university of groningen / faculty of mathematics and natural sciences / chemistry and chemical engineering

YidC in Nanodiscs

Bachelors assignment Biochemistry

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Introduction

Biological cells form basis of every living organism consists on Earth. The number of cells can range from just a single one (bacteria, amoeba) up to 150 trillion (Elephant). These cells contain a number of different components. Some of the most important components are proteins. Being linear polymers of amino-acids, they are folded into specific compact structures and facilitate important functions such as energy production, signal transduction, protein trafficking, molecular transport, and also host-pathogen interactions.[1] An important class of proteins are membrane proteins. These proteins are embedded into anisotropic lipid bilayers at the cell boundaries, where they can be used for the interaction of the cell with the "outer" world. Be it the transport of nutrients across the membrane or signal transduction, they can handle everything. Membrane proteins have a large hydrophobic area. This area is normally located within the hydrophobic core of the membrane formed by lipid acyl chains. Hydrophobic interactions allow the protein to integrate into the membrane and to acquire the correct structure. In figure 1 examples of the membrane protein structures can be seen. Due to the complex environment at the membrane interface, most membrane proteins need some help with folding and positioning inside the membrane. For this insertases are used. In *E.coli* there are two complexes used for this: This is also because it is in itself a membrane protein so it cannot be analyzed outside of a membrane. In a membrane vesicle the exact number and composition of proteins is unknown. The size and shape of the vesicles can differ greatly and it is possible for interactions to occur that can normally not be seen. Also it is hard to analyze both sides of a protein at once since most synthetic bilayers form micelles[2]. This SecYEG and YidC. The SecYEG complex has seen extensive research but YidC is still relatively unknown. is why the structure and mechanism of YidC is still largely unknown.

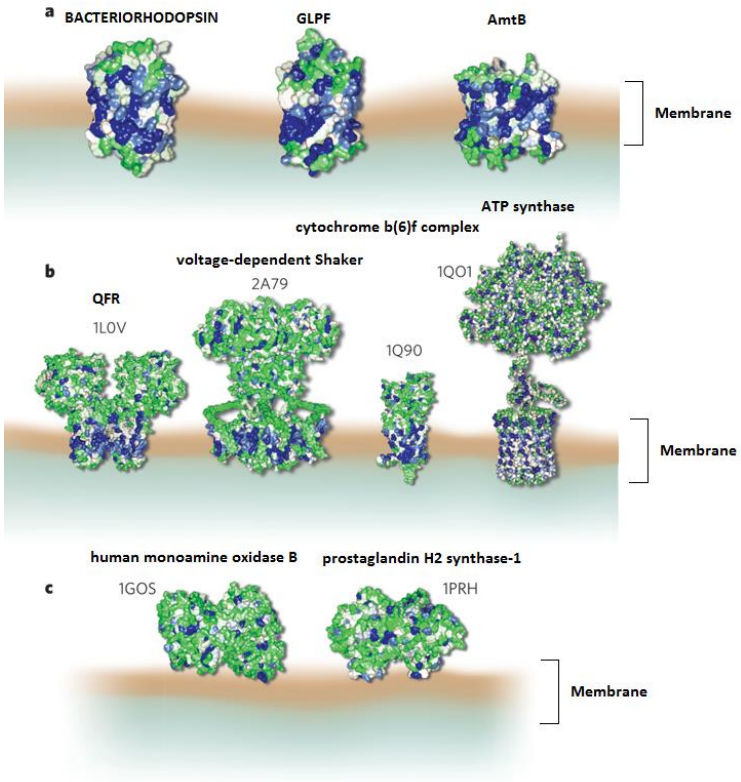

Figure 1: Structure of certain membrane proteins. Green is favorable interaction with water and blue hydrophobic interaction. Adapted from [2]

This is true for most membrane proteins. Normally detergents are used to extract membrane proteins. However, this unnatural environment may destabilize and/or dissociate protein complexes and render the protein non-functional[1]. This makes analytical methods relying on detergent micells very unreliable.
To combat this difficulty a solution has been found. A nanodisc is a small disc of a mixture of lipids surrounded by a scaffold protein to keep the layer intact. This size can be controlled and is homogeneous is shape. A nanodisc (Reference) can imitate a normal membrane while still allowing single molecule analysis. In the following sections more will be explained about YidC, Nanodiscs and the research preformed to incorporate a YidC protein in a nanodisc.

YidC[3]

In *E.coli* more than 900 different cytoplasmic membrane proteins are encoded. This is approximately 20% of all proteins in the cell. The Sec complex inserts most of these proteins but YidC is suspected to be essential for the insertion of 69 membrane proteins (Reference). Also it is suspected that there are more proteins partly dependent on YidC where insertion is facilitated in conjunction with the Sec complex.

