Experimental report

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Proposal: 8-03-848 **Council:** 4/2015

Title: Investigation of PA pore translocation through lipid nanodiscs using SANS and Neutron Reflectometry

Research area: Biology

This proposal is a new proposal

Main proposer: Paul DYER

Experimental team: Beatrice CATTOZ
Susan SHORTER
Paul DYER

Local contacts: Robert BARKER

Samples:

Instrument	Requested days	Allocated days	From	To
D11	2	0		
FIGARO	4	3	30/11/2015	03/12/2015
D17	4	0		
D33	2	0		

Abstract:

Bacillus anthracis produces three virulence factors that lead to profound organismal toxicity. Toxicity is dependent upon the delivery to the cytosol of two enzymatic proteins. The mechanism employed requires a pore forming protein, Protective Antigen (PA). Understanding the mechanism of protein translocation through the PA pore has important implications as it provides opportunities to develop effective neutralising therapies against bioterrorism, as well as novel drug delivery technologies. The hypothesis we wish to test, using neutron reflectometry and small angle scattering, relates to the interaction of the individual components and the capacity for the PA pore to relax allowing for stable macromolecules to translocate accessing the cytosol of a cell. We propose to create a model system to investigate this using lipid nanoparticles.

Investigation of PA pore translocation through lipid nanodiscs by neutron reflectometry

Background

The virulence factor of *Bacillus anthracis* infection, referred to as Anthrax Toxin, consists of three proteins; Oedema factor (EF), Lethal factor (LF), and Protective Antigen (PA). Toxicity is dependent upon EF and LF accessing the cytosol mediated by PA. PA oligomerises as a pre-pore, transition to pore being pH dependent (pH5.5). The decrease in pH (pH5.5) in late endocytic structures also facilitates translocation of LF and EF via a proton gradient (from pH 5.5 to pH7.4). The PA pore has been characterised by crystallography and electron microscopy, and has been described as having a height of 180Å with a "cap" of 130Å diameter.[1] The lumen houses a Φ clamp (Phe^{427}) documented to interact with the N-terminal residues of LF and EF, responsible for pore translocation.[2, 3] The generation of PA pore assembly into lipid nanodiscs has been previously documented.[4]

Method

Specular Neutron Reflectometry (SNR) was used to evaluate the interaction of deuterated LFn-GFP with PA pores stabilised in lipid nanodiscs. A combination of hydrogenated (h) and deuterated (d) nanodiscs (ND) were prepared using either hDMPC or dDMPC stabilised with the hMSPD1 or dMSPD1 respectively. Nanodiscs, both h- and d-, complexed with either dPA or hPA protein (NC), respectively were assembled. ND and NC were adsorbed to the silica surface at 10^{o}C, pH7.4. SNR measurements were performed on the time of flight (TOF) reflectometer, FIGARO. Neutrons of wavelengths ranging between 2 Å and 30 Å and at two incident angles, namely 0.8^{o} and 3.2^{o}, were used giving a Q-range from ~0.002 Å^{-1} to ~0.32 Å^{-1}. Prior to ND adsorption, baseline measurements were performed in D_2O, H_2O, and SiMW. Characterisation of the ND and NC at the solid-liquid interface was performed at pH7.4. Binding and interaction of deuterated LFn-GFP with the both ND and NC was followed in real time at pH7.4 and 37^{o}C mimicking physiological conditions and at pH 5.5 mimicking the acidity of late endosomes. Measurements were performed in a variety of solvents, namely H_2O, D_2O, SiMW and 4MW.

Results

SNR measurements showed good coverage of all nanodiscs and nanocomplexes at the silica/solvent interface. An appreciable change in spectra was observed following addition of dLFn-GFP, and alteration pH from pH7.4 to pH5.5 resulted in additional alteration in the associated spectra in a variety of solvents (Figures 1 and 2). Further analysis of the data will take into account predicted SLD for each component of the system.

