Structure *16*

Supplemental Data

Starch Catabolism by a Prominent Human Gut Symbiont Is Directed by the Recognition of Amylose Helices

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Table S1. Bacterial Strains and Plasmids

Bacterial strains	Features/use	Source or reference
B. thetaiotaomicron ATCC 29148	wild-type parent strain; $FUdR^S$	Jeffrey I. Gordon Laboratory
B. thetaiotaomicron Δ*tdk*	ATCC 29148 with *tdk* deletion; $FUdR^R$	This work
B. thetaiotaomicron Δ*susD*	Derivative of Δ*tdk* strain with *susD* deletion	This work
B. thetaiotaomicron Δ*susD::PsusB-susD*	Δ*susD* strain with complementing *susD* allele	This work

Plasmids	Features/use	Source or reference
pGERM	Source of *bla-ermG* fragment	(Shipman et al., 1999)
pEPJ::N2	source of *att-intN2* from *NBU2*	Abigail Salyers
pKNOCK-cm	*ori* R6K suicide vector; Cml^R	(Alexeyev, 1999)
pKNOCK-*bla-ermG*b	Derivative of pKNOCK-Cm, Erm^R, Amp^R	This study
pExchange-*tdk*	Derivative of pKNOCK-*bla-ermG*b carrying cloned *tdk*	This study
p*NBU2-bla-ermG*b	Derivative of pKNOCK-*bla-ermG*b, *inserts into NBU2 att1* and/or *att2* sites, Erm^R, Amp^R	This study

Table S2. Primers

Primers:	Sequence (5'-3')	Use
susD fwd (*Nhe*I)	ctagctagcatcaacgatctggatattagtccc	*susD* cloning/over-expression
susD rev (*Xho*I)	ccgctcgagttatttatagccttcattttgtg	*susD* cloning/over-expression
bla-ermG fwd (*Mlu*I)	gcgacgcgttatgagtaaacttggtctgacag	pKNOCK-*bla-ermG*b construction
bla-ermG rev (*Mlu*I)	gcgacgcgtaaacatttacagttgcatgtggc	pKNOCK-*bla-ermG*b construction
intN2-att fwd (*Kpn*I)	gcgggtaccaacaaatactttcaggacgatgta	pNBU2-*ermG*b construction
intN2-att rev (*Sal*I)	gcggtcgactaattgcctatcttccagtgatg	pNBU2-*ermG*b construction
Δ*tdk* upstream fwd (*Kpn*I)	gcgggtaccgaaaccattggtagagataccg	*tdk* (*BT2275*) deletion
Δ*tdk* upstream rev (*Sal*I)	gcggtcgactaccatgtgatttttgtgctttc	*tdk* (*BT2275*) deletion
Δ*tdk* downstream fwd (*Sal*I)	gcggtcgacgacggacagaagatataaactc	*tdk* (*BT2275*) deletion
Δ*tdk* downstream rev (*Spe*I)	gcgactagttcgccggacatttcggaaagg	*tdk* (*BT2275*) deletion
tdk fwd (*Kpn*I)	gcgggtaccgcttcttccacaacagtctgc	*tdk* cloning for pExchange-*tdk*
tdk fwd (*Sal*I)	gcggtcgactcgaatgttatcttctttctttcc	*tdk* cloning for pExchange-*tdk*
Δ*susD* upstream fwd (*Sal*I)	gcggtcgacgtcatcatacacaaacttcaagtc	*susD* (*BT3701*) deletion
Δ*susD* upstream rev	catgataaattgatttaatgaatgttag	*susD* (*BT3701*) deletion
Δ*susD* downstream fwd	ctaacattcattaaatcaatttatcatgtaaccaagagttc atccttatataaaag	*susD* (*BT3701*) deletion
Δ*susD* downstream rev (*Xba*I)	gcgtctagatccgcatcctccagtatctgc	*susD* (*BT3701*) deletion
P_{susB} fwd (*Sal*I)	gcggtcgacataagttcacttcaacttatatttatcag	*susD* complementation
P_{susB} rev (*Eco*R V)	gcggatatccattctatttatggtattaaattataagc	*susD* complementation
susD complement fwd (*Eco*R V)	gcggatatcctttcttattaggtttaagtctgc	*susD* complementation
susD complement rev (*Spe*I)	gcgactagtttatttatagccttcattttgtgacat	*susD* complementation
NBU2 *att1* fwd	cctttgcaccgctttcaacg	flanks $tRNA^{ser}$ *NBU2 att1*
NBU2 *att1* rev	tcaactaaacatgagatactagc	flanks $tRNA^{ser}$ *NBU2 att1*
NBU2 *att2* fwd	tatcctattctttagagcgcac	flanks $tRNA^{ser}$ *NBU2 att2*
NBU2 *att2* rev	ggtgtacctggcattgaagg	flanks $tRNA^{ser}$ *NBU2 att2*
susA qRT-PCR fwd	tcggcagtagaaggttttga	*susA* transcript quantification
susA qRT-PCR rev	actccgaggtctgctatgtaatc	*susA* transcript quantification
susB qRT-PCR fwd	gcccgcaatatcctcgcttca	*susB* transcript quantification
susB qRT-PCR rev	cgctggtcagttcgtcggtgtaa	*susB* transcript quantification
susC qRT-PCR fwd	gctattggcggggcattgg	*susC* transcript quantification
susC qRT-PCR rev	cagcggattttggggagagttcg	*susC* transcript quantification
susD qRT-PCR fwd	gtatcgacggctctcctgacctc	*susD* transcript quantification
susD qRT-PCR rev	cccaaacggacgtaaacccactct	*susD* transcript quantification
susE qRT-PCR fwd	gttttccggcagctaccacctac	*susE* transcript quantification
susE qRT-PCR rev	aagtcgagaagcgcattgttgagt	*susE* transcript quantification
susF qRT-PCR fwd	ggctgttgatcccgacggctattat	*susF* transcript quantification
susF qRT-PCR rev	aaggctttggtccagtctatgtcgtt	*susF* transcript quantification
susG qRT-PCR fwd	ccgccggacatccttacatctatta	*susG* transcript quantification
susG qRT-PCR rev	gtccgctaccgttttcacatttttc	*susG* transcript quantification
16s qRT-PCR fwd	ggtagtccacacagtaaacgatgaa	16s transcript quantification
16s qRT-PCR rev	cccgtcaattcctttgagtttc	16s transcript quantification

