

Supplementary Information for:

**ATP-independent control of autotransporter virulence protein transport  
via the folding properties of the secreted protein**

Jonathan P. Renn<sup>†‡</sup>, Mirco Junker<sup>†‡</sup>, Richard N. Besingi,  
Esther Braselmann & Patricia L. Clark<sup>\*†</sup>

Department of Chemistry & Biochemistry and  
<sup>†</sup>Eck Institute for Global Health  
University of Notre Dame, Notre Dame IN 46556-5670 USA

*\*To whom correspondence should be addressed:*

pclark1@nd.edu  
(574)631-8353 [phone]  
(574)631-6652 [fax]

*†These authors contributed equally to this work.*

*‡Current addresses:*

JPR: Department of Molecular Biosciences, Northwestern University, Evanston, IL 60208  
MJ: Department of Cell Biology, Harvard Medical School, Boston, MA 02115

Running title: Folding properties control virulence protein secretion

## **Inventory of Supplementary Information**

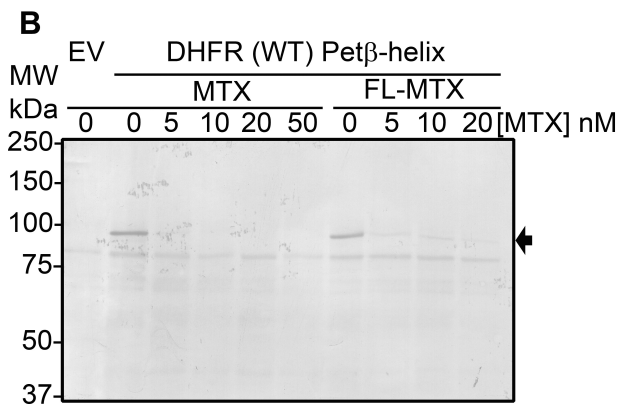
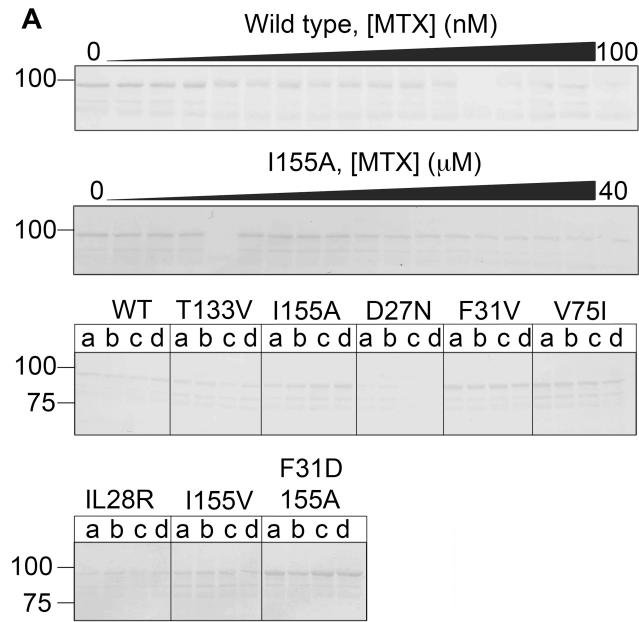
1. Supplementary Figures and Table

Figure S1: Related to Figure 2

Figure S2: Related to Figure 3

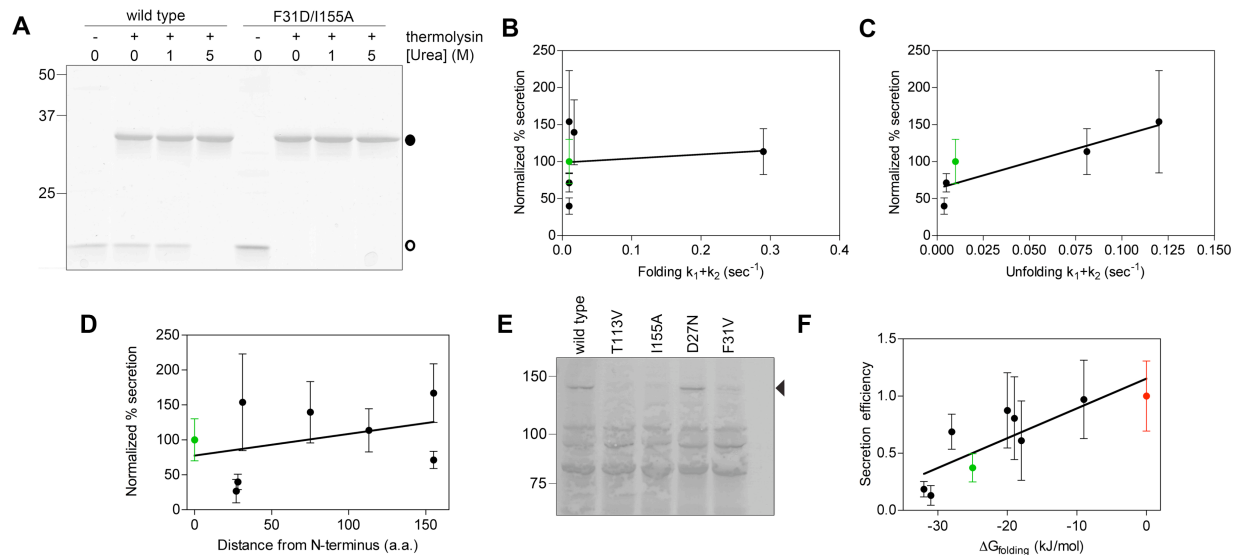
Table 1: Related to Figure 1

2. Supplementary Experimental Procedures



**Supplementary Figure 1:** DFHR stability affects OM secretion efficiency of DFHR-Pet $\beta$ -helix.

**(A) (top)** Anti-Pet western blots of spent culture media from cells expressing DHFR-Pet $\beta$ -helix constructs in the presence of increasing concentrations of MTX, and **(bottom)** anti-Pet western blots of spent culture media from cells expressing DHFR-Pet $\beta$ -helix mutants (four replicate trials (labeled a-d) are shown). **(B)** MTX-FL blocks release of the processed chimera passenger into culture media in a manner analogous to MTX. Numbers on the left correspond to molecular weight in kDa. 'WT' corresponds to DHFR-Pet $\beta$ -helix bearing the wild type *E. coli* DHFR sequence.



