

Supporting Information

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SI Text

Definition of the MexR-Binding Sequence of ArmR. Consistent with its 53-aa size, $^1\text{H}/^{15}\text{N}$ -HSQC NMR measurements indicated that free ArmR adopts a predominantly random coil structure, albeit with residues 31–49 exhibiting α -helical propensity (Fig. S1). Serendipitously, these NMR studies also revealed that MexR-binding protects this C-terminal region of ArmR from proteolytic degradation (see below). Subsequent limited proteolysis experiments confirmed that MexR protects several C-terminal ArmR fragments, corresponding to the last 23–29 residues (Fig. S2). Previously, two-hybrid assays demonstrated an interaction between MexR and an N-terminally truncated ArmR construct (spanning residues 25–53), whereas mutations in this region compromised MexR-binding (1). Using ITC, we compared the dissociation constants between MexR and a variety of truncated ArmR peptides (Table S1). This comparison revealed that ArmR residues 41–53 were sufficient for binding MexR with approximately WT affinity. This fact is supported by the structure of ArmR_C in complex with MexR_{LL}, in which the majority of interactions between ArmR_C and MexR_{LL} are contained within ArmR_C residues 44–53. Together, these experiments indicated that the last 13 residues of ArmR were involved in critical interactions with MexR, whereas residues 1–24 were not contributing to complex formation. This information was used in designing ArmR_C (i.e., residues 29–53) for x-ray crystallography.

Cloning, Expression, and Purification. WT MexR was produced by expression in BL21 (DE3) *E. coli* (Invitrogen) and purified by three chromatographic steps (SP-Sepharose, heparin-agarose, and superdex-75 gel filtration) as described (2). Purified MexR was stored in 20 mM Tris-HCl (pH 7.5), 150 mM NaCl at 50–100 mg/ml (as determined by Bradford assay).

The MexR_{LL}-ArmR_C complex was produced by coexpression of MexR_{LL} with ArmR_C, followed by purification of the intact complex. The MexR_{LL} expression plasmid, pET41a-MexR_{LL}, was obtained through several rounds of QuikChange site-directed mutagenesis, starting from the previously described WT MexR plasmid (2). These modifications produced a construct corresponding to MexR residues 1–142 and two point mutations, Q106L and A110L. The ArmR_C expression plasmid, pTYB12-ArmR_C, was constructed by subcloning ArmR residues 29–53 from plasmid pLC23 (3) into the BsmI-XhoI restriction sites of the IMPACT-CN vector pTYB12 (New England Biolabs). BL21 Star (DE3) *E. coli* (Invitrogen), transformed with both pET41a-MexR_{LL} and pTYB12-ArmR_C plasmids, were grown from an overnight culture at 37°C until $A_{600} \approx 0.6$, cooled to 20°C, and then induced overnight by using 0.1 mM isopropyl β -D-thiogalactopyranoside (IPTG). Purification of the complex was accomplished at 4°C via three chromatography steps: chitin affinity/intein tag cleavage, anion exchange, and gel filtration. Cells were first resuspended in chitin load buffer (20 mM Hepes, 250 mM NaCl, 1 mM EDTA, pH 7.5) and lysed with a French pressure cell press. The soluble cell fraction was loaded onto 50 ml of preequilibrated chitin resin (New England Biolabs), washed with load buffer, and then incubated overnight in elute buffer (20 mM Hepes, 50 mM NaCl, 1 mM EDTA, 50 mM DTT, pH 8.0) at 4°C to reduce the N-terminal tag of ArmR_C to a single Ala residue. The next day, the column was eluted with fresh elute buffer and incubated for a second night to increase yield. The eluate was then diluted with buffer to reduce the NaCl concentration to 83 mM and applied to a Mono Q HR 10/10 anion exchange column (Amersham Biosciences). The complex was

eluted over 100 ml with a linear 83–250 mM NaCl gradient in 20 mM Hepes, 1 mM EDTA, 14 mM β -mercaptoethanol, pH 8.0. Lastly, elution fractions containing protein were further purified via superdex-75 gel filtration (GE Healthcare) in 20 mM Tris-HCl, 150 mM NaCl, 5 mM TCEP-HCl, pH 7.5. The pure complex was concentrated to 9.4 mg/ml by using an Amicon Ultra-15 5K concentrator (Millipore) as estimated by using a predicted $\epsilon_{280} = 12,950 \text{ M}^{-1}\text{cm}^{-1}$ (4), flash frozen in $\text{N}_2(\text{l})$, and stored at -80°C until needed.

MexR_{LL} (without ArmR_C) was produced identically to WT MexR and stored in 20 mM Tris-HCl (pH 7.5), 150 mM NaCl at 50 μM (as determined by Bradford assay). MexR_{LL} demonstrated approximately equivalent DNA and ArmR binding affinities (Fig. S3) as estimated by using an electromobility shift assay (1).

