Monolith Protocol MO-P-080

Critical Micelle Concentration (CMC) of LMNG

Lauryl maltose-neopentyl glycol (LMNG) is a relatively new detergent which solubilizes and stabilizes a wide range of membrane proteins much better than traditional detergents. In this protocol, the critical micelle concentration (CMC) of LMNG is determined by measuring its interaction with a fluorescent dye. As soon as the concentration of LMNG is high enough to form micelles, the dye starts to be incorporated into them, which in turn leads to a more than two-fold fluorescence increase. In addition, dynamic light scattering measurements using the Prometheus Panta confirm the presence of micelles. The determination of the CMC value of surfactants under different environmental conditions is important for many different biological and chemical processes.

critical micelle concentration | LMNG

A1. Target/Fluorescent Molecule

NT-647

A2. Molecule Class/Organism

Fluorescent dye

A3. Sequence/Formula

N/A

A4. Purification Strategy/Source

Monolith Protein Labeling Kit RED – NHS (MO-L001, NanoTemper Technologies GmbH)

A5. Stock Concentration/Stock Buffer

10 µg lyophilized powder

A6. Molecular Weight/Extinction Coefficient

N/A

A7. Dilution Buffer

100 mM Tris pH 7.5

A8. Labeling Strategy

Monolith Protein Labeling Kit RED – NHS (MO-L001, NanoTemper Technologies GmbH)
1* 10 µg NT-647-NHS dye

A9. Labeling Procedure

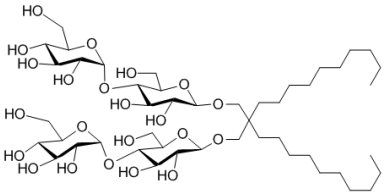
1. Add 282 µL of dilution buffer to the NT-647-NHS dye (10 µg) to obtain a ~50 µM solution. Mix the dye thoroughly by vortexing and make sure that all dye is dissolved.
2. Incubate for at least 30 minutes, so that all active NHS groups will hydrolyze or react with the Tris.

A10. Labeling Efficiency

N/A

B1. Ligand/Non-Fluorescent Binding Partner

Lauryl Maltose Neopentyl Glycol (LMNG)



B2. Molecule Class/Organism

Detergent

B3. Sequence/Formula

$C_{47}H_{88}O_{22}$

B4. Purification Strategy/Source

N/A

B5. Stock Concentration/Stock Buffer

Powder

B6. Molecular Weight/Extinction Coefficient

1005.19 Da

B7. Serial Dilution Preparation

1. Dissolve 20 mg of LMNG in 1 mL of ddH_2O to obtain a 2% (~20 mM) LMNG stock solution.
2. Mix 2 µL of 50 µM NT-647-NHS dye with 198 µL of dilution buffer to obtain 200 µL of 500 nM NT-647.
3. Mix 50 µL of 500 nM NT-647 with 450 µL of ddH2O to obtain 500 µL of 50 nM NT-647 in 10 mM Tris.
4. Take another tube and mix 400 µL of 50 nM NT-647 with 400 µL of ddH_2O to obtain 800 µL of 25 nM NT-647.
5. Prepare a PCR-rack with 24 PCR tubes. Mix 20 µL of the 20 mM LMNG solution with 20 µL of **50 nM** NT-647 in tube **1**. Then, transfer 20 µL of **25 nM** NT-647 into tubes **2** to **24**.
6. Prepare a 1:1 serial dilution by transferring 20 µL from tube to tube. Mix carefully by pipetting up and down.
7. Incubate for 5 minutes at room temperature in the dark before loading capillaries.

