Engineering electroactivity for signalling, energy, and electrosynthesis

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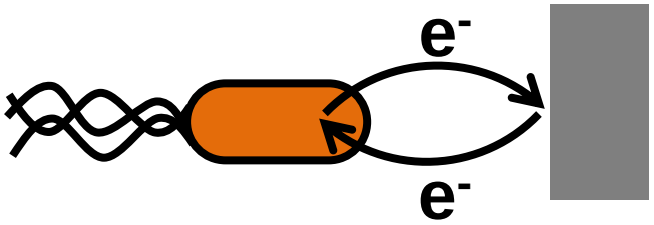
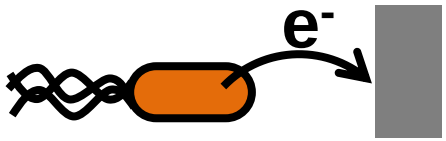
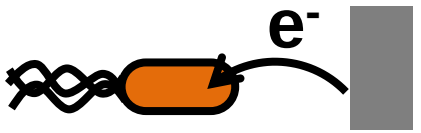
Synthetic biology for extracellular electron transport (EET)

- Electron exchange across the outer membranes of bacteria, between intracellular metabolism and an electrode
- How EET is connected into metabolism







Electrons to anode — **More oxidised products** — **More reduced products** — **Electrons from cathode**

Electron flow

Dissimilatory metal reduction — **Electrofermentation** — **Electrosynthesis; chemolithotrophy**

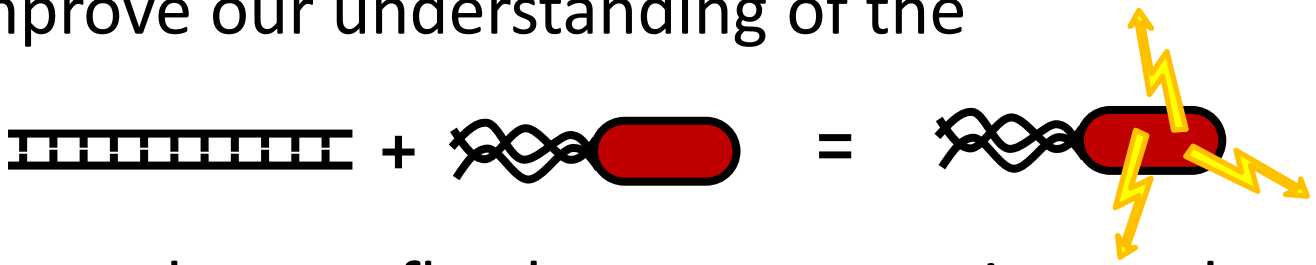
Influence on gene expression…

Synthetic biology for extracellular electron transport

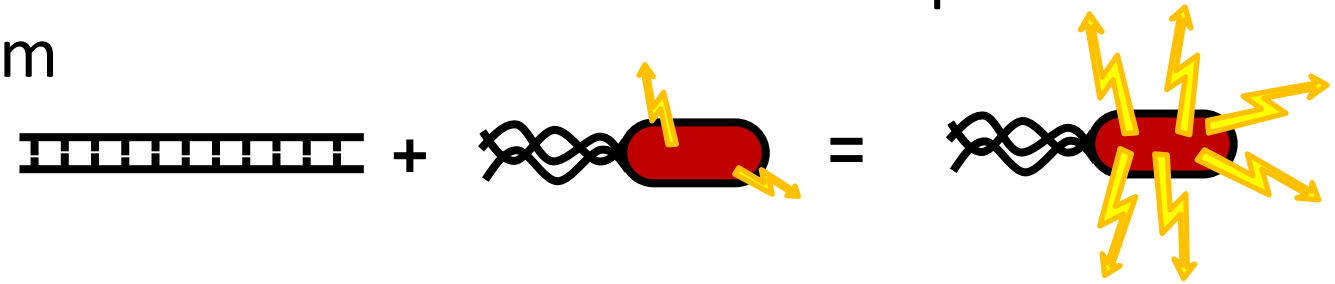
Reconstituting and **adapting** extracellular electron transport mechanisms

