Supplementary material for

Outer membrane targeting of secretin PulD relies on disordered domain recognition by a dedicated chaperone

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Running head: Dedicated chaperone binds unstructured domain

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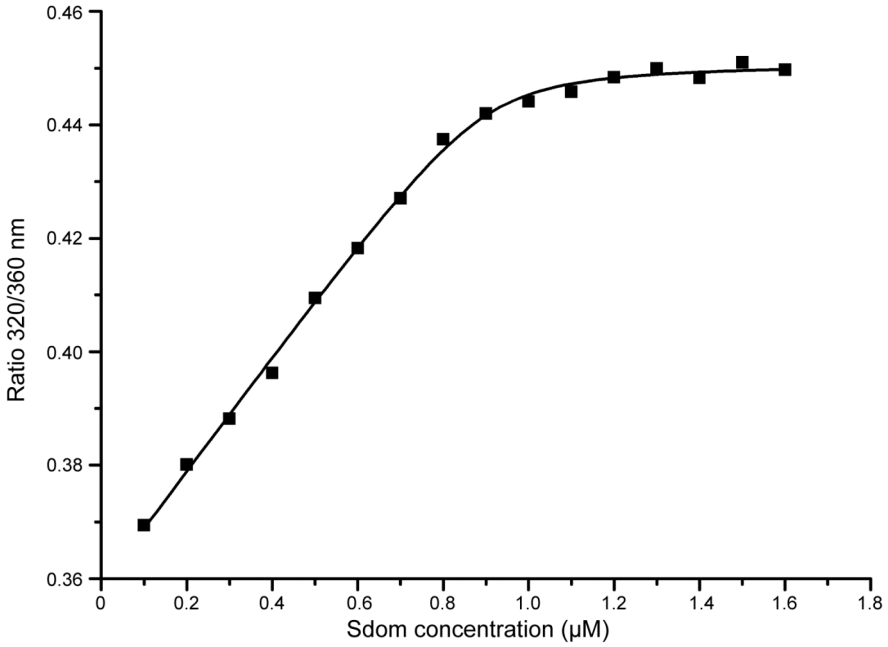
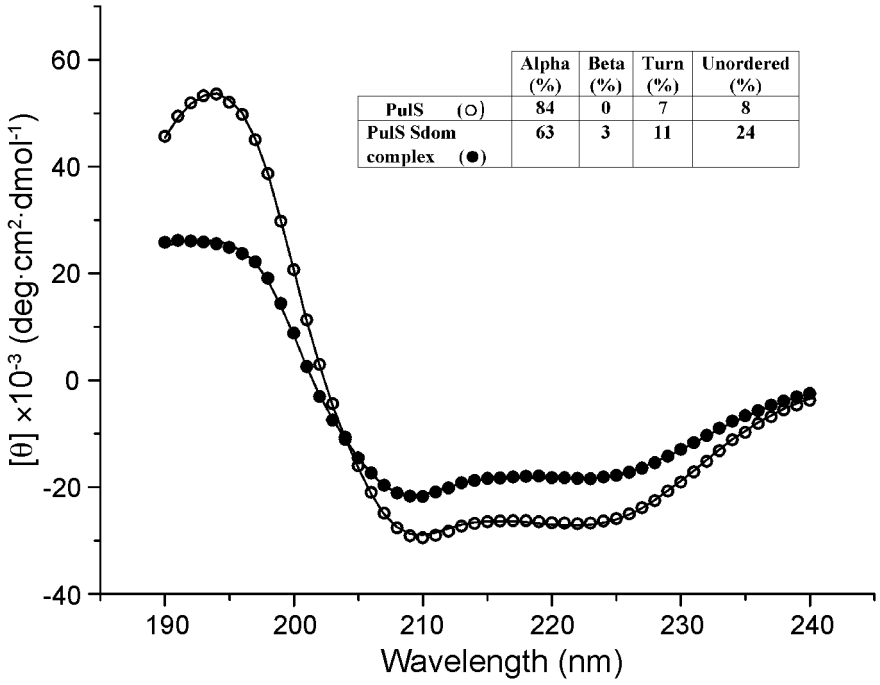
Supplementary Figure S1. The shift in the intrinsic tryptophan fluorescence spectra of 1 μM PulS (sample excited at 295 nm and emission recorded between 310-400 nm) as a function of Sdom titration shows a clear shift in fluorescence until a molar ratio of 1:1 is reached. The shift in tryptophan fluorescence measured by the ratio of 320/360 nm and plotted versus Sdom concentration gave an affinity of 12.6 nM ± 6 nM.

