Supplementary Table 1

Crystal, NMR and Electron Microscopy reconstructions of Mediator in chronological order

Module	Subunits	Org.[a]	Construct	Method	Expression	PDB/EMD	Resol. (Å)	Year	Reference
Head + Middle + Tail		*Sc*	native Mediator	EM	*Sc*	-	40	1999	(*1*)
Head + Middle + Tail		*Sc*	native Mediator[b]	EM	*Sc*	-	30-35	2000	(*2*)
Head + Middle + Tail		*Hs*	native from HeLa cells via SREBP-1a affinity resin (CRSP and ARC-L (with CKM))	EM	*Hs*	-	32	2002	(*3*)
Head + Middle + Tail		*Hs*	native from HeLa cells P1.0M fraction via GST-CTD	EM	*Hs*	-	32	2002	(*4*)
Head + Middle + Tail		*Hs*	native from HeLa cells P0.5M fraction (CRSP/Med2 lacking CKM, MED1 and MED26)	EM	*Hs*	-	31	2004	(*5*)
Head + Middle + Tail		*Hs*	native from HeLa cells P1.0M fraction - TR and VDR	EM	*Hs*	-	29	2004	(*6*)
Middle	7/21	*Sc*	7C(102-205)/21(1-132)	X-ray	*E. Coli*	1YKH	3.0	2005	(*7*)
Kinase	CycC	*Sp*	CyC(5-228)	X-ray	*E. Coli*	1ZP2	3.0	2005	(*8*)
Head	8/18/20	*Sc*	8C(190-210)/18(Δ109-140)/20	X-ray	*E. Coli*	2HZS	2.7	2006	(*9*)
Head	18/20	*Sc*	18(Δ109-140)/20	X-ray	*E. Coli*	2HZM	2.4	2006	(*9*)
Tail	15	*Hs*	15(5-78) KIX domain	NMR	*E. Coli*	2GUT	-	2006	(*10*)
Head + Middle + Tail + Kinase		*Sp*	native Mediator (S-Mediator) and native Mediator with CKM (L-Mediator)	EM	*Sp*	-	25	2006	(*11*)
Head + Middle + Tail		*Hs*	native from HeLa cells + CDK8 module + TRAPP + GCN5L (T/G-Mediator)	EM	*Hs*	-	-	2008	(*12*)
Tail	15	*Sc*	15(6-90) KIX domain	NMR	*E. Coli*	2K0N	-	2008	(*13*)
Head	8/18	*Sp*	8C(180-200)/18	X-ray	*E. Coli*	3C0T	2.4	2008	(*14*)
Middle	7/31	*Sc*	7N(1-83)/31	X-ray	*E. Coli*	3FBI	2.8	2009	(*15*)
Kinase	12/13/CDK8/CycC	*Hs*	12/13/Glu-tagged CDK8/CycC	EM	Baculo	-	38	2009	(*16*)
Head + Middle + Tail		*Sc*	native Mediator harboring Med8-3xHA and Med22-10xHis	CryoEM	*Sc*	-	28	2009	(*17*)
Head	6/8/11/17/18/20/22	*Sc*	6/8/11/17/18/20/22	EM	Baculo	-	30-35	2010	(*18*)
Head + Middle + Tail		*Hs*	native Mediator from HeLa nuclear extract + p53 activation domain	EM	*Hs*	-	34	2010	(*19*)
Head	11/22	*Sc*	11(1-89)/22(5-89)	X-ray	*E. Coli*	3R84	2.1	2011	(*20*)
Head	6/8/11/17/18/20/22	*Sc*	6/8/11(Δ1-16)/17(109-687)/18(Δ109-140)/20/22	X-ray	Baculo	3RJ1	4.3	2011	(*21*)
Tail	15	*Sc*	15(158-238) ABD1 in complex with Gcn4 activation domain (101-134)	NMR	*E. Coli*	2LPB	-	2011	(*22*)
Tail	25	*Hs*	25(391-548) ACID/PTOV domain and characterization of its binding site for VP16 activation domain	NMR	*E. Coli*	2L23, 2XNF, 2L6U, 2KY6	-	2011	(*23-26*)
Kinase	CDK8/CycC	*Hs*	CDK8(1-403)/CyC(1-283)	X-ray	Baculo	3RGF[c]	2.2	2011	(*27*)
Holoenzyme		*Hs*	native Mediator from HeLa nuclear extract + RNAPII + TFIIF + VP16	CryoEM	*Hs*	EMD 5343	36	2011	(*28*)
Head	6/8/11/17/18/22	*Sp*	6(1-180)/8/11/17(77-545)/18/22[d]	X-ray	*E. Coli*	4H63	3.4	2012	(*29*)