YidC is highly conserved in most bacteria and some members of the Archaea with homologues in mitochondria and chloroplasts of eukaryotic cells. Although the protein structure is largely unknown, in figure 2 a model can be seen.[4]

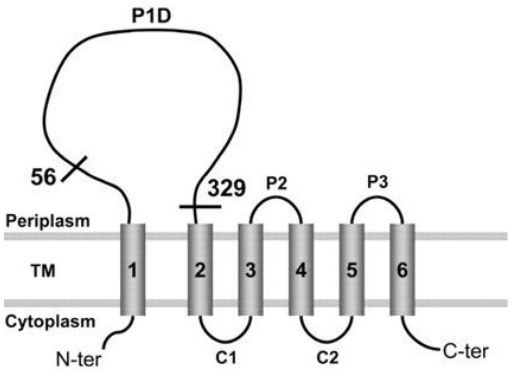

Figure 2: Structure of the periplasmic domain (P1D) of YidC. A, schematic of full-length YidC according to Sääf et al. as shown in Ravaud et al[4]. The domain boundaries of P1D (residues 56–329) are indicated.

Six transmembrane segments can be distinguished[4]. Also one very large periplasmic domain (P1D) which has been structurally characterized in Ravaud at al. (2008) is seen. This P1D domain contains a highly conserved β-supersandwich and an α-helical linker.

Five transmembrane helices are present in all YidC homologues and are therefore considered essential. The P1 domain and the N-terminal transmembrane helix are unique for YidC in Gram-negative bacteria. This P1 domain can interact with SecF thus allowing a interaction with the SecYEG complex. Low resolution cryo-election microscopy shows that YidC can interact with the Sec translocon or assemble with itself to form homo-dimers[4]. The schematic shown in figure 3 demonstrates the expected pathways in which membrane proteins can be integrated into the membrane. Here can be seen that YidC can work on his own to incorporate proteins but can also form a complex with SecYEG.
Further research about whether this is required or optional for YidC to be active is needed.

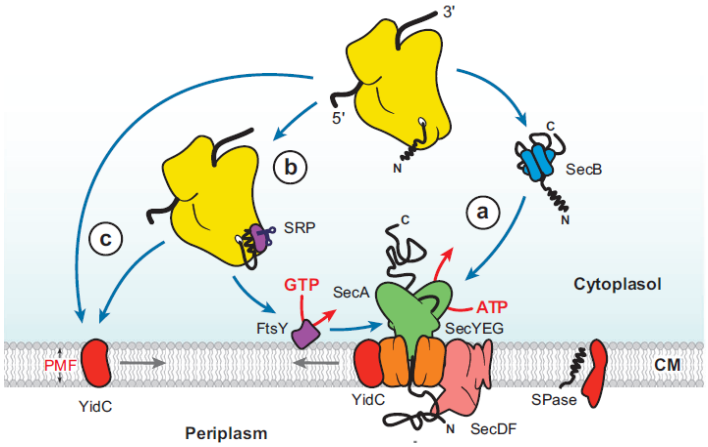

Figure 3: Schematic showing the expected pathways in which membrane proteins are incorporated into the membrane.

Nanodiscs

To conduct research about the structure and functionality of membrane proteins at physiologically-relevant conditions the proteins must be incorporated into a membrane. Only when inside a membrane it will behave as though it were in a living cell. But as stated above, this raises problems to design the experimental system. One way to combat these problems is to employ nanodiscs. These nanodiscs can mimic the cell membrane and still allow for single molecule analysis methods.

Nanodiscs consist of a lipid bilayer about 10 nm in diameter, held together by a scaffold protein. If the scaffold protein is modified different sizes of nanodisc can be achieved. Denisov et al[7] found that nanodiscs with an average size between 9,5 and 12,8 nm can be obtained with a very narrow size distribution (aprox. 3%) by only varying the length of the scaffold protein. The height of the lipid bilayer is found to always be around 5,5 nm and is not dependent on the scaffold protein. A representation of a nanodisc used to unravel the structure of SecYEG is seen in figure 4. Here can be seen that the protein only fills up a partof the nanodisc. This might allow for dimers to be formed. Since a nanodisc of approximately the same size is used in this experiment while YidC is much smaller up to 5 copies of YidC might fit in this nanodisc. Therefore dimers and trimers are expected to appear in the results. But if the reconstitution of YidC works, much more information can be obtained from this development, and oligomeric state may be tuned by refining the preparation procedures.

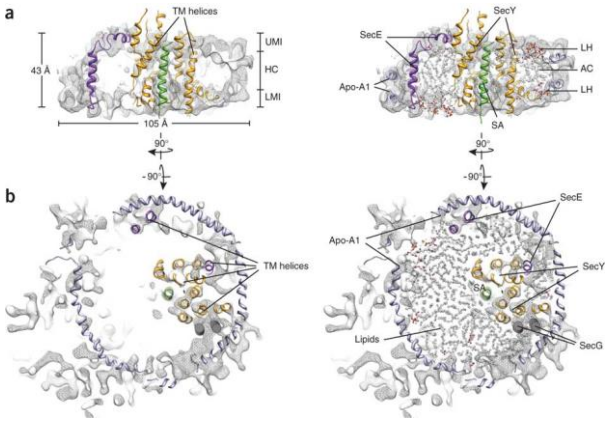

Figure 4: representation of a nanodisc containing SecYEG with Apo-A1 as a scaffold protein.[8]

