Design and implementation of a bacterial signaling circuit

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Chapter 1

Introduction

Living cells sense and respond to changes in their surroundings by networks of molecules that detect environmental cues and process that information. Arguably the simplest biological signaling network is the chemotaxis system of *Escherichia coli,* which has become a paradigm for the molecular understanding of biological signaling and a model system for quantitative experimental and theoretical studies of sensory responses. In this thesis we performed physiological studies of *E. coli* and the closely related species *Salmonella typhimurium*, investigating the signaling and behavioral strategies that arise as a consequence of the design and implementation of the chemotaxis signaling circuit.

Chapter 1

1.1. Environmental sensing and intracellular signaling networks

Living organisms exhibit a number of characteristics that distinguish them from lifeless matter. The fundamental unit of life is the cell: the smallest structure capable of basic life processes, such as growth, metabolism and reproduction. A cell can represent a living organism itself, or it can be a part of a multicellular organism. A defining feature of living organisms is their ability to sense and respond to changes in their surroundings. Organisms detect environmental cues using sensory systems, which can be represented by complex organ structures or molecular receptors that transfer the information into the cell, where the information is processed and a response is generated. Possible responses include changes in the gene expression or behavioral responses that commonly involve movement of the organism.

Cells have developed networks of molecules that link the input from the receptors to behavioral output [29]. These molecular circuits are usually composed of multiple proteins, and each of them has a specific role in the signal transduction process. Some proteins act as transmembrane or soluble receptors that sense the signals; others are messengers or response regulators, the functions of which are to transfer the signals to the protein or protein complex that will generate the behavioral response. Other proteins that act as structural or interaction platforms, and can also take part in regulation of the signaling process, are the signaling scaffold proteins [290]. Sensory pathways in eukaryotes usually rely on serine, threonine or tyrosine protein kinases, whereas prokaryotic sensory pathways most often involve histidine-aspartate phosphorelay systems that involve a dimeric histidine kinase and a response regulator protein [266]. Despite the complexity of biological signaling networks in terms of their molecular organization, they can often be described in terms of functional modules [99], which facilitates characterization of the dynamics of their function.

Bacteria provide an ideal ground for studying signaling networks from their genetic structure, protein-protein interactions, systems-level function, to behavioral outputs. The availability of genomic information [34,100,160,179,257] and ease of genetic manipulations [62] facilitates the discovery and

functional analysis of bacterial network components. Bacteria are amenable to microscopy and functional studies of their signaling systems, and reproduce rapidly, allowing experimental studies of processes that occur in different time scales, such as intracellular and intercellular signaling, behavioral responses and evolutionary processes. In particular, the chemotaxis circuit of *E. coli*, arguably the simplest and best characterized bacterial signaling network, has served as an important paradigm in the understanding of biological signaling [19,244,266]. The *E. coli* chemotaxis circuit is used as a model system in this thesis.

1.2. Tactic responses

A cell or an organism orients in gradients of chemical or physical stimuli in the environment by altering the direction of its movement. This behavioral response is called taxis, and depending on the type of stimulus it can be classified into different categories (Table 1.1). The tactic response can be monotonic, towards increasing or decreasing values of the stimulus (attractant or repellent response) [266] or non-monotonic, towards an optimum intermediate level of the stimulus [113].

Taxis	Stimulus	Organisms/ cells
Chemotaxis, including aerotaxis, pH taxis	Chemicals (organic and inorganic), including O_2, H^+	Bacteria [266], unicellular eukaryotes [109], nematodes [269], sperm cells [74], macrophages [117]
Redox taxis	Redox potential	Bacteria [28]
Thermotaxis	Temperature	Bacteria [210], nematodes [177], amoebae [105]
Phototaxis	Light	Bacteria [278],protozoans [68]
Gravitaxis	Gravity	Protozoans [97]
Rheotaxis	Fluid flow	Bacteria [157], fish [193], sperm cells [172]
Osmotaxis	Osmotic pressure	Bacteria [5]
Galvanotaxis	Electrical current	Bacteria [6], protozoans [16]
Magnetotaxis	Magnetic field	Bacteria [67]

Table 1.1. Examples of tactic responses. Groups of organisms or cells that exhibit these tactic behaviours are shown in the last column.