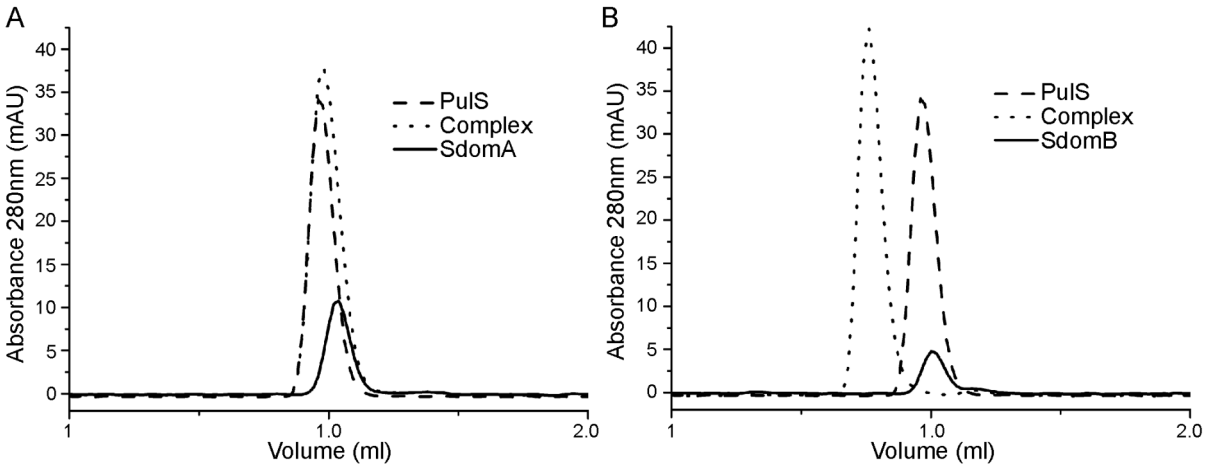


Supplementary Figure S2. PulS and the PulS Sdom complex are mainly α-helical. Far-UV CD spectra and fitted curves of 100 μM PulS (open circles) and the 100 μM PulS Sdom complex (solid circles) from 190-240 nm. The inset shows the relative secondary structure predicted from the deconvolution using the CDSSTR routine of the DICHROWEB server run on the SP175 reference dataset.

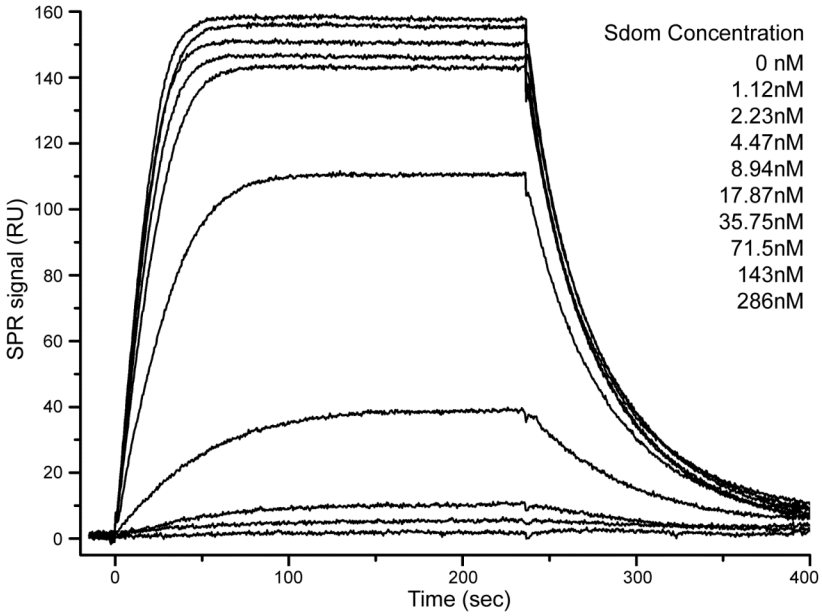


	Alpha (%)	Beta (%)	Turn (%)	Unordered (%)
PulS (○)	84	0	7	8
PulS Sdom complex (●)	63	3	11	24

