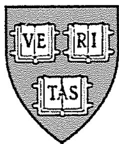

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Yves Brun,
Systems Biology/Microbiology Faculty Search
Department of Biology
Indiana University
Jordan Hall 142
1001 E 3rd St
Bloomington IN 47405-7005

Nov 15, 2005

Dear Professor Brun:

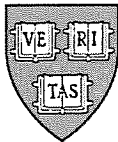
I am writing to apply for a tenure-track assistant professor position at the Department of Biology. I obtained my Ph.D. degree from the Department of Biochemistry and Cell Biology at Rice University. My thesis advisor was Professor Scott F. Singleton and I worked in the area of DNA homologous recombination. Currently I am a postdoctoral fellow working in Professor Sunney Xie's lab in the Department of Chemistry and Chemical Biology at Harvard University. My research focuses on probing gene expression in live cells using single molecule assays. I consider myself a molecular biologist with special interests in using advanced biophysical tools to study the dynamics and functions of cellular processes.

The main objective of my postdoctoral research with Professor Sunny Xie is to develop a reporter system with single molecule sensitivity to monitor gene expression in real time. This research represents pioneering attempts in bringing single molecule approaches into the study of live cells. In collaboration with another postdoctoral fellow in the group, I have demonstrated that membrane-localized yellow fluorescent protein (YFP) can be detected at the single molecule level in live *E. coli* cells. Using the membrane-localized YFP as a gene expression reporter, I was able to track individual protein molecules generated one at a time from a highly repressed gene. I provided first of the kind experimental evidence to show the stochastic nature of gene expression. This study leads to quantitative understanding of the dynamics of gene expression in live cells.

During my stay at Harvard, I have enjoyed mentoring and advising more than five undergraduate and graduate students, collaborating with two postdoctoral fellows and supervising two research assistants. I was the co-author of two successfully funded grants and one pending U.S. patent. As the first and only scientist who came from a biochemistry and molecular biology background to Professor Xie's physical chemistry group, I contributed significantly to the group with my biological background and perspectives. In the mean time, working with the physicists and physical chemists of the group, I have gained not only hands-on experience in single molecule imaging, but also a deeper understanding of the significance of quantitative analysis of biological systems. This experience has placed me at a unique position to tackle biological problems using an interdisciplinary approach.

My Ph.D. research with Professor Scott Singleton at Rice University focused on the study of the mechanism of RecA mediated DNA homologous recombination. By using fluorescence resonance energy transfer (FRET), I made major advances in the understanding of the structure of a key intermediate in the recombination process. I also discovered that the formation of this intermediate follows multiple-step kinetics and is driven by intrinsic properties of the DNA strands. These studies provided valuable insights into the mechanism of DNA repair. In addition, I mentored and advised two undergraduates and two younger graduate students during this period.

My research interest lies in the area of studying the dynamics of cellular processes as they occur in real time at the single molecule and single cell level. Currently my research directions include the signal transduction in *E. coli* two-component signal transduction systems and the assembly of *E. coli* cell division complex. I envision my contribution to your department is to bring an interdisciplinary approach, combining biological, biochemical and biophysical methods to quantitatively address biological problems. I anticipate leading a highly collaborative group and offering unique research opportunities to the faculty and student communities.



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In line with my research plans, I am interested in teaching in the areas of general biology, genetics, microbiology, biochemistry, molecular biology, biophysics and the corresponding lab or seminar courses. I hope that my teaching will bridge these areas and allow students to have comprehensive understandings of modern life science research.

Enclosed please find my CV, representative publications (including one manuscript submitted to *Science*), a statement of past research, a description of future research plan and a teaching statement. I also arranged to have four recommendation letters sent directly to you. They are from Professors. Sunney Xie (postdoctoral advisor, Harvard University), Scott Singleton (Ph.D. thesis advisor, currently at University of North Carolina), Luying Xun (collaborator, Washington State University) and Kathleen Matthews (thesis committee member, Rice University).

Thank you very much for your consideration. If you need any additional materials, please do not hesitate to contact me. I look forward to hearing from you soon.

Sincerely yours

Jie Xiao

Statement of Past Research

Postdoctoral Research (with Professor Sunney Xie, Department of Chemistry and Chemical Biology, Harvard University)

My postdoctoral work focuses on probing real-time gene expression events in individual *E. coli* cells with single protein molecule sensitivity. This research represents pioneering attempts in bringing single molecule approaches into the study of live cells. To achieve single molecule sensitivity, a yellow fluorescent protein (YFP) reporter system was developed. A membrane-targeting sequence fused to the *yfp* gene was used to direct YFP molecules' localization to cell's inner membrane once they are expressed inside the cell. This immobilization effect greatly increases the signal to background ratio and renders the fluorescence of single YFP molecules detectable against cell's autofluorescence background. As a result, individual protein molecules can be counted in real time as they are being expressed inside the cell. Because of the unparalleled sensitivity of this single molecule detection system, I was able to track protein molecules expressed from a highly repressed gene one at a time. The result provided first of the kind experimental evidence for the stochastic nature of gene expression and proved that protein production occurs in randomly distributed bursts and that the number of protein molecules in each burst follows a geometric distribution. This work greatly contributes to the quantitative understanding of the dynamics of gene expression in live cells.