Bacteria can sense and respond to various chemicals (nutrients [4,170], toxins [77], protons [284], oxygen [31] etc.), as well as other stimuli (temperature [210], redox potential [28], blue light [278], fluid flow [157], osmolarity [5], electric and

magnetic fields [6,67] etc) via their chemotaxis signaling systems [2,4,5,210]. Motility of microorganisms was observed as early as 17th century by Anthony van Leeuwenhoek; however the first systematic investigations of directed chemotactic migration of bacteria were made by Wilhelm Pfeffer in the end of the 19th century [171]. Pfeffer studied the attractant response of *Bacterium termo* (rod-shaped bacteria, isolated from putrid fluids) towards meat extract. In the same time Theodor Engelmann described the directed migration of *Bacterium termo* towards oxygen [71].

Chemotaxis of bacteria is involved in more complex behaviors and processes, such as biofilm formation, symbiotic associations and pathogenicity [266]. The development of surface-attached biofilms depends on motility and chemotaxis of bacteria [213,285]. Chemotaxis is also important for some symbiotic associations, such as the association between nitrogen-fixating bacteria and legume plants [48]. Motility and chemotaxis play an important role in invasion and colonization of the host in many bacterial species, such as *Helicobacter pylori* [197], *Vibrio anguillarium* [188] and *Vibrio cholerae* [47]. Understanding bacterial chemotaxis can facilitate the development of biomedical strategies, such as using bacteria to controllably deliver therapeutics to the different tissues in the body [61].

Molecular investigations of bacterial chemotaxis and motility began in the 1960's with the pioneering work of Julius Adler [101]. Adler applied behavioral assays, biochemical and genetic approaches to study the motile response and the underlying molecular mechanisms of *E. coli* chemotaxis [1,2,4-6]. He demonstrated that chemotaxis of *E. coli* does not require metabolism or transport of the sensed compound, but instead the response is generated by recognizing the chemical itself by a specific system for recognition and response. Adler's work initiated the contemporary studies of bacterial chemotaxis, which were greatly expanded in the seventies by the groups of Daniel Koshland, Jr., Melvin Simon, and Howard Berg [101]. Bacterial chemotaxis has been a subject of increasing scientific interest in the following decades. The chemotaxis system of *E. coli* has become a preferred model system for theoretical and experimental studies of sensory responses [120,121,223,225,260]. Other studies of bacterial chemotaxis highlighted the diverse mechanisms of the chemotactic signaling and behavior in various prokaryotic organisms [254,281,283].

1.3. Motility and chemotaxis of enteric bacteria

E. coli and its closely related species *Salmonella typhimurium* swim using organelles called flagella. Each flagellum is a semi-rigid helical filament of repetitive protein (flagellin) units that is several micrometers long but only ~20 nm in diameter, and rotates with rates on the order of 100 Hz [261]. The flagellar filaments exist in different polymorphic forms with distinct curvature and twist [261]. The filaments are linked by a hook to the flagellar motor: a remarkably complex structure consisting of about 50 protein components, which expression is tightly regulated [152,266]. The flagellar motors of *E. coli* and *S. typhimurium* are driven by proton flux: the free energy stored in the electrochemical proton gradient across the cytoplasmic membrane is converted into mechanical work [164]. The protons move through force-generating units, called Mot complexes, which are anchored to the peptidoglycan layer to form the stator of the flagellar motor complex, and interact with a ring of FliG units (rotor) on the cytoplasmic side to drive rotation [266]. The proton-driven flagellar machinery enables bacteria to swim at speeds of 15-100 μm/min [26].

Each bacterium has several flagella, usually 5-8 per cell. When all the flagella rotate counterclockwise (CCW) the flagella form a bundle that propels the cell forwards, i.e. the cell "runs". If one or more flagella change the direction of their rotation to clockwise (CW) the flagellar bundle is disrupted and the bacterium changes its swimming direction, i.e. the cell "tumbles". In this way bacteria swimming in a homogeneous environment perform a random walk [25] (Figure 1.1A). Typical run time for *E. coli* is ~1 s, whereas a tumble lasts ~0.1 s [114]. The direction of the motion during a run fluctuates due to rotational diffusion of the bacteria [232].

