Engineering Microbial Metabolite Dynamics and Heterogeneity

Alexander C. Schmitz, Christopher J. Hartline, and Fuzhong Zhang

As yields for biological chemical production in microorganisms approach their theoretical maximum, metabolic engineering requires new tools, and approaches for improvements beyond what traditional strategies can achieve. Engineering metabolite dynamics and metabolite heterogeneity is necessary to achieve further improvements in product titers, productivities, and yields. Metabolite dynamics, the ensemble change in metabolite concentration over time, arise from the need for microbes to adapt their metabolism in response to the extracellular environment and are important for controlling growth and productivity in industrial fermentations. Metabolite heterogeneity, the cell-to-cell variation in a metabolite concentration in an isoclonal population, has a significant impact on ensemble productivity. Recent advances in single cell analysis enable a more complete understanding of the processes driving metabolite heterogeneity and reveal metabolic engineering targets. The authors emphasize that the ability to control metabolite dynamics and heterogeneity will bring new avenues of engineering to increase productivity of microbial strains.

1. Introduction

Engineering microbes for chemical production is a growing field that has enabled the renewable synthesis of a wide range of target molecules. To obtain high product titers, yields, and productivities, many tools and strategies were developed in recent years, including deletion of competing pathways, directing metabolic flux using regulatory RNAs or CRISPR, synthetic compartmentalization, gene knockouts, and knockdowns along with many others. These tools have strongly facilitated metabolic engineering and increased the titers of many target molecules from an initial scale of micrograms or milligrams per liter to grams per liter. As yield is approaching theoretical maximum, further increases become more challenging but are needed for most products with low margins. New tools that can precisely tune and balance cellular metabolism must be developed to achieve close-to-maximum yield and productivity, and to maintain robust microbial bio-production performance during large-scale fermentation.

Controlling a large population of cells (e.g., more than 10^18 cells in a 100,000 L fermenter) with constantly changing metabolism is extremely daunting and requires deep understanding of the underlying complex mechanisms that drive production. Microbes are highly adaptive living creatures that exhibit dynamic metabolic phenotypes at multiple time scales (seconds to hours) and non-identical behaviors even under identical conditions, so it is important to consider every cell as a unique individual with distinct metabolism.

This review focuses on metabolite dynamics and metabolite heterogeneity, two phenomena that have profound influence on growth and productivity but were rarely considered in traditional metabolic engineering strategies. The term “metabolite dynamics” has been used to describe the change of metabolite concentrations over time in biological systems such as cells, tissues, the body, and fermenters. With the focus on metabolic engineering in this review, we refer to metabolite dynamics as the change of microbial intracellular concentration of a metabolite over time at the ensemble-level. Meanwhile, to differentiate from “metabolic heterogeneity” which is commonly used to describe different metabolic activity (e.g., glycolysis) of cancer cells, we define metabolite heterogeneity as the cell-to-cell variation on the concentration of a metabolite within an isoclonal culture. The goal of this review is to provide information on mechanistic origins of metabolite dynamics and heterogeneity, their impacts to metabolic engineering, and emerging tools that allow researchers to control metabolic dynamics and heterogeneity. Related topics, namely gene expression dynamics and microbial phenotypic heterogeneity, have been reviewed elsewhere, so this review will focus on metabolites.

2. Metabolite Dynamics

Microorganisms are not static in time but instead change their gene expression, protein concentrations, metabolism, and
other cellular activities to respond to their intracellular and extracellular environment. An important aspect of the cellular metabolism response is the transient change in an intracellular metabolite concentration. Metabolite dynamics occur when there is an imbalance in the production and consumption of a particular metabolite which causes a net change in the metabolite concentration over time (Table 1). Once the production and consumption are balanced, the metabolite will be at a steady-state and there are no metabolite dynamics. A common way to study metabolite dynamics is by collecting cells at different time scales after a perturbation.\[22–25\] While the concepts of metabolite dynamics and metabolic flux are related, these concepts should not be confused. Metabolite dynamics refers to changes in metabolite concentration over time while metabolic flux refers to the rate at which material is processed through a metabolic pathway.\[26\] For example, there are no metabolite dynamics (no change on metabolite concentrations) at steady state whereas there are still fluxes through metabolic pathways. While the importance of flux in metabolic engineering has been established,\[27–29\] metabolite dynamics also play a key role in the engineering process. Here we consider the importance of metabolite dynamics, what affects them, and engineering strategies for controlling metabolite dynamics.

