Experimental report

01/12/2021

Proposal: 8-03-957 **Council:** 10/2018

Title: Investigating conformational changes of BmrA reconstituted into invisible-in-SANS artificial nanoscale bilayer disc carriers

Research area: Biology

This proposal is a new proposal

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Samples: LIPIDS
BmrA
dMSP1E3D1

Instrument	Requested days	Allocated days	From	To
D22	2	1	07/02/2020	08/02/2020

Abstract:

The ABC transporter BmrA is involved in the efflux of drugs and antibiotics in Bacillus subtilis (e.g. ethidium, doxorubicin, cervimycin C). Large conformational changes must occur to permit substrate translocation. Our aim is to characterize different conformations of BmrA. We obtained very promising SANS data on detergent-solubilized WT and mutant BmrA blocked in different conformational states. We now aim at studying BmrA reconstituted into artificial nanoscale bilayer discs that mimic the native bilayer environment. In the deuterated form, they should be invisible to neutrons in 100% D2O.

ILL report - BmrA nanodisc experiments

October 2018: BAG-8-37; October 2019: BAG-8-39; Feb 2020: BAG-8-39

Proposal 8-03-932, 8-03-957, 8-03-977

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Annexe table1

			Igor fit (Q x Rg <1.3)					Quality control			
	BmrA Nanodisc Sample	conc.	Io exp	err I(0)	Rg	err Rg	Io/cBmrA		AUC %		
		mg/mL							Ligth ND	Heavy ND	Larger
oct-18	WT Apo 2018	0.72	0.36	0.002	47.1	0.4	0.50				
BAG-8-37	WT ATPVi 2018	0.70	0.346	0.005	46.7	0.8	0.49	after SANS	61	26	13
	WT Doxo 100uM 2018	0.72	0.350	0.004	46.6	0.7	0.49				
	WT Hoechst 100uM 2018	0.72	0.350	0.009	48.0	1.3	0.49				
	WT Doxo 500uM 2018	0.70	aggr	aggr	aggr	aggr					
oct-19	WT Apo 2019	0.99	0.263	0.001	44.9	0.4	0.27	from SEC	86	10	5
BAG-8-39	WT ATPVi 2019	0.96	0.260	0.002	45.5	0.5	0.27	after SANS	81	7	12
	WT Doxo 100 uM 2019	0.99	0.259	0.003	45.9	0.7	0.26				
	WT AD 100 uM 2019	0.97	0.246	0.002	45.0	0.4	0.25				
	WT Reserpine 100 uM 2019	0.99	aggr	aggr	aggr	aggr					
	WT Doxo ATPVi 2019	0.96	0.240	0.004	44.8	0.8	0.25				
	WT AD ATPVi 2019	0.94	0.241	0.002	45.0	0.6	0.26				
	WT Reserpine ATP Vi 2019	0.96	aggr	aggr	aggr	aggr					
	W413F Apo 2019	1.56	aggr	aggr	aggr	aggr		from SEC	83	11	7
	W413F ATPVi 2019	1.52	aggr	aggr	aggr	aggr		after SANS	74	14	11
	W413F Doxo 100 uM 2019	1.55	aggr	aggr	aggr	aggr					
	W413F AD 100 uM 2019	1.53	0.244	0.002	44.6	0.5	0.16				
	W413F Reserpine 100 uM 2019	1.56	aggr	aggr	aggr	aggr					
	W413F Doxo ATPVi 2019	1.51	aggr	aggr	aggr	aggr					
	W413F AD ATPVi 2019	1.48	aggr	aggr	aggr	aggr					
	W413F Reserpine ATP Vi 2019	1.52	aggr	aggr	aggr	aggr					
Feb 2020	WT Apo 2020	0.91	0.149	0.001	42.6	0.5	0.16	After SANS	91	7	1
BAG-8-39	WT ATPVi 2020	0.88	0.153	0.002	43.2	0.7	0.17	After SANS	77	17	5
	WT ADP 2020	0.89	0.140	0.0011	41.7	0.5	0.16				
	WT ADPVi 2020	0.88	0.139	0.001	41.8	0.6	0.16				
	WT Doxo 100 uM 2020	0.91	0.146	0.001	42.0	0.5	0.16				
	WT DoxoATPVi 2020	0.88	0.151	0.001	44.0	0.7	0.17				
	K380A Apo 2020	0.80	0.150	0.001	41.7	0.6	0.19	After SANS	89	9	3
	K380A ATPVi 2020	0.78	0.134	0.003	49.6	1.0	0.17				
	K380A ADP 2020	0.78	0.143	0.002	41.6	0.8	0.18				
	K380A ADPVi 2020	0.77	0.141	0.001	42.2	0.6	0.18				
	K380A Doxo 100 uM 2020	0.80	0.15	0.001	43.0	0.6	0.19				
	K380A DoxoATPVi 2020	0.77	0.149	0.003	42.2	1.1	0.19				
	E504Q Apo 2020	0.49	0.151	0.003	43.3	1.0	0.31				
	E504Q ATP 2020	0.47	0.163	0.004	43.7	1.3	0.35				
	E504Q ADP 2020	0.48	0.145	0.002	42.8	0.9	0.30				
	E504Q ADPVi 2020	0.47	0.148	0.002	42.8	0.8	0.31				
	E504Q Doxo 100 uM 2020	0.49	0.149	0.004	42.9	1.3	0.30				
	E504Q DoxoATP 2020	0.47	0.177	0.005	47.0	1.6	0.38				

Table 1. BmrA Nanodiscs SANS preliminary characterisation. Aggregated samples are indicated by a red color. Non aggregated samples incubated with drug are indicated by a blue color. Concentration are based on the calculated extinction coefficient at 280 nm, calculated considering 2 MSP for 1 BmrA dimer, of 0.992 $cm^{-1}(mg\ BmrA/mL)^{-1}$ for Nanodiscs with WT BmrA, 0.909 $cm^{-1}(mg\ BmrA/mL)^{-1}$ for nanodiscs with W413F BmrA. The calculated I(0) of 1 mg mL^{-1} BmrA dimer is 0.164 cm-1, considering 100% D_2O for the buffer, percentage of labile H exchange of 80%. AUC analysis, with detection with interference and at 280 nm, were done for selected samples. Samples from SEC were used for SANS. Samples after SANS contained absorbing ATP/Vi and were analyzed only with interference optics. The analysis provide sometimes contributions at smaller of generally non-absorbing, detected only in interference, material, which we do not know the relevance. thus in the quality control: 100% = % light nanodisc (ND)+ % heavy ND + % larger species. In 2020, a SEC fraction before the fractions "WT Apo" pooled for SANS, was analyzed by AUC at time 0 and 48h later, contained 89, 8 and 4% of light, heavy, and larger NDs, respectively, without noticeable changes in the relative proportion of light and heavy Nanodiscs.