BESs (energy, bioremediation), bio-sensing, electrosynthesis…

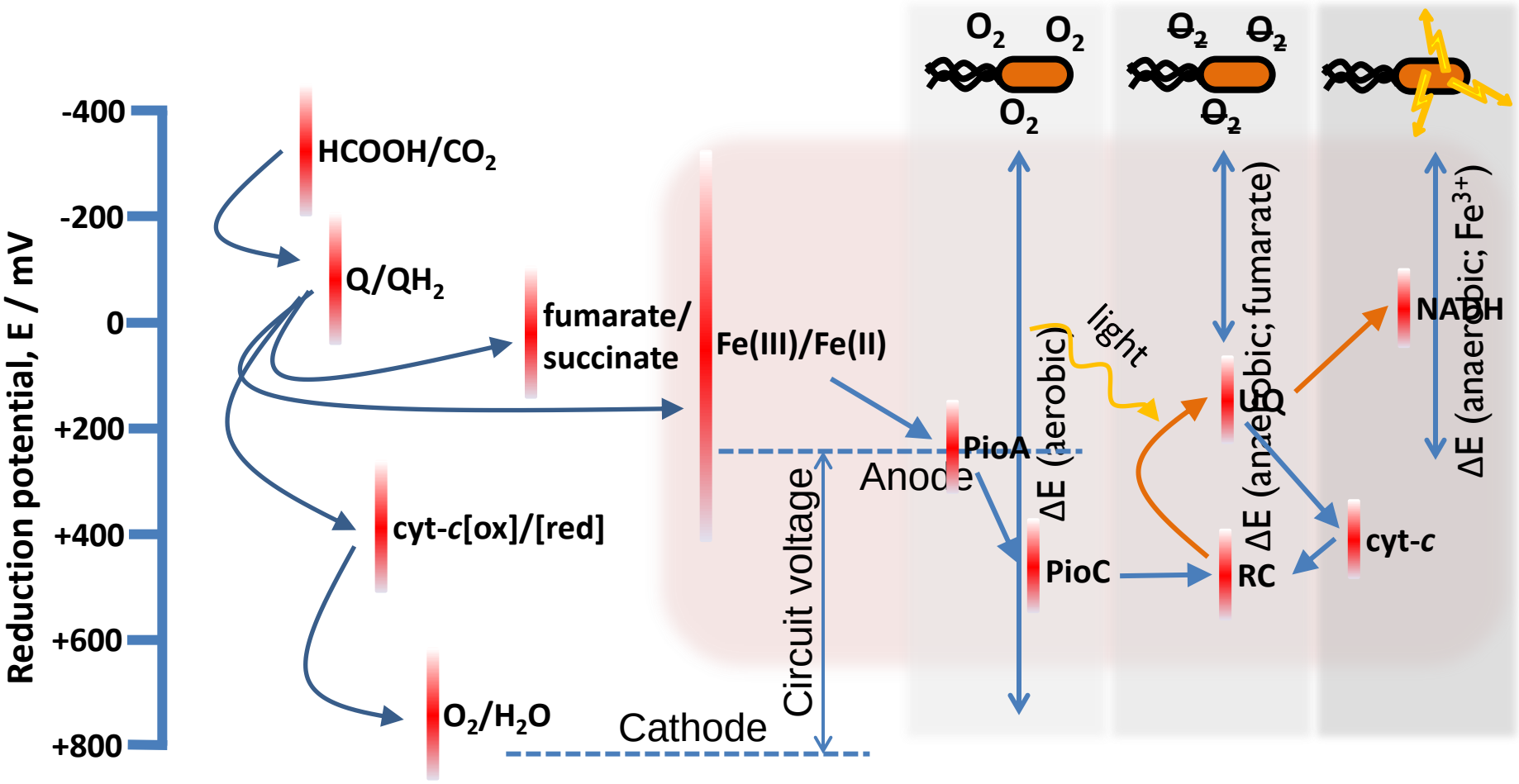
Reconstitute to confer EET capability on other useful species and improve our understanding of the mechanisms