Head	6	*Sp*	6(9-180)	X-ray	*E. Coli*	4H61	2.7	2012	(*29*)
Head	11/17/22	*Sc*	11C(84-115)-22C(96-121)[e]/17C(377-687)	X-ray	*E. Coli*	4H62	3.0	2012	(*29*)
Head	6/8/11/17/18/20/22	*Sc*	native head module harboring 8-TAP/Δ16 + 35-residues peptide (5 CTD repeats)	X-ray	*Sc*	4GWP	4.2	2012	(*30*)
Head + RNAPII	6/8/11/17/18/20/22	*Sc*	Head + RNAPII + TFIIF + TFIIB + TBP + DNA[f]	cryoEM	Mixed	EMD-5407	16	2012	(*31*)
Holoenzyme		*Hs*	native Mediator from HeLa nuclear extract + RNAPII + TFIIF	CryoEM	*Hs*	EMD-5344	32	2012	(*32*)
Kinase	12/13/CDK8/CycC	*Sc*	native CKM module harboring CDK8-TAP	cryoEM	*Sc*	EMD-5588	15	2013	(*33*)
Kinase	12/13/CDK8/CycC	*Sc*	native CKM module harboring CDK8-TAP or Med12-TAP	EM	*Sc*	-	30-35	2013	(*34*)
Head + Middle + Tail		*Sc*	native Mediator harboring 10xHis tag at the C-ter of Med14, 17, 22 or 21	EM	*Sc*	-	32-38	2014	(*35*)
Head + Middle + Tail		*Sc*	native Mediator harboring Med16-MBP and CBP-Med5	CryoEM	*Sc*	EMD-2634	18	2014	(*36*)
Head + Middle + Tail		*Hs*	native Mediator from Hela-S3 cells stably expressing Flag-Med26	EM	*Hs*	EMD-2635	30	2014	(*36*)
Head + Middle + RNAPII	Head/Middle/14/19	*Sc*	Head + Middle + 19 + 14(1-745) + RNAPII + TBP + TFIIB + TFIIF	cryoEM	*Sc/E. Coli*	EMD-2786, 4V1N, 4V1O	9.7	2015	(*37*)
Holoenzyme		*Sc*	native Mediator harboring Med21-Flag-ProteinA	EM	*Sc*	-	-	2016	(*38*)
Holoenzyme		*Hs*	native Mediator from HeLa cells stably expressing Flag-Med7	EM	*Hs*	-	-	2016	(*38*)
Holoenzyme		*Sc*	Mediator + RNAPII + TFIIA + TFIIB + TFIIH + TFIIE + TFIIF + TBP (52 polypeptides)	CryoEM	*Sc*	EMD-8308, 5SVA	21.9	2016	(*39*)
Holoenzyme		*Sp*	native Mediator harboring Med7-3xFlag ΔMed13[g] + RNAPII	CryoEM	*Sp*	EMD-8480, 5U0S	7.8	2017	(*40*)
Holoenzyme		*Sc*	Head/Middle/19/14(1-745) + RNAPII + TBP + TFIIA + TFIIB + TFIIE + TFIIF + TFIIH	CryoEM	Mixed	EMD-3850, 5OQM	5.8	2017	(*41*)
Middle	26	*Hs*	26(1-92)	NMR	*E. Coli*	5ODD	-	2017	(*42*)
Head + Middle	4/6/7/8/9/10/11/14/17/18/19/20/21/22/31	*Sp*	1/4/6/7/8/9/10/11(Δ112-116)/6his-14(Δ581-879)/17/18/19/20/21/22/31[h]	X-ray	*E. Coli*	5N9J	3.4	2017	(*43*)
Tail	15	*Sc*	15(277-368) ABD2	NMR	*E. Coli*	6ALY	-	2018	(*44*)
Tail	23	*Hs*	23(1-1334)[i]	X-ray	Baculo	6H02	2.8	2018	(*45*)

Supplementary Table 1 adapted from (*46*) with permission from Elsevier.

[a] Organism : Sc, *Saccharomyces cerevisiae* ; Sp, *Schizosaccharomyces pombe* ; Hs, *Homo sapiens*

[b] The 3D structures of murine and human Mediator were also reported. The overall architecture is conserved.

[c] Many structures were then reported in complex with small molecule inhibitors of CDK8 such as Cortistatin A (PDB 4CRL (*47*)).

[d] Med20 subunit is not present in the crystals but was unambiguously modelled and positioned by superimposing the previous *Sc* Med18/Med20 structure (*9*).

[e] Med11C/Med22C is a fusion construct with a linker (GAGSGAGSG) inserted between the C terminus of Med11 and residue 96 of Med22.

[f] unable to conclusively identify density corresponding to TFIIB, TBP and DNA. Consequently the cryo-EM map revealed the structure of Mediator Head + RNA polymerase II + TFIIF.

