SUPPLEMENTAL MATERIAL

Structural insights into the unique mechanism of transcription activation by *Caulobacter crescentus* GcrA

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SUPPLEMENTAL TABLES.

Table S1. The statistics for crystal structures of GcrA-SID/σ^{70}_2

	SeMet-GcrA-SID/σ^{70}_2	**GcrA-SID/σ^{70}_2**
Data collection		
Space group	$P6_522$	$P6_522$
Cell dimensions		
a, b, c (Å)	65.0, 65.0, 387.5	65.2, 65.2, 397.9
α, β, γ (°)	90, 90, 120	90, 90, 120
Resolution (Å)	50.00-3.00 (3.05-3.00)	50.00-2.30 (2.34-2.30)
R_{sym} or R_{merge}	0.120 (0.631)	0.113 (0.751)
I/σI	48.5 (8.0)	29.1 (2.8)
Completeness (%)	100 (100)	98.9 (82.0)
Redundancy	35.8 (38.5)	20.2 (14.6)
$CC_{1/2}$ in highest shell	0.98	0.91
Refinement		
Resolution (Å)		50.00-2.30
No. reflections		23395
Rwork/ Rfree		0.221/0.257
No. of atoms		
Protein		2874
Ligand/ion		13
Water		182
B-factors (Å^2)		
Protein		38.1
Ligand/ion		42.1
Water		39.4
R.m.s deviations		
Bond lengths (Å)		0.008
Bond angles (°)		0.947
Ramachandran plot		
Favored (%)		98.4
Allowed (%)		1.6
Disallowed (%)		0

Numbers in parenthesis are for highest resolution.

Table S2. The statistics for crystal structures of GcrA-DBD, GcrA-DBD/m^6A-DNA and GcrA-DBD/DNA

	GcrA-DBD*	**GcrA-DBD/m^6A-DNA (crystal form 1)**	**GcrA-DBD/m^6A-DNA (crystal form 2)**	**GcrA-DBD/DNA (crystal form 2)**
Data collection				
Space group	$P2_12_12_1$	C222	$C222_1$	$C222_1$
Cell dimensions				
a, b, c (Å)	30.4, 37.1, 40.8	124.0, 131.7, 77.7	49.8, 63.7, 126.7	49.9, 64.9, 126.5
α, β, γ (°)	90, 90, 90	90, 90, 90	90, 90, 90	90, 90, 90
Resolution (Å)	50.00-1.42(1.50-1.42)	50.00-2.90(2.95-2.90)	50.00-1.55(1.58-1.55)	50.00-1.60(1.63-1.60)
R_{sym} or R_{merge}	0.08 (0.12)	0.107 (>1)	0.067 (0.742)	0.081 (0.934)
I/σI	20.9 (14.9)	12.4 (1.1)	22.5 (2.5)	22.1 (2.1)
Completeness (%)	99.9 (99.6)	94.3 (92.2)	99.6 (99.9)	99.6(100)
Redundancy	6.2 (6.1)	4.8 (3.6)	7.2 (6.9)	7.2 (7.0)
$CC_{1/2}$ in highest shell	0.98	0.49	0.78	0.92
Refinement				
Resolution (Å)	50.00-1.40	50.00-2.90	50.00-1.55	50.00-1.60
No. reflections	9146	11348	29459	27251
Rwork/ Rfree	0.183/0.211	0.201/0.242	0.205/0.230	0.200/0.224
No. of atoms				
Protein	388	1385	1040	1025
Ligand/ion	0	1416	434	432
Water	50	2	119	173
B-factors (Å2)				
Protein	12.3	52.6	28.2	33.7
Ligand/ion	0	46.5	25.4	30.3
Water	21.2	18.3	32.6	44.2
R.m.s deviations				
Bond lengths (Å)	0.005	0.004	0.018	0.007
Bond angles (°)	0.908	0.728	1.95	0.963
Ramachandran plot				
Favored (%)	100	97.7	96.9	100
Allowed (%)	0	2.3	3.1	0
Disallowed (%)	0	0	0	0

Numbers in parenthesis are for highest resolution.
* Due to detector limitation, the GcrA-DBD dataset was cut at 1.55 Å although the signals at the highest shell are very strong

Table S3. Constructs used in this study.

