Structural proteomics defines a sequential priming mechanism for the progesterone receptor

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Running title: Structural proteomics reveals a unique activation model for PR-A and PR-B

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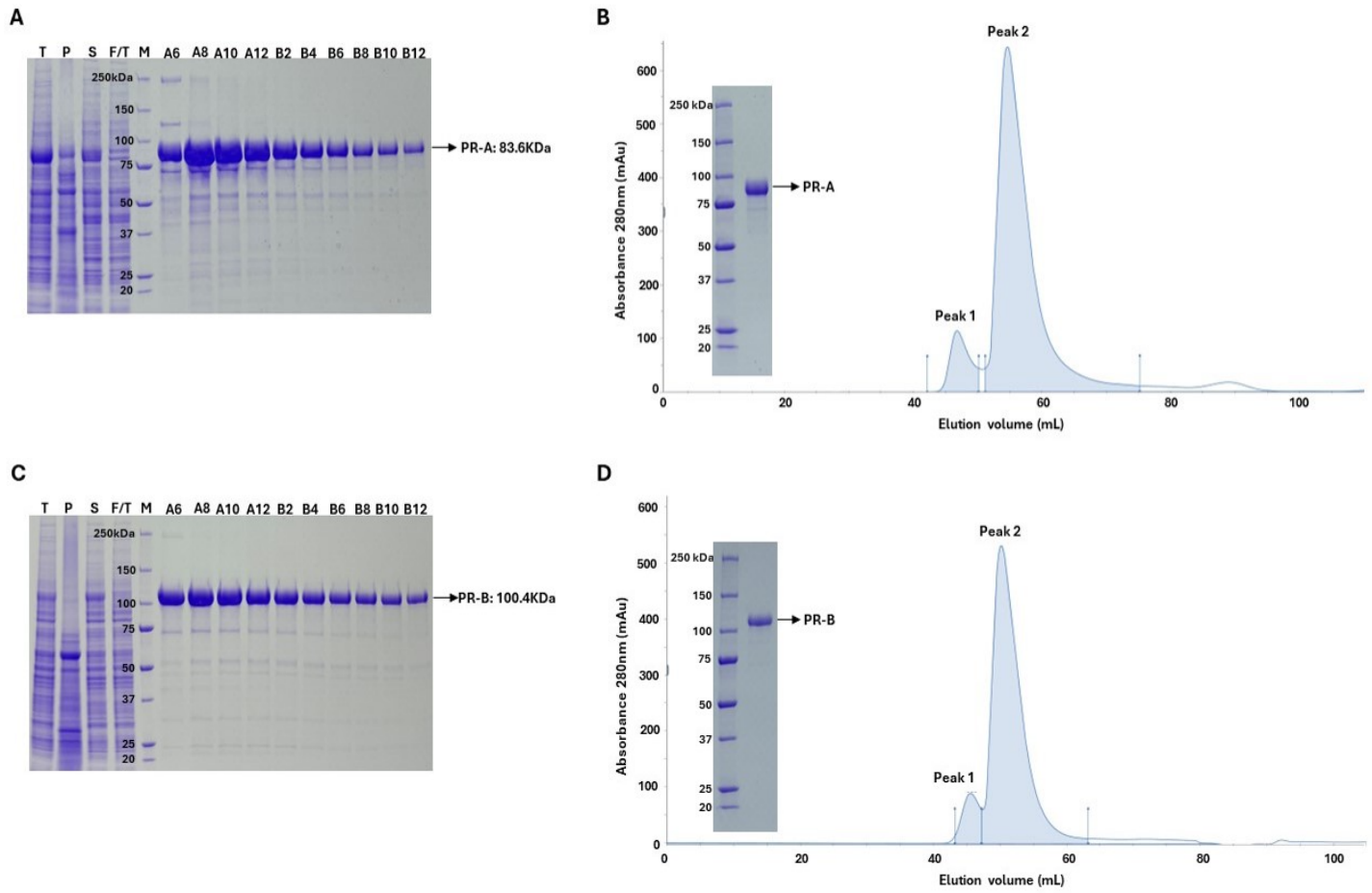

