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# Environmental Fluctuations and Stochastic Resonance in Protein Folding

Kapil Dave,[a] Aram Davtyan,[b] Garegin A. Papoian,[c] Martin Gruebele,*[a, d] and Max Platkov[e]

Stochastic resonance is a mechanism whereby a weak signal becomes detectable through the addition of noise. It is common in many macroscopic biological phenomena, but here we ask whether it can be observed in a microscopic biological phenomenon, protein folding. We investigate the folding kinetics of the protein VlsE, with a folding relaxation time of about 0.7 seconds at 38 °C in vitro. First we show that the VlsE unfolding/refolding reaction can be driven by a periodic thermal excitation above the reaction threshold. We detect the reaction by fluorescence from FRET labels on VlSE and show that accurate rate coefficients and activation barriers can be obtained from modulated kinetics. Then we weaken the periodic temperature modulation below the reaction threshold, and show that addition of artificial thermal noise speeds up the reaction from an undetectable to a detectable rate. We observe a maximum in the recovered signal as a function of thermal noise, a stochastic resonance. Simulation of a small model-protein, analysis in an accompanying theory paper, and our experimental result here all show that correlated noise is a physically and chemically plausible mechanism by which cells could modulate biomolecular dynamics during threshold processes such as signaling.

## 1. Introduction

Weak signals below the detection threshold can be amplified by the addition of noise. The recovered signal is maximized at a certain noise level, resulting in a stochastic resonance.[1] Biological examples range from predating fish generating weak sound waves that are detected by their crayfish prey only when random environmental noise is added to amplification of electrical membrane signals due to membrane channel voltage fluctuations.[2,3] The process is illustrated in Figure 1. It requires a sub-threshold signal to introduce correlation, a detection threshold, and noise that modulates the sub-threshold signal by just the right amount: too little noise, and the signal remains below the threshold; too much noise, and the signal is swamped by the noise.

Many biomolecular reactions exhibit thresholds and are thus candidates for stochastic resonance at the molecular level. Some reactions require correlated noise (such as the sine wave signal + noise we use here). For example, protein folding is a cooperative process with a sharp transition between folded and unfolded state (as a function of pH, denaturant, temperature, crowding, etc).[4] Likewise, biomolecular binding curves have a sigmoid concentration dependence.[5] Other reactions that incorporate feedback can create their own correlated signals, and could be driven by thermal fluctuations, jamming, quinary interactions,[6] and similar random processes. Whether such modulation has adaptive consequences for the cell remains unknown.

Recently, it has been proposed that cooperative kinetics could be driven by a periodic perturbation, and that parameters such as rate coefficients or equilibrium constants could be extracted from such data.[7,8] Indeed, DNA hairpin folding,[9] DNA hybridization inside live cells,[10] and protein folding[11] all have been analyzed by driving the reactions with periodic temperature modulation. From such experiments it is a small step to add artificial noise to the periodic perturbation, or to use colored noise with a frequency cutoff to drive the system. Such "artificial thermal noise" is not limited to the *kT* level, but acts in analogy to thermal noise driving single molecule reactions.

Here we present modulated folding kinetics of the FRET-labeled protein VlsE, a genetically variable extracellular membrane protein used by the Lyme disease agent *B. burgdorferii* during host invasion.[12] We drive the folding reaction experimentally with a periodic temperature perturbation, scanning

[a] *K. Dave, Prof. Dr. M. Gruebele*
*Center for Biophysics and Quantitative Biology*
*University of Illinois, Urbana, IL 61801 (USA)*
*E-mail: mgruebel@illinois.edu*

[b] *Dr. A. Davtyan*
*Department of Chemistry and Institute for Biophysical Dynamics*
*Computation Institute, James Franck Institute*
*University of Chicago, Chicago, IL 60637 (USA)*

[c] *Prof. Dr. G. A. Papoian*
*Department of Chemistry and Biochemistry*
*and Institute for Physical Science and Technology*
*University of Maryland, College Park, MD 20742 (USA)*

[d] *Prof. Dr. M. Gruebele*
*Departments of Chemistry and Physics*
*University of Illinois, Urbana, IL 61801 (USA)*

[e] *Prof. Dr. M. Platkov*
*Department of Physics, Ariel University*
*Ariel, 40770 (Israel)*

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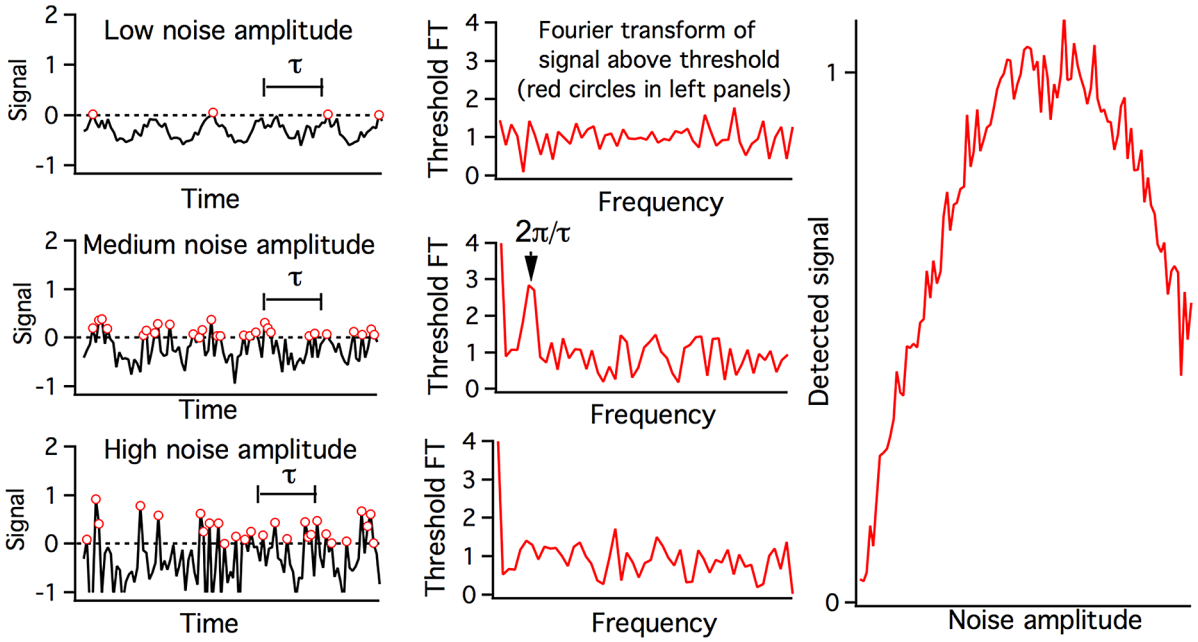