Constructs	Description	Source
pET28a-TEV-*gcrA(1-45)*	Expression vector for HIS_6-GcrA-DBD (1-45)	This study
pTOLO-EX5-*gcrA(88-173)*	Expression vector for SUMO-GcrA-SID (88-173)	This study
pET28a-TEV-*gcrA(1-173)*	Expression vector for HIS_6-GcrA (1-173)	This study
pET28b-σ^{70}	Expression vector for *Cc*σ^{70}	This study
pET28b-σ^{70}*(K273C)*	Expression vector for *Cc*$\sigma^{70}{}_2$ (K273C)	This study
pET28a-TEV-$\sigma^{70}{}_2$*(128-487)*	Expression vector for HIS_6- *Cc*$\sigma^{70}{}_2$ (128-487)	This study
pEASY-*mipZ*-tR2	Template vector for *mipZ* promoter	This study
pEASY-*CCNA-00157*-tR2	Template vector for *CCNA-00157* promoter	This study
P_{xyl}-*gcrA*	Expression vector for WT GcrA	This study
P_{xyl}-*gcrA(R33W)*	Expression vector for GcrA(R33W)	This study
P_{xyl}-*gcrA(I37E)*	Expression vector for GcrA(I37E)	This study
P_{xyl}-*gcrA(I37W)*	Expression vector for GcrA(I37W)	This study
P_{xyl}-*gcrA(G38F)*	Expression vector for GcrA(G38F)	This study
P_{xyl}-*gcrA(G38Y)*	Expression vector for GcrA(G38Y)	This study
P_{xyl}-*gcrA(G38W)*	Expression vector for GcrA(G38W)	This study
pENTR-*gcrA*	Template vector for pENTR-*gcrA* mutants and P_{xyl}-*gcrA*	This study
pENTR-*gcrA(W3A)*	Template vector for P_{xyl}-*gcrA(W3A)*	This study
pENTR-*gcrA(W15A)*	Template vector for P_{xyl}-*gcrA(W15A)*	This study
pENTR-*gcrA(S20A)*	Template vector for P_{xyl}-*gcrA(S20A)*	This study
pENTR-*gcrA(S22A)*	Template vector for P_{xyl}-*gcrA(S22A)*	This study
pENTR-*gcrA(R33A)*	Template vector for P_{xyl}-*gcrA(R33A)*	This study
pENTR-*gcrA(R33W)*	Template vector for P_{xyl}-*gcrA(R33W)*	This study
pENTR-*gcrA(N34A)*	Template vector for P_{xyl}-*gcrA(N34A)*	This study
pENTR-*gcrA(I37A)*	Template vector for P_{xyl}-*gcrA(I37A)*	This study
pENTR-*gcrA(I37E)*	Template vector for P_{xyl}-*gcrA(I37E)*	This study
pENTR-*gcrA(I37W)*	Template vector for P_{xyl}-*gcrA(I37W)*	This study
pENTR-*gcrA(G38F)*	Template vector for P_{xyl}-*gcrA(G38F)*	This study
pENTR-*gcrA(G38Y)*	Template vector for P_{xyl}-*gcrA(G38Y)*	This study
pENTR-*gcrA(G38W)*	Template vector for P_{xyl}-*gcrA(G38W)*	This study
pENTR-*gcrA(K39A)*	Template vector for P_{xyl}-*gcrA(K39A)*	This study

pENTR-*gcrA(H41A)*	Template vector for P_{xyl}-*gcrA(H41A)*	This study
pENTR-*gcrA(R42A)*	Template vector for P_{xyl}-*gcrA(R42A)*	This study
pENTR-*gcrA(R42A, R33A)*	Template vector for P_{xyl}-*gcrA(R42A, R33A)*	This study
pENTR-*gcrA(K39A, R42A)*	Template vector for P_{xyl}-*gcrA(K39A, R42A)*	This study
pENTR-*gcrA(N34A, I37A)*	Template vector for P_{xyl}-*gcrA(N34A, I37A)*	This study
pENTR-*gcrA(R33W)*	Template vector for P_{xyl}-*gcrA(R33W)*	This study
pENTR-*gcrA(I37E)*	Template vector for P_{xyl}-*gcrA(I37E)*	This study
pENTR-*gcrA(I37W)*	Template vector for P_{xyl}-*gcrA(I37W)*	This study
pENTR-*gcrA(G38F)*	Template vector for P_{xyl}-*gcrA(G38F)*	This study
pENTR-*gcrA(G38Y)*	Template vector for P_{xyl}-*gcrA(G38Y)*	This study
pENTR-*gcrA(G38W)*	Template vector for P_{xyl}-*gcrA(G38W)*	This study
pMT375 (pRXMCS-5)	Low-copy vector for xylose-inducible expression (tet^R)	Thanbichler *et al*., 2007
pMT375-*gcrA*	Xylose inducible expression of GcrA	Haakonsen *et al*., 2015
pMT375-*gcrA(W3A)*	Xylose inducible expression of GcrA(W3A)	This study
pMT375-*gcrA(W15A)*	Xylose inducible expression of GcrA(W15A)	This study
pMT375-*gcrA(S20A)*	Xylose inducible expression of GcrA(S20A)	This study
pMT375-*gcrA(S22A)*	Xylose inducible expression of GcrA(S22A)	This study
pMT375-*gcrA(R33A)*	Xylose inducible expression of GcrA(R33A)	This study
pMT375-*gcrA(N34A)*	Xylose inducible expression of GcrA(N34A)	This study
pMT375-*gcrA(I37A)*	Xylose inducible expression of GcrA(I37A)	This study
pMT375-*gcrA(K39A)*	Xylose inducible expression of GcrA(K39A)	This study
pMT375-*gcrA(H41A)*	Xylose inducible expression of GcrA(H41A)	This study
pMT375-*gcrA(R42A)*	Xylose inducible expression of GcrA(R42A)	This study
pMT375-*gcrA(R42A,R33A)*	Xylose inducible expression of GcrA(R42A,R33A)	This study
pMT375-*gcrA(K39A,R42A)*	Xylose inducible expression of GcrA(K39A,R42A)	This study
pMT375-*gcrA(N34A,I37A)*	Xylose inducible expression of GcrA(N34A,I37A)	This study
pMT375-*gcrA(R33W)*	Xylose inducible expression of GcrA(R33W)	This study
pMT375-*gcrA(I37E)*	Xylose inducible expression of G*gcrA(I37E)*	This study