Conclusion

The assembly and adsorption of PA containing nanodiscs to the silica surface was successful. The addition of LFn-GFP resulted in an increased thickness of the nanodisc layer and correspondingly the alteration of pH in the system resulted in a further change in thickness. Measurement of the SLD for the ND and NC will be performed at the Rutherford-Appleton Laboratory, Oxford (RB1610353). Measured SLDs will be used to charaterise the interaction of LFn-GFP with both the ND and NCs.

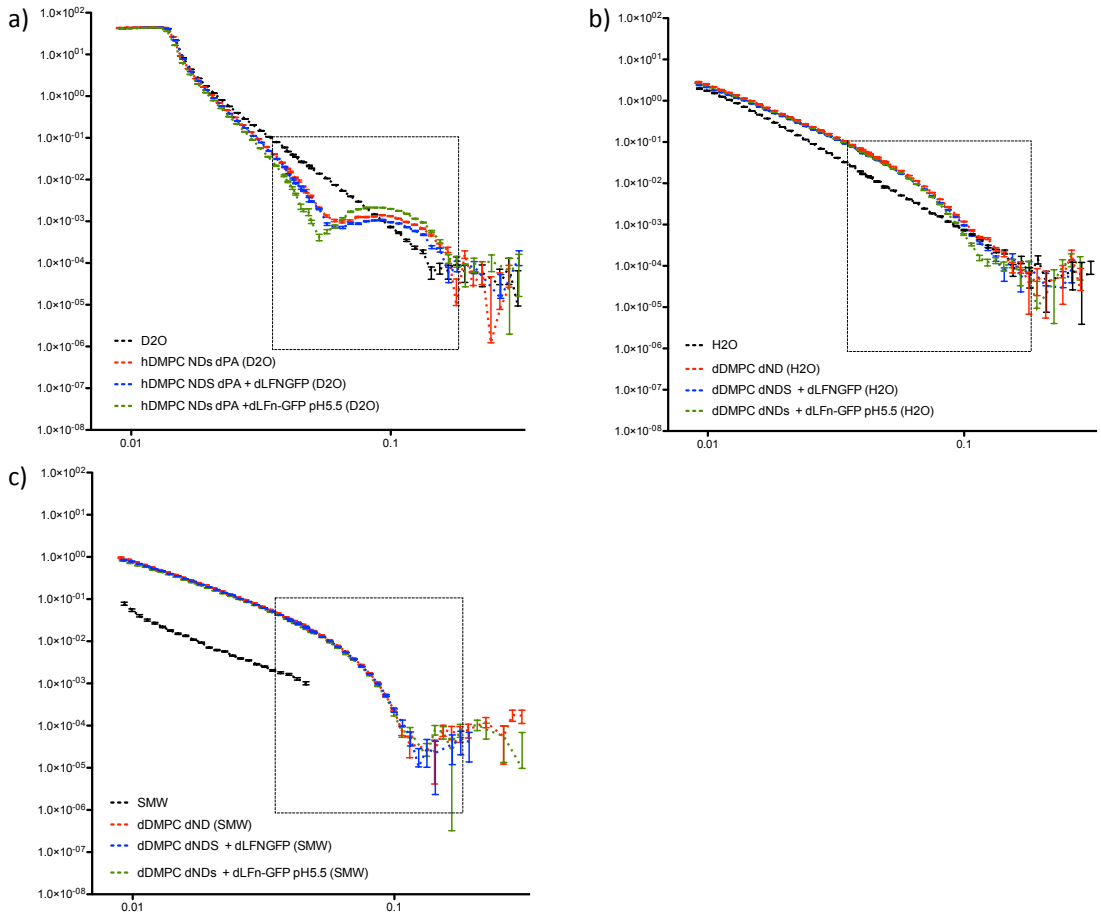

Figure 1. SNR measurement of deuterated DMPC nanodiscs in D_2O (a), H_2O (b) and SiMW (c) solvent at pH7.4, in the presence of LFn-GFP at pH7.4 and pH5.5.

