Science Advances

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Supplementary Materials for

Engineering a nanopore with co-chaperonin function

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Published 11 December 2015, *Sci. Adv.* **1**, e1500905 (2015)
DOI: 10.1126/sciadv.1500905

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Supplementary discussion:

Design of the transmembrane co-chaperonin

Staphylococcal alpha hemolysin nanopore (αHL) and the co-chaperonin GroES both have a homo-heptameric assembly and a flat toroid face with similar dimensions (Figure 1B). We reasoned, therefore, that grafting the ES-loops into the αHL nanopore would produce a transmembrane co-chaperonin that might allow the single-molecule investigation of the GroEL reaction. The GroES-loops are a stretch of 24 amino acids (from R14 to R27) connecting two antiparallel β-sheets (β-hairpin loop) that form the dome shaped GroES. The α-carbons of the R14 residues are spaced 2.7 nm in contiguous protomers and 6.0 nm in opposite protomers (Figure 1B). αHL contains two antiparallel β-hairpin loops that could be used to insert the ES loops. We selected the β-hairpin loop that is located near the C-terminus of the nanopore because the α-carbon of the first amino acid in the loop (D287) is 2.6 nm from the equivalent residues in a contiguous protomer and 6.5 nm apart in a opposite protomer. Therefore, the ES loops in nanopore-GroES chimera will be similarly spaced as in GroES (Figure 1C). Chimera proteins were prepared by introducing the ES-loops between residues D287 and K288 in the αHL gene.

Voltage dependence of the GroEL:αHL-GroES$_L$ interaction.

We performed all our electrical recordings at +100mV. We observed, however, that the interaction of GroEL and αHL-GroES was voltage dependent, with the frequency of the blockades increasing with the applied potential. Experimental and theoretical work indicated that most of the electric potential change occurs inside the β-barrel of αHL and the potential at the *cis* entrance(42, 43) or *trans* exit(52) of the pore is uniform; therefore, the applied potential is not likely to play a direct role in modulating the affinity of GroEL for αHL-GroES$_L$. However, near the pore the flow field can be significantly influenced by electroosmotic forces.(53) WT-αHL nanopores are weakly anion selective and at positive applied potential there is a weak electroosmotic flux of water through the pore from the *cis* to the *trans* entry, which has been shown to facilitate the capture of analytes by the nanopore.(54) Proteins are especially sensitive to electroosmotic forces as observed in both solid-state and biological nanopores.(16, 55) For example, by using a larger ClyA

nanopore that is capable of accommodating folded protein in the nanopore interior, we have shown that the capture of proteins follows the electroosmotic flow rather than the electrical potential, independently on the sign of the net charge of the protein.(16) Therefore, the increased GroEL capture rate with the voltage is likely to reflect the increased electroosmotic flow through the pore as the applied potential is increased.

Additional Experimental Procedures

Construction of the αHL-GroES$_S$ and αHL-GroES$_L$ genes

αHL-GroES$_S$, GroEL and GroES genes were cut from the delivery plasmid (pUC57) and inserted into a pT7 expression plasmid using NdeI and HindIII restriction sites. The αHL-GroES$_S$ was designed to contain the mobile loop of GroES (from Arg-14 to Arg-37 flanked by a Ser-Gly linker on both sides: CGCAAAGAAGTCGAAACGAAATCGGCGGGCGGTATTGTGCTGACGGGCAGTGCGGCAGCAAAATCAACGCGC) between the codons for Glu-288 and Lys-289 in the αHL gene. The αHL-GroES$_L$ gene, which included a larger GroES fragment (from Arg-9 to Ala-42) than αHL-GroES$_S$ and no additional SG linker, was constructed in two steps by homologous recombination using the αHL-GroES$_S$ gene as initial template. In the first step (step 1) we added the first five amino acids at the N-terminus (amino acids RVIVK, GroES residues 9-13). In the second step (step 2) we added the remaining five amino acids at the C-terminus (amino acids GEVLA, GroES residues 38-42). Thus, in the first half reaction of step 1 the αHL-GroES$_S$ gene was digested by NdeI and then amplified by PCR using 5'-CAGAAGTGGTCCTGCAACTTTAT-3' (SC47) and 5'-CGCGTGATTGTAAAGCGCAAAGAAGTCGAAACGAAATCGGCGGGCGGT-3' (αHL-GroES$_L$(RVIVK)/47) primers (the nucleotides complementary to the template are marked in bold). In the second half reaction of the step 1, the αHL-GroES$_S$ gene was digested by HindIII and amplified using 5'-ATAAAGTTGCAGGACCACTTCTG-3' (SC46) and 5'-CTTTACAATCACGCGTTCCCAATCGATTTTATAGCGTTCCGATGAACG-3' (αHL-GroES$_L$(RVIVK)/46) primers. Both half reactions were then transformed together into E. coli DH5α cells (Invitrogen) and the resulting recombinant plasmid DNA purified from

single colonies to obtain the RVIVK-αHL-GroES$_S$ gene, which was used as template in the step 2. In the first half reaction of step 2, the obtained RVIVK-αHL-GroES$_S$ gene was then amplified by PCR using SC47 and 5'-GGCGAAGTGCTGGCTGTCAAAGAAGAAATGACGAACTAAGCTTGGATCCGG-3' (αHL-GroES$_L$(GEVLA)/47) primers, while in the second half reaction the RVIVK-αHL-GroES$_S$ gene was amplified with SC46 and 5'-GACAGCCAGCACTTCGCCGCGCGTTGATTTTGCTGCCGCACTGCCCGTCAG-3' (αHL-GroES$_L$(GEVLA)/46). Both half reactions were combined and transformed into E. coli DH5α cells and the resulting recombinant plasmid DNA purified from single colonies.