pMT375-*gcrA(I37W)*	Xylose inducible expression of GcrA (I37W)	This study
pMT375-*gcrA(G38F)*	Xylose inducible expression of GcrA (G38F)	This study
pMT375-*gcrA(G38Y)*	Xylose inducible expression of GcrA (G38Y)	This study
pMT375-*gcrA(G38W)*	Xylose inducible expression of GcrA (G38W)	This study
pKNT25-σ^{70}	σ^{70}-T25	Haakonsen et al., 2015
pKNT25-σ^{70}*(L128A)*	σ^{70}(L128A)-T25	This study
pKNT25-σ^{70}*(L129A)*	σ^{70}(L129A)-T25	This study
pKNT25-σ^{70}*(G133E)*	σ^{70}(G133E)-T25	This study
pKNT25-σ^{70}*(A136E)*	σ^{70}(A136E)-T25	This study
pKNT25-σ^{70}*(K139A)*	σ^{70}(K139A)-T25	This study
pKNT25-σ^{70}*(R140A)*	σ^{70}(R140A)-T25	This study
pKNT25-σ^{70}*(A143E)*	σ^{70}(A143E)-T25	This study
pKNT25-σ^{70}*(E411A)*	σ^{70}(E411A)-T25	This study
pKNT25-σ^{70}*(R462A)*	$\sigma^{70(}$R462A)-T25	This study
pUT18-*gcrA*	GcrA-T18	Haakonsen *et al*., 2015
pUT18-*gcrA(C118A)*	GcrA(C118A)-T18	This study
pUT18-*gcrA(K119A)*	GcrA(K119A)-T18	This study
pUT18-*gcrA(W120A)*	GcrA(W120A)-T18	This study
pUT18-*gcrA(K119A,W120A)*	GcrA(K119A,W120A)-T18	This study
pUT18-*gcrA(D124A)*	GcrA(D124A)-T18	This study
pUT18-*gcrA(G129D)*	GcrA(G129D)-T18	This study
pUT18-*gcrA(F130A)*	GcrA(F130A)-T18	This study
pUT18-*gcrA(F132A)*	GcrA(F132A)-T18	This study
pUT18-*gcrA(F130A,F132A)*	GcrA(F130A,F132A)-T18	This study
pUT18-*gcrA(Y142A)*	GcrA(Y142A)-T18	This study
pUT18-*gcrA(C143A)*	GcrA(C143A)-T18	This study
pUT18-*gcrA(Y142A ,C143A)*	GcrA(Y142A ,C143A)-T18	This study

pUT18-*gcrA(H146A)*	GcrA(H146A)-T18	This study
pUT18-*gcrA(C143A,H146A)*	GcrA(C143A,H146A)-T18	This study
pUT18-*gcrA(Y151A)*	GcrA(Y151A)-T18	This study
pUT18-*gcrA(Q152A)*	GcrA(Q152A)-T18	This study
pUT18-*gcrA(Y151A, Q152A)*	GcrA(Y151A, Q152A)-T18	This study
pUT18-*gcrA(K158A, K159A)*	GcrA(K158A, K159A)-T18	This study
pET28b-GcrA	Expression vector for purification of HIS_6-GcrA (Fig 5.A)	Haakonsen *et al*., 2015
pET28b-GcrA(1-107)	Expression vector for purification of HIS_6-$GcrA_{1-107}$ (Fig 5.A)	This study
pET28b-GcrA(51-173)	Expression vector for purification of HIS_6-$GcrA_{51-173}$ (Fig 5.A)	This study
pET28b-CcrM	Expression vector for purification of HIS_6-CcrM	Haakonsen *et al*., 2015

Table S4. Primers used in this study.

Constructs	Sequences(5'->3')
pET28a-TEV-*gcrA*(1-45)	F: CCATGGATATGAGCTGGACCGACGAA R: GAATTCTTACAGGCCCAGACGGTGC
pTOLO-EX5-*gcrA*(88-173)	F: AAAACCTGTATTTTCAGGGCGCCATGGATGTTCCGGTCGCCGCG R: TGGTGGTGGTGGTGCTCGAGACTGCAGTTAGATGTAGCGGCGAAGC
pET28a-TEV-*gcrA*(1-173)	F: CCATGGATATGAGCTGGACCGACGAA R: GAATTCTTAGATGTAGCGGCGAAGC
pET28b-σ^{70}	F: AGATCATATGTTGATGAGCAACAATTCCTCGGCC R: AGATAAGCTTTTACGAGTCCAGGAAGCTGCGCAG
pET28b-σ^{70}(K273C)	F: GTCGGCAGCCGCCTGTGCGGCGAGGACC R: GCACAGGCGGCTGCCGACCAGCTTGTCCT
pET21a-TEV-$\sigma^{70}{}_2$(112-487)	F: ATATGCATCACCATCACCATCACGATTACGATATCCCAACGACCGAAA ACCTGTATTTTCAGGGCACCGACGACCCGGTG R: GAATTCTTAAGCCTGGTCGGCGATC
pET21a-TEV-$\sigma^{70}{}_2$(128-487)	F: CATATGCATCACCATCACCATCACGATTACGATATCCCAACGACCGAAAA CCTGTATTTTCAGGGCCTGCTCTCGCGCGAA R: GAATTCTTAAGCCTGGTCGGCGATC
pEASY-*mipZ*-tR2	F: AGGCCCTTAGCCCCCTCGGG R:AAATAAAAAGGCCTGCGATTACCAGCAGGCCCGCGTTTCGGCCATGGCTCG
pEASY-*CCNA-00157*-tR2	F: TGACGCCCAGGGCAAGTTGC R: AAATAAAAAGGCCTGCGATTACCAGCAGGCCGACCGTCACATAGTTCAGCT
pENTR-*gcrA*	F:CACCATGAGCTGGACCGACGAACG R:TTAGATGTAGCGGCGAAGCG
P_{xyl}-*gcrA* and mutants	F: CGGAATTCCATATGAGCTGGACCGACGAACG R: TGTATCGCTAGCTTAGATGTAGCGGCGAAGCG
pENTR-*gcrA(W3A)*	F: GCAACCGACGAACGGGTTTCCACC R: GCTCATGGTGAAGGGGGCGGC
pENTR-*gcrA(W15A)*	F: AAGAAGCTCGCCTTGGACGGCCTTTCGGCCAGCC R: CAGGGTGGAAACCCGTTCGTCG
pENTR-*gcrA(S20A)*	F: GCGGCCAGCCAGATCGCCAAGCAAT R: AAGGCCGTCCAACCAGAGCTTC
pENTR-*gcrA(S22A)*	F: TCGGCCGCGCAGATCGCCAAGCAATTGGGC R: AAGGCCGTCCAACCAGAGCTTC
pENTR-*gcrA(R33A)*	F:GCCAACGCGGTGATCGGCAAGGTG R: CGTCACGCCGCCCAATTGCTTG
pENTR-*gcrA(R33W)*	F: TGGAACGCGGTGATCGGCAAGGTG R: CGTCACGCCGCCCAATTGC
pENTR-*gcrA(N34A)*	F: GCCGCGGTGATCGGCAAGGTGCACC R: ACGCGTCACGCCGCCCAATTG
pENTR-*gcrA(I37A)*	F: AACGCGGTGGCCGGCAAGGTGCACCGTCTGGGCC R: ACGCGTCACGCCGCCCAATTG
pENTR-*gcrA(I37E)*	F: GAAGGCAAGGTGCACCGTCTGG R: CACCGCGTTACGCGTCACG
pENTR-*gcrA(I37W)*	F: TGGGGCAAGGTGCACCGTCTGG R: CACCGCGTTACGCGTCACG
pENTR-*gcrA(G38F)*	F: TTCAAGGTGCACCGTCTGGGCCTG R: GATCACCGCGTTACGCGTCAC
pENTR-*gcrA(G38Y)*	F: CGCGGTGATCTATAAGGTGCACCGTCTGGGCCTG R: GGTGCACCTTATAGATCACCGCGTTACGCGTCAC
pENTR-*gcrA(G38W)*	F: TGGAAGGTGCACCGTCTGGGCCTG R: GATCACCGCGTTACGCGTCAC
pENTR-*gcrA(K39A)*	F: AACGCGGTGATCGGCGCCGTGCACCGTCTGGGCCTGTC R: ACGCGTCACGCCGCCCAATTG
pENTR-*gcrA(H41A)*	F: AACGCGGTGATCGGCAAGGTGGCCCGTCTGGGCCTGTCGGGCCG R: ACGCGTCACGCCGCCCAATTG
pENTR-*gcrA(R42A)*	F: GCGCTGGGCCTGTCGGGCCGCGC R: GTGCACCTTGCCGATCACCGCG