If the bacteria are placed in a gradient of a chemoattractant, the frequency of the tumbling events decreases [35], leading to prolonged runs and therefore the bacteria move in the direction of increasing chemoattractant concentrations (Figure 1.1B). Alternatively, increasing of the concentration of a chemorepellent leads to more frequent tumbles: bacteria change their direction more often, searching for a more favourable environment. Thus bacteria perform temporal comparisons, determining if

the conditions are getting better or worse: if they are getting better the bacteria keep swimming, otherwise they change direction.

The flagellar motility of *E. coli* and *S. typhimurium* is just one of many types of bacterial locomotion that exist in nature. Other flagellated bacteria use different motility strategies, e.g. *Vibrio alginoliticus* have a single flagellum and change direction by flicking its flagellum leading to "run, reverse and flick" swimming rather than "run and tumble" swimming observed in *E. coli* [251]. Bacterial species that move on surfaces often use gliding or twitching that can involve type-IV pili extending from the poles, bind to the surface and pull the cell forward [45].

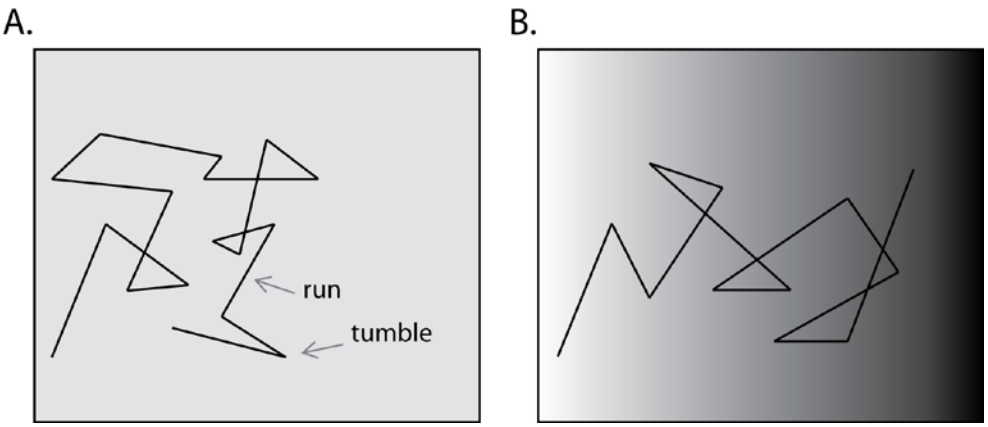

Figure 1.1. Schematic representation of a bacterial trajectory in absence and presence of a gradient of chemoattractant. The trajectory is represented in 2D for simplicity. The straight fragments represent runs, which are separated by tumble events (changing direction). **(A)** Random walk in a homogeneous environment. **(B)** Biased random walk in the presence of a chemoattractant gradient (represented in grey scale, a darker color corresponds to a higher concentration). The runs are prolonged in the direction of the gradient.

Various chemotaxis assays have been developed to evaluate the chemotaxis performance of *E. coli* and other microorganisms in spatial gradients of chemoeffectors [2,4,7,8,25,156,276]. Soft-agar assays are commonly used to evaluate the chemotaxis response to metabolizable chemoattractants [276]. Bacteria are inoculated in a semi-solid agar containing the chemoattractant

of interest, which also serves as a nutrient for the bacteria. The growing colony of bacteria consumes the chemoattractant, and thus generates a radial gradient of the chemoattractant that triggers an outward migration of the bacteria [276]. Another commonly used assay for chemotaxis is the capillary assay: a capillary that contains a chemoattractant is submerged in a solution containing the bacteria; the bacteria that accumulate in the capillary are subsequently counted to quantify the strength of the chemotaxis response [2,171]. Agar-based and capillary-based assays evaluating the negative response to chemorepellents have also been developed [259].

Quantitative studies of bacterial motility and chemotaxis have been greatly facilitated by the development of microfluidics technology, which allows generating precisely controlled gradients of chemoeffectors and observing the behavior of bacteria in these gradients at high spatial and temporal resolution [8]. Microfluidic experiments in combination with theoretical and computational studies of the chemotactic response, enabled investigations of the behavioral strategies used by bacteria, and explaining the observed strategies using the information for the underlying control physiology of the chemotaxis response [137,156,158].