Fig S1. Affinity/SEC two-step purification of SII-tagged PR. SII-tagged PR-A and PR-B(bound to progestin agonist R5020) were expressed in Sf9 cells and cell lysates were prepared and purification performed by two -step affinity Strep Trap XT column and size exclusion chromatography (SEC) S200 as described in Methods. A) SDS-PAGE analysis of PR-A affinity purification fractions T, total cell lysate, P pellet after centrifugation lysate, S supernatant after centrifugation, F/T flow through after binding to Strep TrapXT, M, protein molecular weight markers. Lanes A6-B12 (5uL) biotin eluted fractions with major enriched protein bands at expected size of ~83 kDa for PR-A. B) SEC fractionation of pooled fractions A6-B12 off the Strep Trap XT column. OD tracing at 280nM showsmajor peak 2 of monomeric size for PR-A and peak 1 containing high molecular weight aggregation. Peak 2 fractions were pooled, concentrated and analyzed SDS-PAGE gel showing a single band of >98% purity of the expected size of PR-A. C) A) SDS-PAGE analysis of PR-B affinity purification fractions and D) SEC second step purification by the same set-up and as PR-A except major band and purified product are expected size for monomeric PR-B (~100kDa).

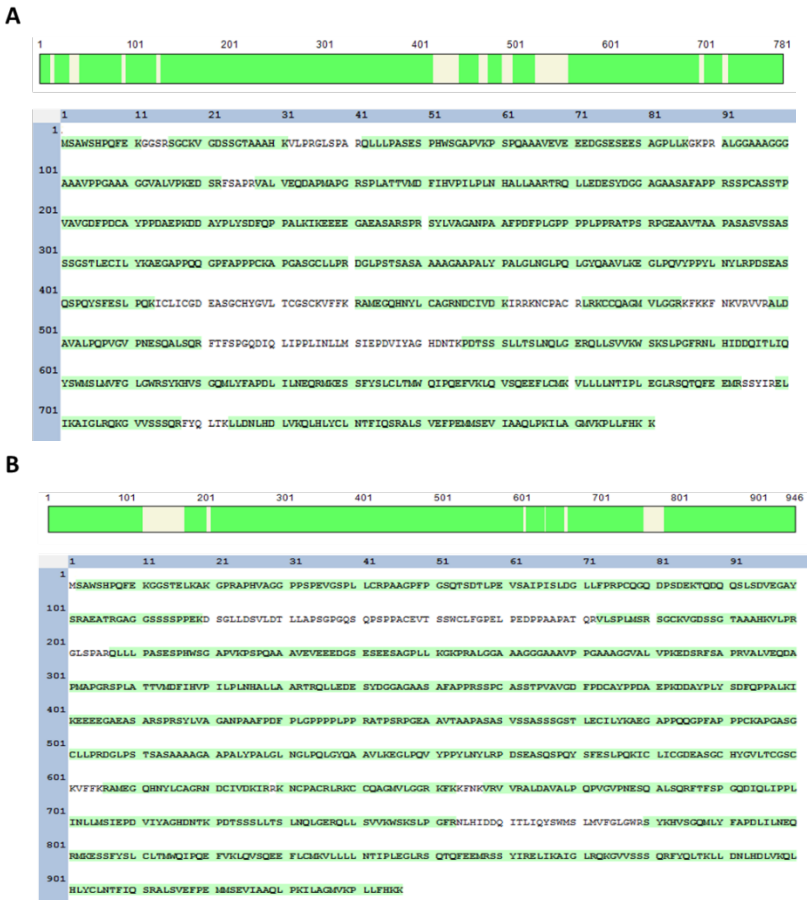

Fig S2. Mass Spectrometry sequencing of purified major PR-A and PR-B bands. In-gel digestion of purified major bands for A) PR-A and B) PR-B (bound to progestin agonist R5020) was performed, followed by LC-MS/MS analysis. The peptide sequences highlighted in green were identified by MS, covering nearly the entire tryptic region

Fig S3. Mass Spectrometry sequencing of minor smaller molecular sized band product with PR-B. A) In-gel digestion of smaller sized PR-B band (bound progestin agonist R5020) was performed, followed by LC-MS/MS analysis. The peptide sequences highlighted in green were identified by MS, suggesting intact full-length PR-B protein. B) The peptide area under curve (AUC) for selected phosphorylated PR-B peptides from major and minor band suggests variable phosphorylation level. The peptide AUC was normalized to the total protein amount in that band.

