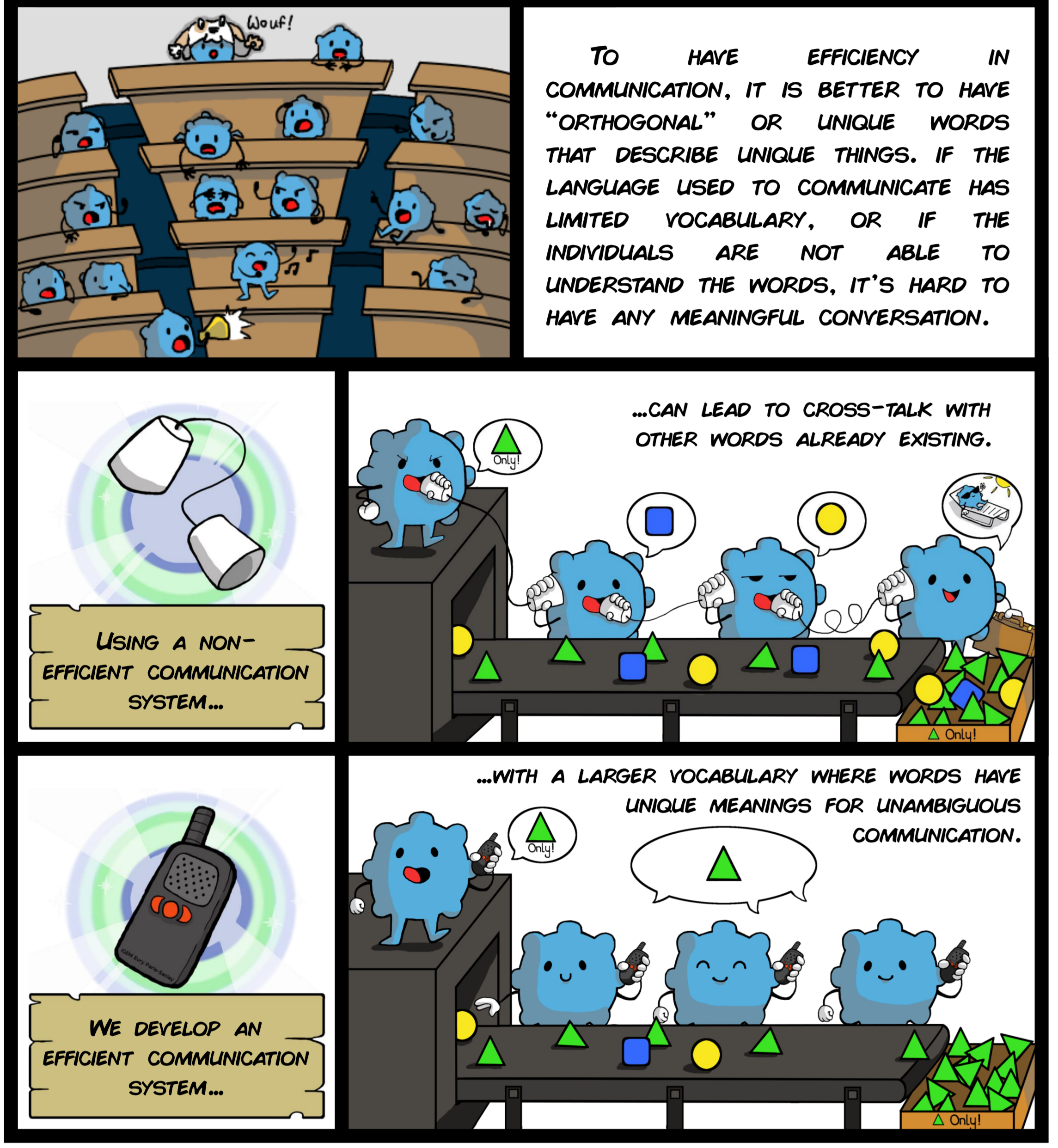


Students: Esteban Lebrun, Axel Radin, Suzanne Phengsay, Paul Ahavi, Adèle Mazelin, Yassine Ajilil, Yann Bourguès, Paul Del Rincon, Solène Castanier, Angelo Cardoso Batista, Marine Podevin, Sara Sadat Aghamiri, Léa Rauscher, Sambavi Markandu, Azim-Berdy Besya, Camille Monteil, Charlotte Deneaux, Nada Fanjaoui, Khawla Achoch; Advisors: Steff Horemans, Nazim Sarica; Supervisors: Ioana Popescu, Manish Kushwaha

BIOLOGICAL ENGINEERING HAS TURNED BACTERIA INTO BIOSENSORS, NANO-ROBOTS AND PRODUCTION FACTORIES. AS THESE APPLICATIONS ARE ORGANISED FOR HIGHER-LEVEL TASKS, MULTIPLE DIFFERENT BACTERIA WILL BE NEEDED TO WORK IN A CONSORTIUM EACH WITH THEIR OWN ASSIGNED SUB-TASK. HOWEVER, SUCH DIVISION OF LABOUR CAN ONLY BE EFFICIENT IF THE BACTERIA CAN COMMUNICATE WITH EACH OTHER USING UNAMBIGUOUS LANGUAGE. THE AIM OF OUR PROJECT IS TO BUILD A SYNTHETIC COMMUNICATION SYSTEM WITH AN EXPANDABLE PEPTIDE VOCABULARY SO THAT BACTERIA CAN SEND DIFFERENT AND SPECIFIC SIGNALS TO COMMUNICATE DIFFERENT THINGS.