pENTR-*gcrA(R42A,R33A)*	F: GCGCTGGGCCTGTCGGGCCGCGC R: GTGCACCTTGCCGATCACCGCG
pENTR-*gcrA(N34A,I37A)*	F: GCCGCGGTGGCCGGCAAGGTGCACC R: ACGCGTCACGCCGCCCAATTG
pENTR-*gcrA(R33W)*	F: TGGAACGCGGTGATCGGCAAGGTG R: CGTCACGCCGCCCAATTGC
pENTR-*gcrA(I37E)*	F: GAAGGCAAGGTGCACCGTCTGG R: CACCGCGTTACGCGTCACG
pENTR-*gcrA(I37W)*	F: TGGGGCAAGGTGCACCGTCTGG R: CACCGCGTTACGCGTCACG
pENTR-*gcrA(G38F)*	F: TTCAAGGTGCACCGTCTGGGCCTG R: GATCACCGCGTTACGCGTCAC
pENTR-*gcrA(G38Y)*	F: CGCGGTGATCTATAAGGTGCACCGTCTGGGCCTG R: GGTGCACCTTATAGATCACCGCGTTACGCGTCAC
pENTR-*gcrA(G38W)*	F: TGGAAGGTGCACCGTCTGGGCCTG R: GATCACCGCGTTACGCGTCAC
pKNT25-σ^{70}*(L128A)*	F: GCGCTCTCGCGCGAAGGCGAAATCG R: TTCCACCGAGCCCATTTCGCGC
pKNT25-σ^{70}*(L129A)*	F: CTGGCGTCGCGCGAAGGCGAAATCGC R: TTCCACCGAGCCCATTTCGCGC
pKNT25-σ^{70}*(G133E)*	F: CTGCTCTCGCGCGAAGAGGAAATCGCCATCGCCAAGCGCATC R: TTCCACCGAGCCCATTTCGCGC
pKNT25-σ^{70}*(A136E)*	F: CTGCTCTCGCGCGAAGGCGAAATCGAGATCGCCAAGCGCATCGAGGCC R: TTCCACCGAGCCCATTTCGCGC
pKNT25-σ^{70}*(K139A)*	F: GCGCGCATCGAGGCCGGCCGCGAC R: GGCGATGGCGATTTCGCCTTCG
pKNT25-σ^{70}*(R140A)*	F: AAGGCCATCGAGGCCGGCCGCGAC R: GGCGATGGCGATTTCGCCTTCG
pKNT25-σ^{70}*(A143E)*	F: AAGCGCATCGAGGAGGGCCGCGACACGATGATCCGC R: GGCGATGGCGATTTCGCCTTCG
pKNT25-σ^{70}*(E411A)*	F: GCGGCCCGTCAGGCCAAGAAGGAA R: GCGCTCGCCCTTCTGCACG
pKNT25-σ^{70}*(R462A)*	F: GCCCGCGGCTACAAGTTCTCGACCTAC R: ATACTCGAACTTATCGACGGCCTTCATC
pUT18-*gcrA(C118A)*	F:GCCAAGTGGCCGATCGGCGATCCG R:CATGTGCGCGCCCAGGGTCAG
pUT18-*gcrA(K119A)*	F:TGCGCATGGCCGATCGGCGATCCGTC R: CATGTGCGCGCCCAGGGTCAG
pUT18-*gcrA(W120A)*	F:TGCAAGGCACCGATCGGCGATCCGTCGTC R: CATGTGCGCGCCCAGGGTCAG
pUT18-*gcrA(K119A,W120A)*	F:TGCGCCGCACCGATCGGCGATCCGTCGTC R: CATGTGCGCGCCCAGGGTCAG
pUT18-*gcrA(D124A)*	F:TGCAAGTGGCCGATCGGCGCGCCGTCGTCGGAAGGCTTCACCTTCTGCG R: CATGTGCGCGCCCAGGGTCAG
pUT18-*gcrA(G129D)*	F:GACTTCACCTTCTGCGGTCGTCGCTC R:TTCCGACGACGGATCGCCGATCG
pUT18-*gcrA(F130A)*	F:GGCGCAACCTTCTGCGGTCGTCGCTCG R: TTCCGACGACGGATCGCCGATCG
pUT18-*gcrA(F132A)*	F:GGCTTCACCGCATGCGGTCGTCGCTCGTCGGAAGG R: TTCCGACGACGGATCGCCGATCG
pUT18-*gcrA(F130A,F132A)*	F:GGCGCAACCGCATGCGGTCGTCGCTCGTCGGAAGG R: TTCCGACGACGGATCGCCGATCG
pUT18-*gcrA(Y142A)*	F:GCATGCGTCGAGCACGCGCGGGTG R:GGGGCCTTCCGACGAGCGACGAC
pUT18-*gcrA(C143A)*	F:TATGCAGTCGAGCACGCGCGGGTGGCC R:GGGGCCTTCCGACGAGCGACGAC
pUT18-*gcrA(Y142A ,C143A)*	F: GCAGCAGTCGAGCACGCGCGGGTGGCC R:GGGGCCTTCCGACGAGCGACGAC
pUT18-*gcrA(H146A)*	F: TATTGCGTCGAGGCAGCGCGGGTGGCCTACCAGCCTC R: GGGGCCTTCCGACGAGCGACGAC

