Molecular anatomy of brain synapses from living psychiatric patients

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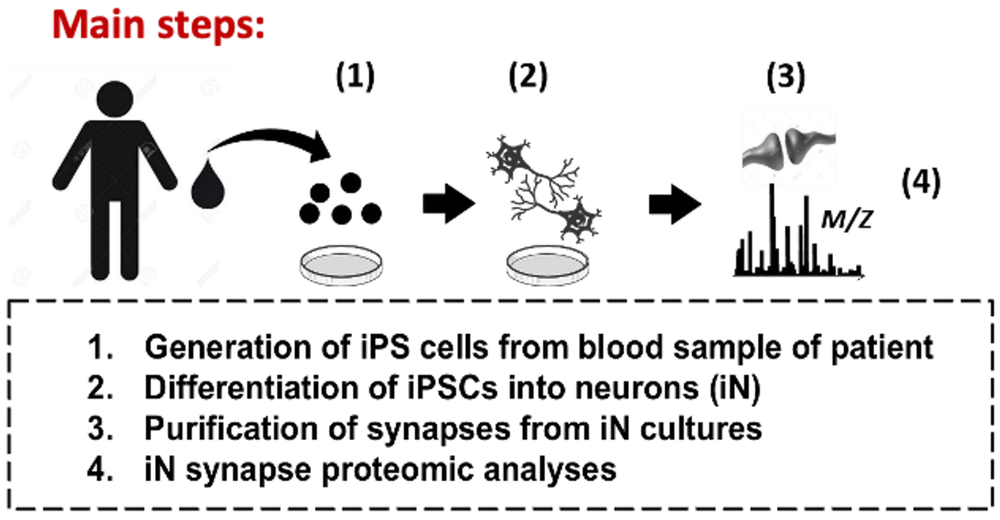
Summary

Synapses are specialized cellular structures connecting neurons that are essential to communication within the brain. They receive-process-store-control all information that flows within neuronal networks. In fact, alteration of synaptic protein expression is often at the root of many brain diseases such as Alzheimer's, autism, ADHD, and schizophrenia. Therefore, there is a tremendous interest in dissecting their proteome.

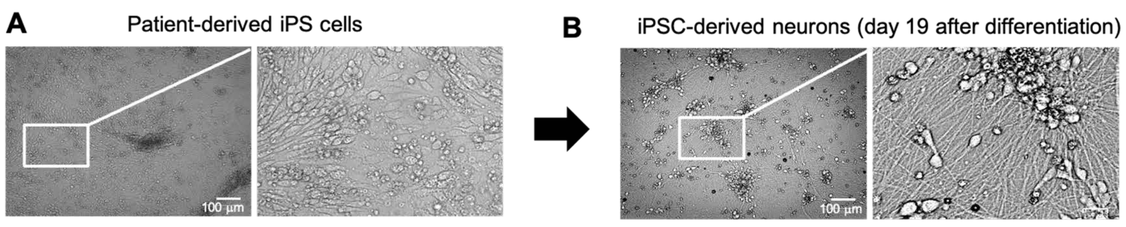
Brain research on living individual is key but unethical. Here, we have developed a noninvasive dissection of brain synapses from living psychiatric patients ('Personalized Synapse Proteomics' (PSP)) technology combining human stem cell reprogramming and our powerful proteomics approach (Taoufiq *et al PNAS* 2020). Our PSP 'ugly prototype' could unveil proteome alterations in brain synapses from a living patient with schizophrenia versus his healthy sibling. These included known schizophrenia disease biomarkers and may potentially encompass dozens of novel ones.

We are planning to expand the use of PSP to many iPS samples. Therefore, **we are now seeking for a future interdisciplinary discussion/collaboration with data scientists** who are using **artificial intelligence and machine learning techniques.** We think this new work will be a gateway toward the development of next-gen personalized and precision therapies against various brain disorders.

