

Supporting Information for

Structure-function correlates of fibrinogen binding by *Acinetobacter* adhesins critical in catheter associated urinary tract infections

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Figures S1 to S5

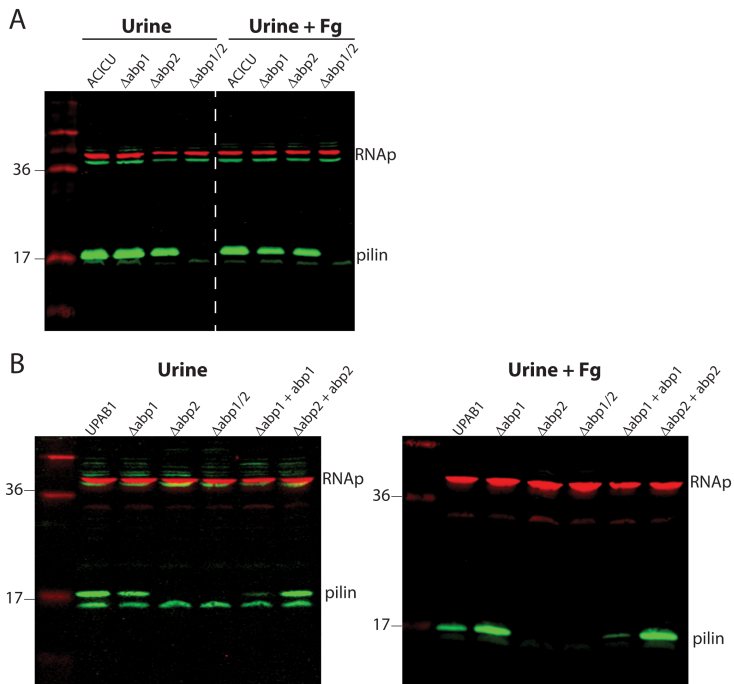


Fig S1: UPAB1 and ACICU expression of Abp1 and Abp2 pili. A) Western blot against the pilin domains of Abp1 and Abp2 pili for WT and mutants of A) ACICU in urine and urine with fibrinogen and B) UPAB1 in urine and urine with fibrinogen.

Percent Identities of CUP adhesin RBDs

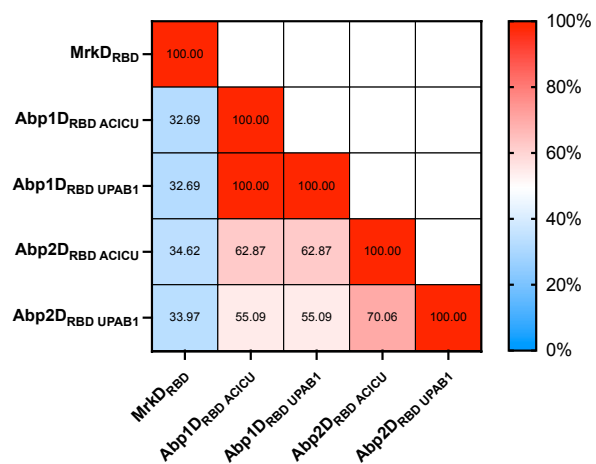


Fig S2: Primary amino acid sequence of various receptor binding domain alleles. Percent identity determined by Clustal Omega from sequences aligned in Figure 3A. MrkD sequence from PDB: 3U4K.