Figure S1. qRT-PCR of *sus* Genes

Wild-type, Δ*susD* and Δ*susD::*P_{susB}*-susD B. thetaiotaomicron* strains were grown to mid-log phase on either glucose or maltose as a sole carbon source. Fold-induction of individual genes in the *susABCDEFG* cluster are expressed as the ratio of expression on maltose divided by expression on glucose. In the Δ*susD* mutant only the *susD* transcript is missing, indicating that the in-frame deletion of this gene does not influence expression of other *sus* genes. Note that despite being controlled by the maltose-inducible P_{susB} promoter, a single-copy of *susD* placed in the genome at one of the two *att NBU2* insertion sites provides ~10% of wild-type *susD* expression. Reduced *susD* expression in the complemented strain is the likely reason for partial recovery of the *susD* dependent growth phenotypes shown in Figure. 2.

Figures S2-S5. These Figures Show the Raw Data and Attempts at Using Origin for Fitting to the Data for Maltoheptaose, α-Cyclodextrin (G6), β-Cyclodextrin (G7), and γ-Cyclodextrin (G8), Respectively

The low affinity of the ligands made for unstable refinement of the various binding parameters and therefore a stepwise binding analysis was performed as described in the Methods section.

Figure S6. Native and SDS PAGE Analysis of SusD Showing a Possible Oligomeric Assembly in Solution

On the right side of this figure is an SDS-PAGE gel demonstrating that the cloned and purified SusD sample is extremely homogeneous. On the left side of this figure is a native gel of the same sample of SusD showing a series of higher molecular weight bands consistent with oligomerization of SusD in solution.

