The earth's rotation around its axis presents us with a challenge, for every day we have to live by the rhythm set by the rising and setting of the sun. Instead of just reacting to the changes in light and temperature, many organisms, like us, have evolved to foretell dusk and dawn and anticipate the impending changes. For instance, the leaves of young sunflower plants track the sun from east to west during the day and, at night, points them towards the east again well before sunrise. Remarkably, when these plants are placed under continuous light, the 24 hour movement of their leaves persists. This shows that the sunflower has an internal 24 hour rhythm, or 'circadian clock' (Latin: *circa* for “around” and *dies* “day”), that is independent of its environment.

I study the clock of *Synechococcus elongatus*, a freshwater cyanobacterium that depends on sunlight for its growth. I use this model organism because it has the simplest known clock in biology, which consists of only three different proteins (the building blocks of the cell), called KaiA, KaiB and KaiC. The central component, KaiC, forms a hexamer structure that contains twelve special sites to which a small phosphate molecule can bind. KaiA can add phosphate molecules to the sites of KaiC and KaiB negates KaiA, such that the bound phosphates fall off again. The antagonistic effects of KaiA and KaiB on KaiC together set up a 24 hour rhythm in the number of phosphate molecules that are bound to KaiC. KaiC thus functions as a register, from which the cyanobacterium can read of the time of the day. However, the speed at which the phosphate molecules are added and removed from KaiC varies over time and strongly depends on the temperature of the surrounding. Furthermore, a cyanobacterium can divide up to four times every 24 hours, whereby all the components that make up the clock are randomly divided between the two daughter cells. In light of these circumstances, it is extraordinarily that the clock is unaffected by the cell division of the cyanobacterium or the temperature of its environment; it always ticks with a constant 24 hour period.

In this thesis I set out to reveal the properties of the biological clock that are essential for having a robust period in the chaotic environment of the cell. To find these properties, I used the large body of experimental data that are available on the circadian clock of *S. elongatus* to design mathematical models of the clock and the cell cycle, which I then simulate using a computer. Computational modeling allows for a systematic study of all the things that can affect the clock's stability, such as the division time of the cell and its environment. Modeling also allows us to dissect each individual component of the circadian clockwork and investigate how it makes the clock more robust.

Starting in chapters 2 and 3, I investigate how circadian clocks and synthetic oscillators (a designed biological clock built into a cell) can have a robust period inside a growing and dividing cell. I find that it is not the division of the cell but rather the copying of its DNA, the blueprint of all the cell's building blocks, that can be detrimental to this robust period. As the cell grows, it has to double all of its components, including its DNA and clock proteins. Each time the DNA is copied, the production of new clock proteins...
components also doubles, which causes a periodic perturbation of the clock by the cell cycle. Just like when you push someone on a swing, by pushing at the right moment, you can change the period and the extent of the swing. Similarly, the rhythmic doubling of the production of clock proteins by the growing cell, can change the clocks period to, for example, twenty hours. I first used a simplified version of the circadian clock to show that a too simple designed clock will be completely enslaved by the cell cycle. Our simplified clock highlights why the clock of *S. elongatus* is far more complicated: A clock that can not maintain a constant period is a bad predictor of time. I find two design features that contribute to the clock’s stability: First, the rhythm of adding and removing of phosphate molecules from the clock protein KaiC is almost unaffected by the production of new KaiC proteins, which stabilizes the clock against the production doubling. Furthermore, *S. elongatus* has multiple copies of its chromosome which are copied at random times during each cell cycle. In this case, when a chromosome is copied, the number does not increase from one to two but, for instance, from four to five, whereby the effect of a single copy event on the protein production rate is relatively smaller.

Subsequently, in chapter 4, I seek to understand what drives the ticking of the clock. In cells, the hydrolysis of the molecule ATP to ADP is the driving force behind many cellular processes. In the clock of *S. elongatus*, ATP is converted in both the CI and CII domain that together make up KaiC. ATP is used in the CII domain for the rhythmic addition and removal of the phosphate molecules while the function of the CI domain is less obvious. It is unclear which of the two domains is the primary driver of the clock. To find out, I used the large body of experimental data to make the most detailed model of the circadian clock of *S. elongatus* to date. I argue that the CI domain is the engine that makes the clock ‘go round’, and that the CII domain acts as a ‘timer’ regulating the hydrolysis in CI.

Finally, in chapter 5, I explore how the clock can fulfill two seemingly contradictory requirements: Its period should be unaffected by the daily changes in the environment, while the phase of the clock needs to be entrained by these daily changes in order for the clock to give the correct time of the day. The amount of ATP in the cell changes during the day with the amount of light the cyanobacterium receives. It has been recently shown that the rhythmic fluctuation in the ATP concentration is the primary input for entrainment, or Zeitgeber, of the clock. Using our new model, I discovered a new mechanism by which the clock can both have a robust 24 hour period and still be entrainable. Inside a cell of *S. elongatus* there are typically thousands of individual KaiC hexamers that all go through their individual cycles. Contrary to earlier models, the cycle that a KaiC complex has to go through is not fixed but depends on the ATP concentration inside the cell. When the ATP concentration is high, an individual KaiC will move faster through its cycle but this cycle will also be longer, such that the time required to traverse the whole cycle is constant. I show that this mechanism stabilizes the period of the clock against fluctuations in the ATP concentration, while still being sensitive to these fluctuations for entrainability.

To recapitulate, I used the well-studied circadian clock of *S. elongatus*, to reveal the mechanisms that are essential for having a robust clock inside a cell and found how a clock can be both robust and entrainable. In doing so, I have increased insight into the minute precision of circadian clocks despite the chaotic environment of the living cell.