**Figure 1.** A cartoon of the stochastic resonance mechanism. The left column shows a periodic signal below the detection threshold (dotted line referenced at 0), with increasing noise added. The middle column shows the Fourier transform of the signal detected above threshold, scaled so the baseline noise in each FT is equal. At low noise amplitude no signal peak is detected within the background noise, at high noise amplitude only a noise spectrum is detected. At medium noise amplitude, the noise is modulated at the signal frequency, and a signal can be detected. Thus there is a stochastic resonance in the detected signal as a function of noise amplitude (right panel).

the frequency of the perturbation.[11] A two-state kinetic model[8,11] is shown to fit the FRET data that monitors the periodic folding/unfolding of VlsE. Reaction parameters such as the activation barrier are extracted from the data. The question then arises whether added noise could accelerate the reaction when thermally modulated below the reaction threshold, that is, whether folding is subject to stochastic resonance. Here we first apply a coarse-grained native structure-based simulation[13,14] to this question. Interesting properties emerge from our simulation: for instance, the mean first passage time for folding decreases the most when a protein is driven by noise with a spectrum peaked just above the folding rate $k_{obs}$. We then follow up on the simulations with analogous experiments. When VlsE is driven with a sub-threshold sine wave of frequency $\nu \approx k_{obs}$, no reaction is seen, but adding noise accelerates the folding/unfolding reaction. A stochastic resonance peak is observed at a specific noise amplitude in the experiments. Thus, it is at least physically and chemically possible, although it remains biologically unproven, that environmental noise in cells can modulate cooperative biomolecular reactions poised near the reaction threshold, and that such modulation could have an adaptive advantage.

## 2. Results

### 2.1. Overview

The extracellular protein VlsE is a large and relatively slow-folding protein [$k_{obs} \approx (0.7\ s)^{-1}$ at 38.3 °C].[12] VlsE is the largest known two-state folder.[15] It should obey simple unimolecular kinetics $\Delta c(t) \sim e^{-k_{obs}t}$, where $k_{obs}$ is the measured rate. This simple behavior is in contrast to the enzyme PGK, a multi-state folder whose modulation kinetics we studied previously.[11] In order to detect modulation of the protein population between the folded and unfolded states, VlsE was FRET-labeled with Ac1GFP at the N-terminus, and with mCherry at the C-terminus along with a His tag for purification. The protein was then subjected to thermal modulation. First VlSE was modulated with variable-frequency sine waves to corroborate its folding rate by the modulation approach. Then stochastic resonance was detected by driving VlSE with a combination of sinusoidal signal and variable thermal noise amplitude, based on encouraging molecular dynamics simulation results for a coarse-grained model protein.

### 2.2. Fluorescence-Detected Thermal Unfolding of VlsE

The optimal temperature range for modulation experiments was determined with the aid of fluorimeter temperature melts. About 2 μM protein solution was used to detect FRET and the donor/acceptor (D/A) fluorescence intensity ratio is plotted in Figure 2. Protein concentrations up to 10 μM showed no signs of aggregation over the temperature range of subsequent thermal modulation experiments (20–38 °C).

Thermal unfolding of VlsE is a nonlinear threshold process. The temperature unfolding data in Figure 2 was fitted to a sigmoidal two-state model (see Methods). The midpoint of the thermal unfolding transition of VlsE-FRET was $T_m = 42 \pm 2$ °C, in agreement with previous work.[12,15] The onset of the unfolding reaction occurs at approximately 38 °C, where the D/A ratio

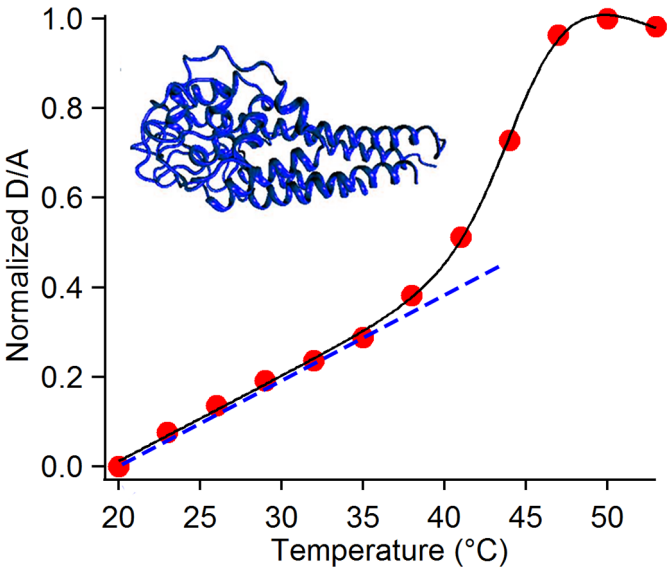


**Figure 2.** Thermal melt of VlsE- FRET (in vitro). Red markers: experimental data; black curve: two-state model fit; blue dashed line: folded state D/A baseline. The reaction threshold for protein unfolding lies at ca. 38 °C, and the equilibrium constant $K_{eq} \approx 1$ at the melting temperature of $42 \pm 2$ °C.

