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crystals MDPI

Structural Study of Serine Chemotaxis Receptor in Nanodisc

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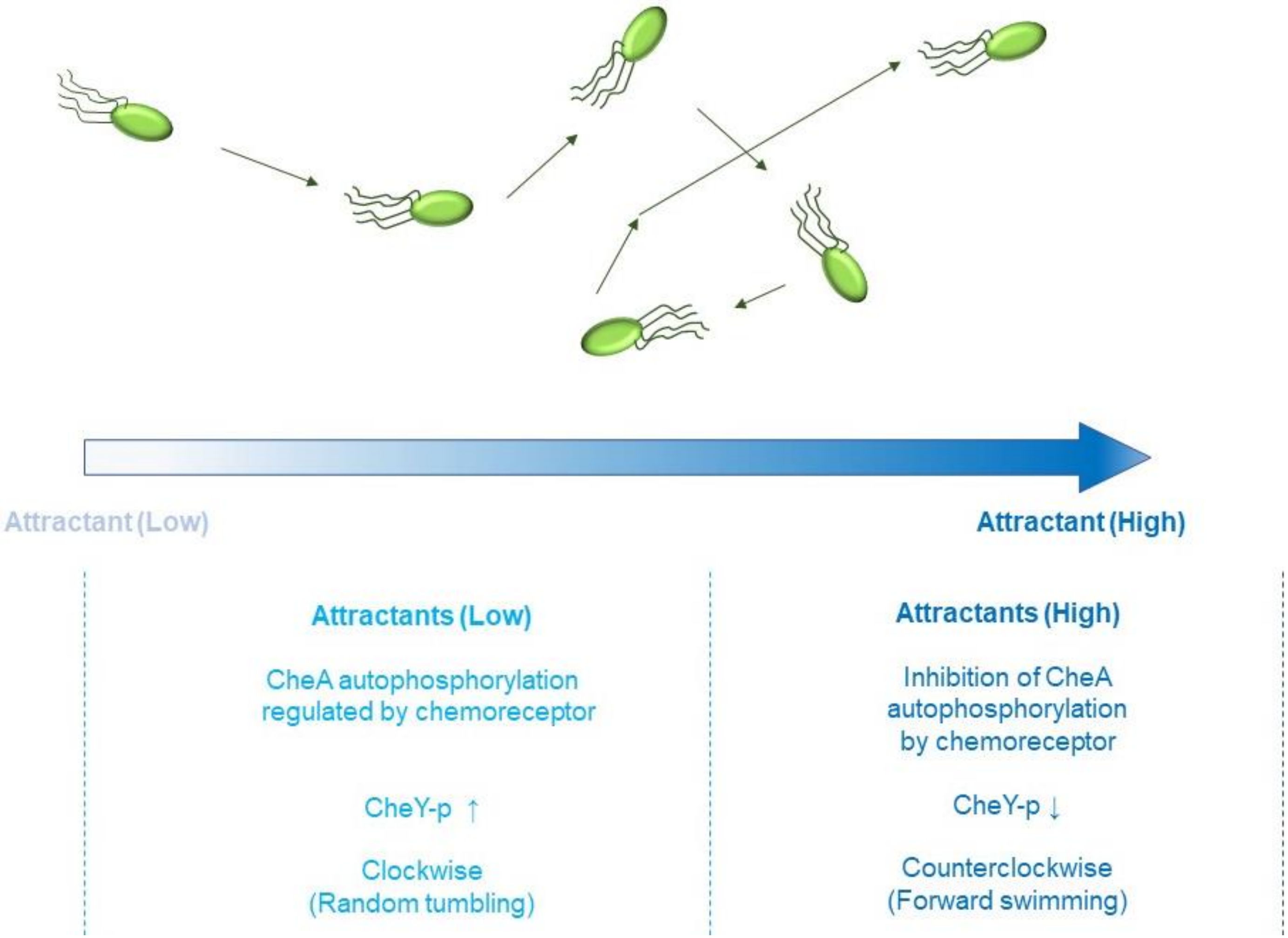
Abstract: Bacterial methyl-accepting chemotaxis proteins (MCP) are the membrane bound receptors responsible for regulating bacterial swimming behavior. Although their structural architecture has been studied in many bacterial species, detailed structural information has not been elucidated yet, especially, in the membrane lipid bilayer. In this study, **Tsr**, a serine chemoreceptor, has been used for the structural study of MCP in the lipid bilayer. The recombinant Tsr was overexpressed in *E. coli* and purified followed by the reconstitution into nanodiscs for providing the lipid bilayer environment. Structural characteristics of Tsr in nanodiscs were first investigated by the transmission electron microscopy (TEM) with negative staining followed by cryo-EM. Microscopic images revealed that one to three Tsr dimers were reconstituted in one nanodisc. However, cytoplasmic tails below HAMP domain showed high flexibility in the micrograph, which resulted in disappearance of the most of tail part during 2D classification. These results suggest that Tsr form a strong dimer with flexible conformation in the cytoplasmic signaling domain. However, trimer of dimer is not stable in the nanodisc although previous studies suggested that dimer forms trimer via interaction among cytoplasmic domains. Further cryo-EM studies of Tsr in complex with other signaling mediators will elucidate the detailed protein interactions and their signaling mechanism.

