of Results*

[0239] Three different time points (2-, 6- and 12- months) that model the pre-diseased state, the intermediate state between AD-related amyloidopathy and cognitive symptoms and finally symptomatic AD (**Fig. 1a** and **b**) were investigated. Considering that human longitudinal studies are challenging due to the limited access to clinical samples at pre-diseased states,[20] this proof-of-concept study was performed using the established transgenic mouse model of AD (APP/PS1), in order to demonstrate the feasibility of systemically monitoring AD and to provide some insight into the dynamic evolution of the plasma proteome with AD progression.

### **Example 2 – AD-specific longitudinal proteomic alterations in blood**

[0240] The goal of the proteomic discovery experiment was to longitudinally monitor and compare the blood proteome of APP/PS1 and WT mice, in order to capture molecular changes indicative of

AD pathophysiology. Equal amounts of total protein from plasma samples (without prior incubation with liposomes) and corona samples (upon *in vivo* recovery and purification of intravenously injected liposomes) were trypsin-digested and subsequently analysed by LC-MS/MS. It should be noted that highly abundant proteins (e.g. albumin and immunoglobulins) were not depleted from plasma and corona samples prior to LC-MS/MS analysis. The extensive purification of unbound plasma proteins from corona samples has been shown to reproducibly increase the range of proteins detected, enabling the identification of low molecular weight and low abundant proteins.[9] Thus, in the case of the corona samples only proteins with high affinity for the liposomal surface or smaller proteins carried by other proteins directly adsorbed onto the liposome surface were analysed.

**[0241]** Processing of the proteomic data generated with Progenesis QI (version 3.0; Nonlinear Dynamics) software was carried out in order to statistically compare the Relative Protein Expression (fold change) and reliability of measured differences between the blood proteome in APP/PS1 and WT mice. The Venn diagrams of **Fig. 2a** and **b** illustrate the number of common and unique differentially abundant proteins between the three different time points, as identified by proteomic analysis of plasma and corona samples, respectively. A significantly higher number of differentially abundant proteins were detected in the corona samples in comparison to the number of proteins identified by plasma control analysis for all three time points of investigation (**Fig. 2a** and **b**). This agrees with previously published work and elucidates the need for novel analytical platforms that can uncover disease-associated molecules in blood, otherwise masked by the predominant signal of albumin.[9]

**[0242]** As shown in **Fig. 2b**, multiple differentially abundant proteins (n=105) were identified between APP/PS1 and WT mice even at the earliest time point of investigation, suggesting that alterations of the blood circulatory proteome may occur at the asymptomatic phase of AD. None of the differentially abundant proteins discovered were found to be common for all the three time points, which demonstrates that the composition of the protein corona is directly affected by the disease stage. A total number of 66 differentially abundant proteins were uniquely found at the earliest stage of AD (2 months), while n=21 and n=73 proteins were uniquely found at the intermediate (6 months) and late (12 months) phases of AD development, respectively. The full lists of differentially abundant proteins are shown in **Tables 7, 8 and 9**. Interestingly, clusterin (apolipoprotein J), one of the most promising candidate blood biomarkers identified in multiple independent discovery studies as an early indicator of amyloid deposition,[22] was found in our study to be upregulated only at the 2-month time point.

**[0243]** **Table 7:** Full list of blood proteins identified by Progenesis analysis to be differentially abundant between 2-months old APP/PS1 and WT mice. Only proteins with  $p < 0.05$  are shown.

| Description                                                                                                      | Accession  | Anova<br>(p value) | Max fold<br>change |
|------------------------------------------------------------------------------------------------------------------|------------|--------------------|--------------------|
| <b>UPREGULATED (n= 101)</b>                                                                                      |            |                    |                    |
| Pancreatic alpha-amylase OS=Mus musculus GN=Amy2 PE=1 SV=2                                                       | AMYP       | 5.71E-04           | Infinity           |
| Rab GDP dissociation inhibitor (Fragment) OS=Mus musculus GN=Gdi2 PE=1 SV=1                                      | A0A1Y7VLG4 | 9.36E-04           | 4295.45            |
| Monocarboxylate transporter 1 OS=Mus musculus GN=Slc16a1 PE=1 SV=1                                               | MOT1       | 1.35E-03           | 72.28              |
| Protein piccolo OS=Mus musculus GN=Pclo PE=1 SV=4                                                                | PCLO       | 5.56E-03           | 28.03              |
| Solute carrier family 29 (Nucleoside transporters), member 1, isoform CRA_a OS=Mus musculus GN=Slc29a1 PE=2 SV=1 | Q3TCZ2     | 2.97E-02           | 16.35              |
| Protein KRI1 homolog OS=Mus musculus GN=Kri1 PE=1 SV=3                                                           | KRI1       | 4.18E-02           | 12.65              |
| Basigin OS=Mus musculus GN=Bsg PE=1 SV=2                                                                         | BASI       | 1.59E-02           | 12.28              |
| Early endosome antigen 1 OS=Mus musculus GN=Eea1 PE=1 SV=2                                                       | EEA1       | 5.06E-03           | 12.21              |
| Uncharacterized protein OS=Mus musculus GN=Cd36 PE=2 SV=1                                                        | Q3TA14     | 3.85E-02           | 12.10              |
| Cytochrome b5 OS=Mus musculus GN=Cyb5a PE=1 SV=2                                                                 | CYB5       | 6.52E-03           | 11.19              |
| Sarcoplasmic/endoplasmic reticulum calcium ATPase 2 OS=Mus musculus GN=Atp2a2 PE=1 SV=2                          | AT2A2      | 1.25E-02           | 11.12              |
| Disintegrin and metalloproteinase domain-containing protein 10 OS=Mus musculus GN=Adam10 PE=1 SV=2               | ADA10      | 2.81E-02           | 10.81              |
| T-complex protein 1 subunit alpha OS=Mus musculus GN=Tcp1 PE=1 SV=3                                              | TCPA       | 1.27E-02           | 9.35               |
| 60S ribosomal protein L18 OS=Mus musculus GN=Rpl18 PE=1 SV=1                                                     | A0A1B0GQU8 | 1.42E-02           | 7.69               |
| Receptor expression-enhancing protein OS=Mus musculus GN=Reep6 PE=1 SV=1                                         | D3Z6V2     | 3.06E-02           | 7.44               |
| E3 ubiquitin-protein ligase UBR4 OS=Mus musculus GN=Ubr4 PE=1 SV=1                                               | UBR4       | 1.11E-03           | 6.98               |
| Uncharacterized protein OS=Mus musculus GN=Psmc5 PE=2 SV=1                                                       | A0A1S6GWH1 | 4.72E-02           | 6.85               |
| 26S proteasome non-ATPase regulatory subunit 1 OS=Mus musculus GN=Psmc1 PE=1 SV=1                                | PSMD1      | 7.99E-03           | 6.57               |
| Filamin-A OS=Mus musculus GN=Flna PE=1 SV=5                                                                      | FLNA       | 1.99E-03           | 6.33               |
| Integrin beta OS=Mus musculus GN=Itgb2 PE=1 SV=1                                                                 | M0QWA7     | 2.71E-03           | 5.99               |
| Predicted gene 45713 OS=Mus musculus GN=Gm45713 PE=2 SV=1                                                        | A0A1B0GS68 | 1.86E-02           | 5.88               |
| ATP synthase subunit beta, mitochondrial OS=Mus musculus GN=Atp5b PE=1 SV=2                                      | ATPB       | 3.90E-02           | 5.78               |
| Ribosomal protein L15 OS=Mus musculus GN=Rpl15 PE=2 SV=1                                                         | Q3U7D2     | 4.80E-02           | 5.58               |
| 26S proteasome regulatory subunit 6A (Fragment) OS=Mus musculus GN=Psmc3 PE=1 SV=6                               | A0A087WPH7 | 3.87E-02           | 5.46               |
| Uncharacterized protein OS=Mus musculus GN=Rpl14 PE=2 SV=1                                                       | Q9CWX0     | 4.59E-02           | 5.42               |
| Long-chain-fatty-acid--CoA ligase 5 OS=Mus musculus GN=Acsl5 PE=1 SV=1                                           | ACSL5      | 3.30E-02           | 5.41               |
| Ribosomal protein S2, pseudogene 6 OS=Mus musculus GN=Rps2-ps6 PE=3 SV=1                                         | A0A140T8L5 | 2.25E-02           | 5.28               |
| Kell blood group glycoprotein homolog OS=Mus musculus GN=Kel PE=1 SV=1                                           | KELL       | 2.69E-02           | 5.24               |
| Calreticulin OS=Mus musculus GN=Calr PE=1 SV=1                                                                   | CALR       | 1.19E-02           | 5.13               |
| Erythrocyte band 7 integral membrane protein OS=Mus musculus GN=Stom PE=1 SV=3                                   | STOM       | 2.40E-02           | 4.93               |

|                                                                                                  |            |          |      |
|--------------------------------------------------------------------------------------------------|------------|----------|------|
| Moesin OS=Mus musculus GN=Msn PE=1 SV=3                                                          | MOES       | 6.20E-03 | 4.69 |
| 60S ribosomal protein L6 OS=Mus musculus GN=Rpl6 PE=1 SV=1                                       | Q3UCH0     | 1.70E-02 | 4.64 |
| 26S proteasome regulatory subunit 10B OS=Mus musculus GN=Psmc6 PE=1 SV=1                         | PRS10      | 3.22E-02 | 4.49 |
| Talin-1 OS=Mus musculus GN=Tln1 PE=1 SV=2                                                        | TLN1       | 1.48E-02 | 4.40 |
| RAS-related C3 botulinum substrate 3, isoform CRA_a (Fragment) OS=Mus musculus GN=Rac3 PE=1 SV=1 | A2AC13     | 8.87E-04 | 4.38 |
| Laminin subunit alpha-2 OS=Mus musculus GN=Lama2 PE=1 SV=2                                       | LAMA2      | 7.84E-03 | 4.26 |
| IgM heavy chain VDJ region (Fragment) OS=Mus musculus PE=2 SV=1                                  | X5J5A8     | 1.33E-03 | 4.19 |
| Aquaporin-1 OS=Mus musculus GN=Aqp1 PE=1 SV=3                                                    | AQP1       | 1.93E-02 | 4.17 |
| Uncharacterized protein OS=Mus musculus GN=Rpl7 PE=2 SV=1                                        | Q3UBI6     | 2.28E-02 | 4.02 |
| Annexin A5 OS=Mus musculus GN=Anxa5 PE=1 SV=1                                                    | ANXA5      | 4.51E-03 | 4.02 |
| Glucosidase 2 subunit beta OS=Mus musculus GN=Prkcsh PE=1 SV=1                                   | GLU2B      | 2.47E-03 | 3.97 |
| VH region (Fragment) OS=Mus musculus PE=2 SV=1                                                   | Q53VQ0     | 2.83E-02 | 3.94 |
| Clathrin heavy chain 1 OS=Mus musculus GN=Cltc PE=1 SV=3                                         | CLH1       | 1.80E-02 | 3.68 |
| Complement C1q tumor necrosis factor-related protein 3 OS=Mus musculus GN=C1qtnf3 PE=2 SV=1      | C1QT3      | 3.68E-02 | 3.59 |
| Proteoglycan 4 OS=Mus musculus GN=Prg4 PE=1 SV=1                                                 | A0A0R4J207 | 1.97E-02 | 3.47 |
| Ezrin OS=Mus musculus GN=Ezr PE=1 SV=3                                                           | EZRI       | 6.84E-03 | 3.46 |
| 26S proteasome non-ATPase regulatory subunit 14 OS=Mus musculus GN=Psmc14 PE=1 SV=2              | PSDE       | 4.20E-02 | 3.31 |
| Immunoglobulin heavy variable 10-1 (Fragment) OS=Mus musculus GN=Ighv10-1 PE=4 SV=1              | A0A0B4J1J6 | 3.80E-02 | 3.30 |
| Ig kappa chain V-III region 50S10.1 OS=Mus musculus PE=1 SV=1                                    | KV3A4      | 1.90E-02 | 3.29 |
| GDH/6PGL endoplasmic bifunctional protein OS=Mus musculus GN=H6pd PE=1 SV=1                      | A2A7A7     | 1.57E-03 | 3.11 |
| Ras-related protein Rap-1b OS=Mus musculus GN=Rap1b PE=1 SV=2                                    | RAP1B      | 3.43E-02 | 3.02 |
| Ig kappa chain V-III region PC 3741/TEPC 111 OS=Mus musculus PE=1 SV=1                           | KV3A8      | 4.38E-02 | 3.02 |
| Endoplasmic reticulum protein OS=Mus musculus GN=Hsp90b1 PE=1 SV=2                               | ENPL       | 2.44E-03 | 3.02 |
| WD repeat-containing protein 90 OS=Mus musculus GN=Wdr90 PE=1 SV=1                               | H7BX49     | 8.74E-03 | 2.99 |
| Proteasome subunit beta type-5 OS=Mus musculus GN=Psmc5 PE=1 SV=3                                | PSB5       | 1.71E-02 | 2.90 |
| Protein Igkv5-48 (Fragment) OS=Mus musculus GN=Igkv5-48 PE=1 SV=2                                | A0A140T8N2 | 5.51E-04 | 2.83 |
| Immunoglobulin lambda variable 3 (Fragment) OS=Mus musculus GN=Iglv3 PE=4 SV=1                   | A0A0B4J1K5 | 1.75E-02 | 2.79 |
| Spectrin alpha chain, non-erythrocytic 1 OS=Mus musculus GN=Sptan1 PE=1 SV=4                     | SPTN1      | 4.22E-02 | 2.77 |
| MCG23377, isoform CRA_b OS=Mus musculus GN=Gm8797 PE=4 SV=1                                      | A0A0A6YW67 | 1.64E-02 | 2.76 |
| Aminopeptidase N (Fragment) OS=Mus musculus GN=Anpep PE=1 SV=1                                   | A0A0U1RNS3 | 4.31E-02 | 2.69 |
| Proteasome subunit beta type-6 OS=Mus musculus GN=Psmc6 PE=1 SV=3                                | PSB6       | 1.20E-03 | 2.68 |
| 40S ribosomal protein S9 OS=Mus musculus GN=Rps9 PE=1 SV=1                                       | D3YWH9     | 4.57E-02 | 2.60 |

|                                                                                             |            |          |      |
|---------------------------------------------------------------------------------------------|------------|----------|------|
| Elongation factor 1-alpha 1 OS=Mus musculus GN=Eef1a1 PE=1 SV=3                             | EF1A1      | 1.39E-02 | 2.57 |
| Myosin-9 OS=Mus musculus GN=Myh9 PE=1 SV=4                                                  | MYH9       | 2.98E-02 | 2.57 |
| Proteasome subunit beta type-4 OS=Mus musculus GN=Psmc4 PE=1 SV=1                           | PSB4       | 1.33E-03 | 2.56 |
| Immunoglobulin kappa chain variable 1-122 (Fragment) OS=Mus musculus GN=Igkv1-122 PE=4 SV=2 | A0A140T8M9 | 3.35E-02 | 2.50 |
| Retinol dehydrogenase 7 OS=Mus musculus GN=Rdh7 PE=1 SV=1                                   | RDH7       | 2.08E-02 | 2.48 |
| Immunoglobulin kappa chain variable 9-124 OS=Mus musculus GN=Igkv9-124 PE=1 SV=7            | A0A075B5K2 | 2.72E-02 | 2.47 |
| Uncharacterized protein OS=Mus musculus GN=Hspa5 PE=2 SV=1                                  | Q3TI47     | 9.21E-03 | 2.41 |
| Uncharacterized protein OS=Mus musculus GN=Thbs1 PE=2 SV=1                                  | Q3TR40     | 4.13E-02 | 2.36 |
| Ig kappa chain V-V region MOPC 149 OS=Mus musculus PE=1 SV=1                                | KV5A4      | 3.72E-02 | 2.35 |
| Uroplakin-3b OS=Mus musculus GN=Upk3b PE=1 SV=1                                             | A0A0R4J0S8 | 1.98E-02 | 2.32 |
| Immunoglobulin heavy variable V1-11 (Fragment) OS=Mus musculus GN=Ighv1-11 PE=4 SV=1        | A0A0A6YW19 | 1.97E-02 | 2.30 |
| Immunoglobulin kappa variable 12-44 (Fragment) OS=Mus musculus GN=Igkv12-44 PE=4 SV=2       | A0A140T8M2 | 1.41E-02 | 2.27 |
| Immunoglobulin kappa variable 8-28 OS=Mus musculus GN=Igkv8-28 PE=1 SV=7                    | A0A075B5N3 | 2.31E-02 | 2.27 |
| Tankyrase-2 OS=Mus musculus GN=Tnks2 PE=2 SV=2                                              | TNKS2      | 6.45E-03 | 2.23 |
| Cartilage oligomeric matrix protein OS=Mus musculus GN=Comp PE=1 SV=2                       | COMP       | 2.39E-02 | 2.22 |
| Immunoglobulin kappa chain variable 8-27 OS=Mus musculus GN=Igkv8-27 PE=4 SV=1              | A0A075B5N4 | 4.33E-02 | 2.17 |
| Proteasome subunit alpha type-6 OS=Mus musculus GN=Psmc6 PE=1 SV=1                          | PSA6       | 9.93E-03 | 2.08 |
| Proteasome subunit alpha type (Fragment) OS=Mus musculus GN=Psmc4 PE=2 SV=1                 | Q3TL95     | 4.15E-02 | 2.05 |
| Immunoglobulin kappa variable 6-17 (Fragment) OS=Mus musculus GN=Igkv6-17 PE=1 SV=2         | A0A140T8Q3 | 4.37E-02 | 2.01 |
| Integrin beta-1 OS=Mus musculus GN=Itgb1 PE=1 SV=1                                          | ITB1       | 1.20E-02 | 2.01 |
| Immunoglobulin kappa variable 2-109 (Fragment) OS=Mus musculus GN=Igkv2-109 PE=4 SV=4       | A0A075B5K6 | 6.31E-03 | 2.00 |
| Immunoglobulin heavy variable V1-59 OS=Mus musculus GN=Ighv1-59 PE=4 SV=1                   | A0A075B5X0 | 1.04E-03 | 1.99 |
| Immunoglobulin kappa variable 4-53 OS=Mus musculus GN=Igkv4-53 PE=1 SV=1                    | A0A075B677 | 2.98E-02 | 1.99 |
| Coagulation factor V OS=Mus musculus GN=F5 PE=1 SV=1                                        | FA5        | 2.48E-03 | 1.99 |
| Single-chain variable OS=Mus musculus GN=Wgn-scFv PE=2 SV=1                                 | A0A1E1GJG6 | 2.64E-02 | 1.98 |
| DNA helicase B OS=Mus musculus GN=Helb PE=1 SV=2                                            | HELB       | 1.46E-03 | 1.98 |
| Immunoglobulin kappa variable 6-23 (Fragment) OS=Mus musculus GN=Igkv6-23 PE=4 SV=2         | A0A140T8N5 | 4.94E-03 | 1.94 |
| Napsin-A OS=Mus musculus GN=Napsa PE=1 SV=1                                                 | NAPSA      | 2.49E-02 | 1.92 |
| Aberrantly recombined kappa chain Vk8/J1 region (Fragment) OS=Mus musculus PE=1 SV=1        | A2N494     | 4.19E-02 | 1.90 |
| Light chain variable region (Fragment) OS=Mus musculus PE=2 SV=1                            | A2NVX0     | 1.11E-02 | 1.87 |
| Adiponectin OS=Mus musculus GN=Adipoq PE=1 SV=2                                             | ADIPO      | 3.69E-02 | 1.83 |
| Long-chain-fatty-acid--CoA ligase 1 OS=Mus musculus GN=Acsl1 PE=1 SV=2                      | ACSL1      | 1.60E-02 | 1.81 |

|                                                                                             |            |          |      |
|---------------------------------------------------------------------------------------------|------------|----------|------|
| Transferrin receptor protein 1 OS=Mus musculus GN=Tfrc PE=1 SV=1                            | TFR1       | 2.17E-04 | 1.81 |
| Anti-VIPase light chain variable region (Fragment) OS=Mus musculus PE=4 SV=1                | Q8K1F1     | 3.96E-02 | 1.78 |
| Maltase-glucoamylase OS=Mus musculus GN=Mgam PE=1 SV=1                                      | B5THE2     | 5.14E-03 | 1.74 |
| Proteasome subunit alpha type-3 OS=Mus musculus GN=Pasma3 PE=1 SV=3                         | PSA3       | 4.56E-02 | 1.70 |
| Anti-myosin immunoglobulin light chain variable region (Fragment) OS=Mus musculus PE=2 SV=1 | Q9JL74     | 3.95E-02 | 1.66 |
| Argininosuccinate lyase OS=Mus musculus GN=Asl PE=1 SV=1                                    | ARLY       | 2.48E-02 | 1.42 |
| Clusterin OS=Mus musculus GN=Clu PE=1 SV=1                                                  | CLUS       | 2.51E-02 | 1.27 |
| <b>DOWNREGULATED (n=4)</b>                                                                  |            |          |      |
| IgG1 heavy chain VDJ region (Fragment) OS=Mus musculus PE=2 SV=1                            | X5J5A8     | 1.83E-02 | 9.03 |
| Colony stimulating factor 1 receptor OS=Mus musculus GN=Csf1r PE=1 SV=1                     | Q0P0S6     | 4.66E-02 | 3.82 |
| Fibronectin OS=Mus musculus GN=Fn1 PE=1 SV=1                                                | A0A087WR50 | 2.56E-02 | 2.35 |
| Serum amyloid A-4 protein OS=Mus musculus GN=Saa4 PE=1 SV=2                                 | SAA4       | 4.57E-02 | 1.69 |

**[0244] Table 8:** Full list of blood proteins identified by Progenesis analysis to be differentially abundant between 6-months old APP/PS1 and WT mice. Only proteins with  $p < 0.05$  are shown.