D1. MST System/Capillaries

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

D2. MST Software

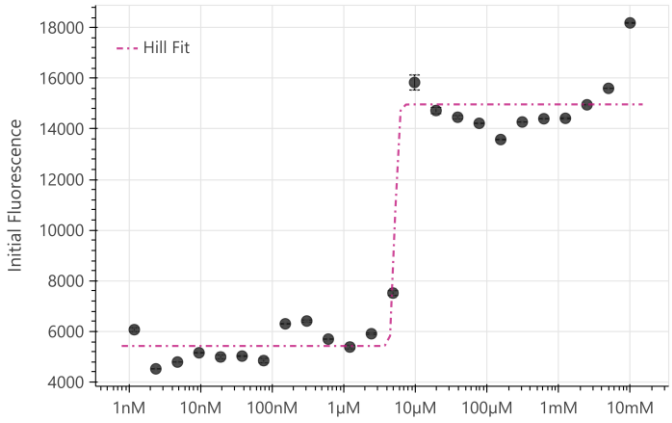
MO.Control v2.1 (NanoTemper Technologies GmbH)
https://nanotempertech.com/monolith-mo-control-software/

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

5 mM Tris pH 7.5
25 nM NT-647 | 10 mM – 1.2 nM LMNG | 25°C | medium MST power | 3% excitation power

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

CMC ~ 10 µM (0.001%)

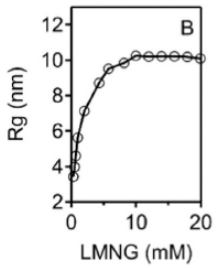


Once, first LMNG micelles are formed, the dye can bind into them, which leads to a sudden increase of the total fluorescence.

D5. Reference Results/Supporting Results

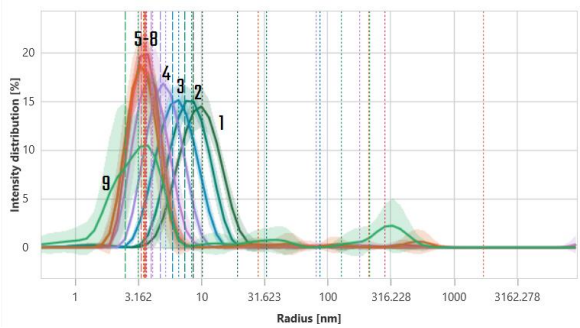
CMC ~ 0.01 mM (0.001%) Hydrophobic dye solubilization
Chae et al., Nature Methods 7, 1003–1008 (2010)

Rg = 3.5 – 10 nm SAXS, radius of gyration
Breyton et al., BBA Biomembranes, 1861, 5, 939-957 (2019)

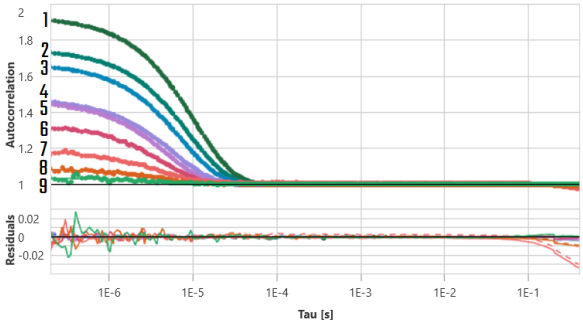


Confirmation of micelle formation by dynamic light scattering measurements with Prometheus Panta: nanotempertech.com/prometheus

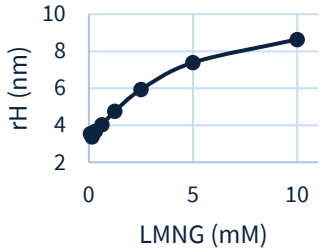
Intensity distribution:



Autocorrelation:



First micelles can be detected above 20µM (rH of ~3.5 nm). Above 500 µM (0.05%) LMNG, micelles become larger.



Capillary	Concentration (µM)	Concentration (%)	rH (nm)	PDI	Scattering Intensity (10^6 cts/s)
1	10,000	1	8.64 ± 0.06	0.19	37.7
2	5,000	0.5	7.39 ± 0.03	0.17	16.3
3	2,500	0.25	5.93 ± 0.03	0.16	6.4
4	1,250	0.125	4.76 ± 0.02	0.15	2.0
5	625	0.0625	4.03 ± 0.04	0.12	1.3
6	313	0.0313	3.67 ± 0.06	0.07	0.69
7	156	0.0156	3.37 ± 0.13	0.15	0.39
8	78	0.0078	3.54 ± 0.32	0.20	0.29
9	39	0.0039	2.49 ± 1.56	0.82	0.24
>9	20	0.0020	n.a.	>1	< 0.24

E. Contributors

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