Construction of the GroEL-LLV gene

GroEL-LLV (containing mutations L234A, L237A and V264A) was constructed by using the MEGAWHOP procedure,(56, 57) which included two PCR reactions. The first PCR reaction was performed using 0.5 µL of the GroEL gene as template (2 ng/µL final concentration), 0.5 µL of a forward primer (0.2 µM final concentration) containing L234A and L237A substitutions (5'-CCAACATCCGCGAAATGGCGCCGGTTGCAGAAGCTGTTGCCAAAGC-3', mutant nucleobases underlined), 0.5 µl reverse primer (0.2 µM final concentration) containing the V264A substitution (5'-CACGATGCCACGCATGGTGTTAGCAACCAGAGTTGC CAGCGCTTCGCC-3'), and Phusion® Hot Start II DNA polymerase (Finnzymes). The entire PCR reaction was then loaded on a 1% agarose gel and the band corresponding to the PCR product (megaprimer) extracted using a PCR purification kit (Qiagen). The megaprimer was then used as primer for the second PCR reaction that used the same GroEL plasmid DNA as template.

Construction of the GroEL-398 gene

GroEL-398 (containing mutation D398A) was constructed by using MEGAWHOP mutagenesis method (see above) using the GroEL gene as template, a forward primer containing D398A substitution (5'-GAGAAAAAAGCACGCGTTGAAGCTGCCCTGCACGCGACCCGTGCTGCG

-3’, mutant nucleobases underlined) and a non-mutagenesis reverse primer (5’-CAGCGGAGCTTCCATTGCACGCAGTGCAACTTTGATACCCACGTTCTGG-3’).

Construction of the SR

SR, containing R452E, E461A, S463A, V464A substitutions, was constructed from the GroEL gene in two mutagenic steps. First, the glutamate at position 452 was introduced by Quik-Change site-directed mutagenesis (Stratagene) by using 5’-CGTGCAATGGAAGCTCCGCTGGAGCAGATCGTATTGAACTGCGGCG-3’ and 5’-GCACGTTACCTTCGAGGCGACCTCGTCTAGCATAACTTGACGCCGC-3’ as forward and reverse primers, respectively. The resulting plasmid DNA (containing R452E mutation) was then used as a template for the simultaneous introduction of the remaining mutations by using the megaprimer method as shown above using 5’-CGTATTGAACTGCGGCGAAGCACCGGCTGCGGTTGCTAACACCGTTAAAGGCGGC-3’ and 5’-AAGCTTCATCATGCCGCCCATGCCACCCATGCCGCCCATA CCGCCAGC-3’ as forward and reverse primers, respectively.

Purification of GroEL, GroEL-LLV, GroEL-398 and SR

GroEL, GroEL-LLV, GroEL-398 were purified following a previously described protocol(58) E. coli BL21(DE3)/pLysS cells (Novagen) harboring the selected constructed plasmid (GroEL, GroEL-LLV, GroEL-398) were grown in 2L of LB medium, containing 100 mg/L ampicillin at 37°C to an optical density of ~0.6 at 600 nm (OD600). Overexpression of proteins was induced by the addition of IPTG (0.5 mM final concentration) after which the temperature was lowered to 28°C and the cells grown for an additional 14-16 hours. Cells were harvested by centrifugation for 20 minutes at 6000 rpm using a Sorvall SLA-1500 rotor and the supernatant discarded. Cell pellets were then re-suspended in 40 ml of lysis buffer A [50 mM Tris-HCl pH 7.5, 50 mM NaCl, 1 mM DTT, 1 mM EDTA and 0.5 mM phenylmethanesulfonylfluoride (PMSF)] supplemented with 0.06 units DNase1 and ~1 mg/ml of lysozyme for 60 min at 4 °C. After sonication, cellular debris was removed by centrifugation for 60 min at 14,000 rpm (4°C) using a Sorvall SS-34 rotor. The pellet was discarded and the supernatant precipitated by the addition of 50% (w/v) ammonium sulphate. After centrifugating for 60 minutes at 14,000 rpm, the pellet was resuspended in

10 ml buffer A and then dialyzed 200-fold in the same buffer overnight. The protein solution was applied to a HiTrap-Q column (GE Healthcare) using a ÄKTAprime plus system (GE Healthcare). A linear gradient of Buffer B (50 mM Tris-HCl pH 7.5, 1M NaCl, 1 mM DTT, 1 mM EDTA) was applied and GroEL proteins eluted at 38%-46% of buffer B. The purity of GroEL fractions was checked by 10% SDS-PAGE gel. Subsequently, the protein samples were pooled together and loaded onto a HiPrep 16/60 Sephacryl S-300 HR column (GE Healthcare) pre-equilibrated with 50 mM Tris-HCl pH 7.5, 50 mM NaCl, 1 mM DTT, 1 mM EDTA containing 10% ethanol. At a flow rate of 0.4 ml/min GroEL protein eluted with 45 to 60 ml of buffer. Aliquotes were collected and concentrated to ~20 mg/mL by 100 kDa cut-off concentrators (Amicon). At this stage, the protein was >98% pure as judged by blue native gel electrophoresis by using a using a 4-20% polyacrylamide gradient gel (Bio-Rad). In order to eliminate minor contaminants of low molecular peptides,(59, 60) ~100 µl of GroEL were incubated for 30 min at 37°C with 3 M urea (final concentration) and then applied onto the desalting column (PD MiniTrap™ G-25, GE Healthcare), which removed the urea and the small molecular weight contaminants. The 100 µL sample was then incubated with $MgCl_2$ (5 mM) and ATP (2 mM) for 90 minutes at room temperature to allow protein reassembly. The protein was loaded onto a HiPrep 16/60 Sephacryl S-300 HR column (see above), the eluted GroEL proteins collected and the buffer exchanged to 50 mM Tris-HCl pH 7.5, 250 mM NaCl, 1 mM DTT, 1 mM EDTA using a Amicon concentrators with 100 kDa cut-off membranes. Finally, the oligomerization of GroEL was checked by blue native page (see above).

