Two SRP signal sequences are present in the amino-terminal domain of the C-tailed protein SciP

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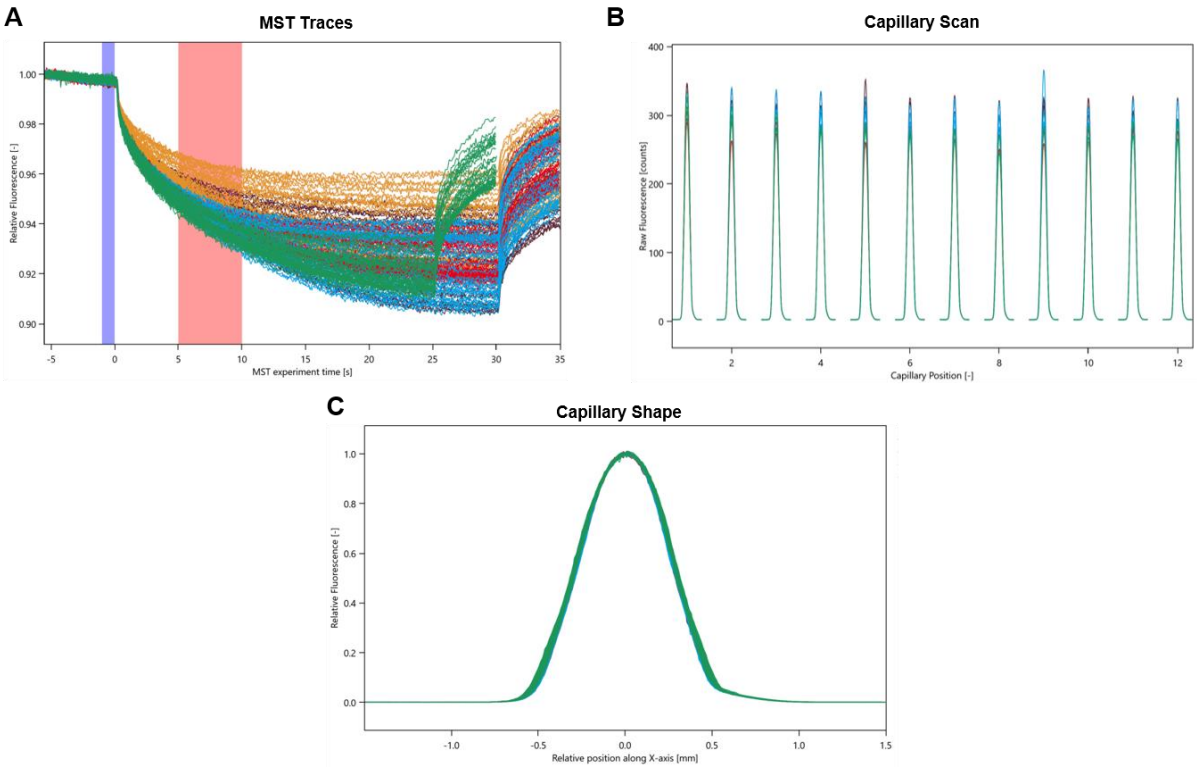
Supplementary Information