pUT18-*gcrA(C143A,H146A)*	F: TATGCAGTCGAGGCAGCGCGGGTGGCCTACCAGCCTC R: GGGGCCTTCCGACGAGCGACGAC
pUT18-*gcrA(Y151A)*	F: GCACAGCCTCAGCAGACCAAGAAGAAGAGC R: GGCCACCCGCGCGTGCTCGAC
pUT18-*gcrA(Q152A)*	F:TACGCACCTCAGCAGACCAAGAAGAAGAGCGGC R:GGCCACCCGCGCGTGCTCGAC
pUT18-*gcrA(Y151A, Q152A)*	F: GCAGCACCTCAGCAGACCAAGAAGAAGAGCGGC R: GGCCACCCGCGCGTGCTCGAC
pUT18-*gcrA(K158A, K159A)*	F: TACCAGCCTCAGCAGACCAAGGCAGCAAGCGGCGGCGCCGAACTGGCC R: GGCCACCCGCGCGTGCTCGAC
pET28b-GcrA(1-107)	F: GATTCATATGAGCTGGACCGACGAACG R: AGATGAGCTCTTACGAGCCGGGTTCTTCGTGACGG
pET28b-GcrA(51-173)	F: AGATCATATGCCCTCGCAACCGGCCCGTCCG R: GTAGGAGCTCTTAGATGTAGCGGCGAAGCG

SUPPLEMENTAL FIGURE LEGEND

Figure S1. GcrA-SID/σ^{70}_2 complex formation. **(A)** The binary complex is separated from individual components on a Superdex S200 column. **(B)** SDS-PAGE results of elution peaks of (A).

Figure S2. The structure analysis of GcrA-SID/σ^{70}_2 complex. **(A)** The anomalous difference map (red) contoured at 4 σ show clear signals for 7 out of 7 Se atoms in σ^{70}_2, and the 2Fo-Fc maps (blue) contoured at 1.0 σ for *Cc*GcrA-SID shows clear density and fitted model. **(B)** The anomalous difference map (red) contoured at 4 σ shows the presence of a Zn atom, and the 2Fo-Fc map (blue) contoured at 1.0 σ shows clear density for the coordination residues. The schematics **(C)** and structures **(D)** of *C. crescentus* and *E. coli* σ^{70} factors (PDB: 3UGO). **(E)** Crystal structures of *Cc*GcrA-SID/*Cc*σ^{70}_2 complex (left), *Mt*RbpA-SID/*Mt*σ^{70}_2 complex (middle; PDB: 4X8K), and superimposition of the two structures. The CR and NCR of *Ec*σ^{70}, black and gray; The CR and NCR of *Cc*σ^{70}, orange and yellow; GcrA, cyan; rbpA, green.

Figure S3. The sequence comparison between *C. crescentus* σ^{70} and σ^{32}. **(A)** The schematics of *C. crescentus* σ^{70} and σ^{32} show that both of them have a conserved $\sigma_{1.2}$ and σ_2, (σ^{70}, orange; σ^{32}, blue) but a radically different non-conserved region (σ^{70}, yellow; σ^{32}, light blue). The gray shadow boxes on σ^{70} indicate GcrA-interacting regions. **(B)** The sequence alignment of *C. crescentus* σ^{70} and σ^{32} by Clustal Omega. Conserved residues, red boxes; σ^{70} residues contacting GcrA, green boxes; GcrA-interacting regions on σ^{70}, gray shadow boxes.

