Supplemental Information for

Sharply tuned pH response of genetic competence regulation in *Streptococcus mutans*: A microfluidic study of environmental sensitivity of *comX*

Minjun Son[1], Delaram Ghoreishi[1], Sang-Joon Ahn[2], Robert A. Burne[2], Stephen J. Hagen[1].

[1]Department of Physics, University of Florida, PO Box 118440, Gainesville FL 32611 USA

[2]Department of Oral Biology, University of Florida, PO Box 100424, Gainesville FL 32611 USA

.

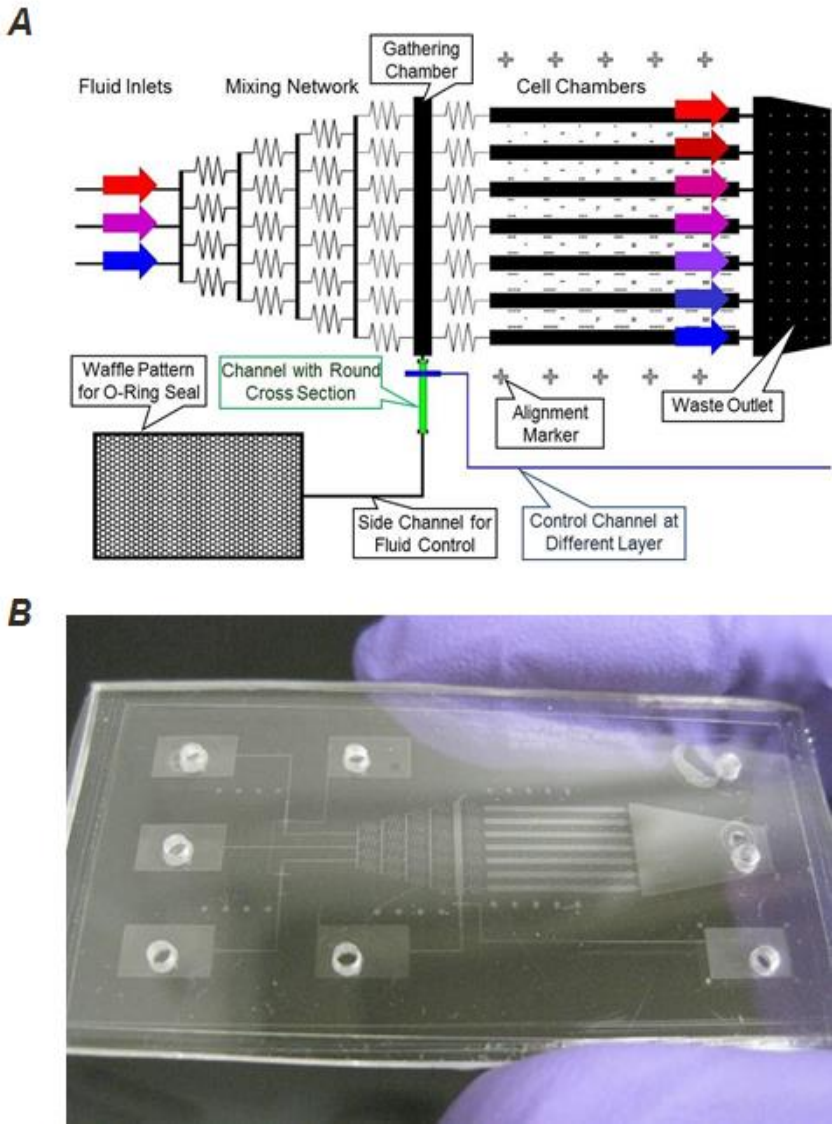

Figure S1

(**A**) Schematic and (**B**) photograph of the microfluidic flow device. Two-layer lithography allows air-actuated valve control of fluid flow in the channels. Fluids supplied at the three inlets are mixed by the network to generate differences (i.e. a gradient) in the peptide signal concentration or pH of the different chambers. A cell culture is initially introduced through the outlet port (at right), so that culture and inlet flows meet in the gathering chamber and exit together through the side channel. When enough cells have adhered in the cell chambers and a stable concentration gradient has formed, the side channel is closed by a control valve. This immediately switches the flow direction so that the cells in the chambers are perfused with mixed, incoming media that provide the desired concentration gradient.

