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Supplementary information

Structure of the decoy module of human glycoprotein 2 and uromodulin and its interaction with bacterial adhesin FimH

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Supplementary Table 1
X-ray data collection, refinement and validation statistics

	GP2 decoy module crystal form I (*P*1) (PDB 7P6R)	**GP2 decoy module crystal form II ($P2_12_12_1$)** (PDB 7P6S)	**GP2 decoy module crystal form III (*C*2)** (PDB 7P6T)
Data collection			
Space group	*P*1 [1]	$P2_12_12_1$ [19]	*C*2 [5]
Cell dimensions			
a, *b*, *c* (Å)	33.04, 46.53, 57.44	33.48, 59.50, 87.04	90.15, 33.66, 59.63
α, *β*, γ (°)	68.750, 75.873, 72.398	90, 90, 90	90, 111.839, 90
Resolution (Å)	52.9–1.90 (1.97–1.90)*	49.1–1.35 (1.39–1.35)	29.0–1.40 (1.47–1.40)
No. unique reflections	22914 (2161)	38991 (2960)	32721 (4653)
Completeness (%)	96.6 (91.4)	99.7 (98.2)	98.4 (96.8)
Redundancy	3.5 (3.2)	6.0 (4.7)	6.9 (6.7)
R_{merge}	0.099 (0.509)	0.153 (2.318)	0.104 (3.379)
R_{meas}	0.118 (0.611)	0.167 (2.612)	0.112 (3.663)
R_{pim}	0.063 (0.333)	0.067 (1.180)	0.042 (1.396)
Wilson B-factor (Å^2)	20.1	13.2	18.9
I / σI	8.8 (2.6)	7.0 (0.7)	8.6 (0.6)
$CC_{1/2}$	0.99 (0.89)	1.00 (0.40)	1.00 (0.48)
CC*	1.00 (0.97)	1.00 (0.75)	1.00 (0.81)
Refinement			
Resolution (Å)	52.9–1.90 (1.97–1.90)	49.1–1.35 (1.39–1.35)	29.0–1.40 (1.47–1.40)
No. reflections	22860 (2157)	38932 (2921)	32522 (4534)
No. free reflections	1579 (152)	2027 (153)	2018 (280)
R_{work}	0.233 (0.275)	0.194 (0.422)	0.194 (0.514)
R_{free}	0.280 (0.313)	0.224 (0.429)	0.223 (0.518)
No. non-H atoms	2400	1389	1288
Protein	2061	1122	1067
Ligand/ion	106	35	87
Water	233	232	134
No. protein residues	265	142	138
B-factors	28.7	18.5	31.3
Protein	27.9	16.5	28.6
Ligand/ion	38.7	18.2	53.4
Water	31.2	28.2	38.4
R.m.s. deviations			
Bond lengths (Å)	0.007	0.004	0.003
Bond angles (°)	0.73	0.72	0.67
Validation			
MolProbity score	1.23	0.95	0.66
Clashscore	4.59	1.83	0.45
Rotamer outliers (%)	0.0	0.0	0.0
Ramachandran plot			
Overall Z-score	-1.29 ± 0.44	-1.47 ± 0.55	-1.12 ± 0.60
Favored (%)	98.4	98.6	98.5
Allowed (%)	1.6	1.4	1.5
Disallowed (%)	0.0	0.0	0.0