| Description                                                                                 | Accession  | Anova (p value) | Max fold change |
|---------------------------------------------------------------------------------------------|------------|-----------------|-----------------|
| <b>UPREGULATED (n= 19)</b>                                                                  |            |                 |                 |
| Immunoglobulin heavy variable V1-43 OS=Mus musculus GN=Ighv1-43 PE=4 SV=1                   | A0A075B5V7 | 5.23E-03        | 1.98            |
| Filamin-B OS=Mus musculus GN=Flnb PE=1 SV=3                                                 | FLNB       | 5.24E-03        | 31.55           |
| Argininosuccinate synthase OS=Mus musculus GN=Ass1 PE=1 SV=1                                | ASSY       | 6.54E-03        | 4.28            |
| Pyrethroid hydrolase Ces2e OS=Mus musculus GN=Ces2e PE=1 SV=1                               | EST2E      | 8.64E-03        | 1.75            |
| Uroplakin-3b OS=Mus musculus GN=Upk3b PE=1 SV=1                                             | A0A0R4J0S8 | 1.07E-02        | 1.75            |
| Major urinary protein 10 OS=Mus musculus GN=Mup10 PE=2 SV=1                                 | A2BIN1     | 1.29E-02        | 3.66            |
| Napsin-A OS=Mus musculus GN=Napsa PE=1 SV=1                                                 | NAPSA      | 1.72E-02        | 1.47            |
| Retinol dehydrogenase 7 OS=Mus musculus GN=Rdh7 PE=1 SV=1                                   | RDH7       | 2.57E-02        | 2.65            |
| Thyrotropin-releasing hormone-degrading ectoenzyme OS=Mus musculus GN=Trhde PE=1 SV=1       | TRHDE      | 2.85E-02        | 3.68            |
| Mepirin A subunit OS=Mus musculus GN=Mep1a PE=1 SV=1                                        | A0A0R4J043 | 2.88E-02        | 3.43            |
| Uncharacterized protein OS=Mus musculus GN=Thbs1 PE=2 SV=1                                  | Q3TR40     | 3.14E-02        | 2.58            |
| Tankyrase-2 OS=Mus musculus GN=Tnks2 PE=2 SV=2                                              | TNKS2      | 3.16E-02        | 2.04            |
| Complement C1q tumor necrosis factor-related protein 3 OS=Mus musculus GN=C1qtnf3 PE=2 SV=1 | C1QT3      | 3.55E-02        | 1.77            |
| Cytosolic 10-formyltetrahydrofolate dehydrogenase OS=Mus musculus GN=Aldh1l1 PE=1 SV=1      | AL1L1      | 3.96E-02        | 5.13            |
| G protein-coupled receptor 179 OS=Mus musculus GN=Gpr179 PE=4 SV=1                          | E9PY61     | 4.08E-02        | 1.32            |
| Thrombospondin-4 OS=Mus musculus GN=Thbs4 PE=1 SV=1                                         | TSP4       | 4.49E-02        | 1.49            |

|                                                                                         |            |          |       |
|-----------------------------------------------------------------------------------------|------------|----------|-------|
| C-type lectin domain family 11 member A OS=Mus musculus<br>GN=Clec11a PE=1 SV=1         | CLC11      | 4.82E-02 | 2.69  |
| MCG5400 OS=Mus musculus GN=Myl12a PE=1 SV=1                                             | Q6ZWQ9     | 4.92E-02 | 4.31  |
| <b>DOWNREGULATED (n=11)</b>                                                             |            |          |       |
| CD5 antigen-like OS=Mus musculus GN=Cd5l PE=1 SV=3                                      | CD5L       | 1.23E-03 | 2.65  |
| Immunoglobulin heavy variable V12-3 (Fragment) OS=Mus<br>musculus GN=Ighv12-3 PE=4 SV=2 | A0A075B5T1 | 1.72E-03 | 3.24  |
| Light chain variable region (Fragment) OS=Mus musculus PE=2<br>SV=1                     | A2NVX0     | 2.48E-03 | 1.77  |
| Oncoprotein-induced transcript 3 protein OS=Mus musculus<br>GN=Oit3 PE=2 SV=2           | OIT3       | 3.38E-03 | 3.81  |
| IgM heavy chain VDJ region (Fragment) OS=Mus musculus PE=2<br>SV=1                      | X5J4G2     | 5.94E-03 | 1.55  |
| Bisphosphoglycerate mutase OS=Mus musculus GN=Bpgm PE=1<br>SV=2                         | PMGE       | 7.21E-03 | 14.62 |
| Properdin OS=Mus musculus GN=Cfp PE=2 SV=2                                              | PROP       | 2.11E-02 | 1.83  |
| Anti-colorectal carcinoma light chain OS=Mus musculus PE=1<br>SV=1                      | Q7TS98     | 2.78E-02 | 2.70  |
| Protein piccolo OS=Mus musculus GN=Pclo PE=1 SV=4                                       | PCLO       | 2.80E-02 | 3.60  |
| Immunoglobulin heavy variable 7-1 (Fragment) OS=Mus musculus<br>GN=Ighv7-1 PE=1 SV=2    | A0A075B5S2 | 4.82E-02 | 1.86  |
| Immunoglobulin heavy variable 1-39 (Fragment) OS=Mus<br>musculus GN=Ighv1-39 PE=4 SV=1  | A0A075B5V5 | 4.98E-02 | 1.39  |

**[0245] Table 9:** Full list of blood proteins identified by Progenesis analysis to be differentially abundant between 12-months old APP/PS1 and WT mice.

| Description                                                                                                                                       | Accession  | Anova<br>(p value) | Max fold<br>change |
|---------------------------------------------------------------------------------------------------------------------------------------------------|------------|--------------------|--------------------|
| <b>UPREGULATED (n= 8)</b>                                                                                                                         |            |                    |                    |
| MRP5 (Fragment) OS=Mus musculus GN=Ighv9-4 PE=2 SV=1                                                                                              | Q925S1     | 7.28E-04           | 3.84               |
| Ig kappa chain V-III region PC 3741/TEPC 111 OS=Mus musculus<br>PE=1 SV=1                                                                         | KV3A8      | 7.86E-04           | 2.61               |
| Immunoglobulin kappa variable 4-80 (Fragment) OS=Mus<br>musculus GN=Igkv4-80 PE=4 SV=2                                                            | A0A075B5L7 | 3.37E-03           | 2.49               |
| Glycogen phosphorylase, muscle form OS=Mus musculus<br>GN=Pygm PE=1 SV=3                                                                          | PYGM       | 3.76E-02           | 6.42               |
| Immunoglobulin heavy variable V9-3 (Fragment) OS=Mus<br>musculus GN=Ighv9-3 PE=1 SV=1                                                             | A0A0B4J1J5 | 3.88E-02           | 1.35               |
| Igh protein OS=Mus musculus GN=Igh PE=2 SV=1                                                                                                      | Q4VAB6     | 3.95E-02           | 4.48               |
| Ig kappa chain V-III region 50S10.1 OS=Mus musculus PE=1<br>SV=1                                                                                  | KV3A4      | 4.00E-02           | 3.00               |
| Immunoglobulin kappa variable 6-23 (Fragment) OS=Mus<br>musculus GN=Igkv6-23 PE=4 SV=2                                                            | A0A140T8N5 | 4.74E-02           | 1.62               |
| <b>DOWNREGULATED (n= 97)</b>                                                                                                                      |            |                    |                    |
| Caveolae-associated protein 1 OS=Mus musculus GN=Cavin1<br>PE=1 SV=1                                                                              | CAVN1      | 4.42E-05           | Infinity           |
| Solute carrier family 2, facilitated glucose transporter member 3<br>OS=Mus musculus GN=Slc2a3 PE=1 SV=1                                          | GTR3       | 5.69E-05           | Infinity           |
| Dihydropyridyllysine-residue acetyltransferase component of<br>pyruvate dehydrogenase complex, mitochondrial OS=Mus<br>musculus GN=Dlat PE=1 SV=2 | ODP2       | 1.64E-04           | 14.72              |

|                                                                                                                  |            |          |          |
|------------------------------------------------------------------------------------------------------------------|------------|----------|----------|
| Bile acyl-CoA synthetase OS=Mus musculus GN=Slc27a5 PE=1 SV=1                                                    | E9PXV4     | 4.41E-04 | Infinity |
| NADPH--cytochrome P450 reductase (Fragment) OS=Mus musculus GN=Por PE=1 SV=1                                     | E9PVT9     | 8.02E-04 | Infinity |
| Uncharacterized protein OS=Mus musculus GN=Epb42 PE=2 SV=1                                                       | Q3T9M7     | 8.63E-04 | 2.23     |
| Uncharacterized protein OS=Mus musculus GN=Cd36 PE=2 SV=1                                                        | Q3TA14     | 1.76E-03 | 28.50    |
| Erythrocyte band 7 integral membrane protein OS=Mus musculus GN=Stom PE=1 SV=3                                   | STOM       | 1.95E-03 | 13.27    |
| Sarcoplasmic/endoplasmic reticulum calcium ATPase 2 OS=Mus musculus GN=Atp2a2 PE=1 SV=2                          | AT2A2      | 3.13E-03 | 9.93     |
| Immunoglobulin lambda variable 3 (Fragment) OS=Mus musculus GN=Iglv3 PE=4 SV=1                                   | A0A0B4J1K5 | 3.30E-03 | 2.48     |
| Fibronectin OS=Mus musculus GN=Fn1 PE=1 SV=1                                                                     | A0A087WR50 | 3.64E-03 | 1.38     |
| Moesin OS=Mus musculus GN=Msn PE=1 SV=3                                                                          | MOES       | 4.90E-03 | 6.57     |
| Tripeptidyl-peptidase 2 OS=Mus musculus GN=Tpp2 PE=1 SV=3                                                        | TPP2       | 5.41E-03 | 2.25     |
| Kell blood group glycoprotein homolog OS=Mus musculus GN=Kel PE=1 SV=1                                           | KELL       | 5.49E-03 | 17.34    |
| Uncharacterized protein OS=Mus musculus GN=Uba1 PE=2 SV=1                                                        | A0A1S6GWH5 | 6.74E-03 | 2.34     |
| Ezrin OS=Mus musculus GN=Ezr PE=1 SV=3                                                                           | EZRI       | 6.87E-03 | 6.60     |
| RAS-related C3 botulinum substrate 3, isoform CRA_a (Fragment) OS=Mus musculus GN=Rac3 PE=1 SV=1                 | A2AC13     | 6.89E-03 | 5.02     |
| Solute carrier family 29 (Nucleoside transporters), member 1, isoform CRA_a OS=Mus musculus GN=Slc29a1 PE=2 SV=1 | Q3TCZ2     | 7.27E-03 | 39.06    |
| Peroxiredoxin-2 OS=Mus musculus GN=Prdx2 PE=1 SV=3                                                               | PRDX2      | 7.94E-03 | 2.59     |
| Immunoglobulin heavy variable 11-1 (Fragment) OS=Mus musculus GN=Ighv11-1 PE=4 SV=1                              | A0A075B5R6 | 7.98E-03 | 3.33     |
| Disintegrin and metalloproteinase domain-containing protein 10 OS=Mus musculus GN=Adam10 PE=1 SV=2               | ADA10      | 1.00E-02 | 36.31    |
| Ras-related protein Rap-1b OS=Mus musculus GN=Rap1b PE=1 SV=2                                                    | RAP1B      | 1.02E-02 | 3.36     |
| ATP synthase subunit beta, mitochondrial OS=Mus musculus GN=Atp5b PE=1 SV=2                                      | ATPB       | 1.07E-02 | 4.06     |
| Heat shock 70 kDa protein 4 OS=Mus musculus GN=Hspa4 PE=1 SV=1                                                   | Q3U2G2     | 1.16E-02 | 2.98     |
| Phospholipid transfer protein OS=Mus musculus GN=Pltp PE=1 SV=1                                                  | PLTP       | 1.23E-02 | 1.61     |
| Integrin beta-3 OS=Mus musculus GN=Itgb3 PE=1 SV=2                                                               | ITB3       | 1.29E-02 | 9.77     |
| Aquaporin-1 OS=Mus musculus GN=Aqp1 PE=1 SV=3                                                                    | AQP1       | 1.44E-02 | 8.30     |
| Annexin A5 OS=Mus musculus GN=Anxa5 PE=1 SV=1                                                                    | ANXA5      | 1.48E-02 | 6.64     |
| Alpha actinin 1a OS=Mus musculus GN=Actn1 PE=1 SV=1                                                              | A1BN54     | 1.53E-02 | 6.43     |
| EMILIN-1 OS=Mus musculus GN=Emilin1 PE=1 SV=1                                                                    | EMIL1      | 1.53E-02 | 11.70    |
| Spectrin alpha chain, non-erythrocytic 1 OS=Mus musculus GN=Sptan1 PE=1 SV=4                                     | SPTN1      | 1.54E-02 | 21.44    |
| Large neutral amino acids transporter small subunit 3 OS=Mus musculus GN=Slc43a1 PE=1 SV=1                       | A2ATS4     | 1.63E-02 | 202.52   |
| Integrin alpha 2b OS=Mus musculus GN=Itga2b PE=2 SV=1                                                            | B2RPR7     | 1.63E-02 | 6.66     |
| Basigin OS=Mus musculus GN=Bsg PE=1 SV=2                                                                         | BASI       | 1.64E-02 | 25.79    |
| Angiopoietin-1 OS=Mus musculus GN=Angpt1 PE=1 SV=2                                                               | ANGP1      | 1.66E-02 | 6.42     |
| Glyceraldehyde-3-phosphate dehydrogenase OS=Mus musculus GN=Gapdh PE=1 SV=1                                      | A0A0A0MQF6 | 1.71E-02 | 2.31     |

|                                                                                                    |            |          |        |
|----------------------------------------------------------------------------------------------------|------------|----------|--------|
| Maltase-glucoamylase OS=Mus musculus GN=Mgam PE=1 SV=1                                             | A0A0J9YVE6 | 1.73E-02 | 1.20   |
| Perilipin-4 OS=Mus musculus GN=Plin4 PE=1 SV=2                                                     | PLIN4      | 1.76E-02 | 64.24  |
| Uncharacterized protein OS=Mus musculus PE=2 SV=1                                                  | I6L9E1     | 1.83E-02 | 2.22   |
| Uncharacterized protein (Fragment) OS=Mus musculus GN=Tpp2 PE=2 SV=1                               | Q3TB11     | 1.91E-02 | 9.38   |
| Microsomal triglyceride transfer protein large subunit OS=Mus musculus GN=Mttp PE=1 SV=2           | MTP        | 1.95E-02 | 25.10  |
| Bifunctional purine biosynthesis protein PURH OS=Mus musculus GN=Atic PE=1 SV=2                    | PUR9       | 1.95E-02 | 5.85   |
| Neuronal membrane glycoprotein M6-a OS=Mus musculus GN=Gpm6a PE=1 SV=1                             | GPM6A      | 2.09E-02 | 14.99  |
| Monocarboxylate transporter 1 OS=Mus musculus GN=Slc16a1 PE=1 SV=1                                 | MOT1       | 2.20E-02 | 10.32  |
| Guanine nucleotide-binding protein G(I)/G(S)/G(T) subunit beta-1 OS=Mus musculus GN=Gnb1 PE=1 SV=3 | GBB1       | 2.27E-02 | 5.66   |
| Glucosidase 2 subunit beta OS=Mus musculus GN=Prkcsh PE=1 SV=1                                     | GLU2B      | 2.28E-02 | 12.09  |
| Hsp90aa1 protein (Fragment) OS=Mus musculus GN=Hsp90aa1 PE=2 SV=1                                  | A0PJ91     | 2.32E-02 | 2.02   |
| ATP synthase subunit alpha, mitochondrial OS=Mus musculus GN=Atp5a1 PE=1 SV=1                      | ATPA       | 2.38E-02 | 15.24  |
| Dimethylaniline monooxygenase [N-oxide-forming] 5 OS=Mus musculus GN=Fmo5 PE=1 SV=4                | FMO5       | 2.39E-02 | 9.17   |
| Coronin OS=Mus musculus GN=Coro1a PE=2 SV=1                                                        | Q3T9L1     | 2.39E-02 | 4.04   |
| Integrin-linked protein kinase OS=Mus musculus GN=Ilk PE=1 SV=2                                    | ILK        | 2.42E-02 | 3.28   |
| Galectin-3-binding protein OS=Mus musculus GN=Lgals3bp PE=1 SV=1                                   | LG3BP      | 2.46E-02 | 5.45   |
| Spectrin beta chain, erythrocytic OS=Mus musculus GN=Sptb PE=1 SV=4                                | SPTB1      | 2.53E-02 | 4.93   |
| RAS oncogene family protein OS=Mus musculus GN=Rab11b PE=2 SV=1                                    | A0A068BFR3 | 2.63E-02 | 3.58   |
| Rab GDP dissociation inhibitor (Fragment) OS=Mus musculus GN=Gdi2 PE=1 SV=1                        | A0A1Y7VLG4 | 2.64E-02 | 151.96 |
| Dipeptidyl peptidase 4 OS=Mus musculus GN=Dpp4 PE=1 SV=3                                           | DPP4       | 2.67E-02 | 10.43  |
| Filamin-A OS=Mus musculus GN=Flna PE=1 SV=5                                                        | FLNA       | 2.77E-02 | 4.44   |
| MCG23377, isoform CRA_b OS=Mus musculus GN=Gm8797 PE=4 SV=1                                        | A0A0A6YW67 | 2.81E-02 | 2.47   |
| Clathrin heavy chain 1 OS=Mus musculus GN=Cltc PE=1 SV=3                                           | CLH1       | 2.83E-02 | 4.86   |
| Adenylyl cyclase-associated protein 1 OS=Mus musculus GN=Cap1 PE=1 SV=4                            | CAP1       | 2.84E-02 | 2.13   |
| Annexin A2 OS=Mus musculus GN=Anxa2 PE=1 SV=2                                                      | ANXA2      | 2.85E-02 | 7.47   |
| CD9 antigen OS=Mus musculus GN=Cd9 PE=1 SV=2                                                       | CD9        | 2.85E-02 | 3.11   |
| Tubulin beta-4A chain OS=Mus musculus GN=Tubb4a PE=1 SV=3                                          | TBB4A      | 2.86E-02 | 3.28   |
| Spectrin beta chain OS=Mus musculus GN=Sptb PE=2 SV=1                                              | B2RX08     | 2.88E-02 | 2.91   |
| Lactadherin OS=Mus musculus GN=Mfge8 PE=1 SV=3                                                     | MFGM       | 2.99E-02 | 4.26   |
| Calnexin OS=Mus musculus GN=Canx PE=1 SV=1                                                         | CALX       | 3.10E-02 | 37.72  |
| 26S proteasome non-ATPase regulatory subunit 2 OS=Mus musculus GN=Psmd2 PE=1 SV=1                  | PSMD2      | 3.29E-02 | 2.24   |
| UDP-glucuronosyltransferase 1-1 OS=Mus musculus GN=Ugt1a1 PE=1 SV=2                                | UD11       | 3.47E-02 | 9.57   |
| Decorin OS=Mus musculus GN=Dcn PE=1 SV=1                                                           | PGS2       | 3.48E-02 | 5.23   |

|                                                                                                                                                           |            |          |       |
|-----------------------------------------------------------------------------------------------------------------------------------------------------------|------------|----------|-------|
| Leucine-rich repeat neuronal protein 4 OS=Mus musculus<br>GN=Lrrn4 PE=1 SV=2                                                                              | LRRN4      | 3.53E-02 | 6.74  |
| Annexin A6 OS=Mus musculus GN=Anxa6 PE=1 SV=3                                                                                                             | ANXA6      | 3.65E-02 | 80.55 |
| Mannan-binding lectin serine protease 2 OS=Mus musculus<br>GN=Masp2 PE=1 SV=1                                                                             | MASP2      | 3.68E-02 | 1.50  |
| Filamin-B OS=Mus musculus GN=Flnb PE=1 SV=3                                                                                                               | FLNB       | 3.74E-02 | 5.37  |
| Integrin beta-1 OS=Mus musculus GN=Itgb1 PE=1 SV=1                                                                                                        | ITB1       | 4.00E-02 | 5.08  |
| Immunoglobulin kappa variable 8-34 (Fragment) OS=Mus<br>musculus GN=Igkv8-34 PE=4 SV=4                                                                    | A0A0G2JDU0 | 4.03E-02 | 15.10 |
| Aminopeptidase N (Fragment) OS=Mus musculus GN=Anpep<br>PE=1 SV=1                                                                                         | A0A0U1RNS3 | 4.04E-02 | 4.95  |
| 55 kDa erythrocyte membrane protein OS=Mus musculus<br>GN=Mpp1 PE=1 SV=1                                                                                  | B7ZCL8     | 4.07E-02 | 46.48 |
| Dihydrolipoyllysine-residue succinyltransferase component of 2-<br>oxoglutarate dehydrogenase complex, mitochondrial OS=Mus<br>musculus GN=Dlst PE=1 SV=1 | ODO2       | 4.12E-02 | 2.62  |
| Transketolase (Fragment) OS=Mus musculus GN=Tkt PE=1 SV=1                                                                                                 | A0A286YE28 | 4.15E-02 | 2.85  |
| Protein Igkv5-48 (Fragment) OS=Mus musculus GN=Igkv5-48<br>PE=1 SV=2                                                                                      | A0A140T8N2 | 4.17E-02 | 1.54  |
| Serglycin OS=Mus musculus GN=Srgn PE=1 SV=1                                                                                                               | SRGN       | 4.34E-02 | 7.41  |
| Laminin subunit gamma-1 OS=Mus musculus GN=Lamc1 PE=1<br>SV=1                                                                                             | F8VQJ3     | 4.41E-02 | 2.26  |
| Anti-myosin immunoglobulin light chain variable region (Fragment)<br>OS=Mus musculus GN=Igkv4-91 PE=2 SV=1                                                | Q9JL78     | 4.49E-02 | 3.51  |
| Uncharacterized protein OS=Mus musculus GN=Cyp2c37 PE=2<br>SV=1                                                                                           | Q9DBD9     | 4.50E-02 | 19.04 |
| Mannan-binding lectin serine protease 1 OS=Mus musculus<br>GN=Masp1 PE=1 SV=2                                                                             | MASP1      | 4.56E-02 | 1.35  |
| Vinculin OS=Mus musculus GN=Vcl PE=1 SV=4                                                                                                                 | VINC       | 4.57E-02 | 2.39  |
| Ankyrin-1 OS=Mus musculus GN=Ank1 PE=1 SV=1                                                                                                               | A0A0R4J1N7 | 4.60E-02 | 5.50  |
| Protein KRI1 homolog OS=Mus musculus GN=Kri1 PE=1 SV=3                                                                                                    | KRI1       | 4.61E-02 | 28.14 |
| UTP--glucose-1-phosphate uridylyltransferase OS=Mus musculus<br>GN=Ugp2 PE=1 SV=3                                                                         | UGPA       | 4.66E-02 | 3.26  |
| Fermitin family homolog 3 OS=Mus musculus GN=Fermt3 PE=1<br>SV=1                                                                                          | URP2       | 4.70E-02 | 1.78  |
| Retinal dehydrogenase 1 OS=Mus musculus GN=Aldh1a1 PE=1<br>SV=5                                                                                           | AL1A1      | 4.73E-02 | 2.46  |
| Laminin subunit gamma-1 OS=Mus musculus GN=Lamc1 PE=1<br>SV=2                                                                                             | LAMC1      | 4.81E-02 | 3.21  |
| Serum paraoxonase/arylesterase 1 OS=Mus musculus GN=Pon1<br>PE=1 SV=2                                                                                     | PON1       | 4.92E-02 | 1.59  |
| L-lactate dehydrogenase OS=Mus musculus GN=Ldha PE=1<br>SV=1                                                                                              | A0A1B0GSR9 | 4.92E-02 | 1.57  |
| Uncharacterized protein OS=Mus musculus GN=Hspa5 PE=2<br>SV=1                                                                                             | Q3TI47     | 4.93E-02 | 1.85  |
| ADP/ATP translocase 2 OS=Mus musculus GN=Slc25a5 PE=1<br>SV=3                                                                                             | ADT2       | 4.95E-02 | 7.18  |
| Annexin OS=Mus musculus GN=Anxa7 PE=1 SV=1                                                                                                                | A0A2C9F2D2 | 4.98E-02 | 10.61 |

**[0246]** Among the differentially abundant proteins identified by comparing the protein coronas formed in APP/PS1 and WT mice, pancreatic alpha amylase (Amy2) and T-complex protein 1 (Cct3) were exclusively identified in APP/PS1 mice at 2- and 6-months of age, respectively, while caveolae-

associated protein 1 (Cavin1), solute carrier family 2 facilitated glucose transporter member 3 (Slc2a3), bile acyl-CoA synthetase (Slc27a5) and NADPH-cytochrome P450 reductase (Por) were uniquely identified in WT mice and were completely absent in the corona samples recovered from 12-month old APP-PS1 mice (**Fig. 2c-e**). The 'presence' or 'absence' of certain proteins from the coronas formed in AD mice in comparison to control mice of the same age indicate clear differences between the two groups which further supports our hypothesis that blood-circulating liposomes can capture molecular changes indicative of AD pathophysiology.