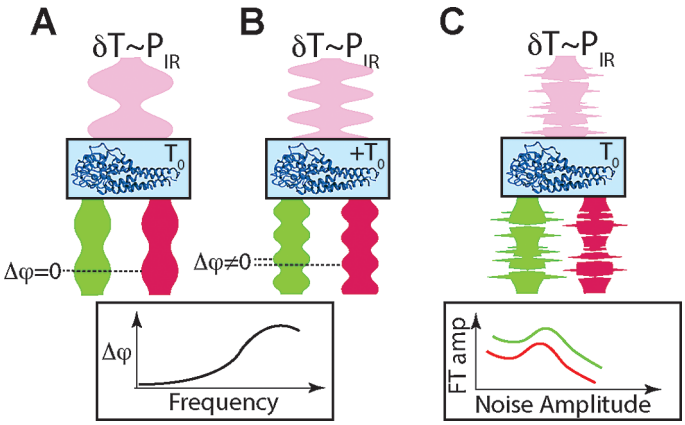


**Figure 3.** Schematic of the experiment. A 2200 nm IR pulse (pink) modulates the sample temperature around the average temperature $T_0$. The green (donor) and red (acceptor) fluorescence of the labeled VlsE (sample in blue box) oscillates as a result. A) The average temperature "$T_0$" is too far below the reaction threshold to allow significant reaction. Donor and acceptor fluorescence signals are modulated in phase because both have a quantum yield that decreases with temperature.[34] B) The average temperature "$+T_0$" is sufficiently high for reaction to occur, and the modulation frequency is comparable to or faster than the reaction rate $k_{obs}$. Now the green and red fluorescence contain components shifted by 180° relative to one another, and up to 90° with respect to the temperature modulation. The 90° shift is a "low pass filter" effect when the reaction is driven too fast. The plot of phase shift vs. applied modulation frequency can be fitted to a model to obtain $k_{obs}$ and the activation barrier. C) If the IR modulation is below the reaction threshold, but an increasing amount of temperature noise is added, stochastic resonance can be detected above the background caused by quantum yield modulation.

begins to differ substantially for the almost linear native state baseline (dashed blue line in Figure 2). The baseline is due to temperature-dependent quantum yields of AcGFP1 and mCherry donor and acceptor labels.[12,16]

### 2.3. Periodic Thermal Modulation

In our earlier study of the enzyme PGK, we showed that thermal modulation can be used to study protein-folding reaction kinetics.[11] VlsE folding can be driven by a periodic waveform, and kinetic parameters such as the activation free energy $\Delta G^{\dagger}$ can be obtained, in analogy to measuring fluorescence lifetimes by periodic modulation instead of a fast excitation pulse.[17,18]

The experiment is illustrated in Figure 3 (A and B). Thermal modulation was performed with a waveform-controlled 2200 nm infrared laser about an average temperature of $T_0 =$ 38 °C to maximize the signal without inducing protein aggregation. The ≈10 μM protein solution was subjected to a periodic temperature waveform [Eq. (1)]:

$$T(t) = T_0 + \delta T(t) = T_0 + \frac{1}{2}\Delta T \sin(2\pi\nu t + \phi) \qquad (1)$$

at the sample slit. As discussed in detail previously,[11] the green donor and red acceptor fluorescence signals collected at the CCD camera are affected by two processes: 1) The quantum yield of the fluorescence labels decreases linearly with temperature.[16] This process causes each of the green and red signals to be 180° out of phase with $T(t)$. In that case, the relative phase $\Delta\varphi$ between green and red is 0° (Figure 3 A). 2) The folding reaction causes green and red FRET signals to be 180° out of phase relative to each other (unfolding = more green/less red, refolding = less green/more red). Moreover, the red signal is in phase with $T(t)$ for slow modulation frequency $\nu \ll k_{obs}$, but up to 90° out of phase with $T(t)$ for fast modulation frequency $\nu \gg k_{obs}$ as the protein folding reaction cannot track rapid variations in temperature, which is further elaborated elsewhere.[19]

The resulting phase shift $\Delta\varphi(\nu)$ between the red and green output signals (Figure 3 B) can be used to extract the folding/unfolding kinetics from the data. We use a kinetic two-state model with time-dependent free energy and quantum yields.[11] From the model parameters, a two-state kinetic master equation is solved, the time-dependent rate coefficients can be calculated, and donor and acceptor fluorescence signals are calculated. Finally, the simulated phase shift $\Delta\varphi(\nu)$ is calculated from the simulated fluorescence signals and compared with the obtained experimental phase shift between the green and red acquired signals (see Methods). The suitability of such simple kinetic master equation models[8] has been tested by comparison with Brownian dynamics simulations.[19]

Figure 4 shows the experimental data (red) and the computed phase shift (black) after the model parameters have been optimized by least squares fitting. Table 1 shows the optimized model parameters and $1\sigma$ uncertainty for the model fit in Figure 4 (see Methods, Data Analysis). As expected, the phase difference $\Delta\varphi$ between green and red is small at low driving frequency. $\Delta\varphi$ increases as $\nu$ approaches the unimolecular reaction rate $k_{obs}$. Eventually $\Delta\varphi$ decreases again: If the reaction