## COMMUNICATION IS KEY



**TO HAVE EFFICIENCY IN COMMUNICATION, IT IS BETTER TO HAVE "ORTHOGONAL" OR UNIQUE WORDS THAT DESCRIBE UNIQUE THINGS. IF THE LANGUAGE USED TO COMMUNICATE HAS LIMITED VOCABULARY, OR IF THE INDIVIDUALS ARE NOT ABLE TO UNDERSTAND THE WORDS, IT'S HARD TO HAVE ANY MEANINGFUL CONVERSATION.**

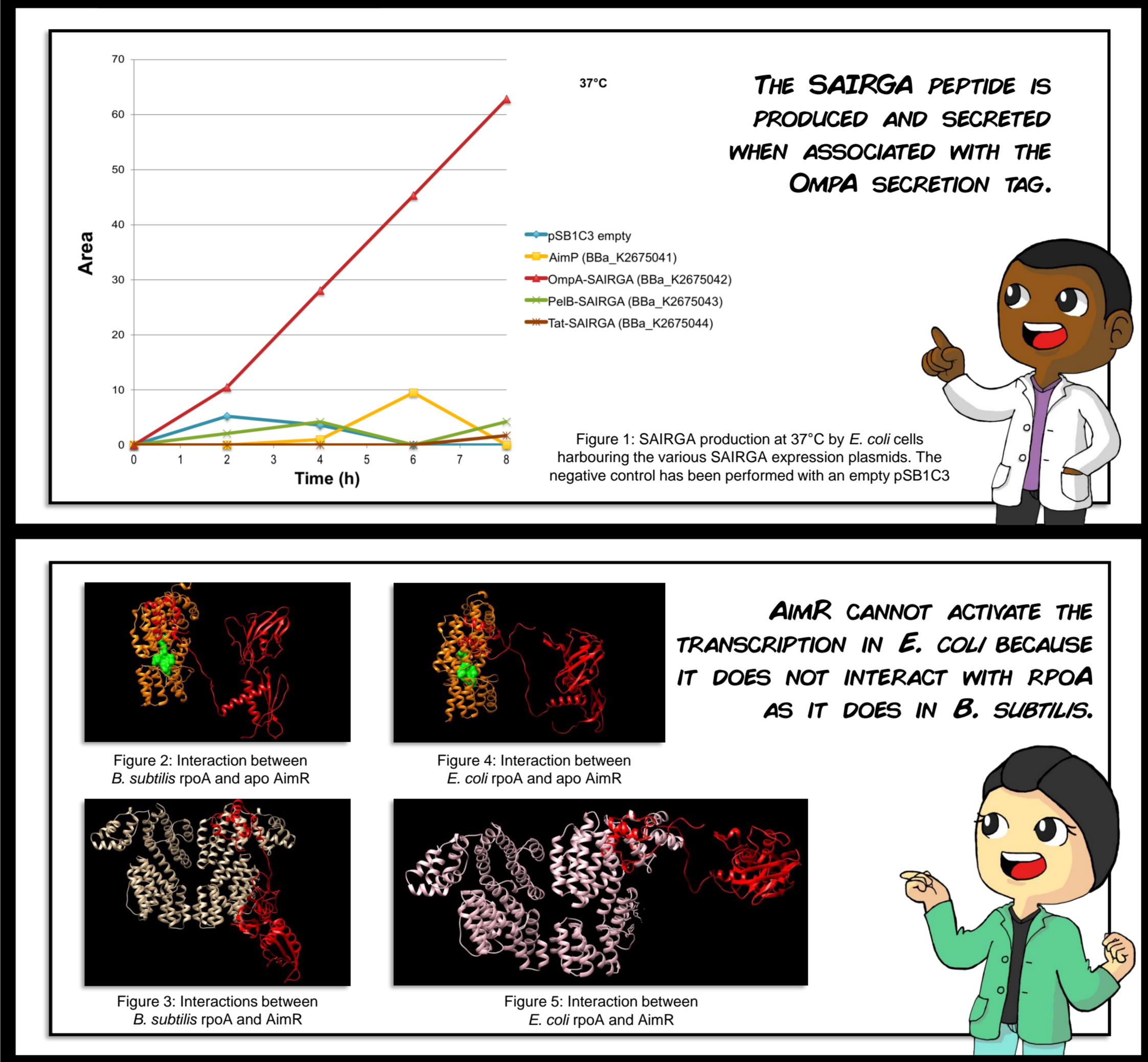
**...CAN LEAD TO CROSS-TALK WITH OTHER WORDS ALREADY EXISTING.**

**USING A NON-EFFICIENT COMMUNICATION SYSTEM...