Ph. D Thesis Research (with Professor Scott Singleton, Department of Biochemistry and Cell Biology, Rice University)

My Ph.D. thesis research with Professor Scott Singleton at Rice University focused on the study of the mechanism of RecA mediated DNA homologous recombination. In this process, a RecA-coated single-stranded DNA pairs with a homologous double-stranded DNA and forms a RecA-three stranded DNA complex (RecA-tsDNA). By using fluorescence resonance energy transfer (FRET), I systematically characterized the helical geometries of the three DNA strands inside the RevA-tsDNA complex. I demonstrated that the three DNA strands adopt extended and unwound conformations similar to that of RecA-bound dsDNA. I also discovered that the displaced strand lies in the major groove of the newly formed heteroduplex DNA. Furthermore, I characterized the kinetics of the formation of the RecA-tsDNA complex by using stopped-flow spectrofluoremetry. I found that the formation of the complex follows multiple-step kinetics and discovered an important intermediate, the formation of which is crucial in the kinetic discrimination of homologous and heterologous sequences. These results provided valuable insights into the mechanism of DNA repair.

Research Overview

The overall objective of my research is to take the single molecule approaches into live cells to study the dynamics of cellular processes as they occur in real time. More specifically, I propose to use single molecule fluorescence microscopy methods, in combination with statistical analysis to study the signal transduction in *E. coli* two-component systems and the assembly of the *E. coli* division complex. The results will not only complement traditional population studies, but also shed new lights on the mechanisms of these cellular processes at an unprecedented level. The methodology developed in the proposed research will open a new dimension in characterizing biological systems in live cells.

The first part of my proposal will focus on the two-component signal transduction systems in *E. coli*. By using single molecule tracking techniques at the single cell level, my initial effort will be concentrated on the dynamics of the membrane sensor kinase protein and the transient interactions between the kinase and the cytoplasmic response regulator proteins. The research can be further extended to the study of the interactions between the response regulator, which is often a transcription factor, and its target DNA sequence. With this methodology established, the entire collection of *E. coli* two-component systems (29 sensor kinases, and 32 response regulators [1]) will be studied systematically.

The second part of my proposal will focus on the dynamic assembly of the *E. coli* division complex, which contains at least ten proteins. Specifically, I intend to investigate (1) the temporal order of the arrivals of each division proteins at the division site (midcell) by using highly sensitive single molecule fluorescence microscopy method and (2) the spatial organization of the assembly by using time lapse fluorescence resonance energy transfer (FRET) imaging. All possible combinations of FRET pairs within the assembly will be examined systematically to map the protein-protein interactions in the assembly. A comprehensive table for the temporal and spatial orders of the assembly will be complied.

The proposed research is highly exploratory in nature. In addition to the stated objectives, the real time imaging will also be a rich source for other information such as cell morphology and protein distribution patterns. Therefore, I believe that these studies will not only offer deep insights into the mechanisms of cellular processes, but also lead to intriguing findings which are not accessible through traditional population based biochemical or genetic assays.

In summary, I foresee the major contribution of my research program will be the unraveling of dynamic cellular processes at the single molecule and single cell levels using a combination of biological, biochemical and biophysical methods. With the proposed methodology established in my group, I also expect to extend my research into the area of transcriptional control of gene expression, which is another crucial cellular process, involving transient interactions between DNA and protein transcription factors. With my biochemistry and molecular biology background, coupled with my postdoctoral experience in a single molecule physical chemistry group, I believe that I am at a unique position to tackle these fundamental biological problems with a multidisciplinary approach. I anticipate leading a highly collaborative group and offering unique research opportunities to the faculty and student communities.

I. Signal transduction dynamics at the single molecule level

Specific Aims

The objective of this research proposal is to study the real time dynamics of the sensor and response regulator proteins in bacterial two-component systems at the single molecule level. Using the *E. coli* sugar-phosphate uptake system Uhp as a model system, the specific aims of this proposal are: (1) to investigate the temporal and spatial distributions of the membrane embedded sensor protein UhpC and histidine kinase UhpB; and (2) to investigate the transient interactions between UhpBC and the response regulator protein UhpA.

Fluorescent protein fused to UhpC, UhpB and UhpA proteins will be made. The movements of the membrane bound individual UhpC and UhpB molecules and the recruitment of the UhpA molecules from cytoplasm to membrane will be tracked in real time upon stimulation. These time trajectories of individual molecules will offer invaluable kinetic information about the signal transduction mechanism. With the methodology established, the entire collection of *E. coli* two-component systems (29 sensor kinases, and 32 response regulators [1]) will be studied systematically.