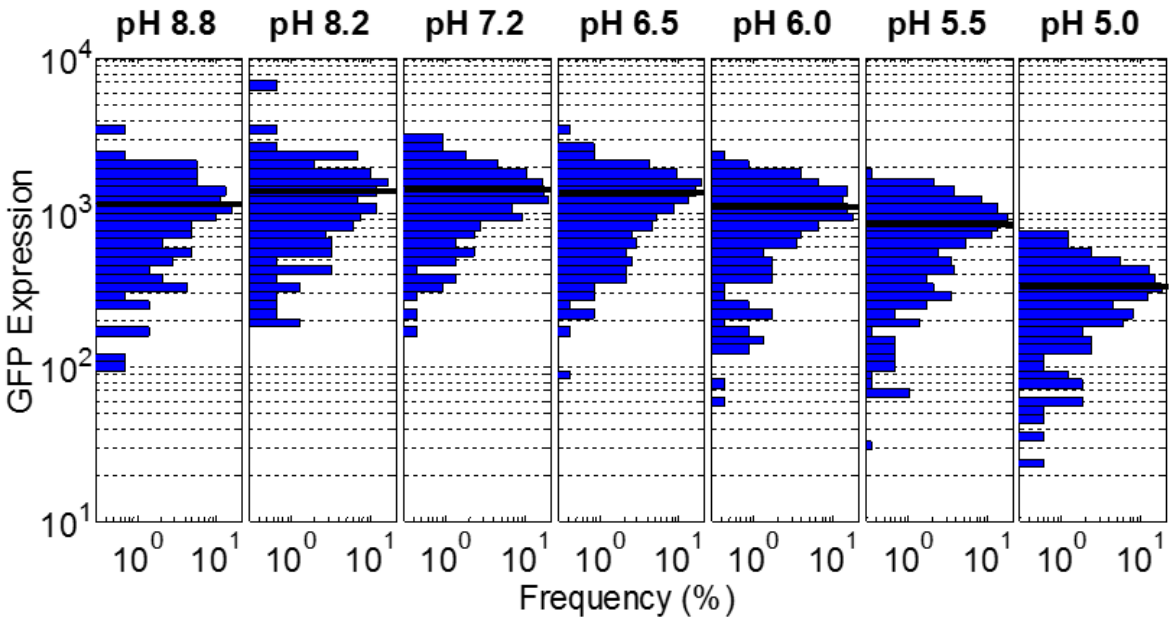

Figure S2

Effect of pH on the fluorescence of a GFP reporter. To estimate the significance of pH alone on the brightness of a GFP reporter, the fluorescence of individual cells of an *S. mutans* strain constitutively expressing *gfp* was measured. The pH of a bulk culture in early exponential phase was adjusted to the values shown and, 30-60 minutes later, GFP fluorescence images were collected and analyzed. Histograms show GFP levels in individual cells, and black horizontal bars indicate the mean values. The impact of pH on fluorescence is modest in the range pH 6 – pH 8 that is the focus of the present study. Fluorescence was more significantly suppressed below pH 6.0. The mean fluorescence at its peak (near pH 7.2) is approximately 1.27X larger than at pH 6.0, but 4.3X larger than at pH 5.0.

