INTERFACING *IN VIVO* AND *IN SILICO* EVOLUTION

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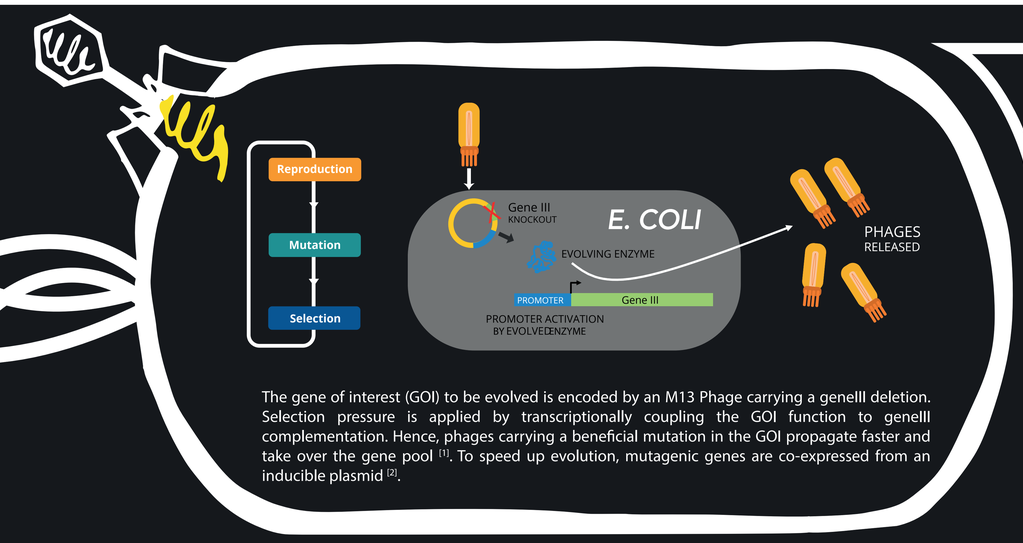
iGEM TEAM HEIDELBERG 17

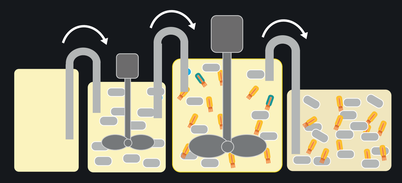
THE PHAGE AND THE FURIOUS

The iGEM Team Heidelberg provides a new foundational advance by introducing an innovative *in vivo* and *in silico* evolution interface as novel engineering paradigm to Synthetic Biology. We present a standardized evolution toolbox that highly simplifies the complex phage-assisted continuous evolution (PACE) [1] method and widely expands its utility towards various new application areas, including the biological production of organosilicons. To simplify the generation of desired functionalities *de novo*, we created AiGEM, our Artificial Intelligence for Genetic Evolution Mimicking software suite.



The gene of interest (GOI) to be evolved is encoded by an M13 Phage carrying a geneIII deletion. Selection pressure is applied by transcriptionally coupling the GOI function to geneIII complementation. Hence, phages carrying a beneficial mutation in the GOI propagate faster and take over the gene pool [1]. To speed up evolution, mutagenic genes are co-expressed from an inducible plasmid [2].

PACE Phage-Assisted Continuous Evolution

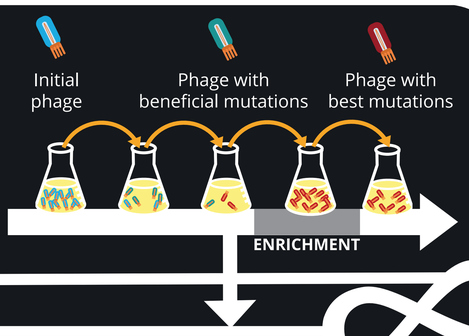


Continuous evolution can be realized in a complex bioreactor setup [1].

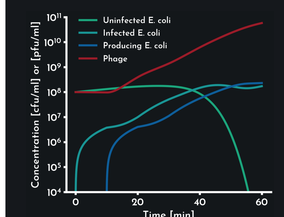
SIMPLIFY

PREDCEL

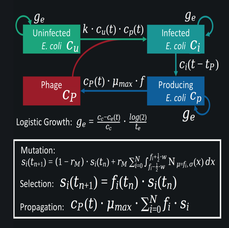
To simplify PACE, we developed a novel method named "phage-related discontinuous evolution" (PREDCEL). This simple batch-mode procedure [3] is trivial to set up in any lab, cheap and easy to control and adapt.