Keywords: serine chemotaxis receptor; nanodisc; cryo-EM

Introduction

1. Chemotaxis

<Chemotaxis system in *E.coli*>



Attractant (Low)

Attractant (High)

Attractants (Low)

CheA autophosphorylation
regulated by chemoreceptor

CheY-p ↑

Clockwise
(Random tumbling)

Attractants (High)

Inhibition of CheA
autophosphorylation
by chemoreceptor

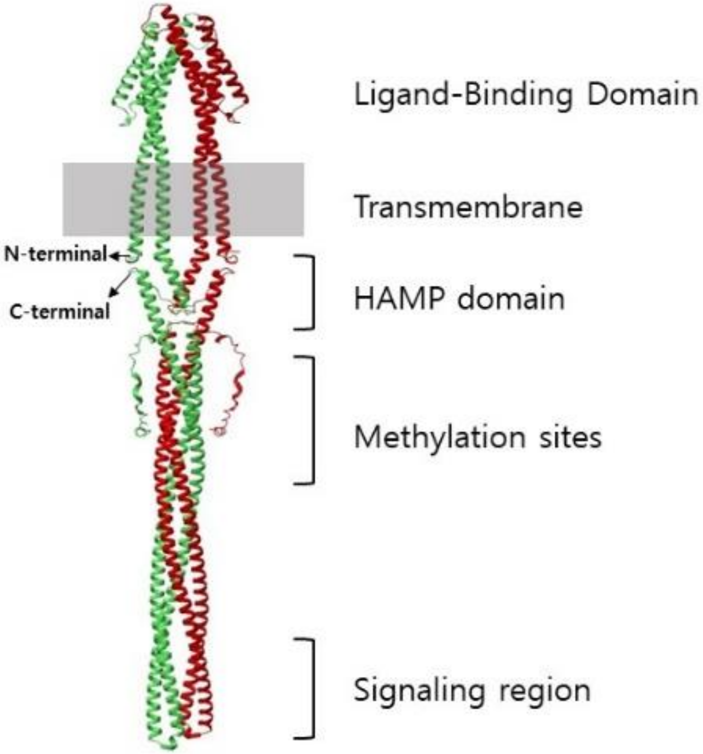
CheY-p ↓

Counterclockwise
(Forward swimming)

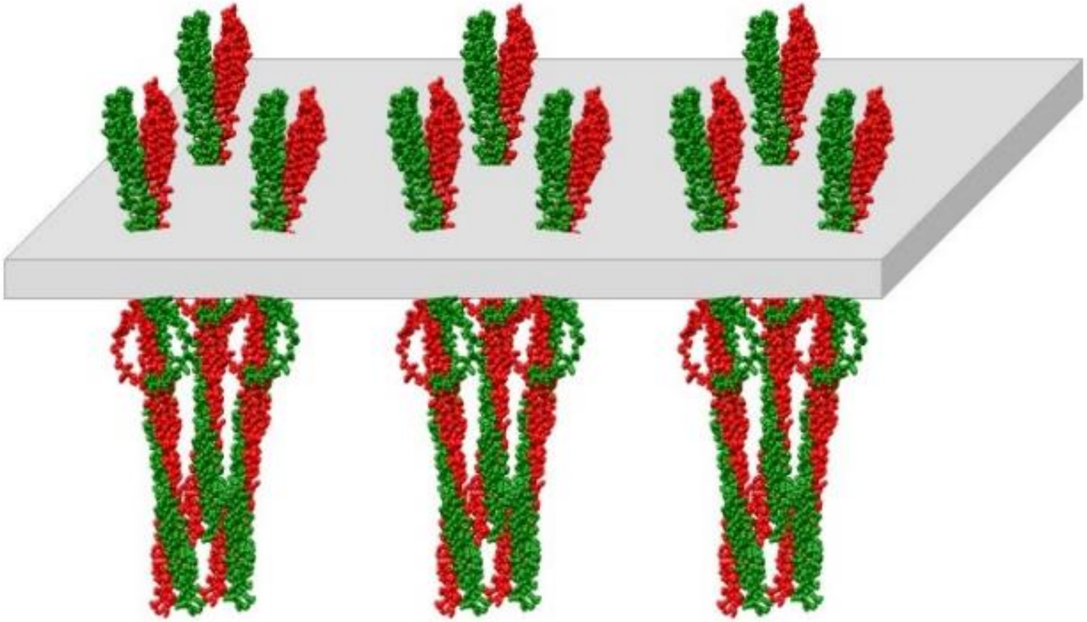
Introduction

2. Structures of Chemoreceptors

<Schematic model of Tsr homodimer>



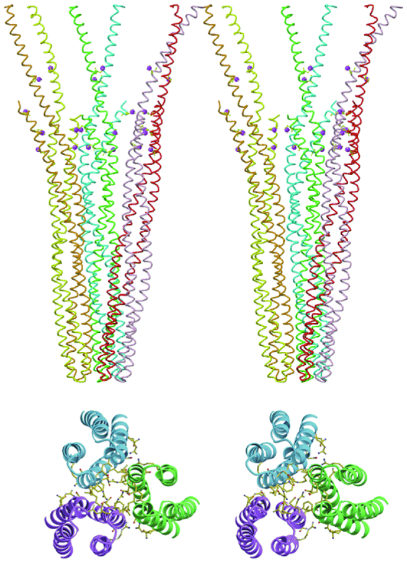
<Schematic model of core signaling unit (Trimer of dimer) in *E.coli*>