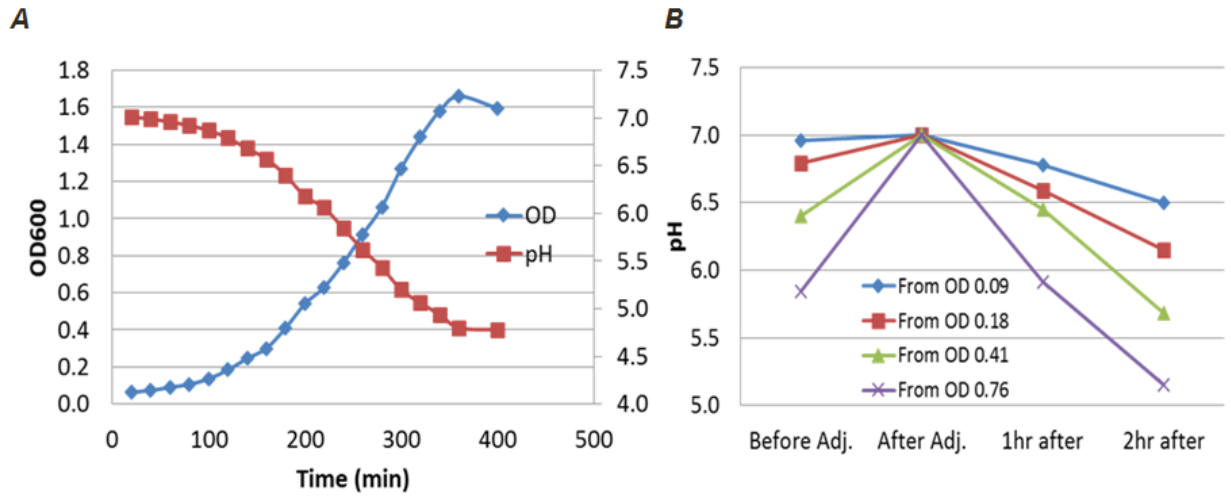

Figure S3

Rapid acidification of growth medium by a bulk culture of *S. mutans*. An overnight culture of *S. mutans* was diluted 40X into a fresh medium of ⅓ BHI and ⅔ FMC (v:v). (**A**) The pH and OD (1 cm at 600 nm) of the culture were measured every 20 minutes. The pH falls rapidly during growth, especially during the mid-exponential phase when the OD increases most quickly. (**B**) To estimate the rate of acidification and compare it to the response time for CSP or XIP, a sample of the bulk *S. mutans* culture was taken at four different growth stages (OD = 0.09, 0.18, 0.41, and 0.76) and its pH was adjusted to 7.0. The figure shows the pH after 1-2 h of further growth. After 1 h, the pH of all samples had dropped roughly to its pre-adjustment value.

In our P_{comX}-*gfp* reporter strain, CSP required 1−1.5 h of incubation to induce a strong response from P_{comX}. Adjusting the pH of a mid- or late-exponential phase bulk culture to a neutral value would not then be sufficient P_{comX} response to recover before the culture becomes acidified. By contrast, XIP induces a strong P_{comX} response within 30 min of incubation (Son *et al.*,2012). Consequently, adjusting the pH of a mid- or late-exponential phase culture to neutral pH may permit XIP to induce detectable P_{comX} activity before the culture becomes too acidified to respond.

