

Reporting Summary

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Statistics

For all statistical analyses, confirm that the following items are present in the figure legend, table legend, main text, or Methods section.

n/a Confirmed

- ☐ ☒ The exact sample size (n) for each experimental group/condition, given as a discrete number and unit of measurement
- ☐ ☒ A statement on whether measurements were taken from distinct samples or whether the same sample was measured repeatedly
- ☒ ☐ The statistical test(s) used AND whether they are one- or two-sided
Only common tests should be described solely by name; describe more complex techniques in the Methods section.
- ☒ ☐ A description of all covariates tested
- ☒ ☐ A description of any assumptions or corrections, such as tests of normality and adjustment for multiple comparisons
- ☐ ☒ A full description of the statistical parameters including central tendency (e.g. means) or other basic estimates (e.g. regression coefficient) AND variation (e.g. standard deviation) or associated estimates of uncertainty (e.g. confidence intervals)
- ☒ ☐ For null hypothesis testing, the test statistic (e.g. F , t , r) with confidence intervals, effect sizes, degrees of freedom and P value noted
Give P values as exact values whenever suitable.
- ☒ ☐ For Bayesian analysis, information on the choice of priors and Markov chain Monte Carlo settings
- ☒ ☐ For hierarchical and complex designs, identification of the appropriate level for tests and full reporting of outcomes
- ☒ ☐ Estimates of effect sizes (e.g. Cohen's d , Pearson's r), indicating how they were calculated

Our web collection on [statistics for biologists](#) contains articles on many of the points above.

Software and code

Policy information about [availability of computer code](#)

Data collection Serial EM 3.8 beta 8

Data analysis RELION v3.1.0, UCSF Chimera 1.13, UCSF ChimeraX v1.11, Pymol 2.3.4, Coot 0.8.9.2, Warp v1.0.7-1.0.9, PHENIX 1.18.2, cryoSPARC 3.2.0, RStudio, R version 3.6.1, ggplot2, ggbio.

For manuscripts utilizing custom algorithms or software that are central to the research but not yet described in published literature, software must be made available to editors and reviewers. We strongly encourage code deposition in a community repository (e.g. GitHub). See the Nature Research [guidelines for submitting code & software](#) for further information.

Data

Policy information about [availability of data](#)

All manuscripts must include a [data availability statement](#). This statement should provide the following information, where applicable:

- Accession codes, unique identifiers, or web links for publicly available datasets
- A list of figures that have associated raw data
- A description of any restrictions on data availability

The cryo-EM density reconstructions were deposited to the EMDDB under accession codes EMD-14996 (U5-CC), -14997 (U5-local), -15006 (U1-CC), -15007 (U1-OC), -15009 (U1-local) and the respective atomic coordinates were deposited to the PDB under the accession codes PDB-7ZWC, -7ZWD, -7ZX7, -7ZX8, -7ZXE. The mass spectrometry proteomics data have been deposited to the ProteomeXchange Consortium via the PRIDE partner repository with the dataset identifier PXD033638. All data is available in the main text or the supplementary materials.

Field-specific reporting

Please select the one below that is the best fit for your research. If you are not sure, read the appropriate sections before making your selection.

☒ Life sciences ☐ Behavioural & social sciences ☐ Ecological, evolutionary & environmental sciences

For a reference copy of the document with all sections, see [nature.com/documents/nr-reporting-summary-flat.pdf](https://www.nature.com/documents/nr-reporting-summary-flat.pdf)

Life sciences study design

All studies must disclose on these points even when the disclosure is negative.

Sample size	No Sample size calculations were performed. For cryo-EM samples, at least eleven grids of SNAPc-PIC (U1/U5 promoter) sample were pre-screened to identify the optimal grid for data collection. The number of grids screened were random and was not limited by any experimental parameter. Biochemical experiments were performed with three sample replicates and each experiment was repeated minimum thrice. This is a standard in the field and the sample size was sufficient to observe the effect and binary outcome of this experiment. i.e. SNAPc activation of Pol II PIC in the in vitro transcription assay
Data exclusions	No data were excluded from the analyses.
Replication	All attempts at replication were successful. Cryo-EM single particle analysis inherently relies on averaging over a large number of independent observations. During the processing pipeline, replicate reconstructions were calculated over 3 times during the polishing and other related refinement procedures, yielding the same results at different resolutions. The in vitro transcription assay and EMSA were repeated minimum thrice.
Randomization	Samples were not allocated to groups.
Blinding	Blinding is not applicable for this study, as group allocation is not used.

Reporting for specific materials, systems and methods

We require information from authors about some types of materials, experimental systems and methods used in many studies. Here, indicate whether each material, system or method listed is relevant to your study. If you are not sure if a list item applies to your research, read the appropriate section before selecting a response.

Materials & experimental systems

n/a	Involved in the study
☒	☐ Antibodies
☐	☒ Eukaryotic cell lines
☒	☐ Palaeontology and archaeology
☒	☐ Animals and other organisms
☒	☐ Human research participants
☒	☐ Clinical data
☒	☐ Dual use research of concern

Methods

n/a	Involved in the study
☒	☐ ChIP-seq
☒	☐ Flow cytometry
☒	☐ MRI-based neuroimaging

Eukaryotic cell lines

Policy information about [cell lines](#)

Cell line source(s)	Hi5 cells: Expression Systems, Tni Insect cells in ESF921 media, item 94-002F Sf9 cells: ThermoFisher, Catalogue Number 12659017, Sf9 cells in Sf-9000TM III SFM
Authentication	None of the cell lines were authenticated.
Mycoplasma contamination	Cell lines were not tested for mycoplasma contamination.
Commonly misidentified lines (See ICLAC register)	No commonly misidentified cell lines were used.