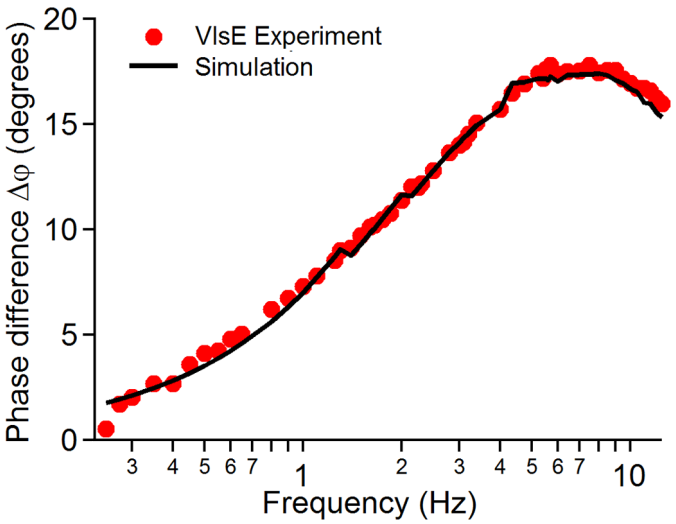


**Figure 4.** Experimental phase shift between the donor and acceptor fluorescence (red circles) of the VLSE protein. The least-squares fit to a two-state model including temperature-dependent donor and acceptor quantum yield is shown as a black curve. Small glitches in the black fit are numerical errors due to sampling the phase at 0.5° increments in the simulation (see SI).

**Table 1.** Experimental folding parameters and FRET parameters for VlsE, obtained by least-squares fitting of the measured denaturation curve in Figure 2 and the measured phase curve in Figure 4.

| Fitting parameter | Value and fitting uncertainty (1 standard deviation) |
|---|---|
| $T_m$ | $42 \pm 2$ °C |
| $\delta g_1$ | $1380 \pm 180$ kJ mole$^{-1}$ K$^{-1}$ |
| $k_m$ | 5 μs$^{-1}$ (fixed) |
| $\Delta G^{\dagger}$ | $22.3 \pm 0.1$ kJ mole$^{-1}$ |
| $A_F$ | $0.4 \pm 0.1$ |
| $D_F$ | $0.6 \pm 0.1$ |
| $A_U$ | $0.17 \pm 0.05$ |
| $D_u$ | $0.83 \pm 0.05$ |

is driven at $\nu > k_{obs}$, the reaction amplitude decreases, so the quantum yield modulation, which has $\Delta\varphi = 0$ dominates the signal. The activation barrier determined for this reaction is $\Delta G^{\dagger} = 22.3 \pm 0.1$ kJ mole$^{-1}$, assuming a prefactor of $k_m \approx (5\ \mu s)^{-1}$ in the equation $k_i = k_m \exp(-\Delta G^{\dagger}/RT)$ for the rate coefficients. The value of $k_m$ is chosen close to the "speed limit" of protein folding,[20,21] with an upwards adjustment because VlsE is much larger than the mini proteins for which the speed limit has been estimated.[22,23] At 38 °C, the fitted kinetic and thermodynamic parameters yield a reaction rate $k_{obs} \approx (0.7\ s)^{-1}$, in agreement with conventional T-jump measurements.[12]

### 2.4. Computational Prediction of Stochastic Resonance in a Folding Reaction

So far, our results are analogous to what was found previously for PGK.[11] To see if folding/unfolding can be accelerated by application of artificial thermal noise, we performed molecular dynamics simulations on a small model protein with a temperature-dependent native structure-based model potential.[13,14] The goal was not to simulate VlsE, which folds far too slowly for realistic simulation with an all-atom force field, but to obtain in general the effect of noise amplitude and correlation time on reaction rate (see Methods and accompanying paper).[19]

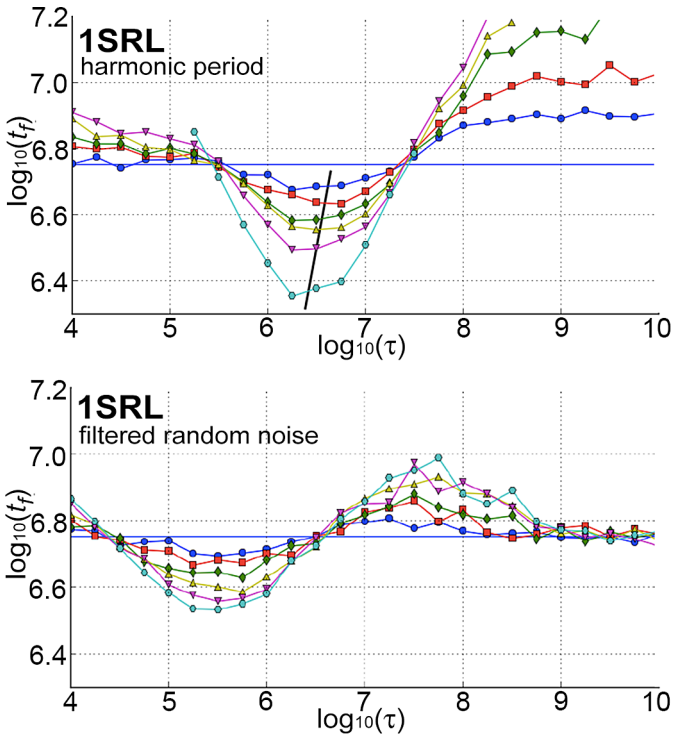


**Figure 5.** Computational prediction of periodic-driven response and noise-driven response of a model protein. Top: For reference, a native structure-based (Gō-like) model protein (PDB code: 1SRL, SH3 domain) is subject to harmonic temperature modulation $\varepsilon(t) = \varepsilon + \delta\varepsilon\sqrt{2}\sin(2\pi t/\tau + \phi)$. The average first-passage time vs. period $\tau$ of the driving waveform is plotted. Several modulation amplitudes are shown. $\sqrt{\delta\varepsilon^2/\varepsilon^2}$ covers the range 0.03 (dark blue), 0.04, 0.05, 0.06, 0.07, 0.1 (light blue). The black line shows that optimal driving frequency and amplitude are correlated. Bottom: Same model, but driven by Gaussian noise with a correlation time $\tau$, obtained by solving the Langevin Equation (2) in the text. When $\tau$ is equal or faster than the natural rate $k_{obs}$ of the unperturbed system, the first passage time of the driven system decreases (reaction rate $k_f$ increases) as the amplitude increases. $t_f$ and $\tau$ on those plots are in the units of femtoseconds.