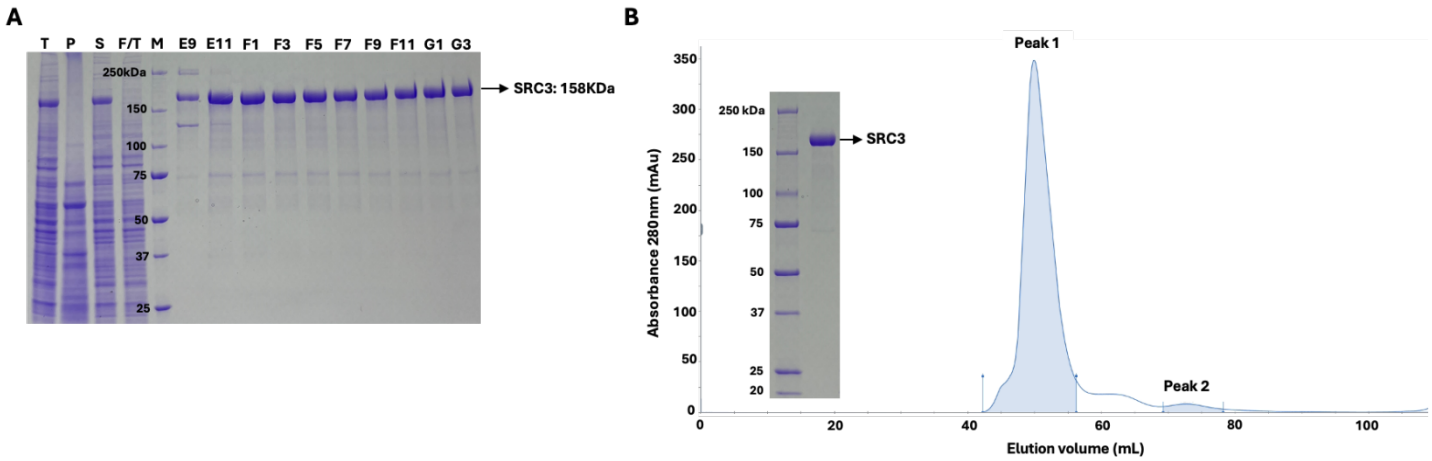

Fig S4. Affinity/SEC two-step purification for SRC3 tagged SII. SII-tagged SRC3 was expressed in Sf9 insect cells and purification performed by two -step affinity by StrepTrap XT and size exclusion chromatography (SEC) S200 as described in Methods. A) SDS-PAGE analysis of affinity purification fractions T, total cell lysate, P pellet after centrifugation, S supernatant after centrifugation, F/T flow through after binding to Strep TrapXT M, protein molecular weight markers. Lanes E9-G3 are SDS-PAGE analysis (5uL) of biotin eluted fractions with a major enriched band of expected size of ~158kDa for SRC3. B) SEC (S200) fractionation of pooled fractions E11-G3 off the StrepTrap XT column. OD tracing at 280nM shows a major peak (peak 1) of the native size expected for monomeric SRC3 and a minor peak (peak 2) of lower molecular weight material. Peak 1 fractions were pooled, concentrated

and analyzed by SDS-PAGE(left panel) showing a single band of >98% purity of the expected size of monomeric SRC3

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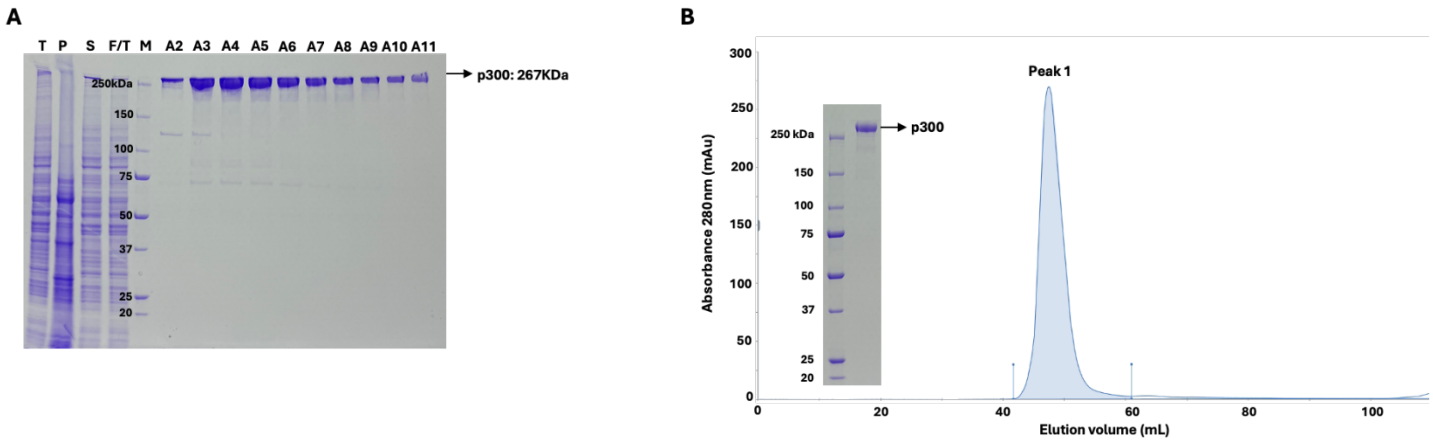

