Monolith Protocol MO-P-027

# 30S Ribosomal Subunit – YjeQ

The ribosome is responsible for translating the genetic code into functional proteins and is often a target for antimicrobial research. Specifically, the 30S ribosomal subunit decodes mRNA to ensure high fidelity during protein synthesis. Although much is known about protein translation, the details surrounding ribosome biogenesis remain largely elusive. Ribosome biogenesis is a complex process that requires the assembly of multiple proteins and rRNA molecules to form a functional 70S ribosome in bacteria. This process is aided by multiple assembly factors that transiently interact with assembling ribosome particles to aid in their maturation. A central protein involved in this process is the GTPase YjeQ, which has been associated with aiding in the later stages of 30S maturation.

protein – protein interaction | ribosome assembly | GTPase | biogenesis

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## A1. Target/Fluorescent Molecule

Prokaryotic small ribosomal subunit (30S ribosomal subunit)

## A2. Molecule Class/Organism

Ribonucleoprotein
*Escherichia coli*

## A3. Sequence/Formula

N/A[1]

## A4. Purification Strategy/Source

Isolated from wild type E. coli (BW25113)
Purified using ultracentrifugation over sucrose cushions and gradients[2]

## A5. Stock Concentration/Stock Buffer

4.25 mg/mL | 5 µM
10 mM Tris-HCl, pH 7.5, 1.1 mM magnesium acetate, 60 mM $NH_4Cl$, 0.5 mM EDTA, 2 mM 2-mercaptoethanol

## A6. Molecular Weight/Extinction Coefficient

850 kDa
13,700,000 $M^{-1}cm^{-1}$ ($\varepsilon_{260}$)

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[1] The 30S ribosomal subunit is a complex of the 16S ribosomal RNA (rRNA) and 19 proteins.
[2] See Leong et al., RNA, 19, 789-802 (2013) for further details.

## A7. Dilution Buffer

20 mM Tris-HCl, pH 7.5, 150 mM NaCl, 10 mM $MgCl_2$, 1 mM DTT, 0.05% TWEEN® 20, 0.4 mg/mL BSA, 1 mM GMP-PNP[3]

## A8. Labeling Strategy

Monolith Protein Labeling Kit RED – Maleimide (MO-L004, NanoTemper Technologies GmbH)
1x Labeling Buffer NHS | 1x A-Column | 1x 10 µg NT-647-MALEIMIDE dye | 1x B-Column

## A9. Labeling Procedure

1. Prepare 100 µL of a 5 µM 30S ribosomal subunit solution.
2. Use Spin Column A to perform a buffer exchange into Labeling Buffer NHS **supplemented** with 10 mM $MgCl_2$.
   a. Invert Spin Column A to suspend slurry and twist off bottom (twist slightly in both directions).
   b. Loosen the cap of the column and place it in a 1.5 mL microcentrifuge collection tube.
   c. Centrifuge at **1500 × g** for **1 min** to remove excess liquid.
   d. Add 300 µL of Labeling Buffer NHS with $MgCl_2$ and centrifuge at **1500 × g** for **1 min** (3x).
   e. Place 100 µL of the 5 µM 30S ribosomal subunit solution in the center of the resin.
   f. Place the sample in a **new** microcentrifuge collection tube and centrifuge at **1500 × g** for **2 min**.

   The collected flow-through should yield around 100 µL of ~4 µM 30S ribosomal subunit (~80 % recovery).
3. Add 30 µL of DMSO to 10 µg NT-647-MALEIMIDE dye to obtain a ~470 µM solution. Mix the dye thoroughly by vortexing and make sure that all dye is dissolved.
4. Mix 3.2 µL of the 470 µM dye solution with 96.8 µL of Labeling Buffer NHS with $MgCl_2$ to obtain 100 µL of a 15 µM dye solution (~4x protein concentration).
5. Mix 30S ribosomal subunit and dye in a 1:1 volume ratio (200 µL final volume).
6. Incubate for 30 minutes at room temperature in the dark.
7. In the meantime, remove the top cap of the B-Column and pour off the storage solution. Remove the bottom cap and place with adapter in a 15 mL tube.
8. Fill the column with dilution buffer and allow it to enter the packed resin bed completely by gravity flow. Discard the flow through collected. Repeat this step 3 more times.
9. Add 200 µL of the labeling reaction from step 5 to the center of the column and let sample enter the bed completely.
10. Add 400 µL of dilution buffer after the sample has entered and discard the flow through.
11. Place column in a new collection tube, add 500 µL of dilution buffer and collect the eluate.
12. Keep labeled 30S ribosomal subunit (~0.8 µM) on ice in the dark.