Background and Significance

The two-component system is the predominant form of signal transduction used by bacteria to respond to environmental stresses. The prototypical system consists of a sensor protein, which usually is a histidine kinase protein (HK) containing a sensory domain and a kinase domain, and a response regulator protein (RR), containing a conserved receiver domain and a regulatory domain (Figure 1.1). Sensor proteins use adenosine triphosphate (ATP) to autophosphorylate at a conserved histidine residue, often in response to a specific signal. The high energy phosporyl group is then transferred to a conserved aspartate residue in the receiver domain of the RR. The phosphorylation often alters the regulator domain's ability to bind its target DNA sequences, which in turn regulates the expression of the effecter gene (for a review, see [2]).

Since its discovery more than a decade ago [3, 4], large amount of information about the general structures of HKs and RRs and the regulations of some specific effecter genes have been obtained through biochemical and genetic assays (reviewed in [2]). However, due to the highly dynamic nature of signal transduction [5], the kinetic information about the immediate response of HKs to signals and the subsequent interactions with RRs are not available from these methods. In addition, many RRs are transcription factors that are expressed at low levels [2], which require highly sensitive detection methods.

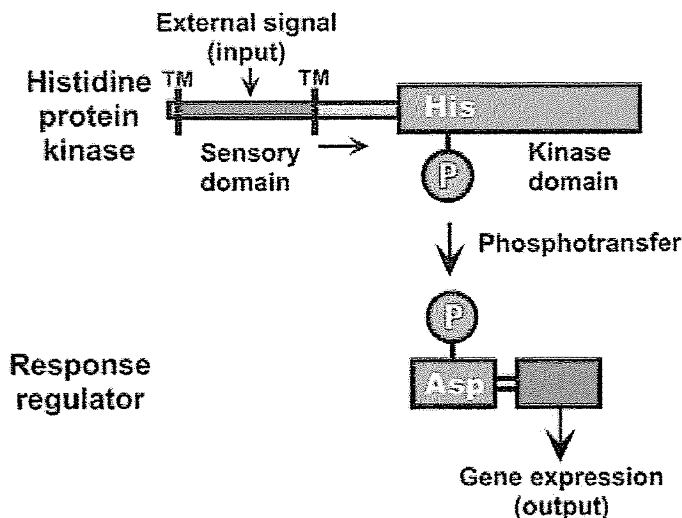

Figure 1.1: A schematic drawing of the bacterial two-component system.

In this research, I propose to use single molecule fluorescence microscopy method to study the dynamics of HKs and RRs in response to environmental stimuli. The single molecule approach has changed the way many biological problems are addressed [6-9]. The power of single molecule techniques lies in their ultimate sensitivities to detect individual molecules, which allows the researchers to resolve and analyze subpopulations, to follow dynamic trajectories of individual molecules and to capture and identify transient intermediates. As discussed above, the two-component signal transduction systems are well suited for these studies.

I will start with *E. coli* sugar update system Uhp (reviewed in [10]). Uhp system is a typical two-component system in that upon the addition of the signaling molecule glucose 6-phosphate (G6p), the signal is transduced from the membrane bound histidine kinase UhpB to its cytoplasmic response regulator UhpA and then passed on to the effecter gene *uhpT*. This results in the expression of UhpT and the intake of a broad range of phosphorylated sugars. The uniqueness of this system lies in the presence of a third protein UhpC which is the actual G6p binding protein on the membrane. It is postulated that the interaction between UhpB and UhpC leads to UhpB's autophosphorylation. Thus the Uhp system not only allows the study of the dynamics of the HK and RR proteins, but also offers a unique opportunity to study the activation of membrane receptors, the presence of which is the norm in many other signal transduction pathways.

Experimental Design

Single molecule tracking method will be used as the main tool in the proposed research. Wide field fluorescence microscope equipped with a deep-cooled charge coupled device (CCD) camera at video rate (33 ms/frame) will be used (Figure 1.2a). Fluorescent protein fusions to the G6p receptor UhpC, HK UhpB and RR UhpA will be made separately. *E. coli* cells expressing each of these fluorescently-labeled proteins will be immobilized on a coverslip using anit-FliC antibody [11]. The coverslip will be mounted in a temperature-controlled flow cell (Bioptech Inc.) as shown in Figure 1.2b. The flow cell allows steady growth of the bacteria culture and rapid change of growth medium. The fusion proteins will be excited with an appropriate line of an argon ion laser and short time series (a few seconds) of fluorescent images of cells will be recorded at video rate. These images will then be analyzed to track the movement of individual