Experimental

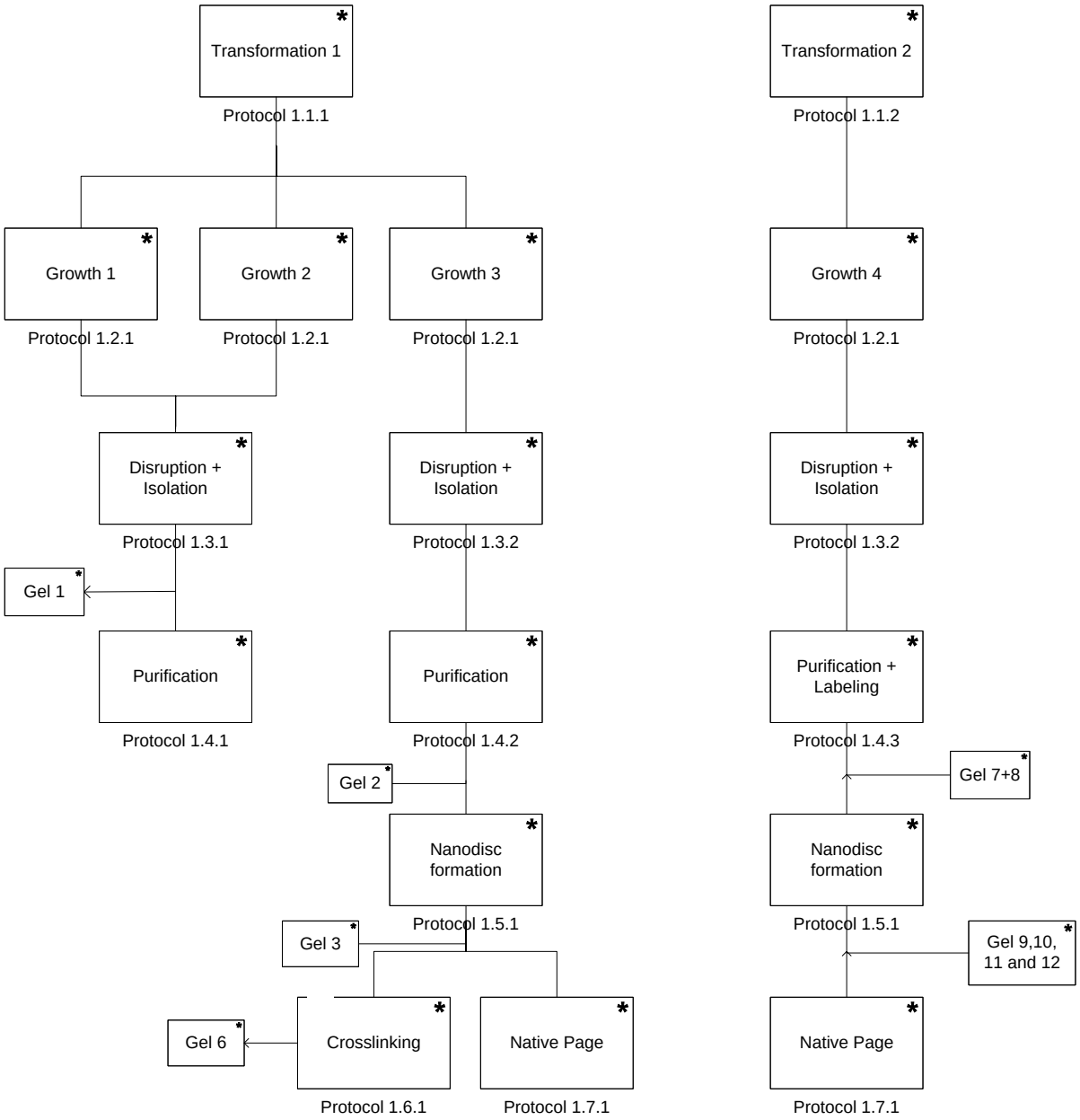

Figure 5: Visualization of all experiments done during this research including which protocol used and when gels were run.

Growing of a YidC overproduction mutant

A YidC overproduction plasmid called YEH1YidCHis and a SF100 *E. coli* strain were used for transformation following the protocol in Appendix 1.1.1. Protocol 1.1.2 was used for the transformation for the YidC overproduction mutant where the YidC would later be labeled. After transformation protocol 1.2.1 was used to facilitate growth and induction. In protocol 1.2.1 the first result is shown. In appendix 2: *"Additional Growth and Induction results"* more results of following experiments are shown.

Isolation of YidC

In protocol 1.3.1 growth 1 is used. But due to a fault during the sucrose gradient no further analysis could be made. Growth 2 was partly black after cell disruption and centrifugation. Protocol 1.4.1 was used but only a very small amount of YidC was detected in the elution fraction (as seen on Gel 1 in Appendix 3). This was probably due to the cell death during growth and protein over-expression. For disruption and isolation of growth 3 and 4 protocol 1.3.2 was used. Here, only small streaks of the black pellet were found in the disrupted cells.

After purification the yield of YidC from growth 2 was low, so no further tests were possible. Therefore the tests were continued with growth 3. The results of that purification can be seen in gel 2 in appendix 3. On the gel 2 bands can be seen. The lower band is probably the monomer while the top band is expected to be a dimer. Two elution fractions were obtained, the large fraction was about 1,2 mL and the small fraction about 500 µL. The obtained concentrations (through absorbance at 280 nm) are 7,0 µM and 5,4 µM respectively. Calculated extinction coefficient of YidC based on amino acid sequence is 96.000 M^{-1} cm^{-1}

Growth 4 was grown using protocol 1.2.1, isolated using protocol 1.3.2 and 1.4.3.