**[0247]** Given the co-occurrence of numerous systemic abnormalities in AD, monitoring of multiple blood analytes is needed to collectively reflect AD-related processes in the brain. Distribution of the corona proteins identified by statistical significance and magnitude of change revealed that while the majority of differentially abundant proteins were upregulated in APP/PS1 mice in comparison to WT mice at the 2-month time point, 97 out of 105 differentially abundant proteins were found to be downregulated at the 12-month time point (**Fig. 2c-e**).

**[0248]** The above observation prompted us to further investigate the kinetics of the blood alterations as the disease progresses. A closer comparison between the 2- and 12- month time points revealed 31 common proteins with differential abundance between APP/PS1 and WT mice, of which 26 were upregulated at the earliest time point and gradually became downregulated, as pathophysiological changes culminated in cognitive impairment (**Fig. 2f** and **Fig. 2h**). Interestingly, this downregulation effect was observed for the majority of the upregulated proteins identified at the 2-month time point. As illustrated in **Fig. 2g**, the progressive increase of amyloid plaque deposits in the cortex and hippocampus of APP/PS1 mice resulted in the gradual reduction in the blood concentration of the proteins upregulated at 2-months. This explains the significantly lower number (n= 30) of differentially abundant proteins identified at the intermediate stages of AD progression and indicates that the development of brain amyloidopathy is systemically mirrored beyond the brain.

#### *Discussion of results*

**[0249]** To prove our hypothesis, the nanoparticle-recovered blood proteome was subjected to LC-MS/MS analysis. The subsequent comparison of the resultant protein coronas, formed in APP/PS1 and WT control mice, revealed the identification of multiple disease-specific signatures in blood, even at the earliest time point (before A $\beta$  plaque deposition). The advantage of using this nanoparticle-enrichment approach was demonstrated by the significantly higher number of differentially abundant proteins identified by the analysis of the corona samples in comparison to plasma control analysis (**Fig. 2a** and **b**). The distinct proteomic fingerprints observed at the three different time points of

investigation (before and after plaque formation and cognitive impairment; **Fig. 2c-e**), suggest a clear connection between the nanoparticle-harvested proteome and the disease development in the brain. **[0250]** Remarkably, although the majority of differentially abundant proteins were found to be upregulated in APP/PS1 mice in comparison to WT mice at the asymptomatic stage, 97 out of the 105 differentially abundant proteins identified at the late symptomatic stage were found to be downregulated (**Fig. 2f**). Prompted by the above observation, we monitored the temporal evolution of the differentially abundant proteins identified at the earliest time point and our data revealed an overall downregulation effect with disease progression (**Fig. 2g**). The disease stage-dependent fluctuation of the AD-specific blood proteome observed, could explain the lack of reproducibly identified blood-based biomarkers for AD.[7]

### **Example 3 – systemic monitoring of AD progression**

**[0251]** The above proteomic comparison between the liposomal coronas formed in APP/PS1 and WT mice suggested that intravenously administered nanoparticles can capture numerous systemic signatures that reflect AD-related processes in the brain even at the onset of AD. To assess the enrichment of AD-monitoring proteomic signatures in blood, that could distinguish the asymptomatic phase from mild amyloidopathy and cognitive deterioration, we further investigated the temporal evolution of corona formation in APP/PS1 mice at 2-, 6- and 12 months of age, by statistically comparing the respective corona profiles.

**[0252]** As illustrated in the Volcano plots of **Fig. 3a and 3b**, proteomic comparison of the ‘diseased’ coronas at the three different time points revealed statistically significant differences. While 75 proteins were differentially abundant between the 2- and 6- month time points (**Fig. 3a**), 71 proteins were found to be differentially abundant between the 6- and 12- month time points (**Fig. 3b**). It should be noted that ‘healthy’ corona profiles were also compared between WT mice of different age as a control, in order to exclude any ageing-related differences and to identify only AD-specific monitoring proteins.

**[0253]** To gain some further understanding of the protein binding kinetics as AD progresses, we classified the above differentially abundant proteins into three groups according to the fluctuation of their normalized protein abundance value over time: a) proteins with increased abundance with AD progression, b) proteins with decreased abundance with AD progression, and c) proteins characterized by lower abundance at the pre-diseased state (2 months) and at later time points (12 months), but displaying peak abundance at the intermediate state (6 months) or vice versa. As depicted in **Fig. 3c**, the majority of the disease-altered proteins identified, displayed decreased plasma levels with disease progression, which in some cases resulted in the complete absence of

these proteins from the corona samples recovered from 12-month old APP/PS1 mice. It should be noted that the majority of downregulated and upregulated proteins displayed a linearly-altered abundance with disease progression (**Fig. 3c**).

**[0254]** Among the above AD-stage specific protein signals, we identified a group of 18 proteins which could differentiate not only the pre-diseased state from mild plaque burden but could also discriminate cognitive deterioration (**Fig. 3d**). Fourteen out of the eighteen proteins displayed a gradual reduction in their abundance with disease progression, while only 3 exhibited increased abundance. Interestingly, inverse peak-shaped abundance kinetics were observed for properdin (cfp), a component of the alternative complement pathway previously associated with plaque deposition in transgenic mouse models of AD (**Fig. 3d**).[23]

**[0255]** Collectively, our findings here reveal AD stage-specific alterations of the plasma proteome. The complex kinetics of the plasma proteome observed (**Fig. 3c**), suggest a direct connection between the brain neurodegeneration and the blood proteome, which necessitates the need for longitudinal rather than cross sectional biomarker discovery studies.

**[0256]** The full list of blood proteins identified by Progenesis analysis to be differentially abundant between 2- and 6-months old APP/PS1 mice and between 6- and 12-months old APP/PS1 mice are shown in **Tables 10 and 11** respectively.

**[0257]** **Table 10:** Full list of blood proteins identified by Progenesis analysis to be differentially abundant between 2- and 6-months old APP/PS1 mice.

| Description                                                                             | Accession  | Anova<br>(p value) | Max fold<br>change |
|-----------------------------------------------------------------------------------------|------------|--------------------|--------------------|
| <b>UPREGULATED (n= 10)</b>                                                              |            |                    |                    |
| HC protein OS=Mus musculus GN=HC PE=2 SV=1                                              | A0A0C6E3V3 | 8.39E-06           | Infinity           |
| Immunoglobulin heavy variable V1-43 OS=Mus musculus<br>GN=Ighv1-43 PE=4 SV=1            | A0A075B5V7 | 9.00E-06           | 2.12               |
| Thyroxine-binding globulin OS=Mus musculus GN=Serpina7<br>PE=2 SV=1                     | THBG       | 2.18E-04           | Infinity           |
| Uncharacterized protein OS=Mus musculus PE=2 SV=1                                       | Q2TAW9     | 2.42E-03           | 17.22              |
| Complement component C8 alpha chain OS=Mus musculus<br>GN=C8a PE=1 SV=1                 | CO8A       | 1.14E-02           | 3.60               |
| IgG1 heavy chain VDJ region (Fragment) OS=Mus musculus<br>PE=2 SV=1                     | X5J4T6     | 1.78E-02           | 46.85              |
| Serum amyloid A-4 protein OS=Mus musculus GN=Saa4 PE=1<br>SV=2                          | SAA4       | 2.24E-02           | 1.94               |
| Uncharacterized protein (Fragment) OS=Mus musculus<br>GN=Rbp4 PE=2 SV=1                 | Q3TF08     | 3.03E-02           | 5.85               |
| Phospholipid transfer protein OS=Mus musculus GN=Pltp PE=1<br>SV=1                      | PLTP       | 3.91E-02           | 1.95               |
| IgA heavy chain variable region (Fragment) OS=Mus musculus<br>PE=2 SV=1                 | K7THF9     | 4.13E-02           | 19.88              |
| <b>DOWNREGULATED (n= 65)</b>                                                            |            |                    |                    |
| Immunoglobulin heavy variable V1-11 (Fragment) OS=Mus<br>musculus GN=Ighv1-11 PE=4 SV=1 | A0A0A6YWI9 | 3.57E-02           | 2.71               |

|                                                                                                                                               |            |          |       |
|-----------------------------------------------------------------------------------------------------------------------------------------------|------------|----------|-------|
| Uncharacterized protein OS=Mus musculus GN=Serinc3 PE=2 SV=1                                                                                  | Q3TWZ3     | 3.63E-02 | 4.22  |
| Uncharacterized protein OS=Mus musculus GN=Hspa5 PE=2 SV=1                                                                                    | Q3TI47     | 3.64E-02 | 2.40  |
| Immunoglobulin kappa variable 8-34 (Fragment) OS=Mus musculus GN=Igkv8-34 PE=4 SV=4                                                           | A0A0G2JDU0 | 3.69E-02 | 9.15  |
| 14-3-3 protein zeta/delta OS=Mus musculus GN=Ywhaz PE=1 SV=1                                                                                  | 1433Z      | 3.83E-02 | 5.45  |
| Tenascin OS=Mus musculus GN=Tnc PE=1 SV=1                                                                                                     | TENA       | 3.93E-02 | 1.87  |
| Ig heavy chain V region 6.96 OS=Mus musculus PE=4 SV=1                                                                                        | HVM57      | 4.02E-02 | 3.06  |
| Acylamino-acid-releasing enzyme (Fragment) OS=Mus musculus GN=Apeh PE=1 SV=1                                                                  | A0A0R4J107 | 4.05E-02 | 2.31  |
| Early endosome antigen 1 OS=Mus musculus GN=Eea1 PE=1 SV=2                                                                                    | EEA1       | 4.11E-02 | 2.76  |
| Proteasome subunit beta type-4 OS=Mus musculus GN=Psmb4 PE=1 SV=1                                                                             | PSB4       | 4.22E-02 | 1.62  |
| Proteasome subunit alpha type-3 OS=Mus musculus GN=Pma3 PE=1 SV=3                                                                             | PSA3       | 4.31E-02 | 1.68  |
| Protein disulfide-isomerase OS=Mus musculus GN=P4hb PE=1 SV=2                                                                                 | PDIA1      | 4.35E-02 | 3.73  |
| Cytochrome P450 1A2 OS=Mus musculus GN=Cyp1a2 PE=1 SV=1                                                                                       | CP1A2      | 4.40E-02 | 13.40 |
| Annexin A5 OS=Mus musculus GN=Anxa5 PE=1 SV=1                                                                                                 | ANXA5      | 4.40E-02 | 3.74  |
| Collagen, type VI, alpha 3 OS=Mus musculus GN=Col6a3 PE=1 SV=1                                                                                | A0A087WS16 | 4.47E-02 | 3.85  |
| Protein disulfide-isomerase A3 OS=Mus musculus GN=Pdia3 PE=1 SV=2                                                                             | PDIA3      | 4.55E-02 | 4.50  |
| Immunoglobulin heavy variable V3-4 (Fragment) OS=Mus musculus GN=Ighv3-4 PE=4 SV=1                                                            | A0A0A6YXF1 | 4.57E-02 | 6.85  |
| MCG23377, isoform CRA_b OS=Mus musculus GN=Gm8797 PE=4 SV=1                                                                                   | A0A0A6YW67 | 4.61E-02 | 2.58  |
| Immunoglobulin heavy variable 1-39 (Fragment) OS=Mus musculus GN=Ighv1-39 PE=4 SV=1                                                           | A0A075B5V5 | 4.64E-02 | 2.27  |
| Tyrosine 3-monooxygenase/tryptophan 5-monooxygenase activation protein, epsilon polypeptide, isoform CRA_c OS=Mus musculus GN=Ywhae PE=1 SV=1 | Q5SS40     | 4.80E-02 | 4.77  |
| Collagen alpha-1(VI) chain OS=Mus musculus GN=Col6a1 PE=1 SV=1                                                                                | CO6A1      | 4.90E-02 | 6.45  |
| Clathrin heavy chain 1 OS=Mus musculus GN=Cltc PE=1 SV=3                                                                                      | CLH1       | 4.93E-02 | 3.10  |
| Proteasome subunit beta type-6 OS=Mus musculus GN=Psmb6 PE=1 SV=3                                                                             | PSB6       | 4.94E-02 | 1.70  |

**[0258] Table 11:** Full list of blood proteins identified by Progenesis analysis to be differentially abundant between 6- and 12-months old APP/PS1 mice.

| Description                                                                                     | Accession | Anova (p value) | Max fold change |
|-------------------------------------------------------------------------------------------------|-----------|-----------------|-----------------|
| <b>UPREGULATED (n= 22)</b>                                                                      |           |                 |                 |
| Zinc finger protein 280D (Fragment) OS=Mus musculus GN=Zfp280d PE=1 SV=1                        | V9GXF6    | 4.36E-04        | 2.62            |
| CD5 antigen-like OS=Mus musculus GN=Cd5l PE=1 SV=3                                              | CD5L      | 1.85E-03        | 2.74            |
| MRP5 (Fragment) OS=Mus musculus GN=Ighv9-4 PE=2 SV=1                                            | Q925S1    | 2.31E-03        | 6.21            |
| Translation initiation factor IF-3, mitochondrial (Fragment) OS=Mus musculus GN=Mtif3 PE=1 SV=1 | D3YWL2    | 2.51E-03        | 43.85           |

|                                                                                                   |            |          |          |
|---------------------------------------------------------------------------------------------------|------------|----------|----------|
| Fibrinogen gamma chain OS=Mus musculus GN=Fgg PE=1 SV=1                                           | FIBG       | 2.54E-03 | 2.46     |
| Fibrinogen beta chain OS=Mus musculus GN=Fgb PE=1 SV=1                                            | FIBB       | 2.99E-03 | 2.95     |
| Immunoglobulin heavy variable 1-69 OS=Mus musculus GN=Ighv1-69 PE=4 SV=1                          | A0A075B5X7 | 4.17E-03 | 25.59    |
| Fibrinogen alpha chain OS=Mus musculus GN=Fga PE=1 SV=1                                           | FIBA       | 6.17E-03 | 2.44     |
| Casein kinase II subunit alpha' (Fragment) OS=Mus musculus GN=Csnk2a2 PE=4 SV=1                   | A0A1D5RLQ2 | 7.08E-03 | 33.06    |
| Immunoglobulin kappa variable 4-80 (Fragment) OS=Mus musculus GN=Igkv4-80 PE=4 SV=2               | A0A075B5L7 | 1.21E-02 | 3.75     |
| Glycogen phosphorylase, muscle form OS=Mus musculus GN=Pygm PE=1 SV=3                             | PYGM       | 1.36E-02 | 8.03     |
| Major vault protein OS=Mus musculus GN=Mvp PE=1 SV=4                                              | MVP        | 1.42E-02 | 2.62     |
| Serine palmitoyltransferase 2 OS=Mus musculus GN=Sptlc2 PE=1 SV=2                                 | SPTC2      | 1.51E-02 | 4.25     |
| MCG140437, isoform CRA_d OS=Mus musculus GN=Myh2 PE=1 SV=1                                        | G3UW82     | 1.82E-02 | 1.87     |
| Intraflagellar transport protein 172 homolog (Fragment) OS=Mus musculus GN=Ifit172 PE=1 SV=1      | A0A0J9YU41 | 2.34E-02 | 15.28    |
| Anti-norfloxacin MAb light chain variable region (Fragment) OS=Mus musculus PE=2 SV=1             | A0A0M3RM39 | 2.52E-02 | 42.06    |
| Low density lipoprotein receptor-related protein 1 OS=Mus musculus GN=Lrp1 PE=1 SV=1              | A0A0R4J0I9 | 2.86E-02 | 1.65     |
| Properdin OS=Mus musculus GN=Cfp PE=2 SV=2                                                        | PROP       | 3.16E-02 | 1.91     |
| Uncharacterized protein OS=Mus musculus PE=2 SV=1                                                 | Q2TAW9     | 3.76E-02 | 8.26     |
| Protein-glutamine gamma-glutamyltransferase 2 OS=Mus musculus GN=Tgm2 PE=1 SV=4                   | TGM2       | 3.77E-02 | 4.33     |
| Anti-H5N1 hemagglutinin monoclonal antibody H5M9 heavy chain (Fragment) OS=Mus musculus PE=1 SV=1 | U5LP42     | 4.44E-02 | 6.03     |
| Haptoglobin OS=Mus musculus GN=Hp PE=1 SV=1                                                       | HPT        | 4.54E-02 | 10.76    |
| <b>DOWNREGULATED (n= 49)</b>                                                                      |            |          |          |
| T-complex protein 1 subunit gamma OS=Mus musculus GN=Cct3 PE=1 SV=1                               | TCPG       | 5.66E-05 | Infinity |
| Kell blood group glycoprotein homolog OS=Mus musculus GN=Kel PE=1 SV=1                            | KELL       | 1.62E-03 | 5.38     |
| Immunoglobulin heavy variable 7-1 (Fragment) OS=Mus musculus GN=Ighv7-1 PE=1 SV=2                 | A0A075B5S2 | 2.41E-03 | 2.01     |
| Proteasome subunit alpha type-6 OS=Mus musculus GN=PsmA6 PE=1 SV=1                                | PSA6       | 3.37E-03 | 2.02     |
| Transferrin receptor protein 1 OS=Mus musculus GN=Tfrc PE=1 SV=1                                  | TFR1       | 5.94E-03 | 1.88     |
| WD repeat-containing protein 90 OS=Mus musculus GN=Wdr90 PE=1 SV=1                                | H7BX49     | 6.15E-03 | 7.75     |
| Proteasome subunit beta type-3 OS=Mus musculus GN=PsmB3 PE=1 SV=1                                 | PSB3       | 6.30E-03 | 2.67     |
| Serpin OS=Mus musculus GN=Srgn PE=1 SV=1                                                          | SRGN       | 6.31E-03 | 4.01     |
| Proteasome subunit alpha type OS=Mus musculus GN=PsmA7 PE=2 SV=1                                  | Q3TN31     | 8.31E-03 | 2.16     |
| Peroxiredoxin-2 OS=Mus musculus GN=Prdx2 PE=1 SV=3                                                | PRDX2      | 1.01E-02 | 4.24     |
| Heat shock 70 kDa protein 4 OS=Mus musculus GN=Hspa4 PE=1 SV=1                                    | Q3U2G2     | 1.06E-02 | 2.75     |
| 26S proteasome non-ATPase regulatory subunit 3 OS=Mus musculus GN=PsmD3 PE=1 SV=3                 | PSMD3      | 1.25E-02 | 4.08     |
| Proteasome subunit beta type-6 OS=Mus musculus GN=PsmB6 PE=1 SV=3                                 | PSB6       | 1.25E-02 | 2.17     |