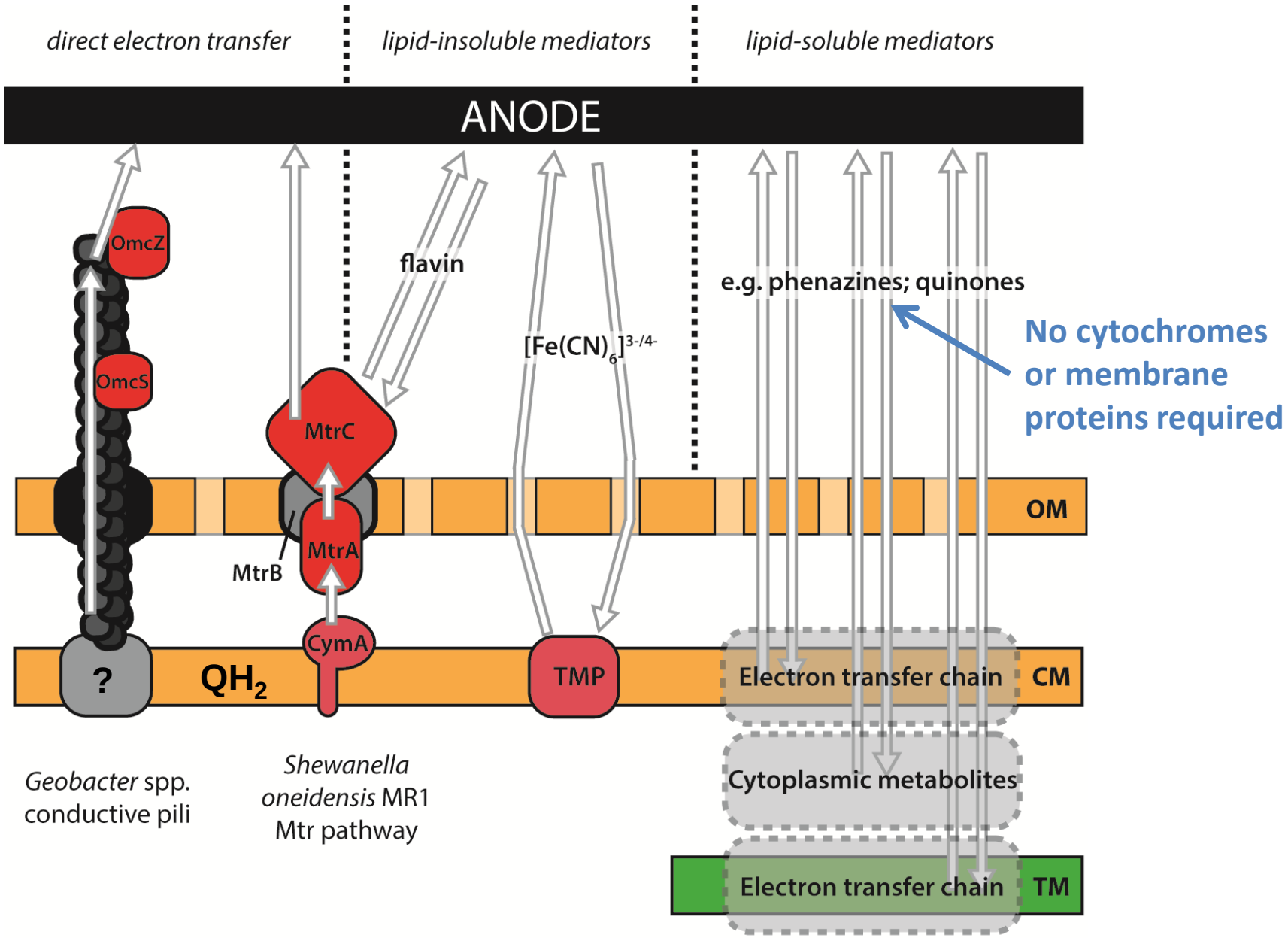
Adapt to increase electron flux between organism and electrode or to connect EET to desirable parts of metabolism



EET for life


Reduction potential, E / mV
-400
-200
0
+200
+400
+600
+800
HCOOH/CO2
Q/QH2
fumarate/
succinate
cyt-c[ox]/[red]
O2/H2O
Fe(III)/Fe(II)
Anode
Cathode
Circuit voltage
PioA
PioC
RC
cyt-c
NATH
light
ΔE (aerobic)
ΔE (anaerobic; fumarate)
ΔE (anaerobic; Fe3+)
O2
O2
O2

Mechanisms of extracellular electron transport



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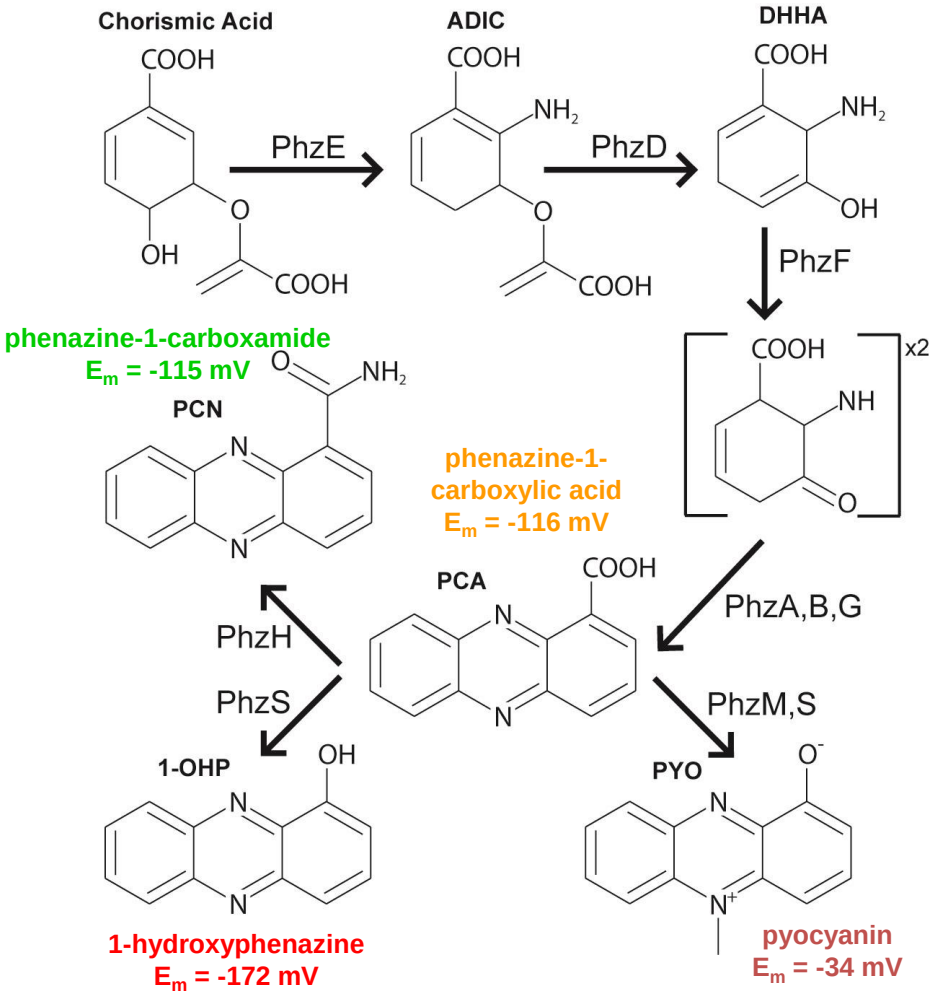
Phenazine production in *E. coli*