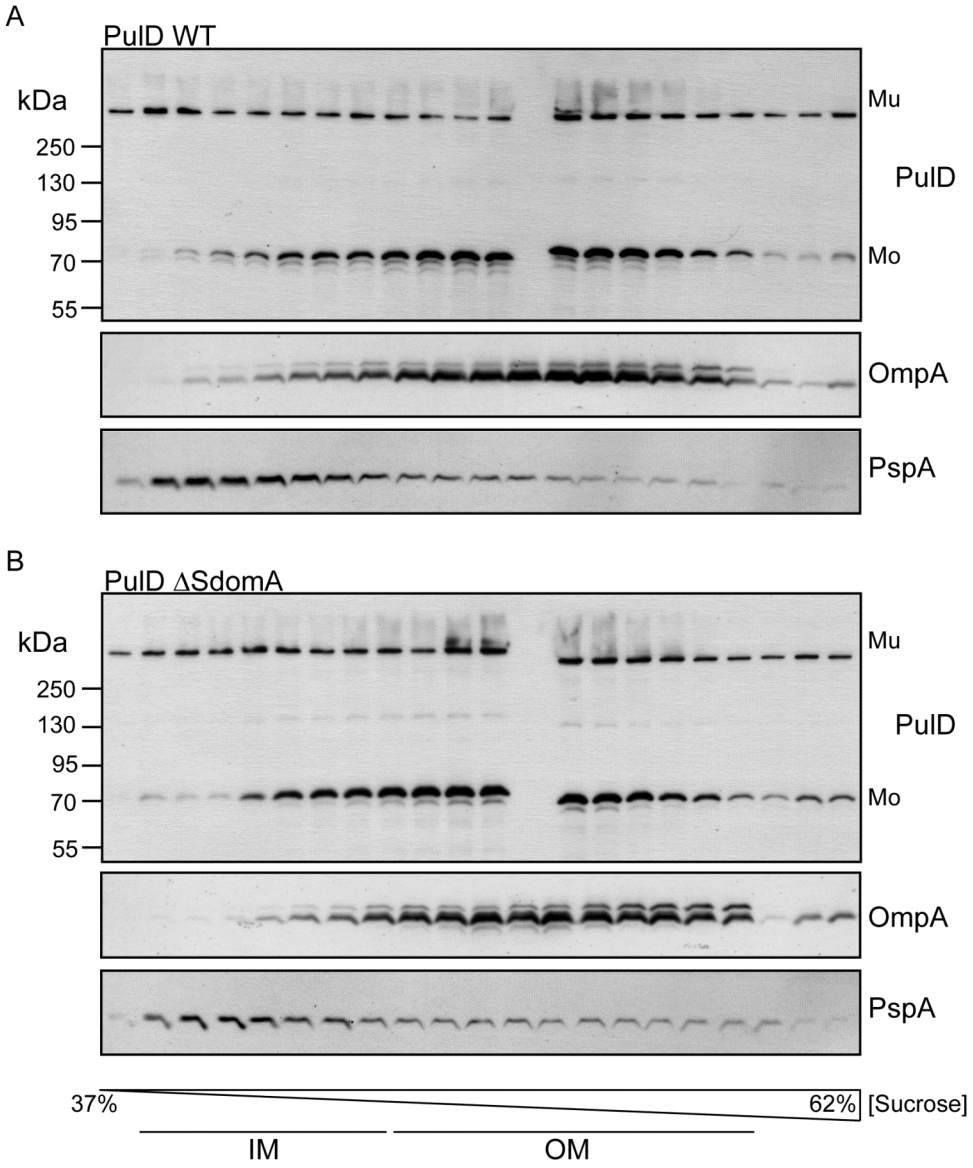
Supplementary Figure S3. Formation of complexes between PulS and SdomA or SdomB peptides. Size exclusion chromatography elution profiles with SdomA (**A**) or SdomB (**B**) on Superdex 75 column. Elution of 20 μl of loaded sample corresponding to 40 μM of protein was monitored at A_{280} nm.