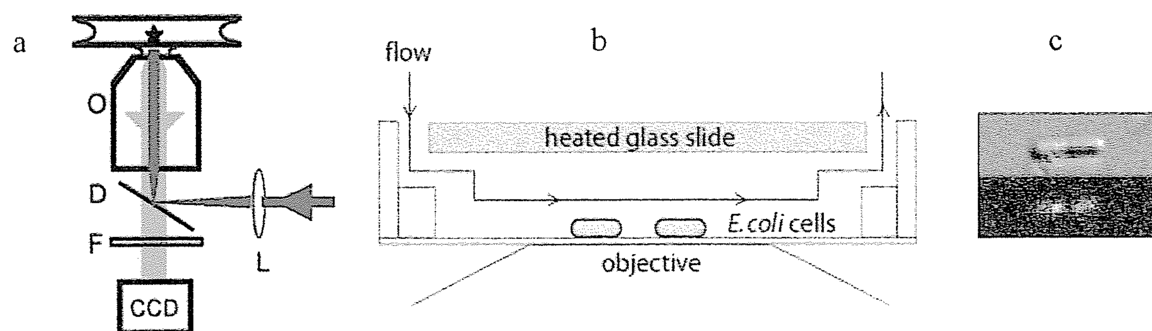

Figure 1.2. a: Wide field fluorescence microscopy setup. L: laser; O: objective; D: dichroic mirror and F: focal plane. b: Schematic drawing of the flow cell. The flow cell allows rapid change of growth medium and can be maintained at constant temperature. c: An example of the DIC (top panel) and fluorescent (bottom panel) images of cells recorded simultaneously. The bright dot on the left in the fluorescent image is a single YFP molecule on membrane [19].

fluorescent fusion protein molecules. The same time series will be repeated with fixed time interval (every few minutes) during cells' growth. Differential interference contrast (DIC) images of the cells will be taken simultaneously to record cells' contour during growth (Figure 1.2c). This allows the correlation of the dynamic behaviors of the molecules of interest with cell cycle.

Current choice of fluorescent protein for the proposed research is yellow fluorescent protein (YFP) due to its brightness, resistance to photobleaching and red-shifted spectra from cell autofluorescence background [12, 13]. It was shown that YFP is a superior choice over green or red fluorescent proteins for applications in dynamics of individually labeled fluorescent fusion proteins such as single-particle tracking, conformational dynamics, and fluorescence resonance energy transfer [14]. However, since the field evolves rapidly with new fluorescent proteins of different improved properties being discovered or engineered continuously [15, 16], the option will remain open and other fluorescent proteins may be examined as well.

Dynamics of membrane bound UhpB and UhpC

It has been shown that receptors and sensor proteins involved in signal transduction pathways often exhibit dynamic movements on the membrane. A recent study of the PleC sensor protein of *Caulobactor* using single molecule tracking technique revealed that the sensor protein undergoes dynamic movement throughout the membrane and provided evidence against previously proposed positive transport mechanism [17]. Another single molecule imaging study on the small G protein Ras demonstrated that its diffusion is greatly suppressed upon activation, suggesting the formation of large signaling complexes [18]. By tracking an individual molecule on the membrane before, during and after stimulation, diffusion coefficients (D) of the molecule can be calculated from the time trajectories, and specific information about the interactions of the molecule with its surrounding lipid membrane or other protein partners can be inferred.

YFP fusions will be made to the cytoplasmic domains (C termini) of UhpB and UhpC proteins respectively. The movements of individual UhpC or UhpB molecules on membrane will be tracked upon the supply of the signaling molecule G6p in the flowing medium. The diffusion coefficients of these individual molecules in the absence and presence of G6p will be calculated from the time trajectories and compared. The use of wide field illumination allows multiple cells at different stages of cell cycle to be imaged simultaneously, thus the correlation between the diffusion behaviors of the molecules and the cell cycle can be analyzed. Moreover, the spatial distribution (or redistribution if there is any) of the molecules on the membrane upon stimulation can be studied as the correlation between the diffusion coefficients and cell positions can be analyzed. These measurements will provide valuable information about the dynamics of the activation of the membrane receptor and reveal any possible interactions between UhpC and UhpB.

Dynamics of the recruitment of UhpA to membrane

It is especially interesting to look at the diffusive behavior of UhpA. Upon stimulation, UhpA, a cytoplasmic transcription factor expressed at low level [10], needs to make physical contacts with the membrane-embedded histidine kinase UhpB to be phosphorylated. Thus it has to be recruited to the cell membrane and interact with UhpB to allow signal transduction. To monitor the recruiting process, UhpA will be fused to YFP. Without stimulation, UhpA stays in cytosol. Because of its rapid diffusion, fluorescence of individual YFP-tagged UhpA molecules will be spread to the entire cytoplasm and therefore is not detectable above the cell's

autofluorescence background. However, upon stimulation, recruitment of UhpA to the membrane significantly slows down its diffusion, thus the fluorescence of individual UhpA-YFP molecule will be concentrated to a restricted area and appear as a diffraction-limited spot on the membrane. Therefore, the relocation of UhpA to the membrane will be detected as sudden appearance of fluorescent dot on the membrane. The subsequent movement of the fluorescent dot on membrane will be monitored as proposed in the previous section. A recent study monitoring the recruitment of a Ras binding protein to the membrane using single molecule tracking technique [18] demonstrated the feasibility of this method.