2.1. Understanding the Importance of Metabolite Dynamics

Understanding and controlling metabolite dynamics is a key undertaking for advancing metabolic engineering from at least three aspects: 1) studying metabolite dynamics provides insights to metabolic engineering; 2) metabolite dynamics affects cell growth and fitness; and 3) controlling metabolite dynamics could improve productivity of target chemicals.

2.1.1. Studying Metabolite Dynamics Informs Engineering Strategies

Studying metabolite dynamics provides insights that can guide metabolic engineering efforts. For example, metabolite dynamics can be used to identify in vivo kinetic properties of enzymes.\[30\] Since the in vitro properties of enzymes may not correspond with their in vivo values, models constructed from in vitro kinetic data may be inaccurate.\[31,32\] Studying metabolite dynamics provides a way to assess the in vivo kinetic parameters and to assemble more accurate models for metabolic engineering.\[33–35\] Furthermore, metabolite dynamics can be used to identify unknown network interactions including those that cause pathway bottlenecks.\[36\] Different bottlenecks produce different metabolite dynamics which allows rapid bottleneck identification by comparing metabolite time course data to a kinetic model. A similar approach was used to identify unknown allosteric interactions in vivo by comparing dynamic metabolite data to an ensemble of structurally distinct models.\[37\] These unknown interactions are difficult to identify through other means, which makes studying metabolite dynamics a powerful metabolic engineering tool.

2.1.2. Metabolite Dynamics Affects Cell Growth and Fitness

The speed of response to the environment is important for cell survival and growth. Under changing environmental conditions cells that adapt their metabolism the quickest will be able to better capitalize on transitions to favorable conditions or survive unfavorable conditions compared to those cells with slow response. *Escherichia coli*, for example, changes the concentrations of 55% of glycolysis metabolites by more than twofold within 5–10 s of a pulse of glucose to resume growth after carbon limitation.\[38\] Metabolite dynamics serve as a driver of cellular response to the environment,\[39\] rather than what was traditionally thought as just an output of the cellular function.\[40\]
One major way that metabolite dynamics drives cell response is through metabolite-responsive transcription factors to continuously control gene expression profiles. Nearly half of all transcription factors in *E. coli* are regulated directly through binding to metabolites. Metabolites binding to RNA aptamers also regulate the translation of proteins, which could in turn affect metabolite concentrations in some cases. These examples indicate that natural systems control and utilize metabolite dynamics to achieve robust growth and survival.

### 2.1.3. Metabolite Dynamics Affects Cellular Productivity

Metabolite dynamics are also important to consider in industrial fermentations. Cells face two types of environmental variation in batch processes: slow global environmental changes due to process progression, and local environment heterogeneity. Traditionally, cellular productivity is optimized for production in a particular environment and at exponential or stationary growth phase, which leads to sub-optimal productivity as the environment or cell growth phase changes during a production process. Due to the difficulty in maintaining a homogenous environment in a large fermenter, cells can pass through regions with varied microenvironments, for instance different pH and different oxygen or substrate concentrations, resulting in changes in metabolite concentrations affecting bioproduction. Controlling metabolite dynamics can address these challenges by altering output of both natural and engineered metabolic pathways in response to environmental changes, which has been done through the dynamic regulation of fatty acid ethyl ester and fatty acid producing pathways to achieve high productivity and yield. From both fundamental science and metabolic engineering perspectives, it is thus important to understand and have the ability to control metabolite dynamics.

### 2.2. What Affects Metabolite Dynamics?

Several factors could affect metabolite dynamics: 1) the concentration of active enzymes that catalyze the reaction; 2) the concentration of the precursor and cofactors needed to produce the metabolite; and 3) the effective kinetic parameters such as the enzyme turnover rates and Michaelis–Menten constants of those enzymes. Regulatory mechanisms can modify these factors to produce dynamic responses in metabolite concentrations. Here, we focus on how changes in enzyme concentration due to transcriptional regulation and changes in kinetic parameters due to allosteric regulation affect metabolite dynamics.

#### 2.2.1. Transcriptional Regulation of Metabolite Dynamics

According to metabolic control theory, the synthesis of new enzymes can cause changes in the metabolite concentrations. The transcriptional program of the cell plays a major role in
regulating enzyme concentrations over time. For natural metabolic pathways, it is thought that transcriptional network architectures and parameters are chosen to control the dynamic response of metabolic pathways to environmental conditions. For example, the Saccharomyces cerevisiae uses several feedback mechanisms including intermediate metabolite activation to control expression of the leucine biosynthesis pathway.\footnote{53} Models show that altering leucine pathway regulation by increasing the maximum expression rate of leu1 and leu2 leads to a faster recovery of leucine in leucine-limited media, while the steady-state concentration of leucine remains unchanged.