Supplementary Figure S4. Real-time SPR sensorgrams of the PulS-Sdom interaction. Representative sensorgrams of the real time association and dissociation profiles corresponding to the injection of PulS +/- competitor over the surface-immobilized MalE antibody loaded with MalE-Sdom. SPR signal (RU) is plotted against time for the indicated concentrations of competitor (1.12 nM – 286 nM, Sdom).



Supplementary Figure S5. PulD ΔSdomA is localized in the outer membrane. Membranes from strains producing PulD **(A)** and PulD ΔSdomA **(B)** in the presence of PulS were separated by floatation in a sucrose gradient (1). Fractions were analyzed by SDS-PAGE and immunoblotting with PulD-, OmpA- (outer membrane marker) and PspA- (inner membrane marker) specific antibodies. Mu, PulD multimer; Mo, PulD monomer.



1. Robichon, C., Vidal-Ingigliardi, D., and Pugsley, A. P. (2005) *J. Biol. Chem.* **280**, 974-983

Supplementary Table 1. Primer Sequences

Name	Sequence[a] (5′-3′)	Restriction site
PNN19	ccctacgtaCAGCAAAATCGCCCGACAAC	SnaBI
PNN12	cccaagcttCTATTTATTGTCGCGCAGGC	HindIII
PNN28	cgcggatccCGCCCGACGGTGATCCGC	BamHI
PNN29	cccaagcttTCATAGATTGCCTCCCAGATTG	HindII
PNN55	ccggaattcCTGGTGCCGCGCGGCAGCATCCGCGACCGCGACGAG	EcoRI
PNN56	cccaagcttTCACAGATTGAACGCGTCGATAGC	HindIII
PNN57	ccggaattcCTGGTGCCGCGCGGCAGCAATCAGGATCTGCTGGAGATC	EcoRI
PNN58	cccaagcttTCACAGCATCGCGTCGTTGTTCTC	HindIII
PNN67	GGTGATCCGCGACCGCGACAATCAGGATCTGCTGGAGATCTAC	
PNN68	GTAGATCTCCAGCAGATCCTGATTGTCGCGGTCGCGGATCACC	
PNN69	GGTGATCCGCGACCGCGACgagaattcgagctcgaacaacaacaacaataacaat-aacaacaacctcgggatcgagggaaggatttcagaattcggatcctctagagtcAATCAGGA-TCTGCTGGAGATCTAC	
PNN70	GTAGATCTCCAGCAGATCCTGATTgactctagaggatccgaattctgaaatcct-tccctcgatcccgaggttgttgttattgttattgttgttgttcgagctcgaattctcGTCGCGGT-CGCGGATCACC	

[a] Lower case letters correspond to either the sequence of restriction enzymes or the linker region from pMal-c2 vector and upper case letters denote sequence of target gene.

