Monolith X Protocol MOX-P-113

SecYEG DIBMA nanodisc – SecA

SecY is the main transmembrane subunit of the bacterial Sec pathway and a protein-secreting ATPase complex. Homologs of the SecYEG complex are found in eukaryotes and in archaea. SecA is a cell membrane associated subunit. Within this system the SecA ATPase forms a translocase complex with the SecYEG channel, thereby driving the movement of the protein substrate across the membrane.

protein – protein | membrane protein | nanodisc

A1. Target/Fluorescent Molecule

SecYEG DIBMA nanodisc

SecY
uniprot.org/uniprot/P0AGA2

SecE
uniprot.org/uniprot/P0AG96

SecG
uniprot.org/uniprot/P0AG99

A2. Molecule Class/Organism

Translocon
E.coli

A3. Sequence/Formula

SecY

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MAKQPGLDFQ SAKGGLGELK RRLLFVIGAL IVFRIGSFIP IPGIDAAVLA KLLEQQRGTI IEMFNMFSGG ALSRASIFAL
GIMPYISASI IIQLLTVVHP TLAEIKKEGE SGRRKISQYT RYGTLVLAIF QSIGIATGLP NMPGMQGCVI NPGFAFYFTA
VVSLVTGTMF LMWLGEQITE RGIGNGISII IFAGIVAGLP PAIAHTIEQA RQGDLHFLVL LLVAVLVFAV TFFVVFVERG
QRRIVVNYAK RQQGRRVYAA QSTHLPLKVN MAGVIPAIFA SSIILFPATI ASWFGGGTGW NWLTTISLYL QPGQPLYVLL
YASAIIFFAF FYTALVFNPR ETADNLKKSG AFVPGIRPGE QTAKYIDKVM TRLTLVGALY ITFIALIPEF MRDAMKVPFY
FGGTSLLIVV VVIMDFMAQV QTLMMSSQYE SALKKANLKG YGR
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SecE

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MSANTEAQGS GRGLEAMKWV VVVALLLVAI VGNYLYRDIM LPLRALAVVI LIAAAGGVAL LTTKGKATVA FAREARTEVR
KVIWPTRQET LHTTLIVAAV TAVMSLILWG LDGILVRLVS FITGLRF
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SecG

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MYEALLVVFL IVAIGLVGLI MLQQGKGADM GASFGAGASA TLFGSSGSGN FMTRMTALLA TLFFIISLVL GNINSNKTNK
GSEWENLSAP AKTEQTQPAA PAKPTSDIPN
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A4. Purification Strategy/Source

L148C single-cysteine mutant
Institute of Biochemistry, Heinrich-Heine-Universität Düsseldorf

A5. Stock Concentration/Stock Buffer

0.54 mg/mL | 7.5 µM
50 mM HEPES/KOH, pH 7.4, 150 mM KCl, 5 % Glycerol

A6. Molecular Weight/Extinction Coefficient

72 kDa
72,000 $M^{-1}cm^{-1}$ (ε_{280})

A7. Dilution Buffer

50 mM HEPES, pH 7.4, 150 mM KCl, 5 mM Magnesium acetate[1]

A8. Labeling Strategy

Monolith Protein Labeling Kit RED – MALEIMIDE 2nd Generation (MO-L014, NanoTemper Technologies GmbH)
1* Dye RED-MALEIMIDE 2nd Generation (10 µg) | 1* B-Column

A9. Labeling Procedure

1. Prepare 40 µL of 7.5 µM SecYEG.
2. Add 22 µL of DMSO to Dye RED-MALEIMIDE 2nd Generation (10 µg) to obtain a ~600 µM solution. Mix the dye thoroughly by vortexing and make sure that all dye is dissolved.
3. Mix 2 µL of the 600 µM dye solution with 38 µL of dilution buffer to obtain 40 µL of a 30 µM dye solution (4x protein concentration).
4. Mix SecYEG and dye in a 1:1 volume ratio (80 µL final volume, 2.5% final DMSO concentration).
5. Incubate for 60 minutes at room temperature in the dark.
6. 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.
7. 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.
8. Add 80 µL of the labeling reaction from step 4 to the center of the column and let sample enter the resin bed completely.
9. Add 600 µL of dilution buffer after the sample has entered and discard the flow through.
10. Place column in a new collection tube, add 400 µL of dilution buffer and collect the eluate.
11. Prepare a 5 mg/mL BSA solution in dilution buffer. Then, add 8 µL of the 5 mg/mL BSA solution to the 400 µL labeled SecYEG (~1 µM) to obtain a final BSA concentration of 0.1 mg/mL.
12. Prepare 10 µL aliquots and store at -80°C.