Core *phzAG* operon produces PCA which can be further modified to other phenazine types:
PYO, PCN, 1-OHP, 2-OHP

Genes cloned in a modular way (BioBricks)

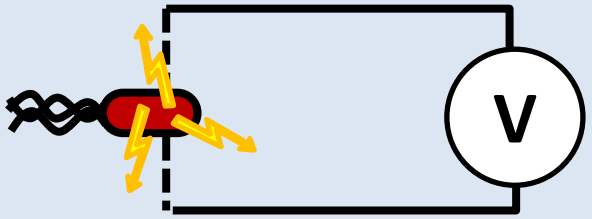
Electroactivity for biosensing

Interaction is communication

Environmental change → **Change in electroactivity** → **Measure with external circuitry**

What can we measure?

- Current exchange
- Voltage change
- Voltammetry to characterise/quantify electron carriers

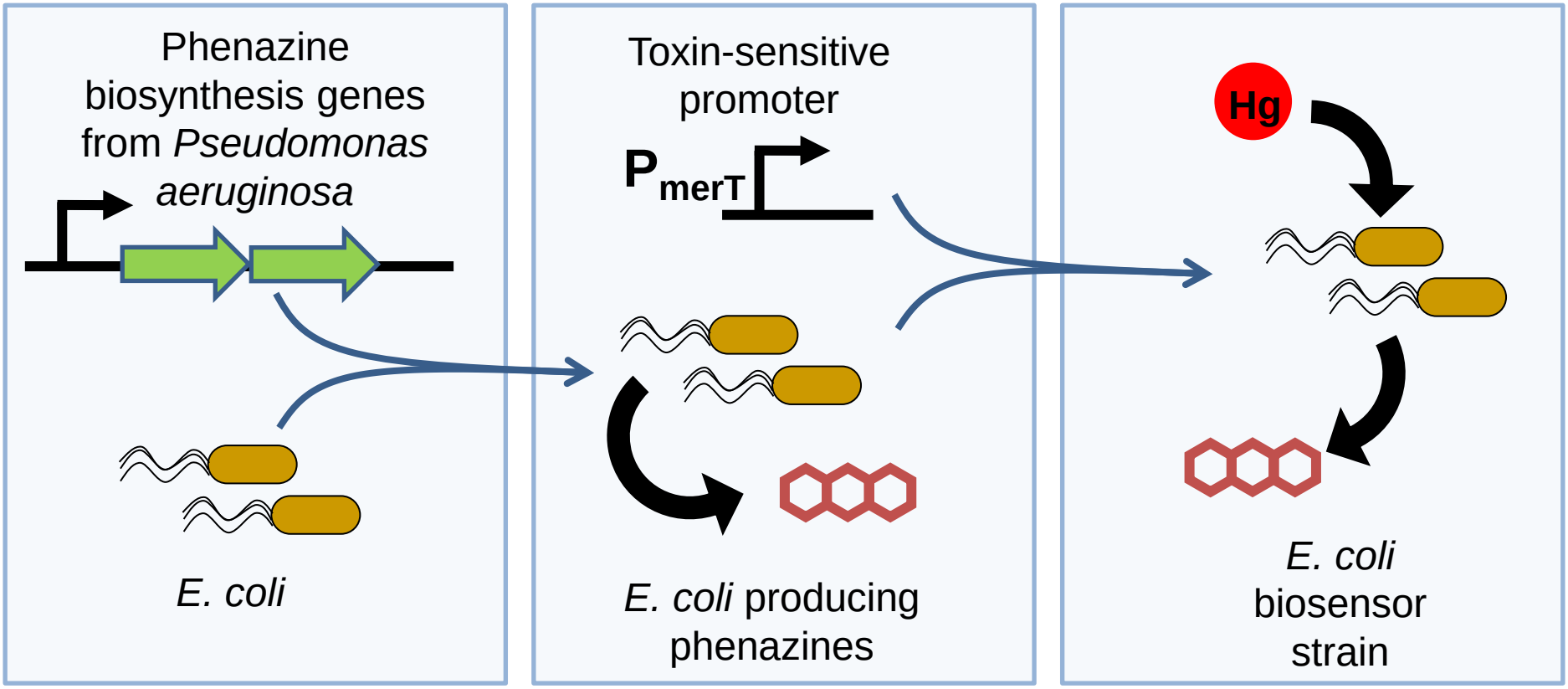


Electroactive biosensing organism

Integrate electrodes with culturing environment

Engineering *E. coli* to make an electroactive biosensor

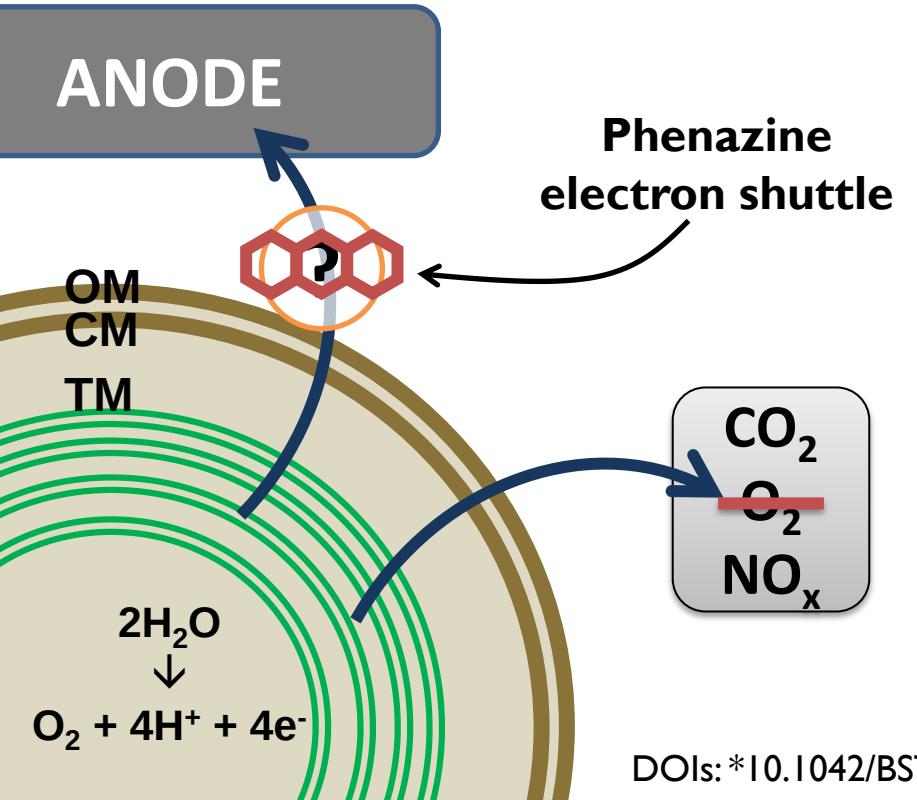
Use toxin-responsive promoters to create whole-cell biosensors with electrical outputs

