Data Article

Nuclear magnetic resonance spectral data of the USP7 TRAF and UBL1-2 domains in complex with DNA polymerase \( \iota \) peptides

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**A B S T R A C T**

This data article is related to the publication 'DNA polymerase \( \iota \) interacts with both the TRAF-like and UBL1-2 domains of USP7'' [1]. Ubiquitin-specific protease 7 (USP7) is an essential deubiquitinating enzyme with characterized substrates in many cellular pathways. Established USP7 substrates interact with one of two major binding sites, located on the N-terminal TRAF-like (TRAF) domain and the first and second UBL domains (UBL1-2) within the C-terminal tail. In this article, we present complete nuclear magnetic resonance (NMR) spectroscopy data used to characterize direct interactions between USP7 and its novel substrate DNA polymerase \( \iota \) (Pol \( \iota \)), that binds both TRAF and UBL1-2 domains. The detailed description of the NMR data, and the methodology used for processing and analysis, will add to the reproducibility and transparency of the companion research article, as well as aid in the reuse of these data.

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Specifications Table

| Subject                              | Biological sciences |
|--------------------------------------|---------------------|
| Specific subject area                | Biophysics          |
| Type of data                         | Structural biology  |
| How data were acquired               | NMR spectral data   |
|                                      | NMR chemical shift assignments |
|                                      | Figures             |
| Data format                          | Raw NMR data: Free induction decay (FID) time domain datasets (.fid files) |
|                                      | Scripts             |
|                                      | FID conversion and processing scripts (.com files) |
| Processed NMR data:                  | Fourier transformed (FT) frequency domain datasets (.ft2, .ft3, and .ft4 files) |
| Analyzed NMR data:                   | 1) Backbone chemical shift assignment in BMRB star format (.str files) |
|                                      | 2) \(^1\)H/\(^15\)N HSQC peak lists in SPARKY format (.list files) |
|                                      | 3) Per-residue chemical shift perturbations in text format (.txt) |
| Parameters for data collection       | Prior to all NMR experiments, the respective NMR spectrometer was tuned on hydrogen, nitrogen and carbon channels, the sample was locked using D\(_2\)O, and the magnetic field was shimmed. |
| Description of data collection       | Data for USP7 TRAF domain used for NMR backbone resonance assignment were collected on 800 MHz \(^1\)H Agilent VNMRS spectrometer at 30 °C. |
|                                      | Data for USP7 TRAF- Pol i NMR titration experiments were collected at 500 MHz \(^1\)H Agilent VNMRS spectrometer at 30 °C. |
|                                      | Data for USP7 UBL 1–2- Pol i NMR titration experiments were collected at 800 MHz \(^1\)H Agilent VNMRS spectrometer at 25 °C. |
|                                      | Experimental set parameters for each NMR experiment can be found in the prepro file deposited to BMRbig (accession BMRbig5). |
| Backbone NMR resonance assignment:  | Data for USP7 TRAF domain used for NMR backbone resonance assignment, including standard two-dimensional \(^1\)H-\(^15\)N HSQC and \(^1\)H–\(^13\)C HSQC and three-dimensional HNCA, HNCA CB, HNCO and \(^15\)N-NOESY NMR experiments [5], were collected on the 800 MHz \(^1\)H Agilent VNMRS spectrometer at 30 °C. |
|                                      | The sample of USP7 TRAF domain used for NMR backbone resonance assignment contained 0.9 mM \(^15\)N–\(^13\)C TRAF in a buffer of 20 mM phosphate buffer, pH 7.5, 250 mM NaCl, 10 mM DTT and 10% D\(_2\)O (v/v). |
| USP7 TRAF/Pol i NMR titrations:      | USP7 TRAF was titrated with Pol i peptides containing P/A/Exxs TRAF-binding motifs. In total, four peptides were tested, including Pol i peptides 421–442 (KGLIDYYLMPSLTTTSRC), 491–501 (NEPLCSLPEG), peptide 513–523 (DIQEEILSGKS) and 573–584 (SPCEPTSGFNS), TRAF domain and the peptides were in a buffer containing 20 mM phosphate buffer pH 7.5, 250 mM NaCl, 10 mM DTT and 10% D\(_2\)O (v/v). NMR titration experiments were collected on the 500 MHz \(^1\)H Agilent VNMRS spectrometer at 30 °C. For these experiments, a 3 mM stock of unlabeled peptide was gradually added to 0.25 mM \(^15\)N TRAF to a molar ratio of 1:5 (protein:peptide). \(^1\)H–\(^15\)N HSQC spectrum was collected at each of the six titration points. |
USP7 TRAF/Pol • NMR titrations:

USP7 TRAF was titrated with Pol • peptides containing P/A/ExxS TRAF-binding motifs. In total, four peptides were tested, including Pol • peptides 421–442 (KGLDDYLYMPSLSTTSRGK), 491–501 (NEFPLCSLPEG), peptide 513–523 (DIQEEILSGKS) and 573–584 (SPCETSGFNS). TRAF domain and the peptides were in a buffer containing 20 mM phosphate buffer pH 7.5, 250 mM NaCl, 10 mM DTT and 10% D2O (v/v). NMR titration experiments were collected on the 500 MHz (1H) Agilent VNMR5 spectrometer at 30 °C. For these experiments, a 3 mM stock of unlabeled peptide was gradually added to 0.25 mM 15N TRAF to a molar ratio of 1:5 (protein:peptide). 1H-15N HSQC spectrum was collected at each of the six titration points.

USP7 UBL1-2/Pol • titrations:

USP7 UBL1–2 was titrated with Pol • peptides containing (R/K)xKxxxK UBL 1–2-binding motifs. In total, two peptides were tested, including 438–448 (SGKHSFKMKD) and 523–532 (SREKFQGKGS). UBL 1–2 and the peptides were in a buffer containing 20 mM phosphate buffer pH 7, 100 mM NaCl, 10 mM DTT and 10% D2O (v/v). NMR titration experiments were collected on the 800 MHz (1H) Agilent VNMR5 spectrometer at 25 °C, using a 3 mM stock of unlabeled peptide that was gradually added to 0.25 mM 15N UBL 1–2 to a molar ratio of 1:5 (protein:peptide). 1H-15N HSQC spectrum was collected at each of the six titration points. The raw time domain fid datasets, the processed Fourier transformed datasets, and the peak assignments for all NMR experiments have been deposited to BMRB data bank (accession: 50080) and BMRbig (accession: BMRbig5).

Data source location
Institution: UConn Health
City/Town/Region: Farmington, CT
Country: USA
Latitude and longitude for collected samples/data: Latitude: 41.7300999, Longitude: -72.7909308

Data accessibility
Repository name: Biological Magnetic Resonance Data Bank (BMRB)
Data identification number: 50080
Direct URL to data: https://bmrb.io/
Repository name: BMRbig
Data identification number: BMRbig5
Direct URL to data: https://bmrb.org/released

Related research article
N.W. Ashton, G.J. Valles, N. Jaiswal, I. Bezsonova, R. Woodgate, DNA polymerase • interacts with both the TRAF-like and UBL1-2 domains of USP7. J. Mol. Biol. DOI: 10.1016/j.jmb.2020.166733

Value of the Data

• Data presented here underpin the first biophysical investigation of USP7 interactions with Pol •.
• These data will be of use and importance to researchers studying USP7 substrate-binding, as well as those interested in the regulation of Pol • by post-translation modifiers.
• Our description of the NMR spectra observed here, as well as our detailed description of how these data were generated, will be useful as a reference for researchers studying the interaction of USP7 domains with other substrates.

1. Data Description

The following data were generated using solution NMR spectroscopy to map the binding site of Pol • peptides with the USP7 TRAF domain. All experimental data has been deposited to BMRB data bank (accession: 50080) and BMRbig (accession: BMRbig5).
1.1. USP7 TRAF NMR backbone resonance assignment

The backbone resonance assignment of USP7 TRAF domain was completed using a standard set of NMR experiments including 2D $^1$H-$^{15}$N HSQC and 3D HNCA, HNCACB, HNCO and $^{15}$N-NOESY [5]. The assigned $^1$H-$^{15}$N HSQC spectrum is shown in Fig. 1.