The rational construction and study of synthetic gene-metabolite circuits provides another avenue for elucidating the connection between transcriptional control of enzyme concentration and metabolite dynamics, for instance, in the construction of an oscillating metabolite-driven gene circuit.\footnote{54,55} In this circuit, oscillation on the concentrations of enzymes that produce and consume acetyl phosphate (AcP) correlated to the oscillation of AcP metabolite concentration as observed from an AcP sensor. Systems like these show how changes in translational programs can be used to produce varied metabolite dynamics.

2.2.2. Post-Translational Regulation of Metabolite Dynamics

Upon entering new environmental conditions, the expression of new proteins can take several minutes, and the dilution of old proteins through cell growth can take several hours, which means that cells need mechanisms for controlling metabolite dynamics at fast time scales.\footnote{56,57} In order to respond to fast changes in environmental conditions, prokaryotes use passive and active regulatory mechanisms. Passive mechanisms include maintaining metabolite concentrations close to the enzyme’s $K_m$ and near equilibrium for some reversible reactions including in lower glycolysis.\footnote{58–60} Active regulation includes allosteric mechanisms and post-translational modification which lead to changes in the effective enzyme kinetic parameters. One important type of allosteric interaction is product feedback inhibition (PFI) where the end product of a pathway inhibits the activity of the enzymes that produce it. The $E. coli$ valine and tryptophan biosynthesis pathway was observed to use PFI to control the dynamics of metabolically expensive amino acids in order to quickly recover growth after a period of carbon starvation.\footnote{61} Other allosteric interactions, such as feed-forward activation found in $E. coli$ glycolysis\footnote{37} may be needed to help the cell to achieve ultrasensitive responses to control changes in metabolite concentration.\footnote{62,63} These examples imply that microbes employ different allosteric regulations to control the rate of change in metabolite concentrations at short time scales. Thus, both transcriptional and allosteric regulation can be used to produce appropriate metabolite dynamics, for example, a faster rate of change in metabolite pool size. These regulatory strategies can be harnessed to control metabolite dynamics for engineering purposes.

2.3. Engineering Strategies for Controlling Metabolite Dynamics

Many of the strategies for controlling metabolite dynamics focus on controlling enzyme concentration.\footnote{63,64} The use of transcriptional dynamic metabolic control is a way to cause metabolite concentrations to change over the course of production by changing enzyme concentrations (Figure 1). In this strategy a biosensor is used to detect a cellular or an environmental signal and use the sensor to regulate the expression of rate-limiting genes, so that the metabolite concentration can be constantly adjusted to meet bio-production needs according to the detected signal. This strategy was used to increase the production of lycopene, fatty acids, and fatty acid ethyl esters.\footnote{49–51,65} For example, malonyl-CoA was used as a signal in the production of fatty acids in $E. coli$ to control the expression of acetyl-CoA carboxylase.\footnote{51} The malonyl-CoA concentration was sensed by FapR from Bacillus subtilis which, either through an engineered promotor\footnote{50} or through an inverter,\footnote{53} repressed acc once a sufficient malonyl-CoA pool size was reached. By balancing Acc toxicity and fatty acid productivity, the final fatty acid titer increased by 34% compared to the strain without regulation of Acc.\footnote{51} Although this strategy has been shown to be successful in reaching higher titers, productivities, and yields, finding a transcription factor that acts as a metabolite biosensor for a critical pathway intermediate could be challenging for some chemicals.\footnote{66} This challenge prompted the development of a response system that uses quorum sensing to control the timing of switch between growth and production of a chemical, which led to a 3.5-fold increase in myo-inositol production in $E. coli$.\footnote{60} Despite these successes, transcriptional control strategies may be too slow in controlling metabolite dynamics when cells are transiently exposed to new microenvironments in bioreactors, so strategies for post-translational control of metabolite dynamics need to be developed to maximize chemical production.