|                                                                                                                                                    |            |          |       |
|----------------------------------------------------------------------------------------------------------------------------------------------------|------------|----------|-------|
| Bifunctional purine biosynthesis protein PURH OS=Mus musculus GN=Atic PE=1 SV=2                                                                    | PUR9       | 1.31E-02 | 3.79  |
| Uncharacterized protein OS=Mus musculus GN=Psmc5 PE=2 SV=1                                                                                         | A0A1S6GWH1 | 1.32E-02 | 23.17 |
| Glyceraldehyde-3-phosphate dehydrogenase OS=Mus musculus GN=Gapdh PE=1 SV=1                                                                        | A0A0A0MQF6 | 1.49E-02 | 2.46  |
| Vimentin OS=Mus musculus GN=Vim PE=1 SV=1                                                                                                          | A0A0A6YWC8 | 1.57E-02 | 2.37  |
| Clusterin OS=Mus musculus GN=Clu PE=1 SV=1                                                                                                         | CLUS       | 1.59E-02 | 1.88  |
| Tripeptidyl-peptidase 2 OS=Mus musculus GN=Tpp2 PE=1 SV=3                                                                                          | TPP2       | 1.74E-02 | 2.26  |
| Thyrotropin-releasing hormone-degrading ectoenzyme OS=Mus musculus GN=Trhde PE=1 SV=1                                                              | TRHDE      | 1.78E-02 | 9.31  |
| EG214403 protein OS=Mus musculus GN=Gm4788 PE=2 SV=1                                                                                               | B2RUG5     | 1.82E-02 | 2.61  |
| Immunoglobulin heavy variable V1-11 (Fragment) OS=Mus musculus GN=Ighv1-11 PE=4 SV=1                                                               | A0A0A6YWI9 | 1.92E-02 | 4.34  |
| Proteasome subunit alpha type-3 OS=Mus musculus GN=Pasma3 PE=1 SV=3                                                                                | PSA3       | 2.16E-02 | 1.68  |
| Acylamino-acid-releasing enzyme (Fragment) OS=Mus musculus GN=Apeh PE=1 SV=1                                                                       | A0A0R4J107 | 2.51E-02 | 10.22 |
| 26S proteasome non-ATPase regulatory subunit 6 (Fragment) OS=Mus musculus GN=Psmc6 PE=1 SV=1                                                       | A0A286YDW8 | 2.75E-02 | 6.07  |
| Proteasome subunit alpha type OS=Mus musculus GN=Pasma5 PE=2 SV=1                                                                                  | Q3TUI9     | 2.77E-02 | 2.95  |
| Uncharacterized protein OS=Mus musculus GN=Epb42 PE=2 SV=1                                                                                         | Q3TYT5     | 2.86E-02 | 40.43 |
| Carboxypeptidase N catalytic chain OS=Mus musculus GN=Cpn1 PE=1 SV=1                                                                               | CBPN       | 3.11E-02 | 1.71  |
| Integrin alpha 2b OS=Mus musculus GN=Itga2b PE=2 SV=1                                                                                              | B2RPR7     | 3.13E-02 | 2.41  |
| 26S proteasome non-ATPase regulatory subunit 2 OS=Mus musculus GN=Psmc2 PE=1 SV=1                                                                  | PSMD2      | 3.19E-02 | 2.21  |
| Uncharacterized protein OS=Mus musculus GN=Thbs1 PE=2 SV=1                                                                                         | Q3TR40     | 3.27E-02 | 4.69  |
| Ig heavy chain V region 3-6 OS=Mus musculus GN=Ighv3-6 PE=1 SV=1                                                                                   | HVM60      | 3.31E-02 | 5.12  |
| Galectin-3-binding protein OS=Mus musculus GN=Lgals3bp PE=1 SV=1                                                                                   | LG3BP      | 3.32E-02 | 2.93  |
| 26S proteasome regulatory subunit 10B OS=Mus musculus GN=Psmc6 PE=1 SV=1                                                                           | PRS10      | 3.44E-02 | 5.81  |
| Myosin-9 OS=Mus musculus GN=Myh9 PE=1 SV=4                                                                                                         | MYH9       | 3.50E-02 | 3.36  |
| Glucosidase 2 subunit beta OS=Mus musculus GN=Prkcsh PE=1 SV=1                                                                                     | GLU2B      | 3.63E-02 | 6.90  |
| Dihydrolipoyllysine-residue succinyltransferase component of 2-oxoglutarate dehydrogenase complex, mitochondrial OS=Mus musculus GN=Dist PE=1 SV=1 | ODO2       | 3.83E-02 | 5.83  |
| Erythrocyte band 7 integral membrane protein OS=Mus musculus GN=Stom PE=1 SV=3                                                                     | STOM       | 3.93E-02 | 6.71  |
| Lumican OS=Mus musculus GN=Lum PE=1 SV=2                                                                                                           | LUM        | 4.00E-02 | 2.94  |
| Complement component 7 OS=Mus musculus GN=C7 PE=1 SV=2                                                                                             | D3YXF5     | 4.01E-02 | 2.35  |
| RIKEN cDNA 1300017J02 gene OS=Mus musculus GN=1300017J02Rik PE=1 SV=1                                                                              | D3YY36     | 4.10E-02 | 2.99  |
| Inter-alpha-trypsin inhibitor heavy chain H2 OS=Mus musculus GN=Itih2 PE=1 SV=1                                                                    | ITIH2      | 4.11E-02 | 1.66  |
| Epidermal growth factor receptor OS=Mus musculus GN=Egfr PE=1 SV=1                                                                                 | EGFR       | 4.12E-02 | 3.06  |
| Uroplakin-3b OS=Mus musculus GN=Upk3b PE=1 SV=1                                                                                                    | A0A0R4J0S8 | 4.22E-02 | 2.01  |

|                                                                                            |            |          |       |
|--------------------------------------------------------------------------------------------|------------|----------|-------|
| Metalloproteinase inhibitor 3 OS=Mus musculus GN=Timp3 PE=1 SV=1                           | TIMP3      | 4.41E-02 | 6.47  |
| Large neutral amino acids transporter small subunit 3 OS=Mus musculus GN=Slc43a1 PE=1 SV=1 | A2ATS4     | 4.62E-02 | 31.86 |
| Aminopeptidase N (Fragment) OS=Mus musculus GN=Anpep PE=1 SV=1                             | A0A0U1RNS3 | 4.62E-02 | 2.47  |
| T-complex protein 1 subunit alpha OS=Mus musculus GN=Tcp1 PE=1 SV=3                        | TCPA       | 4.64E-02 | 3.95  |
| Immunoglobulin heavy variable 1-52 OS=Mus musculus GN=Ighv1-52 PE=4 SV=1                   | A0A075B5W2 | 4.79E-02 | 2.19  |

### Discussion of results

**[0259]** Furthermore, to identify blood protein signatures that could systemically monitor amyloid burden and cognitive impairment, we statistically compared the blood proteome fingerprints recovered from APP/PS1 mice of 2-, 6- and 12-months of age. We identified three kinetically-defined groups of disease-monitoring proteins: a) proteins with increased abundance with AD progression; b) proteins with decreased abundance with AD progression; and c) proteins characterized by lower abundance at the pre-diseased state (2 months) and at later time points (12 months), but displaying peak abundance at the intermediate state (6 months) or vice versa (**Fig. 3c**). Interestingly, the majority of disease-monitoring proteins identified displayed decreased plasma levels with AD progression (**Fig. 3c and d**). These findings could be explained by the increased permeability of the BBB and subsequent translocation of blood proteins into the brain tissue and/or by the compromised diffusion of molecules from the brain to the blood circulation, as a result of protein misfolding and aggregation.

**[0260]** The complex protein kinetics observed, suggest a direct connection between the blood proteome and brain neurodegeneration, which necessitates the need for longitudinal rather than cross sectional biomarker discovery studies.[25] Considering the comorbidities associated with neurodegeneration, it is now increasingly accepted that multiple biomarkers will be needed to provide adequate specificity and sensitivity for AD diagnosis. Our data demonstrate that the analysis of the nanoparticle protein corona has the potential to uncover and monitor the kinetics of multiple molecules in blood that reflect AD-related processes in the brain.

### Example 4 - Molecular pathway enrichment analysis

**[0261]** In order to gain some insight into the molecular pathways that were activated systemically in response to amyloidopathy, we performed pathway enrichment analysis for all the differentially abundant proteins identified between APP/PS1 and WT mice, using the Enrichr's analysis tool. Proteins were classified by Kyoto Encyclopaedia of Genes & Genomes (KEGG) database. As shown in **Table 12**, the identified differentially abundant proteins were found to act in 32 major pathways

(adjusted  $p$  value  $< 0.05$ ). Proteasome ( $p$  value= $5.29E-15$ ), focal adhesion ( $p$  value= $3.50E-13$ ), phagosome ( $p$  value= $1.27E-10$ ), ECM-receptor interaction ( $p$  value= $3.89E-09$ ), PPAR signaling ( $p$  value= $3.80E-07$ ), regulation of actin cytoskeleton ( $p$  value= $6.78E-07$ ), PI3K-Akt signalling ( $p$  value= $9.73E-07$ ), hematopoietic cell lineage ( $p$  value= $3.04E-06$ ), leukocyte transendothelial migration ( $p$  value= $8.91E-06$ ) and proteoglycans ( $p$  value= $1.68E-05$ ) were found to be the 10 most significantly enriched pathways (**Fig. 4a and b**).

**[0262]** Moreover, the following five proteins were found to be directly involved in the AD KEGG pathway (**Fig. 4a**), namely glyceraldehyde-3-phosphate dehydrogenase (GAPDH), disintegrin and metalloproteinase domain-containing protein 10 (ADAM10), ATP synthase subunit beta, mitochondrial (ATP5B), sarcoplasmic/endoplasmic reticulum calcium ATPase 2 (ATP2A2) and ATP synthase subunit alpha, mitochondrial (ATP5A1). All five proteins were identified to be downregulated in APP/PS1 mice in comparison to WT mice at the 12-month time point and three of them (ADAM10, ATP5B, ATP2A2) were also found to be upregulated at the 2-month time point (**Tables 6 and 7**).

**[0263]** As illustrated in **Fig.4a**, the majority of proteins associated with the 10 most enriched pathways displayed a decreased abundance with disease progression. Among the differentially abundant proteins, integrin beta-3 (Itgb3) and integrin beta-1 (Itgb1) were found to be the most frequently identified proteins, involved in 7 out of the 10 most enriched pathways. Interestingly, cellular component enrichment analysis revealed that nine proteins were constituents of myelin sheath (ATP synthase subunit beta, mitochondrial, ATP5B; ATP synthase subunit alpha, mitochondrial, ATP5A1; calnexin, CANX; guanine nucleotide-binding protein G(I)/G(S)/G(T) subunit beta-1, GNB1; tubulin beta-4A chain, TUBB4A; ezrin, EZR; moesin, MSN; integrin beta-1, Itgb1 and heat shock protein HSP 90-alpha, HSP90AA1).

**[0264]** Based on the above analysis, a protein interaction network was constructed using the Gephi visualization platform. As illustrated in **Fig. 4b**, the focal adhesion pathway, previously identified to play a key role in synaptic plasticity and activity,[24] was identified as the central node with 18 differentially abundant proteins being involved.

**[0265]** **Table 12:** Molecular Pathway Enrichment Analysis. The 37 enriched pathways and the respective differentially abundant proteins involved according to Kyoto Encyclopaedia of Genes & Genomes (KEGG) database using the Enrichr analysis tool. The p-value is computed using the Fisher's exact test. The q-value is an adjusted p-value using the Benjamini-Hochberg method for correction for multiple hypotheses testing.

| Pathway name | Differentially abundant proteins | P-value | q-value |
|--------------|----------------------------------|---------|---------|
|              |                                  |         |         |

|                                                               |                                                                                                    |          |          |
|---------------------------------------------------------------|----------------------------------------------------------------------------------------------------|----------|----------|
| <b>Proteasome</b>                                             | PSMB6;PSMA6;PSMB4;PSMC5;PSMA3;PSMB5;PSMC6;PSMA4;PSMD14;PSMC3;PSMD2;PSMD1                           | 5.29E-15 | 1.63E-12 |
| <b>Focal adhesion</b>                                         | ITGB1;LAMA2;ACTN1;ITGB3;ITGA2B;FN1;ILK;LAMC1;THBS1;MYL12A;THBS4;COMP;RAP1B;RAC3;FLNA;FLNB;TLN1;VCL | 3.50E-13 | 5.38E-11 |
| <b>Phagosome</b>                                              | ITGB1;TFRC;ITGB3;ITGB2;IGH;CORO1A;THBS1;TUBB4A;THBS4;COMP;EEA1;CANX;CALR;CD36                      | 1.27E-10 | 1.30E-08 |
| <b>ECM-receptor interaction</b>                               | ITGB1;COMP;LAMA2;ITGB3;ITGA2B;FN1;LAMC1;CD36;THBS1;THBS4                                           | 3.89E-09 | 2.99E-07 |
| <b>PPAR signaling pathway</b>                                 | ACSL1;ADIPOQ;PLIN4;ILK;ACSL5;CD36;PLTP;SLC27A5                                                     | 3.80E-07 | 2.34E-05 |
| <b>Regulation of actin cytoskeleton</b>                       | ITGB1;ACTN1;ITGB3;ITGA2B;ITGB2;FN1;MSN;RAC3;MYH9;EZR;VCL;MYL12A                                    | 6.78E-07 | 3.48E-05 |
| <b>PI3K-Akt signaling pathway</b>                             | ITGB1;CSF1R;HSP90AA1;LAMA2;ANGPT1;ITGB3;ITGA2B;FN1;LAMC1;IGH;THBS1;THBS4;HSP90B1;COMP;GNB1         | 9.73E-07 | 4.28E-05 |
| <b>Hematopoietic cell lineage</b>                             | CSF1R;TFRC;ANPEP;ITGB3;ITGA2B;CD9;CD36;IGH                                                         | 3.04E-06 | 1.17E-04 |
| <b>Leukocyte transendothelial migration</b>                   | RAP1B;ITGB1;ACTN1;ITGB2;MSN;EZR;VCL;MYL12A                                                         | 8.91E-06 | 3.05E-04 |
| <b>Proteoglycans in cancer</b>                                | ITGB1;ITGB3;FN1;MSN;FLNA;FLNB;EZR;ANK1;THBS1;DCN                                                   | 1.68E-05 | 5.19E-04 |
| <b>Rap1 signaling pathway</b>                                 | RAP1B;ITGB1;CSF1R;ANGPT1;ITGB3;ITGA2B;ITGB2;RAC3;TLN1;THBS1                                        | 2.08E-05 | 5.83E-04 |
| <b>Amoebiasis</b>                                             | LAMA2;ACTN1;ITGB2;FN1;LAMC1;IGH;VCL                                                                | 2.87E-05 | 7.38E-04 |
| <b>Malaria</b>                                                | COMP;ITGB2;CD36;THBS1;THBS4                                                                        | 8.36E-05 | 1.98E-03 |
| <b>Platelet activation</b>                                    | RAP1B;ITGB1;ITGB3;ITGA2B;TLN1;MYL12A;FERMT3                                                        | 1.46E-04 | 3.22E-03 |
| <b>Dilated cardiomyopathy (DCM)</b>                           | ITGB1;LAMA2;ITGB3;ITGA2B;ATP2A2;IGH                                                                | 1.89E-04 | 3.89E-03 |
| <b>Arrhythmogenic right ventricular cardiomyopathy (ARVC)</b> | ITGB1;LAMA2;ITGB3;ITGA2B;ATP2A2                                                                    | 5.17E-04 | 9.96E-03 |
| <b>Epstein-Barr virus infection</b>                           | PSMC5;PSMC6;PSMD14;PSMC3;PSMD2;PSMD1;CALR;IGH                                                      | 5.40E-04 | 9.79E-03 |
| <b>Bacterial invasion of epithelial cells</b>                 | ITGB1;CLTC;FN1;ILK;VCL                                                                             | 5.87E-04 | 1.00E-02 |
| <b>Antigen processing and presentation</b>                    | HSP90AA1;HSPA5;HSPA4;CANX;CALR                                                                     | 7.04E-04 | 1.14E-02 |
| <b>Complement and coagulation cascades</b>                    | ITGB2;MASP2;MASP1;CLU;F5                                                                           | 7.92E-04 | 1.22E-02 |
| <b>Human papillomavirus infection</b>                         | COMP;ITGB1;LAMA2;ITGB3;ITGA2B;UBR4;FN1;LAMC1;THBS1;THBS4                                           | 9.54E-04 | 1.40E-02 |
| <b>Tight junction</b>                                         | ITGB1;HSPA4;ACTN1;MSN;MYH9;EZR;MYL12A                                                              | 9.84E-04 | 1.38E-02 |
| <b>Hypertrophic cardiomyopathy (HCM)</b>                      | ITGB1;LAMA2;ITGB3;ITGA2B;ATP2A2                                                                    | 1.10E-03 | 1.48E-02 |

|                                             |                                         |          |          |
|---------------------------------------------|-----------------------------------------|----------|----------|
| Small cell lung cancer                      | ITGB1;LAMA2;ITGA2B;FN1;LAMC1            | 1.65E-03 | 2.11E-02 |
| Fluid shear stress and atherosclerosis      | HSP90AA1;ITGB3;ITGA2B;RAC3;ASS1;HSP90B1 | 1.78E-03 | 2.19E-02 |
| Viral myocarditis                           | LAMA2;ITGB2;RAC3;IGH                    | 2.09E-03 | 2.47E-02 |
| Ribosome                                    | RPS9;RPL14;RPL15;RPL18;RPL6;RPL7        | 2.88E-03 | 3.28E-02 |
| Staphylococcus aureus infection             | ITGB2;MASP2;MASP1;IGH                   | 3.50E-03 | 3.85E-02 |
| Glycolysis / Gluconeogenesis                | LDHA;DLAT;BPGM;GAPDH                    | 3.50E-03 | 3.72E-02 |
| Adipocytokine signaling pathway             | ACSL1;ADIPOQ;ACSL5;CD36                 | 3.69E-03 | 3.79E-02 |
| Protein processing in endoplasmic reticulum | HSP90AA1;HSPA5;PRKCSH;CANX;CALR;HSP90B1 | 4.17E-03 | 4.14E-02 |
| Starch and sucrose metabolism               | MGAM;UGP2;PYGM                          | 4.31E-03 | 4.15E-02 |

### Discussion of results

[0266] The connection between the brain tissue and blood during AD progression and the subsequent bidirectional transport of cells and proteins across the BBB are still poorly understood.[25] In addition to the accumulation of amyloid- $\beta$  plaques and degeneration of memory, recent high throughput genomic approaches revealed a complex network of dysregulated pathways in the brain, including mitochondrial dysfunction, deficits in glucose availability, neuronal damage, synapse loss and inflammatory activation of microglia and astrocytes.[24] In order to gain some insight into the underpinning mechanisms of brain amyloidopathy that are reflected in the blood proteome we performed molecular pathway enrichment analysis. Our results revealed the focal adhesion cascade as a central hub in the disease development (**Fig. 4a and b**). This finding is in line with the recently proposed genetic landscape of AD, in which dysfunction of the focal adhesion pathway and the related cell signalling are key elements in AD pathogenesis.[24] To the best of our knowledge, this is the first study to confirm the involvement of focal adhesion cascade proteins in AD progression at a systemic level.

[0267] The potential use of intravenously-injected nanoparticles to uncover information in the blood that is directly connected with the molecular cascade of neurodegeneration in the brain, was further exemplified by the identification of five proteins that are involved in the AD pathway namely glyceraldehyde-3-phosphate dehydrogenase (GAPDH), disintegrin and metalloproteinase domain-containing protein 10 (ADAM10), ATP synthase subunit beta, mitochondrial (ATP5B), sarcoplasmic/endoplasmic reticulum calcium ATPase 2 (ATP2A2) and ATP synthase subunit alpha, mitochondrial (ATP5A1) (**Fig. 4a**). It should be noted that a significant decrease of ADAM10 in AD cerebrospinal fluid and platelets and an upregulation of GAPDH in circulating leukocytes have been previously reported, however their use as potential plasma biomarkers has not been proposed and

requires further investigation.[26]. Despite the documented significant role of the above-mentioned proteins in AD development, their simultaneous recovery from plasma, dynamic monitoring and their potential value in systemically monitoring AD progression has not been previously shown.

## 5 **Example 5: Experimental Study using Human serum samples.**

### Materials and Methods

**[0268] Human serum samples.** Eligible cases for the study included patients with confirmed Alzheimer's disease (n=40; n=20 females, n=20 males). Age- and sex-matched serum control samples were also used.

10 **[0269] Preparation of liposomes:** HSPC:Chol:DSPE-PEG2000 (56.3:38.2:5.5) liposomes were prepared, by thin lipid film hydration method followed by extrusion. The physicochemical properties of the liposomes employed, were measured using Zetasizer Nano ZS (Malvern, Instruments, UK) and are shown in Figure 1e.

15 **[0270] Ex vivo protein corona formation.** The protein corona was allowed to form by adding 180 µL of 12.5 mM liposomes into 820 µL of human serum obtained from Alzheimer's disease patients (n=40) and controls (n=40). Liposomes interacted with plasma for 10 minutes at 37°C in orbital shaker (ThermoFisher, MaxQ™ 4450 Benchtop Orbital Shaker) at 250 rpm.

20 **[0271] Separation of corona-coated liposomes from unbound and weakly bound proteins.** Corona-coated liposomes were separated from excess plasma proteins by size exclusion chromatography followed by membrane ultrafiltration.

**[0272] Mass Spectrometry. Enzymatic digestion of corona proteins with suspension – trapping method (S – trap).** Digestion of corona proteins was performed prior to LC – MS/MS analysis by suspension – trapping (S-trap) method. Briefly, 10 µg of corona proteins were mixed overnight with 10 µL of S-trap lysis buffer (5% SDS, 50 mM triethylammonium bicarbonate TEAB pH 7.5) to allow protein solubilisation. Samples were reduced with 5 mM dithiothreitol (DTT, Fisher, UK) and alkylated with 15 mM iodoacetamide (IAM, Sigma Aldrich, UK). 5 mM of DTT were added again to the samples to quench the alkylation reaction and samples were then centrifuged at 14,000 rcf for 10 min. Protein lysates were collected to clean Eppendorf tubes and were acidified by adding 2.5 µL of 12% aqueous phosphoric acid. 165 µL of S-trap binding buffer (90% aqueous methanol containing a final concentration of 100 mM TEAB, pH 7.1) were subsequently added to the acidified protein lysates to form colloidal protein particulate. Samples were then loaded onto S-Trap columns (ProtiFi, LLC, USA) to allow the capture of the protein within the protein-trapping matrix of the column and were washed 4 times with S-trap binding buffer. Captured protein was then digested by 0.1 µg/µL trypsin at 47 °C for 1 hour and the digested peptides were collected in three elutions following the

25

30

sequential addition of 65  $\mu$ L of digestion buffer (50 mM TEAB), 65  $\mu$ L of 0.1% aqueous formic acid and 30  $\mu$ L of 30% aqueous acetonitrile containing 0.1% formic acid. Finally, peptide samples were desalted by oligo R3 beads in 50% acetonitrile, dried using a vacuum centrifuge (Heto Speedvac) and stored in the fridge until MS analysis. Liquid – chromatography tandem mass spectrometry (LC – MS/MS). Dried samples were reconstituted in 10  $\mu$ L 5% acetonitrile 0.1% formic acid and analysed by LC-MS/MS using an UltiMate® 3000 Rapid Separation LC (RSLC, Dionex Corporation, Sunnyvale, CA) coupled to a Q Exactive™ Hybrid Quadrupole-Orbitrap™ (ThermoFisher Scientific, Waltham, MA) mass spectrometer.

**[0273] LC – MS/MS data analysis.** MS data analysis was carried out by Progenesis QI for proteomics software tool (version 4.1; Nonlinear Dynamics, Waters). Briefly, RAW files were imported into the software and relative protein quantitation using Hi-N with N=3 peptides to measure per protein (Hi-3) was performed. A local Mascot server (Matrix Science, London, UK; version 2.7.) was used for the search of MS spectra against SwissProt\_2019\_10 fasta; Trembl\_2019\_10 databases (selected for Homo sapiens, 565254 entries). Progenesis generated tables with the normalised protein abundances in each sample and calculated the max fold-change and p value (ANOVA) of each identified protein.