Supplemental Experimental Procedures

B. thetaiotaomicron Growth and Culture Conditions

Bacterial strains, plasmids and primers are summarized in Tables S3 and S4. *B. thetaiotaomicron* was routinely grown in TYG medium (Holdeman et al., 1977) or on brain-heart infusion (BHI; Beckton dickinson, co.) agar supplemented with 10% horse blood and stored at -80°C in TYG supplemented with 25% glycerol. Antibiotics (Sigma) were added as appropriate: erythromycin (25 μg ml^{-1}), gentamycin (200 μg ml^{-1}) and 5-fluor-2'-deoxyuridine (FUdR, 200 μg ml^{-1}). *E. coli* S17-1 λ *pir* (Simon et al., 1983) was used for conjugation of constructs into *B. thetaiotaomicron*.

Quantitative Real-Time PCR

Quantification of *susABCDEFG* transcripts was conducted by quantitative real-time PCR (qRT-PCR) using total cellular RNA extracted from the glucose- and maltose-grown cultures of Figure 2 (n=3 per strain and growth condition). Bacteria were harvested during logarithmic growth between OD_{600} values of 0.5-0.57 and preserved in RNAProtect (Qiagen) according to the manufacturer's instructions. RNA was extracted using an RNAeasy purification kit (Qiagen) and 2 μg of RNA from each sample was reverse-transcribed into cDNA using Superscript II reverse transcriptase (Invitrogen). qRT-PCR was conducted on a Stratagene model MX3000P instrument. PCR conditions were 40 cycles of 15 sec at 95^{o}C, 45 sec at 55^{o}C, 30 sec at 72^{o}C. The 25 μL PCR reactions contained 12.5 μL ABsolute™ QPCR SYBR® Green ROX (ABgene House, Epsom, UK), 0.25 μL UDP-N-glycosidase (1 unit/μL; Invitrogen), 10 ng of cDNA and gene-specific primers (0.3 μM each). Individual samples were normalized by 16s rDNA quantification.

New *Bacteroides* spp. Plasmid Constructs

Three new plasmid constructs were created during this work (Table S3) that are derivatives of the *ori*R6K suicide vector pKNOCK-Cm (Alexeyev, 1999). Plasmid pKNOCK-*bla-ermG*b was constructed by removing an *Mlu*I fragment carrying the *cat* (chloramphenicol resistance) gene from pKNOCK-Cm and replacing it with an *Mlu*I ended *bla-ermG* fragment (containing β-lactamase for ampicillin selection in *E. coli* and *ermG* for erythromycin selection in *Bacteroides* spp.) that was amplified from the *Bacteroides* spp. suicide vector pGERM (Shipman et al., 1999).

Two additional plasmids were constructed by elaborating upon pKNOCK-*bla-ermG*b. The counter-selectable suicide vector for *B. thetaiotaomicron* allelic exchange, pExchange-*tdk*, was constructed by amplifying *BT2275* (promoter and ORF) and ligating it into the *Kpn*I and *Sal*I sites of pKNOCK-*bla-ermG*b. The NBU2-based genomic insertion vector, pNBU2-*bla-ermG*b, was constructed by amplifying a fragment containing the integrase (*intN2*) and attachment site (*attN2*) from the non-replicating *Bacteroides* element NBU2 using plasmid pEPJ::N2 (a kind gift from Abigail Salyers, University of Illinois, Urbana-Champagne) as a template and ligating it into the *Kpn*I and *Sal*I sites of pKNOCK-*bla-ermG*b. The pNBU2-*bla-ermG*b carries cloned DNA fragments into the *B. thetaiotomicron* genome in single-copy by integrating into one of two $tRNA^{ser}$ attachment sites.

Supplemental References

1. Alexeyev, M.F. (1999). The pKNOCK series of broad-host-range mobilizable suicide vectors for gene knockout and targeted DNA insertion into the chromosome of gram-negative bacteria. BioTechniques *26*, 824-826, 828.

2. Holdeman, L.V., Cato, E.D., and Moore, W.E.C. (1977). Anaerobe Laboratory Manual. (Blacksburg, Va., Virginia Polytechnic Institute and State University Anaerobe Laboratory).

