Supplement to

Acetylation of the Response Regulator RcsB Controls Transcription from a Small RNA Promoter

Linda I. Hu[1], Bui Khanh Chi[2], Misty L. Kuhn[3], Ekaterina V. Filippova[3], Arti J. Walker-Peddakotla[1], Katrin Bäsell[2], Dörte Becher[2], Wayne F. Anderson[3], Haike Antelmann[2], and Alan J. Wolfe[1#]

[1]Department of Microbiology and Immunology Loyola University Chicago Stritch School of Medicine 2160 S. First Ave. Bldg. 105 Maywood, IL 60153, USA

[2]Institute for Microbiology Ernst-Moritz-Arndt-University of Greifswald, F.-L.-Jahn-Str. 15, D-17487 Greifswald, Germany

[3]Center for Structural Genomics of Infectious Diseases Northwestern University, Feinberg School of Medicine, Department of Molecular Pharmacology and Biological Chemistry, Chicago, IL 60611, USA

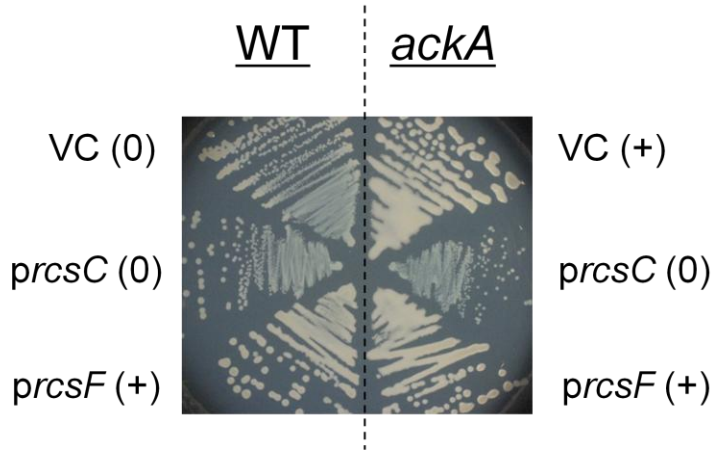

Figure S1. RcsC and RcsF overexpression can manipulate the mucoid phenotype on TB. AJW678 WT or the isogenic *ackA* mutant transformed with the vector control (pTrc99a), an RcsC overexpression plasmid (pPSG980), or an RcsF overexpression plasmid (pMH300) were grown on TB plates supplemented with ampicillin and 50 µM IPTG at 22°C for 2 days. The image was taken on a light box. "+" indicates mucoidy and "0" indicates no observable mucoidy.