In our model, the strength $\varepsilon$ of the native contact terms in the protein interaction potential was modulated either periodically or by correlated random noise, where the deviation $\delta\varepsilon(t)$ of $\varepsilon$ from its average value is determined by solving the following the Langevin equation [Eq. (2)]:

$$\frac{\partial\delta\varepsilon(t)}{\partial t} = -\frac{\delta\varepsilon(t)}{\tau} + \frac{<\delta\varepsilon^2>}{\tau}G(t) \qquad (2)$$

where $G(t)$ is Gaussian white noise. The resulting $\delta\varepsilon(t)$ simulates artificial thermal noise with amplitude $\sqrt{<\delta\varepsilon^2>}$ and correlation time $\tau$. Frequency components higher than $1/\tau$ rapidly diminish in such noise. Figure 6 shows the similar results obtained when the protein is driven periodically at period τ, or by artificial thermal noise with frequency content up to $1/\tau$.

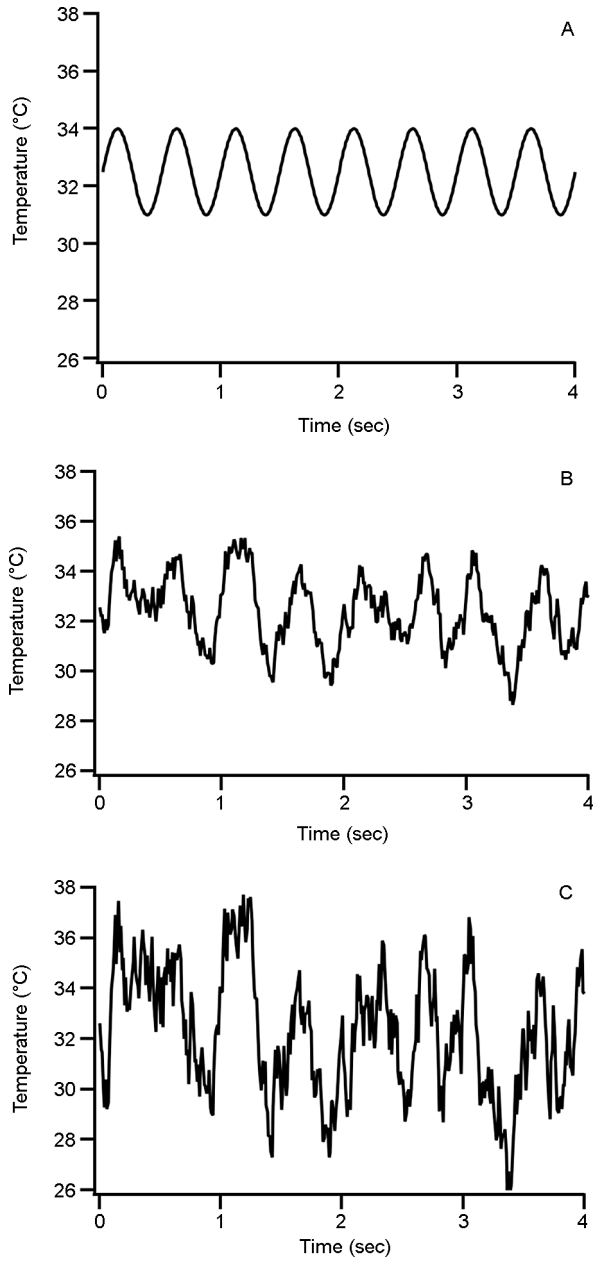


**Figure 6.** The 2 Hz sine wave + noise signal used to drive VlsE into stochastic resonance as shown in Figure 7: A) with 0 °C RMS temperature noise, B) with 1 °C RMS temperature noise (near stochastic resonance when $T_0 = 32$–33 °C), and C) with 2.25 °C RMS temperature noise (above stochastic resonance maximum).

The first passage time ($\tau_{MFPT} = 1/k_f$ in our experiments discussed above) decreases (i.e. the reaction speeds up) when the reaction is driven at frequencies comparable to the reaction rate. Additionally, as the noise level $\sqrt{<\delta\varepsilon^2>}$ increases, at driving frequencies just above resonance, the rate acceleration is continuously enhanced, indicating that the protein approaches stochastic resonance driven by the noise. Therefore it is possible that such a noise-driven rate increase can be observed experimentally for protein folding.

### 2.5. Experimental Addition of Artificial Thermal Noise

Next we tested the idea that noise can be utilized to amplify a sub-threshold folding reaction to increase its rate to the detectability limit. We thermally modulated VlsE again as described by Equation (1), slightly above and below the folding rate (2 Hz and 1 Hz, respectively), but this time we kept the average temperature and periodic modulation well below the reaction threshold of about 38 °C (Figure 6 A). We then added increasing amounts of noise to the thermal waveform to drive the system towards the reaction threshold as follows [Eq. (3)]:

$$T(t) = T_0 + \frac{1}{2}\Delta T \sin(2\pi\nu t + \phi) + \frac{1}{2}\Delta T_{rand} G_{lp}(t) \quad (3)$$

The random component $G_{lp}(t)$ was obtained by computing Gaussian-distributed pseudo-random numbers $G(t)$ and passing $G(t)$ through a 6dB/octave low-pass filter with a cut-off frequency of 20 Hz. The values of $\Delta T$ (3 °C) and $T_0$ (28 to 33 °C) were chosen so $T$ would remain below the reaction threshold of ~38 °C at all times unless assisted by noise. The Gaussian random noise amplitude was tuned so the root-mean-squared temperature fluctuations (RMS temperature noise in Figure 7) ranged from 0 to 2.25 °C. Figure 6 shows examples of the periodic + noise waveforms driving the VlsE folding reaction for $T_0 = 32.5$ °C and RMS thermal noise of 0, 1, and 2.25 °C.