Peer Review Information

Journal: Nature Structural and Molecular Biology

Manuscript Title: Structural basis of SNAPc-dependent snRNA transcription initiation by RNA polymerase II

Corresponding author name(s): Professor Patrick Cramer

Editorial Notes:

Reviewer Comments & Decisions:

Decision Letter, initial version:
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Our ref: NSMB-A46118

29th Apr 2022

Dear Patrick,

Thank you again for submitting your manuscript "Structural basis of SNAPc-dependent snRNA transcription initiation by RNA polymerase II". The reports of the referees are below, and based on these comments, we are happy to accept your paper, in principle, for publication as an Article in Nature Structural & Molecular Biology, on the condition that you revise your manuscript in response to the comments of the referees and our editorial requirements.

I hope that you will be pleased to see that both reviewers are positive about the work, and that Reviewer #2 offers a particularly complementary summary of the insights provided by the findings. Each reviewer queries aspects of the in vitro transcription assays presented in Figure 1 that can be addressed by text and figure modifications. Reviewer #1 requests an expanded discussion of why TFIIE/TFIIH does not increase promoter-specific transcription in these assays and requests that a quantitative comparison of specific and non-specific transcripts be provided. Reviewer #2 similarly requests that the low in vitro stimulatory activity of SNAPc be noted and addressed in the text. Reviewer #1 also suggests including a panel showing SNAPc:IIA/IIB interactions in context of the full PIC. Editorially, we agree that these suggestions would strengthen the presentation of the findings and ask that they be included in a revised manuscript.

Note that, within a few days, we will send you detailed instructions for the final revision, along with information on editorial and formatting requirements. We recommend that you do not start revising the manuscript until you receive this additional information.

*****To facilitate our work at this stage, we would appreciate if you could send us the main text as a Word file. Please make sure to copy the NSMB account (cc'ed above).*****

TRANSPARENT PEER REVIEW

Nature Structural & Molecular Biology offers a transparent peer review option for new original research manuscripts submitted from 1st December 2019. We encourage increased transparency in peer review by publishing the reviewer comments, author rebuttal letters and editorial decision letters if the authors agree. Such peer review material is made available as a supplementary peer review file. **Please state in the cover letter 'I wish to participate in transparent peer review' if you want to opt in, or 'I do not wish to participate in transparent peer review' if you don't.** Failure to state your preference will result in delays in accepting your manuscript for publication.

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If you have any questions, please do not hesitate to contact me directly.

With kind regards,

Beth

Beth Moorefield, Ph.D.
Senior Editor
Nature Structural & Molecular Biology

Reviewer #1 (Remarks to the Author):

Rengachari et al present several cryo-EM structures of SNAPc in complex with minimal Pol II preinitiation complex on U1 and U5 snRNA promoters. Using structural data complemented by in vitro transcription assays and EMSA the authors show that SNAPc in complex with minimal PIC allows for DNA melting in the absence of TFIIE and TFIIH. The authors also highlight the role of SNAPc extensions named wing-1 and wing-2 in binding TFIIA and TFIIIB, respectively, and coordinating SNAPc-PIC-promoter interaction. The authors compare open and closed promoter complexes and propose a mechanism of Pol II DNA opening in the absence of TFIIH.

Comments:

TFIIE density is not seen in structures, and this is supported by IVT experiments. However, after sucrose gradient ultracentrifugation it seems that both TFIIE subunits are present in complex with PIC-SNAPc in stoichiometric amounts (Fig 1 panel c). This needs more discussion.

Discuss why adding TFIIE/TFIIH does not increase transcription activity and leads to non-specific transcripts, even though their binding potentially is not restricted in the presented structures.

Extended Data Figure 5: typo, panel d) should be labeled as panel k)

Fig 6. Consider include a panel showing the interactions between SNAP and TFIIA/B in the context of the full complex.

Fig 1e. Show everything relative to PIC (w/o TFIIE/H). Quantitate the percentage of background products.

Ext Fig 6A. If you used a software to generate this figure please cite, otherwise disregard this comments.

In the methods please better define "scoop of DNase I"

Reviewer #2 (Remarks to the Author):

Rengachari et al have resolved cryo-EM structures of the SNAPc-containing Pol II preinitiation complex (PIC) assembled on U1 and U5 snRNA promoters. The structures show that the core of SNAPc binds two turns of DNA and recognizes the snRNA promoter-specific proximal sequence element (PSE) located upstream of the TATA box-binding protein TBP. Interestingly, SNAPc defines the TSS very precisely. The structures also show that two extensions of SNAPc called wing-1 and wing-2 bind TFIIA and TFIIB, respectively, explaining how SNAPc directs Pol II to snRNA promoters. Comparison of structures of closed and open promoter complexes indicate and explain that DNA opening is TFIIF-independent, unlike DNA opening for protein-coding gene initiation.