The deposited $^1$H-$^{15}$N HSQC data contains everything necessary for reconstruction of the spectrum shown in Fig. 1, including the FID file (HSQCfid), the processing scripts (TRAF-hsqc-conversion-script-fid.com and TRAF-hsqc-processingscript-nmr_ft.com), and the resulting Fourier transformed dataset (15Nhsqc-TRAF.ft). The $^1$H-$^{15}$N HSQC peak list in SPARKY [4] format can be found in the FINAL-15Nhsqc-TRAF.list file. The TRAFusp7bh.str file contains NMR backbone chemical shift assignment of the USP7 TRAF domain in BMRB NMR-STAR format.

The deposited HNCA data includes the raw time domain dataset (HNCAfid) of USP7 TRAF domain, the scripts used to process the data (TRAF-HNCA-conversionscript-fid.com and TRAF-HNCA-processingscript-nmr_ft.com), and the resulting Fourier transformed processed spectrum (HNCA-TRAF.ft3). The FINAL-HNCA-TRAF.list is the HNCA peak list in SPARKY [4] format.

The deposited HNCACB data include the time domain dataset (HNCACBfid) of TRAF domain, the scripts used to process the data (TRAF-HNCACB-conversionscript-fid.com and TRAF-HNCACB-processingscript-nmr_ft.com), and the resulting processed spectrum (HNCACB.ft3). The FINAL-HNCACB.list is the HNCACB peak list in SPARKY [4] format.

The deposited HNCO data include the time domain dataset (HNCOfid), the scripts used to process the data (TRAF-HNCO-conversionscript-fid.com and TRAF-HNCO-processingscript-nmr_ft.com), and the resulting processed HNCO spectrum (HNCO-TRAF.ft3). The FINAL-HNCO-TRAF.list file is the HNCO peak list in SPARKY [4] format.

The deposited $^{15}$N-NOESY data include the time domain dataset (NOESYfid), the scripts used to process the data (TRAF-NOESY-conversionscript-fid.com and TRAF-NOESY-processingscript-nmr_ft.com), and the resulting processed $^{15}$N-NOESY spectrum (Noesy-traf-3D.ft4).
1.2. NMR titrations of USP7 TRAF with Pol ι peptides

The molecular interactions between Pol ι and USP7 TRAF domain were characterized using NMR chemical shift perturbation experiments, where 15N-labeled USP7 TRAF domain was titrated with increasing concentrations of Pol ι peptides up to a 1:5 molar ratio of protein to peptide. These Pol ι peptides include 421–442 (KGLIDYYLMPSLSTTSRSGK), 491–501 (NEFPLCSLPEG), 513–523 (DIQEEILSGKS) and 573–584 (SPCEPGTSGFNS). Analyzed data resulting from USP7 TRAF binding to Pol ι peptide 573–584, as well as models generated from these data, are presented in Fig. 5 of our companion article [1].

All data necessary to reproduce 1H-15N HSQC spectra of USP7 TRAF domain alone and in presence Pol ι peptides are deposited to BMRB data bank (accession: 50080) and the BMRbig database (accession: BMRbig5). The data include FIDs, scripts for processing the raw data and the resulting processed spectra in NMRPipe format as described below.

**Free TRAF domain:**

The **USP7TRAFdomainfree-fid** is the 1H-15N HSQC FID of the free TRAF domain, **TRAF-titration hsqc-conversionscript-fid.com** and the **TRAF-titration-processingscript-hsqc.com** are scripts for processing the raw data, and the **USP7TRAF-free.ft2** is the resulting processed 1H-15N HSQC spectrum. The **USP7TRAF.list** is the corresponding peak list in SPARKY [4] format.

**USP7 TRAF domain in complex with Pol ι peptide 573–584:**

The **USP7TRAFxPolipeptide573–584_1 × 5-fid** data file is the 1H-15N HSQC FID of USP7 TRAF domain in complex with Pol ι peptide 573–584 (1:5 protein to peptide molar ratio). The **TRAFxPolipeptide573–584-conversionscript-fid.com** and the **TRAFxPolipeptide573–584-processingscript-hsqc.com** are scripts used to process the raw data, and the **USP7TRAFxPolipeptide573–584_1 × 5.ft2** is the resulting processed 1H-15N HSQC spectrum. The **TRAFpoliota573–584_complex.list** is the corresponding peak list in SPARKY [4] format. **USP7TRAF-like_domain_DNApolymeraselota573–584complex.str** is the NMR chemical shift assignment of TRAF domain in complex with Pol ι 573–584 in BMRB format. **Fig. 2**
USP7 TRAF domain in complex with Pol \( \tau \) peptide 421–440:

