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| הפקולטה להנדסה כימיתע"ש וולפסון |  |  |
| The Wolfson Department of Chemical Engineering |  |  |

**Wolfson Department of Chemical Engineering Seminar**

**Monday, October 8th, 2024 at 13:30**

**ZOOM:** **https://technion.zoom.us/j/96298325147**

**Reconstruction of Indigo Biosynthetic Pathway in *Yarrowia lipolytica***

**Han CHEN**

**MSc Seminar**

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Abstract:

Indigo is a widely used colorant in the textile industry, known for its deep blue color, which stems from its unique chemical structure—a chromophore with a small conjugated π-network. While indigo was originally derived from plants, today’s commercial indigo is chemically synthesized from N-phenylglycine, making it relatively inexpensive but posing environmental concerns. In this work, we report our effort to reconstitute the indigo biosynthetic pathway in oleaginous yeast *Yarrowia lipolytica*.

In the first part of our study, we optimized the heterologous expression conditions for indigo production using a flavin-containing monooxygenase from *Methylophaga sp. strain SK1* (MaFMO). We tested the in vivo conversion efficiency of MaFMO, as well as that of a heterologous tryptophanase from *Escherichia coli* (EcTnaA), addressing the bottleneck of indigo biosynthesis in *Yarrowia lipolytica* is the low accumulation of tryptophan in the yeast.

In the second part, we employed the Cre-loxP system to regulate homologous genes and boost tryptophan accumulation in order to improve biosynthesis of indigo in the yeast. LC-MS analysis revealed the accumulation of indole-3-lactic acid, a by-product of elevated tryptophan levels, which prompted us to perform targeted gene knockouts. By knocking out ARO8, we achieved the first indigo biosynthesis using glucose as substrate in *Yarrowia lipolytica*. Further targeting of BNA2, a gene involved in converting excess tryptophan to anthranilate, resulted in a threefold increase in molar conversion efficiency, reaching an average molar conversion ratio of 16.44%. The titer of de-novo synthesized indigo reached 524 μg/L. And 21.5 mg/L of indigo titer was achieved when 1 mM tryptophan supplied.