Perspectives Article

Carbon monoxide biosensor coupled with conceptual electrochemical flow reactor for applications in an aerobic environment *

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An extension of research, summarized in the article, introduces a bacteria-based fluorescence iodinated carbon monoxide (CO) biosensor, applicable to aerobic conditions. Upon finding that the bacteria Rhodospirillum rubrum is able to bind to CO and oxygen, and when bonded with oxygen is no longer able to bind with CO. R. rubrum can be used to induce expression of a red fluorescent protein (RFP) in an environment which is strictly CO composed. In order to make this concept applicable to a real world environment, where CO and oxygen compete to bind to the heme center of R. rubrum, an electrochemical flow reactor can be used. In order to reduce the oxygen bound to the R. rubrum heme complex which has a Fe3- center, to Fe2-, which is capable of binding with CO and expressing the RFP. This creates a carbon monoxide biosensor applicable to a real world environment.

Keywords: Carbon monoxide biosensor, *Rhodospirillum rubrum*, CO detector, synthetic biology

Carbon monoxide is a colorless and odorless gas which can cause death when inhaled in large quantities. According to the Center of Disease Control (CDC), deaths caused by carbon monoxide poisoning are the largest non-drug poisoning deaths globally (CDC, 2013). The lack of odor and color contributes to the high level of difficulty in sensing carbon monoxide without a sensor. Carbon Monoxide is emitted from the burning of fossil fuels, it is released in numerous applications including - but not limited to - gas powered vehicles, gas or wood-burning stoves, fireplaces, and other fuel burning applications. The lack of information regarding CO poisoning in regions of low socioeconomic status, which often rely on biofuels and coal for heating and cooking inside of poorly ventilated homes, are at higher risk for CO poisoning. A study conducted by researchers at the First Hospital of China Medical University, and published in the National Library of Medicine found a higher prominence of CO poisoning in areas of lower socioeconomic status such as rural China, when compared to western- electricity reliant regions. This disparity was attributed to a lack of information and means of

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detection in low status regions. (Cui et al., 2022). The National Library of Medicine finds that CO has a 210 times greater affinity for hemoglobin (a heme protein found in red blood cells) than oxygen. Inhalation of CO causes the heme complexes in hemoglobin to prioritize the binding of the Fe2+ with CO over the binding of Fe2+. Once CO has binded with the Fe2+ in the center of the heme protein complex in hemoglobin, oxygen will not be delivered to the tissues throughout the body. As discussed in the Angew Chem Int Ed Engl., this would lead to reduced blood oxygen levels and failure of organs (Savagatrup et al., 2017).

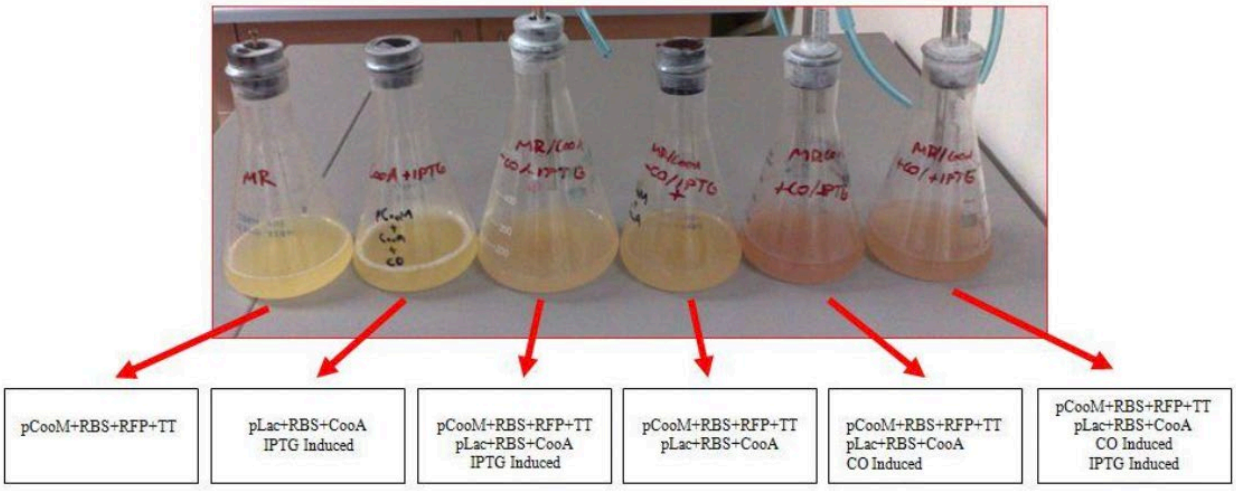
Creating a carbon monoxide sensor that functions independent of environmental conditions can prove instrumental in combating carbon monoxide poisoning. A journal titled Nature Reviews Microbiology discusses a CO oxidizing bacteria that functions aerobically (King & Weber, 2007). With the ability to efficiently detect CO, through utilization of a biosensor, such innovations can be used to improve air quality and reduce concentration of CO. The use of a biosensor requiring little electricity with means of detection through color change is easily accessible to those in destitute regions. Furthermore, this sensor can be installed without the use of home electricity making it easy to employ. The gradual change of color with the use of RFP in the biosensor promotes the understanding of CO released within homes at moderate to dangerous levels.