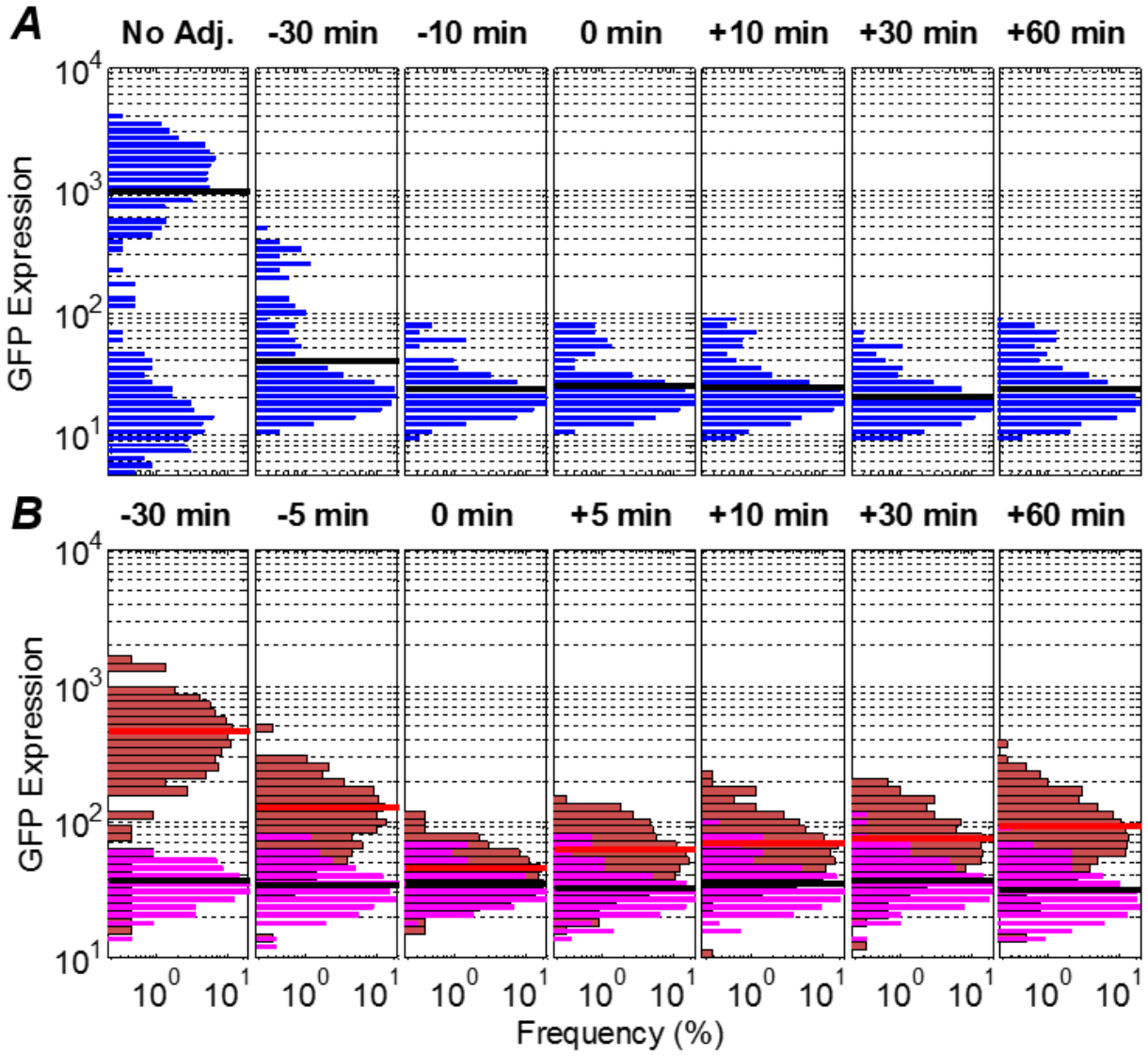

Figure S4

Kinetics of P_{comX} stimulation by CSP/XIP and suppression at low pH. (**A**) A bulk culture of *S. mutans* carrying a P_{comX}-*gfp* reporter was divided into seven samples. Each sample was grown to early exponential phase (OD = 0.1–0.2) and then adjusted to pH 5.5 at a time designated $t = 0$. Further, at a subsequent time τ, 1 µM CSP was added to each sample. (Thus a negative τ indicates that CSP was added to the sample prior to pH adjustment.) Each sample was incubated for 2 h after addition of CSP (i.e. until $t = \tau + 2$ h), and then the GFP fluorescence of individual cells was imaged and measured. The sample labeled as 'No Adj.' was not pH adjusted and serves as a control. When CSP was supplied 30 minutes (or less) prior to pH adjustment, only a weak P_{comX} response was observed. Horizontal bars

indicate mean GFP expression levels; (**B**) A similar experiment is performed with XIP stimulation. The red histograms show P_{comX} activity in cells that were grown in FMC medium and adjusted to pH 5.5 at $t = 0$ (at OD = 0.1 - 0.2), with 1 µM XIP then added at time $t = \tau$. Magenta histograms show P_{comX} activity in control samples, which were pH adjusted at time $t = 0$ but did not receive XIP. Red and black horizontal bars indicate mean expression for +XIP and –XIP data respectively. When XIP was supplied 30 minutes prior to pH adjustment, a roughly tenfold increase in the median P_{comX} activity was observed.

