Supplementary information for Huai *et al.* "Crystal structure of a human vitronectin, urokinase, and urokinase receptor complex"

Fig. S1. Stereo view of the electron density map of suPAR-ATF-SMB complex (σ_A-weighted 2Fo-Fc map contoured at 1σ level) in the uPAR-SMB interface. This figure is in the same orientation and has the same labels as the Fig. 2A. The figure was made with pymol[1].

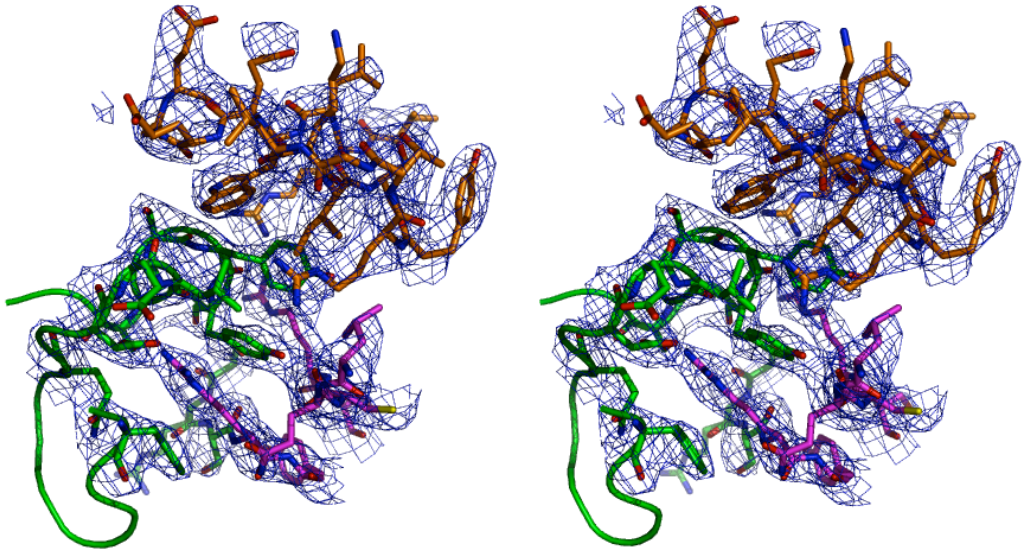

Table 1 Data collection and model refinement statistics of suPAR-ATF-SMB-ATN615 and suPAR-ATF-SMB complexes.

	suPAR-ATF-SMB-ATN615	suPAR-ATF-SMB
Data collection		
Space group	P21	P21212
Cell dimensions		
a, *b*, *c* (Å)	52.10, 87.20, 124.27	97.35, 105.19, 55.36
α, *β*, *γ* ( )	90.00, 94.31, 90.00	90.00, 90.00, 90.00
Resolution (Å)	2.50(2.59-2.50) *	2.80(2.90-2.80) *
R_{sym} or R_{merge}	0.077 (0.356) *	0.081 (0.803)
$I / \sigma I$	24.1 (2.8) *	33.1 (2.6) *
Completeness (%)	99.2% (96.0%)*	99.5% (100.0%)*
Redundancy	5.3 (4.1) *	5.7 (5.9) *
Refinement		
Resolution (Å)	124.0-2.50 (2.56-2.50) *	31.01-2.90 (2.97-2.90) *
No. reflections	37032	12448
R_{work} / R_{free}	0.228 (0.267) / 0.272 (0.344)	0.237 (0.354) / 0.316 (0.416)
No. atoms		
Protein	6601	3463
Ligand/ion	0	0
Water	57	0
B-factors	72.2	75.9
Protein	72.5	75.9
Ligand/ion	0	0

Water	39.8	0
R.m.s. deviations		
Bond lengths (Å)	0.018	0.015
Bond angles ( )	1.740	1.747

*Note: data in parentheses are for the highest resolution shell indicated in "Resolution"