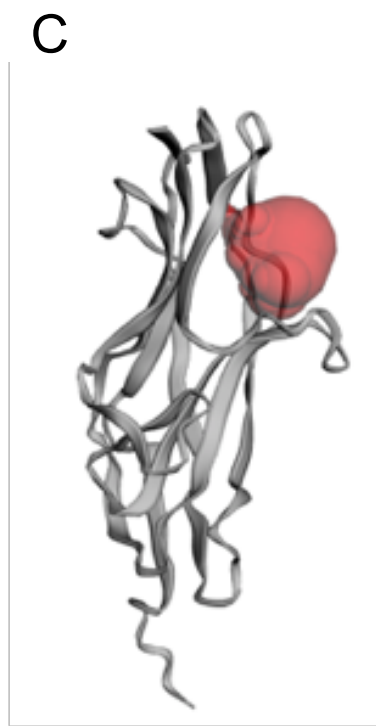
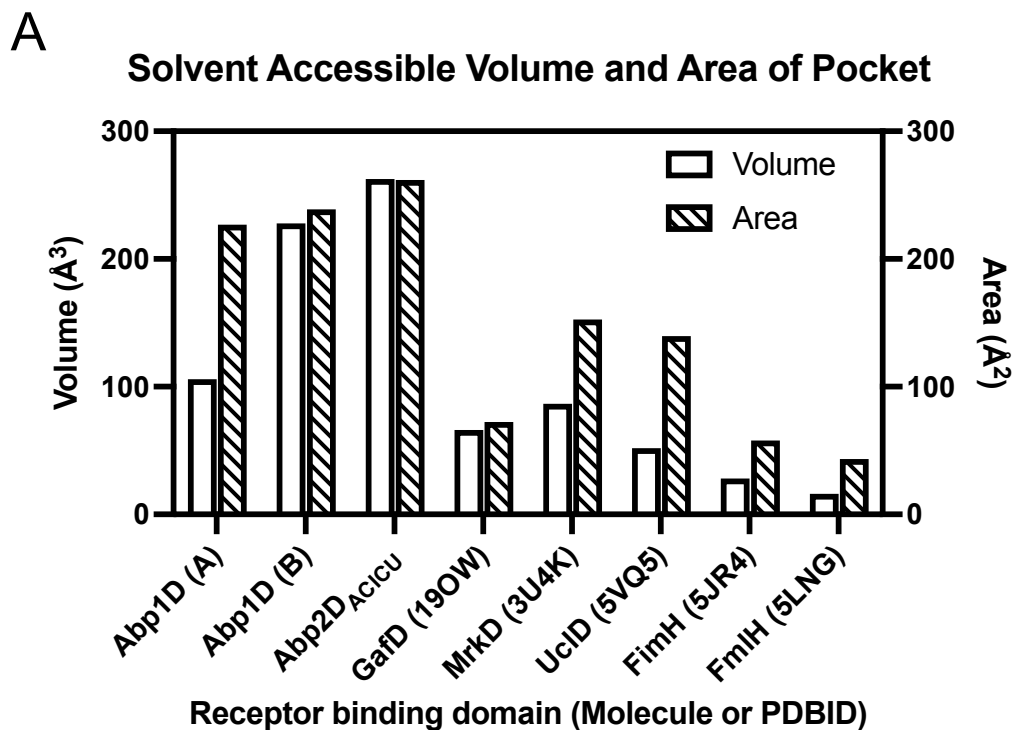


Fig S3: Binding Pocket Volume of $\gamma 4$ and $\gamma 1$ CUP adhesin lectin/receptor binding domains. *In silico* calculation by CASTp. A) Quantification of solvent accessible volume and surface area. Visual representation of binding pocket volume in Red of B) two molecules of Abp1D_{RBD} (Molecules A and B from left to right) and C) Abp2D_{RBD}.