ArmR and truncated ArmR constructs were either expressed recombinantly or synthesized at $>95\%$ purity without using chemical ligation by C. S. Bio Company) or the Brain Research Center (Vancouver). Recombinant ArmR constructs were separately cloned into the IMPACT-CN vector pTYB12 as described above. BL21 (DE3) cells carrying pTYB12-armR or pTYB12-armR_C were grown from an overnight culture at 37°C until $A_{600} \approx 0.5$, cooled to 20°C, and then induced overnight by using 0.1 mM IPTG. The cells were lysed in load buffer (20 mM Hepes, 250 mM NaCl, 1 mM EDTA, pH 7.5) by passing the resuspended cells four times through a French pressure cell press. After ultracentrifugation for 1 h at $40,000 \times g$ to pellet insoluble cell debris, the soluble cell lysate was loaded onto 25 ml of preequilibrated chitin resin (New England Biolabs) and washed with 15–20 column volumes of load buffer. The column was quickly flushed with three column volumes of elute buffer (20 mM Hepes, 50 mM NaCl, 1 mM EDTA, 50 mM DTT, pH 8.0) and incubated overnight at room temperature to induce cleavage of the N-terminal CBD. The next day, the column was eluted with just over a column volume of elute buffer. The eluate was further purified by using a Mono S HR 5/5 cation exchange column (Amersham Biosciences) and a 0.05–1.0 M NaCl elution gradient in 20 mM Hepes, pH 8.0. Elution fractions corresponding to the principal A_{280} peak were combined and purified by semipreparative C18 reversed-phase HPLC using a 5–80% CH_3CN gradient in 0.1% TFA. The major A_{280} peak was collected, lyophilized to dryness, and then dissolved in 20 mM Tris-HCl (pH 7.5), 50 mM NaCl at a concentration of 0.3–2.0 M as estimated by using a predicted 280-nm molar absorption coefficient of $9,970 \text{ M}^{-1}\text{cm}^{-1}$ (4). The identities of ArmR and ArmR_C were confirmed by MALDI-TOF mass spectrometry and frozen at -80°C .

NMR Spectroscopy. Full-length $^{15}\text{N}/^{13}\text{C}$ -labeled ArmR was produced by expression in BL21 (DE3) *E. coli* grown in M9 medium containing 3 g of $^{13}\text{C}_6\text{-D-glucose}$ and 1 g of $^{15}\text{NH}_4\text{Cl}$ per liter. $^{15}\text{N}/^{13}\text{C}$ -ArmR was purified and lyophilized as described for unlabeled ArmR and then resuspended in 20 mM Tris-HCl (pH 7.5), 50 mM NaCl, and 10% D_2O to a concentration of 1 mM. NMR analysis of full-length $^{15}\text{N}/^{13}\text{C}$ -ArmR indicated that ArmR adopts a predominantly random coil structure with diagnostic amide chemical shifts in the range of 8 to 8.5 ppm. No signals were detected for MexR-bound $^{15}\text{N}/^{13}\text{C}$ -ArmR, presumably from the high molecular weight of the complex. $^{15}\text{N}/^{13}\text{C}$ -ArmR_{25–53} was produced serendipitously from a sample containing unlabeled MexR and $^{15}\text{N}/^{13}\text{C}$ -ArmR that apparently degraded via an unknown proteolytic contaminant. The surviv-

ing $^{15}\text{N}/^{13}\text{C}$ -ArmR_{25–53} peptide (protected by MexR binding) was purified by reversed-phase HPLC and identified by MALDI-TOF mass spectrometry and $^1\text{H}/^{15}\text{N}$ -HSQC spectroscopy. $^1\text{H}/^{15}\text{N}$ -HSQC spectra were acquired on $^{15}\text{N}/^{13}\text{C}$ -ArmR at pH 7.5 by using Varian 500 Unity and 600 Inova spectrometers at 30°C (5, 6). Subsequent $^1\text{H}/^{15}\text{N}$ -HSQC and 3D spectra (CBCACONH, HNCACB, HNCACO, HNCO) were recorded on the resulting $^{15}\text{N}/^{13}\text{C}$ -ArmR_{25–53} fragment at pH 6.5. NMR spectra were processed with NMRPipe (7) and assigned (Fig. S1) with Sparky software (8). Secondary structure propensity was determined by using the SSP algorithm (9).