The results of this tour de force of structural and in vitro transcription analysis clearly provide the structural basis of Pol II initiation at PSE-containing promoters. As such, this is a timely and important piece of work.

I only have one quibble-in Figure 1d there is not a huge increase in transcription when SNAPc is added to the reaction, indicating that the in vitro conditions used are not great for PSE/SNAPc-dependent initiation. The authors should address why this might be.

Decision Letter, final checks:

Our ref: NSMB-A46118

16th May 2022

Dear Dr. Cramer,

Thank you for your patience as we've prepared the guidelines for final submission of your Nature Structural & Molecular Biology manuscript, "Structural basis of SNAPc-dependent snRNA transcription initiation by RNA polymerase II" (NSMB-A46118). Please carefully follow the step-by-step instructions provided in the attached file, and add a response in each row of the table to indicate the changes that you have made. Ensuring that each point is addressed will help to ensure that your revised manuscript can be swiftly handed over to our production team.

We would like to start working on your revised paper, with all of the requested files and forms, as soon as possible (preferably within two weeks). Please get in contact with us if you anticipate delays.

When you upload your final materials, please include a point-by-point response to any remaining reviewer comments.

If you have not done so already, please alert us to any related manuscripts from your group that are under consideration or in press at other journals, or are being written up for submission to other journals (see: <https://www.nature.com/nature-research/editorial-policies/plagiarism#policy-on-duplicate-publication> for details).

In recognition of the time and expertise our reviewers provide to Nature Structural & Molecular Biology's editorial process, we would like to formally acknowledge their contribution to the external peer review of your manuscript entitled "Structural basis of SNAPc-dependent snRNA transcription initiation by RNA polymerase II". For those reviewers who give their assent, we will be publishing their names alongside the published article.

Nature Structural & Molecular Biology offers a Transparent Peer Review option for new original research manuscripts submitted after December 1st, 2019. As part of this initiative, we encourage our authors to support increased transparency into the peer review process by agreeing to have the reviewer comments, author rebuttal letters, and editorial decision letters published as a Supplementary item. When you submit your final files please clearly state in your cover letter whether or not you would like to participate in this initiative. Please note that failure to state your preference will result in delays in accepting your manuscript for publication.

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We accept TIFF, JPEG, PNG or PSD file formats (a layered PSD file would be ideal), and the image should be at least 300ppi resolution (preferably 600-1200 ppi), in CMYK colour mode.

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Please submit your suggestions, clearly labeled, along with your final files. We'll be in touch if more information is needed.

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Please use the following link for uploading these materials:
[Redacted]

If you have any further questions, please feel free to contact me.

Best regards,

Sophia Frank
Editorial Assistant
Nature Structural & Molecular Biology
nsmb@us.nature.com

On behalf of

Carolina Perdigoto, PhD
 Chief Editor
 Nature Structural & Molecular Biology
orcid.org/0000-0002-5783-7106

Reviewer #1:

Remarks to the Author:

Rengachari et al present several cryo-EM structures of SNAPc in complex with minimal Pol II preinitiation complex on U1 and U5 snRNA promoters. Using structural data complemented by in vitro transcription assays and EMSA the authors show that SNAPc in complex with minimal PIC allows for DNA melting in the absence of TFIIE and TFIIH. The authors also highlight the role of SNAPc extensions named wing-1 and wing-2 in binding TFIIA and TFIIB, respectively, and coordinating SNAPc-PIC-promoter interaction. The authors compare open and closed promoter complexes and propose a mechanism of Pol II DNA opening in the absence of TFIIH.

Comments:

TFIIE density is not seen in structures, and this is supported by IVT experiments. However, after sucrose gradient ultracentrifugation it seems that both TFIIE subunits are present in complex with PIC-SNAPc in stoichiometric amounts (Fig 1 panel c). This needs more discussion.

Discuss why adding TFIIE/TFIIH does not increase transcription activity and leads to non-specific transcripts, even though their binding potentially is not restricted in the presented structures.

Extended Data Figure 5: typo, panel d) should be labeled as panel k)

Fig 6. Consider include a panel showing the interactions between SNAP and TFIIA/B in the context of the full complex.

Fig 1e. Show everything relative to PIC (w/o TFIIE/H). Quantitate the percentage of background products.

Ext Fig 6A. If you used a software to generate this figure please cite, otherwise disregard this comments.

In the methods please better define "scoop of DNase I"

Reviewer #2:

Remarks to the Author:

Rengachari et al have resolved cryo-EM structures of the SNAPc-containing Pol II preinitiation complex (PIC) assembled on U1 and U5 snRNA promoters. The structures show that the core of SNAPc binds two turns of DNA and recognizes the snRNA promoter-specific proximal sequence element (PSE) located upstream of the TATA box-binding protein TBP. Interestingly, SNAPc defines the TSS very precisely. The structures also show that two extensions of SNAPc called wing-1 and wing-2 bind TFIIA and TFIIB, respectively, explaining how SNAPc directs Pol II to snRNA promoters. Comparison of

structures of closed and open promoter complexes indicate and explain that DNA opening is TFIIF-independent, unlike DNA opening for protein-coding gene initiation.

The results of this tour de force of structural and in vitro transcription analysis clearly provide the structural basis of Pol II initiation at PSE-containing promoters. As such, this is a timely and important piece of work.