Purification of αHL, αHL-GroESs and αHL-GroES$_L$

αHL, αHL-GroES$_S$ and αHL-GroES$_L$ were overexpressed in E. coli BL21(DE3)/pLysS cells as described above for GroEL. The cultured cells were lysed in lysis buffer as shown above supplemented with 1% v/v Triton X-100. Proteins were then precipitated with 70% (w/v) ammonium sulfate and the pellet treated as previously described for GroEL. Protein solutions (in buffer A: 20 mM sodium acetate, pH 5.2) were loaded onto Hi-Trap S cation exchanger column (GE Healthcare) and eluted with a linear gradient of 1 M NaCl in buffer A. The fractions were run on a 12% SDS-PAGE and the fractions corresponding to the αHL monomers were loaded onto a 10 kDa cut-off Amicon concentrator, were the solution

was exchanged with 10 mM Tris–HCl, pH 8.0, 150 mM NaCl. Oligomer proteins were formed by dropwise addition of a stock solution of deoxycholate (DOC, 100 mM, in 100 mM Tris–HCl, pH 8.3) while stirring at room temperature to a final concentration of 6.25 mM DOC and then incubated overnight at 4°C. Heptamers were separated from monomers by using a HiPrep 16/60 Sephacryl S-300 HR column, equilibrated with 10 mM Tris–HCl, pH 8.0, 150 mM NaCl, 1.25 mM DOC. Fractions were collected and analyzed by loading into a 5% SDS-PAGE.

Purification of GroES

GroES containing an N-terminal hexahistidine tag was overexpressed in E. coli BL21(DE3)/pLysS cells as described above for GroEL. The cultured cells were lysed in lysis buffer (50 mM Tris-HCl, 250 mM NaCl, pH 8.0) supplemented with ~1 mg/ml lysozyme, 0.06 units DNaseI and 0.5 mM PMSF. After sonication, cellular debris was removed by centrifugation as described above. The pellet was discarded and the supernatant was purified using Ni-NTA affinity chromatography. The fractions were run on a 12% SDS-PAGE and the samples were concentrated using a 10 kDa cut-off Amicon concentrator.

Hemolytic assay

The haemolytic activity of αHL, αHL-GroES$_S$ and αHL-GroES$_L$ was measured by observing the decrease in the absorbance at 595 nm after incubating αHL with diluted solutions of rabbit red blood cells (rRBCs). 450 μL of rRBCs were diluted into a 900 μL of MBSA buffer (10 mM MOPS, 150 mM NaCl, pH 7.4, containing 1 mg/ml bovine serum albumin (BSA)), spun down for 2 minutes at 1200 RPM and the supernatant discarded. The rRBCs were then re-washed for 2-3 times until the OD595 was 0.6 - 0.8. 100 μL of washed rRBCs were quickly added to the each well of a 96 well microwell plate containing 50 μL of monomer αHL, αHL-GroES$_S$ or αHL-GroES$_L$ proteins (0.0625 uM, final concentration). The hemolytic activity of proteins was assayed by monitoring the decrease in light scattered at 595 nm with a TECAN XFluor4 Safire II microplate reader.

ATPase assay

The ATPase assay of GroEL was measured spectrophotometrically with an ATP regeneration system. The assay is based on the conversion of ADP to ATP by pyruvate kinase (PK), coupled to the conversion of phosphoenolpyruvate (PEP) to pyruvate. Pyruvate will then be converted to lactate by lactate dehydrogenase (LDH). The latter step requires NADH, which is oxidized to NAD+ and can be followed by monitoring absorbance at 340 nm. The assay mixture (150 µL) contained 250 µM NADH, 2 mM PEP, 100 µg/ml PK, 2 µM LDH, 1 mM DTT in a 50 mM Tris-HCl pH 7.5, 5 mM $MgCl_2$, 50 mM or 1 M KCl buffer. The final concentration of GroEL was 50 nM while GroES, αHL-$GroES_L$, αHL-$GroES_S$ or αHL final concentrations were 200 nM. The assay solution was pre-incubated for 10 min at 25 °C prior the addition of ATP (1 mM, final concentration). The decrease in the absorbance at 340 nm was monitored continuously with a spectrophotometer (TECAN XFluor4 SafireII microplate reader) and the ATPase activity was determined from the slope of the NADH absorbance decrease.

MDH refolding assay

MDH (EC number: 1.1.1.37) from porcine heart (300 µM) was denatured with 3 M guanidine hydrochloride in the presence of 5 mM DTT for 2 hr at 37 °C. Unfolded MDH (2 µM, final concentration) was then diluted in 150 µL of refolding buffer [20 mM 3-[N-morpholino]propane-sulfonic acid (MOPS.KOH), pH 7.4, 50 mM or 1 M KCl, 5 mM $Mg(OAc)_2$, 100 µg/ml PK, 5 mM PEP), which contained GroEL (50 nM, final concentration) with or without GroES, αHL-$GroES_L$, αHL-GroESs or αHL (200 nM, final concentration). The protein solution was then incubated at 37 °C for at least 5 min prior to addition of 2 mM of ATP after which the temperature was switched to 25 °C. After 3 hours the reaction was quenched by the addition of 0.45 M (final concentration) of trans-1,2-diaminocyclohexane-N,N,N',N'-tetraacetic acid (CDTA). The protein solution was further incubated for 3 hours to allow MDH dimerization at 25°C. The refolding of MDH was observed by monitoring changing of the signal at 340 nm in the MDH assay buffer: 20 mM MOPS-KOH, pH 7.4, 50 mM or 1 M KCl, 5 mM $Mg(OAc)_2$, 0.5 mg/mL BSA, 250 µM NADH, 1 mM oxaloacetate.