The research can be further extended to the study of the interaction between UhpA and its target DNA sequence based on the same principle. The research can also be carried out in different mutant backgrounds to study the structure-function relationships of HKs and RRs. With the methodology and analysis established, the entire collection of *E. coli* two-component systems (29 sensor kinases, and 32 response regulators [1]) will be studied systematically.

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II. Dynamics of the Assembly of *E. coli* Division Complex

Specific Aims

The overall objective of this proposal is to study the dynamic assembly of the *E. coli* division complex (divisome). The specific aims are: (1) to investigate the temporal order of the arrivals of division proteins at the division site and (2) to investigate the spatial organization of the assembly.

To study the temporal order of the assembly, division proteins will be tagged with fluorescent protein one at a time and the appearance of the fusion protein molecules at midcell will be tracked during cell's growth. The arrival time of this division protein will be extracted from the recorded time trajectories and correlated with cell age (referred from cell length) or compared with that of the FtsZ protein, which will be labeled with a fluorescent protein at a different color in the same cell. Similar measurements will be carried out for all the division proteins and a time table for the temporal order of assembly will be compiled.

To study the spatial organization of the assembly, the division proteins will be labeled in pairs with different fluorescent proteins to allow fluorescence resonance energy transfer (FRET) analysis. FRET signal between each pair will be measured in real time. An interaction network inferred from the FRET measurements will be constructed.

The research can be further extended to the study of the dynamics of cell division in conditional mutant background to elucidate the structure-function relationships of different division proteins.

Background and Significance

Cell division is essential for the survival of the organism, yet it is one of the most complex and least understood phenomena in the cell biology of bacteria. In *E. coli,* this process requires a set of at least ten proteins: FtsZ, FtsA, ZipA, FtsK, FtsQ, FtsL, FtsB, FtsW, FtsI and FtsN (Figure 2.1), all of which localize to the midcell constriction site or septum (reviewed in [1]). Among these proteins, with the exception of FtsZ and FtsA, all the others are membrane proteins [2]. Cell division is initiated by the polymerization of FtsZ into a membrane-associated ring-like structure called Z-ring at midcell [3]. The Z-ring serves as a scaffold for the assembly of other division proteins. The recruitment of other division proteins in an ordered fashion in a time span of ten to twenty minutes [4] results

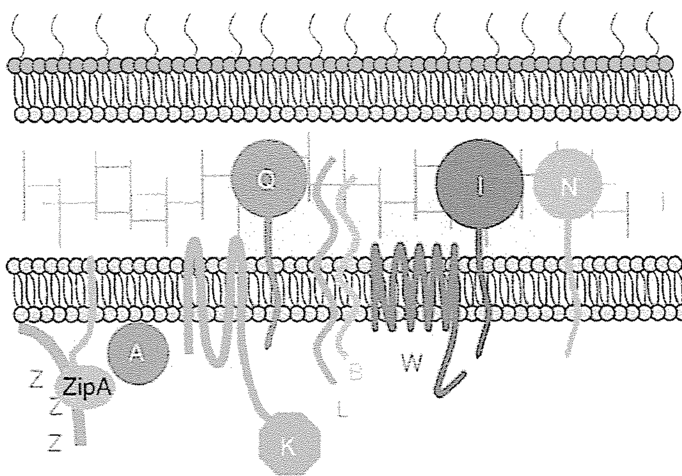

Figure 2.1: Schematic drawing of the ten division proteins in divisome (adapted from [1]). Please note that this drawing does not imply the spatial organization of the divisome. The relative positions of the FtsZ, ZipA and FtsA are drawn based on the results of a few previous studies [10-12]. The other division proteins are drawn based on their topology in membrane and the locations of them in the divisome are unknown.

in a division complex termed 'divisome'. This divisome is capable of carrying out "cytokinesis" in *E. coli*, which causes invagination of the cell membrane, inward growth of the cell wall, and, finally, separation of daughter cells.

Despite decades of work, however, little is known about the dynamics of the assembly of the divisome. In particular, the temporal order and the spatial organization of the assembly remain to be illustrated. The challenge lies in the facts that with the exception of FtsZ (~ 15,000 molecules per cell) [3, 5] and ZipA (100-1000) [6], the majority of the cell division proteins are present at low levels (~25 to 100) [1] and that the whole process is highly dynamic (for an example, the turn over rate of the FtsZ ring is about 30s [7]). Because of the low copy numbers of the division proteins and the fast dynamics, a highly sensitive detection method with high time resolution is required.

In this research, I propose to take advantage of the high sensitivity and high time resolution of time lapse single molecule fluorescence microscopy to study the assembly of the *E. coli* divisome. In particular, I propose to study the temporal order of the assembly of the divisome by tracking the appearance of YFP fusion protein molecules at mid cell, and to study the spatial organizations of the divisome by monitoring FRET signal between protein pairs.

