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Supporting information for article:

Crystallization of and selenomethionine phasing strategy for a SETMAR–DNA complex

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Supporting Information

Table S1: Primers for site directed mutagenesis

Name	Primer	Sequence (5' – 3')
C381R	Forward	GCAGTGGTGGTTCAAGAAGTTTCGCAAAGGAGATG
	Reverse	CATCTCCTTTGCGAAACTTCTTGAACCACCACTGC
C381S	Forward	GCAGTGGTGGTTCAAGAAGTTTAGCAAAGGAGATG
	Reverse	CATCTCCTTTGCTAAACTTCTTGAACCACCACTGC
L343M	Forward	AAGCAAATTCGAGCAATTTTCATGTTCGAGTTCAAAATGGGTCGT
	Reverse	ACGACCCATTTTGAACTCGAACATGAAAATTGCTCGAATTTGCTT
I359M	Forward	CAGAAACAACTCGCAACATGAACAATGCATTTGGCC
	Reverse	GGCCAAATGCATTGTTCATGTTGCGAGTTGTTTCTG
L404M	Forward	GAAGTTGACAACGACCAGAT GAGAGCAATCATCGAAG
	Reverse	CTTCGATGATTGCTCTCATCTGGTCGTTGTCAACTTC
L423M	Forward	CACGAGAAGTTGCTGAAGAAATGAATGTCAACCATTCTACGGT
	Reverse	ACCGTAGAATGGTTGACATTCATTTCTTCAGCAACTTCTCGTG

Forward and reverse oligonucleotides for each site were used in the same PCR reaction.

Underlined bases represent the mutated codon.

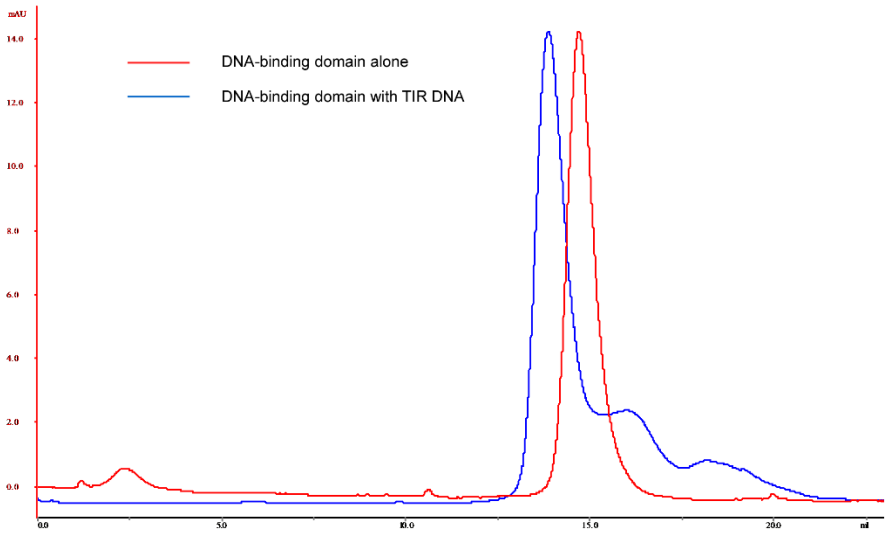

Figure S1. Elution profiles at 280 nm are shown for SETMAR DBD (red) and SETMAR DBD complexed with a 25-mer TIR DNA duplex (blue). For this experiment, 50 μL of 50 μM SETMAR DBD in the presence and absence of 50 μM TIR DNA (25-mer duplex, 5'-AACCGCAATTACTTTTGCACCAACC and complementary strand) was loaded on a Superdex 200, 10/300 GL column buffered in 50 mM MES pH 6.5, 150 mM NaCl, 1 mM DTT. The protein alone eluted at 14.7 ml as a single peak (red), while its complex with DNA eluted as a similar sized peak at 13.9 ml (blue), consistent with a higher molecular weight complex.