1.4. Molecular organization of the chemotaxis system

The frequency of clockwise rotation of the flagellar motors of *E. coli* and *S. typhimurium* is determined by the activity of the chemotaxis signaling network (Figure 1.2) [266]. Chemoeffector molecules are detected by transmembrane chemoreceptors, called methyl-accepting chemotaxis proteins (MCPs). Bacteria express MCPs with different specificities and affinities for ligands. The most commonly used laboratory strains of *E. coli* K12 and *S. typhimurium* LT2 have five and nine chemoreceptor species respectively [30,129,154,170]. However, variations exist even within each species: e.g. uropathogenic strains of *E. coli* lack two out of five chemoreceptors present in *E. coli* K12 [133]. MCPs exist as dimers and are coupled to the dimers of the histidine kinase CheA via scaffolding proteins: CheW in *E. coli* [266] and CheW and CheV in *S. typhimurium* [10,86,267]. Each monomer of CheA transfers the γ-phosphoryl group of an ATP molecule, bound to its kinase domain to a His residue on the opposing monomer. This phosphoryl

group then can be transferred to an Asp residue of the response regulator CheY, which transduces the signal to the flagellar motors and biases the direction of their rotation. In the absence of phosphorylated CheY, the flagellar motors rotate counterclockwise [75]. Phoshorylated CheY molecules bind to FliM molecules in the switch complexes of the flagellar motors, biasing the rotation of the motors towards the clockwise direction [33]. The phosphataze CheZ accelerates CheY dephosphorylation and allows a rapid termination of the signal [161]. CheA also transfers its phosphoryl groups to other molecules of the chemotaxis system that has phospho-receiver domain: the methylesterase / deamidase CheB [233] and the scaffolding protein CheV [10] (although CheV phosphorylation has not been shown experimentally in *S. typhimurium*).

An increased concentration of chemoattractant (or decreased concentration of chemorepellent) inhibits the CheA autophosphorylation activity, thus the concentration of phosphorylated CheY decreases and respectively the probability of switching the flagellar motors to clockwise direction decreases, causing prolonged runs. The methyltransferase CheR methylates the receptors at multiple sites increasing their ability to stimulate CheA autophosphorylation. In this way the CheA autophosphorylation returns to prestimulus level even in the continued presence of chemoattractant, *i.e.* the bacteria adapt to the stimulus. Another enzyme, the methylesterase / deamidase CheB removes methyl or glutamyl groups from the chemoreceptors, reducing their ability to induce CheA autophosphorylation. Thus, CheR and CheB introduce a negative feedback in the chemotaxis system [243,266]. There is also phosphorylation-dependent negative feedback on the activity of CheB: CheA phosphorylates CheB, stimulating its methylesterase activity [15].

Additional chemotaxis proteins that are not present in the *E. coli* and *S. typhimurium*: the phosphatases CheX and CheC, and the deamidase CheD, exist in other bacterial species [281]. The number of chemoreceptor genes also vary significantly between different bacterial species: from 1 in *Mezorhizobium loti* to >60 in *Magnetospirillum magnetotacticum* [266].