A10. Labeling Efficiency

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

Absorbance A_{280}	0.072	Protein concentration	~0.96 µM
Absorbance A_{650}	0.094	Degree-of-labeling (DOL)	~0.51

[1] Note that no detergent can be used due to the nanodisc. Instead, BSA is added after the labeling reaction is completed.

B1. Ligand/Non-Fluorescent Binding Partner

SecA
uniprot.org/uniprot/P10408

B2. Molecule Class/Organism

ATPase
E.coli

B3. Sequence/Formula

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MLIKLLTKVF GSRNDRTLRR MRKVVNIINA MEPEMEKLSD EELKGKTAEF RARLEKGEVL ENLIPEAFAV VREASKRVFG
MRHFDVQLLG GMVLNERCIA EMRTGEGKTL TATLPAYLNA LTGKGVHVVT VNDYLAQRDA ENNRPLFEFL GLTVGINLPG
MPAPAKREAY AADITYGTNN EYGFDYLRDN MAFSPEERVQ RKLHYALVDE VDSILIDEAR TPLIISGPAE DSSEMYKRVN
KIIPHLIRQE KEDSETFQGE GHFSVDEKSR QVNLTERGLV LIEELLVKEG IMDEGESLYS PANIMLMHHV TAALRAHALF
TRDVDYIVKD GEVIIVDEHT GRTMQGRRWS DGLHQAVEAK EGVQIQNENQ TLASITFQNY FRLYEKLAGM TGTADTEAFE
FSSIYKLDTV VVPTNRPMIR KDLPDLVYMT EAEKIQAIIE DIKERTAKGQ PVLVGTISIE KSELVSNELT KAGIKHNVLN
AKFHANEAAI VAQAGYPAAV TIATNMAGRG TDIVLGGSWQ AEVAALENPT AEQIEKIKAD WQVRHDAVLE AGGLHIIGTE
RHESRRIDNQ LRGRSGRQGD AGSSRFYLSM EDALMRIFAS DRVSGMMRKL GMKPGEAIEH PWVTKAIANA QRKVESRNFD
IRKQLLEYDD VANDQRRAIY SQRNELLDVS DVSETINSIR EDVFKATIDA YIPPQSLEEM WDIPGLQERL KNDFDLDLPI
AEWLDKEPEL HEETLRERIL AQSIEVYQRK EEVVGAEMMR HFEKGVMLQT LDSLWKEHLA AMDYLRQGIH LRGYAQKDPK
QEYKRESFSM FAAMLESLKY EVISTLSKVQ VRMPEEVEEL EQQRRMEAER LAQMQQLSHQ DDDSAAAAAL AAQTGERKVG
RNDPCPCGSG KKYKQCHGRL Q
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B4. Purification Strategy/Source

Institute of Biochemistry, Heinrich-Heine-Universität Düsseldorf

B5. Stock Concentration/Stock Buffer

1.6 mg/mL | 16 µM
20 mM Tris/HCl, pH 7.5, 50 mM potassium acetate, 20 % glycerol, 5 mM Magnesium acetate, 1 mM DTT

B6. Molecular Weight/Extinction Coefficient

102 kDa
76,000 $M^{-1}cm^{-1}$ (ε_{280})