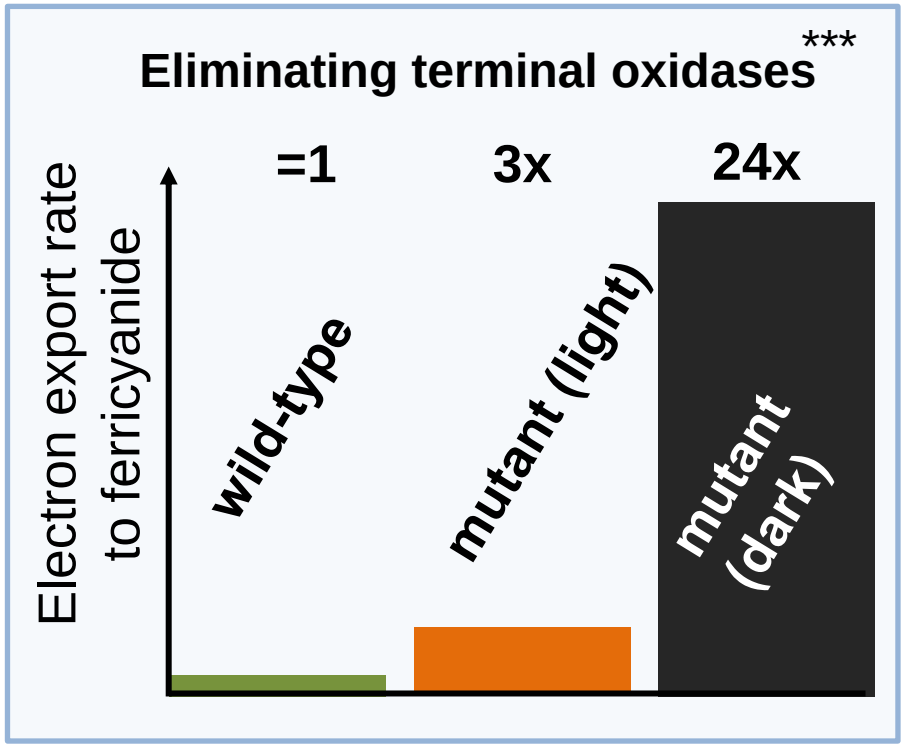


Photosynthetic microbial fuel cells

Cyanobacteria as potential electricigens

- Generate current from water and light energy
  - ~10 pA/cell * or >3 A m^{-2} **
- Many alternative electron sinks
- No electron export mechanisms





DOIs: *10.1042/BST20120118 ; **10.1039/C4EE03875D; *** 10.1039/C3CP52438H

