

SUPPLEMENTARY INFORMATION TO

**Signal-sequence-independent SRP-SR complex formation at the membrane suggests an
alternative targeting pathway within the SRP cycle**

David Braig^{*‡}, Miryana Mircheva^{*‡}, Ilie Sachelaru^{*†‡}, Eli O. van der Sluis[§], Lukas Sturm^{*},
Roland Beckmann[§] and Hans-Georg Koch^{*}

^{*}Institut für Biochemie und Molekularbiologie, ZBMZ, and [†]Fakultät für Biologie, Albert-Ludwigs-Universität Freiburg, Stefan-Meier-Strasse 17, 79110 Freiburg, and [§]Gene Center and Center of integrated Protein Science Munich CiPS-M, Dept. for Biochemistry, Ludwigs-Maximilian-Universität Muenchen, 81377 Muenchen, FR Germany

[‡]These authors contributed equally to this study

Correspondence to:

Hans-Georg Koch

Hans-Georg.Koch@biochemie.uni-freiburg.de

Phone 0049-761-2035250

Fax 0049-761-2035289

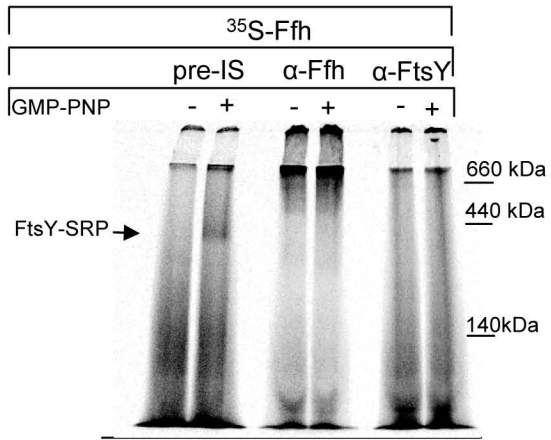
Running head: Targeting by a pre-formed SRP-SR complex

Supplementary Table S1: Sequences of oligonucleotides used for PCR-based mutagenesis and cloning.

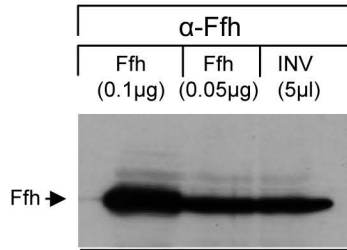
name	sequence
FtsY_fusion_r	5'-gtg gct gaa ttc tgg act tgg act tgg act tgg act gcc atc ctc tgc ggc aaa aag tgc-3'
FtsY_fusion_f	5'-ata cat acc atg gcg aaa gaa aaa aaa cgt ggc-3'
Ffh_fusion_f	5'-agc cac gaa ttc agt cca agt cca agt cca agt cca ggc ttt gat aat tta acc gat cgt-3'
Ffh_fusion_r	5'-gtg gct aag ctt tca gtg gtg gtg gtg gtg ggc acc agg gaa gcc tgg ggg-3'
CM_p15A_pBAD_for	5'-tag aca taa gcg gct att taa cga ccc tgc -3'
CM_p15A_pBAD_rev	5'-gcg cca cag gtg cgg ttg ct-3'
pTrc99a_Amp_for	5'-agc cga acg acc gag cg-3'
pTrc99a_Amp_rev	5'-gga tac ata ttt gaa tgt att tag aaa aat-3'
Ffh_A144W_for	5'-ccg gcg tgg atc aaa cag ctt gag ac-3'
Ffh_A144W_rev	5'-gcg ata aac gtc ggc aga aac ca-3'
FtsY_A336W_for	5'-ttc cgt gca gct tgg gtt gaa cag c-3'
FtsY_A336W_rev	5'-agt atc acc cgc cgc cag cat c-3'
Ffh_YFP_XbaI	5'-gtg tac tct aga agg agt gtg cct tga tgt ttg at-3'
Ffh_YFP_pBad33_rev	5'-aaa tct tct ctc atc cgc caa aac-3'
GFP_EcoRI_for	5'-tag cca cga att cct atg cgg ccg cag taa agg aga a-3'
GFP_EcoRI_rev	5'-tgg cat tga att ctt tgt ata gtt cat cca tgc cat gtg taa tcc c-3'
Dav_Hf	5'-tta aaa acc aaa gaa aat ctc gg ttcc-3'
Dav_Hr	5'-acc ttc ttt ggt cgg ttt ttc ctg-3'
K198_Dav_f	5'-ttt ttc gcg aag ctg aaa cgc-3'
D198_Dav_f	5'-ttt ttc gcg gac ctg aaa cgc-3'
A198_Dav_f	5'-ttt ttc gcg gca ctg aaa cgc-3'
R198D+K200D_f_Dav	5'-gcg gac ctg gac cgc agc ctg-3'
Fw-NcoI-TatC	5'- gcg ccc atg gct gta gaa gat act caa ccg c-3'
Rev-NcoI-TatC	5'- gcg ccc atg ggt tct tca gtt ttt tgc ctt tc-3'

Legend to Supplementary Figure S1: The 400 kDa complex is also observed with in ³⁵S-Ffh and recognized by both α -Ffh and α -FtsY antibodies. (A) *In vitro* synthesized Ffh was incubated with inner membrane vesicles in the presence or absence of the non-hydrolysable GTP-analogue GMP-PNP. After solubilisation with dodecyl maltoside, the samples were treated either with pre-immune serum (pre-IS) or with antibodies against Ffh (α -Ffh) or FtsY (α -FtsY) and separated on a 5-10% BN-PAGE gel. Note that Ffh in contrast to FtsY (*c.f.* Fig. 1) does not show a distinct band on BN-PAGE. (B) Determination of the SRP content in INV. Different amounts of purified Ffh and 5 μ l INV were TCA-precipitated and separated on a 13% SDS-PAGE before western blotting and immune detection using α -Ffh antibodies.

A



B



(Braig et al., Fig. S1)