LDH refolding assay

LDH (EC number: 1.1.1.28) from bovine heart (200 μM) was denatured in 3 M guanidine hydrochloride and in the presence of 5 mM DTT for 2 hr at 37 °C. Unfolded LDH was then diluted 100-fold into the refolding buffer (20 mM MOPS-KOH pH 7.4, 50 mM KCl, 5 mM $Mg(OAc)_2$, and 1 mM DTT), which also contained GroEL (50 nM), and GroES, αHL-$GroES_L$, αHL-GroESs and αHL (200 nM). The protein solutions were incubated at 37 °C for at least 5 min prior addition of ATP (2 mM), after which the temperature was switched to 25 °C. After waiting for ten minutes, the refolding of LDH was monitored by observing the absorbance decrease at 340 nm upon addition of the LDH assay solution (50 mM Tris-HCl, pH 7.5, 50 mM KCl, 5 mM $MgCl_2$, 250 μM NADH and 500 μM sodium pyruvate).

GroEL/GroES and GroEL/αHL-$GroES_L$ interaction monitored by proteinase K protection

GroEL (0.2 μM) was incubated at 25 °C with GroES or αHL-$GroES_L$ (1 μM, final) in 20 μL of 20 mM MOPS buffer, pH 7.4, 50 mM KCl and 5 mM $MgCl_2$ in the presence of 1 mM ATP. After 20 minutes, proteinase K (final concentration 10 μg/mL) was added to the samples for an additional 10 min at 25 °C. Proteinase K activity was then quenched by addition of 1 mM PMSF (final concentration). Digested mixtures were finally run on a 10% SDS-PAGE. The protein were visualized with silver-staining.

Additional Figures

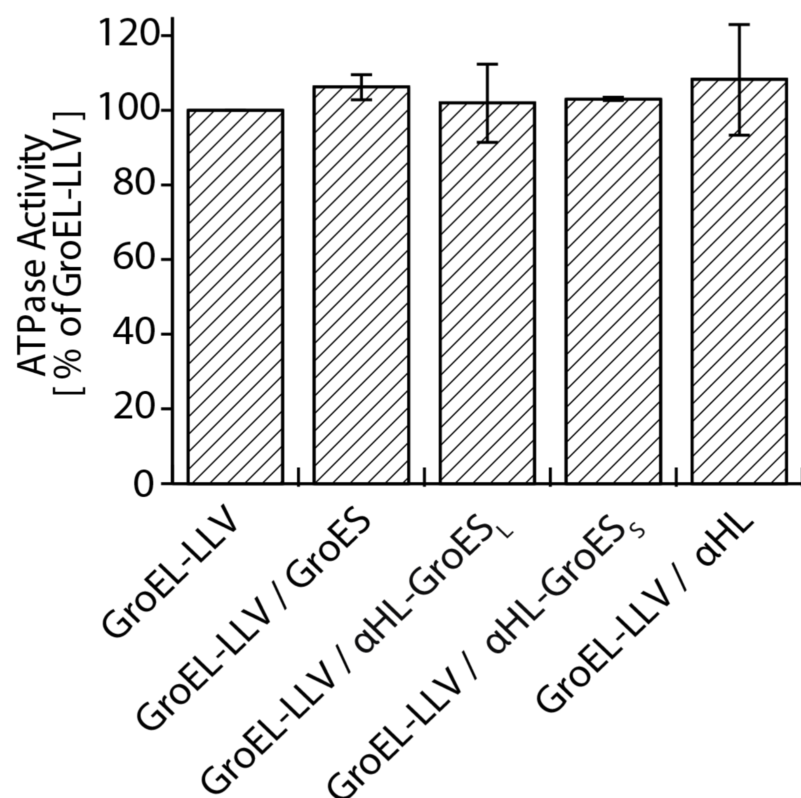

Figure S1. GroEL-LLV ATPase activity. GroEL-LLV (50 nM, final) was added to the reaction buffer (50 mM Tris-HCl pH 7.5, 50 mM KCl, 1 mM ATP and 5 mM $MgCl_2$) containing GroES or αHL proteins (200 nM, final). αHL proteins were solubilized in 0.125 mM DOC. The values are shown in Table S3.

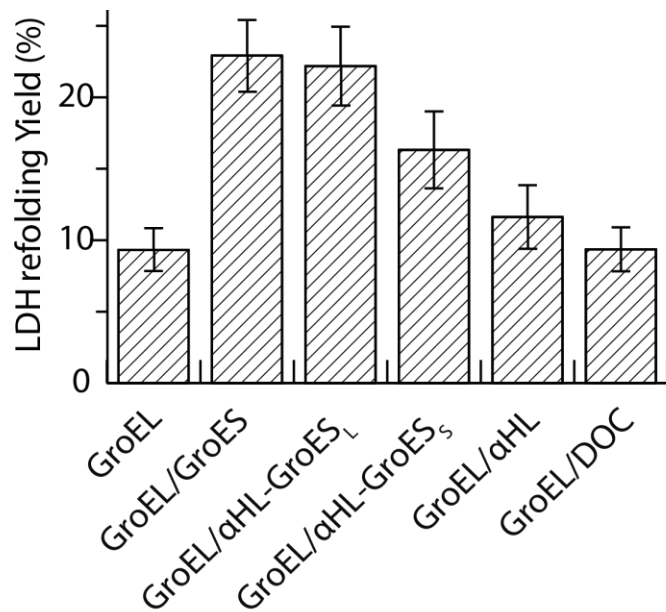

Figure S2. LDH refolding assay. LDH (2 μM, final), unfolded with 3M guanidinium.HCl, was added to the refolding buffer [20 mM MOPS, pH 7.4, 50 mM KCl, 5 mM $Mg(OAc)_2$ and 5 mM DTT] containing GroEL (50 nM) and GroES, αHL-GroES$_L$, αHL-GroES$_s$ or αHL (200 nM). The protein solutions were incubated for at least 5 min prior addition of ATP (2 mM). Then, after incubating for 10 minutes, the refolding of LDH was monitored by observing the absorbance decrease at 340 nm upon addition of the LDH assay solution (50 mM Tris-HCl, pH 7.5, 50 mM KCl, 5 mM $MgCl_2$, 250 μM NADH and 500 μM sodium pyruvate). The values are shown in Table S4.