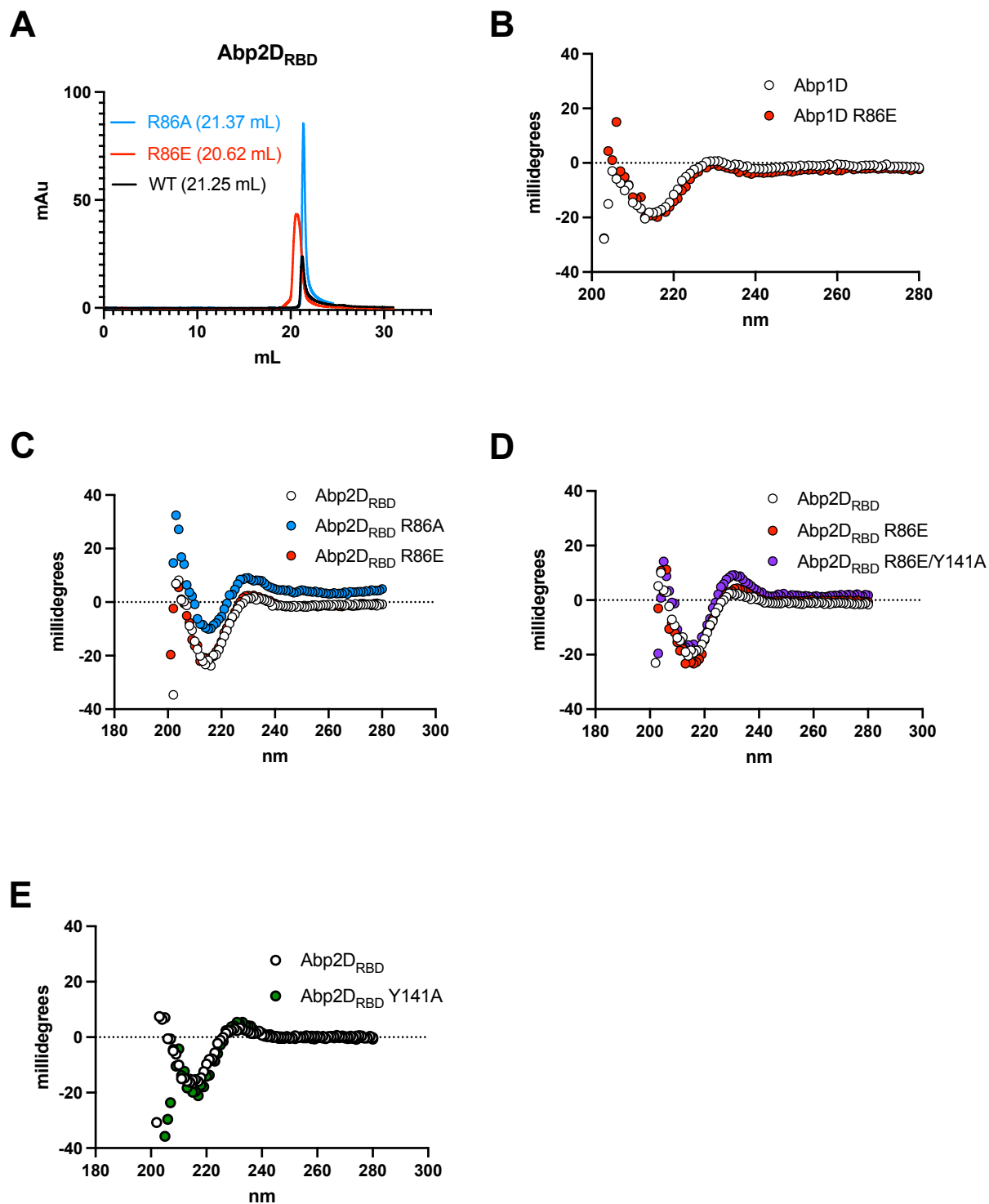


Fig S4: Mutants of the Abp1D and Abp2D RBDs are appropriately folded. A) Size exclusion chromatography profiles of Abp2D_{RBD} WT and R86 mutants. Proteins suspended 20 mM MES 5.79 + 50 mM NaCl. Assayed on Superdex 200 column. In parentheses, elution volume of respective peaks. B-E) Circular dichroism spectra of Abp1D and Abp2D_{ACICU} RBD mutants in 20 mM Phosphate 7.00.