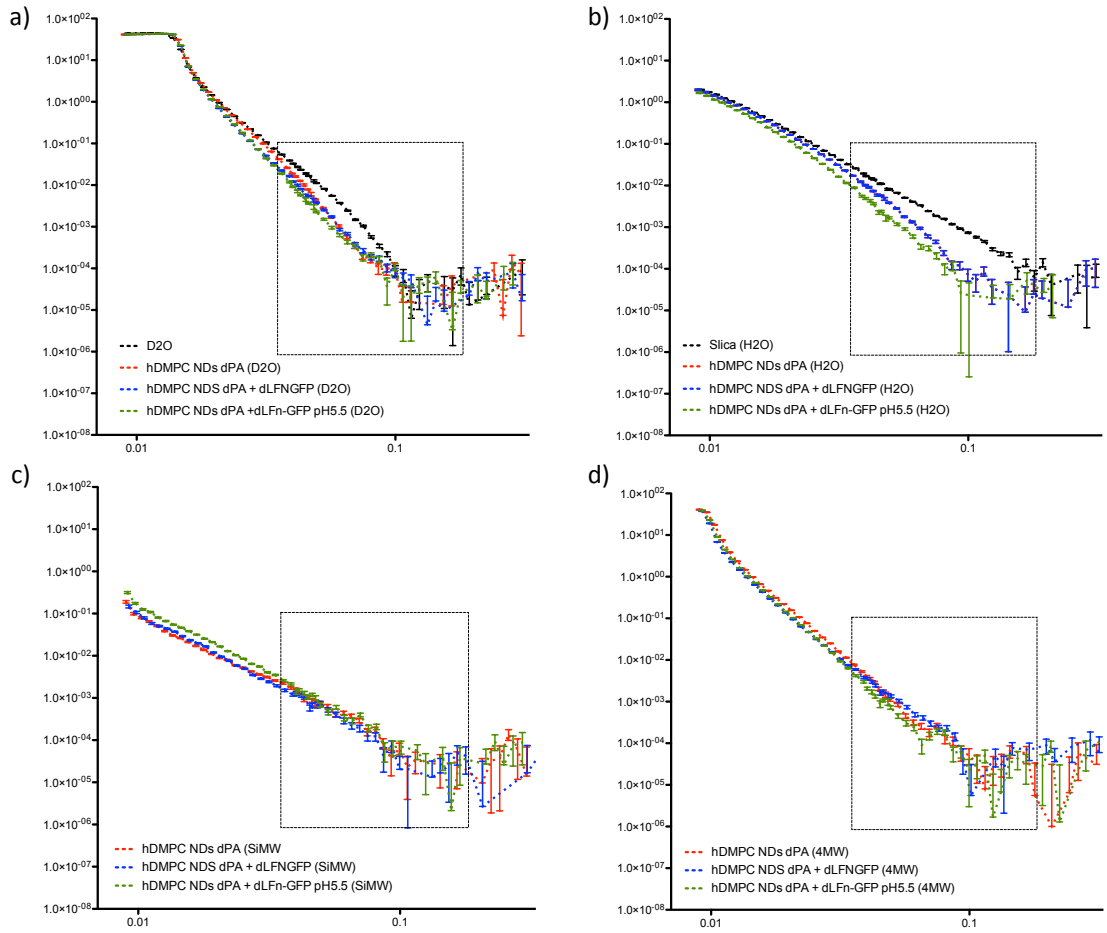

Figure 2. SNR measurement of hDMPC nanodiscs complexed with dPA in D_2O (a), H_2O (b), SiMW (c) and 4MW (d) solvent at pH7.4, in the presence of LFn-GFP at pH7.4 and pH5.5.

References:

1) Rynkiewicz D, *et al.*, (2011) *Vaccine*. **29**(37):6313-20.
2) Petosa C, *et al.,* (1997) *Nature*, 385:833–838.
3) Jennings-Antipov L. D., *et al.,* (2011) *PNAS* **108**(5): 1868–1873.
4) Katayama H., *et al.,* (2010) *PNAS* **107**(8); 3453–3457.