### 2.6. Observation of Stochastic Resonance

We measured the resulting donor and acceptor FRET amplitudes $D(t)$ and $A(t)$, and computed their Fourier transform amplitude at the driving frequency of 2 Hz (see Figure S1 of the Supporting Information, SI), slightly faster than the natural relaxation rate $k_{obs} \approx 0.7\ s^{-1}$ measured in the previous section and in ref. [12] at 38.3 °C. The sub-threshold periodic modulation alone produces only a slope as a function of RMS temperature noise, due to the modulation of the quantum yields of donor and acceptor, which depend linearly on temperature (Figure 7 top). However, as the average temperature is increased from 11 °C below reaction threshold to 5 °C below reaction threshold, a peak can be seen in the signal at 2 Hz at an artificial thermal noise of ≈1.3 °C RMS. At higher noise amplitude, the signal disappears again and returns to the baseline (Figure 7 middle and bottom). A much weaker effect is seen at 1 Hz ($\nu < k_{obs}$) sub-threshold modulation with the same added noise levels (SI Figure S2).

We assign the peak in the 2 Hz signal versus noise amplitude to a stochastic resonance of the folding/unfolding reaction of VlsE, driven by a sub-threshold periodic modulation that produces no detectable reaction on its own, but induces a reaction rate above our detection threshold when noise is added. Adding too much noise (>2 °C RMS) still produces reaction, but swamps the periodic sub-threshold modulation so the Fourier transform no longer peaks at 2 Hz (see SI Figure 1 for examples of the full Fourier spectra).

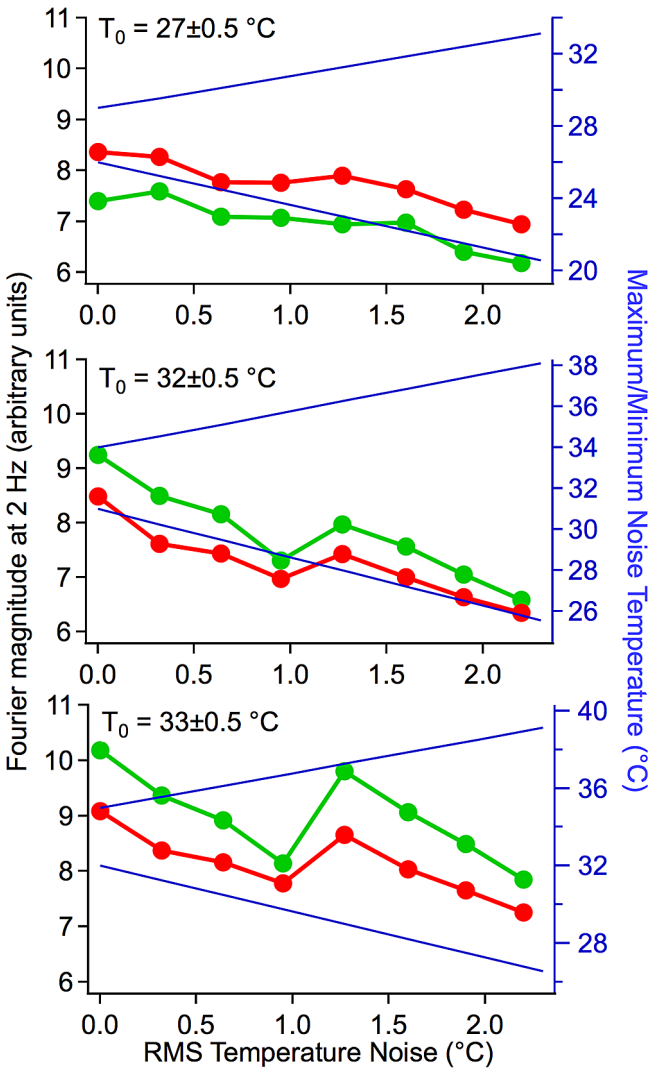


**Figure 7.** Stochastic resonance, detected by Fourier transform magnitude of the donor (green) and acceptor (red) signals grows in when a root-mean-squared (RMS) temperature noise of about 1.2 °C is superimposed on the sub-threshold sine wave modulation in Figure 6 A. (A) $T_0 = 28$ °C. (B) $T_0 = 32$ °C. (C) $T_0 = 33$ °C. Stochastic resonance grows in at $\sqrt{\delta(T - T_0)^2} \approx 1.25$ °C as the average temperature $T_0$ is increased. The baseline (ca. six units of the FT *y*-axis) is due to modulation of the quantum yield of donor and acceptor. The blue decreasing line is the minimal noise temperature applied on the protein at each RMS temperature, and the blue increasing line is the maximal noise temperature thereof.

## 3. Discussion and Conclusion

Stochastic resonance has been observed in a variety of natural phenomena. Macroscopic phenomena include mechanoreceptors in rats[24] and electroreceptors in paddlefish that receive signals more sensitively due to added environmental noise.[25] It also plays a role in biological signal processing, from visual enhancement[26] to neuronal signaling.[27]

Stochastic resonance can also affect chemical reactions, which have intrinsically nonlinear rate and equilibrium behavior. This effect is generally observed near unstable points of the reaction's state space as a function of perturbation parameters.[28] Examples include pulsing Belousov-Zhabotinsky reactions,[29] as well as electron transfer reactions.[30] In particular, stochastic resonance plays a role in biochemical reactions that are part of feedback cycles, such as cell signaling, where noise due to a small number of signaling molecules can control gene silencing.[31,32]