FigS5 Affinity/SEC two-step purification for p300 tagged SII. SII-tagged p300 was expressed in Sf9 insect cells and purification performed by two -step affinity by StrepTrap XT and size exclusion chromatography (SEC) S200 as described in Methods. *A)* SDS-PAGE analysis of affinity purification fractions T, total cell lysate, P pellet after centrifugation, S supernatant after centrifugation, F/T flow through after binding to Strep TrapXT M, protein molecular weight markers. Lanes A2-A11 are SDS-PAGE (5uL) of biotin eluted fractions with a major enriched band of the expected size for monomeric p300 (~267kDa). *B)* SEC (S200) fractionation of pooled fractions A3-A8 from Strep TrapXT shows a single major peak (OD tracing at 280nM) of expected size for native monomeric p300. The pooled SEC fractions from peak 1 on SDS-PAGE (left panel) analysis shows a single major band of the expected sized for monomeric p300.

A

B

Fig S6. Mass Spectrometry of purified SRC3 and p300. In-gel digestion of purified A) SRC3 and B) p300 bands was performed, followed by LC-MS/MS analysis. The peptide sequences highlighted in green were identified by MS, covering nearly the entire tryptic region.

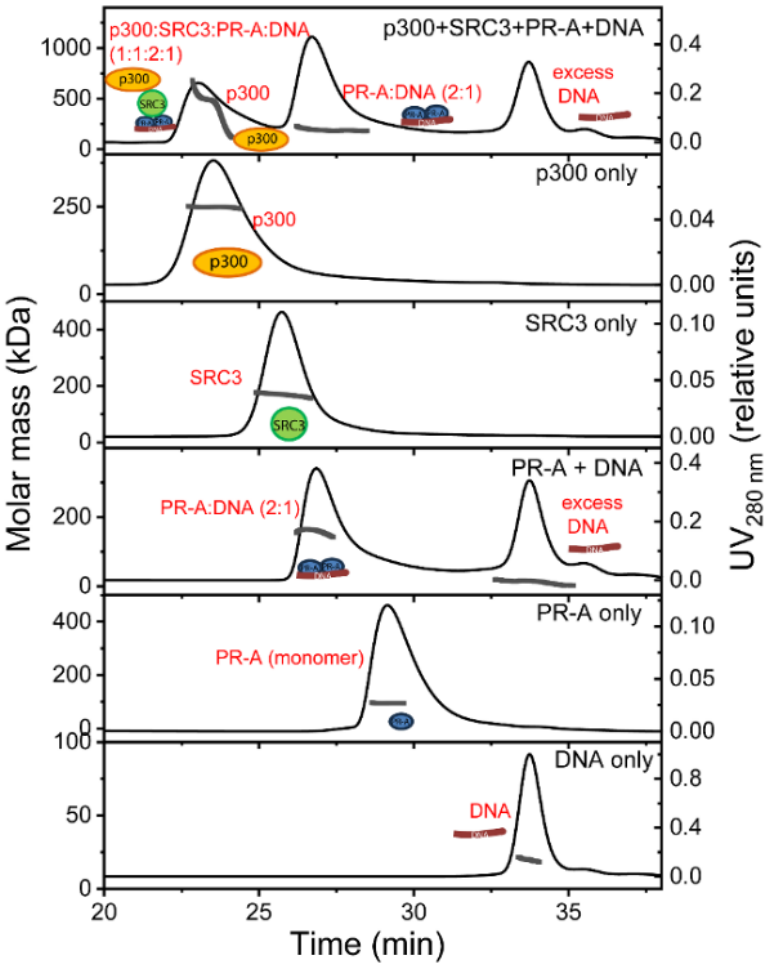

Fig S7 SEC-MALS chromatograms with molar mass distribution for various proteins (PR-A, SRC3, p300) and DNA alone or in complexes. Shown are double y-plots (left y-axis, molar mass; right y-axis, UV absorbance at 280 nm) vs time (x-axis). Molar mass distribution is displayed as gray dots across the peaks. PR-A (with agonist R5020) assembles only to a dimer in the presence of DNA. A multi-complex of