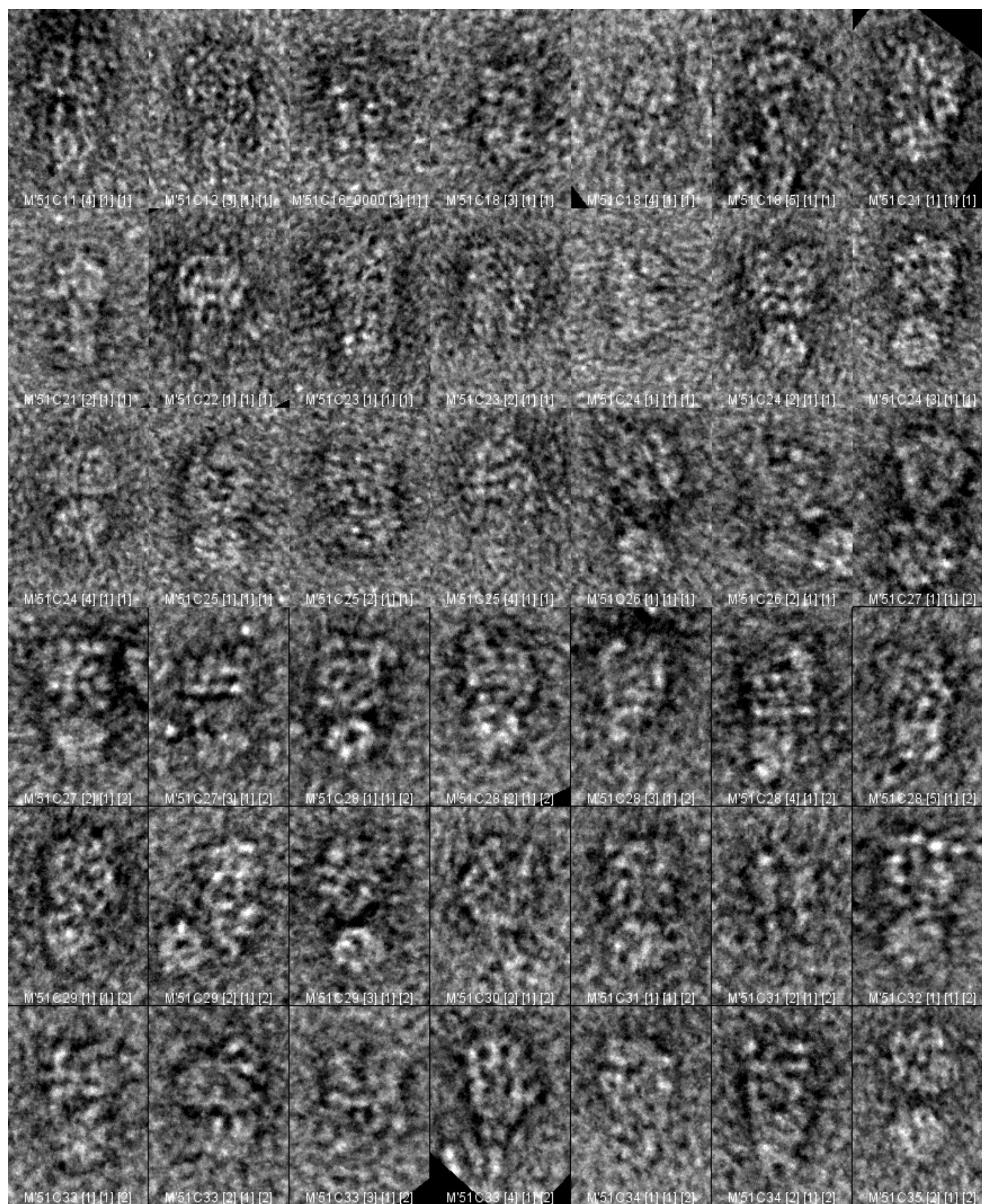

Figure S3. Representative EM micrograph of single αHL-GroES$_L$-GroEL complexes. Negative stained EM image of GroEL-398 bound to αHL-GroES$_L$ formed by pre-incubating GroEL-398 (0.5 μM) with αHL-GroES$_L$ (1 μM) for 20 minutes before applying a 100-fold dilution to negatively-stained EM grids.