Here we add protein folding to the list of chemical reactions that exhibit stochastic resonance when driven by correlated noise. The analysis of a small protein using native structure-based coarse-grained simulations shows that when a folding reaction is driven either by periodic perturbation with period $\tau$, or by colored noise with a characteristic cutoff time $\tau \sim 1/k$, a significant decrease in the first passage time (or increase in the forward rate) can be observed. Thus protein folding has sufficiently nonlinear equilibrium curves (Figure 2) to exhibit stochastic resonance. At first, our modulation experiment confirmed that VlsE behaves as a two-state folder under periodic modulation above threshold. We then subjected VlsE to a combination of a sub-threshold temperature perturbation with period $\tau$ plus artificial thermal noise. When monitored by Fourier transform at the frequency $\nu = 1/\tau$, the fluorescence output signal peaks as a function of noise amplitude, but only when the periodic modulation is close to threshold (average temperatures of 32 or 33 °C vs. 27 °C in Figure 7). We also measured the response at $\nu = 1$ Hz, a factor of 2 below the reaction rate $k_{obs}$, (SI Figure S2), but there the response is not as evident, in keeping with a slowdown of the reaction when the noise correlation time is slower than the reaction rate (Figure 5).

As discussed in the accompanying paper,[19] stochastic resonance can also be seen via the phase shift $\Delta\varphi$ of the red and green signal. The predicted phase shifts with realistic FRET input parameters are small (see accompanying theory paper), but we were able to identify stochastic resonance by comparing the experimental phase shift with a Brownian dynamics simulation. Thus two complementary approaches identified stochastic resonance in the folding of two different proteins.

It is not known at present whether cells use stochastic resonance to modulate biomolecule function outside the cases of signaling or visual signal enhancement that have been studied.[26,31] However, our results show that it is a physically plausible process. There is growing evidence that many proteins in the cell, for example certain intrinsically disordered protein (IDPs), can switch conformation based on small perturbations. Lymphotactin is an example of such a protein whose structure and function are modulated by a small environmental perturbation.[33] It is possible that correlated environmental fluctuations are accelerating protein folding and association reactions, and thus contribute to the cellular control of structure and function of such proteins.

## Experimental and Computational Section

### Sample

Protein expression was reported previously,[12] so we describe mainly slight differences here. An Ac1GFP-VlsE-mCherry plasmid obtained from Genscript was transformed into *E. Coli* P-lysis cells. The bacterial colonies were later grown into lysogeny media (LB) containing chloramphenicol antibiotics to an OD of around 0.6. At

this OD isopropyl thiogalactopyranoside (1 mM IPTG) was added to induce protein expression. Cells were left to grow overnight (≈12 h) at room temperature. Later the cells were collected by centrifugation and sonicated to get cell lysate. Cell lysate was applied to a nickel-nitrilotriacetic acid (Ni-NTA) column which has high affinity towards the histidine tag, protein was purified according to the Qiagen protocol.[12]

### Apparatus and Measurement Procedure

The experimental setup was developed in-house on our live-cell instrument,[16] as described previously.[11] Briefly, a blue LED (470 nm, 400 mW) excites the GFP donor; an inverted epifluorescent microscope with a 40× objective illuminates the protein sample, and collects the donor and acceptor fluorescence separately after splitting by a dichroic filter. A frame-rate of 110 Hz was used and data was collected for 11 sec in order to probe and compare the dynamic range of VlsE folding/unfolding kinetics.

The sample chamber was made using double-sided tape of approximately 120 μm height (Grace, Secureseal 654006) on a glass slide and coverslip. The experiments were conducted using VlsE protein concentration of up to 10 μM, with no signs of aggregation over the entire average temperature range ($T_0$=25–39 °C).

Temperature modulation was performed near the reaction threshold of the protein for the sine wave-driven experiment in Figure 4, and below the reaction threshold of about 38 °C (Figure 2) for the stochastic resonance experiment, where added temperature noise makes the harmonic modulation detectable. Periodic and random temperature modulation (see Figure 6) was achieved by heating the sample with an infrared laser (m2K Lasers, $\lambda$=2200 nm, up to 700 nm) which is attached to a computer-controlled power supply (LDC340). The sample base-temperature was set by using two PID-controlled heating-resistors and a Peltier chip to within 0.1° of a user-selected setpoint temperature target in a range of 12 °C up to 50 °C. These resistors, the Peltier chip and heat-conducting copper ribbons as well as fan-cooled heat dissipation fins were attached to the sample chamber through a layer of heat-conduction compound, and the assembly was mounted on the microscope stage.

It is known that the protein VlsE-FRET folds with a folding time $\tau_{obs}=k_{obs}^{-1}\approx 0.7\ s^{-1}$ at 38.3 °C.[12] Based on that and the calculations, we chose to induce stochastic resonance by modulating the temperature on the VlsE-FRET protein with a sine-wave whose period is slightly faster than the folding rate (2 Hz) and below the folding rate (1 Hz), to see if the stochastic resonance weakens or shifts with driving frequency.

The green and red fluorescence coming from the protein were imaged onto a charge-coupled device camera. The fluorescence recorded by the camera exhibited a photobleaching and quantum yield temperature dependence of the donor and acceptor fluorophores. Photobleaching resulted in a linear decrease of signal over the 11 s time scale of the experiment, and was taken into account by a linear scaling, after which the output could be fitted to phase shifted sine waves. The same correction was used for analogous noise experiments taken under the same conditions. The quantum yield of Ac1GFP and mCherry depends linearly on temperature over the small temperature range used here (20–40 °C). As a result intensity modulation occurs together with temperature modulation, and was taken into account by the fitting model.