p300:SRC3:PR-A:DNA at a 1:1:2:1 ratio is observed (top chromatogram). The presence of DNA in the complex is confirmed by deconvolution of the protein and DNA fractions in the peak. The full complex showed a heterogeneous distribution of molar mass due to overlap with the p300 monomeric peak.

Table S1. SEC-MALS Experimental vs Theoretical MWs of individual purified proteins and various mixtures in the presence and absence of DNA.

Table 1. SEC-MALS derived molecular weights for proteins, DNA and the complexes formed

	Theoretical MW	Actual MW[a]	
Protein/DNA		**by UV**	**by dRI**
DNA	19.6	18.2 ± 0.9	20.1 ± 1.0
PR-A(R5020)	83.6	94.9 ± 4.8	91.2 ± 4.6
PR-A(RU486)	83.6	98.8 ± 4.7	95.3 ± 4.8
PR-B(R5020)	100.4	116.4± 5.8	101.6± 5.1
PR-B(RU486)	100.4	96.3 ± 4.8	97.7 ± 4.10
p300	267	249.2 ± 12.5	200.5 ± 9.2
SRC3	158	170.3 ± 8.5	159.7 ± 8

Complexes	**Theoretical MW**	**Actual MW**
PR-A(R5020):DNA (2:1)	186.8	161.6 ± 8.1
Protein component	167.2	144.8 ± 7.2
DNA component	19.6	16.8 ± 0.8
PR-A(RU486):DNA (2:1)	186.8	189.3 ± 9.5
Protein component	167.2	167.5 ± 8.4
DNA component	19.6	21.8 ± 1.1
PR-B(R5020):DNA (2:1)	220.4	224.0 ± 11.2
Protein component	200.8	201.7± 10.1
DNA component	19.6	22.3 ± 1.1
PR-B(RU486):DNA (2:1)	220.4	221.8 ± 11.1
Protein component	200.8	197.7± 9.9
DNA component	19.6	24.1 ± 1.2
p300:SRC3:PR-A(R5020):DNA (1:1:2:1)	611.8	536.5 ± 26.8[b]
Protein component	592.2	512.7 ± 25.6
DNA component	19.6	23.7 ± 1.2
PR-A(R5020):DNA (2:1)[c]	186.8	190.7 ± 9.5
Protein component	167.2	161.4 ± 8.1
DNA component	19.6	29.3 ± 1.5

[a] errors are 5% of calculated MW; dn/dc based off BSA values in similar buffer condition