Electrosynthesis with *Rh. palustris*

We aim to modify *Rh. palustris* to *c*ontrol its diverse metabolism, including electron uptake

- Maximise current uptake
- Direct flux to desirable pathways

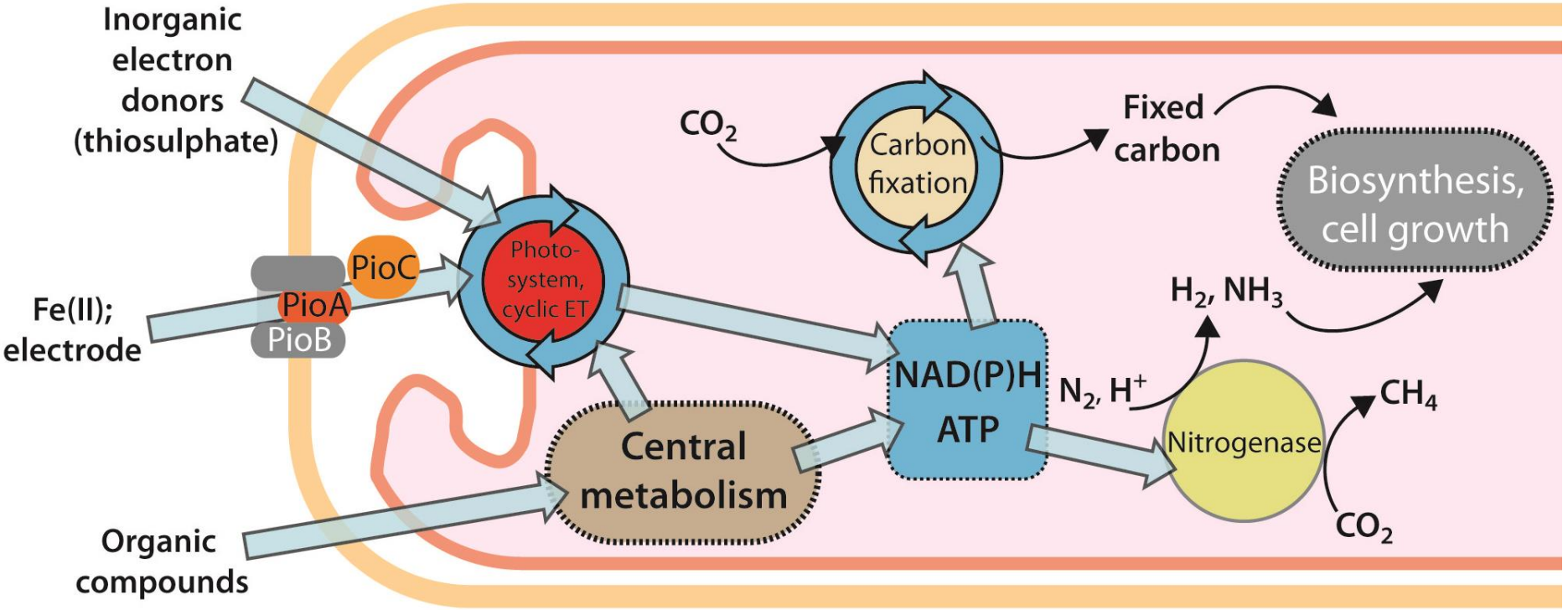
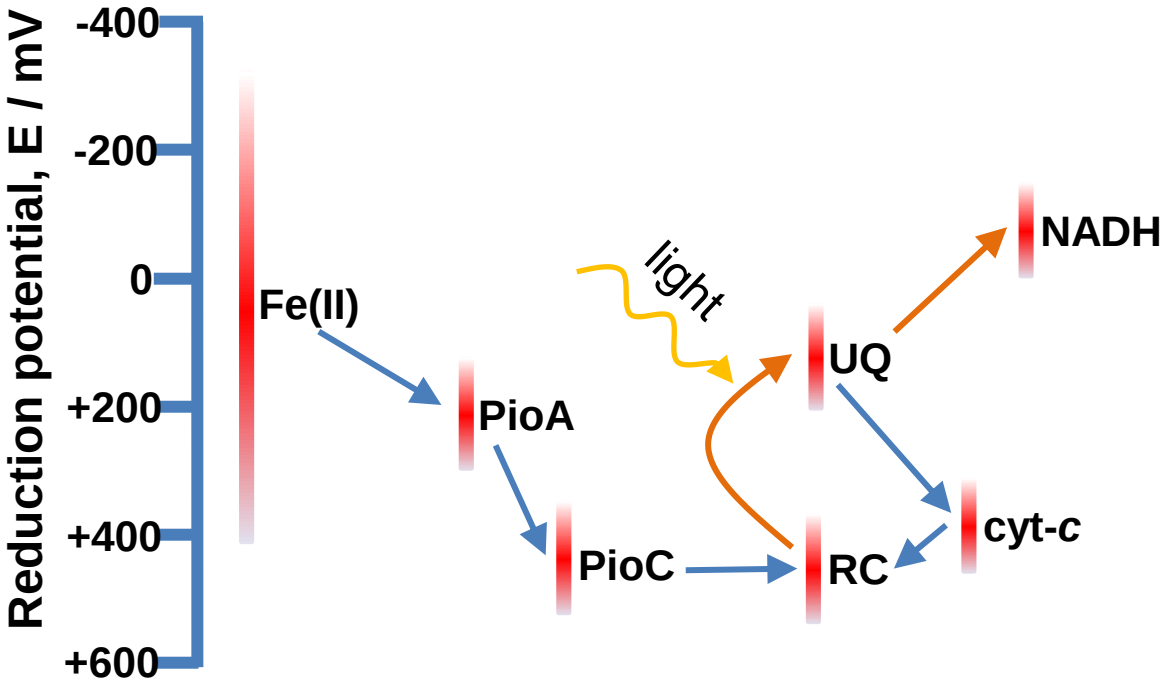


