Ultrasensitive **BIO**sensing platform for multiplex **CELL**ular protein **PHE**notyping at single-cell level

Engineered *E. coli* to produce Raman Reporters (RaRs) that can be detected by Surface-enhaced Raman Scattering (SERS)

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Introduction

- BIOCELLPHE project proposes the generation of an engineered *E.coli* bacteria that binds to circulating tumor cells (CTCs).
- The binding transduces the signal to activate the biosynthesis of small diffusible metabolites (i.e., RaRs) and can be detected by surface-enhaced Raman scattering (SERS).
- Three criteria were applied in order to select appropriate RaRs:
  I. The molecules must display high SERS activity.
  II. The molecules must be unambiguously identified in mixtures.
  III. The biosynthetic pathway must be expressed and optimized in *E.coli.*
- SERS advantages:

- Highly specific and sensitive.
- Achieve single molecule detection level under optimal conditions.
- The narrow bandwidths of Raman spectra facilitate the simultaneous detection of up to 100 distinct molecules.

Selection of output RaRs for multiplex SERS

- We collected a set of 113 molecules detectable by SERS from various literature sources [1] [2] [3].
- We ran a machine learning programs trained on selected bacterial metabolites and retrosynthesis software from the Galaxy-SynBioCAD portal [4] for engineering and optimizing their biosynthesis pathways in *E.coli.*
- We built a negative set by randomly drawing molecules from HMBD [5].

Molecule	ML score
violacein	0.97±0.02
deoxyviolacein	0.96±0.03
Cysteamine	0.95±0.04
Tryptamine	0.95±0.04
Phenethylamine	0.95±0.03
prodeoxyviolacein	0.94±0.03
Norspermidine	0.93±0.06
oxyviolacein	0.93±0.03
aurantinidin	0.91±0.05
cyanidin	0.91±0.05
delphinidin	0.91±0.05
proviolacein	0.91±0.04
pelargonidin	0.91±0.04
Tyramine	0.91±0.04
Pterin	0.91±0.07
1-Naphthylamine	0.9±0.09
Homocystine	0.9±0.05
L-Tryptophanamide	0.9±0.05
4-Imidazoleacetic Acid	0.89±0.03
Indole-3-Acetic Acid	0.88±0.11
1-Methylnicotinamide	0.88±0.05
peonidin	0.87±0.04
Methylguanidine	0.87±0.08
Dimethyl-1,4-Phenylenediamine	0.84±0.08
malvidin	0.83±0.06
Dopamine	0.82±0.05
2-Quinolinecarboxylic Acid	0.81±0.1
Glutathione	0.79±0.11
Pipecolate	0.79±0.08
Octopamine	0.77±0.07
Lumichrome	0.74±0.09
rosinidin	0.74±0.05
N-Methyl-D-Aspartic Acid	0.74±0.07
europinidin	0.74±0.05
Kynurenine	0.73±0.09
caffeine	0.72±0.09
N-Acetyl-L-Cysteine	0.69±0.07
Lipoamide	0.68±0.1
N-Methyltryptamine	0.68±0.08
3-Methoxytyramine	0.66±0.06
scytonemin	0.64±0.08
Dethiobiotin	0.63±0.08
pyocyanin	0.62±0.07
Riboflavin	0.6±0.07
L-Methionine Sulfoximine	0.59±0.07
Biliverdin	0.58±0.08
Mandelic Acid	0.57±0.06

Table 1: Metabolic pathways producing SERS detectable molecules in *E.coli* DH5α

SERS of the RaRs candidates

- After shortlisting, the promising metabolites were analyzed in order to identify those with:
  I. Higher SERS activity
  II. Amenable to simultaneous detection based on their Raman fingerprints.
- 5 candidates were selected and analyzed by SERS following published procedures developed by University of Vigo (UVIGO).

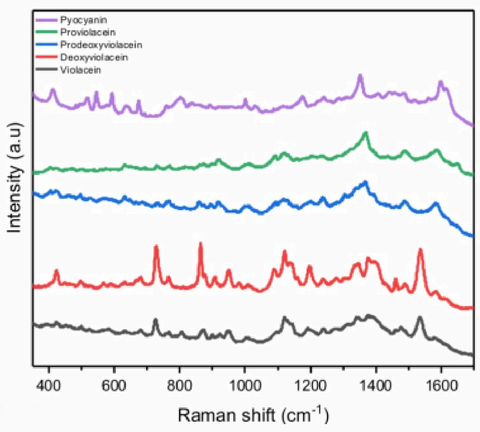

Figure 1 : Representative SERS spectra of candidate RaRs.