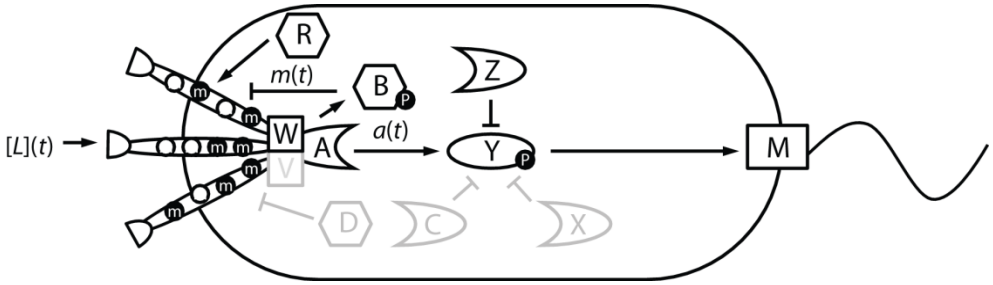

Figure 1.2. Molecular organization of the bacterial chemotaxis network. The transmembrane chemoreceptor clusters regulate the autophosphorylation activity of the kinase CheA (A), coupled to the receptors via scaffolding proteins CheW (W) and CheV (V) (CheW exists in both *E. coli* and *S. typhimurium*, whereas CheV is present in *S. typhimurium* but not in *E. coli*). CheA phosphorylates the response regulator CheY (Y), whereas CheZ (Z) accelerates its dephosphorylation. Phosphorylated CheY interacts with the flagellar motor (M) biasing its rotation in a clockwise direction. The adaptation enzymes CheR (R) and CheB (B) are involved in reversible methylation of the chemoreceptors at multiple sites. CheB methylesterase activity is activated by phosphorylation by CheA. The ligand input, kinase output and methylation feedback are indicated with $[L](t)$, $a(t)$ and $m(t)$ respectively. Components that do not exist in *E. coli* but exist in other bacteria are indicated in gray: the scaffolding protein CheV (V), the phosphatases CheC (C) and CheX (X), and the deamidase CheD (D) [281].

1.5. Functional organization of the chemotaxis system

The chemotaxis signaling pathway of *E. coli* is very well understood at the molecular level: structural and biochemical data are available for every step of the pathway [266]. Thus, it has provided an ideal system for quantitative theoretical and experimental studies, which enabled better understanding of functional properties of chemotaxis and other sensory systems such as signal amplification, perfect adaptation, and wide dynamic range [14,78,113,114,137,158,185,186,192,222,225,226,232,234,240-242,260,263-266]. For example, experiments and computational modeling showed that cooperative interactions between the clustered chemoreceptors can explain the high sensitivity of the chemotaxis response of *E. coli* [42,168].

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A coarse-grained model that describes the chemotaxis system as a modular system (Figure 1.3) has been recently developed [260], and it has been shown to explain consistently the experimental data for *E. coli*'s signaling response to time-varying chemoeffector stimuli [226]. In this model the molecular details are omitted and the chemotaxis system is simplified to two modules: a receptor module, representing the activity of the receptor-kinase complexes, which detect signals from the environment and generate excitatory response, and an adaptation module, representing the methylation-dependent feedback by CheR and CheB to maintain the steady-state activity of CheA independent of the background concentration of the stimulus [260]. The system-level dynamics of the chemotaxis signaling response is described by three dynamic variables: the concentration of the chemoeffector, $[L](t)$, the activity of the kinase representing the output of the system, $a(t)$, and the methylation level of the chemoreceptors, $m(t)$. The timescales for ligand binding (τ_L), kinase response (τ_a), and receptor methylation (τ_m) are well separated: $\tau_m >> \tau_a >> \tau_L$. The pathway kinetics is studied at the methylation timescale [260], which is most relevant to the bacterial motility since the typical run time is ~1 s. The ligand binding and kinase response timescales are treated with a quasi-equilibrium approximation [260].

The three dynamic variables are linked by two transfer functions. The transfer function G of the receptor module, $G([L], m) = a$, takes $[L](t)$ and $m(t)$ as inputs to produce an output $a(t)$, connecting the linear pathway downstream of the kinase toward motor output. This transfer function represents the cooperative modulation of CheA activity by chemoreceptors. G can be expressed by using a two-state model, in which the dependence on m and $[L]$ can be accounted for specifying a functional form of the free energy difference, f_t, between active and inactive receptor clusters:

$$G(m, [L]) = \left(1 + e^{f_t}\right)^{-1},$$

where f_t is an additive function of two linearly independent terms:

$$f_t([L], m) = N\left(f_L([L]) + f_m(m)\right),$$

where $f_L([L])$ depends on the ligand binding and is characterized by the dissociation constants K_A and K_I of the active and inactive receptors, $f_m(m)$ depends linearly on the methylation level of the receptors and determines the kinase activity in the absence of ligand, and the amplification factor N

corresponds to the number of ligand-binding receptor dimers in a functional receptor cluster [226,260].

The transfer function F of the adaptation module, $F(a) = dm/dt$, converts $a(t)$ to rate of change of methylation level, dm/dt, and integrates it over time [260]. This transfer function represents the net methylation rate (methylation rate-demethylation rate) driven by the activities of CheR and CheB. Perfect adaptation of the kinase activity, i.e. adapting to the same level a_0 at steady-state, regardless of the background ligand concentration [22,167,286], is a property embedded in the design of the chemotaxis signaling system as $F(a)$ has a single fixed point at $a=a_0$ [260]. Note that imperfect adaptation has also been observed for some chemoeffectors, and explained with the finite number of methylation sites possessed by the chemoreceptors [132,163].