Labeling YidC

Growth 4 was fluorescently labeled using protocol 1.4.3 and after purification the concentration was measured.

Concentration protein through absorbance at 280 nm was 11,6 µM. Alexa 488 concentration was 4,1 µM and the concentration of ATTO 647 was 10,0 µM . This shows that the labeling of ATTO 647 was more than twice as effective and some of the YidC was double labeled. Results after purification, labeling and incorporation in a nanodisc can been seen on gels 7 (UV) and 8 (Coomassie Brilliant Blue stained) in appendix 3.

Crosslinking

The non-labeled YidC from growth 3 was used to perform a crosslinking experiment using protocol 1.6.1. Results of this can be seen on gel 6 in appendix 3. The top band is the crosslinked YidC and the lower band the non-cross-linked YidC. As can been seen, most of the YidC is not crosslinked. The reason for this is not known and the low efficiency means it is not interesting for further research.

Incorporation of YidC in Nanodiscs

The incorporation of YidC in the nanodiscs was preformed according to protocol 1.5.1. The results after FPLC are shown in gel 3 for the non-labeled YidC and the native-PAGE gels 9 (UV), 10 (stained), 11 (UV) and 12 (stained) for the labeled YidC in appendix 3. In gel 7 and 8 the SDS-PAGE results of the labeled nanodiscs can be seen. The top band is the YidC and the lower band is MSP. Because it is SDS-PAGE the nanodiscs are denatured so no complex can be seen.

Analysis of Nanodiscs containing YidC

Native PAGE

In gel 12 from appendix 3 two clear bands can be seen in fraction 14 and 15. When comparing to gel 11 the lower band does not contain YidC, but the top band does. Because MSP was added in a 10:1

ratio compared to YidC we can assume that on gel 12 the visible bands consist mainly of MSP. Therefore the lower band probably consists of empty discs. The top band is heavier and probably contains discs and YidC. In fraction 11 some free YidC is found. But in fraction 16 both the top band and the lower band are both seen while gel 11 suggests that fraction 16 does not contain any YidC. Two possible explanations for this are: fraction 16 does contain YidC but in such a low concentration it cannot be seen on the gel. Or the top bands seen in fraction 14 and 15 are not YidC in discs. Alami et al suggests the double bands are empty Nanodiscs.

Confocal microscope

Labeled reconstituted YidC fractions 9, 11 and 15 were measured in a confocal microscope. Fraction 9 contained only aggregates so this could not be accurately measured and therefore not included in this paper. The graph found for fractions 11 and 15 can be seen in figure 6. When comparing the results the most important thing is the height of the cross-correlation signal (black line) compared to auto-correlation traces for YidC-AlexaFluor 488 and YidC-ATTO 647N (blue and red lines, respectively). In fraction 15 the difference is the largest. This suggests that mostly monomers are present. The residual cross-correlation signal is probably mostly the double labeled YidC. This supports the suggestion that in earlier fractions, e.g. fraction 9, more aggregates are present.

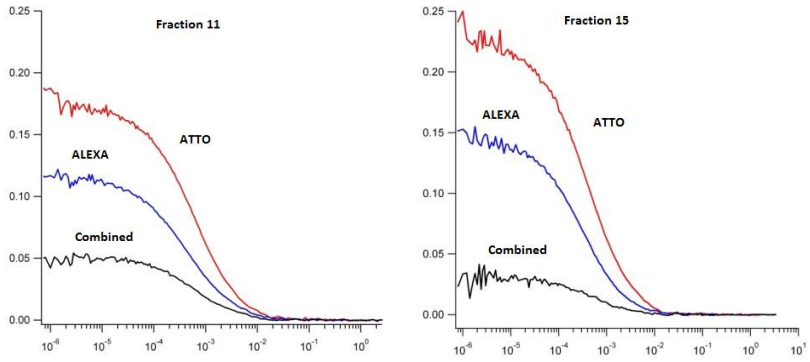

Figure 6: left: confocal microscope result of fraction 11. Right: confocal microscope result of fraction 15. The red line is the ATTO dye, the blue line the Alexa dye and the black line is both dyes in a single nanodisc.

Discussion

YidC was purified from a E. *coli* overexpression mutant in quantities from which further research could be conducted. The SDS-PAGE and Native PAGE showed clear results that YidC was present in some form and up to 7 µM purified YidC concentration was reached. After incorporation and labeling the confocal microscope showed a large portion of fraction 11 to 15 are single molecules and only a few aggregates.

During the over-expression of a YidC mutant some problems were encountered. It was found that when adding a very small amount of glucose just before incubation the cells are destroyed before the amount of cells has reached harvestable amounts. Also due to some error a black spot if seen after disruption. This is caused by cell death during growth and/or after induction of YidC synthesis. It is unknown what the cause of this phenomenon is. Another problem was encountered during purification. When using the sucrose gradient to separate outer and inner membrane vesicles it is

sometimes very hard to discern the layers that contain the YidC in inner membranes. The obtained concentrations of purified YidC were very different each time, but in all cases high enough to continue with the experiments. Some samples contained a small portion of dimeric YidC but that could be combated by added a small amount of DTT. During the crosslinking experiment the efficiency was very low (about 10% or lower). The cause of the low efficiency during the crosslinking experiment is unknown and might be a subject open to more research.