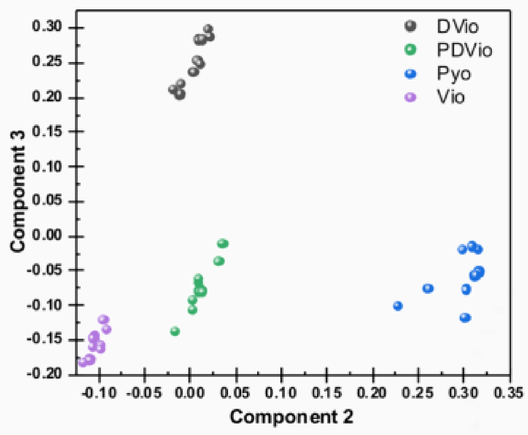

Figure 2 : PCA plot of SERS spectra of candidate RaRs.

Generation of biosynthesis pathways for output RaRs

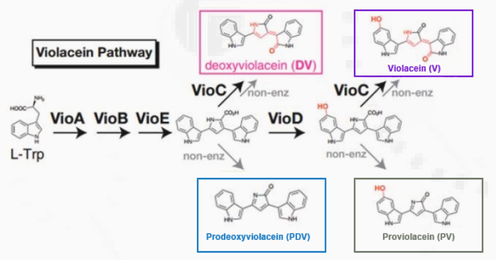

Figure 3 : Metabolic pathway of Violacein and its derivatives.

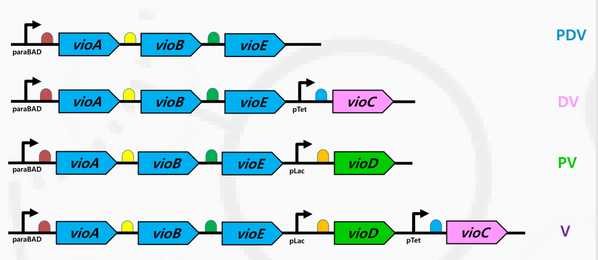

Figure 4 : Engineering of different constructs.

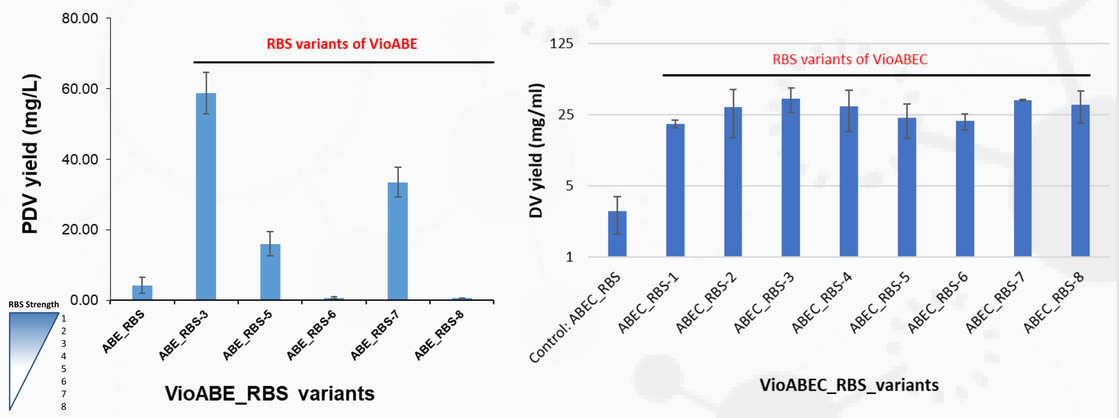

Figure 5 : Histograms shows the yield production of Prodeoxyviolacein and Desoxyviolacein after optimization.

- From the histogram it is evident that RBS-3 has the highest yield of PDV (60 mg/ml) and DV (35,89 mg/ml).

Conclusions

- By using machine learning approach, RaRs are shortlisted from a large pool of candidates and then screened by retrosynthesis software (table 1).
- UVIGO showed that 5 molecules are SERS-active (Figure 1) and four of them (prodeoxyviolacein, desoxyviolacein, violacein and pyocyanin) can de distinguished in mixtures by PCA analysis.
- After the selection of the RaRs, the next step is to create the pathways for these molecules in *E.coli*

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