### Data Analysis

For the analysis of periodic modulation data, we used the same algorithm presented previously for the analysis of PGK.[11] This is similar to algorithms proposed by Lemarchand and coworkers,[8] and verified by Brownian dynamics in the accompanying paper in this issue.[19] The new addition to the model is the capability to optimize model parameters by a least-squares algorithm, and the code used in this paper is available in SI. Briefly, the donor and acceptor signals at each driving frequency $\omega=2\pi\nu$ were least-square-fitted to a sine wave [Eq. (4)]:

$$S(t) = A\ \sin(2\pi\nu t + \varphi) \tag{4}$$

Then the phase difference $\Delta\varphi\ (\nu)$ was calculated, as plotted in Figure 4. This phase difference was simulated as follows [Eqs. (5)–(7)]:[11]

$$\Delta G(t) = \delta g_1 (T(t) - T_m) \tag{5}$$

$$\Delta G^{\dagger}(t) = \delta g_0^{\dagger} + \delta g_1^{\dagger} (T(t) - T_m) \tag{6}$$

$$QY_i(t) = 1 + QY_{1i}(T(t) - T_m) \tag{7}$$

for the folding free energy, activation barrier, and relative quantum yield of "$i$"=donor or acceptor. $\delta g_1$ and $T_m$ were obtained from a thermodynamic fit to Figure 2. $\delta g_0^{\dagger}$, $\delta g_1^{\dagger}$ are kinetic fitting parameters, and $QY_{1D} =< M- >0.011$ and $QY_{1D} =< M- >0.010$ were fixed at the known relative quantum yield slopes of Ac1GFP and mCherry.[34] For a two-state folder the observed rate coefficient equals the sum of the rates of folding and unfolding, or $k_{obs}=k_f+k_u$. Rate coefficients for the forward/backward reactions were calculated as $k_m \exp[-(\Delta G^{\dagger}(t) \pm {}^1/_2 \Delta G(t))/RT$. The two-state kinetic master equation $[\dot{F}] = -k_u(t)[F] + k_f[U]$) was then solved, where $[U]=C-[F]$ is the unfolded protein concentration and $C$ is the total protein concentration. From the folded and unfolded concentrations, the observed donor and acceptor fluorescence signals were computed as $D(t) = QY_D \cdot (D_F[F](t) + D_U[U](t))$ and $A(t) = QY_A \cdot (A_F[F](t) + A_U[U](t))$. Here $D_i$ and $A_i$ are four constants between 0 and 1 to account for the relative donor and acceptor fluorescence in the folded and unfolded states. The signals $A(t)$ and $D(t)$ were fitted to sine waves, just like the experimental data [Eq. (4)], and the phase difference of the resulting sine waves was evaluated. Adjustable parameters were then optimized by least-squares fitting.

For the sub-threshold modulation + noise experiments (Figure 6), the fast Fourier Transform amplitude of the donor and acceptor signals was calculated using Matlab (Mathworks). This results in a baseline from quantum yield modulation, but any stochastic resonance of comparable magnitude can be seen easily when the FT is plotted as a function of noise amplitude (Figure 7). The baseline is due to fast (<0.1 s) response of the fluorophores to temperature, whereas the ≥0.5 s response of the reaction is delayed. Thus one could enhance the stochastic resonance signal further by zeroing out the in-phase component of the FT. However, when unknown sub-threshold modulation waveforms are to be detected, this phase is not known, and thus we did not make use of this information in Figure 7, although the full phase information is available in Figure 4.

### Native Structure-Based Model Potential and Dynamics with Periodic and Random Environmental Modulation

More details on the native structure-based model and molecular dynamics simulation and its comparison with kinetic master equation models can be found in the companion computational theory paper.[19] Here we focus on the native structure-based model and folding rate simulations for 1SRL, a small beta sheet test protein. Similar results were found for the larger protein PGK,[19] supporting the idea that the observed resonance effect is universal.

For this study we used a Gō-like (native structure-based) model developed by Onuchic and coworkers.[14] According to this model, the energy of a specific conformation of a protein is given by a sum of bond distance/angle/contact potential terms shown in the accompanying paper.[19]

Molecular dynamics simulations of the PDB model protein 1SRL (an SH3 domain from tyrosine kinase) were carried out with the Go-like potential described above, but with $\varepsilon$ in the native contact energy term modulated as follows about its average value $\varepsilon_0$: A sinusoidal wave with amplitude $\delta\varepsilon$ and period $\tau$, or Gaussian noise with amplitude $\delta\varepsilon$ and correlation time $\tau$ was added at each time step, to act like the modulation and artificial thermal noise in the experiments. Each folding time (average mean first passage time) was computed from 1000 trajectories as a function of $\delta\varepsilon$ and $\tau$. All simulations were started in random conformations with low native contact order $Q$. A fraction of native contacts above $Q=0.8$ was considered folded and yielded one mean first passage time for each trajectory.

For 1SRL we used the following model parameter: $\varepsilon_0=$ 2.3 kJ mol$^{-1}$e. This results in 2.5 $k_BT$ free energy barrier for folding versus about 9 $k_BT$ barrier for VISE used in the experiments (see Table 1) and ensures that the folding reaction can be seen over the course of computationally feasible simulations. Thus the absolute time scales of the simulation in Figure 5 and experiment in Figure 7 cannot be compared. Additionally, the reduction in the number of degrees of freedom in the coarse grained native structure-based model results in smoother free energy landscape and thus even faster dynamics for 1SRL protein. Consequently, the times in Figure 5 that are on the sub-picosecond time scale cannot be directly related to the experimental times, and only the trends as a function of $\delta\varepsilon$ and $\tau$ should be considered.

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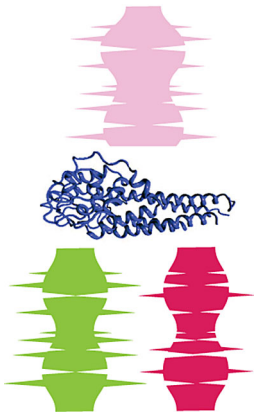
# ARTICLES

**The power of noise:** The folding of protein VlsE can be enhanced by stochastic resonance when noise is added to a weak periodic thermal modulation that by itself is not sufficient to drive the folding reaction.



*K. Dave, A. Davtyan, G. A. Papoian, M. Gruebele,* M. Platkov*

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**Environmental Fluctuations and Stochastic Resonance in Protein Folding**