The USP7TRAFxPolipeptide421–440_1 \times 5-fid is the \(^{1}H\)-\(^{15}N\) HSQC FID of USP7 TRAF domain in complex with Pol \( \tau \) peptide 421–440 (1:5 protein to peptide molar ratio). The TRAFxPolipeptide421–440-conversionscript-fid.com and the TRAFxPolipeptide421–440-processingscript-hsqc.com are scripts used to process raw data, and the USP7TRAFxPolipeptide421–440_1 \times 5.ft2 is the resulting 2D \(^{1}H\)-\(^{15}N\) HSQC spectrum. TRAFxPolipeptide421–440 list is the corresponding peak list in SPARKY [4] format. Fig. 3 displays an overlay of free and 421–440 peptide-bound \(^{1}H\)-\(^{15}N\) HSQC spectra of the TRAF domain. No chemical shift perturbations were observed.

USP7 TRAF domain in complex with Pol \( \tau \) peptide 491–501:

The USP7TRAFxPolipeptide491–501_1 \times 5-fid is \(^{1}H\)-\(^{15}N\) HSQC FID of USP7 TRAF domain in complex with Pol \( \tau \) peptide 491–501 (1:5 protein:peptide molar ratio). The TRAFxPolipeptide491–501-conversionscript-fid.com and the TRAFxPolipeptide491–501-processingscript-hsqc.com are scripts used to process the raw data, and the USP7TRAFxPolipeptide491–501_1 \times 5.ft2 is the resulting 2D \(^{1}H\)-\(^{15}N\) HSQC spectrum. Fig. 4 shows an overlay of free and 491–501 peptide-bound \(^{1}H\)-\(^{15}N\) HSQC spectra of the TRAF domain. No chemical shift perturbations were observed.

USP7 TRAF domain in complex with Pol \( \tau \) peptide 513–523:

The USP7TRAFxPolipeptide513–523_1 \times 5-fid is \(^{1}H\)-\(^{15}N\) HSQC FID of USP7 TRAF domain in complex with DNA Pol \( \tau \) 513–523 (1:5 protein to peptide molar ratio). The TRAFxPolipeptide513–523-conversionscript-fid.com and the TRAFxPolipeptide513–523-processingscript-hsqc.com are the scripts used for processing raw data, and USP7TRAFxPolipeptide513–523_1 \times 5.ft2 is the resulting processed \(^{1}H\)-\(^{15}N\) HSQC spectrum. Fig. 5 shows an overlay of free and 513–523 peptide-bound \(^{1}H\)-\(^{15}N\) HSQC spectra of the TRAF domain. No chemical shift perturbations were observed.

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**Fig. 3.** NMR spectra of the USP7 TRAF domain alone and in complex with Pol \( \tau \) peptide 421- KGLIDYLYMP- SLSTTSRSGK – 440. Overlay of \(^{1}H\)-\(^{15}N\) HSQC spectra of free \(^{15}N\) TRAF (black) and \(^{15}N\) TRAF in complex with unlabeled Pol \( \tau \) 421–440 (blue) at a 1:5 protein to peptide molar ratio.
To test molecular interactions between Pol ι and USP7 UBL 1–2 domains, NMR chemical shift perturbation experiments were used. 15N-labeled USP7 UBL 1–2 domain was titrated with increasing concentrations of unlabeled Pol ι peptides up to a 1:5 molar ratio of protein to peptide. The examined Pol ι peptides included Pol ι 438–448 (SGKHSFKMKT) and Pol ι 523–532 (SREKFQGKGS). All data necessary to reproduce the 1H–15N HSQC spectra of USP7 UBL1-2 alone and in the presence of Pol ι peptides are deposited to BMRB data bank (accession: 50080) and the BM-Rbig database (accession: BMRbig5). The data include FIDs, scripts for processing the raw data and the resulting processed spectra in NMRPipe format. Chemical shift perturbation analysis of
The USP7 served displays data, UBL12xPolipeptide438–448-processingscript-nmr_ft.com protein USP7 438–448 converted to a molar ratio of 1:5 (protein to peptide). USP7 UBL 1-2 binding to Pol ι peptide 438–448, as well as structural models of the complex generated from these data, are presented in Fig. 7 of our companion article [1]. NMR data generated from USP7 UBL1–2 binding to Pol ι peptide 523 – 532, do not appear in the companion article. The following data files were deposited in the BMRbig database (accession: BMRbig5):

