Supplementary Information

Mediator Head module Structure and Functional Interactions

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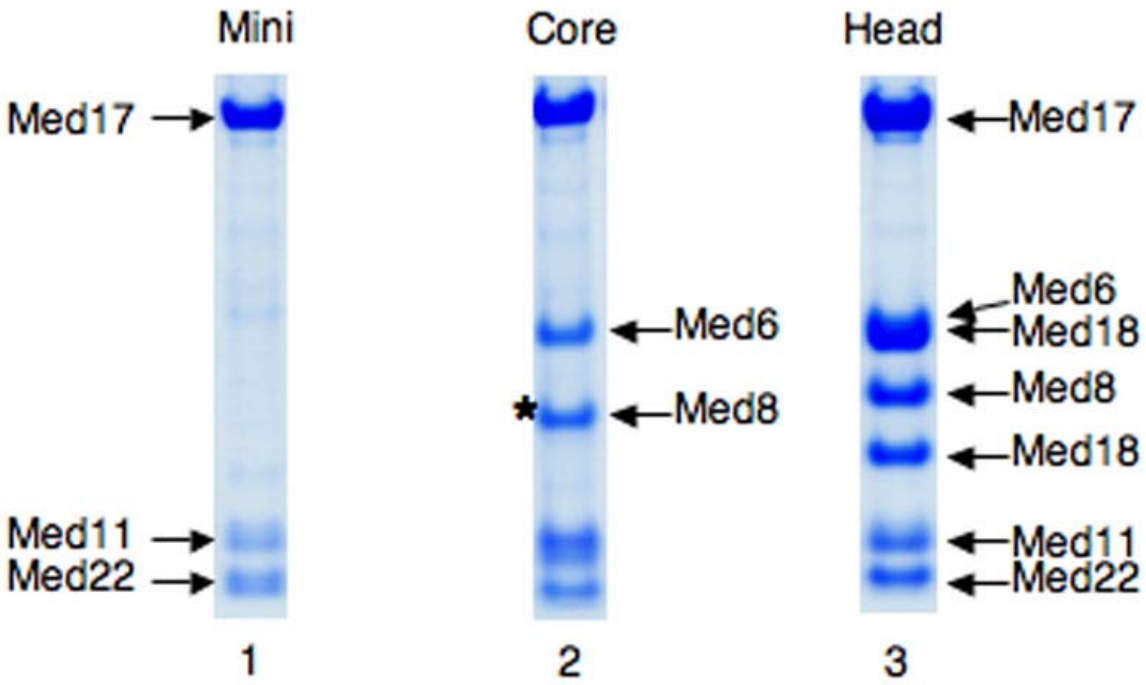
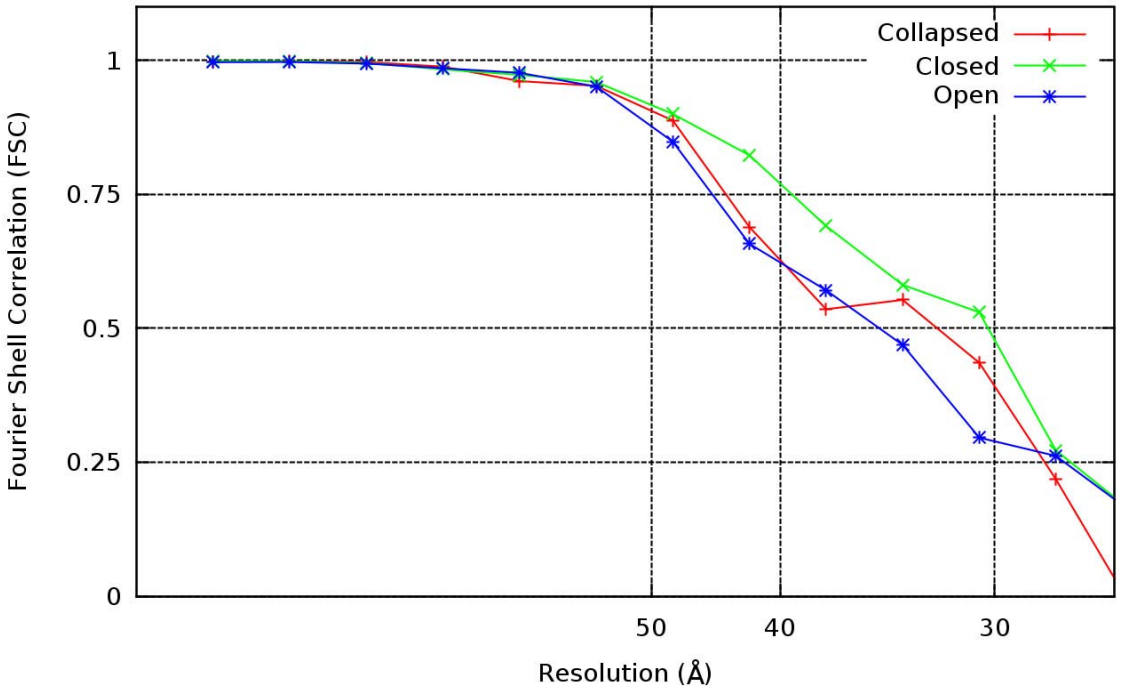
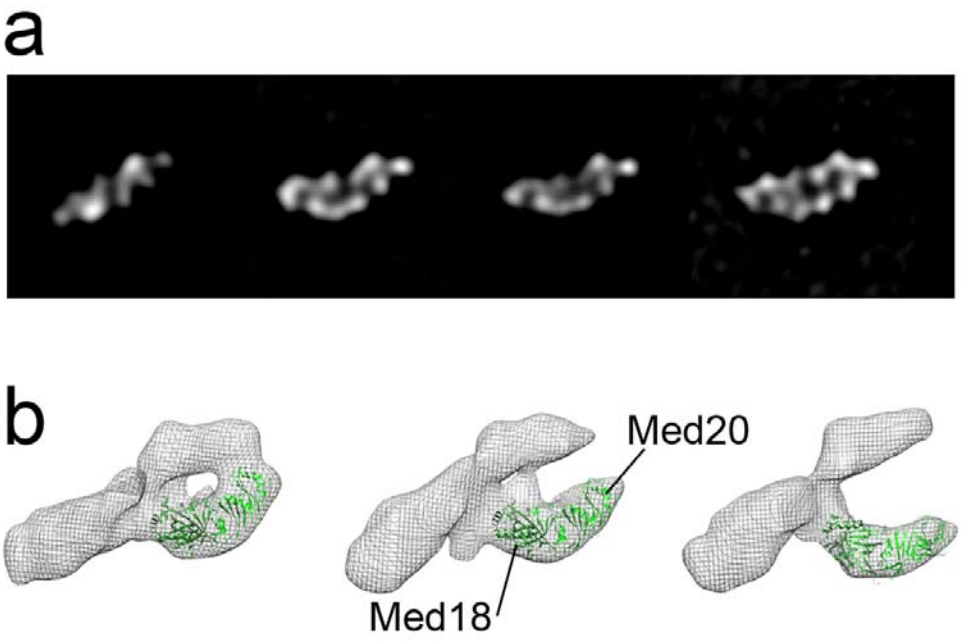
SUPPLEMENTARY FIGURES