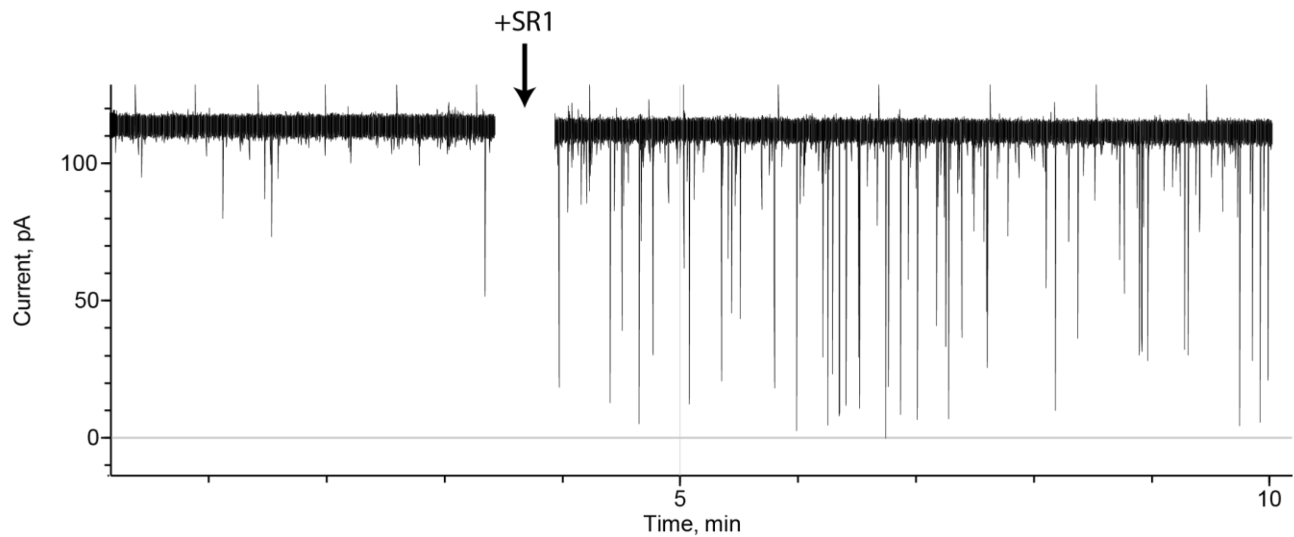

Figure S4. Nucleotide dependency of SR1 interaction with αHL-GroES$_L$.

In the absence of nucleotide, the addition of 100 nM of SR1 under +100 mV applied potential induced only fast current blockades, indicating that the interaction of SR1 and αHL-GroES$_L$ is nucleotide dependent. The electrical recordings were carried out in 1 M KCl, 50 mM Tris.HCl, pH 7.5, and 5 mM $MgCl_2$, 23°C applying a 10 kHz low-pass Bessel filter with 50 kHz sampling rate. An additional digital Gaussian filter at 2 kHz was applied to current traces.

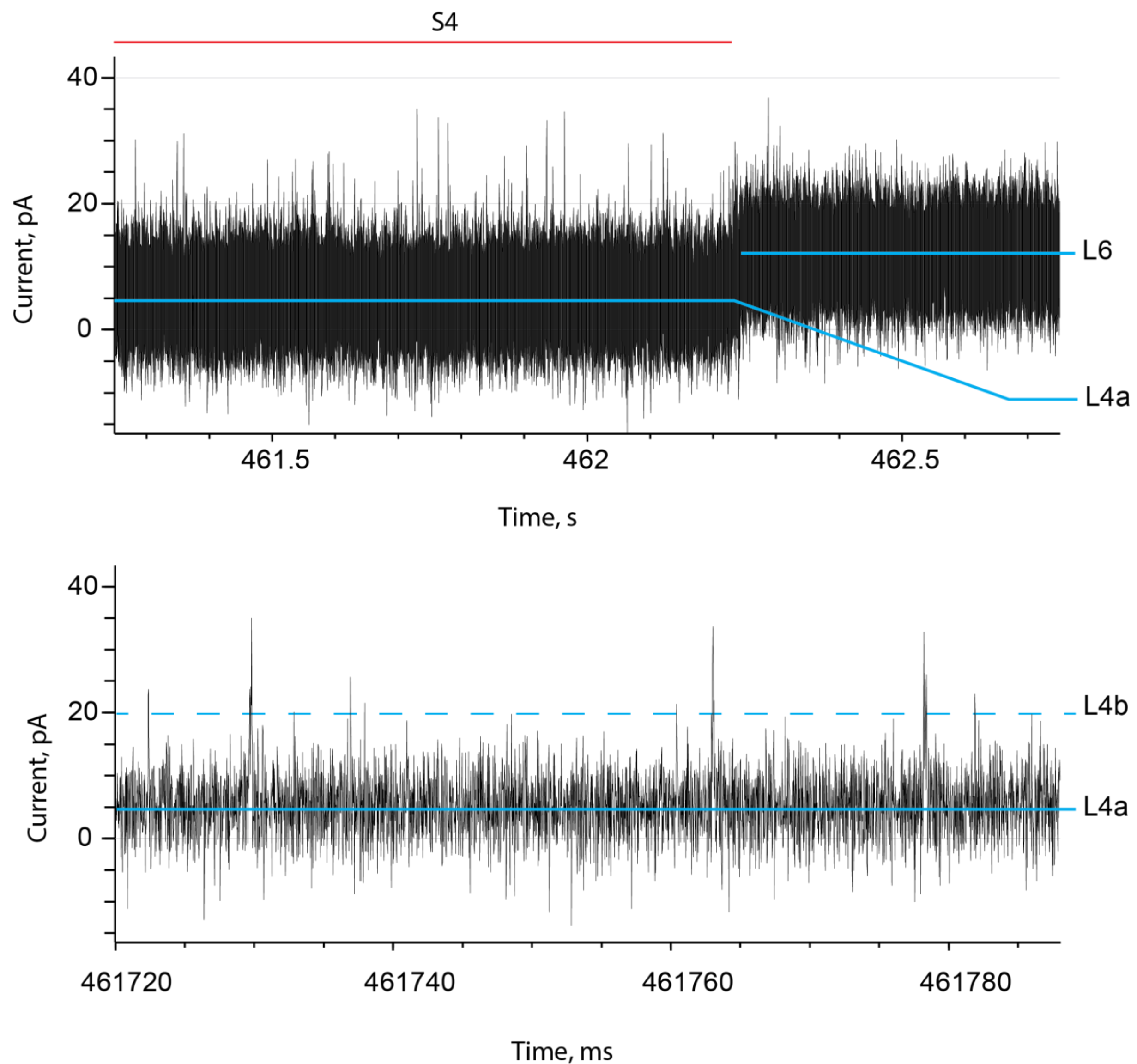

Figure S5. Expansions of an SR1-induced current blocked.