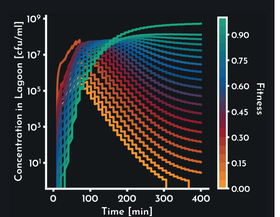
MODELING



Simulation of phage titer development during a single PREDCEL evolution round.

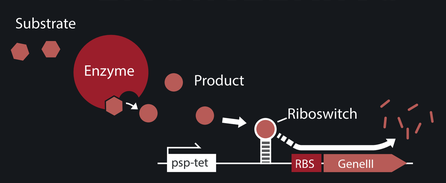


Comprehensive delayed differential eqation model describing the processes underlying our phage - assisted *in vivo* evolution.



Distribution of phage-producing *E. coli* concentrations with different fitness values.

CYTOCHROME ENGINEERING



An innovative concept for enzyme engineering. The catalysis of a desired product is linked to the selection of improved enzymes via a riboswitch. We applied this concept on Cytochrome P450 1A2 to direct the conversion of caffeine towards theophylline instead of paraxanthine [5].

EVOLUTION TOOLBOX

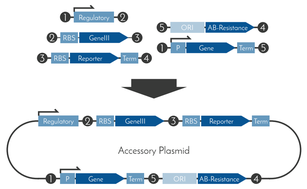
1 OPTIMIZE PARENTAL SEQUENCE
2 GET THE PHAGE
3 ADD SELECTION PRESSURE
4 INDUCE MUTA-GENESIS
5 OPTIMIZE EXPERIMENT CONDITIONS

Your workflow

LEARN. EVOLVE. PREDCEL.

Now it's on you to start your own evolution with PREDCEL!

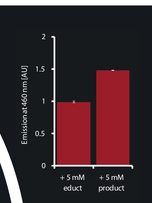
Our toolbox contains all parts and protocols required to make the phages and accessory plasmids for your experiment. We also provide validated selection circuits and engineering scaffolds for various applications. Our online toolbox guide and accompanying RFC (# pending) make *in vivo* evolution easy for everybody.



Organosilicons | Protein Interactions | Optogenetics | Enzymes | CRISPR/Cas9

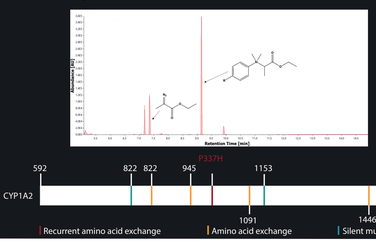
IN VIVO EVOLUTION

IN SILICO EVOLUTION



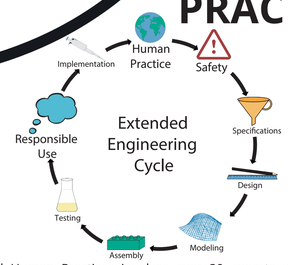
Organosilicon production in whole cell lysates by an engineered cytochrome C (left) [4].

Using the MAWS software (iGEM Heidelberg 2015), we also created the first riboswitch for organosilicon detection *in vivo* (right) [6, 12].



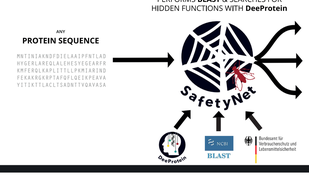
AiGEM

FACING HUMAN PRACTICES



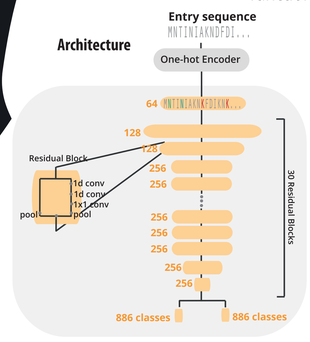
Our Integrated Human Practices involves over 20 experts, 4 surveys including more than 160 participants, over 20 collaborations, and engages ~50 high school students.
We conclude that we have to make our (i) technologies safe, (ii) stimulate their responsible use, and (iii) apply them to address urgent human needs.