Experimental Design

A similar microscope setup discussed in Proposal I will be used in this study. In addition, another laser line will be added to excite a second fluorescent protein which has a different color than YFP. Fluorescence from YFP and the other fluorescent protein will be recorded simultaneously. Current choice for the second color fluorescent protein is the cyan fluorescent protein (CFP) due to the fact that it is a good FRET donor for YFP for live cell imaging [8]. However, as indicated before, the option will remain open and other fluorescent proteins or labeling with organic dyes in periplasmic side of the division proteins may be tested [9].

Temporal order of the assembly

Studies on the localization of cell division proteins in conditional mutants revealed that there is a defined linear hierarchy of dependence (reviewed in [2]) (Figure 2.2a). In this hierarchy, localization of a given protein to the midcell requires the presence of all upstream proteins and its presence is in turn the prerequisite for the localization of all downstream proteins. A simple explanation for this hierarchy is that each division protein is added sequentially in time to the divisome. However, since it is also possible that the localization of a downstream protein only requires the existence but not necessarily the localization of its upstream proteins, this hierarchy does not necessarily reflect a linear temporal order of the assembly. Another possibility could be that a complex or subcomplex of proteins can form independently of other division proteins and recruited to midcell together. Indeed, it was shown that FtsQ, L, and B form a stable complex that is independent of other known division proteins [13].

It is of the research community's great interest to distinguish these possibilities to generate a time table for the assembly of the divisome. However, to date there is no comprehensive report available. Only one recent study showed that the delay between the localization of the first protein FtsZ and the last protein FtsN in the hierarchy is between 14 to 21 minutes depending on the growth conditions [4]. This work was done by counting the relative frequency of cells showing FtsZ localization and those with detectable localization of the other proteins using immunofluorescence and GFP fluorescence microscopy. However, due to the

significantly lower levels of other division proteins (10^2 copies per cell) comparing to that of FtsZ (10^4) and the limited sensitivity of the detection methods, the observed differences in localization frequency could be due to the variation in the detection efficiencies of these proteins. In addition, the static nature of the methods used in the study does not allow precise timing of the assembly.

In this proposed study, with the ability to detect single YFP fluorophore on membrane in real time [14], the low expression levels of many division proteins and the fast assembly of the divisome will no longer be technical barriers. Multiple *E. coli* strains, each of them expressing YFP fusion protein driven by that particular division protein's native promoter will be made. The movement of the fluorescent fusion proteins on membrane and their appearance at midcell will be tracked during cells' growth. The arrival time of the division protein at midcell in relative to the cell's age (inferred from cell's length) will be extracted from the recorded time trajectories and compared with those of other division proteins to generate a time table of the assembly.

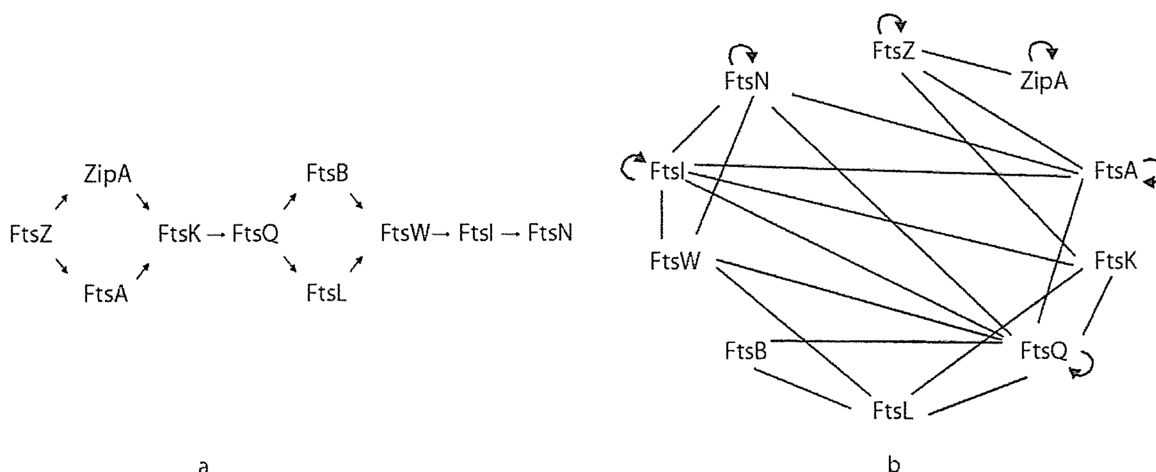

Figure 2.2. a: Linear hierarchy of the interdependence of the ten division proteins. b: protein-protein interactions as probed by bacteria two hybrid system or protein co-purification methods. The lines connecting two proteins indicate the interaction between them and the circular arrows indicate potential self-interaction. Both a and b are adapted from [1].