Literature review (Summarized)

A summary of the BBa_K352001: Experience conducted by iGEM10_METU_Turkey, which is an initial design for a carbon monoxide biosensor includes the coding sequence (part BBa_K352001 in the iGEM catalog parts registry), entails for a transcriptional activator derived from the bacteria *R. rubrum*. *R. rubrum* is able to oxidize CO with the use of a protein called CooA, a CO sensing protein that upon detection of CO, is able to activate the transcription of genes which encode for carbon monoxide oxidation and the coo regulon. These genes are essential to the creation of proteins needed for the harnessing of CO in *R. rubrum*. This notion was supported in another study published in the Food and Agriculture Organization of the United Nations, "The heme-based CO sensor from *R. rubrum*, CooA, was designed to report CO levels in vitro and in vivo." (Gondim et al., 2022). This study in conjunction with the experience conducted by iGEM10_METU_Turkey showcased a broader use of the *R. rubrum* Bacteria. iGEM10_METU_Turkey found that the Coo regulon has three main products, an oxygen sensitive CO dehydrogenase (CooS), the associated Fe-S protein CooF, and a carbon monoxide forbrearing hydrogenase (CooH). All of these products are O2-sensitive and can be attributed as reliant on CooA sensing CO for expression strictly in anaerobic environments. They utilized the CooA transcriptional activator in conjunction with a pCooF and pCooM promoter sequence and a red fluorescent protein (RFP).

CooA poses two tryptophan units, upon binding to DNA (pCooF promoter in this instance) conformational changes through alternated alignment of tryptophan in the CooA protein occur. This team chose to conduct fluorescence through internal tryptophan consequence. They expected fluorescent changes to occur in tryptophan units upon binding of the pCooF promoter (BBa_K352002) with the CooA transcriptional activator (BBa_K352001) - given the CooA protein senses CO, the tryptophan protein will orient in a way which allows for the expression of RFP. The BioBrick sequence elaborated on in this experience consists of a pCooM promoter, ribossome binding site (RBS), RFP and a double terminator in that order, active upon the CooA transcriptional activator binding with carbon monoxide in the atmosphere. They performed a gel electrophoresis assay and reported their findings regarding conditions which hinder fluorescence.

Results indicated there was little slowing of DNA progression. They found that abnormal slowing of DNA is expected when CooA bound CO binds to pCooF although the gel image did show abnormal slowing of DNA progression when CooA binds with either promoter. The applications of part BBa_K352001 with pCooM promoters were



shown in a Culturing SO Sensor *Escherichia Coli* with CO induction through cultures of LB 80 mL in six flasks, the contents of each are depicted in the image.

The CO induced beakers show the expression of the red fluorescent protein. In the Culturing CO Sensor *E. coli* with CO induction conducted by the iGEM10_METU_Turkey team, they failed to account for sensing CO binding with CooA in an environment which is not strictly CO. As the CO induced beakers with only carbon monoxide being emitted into them did not account for applications in a real-world environment where oxygen exists with carbon monoxide when the sensor will be applicable. An article published by the National Library of Medicine describes the limited function of CooA as a CO sensor in the presence of oxygen; "CooA is unable to bind CO when the Fe heme is oxidized, consistent with the fact that some of the regulated gene products are oxygen-labile." (Roberts et al., 2001). As CooA serves as both a redox state and CO indicator the competition between CO and oxygen to bind with the heme center of this transcriptional sensor complicates CO sensing in aerobic environments.