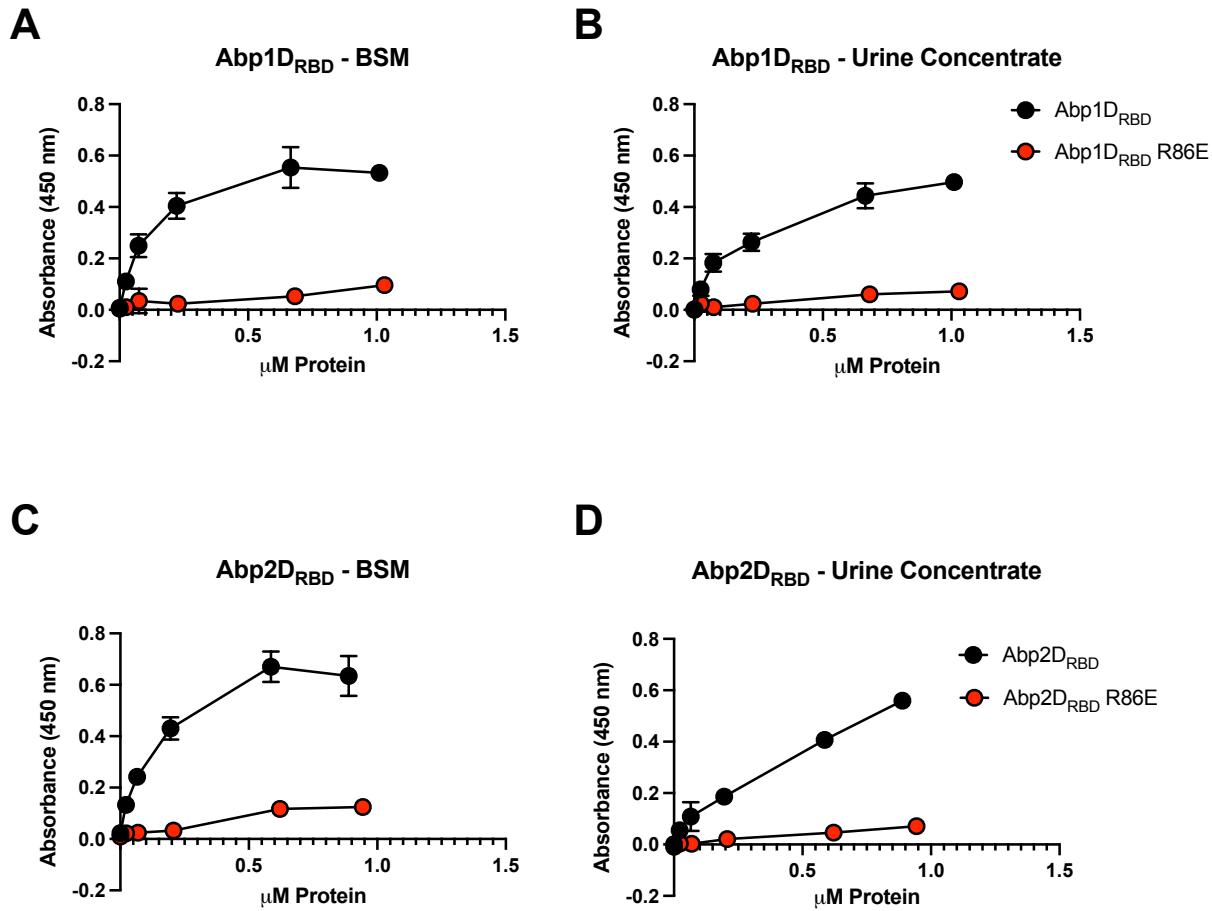


Fig S5: Repertoire of RBD binding. Abp2D_{RBD} binding to A) bovine submaxillary mucin and B) urine concentrate and Abp1D_{RBD} binding to C) bovine submaxillary mucin and D) urine concentrate. Biotinylated Abp2D_{RBD} and Abp2D_{RBD} R86E tested for binding to set of glycoproteins by ELISA. n=4 with a background (n=1) subtracted.

Table S1: Refinement Statistics.

	Abp1D RBD	Abp2D RBD	Abp2D R86E RBD
Resolution range	44.51 - 2.1 (2.175 - 2.1)	47.53 - 1.29 (1.336 - 1.29)	43.8 - 1.9 (1.968 - 1.9)
Space group	P 42 2 2	P 41 21 2	P 21 21 21
Unit cell	91.97 91.97 176.915 90 90 90	51.176 51.176 128.236 90 90 90	36.438 51.847 81.828 90 90 90
Total reflections	629264 (61626)	505499 (19435)	89401 (8836)
Unique reflections	45137 (4370)	42203 (2806)	12772 (1230)
Multiplicity	13.9 (14.0)	12.0 (6.0)	7.0 (7.2)
Completeness (%)	99.76 (99.36)	94.96 (65.35)	99.95 (99.92)
Mean I/sigma(I)	11.90 (0.97)	15.92 (0.63)	15.46 (3.17)
Wilson B-factor	33.97	10.38	22.32
R-merge	0.2715 (2.938)	0.1305 (2.07)	0.1146 (0.8869)
R-meas	0.2818 (3.049)	0.1364 (2.266)	0.124 (0.9574)
R-pim	0.07507 (0.8113)	0.03882 (0.8954)	0.04693 (0.3571)
CC1/2	0.996 (0.434)	0.999 (0.41)	0.998 (0.804)
CC*	0.999 (0.778)	1 (0.763)	0.999 (0.944)
Reflections used in refinement	45041 (4370)	41657 (2804)	12768 (1230)
Reflections used for R-free	2257 (212)	2041 (141)	581 (61)
R-work	0.2258 (0.2972)	0.2028 (0.3137)	0.2117 (0.2518)
R-free	0.2394 (0.3031)	0.2073 (0.3522)	0.2403 (0.2545)
CC(work)	0.945 (0.716)	0.952 (0.710)	0.950 (0.859)
CC(free)	0.936 (0.791)	0.958 (0.616)	0.927 (0.859)
Number of non-hydrogen atoms	2575	1507	1282
macromolecules	2524	1372	1257
ligands	51	31	
solvent	335	104	25
Protein residues	0.01	175	165

