# Experimental report

21/06/2022

**Proposal:** 8-02-936 **Council:** 10/2020

**Title:** Characterization of a Model Post-Synaptic Density

**Research area:** Biology

**This proposal is a new proposal**


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**Samples:** PSD-95 protein (Uniprot P78352) 78kDa Formula weight

| Instrument | Requested days | Allocated days | From | To |
|---|---|---|---|---|
| D17 | 2 | 2 | 05/07/2021 | 07/07/2021 |
| FIGARO | 2 | 0 | | |


## 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. We propose to use the technique of Neutron Reflectometry to explore this self-assembling condensate. In the long term the use of neutrons will allow isotopic labelling of components and therefore the interrogation of the dense scaffold structure.

**Objectives:**

This project builds upon recent work resolving the substructure of a key component of neuronal synapses[1]. 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 subsequently used Neutron Reflectometry to explore the resulting layer.

**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. 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 7 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.

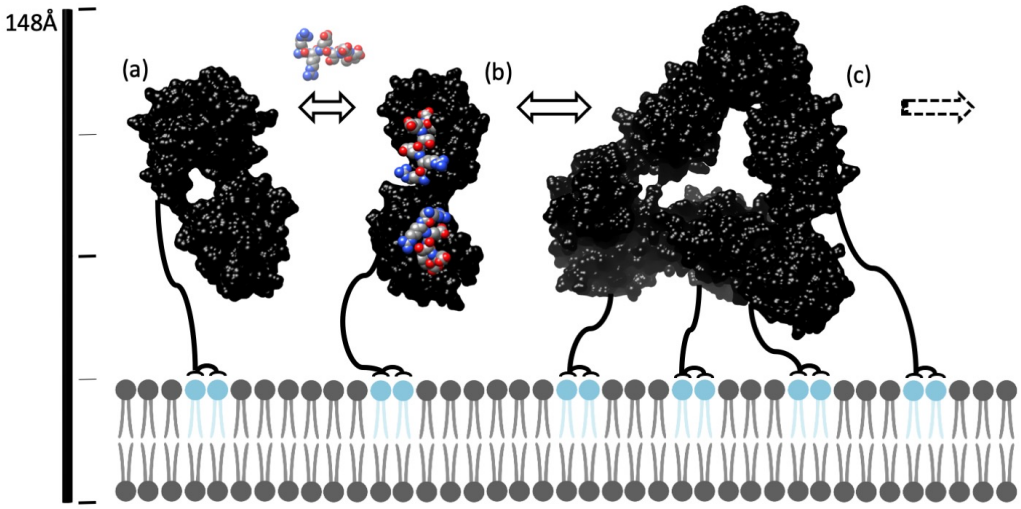


**Figure 1.** Premise outlined above for N terminally anchored PDZ12 protein (black surface representation) containing 2 PDZ domains. Double His tag adheres to one or more NiNTA lipids (blue). Ligand peptide (RRESEI, space-fill atom colours) enhances interdomain contacts. (a) PDZ12 in compact conformation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

**Measurement**:

All measurements were made on the D17 reflectometer. 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% $D_2O$), proteins solutions were then added to the solution bathing the model membranes The protein layer was then successively

characterised using $D_2O$, SiMW, 4MW and $H_2O$ solvent contrasts. We additionally measured a final contrast in $D_2O$ to check that the integrity of the sample was maintained throughout. We were able to see clear difference in the Reflectometry signal on the addition of each protein to the model bilayer.

**Preliminary Data Analysis**

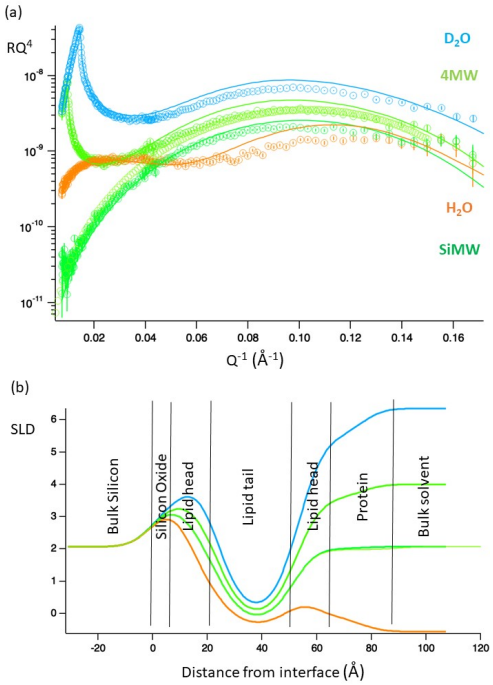


**Figure 2.** (Preliminary) neutron reflectometry analysis for PDZ123 (a) fit to transformed reflectometry data, (b) corresponding Scattering length Density model with annotation.

The reflectivity profiles of the two deposited proteins are presently being analysed using the optical matrix method. The analysis of the PDZ123 data is shown: The fit is shown in figure 2(a) the corresponding laminar model is shown figure 2(b).

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. We are able to assign a layer for the protein in our preliminary models.

**Future plans**

We are working to produce a refined laminar model of PSD95 domains affixed to a membrane surface based upon these data. We are conducting additional experiments (including Surface Plasmon Resonance and Small Angle X-ray Scattering).

The reflectometery signal for the protein can be modified through the use of isotopic labelling and this will be our next priority. We will use codon optimized clones for efficient expression of isotopically labelled proteins. We will seek ancillary support for sample isotopic labelling (per-deuteration) through the ILL D-LAB and or the ESS DMAX platform.


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.