**

**...WITH A LARGER VOCABULARY WHERE WORDS HAVE UNIQUE MEANINGS FOR UNAMBIGUOUS COMMUNICATION.**

**WE DEVELOP AN EFFICIENT COMMUNICATION SYSTEM...**

## RESULTS



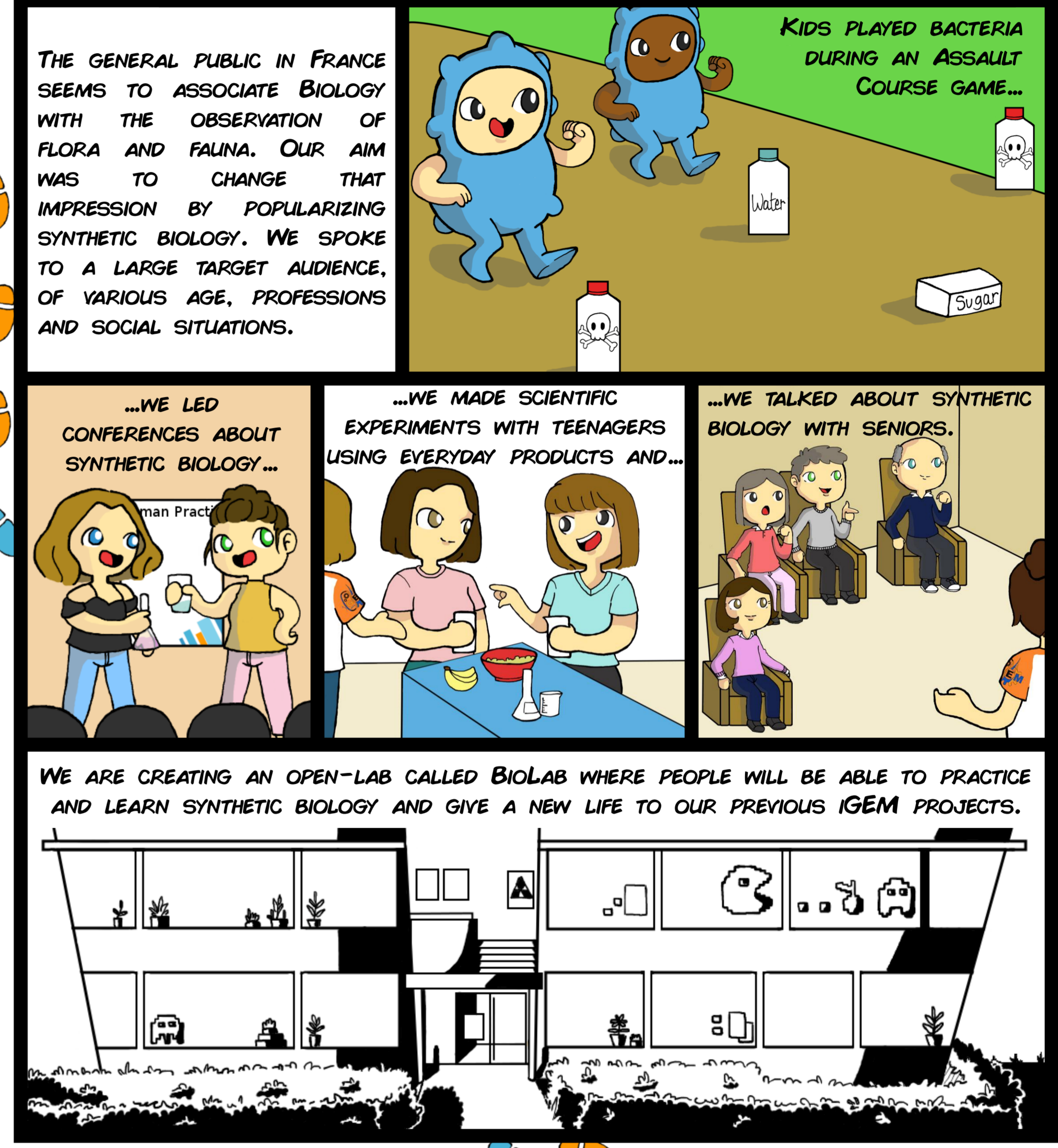
**THE SAIRGA PEPTIDE IS PRODUCED AND SECRETED WHEN ASSOCIATED WITH THE OMPA SECRETION TAG.**

Figure 1: SAIRGA production at 37°C by *E. coli* cells harbouring the various SAIRGA expression plasmids. The negative control has been performed with an empty pSB1C3