3. Shipman, J.A., Cho, K.H., Siegel, H.A., and Salyers, A.A. (1999). Physiological characterization of SusG, an outer membrane protein essential for starch utilization by Bacteroides thetaiotaomicron. Journal of bacteriology *181*, 7206-7211.

4. Simon, R., Priefer, U., and Puhler, A. (1983). A broad host range mobilization system for *in vivo* genetic engineering: transposon mutagenesis in Gram-negative bacteria. Biotechnology *1*, 784-791.

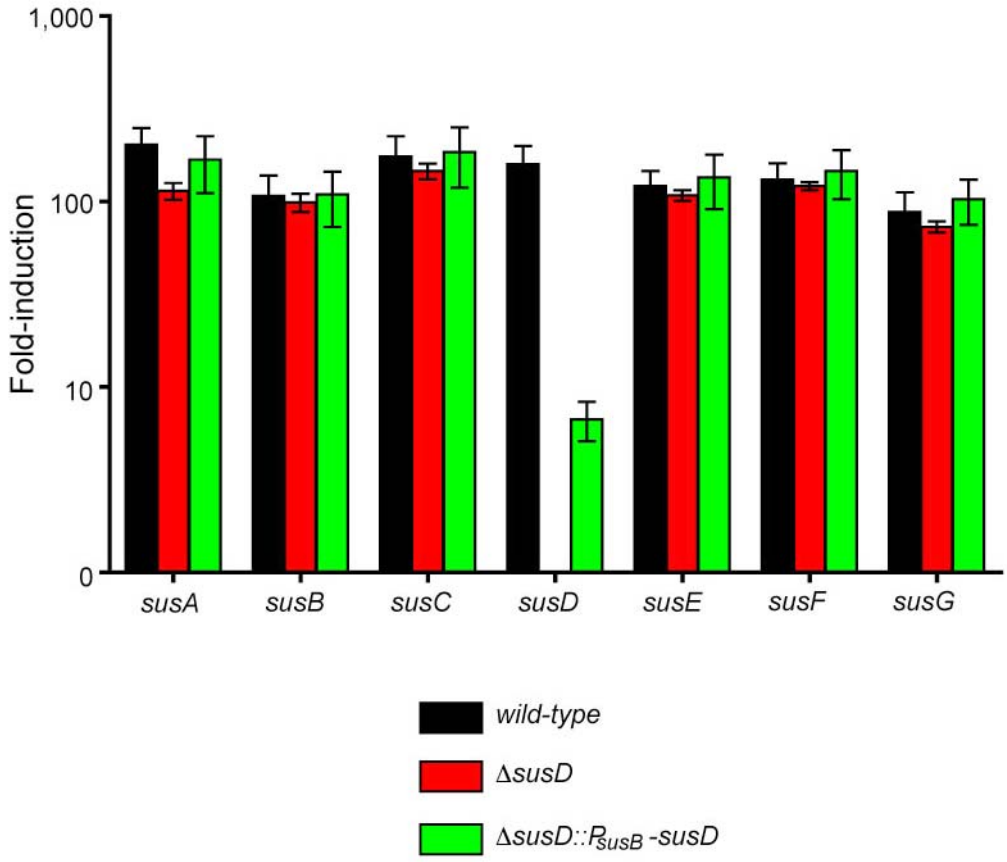
1,000
100
10
0
Fold-induction
susA
susB
susC
susD
susE
susF
susG
wild-type
ΔsusD
ΔsusD::P$_{susB}$ -susD