I only have one quibble—in Figure 1d there is not a huge increase in transcription when SNAPc is added to the reaction, indicating that the in vitro conditions used are not great for PSE/SNAPc-dependent initiation. The authors should address why this might be.

Author Rebuttal, First Revision:

Detailed list of responses to reviewer comments

NSMB-A46118

Responses are in blue italics

Referees' comments:

Reviewer #1 (Remarks to the Author):

Rengachari et al present several cryo-EM structures of SNAPc in complex with minimal Pol II preinitiation complex on U1 and U5 snRNA promoters. Using structural data complemented by in vitro transcription assays and EMSA the authors show that SNAPc in complex with minimal PIC allows for DNA melting in the absence of TFIIE and TFIIF. The authors also highlight the role of SNAPc extensions named wing-1 and wing-2 in binding TFIIA and TFIIB, respectively, and coordinating SNAPc-PIC-promoter interaction. The authors compare open and closed promoter complexes and propose a mechanism of Pol II DNA opening in the absence of TFIIF.

We thank the reviewer for their work. We have addressed the minor issues as described below.

TFIIE density is not seen in structures, and this is supported by IVT experiments. However, after sucrose gradient ultracentrifugation it seems that both TFIIE subunits are present in complex with PIC-SNAPc in stoichiometric amounts (Fig 1 panel c). This needs more discussion.

The approximate molecular weight of the Pol II PIC containing either SNAPc or TFIIE are similar. The resolution of the sucrose gradient is not high enough to distinguish between these two variants of the PIC and hence they co-migrate and are collected in the same gradient fractions. We observed this very early in our cryoEM data processing steps, where the PIC containing SNAPc or TFIIE separate as distinct sets of particles. The revised version of the manuscript includes a statement explaining this better.

Discuss why adding TFIIE/TFIIH does not increase transcription activity and leads to non-specific transcripts, even though their binding potentially is not restricted in the presented structures.

The exacerbated turnover of non-specific products evident for the PIC assembly containing both TFIIE and TFIIH are also mildly observed across the entire IVT experiment. This suggests the presence of a few putative non-specific binding events and start sites for Pol II PIC in the IVT template. In the absence of TFIIE and TFIIH, promoter opening and snRNA transcription occurs preferentially from the original PSE-directed start site. Once TFIIE and TFIIH are present, they appear to over-ride this start site preference because the sophisticated translocase machinery of TFIIH can enable opening of all the ungainly start sites. To a lesser extent, TFIIE alone is able to generate a similar effect, as has been observed in the yeast system (Plaschka, Hantse et al 2016, Dienemann et al 2019), on promoters with high meltability. A statement addressing this has been included in the updated version of the manuscript. With respect to the second point, TFIIE and TFIIH are not occluded sterically.

Extended Data Figure 5: typo, panel d) should be labeled as panel k)

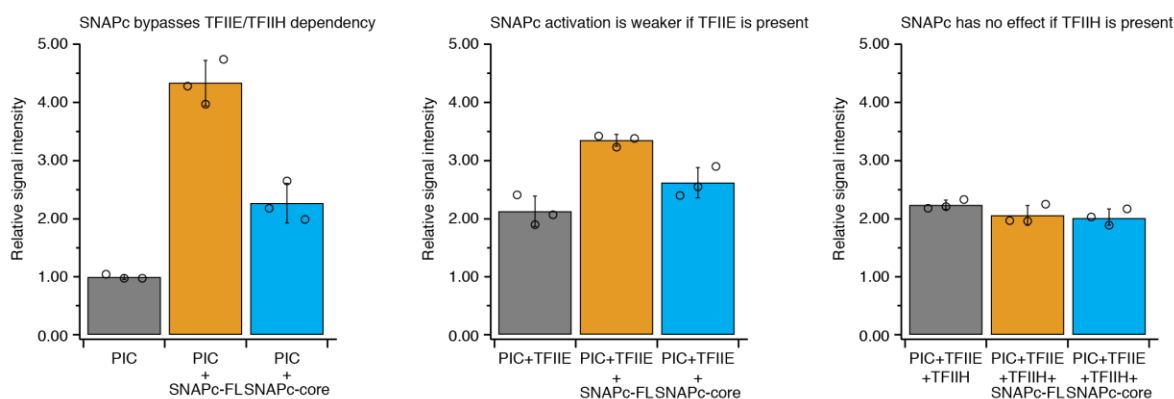
The updated Extended Data Figure 5 has the suggested change incorporated.

Fig 6. Consider include a panel showing the interactions between SNAP and TFIIA/B in the context of the full complex.

This fine suggestion can help the reader with the bigger picture. SNAPC-TFIIA/TFIIB interaction belongs to Figure 5 and it has been updated accordingly.