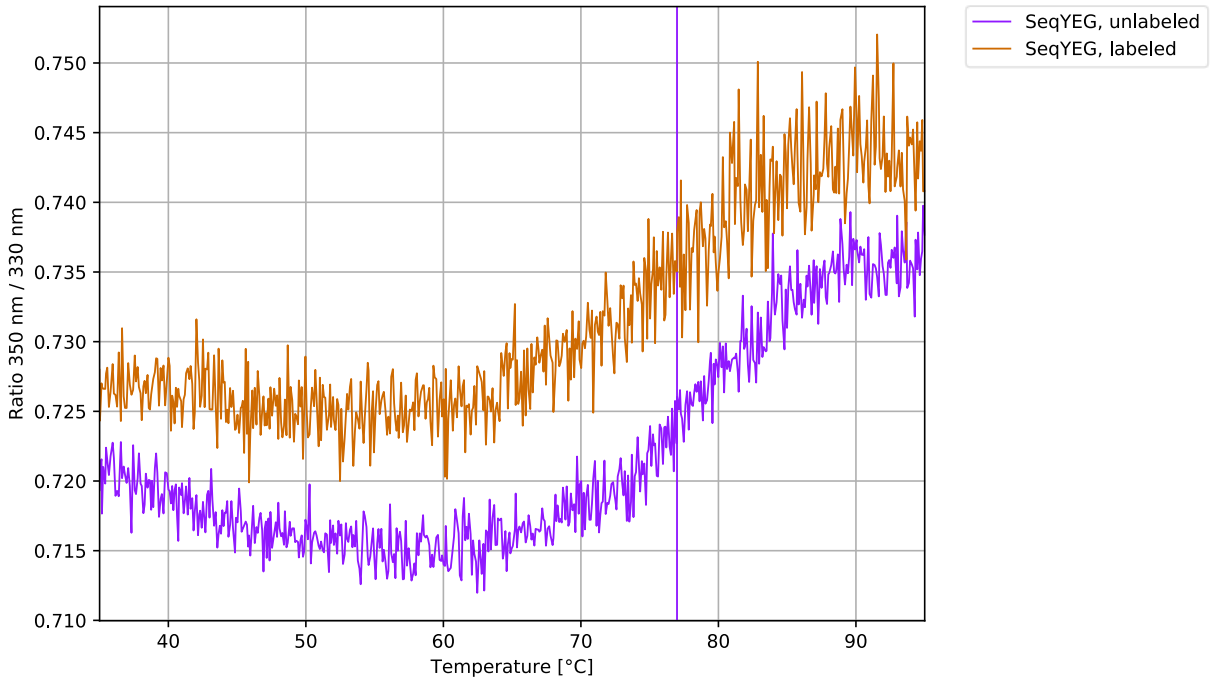
B7. Serial Dilution Preparation

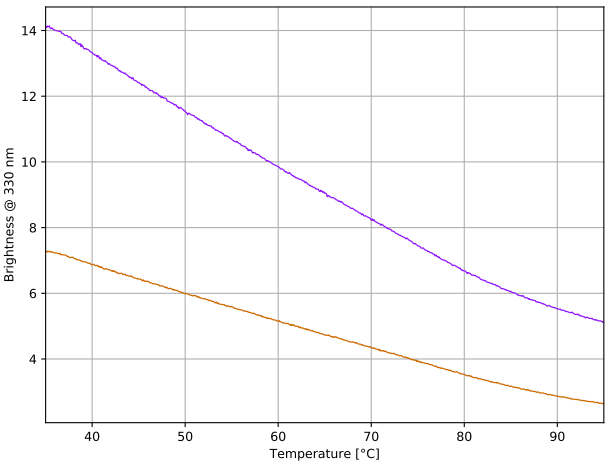
1. Mix 4 µL of the labeled SecYEG DIBMA nanodisc (~1 µM) with 96 µL of dilution buffer supplemented with 0.1 mg/mL BSA to obtain 100 µL of a ~40 nM SecYEG solution.
2. Take a fresh PCR tube and mix 80 µL of dilution buffer with 80 µL of the 40 nM SecYEG solution to obtain 160 µL of a 20 nM SecYEG solution in dilution buffer containing 0.05 mg/mL BSA.
3. Prepare a PCR-rack with 16 PCR tubes. Transfer 10 µL of the 10 nM SecYEG solution into tubes **2** to **16**. Then, mix 10 µL of 40 nM SecYEG with 1.25 µL of 16 µM SecA and 8.75 µL of dilution buffer in tube **1**.
4. Prepare a 1:1 serial dilution by transferring 10 µL from tube to tube. Mix carefully by pipetting up and down.
5. Incubate for 30 minutes at room temperature in the dark before loading capillaries.