Individual extension

This article highlights the inability of CooA to bind with CO upon the binding of oxygen to the Fe2+ in the heme complex as Fe2+ becomes Fe3+ post undergoing an oxygen-dependent oxidation, the products for gene expression initiated by CooA are oxygen subject. The Fe3+ is unable to bind with carbon monoxide due to an abnormal alteration of ligands to the "six-coordinate heme", as directly stated in the Journal of Biological Chemistry (Yamamoto et al., 2001). Because the environmental application of a carbon monoxide biosensor would realistically never be applicable in a completely anaerobic environment, this extension was done to account for the changing of Fe3+ in the heme complex of CooA, back to Fe2+ allowing CooA to continue binding with carbon monoxide. "Enhanced electro-reduction of Fe3+ to Fe2+ by acidified carbon nanotube-modified graphite cathode and its application in a novel Fenton process for p-nitrophenol degradation." (Chu et al., 2021). This study entails a process that utilizes the use of a simple Electrochemical Flow Reactor supplying a direct current to Fe3+, constantly reducing it to Fe2+, specifically through the use of a graphite cathode.

A study found results consistent with this notion as stated, "The sensors show a significant increase in sensitivity toward CO when negative gate voltage is applied. The dosimetric sensors are selective to ppm levels of CO and functional in air." (Savagatrup et al., 2017). In the application of this context, the use of a batch electrochemical reactor inserted into a replication of the Luria-Bertani (LB) culture beakers, consisting of an anode and cathode inserted into the culture, with a (direct current) DC power supply (essentially a battery) outside of the culture

connecting the anode and cathode. By adding this simple DC to constantly convert Fe3+ into Fe2+, CO introduction can be unrestricted from strictly CO- induced to CO in an environmental context, where it will have to compete with oxygen to bind to the Fe2+ heme center of the CooA protein. By supplying the Fe3+ heme centers in CooA complexed with DC the heme complex Fe3+ center will be reduced to Fe2+ even after oxidation as stated by professors of bacteriology at the University of Wisconsin, “The basis of this redox-sensing is that CO binds only to the reduced form of the CooA heme” (Roberts et al., 2004). As a reduction allows the CooA to continue binding with CO, expressing genes in the pCooF promoters which will forward the tryptophan internalized fluorescence even in an aerobic environment.

The Journal of Fluorescence explores the use of bovine serum albumin to accelerate fluorescence under aerobic conditions (Xu et al., 2007). This protein supplement can be considered in future research trials if fluorescence is less pronounced and restricts the ability to conduct observations. Although CO does have a higher affinity for Fe2+ centers in CooA heme complexes than oxygen, an environment where CO enters is gradual and will exist with O2 while still having negative implications on human health.

Methodology

The project entailed in the literature review will be replicated with the pCooF promoter instead of the pCooM promoter in order to gauge any discrepancies between promoters. The RFP will still be utilized to measure CO sensing, owever instead of introducing strictly CO, the CO will be introduced alongside oxygen, in equal parts within a beaker containing the batch electrochemical flow reactor deployed. For the purpose of this experiment, it would be most beneficial to use specifically a batch reactor with an anode and cathode connected to a power supply submerged in the beaker containing the bacteria as described by researchers at Baran Laboratory (Chen, 2020). There will be a control without the reactor but will still have parts BBa_k352002 (pCooF promoter) and BBa_K352001 (CooA transcriptional activator) with RFP in the same sequence as entailed in the literature review for a biobrick with all of these parts on a standard plasmid backbone.

Next steps

We intend to apply the individual extension coupled with the methodology to create a working CO biosensor applicable to an aerobic environment. We intend to use a model of a batch reactor described earlier by researchers at Baran Laboratory with a simple cathode and anode connected to a power supply, such as a battery, submerged in the solution while CO mixed with oxygen is being pumped in through an enclosed tubing: pumping through enclosure decreases risk for confounders and external disruptors. Once the biosensor is functioning, we intend to create a smaller, marketable model for applications in real world contexts. We aim to reach out to biotechnology companies in Georgia and determine the viability of the biosensor in the business context. This will enable us to determine the plausibility of product distribution and accessibility to those in regions which employ biofuels for cooking and heating within the home, due to factors such as lack of electricity, or lack of knowledge regarding carbon monoxide poisoning.

Author Contributions

R.V., K.A., R.P., M.J., S.D., K.S., and R.S. served major roles in the development of research questions and construction of experimental design. R.V., K.A., R.P., M.J., and S.D. provided feedback throughout the design process along with guidance on potential areas of improvement regarding future research. R.S and K.S in the direct development of this paper, as they analyzed sources and communicated to the research team and younger authors, as well as adding to the growth of this paper directly.

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