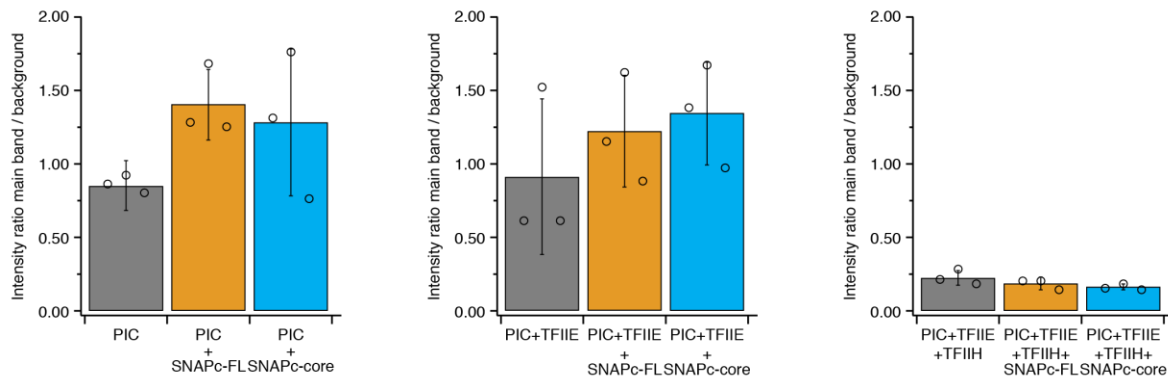
Fig 1e. Show everything relative to PIC (w/o TFIIH). Quantitate the percentage of background products.

To address the reviewer's concern, we have prepared a version of Fig. 1e in which all bars are shown relative to PIC (w/o TFIIH) (see below). However, we refrain from the use of this version as it may confuse the reader and distract from the original message of the figure, which is to allude to the stimulatory effect of SNAPc on transcription.



We have quantified the background products and present the ratio between the main transcript and the background products in another separate figure (see below). There are two major observations. First, background transcription (all transcription that does not result in production of the main transcript) is increased by 4-7-fold in the presence of TFIIH. This points to the effectiveness of TFIIH in inducing DNA opening and transcription initiation also at such promoter sites as discussed above. Second, for the samples without TFIIH, background transcription is decreased for SNAPc-containing samples. This reflects

the ability of SNAPc to direct the Pol II machinery to the preferred TSS within the promoter sequence and assist in DNA opening at that position as discussed in the manuscript.



However, we would like to note that due to the nature of the experiment, which relies on quantification of bands from different urea-PAGE gels, the assignment of a defined baseline can vary between gels. Whereas this has no significant effect on quantification of a defined single band in the gel (such as the main transcript), this effect is much stronger for quantification of the background signal, which is the sum of all additional signal from a respective lane in the gel. This inconsistency is reflected in larger error bars. In order not to confuse the reader we would suggest not to include the background quantification figure in the manuscript. We also believe that it does not add any additional insights that are not obvious from the gel image itself. To this end, we have included a statement mentioning the 4-7-fold increased background signal in the presence of TFIIE in the legend for Figure 1e.

Ext Fig 6A. If you used a software to generate this figure please cite, otherwise disregard this comments.

We have cited all the software used for the generation of Ext Fig 6 in the methods section.

In the methods please better define "scoop of DNase I"

We have changed the text in the methods.

Reviewer #2 (Remarks to the Author):

Rengachari et al have resolved cryo-EM structures of the SNAPc-containing Pol II preinitiation complex (PIC) assembled on U1 and U5 snRNA promoters. The structures show that the core of SNAPc binds two turns of DNA and recognizes the snRNA promoter-specific proximal sequence element (PSE) located upstream of the TATA box-binding protein TBP. Interestingly, SNAPc defines the TSS very precisely. The structures also show that two extensions of SNAPc called wing-1 and wing-2 bind TFIIA and TFIIB, respectively, explaining how SNAPc directs Pol II to snRNA promoters. Comparison of structures of closed and open promoter complexes indicate and explain that DNA opening is TFIIF-independent, unlike DNA opening for protein-coding gene initiation.

The results of this tour de force of structural and in vitro transcription analysis clearly provide the structural basis of Pol II initiation at PSE-containing promoters. As such, this is a timely and important piece of work.

We thank the reviewer for a very positive assessment of our work.

I only have one quibble-in Figure 1d there is not a huge increase in transcription when SNAPc is added to the reaction, indicating that the in vitro conditions used are not great for PSE/SNAPc-dependent initiation. The authors should address why this might be.

Although SNAPc does not induce transcription in the presence of TFIIIE and TFIIH, there is a pronounced increase in transcript formation in the absence of these two factors, especially for SNAPc-FL (Figure 1d, 1e). The effect observed in the presence of TFIIIE and TFIIH could be the result of non-specific initiation (please see our response to reviewer #1). The effect of TFIIIE alone increasing transcription of PIC has been well documented also in Pol II mRNA transcription on promoters with high meltability (Plaschka, Hantse et al 2016, Dienemann et al 2019). However, the lack of an additive effect of TFIIIE and SNAPc together and the absence of TFIIIE in the cryoEM structures, supports the non-cooperative role of TFIIIE in Pol II snRNA transcription.

Final Decision Letter:

Dear Dr. Cramer,

We are now happy to accept your revised paper "Structural basis of SNAPc-dependent snRNA transcription initiation by RNA polymerase II" for publication as a Article in Nature Structural & Molecular Biology.

Acceptance is conditional on the manuscript's not being published elsewhere and on there being no announcement of this work to the newspapers, magazines, radio or television until the publication date in Nature Structural & Molecular Biology.