Figure S1



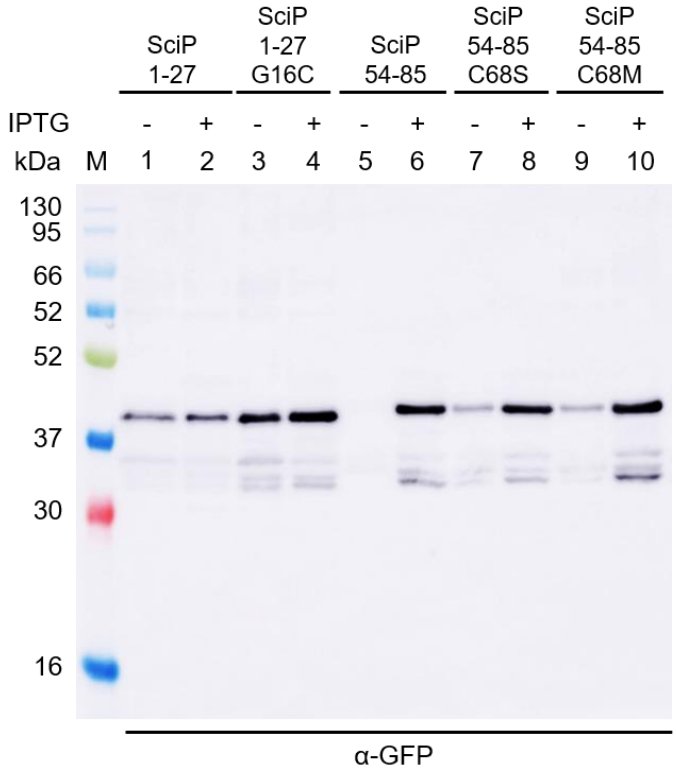
Supplementary Figure S1: Raw data of the MST measurements with FtsQ4-85, SciP2-54, SciP54-100, SciP54-100 C68S and Luc2-50 as a nascent chain with SRP.

Microscale thermophoresis measurements of unlabelled SRP (1 µM to 0.49 nM) with labelled RNCs (5 nM) of FtsQ4-85 (blue), SciP2-54 (brown), SciP54-100 (orange), SciP54-100 C68S (red) and Luc2-50 (green). The samples were incubated for 5 min on ice and filled into Premium capillaries (NanoTemper Technologies) for the MST measurements. In A, the MST traces (relative fluorescence [-] are plotted against the MST experiment [s]), in B, the Capillary Scan (raw fluorescence [counts] is plotted against the capillary position [-]) and in C, the Capillary Shape (relative fluorescence [-] is plotted against the relative position along x-axis [mm].

Supplementary Table S1: Dataset overview of the MST measurements with FtsQ4-85, SciP2-54, SciP54-100, SciP54-100 C68S and Luc2-50 as a nascent chain with SRP.

Name	FtsQ4-85 vs SRP	SciP2-54	SciP54-100	SciP54-100 C68S	Luc2-50
Graph Color:	●	●	●	●	●
Target Concentration:	5 nM	5 nM	5 nM	5 nM	5 nM
Ligand Concentration:	1E+03 to 0.488 nM	1E+03 to 0.488 nM	1E+03 to 0.488 nM	1E+03 to 0.488 nM	1E+03 to 0.488 nM
n:	3	3	3	3	3
Excitation Power:	50%	50%	50%	50%	40%
MST Power:	20%	20%	20%	20%	20%
Temperature:	22.0°C	22.0°C	22.0°C	22.0°C	22.0°C
Kd:	30.552 nM	49.716 nM	56.984 nM	112.07 nM	22[Fixed]
Kd Confidence:	± 5.9552 nM	± 12.034 nM	± 7.6201 nM	± 33.187 nM	
Response Amplitude:	9.6515643	13.166952	16.78356	4.3734844	0
Target Conc.:	5 nM [Fixed]	5 nM [Fixed]	5 nM [Fixed]	5 nM [Fixed]	5 nM [Fixed]
Unbound:	941.45	944.15	950.7	946.1	942.9[Fixed]
Bound:	951.1	957.32	967.49	950.47	942.9[Fixed]
Std. Error of Regression:	0.49673909	0.82411441	0.57370651	0.29939916	1.0688193
Reduced χ^2:	3.5214633	1.2164738	9.1310442	0.71247396	10.606039
Signal to Noise:	21.480504	17.663341	32.342187	16.14924	0