The arrival time of each division protein can also be measured in relative to that of FtsZ, the first protein who localizes to midcell [3]. In this alternative method, a parental *E. coli* strain in which FtsZ is fused with a different fluorescent protein such as CFP will be constructed first. Different division protein fused to YFP will then be constructed using this parental strain so that the daughter strain will express both FtsZ-CFP and a particular type of division protein fused to YFP. Two-color imaging will be performed and the arrival time for the YFP fused division protein will be measured in relative to that of FtsZ in the same cell. Localization of FtsZ-CFP in the same cell serves as an internal standard to minimize the cell to cell variations in the cell length measurement described in the first method.

By using the highly sensitive time lapse single molecule fluorescence microscopy method, large scale statistical analysis can be performed on multiple cells and therefore the arrival time for each division protein can be accurately determined. Moreover, because this method can be used to record the time course of the movement of each division protein along the cell membrane, additional information about the dynamics of the assembly can be obtained.

Spatial organization of the assembly

Recent studies using the bacterial two hybrid system and the protein co-purification method suggest that the cell division proteins interact with at least one other cell division protein, and that several proteins may interact with multiple proteins within the assembly [13, 15, 16] (Figure. 2.2b). Though caution should be taken to interpret these data due to the facts that they often generate false positive results because of the over expression of the proteins and that sometimes they reflect indirect interactions between proteins belonging to the same complex, nevertheless the data have shown that the assembly is complicated and there is no doubt that protein-protein interactions play important roles in the organization of the assembly.

To understand how these proteins are organized and interact with each other inside the assembly, I propose to measure all pair wise interactions between these proteins during the cell cycle using FRET microscopy. FRET is the radiationless energy transfer from an excited donor fluorophore to a suitable acceptor fluorophore, a physical process that is extremely sensitive to the distance between the pair of fluorophores, with FRET efficiency decreasing with the sixth power of the distance [17]. The occurrence of FRET is characterized by a decrease in the donor emission and a simultaneously increased (sensitized) acceptor emission (Figure 2.3). So far the most popular pair of fluorescent proteins for FRET analysis in cell biology is CFP (donor) and YFP (acceptor) [8]. The characteristic distance, or Forster radius, at which the FRET efficiency is 50% of its maximum of this FRET pair, is 49 – 52 Å [8]. Because this distance matches the sizes of most proteins, when two proteins are tagged with CFP and YFP respectively, FRET will only be observed if they are so close to each other that they almost certainly interact. In another word, if two proteins belong to the same complex but do not directly interact with each other, little or no FRET signal will be observed. Possible false positive results obtained from bacterial two hybrid system or protein co-purification methods can be avoided by using this method.

In the proposed study, a systematic approach to address the interactions among the division proteins will be used. Because there are at least ten division proteins in the assembly, there are a total of 45 possible combinations (not counting self interactions) of FRET pairs with one division protein fused to CFP as the FRET donor and another one fused to YFP as the acceptor (assuming that a combination of A fused to CFP and B fused to YFP is the same as that of A fused to YFP and B fused to CFP). Thus multiple *E. coli* strains with each one of them co-

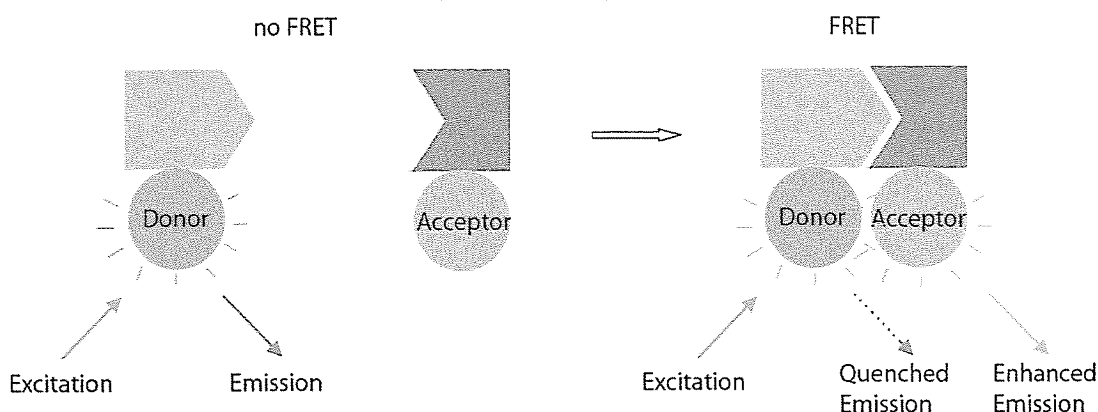

Figure 2.3. Schematic drawing showing that when two proteins, one attached with a FRET donor, and the other one attached with FRET acceptor, do not associate with each other, there is no FRET occurring. When the two proteins are in close proximity, energy transfer occurs thus decreased donor emission and enhanced acceptor emission are observed.

expressing a particular pair of division proteins will be made. The occurrence of FRET for the pair of labeled proteins will be imaged through cell cycle using time lapse fluorescence microscopy. With experiments carefully designed and data critically analyzed [18, 19], detailed information about the interactions between each protein pair can be extracted and the spatial organization of the divisome can be inferred.