Introduction

2. Structures of Chemoreceptors (continued)

<Previously solved structures of chemoreceptors>

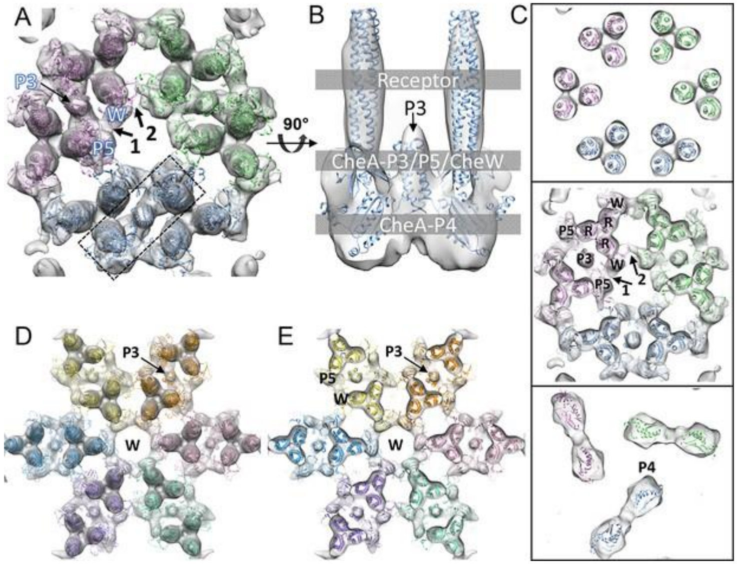


2.6 Å

Crystal structure
(Cytoplasmic domain)

2.5 Å

Crystal structure
(Ligand binding domain)



11.3 Å

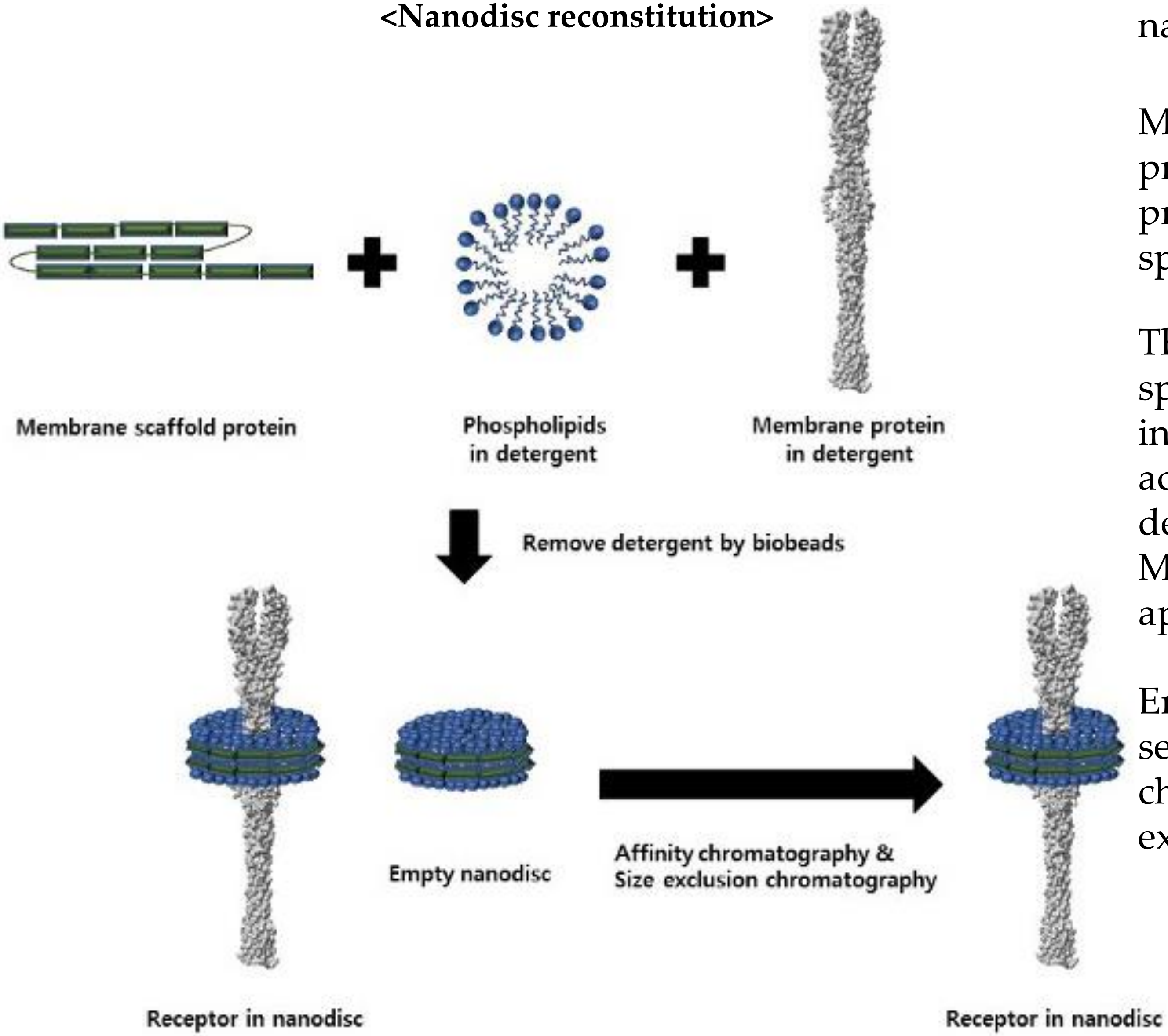
Cryo-electron tomography
(Chemoreceptor-CheA-CheW complex)

Nature 400, 787-792 (1999)
Journal of Biological Chemistry 286, 42200-42210 (2011)
Elife 4, e08419 (2015)

Introduction

3. Nanodisc

<Nanodisc reconstitution>



The structural properties of receptors in native-lipid bilayer have been studied by nanodisc method.

Mix the purified membrane protein, membrane scaffold protein (MSP), and lipid at specific ratio.