The model of reference [260] is used in this thesis to explain the experimental results obtained for both *E. coli* and *S. typhimurium*, which have very similar chemotactic networks [66].

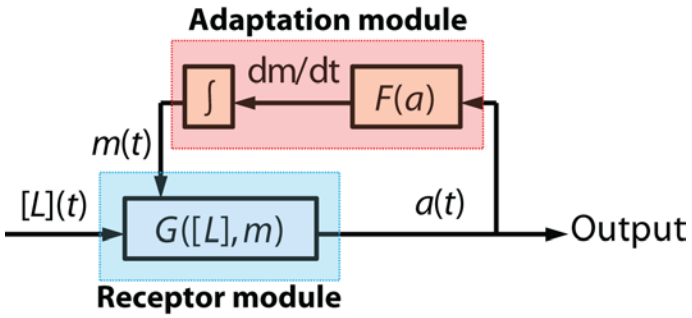

Figure 1.3. Modular organization of the bacterial chemotaxis network. The block diagram represents the functional modules rather than molecular components of the network (adapted from reference [226]). The dynamic variables are viewed as inputs or outputs (represented along wires) of two discrete functional modules (represented as boxes). The input-output relationship of the receptor and adaptation modules are described by functions $G([L],m)$ and $F(a)$ respectively.

1.6. *In vivo* fluorescence resonance energy transfer (FRET) studies of the chemotactic signaling response

Understanding of the quantitative features of the chemotaxis signaling has been greatly facilitated by the development of experimental techniques that enabled following the signal processing dynamics in live bacteria [224,242,264]. Most of these methods rely on fluorescence resonance energy transfer (FRET) between components of the chemotaxis pathway, which permit monitoring the activity-dependent interactions between these components in real time in live cells [242].

FRET assays rely on a nonradiative distance-dependent energy transfer from one fluorescent molecule, called donor, to another fluorescent molecule, called acceptor [277]. FRET measurements are widely used in biology to study interactions between proteins or parts of the same protein since the FRET technique is non-invasive, and allows observations of these interactions in real time [17,181,207,239,242,277]. *In vivo* labeling with fluorescent dyes is difficult in bacteria due to their small size and low permeability of the cellular envelope; thus the most commonly used approach for bacterial FRET studies is genetically fusing fluorescence proteins (donor and acceptor) to the protein pair of interest. The efficiency of energy transfer, E_{FRET}, depends strongly on the distance between the donor and acceptor fluorophores: $E_{FRET} = R_0^6/(R^6 + R_0^6)$, where R is the distance between the fluorophores and R_0 is the Föster radius, representing the distance at which the energy transfer between the donor and the acceptor have 50% efficiency [242]. R_0 for the cyan and yellow fluorescence protein pair (CFP/YFP) in the bacterial cytoplasm is 4.9 nm, and very little energy transfer occurs if the donor and the acceptor are >10 nm apart [242].

The most widely used FRET assay for studying the chemotactic signaling dynamics in *E. coli* utilizes a FRET pair consisting of CheZ-CFP and CheY-YFP fusion proteins, serving as a donor and acceptor respectively [185,192,239-242,284] (Figure 1.4A). The steady-state concentration of the phosphorylated CheY (CheY-P), and the concentration of the CheZ·CheY-P complex respectively, is determined by the balance between the phosphorylation of CheY by CheA and dephosphorylation of CheY-P by CheZ. These two enzymatic reactions have equal rates at steady-state, thus

the activity of the kinase CheA, $a(t)$, is proportional to the concentration of the protein complex CheZ·CheY-P. This concentration, which can be determined by FRET, therefore serves as a measure of the kinase output $a(t)$ on time scales that are shorter than the relaxation time of CheY phosphorylation cycle.

