Experimental report

07/09/2023

Proposal: **8-02-978** **Council:** 10/2022

Title: Characterization of a Model Post-Synaptic Density

Research area: Biology

This proposal is a continuation of 8-02-936

Main proposer: **Stephen M. PRINCE**

Experimental team: Giovanna FRAGNETO
USAME AKTAS
Stephen M. PRINCE
Margaret Jayne LAWRENCE

Local contacts: Philipp GUTFREUND
Nicolo PARACINI

Samples: POPC lipids
Multidomain fragments of PSD-95 protein (Uniprot P78352)
Ni-NTA lipid
DPPC lipids

Instrument	Requested days	Allocated days	From	To
D17	3	0		
FIGARO	3	3	09/06/2023	12/06/2023

Abstract:

There are approximately 100 trillion chemical synapses in the human brain. Synapses mediate communication between electrically active cells. The ionotropic channels in the postsynaptic membrane are primarily responsible for the electrical response of the synapse. These channels operate under the executive control of the post-synaptic density (PSD). The PSD is a membrane associated, protein-rich volume with an approximate diameter of 300nm and depth of 30nm. Our recent work has revealed that the PSD scaffold can be organized along the principles of a crystalline lattice. We aim to recapitulate this scaffold at a membrane surface and subsequently explore the incorporation of various components of the PSD. Our preliminary experiments have confirmed the establishment of a protein layer on a bilayer surface we now aim to use isotopic labelling of protein components to interrogate the scaffold structure.

Introduction: The nervous system operates via the transfer of a signal within and between electrically active cells termed neurons. The transfer of the signal commonly occurs at cellular junctions known as chemical synapses. Ionotropic receptor channels at the receiving membrane elicit an electrical response. These channels operate under the executive control of the post-synaptic density (PSD). We aim to improve our understanding of the syapse by examining a model PSD. The technique of specular neutron reflectometry in combination with contrast variation is ideal for the detailed analysis of the membrane localized PSD condensate.

This project builds upon recent work resolving the substructure of a key component of neuronal synapses[1]. We performed initial experiments on the D17 reflectometer (8-02-936) using hydrogenated proteins, and a signal for synaptic proteins was resolved using neutron reflectometry measurements in combination with D_2O contrast. This experiment (8-02-978) built upon the previous experiment in two ways: (i) Using isotopically labelled protein to enhance the signal of the initial protein layer. (ii) Adding a further layer of untagged, hydrogenated proteins to explore the effect of the addition of more laminae of the PSD.

Objectives: We aimed to recapitulate the intial layer of the Post-Synaptic Density (PSD) scaffold at a membrane surface. This layer is formed by the multidomain protein PSD95. We used neutron Reflectometry measurements on FIGARO with solution contrast to explore the structure of a PSD95 protein layer deposited on a memebrane.

Experiment: We used two fragments of the PSD95 protein (UniProt P78352). Each protein component consisted of a dual His_6 tag sequence followed by residues 6-249 (including PDZ domains 1&2) or 6-415 (PDZ 1,2 & 3) of the sequence of PSD95. The experiment was conducted using the his-tag to localize the protein to a lipid membrane in. The premise of the experiment is shown in Figure 1. In the PCSM a Langmuir trough with Blodgett capability was used to deposit, on a silicon block, an asymmetric lipid membrane composed of a first layer (i.e. in direct contact with the block) of dipalmitoylphosphatidylcoline (DPPC) and a second layer composed primarily of palmitoyloleylphosphatidylcoline (POPC) containing 10 mol% of a Ni-NTA lipid to allow the interaction with the his-tag protein, thereby modelling the membrane localized (via palmitolylation [2]) protein *in vivo*. Bilayer deposition was performed at room temperature. Scaffold promoting peptides representative of an ion channel C-terminal sequence (RRESEI) were present in the solution bathing the model lipid membrane to promote scaffold formation.

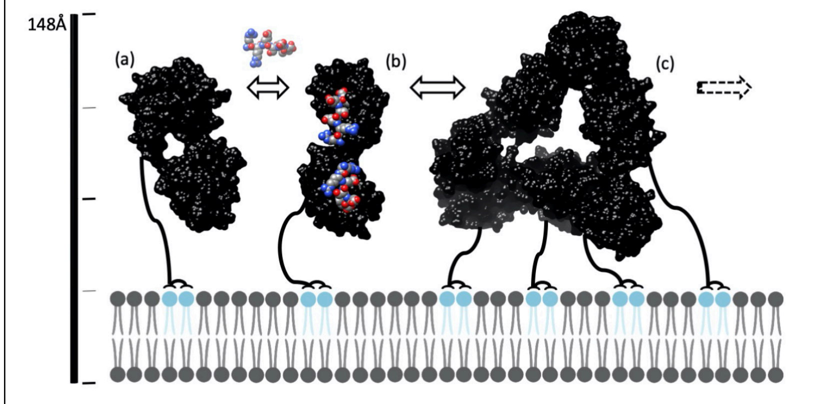


Figure 1. Premise outlined above for N terminally anchored PDZ12 protein (black surface representation) containing 2 PDZ domains. Double His tag adheres to one or more NiNTA lipids (blue). Ligand peptide (RRESEI, space-fill atom colours) enhances interdomain contacts. (a) PDZ12 in compact conformation (b) PDZ12:$(RRESEI)_2$ monomer in extended conformation (c) tetramer of PDZ12:$(RRESEI)_2$. Scaffold formation (c).