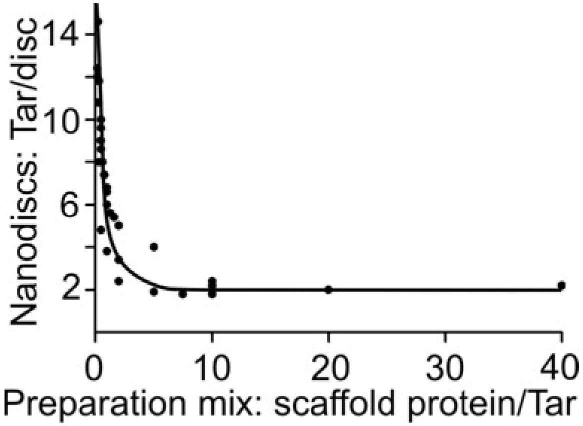
The membrane protein is spontaneously reconstituted into lipid surrounded by MSP according to absorption of detergents with biobead since MSP is derived from an apolipoprotein.

Empty nanodisc can be separated by affinity chromatography or size exclusion chromatography.

Introduction

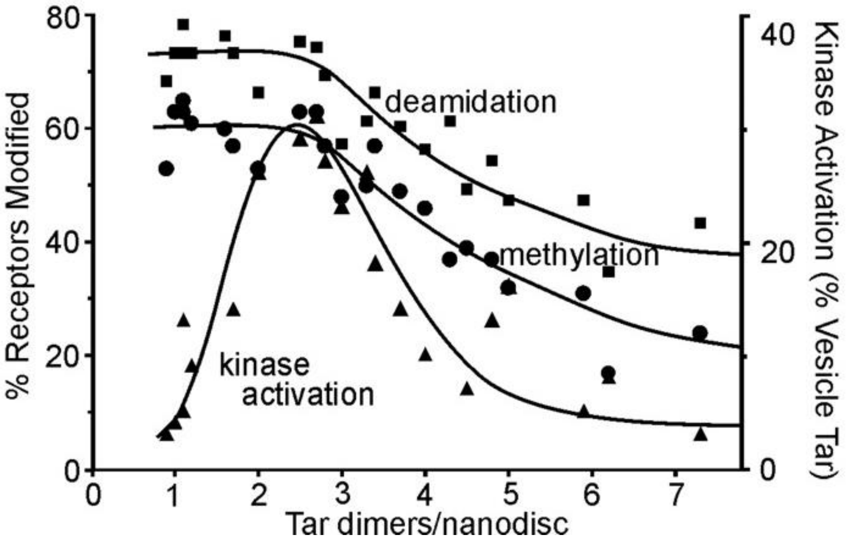
4. Characteristics of nanodisc-embedded chemoreceptor

<Tar per disc as increasing ratio of MSP/receptor>



Previous studies showed that
the number of chemoreceptors per disc
depends on MSP/receptor ratio.

<CheA kinase activity assays by Nanodisc-embedded chemoreceptor Tar>



CheA kinase activity is
much higher by Tar nanodisc containing three Tar dimers/disc
than Tar dimer/disc.
However, structural characteristics of three chemoreceptors dimers/disc are still unclear

Proceedings of the National Academy of Sciences
103, 11509-11514 (2006)