Over the next few weeks, your paper will be copyedited to ensure that it conforms to Nature Structural & Molecular Biology style. Once your paper is typeset, you will receive an email with a link to choose the appropriate publishing options for your paper and our Author Services team will be in touch regarding any additional information that may be required.

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Figure 1-a

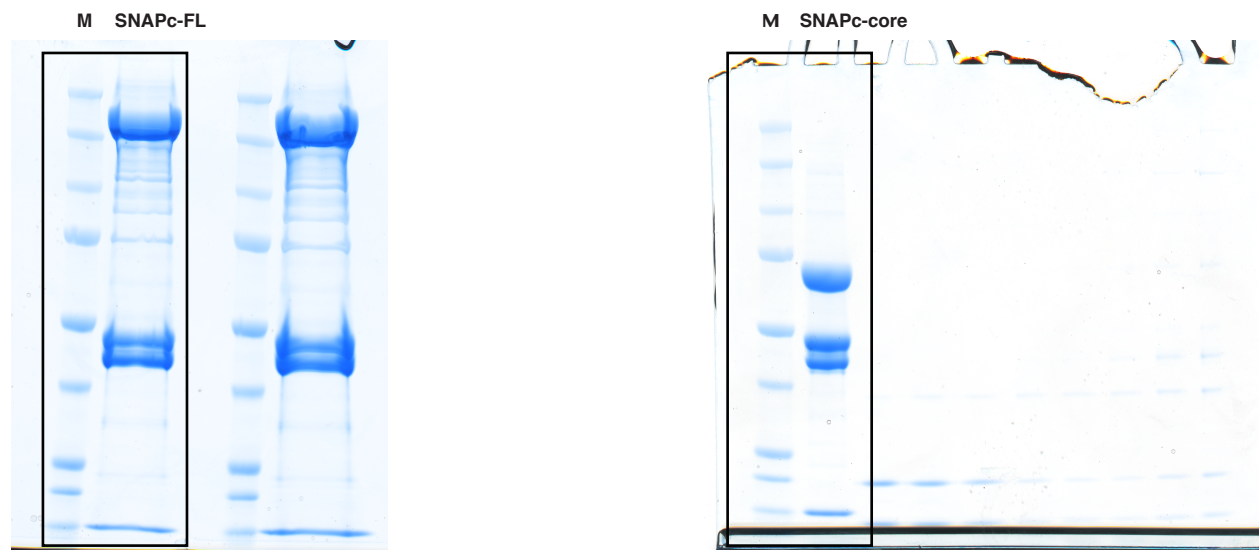
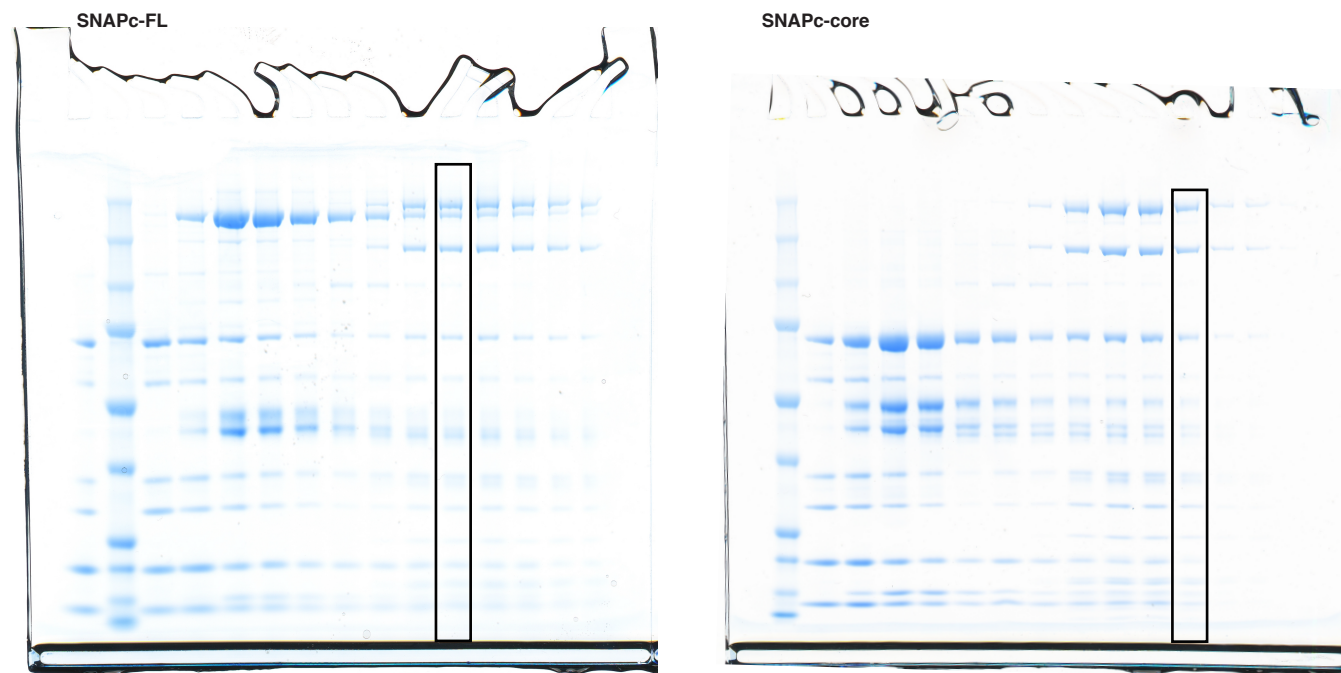
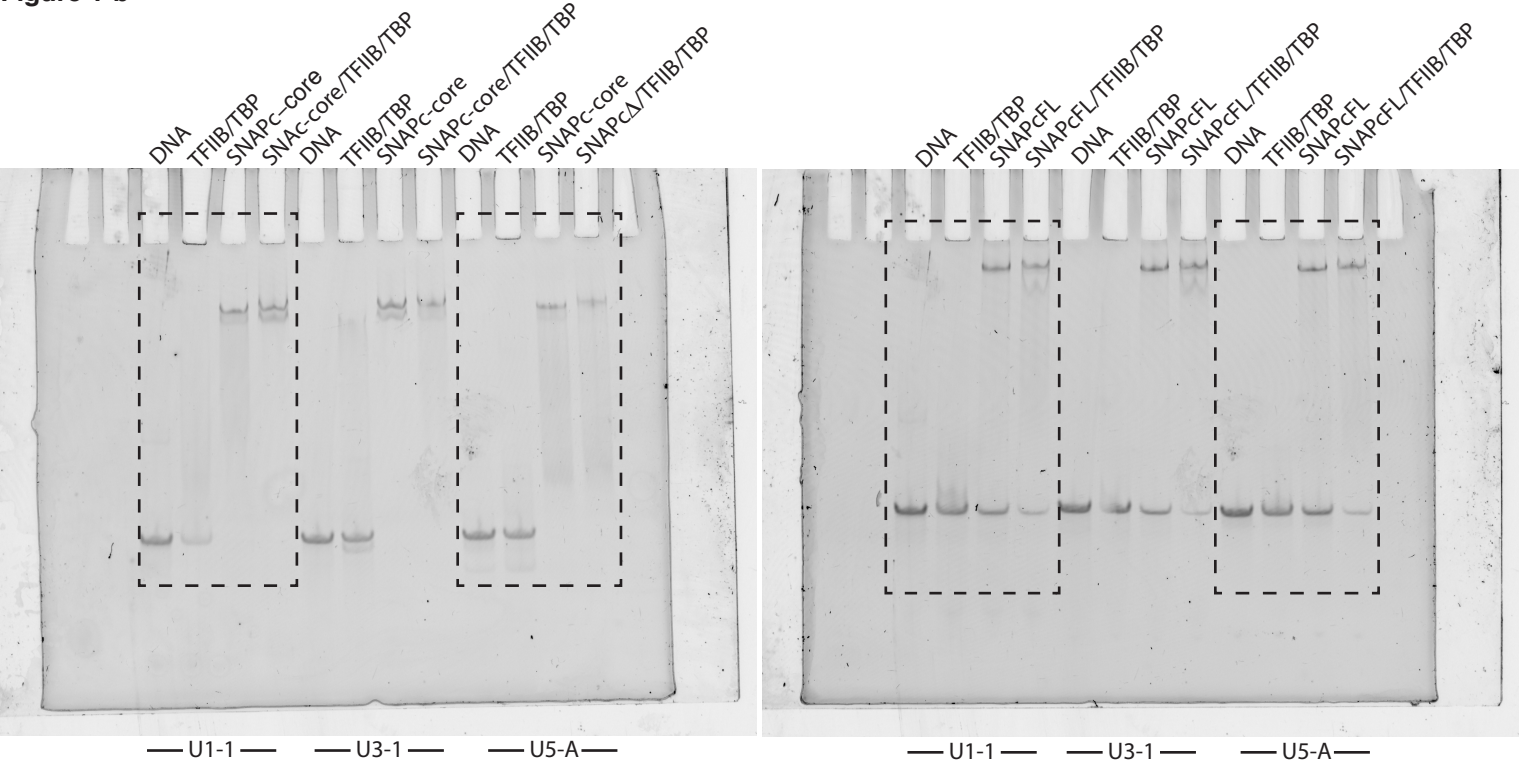