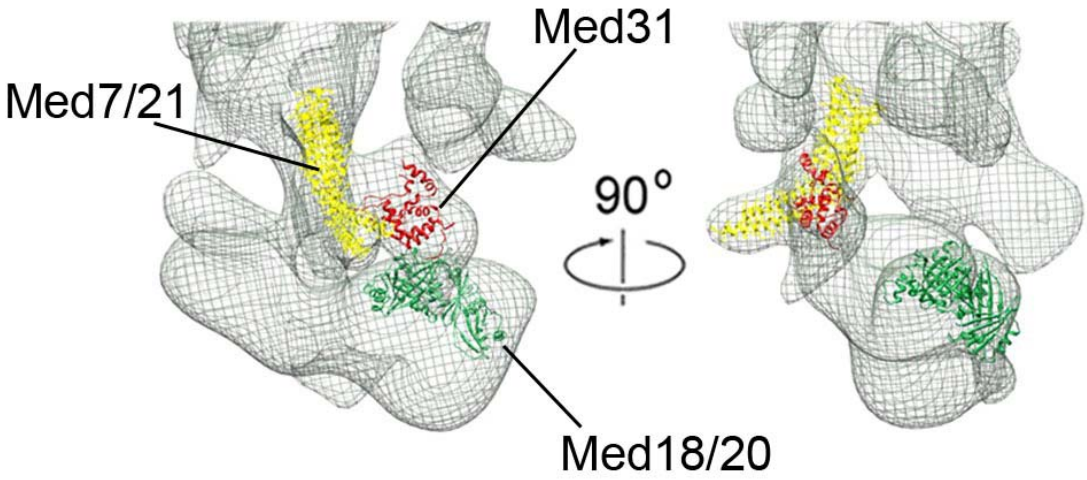
Supplementary Figure 1. SDS-PAGE analysis of a recombinant Head module and Head module sub-complexes. The Mediator Head module and its sub-complexes were expressed in insect cells and purified by means of a 10xHis tag on Med17. Protein complexes were resolved by 4-12% NuPAGE and stained with Coomassie blue. The Mini Head module is composed of three subunits: Med17, Med11, and Med22 (lane 1). The Core Head module includes five subunits: Med17, Med6, Med8, Med11, and Med22 (lane 2). The complete Head module is composed of 7 subunits: Med17, Med6, Med18, Med8, Med20, Med11 and Med22 (lane 3). Subunit bands are indicated by arrows. Asterisk indicates apparent slight proteolysis of Med8 In the absence of Med18.