Main steps:

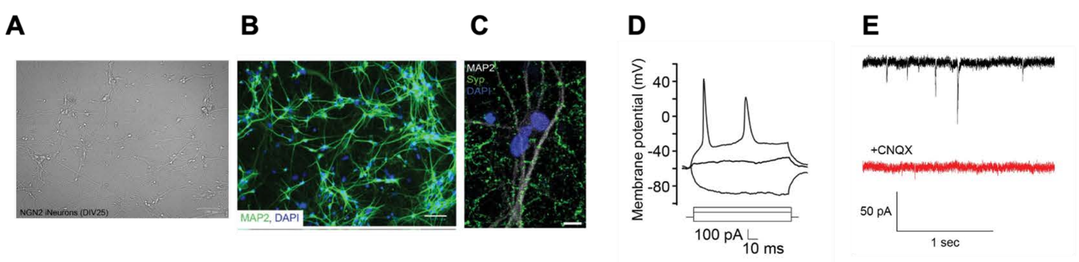


I. Rapid and efficient reprogramming of patient-derived iPScells into neurons:



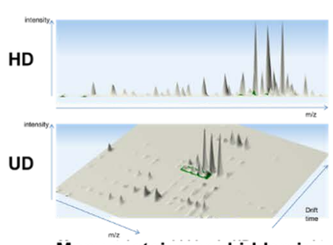
A) DIC image of iPS cells generated from patient, depicting the flat colony structure with cobblestone morphology. Scale bar:100um. **B)** DIC image of patient's iPSCs differentiated into neurons (iN), 19 days after reprogramming with lentiviral-based delivery of Neurogenin-2. iN cells show extensive features of neuronal morphology, such as branching axons and dendrites. Scale bar:100um.

II. The induced neurons (iN) in culture are making functional synapses:

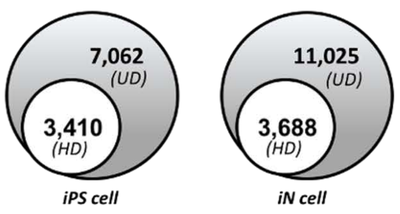


A) DIC image of human ipsc-derived neurons (iN) in culture at DIV25. Scale bar:100um. **B)** Immunofluorescence imaging of iN at DIV25 (neuron-specific marker MAP2 (green); DAPI-stained cell nuclei (blue); Scale bar: 50um). **C)** Immunofluorescence imaging showing iN synapses (synaptic marker synaptophysin (green); neurite marker MAP2 (white); DAPI cell nuclei (blue); Scale bar: 10um). **D)** Action potentials induced in iN by current injection demonstrating normal basic neuronal membrane properties of iN. **E)** Spontaneous synaptic currents in iN (black trace) abolished by addition of glutamate neurotransmitter receptor inhibitor CNQX (red trace) demonstrating the formation of functional excitatory synapses in iN cultures.

III. Our UD proteomics workflow unveils a large number of hidden proteins in human iPS and induced neuronal (iN) cells:



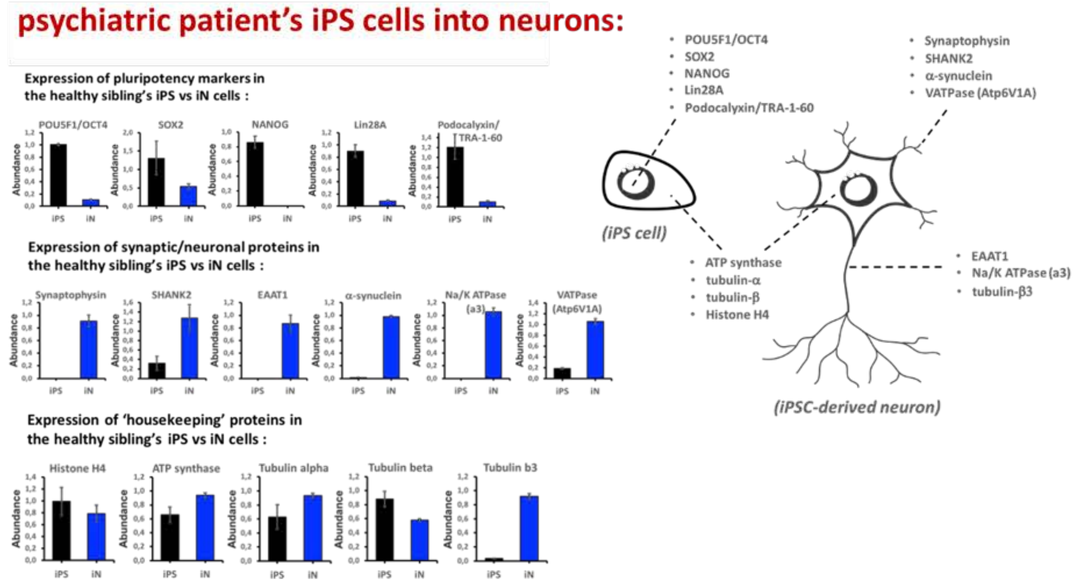
Many proteins are hidden in HD compared to UD
(Taoufiq et al PNAS 2020)