Upon a step addition of attractant (or a removal of repellent) the FRET level decreases, indicating a decrease in the kinase activity (Figure 1.4B). A step addition of repellent (or a removal of attractant) induces an opposite effect (Figure 1.4C). In adaptation-proficient bacteria, the kinase activity recovers toward the pre-stimulus level due to the activity of the methylation / demethylation pair of enzymes, CheR and CheB.

The described FRET system can be used to monitor the signaling activity of the chemotaxis pathway of single cells or populations of cells, attached to a coverslip, using fluorescence microscopy [242]. In this thesis, we implemented population-based FRET measurements, which provide better sensitivity and temporal resolution compared to single-cell FRET measurements. Since the fluorescence emission is collected from hundreds of cells, attached to a coverslip, using sensitive photomultiplier tubes, we used relatively low intensities to excite fluorescence so as to minimize the photobleaching and photodamage to the cells, while achieving high signal-to-noise ratios. Input profiles smoothly varying in time were achieved by mixing a concentrated solution of chemoeffector with motility buffer using a fluid-mixing device of a design described in reference [226]. Using different time-varying inputs (temporal steps, up and down ramps, or sinusoids) has allowed characterization of both the receptor and adaptation modules of the chemotactic signaling system of *E. coli* [226], and in this thesis we have explored in a similar manner the response of *S. typhimurium*.