The current trace was recorded in 1 M KCl, 50 mM Tris.HCl, pH 7.5, at 23°C in the presence of 1 mM ATP, 100 nM SR1 and 5 mM $MgCl_2$ by applying a 10 kHz low-pass Bessel filter and using a 50 kHz (20 μs) sampling rate. Contrary to all the traces showed in the main text and supporting information, the current trace was *not* additionally filtered. The bottom trace shows that single-channel recordings cannot accurately measure the L4b level.

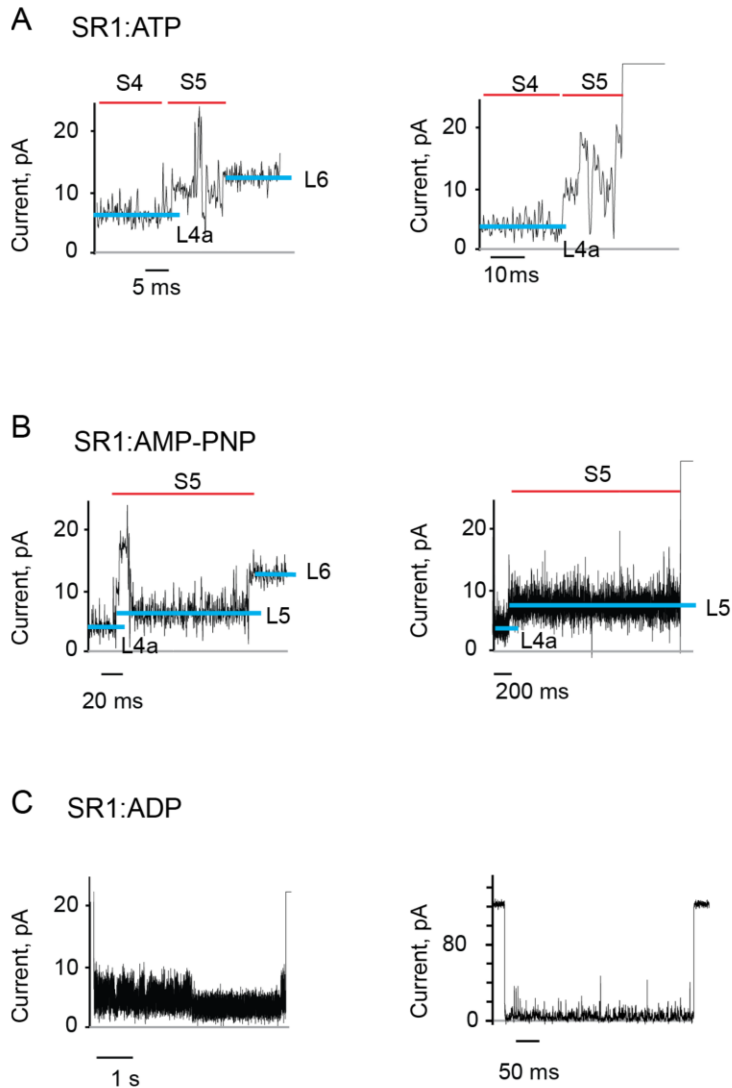

Figure S6. Release of the binary SR1 current.

Current blockades provoked by SR1 to αHL-GroES$_L$ in the presence of 1 mM nucleotide (*cis*) at +100 mV. (A) All ATP induced blockades showed S5 transitions that either released the complex (right) or led to the formation of a L6 current level (left). (B) The current transitions induced by AMP-PNP were very similar to that observed with ATP, although showed noticeable longer S5 current transition with a well-defined current level (L5). (C) Details of the release from the ADP the binary complex. The electrical recordings were carried out in 1 M KCl, 50 mM Tris.HCl, pH 7.5, and 5 mM $MgCl_2$, 23°C and +100 mV applying a 10 kHz low-pass Bessel filter with 50 kHz sampling rate. An additional digital Gaussian filter at 2 kHz was applied to current traces.