Figure S1

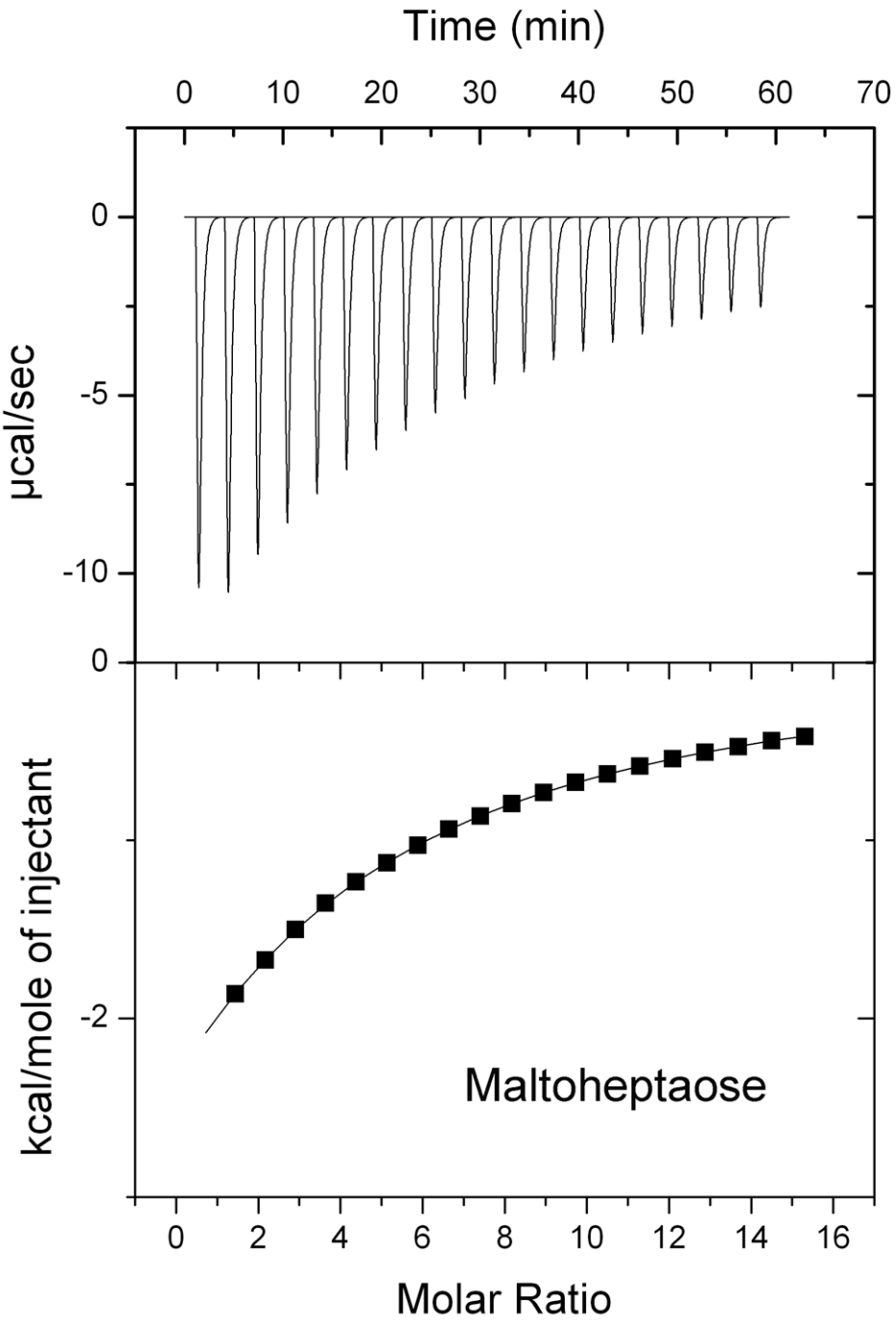
Time (min)
0
10
20
30
40
50
60
70
0
-5
-10
0
µcal/sec
kcal/mole of injectant
-2
Maltoheptaose
0
2
4
6
8
10
12
14
16
Molar Ratio

