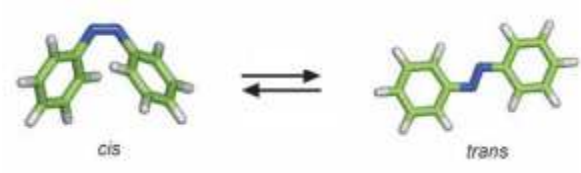
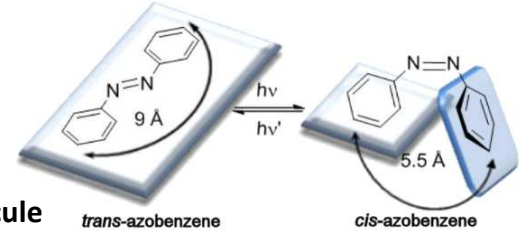




Azobenzene photo-induced magical molecule

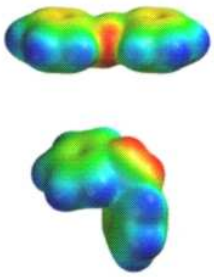
From NPs aggregation to synthetic biofilm

Azobenzene molecule is an organic molecule which responds to light and photoswitches selectively from an extended Trans to a more compact Cis conformation.

In general:

In the dark, at equilibrium, azobenzene is >99% trans. Irradiation at specific short wavelength leads to the production of up to ~90% of the cis isomer. Irradiation at longer wavelengths and/or thermal relaxation returns the trans isomer. Cis and trans isomers of azobenzene differ significantly in structure and dipole moment. Scientists and researchers have used each of these differences to control biomolecular structure and activity.

Azobenzenes are certainly one of the most used organic chromophores for optical switching applications. In this process, the photoreaction simply causes the rearrangement of the electronic and nuclear structure of the molecule without any bonds breaking. Moreover, the totally clean reverse *cis*-to-*trans* conversion also takes place thermally in the dark, spontaneously (Figure 1). It should be also noted that the *cis*-to-*trans* back reaction can be induced by visible-light excitation as well as thermally.



The trans (top) and cis (bottom) forms of azobenzene.

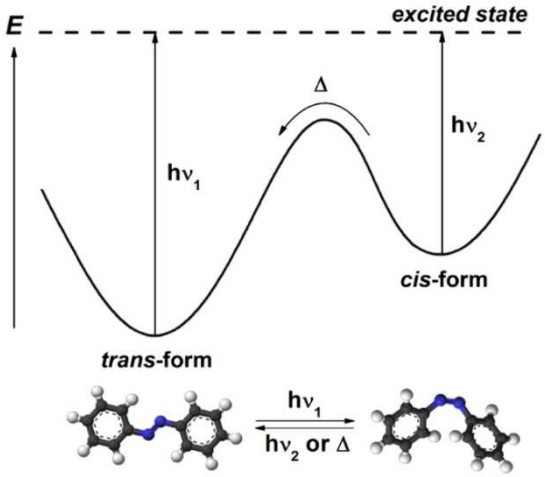


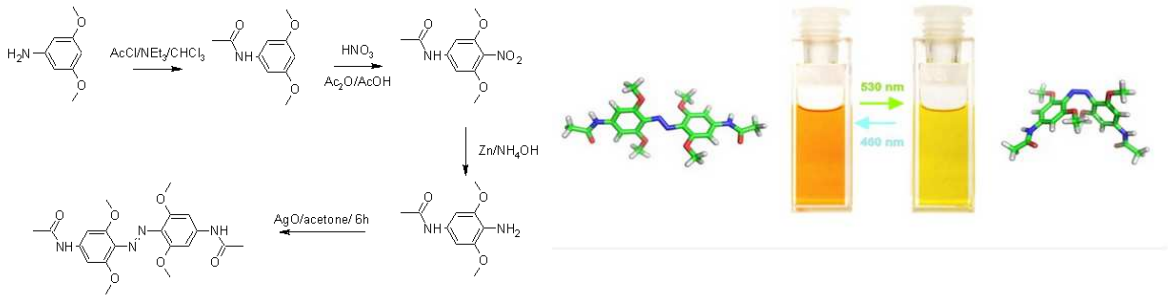
Figure1: Photochromism of azobenzene derivatives and energetic profile for the switching process.