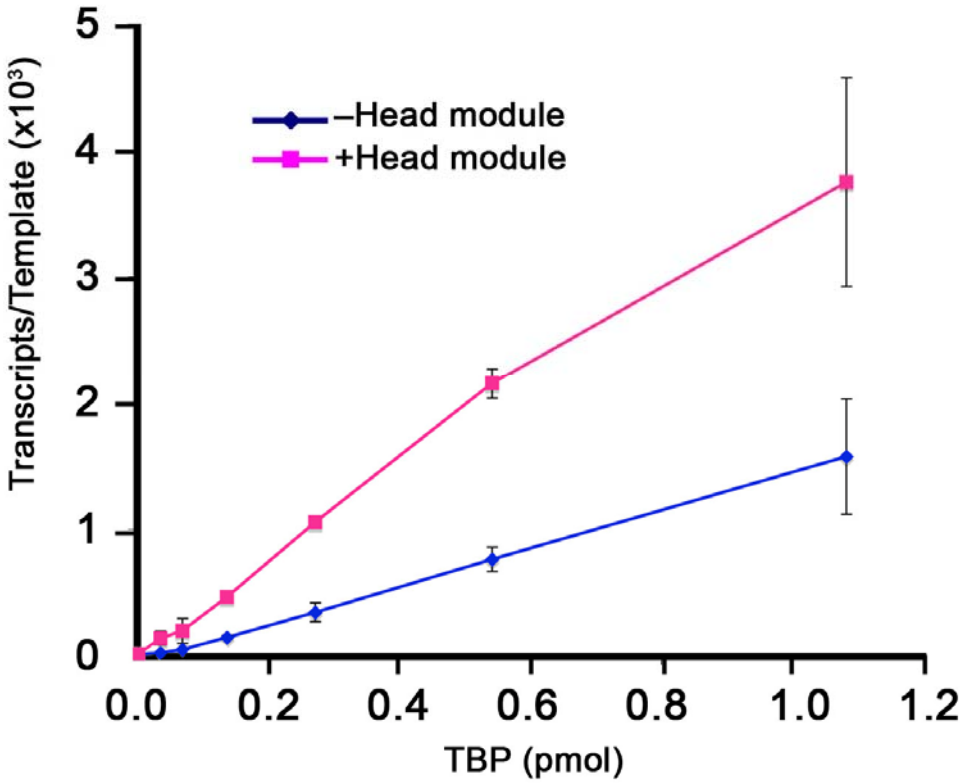
Supplementary Figure 2. Fourier Shell Correlation (FSC) curves for 3D structures of the different Head module conformations. Based on the FSC values[1], the resolution of the reconstructions is estimated to be in the 30-35 Å range.