## A10. Labeling Efficiency

Measurement of protein concentration and degree of labeling (DOL) using a NanoDrop™:
nanotempertech.com/dol-calculator

| | | | |
|---|---|---|---|
| Absorbance $A_{260}$ | 7.0 | Protein concentration | 0.51 µM |
| Absorbance $A_{650}$ | 0.06 | Degree-of-labeling (DOL) | 0.47 |

[3] GMP-PNP was substituted with GDP for the negative control.

## B1. Ligand/Non-Fluorescent Binding Partner

YjeQ (RsgA)
uniprot.org/uniprot/P39286

## B2. Molecule Class/Organism

GTPase
*Escherichia coli*

## B3. Sequence/Formula

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MSKNKLSKGQ QRRVNANHQR RLKTSKEKPD YDDNLFGEPD EGIVISRFGM HADVESADGD VHRCNIRRTI RSLVTGDRVV
WRPGKPAAEG VNVKGIVEAV HERTSVLTRP DFYDGVKPIA ANIDQIVIVS AILPELSLNI IDRYLVACET LQIEPIIVLN
KIDLLDDEGM AFVNEQMDIY RNIGYRVLMV SSHTQDGLKP LEEALTGRIS IFAGQSGVGK SSLLNALLGL QKEILTNDIS
DNSGLGQHTT TAARLYHFPH GGDVIDSPGV REFGLWHLEP EQITQGFVEF HDYLGLCKYR DCKHDTDPGC AIREAVEEGK
IAETRFENYH RILESMAQVK TRKNFSDTDD
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## B4. Purification Strategy/Source

YjeQ was overexpressed in BL21-AI competent cells using the pDEST17-*yjeQ* plasmid with an N-terminal $His_6$-tag cleavable by tobacco etch virus (TEV) protease[4]. One liter of LB medium was inoculated with 10 mL of saturated overnight culture and cells were grown to OD600 = 0.6 by incubation at 37 °C and shaking at 225 rpm in an Excella E24 incubator (New Brunswick). Expression was induced with 0.2% L-arabinose. Cells were then induced for 3 hours at 37 °C and harvested by centrifugation at 3700 × g for 15 min. Cell pellets were washed with 1X PBS buffer (137 mM NaCl, 2.7 mM KCl, 8.1 mM $Na_2HPO_4$ at pH 7.4) and resuspended in 20 mL of lysis buffer (50 mM Tris-HCl at pH 8.0, 10% [w/v] sucrose, 100 mM NaCl) containing a protease inhibitor cocktail (cOmpleteTM Mini Protease Inhibitor Cocktail Tablets, Roche).

The cell suspension was passed through a French pressure cell at 1400 kg/cm$^2$ three consecutive times to lyse the cells. The lysate was spun at 39,200 × g for 45 min to clear cell debris and the supernatant was collected. All the following steps were performed at 4 °C. NaCl was added to the supernatant of the YjeQ overexpression cells to a concentration of 0.5 M. Clarified cell lysates of YjeQ were passed through a 0.45-µm syringe filter (Millipore) and loaded to a HiTrap Metal Chelatin2 Column (GE Healthcare Life Sciences) previously equilibrated with 50 mM Tris-HCl at pH 8.0, 0.5 M NaCl and 5% [v/v] glycerol. Nonspecifically bound proteins were washed with incremental step-wise increases in the concentration of imidazole from 45 mM to 90 mM. YjeQ was eluted with 240 mM imidazole. Purity of the fractions was monitored by SDS-PAGE and fractions containing pure (>95%) YjeQ protein were collected and pooled together.

The N-terminal $His_6$-tag was removed by digestion with TEV protease at a ratio of 10:1 (YjeQ:TEV) during overnight dialysis against buffer containing 50 mM Tris-HCl at pH 8.0, 60 mM imidazole and 0.2 M NaCl. Any overnight precipitate was removed by spinning at 12,000 × g for 10 min and the supernatant was collected and loaded onto a HiTrap Metal Chelating Column previously equilibrated with 50 mM Tris-HCl at pH 8.0, 0.2 M NaCl and 60 mM imidazole. Fractions were collected, and their purity evaluated by SDS-PAGE and Coomassie Brilliant Blue staining. Fractions containing pure untagged YjeQ were pooled and dialyzed against 50 mM Tris-HCl at pH 8.0, 5% [v/v] glycerol overnight. A 10 kDa-cutoff filter (Amicon) was used to concentrate the protein and the purified YjeQ was frozen in liquid nitrogen and stored at -80 °C.

[4] See Jomaa et al., RNA, 17, 2026-2038 (2011) for further details.