The goal of the experiments was to obtain pure monomeric YidC reconstituted in a nanodisc. The nanodisc reconstitution procedure was successfully established on YidC. However, it is unknown if monomeric YidC incorporated in a nanodisc. Further research could include the following analysis: Atomic force microscopy, UV/Vis spectroscopy, transmission electron microscopy and fluorescence-detected linear dichroism measurements.[6] The protocol used for reconstitution in nanodiscs is a protocol for the incorporation of bacteriorhodopsin as used by Bayburt and Sligar (2003)[6]. It could very well be that the process is not ideal for this protein and therefore should be a subject for further research.

The conclusion is that monomeric reconstituted YidC was probably formed but no conclusive evidence was found. Also purity of the obtained samples was not checked. The method of producing pure YidC is not perfect, but efficiently enough to obtain pure YidC in quantities high enough for further research. The method of reconstituting YidC in a nanodisc might have room for improvement and the final results need further analysis.

References

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4) *"The Crystal Structure of the Periplasmic Domain of the Escherichia coli Membrane Protein Insertase YidC contains a Substrate Binding Cleft*", Ravaud et al, April 4, 2008 The Journal of Biological Chemistry, 283, 9350-9358.
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Appendix 1: Protocols

1.1.1 Transformation

Prepared 200 µL SF100 E. *coli* culture (100 µL control and 100 µL sample)
Added 5µL of 10 times diluted plasmid (YEH1YidCHis) solution to sample.
Both sample + control:
5' on ice, 1.5' @ 42°C, 5' on ice. Added 500 µL LB medium to sample and control. 30' incubation @ 37°C.
Short spin and then took out the first 400 µL.
Poured 4 20 mL LB-Agar plates with 25µg/mL kanamycin.

Plate #	Amount of liquid plated out	# of colonies after overnight growth
1	50µL control	Nothing
2	150µL control	Nothing
3	50µL sample	Some
4	150µL sample	More colonies

Selected 2 colonies on plate 4 for overday preculture. 10 mL LB preculture with 25µg/mL kanamycin.
After 6 hours 3xovernight preculture was made:
100mL LB
2.5mL 20% glucose (final conc. 0.5%)
100µL kanamycin (final conc. 25µg/mL)
~6 mL of the overday preculture
OD after overnight growth:

Preculture #	OD measured	Amount needed for 1L culture (starting OD 0.05)
1	2.10	24.29
2	2.35	21.70
3	2.31	22.08

1.1.2 Transformation

0.2 µL plasmid solution (YEH1YidCHis) and 100 µL SF100 E. *coli* cells mixed together
1' 42°C, 5' ice. Added 800 LB then 30' incubation @ 37°C.
Plated 100µL of mixture on 20 mL LB-Agar plates with 25µg/mL kanamycin.
After overnight growth selected 2 cultures for 100mL preculture (overday + overnight).
25mL preculture needed for 1L culture.

1.2.1 Growth and induction

This step was preformed multiple times and the results of the first time are displayed. See Appendix 2 for the other results.

Made 6x 1L cultures with 1L LB and 25µg/mL kanamycin.

Added to 3 1L cultures 25mL 20% glucose.

Incubation @ 37°C, started shaking after 30 min.

Sample #	Time (min)						Final OD
	0	30	60	90	120	150	
1 with glu	0.074	0.108	0.185	0.270	0.039		0.053
2 with glu	0.065	0.088	0.145	0.280	0.553	*0.884*	0.177
3 with glu	0.078	0.111	0.205	0.394	*0.758*	1.138	0.545
1	0.076	0.102	0.175	0.319	0.536	*0.707*	0.911
2	0.063	0.082	0.138	0.265	0.470	*0.677*	0.840
3	0.074	0.126	0.203	0.344	0.586	*0.653*	0.878

Induction with 1mL 1M IPTG (for cultures with glucose) and 500µL 1M IPTG for cultures without glucose. Underlined OD is time of induction. After induction 2.5 hours incubation. After 2,5 hours OD was checked resulting in the final OD column.

Centrifuged cultures using JLA 8.1000, 4°C for 15 min @ 7000rpm.

Resuspended pellets in Tris/HCl 50mM pH 8.0 (100mL total) and combined fractions without glucose. (discarded fractions with glucose)

Centrifuged for 10'@7000rmp, 4°C.

Discarded supernatant.

Resuspended pellet in 30mL Tris/HCl 50mM pH 8.0, 20% sucrose. Stored in -80°C freezer.

1.3.1 Disruption and isolation

Thawed Cells and added 100µL 1M DNAse.

Added PMSF (0.1mM end concentration)

One shot French press with 13000 psi.

Centrifuged 15' 8k rpm SS34 (4°C)

Centrifuged supernatant 45' 40000 rpm Ti 45 (4°C)

Pellet brown with large black patches.