In order to more clearly delineate the initial protein layer from the membrane prioir to these experiments we obtained perdeuterated versions of the PSD95 PDZ domains from DLAB (proposals DL-03-272 and DL-03-273). Perdeuterated *E. coli* cell-paste was provided by DLAB and PSD95 domains were purified to homogenaity in our home lab in Manchester.

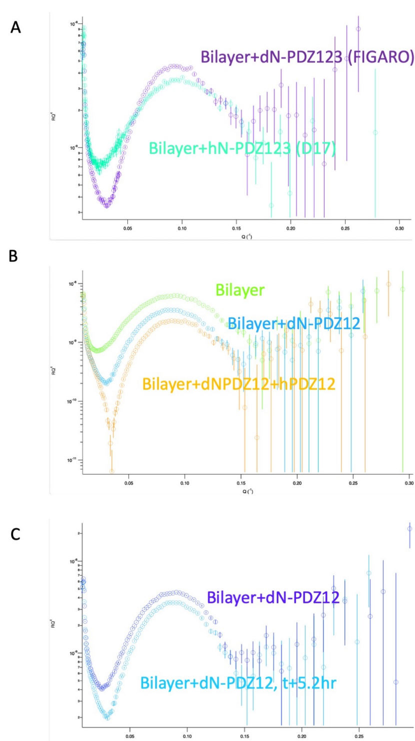
Measurement: A control silicon block was first characterised using D_2O and H_2O. For the experiment proper two Si blocks were used for sample deposition. Initially the bilayers were characterized using four D_2O/H_2O contrasts corresponding to the full range of aqueous solution contrast including the match point of the Si substrate namely: D_2O (100% D_2O), SiMW (38% D_2O), 4MW (66% D_2O), and H_2O (0% D_2O). Contrasts were established using the HPLC pumps in the FIGARO sample set-up. Proteins solutions were then added to the solution bathing the model membranes using the syringe pump in the FIGARO sample set up. Each proteins layer was then successively characterised using D_2O, SiMW, 4MW and H_2O solvent contrasts. A further proteins layer consisting of tag-cleaved hydrogenated PSD95 domains (hPDZ12) was overlaid onto the layer 1 proteins. The untagged hydrogenated protein cannot interact with the NiNTA-lipid and can only form non-covalent interactions with other components in the model PSD. We remeasured a final contrast in SMW to check that the integrity of the sample was maintained throughout.

Table 1. Reflectometry measurements of bilayers + perdeuterated proteins (dN-PDZ12, dN-PDZ123) and hydrogenated protein (hPDZ12) on FIGARO using D_2O, SiMW, 4MW and H_2O buffers (× indicates a single high+low angle reflectometry measurement).

Sample (Position)	Solution Contrast			
	H2O	SMW	4MW	D2O
Si block (P1)	×			×
Bilayer1 (P2)	×	×	×	×
Bilayer2 (P3)	×	×	×	×
Bilayer1+dN-PDZ12 (P2)	××	××	××	××
Bilayer2+dN-PDZ123 (P3)	××	××	××	××
Bilayer1+dN-PDZ12+hPDZ12 (P2)	×	××	×	×
Bilayer2+dN-PDZ123+hPDZ12 (P3)	×	××	×	×

Initial observations on the data collected:

Figure 2. Comparson plots of selected data. Momemtum transfer (Q) on the independent axis, Reflectivity × Q^4 on the dependent axis. All data shown are recorded at 4MW (66% D_2O) contrast. (A) shows a comparison of the signal arising from perdeuterated proteins (on FIGARO) versus hydrogenated proteins (on D17). (B) compares the various layers deposited on the Silicon substrate. (C) Show the time dependent (dynamic) effect observed after proteins deposition.



On comparison between the bilayer only and the bilayer plus protein reflectometry profiles a signal for the protein was seen in the neutron reflectometry experiments for both PDZ12 and PDZ123. Compared to our initial experiment (8-02-936) the perdeuterated proteins showed much stronger effects on the reflectometry signal (see figure 2A).