Figure 1-c



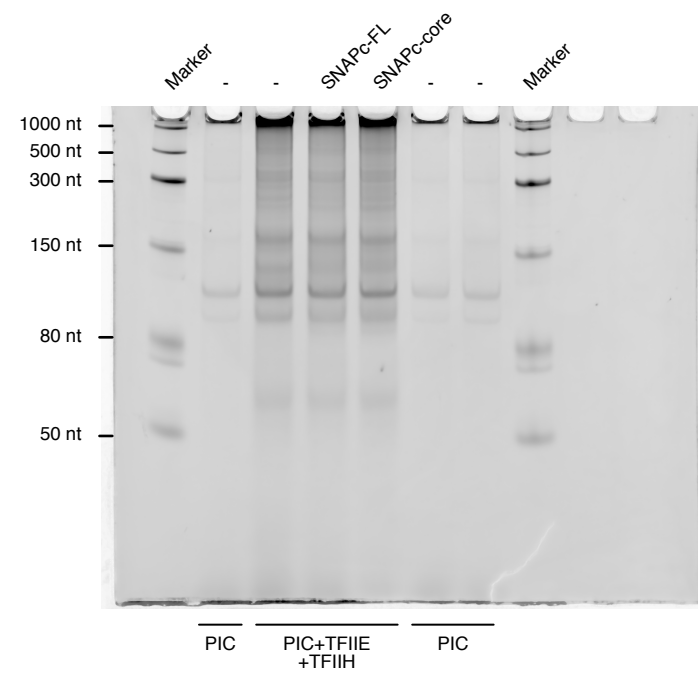
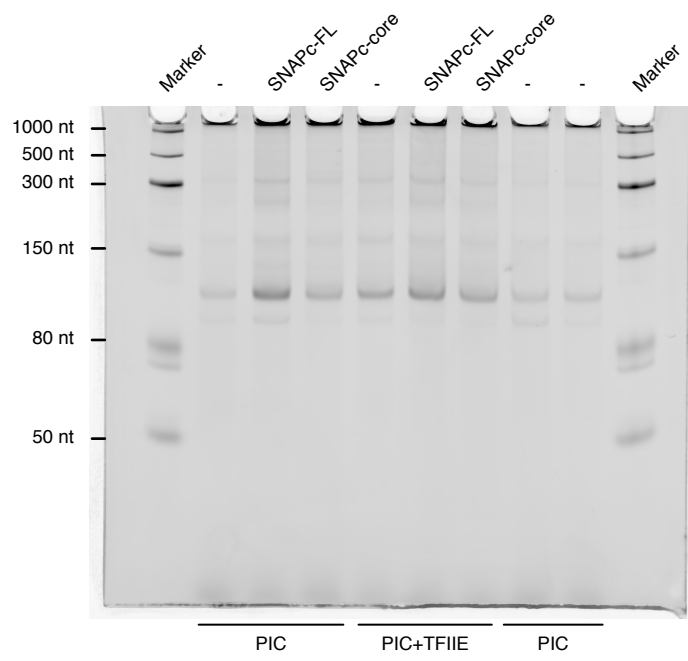
Figures: Source data of SDS_PAGE gels presented in Figures 1a, 1c. Black boxes represent the lane used in the in the respective figure.

Figure 1-b

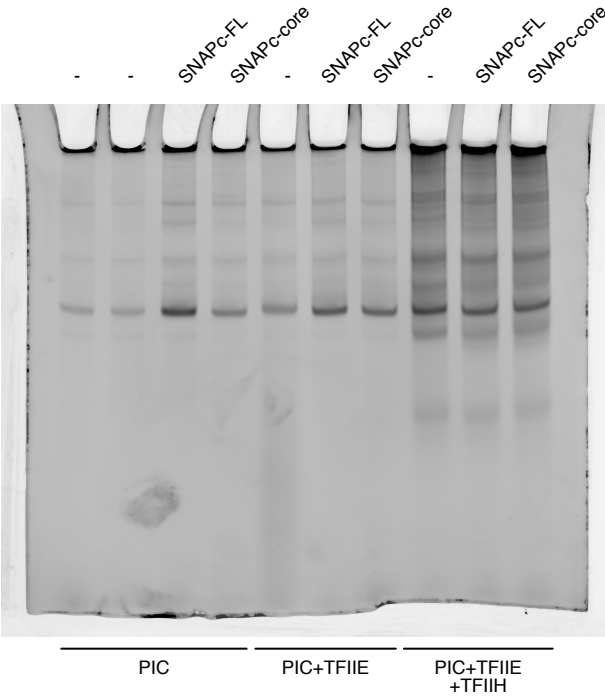


Figures: Source data of SDS_PAGE gels presented in Figures 1b. Dashed boxes represent the lanes used in the in the figure.

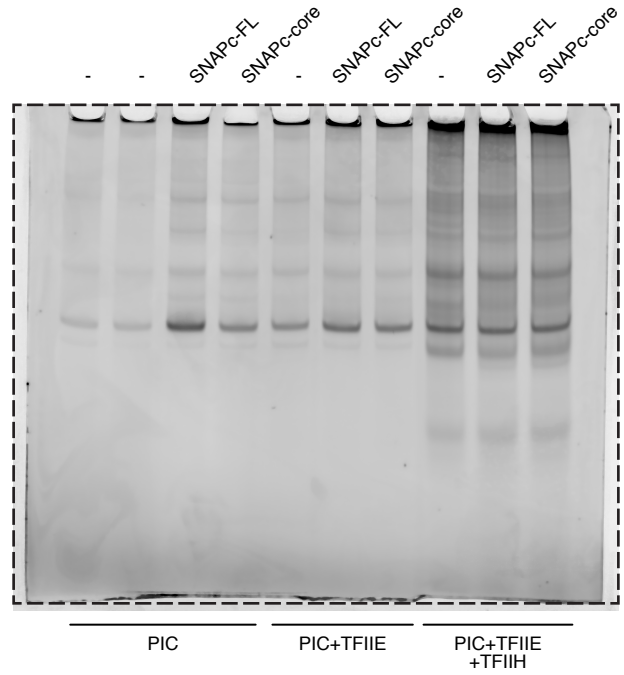
Replicate 1:



Replicate 2:



Replicate 3:



Figures: Source data of Urea-PAGE gels presented in Figures 1d, and the three replicates used for quantification in Figure 1e. Replicate 3 used in Figure 1d is highlighted in a dashed black box.