Figure S2

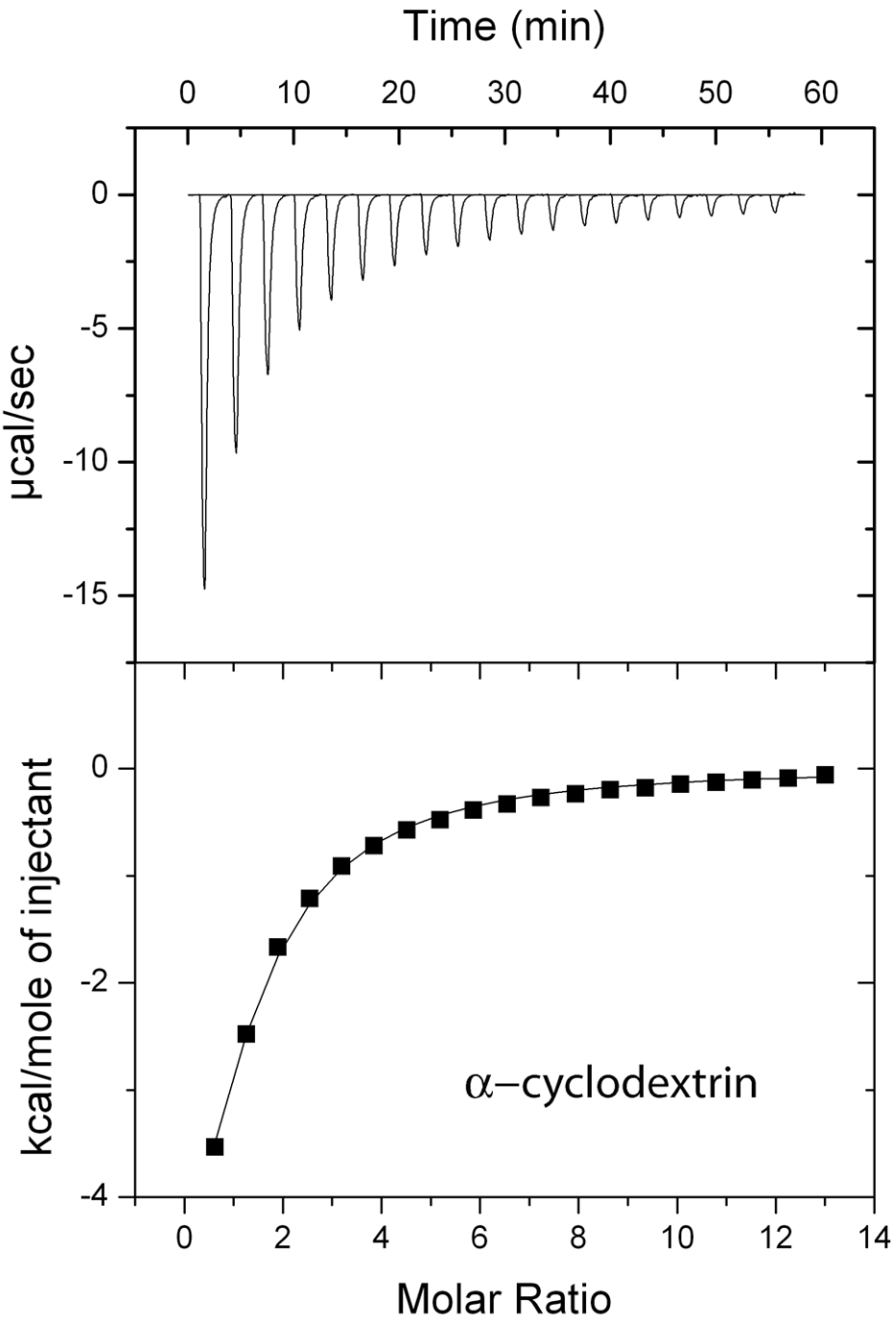
Time (min)
0
10
20
30
40
50
60
0
-5
-10
-15
µcal/sec
0
-2
-4
kcal/mole of injectant
α–cyclodextrin
0
2
4
6
8
10
12
14
Molar Ratio