Materials and Method

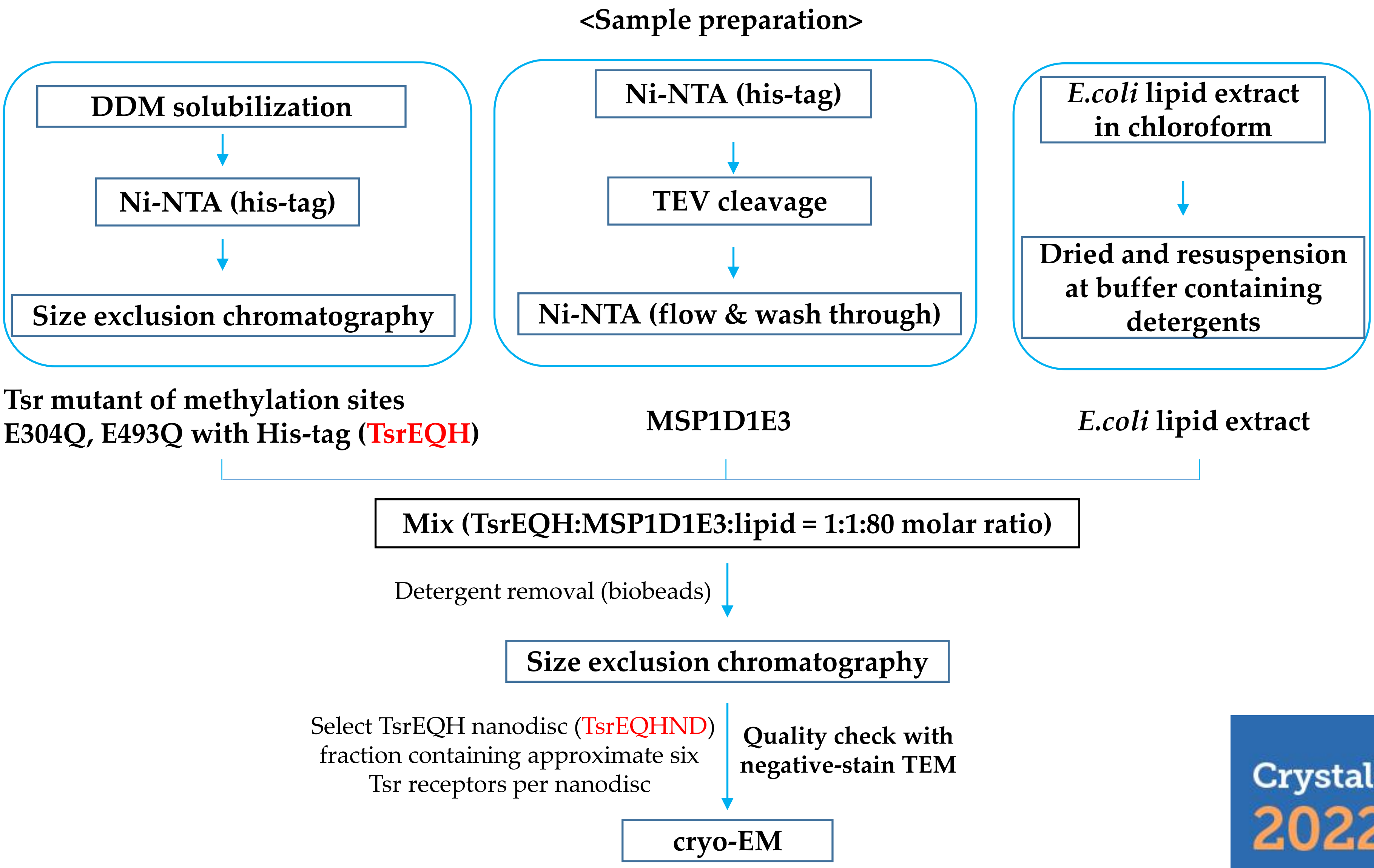
1. Materials

<Key resources table>

Reagent or Resource	Source	Identifier
E.coli BL21 (DE3)	Agilent	Cat# 200131
n-Dodecyl-β-D-Maltopyranoside (DDM)	Anatrace	Cat# D310 5 GM
n-Octyl- β-Glucopyranoside (OG)	Anatrace	Cat# O311S
Sodium cholate hydrate	Sigma Aldrich	Cat# C6445-25G
Triton X-100	affymetrix	Cat# 22686
E.coli polar lipid extract, chloroform	Avanti	Cat# 100600C
Ni-NTA Superflow Cartridges	QIAGEN	Cat# 30760
Bio-Beads SM-2	Bio-Rad	Cat# 1523920
Superose 6 Increase 10/300 GL	GE Healthcare	Cat# 29-0915-96
PD-10 Desalting Columns	GE Healthcare	Cat# 17-0851-01
Uranylacetat	Merck	Cat# 8473
cOmplete™, EDTA-free Protease inhibitor Cocktail	Sigma Aldrich	Cat# 11873580001
R1.2/1.3 Holey Carbon Grids, 200 Mesh Copper	Quantifoil	4220C-XA

Materials and Method

2. Method



<Sample preparation>

DDM solubilization
↓
Ni-NTA (his-tag)
↓
Size exclusion chromatography

Tsr mutant of methylation sites E304Q, E493Q with His-tag (TsrEQH)

Ni-NTA (his-tag)
↓
TEV cleavage
↓
Ni-NTA (flow & wash through)

MSP1D1E3

***E.coli* lipid extract in chloroform**
↓
Dried and resuspension at buffer containing detergents

***E.coli* lipid extract**

Mix (TsrEQH:MSP1D1E3:lipid = 1:1:80 molar ratio)

Detergent removal (biobeads) ↓

Size exclusion chromatography

Select TsrEQH nanodisc (TsrEQHND) fraction containing approximate six Tsr receptors per nanodisc

Quality check with negative-stain TEM

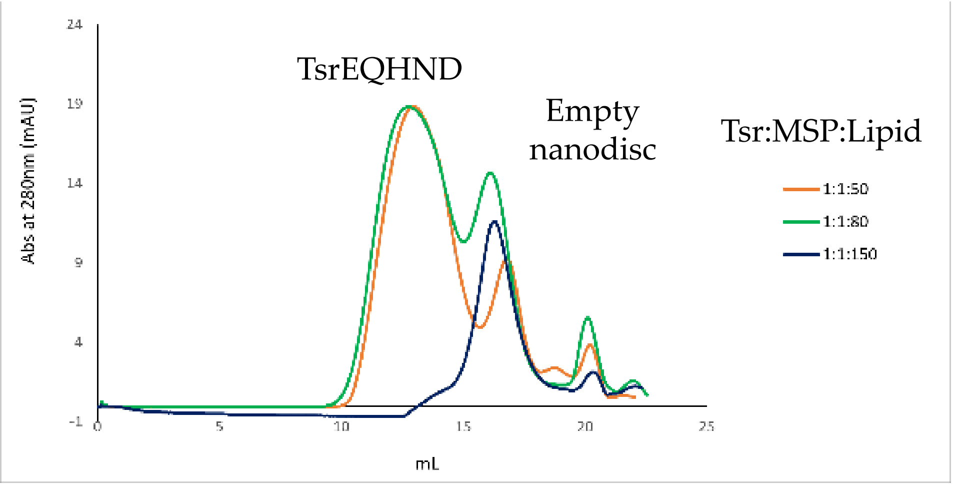
↓

cryo-EM

Results and Discussion

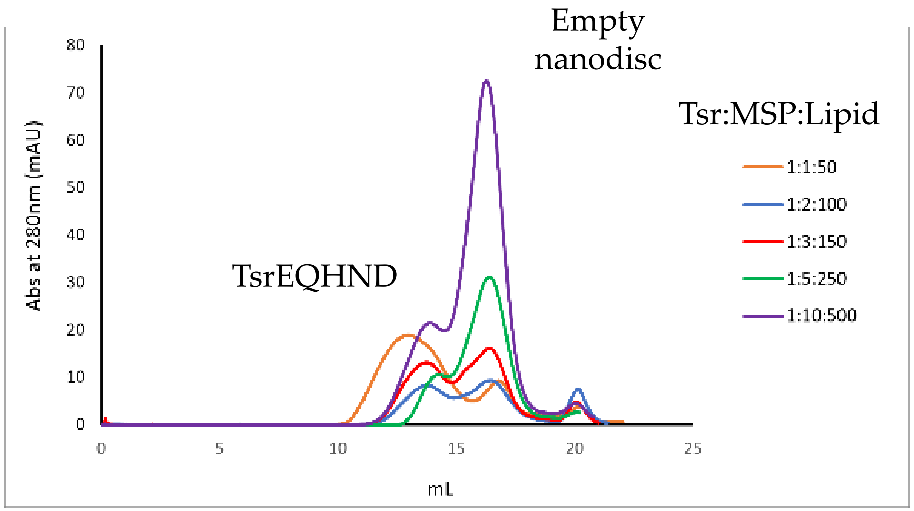
1. Optimization of nanodisc reconstitution ratio