RMS(bonds)	1.35	0.008	0.009
RMS(angles)	99.09	1.27	1.16
Ramachandran favored (%)	0.91	97.66	98.76
Ramachandran allowed (%)	0	2.34	1.24
Ramachandran outliers (%)	2.1	0	0
Rotamer outliers (%)	1.59	1.95	0
Clashscore	38.82	0	4.03
Average B-factor	38.93	13.71	28.45
macromolecules	33.58	13.11	28.55
ligands	1	22.58	
solvent		19.03	23.62
Number of TLS groups		1	1

Table S2: Primer list

Abp1 DS F FRT	ATAATGTGTATGATCATTGACTTTTTTAGTATTAATTAATAAATTATTTCATTCTAT AAAGCCTACTACTTACTACTAAGATCTAGTGTTTAGAAGAAATA
Abp1 US R FRT	AAAATAATTTTCATGATGTTTTACCATATGGTATAAATTAATAAATAAAAACCGCATT ATCAATAAAAAATAACCAATCAAATACATAAAAAATATAATGTT
Abp1 out F	GCAAATTGTGATCTATTTCCC
Abp1 ut R	CCAAACTCCAGATGATCTTTTATTG
Abp2 US R Frt	TTTGTTTTAAAAAGTGTTAGCATTGTTACACTTTTCAAGATGTCAAACACTGGA AAAGTAAGGAATTAACCTTTTATCCAGACTTTTGAGAAAACACT
Abp2 DS F frt	ATATTCCTATCGAGTTAAGTATGAAATTTTTAGAAAGTAAAAGTTATTATTAATAT TTAATTATTTGAAAATTATTAATTTTATTAACCTATTGAATATGA
Abp2 out F	ATGTGACTTTTTGGCTAGCC
Abp2 out R	TCTTATCTCTATGATTCCTTTCTTC
Abp1prom inf Fw	CTCACTAGTGGATCCCAAACCTCCAGATGATCTTTTATTG
Abp1comp inf Rv	ACCGGGCCCAAGCTTCTAATCATATTGGATCGTTACCG
Abp2 prom Fw	CTCACTAGTGGATCCGAAGCTGGCAAAAGAGTG
Abp2 comp rv	ACCGGGCCCAAGCTTATTCCTATCGAGTTAAGTATG
BamHI pEX18 Fw	GGATCCCCGGGTACC
HindIII pEX18 Rv	AAGCTTGGCACTGGC
US Fw Abp1_ACICU	GCCAGTGCCAAGCTTGAAGAATTGCCAGTTAGAAG
US Rv Abp 1_ACICU	ATTGAATATTTTGGGCATTTCTTATAAACGCTAGAGC
DS Fw Abp 1_ACICU	CCCAAAATATTCAATAAATATCGC

DS Rv Abp 1_ACICU	GGTACCCGGGGATCCTTAGGTGAAAGCCGTGAAG
US Fw Abp 2_ACICU	GCCAGTGCCAAGCTTTGCTACAGATTTCTTAAATCC
US Rv Abp 2_ACICU	TCATATTCAATAGTTTTTAAACC
DS Fw Abp 2_ACICU	AACTATTGAATATGATATGTGACCCGTATCTCAC
DS Rv Abp 2_ACICU	GGTACCCGGGGATCCATCAGTAAAAGCCGATACTC