The proposed research can be further extended into the study of cell division conditional mutants to elucidate the structure-function relationships of different division proteins. As to date the functions of the majority of the division proteins are still unknown, direct observation of their dynamics during cell division under mutant background will provide valuable information towards the understanding of their functions.

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Teaching Statement

I view teaching as an interactive, student centered and teacher-assisted self learning process instead of merely transferring knowledge from the teacher to the students. Ultimately, success of teaching is not judged by the test scores of students, but by the interest they maintain in the subject, and the ability to apply the knowledge long after the course is completed. Coming from a family which values teaching, and inspired by my mother, who has been a respected teacher for more than thirty years, I have long time passion for teaching and I am willing to commit myself to its excellence.

As described in my research plan, my research is highly interdisciplinary in nature. In line with my research, I intend to teach in the areas of general biology, genetics, microbiology, biochemistry, molecular biology, biophysics and the corresponding lab or seminar courses. Advanced topics in the latter two subjects could be at the senior undergraduate and graduate level. My teaching will bridge these areas and allow students to have comprehensive understandings of modern life science.

During my Ph.D. program, I had one year experience of being a teaching assistant (TA) for general biochemistry. I was responsible for leading discussion sessions and grading tests. Because of the large class size, I had the opportunity to interact with a wide variety of students and this allowed me to understand the needs of different individuals. In discussion sessions, I guided students through problem solving steps and encouraged them to find the answers by themselves. Such exercise not only helped students to understand the subject better, but also made the learning experience more enjoyable and exciting. I also paid close attention to my students' feedback and asked for their comments so that I can constantly improve my teaching style. As for grading, I did not treat it just as a repetitive duty. Instead, I viewed grading as an important feedback to a student's learning effort. In grading I rewarded not only accuracy and skillfulness, but also improvement and creativity. I often wrote thorough, personalized and constructive comments to motivate students to learn more and learn better.

During my postdoctoral research period, I have led multiple individuals or teams of undergraduate and graduates students in conducting scientific researches. Because of the interdisciplinary nature of the projects I worked, I taught students with physics or chemistry background to conduct molecular biology experiments, and students majored in biology to learn biophysics and fluorescence microscopy. As a mentor in these small groups or face-to-face settings, I was able to hone my skills in teaching and inspiring students on individual basis.

Based on these experiences, I realize that teaching is most effective when students are drawn to the topics by their interests instead of their needs for credits. Therefore, in my classes, I will emphasize the real world relevance of my teaching subjects. Given that life science has an ever increasing impact on our way of life, I will develop extensive course readers with concrete examples to elucidate the scientific concepts and their applications in day-to-day living. Frontier topics in current research fields will be cited to stimulate students' interests and curiosity. In addition, I will use molecule models, 3-D computer animations and movies to give students vivid visualization of proteins and DNA's chemical structures, cells and organisms' compositions and various cellular processes. One of the obstacles for students to learn biochemistry and molecular biology is the

existence of complicated interaction networks and numerous pathways and proteins. It's a daunting task to memorize all of the names and functions. To help students to learn, I will emphasize on teaching in a holistic way, linking individual concepts by their discovery histories, interaction mechanisms and biomedical applications. Primary literatures for key discoveries and breakthroughs of specific areas will also be introduced to help students achieve a more thorough understanding of the subject. Lastly, I would like to be actively engaged in interactions with students. In classroom I will ask well-formatted questions and encourage different opinions among students. In lab I will work with students closely and discuss their progress with them on daily bases. I will also encourage students' feedback so that I can adjust my teaching strategies to meet variable student needs. Versatile course websites including course forums and discussion groups will be setup to communicate and connect with students.

My major teaching goal at general undergraduate level is to equip students with not only the knowledge of fundamentals of modern life science, but also critical thinking and problem-solving skills, so that students can benefit from the teaching even if they pursue non-scientific careers. To achieve this, I will prepare extensive course reading materials, which will focus on particular scientific problems and include journal articles, book sections and even news coverage. Students will be asked to analyze the materials and write reviews based on their understanding of these materials.

For more advanced undergraduate and graduate level, in addition to the above methods, I will encourage active involvement in research. I believe research is the ultimate teaching tool to convey knowledge. For the advanced topics in molecular biology and biophysics, hands-on experience in laboratory through active research is the best way to obtain solid understandings of abstract concepts. My research projects, which will adopt an interdisciplinary approach to investigate the dynamics of cellular processes, provide an excellent opportunity for students who have the desire to pursue a scientific career to practice their analyzing and problem-solving skills. I expect that my lab will attract students from various backgrounds, including biology, chemistry and physics. I will encourage students to read primary literatures about specific areas, to think independently and to realize these ideas through active research.

I am deeply indebted to all of my teachers and mentors who encouraged me to pursue a scientific career and showed me how to be a successful teacher and researcher. Now with the opportunity to establish my own research lab and become a faculty member, it is time for me to continue the tradition: to encourage, to enable and to inspire future generations to carry on the noble spirit of scientific learning and exploring.