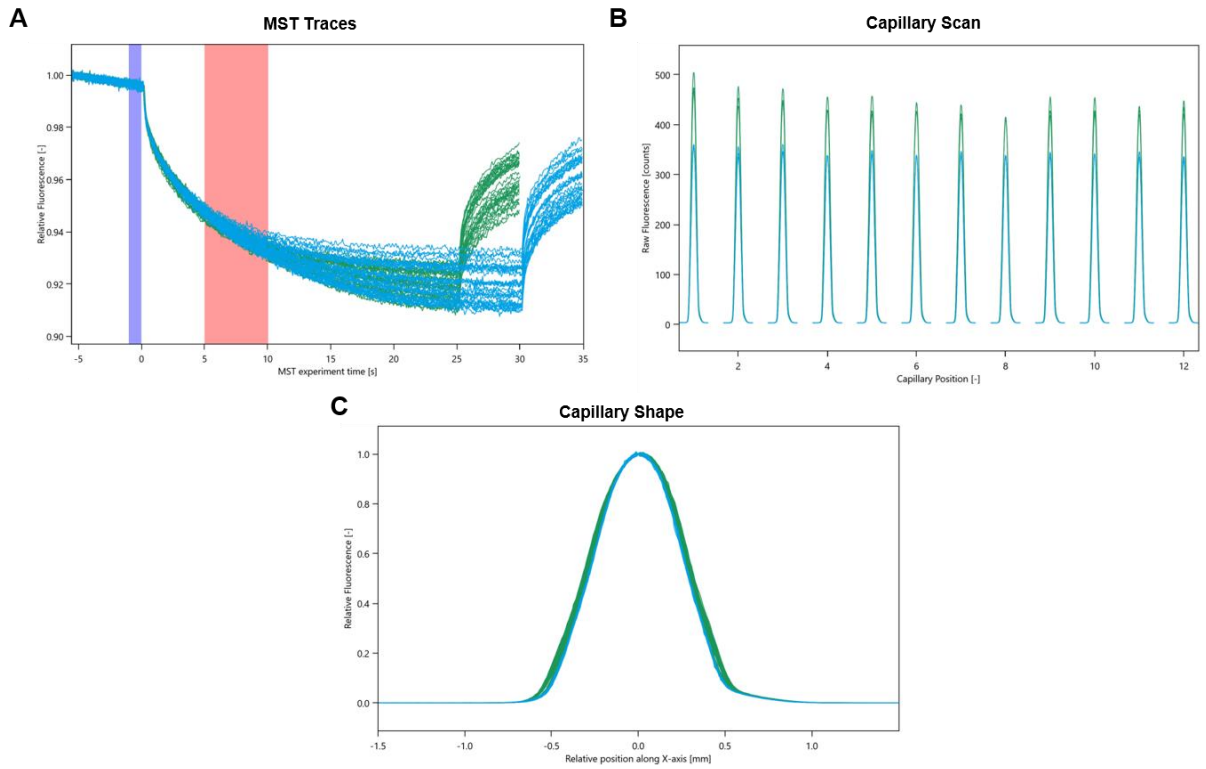
Figure S2



Supplementary Figure S2: Expression of the different SciP-sfGFP fusion proteins in *E. coli* MC4100

The sequences encoding the peptides 1-27 or 54-85 of SciP and their variants G16C, C68S and C68M were fused to the N-terminus of sfGFP extended with residues 96-217 of SciP at the C-terminus. The expression was induced with 1 mM IPTG at an OD_{600} of 0.5 for 1h at 30°C. The cells were TCA precipitated and loaded on a 12% SDS-PAGE. After Western transfer, immunodetection was carried out with α-GFP and α-rabbit antibodies. After induction a band between 37 and 52 kDa was detected, corresponding to the respective SciP-sfGFP fusion protein.