<Optimization of lipid/MSP1D1E3 ratio >



The best yield of Tsr nanodisc (TsrEQHND) at 1:1:80 (Tsr:MSP1D1E3:*E.coli* lipid extract)

<Optimization of MSP1D1E3/Tsr ratio >

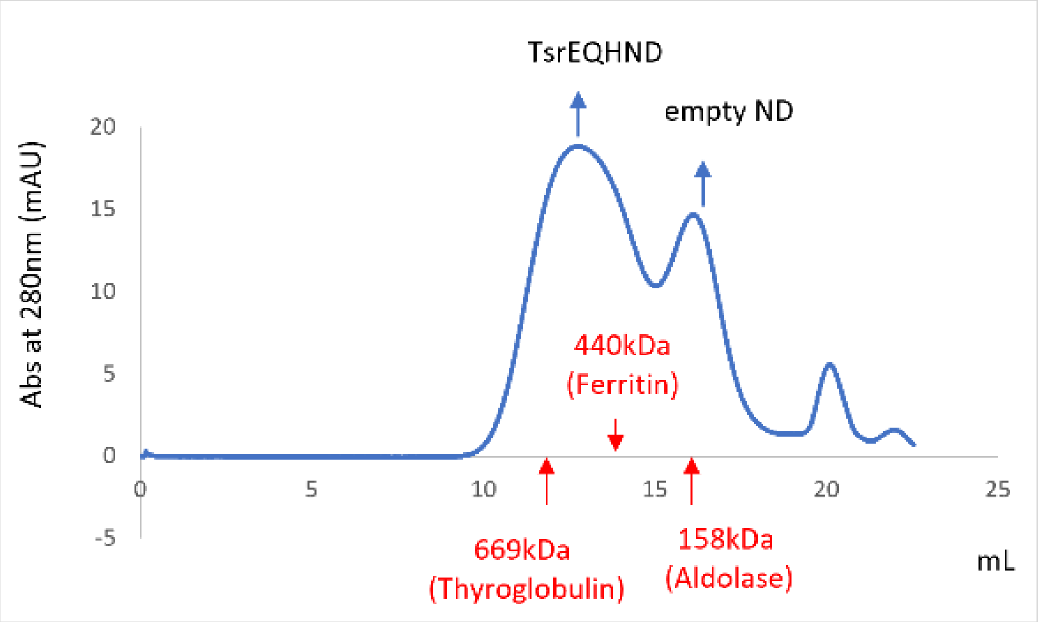


The gradual peak shift as the ratio of MSP to Tsr increases suggests that the major number of Tsr per nanodisc decreases

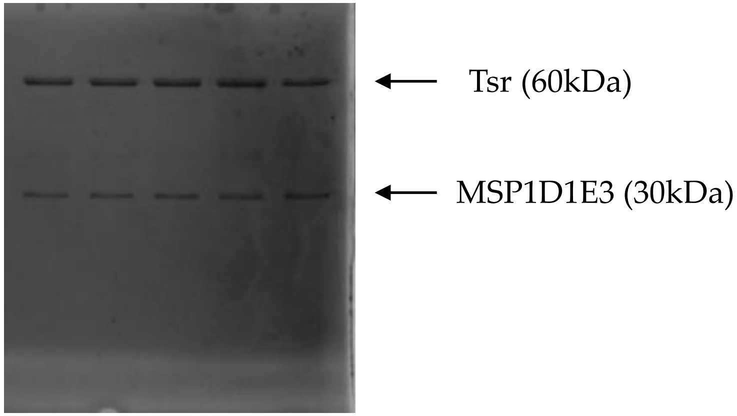
Results and Discussion

1. Optimization of nanodisc reconstitution ratio (continued)

<TsrEQHND containing six Tsr per nanodisc at 1:1:80>



<SDS-PAGE of TsrEQHND fractions>



TsrEQHND fractions contatining approximate six Tsr receptors
are selected by comparing to standard proteins with exact molecular weight

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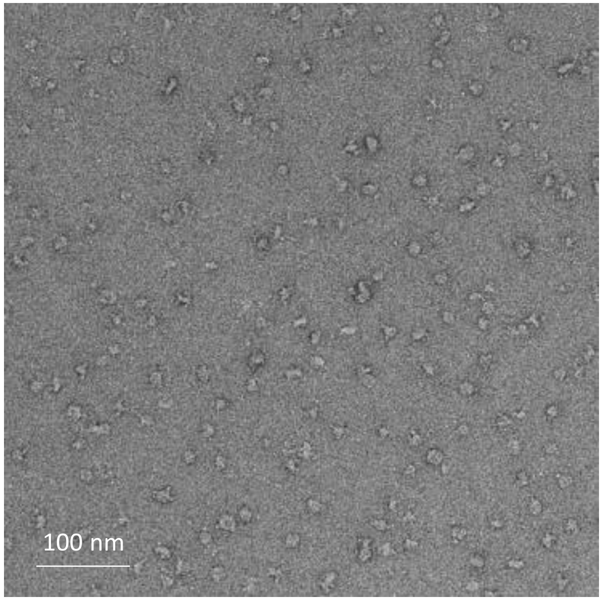
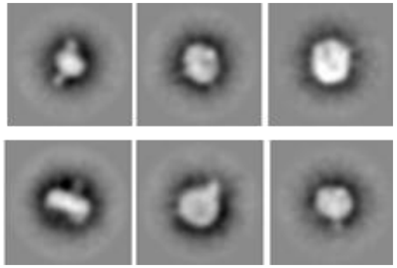
Results and Discussion

