Modular Nanoparticle-Enzyme Systems For Designer Biosynthesis Of High-Value Products From Agriculturally-Derived Feedstock

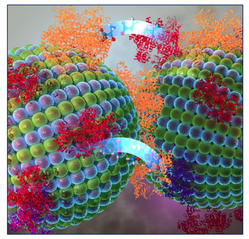
U.S. NAVAL RESEARCH LABORATORY

Joyce C. Breger, Shelby L. Hooe, Eunkeu Oh, Michael H. Stewart, Kimihiro Susumu, Scott A. Walper, Sebastián A. Díaz, Meghna Thakur, Mario G. Ancona, Igor L. Medintz, and Gregory A. Ellis

Center for Bio/Molecular Science and Engineering, Code 6900, Division of Optical Sciences, Code 5611, Electronic Science and Technology Division, Code 6876, U.S. Naval Research Laboratory; Research Associateship Program, National Research Council; George Mason University

Research Overview

Self-contained nanosystem
High enzyme density



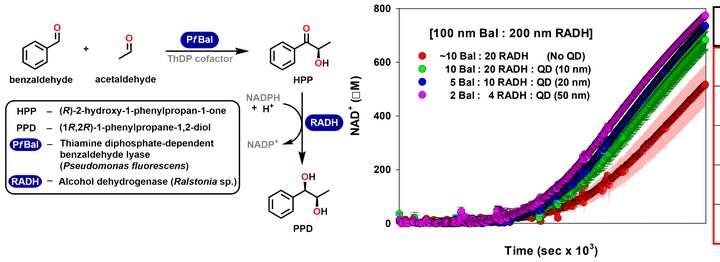
Goal: Achieve Enhanced Bioctalysis *without* Cells

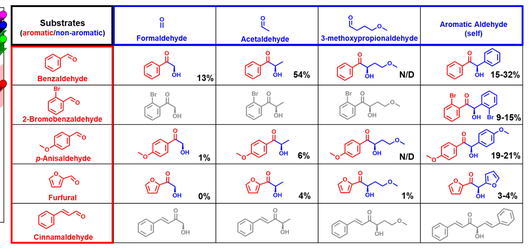
- Build a functional *ex vivo* enzyme pathway on a nanoparticle cluster
- Completely artificial *ex vivo* biosynthetic system
- Access channeling (fastest multistep enzyme catalysis)

Efficient multistep biosynthesis
Confined system + toxicity

Efficient multistep biosynthesis
Soft confinement

Exploring Channeling and Substrate Scope when Immobilized on Semiconductor Quantum Dots

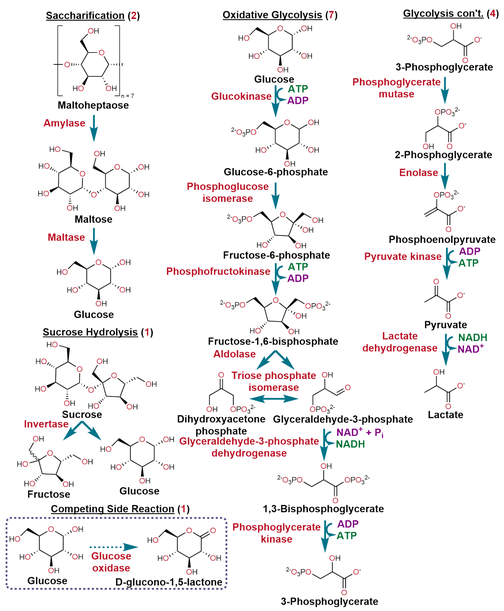


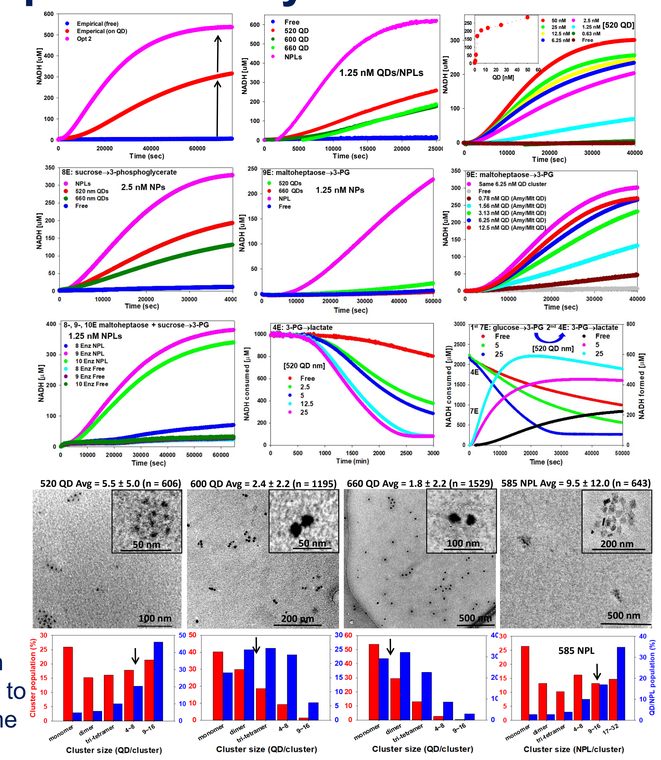


~50% increase in the coupled enzymatic flux despite the two enzymes displaying a 10,000x difference in k_{cat} and 3 orders of magnitude difference in their respective K_M. Multiple non-natural products synthesized.