Figure S3

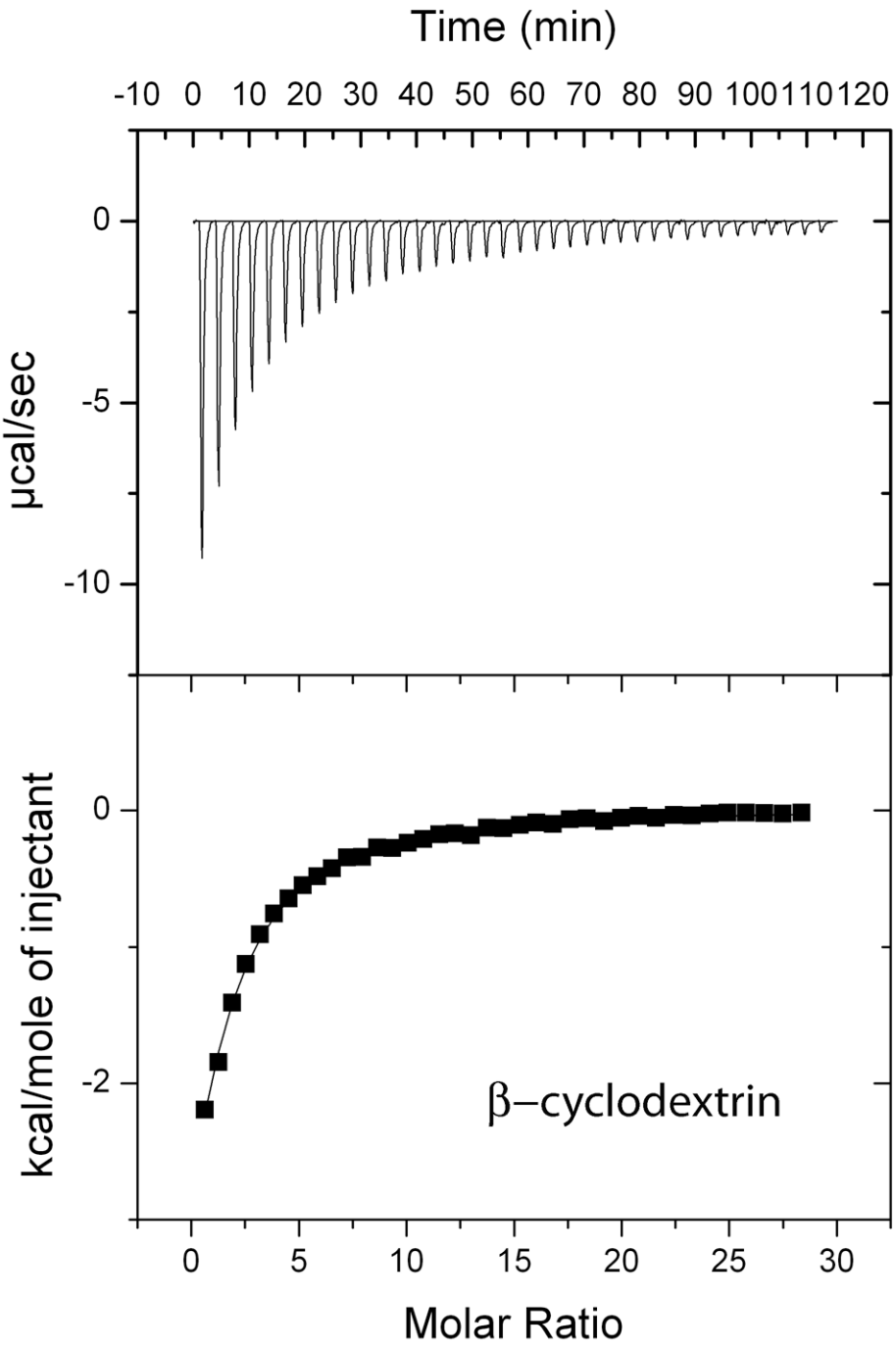
Time (min)
-10 0 10 20 30 40 50 60 70 80 90 100 110 120
0
-5
-10
µcal/sec
0
-2
kcal/mole of injectant
β–cyclodextrin
0 5 10 15 20 25 30
Molar Ratio