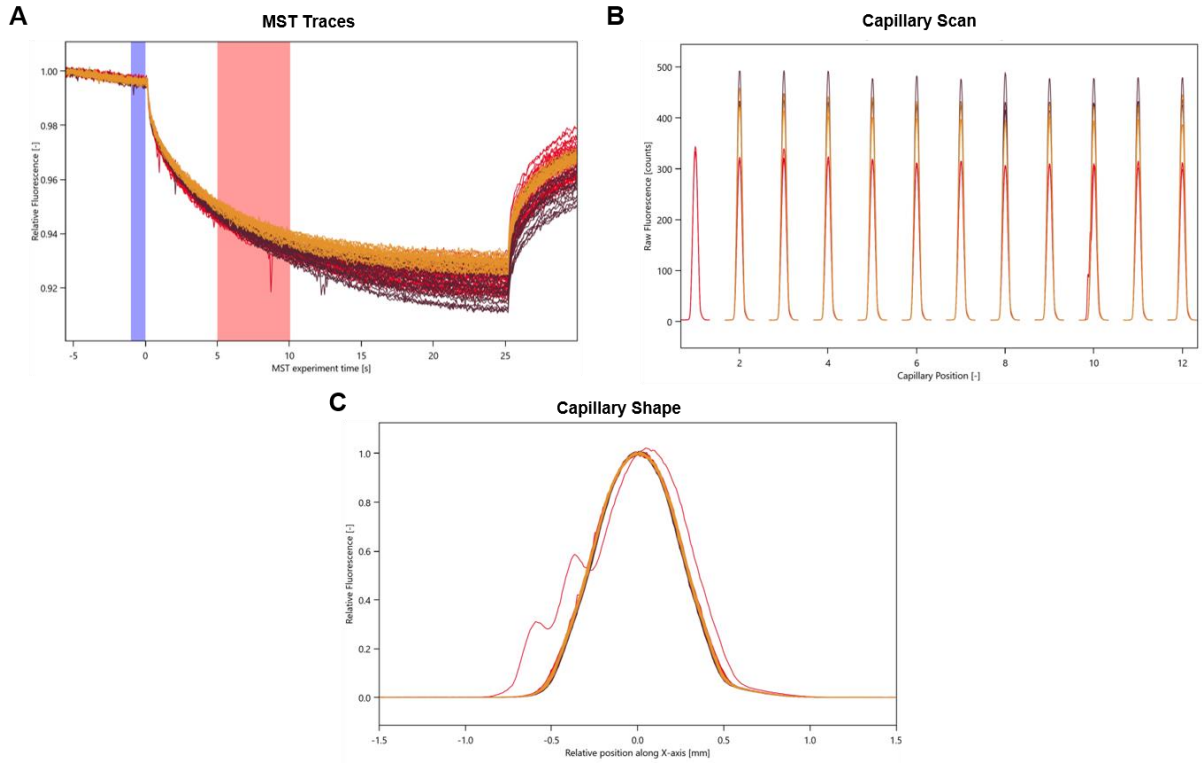
Figure S3



Supplementary Figure S3: Raw data of the MST measurements with FtsQ4-85 and Luc2-50 as a nascent chain with SRP-FtsY.

Microscale thermophoresis measurements of unlabelled SRP-FtsY (500 / 250 nM to 0.244 / 0.122 nM) with labelled RNCs (5 nM) of FtsQ4-85 (blue) and Luc2-50 (green). The samples were incubated for 5 min on ice and filled into Premium capillaries (NanoTemper Technologies) for the MST measurements. In A, the MST traces (relative fluorescence [-] are plotted against the MST experiment [s]), in B, the Capillary Scan (raw fluorescence [counts] is plotted against the capillary position [-]) and in C, the Capillary Shape (relative fluorescence [-]) is plotted against the relative position along x-axis [mm].