Resuspended in 1 mL 50mM Tris/HCl pH 8.0, 20% glycerol.

Prepared sucrose gradient (36%, 45%, 51%, 55%), 6 vials and loaded the resuspended pellet on the 6 vials.

30' 100000 rpm TLA 100.4 (4°C)

No visible band found in the gradient so stopped at that point.

1.3.2 Disruption and isolation

Thawed Cells and added 100µL 1M DNAse.

Added PMSF (0.1mM end concentration)

One shot French press with 13000 psi.

Centrifuged 15' 8k rpm SS34 (4°C)

Centrifuged supernatant 45' 40000 rpm Ti 45 (4°C)
Pellet is brown with a streak of black.
Resuspended in 1 mL 50mM Tris/HCl pH 8.0, 20% glycerol.
Prepared sucrose gradient (36%, 45%, 51%, 55%), 6 vials and loaded the resuspended pellet on the 6 vials.
30' 100000 rpm TLA 100.4 (4°C)
Collected brown fraction
Dilution with 1.5mL 50mM Tris/HCl pH 8.0, 20% glycerol
30' 100000 rpm TLA 100.4
Pellet (IMV's) resuspended in 300 µL 50mM Tris/HCl pH 8.0, 20% glycerol
Frozen in -80°C freezer

1.4.1 Purification

2mg IMV's diluted to 480µL in buffer A (50mM Tris/HCl pH 8.0, 20% glycerol).
Added: 20µL 4% DDM in buffer A (end conc. 2%)
30' rollerbank to solubelize.
30' 13k centrifugation @ 4°C

During solubelization and centrifugation:
Washing 150µL beads with 1mL MQ, 5 min centrifugation @ 7600g. (3x)
Washing beads with 1mL 50mM Tris/HCl pH 8.0, 20% Glycerol, 100mM NaCl, 0.05% DDM, 50mM Imidazol (buffer W), 5 min centrifugation @ 7600g (3x)

Mixed beads with solubelized IMV's and kept on rollerbank for 1h15min.
Poured on biospin column
Washed with 1 mL buffer W (4x)
Closed column, added 800µL buffer E (50mM Tris/HCl pH7.0, 20% glucerol, 100mM NaCl, 0.05% DDM, 400mM Imidazole)
10 min rollerbank, then collected Elution sample

1.4.2 Purification

2mg IMV's diluted to 480µL in buffer A (50mM Tris/HCl pH 8.0, 20% glycerol).
Added: 20µL 4% DDM in buffer A (end conc. 2%)
30' rollerbank to solubelize.
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Mixed beads with solubelized IMV's and kept on rollerbank for 1h15min.
Poured on biospin column

Washed with 1 mL buffer W (4x)
Closed column, added 800µL buffer E (50mM Tris/HCl pH7.0, 20% glucerol, 100mM KPi, 0.05% DDM, 400mM Imidazole)
10 min rollerbank, then collected Elution in eppendorf cup and concentrated the sample with 30kDa filters. 5x5' 13k rpm 4°C, about 100µL per run through filter so about 300µL left over after concentrating.

1.4.3 Purification and labeling

2mg IMV's diluted to 480µL in buffer A (50mM Tris/HCl pH 7.0, 20% glycerol).
Added: 20µL 4% DDM in buffer A (end conc. 2%)
4µL TCEP
15µL ATTO 647 (20mM)
30µL Alexa 488 (10mM)

90' rollerbank to solubelize.
10' 13k centrifugation @ 4°C

During solubelization and centrifugation:
Washing 150µL beads with 1mL MQ, 5 min centrifugation @ 7600g. (3x)
Washing beads with 1mL 50mM Tris/HCl pH 8.0, 20% Glycerol, 100mM NaCl, 0.05% DDM, 50mM Imidazol (buffer W), 5 min centrifugation @ 7600g (3x)

Mixed beads with solubelized IMV's and kept on rollerbank for 1h15min.
Poured on biospin column
Washed with 1 mL buffer W (4x)
Closed column, added 800µL buffer E (50mM Tris/HCl pH8.0, 20% glucerol, 100mM KPi, 0.05% DDM, 400mM Imidazole)

Collected Elution in eppendorf cup and concentrated the sample with 30kDa filters
5x5' 13k rpm 4°C, about 100µL per run through filter so about 300µL left over after concentrating.

1.5.1 Formation of nanodiscs

Mixed in a ratio of about 10:800:1 MSP: lipid mixture*:YidC
40µL 350µM MSP, 142µL 7mM lipids and 200µL 1.4nmol YidC.
Then 1h 4°C rollerbank.
Added 200mg Biobeads (SM-2) and put overnight on a rollerbank (4°C)
Separated liquid from Biobeads using a syringe.
FPLC with Superdex 200 HR 10/30 column. Measured fractions for protein concentration.

* Lipid mixture containing 10% cardiolipin, 20% DOPG, 30% DOPE, 40% DOPC.

1.6.1 Cross linking

Mixed 5µL 0.24mM $CuSO_4$ and 10µL 0,36mM Phenanthroline. Added to YidC filled nanodiscs (final concentration 1mM) and incubated for 1h @ 37°C.
Split sample in half and added to one half DTT (10mM final concentration)
Also made a proteoliposome sample and added DTT to half of it.
Loaded on gel without DTT present.