**AIMR CANNOT ACTIVATE THE TRANSCRIPTION IN *E. COLI* BECAUSE IT DOES NOT INTERACT WITH RPOA AS IT DOES IN *B. SUBTILIS*.**

Figure 2: Interaction between *B. subtilis* rpoA and apo AimR  
Figure 3: Interactions between *B. subtilis* rpoA and AimR  
Figure 4: Interaction between *E. coli* rpoA and apo AimR  
Figure 5: Interaction between *E. coli* rpoA and AimR

## EDUCATION AND PUBLIC ENGAGEMENT



**THE GENERAL PUBLIC IN FRANCE SEEMS TO ASSOCIATE BIOLOGY WITH THE OBSERVATION OF FLORA AND FAUNA. OUR AIM WAS TO CHANGE THAT IMPRESSION BY POPULARIZING SYNTHETIC BIOLOGY. WE SPOKE TO A LARGE TARGET AUDIENCE, OF VARIOUS AGE, PROFESSIONS AND SOCIAL SITUATIONS.**

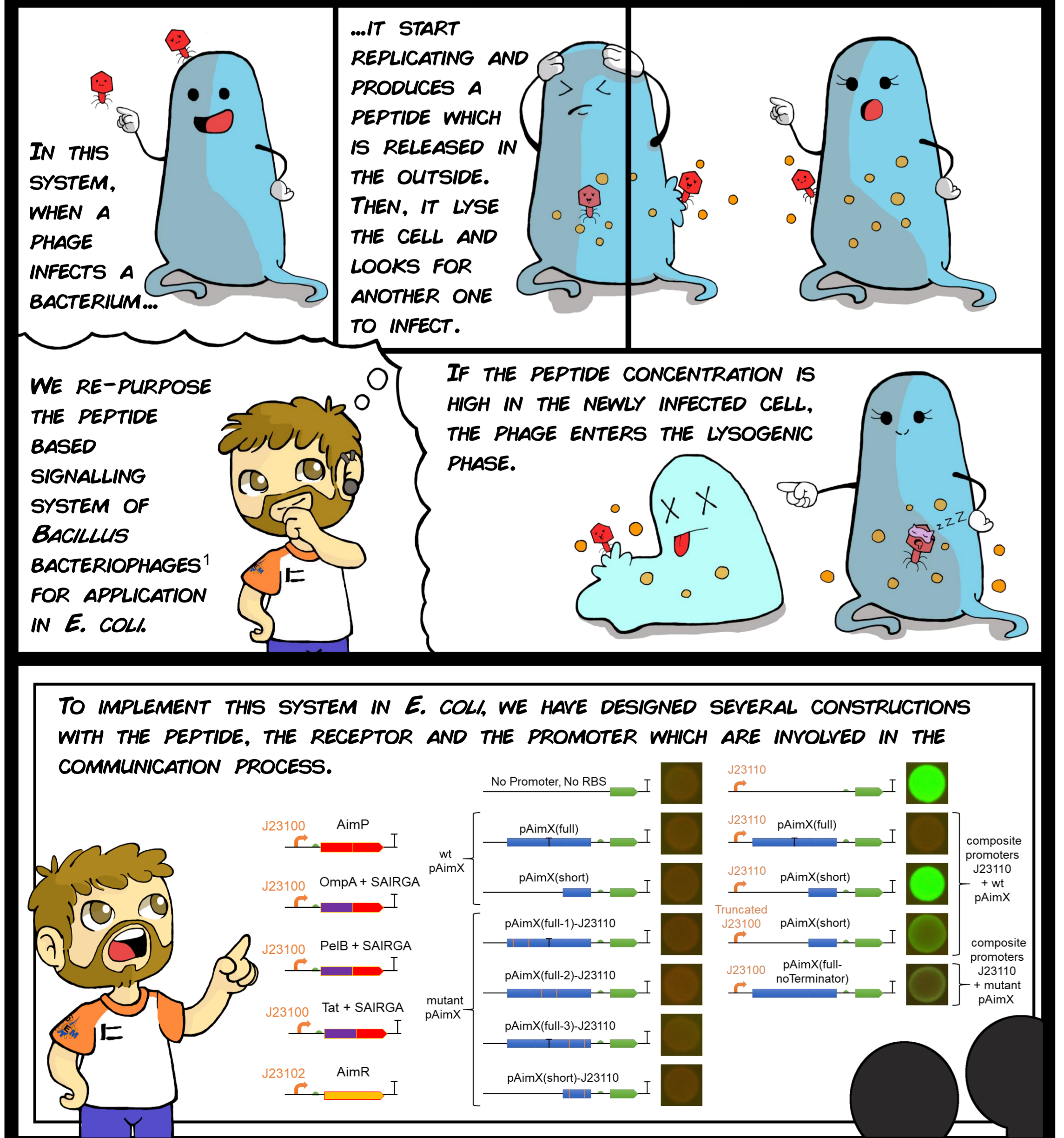