(POC project 2021)

Mass spectrometry-based proteomics was conducted using HD versus UD workflows. HD proteomics seems to be limited in both iPS and iN cells to ~ 4,000 identified protein species, whereas UD proteomics unveils a significantly larger number of proteins. Many of the proteins introduced in the next figures remain 'hidden' in the samples when using HD proteomics...
*('**HD proteomics**' = conventional method prior to use of Orbitrap Fusion Lumos MS device (Thermo). '**UD proteomics**' = (Taoufiq et al PNAS 2020) method prior to use of Orbitrap Fusion Lumos MS device (Thermo))*

IV. Our UD proteomics quantification reliably measures reprogramming of psychiatric patient's iPS cells into neurons:

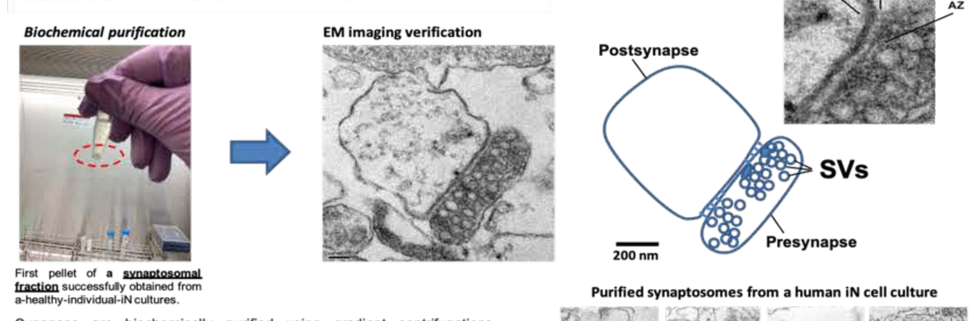


(NB: tubulin beta3 is a microtubule element of the tubulin family found almost exclusively in neurons and testis cells)

Pluripotency proteins are found highly expressed in iPS compared to iN, confirming the establishment of the stem cell lines. In contrast, neuronal and synaptic specific proteins are found highly expressed in iN compared to iPS cells, confirming the successful differentiation induced by NGN2 lentivirus.

Bar graphs represent normalized-to-actin label-free quantifications (LFQ) in UD proteomic analyses of proteins extracted from iPS cell versus iPSC-derived neuron (iN) (mean LFQ ± SEM, n = 3).

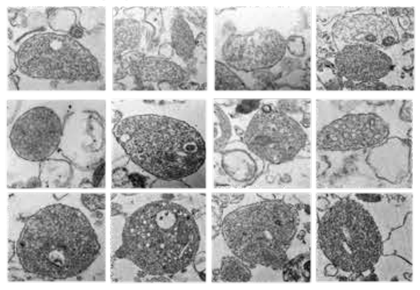
V. Successful purification of synapses from patient-derived iN cell cultures:



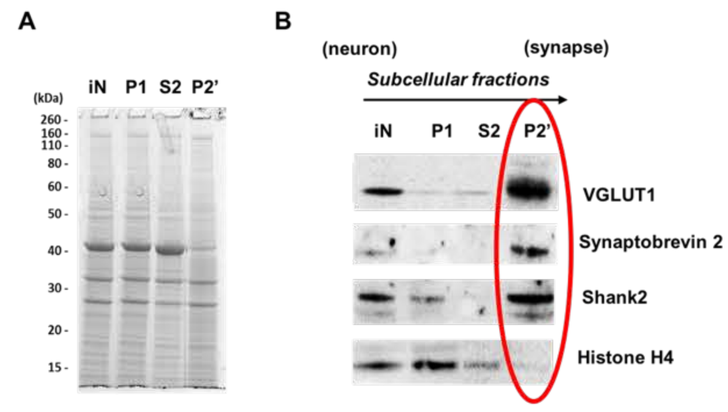
First pellet of a synaptosomal fraction successfully obtained from a-healthy-individual-iN cultures.