## Results

**[0274]** In total 202 differentially abundant proteins with a *p* value <0.05 were identified, of which 127 were upregulated and 75 downregulated (Figure 5 and Table 13). Of these 202 proteins, 27 were considered promising as potential biomarkers for AD (Figure 6 and Table 6a).

**Table 13** - Full list of blood proteins identified by Progenesis analysis to be differentially abundant between patients with AD and control. Only proteins with *p*<0.05 are shown.

| Protein name                                             | Gene name | Accession Number | p-value  | Max fold change |
|----------------------------------------------------------|-----------|------------------|----------|-----------------|
| KIAA0100, isoform CRA_a                                  | KIAA0100  | K7EQ86           | 7.23E-08 | 2.28            |
| Immunoglobulin kappa joining 3 (Fragment)                | IGKJ3     | A0A0A0MT96       | 3.42E-07 | 361.76          |
| Anoctamin (Fragment)                                     | ANO7      | H7C220           | 1.34E-06 | 2.21            |
| Apolipoprotein E (Fragment)                              | APOE      | Q8TCZ8           | 8.65E-06 | 3.75            |
| Pancreas transcription factor 1 subunit alpha (Fragment) | PTF1A     | A0A1W2PRT5       | 1.89E-05 | 17.41           |
| MS-D2 light chain variable region                        |           | A0A0X9USL5       | 1.32E-04 | 1.84            |

|                                                                                                                   |                |            |          |        |
|-------------------------------------------------------------------------------------------------------------------|----------------|------------|----------|--------|
| Proprotein convertase subtilisin/kexin type 9                                                                     | PCSK9          | PCSK9      | 1.62E-04 | 9.11   |
| Superoxide dismutase                                                                                              |                | A0A140VJU8 | 1.75E-04 | 140.47 |
| Uncharacterized protein                                                                                           | DKFZp686K03196 | Q6N095     | 2.32E-04 | 2.12   |
| Pyruvate kinase                                                                                                   | PKM            | B4DNK4     | 3.27E-04 | 1.04   |
| Hepatocyte growth factor activator                                                                                | HGFAC          | HGFA       | 5.82E-04 | 1.63   |
| Plasma kallikrein (Fragment)                                                                                      | KLKB1          | H0YAC1     | 6.39E-04 | 1.54   |
| Latent-transforming growth factor beta-binding protein 1                                                          | LTBP1          | LTBP1      | 6.60E-04 | 1.77   |
| Cathepsin D                                                                                                       | CTSD           | A0A1B0GV23 | 7.00E-04 | 1.74   |
| Angiopoietin-like 6, isoform CRA_a                                                                                | ANGPTL6        | A0A024R7A9 | 8.64E-04 | 1.68   |
| Histone H1.5                                                                                                      | HIST1H1B       | H15        | 1.14E-03 | 2.72   |
| Catalase                                                                                                          | CAT            | CATA       | 1.38E-03 | 1.50   |
| Uncharacterized                                                                                                   |                | Q7Z2U7     | 1.63E-03 | 1.81   |
| V1-22 protein (Fragment)                                                                                          | V1-22          | Q5NV88     | 2.36E-03 | 2.24   |
| cDNA FLJ45477 fis, clone BRSTN2018692, moderately similar to Complement C1q tumor necrosis factor-related protein |                | B3KXI7     | 2.61E-03 | 2.59   |
| Proteoglycan 4, isoform CRA_a                                                                                     | PRG4           | A0A024R930 | 2.62E-03 | 1.54   |
| cDNA FLJ52761, highly similar to Actin, aortic smooth                                                             |                | B4DUI8     | 2.80E-03 | 26.61  |
| Adipocyte plasma membrane-associated protein                                                                      | APMAP          | APMAP      | 2.99E-03 | 1.49   |
| Leukocyte elastase inhibitor                                                                                      | SERPINB1       | ILEU       | 3.03E-03 | 5.88   |
| Anti-streptococcal/anti-myosin immunoglobulin kappa light chain variable region                                   |                | Q96SA9     | 3.31E-03 | 1.16   |
| Transmembrane protein 163                                                                                         | TMEM163        | TM163      | 3.87E-03 | 1.71   |
| Serpin peptidase inhibitor, clade C (Antithrombin), member 1, isoform CRA_a                                       | SERPINC1       | A0A024R944 | 3.91E-03 | 1.43   |
| Neurogenic locus notch homolog protein 3                                                                          | NOTCH3         | NOTC3      | 3.95E-03 | 1.62   |

|                                                                                |                |            |          |       |
|--------------------------------------------------------------------------------|----------------|------------|----------|-------|
| Leucine-rich alpha-2-glycoprotein                                              | LRG1           | A2GL       | 4.29E-03 | 1.27  |
| Myc target protein 1 (Fragment)                                                | MYCT1          | H0YDV5     | 4.82E-03 | 2.03  |
| IgGfc-binding protein                                                          | FCGBP          | FCGBP      | 5.70E-03 | 1.39  |
| Coagulation factor IX OS=Homo                                                  | F9 p22         | F2RM37     | 5.91E-03 | 1.46  |
| IGL@ protein                                                                   | IGL@           | Q6IN99     | 2.29E-02 | 1.50  |
| Heparan sulfate proteoglycan 2 (Perlecan), isoform CRA_b                       | HSPG2          | A0A024RAB6 | 6.65E-03 | 60.03 |
| Transforming growth factor, beta 1 (Camurati-Engelmann disease), isoform CRA_a | TGFB1          | A0A024R0P8 | 7.68E-03 | 1.53  |
| Uncharacterized protein DKFZp686K18196 (Fragment)                              | DKFZp686K18196 | Q6N092     | 7.88E-03 | 2.09  |
| cDNA FLJ41552 fis, clone COLON2004478, highly similar to Protein Tro alpha1    |                | Q6ZW64     | 7.88E-03 | 2.09  |
| Immunoglobulin lambda variable 10-54                                           | IGLV10-54      | A0A1W2PQ80 | 8.73E-03 | 1.27  |
| Histone                                                                        |                | A2RUA4     | 9.28E-03 | 59.08 |
| Podocalyxin                                                                    | PODXL          | PODXL      | 9.39E-03 | 1.36  |
| Coagulation factor XI                                                          | F11            | FA11       | 9.41E-03 | 1.70  |
| von Willebrand factor                                                          | VWF            | VWF        | 1.05E-02 | 1.43  |
| von Willebrand factor                                                          | VWF            | L8E853     | 1.05E-02 | 1.43  |
| Platelet factor 4 variant                                                      | PF4V1          | PF4V       | 1.07E-02 | 1.58  |
| Annexin                                                                        | ANXA2          | A0A024R5Z7 | 1.09E-02 | 1.42  |
| Immunoglobulin lambda variable 5-39                                            | IGLV5-39       | LV539      | 1.17E-02 | 3.26  |
| Uncharacterized                                                                |                | Q6DHW4     | 1.21E-02 | 1.58  |
| Protein tweety homolog                                                         | TTYH3          | A0A024R816 | 1.27E-02 | 1.63  |
| Fc of IgG low affinity IIIa receptor isoform 2 (Fragment)                      | FCGR3A         | A0A0S2Z3S7 | 1.36E-02 | 3.71  |
| Lysophospholipid acyltransferase 1                                             | MBOAT1         | MBOA1      | 1.54E-02 | 44.68 |

|                                                                                |                |            |          |       |
|--------------------------------------------------------------------------------|----------------|------------|----------|-------|
| Anti-Mpl scFv                                                                  |                | A2J423     | 1.75E-02 | 1.86  |
| Leukocyte surface antigen CD53                                                 | CD53           | CD53       | 1.84E-02 | 1.65  |
| Platelet factor 4                                                              | PF4            | PLF4       | 1.84E-02 | 1.51  |
| Cold agglutinin FS-1 L-chain                                                   |                | A2NB45     | 1.97E-02 | 3.05  |
| Mucin-2                                                                        | MUC2           | A0A0G2JM87 | 1.98E-02 | 6.83  |
| Extracellular glycoprotein lacritin                                            | LACRT          | LACRT      | 2.00E-02 | 19.70 |
| Lysozyme                                                                       | LYZ            | A0A0B4J259 | 2.19E-02 | 3.04  |
| Keratinocyte proline-rich protein                                              | KPRP           | KPRP       | 2.35E-02 | 1.76  |
| GRB2-related adapter protein                                                   | GRAP           | I3L2P9     | 2.57E-02 | 1.57  |
| Coiled-coil domain-containing protein 187                                      | CCDC187        | A0A075B733 | 2.65E-02 | 1.19  |
| NADH dehydrogenase [ubiquinone] 1 alpha subcomplex subunit 3 (Fragment)        | NDUFA3         | H7C2R1     | 2.81E-02 | 3.48  |
| Vimentin                                                                       | VIM            | VIME       | 2.86E-02 | 20.39 |
| cDNA FLJ39539 fis, clone PUAEN2008228, highly similar to Platelet glycoprotein |                | B3KUB8     | 2.99E-02 | 1.29  |
| ITIH4 protein                                                                  | ITIH4          | B7ZKJ8     | 3.01E-02 | 1.42  |
| Inter-alpha (Globulin) inhibitor H4 (Plasma Kallikrein-sensitive glycoprotein) | ITIH4          | B2RMS9     | 3.01E-02 | 1.42  |
| Fibrinogen alpha chain                                                         | FGA            | FIBA       | 3.06E-02 | 4.28  |
| Transcobalamin-1                                                               | TCN1           | TCO1       | 3.44E-02 | 1.17  |
| Immunoglobulin alpha-2 heavy                                                   |                | IGA2       | 3.57E-02 | 1.48  |
| Alpha-2-macroglobulin                                                          | A2M            | A2MG       | 3.82E-02 | 1.31  |
| Collagen alpha-1(II) chain                                                     | COL2A1         | CO2A1      | 4.59E-02 | 2.37  |
| Protein HEG homolog 1                                                          | HEG1           | HEG1       | 4.60E-02 | 1.36  |
| Uncharacterized protein DKFZp686O16217 (Fragment)                              | DKFZp686O16217 | Q6N041     | 4.75E-02 | 1.97  |
| IgG L                                                                          |                | S6B2A1     | 4.91E-02 | 1.06  |

|                                                     |          |            |          |        |
|-----------------------------------------------------|----------|------------|----------|--------|
| HRV Fab 027-VL                                      |          | A2IPI6     | 4.97E-02 | 1.92   |
| Platelet endothelial cell adhesion molecule         | PECAM1   | A0A075B738 | 5.00E-02 | 2.97   |
| Testicular secretory protein Li                     |          | A0A140VK24 | 3.55E-10 | 2.88   |
| Beta-2-glycoprotein I                               |          | D9IWP9     | 9.96E-09 | 2.81   |
| Hyaluronan-binding protein 2                        | HABP2    | HABP2      | 1.33E-08 | 2.33   |
| Apolipoprotein C-III                                | APOC3    | B0YIW2     | 1.02E-07 | 2.44   |
| Apolipoprotein A-II                                 | APOA2    | APOA2      | 2.95E-07 | 2.03   |
| Apolipoprotein B variant                            |          | Q59HB3     | 1.96E-06 | 2.62   |
| Serum paraoxonase/arylesterase 1                    | PON1     | PON1       | 4.27E-06 | 1.68   |
| Prenylcysteine oxidase 1                            | PCYOX1   | PCYOX      | 4.30E-06 | 1.68   |
| Apolipoprotein A-IV                                 | APOA4    | APOA4      | 6.81E-06 | 2.35   |
| Hemopexin                                           | HPX      | HEMO       | 8.25E-06 | 1.49   |
| Ankyrin repeat domain-containing protein 61         | ANKRD61  | ANR61      | 1.66E-05 | 1.46   |
| Protein piccolo                                     | PCLO     | PCLO       | 1.83E-05 | 123.21 |
| Alpha-1-antitrypsin                                 | SERPINA1 | A0A024R6I7 | 2.58E-05 | 1.65   |
| Alpha-1-antitrypsin                                 | SERPINA1 | A1AT       | 2.58E-05 | 1.65   |
| Glutathione peroxidase                              | GPX3     | A0A087X1J7 | 2.95E-05 | 2.14   |
| LINE-1 type transposase domain-containing protein 1 | L1TD1    | LITD1      | 5.50E-05 | 1.42   |
| GCT-A3 heavy chain variable region                  |          | A0A0X9TD88 | 7.23E-05 | 1.72   |
| Slit homolog 1 protein                              | SLIT1    | SLIT1      | 7.52E-05 | 2.86   |
| Anti-thyroglobulin heavy chain variable region      |          | Q8IZD7     | 8.38E-05 | 2.95   |
| Apolipoprotein B (Including Ag(X) antigen)          | APOB     | C0JYY2     | 1.16E-04 | 1.39   |
| Corticosteroid-binding globulin                     | SERPINA6 | CBG        | 1.37E-04 | 1.63   |
| Immunoglobulin kappa variable 4-1                   | IGKV4-1  | KV401      | 1.57E-04 | 1.68   |

|                                                          |            |            |          |      |
|----------------------------------------------------------|------------|------------|----------|------|
| Vitamin D-binding protein                                | GC         | D6RF35     | 2.16E-04 | 1.49 |
| Apolipoprotein A-V, isoform CRA_a                        | APOA5      | A0A0B4RUS7 | 2.34E-04 | 1.97 |
| Apolipoprotein A-I, isoform CRA_a                        | APOA1      | A0A024R3E3 | 2.55E-04 | 2.02 |
| Leucine-rich repeat-containing protein 7 (Fragment)      | LRRC7      | K7ELH5     | 2.59E-04 | 3.75 |
| Apolipoprotein D                                         | APOD       | APOD       | 3.02E-04 | 1.71 |
| Serum paraoxonase/lactonase 3                            | PON3       | PON3       | 3.66E-04 | 1.48 |
| Immunoglobulin heavy variable 4-38-2                     | IGHV4-38-2 | HVD82      | 3.67E-04 | 3.38 |
| Kelch-like protein 34                                    | KLHL34     | KLH34      | 3.97E-04 | 2.26 |
| Immunoglobulin heavy variable 3-64D                      | IGHV3-64D  | HV64D      | 3.97E-04 | 1.63 |
| Serum albumin                                            | ALB        | ALBU       | 3.99E-04 | 1.34 |
| cDNA FLJ53691, highly similar to                         |            | B4E1B2     | 4.05E-04 | 1.48 |
| Immunoglobulin heavy chain variable region               |            | Q0ZCI6     | 4.07E-04 | 1.72 |
| Tissue factor pathway inhibitor (Fragment)               | TFPI       | C9JKV3     | 4.24E-04 | 1.98 |
| Phosphatidylinositol-glycan-specific phospholipase D     | GPLD1      | PHLD       | 4.53E-04 | 1.60 |
| Selenoprotein P (Fragment)                               | SELENOP    | A0A182DWH7 | 6.38E-04 | 1.98 |
| Alpha-2-HS-glycoprotein                                  | AHSG       | FETUA      | 6.59E-04 | 1.43 |
| Anthrax toxin receptor 2                                 | ANTXR2     | A0A0C4DG84 | 7.09E-04 | 1.60 |
| GCT-A9 heavy chain variable region                       |            | A0A109PT03 | 7.61E-04 | 1.71 |
| cDNA FLJ55673, highly similar to Complement factor B (EC |            | B4E1Z4     | 7.63E-04 | 1.35 |
| Transthyretin                                            | TTR        | TTHY       | 9.73E-04 | 1.37 |
| NADH-ubiquinone oxidoreductase chain 5                   | ND5        | A0A059RS62 | 1.01E-03 | 2.10 |
| Epididymis luminal protein 180 (Fragment)                | HEL180     | B6EDE2     | 1.16E-03 | 2.33 |
| C-reactive protein                                       | CRP        | CRP        | 1.52E-01 | 1.33 |
| Transferrin variant                                      |            | Q53H26     | 1.44E-03 | 1.23 |

|                                                                                                    |             |            |          |       |
|----------------------------------------------------------------------------------------------------|-------------|------------|----------|-------|
| IgG H                                                                                              |             | S6C4S4     | 1.70E-03 | 1.91  |
| Immunoglobulin lambda variable 3-1                                                                 | IGLV3-1     | LV301      | 1.86E-03 | 2.63  |
| Single-chain Fv (Fragment)                                                                         | scFv        | Q65ZC9     | 2.23E-03 | 1.74  |
| Serpin peptidase inhibitor, clade A (Alpha-1 antiproteinase, antitrypsin), member 4, isoform CRA_a | SERPINA4    | A0A024R6I9 | 2.36E-03 | 1.63  |
| IGK@ protein                                                                                       | IGK@        | Q6P5S8     | 2.49E-03 | 2.11  |
| SH3 domain-binding glutamic acid-rich-like protein 3                                               | SH3BGRL3    | Q5T123     | 3.09E-03 | 29.38 |
| Immunoglobulin epsilon heavy                                                                       |             | IGE        | 3.40E-03 | 3.31  |
| N-acetylmuramoyl-L-alanine amidase                                                                 | PGLYRP2     | PGRP2      | 3.45E-03 | 1.35  |
| SAA2-SAA4 readthrough                                                                              | SAA2-SAA4   | A0A096LPE2 | 3.68E-03 | 1.24  |
| Serum amyloid A                                                                                    |             | B2R5G8     | 3.68E-03 | 1.24  |
| Coiled-coil domain-containing protein 40                                                           | CCDC40      | CCD40      | 3.71E-03 | 1.76  |
| APOC4-APOC2 readthrough (NMD candidate)                                                            | APOC4-APOC2 | K7ER74     | 3.90E-03 | 1.45  |
| Complement decay-accelerating factor                                                               | CD55        | A0A172WBW8 | 4.19E-03 | 1.56  |
| IGHV5-51 protein (Fragment)                                                                        | IGHV5-51    | A0A0F7T891 | 4.28E-03 | 5.83  |
| Histidine-rich glycoprotein                                                                        | HRG         | HRG        | 4.40E-03 | 1.42  |
| cDNA, FLJ93914, highly similar to Homo sapiens histidine-rich glycoprotein (HRG),                  |             | B2R8I2     | 4.40E-03 | 1.42  |
| APOB protein                                                                                       | APOB        | Q7Z7Q0     | 4.43E-03 | 1.36  |
| L-lactate dehydrogenase A chain                                                                    | LDHA        | LDHA       | 4.88E-03 | 1.15  |
| Lipoprotein B (Fragment)                                                                           | APOB        | S5FLF7     | 5.38E-03 | 2.35  |
| MS-D1 heavy chain variable region                                                                  |             | A0A109PS45 | 5.52E-03 | 1.65  |
| Immunoglobulin kappa variable 1D-16 (Fragment)                                                     | IGKV1D-16   | KVD16      | 6.64E-03 | 1.51  |
| Immunoglobulin heavy variable 3-9                                                                  | IGHV3-9     | HV309      | 6.82E-03 | 2.75  |

|                                                                        |                |            |          |      |
|------------------------------------------------------------------------|----------------|------------|----------|------|
| Alpha-actinin-4                                                        | ACTN4          | ACTN4      | 7.15E-03 | 3.27 |
| Apolipoprotein C-I, isoform CRA_a                                      | APOC1          | A0A024R0T8 | 7.29E-03 | 1.25 |
| cDNA, FLJ93383, highly similar to Homo sapiens semenogelin II (SEMG2), |                | B2R7C8     | 7.33E-03 | 3.69 |
| Complement component C8 gamma chain                                    | C8G            | CO8G       | 7.58E-03 | 1.30 |
| IgG H                                                                  |                | S6AWF0     | 8.32E-03 | 2.11 |
| Hemoglobin subunit beta                                                | HBB            |            | 8.40E-03 | 2.32 |
| Ig heavy chain variable region                                         |                | A0A068LKQ2 | 9.29E-03 | 1.35 |
| Ig heavy chain variable region                                         |                | A0A068LN03 | 9.85E-03 | 1.51 |
| Alpha-1B-glycoprotein                                                  | A1BG           | A1BG       | 9.99E-03 | 1.17 |
| Rheumatoid factor RF-ET9                                               |                | A2J1N6     | 1.01E-02 | 2.13 |
| V2-17 protein (Fragment)                                               | V2-17          | Q5NV90     | 1.08E-02 | 1.65 |
| Complement factor H-related protein 4                                  | CFHR4          | FHR4       | 1.09E-02 | 1.72 |
| Alpha-1-antitrypsin (Fragment)                                         | SERPINA1       | G3V2B9     | 1.11E-02 | 1.19 |
| MS-A3 light chain variable region                                      |                | A0A120HF56 | 1.17E-02 | 2.69 |
| Biotinidase                                                            | BTD            | BTD        | 1.19E-02 | 2.22 |
| Anti-staphylococcal enterotoxin D heavy chain variable region          |                | A0A1L2BU33 | 1.21E-02 | 1.51 |
| Polymeric immunoglobulin receptor                                      | PIGR           | PIGR       | 1.22E-02 | 1.43 |
| Histone PARylation factor 1                                            | HPF1           | HPF1       | 1.22E-02 | 2.55 |
| IBM-A3 heavy chain variable region                                     |                | A0A0X9UWM4 | 1.23E-02 | 1.65 |
| Rheumatoid factor RF-IP14                                              |                | A2J1N0     | 1.26E-02 | 1.50 |
| Pescadillo homolog                                                     | PES1           | PESC       | 1.28E-02 | 1.21 |
| Mannan-binding lectin serine protease 2                                | MASP2          | MASP2      | 1.30E-02 | 1.57 |
| MS-F1 light chain variable region                                      |                | A0A0X9V9B3 | 1.53E-02 | 2.46 |
| Uncharacterized protein DKFZp686M08189                                 | DKFZp686M08189 | Q6MZX9     | 1.65E-02 | 1.54 |