**...WE LED CONFERENCES ABOUT SYNTHETIC BIOLOGY...**

**...WE MADE SCIENTIFIC EXPERIMENTS WITH TEENAGERS USING EVERYDAY PRODUCTS AND...**

**...WE TALKED ABOUT SYNTHETIC BIOLOGY WITH SENIORS.**

**WE ARE CREATING AN OPEN-LAB CALLED BIOLAB WHERE PEOPLE WILL BE ABLE TO PRACTICE AND LEARN SYNTHETIC BIOLOGY AND GIVE A NEW LIFE TO OUR PREVIOUS IGEN PROJECTS.**

## DESIGN



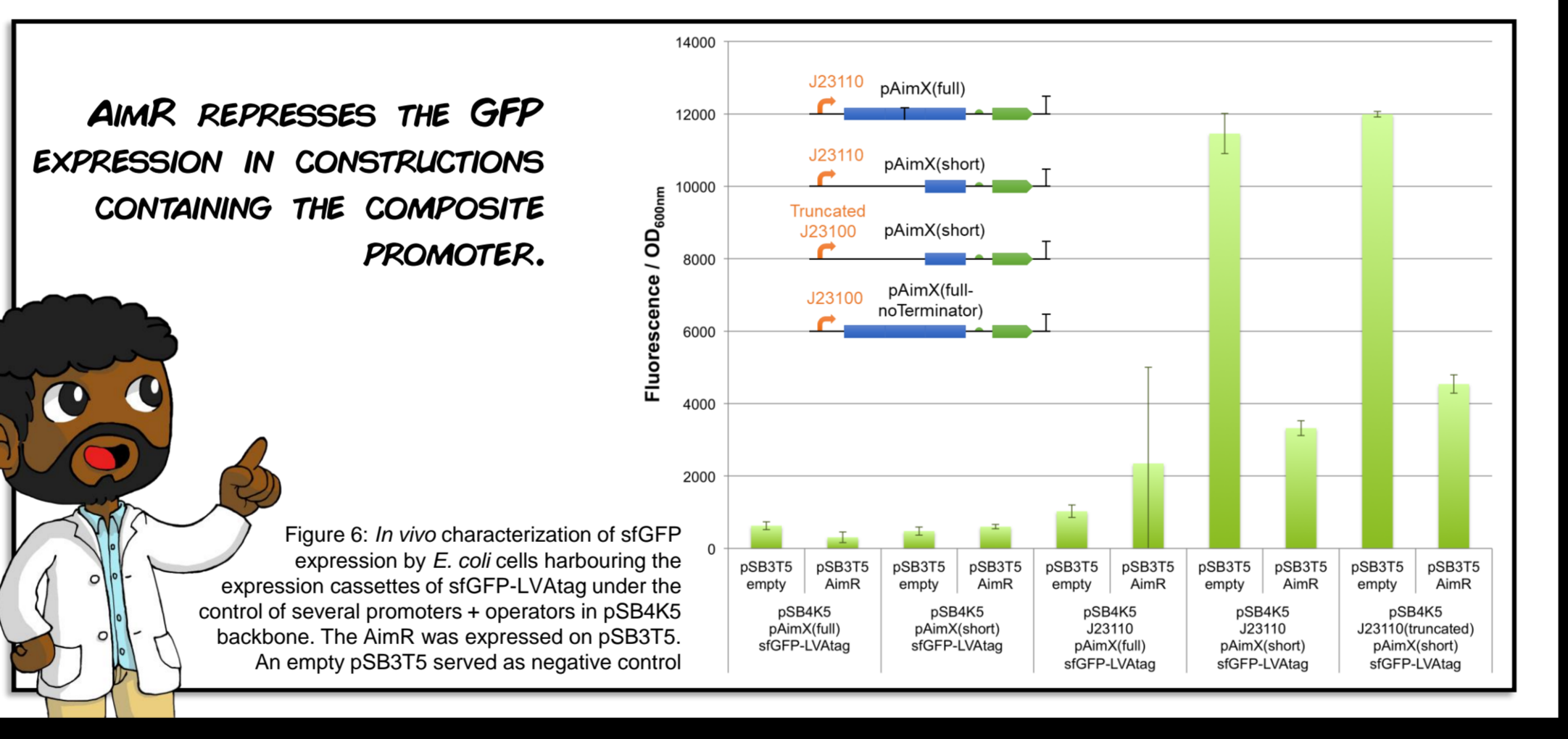
**...IT START REPLICATING AND PRODUCES A PEPTIDE WHICH IS RELEASED IN THE OUTSIDE. THEN, IT LYSE THE CELL AND LOOKS FOR ANOTHER ONE TO INFECT.**