Figure S4. The GcrA-DBD/m^{6}A-DNA structures. **(A)** The asymmetry unit of crystal form 1 contains four GcrA-DBD molecules and four dsDNA molecules.

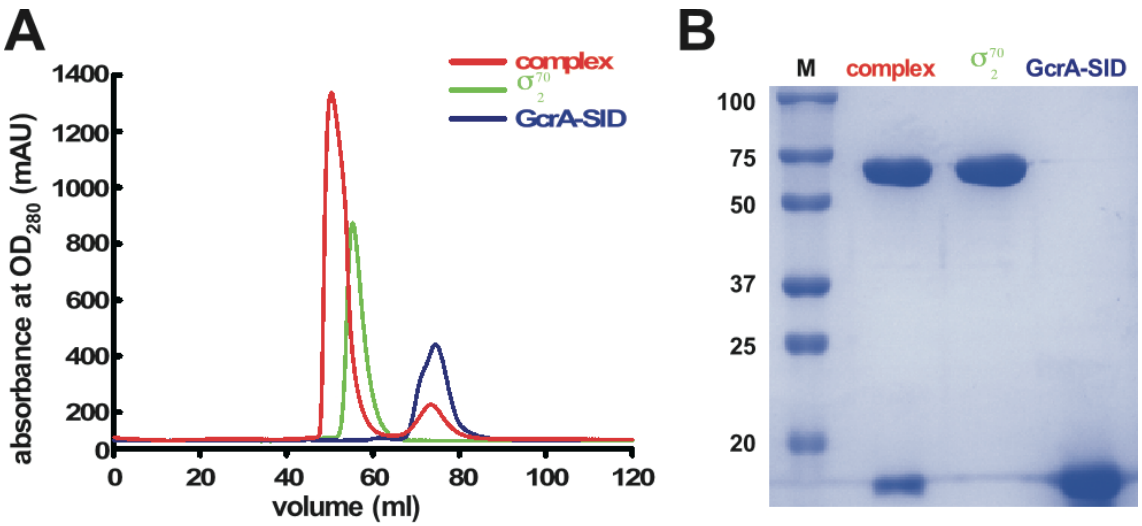
Superimposition of four GcrA-DBD/DNA structures shows an identical interaction between GcrA-DBD and DNA (circle in the middle). **(B)** The asymmetry unit of crystal form 2 contains three GcrA-DBD molecules and one dsDNA. Molecule 1 of GcrA-DBD interacts with the major groove of dsDNA; molecule 2 of GcrA-DBD makes no interaction with dsNA; and molecule 3 of GcrA-DBD makes non-specific interaction with the minor groove of dsDNA. **(C)** Superimposition of the complex structure from crystal form 1 (red) and the complex structure from crystal form 2 (cyan) suggests that the interaction between GcrA-DBD molecule 1 and DNA in crystal 2 is essentially the same as the interaction between GcrA-DBD and DNA in crystal form 1. **(D and E)** The 2Fo-Fc map contoured at 2 σ shows clear density for DNA and m^6A on both strands. **(F)** Structure superimposition of *C. crescentus* GcrA-DBD (this study; cyan) and *T. aquaticus* σ^{70}_4 (yellow; PDB: 1KU7). **(G)** Structure superimposition between *C. crescentus* GcrA-DBD/m^6A-DNA (this study; cyan and gray) and *T. aquaticus* σ^{70}_4/-35 element (yellow). **(H)** Sequence alignment between *C. crescentus* GcrA-DBD and σ^{70}_4 from various bacterial species. Identical or similar residues, red; residues contacting DNA, green. **(I)** The western blot results of GcrA mutants in Figure 3G. **(J)** The western blot results of GcrA mutants in Figure 4D. The RNAP α subunit (RpoA) was used as a loading control. Surface presentation of GcrA-DBD shows the pockets for methyl groups on '+' **(K)** or '-' **(L)** strand of unmethylated dsDNA. The colors are as in previous figures. (M) Superimposition of GcrA-DBD/m^6A-DNA structure and GcrA-DBD/DNA structure (orange).

Figure S5. Structure models of GcrA-RPo complexes. **(A)** The GcrA-DBD at promoter region centered at -30 (middle) has no clash with RNAP, however GcrA-DBD centered at promoter region centered at -27 has potential clash with RNAP β subunit and GcrA-DBD at promoter region centered at -33 has potential clash with