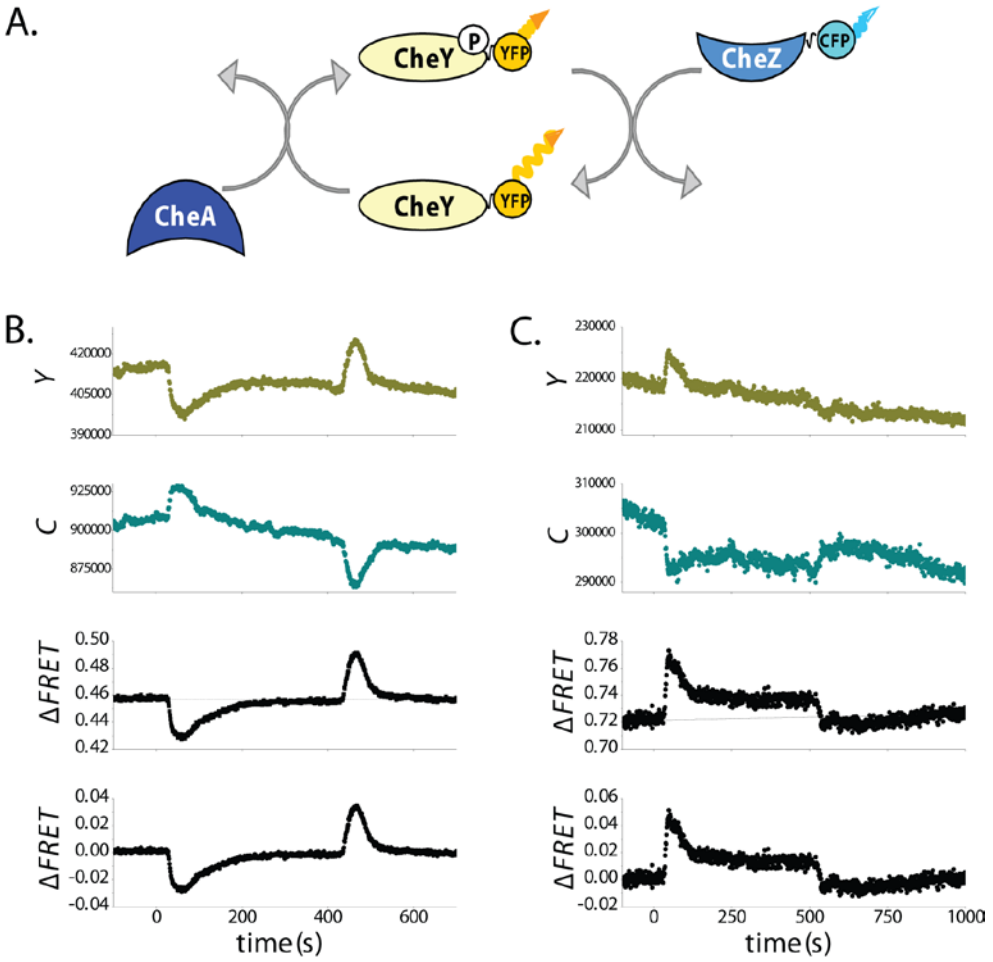

Figure 1.4. Description of the FRET system and typical responses to a chemoattractant and a chemorepellent. (A) Schematic representation of the FRET system used in this thesis. **(B)** and **(C)** From top to bottom: changes in the yellow (*Y*) fluorescence channel, cyan (*C*) fluorescence channel, *Y*/*C* ratio and *Y*/*C* ratio corrected for baseline drift: **(B)** at 0 s a chemoattractant (200 μM cysteine) is added, removed after 400s; **(C)** at 0 s a chemorepellent (200 μM cystine) is added, removed after 500 s.

1.7. This thesis

The scope of this thesis has been to understand the design and implementation of the sensory system controlling the chemotaxis response of the enterobacteria *E. coli* and *S. typhimurium*. We characterized the input-output relationships of the chemotactic signaling response using FRET and studied the behavioral responses of bacteria in spatial gradients using microfluidics. We identified novel response strategies (Chapter 2), and performed a complete systems-level comparison of chemotactic signaling and behavior of *S. typhimurium* and *E. coli* (Chapter 3). We furthermore studied the response to previously uncharacterized chemoeffectors (Chapter 4) and the function of previously uncharacterized chemoreceptors (Chapter 4, Appendix A) and scaffolding proteins (Chapter 5), as outlined below.

In Chapter 2 we showed the first experimental proof that a sensory system implements a fold-change detection (FCD) strategy, *i.e.* responding faithfully to the shape of the input profile irrespective of its absolute intensity. We used *in vivo* FRET measurements on immobilized populations of *E. coli* to study the response-rescaling properties of chemotactic signaling, and showed that the entire time series of the response during stimulation with complex temporal waveforms depends only on fold-changes in input and not on its absolute level. Intensity-independent responses to spatial gradients were also observed using microfluidics-based assay of swimming bacteria. By theoretical analysis we identified a set of sufficient conditions for FCD.

In Chapter 3 we performed a FRET-based systems-level characterization of the chemotactic signaling transfer functions of *S. typhimurium* LT2, a closely related species to the model organism *E. coli* K12. The comparison of chemotactic signaling in the two species revealed conserved and divergent features. *S. typhimurium* showed a lower apparent cooperativity of its response to the chemoattractant α-methylaspartate (MeAsp), and a faster kinetics of adaptation. We evaluated the consequences of the differences in the signaling on the chemotactic behavior in spatial gradients created in microfluidic platforms. We were able to predict the behavioral response of bacteria from the transfer

functions of their sensory response, which could provide a basis for studying organism's behavioral strategies at the level of physiological measurements. In Appendix B we set the foundations of a future comparative physiology study of the chemotactic properties of natural *E. coli* strains.

In Chapter 4 and Appendix A, we studied the chemotactic signaling response of *S. typhimurium* to the cystine / cysteine redox pair. In Chapter 4 we used *in vivo* FRET to show that the previously uncharacterized chemoreceptors McpB and McpC mediate a repellent response to the oxidized form, cystine, in *S. typhimurium* 14028, whereas Tsr and Tar mediate an attractant response to the reduced form, cysteine. In Appendix A, we measured the dose-dependent response to both cysteine and cystine, and discovered McpB / C-independent responses to cystine in *S. typhimurium* LT2 that are likely to be redox-dependent.

In Chapter 5 we tested the role of the phosphorylatable scaffolding protein CheV in the chemotaxis system of *S. typhimurium*. We observed CheV-dependent methylation-independent weak partial adaptation to MeAsp. Imaging revealed that the CheV molecules form clusters localized predominantly at the cell poles, and the number of the receptor clusters decreases in *cheV* knockout, and to a lesser extent, in the phosphorylation-deficient CheV mutants.

In Appendix C, we explored another phospho-dependent interaction: the phosphorylation-dependent feedback on CheB methylesterase activity on the kinetics of chemoreceptor methylation. We found that the strong nonlinearity in the transfer function characterizing the adaptation module in *E. coli* could be a consequence of the phosphorylation feedback on CheB activity.

In Chapter 6, we summarized our findings, and proposed experiments (including preliminary data) that can provide a continuation of the research topics explored in this thesis.