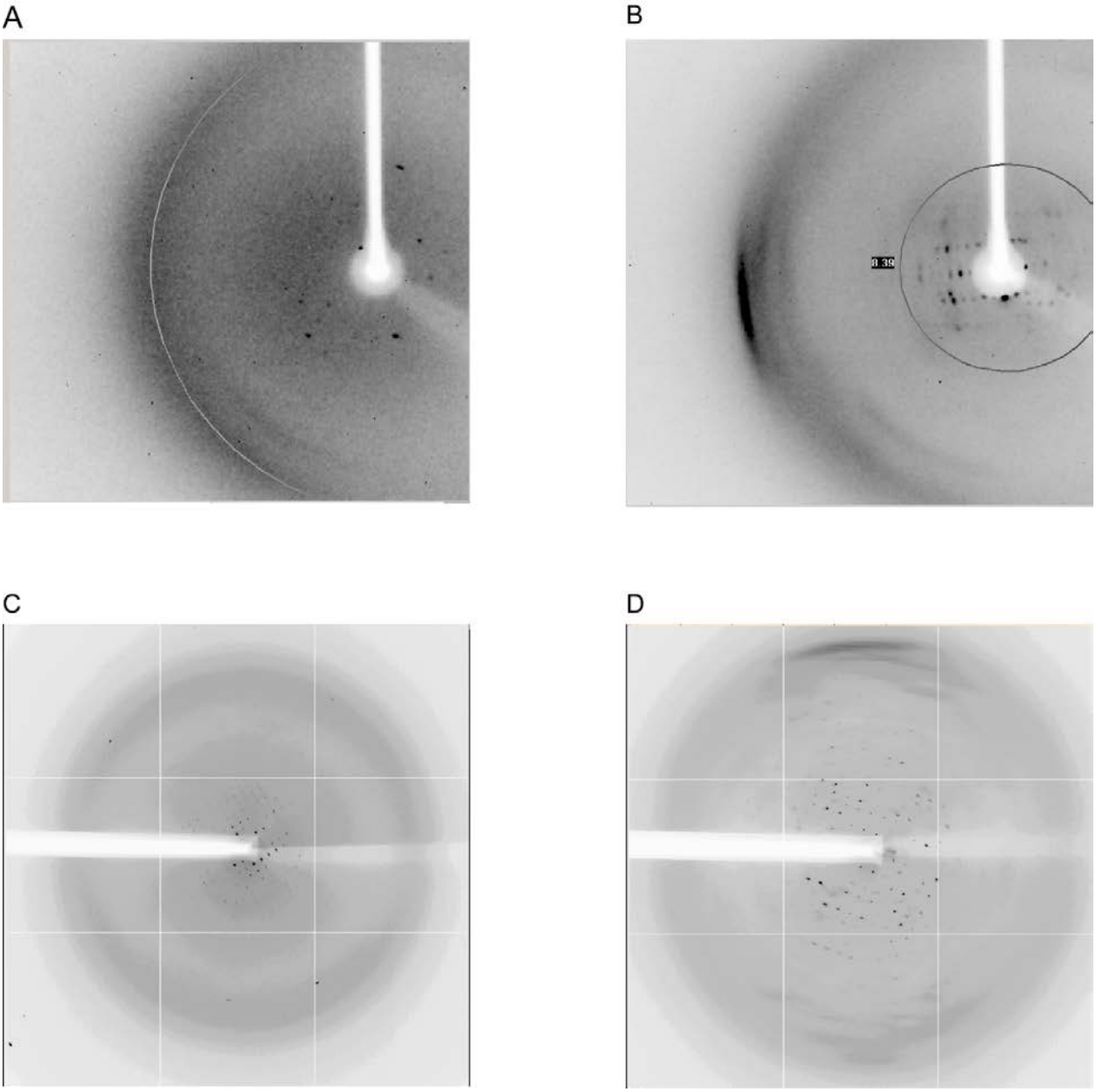

Figure S2. Representative still X-ray diffraction patterns of SETMAR DBD-DNA crystals are shown for (A) 329-440(wt) in complex with TIR2, crystals shown in Figure 2A, (B) 329-440(wt) in complex with TIR2 at the presence of TCEP, crystals shown in Figure 2B, (C) 316-440(C381S) in complex with variant-TIR1, crystals shown in Figure 2E, and (D) 329-440(C381R) in complex with TIR2, crystals shown in Figure 2F. Diffraction images for (A) and (B) were taken on a Bruker X8 Prospector with Cu IμS Microfocus source**,** QUAZAR MX optics, kappa goniometer, and APEX II CCD detector at 1.54 Å. Diffraction images for (C) and (D) were taken at the Advanced Photon Source 19-ID beamline at 0.97915 Å.

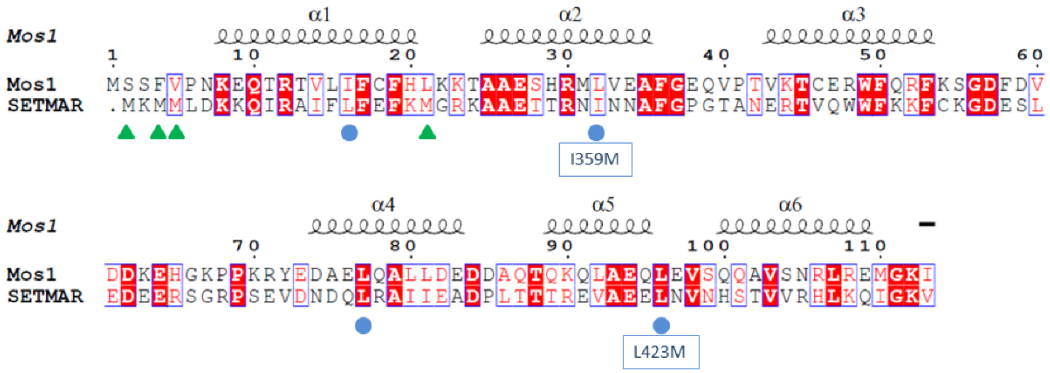

Figure S3. The pairwise sequence alignment of Mos1 transposase and SETMAR DNA-binding domains, 1-113 and 329-440, respectively. The secondary structure shown is for the Mos1 DNA-binding domain. Green triangles indicate the positions of intrinsic Met residues within SETMAR. Blue circles indicate the positions of selected Leu or Ile residues for substitution with Met. Pairwise combinations were generated with one new Met in the N-terminal half and a second in the C-terminal half of the protein.