[g] Cryo-EM map was masked to hide the Tail. The atomic model includes 16 subunits (Med4/Med6/Med7/Med8/Med9/Med10/Med11/Med14/Med17/Med18/Med19/Med20/Med21/Med22/Med27/Med31).

[h] The core Mediator structure comprises 15 subunits (Head + Middle + Med14) and only lacks Med1 which probably dissociated during crystallization.

[i] The structure of Med23 was determined in complex with a stabilizing nanobody.

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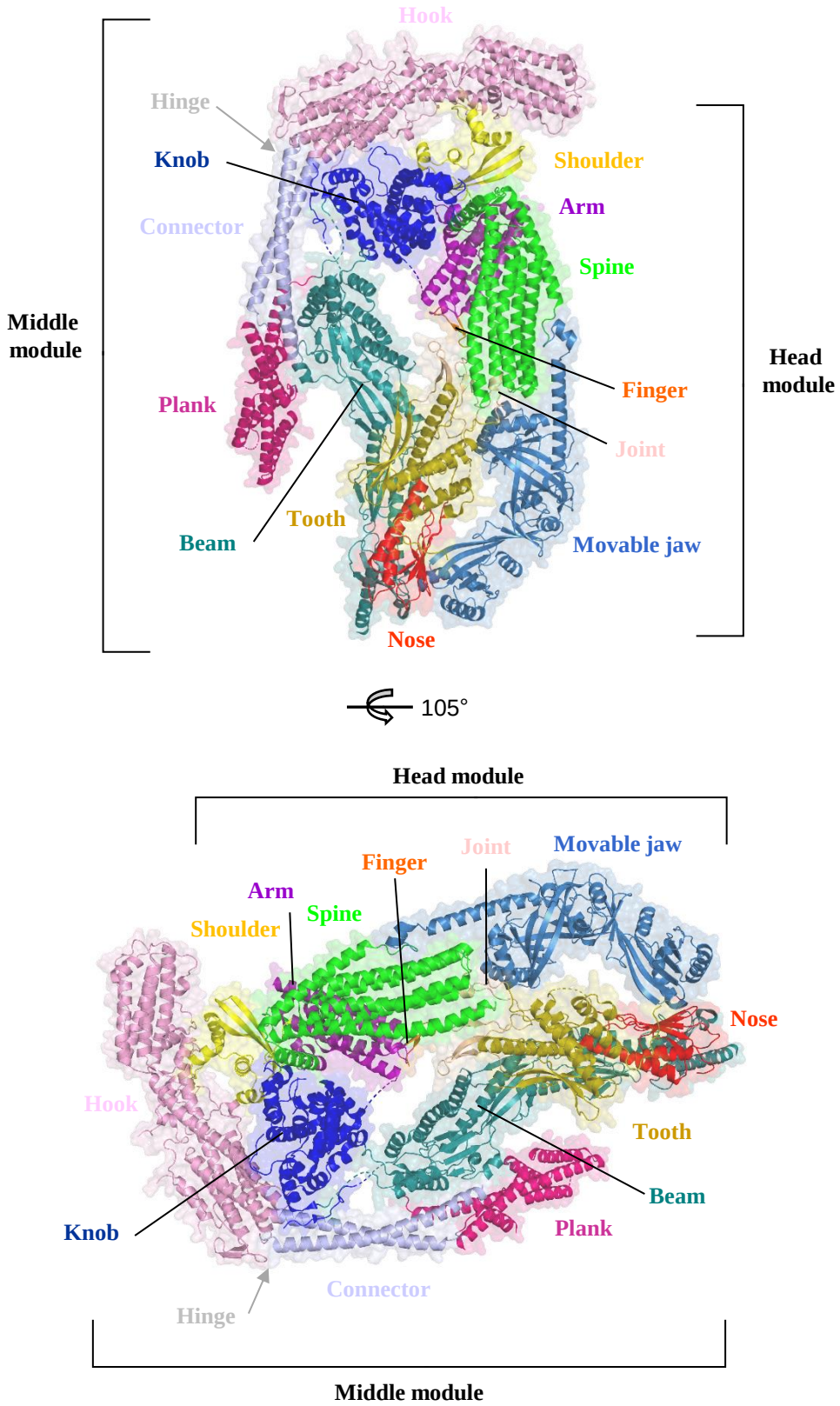
Supplementary Figure 1. Submodule architecture of core yeast Mediator. The 13 submodules which were defined in 2017 (*47*) are indicated and colored: 8 in the Head module (shoulder, arm, spine, finger, joint, movable jaw, tooth and nose) and 5 in the Middle module (hook, knob, connector, plank and beam). The hinge in the Med7C/Med21 subcomplex that flexibly links the hook to the connector is indicated (see also Figure 1A). The Middle module Med1 subunit was included in recombinant core Mediator but is lacking in the crystal structure (*47*). The second view is obtained by a -105° rotation around the z axis.



Supplementary Figure 1