Monolith Protocol MO-P-066

Bicelles – Dystrophin (R1-3)

Dystrophin is a peripheral membrane protein, supporting the plasma membrane of muscle cells. Gene mutations lead either to a total deficit of dystrophin or to the presence of the protein in truncated forms and are responsible of Duchenne (DMD) and Becker (BMD) muscular dystrophies, respectively. Here, the authors focused on the three first spectrin-like repeats (R1-3) of dystrophin's central domain which are known to interact with membrane lipids and are found in the potential therapeutic mini-dystrophin.

lipid – protein interaction | bicelles | lipidic target

A1. Target/Fluorescent Molecule

Zwitterionic bicelles (DMPC /DHPC)

A2. Molecule Class/Organism

Lipidic object

A3. Sequence/Formula

N/A

A4. Purification Strategy/Source

1,2-dimyristoyl-sn-glycero-3-phosphocholine (DMPC) and 1,2-dihexanoyl-sn-glycero-3-phosphocholine (DHPC), conditioned in chloroform, were purchased from Avanti Polar Lipids (Alabaster, AL) and used without any further purification. Chloroform solutions containing the appropriate amounts of zwitterionic lipid mixtures were dried overnight under vacuum. The lipids were then rehydrated in TNE buffer solution (20 mM Tris, pH 7.5, 150 mM NaCl, 0.1 mM EDTA) to reach a total lipid concentration of at least 200 mM. Then, the solution was frozen in liquid N_2, thawed (10 min at 40°C), vigorously shaken with a vortex (1 min), and then centrifuged (1.5 min, 6000 rpm, Eppendorf MiniSpin). This procedure was repeated twice to obtain a clear suspension of hydrogenated zwitterionic bicelles (HZB).

The molar ratio of the two phospholipids, denoted 'q' is the main parameter governing the size of the bicelles. The effective molar ratio is denoted 'q_{eff}', taking into account the proportion of free DHPC, and is defined by:

$$q_{eff} = \frac{[DMPC]}{[DHPC]_{tot} - [DHPC]_{free}}$$

A5. Stock Concentration/Stock Buffer

200 mM total lipid concentration (DMPC/DHPC 1:2 mol/mol, q_{eff} = 0.5)
20 mM Tris, pH 7.5, 150 mM NaCl, 0.1 mM EDTA

A6. Molecular Weight/Extinction Coefficient

N/A

A7. Dilution Buffer

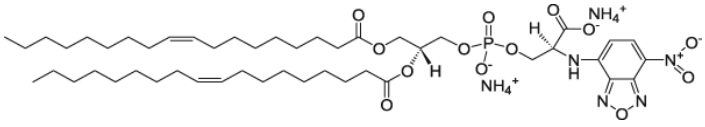
20 mM Tris, pH 7.5, 150 mM NaCl, 0.1 mM EDTA

A8. Labeling Strategy

Incorporation of 1,2-dioleoyl-sn-glycero-3-phospho-Lserine-N-(7-nitro-2-1,3-benzoxadiazol-4-yl) (also called 18:1 NBD PS) in the above described bicelles preparation.

A9. Labeling Procedure

Bicelles were labeled using 1 µM of 1,2-dioleoyl-sn-glycero-3-phospho-Lserine-N-(7-nitro-2-1,3-benzoxadiazol-4-yl) (**18:1 NBD PS**) for a final concentration of 15 mM of lipids (~50 µM of bicellar objects).



Avanti Polar Lipids, Inc.
810198

A10. Labeling Efficiency

N/A

B1. Ligand/Non-Fluorescent Binding Partner

Dystrophin R1–3
uniprot.org/uniprot/P11532

B2. Molecule Class/Organism

Homo sapiens (Human)

B3. Sequence/Formula

MKHHHHHHHL VPR*[1]
GSEVNLDRYQ TALEEVLSWL LSAEDTLQAQ GEISNDVEVV KDQFHTHEGY MMDLTAHQGR VGNILQLGSK LIGTGKLSED
EETEVQEQMN LLNSRWECLR VASMEKQSNL HRVLMDLQNQ KLKELNDWLT KTEERTRKME EEPLGPDLED LKRQVQQHKV
LQEDLEQEQV RVNSLTHMVV VVDESSGDHA TAALEEQLKV LGDRWANICR WTEDRWVLLQ DILLKWQRLT EEQCLFSAWL
SEKEDAVNKI HTTGFKDQNE MLSSLQKLAV LKADLEKKKQ SMGKLYSLKQ DLLSTLKNKS VTQKTEAWLD NFARCWDNLV
QKLEKSTAQI SQA*

B4. Purification Strategy/Source

The His-tagged protein was produced in the BL21 *Escherichia coli* (DE3) strain and purified by immobilized metal affinity chromatography on Ni-Sepharose column (HisTrap, GE Healthcare) according to the manufacturer's instructions. The tag was removed by thrombin cleavage, and the protein was further purified with a size-exclusion chromatography column (HiLoad 16/600 Superdex 200 prep grade, GE Healthcare) equilibrated with TNE buffer (20 mM Tris pH 7.5, 150 mM NaCl, 0.1 mM EDTA).