Supplementary Table 2. Peptides identified by mass spectrometry

Experimental, m/z (Da)	Charged State	Theoretical MW (Da)	Standard Deviation (%)	His-Sdom[a] Start	His-Sdom[a] End	PulD[b] Start	PulD[b] End	Modification	Sequence
Sdom Digestion									
Peptides identified without modifications									
2298.186	3	6886.424	0.0742	18	77	595	654	-	RDRDEYRQASSG....AFRQVSAAIDAF
2952.103	3	8851.618	0.0188	1	77	590	654	-	MRGSHHHHHHGS....AFRQVSAAIDAF
3108.380	2	6210.685	0.0653	18	70	595	647	-	RDRDEYRQASSG....YPRQDTAAFRQV
3167.821	2	6323.844	0.1544	17	70	594	647	-	IRDRDEYRQASS....YPRQDTAAFRQV
3801.562	1	3795.118	0.1430	35	67	612	644	-	NDAQSKQRGKEN....LEIYPRQDTAAF
3887.156	2	7792.428	0.2589	1	67	590	644	-	MRGSHHHHHHGS....LEIYPRQDTAAF
4055.160	1	4064.338	0.2512	17	51	594	628	-	IRDRDEYRQASS....KQRGKENNDAML
6326.702	1	6323.844	0.0292	17	70	594	647	-	IRDRDEYRQASS....YPRQDTAAFRQV
6383.491	1	6386.855	0.0685	1	55	590	650	-	MRGSHHHHHHGS....KENNDAMLNQDL
Peptides identified with modifications									
3816.642	1	3795.118	0.0648	35	67	612	644	Sodium	NDAQSKQRGKEN....LEIYPRQDTAAF
3872.044	1	3795.118	0.0517	35	67	612	644	Potassium	NDAQSKQRGKEN....LEIYPRQDTAAF
3929.623	2	7792.428	0.1669	1	67	590	644	Potassium	MRGSHHHHHHGS....LEIYPRQDTAAF
3973.111	1	3951.178	0.0520	18	51	595	628	Sodium	RDRDEYRQASSG....KQRGKENNDAML
4231.715	1	4178.569	0.1455	35	70	612	647	Sodium	NDAQSKQRGKEN....YPRQDTAAFRQV
4302.385	2	8518.274	0.0763	1	74	590	651	Potassium	MRGSHHHHHHGS....DTAAFRQVSAAI
5895.253	1	5827.234	0.1850	18	67	595	644	Potassium	RDRDEYRQASSG....LEIYPRQDTAAF
7649.621	1	7568.214	0.0324	17	83	594	660	Potassium	IRDRDEYRQASS....AAIDAFNLGGNL
Sdom-PulS complex digestion									
Peptides identified without modifications									
2144.874	1	2149.434	0.2596	56	74	633	651	-	LEIYPRQDTAAFRQVSAAI
2182.752	3	6553.080	0.1198	18	74	595	651	-	RDRDEYRQASSG....DTAAFRQVSAAI
3170.020	2	6323.844	0.2237	17	70	594	647	-	IRDRDEYRQASS....YPRQDTAAFRQV
3800.958	1	3795.118	0.1271	35	67	612	644	-	NDAQSKQRGKEN....LEIYPRQDTAAF
4054.650	1	4064.338	0.2638	17	51	594	628	-	IRDRDEYRQASS....KQRGKENNDAML
Peptides identified with modifications									
2175.131	3	6500.014	0.0097	1	56	590	633	Sodium	MRGSHHHHHHGS....ENNDAMLNQDLL
3640.063	2	7226.847	0.0726	17	79	594	656	Sodium	IRDRDEYRQASS....RQVSAAIDAFNL
3871.206	1	3795.118	0.0733	35	67	612	644	Potassium	NDAQSKQRGKEN....LEIYPRQDTAAF
7669.001	1	7568.214	0.2850	17	83	594	660	Potassium	IRDRDEYRQASS....AAIDAFNLGGNL

Samples were desalted on C18-µZipTip (Millipore) and eluted directly onto a 96-well stainless steel MALDI target plate (ABSCIEX) with 0.5 µl of CHCA matrix (2.5 mg/ml in 70% CAN / 30 % H_2O / 0.1 % TFA). MS spectra were acquired on the 4800 Proteomics Analyzer (ABSCIEX, USA) using positive-ion linear mode, calibrated using the calMix1 kit of LaserBio Labs (bovine insulin $[M+H]^+$ = 5734.8; horse heart cytochrome C $[M+H]^+$ = 12361; horse myoglobin $[M+H]^+$ = 16952.5). Sample mass spectra were acquired using a mass range window of 2000-12000 m/z and an average of 3000 laser shots per spectrum. Mass search against undigested protein sequence was performed using GPMAW 9.0 software (Lighthouse, Odense, Denmark) with the following parameters: thermolysin, average mass ($[M+H]^+$), mass tolerance of 0.05 %, possibility of multi-charged ions and Na^+ and K^+ adducts.

[a] Amino acid residue corresponding to His-tagged Sdom peptide identified by mass spectrometry.

[b] Corresponding amino acid residue of PulD.