**IN THIS SYSTEM, WHEN A PHAGE INFECTS A BACTERIUM...**

**WE RE-PURPOSE THE PEPTIDE BASED SIGNALLING SYSTEM OF BACILLIUS BACTERIOPHAGES<sup>1</sup> FOR APPLICATION IN *E. COLI***

**IF THE PEPTIDE CONCENTRATION IS HIGH IN THE NEWLY INFECTED CELL, THE PHAGE ENTERS THE LYSOGENIC PHASE.**

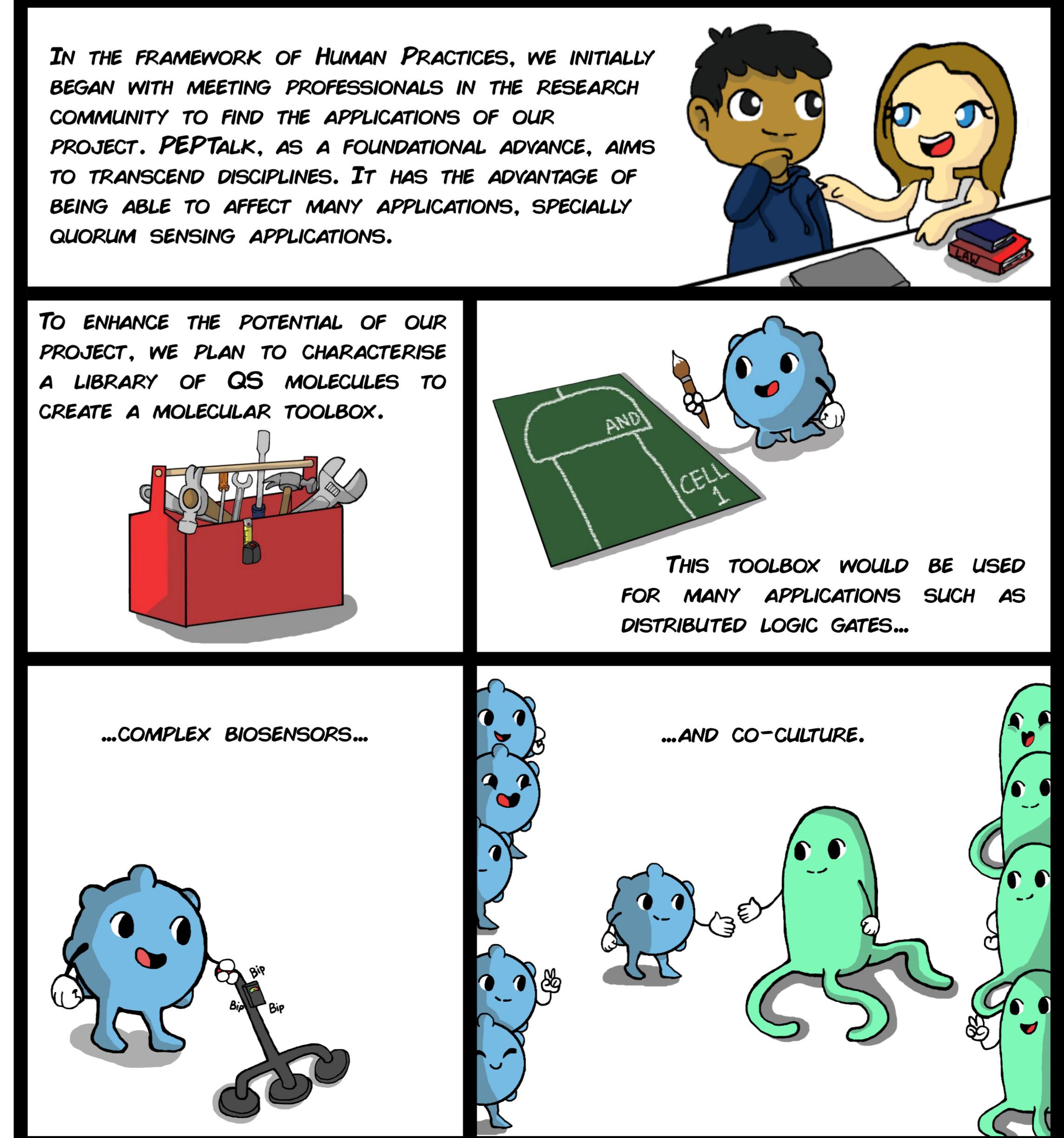
**TO IMPLEMENT THIS SYSTEM IN *E. COLI*, WE HAVE DESIGNED SEVERAL CONSTRUCTIONS WITH THE PEPTIDE, THE RECEPTOR AND THE PROMOTER WHICH ARE INVOLVED IN THE COMMUNICATION PROCESS.**



**AIMR REPRESSES THE GFP EXPRESSION IN CONSTRUCTIONS CONTAINING THE COMPOSITE PROMOTER.**

Figure 6: *In vivo* characterization of stGFP expression by *E. coli* cells harbouring the expression cassettes of stGFP-LVtag under the control of several promoters + operators in pSB4K5 backbone. The AimR was expressed on pSB3T5. An empty pSB3T5 served as negative control