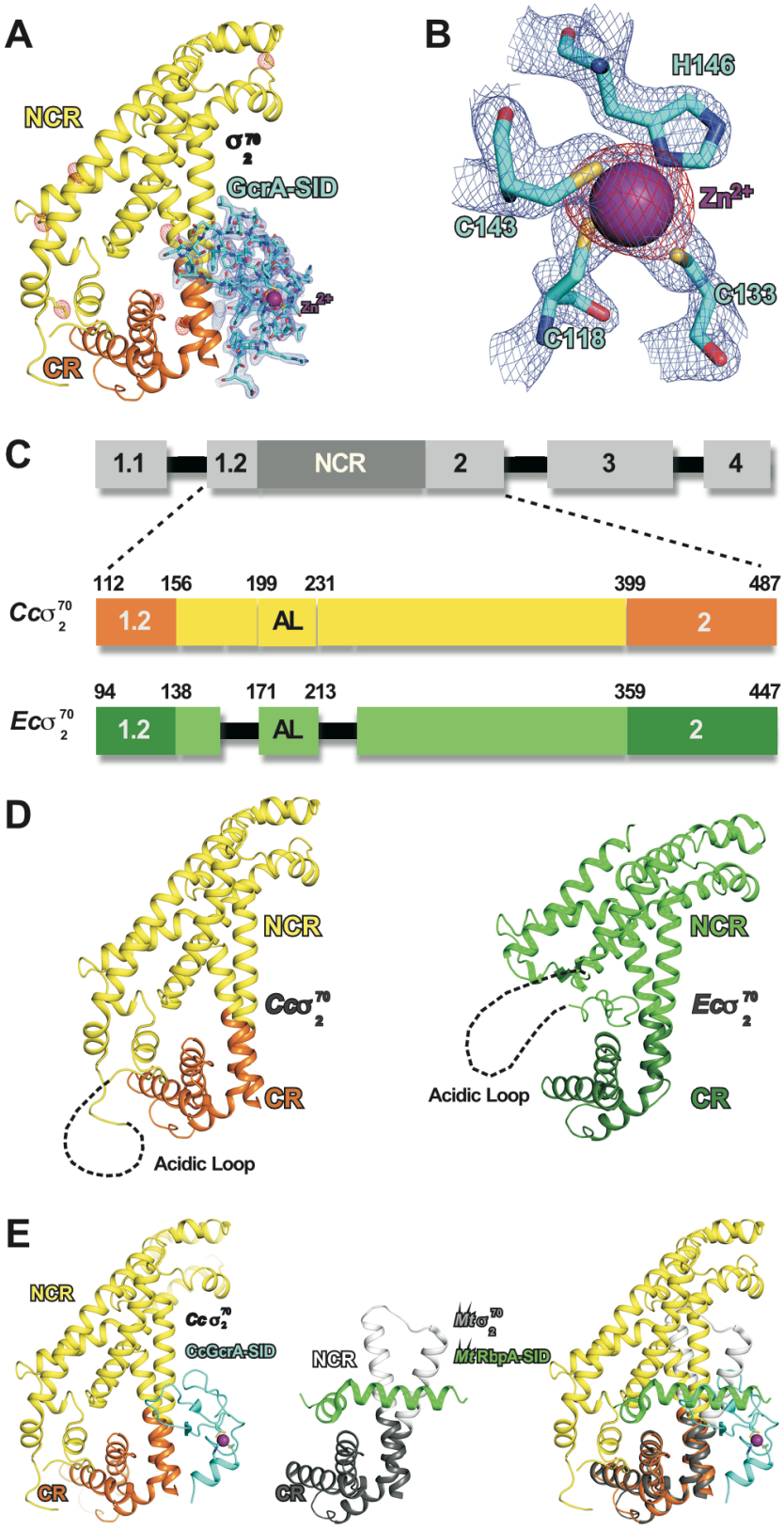
σ^{70}_4 and RNAP β subunit. **(B)** The GcrA-DBD at promoter region centered at -20 (middle) has no clash with RNAP, however GcrA-DBD at promoter region centered at -18 has potential clash with σ^{70}_3 and GcrA-DBD at promoter region centered at -22 has potential clash with σ^{70}_{NCR}. Blue arrows point to the potential clashes. **(C)** Structures models of GcrA-DBD at different locations of downstream promoter DNA suggest that GcrA-DBD is able to bind on a broad range of locations. The estimated distances between GcrA-DBD at various positions and GcrA-CTD bound on σ^{70}_2 are listed. **(D)** The top panel shows the conserved basic patch at C-terminal domain of GcrA from an alignment of ~1000 GcrA protein sequences, and the bottom panel shows that the C-terminal basic patch is physically close to the backbone of -10 element nontemplate ssDNA. The dash indicates a possible path for the C-terminal basic patch and potential interactions between K157/K158 with the phosphate of -8/-9 nucleotides.