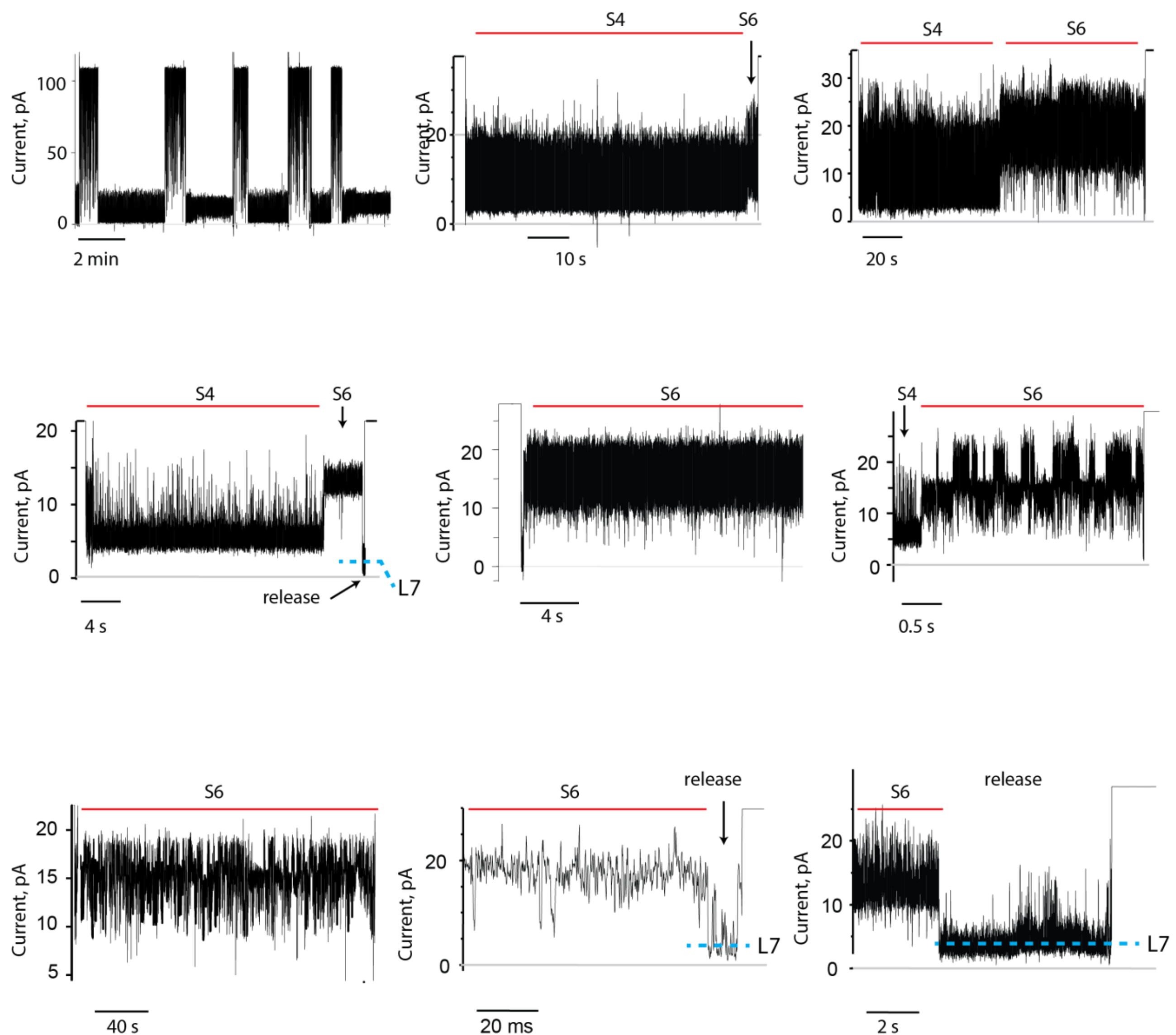

Figure S7. Ternary SR1 current blockades.

Selected examples of ternary complexes ATP:SR1:unfolded protein:αHL-GroES$_L$. DHFR or rhodanase were urea unfolded and incubated with SR1 prior addition to the *cis* chamber. S6 current state showed many different current levels. Such blockades were not observed in the absence of unfolded substrates and could reflect the random motions / interactions of the unfolded protein inside GroEL. The release of the current blockade was most often associated to the visit of a current state L7 (I_B=4.3±0.9 pA, τ=60±29 ms). Traces were recorded in 1 M KCl, 15 mM Tris.HCl, pH 7.5, at 23°C in the presence of 1 mM ATP and 5 mM $MgCl_2$ by applying a 10 kHz low-pass Bessel filter and using a 20 μs

(50 kHz) sampling rate. An additional Gaussian filter at 2 kHz was then applied to current traces.

Additional Tables

Table S1. Percentage of GroEL-LLV ATPase activity in the presence of GroES or αHL constructs. Conditions as in Figure S1. Errors as quoted as S.D.

	GroEL-LLV, GroES (%)	GroE-LLV L, DOC, αHL-$GroES_L$ (%)	GroEL-LLV, DOC, αHL-$GroES_S$ (%)	GroEL-LLV, DOC, αHL (%)
50 mM KCl	107.6±2.7 *n*=3	104.7±12.4 *n*=3	105.2±2.42 *n*=3	110.1±14.9 *n*=3

Table S2. Percentage of GroEL ATPase activity in the presence of GroES, αHL constructs, and/or DOC. Conditions as in Figure2b. Errors as quoted as S.D.

	GroEL, GroES (%)	GroEL, DOC, αHL-$GroES_L$ (%)	GroEL, DOC, αHL-$GroES_S$ (%)	GroEL, DOC, αHL (%)	GroEL, DOC (%)
50 mM KCl	39.3±6.0 *n*=12	57.2±7.1 *n*=10	63.6±0.5 *n*=3	87.3±8.7 *n*=5	98.5±1.6 *n*=3
1 M KCl	43.7±9.7 *n*=7	67.2±10.5 *n*=7	77.8±9.4 *n*=3	97.5±9.3 *n*=4	95.9±3.9 *n*=5

Table S3. MDH refolding yield catalyzed by GroEL in the presence and absence of GroES, αHL constructs, and/or DOC. Conditions as in Figure2c. Errors as quoted as S.D.

	GroEL (%)	GroEL, GroES (%)	GroEL, DOC, αHL-$GroES_L$ (%)	GroEL, DOC, αHL-$GroES_S$ (%)	GroEL, DOC, αHL (%)	GroEL, DOC (%)
50 mM KCl	13.2±2.3 *n*=10	28.6±1.8 *n*=9	32.6±1.7 *n*=6	25.8±0.6 *n*=4	19.1±3.4 *n*=6	15.1±2.1 *n*=8
1 M KCl	14.5±2.8 *n*=6	33.9±3.0 *n*=6	34.8±3.1 *n*=6	30.6±0.7 *n*=6	20.5±3.0 *n*=6	14.3±1.1 *n*=6

Table S4. LDH refolding yield catalyzed by GroEL in the presence and absence of GroES, αHL constructs, and/or DOC. Conditions as in Figure S2.

	GroEL (%)	GroEL, GroES (%)	GroEL, DOC, αHL-$GroES_L$ (%)	GroEL, DOC, αHL-$GroES_S$ (%)	GroEL, DOC, αHL (%)	GroEL, DOC (%)
50 mM KCl	9.3±1.6 *n*=6	22.8±2.5 *n*=6	21.8±2.9 *n*=6	16.2±2.7 *n*=4	11.5±2.1 *n*=4	9.1±1.8 *n*=4