3. Metabolite Heterogeneity

While variations in microenvironments and random mutations lead to phenotypic heterogeneity, non-genetic heterogeneity under identical conditions exist due to the inherent stochasticity of biology: random division of molecules between daughter cells, stochastic gene expression, and enzyme catalysis, and a few other processes. Metabolite heterogeneity has only recently been experimentally revealed due to difficulties in analyzing metabolite concentrations at single cell levels, particularly during cell growth.\footnote{68–71}

3.1. Understanding Metabolite Heterogeneity is Important for Controlling It

Variation of single cell metabolite concentration affects the collective behavior of a culture in both ensemble growth rate and chemical productivity. Flux balance analysis using experimentally measured single-cell protein copy numbers\footnote{72} predict that there is a large variation on single cell metabolic activities, that could lead to single cell growth rate variation of as much as fivefold.\footnote{73} Furthermore, experimentally measured coefficient of variation (CV) (standard deviation divided by mean) of single cell growth rate ranges from 0.2 to 0.4.\footnote{74} Both the size and the frequency of growth rate fluctuation is likely caused by
fluctuations in the concentration of cellular components, including metabolites.\textsuperscript{[74]} Metabolite heterogeneity also causes large cell-to-cell variation on productivity of engineered microbes as observed in the biosynthesis of TPP (5'-pyrophosphate) in \textit{E. coli},\textsuperscript{[70]} the production of free fatty acids (FFA) in \textit{E. coli},\textsuperscript{[75]} and the production of L-valine, L-leucine, and L-isoleucine in \textit{Corynebacterium glutamicum}.\textsuperscript{[71]} The size of metabolite variation was recently quantified in an engineered FFA-producing \textit{E. coli} strain, where mean FFA concentration between four isolated subpopulations was as large as ninefold. This metabolite

\begin{center}
\textbf{Figure 1.} A summary of engineering strategies for controlling metabolite dynamics and metabolite heterogeneity. A) Acyl Phosphate (AcP) accumulates when there is excess flux from glycolysis due to under-expression of the lycopene production pathway. AcP concentration is sensed by NRI which promotes expression of idi and pps. Increased concentrations of Idi and Pps direct the excess glycolytic flux towards lycopene, which allows AcP concentrations to decrease. B) Excess malonyl-CoA is sensed by binding to FapR, which ultimately represses expression of the acc operon, leading to a decrease in malonyl-CoA. C) Cell growth and myo-inositol production compete for glucose. The QS promoter, $P_{\text{esaS}}$, controls expression of a degradation-tag-fused $pfkA$ which produces Pfk-1. This allows carbon to initially be used for cell growth. As cells grow, acyl-homoserine lactone (AHL) accumulates, repressing $pfkA$ expression. Pfk-1 then degrades, allowing carbon to be used for myo-inositol production. D) The Lsr QS system forms an intracellular positive feedback loop and an intercellular negative feedback loop that can lead to bimodal concentrations of AI-2. E) PopQC promotes the survival of high producing cells by allowing them to adequately express a tetracycline transporter, which confers a growth advantage over low production cells that cannot express the transporter.
\end{center}
heterogeneity strongly affects the ensemble FFA titer and productivity as more than half of the FFA was produced from a subpopulation consisting of only 15% of the bacteria. The rest of the population consumed nutrient without producing a high concentration of FFA. Low producing cells probably devote more resources to growth thus could outcompete high-producing cells, dominating the culture over time, leading to an overall decrease in productivity as demonstrated by bimodal L-valine production in C. glutamicum. These findings demonstrate that it is essential to understand what affects metabolic heterogeneity and to control it in engineered cells.

3.2. What Affects Metabolite Heterogeneity?

Metabolite heterogeneity is affected by both extrinsic (global factors) and intrinsic (metabolite- or metabolic pathway-specific) reasons. This approach to describe metabolic heterogeneity is analogous to the definition of extrinsic and intrinsic sources of gene expression noise, which is well studied and extensively described. Extrinsic sources include cell-to-cell variations in growth rate, variations in shared resources such as amino acids, RNA polymerases, ribosomes, ATP, and other pathway- nonspecific cellular processes such as global regulation. Intrinsic sources are cell-to-cell variations in pathway specific factors such as enzymes and substrates involved in the target pathway. Here we only focus on intrinsic sources of metabolite heterogeneity, specifically 1) stochastic gene expression and turnover; 2) substrate heterogeneity; and 3) pathway specific metabolic regulation, since intrinsic sources provide a clear target for metabolic engineering.