|                                                                        |          |            |          |      |
|------------------------------------------------------------------------|----------|------------|----------|------|
| Immunoglobulin lambda variable 3-9                                     | IGLV3-9  | LV39       | 1.69E-02 | 1.81 |
| Tenascin-X                                                             | TNXB     | A0A087X0I0 | 1.71E-02 | 1.28 |
| Insulin-like growth factor-binding protein complex acid labile subunit | IGFALS   | ALS        | 1.74E-02 | 1.24 |
| Immunoglobulin heavy variable 3-38 (non-functional) (Fragment)         | IGHV3-38 | A0A0C4DH36 | 1.74E-02 | 1.75 |
| Apolipoprotein C-IV                                                    | APOC4    | APOC4      | 1.86E-02 | 1.44 |
| Apolipoprotein C-IV                                                    | APOC4    | A5YAK2     | 1.86E-02 | 1.44 |
| Variable immunoglobulin anti-estradiol heavy chain                     |          | A2NZ55     | 2.03E-02 | 1.81 |
| IgG H                                                                  |          | S6BGD4     | 2.10E-02 | 1.29 |
| Plexin domain-containing protein 2                                     | PLXDC2   | PXDC2      | 2.21E-02 | 2.33 |
| Fermitin family homolog 3                                              | FERMT3   | URP2       | 2.24E-02 | 1.40 |
| IGL@ protein                                                           | IGL@     | Q6PIK1     | 2.29E-02 | 1.50 |
| Cathelicidin antimicrobial peptide                                     | CAMP     | CAMP       | 2.33E-02 | 1.31 |
| Probable G-protein-coupled receptor 179                                | GPR179   | A0A087VW83 | 2.37E-02 | 2.58 |
| Apolipoprotein M                                                       | APOM     | APOM       | 2.40E-02 | 1.15 |
| Fab Hu 15C1 Heavy                                                      |          | A0A0M3KKW6 | 2.41E-02 | 2.45 |
| Phospholipid transfer protein                                          | PLTP     | PLTP       | 2.54E-02 | 1.26 |
| Semenogelin-1                                                          | SEMG1    | SEMG1      | 2.64E-02 | 4.23 |
| Immunoglobulin kappa variable 3-11                                     | IGKV3-11 | KV311      | 2.67E-02 | 1.56 |
| Thyroxine-binding globulin                                             | SERPINA7 | THBG       | 2.70E-02 | 1.67 |
| Immunoglobulin heavy variable 4-59                                     | IGHV4-59 | HV459      | 2.74E-02 | 2.34 |
| Neutrophil defensin 1                                                  | DEFA1    | DEF1       | 2.80E-02 | 1.30 |
| Complement C1r subcomponent-like protein                               | C1RL     | C1RL       | 2.88E-02 | 1.49 |
| Beta-Ala-His dipeptidase                                               | CNDP1    | CNDP1      | 2.90E-02 | 1.34 |
| Vitamin D binding protein (Fragment)                                   | Gc       | A0A1B1CYC8 | 3.08E-02 | 2.55 |

|                                                                    |                       |            |          |      |
|--------------------------------------------------------------------|-----------------------|------------|----------|------|
| MS-A2 heavy chain variable region                                  |                       | A0A125U0U3 | 3.12E-02 | 1.08 |
| Anti-folate binding protein (Fragment)<br>OS=Homo                  | HuC4lambda<br>Vlambda | A2NYQ7     | 3.23E-02 | 1.46 |
| Uncharacterized protein DKFZp686C02218<br>(Fragment)               | DKFZp686C<br>02218    | Q7Z374     | 3.71E-02 | 3.12 |
| Ceruloplasmin                                                      | CP                    | CERU       | 3.81E-02 | 1.20 |
| Immunoglobulin heavy variable 3-74                                 | IGHV3-74              | HV374      | 3.87E-02 | 1.35 |
| Angiotensinogen                                                    | AGT                   | ANGT       | 4.17E-02 | 1.38 |
| Cholesteryl ester transfer protein plasma<br>isoform 1 (Fragment)  | CETP                  | A0A0S2Z3F6 | 4.54E-02 | 1.57 |
| Immunoglobulin heavy variable 3-43                                 | IGHV3-43              | HV343      | 4.65E-02 | 2.21 |
| Immunoglobulin heavy variable 3-35 (non-<br>functional) (Fragment) | IGHV3-35              | A0A0C4DH35 | 4.72E-02 | 1.34 |
| Alpha-1-acid glycoprotein 2                                        | ORM2                  | A1AG2      | 4.73E-02 | 2.30 |
| Testicular tissue protein Li                                       |                       | A0A140VK00 | 4.89E-02 | 1.37 |

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**CLAIMS**

1. A method of assessing the likelihood of developing Alzheimer's disease or of determining Alzheimer's disease or of monitoring Alzheimer's disease progression in a subject, comprising the step of determining the presence and/or amount of one or more protein biomarkers in the subject or biofluid taken from the subject, the one or more protein biomarkers being independently selected from those listed in Table 6a, Table 1 or Table 1a.
2. A method of testing a patient's biofluid sample for one or more biomarkers of Alzheimer's disease, comprising the steps of:
  - a. contacting a plurality of nanoparticles in a biofluid sample taken from a subject to allow a biomolecule corona to form on the surface of said nanoparticles;
  - b. optionally isolating the nanoparticles and surface-bound biomolecule corona;
  - c. determining the abundance of one or more protein biomarkers in the corona, the one or more protein biomarkers being independently selected from those listed in Table 1a or Table 1; and
  - d. comparing the abundance of the one or more protein biomarkers in the corona to the abundance of the same one or more protein biomarkers in a non-diseased control reference.
3. A method as claimed in claim 2, wherein the biomolecule corona comprises a protein corona.
4. A method as claimed in claim 2 or 3, wherein the nanoparticles are selected from liposomes, gold nanoparticles, polymeric nanoparticles, carbon nanotubes and graphene oxide nanoparticles, or any combination thereof.
5. A method as claimed in any one of claims 2 to 4, wherein the nanoparticles comprise liposomes.
6. A method as claimed in any one of claims 2 to 5, wherein the biofluid is selected from blood, plasma, serum, urine, saliva, sputum, lacrimal, cerebrospinal and ocular fluids.
7. A method as claimed in any one of claims 2 to 6, wherein step a) comprises administering a plurality of nanoparticles to a subject by intravenous, oral, intracerebral, spinal, intraperitoneal or intra-ocular administration, or any combination thereof.

8. A method as claimed in any one of claims 2 to 7, wherein step a) comprises incubating a plurality of nanoparticles in a biofluid sample taken from a subject under conditions to allow a biomolecule corona to form on the surface of said nanoparticles.
- 5 9. A method as claimed in any one of claims 2 to 6, wherein the method comprises isolating the nanoparticles with surface-bound biomolecule corona from the biofluid and purifying them to remove unbound and highly abundant biomolecules, for example albumin and/or immunoglobins.
- 10 10. A method as claimed in any one of claims 2 to 9, wherein step b) comprises performing size exclusion chromatography and/or ultrafiltration.
11. A method as claimed in claim 10, wherein step b) comprises performing size exclusion chromatography followed by ultrafiltration.
12. A method as claimed in any one of claims 2 to 11 wherein step c) and/or step d) comprises a step of identifying the one or more Alzheimer's disease biomarkers in the corona and a step  
15 of quantifying the one or more Alzheimer's disease biomarkers in the corona.
13. A method as claimed in any one of claims 2 to 12, wherein step c) and/or step d) comprises performing gel electrophoresis, mass spectrometry, an immunoassay, UV-Vis. absorption, fluorescence spectroscopy, chromatography, liquid chromatography or NMR methodology, or any combination thereof.
- 20 14. A method as claimed in claim 13, wherein step c) and/or step d) comprises performing mass spectrometry or LC/MS.
15. A method as claimed in any one of claims 2 to 14, wherein the non-diseased control reference comprises a biomolecule corona or protein corona obtained from a healthy subject or a sample of biofluid obtained therefrom.
- 25 16. A method as claimed in claim 15, wherein the corona obtained from the healthy subject is obtained by a method substantially the same as or identical to steps a) and b) and/or the protein biomarker abundance thereof is analysed by a method substantially the same as or identical to step c).

17. A method as claimed in any one of claims 2 to 16, wherein step d) comprises determining and/or calculating relative or differential abundance between the corona and the non-diseased control reference with respect to the or each of the one or more protein biomarkers.

18. A method as claimed in any one of claims 2 to 17, wherein the plurality of nanoparticles is a homogeneous population of nanoparticles.

19. A method as claimed in any one of the preceding claims, wherein the subject is a human.

20. A method as claimed in any one of the preceding claims, comprising determining the presence and/or amount and/or abundance of one or more protein biomarkers independently selected from Table 6a or Table 6 and/or comparing the presence and/or amount and/or abundance of the same one or more protein biomarkers in a non-diseased control reference.

21. A method for monitoring Alzheimer's disease (AD) progression in a subject, comprising the steps of:

- a. contacting a plurality of nanoparticles with a biofluid sample from a subject with AD to allow a biomolecule corona to form on the surface of said nanoparticles;
- b. optionally isolating the nanoparticles and surface-bound biomolecule corona;
- c. determining the abundance of one or more protein biomarkers in the corona, the one or more protein biomarkers being independently selected from those listed in Table 1a, Table 6a or Table 2; and
- d. comparing the abundance of the one or more protein biomarkers in the corona to the abundance of the same one or more protein biomarkers in a non-diseased control reference;

wherein down regulation of one or more of the proteins in Table 1a, Table 6a or Table 2 relative to a previous measurement in the same subject indicates AD progression.

22. A method for assessing the stage of Alzheimer's disease (AD) in a subject, comprising the steps of:

- a. contacting a plurality of nanoparticles with a biofluid sample from the subject to allow a biomolecule corona to form on the surface of said nanoparticles;
- b. optionally isolating the nanoparticles and surface-bound biomolecule corona;

- c. determining the abundance of one or more protein biomarkers in the corona, the one or more protein biomarkers being independently selected from those listed in Table 1; and
- d. comparing the abundance of the one or more protein biomarkers in the corona to the abundance of the same one or more protein biomarkers in a non-diseased control reference;

wherein a change in abundance relative to a non-diseased control reference of one or more proteins in Table 3 indicates that the patient has early stage asymptomatic AD, or the presence of one or more proteins in Table 4 indicates that the patient has intermediate stage AD, or the presence of one or more proteins in Table 5 indicates that the patient has advanced stage AD.

23. A method for assessing the stage of Alzheimer's disease (AD) in a subject, comprising the steps of:

- a. contacting a plurality of nanoparticles with a biofluid sample from the subject to allow a biomolecule corona to form on the surface of said nanoparticles;
- b. optionally isolating the nanoparticles and surface-bound biomolecule corona;
- c. determining the abundance of one or more protein biomarkers in the corona, the one or more protein biomarkers being independently selected from those listed in Table 1a; and
- d. comparing the abundance of the one or more protein biomarkers in the corona to the abundance of the same one or more protein biomarkers in a non-diseased control reference;

wherein a change in abundance relative to a non-diseased control reference of one or more proteins in Table 3a indicates that the patient has early stage asymptomatic AD, or the presence of one or more proteins in Table 4a indicates that the patient has intermediate stage AD, or the presence of one or more proteins in Table 5a indicates that the patient has advanced stage AD.

24. A method as claimed in any preceding claim wherein the method comprises an extra step of administering a therapy to the subject diagnosed with AD or whose AD has progressed.

25. A method for identifying neurodegeneration-associated protein biomarkers in the blood of a subject, comprising (a) (i) contacting a plurality of nanoparticles with a blood-derived fluid sample from one or more healthy subjects; and (ii) contacting a plurality of nanoparticles with

a blood-derived fluid sample from one or more subjects with neurodegeneration, under conditions to allow a biomolecule corona to form on the surface of said nanoparticles; (b) optionally isolating the nanoparticles and surface-bound biomolecule corona; (c) determining the abundance of one or more protein biomarkers in the corona from each subject; (d) comparing the abundance of the one or more protein biomarkers in the corona of subjects in (i) to the abundance of the same one or more protein biomarkers in the corona of subjects in (ii); wherein protein biomarkers which display statistically significant differential expression between (i) and (ii) are identified as neurodegeneration-associated protein biomarkers.

26. A method according to any preceding claim, wherein the protein biomarker is part of a panel of AD-specific biomolecule biomarkers.

27. A method according to claim 26, wherein the panel comprises unknown and known AD-specific biomolecule biomarkers.

28. A method according to claims 27, wherein the unknown biomarker is a unique biomolecule, meaning that it is a biomolecule that would not have been detected if analysis was carried out directly on biofluid, such as plasma, isolated from the subject.

29. A method according to any preceding claim, wherein a change in a biomarker in response to therapy is monitored.

30. A method according to claim 29, wherein the therapy is administered to the subject prior to testing is a drug molecule.