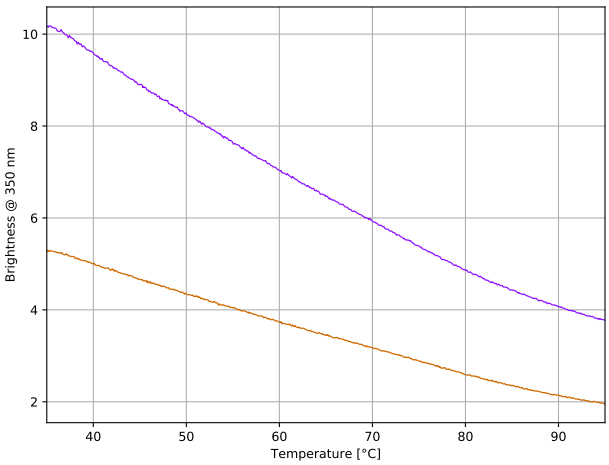
C. Tycho

Validation of structural integrity of labeled SecYEG using Tycho NT.6:
nanotempertech.com/tycho

SecYEG, unlabeled	0.35 µL of 7.5 µM SecYEG + 9.7 µL of dilution buffer	T_i = 77.0°C
SecYEG, labeled	5 µL of B-Column eluate (~1 µM) + 5 µL of dilution buffer	T_i = 77.0°C