Photo-ferrotrophy

Uptake of electrons from Fe(II) and conversion to NADH using light energy



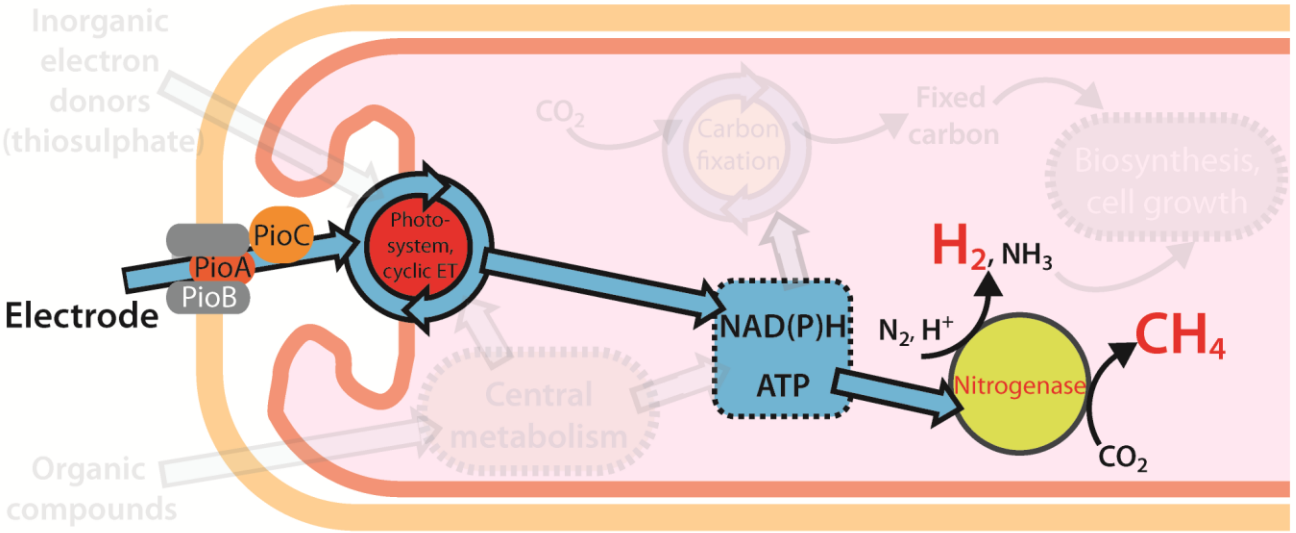
Direct uptake from carbon electrode observed but full mechanism unknown