Free UBL1-2:
- The UBL12-free-fid file contains the $^1$H-$^1$N HSQC FID of USP7 UBL 1–2 domains. The UBL12-conversionsscript-fid.com and the UBL12processingsscript-nmr_ft.com are the scripts used for processing raw data, and 15nUBL12_freepH7.5_25c.ft2 file is the resulting processed $^1$H-$^1$N HSQC spectrum. UBL12-free.list is the corresponding peak list in SPARKY [4] format. The NMR backbone resonance assignment of UBL1-2 has been previously reported [6] (BMRB accession: 26782).

USP7 UBL 1–2 in complex with Pol ι peptide 438–448:
- The UBL12xPolipeptide438–448_1 × 5-fid file is the $^1$H-$^1$N HSQC FID of $^15$N-labeled USP7 UBL 1–2 domains in complex with unlabeled Pol ι peptide 438–448 at a 1:5 protein to peptide molar ratio. The UBL12xPolipeptide438–448-conversionsscript-fid.com and the UBL12xPolipeptide438–448-processingscript-nmr_ft.com are scripts used for processing raw data, and UBL12xPolipeptide438–448_1 × 5.ft2 is the resulting processed $^1$H-$^1$N HSQC spectrum. UBL12xPolipeptide438–448.list is the corresponding peak list in SPARKY [4] format. Fig. 6 displays an overlay of free and 438–448 peptide-bound $^1$H-$^1$N HSQC spectra of UBL1–2. The observed chemical shift perturbations are analyzed in our companion article [1].

USP7 UBL 1–2 in complex with Pol ι peptide 523–532:
- The UBL12xPolipeptide523–532_1 × 5-fid file is the $^1$H-$^1$N HSQC FID of $^15$N-labeled USP7 UBL 1–2 domains in the presence of 5 molar excess of Pol ι peptide 523–532. The UBL12xPolipeptide523–532-conversionsscript-fid.com and the UBL12xPolipeptide523–532-processingscript-nmr_ft.com are the scripts used for processing raw data, and UBL12xPolipeptide523–532_1 × 5.ft2 is the resulting processed $^1$H-$^1$N HSQC spectrum. Fig. 7

Fig. 6. NMR spectra of the USP7 UBL1-2 domains alone and in complex with Pol ι peptide 438 - SGKHSFKMKDT – 448. Overlay of $^1$H-$^1$N HSQC spectra of free $^15$N-UBL 1-2 (black) and $^15$N-UBL 1-2 in the presence of unlabeled Pol ι 438–448 (red) at a molar ratio of 1:5 (protein to peptide).
Fig. 7. NMR spectra of the USP7 UBL 1-2 domains alone and in complex with Pol i peptide 523- SREKFQGKGS - 532. Overlay of $^1$H-$^{15}$N HSQC spectra of free $^{15}$N-UBL 1-2 (black) and $^{15}$N-UBL 1-2 in the presence of unlabeled Pol i 523–532 (red) at a molar ratio of 1:5 (protein to peptide).

shows an overlay of free and 523–532 peptide-bound $^1$H-$^{15}$N HSCQ spectra of UBL1–2. No chemical shift perturbations were observed.

2. Experimental Design, Materials and Methods

Methodology for the purification of the recombinant USP7 TRAF and UBL1–2 domains, as well the preparation of peptides, are described in our companion publication [1]. Experimental set up parameters for each NMR experiment can be found in the procpar files deposited to BMRbig (accession: BMRbig5). The conversion and processing scripts for data processing that were described in the Data Description section were deposited to the BMRbig database (accession: BMRbig5). Here, we present a detailed methodology for NMR assignment and titration experiments.