2. Negative-stain TEM of TsrEQHND

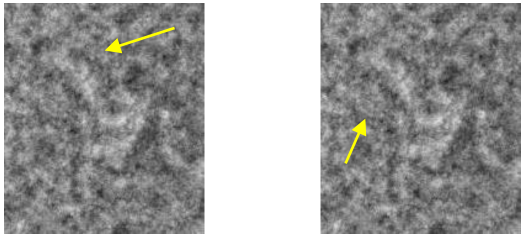
<Representative micrograph>



<Representative 2D class averages>

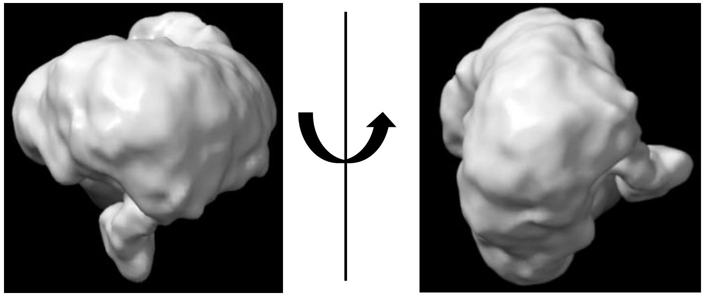


<Magnified view of particles>



Cytoplasmic tails are pointed out by yellow arrow.

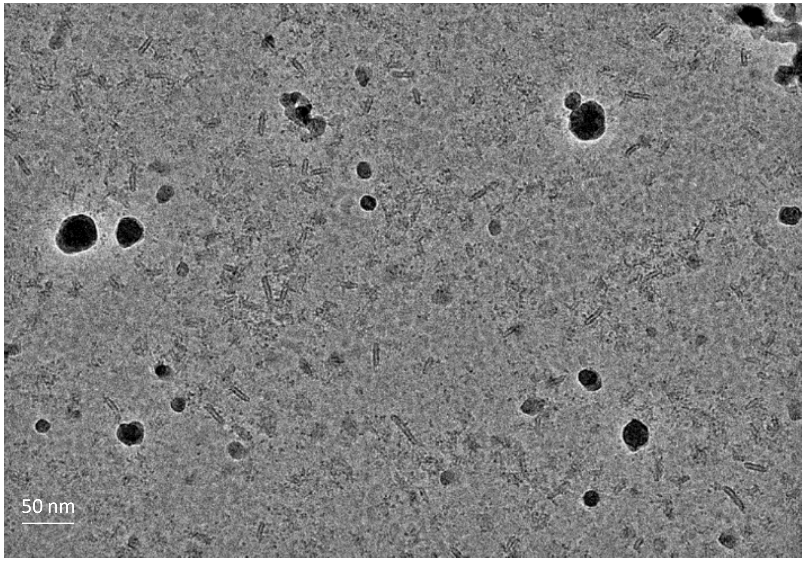
<Ab initio 3D model>



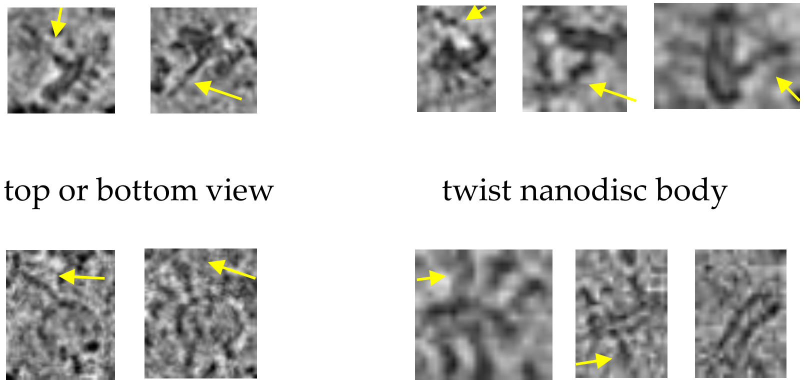
Results and Discussion

3. Cryo-EM images and data processing of TsrEQHND

<Representative micrograph>



<Magnified view of particles>


straight long tails

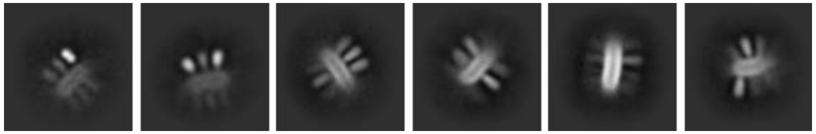
twist tails

top or bottom view

twist nanodisc body

Cytoplasmic tails are pointed out by yellow arrow

<Representative 2D class avrages>

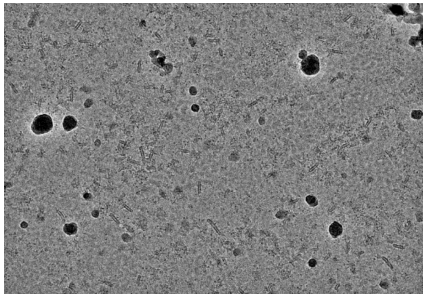


Results and Discussion

3. Cryo-EM images and data processing of TsrEQHND

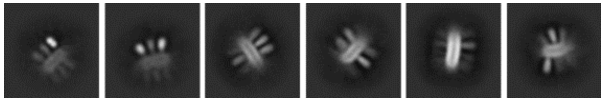
<Cryo-EM data processing>

2030 movies



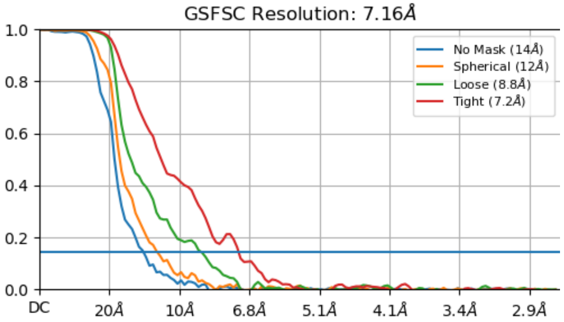
310167 particles auto-picked with the template that were created by manually picked particles