[b] heterogeneous MW distribution, overlap with p300

[c] in same buffer conditions as above but with 0M Urea

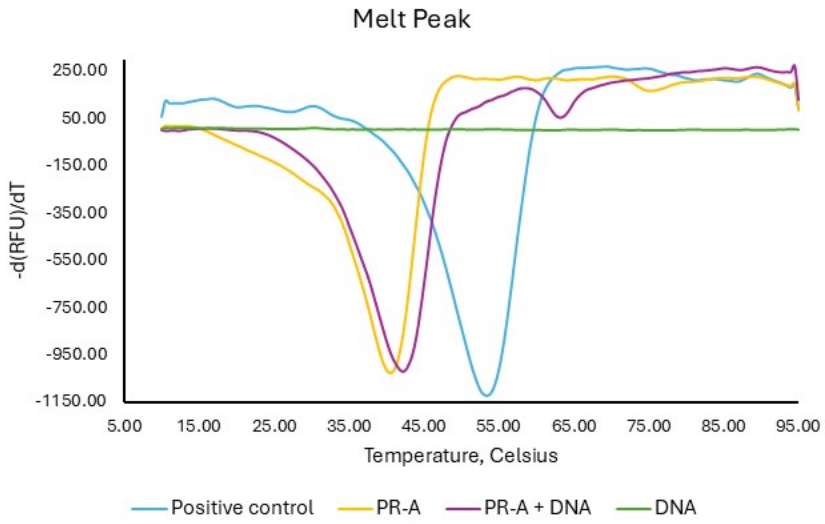

Fig S8. Differential scanning fluorimetry (DSF) analysis of PR-A with and without DNA. DSF analysis was performed with 4 μM PR-A and 25× SYPRO Orange in 20 mM HEPES, pH 7.5, 200mM NaCl, 5% Glycerol, 1M Urea, 1mM TCEP with (purple) or without 3uM PRE DNA (yellow), compared with the same concentration of TLX-WT as a positive control (blue). Fluorescent intensities and the derivative of the fluorescent intensities (dF/dT) were plotted against temperature (Tm).

Table S2. Transition melting temperatures (T_M) of each purified protein as determined by DSF. Samples were analyzed as three technical replicates and the average Tm was calculated with standard error of the mean.

Protein	T_m (°C)
PR-A	40.7±0.3
PR-A + DNA	42.2±0.3
PR-B	43.5±0
PR-B + DNA	45.0±0
p300	49.1±0
SRC3	46.5±0.1

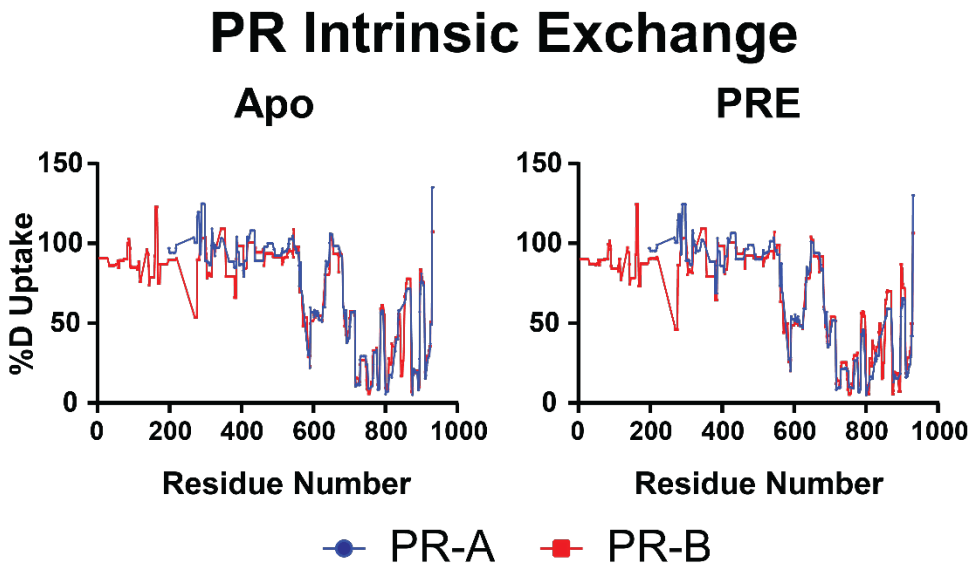

Figure S9. **Intrinsic deuterium exchange for PR-A and PR-B**. Woods plots showing the percent deuterium exchange (%D Uptake) for both PR-A (blue) and PR-B (red) across each residue across the protein in both non-DNA-bound (Apo) and DNA-bound (PRE) states. Graphs made using GraphPad Prism 10.