Figure S4

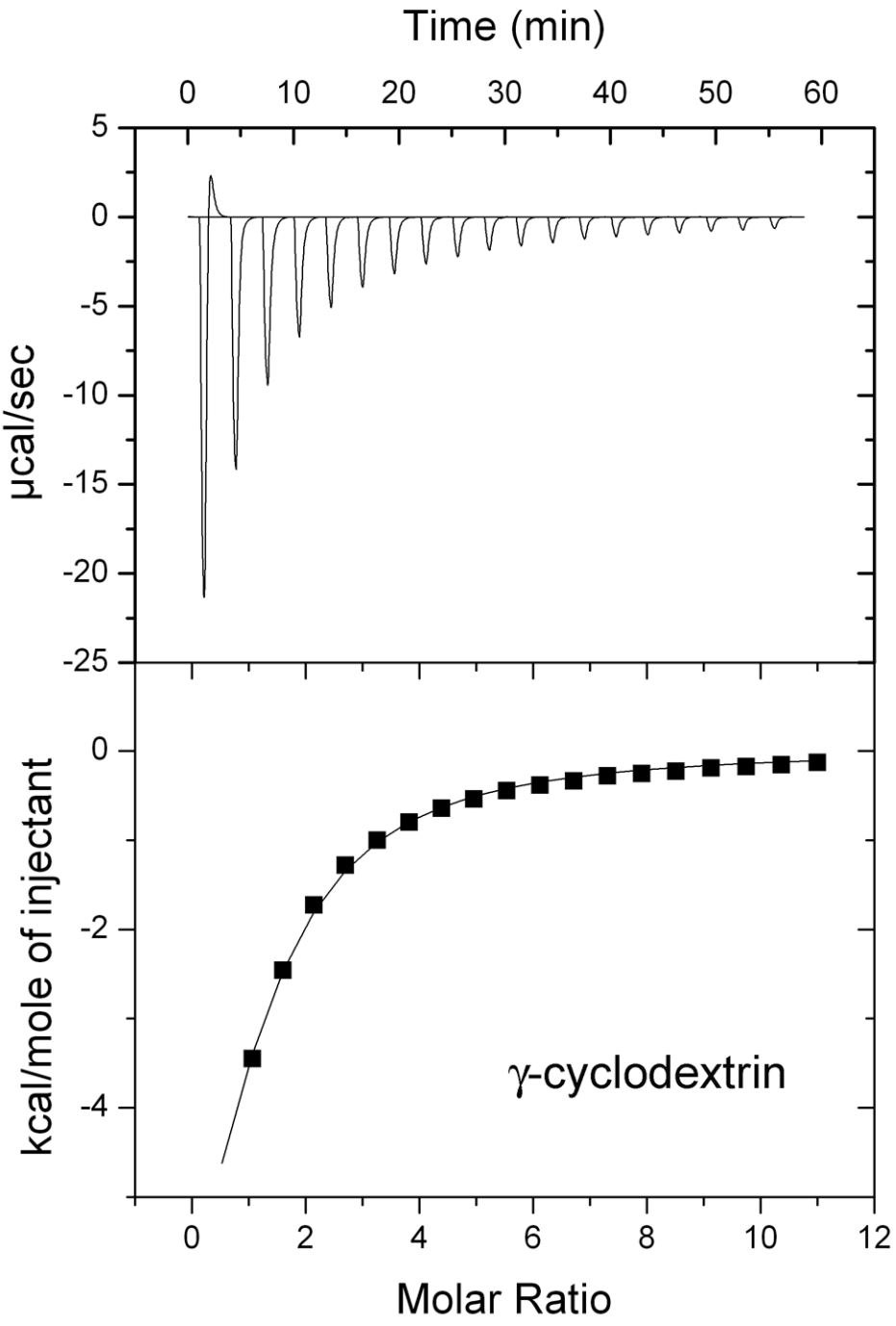
Time (min)
0
10
20
30
40
50
60
5
0
-5
-10
-15
-20
-25
µcal/sec
0
-2
-4
kcal/mole of injectant
γ-cyclodextrin
0
2
4
6
8
10
12
Molar Ratio

Figure S5

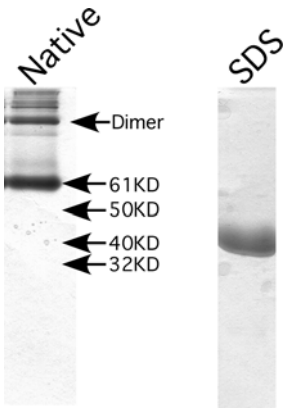

Figure S6