SUPPLEMENTAL FIGURES

Supplemental figure 1



Supplemental figure 2



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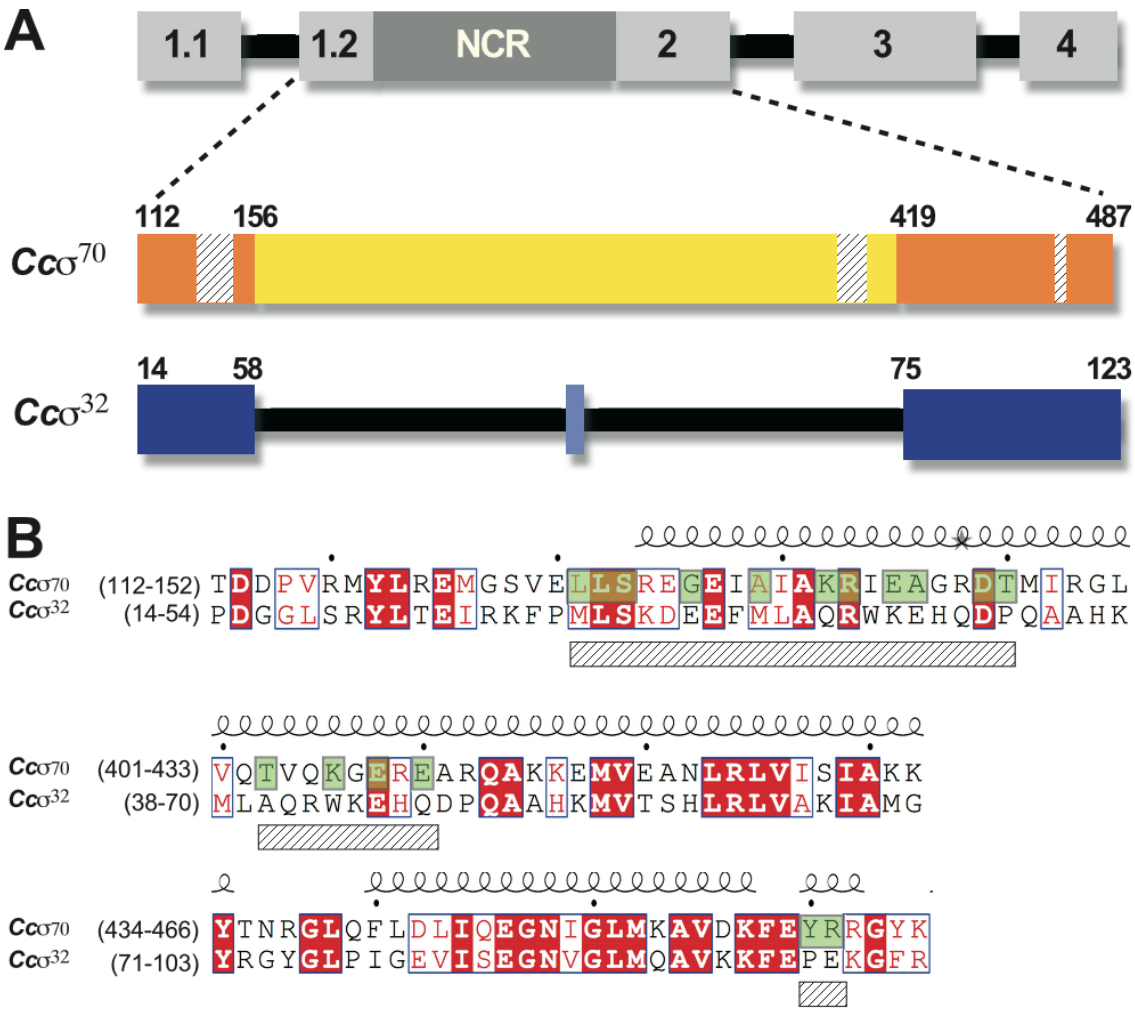
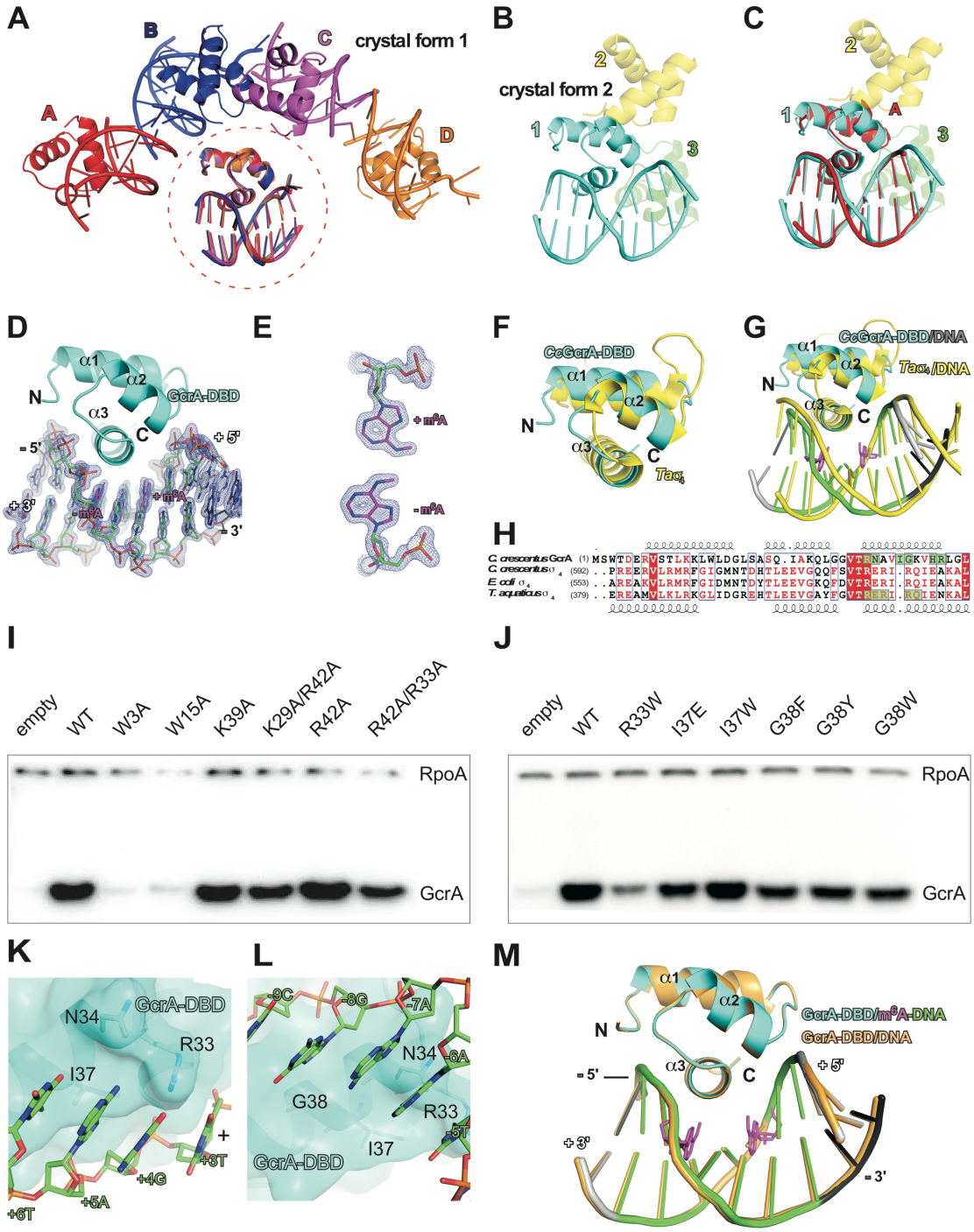


Supplemental figure 4



Supplemental figure 5