## B5. Stock Concentration/Stock Buffer

2.0 mg/mL | 50 µM
50 mM Tris-HCl, pH 8.0, 5% glycerol

## B6. Molecular Weight/Extinction Coefficient

39.1 kDa
24,410 $M^{-1}cm^{-1}$ ($\varepsilon_{280}$)

## B7. Serial Dilution Preparation

1. Centrifuge YjeQ and 30S ribosomal subunits for 14 000 × g for 10 min and carefully remove supernatant.
2. Add 2 µl of unlabeled YjeQ to 48 µl dilution buffer to obtain 50 µl of a 2 µM solution.
3. Prepare a PCR-rack with 16 PCR tubes. Transfer 20 µL of the 2 µM YjeQ solution into tube **1**. Then, transfer 10 µL of dilution buffer into tubes **2** to **16**.
4. Prepare a 1:1 serial dilution by transferring 10 µL from tube to tube. Mix carefully by pipetting up and down. Remember to discard 10 µL from tube **16** to get an equal volume of 10 µL for all samples.
5. Mix 20 µL of labeled 30S ribosomal subunit with 180 µL of dilution buffer to obtain 200 µL of ~80 nM 30S.
6. Add 10 µL of 80 nM labeled 30S ribosomal subunit to each tube from **16** to **1** and mix by pipetting.
7. Incubate for 10 minutes at room temperature in the dark before loading capillaries.

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## D1. MST System/Capillaries

Monolith NT.115 Red (NanoTemper Technologies GmbH)
Hydrophobic Capillaries Monolith NT.115 (NanoTemper Technologies GmbH)

## D2. MST Software

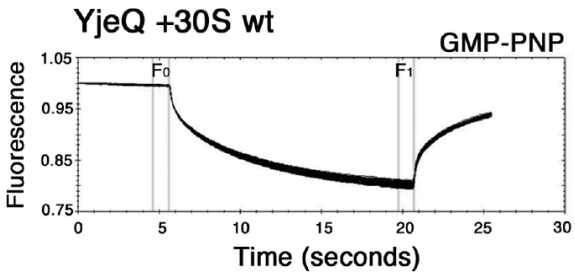
MO.Control v1.6 (NanoTemper Technologies GmbH)
nanotempertech.com/monolith-mo-control-software

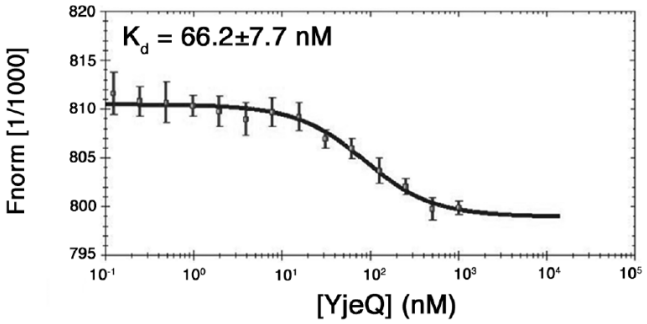
## D3. MST Experiment (Assay Buffer/Concentrations/Temperature/MST Power/Excitation Power)

20 mM Tris-HCl, pH 7.5, 150 mM NaCl, 10 mM $MgCl_2$, 1 mM DTT, 0.05% TWEEN® 20, 0.4 mg/mL BSA, 1 mM GMP-PNP
40 nM 30S ribosomal subunit | 0.03 nM – 1 µM YjeQ | 25°C | high MST power | 70% excitation power

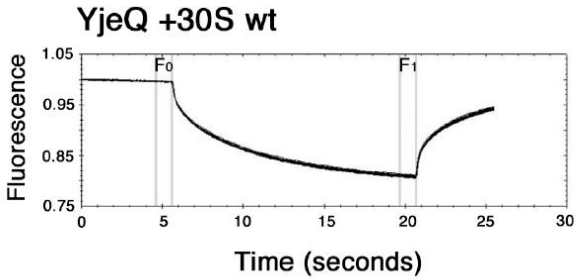
## D4. MST Results (Capillary Scan/Time Traces/Dose Response)

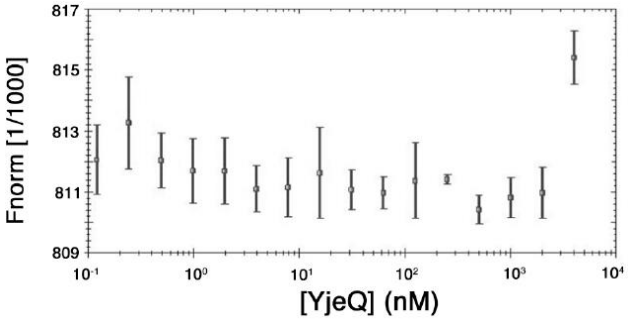
$K_d$ = 66.2 ± 7.7 nM (with GMP-PNP) Thurlow et al. (2016), Nucleic Acids Research, 44(20), 9918-9932




$K_d$= N/A (with GDP, negative control)




## D5. Reference Results/Supporting Results

Interaction of YjeQ with the 30S subunit

Daigle et al., Journal of Bacteriology, 186, 1381-1387 (2004) | Jeganathan et al., RNA, 21, 1203-1216 (2015) | Razi et al., PNAS, 114, 3396-3403 (2017)

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## E. Contributors

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