Synapses are biochemically purified using gradient centrifugations (traditional method adapted for 'mini scale' samples). Shear forces cause the nerve terminals to detach from axons and dendrites, the membrane of which then reseals to form a spherical structure keeping synaptic content intact: 'synaptosomes'.

Purified synaptosomes from a human iN cell culture

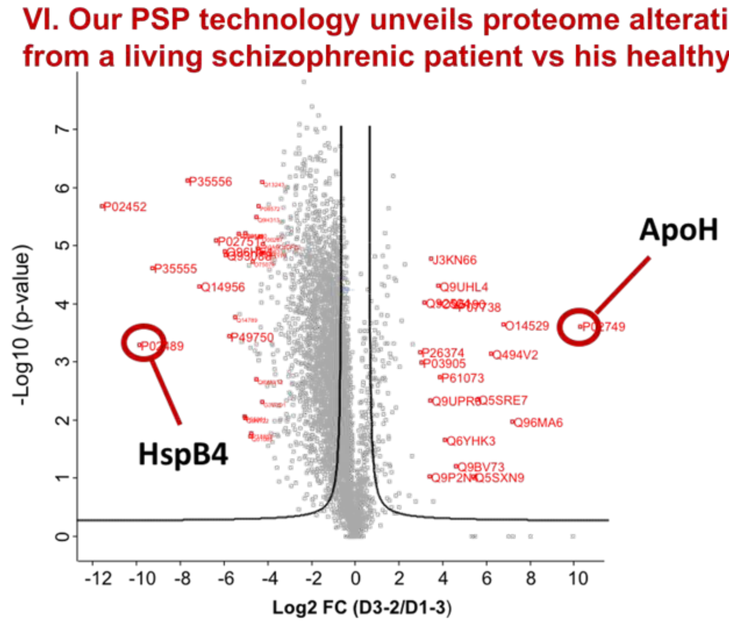


Electron microscopy imaging of a healthy-individual iN synaptosomal sample. The 'pre-synaptosomes' are distinguished from the 'post-synaptosomes' by the presence of numerous synaptic vesicles (SVs, 40-60 nm diameter). Synaptosomes have homogenous sizes between 0.5- and 1.5-µm diameter.



A)Total staining of proteins extracted from subcellular fractions of iPSC-derived neurons (iN). P1: fraction containing cell nuclei; S2: cytoplasmic fraction; P2': synaptosomal fraction. The image shows an effective equal loading of samples into the gel prior to immunoblot analysis. **B)** Immunoblot analyses of cell nucleus (Histone H4), postsynaptic (Shank2) and presynaptic (VGLUT1, Synaptobrevin2) marker proteins in the subcellular fractionation. The high enrichment of synaptic proteins observed in the P2' fraction demonstrates the high efficiency of the synapse purification from a healthy individual's iPSC-derived neuronal culture.

VI. Our PSP technology unveils proteome alterations in synapses from a living schizophrenic patient vs his healthy siblings:



Volcano plot showing differential expression of synaptic proteins in a schizophrenic patient ('D3-2') versus his healthy sibling ('D1-3'). X axis: log2 scale, mean iBAQ ('D3-2'/'D1-3') ratio; Y axis: log10 scale, probability of statistical significance (p-value) from three independent experiments.

In the Literature:

APOH gene is described as antigenic target for antiphospholipid antibodies, leading to antiphospholipid syndrome. Memory alterations, cognitive impairment, mood disorders and psychosis are known to precede the onset of primary antiphospholipid syndrome. Therefore, the role of APOH mutations in neuropsychiatric phenotypes warrants further study...
Desequilibrium of ApoH level is associated with negative health outcomes...
ApoH gene is a risk variant for schizophrenia...

HSPB4 acts as a chaperone, preventing aggregation of various proteins under a wide range of stress conditions.
It is expressed in a highly brain region-specific manner and was discovered to be an essential survival factor for the dopaminergic interneurons.

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