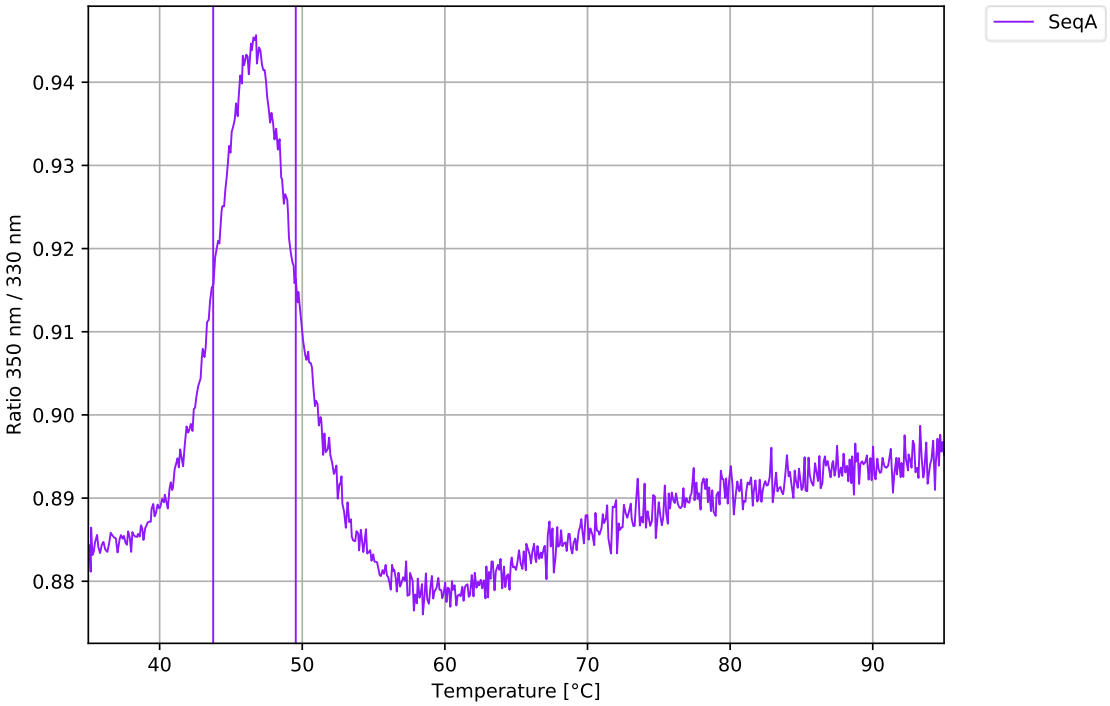


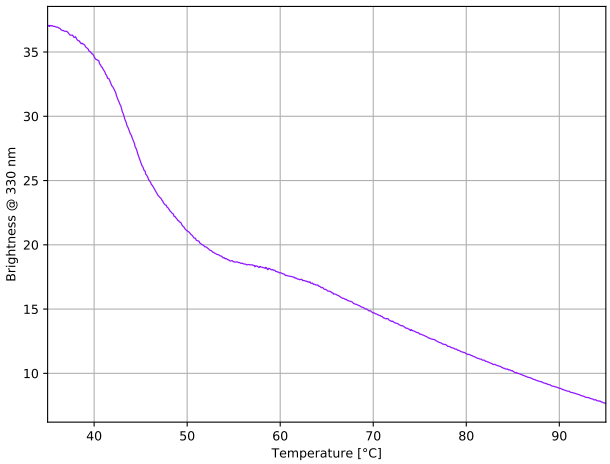


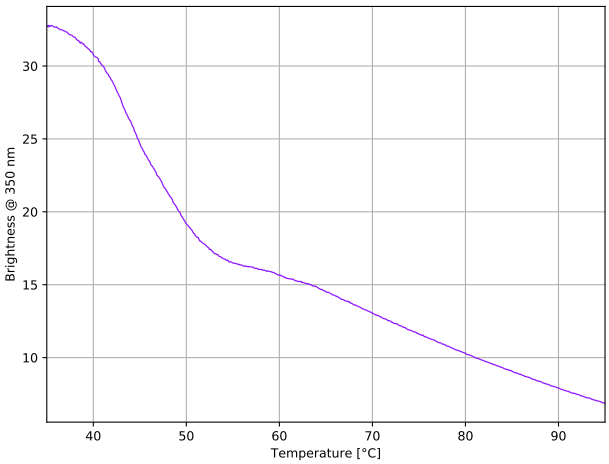
Validation of structural integrity of SecA using Tycho NT.6:

nanotempertech.com/tycho

SecA	0.6 µL of 16 µM SecA + 9.4 µL of dilution buffer	$T_{i,1}$ = 43.8°C | $T_{i,2}$ = 49.5°C







D1. Monolith System/Capillaries

Monolith X (NanoTemper Technologies GmbH)
Monolith Premium Capillaries (MO-K025, NanoTemper Technologies GmbH)

D2. Monolith Software

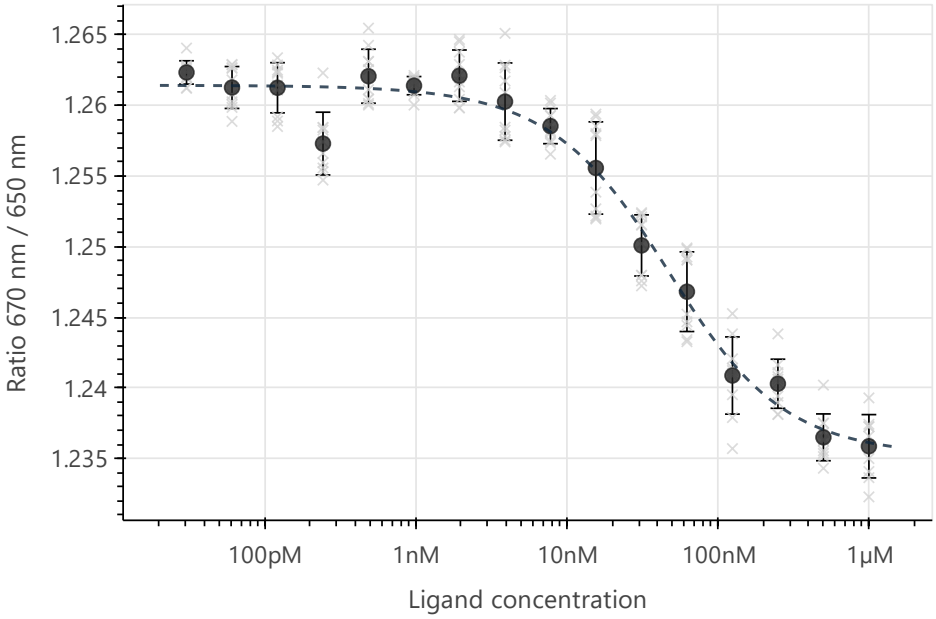
MO.Control v2.4.2 (NanoTemper Technologies GmbH)
nanotempertech.com/monolith-mo-control-software

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

50 mM HEPES, pH 7.4, 150 mM KCl, 5 mM Magnesium acetate, 0.05 mg/mL BSA
20 nM SecYEG | 1 µM – 30.6 pM SecA | 20°C | 100% excitation power

D4. Monolith Results (Dose Response)

K_d = 36.8 ± 7.9 nM (S/N = 19.2)



D5. Reference Results/Supporting Results

K_d = 50 nM	MicroScale Thermophoresis (MST) unpublished results
K_d = 4.5 nM	Surface Plasmon Resonance (SPR) unpublished results

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