31. A method according to any preceding claim wherein the protein biomarker has a molecular weight of less than 80 kDa.

Figure 1a

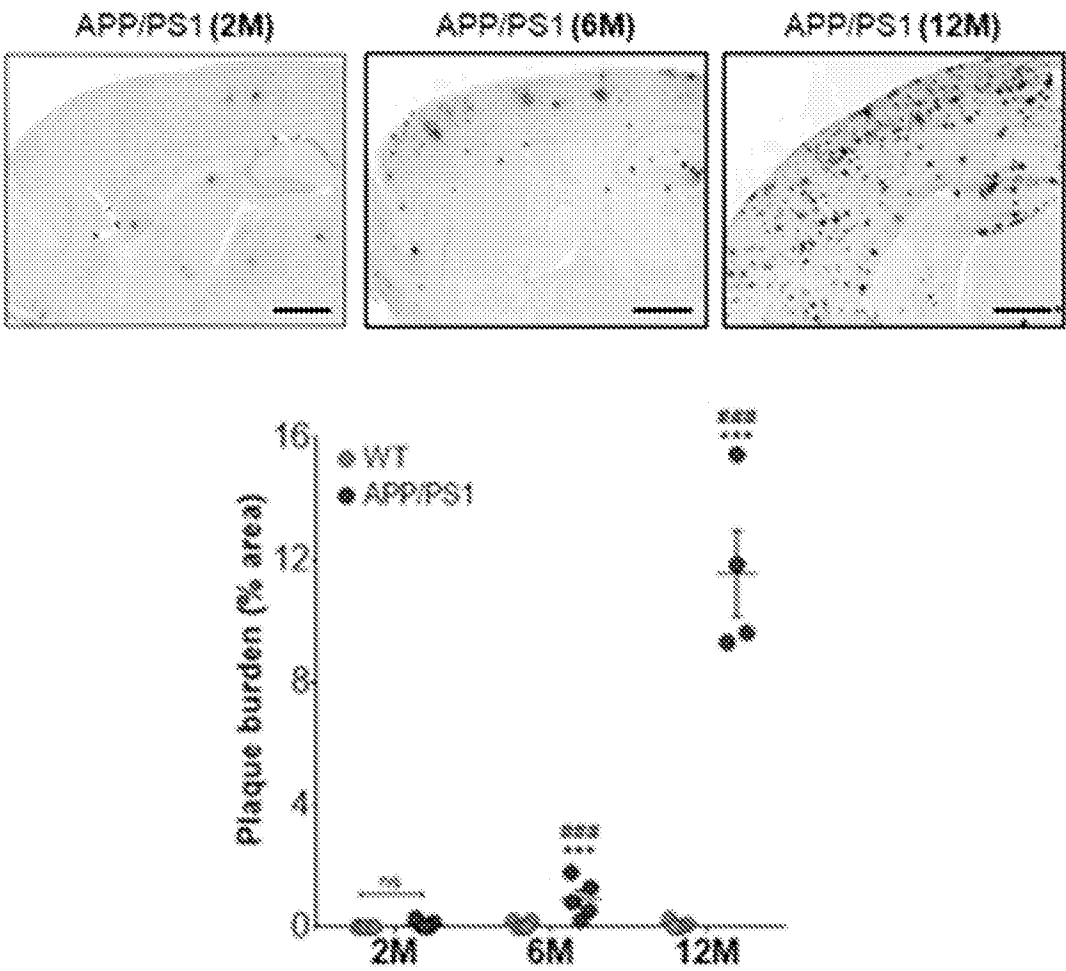


Figure 1b

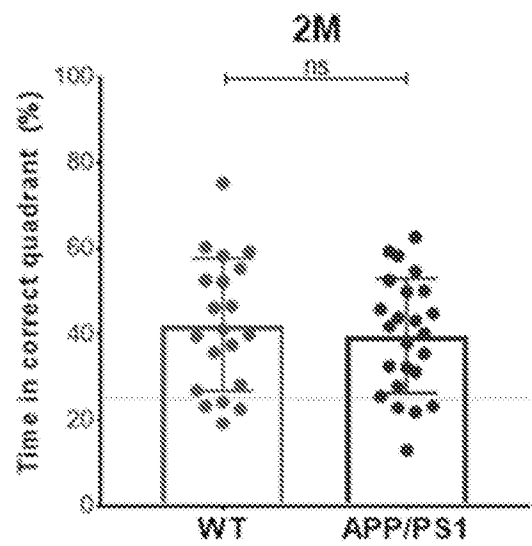


Figure 1c

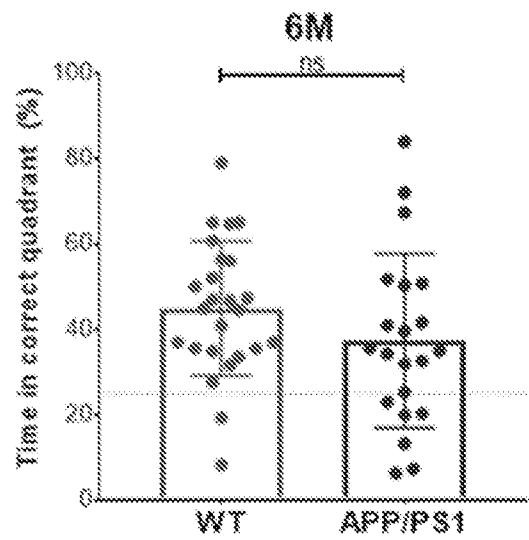




Figure 1f

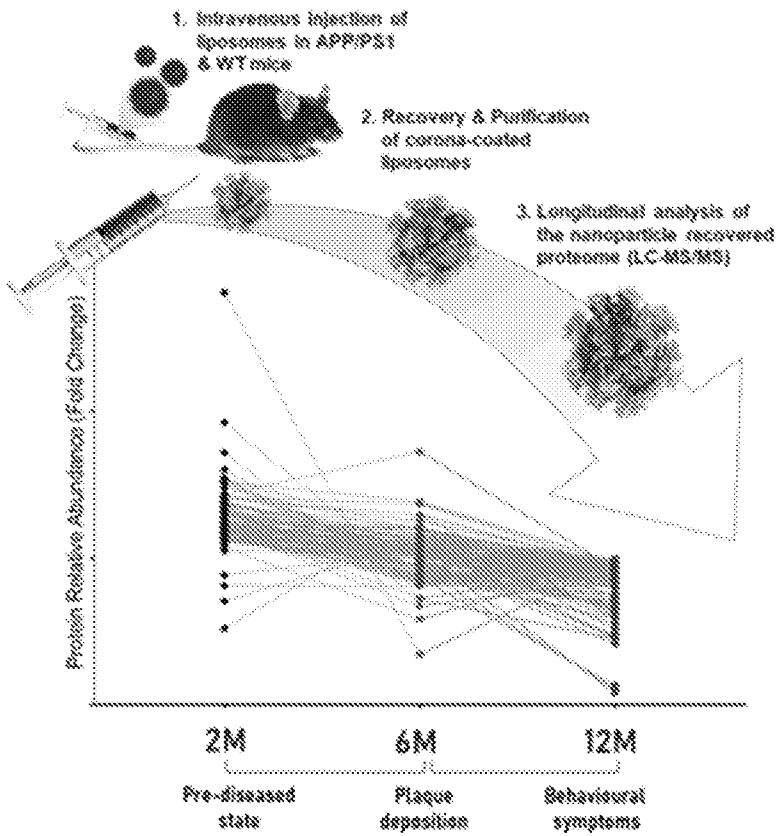


Figure 1g

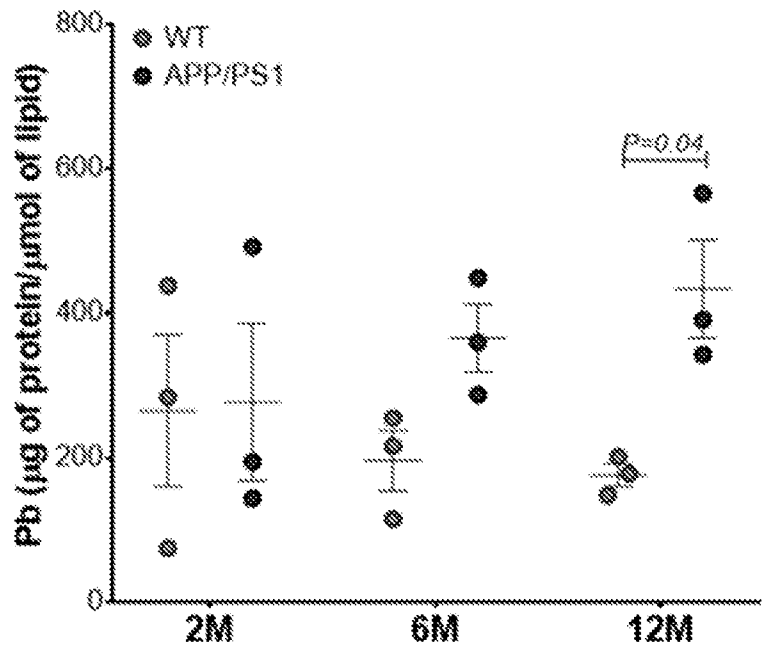


Figure 1h

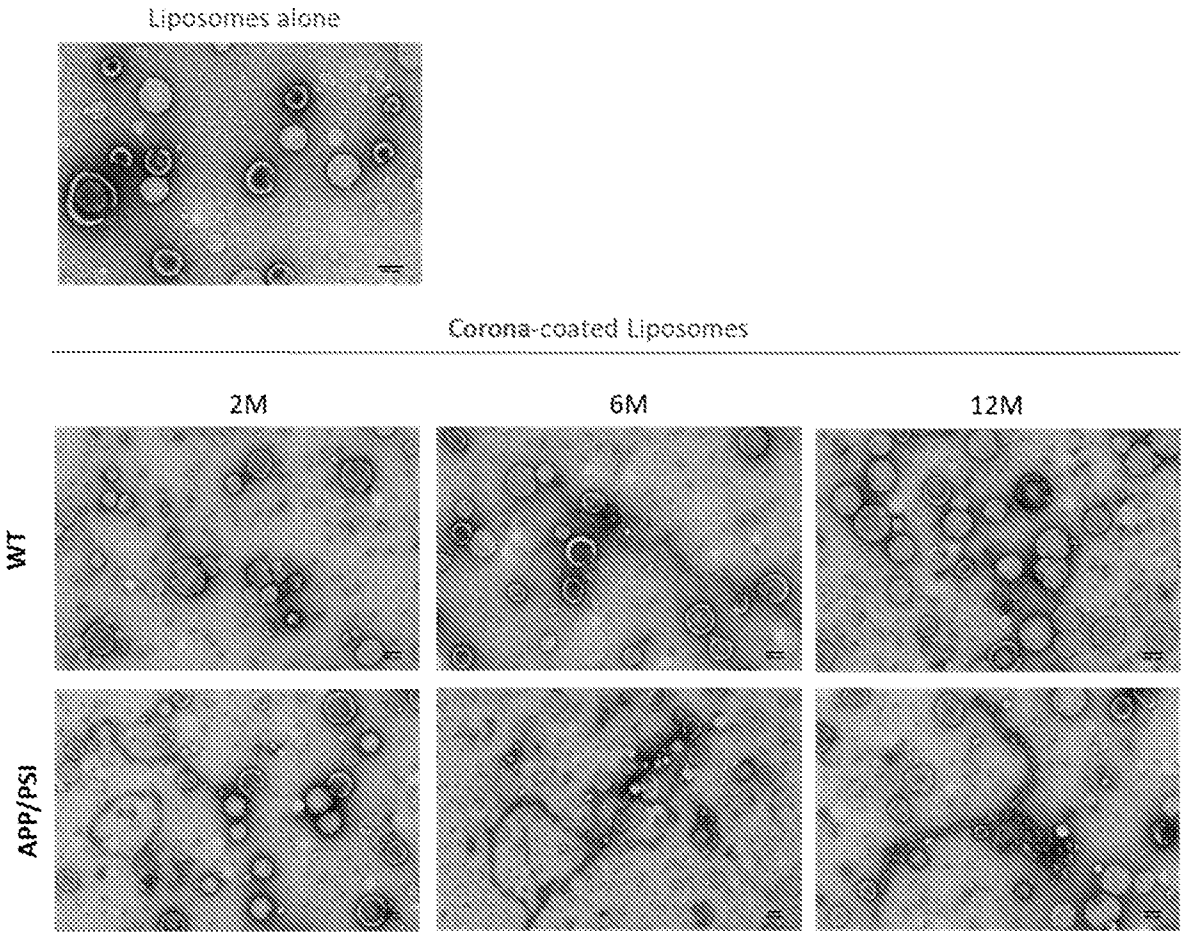


Figure 2a

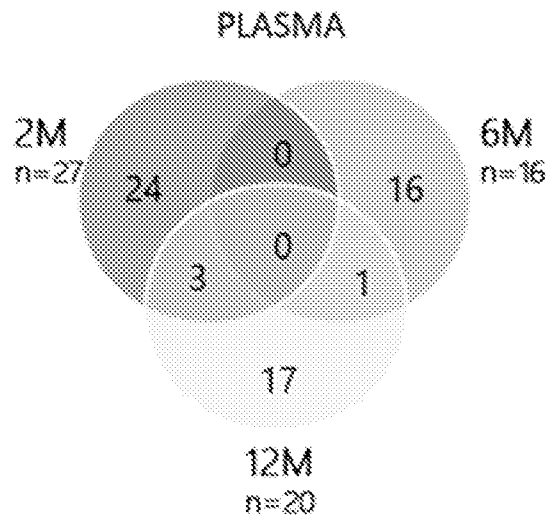


Figure 2b

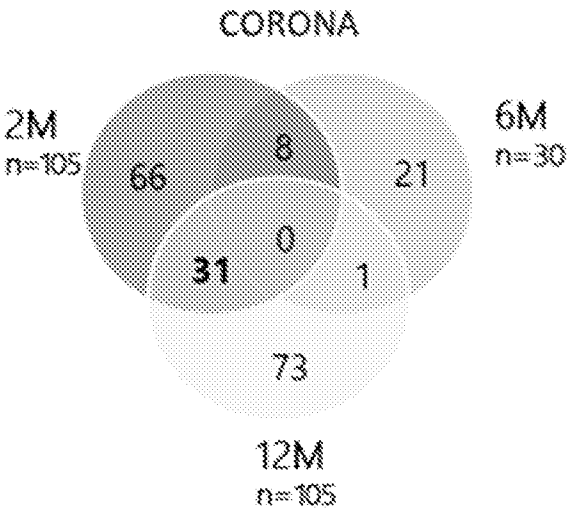


Figure 2c

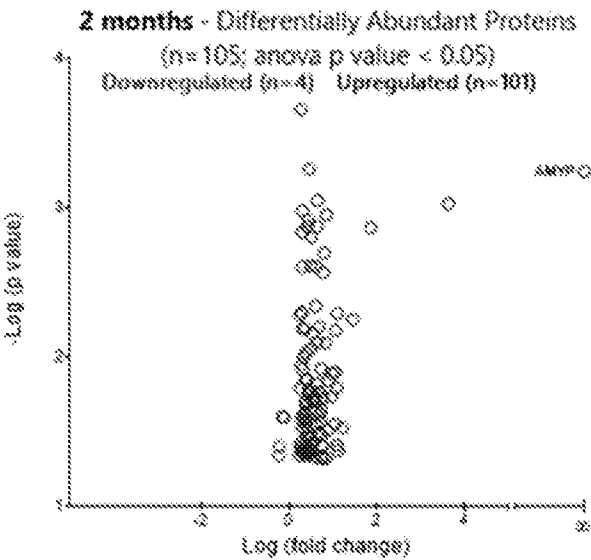


Figure 2d

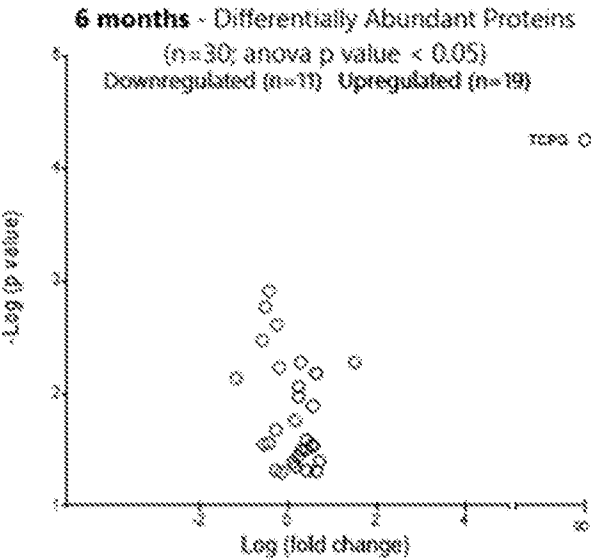


Figure 2e

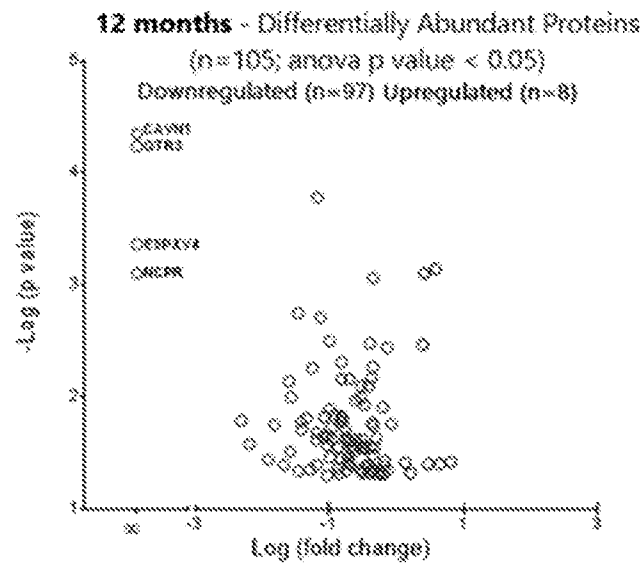


Figure 2f

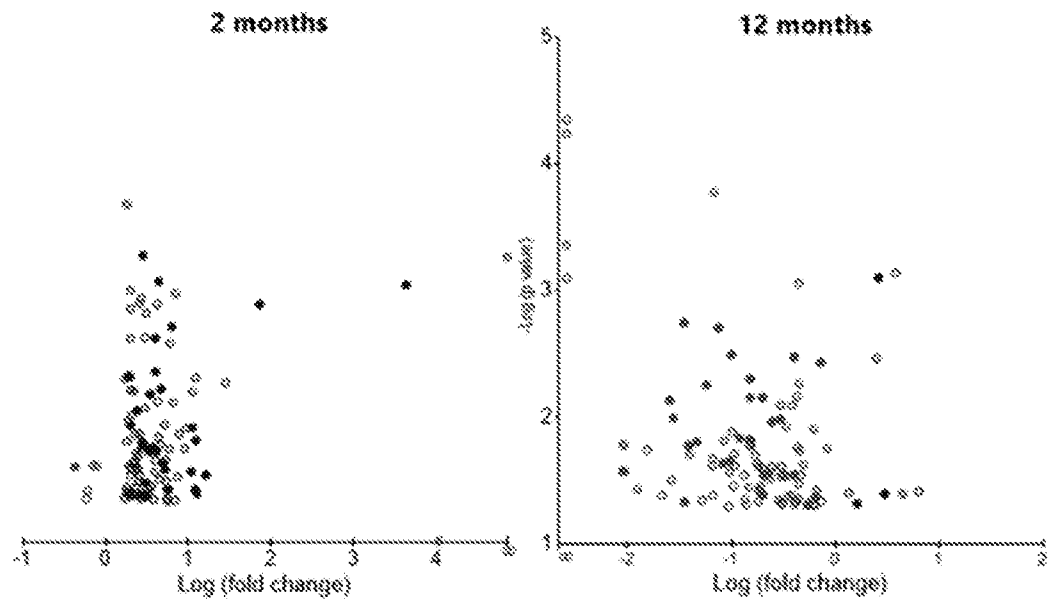


Figure 2g

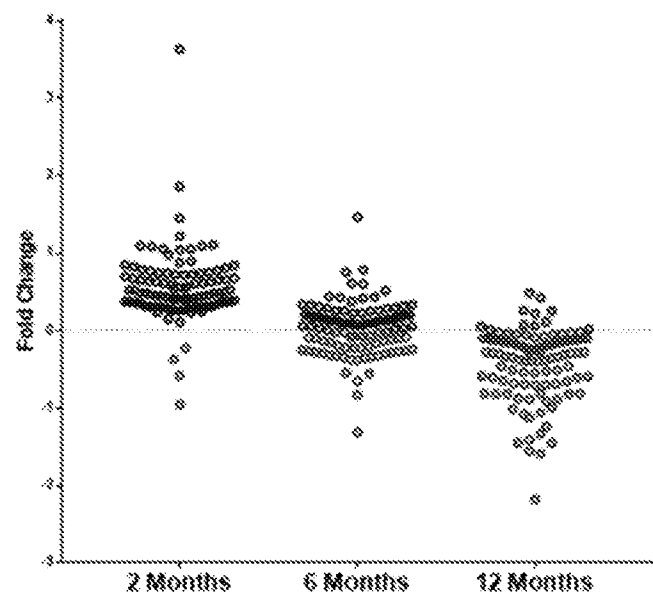
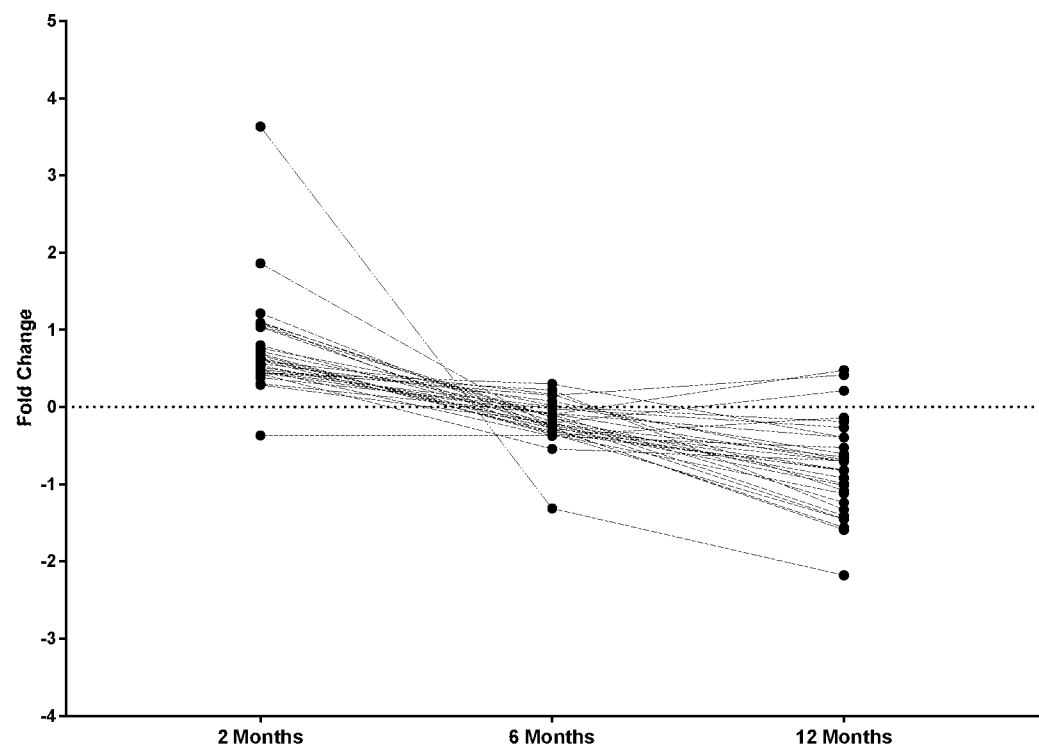
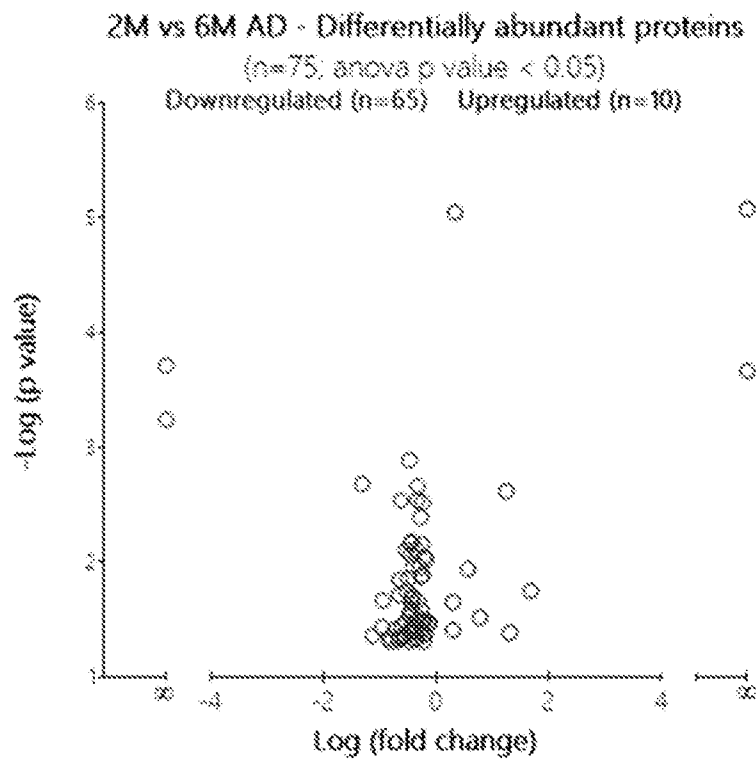