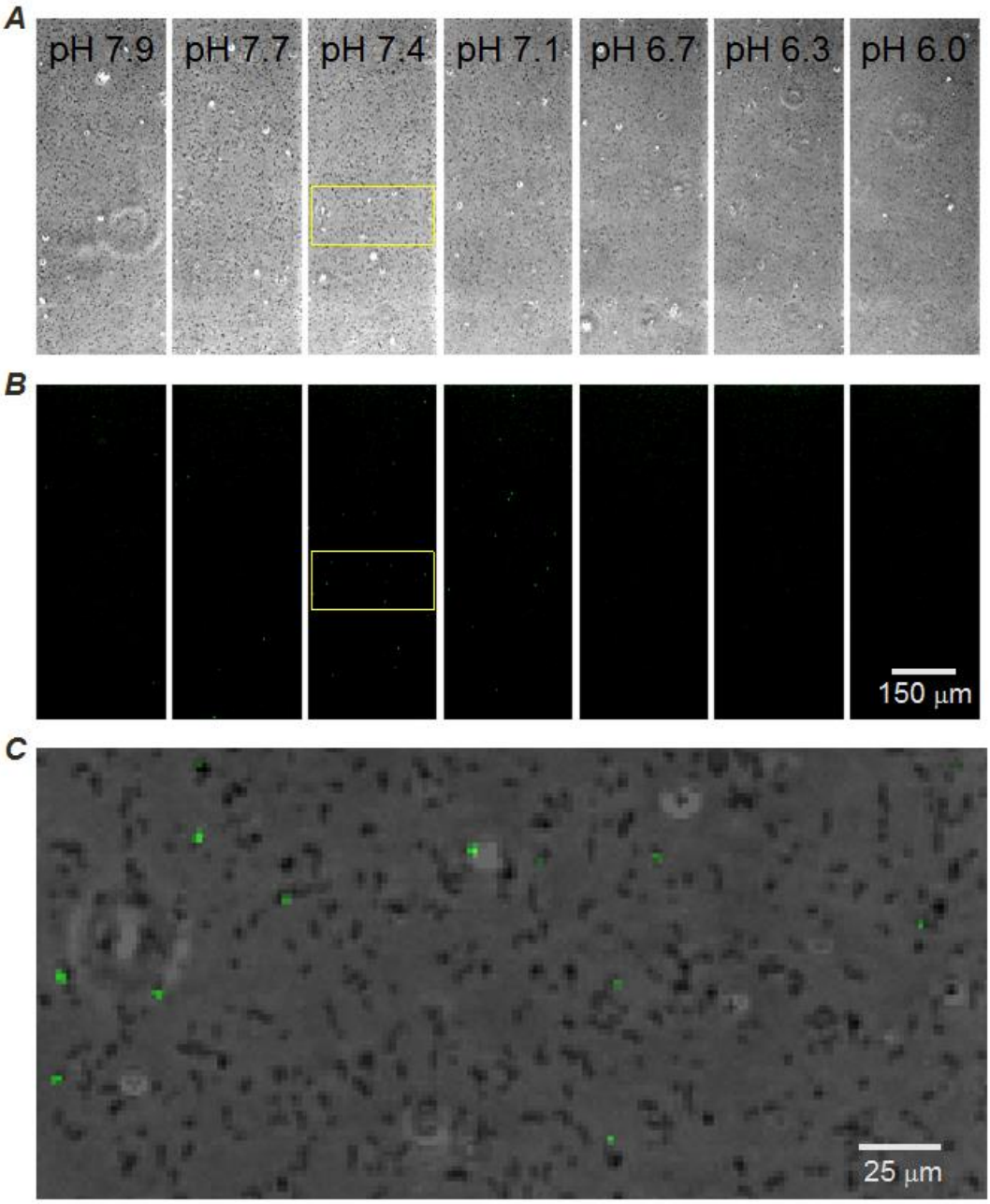

Figure S5

pH dependence of P_{comX} activity in late exponential phase *S. mutans.* Cells grown to OD = 0.8 were supplied with filtered OD = 0.8 supernatant which had been pH adjusted as indicated, with 1 μM CSP + 10 mM glucose added. The figure shows phase contrast (**A**) and green fluorescence (**B**) images collected in the microfluidic chamber. The small region of the pH 7.4 channel indicated by the yellow box in (**A**) and (**B**)) is enlarged in (**C**), and its phase contrast and green fluorescence images overlaid. Although the number of P_{comX} -active cells was diminished relative to fresh medium, bimodal activation of P_{comX} activity was observed in channels at near neutral pH (but absent at pH 7.9 or pH 6.0).

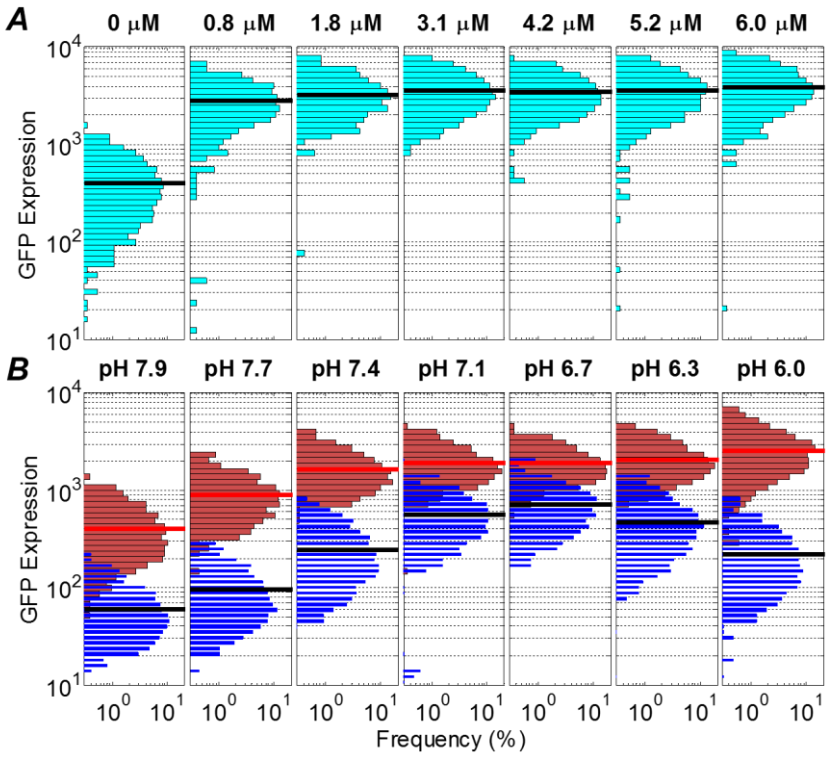

Figure S6

Effect of CSP and acid pH on activity of P_{cipB}. *cipB* encodes a bacteriocin in response to CSP stimulation of ComD/E, and is required for CSP-induced transformation of *S. mutans.* Histograms show population-wide response of a P_{cipB}-*gfp* reporter when cells were stimulated by exogenous CSP at acid pH in microfluidic flow. Solid bars indicate means of histograms. Activation of P_{cipB} was unimodal: all cells in the population expressed the reporter gene in response to CSP. (**A**) A P_{cipB}-*gfp* reporter strain was grown to OD = 0.1 and loaded into the microfluidic chambers, where it was supplied with media adjusted to pH 6.0 and containing CSP concentrations spanning 0 - 6 μM. (**B**) P_{cipB} activity in the same strain was studied in fresh media adjusted to the pH values shown, and either containing 1 μM CSP (red) or lacking exogenous CSP (blue). Although there was significant basal activity of P_{cipB} in the absence of exogenous CSP, addition of CSP consistently enhanced P_{cipB} activity roughly three-fold at all pH values, indicating that the CSP/ComDE signal transduction pathway remained active at low pH.