B5. Stock Concentration/Stock Buffer

~17.3-18.8 mg/ml | ~450-490 µM
20 mM Tris pH 7.5, 150 mM NaCl, 0.1 mM EDTA

B6. Molecular Weight/Extinction Coefficient

38.5 kDa
59,720 $M^{-1}cm^{-1}$ (ε_{280})

B7. Serial Dilution Preparation

1. Prepare a PCR-rack with 16 PCR tubes. Transfer 60 µL of the 450 µM R1-3 solution into tube **1**. Then, transfer 15 µL of dilution buffer into tubes **2** to **16**.
2. Prepare a 3:1 serial dilution by transferring 45 µL from tube to tube. Mix carefully by pipetting up and down. Remember to discard 45 µL from tube **16** to get an equal volume of 15 µL for all samples.
3. Add 5 µL of 60 mM (lipid concentration) of labeled bicelles (4 µM 18:1 NBD PS) to each tube from **16** to **1** and mix by pipetting.
4. Incubate for 5 minutes at room temperature in the dark before loading capillaries.

[1] The exact protein sequence used is surrounded by stars. The beginning of the sequence was added for purification and cleavage.

C. Applied Quality Checks

SANS measurement

D1. MST System/Capillaries

Monolith NT.115 Blue (NanoTemper Technologies GmbH)
Premium Capillaries Monolith NT.115 (MO-K025, NanoTemper Technologies GmbH)

D2. MST Software

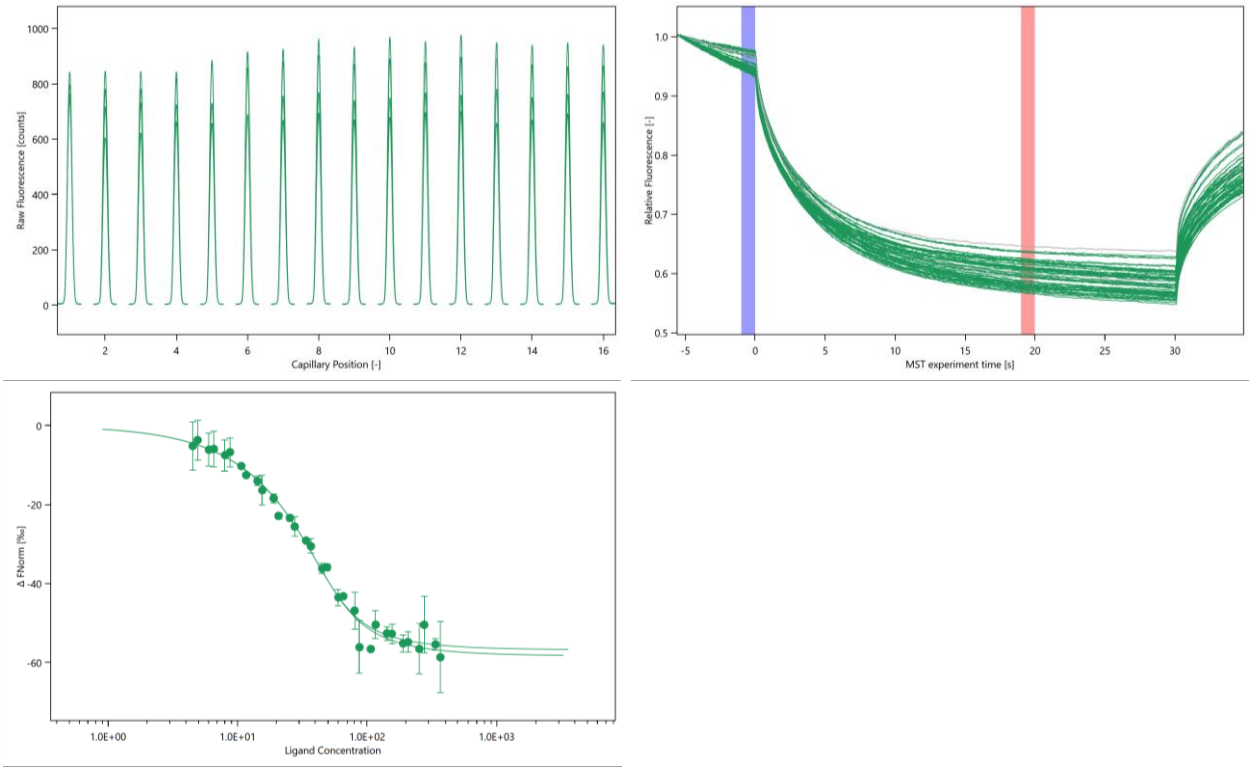
MO.Affinity Analysis software v2.1 (NanoTemper Technologies GmbH)
nanotempertech.com/monolith/#monolith-software

D3. MST Experiment (Assay Buffer/Concentrations/Temperature/MST Power/Excitation Power)

20 mM Tris, pH 7.5, 150 mM NaCl, 0.1 mM EDTA
50 µM bicelles[2] | 338-368 µM – 4.5-4.9 µM R1-3 | 22°C | medium MST power | 20-30% excitation power

D4. MST Results (Capillary Scan/Time Traces/Dose Response)

K_d ≈ 10 µM Dos Santos Morais et al., Biophysical J, 115 (7) 1231-1239 (2018)



[2] DMPC/DHPC, q_{eff} ≈ 1.3

D5. Reference Results/Supporting Results

SANS, CD and tryptophan fluorescence data from the same publication clearly prove the interaction, although no K_d determination could be performed using those techniques.
Dos Santos Morais et al., Biophysical J, 115 (7) 1231-1239 (2018)

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