Limited Proteolysis of Free and MexR-Bound ArmR. Reactions were carried out on ice in 50 μl of cleavage buffer [20 mM Hepes (pH 7.5), 50 mM KCl, 10 mM MgSO_4]. MexR (2.9 nmol) or BSA (Sigma; 0.7 nmol i.e., equivalent weight to MexR) was combined with synthetic ArmR (1.45 nmol), and the reaction was started by addition of 0.5 μl of 5 mg/ml trypsin (Sigma). Reactions were stopped at various time points ($t = 0$ min to overnight) by transferring 1–9 μl of matrix solution (10 mg/ml α -cyano 4-hy-

droxycinnamic acid in 50% acetonitrile, 0.1% TFA) and analyzed by using a Voyager DESTRA MALDI-TOF mass spectrometer (Applied Biosystems) at the University of British Columbia MSL/LMB Proteomics Core Facility. Peptides were identified by comparison with a simulated tryptic digest.

ITC. ITC titrations were performed by using a VP-ITC (MicroCal) in 20 mM Hepes (pH 7.5), 150 mM NaCl by injecting consecutive 10- μl aliquots of 0.12–0.18 mM ArmR (or truncated ArmR construct) into the ITC cell (volume = 1.4 ml) containing 13–29 μM MexR. Except for ArmR_C, the ITC data were corrected for the heat of dilution of the titrant by subtracting mixing enthalpies for 10- μl injections of ArmR solution into buffer. For ArmR_C, the average heat of the last four data points was subtracted as background to correct for a slight difference in pH between the two solutions. The titration experiments were performed at 25°C to determine the binding constant of ArmR to MexR. Binding stoichiometry (N), binding enthalpy (ΔH), and equilibrium association constants (K_a) were determined by fitting the corrected data to a single-site interaction model (MicroCal Origin software).

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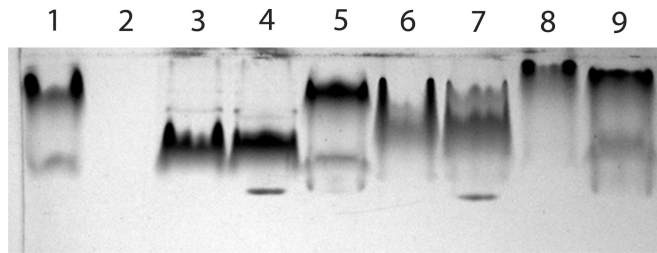
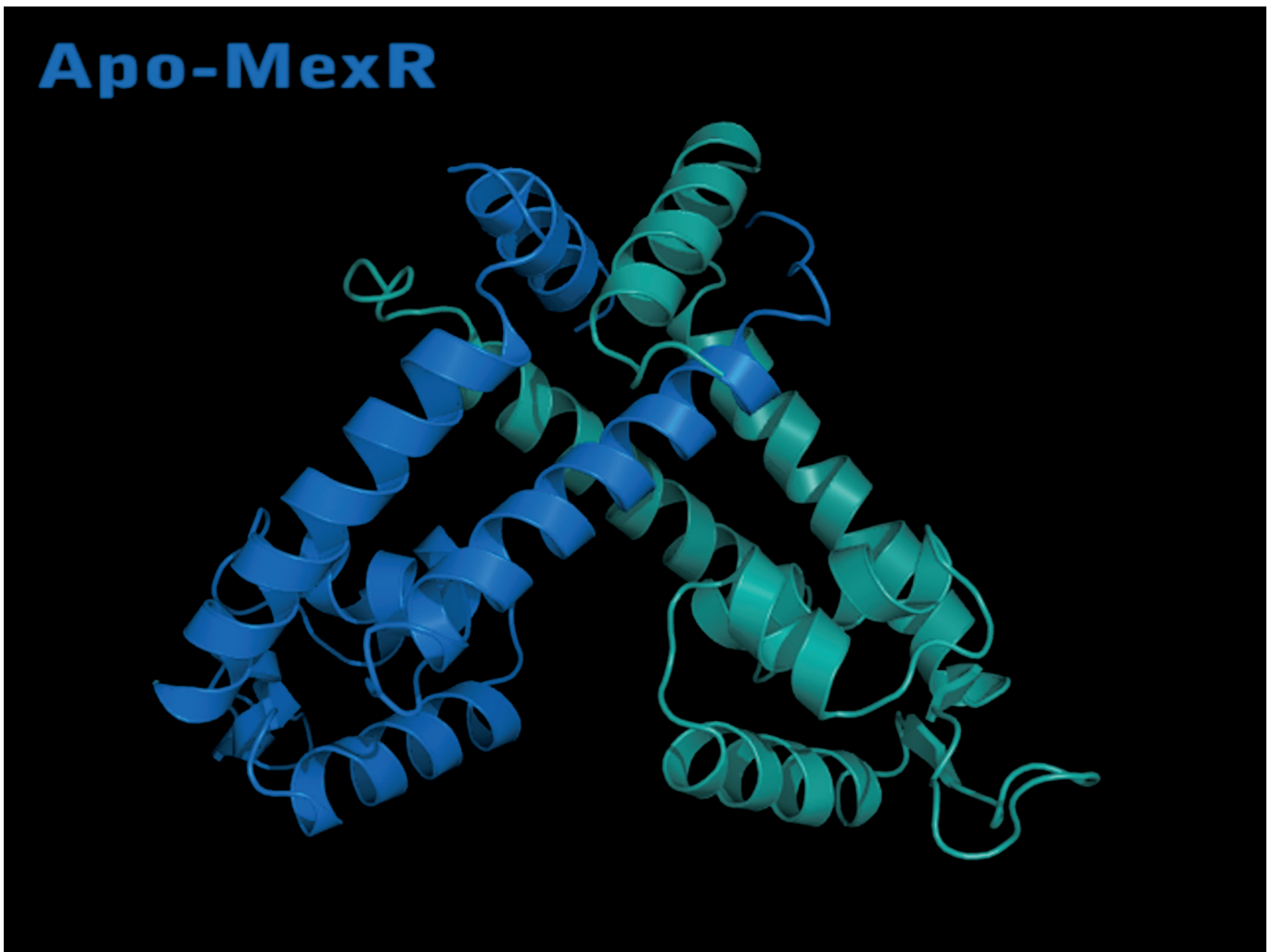