Table 2A Residues in uPAR-SMB interface and their contact area and exposed area for suPAR-ATF-SMB complex

uPAR	Contact Area,Å^2	Exposed Area,Å^2	Percent Contact	SMB	Contact Area,Å^2	Exposed Area,Å^2	Percent Contact
Arg91	93.7	183.0	51	Tyr27	94.1	148.0	64
Trp32	54.8	171.0	32	Tyr28	84.8	132.8	64
Arg116	44.7	182.0	25	Leu24	33.4	96.7	35
Arg30	30.8	174.6	18	Phe13	29.8	134.3	22
Ile63	26.7	107.9	25	Ser26	21.9	76.4	29
Gln114	23.3	124.1	19	Asp22	15.3	56.9	27
Arg58	10.1	204.1	5	Gln29	14.9	128.4	12
Ser88	9.6	93.8	10	Glu23	5.9	131.7	5
Ser56	8.5	112.5	8	Ser30	5.0	44.9	11
Ser65	7.1	86.1	8	Gln20	3.7	41.2	9

Table 2B Hydrogen bonds in suPAR-SMB interface

uPAR		SMB		Distance, Å
Arg91	NH2	Asp22	OD1	2.99
Arg91	NH1	Asp22	OD2	2.94
Arg91	NH2	Ser30	OG	3.19
Arg30	NH2	Ser26	O	3.09
Arg30	NH1	Tyr27	O	2.96
Arg116	NH1	Tyr27	O	3.22
Arg116	NH2	Gln29	OE1	3.11
Gln14	N	Tyr28	OH	2.75
Ser56	OG	Tyr27	OH	2.82
Glu34	OE1	Lys40	NZ	2.74

Supplementary Methods

Both recombinant soluble uPAR (suPAR, residue 1-277) and ATF (amino acid residue 1-143 of uPA) were produced in Drosophila S2 cells as secreted proteins and were purified as described[2,3]. The suPAR-ATF complex was formed by incubating ATF with suPAR at room temperature in 50 mM HEPES and 100 mM NaCl pH 7.4 and was purified on a Superdex75 gel filtration column. SMB domain (amino acid residue 1-50 of vitronectin) was expressed in *E.Coli* and purified as described[4].

To crystallize suPAR-ATF-SMB complex, the suPAR-ATF complex (about

10 mg/ml) was mixed with SMB domain of vitronectin at 1:3 molar ratio, and the crystals were generated by the microdialysis method using a precipitant solution of 12% PEG3350, 50mM HEPES at pH 7.5. For X-ray diffraction data collection, the crystals were frozen using a cryo-protectant solution of 25% PEG3350, 25% glycerol, 50 mM HEPES pH 7.5.

The quaternary protein complex, suPAR-ATF-SMB-ATN-615, where ATN-615 is a Fab fragment of an anti-uPAR antibody, were also formed by mixing the purified suPAR-ATF-ATN615 with SMB at molar ratio of 1:3 to 1:5. The crystals of the quaternary protein complex were also generated by microdialysis with a precipitant solution of 8% PEG4K, 2.5% ethanol, 0.05% sodium azide, 50 mM cacodylate pH 6.5. These crystals typically appeared in 3 to 7 days, and grew to a maximal size of 0.05 x 0.2 x 0.3 mm^3. The crystals were frozen using the cryoprotectant as 25% glycerol, 20% PEG4K, 50 mM cacodylate pH 6.5 for X-ray data collection. The X-ray diffraction data (Table 1) for all these crystals was collected at 100K using synchrotron radiation at the Advanced Photon Source (APS), Argonne National Laboratory. The suPAR-ATF or the suPAR-ATF-ATN-615 model (from the PDB entry 1fd6[5]) were each positioned into the crystals of the ternary or the quaternary complexes by molecular replacement methods (molrep[6]). After refinement using the CNS program[7], the Fo-Fc difference electron density showed the electron density for SMB domain of vitronectin, which was added to the model according to the X-ray structure of SMB[4]. The resulting models were refined using CNS[7] and REFMAC[8], and manual model fitting was carried out using the program O[9]. The final structures were analyzed by PROCHECK[10], PYMOL[1] and MOLSOFT ICM[11]. Ramachandran plots of the structures showed that 84.5%, 13.2%, 1.5%, 0.8% of residues are in the core, allowed, generally allowed, and disallowed regions for suPAR-ATF-SMB-ATN615 structure,, respectively. For suPAR-ATF-SMB structure, the corresponding distributions are 70.4%, 23.3%, 3.4%, and 2.9%, respectively.

The ternary structure suPAR-ATF-SMB contains more ordered residues (uPAR 1-82, 87-275; SMB: 2-41; ATF: 8-132) compared with the quaternary structure (uPAR 1-80, 87-130, 139-275; SMB: 2-41; ATF: 9-132; H2O: 1-57), and was used for further analysis.

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