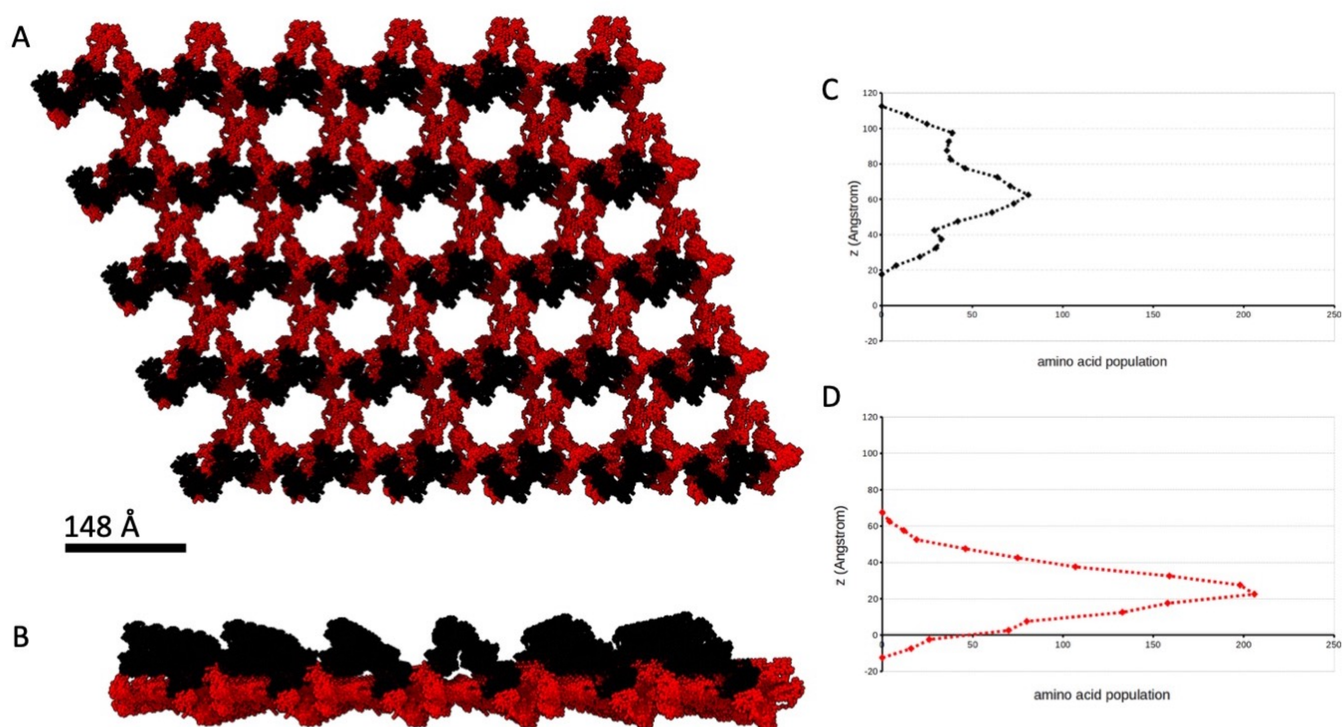
We were able to see pronounced and clear differences in the Reflectometry signal on the addition of each protein to the model bilayer (figure 2B). The reflectivity profiles of the two deposited proteins layers are presently being analysed using the optical matrix method.

We observed some time-dependent effects after addition of the first layer of protein when comparing remeasured these data (see example in figure 2C). The period of these effects was over several hours and we aim to further explore these dynamics in future measurements.

Future plans: We are working to produce a refined laminar model of PSD95 domains affixed to a membrane surface based upon these data. We have now obtained comprehensive reflectometry data sets representing layer 1 of the model PSD which will allow us to develop a robust model. We are exploring the deployment of a detailed domain model based upon our observations from SAXS [1] of the formation of a scaffold (see figure 3) for fitting or comparison. We anticipate that the data collected in this experiment will therefore lead to a publication in the coming months.

We will seek to extend our model by exploring the next layer of the PSD. We hypothesise that this layer is formed from a protein named SAP97 (UniProt Q12959). SAP97 is a relative of PSD95 and has similar domains, but lacks the capacity for direct incorporation into a membrane. We aim to establish a membrane plus layer 1 protein substrate, then introduce perdeuterated SAP97. In effect repacing the hydogenated PSD95 in these measurements. The use of SAP97 will allow us to begin to explore the diversity of components of the PSD. We will continue to use codon optimized clones for efficient expression of isotopically labelled proteins. We will seek ancillary support for SAP97 isotopic labelling (per-deuteration) through the ILL D-LAB.

Figure 3. A/B show a plan/elevation view of a model protein layers with a scalebar (applies to A). The layer in contact with the membrane surface is represented by the Cα coordinates of PDZ domains and is shown in red. A second protein layer is shown in black. C/D show a histograms of populations of amino acids (Cα coordinates) along the (z) coordinate noremal to the membrane.



References

1. Rodzli, N.A., et al., *The Dual PDZ Domain from Postsynaptic Density Protein 95 Forms a Scaffold with Peptide Ligand.* Biophys J, 2020. **119**(3): p. 667-689.
2. El-Husseini, A.E., et al., *Dual palmitoylation of PSD-95 mediates its vesiculotubular sorting, postsynaptic targeting, and ion channel clustering.* J Cell Biol, 2000. **148**(1): p. 159-72.