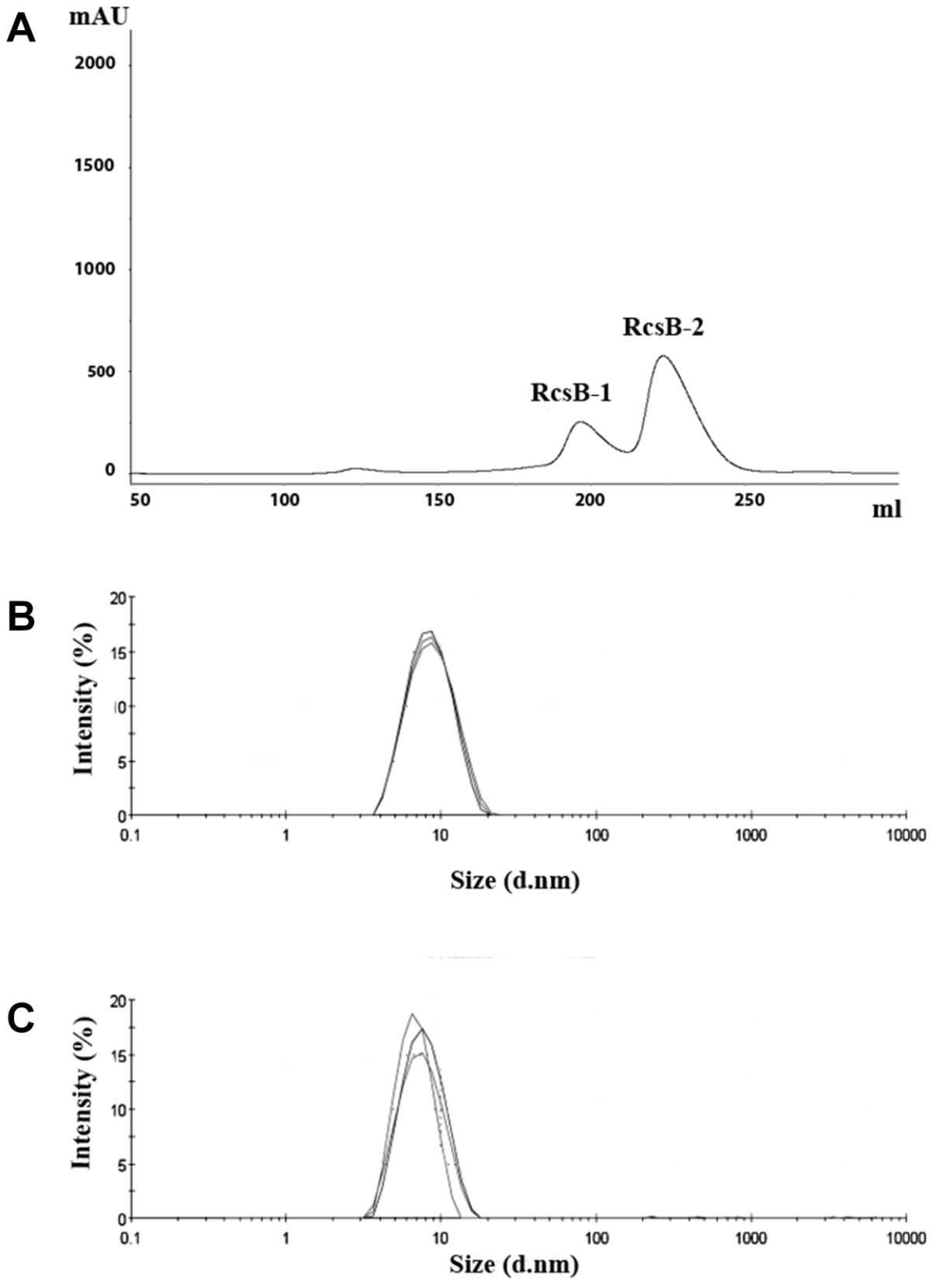
A
mAU
2000
1500
1000
500
0
RcsB-2
RcsB-1
50
100
150
200
250
ml
B
Intensity (%)
20
15
10
5
0
0.1
1
10
100
1000
10000
Size (d.nm)
C
Intensity (%)
20
15
10
5
0
0.1
1
10
100
1000
10000
Size (d.nm)

Figure S2. Gel filtration and dynamic light scattering analysis of RcsB.

A) Elution profile at 280 nm of the purified RcsB protein in 10 mM Bis–Tris, 500 mM Sodium Chloride, pH 8.3. Flow rate 3.2 ml/min on a gel filtration column (Superdex) attached to FPLC system.

B) Distribution of scattering intensity for RcsB peak 1 (RcsB-1) at ~ 1.0 mg/ml. The estimated molecular weight of RcsB-1 is 110 kDa (tetramer).

C) Distribution of scattering intensity for RcsB peak 2 (RcsB-2) at ~ 1.0 mg/ml. The estimated molecular weight of RcsB-2 is 67 kDa (dimer).

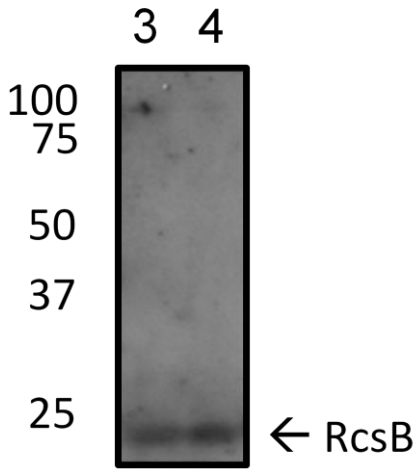

Figure S3. Purified RcsB acetylation is detectable by anti-acetyl lysine Western immunoblot analysis.

A longer exposure time (5 minutes) of the blot from Figure 6A, showing lanes 3 and 4, which contains purified RcsB peak 1 and peak 2, respectively.