Supplementary Figure 3. Structure of the Core subcomplex and localization of subunits Med18 and Med20. (**a**) Class averages calculated after alignment and classification of Core single particle images show that the Core is found in several distinct orientations on the EM grids. The left-most average corresponds to an orientation similar to that displayed by Head module particles in the collapsed conformation. (**b**) Docking the X-ray model of the Med18/Med20 subcomplex[2] into the corresponding portion of the Head module 3D EM volumes shows that the X-ray structure closely matches the size and shape of the extended, mobile portion of the Head module volumes.



Supplementary Figure 4. Docking of the Med18/Med20, Med7/Med21, and Med7(N-terminus)/Med31 X-ray structures into the cryo-EM Mediator structure. X-ray structures of Med18/Med20[2] (green ribbons), Med7/Med21[3] (yellow ribbons), and Med7(N-terminus)/Med31[4] (red ribbons) were docked by matching the respective structures to the Mediator cryo-EM structure[5] (gray mesh). The location of Head module subunits and biochemical information about subunit interactions were also considered in determining the overall location of the Med7/Med21 and Med7(N-terminus)/Med31 subcomplexes.



Supplementary Figure 5. Head module lowers an effective TBP concentration on basal transcription *in vitro*. *In vitro* reconstituted transcription assay results showing the transcription level dependence on TBP in the absence and in the presence of a recombinant Head Mediator module. Transcription measurements were carried out with purified proteins and a pGCN4 DNA template[6] with increasing amounts of TBP (0, 0.033, 0.067, 0.135, 0.27, 0.54, and 1.08 pmol) in the absence or presence of recombinant Head module. Transcripts (~360 bp) were separated by 6% denaturing PAGE and quantified using fluorescence image analysis. The plot shows the level of transcription activity as a function of the amount of TBP added. The assay was performed in duplicate and standard deviations are indicated as error bars in the figure.

SUPPLEMENTARY TABLES

Supplementary Table 1. *S. cerevisiae* strains used in this study

Strain	Genotype	Source
BY4742	*MAT*a	Open Biosystems
YT202	*MAT*a *rpb4Δ::KanMX*	Open Biosystems
YT204	*MAT*a *rpb4Δ::KanMX pYT505 (pRS316-RPB4)*	This study
YT205	*MAT*a *rpb4Δ::KanMX med20Δ::LEU2 pYT505 (pRS316-RPB4)*	This study
YT206	*MAT*a *rpb4Δ::KanMX med18Δ::LEU2 pYT505 (pRS316-RPB4)*	This study
YT218	*MAT*a *rpb4Δ::KanMX med1Δ::LEU2 pYT505 (pRS316-RPB4)*	This study
YT219	*MAT*a *rpb4Δ::KanMX med16Δ::LEU2 pYT505 (pRS316-RPB4)*	This study
YT220	*MAT*a *rpb4Δ::KanMX med31Δ::LEU2 pYT505 (pRS316-RPB4)*	This study
YT221	*MAT*a *rpb4Δ::KanMX med9Δ::LEU2 pYT505 (pRS316-RPB4)*	This study
YT222	*MAT*a *rpb4Δ::KanMX med5Δ::LEU2 pYT505 (pRS316-RPB4)*	This study
YT227	*MAT*a *rpb4Δ::KanMX pYT505 (pRS316-RPB4) pRS415* (empty)	This study
YT228	*MAT*a *pRS316* (empty) *pRS415* (empty)	This study

Note: all strains are *his3Δ1 leu2Δ0 lys2Δ0 ura3Δ0*.

Supplementary References

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2. Lariviere, L. et al. Structure and TBP binding of the Mediator head subcomplex Med8-Med18-Med20. *Nat Struct Mol Biol* **13**, 895-901 (2006).
3. Baumli, S., Hoeppner, S. & Cramer, P. A conserved mediator hinge revealed in the structure of the MED7.MED21 (Med7.Srb7) heterodimer. *J Biol Chem* **280**, 18171-8 (2005).
4. Koschubs, T. et al. Identification, structure, and functional requirement of the Mediator submodule Med7N/31. *Embo J* **28**, 69-80 (2009).
5. Cai, G., Imasaki, T., Takagi, Y. & Asturias, F.J. Mediator structural conservation and implications for the regulation mechanism. *Structure* **17**, 559-67 (2009).
6. Takagi, Y. & Kornberg, R.D. Mediator as a general transcription factor. *J Biol Chem* **281**, 80-9 (2006).