Figure 2h



**Figure 3a**



### Figure 3b

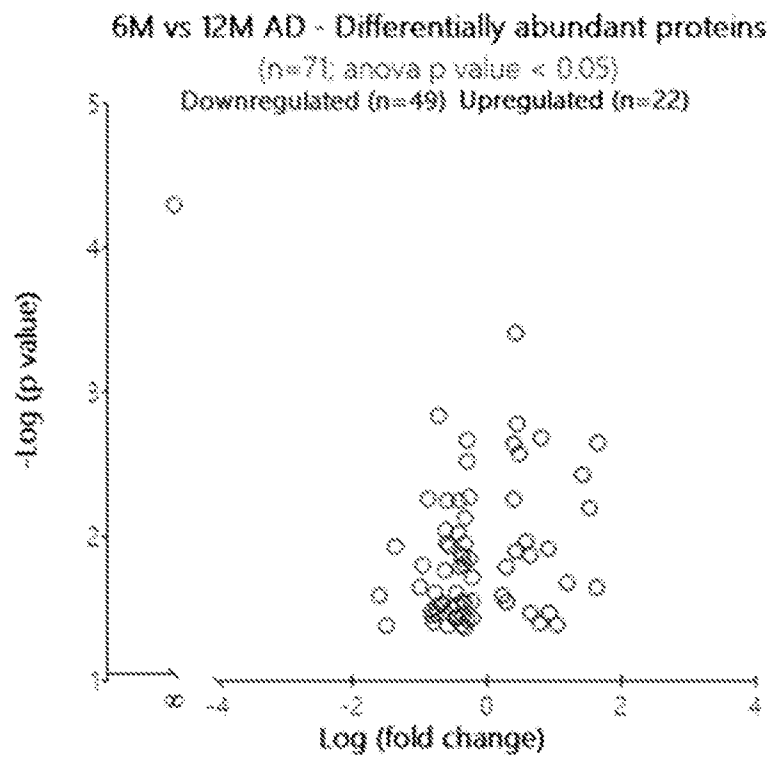


Figure 3c

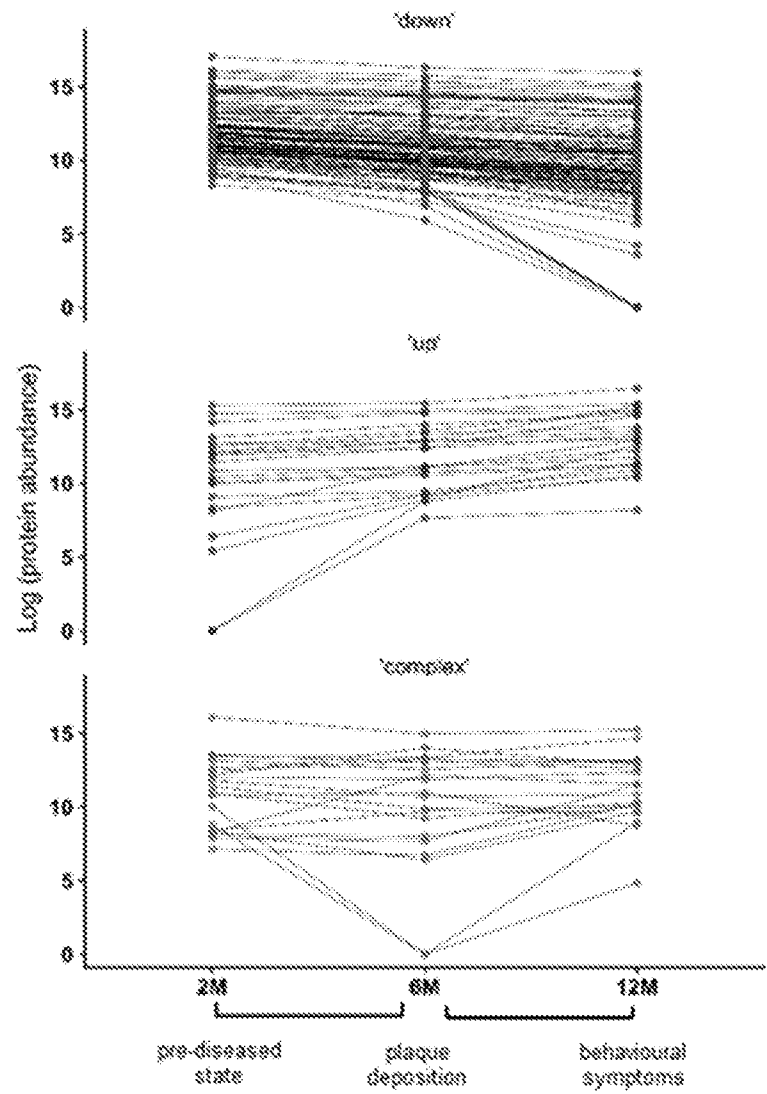


Figure 3d

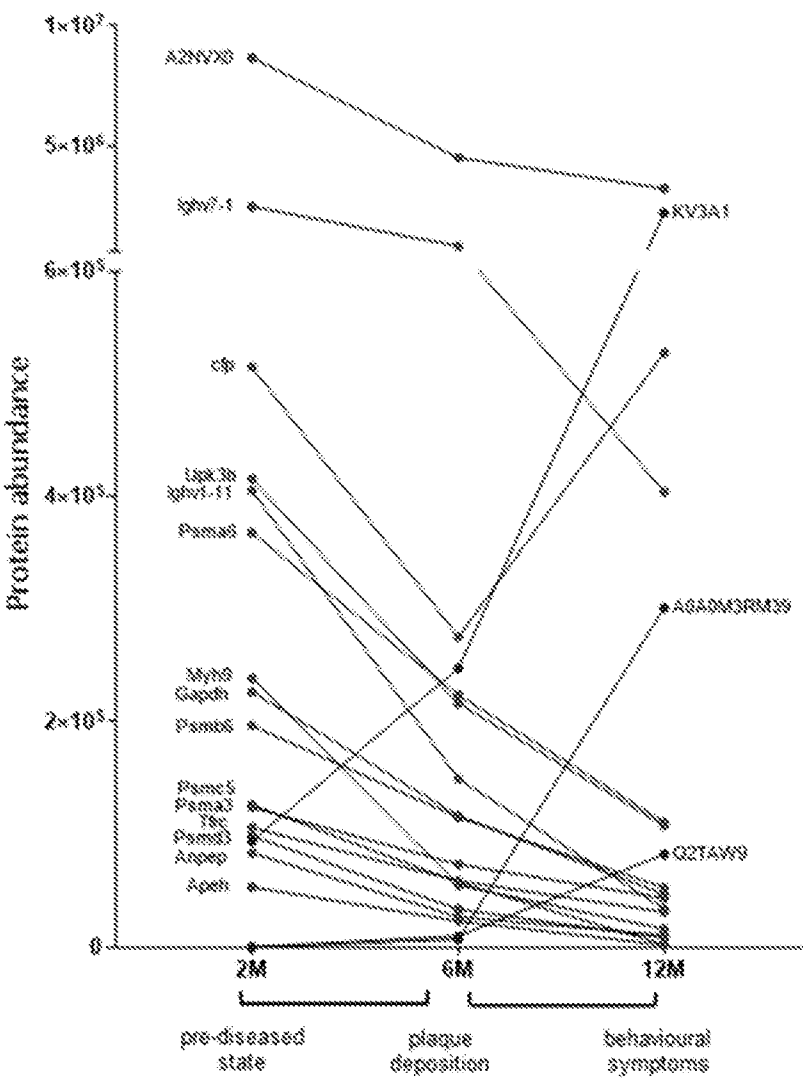




Figure 4b

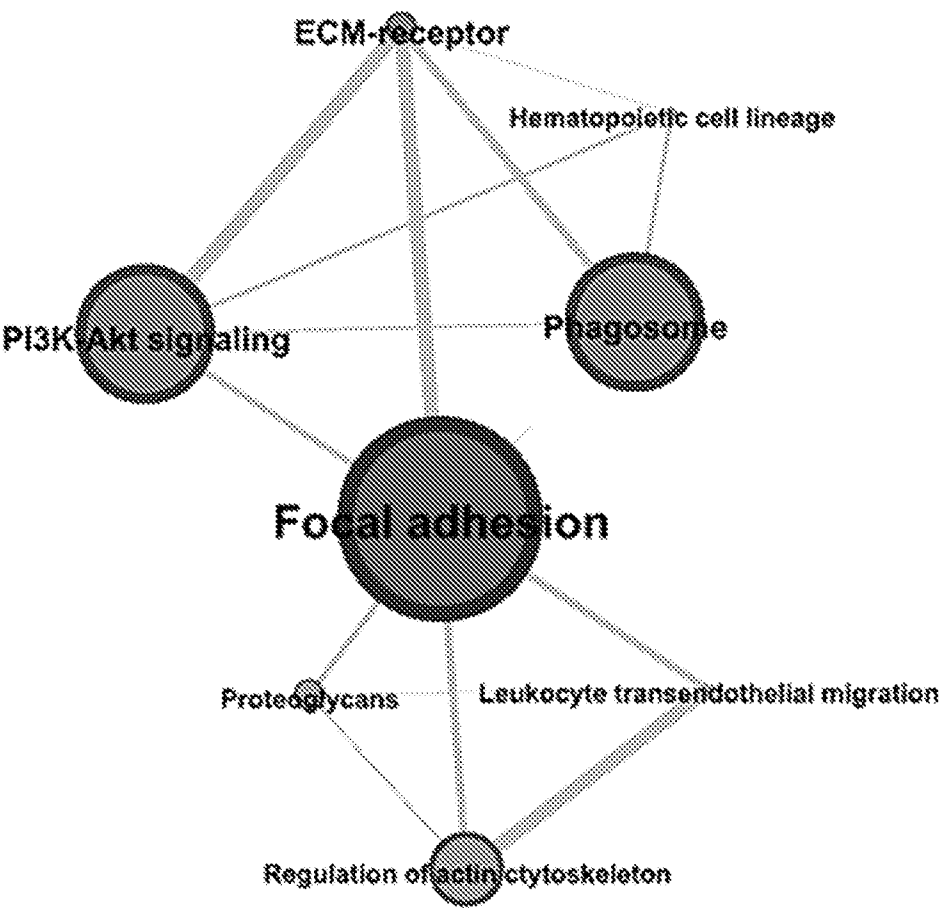


Figure 5

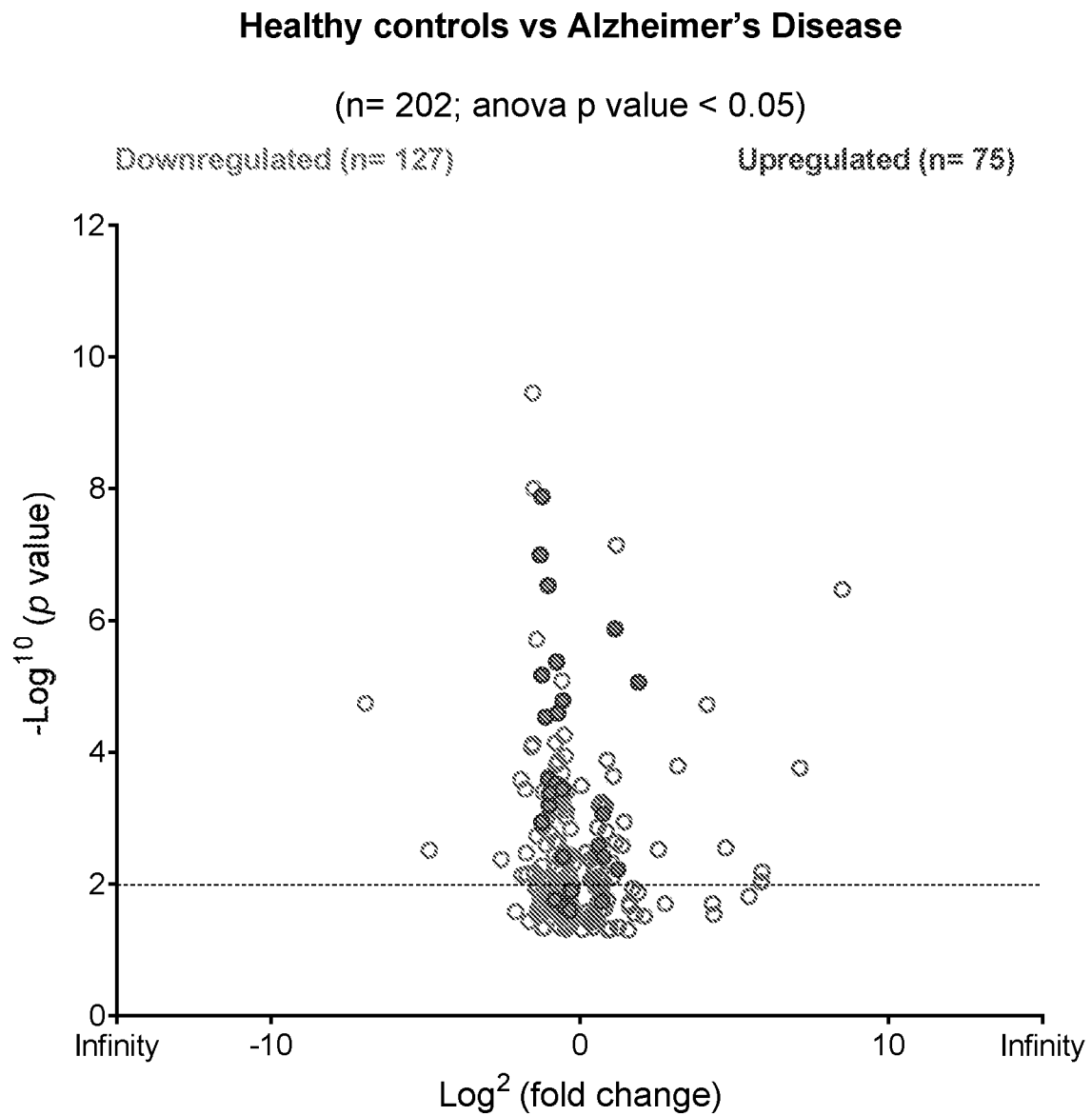
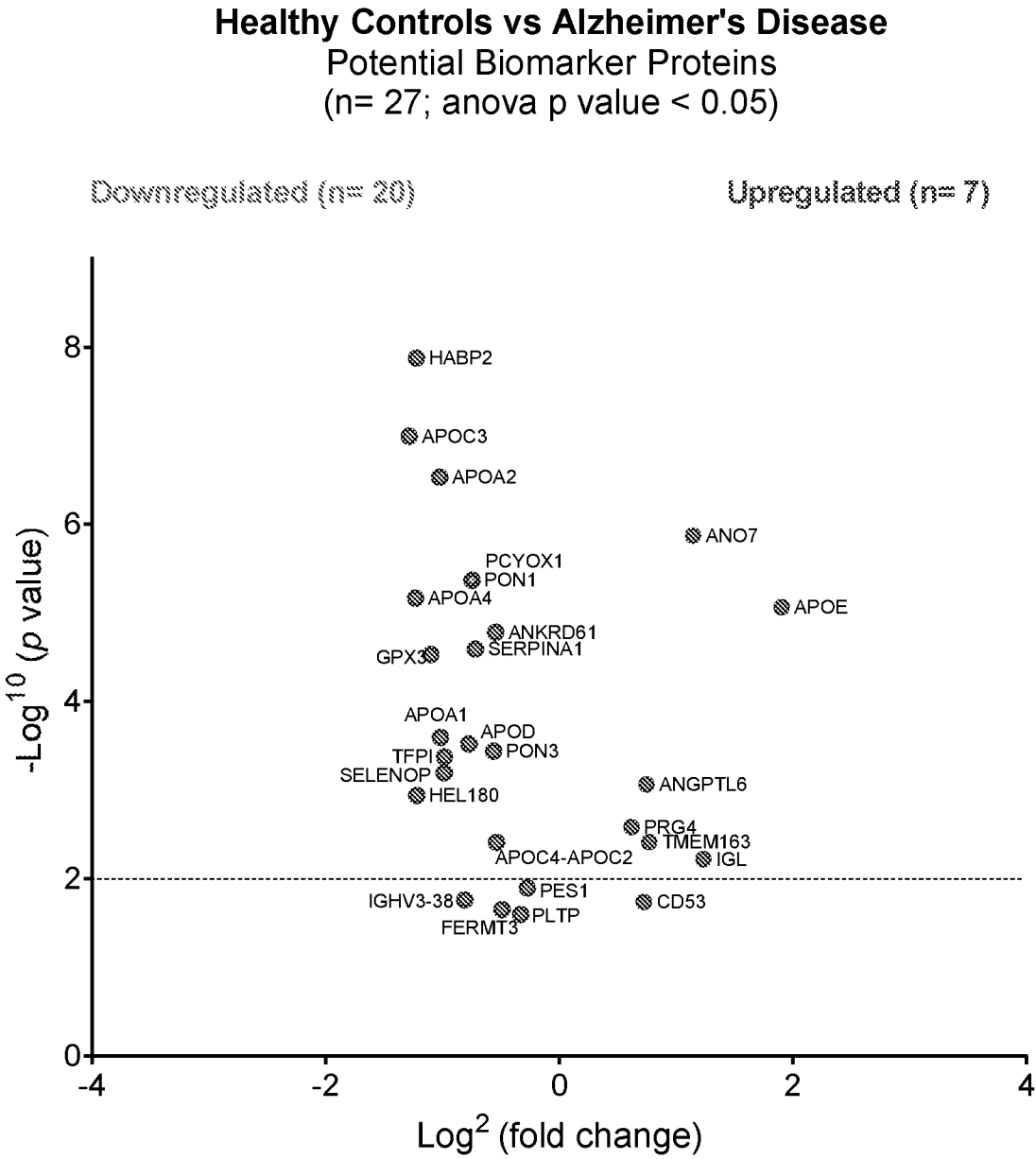


Figure 6



# INTERNATIONAL SEARCH REPORT

International application No  
**PCT/GB2022/050442**

|                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                      |                                                                                                                                                                                                                                                                                                                                                                                        |                                                                                                                    |
|----------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------|----------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------|--------------------------------------------------------------------------------------------------------------------|
| <b>A. CLASSIFICATION OF SUBJECT MATTER</b><br><b>INV. G01N33/543 G01N33/68</b><br><b>ADD.</b>                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                        |                                                                                                                                                                                                                                                                                                                                                                                        |                                                                                                                    |
| According to International Patent Classification (IPC) or to both national classification and IPC                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                    |                                                                                                                                                                                                                                                                                                                                                                                        |                                                                                                                    |
| <b>B. FIELDS SEARCHED</b><br>Minimum documentation searched (classification system followed by classification symbols)<br><b>G01N</b>                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                |                                                                                                                                                                                                                                                                                                                                                                                        |                                                                                                                    |
| Documentation searched other than minimum documentation to the extent that such documents are included in the fields searched                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                        |                                                                                                                                                                                                                                                                                                                                                                                        |                                                                                                                    |
| Electronic data base consulted during the international search (name of data base and, where practicable, search terms used)<br><b>EPO-Internal, WPI Data, BIOSIS, EMBASE</b>                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                        |                                                                                                                                                                                                                                                                                                                                                                                        |                                                                                                                    |
| <b>C. DOCUMENTS CONSIDERED TO BE RELEVANT</b>                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                        |                                                                                                                                                                                                                                                                                                                                                                                        |                                                                                                                    |
| Category*                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                            | Citation of document, with indication, where appropriate, of the relevant passages                                                                                                                                                                                                                                                                                                     | Relevant to claim No.                                                                                              |
| <b>A</b>                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                             | <b>CUI J G ET AL: "Micro-RNA-128 (miRNA-128) down-regulation in glioblastoma targets ARP5 (ANGPTL6), Bmi-1 and E2F-3a, key regulators of brain cell proliferation", JOURNAL OF NEURO-ONCOLOGY, KLUWER ACADEMIC PUBLISHERS, BO, vol. 98, no. 3, 26 November 2009 (2009-11-26), pages 297-304, XP019827180, ISSN: 1573-7373 abstract</b><br><div style="text-align: center;">-----</div> | <b>1, 24, 26-31</b>                                                                                                |
| <b>A</b>                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                             | <b>WO 2016/146783 A1 (ELECTROPHORETICS LTD [GB]) 22 September 2016 (2016-09-22) claims 1-12; table 2</b><br><div style="text-align: center;">-----</div> <div style="text-align: center;">-/--</div>                                                                                                                                                                                   | <b>1, 24, 26-31</b>                                                                                                |
| <div style="display: flex; justify-content: space-between;"> <div> <input checked="" type="checkbox"/> Further documents are listed in the continuation of Box C.         </div> <div> <input checked="" type="checkbox"/> See patent family annex.         </div> </div>                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                            |                                                                                                                                                                                                                                                                                                                                                                                        |                                                                                                                    |
| <div style="display: flex;"> <div style="flex: 1;"> <p>* Special categories of cited documents :</p> <p>"A" document defining the general state of the art which is not considered to be of particular relevance</p> <p>"E" earlier application or patent but published on or after the international filing date</p> <p>"L" document which may throw doubts on priority claim(s) or which is cited to establish the publication date of another citation or other special reason (as specified)</p> <p>"O" document referring to an oral disclosure, use, exhibition or other means</p> <p>"P" document published prior to the international filing date but later than the priority date claimed</p> </div> <div style="flex: 1;"> <p>"T" later document published after the international filing date or priority date and not in conflict with the application but cited to understand the principle or theory underlying the invention</p> <p>"X" document of particular relevance;; the claimed invention cannot be considered novel or cannot be considered to involve an inventive step when the document is taken alone</p> <p>"Y" document of particular relevance;; the claimed invention cannot be considered to involve an inventive step when the document is combined with one or more other such documents, such combination being obvious to a person skilled in the art</p> <p>"&amp;" document member of the same patent family</p> </div> </div> |                                                                                                                                                                                                                                                                                                                                                                                        |                                                                                                                    |
| Date of the actual completion of the international search<br><br><div style="text-align: center;"><b>11 May 2022</b></div>                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                           |                                                                                                                                                                                                                                                                                                                                                                                        | Date of mailing of the international search report<br><br><div style="text-align: center;"><b>12/07/2022</b></div> |
| Name and mailing address of the ISA/<br>European Patent Office, P.B. 5818 Patentlaan 2<br>NL - 2280 HV Rijswijk<br>Tel. (+31-70) 340-2040,<br>Fax: (+31-70) 340-3016                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                 |                                                                                                                                                                                                                                                                                                                                                                                        | Authorized officer<br><br><div style="text-align: center;"><b>Moreno de Vega, C</b></div>                          |

## INTERNATIONAL SEARCH REPORT

International application No

PCT/GB2022/050442

| C(Continuation). DOCUMENTS CONSIDERED TO BE RELEVANT |                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                       |                       |
|------------------------------------------------------|-------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------|-----------------------|
| Category*                                            | Citation of document, with indication, where appropriate, of the relevant passages                                                                                                                                                                                                                                                                                                                                                                                                                    | Relevant to claim No. |
| Y                                                    | <p>WO 2018/112460 A1 (BRIGHAM &amp; WOMENS HOSPITAL INC [US])<br/> 21 June 2018 (2018-06-21)<br/> paragraphs [0267] - [0277]; claims 1-72;<br/> figures 54, 56; examples 6-8</p> <p>-----</p>                                                                                                                                                                                                                                                                                                         | 1, 24,<br>26-31       |
| Y                                                    | <p>CORBO CLAUDIA ET AL: "Analysis of the Human Plasma Proteome Using Multi-Nanoparticle Protein Corona for Detection of Alzheimer's Disease",<br/> ADVANCED HEALTHCARE MATERIALS<br/> ,<br/> vol. 10, no. 2<br/> 9 November 2020 (2020-11-09), page<br/> 2000948, XP055854100,<br/> DE<br/> ISSN: 2192-2640, DOI:<br/> 10.1002/adhm.202000948<br/> Retrieved from the Internet:<br/> URL:https://onlinelibrary.wiley.com/doi/full-xml/10.1002/adhm.202000948<br/> the whole document</p> <p>-----</p> | 1, 24,<br>26-31       |
| Y,P                                                  | <p>HADJIDEMETRIOU MARILENA ET AL:<br/> "Nanoparticle-Enabled Enrichment of Longitudinal Blood Proteomic Fingerprints in Alzheimer's Disease",<br/> ACS NANO<br/> ,<br/> vol. 15, no. 4<br/> 27 April 2021 (2021-04-27), pages<br/> 7357-7369, XP055853987,<br/> US<br/> ISSN: 1936-0851, DOI:<br/> 10.1021/acsnano.1c00658<br/> Retrieved from the Internet:<br/> URL:https://pubs.acs.org/doi/pdf/10.1021/acsnano.1c00658<br/> the whole document</p> <p>-----</p>                                   | 1, 24,<br>26-31       |

# INTERNATIONAL SEARCH REPORT

International application No.  
**PCT/GB2022/050442**

## Box No. II Observations where certain claims were found unsearchable (Continuation of item 2 of first sheet)

This international search report has not been established in respect of certain claims under Article 17(2)(a) for the following reasons:

1. ☐ Claims Nos.:  
because they relate to subject matter not required to be searched by this Authority, namely:
2. ☐ Claims Nos.:  
because they relate to parts of the international application that do not comply with the prescribed requirements to such an extent that no meaningful international search can be carried out, specifically:
3. ☐ Claims Nos.:  
because they are dependent claims and are not drafted in accordance with the second and third sentences of Rule 6.4(a).

## Box No. III Observations where unity of invention is lacking (Continuation of item 3 of first sheet)

This International Searching Authority found multiple inventions in this international application, as follows:

**see additional sheet**

1. ☐ As all required additional search fees were timely paid by the applicant, this international search report covers all searchable claims.
2. ☐ As all searchable claims could be searched without effort justifying an additional fees, this Authority did not invite payment of additional fees.
3. ☐ As only some of the required additional search fees were timely paid by the applicant, this international search report covers only those claims for which fees were paid, specifically claims Nos.:
4. ☒ No required additional search fees were timely paid by the applicant. Consequently, this international search report is restricted to the invention first mentioned in the claims;; it is covered by claims Nos.:  
**1, 24, 26–31 (all partially)**

### Remark on Protest

- ☐ The additional search fees were accompanied by the applicant's protest and, where applicable, the payment of a protest fee.
- ☐ The additional search fees were accompanied by the applicant's protest but the applicable protest fee was not paid within the time limit specified in the invitation.
- ☐ No protest accompanied the payment of additional search fees.

## FURTHER INFORMATION CONTINUED FROM PCT/ISA/ 210

This International Searching Authority found multiple (groups of) inventions in this international application, as follows:

1. claims: 1, 24, 26-31(all partially)

Method of assessing the likelihood of developing Alzheimer's disease or of determining Alzheimer's disease or of monitoring Alzheimer's disease progression in a subject, comprising determining the presence and/or amount of one or more protein biomarkers selected from those listed in Table 6a, Table 1 or Table 1a in a sample from a subject, the marker being ANGPTL6 (Angiopietin-like 6, isoform CRA\_a).

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2-300. claims: 1, 24, 26-31(all partially)

Method of assessing the likelihood of developing Alzheimer's disease or of determining Alzheimer's disease or of monitoring Alzheimer's disease progression in a subject, comprising determining the presence and/or amount of one or more protein biomarkers selected from those listed in Table 6a, Table 1 or Table 1a in a sample from a subject, the marker being any one of the markers of said tables and their combinations excepting ANGPTL6 alone.

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301-500. claims: 2-20, 24, 26-31(all partially)

Method of testing a patient's biofluid sample for one or more biomarkers of Alzheimer's disease, comprising contacting a plurality of nanoparticles in a biofluid sample taken from a subject to allow a biomolecule corona to form on the surface of said nanoparticles; and determining the abundance of one or more protein biomarkers in the corona, the one or more protein biomarkers being independently selected from those listed in Table 1a or Table 1.

---

501-700. claims: 21, 24, 26-31(all partially)

Method for monitoring Alzheimer's disease (AD) progression in a subject, comprising contacting a plurality of nanoparticles with a biofluid sample from a subject with AD to allow a biomolecule corona to form on the surface of said nanoparticles; and determining the abundance of one or more protein biomarkers in the corona, the one or more protein biomarkers being independently selected from those listed in Table 1a, Table 6a or Table 2.

---

701-900. claims: 22, 24, 26-31(all partially)

Method for assessing the stage of Alzheimer's disease in a subject, comprising contacting a plurality of nanoparticles with a biofluid sample from the subject to allow a

## FURTHER INFORMATION CONTINUED FROM PCT/ISA/ 210

biomolecule corona to form on the surface of said nanoparticles; and determining the abundance of one or more protein biomarkers in the corona, the one or more protein biomarkers being independently selected from those listed in Table 1.

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901-999. claims: 23, 24, 26-31 (all partially)

Method for assessing the stage of Alzheimer's disease in a subject, comprising contacting a plurality of nanoparticles with a biofluid sample from the subject to allow a biomolecule corona to form on the surface of said nanoparticles; and determining the abundance of one or more protein biomarkers in the corona, the one or more protein biomarkers being independently selected from those listed in Table 1a.

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1000. claims: 25 (completely); 26-31 (partially)

Method for identifying neurodegeneration-associated protein biomarkers in the blood of a subject, comprising contacting a plurality of nanoparticles with a blood-derived fluid sample from one or more healthy subjects; and with a blood-derived fluid sample from one or more subjects with neurodegeneration, and determining the abundance of one or more protein biomarkers in the corona from each subject.

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# INTERNATIONAL SEARCH REPORT

Information on patent family members

International application No

PCT/GB2022/050442

| Patent document<br>cited in search report | Publication<br>date | Patent family<br>member(s) | Publication<br>date |
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|                                           |                     | DE 202017107858 U1         | 28-05-2018          |
|                                           |                     | DK 3554681 T3              | 21-03-2022          |
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|                                           |                     | PL 3554681 T3              | 16-05-2022          |
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|                                           |                     | WO 2018112460 A1           | 21-06-2018          |
| -----                                     |                     |                            |                     |