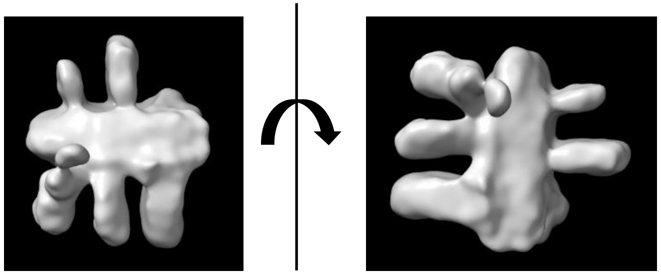
48047 particles in selected 2D classes



Ab-initio reconstruction

Homogeneous refinement

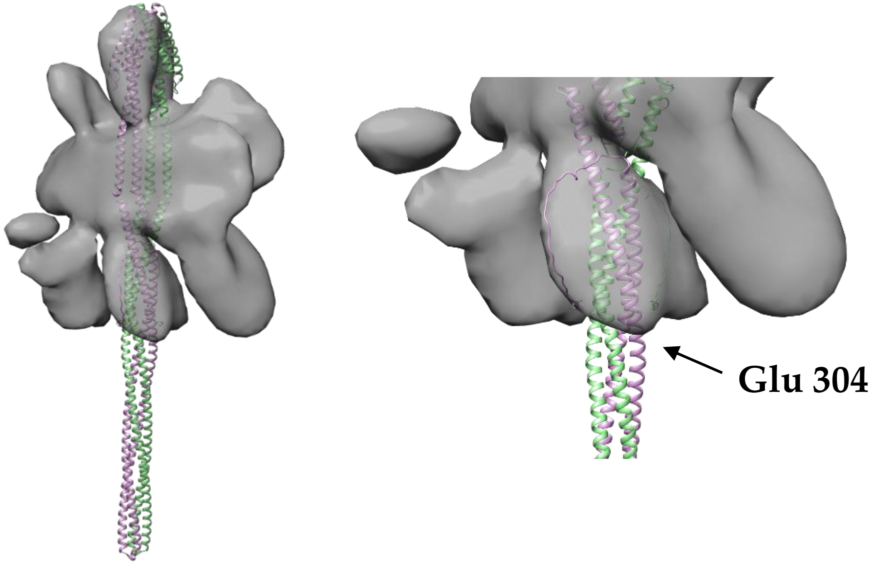




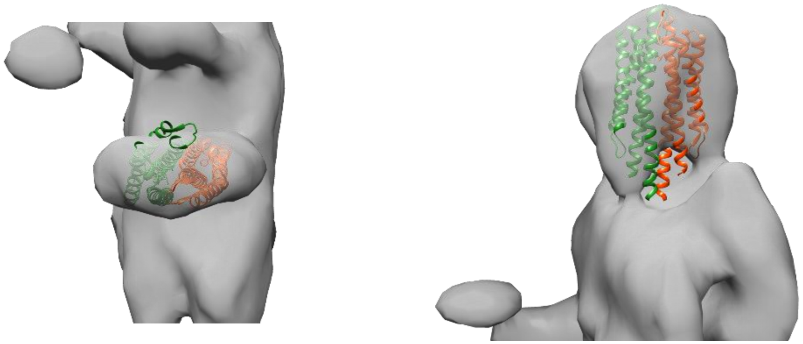
Results and Discussion

4. Fitting of Tsr dimer into cryo-EM map of TsrEQHND

<Alphafold Tsr fit>



<Ligand binding domain Tsr dimer (PDB:3ATP) fit>



<Fitting workflow>

The alphafold (AF-P02942-F1) predicted structure of Tsr monomer were docked into cryo-EM map

↓

The previous solved structure of cytoplasmic domain Tsr dimer (PDB:1QU7) were matched to the alphafold Tsr monomer

↓

The another alphafold Tsr monomer were matched to the cytoplasmic domain Tsr dimer

↓

Finally, the alphafold Tsr dimer fitted to cryo-EM map were created based on the cytoplasmic domain Tsr dimer

Conclusions

- The peak shift and sharpening elution range of TsrEQHND as the MSP/Tsr ratio increases indicate that excessive MSPs relative to Tsr causes Tsr to be more divided into one nanodisc and consequently reaches a lower limit of core unit.

- The alignment of particles during 2D classification and fitting the density map to atomic model suggest that the middle fractions of TsrEQHND reconstituted at 1:1:80 ratio contain three Tsr homodimer dominantly. However, trimer of dimers is not stable and each Tsr homodimer in nanodisc is very flexible.

- The validation whether oligomeric form of membrane proteins on nanodisc is built by recovered interaction among each of core units or by the force that MSPs and lipid bind them together is necessary.

- Further cryo-EM studies of Tsr complex with other signaling molecules such as CheA and CheW will elucidate how interactions among cytoplasmic domains and signaling molecules play important roles in heterogeneous tails.

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