**Supplementary Figure 2:** Control experiments supporting the correlation between low stability in the globular N-terminal domain of DHFR-Pet $\beta$ -helix and high OM secretion efficiency. **(A)** F31D/I155A DHFR is not stably folded in buffer ( $\Delta G_{\text{folding}} > 0$ ). Pulse proteolysis of purified wild type and F31D/I155A DHFR. Samples were equilibrated in 0, 1 or 5 M urea and treated with 0.2 mg/ml thermolysin for 1 min. Samples were separated on a 16% SDS-polyacrylamide gel, and silver stained. *Open circle*, full length DHFR; *closed circle*, thermolysin. Numbers on the left correspond to the position of molecular weight markers, in kDa. **(B-D)** Results of attempts to correlate DHFR-Pet $\beta$ -helix secretion with other DHFR structural and folding properties. Relationships between OM secretion efficiency and **(B)**, kinetics for the rate limiting step of DHFR folding or **(C)** unfolding, or **(D)**, the distance of the point mutation site from the N-terminus of DHFR (number of residues). The best linear fit (line) results in  $R = 0.1, 0.68,$  and  $0.3,$  respectively. Error bars represent the standard deviation of at least 4 replicates. Wild type

DHFR is shown in green. **(E-F)** Accumulation of DHFR-Pet $\beta$ -helix pre-protein in cells bearing wild type and DHFR mutants, but not less stable mutants. **(E)** Anti-Pet western blots of whole cell lysates from cell expressing the DHFR-Pet $\beta$ -helix chimera bearing the wild type DHFR sequence, or T113V, I155A, D27N, or F31V point mutations. D27N is more stable than wild type DHFR, while T113V, I155A, and F31V are less stable. The filled arrowhead corresponds to the location of the DHFR-Pet $\beta$ -helix unprocessed precursor. Numbers on the left correspond to molecular weight in kDa. **(F)** The correlation between passenger domain N-terminal stability and overall secretion efficiency, calculated as [amount secreted (as determined in Fig. 3)]/[total protein detected in media and cells (amount secreted plus amount of protein detected in whole cell lysates (see (E))]. The green symbol corresponds to the chimera bearing wild type DHFR, the blue symbol corresponds to the F31D/I155A chimera. The best linear fit (line) resulted in  $R = 0.83$ .

**Supplementary Table 1: Folding and stability properties of DFHR mutations.**

	$\Delta G_{H2O}$ (kJ/mol)	Refolding rates		Unfolding rates		Reference
		$k_2$ (sec <sup>-1</sup> )	$k_1$ (sec <sup>-1</sup> )	$k_2$ (sec <sup>-1</sup> )	$k_1$ (sec <sup>-1</sup> )	
wild type	-25	0.027	0.006	0.008	0.002	<i>a</i>
D27N	-31	0.027	0.006	0.004	0.001	<i>b</i>
I155A	-9	0.026	N/D	N/D	N/D	<i>c</i>
T113V	-20	0.270	0.024	0.08	0.010	<i>b</i>
V75I	-19	0.011	0.006	N/D	N/D	<i>d</i>
L28R	-32	0.027	0.006	0.0027	0.00081	<i>b</i>
F31V	-18	0.027	0.006	0.10	0.019	<i>b</i>
I155V	-28	0.027	N/D	N/D	N/D	<i>c</i>
F31D/I155A	>0	N/D	N/D	N/D	N/D	this study

*a* Jennings et al., 1993.

*b* Perry et al., 1987.

*c* Arai et al., 2003.

*d* Garvey et al., 1989.

## **Supplementary Experimental Procedures**

### **Preparation of DHFR F31D/I155A**

F31D and I155A mutations were introduced into pTZ-DHFR plasmid by site-directed mutagenesis. Wild type and F31D/I155A DHFR were expressed in *E. coli* BL21(DE3)pLysS. Typically an overnight culture was prepared in LB supplemented with 100 µg/mL ampicillin. One hundred milliliters of the overnight culture was centrifuged and resuspended in 20 mL 10 mM Tris pH 8.0. Cells were lysed by 3 freeze thaw cycles, followed by sonication. The sonication lysates were centrifuged for 10 min at 20,000xg. Wild type DHFR was found primarily in the soluble fraction, while the mutant F31D/I155A was found in inclusion bodies.

### **DHFR F31D/I155A refolding and pulse proteolysis**

DHFR F31D/I155A inclusion bodies were resuspended in 6 M urea. Inclusion bodies were refolded in buffer to a final concentration of 0.09 M urea. Pulse proteolysis was performed as described (see ref. 32 in the main text). Briefly, wild type and F31D/I155A DHFR were equilibrated in 0, 1 or 5 M urea for 10 min before treatment with thermolysin (Sigma). A 10 mg/mL thermolysin stock solution was prepared in 10 mM Tris pH 8.0, 2.5 M NaCl, 10 mM CaCl<sub>2</sub>. This stock solution was added to each DHFR sample to a final concentration of 0.2 mg/mL thermolysin. After 1 min, the reaction was quenched by addition of EDTA (final concentration 6.7 mM).