3.2.1. Stochastic Gene Expression and Enzyme Turnover

As metabolites are products of enzymes, stochastic expression of genes could presumably lead to metabolite heterogeneity. Cell-to-cell variation in protein abundance has been extensively studied. Variation in protein copy numbers is strongly tied to their mean expression levels. As mean protein copy number increases, cell-to-cell protein variation decreases, and reaches a minimum CV of approximately 30% at high mean expression levels for both prokaryotes and eukaryotes. The observed cross-correlation between the expression of lactulose catabolic enzymes (lacZ and lacY) and the growth rate of E. coli suggests that gene expression heterogeneity can pass to metabolite, however more direct experimental proof is needed to confirm this.

Even for cells with the same copy number of a specific enzyme, the metabolite product could be synthesized at a different rate due to stochastic enzyme turnover. However, in the regime of high gene expression, the case most applicable to an engineered system, the ensemble enzyme turnover rate matches the simple Michaelis–Menten kinetics due to the law of large numbers, meaning there are negligible effects on metabolite heterogeneity arising from stochastic enzyme turnover. Additionally, when considering time-scales of seconds or longer, heterogeneity in single enzyme turnover is negligible.

Engineering metabolite heterogeneity begins with strategies to control gene expression. Decreasing gene expression variation of metabolic enzymes might homogenize metabolism between cells, but there is limited information on the connection between enzyme heterogeneity and metabolite heterogeneity except indirectly via growth rate variation.

3.2.2. Substrate Heterogeneity

As every metabolite is synthesized from its precursor substrate, variation of a metabolite could be affected by cell-to-cell variation of its enzyme’s substrate and cofactors, allowing metabolic variation to propagate along metabolic pathways from upstream substrates and intermediates to downstream metabolites. Substrate and cofactor concentrations can differ from cell to cell due to variations in substrate transport, biosynthesis, utilization, and uneven cell division. A stochastic model predicts that there is little variation propagation from one metabolite to the next metabolite in a pathway, especially if that metabolite is shared by multiple pathways. The model prediction was explained by the presence of metabolite pools between enzymatic reactions that eliminate the variation of metabolite coming from substrate variation. The authors believe that substrate pooling is a strategy utilized by the cell to reduce metabolic noise propagated from upstream of a target metabolite. Supporting this idea, Bennett et al. found that a majority of enzymatic substrates are present at quantities exceeding the enzyme’s Kin so that no active sites are wasted with an exception being enzymes in lower glycolysis. This natural phenomenon allows metabolic flux over an enzymatic step to be insensitive to small fluctuations in substrate concentration. These findings imply the main driving force for variation in metabolism would be the variation of gene expression when substrate concentration is not limiting. On the other hand, for enzymes whose substrate concentration is near or below the Kin, cell-to-cell variations in substrate concentration could allow metabolic variation to propagate through a pathway and lead to heterogeneity of downstream metabolites. However, further experimentation is needed to confirm this.

3.2.3. Metabolic Regulation Exaggerates Metabolite Heterogeneity, Leading to Multimodality

Under some regulation topology, such as a positive feedback loop, heterogeneity could lead to metabolic multimodality, meaning the co-existence of more than one, distinct, metabolic states characterized by drastically different concentrations in a metabolite (Table 1). One famous example is arabinose catabolism. In E. coli, transcription of the arabinose transporter AraE is activated by arabinose via the regulator AraC, thus forming a positive feedback loop. Due to stochastic transport of arabinose into the cell at low extracellular arabinose concentration, cells that initially transport arabinose are able to express AraE, thus transporting more arabinose and activate the arabinose catabolism pathway, displaying two distinct subpopulations with low and high arabinose catabolism. Such bimodality in transporting extracellular nutrient can be
potentially eliminated by replacing the regulated AraE promoter with a constitutive promoter. Bimodal metabolic pathway activation is also seen in quorum-sensing-regulated systems. Bacteria use quorum sensing to coordinate gene expression across a culture allowing them to respond to cell density and their environment. Quorum sensing induction is inherently a stochastic process; random fluctuations in intracellular auto-inducer concentration caused by stochastic transport of the auto-inducing signal can initiate the quorum response. Quorum sensing signals often induce significant metabolic shifts so variation in cell-to-cell quorum sensing induction can lead to drastically different metabolic phenotypes. A well-studied quorum sensing system is the Lsr QS system, which acts through autoinducer-2 (AI-2) and displays fractional activation in both Gram-negative and Gram-positive bacteria. Lsr activation produces AI-2 importer (LsrABC) and a kinase (LsrK), LsrABC and LsrK import and phosphorylate AI-2 which de-represses Lsr, making more transporter and kinase and forming an intracellular positive feedback loop. The AI-2 import occurs through low affinity transporters leading to variability in uptake between cells. The interaction of the positive feedback loops plus a low affinity transporter lead to bimodal Lsr activation and cell-to-cell variation in concentration of AI-2. Bimodal Lsr activation is a natural strategy for cells to differentiate their metabolic phenotype to specialize or prepare for changes in the environment such as forming biofilms.