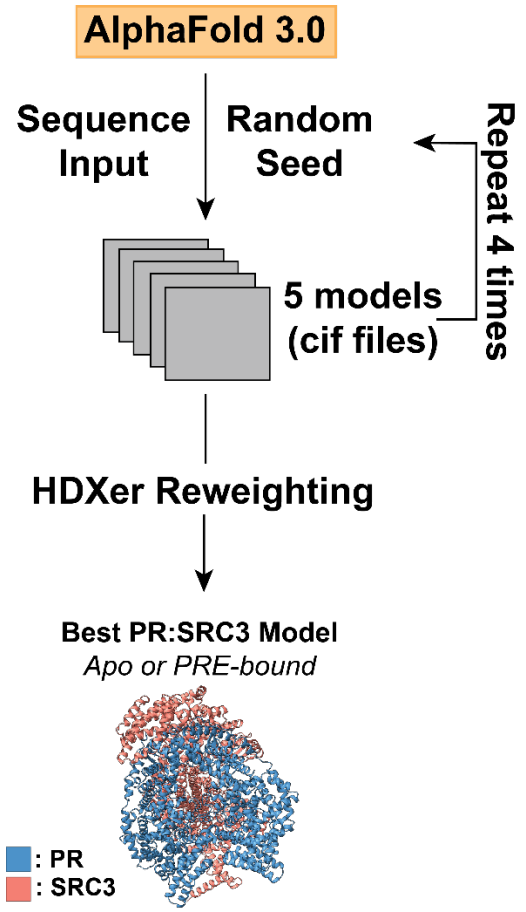

Figure S10 Workflow for PR:SRC3 model generation and selection. Workflow detailing the model generation for ternary complexes. AlphaFold3.0 webserver was used to generate PR:SRC3 models in quintuplicate. Models were ensemble reweighted using HDXer (see methods) to determine best representation based on HDX data.

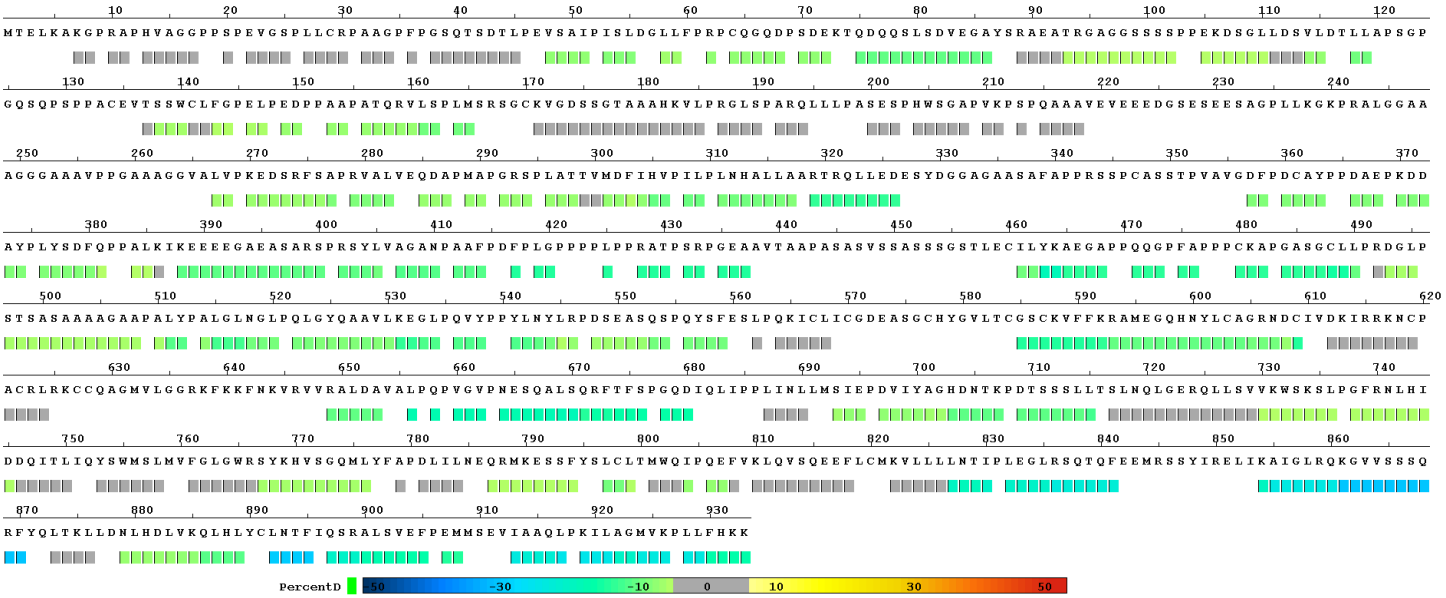

Figure S11 PR-B vs. PR-B:PRE consolidated HDX-MS data. *Consolidated data output from HDXWorkbench for PR-B ± DNA HDX-MS experiments when RU-486 bound. This illustrates the concerted decrease in deuterium exchange when DNA bound, compared to non-DNA-bound protein. Each point represents an average across each peptide detected, with the differential HDX data following the color scheme shown at the bottom.*