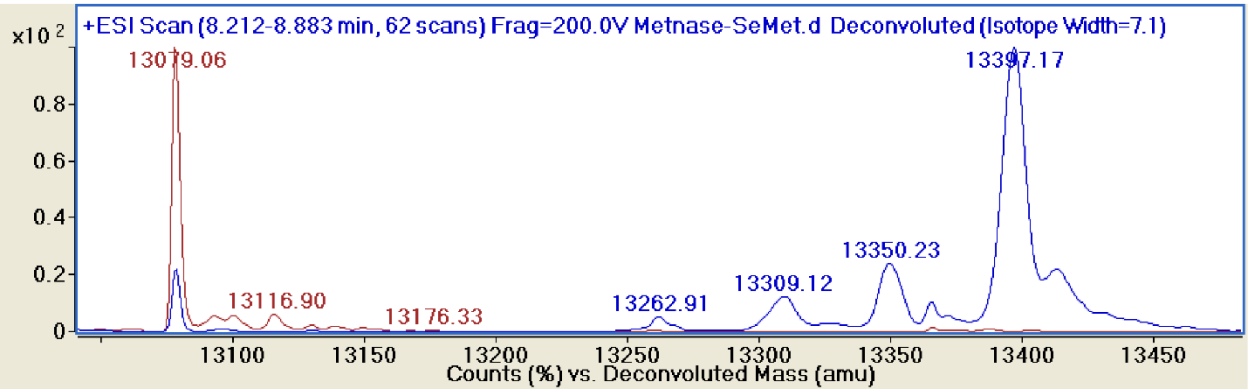

Figure S4. Mass spectrometric analysis of SETMAR DBD 329-440(C381R) and SeMet substituted SETMAR DBD 329-440(C381R) (I359M)(L423M). Samples were diluted in 50 mM HEPES pH 7.5, 50 mM NaCl, 1 mM DTT, and approximately 6 μg of each protein was used for analysis on an Agilent Tech 6520, Accurate-Mass Q-TOF LC/MS system. A single large peak is observed for SETMAR DBD (C381R) in the deconvoluted mass spectrum (red) with a MW of 13079.06 Da, a close match with its calculated MW of 13078.8 Da. The deconvoluted spectrum for DBD 329-440(C381R)(I359M)(L423M) with incorporated SeMet residues has a large peak for incorporation of 6 SeMet residues of 13397.2 Da, consistent with the calculated MW of 13396.5.

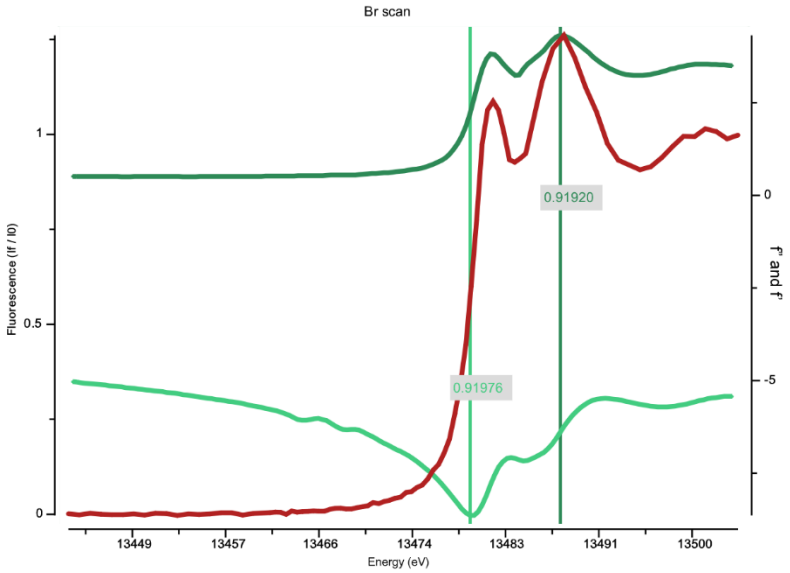

Figure S5. X-ray absorption scan for DBD 316-440 (C381S)- variant BrdU-TIR1 complex crystals measured at the Advanced Photon Source 19-ID beamline. An absorption peak at 13487.52 eV (0.91922 Å) is consistent with the presence of Br in the crystal.