3.3. Engineering Metabolite Heterogeneity

Several strategies for engineering gene expression variation have been recently reported, including negative feedback regulation and regulating the translational efficiency of a gene. These strategies allow target genes to have more homogeneous expression levels across a population. If used to control enzyme concentrations, these strategies may also be able to reduce metabolite variation, but need to be tested experimentally. Furthermore, reducing metabolite heterogeneity is not the only goal of engineering metabolite heterogeneity. Being able to precisely control metabolite heterogeneity may be important for processes that benefit from phenotypic diversity such as bet hedging for rapid response to a changing environment and division of labor to help a community.

As the overall goal of metabolic engineering is to improve chemical production, engineering cells to favorable chemical production is a more attractive approach. A practical method to enhance ensemble productivity is to enrich high-producer cells in a population, as demonstrated by the strategy called Population Quality Control (PopQC). In this strategy, a metabolite biosensor was used to detect the single-cellular concentration of a target product FFA. The sensor’s signal was then used to control the expression of a survival gene, so that high-producing cells were given a growth advantage from the added selection pressure compared to low-producing variants. PopQC led to an enrichment of the high-producing subpopulation, which resulted in enhanced ensemble yield by repressing the growth of low-producing subpopulations.

These engineering strategies provide potential improvements to ensemble productivity and homogenizing metabolic phenotypes. These strategies could even be combined along with other engineering strategies not explored in this review for further bio-production improvements.

4. Conclusion

Metabolite dynamics and heterogeneity are emerging fields of interest in metabolic engineering. Until recently, robust, unobtrusive, and useful biosensors that track metabolites directly have been difficult to design and implement, and presented a major limitation when assaying metabolite dynamics and heterogeneity. It is possible with recent advances in molecular and analytical tools such as microfluidic devices, single cell metabolite biosensors, and other single cell technologies, to more accurately measure single cell metabolite dynamics and heterogeneity.

Controlling the timing and induction level of the expression of new enzymes can lead to increased control over metabolite dynamics, although strategies for speeding up metabolite dynamics through transcriptional and post-translational control need to be explored. To homogenize metabolite concentration between cells and increase productivity, negative feedback loops, quorum sensing motifs, and selection strategies can be applied, but more research needs to be conducted on the connection between enzymes variation and metabolite variation to determine the best engineering strategies. As strategies for controlling metabolite dynamics and heterogeneity improve, increases in biochemical productivity will be possible and new insights into maximizing strain productivity will be discovered.

Abbreviations

AcP, acetyl phosphate; AI-2, autoinducer-2; CV, coefficient of variation; FFA, free fatty acid; Lsr, LuxS-regulated; PFI, product feedback inhibition; PopQC, population quality control; QS, quorum sensing; TPP, 5′-pyrophosphate.

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Conflict of Interest

The authors declare no financial or commercial conflict of interest.

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Y. Xiao, C. H. Bowen, D. Liu, F. Zhang, Nat. Chem. Biol. 2016, 12, 339.

N. Mustafi, A. Grünberger, R. Mahr, S. Helfrich, K. Noh, B. Blombach, D. Kohlheyer, J. Frunzke, PLoS ONE 2014, 9, e85731.

A. Raj, A. van Oudenaarden, Cell 2008, 135, 216.

J. M. Raser, E. K. O’Shea, Science 2005, 309, 2010.

A. Bar-Even, J. Paulsson, N. Maheshri, M. Carmi, E. O’Shea, Y. Pilpel, N. Barkai, Nat. Genet. 2006, 38, 636.

O. Pulkkinen, R. Metzler, Sci. Rep. 2015, 5, 17820.

B. P. English, W. Min, A. M. van Oijen, K. T. Lee, G. Luo, H. Sun, B. J. Cherayil, S. C. Kuo, X. Sunney Xie, Nat. Chem. Biol. 2006, 2, 87.

E. Levine, T. Hwa, Proc. Natl. Acad. Sci. USA 2007, 104, 9224.

J. A. Megerle, G. Fritz, U. Gerland, K. Jung, J. O. Rädler, Biophys. J. 2008, 95, 2103.

J. D. Keasling, ACS Chem. Biol. 2008, 3, 64.