* Values in parentheses are for highest-resolution shell

Supplementary Table 2
Pathogenic UMOD D10C domain missense mutations

UMOD mutation	Equivalent GP2 residue*	Predicted mutation effect based on structural information	Disease reported[§]	Reference
D172H	**D61**	Affects the relative orientation of the β-hairpin and D10C domain by disrupting the salt bridge between D172 and K265 (K155 in GP2)	TN	66
P173L P173R	**P62**	The mutated residue clashes against invariant W202 (W92 in GP2), affecting the interface between the D10C domain and the β-hairpin	UAKD FJHN	4 67
C174R	**$C_1$63**	Destroys conserved disulfide bond C_1-C_8	UAKD	68
R185C R185G R185H R185L R185S	**R74**	Disrupts the interaction between helix 3_{10}B and loop 3_{10}B-βB	TN FJHN TN ADTKD FJHN	66 69 66 70 71
C195F C195Y	**$C_2$85**	Destroys conserved disulfide bond C_2-C_9	FJHN FJHN	72 73
D196N D196Y	**D86**	Disrupts the interaction between loop 3_{10}B-βB and helix 3_{10}B	FJHN FJHN	69 74
W202C W202S	**W92**	Disrupts the interaction between the D10C domain and the β-hairpin	UAKD FJHN	4 72
R204G R204P	**R94**	Disrupts the cation-π interaction with βG Y271 (Y161 in GP2) and affects the interface between the 3_{10}A-βA region and the D10C domain β-strand core	FJHN TN	71 66
G210D G210S	G100	The mutated residue clashes against the C-terminal end of D10C that includes β-strand I	UAKD TN	4 66
R212C	R102	Interferes with correct disulfide bond formation	UAKD	75
C217G C217R C217W	$C_3$107	Destroys conserved disulfide bond C_3-C_6	FJHN, TN FJHN FJHN	66,71 76 69
C223R C223Y	**$C_4$113**	Destroys conserved disulfide bond C_4-C_{10}	FJHN FJHN	69 77
T225K T225M	T115	Disrupts hydrogen bonding between the Thr hydroxyl group and main chain atoms; introduces clashes with β-strands D/H	MCKD2 FJHN	78 71
M229R	M119	Disrupts D10C hydrophobic core	FJHN/MCKD	79
W230R	W120	Disrupts a key D10C residue whose aromatic side chain lies between the C_3-C_6 and C_5-C_7 disulfides	UAKD	80
P236L P236Q P236R P236S	P126	Disrupts the interaction between loop βD-βE and the D10C domain β-strand core	FJHN FJHN FJHN UAKD	72 81 82 4
C248S C248W	$C_5$138	Destroys conserved disulfide bond C_5-C_7	UAKD MCKD2	80 78
H250L H250Q	H140	Disrupts the packing of the His ring against the C_3-C_6 disulfide (on the opposite side of W230 (GP2 W120)	TN TN	66 83
C255Y	$C_6$145	Destroys conserved disulfide bond C_3-C_6	FJHN	84
C256G C256Y	$C_7$146	Destroys conserved disulfide bond C_5-C_7	FJHN UAKD	85 75
C267F	**$C_8$157**	Destroys conserved disulfide bond C_1-C_8	MCKD2, FJHN	86
G269C	G159	Interferes with correct disulfide bond formation and the β-turn between strands βF and βG	UAKD	4
G270C	G160	Interferes with correct disulfide bond formation and the β-turn between strands βF and βG	UAKD	87
V273F V273L	**V163**	Introduces clashes into the hydrophobic core	FJHN/MCKD TN	79 66
Y274C Y274H	**Y164**	Destabilizes the structure of the βG strand, carrying the UMOD high-mannose glycan and, in the case of Y274C, may also interfere with correct disulfide bond formation	UAKD	80
C282R C282S	$C_9$172	Destroys conserved disulfide bond C_2-C_9	FJHN UAKD	71 4
L284P	L174	Affects closely located disulfide bond C_2-C_9	UAKD	4
C287F	**C_{10}177**	Destroys conserved disulfide bond C_4-C_{10}	ADTKD	88

* Residues shown in Fig. 1c-g and Extended Data Fig. 5c-g are highlighted in bold

[§] ADTKD, Autosomal Dominant Tubulointerstitial Kidney Disease; FJHN, Familial Juvenile Hyperuricemic Nephropathy; MCKD, Medullary Cystic Kidney Disease; TN, Tubulointerstitial Nephritis; UAKD, Uromodulin-Associated Kidney Disease

Supplementary Table 3
Cryo-EM data collection, refinement and validation statistics

	Full-length UMOD (EMD-10553 + EMD-13378) (PDB 7PFP)		**UMOD branch + EGF IV/ $FimH_L$ complex** (EMD-13794) (PDB 7Q3N)
Data collection and processing			
Magnification	130,000x		105,000x
Voltage (kV)	300		300
Electron exposure (e–/Å^2)	39.6		40
Defocus range (μm)	-1.5 to -3.5		-1 to -3
Pixel size (Å)	1.06		0.84
Body	Filament core + D10C domain	Branch	
Symmetry imposed	Helical (with 62.5 Å rise, 180.0° twist)	Non-helical	Helical (initial; with 65.2 Å rise, 180.0° twist); non-helical (final)
Initial particle images (no.)	412,322	412,322	3,767,790
Final particle images (no.)	288,403	114,206	225,819
Map resolution (Å)	3.35	6.1	7.4
FSC threshold	0.143	0.143	0.143
Map resolution range (Å)	3.0–4.2	5.0-6.8	6.4–7.9
Refinement			
Initial models used (PDB codes)	PDB 6TQK, AlphaFold2 model, PDB 7P6R/7P6S/7P6T		PDB 7PFP, PDB 6GTW
Model resolution (Å)			
masked	4.1		8.3
unmasked	4.4		8.5
FSC threshold	0.143		0.143
Map sharpening *B* factor (Å^2)	-200		-150
Model composition			
Non-hydrogen atoms	9,582		3,599
Protein residues	1,127		451
Carbohydrate residues	84		20
B factors (Å^2)			
Protein	315		404
Carbohydrate residues	406		291
R.m.s. deviations			
Bond lengths (Å)	0.005		0.003
Bond angles (°)	0.845		0.672
Validation			
MolProbity score	1.83		1.71
Clashscore	4.08		9.47
Poor rotamers (%)	2.4		0.5
Ramachandran plot			
Overall Z-score	-1.66 ± 0.24		-0.74 ± 0.37
Favored (%)	94.9		96.6
Allowed (%)	5.1		3.4
Disallowed (%)	0.0		0.0

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