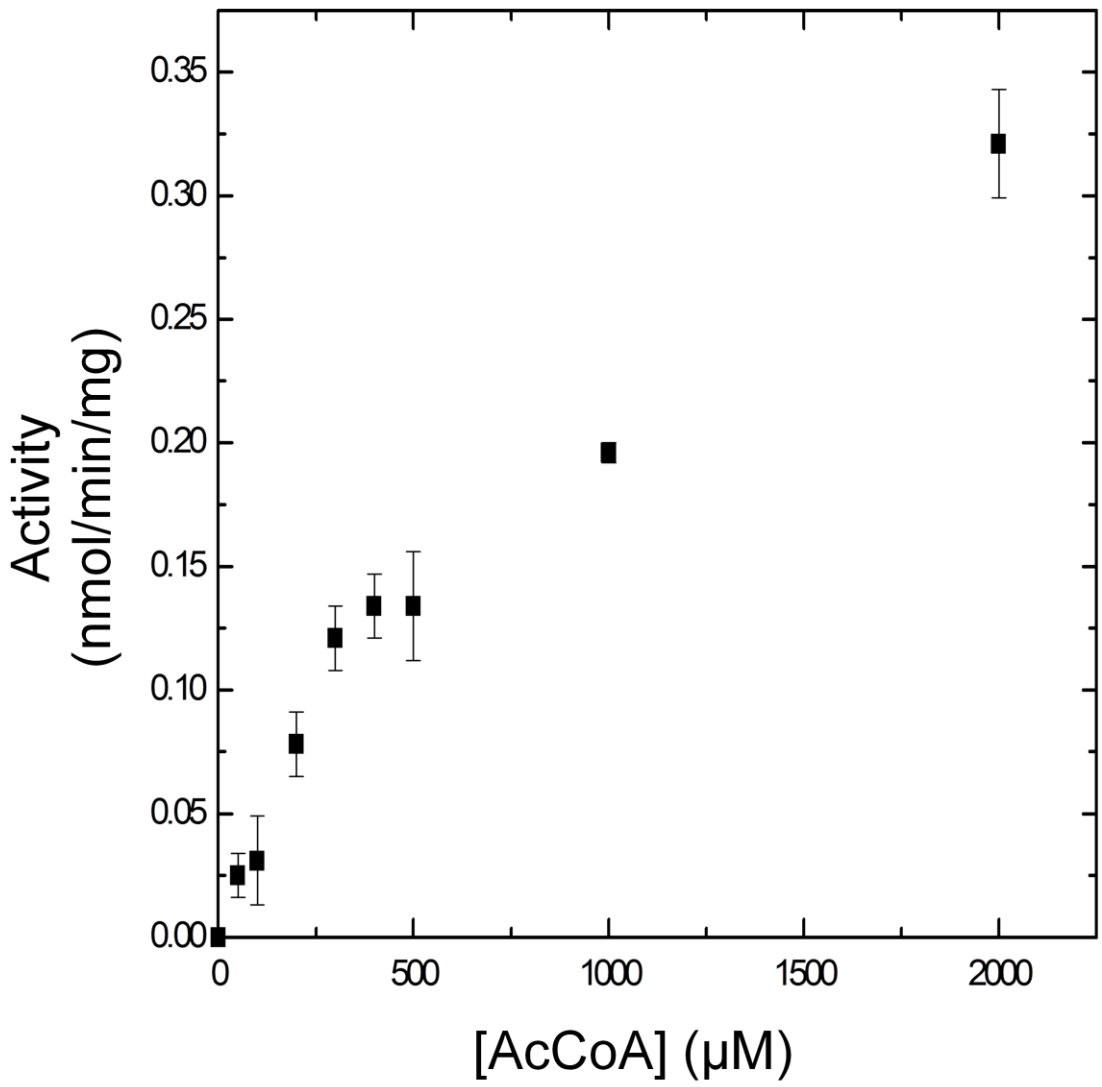

Figure S4. Concentration-dependent hydrolysis of AcCoA by YfiQ

Activity of YfiQ versus increasing concentrations of AcCoA. Reactions were performed at 25 degrees Celsius for one hour in a 50 µl reaction volume at 0-2 mM concentrations of AcCoA. YfiQ (0.02 mM) was used to initiate the reaction. Activity (nmol/min/mg) is the average of two separate trials.