We aim to understand and exert control over electron uptake mechanism

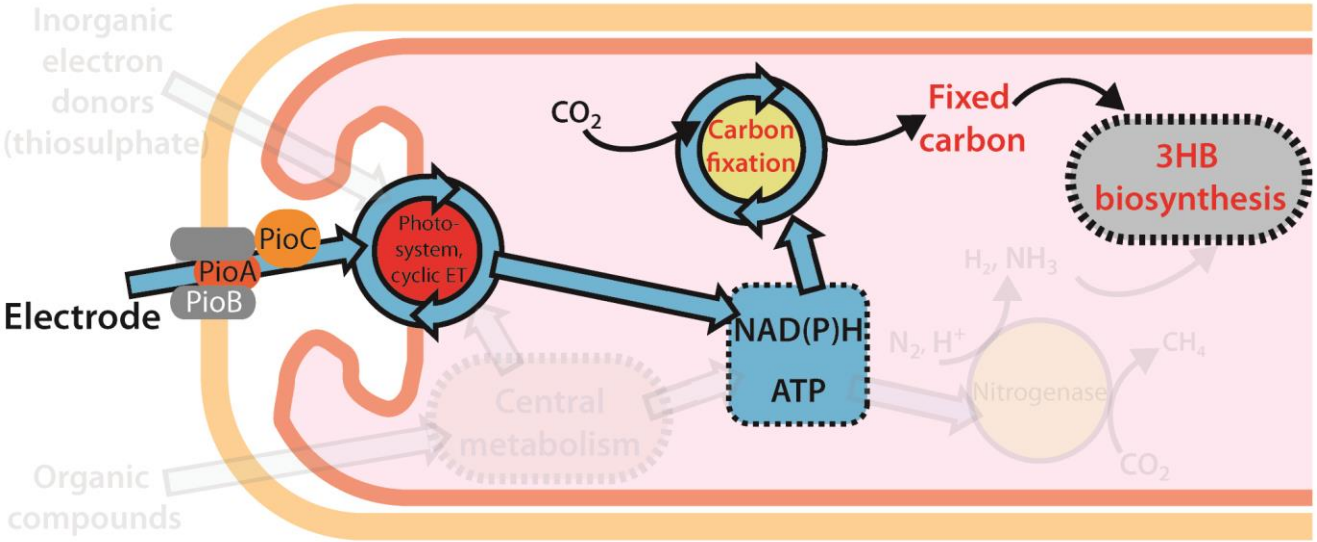
Electrosynthesis

Control expression of electron import machinery and direct reducing power to…

- the nitrogenase for H_2, NH_3, CH_4 production



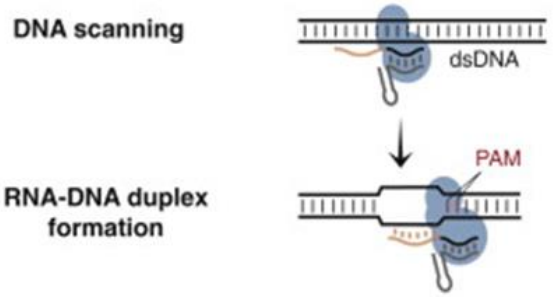
- carbon fixation and then desirable chemical production



Genetic tools

To achieve effective electrosynthesis we need genetic tools for metabolic engineering

- Basic part libraries: promoters, RBSs, transcription terminators
  - Reporters: GFP/RFP, iLov, B-gal, B-glucuronidase
- Tools for systems engineering:
  - CRISPR-dCas9
  - TetR repressors
  - Mf-lon inducible protease



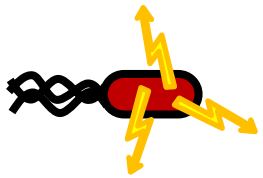
- Improvements in transformation speed and efficiency
  - Native restriction-modification system
  - Inhibit capsule formation?

BEE community-wide projects

- **Standardised tools**: bio-electrochemical devices
  - Enabling easier comparison between studies
  - Encouraging involvement
    - Improve access to equipment
    - Student competitions?

- **Techno-economic assessments**: electrosynthesis/electrofermentation
  - Identify priority molecules for production
  - Where is value added vs conventional fermentation?

Thank you!



Bio-photovoltaics
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David Lea-Smith *(UEA)*
Alistair McCormick *(Uni. Edinburgh)*
Mark Bennett *(ICL - DoLS)*
Jenny Zhang (*Uni. Cambridge*)
Laura Wey (*Uni. Cambridge*)
Eleanor Clifford (*Uni. Cambridge*)

Bio-electronic sensors
Martin Buck *(ICL - DoLS)*
Martin Buck lab *(ICL - DoLS)*
Baojun Wang *(Uni. Edinburgh)*
Firat Guder *(ICL - Bioeng.)*
Estefania Nunez Bajo *(ICL - Bioeng.)*

Electrosynthesis in *Rh. palustris*
Martin Buck *(ICL - DoLS)*
Patrik Jones *(ICL - DoLS)*
Robbie Pott *(Stellenbosch Uni.)*

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