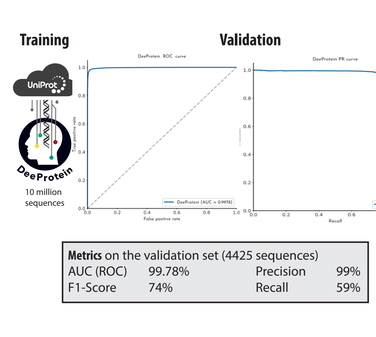
SAFETY NET



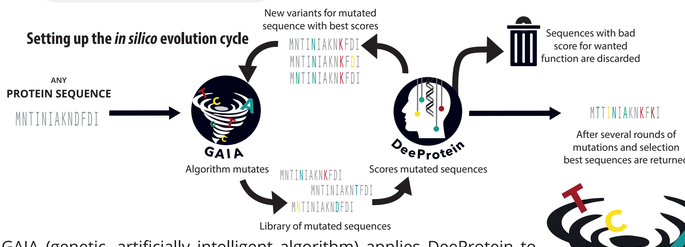
DeeProtein

is a deep neural network for multi-label protein function prediction from sequence [8, 9, 10].





Metrics on the validation set (4425 sequences)			
AUC (ROC)	99.78%	Precision	99%
F1-Score	74%	Recall	59%

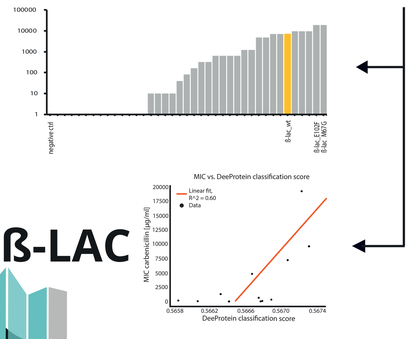


GAIA (genetic, artificially intelligent algorithm) applies DeeProtein to score the fitness of the progeny after each in silico evolution cycle. The fittest sequences are kept and the next evolution cycle is started.

GAIA

Modulating protein activity

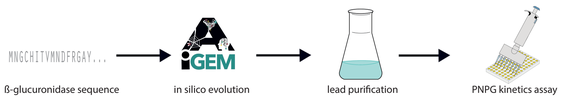
Using AiGEM, we generated a ß-lactamase library with a diverse DeeProtein classification score and determined the minimum inhibitory concentration (MIC) of carbenicillin.

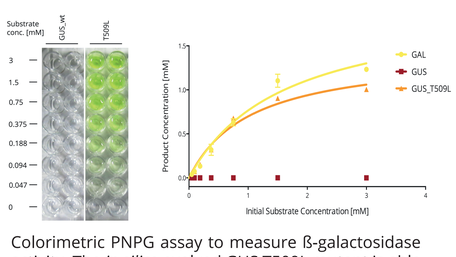


ß-LAC

Generating function *de novo*

We successfully reprogrammed ß-glucoronidase towards ß-galactosidase activity [7].





Colorimetric PNPG assay to measure ß-galactosidase activity. The *in silico* evolved GUS T509L mutant is able to convert the substrate almost wildtype efficiency.

GUS/GAL

REFERENCES

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[3] Brodel et al. (2017), DOI: 10.1038/nprot.2017.084
[4] Kan et al. (2016), DOI: 10.1126/science.aah6219
[5] Ahn et al (2004), DOI: 10.1016/j.pep.2004.03.005
[6] Henkin (2008), DOI: 10.1101/gad.1747308
[7] Matsumura et al. (2001), DOI:10.1006/jmbi.2000.4259
[8] Szalkai et al. (2017), DOI:10.1016/j.ymeth.2017.06.034
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[10] He et al (2016), DOI: 10.1109/CVPR.2016.90
[11] Liu et al (2017), arXiv:1701.08318
[12] Jenison et al (1994), DOI: 10.1126/science.7510417

ACHIEVEMENTS

- **Introduced:** A unique *in vivo* and *in silico* directed evolution interface as novel engineering paradigm to synthetic biology.
- **Developed:** PREDCEL, as simple, cheap and easy to implement PACE alternative.
- **Established:** *In vivo* enzyme evolution based on computationally predicted riboswitches.
- **Established:** First iGEM team to use an engineered cytochrome to catalyze carbon-silicon bond formation.
- **Integrated:** SafetyNET to safeguard directed evolution experiments.
- **Implemented:** First iGEM team to apply deep learning to protein engineering.
- **Created and validated:** AiGEM, an intelligent software for generating protein functionality *de novo*.
- ***In silico* evolved:** ß-glucuronidase towards ß-galactosidase activity.

ATTRIBUTIONS

Kai Ludwig, Lara Schlegel, Paul Rothhaar, Sebastian Degner, Maik Schauerte, Tobias Stadelmann, Jana Tegethoff, Fabian Ebner, Jonas Koneczny, Alex Mattausch, Jakob Kreft, Ilia Kats , Dr. Lutz Greb, Dr. Kevin Esvelt, Dickinson lab, Liu lab