## FUTURE APPLICATIONS



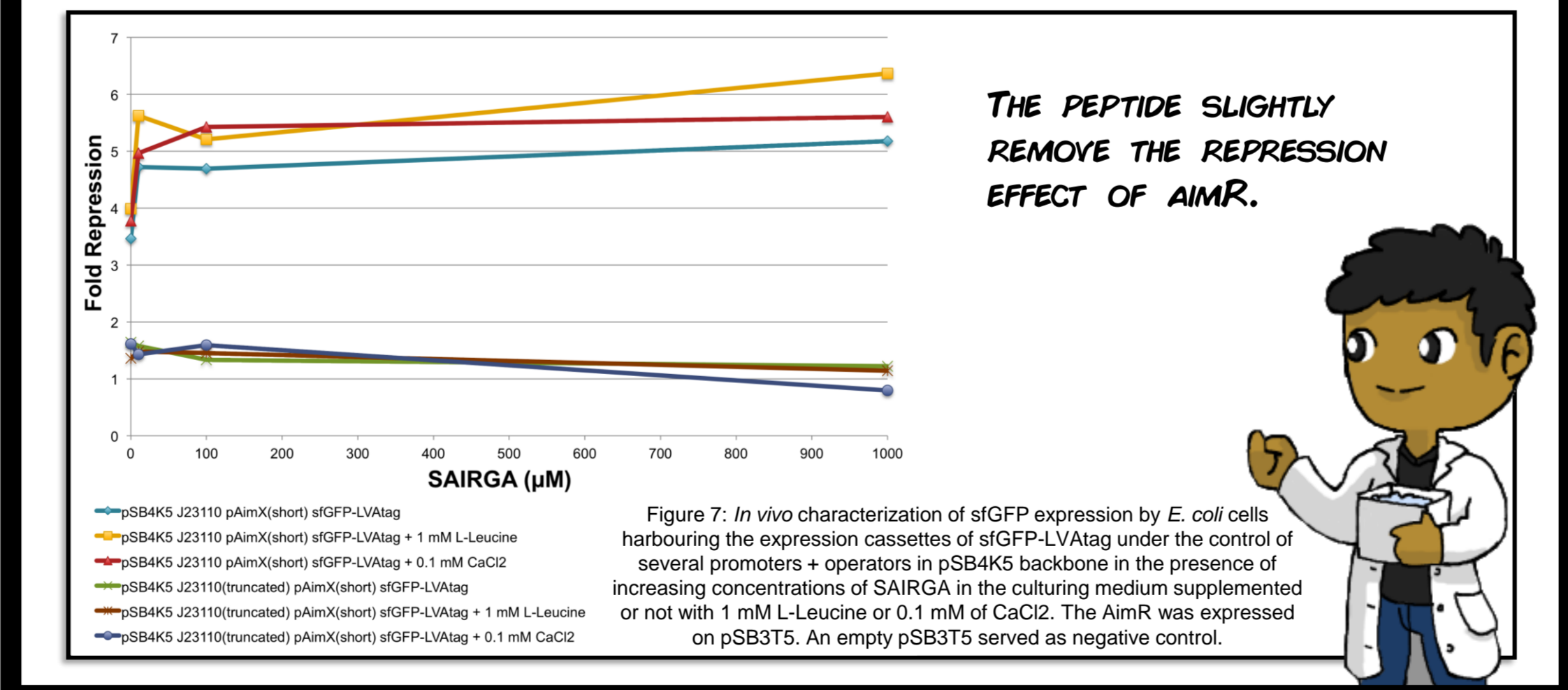
**IN THE FRAMEWORK OF HUMAN PRACTICES, WE INITIALLY BEGAN WITH MEETING PROFESSIONALS IN THE RESEARCH COMMUNITY TO FIND THE APPLICATIONS OF OUR PROJECT. PEPTALK, AS A FOUNDATIONAL ADVANCE, AIMS TO TRANSCEND DISCIPLINES. IT HAS THE ADVANTAGE OF BEING ABLE TO AFFECT MANY APPLICATIONS, SPECIALLY QUORUM SENSING APPLICATIONS.**

**TO ENHANCE THE POTENTIAL OF OUR PROJECT, WE PLAN TO CHARACTERISE A LIBRARY OF QS MOLECULES TO CREATE A MOLECULAR TOOLBOX.**