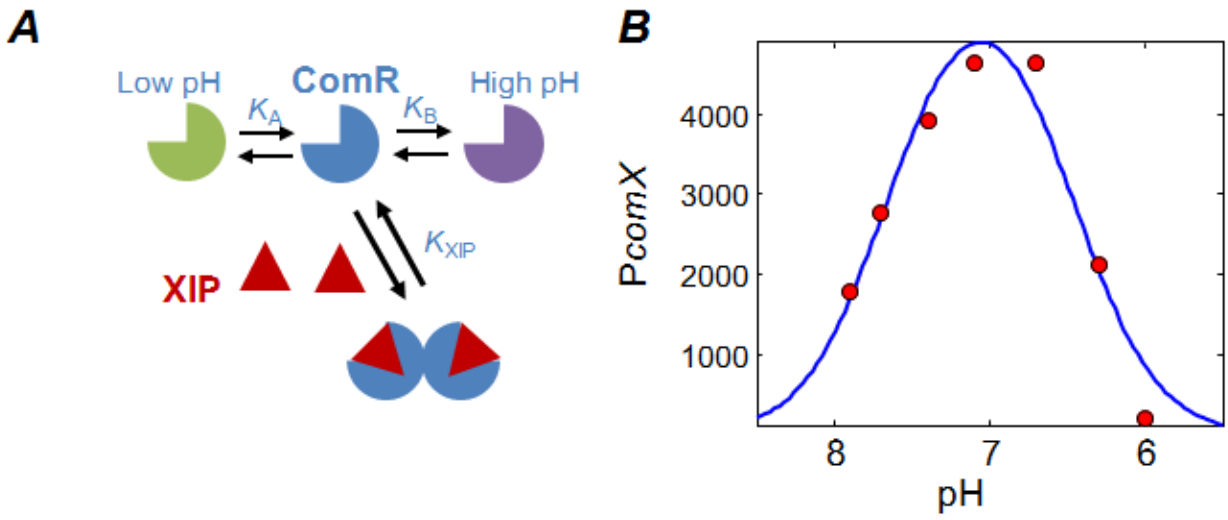

Figure S7

(**A**) Schematic of a model of type 3 (Figure 7) in which acid or basic pH inhibit association of ComR with XIP. (**B**) To test whether the observed pH-dependence of P_{comX} response to XIP is compatible with a pH-dependent ComR association model, a mathematical model of the schematic in (**A)** was implemented in Matlab. In the model, ComR has three successive protonation states, $ComR_1$ (at low pH), $ComR_2$, and $ComR_3$ (at high pH). These states interconvert with dissociation constants K_A and K_B: $ComR_2 + H^+ \Leftrightarrow ComR_1$, and $ComR_3 + H^+ \Leftrightarrow ComR_2$. Only the intermediate state $ComR_2$ is presumed to interact (with dissociation constant K_{XIP}) with intracellular XIP to form a dimeric complex that activates P_{comX}. The activation of P_{comX} in response to XIP was assumed to be proportional to concentration of dimeric ComR. Fitting this model to the experimentally measured (population average) activation of P_{comX} of Figure 2 gives the solid curve shown here with $\log_{10}(K_A) = -6.7$, $\log_{10}(K_B) = -7.4$, $K_{XIP} = 128$ nM if the total intracellular ComR concentration is 10 nM. The calculated concentration of the ComR/XIP dimer is scaled (blue curve) to overlay the P_{comX} expression data (red circles) of Figure 2.