Azobenzene-based photochromic systems are under kinetic control; that is, after a photochemical conversion, the rate of which depends mainly on the intensity of the excitation beam, the spontaneous thermal back reaction occurs. While the photo-induced *trans*-to-*cis* isomerisation reaction can be performed in a few femtoseconds with a light source that is powerful enough, the rate of the thermal *cis*-to-*trans* back reaction depends greatly on the chemical architecture of the system. It is well known that the

appropriate modification of the substitution of the azobenzene core is one of the main factors that allows modulating the thermal relaxation rate of azo-dyes and, therefore, determines the response time of the photochromic molecular switch. The response time of the photochromic switch is a key feature in its overall performance. This parameter is directly related to the thermal isomerisation rate of the photo-sensitive azo-dye in the dark, that is, to the relaxation time of the *cis* isomer of the azo-moiety. Slow, thermally back-isomerising azo derivatives are valuable photoactive basic materials for information storage (memory) purposes. A molecular-level memory should be stable and easy to write, and, moreover, its switched form should be stable but readily erasable when necessary. However, for azobenzene-based photochromic switches that can be used in real-time information-transmitting systems as well as optical oscillators, it is essential that the return to the thermodynamically stable *trans* form in the dark occurs as fast as possible; as soon as the optical stimulation is removed the molecule should revert to its initial state. In fact, the information is expected to be transmitted at the molecular scale with response times ultimately within the nanosecond or picosecond range. Even though some chromophores, such as spiropyranes, have been already proved to show very fast thermal back reactions occurring within hundreds of nanoseconds, reaching such fast relaxation times for azobenzene-based photochromic molecular switches is still a challenge. This aim has attracted a great deal of attention over the past few years due to the potential application of fast photoactive azobenzene-based materials in micropumps and autonomous valves that simulate the beating of the heart; photoactive polymers that mimic cilia movement; and artificial muscles for robotics or molecular rotary motors, among others . Moreover, aside from photochromic switching, azobenzenes with different isomerisation rates have been successfully applied very recently for both photoelectronic and photomagnetic actuating purposes.

iGEM new azobenzene synthesis breakthrough :

Most azobenzene-based photoswitches use UV light for photoisomerization. This can limit their application in biological systems, where UV light can trigger unwanted responses, including cellular apoptosis. We have seen that substitution of all four ortho positions with methoxy groups in an amidoazobenzene derivative leads to a substantial (~35 nm) red shift. This red shift makes trans-to-cis photoswitching possible using green light (530-560 nm). The cis state is thermally stable with a half-life of ~2.4 days in the dark in aqueous solution. Reverse (cis-to-trans) photoswitching can be accomplished with blue light (460 nm), so bidirectional photoswitching between thermally stable isomers is possible without using UV light at all. We must underline that our group did all of the synthesis in order to get the final azobenzene molecule.



Bacteria-azobenzene connection:

We succeeded to attach the azobenzene molecule to modified lipopolysaccharides (LPS) on the outer-membrane of bacteria. Azobenzene reacts with the Kdo sugars part of the LPS giving us stable amide bonds of azo-bacteria connections. Amide bonds cannot be formed in room temperature; hence, in organic chemistry they use DCC catalyst molecules in order to make this procedure possible at room temperature. The problem is that DCC is not water-soluble and slightly toxic to *E. coli* bacteria, so, we planned to use EDC catalyst which is a DCC-derivative for biological applications like protein labeling. EDC is water-soluble and is used to form amide bonds between peptides, however we could not find any information about how much EDC molecules are safe for *E. coli* cells, so, we made a series of experiments to find the safe concentration range of EDC for bacteria culture and then we used it with azobenzene in order to attach it to the LPS of *E. coli*.

Synthetic biofilm formation:

After mixing azobenzene with bacteria, they were exposed to 530-560 nm wavelength and we saw that the sticky bacteria aggregated to form a biofilm and by quorum sensing. The AHL molecules can diffuse faster between them causing faster information exchanging and processing.

Bacteria cannot form aggregates spontaneously because of their negative membrane charge, but with azobenzene the dipole forces between azobenzene rings are more powerful and dominant over the negative repulsion of bacteria.

We should also emphasise that we are the first iGEM group and research group who have used azobenzene to trigger this behaviour, and our discovery may help many researchers study the kinetics of biofilm formation in real time.

Signal focusing by azobenzene:

Quorum sensing bacteria produce and release chemical signal molecules called autoinducers (AHL molecules) that increase in concentration as a function of cell density.

In our group, we are planning to make the detection process as fast as possible. To do this we must make the bacteria attract each other in order to exchange AHL molecules faster, because the diffusion process is too slow.

Therefore, we engineered bacteria that on detecting allergens or toxic materials emit a maximum wavelength range at 530-560nm (green light) which can be absorbed by

azobenzene. Hence, the bacteria aggregate and form a reversible biofilm and so the strong signal can be seen by the naked eye.

Azobenzene aggregate Nano-Particles (NPs):

We established our iGEM azobenzene biological conceptions based on the Nano-word. We collaborated with Weizmann institute to test azobenzene molecules and tried to get some conclusions, we have seen that azobenzene can aggregate various NPs like iron oxide, gold and big particles like silica (see reference and TEM figures), and based on this we established our concept to use azobenzene as a photo-induced molecule to aggregate bacteria forming a synthetic biofilm.