Fig. S3. Coomassie-stained native 12% polyacrylamide gel demonstrating approximately equivalent DNA and ArmR-binding affinities for MexR_{LL} and WT MexR: MexR, lane 3; MexR_{LL}, lane 6; DNA, lane 2, no band; MexR + DNA, lane 4; MexR_{LL} + DNA, lane 7; MexR + ArmR, lane 1; MexR_{LL} + ArmR, lane 8; MexR + DNA + ArmR, lane 5; MexR_{LL} + DNA + ArmR, lane 9.



Movie S1. Allosteric mechanism of MexR antirepression. The movie begins with apo MexR dimer CD (Protein Data Bank ID code 1LNW; shown in blue). The DNA operator from the OhrR-DNA structure (Protein Data Bank ID 1Z9C, shown in yellow) is then fit to apo MexR by overlapping OhrR to MexR (OhrR not shown). DNA disappears and ArmR (shown in orange) is added by superimposing the dimerization domain of MexRLL–ArmRC (MexRLL not shown) to apo MexR dimer CD. The resulting steric clashes between MexR and ArmR are then relieved by morphing the structure of apo MexR dimer CD to the ArmR-bound conformation of MexR. DNA is then fit to one of the DNA-binding domains of MexR, resulting in severe steric clashes between DNA and the second DNA-binding domain.

[Movie S1](#)

Table S1. ITC results for titrations of MexR and ArmR-derived peptides

Peptide (ArmR residues)	<i>N</i>	ΔH , kcal/mol	ΔS , cal/mol·K	K_a , M ⁻¹	K_d , nM
1–53 (ArmR)	0.48 (± 0.05)*	–14.5 (± 0.1)	18.8 (± 0.2)	$3.4 (\pm 0.1) \times 10^6$	290 (± 30)
29–53 (ArmR _C)	0.48	–9.8	–3.1	3.3×10^6	300 (± 70)
29–47			No binding observed ($K_a < 10^4$ M ⁻¹)		
41–53	0.50 (± 0.05) [†]	–10 (± 1)	–5 (± 5)	$4 (\pm 2) \times 10^6$	190 (± 30)
48–53			No binding observed ($K_a < 10^4$ M ⁻¹)		

*Where provided, errors refer to the standard deviations of replicate experiments.

[†]Because of uncertainty in the MexR concentration, thermodynamic parameters for ArmR_{41–3} are based on adjusting the MexR concentration to give *N* = 0.50 (consistent with structural data).

Table S2. X-ray data collection and refinement statistics

Crystal parameters	
Space group	$P2_12_12_1$
Cell dimensions: $a \times b \times c$, Å	$52.2 \times 57.2 \times 91.9$
Resolution, Å	1.8 (1.86–1.80)
Wavelength, Å	1.00000
No. reflections	207,187
No. unique reflections	26,074
Redundancy	7.9 (8.0)
Completeness, %	100 (100)
$I/\sigma I$	35.1 (3.0)
R_{sym} %	6.1 (5.6)
Refinement statistics	
Resolution range, Å	48.56–1.80
R_{work}/R_{free} , %	17.6/22.9
rmsd bond lengths, Å	0.014
rmsd bond angles, °	1.401
Average B factor, Å ²	
MexR	14.6
ArmR	18.2
H ₂ O	34.4

Highest resolution shell is shown in parentheses. $R_{sym} = \sum |I(hkl) - \langle I \rangle| / \sum I(hkl)$. Five percent of reflections was excluded from refinement.