1.7.1 Native PAGE

Used a Criterion precast 4-20% Tris/HCl gradient gel.
For the electrophoresis buffer the following mixture was used:
25mM Tris, 192mM glycine and added water to 1L, pH=8.8.
For the 5x sample buffer the following mixture was used:
312.5mM Tris/Cl (pH=6.8), 50% glycerol, 0.05% bromophenol blue, 14% H_2O.
Added 30µL 5x sample buffer to unused wells and 7µL to used wells. Added 30µL sample to each well except the pure YidC and pure MSP well. There 10µL and 1µL was used respectively.

Appendix 2: Additional results

Additional Growth and Induction results

Growth 2:

Sample #	Time (min)			Final OD
	0	60	90	
1	0.310	0.566	0. 745	0.989
2	0.350	0.678	0.920	1.207
3	0.367	0.632	0.860	1.120
4	0.361	0.610	0.826	1.057

4x1L Grown

Growth 3:

Sample	Time (min)			Final OD
	0	60	120	
Wild type	0.102	0.303	0.736	0.858
YidC^{269} Amp	0.081	0204	0.637	1.701

2x3L grown

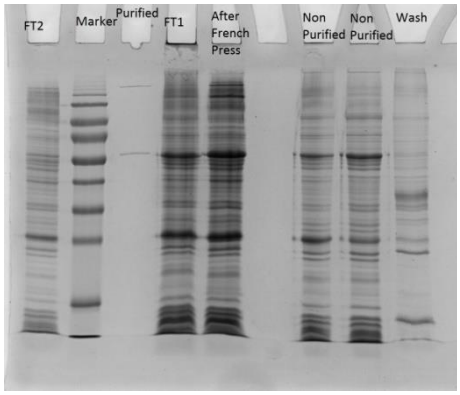
Growth 4 (Used for labeling):

Sample #	Time (min)			Final OD
	0	60	120	
1	0.092	0.205	0.834	Unknown

Only 1 flask measured but 6x1L grown.

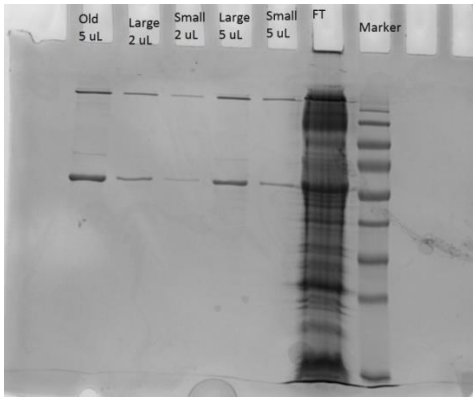
Appendix 3: Gel Samples

3.1



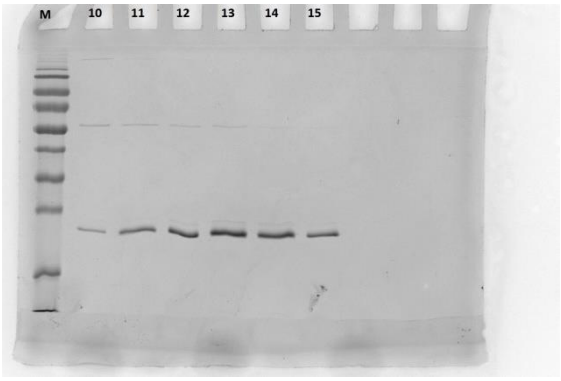
Slot	Content
FT2	Second part of Flow through
Marker	Standard marker
Purified	After Purification
FT1	First part of Flow through
After French Press	After French Press
Non Purified	Before purification
Non Purified	Duplo
Wash	After wash step

Figure 1: Gel Sample 1 , samples from growth 1 during different steps of the disruption and purification procedure.

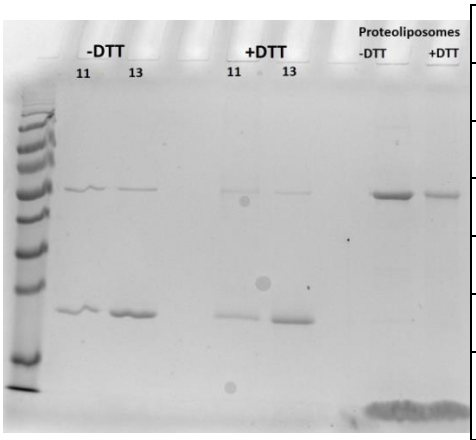


Slot	Content
Old 5 uL	older sample with error in purification
Large 2uL	2 uL of the larger elution fraction
Small 2 uL	2 uL of the smaller elution fraction
Large 5 uL	5 uL of the larger elution fraction
Small 5 uL	5 uL of the smaller elution fraction
FT	Flow through during purification
Marker	Standard Marker

Figure 2: Gel Sample 2, multiple samples after the purification proces.