**THIS TOOLBOX WOULD BE USED FOR MANY APPLICATIONS SUCH AS DISTRIBUTED LOGIC GATES...**

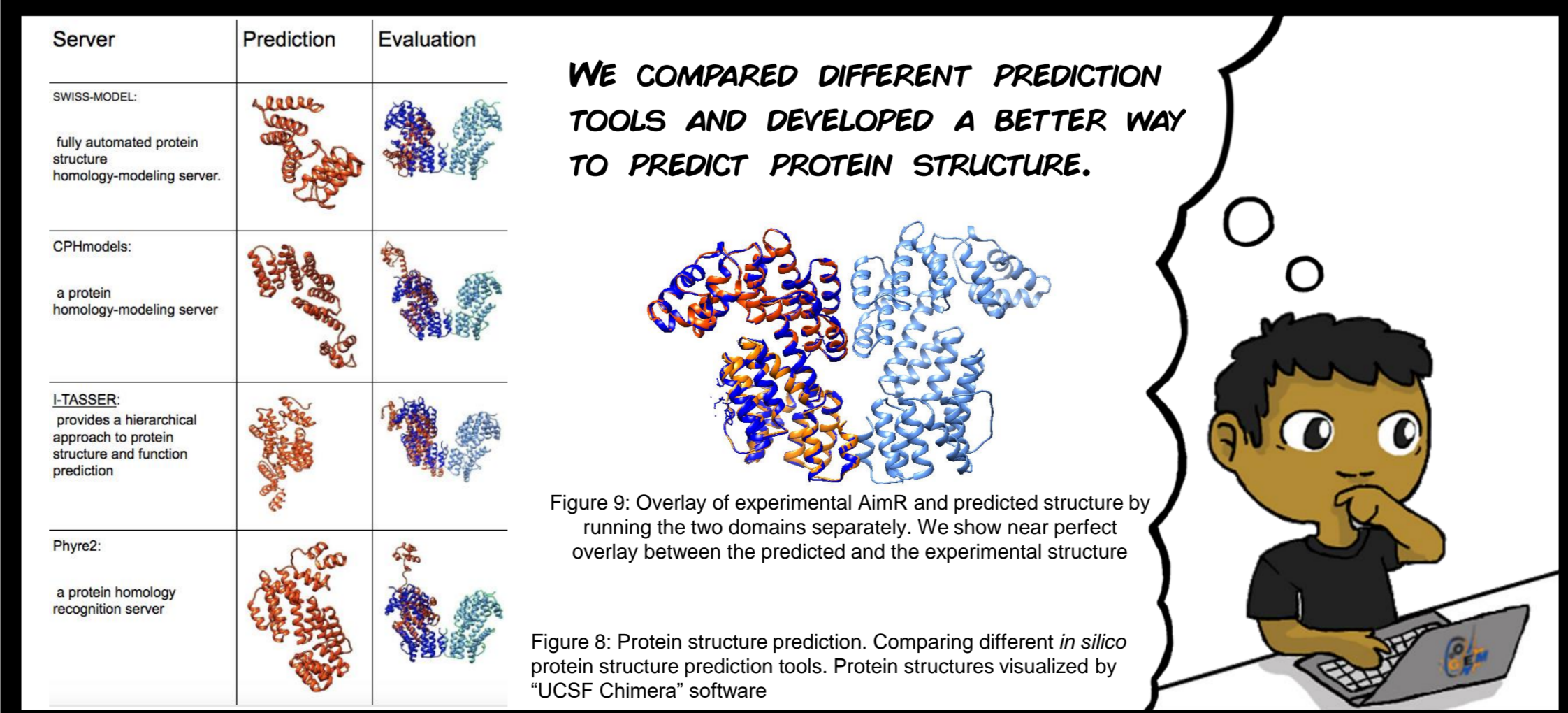
**...COMPLEX BIOSENSORS...**

**...AND CO-CULTURE.**



**THE PEPTIDE SLIGHTLY REMOVE THE REPRESSION EFFECT OF AIMR.**

Figure 7: *In vivo* characterization of stGFP expression by *E. coli* cells harbouring the expression cassettes of stGFP-LVtag under the control of several promoters + operators in pSB4K5 backbone in the presence of increasing concentrations of SAIRGA in the culturing medium supplemented or not with 1 mM L-Leucine or 0.1 mM of CaCl<sub>2</sub>. The AimR was expressed on pSB3T5. An empty pSB3T5 served as negative control.



**WE COMPARED DIFFERENT PREDICTION TOOLS AND DEVELOPED A BETTER WAY TO PREDICT PROTEIN STRUCTURE.**

Server	Prediction	Evaluation
SWISS-MODEL	fully automated protein structure homology-modelling server	
CPHmodels	a protein homology-modelling server	
I-TASSER	provides a hierarchical approach to protein structure and function prediction	
Phyre2	a protein homology recognition server	

Figure 8: Protein structure prediction. Comparing different *in silico* protein structure prediction tools. Protein structures visualized by "UCSF Chimera" software

Figure 9: Overlay of experimental AimR and predicted structure by running the two domains separately. We show near perfect overlay between the predicted and the experimental structure



<sup>1</sup> Erez et al., Nature (2017) 541, 488-493.