Figure S4



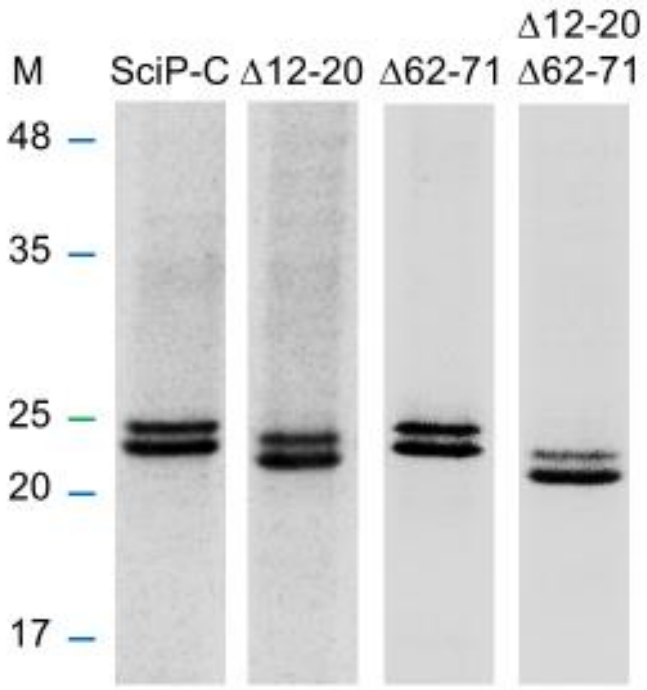
Supplementary Figure S4: Raw data of the MST measurements with SciP2-54, SciP54-100 and SciP54-100 C68S as a nascent chain with SRP-FtsY.

Microscale thermophoresis measurements of unlabelled SRP-FtsY (500 / 250 nM to 0.244) with labelled RNCs (5 nM) of SciP2-54 (brown), SciP54-100 (orange) and SciP54-100 C68S (red). The samples were incubated for 5 min on ice and filled into Premium capillaries (NanoTemper Technologies) for the MST measurements. In A, the MST traces (relative fluorescence [-] are plotted against the MST experiment [s]), in B, the Capillary Scan (raw fluorescence [counts] is plotted against the capillary position [-]) and in C, the Capillary Shape (relative fluorescence [-] is plotted against the relative position along x-axis [mm].

Supplementary Table S2: Dataset overview of the MST measurements with FtsQ4-85, SciP2-54, SciP54-100, SciP54-100 C68S and Luc2-50 as a nascent chain with SRP-FtsY.

Name	FtsQ4-85 vs SRP	SciP2-54	SciP54-100	SciP54-100 C68S	Luc2-50
Graph Color:	●	●	●	●	●
Target Concentration:	5 nM	5 nM	5 nM	5 nM	5 nM
Ligand Concentration:	250 to 0.122 nM	250 to 0.244 nM	250 to 0.244 nM	500 to 0.244 nM	500 to 0.244 nM
n:	3	3	3	3	3
Excitation Power:	60%	60%	60%	60%	60%
MST Power:	20%	20%	20%	20%	20%
Temperature:	22.0°C	22.0°C	22.0°C	22.0°C	22.0°C
Kd:	22.932 nM	0.79589 nM	10.763 nM	11[Fixed]	7.8[Fixed]
Kd Confidence:	± 10.75 nM	± 1.1838 nM	± 3.5898 nM		
Response Amplitude:	4.8508007	3.0604397	2.3097048	0	0
Target Conc.:	5 nM [Fixed]	5 nM [Fixed]	5 nM [Fixed]	5 nM [Fixed]	5 nM [Fixed]
Unbound:	941.56	942.08	946.23	942.68[Fixed]	942.59[Fixed]
Bound:	946.41	945.14	948.54	942.68[Fixed]	942.59[Fixed]
Std. Error of Regression:	0.50948147	0.61162167	0.174534	0.60178988	0.46206252
Reduced χ^2:	6.8864189	3.9633787	0.9442135	0.71951065	2.5531429
Signal to Noise:	10.525922	5.5944316	14.795562	0	0

Figure S5



Supplementary Figure S5: Cysteine accessibility assay of SciP-C and the mutant proteins with AMS. The C-tail of the SciP variants with deletions of one or both hydrophobic regions was extended by a cysteine residue at position 218 and expressed in *E. coli* MC4100 cells. The expression was induced with 1 mM IPTG for 10 min, 2.5 mM AMS was added 1 min prior to pulse labeling with ^{35}S-methionine / cysteine (15-30 µCi/ml culture) for 2 min. The cells were chased for 10 min with non-labeled methionine / cysteine, immunoprecipitated against the N-terminal His_{10}-Tag and analyzed on a 14% SDS-PAGE. The modification of the cysteine residue at position 218 leads to a mobility shift if its exposed in the periplasm.