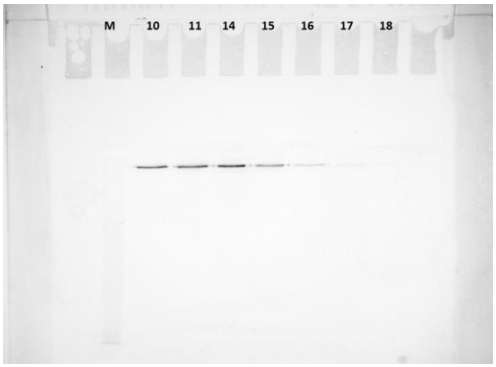


Figuur 3: Gel sample 3, the FPLC fractions after nanodisc formation. Marker in the first lane, lane 2 till 7 contain FPLC fraction 10-15.

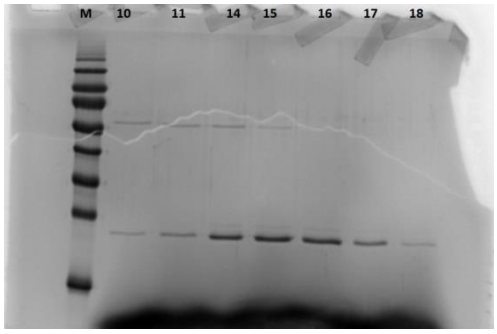


Slot	Content
First	Standard Marker
-DTT 11	Fraction 11 without added DTT
-DTT 13	Fraction 13 without added DTT
+DTT 11	Fraction 11 with added DTT
+DTT 13	Fraction 13 with added DTT
Proteoliposomes -DTT	Sample proteolipsomes for comparison without DTT
Proteoliposomes +DTT	Sample proteolipsomes for comparison with DTT

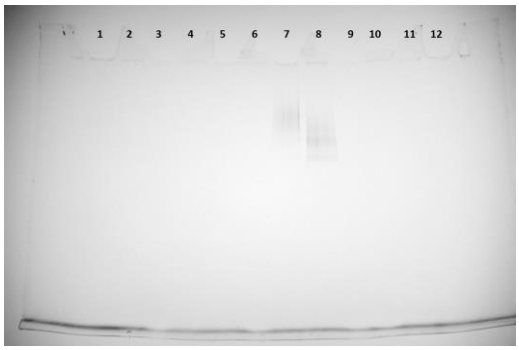
Figuur 4: Gel sample 6, result crosslinking with $CuSO_4$ and Phenanthroline, as can been seen the crosslinking efficiency (top band compared to the lower band) is lower than 10% so no further research was performed.



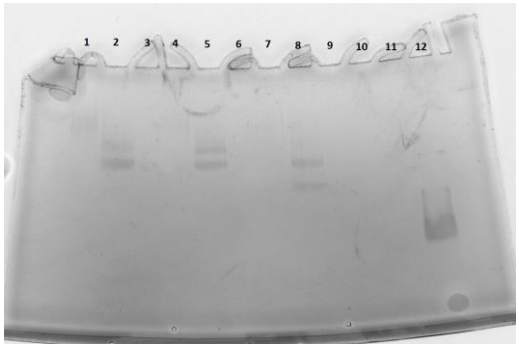
Figuur 5: Gel sample 7, Fluorescence image of labeled YidC in nanodiscs after FPLC (fraction 10 to 18). Lane 2 contains a standard non-fluorescent marker.



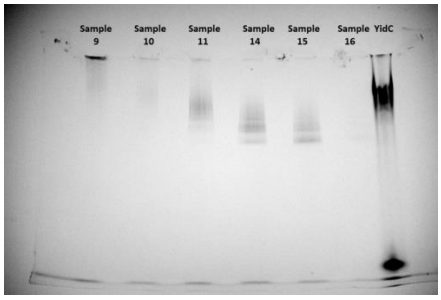
Figuur 6: Gel sample 8, Visible light image of labeled YidC in nanodiscs after FPLC, fraction 10 to 18 (Coomassie stained). Lane 2 contains a standard Marker.



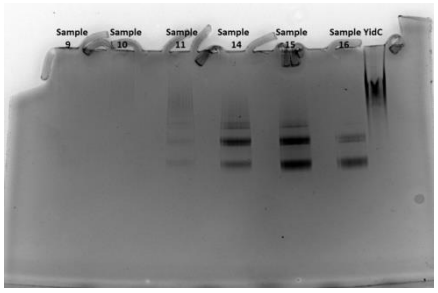
Figuur 7: Gel sample 9, Fluorescence image where slot 1,2,4 and 5 contain non labeled YidC in nanodiscs, 7 and 8 contain labeled YidC in nanodiscs, slot 11 contains pure YidC (non labeled) and slot 12 pure MSP.



Figuur 8: Gel sample 10, Visible light image after Coomassie staining where slot 1,2,4 and 5 contain non labeled YidC in nanodiscs, 7 and 8 contain labeled YidC in nanodiscs, slot 11 contains pure YidC (non labeled) and slot 12 pure MSP.



Figuur 9: Gel sample 11, Fluorescence image from Native PAGE containing fraction 10 to 16 from FPLC with labeled YidC in nanodiscs. YidC lane contains reference non treated but fluorescent labeled YidC.



Figuur 10: Gel Sample 12, Visible light image after Coomassie staining, Native page containing fraction 10 to 16 from FPLC with labeled YidC in nanodiscs. YidC lane contains reference non treated but fluorescent labeled YidC.