2.1. NMR assignment for USP7 TRAF domain

The sample of USP7 TRAF domain used for NMR backbone resonance assignment contained 0.9 mM $^{15}$N-$^{13}$C TRAF in a buffer of 20 mM phosphate buffer, pH 7.5, 250 mM NaCl, 10 mM DTT and 10% D$_2$O (v/v). Standard two-dimensional $^1$H-$^{15}$N HSQC and $^1$H–$^{13}$C HSQC and three-dimensional HNCA, HNCACB, HNCO and $^{15}$N-NOESY NMR experiments [5] were collected on 800 MHz ($^1$H) Agilent VNMRS spectrometer at 30°C. Data was processed using NMRPipe [3],
analyzed with SPARKY [4] and deposited to BMRB data bank (accession: 50080) and BMRbig (accession: BMRbig5).

2.2. USP7 TRAF-Pol \(\iota\) NMR titration experiments

All \(^{15}\)N USP7 TRAF NMR titration experiments were collected at 500 MHz \(^1\)H Agilent VNMR spectrometer at 30 °C. To test TRAF binding to Pol \(\iota\) peptides containing P/A/ExxS motif, a 3 mM stock of unlabeled peptide was gradually added to 0.25 mM \(^1\)H-\(^{15}\)N TRAF to a molar ratio of 1:5 (protein to peptide). \(^1\)H-\(^{15}\)N HSQC spectrum was collected at each of the six titration points. TRAF domain and the peptides were in a buffer containing 20 mM phosphate buffer pH 7.5, 250 mM NaCl, 10 mM DTT and 10% D\(_2\)O (v/v).

Observed NMR frequency perturbations for each amino acid residue during the course of titration were calculated using Eq. (1):

\[
\Delta \omega_{\text{obs}} = (\Delta \omega_N^2 + \Delta \omega_H^2)^{1/2}
\]

where \(\Delta \omega_N\) and \(\Delta \omega_H\) are the frequency differences between free and peptide-bound samples measured in Hz for \(^{15}\)N and \(^1\)H, respectively. The frequency perturbations were converted to chemical shift perturbations by dividing \(\Delta \omega_{\text{obs}}\) by \(^1\)H frequency of the spectrometer.

2.3. USP7 UBL1-2-Pol \(\iota\) NMR titration experiments

All \(^{15}\)N USP7 UBL1-2 NMR titration experiments were collected at 800 MHz \(^1\)H Agilent VNMR spectrometer at 25 °C. To test UBL1-2 binding to Pol \(\iota\) peptides containing forward and reverse KxxxKxxK motifs, a 3 mM stock of unlabeled peptide was gradually added to 0.25 mM \(^{15}\)N UBL 1-2 to a molar ratio of 1:5 (protein:peptide). \(^1\)H-\(^{15}\)N HSQC spectrum was collected for each of the six titration points. Both UBL 1-2 and the peptides were in a buffer containing 20 mM phosphate buffer pH 7, 100 mM NaCl, 10 mM DTT and 10% D\(_2\)O (v/v).

NMR backbone resonance assignment for USP7 UBL1-2 was transferred from previously reported [6] (BMRB accession: 26782). The observed frequency perturbations for each amino acid residue during the titration were calculated as described in Eq. (1).

CRediT Author Statement

Gabrielle J. Valles: Methodology, Investigation, Formal analysis, Visualization, Writing - original draft. Nicholas W. Ashton: Conceptualization, Visualization, Writing - original draft. Nancy Jaiswal: Validation, Investigation. Roger Woodgate: Writing - review & editing, Project administration, Funding acquisition. Irina Bezsonova: Conceptualization, Writing - review & editing, Visualization, Supervision, Project administration, Funding acquisition.

Declaration of Competing Interest

The authors declare that they have no known competing financial interests or personal relationships which have or could be perceived to have influenced the work reported in this article.

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and TRAF plasmids and Dmitry Korzhnev (UConn Health) for his help with NMR experiments. This work was supported by funds from the National Institute of Child Health and Human Development (NICHD)/National Institutes of Health (NIH) Intramural Research Program [to RW], as well by NIH grants [R35 GM128864, P41 GM111135 and R01 GM123249 to IB] and funding from the National Science Foundation [NSF 1616184 to IB]. Funding for open access charge: NICHD/NIH Intramural Research Program.

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