S.L. Hooe, J. Breger, S. Dean, K. Susumu, E. Oh, S. Walper, G.A. Ellis and I.L. Medintz; *ACS Appl. Nano Mater.* **2022**, 5, 10900-10911

Self-Assembled Nanoparticle Enzyme Clusters

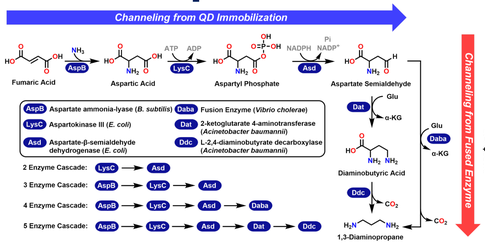




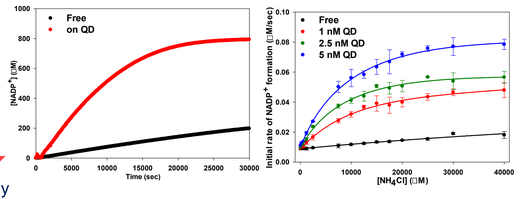
Channeling efficiency is **enhanced** several hundred fold more by optimizing enzymatic stoichiometry with numerical simulations, switching from spherical QDs to 2-D planar nanoplatelets, and by ordering the enzyme assembly

Breger, J.C.; Vranish, J.N.; Oh, E.; Stewart, M.H.; Susumu, K.; Aragonés G.L.; Ellis, G.A.; Walper, S.A.; Díaz, S.A.; Hooe, S.L.; Klein, W.P.; Ancona, M.G.; Medintz, I.L.; *Nat. Commun.* **2023**, 14, 1757

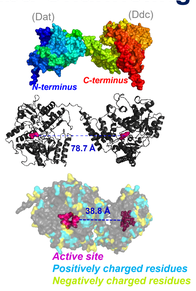
Classical Fusion Enzyme Channeling Complements Nanoparticle-Clustered Intermediary Channeling



Channeling in the 3E Cascade



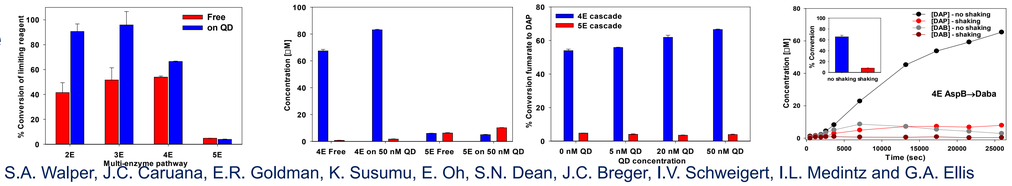
Daba Channeling



- Two channeling processes coexist and manifest independently
- **Dramatic** increase in flux and yield of DAP (>80% conversion)
- **Achieved** an initial rate 43× that of the freely diffusing and non-fused enzyme cascade yielding ~10× the amount of DAP.

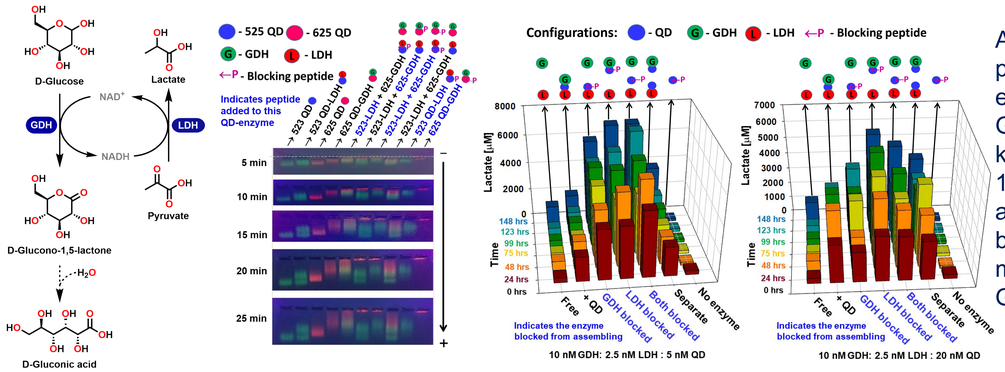
Comparing Channeling Between the 5E and 4E Cascade

Comparing the Different Cascades



S.L. Hooe, T. Tschirhart, S.A. Walper, J.C. Caruana, E.R. Goldman, K. Susumu, E. Oh, S.N. Dean, J.C. Breger, I.V. Schweigert, I.L. Medintz and G.A. Ellis (*under review*)

Enzyme Assembly for Enhanced Cofactor Recycling



A 200-fold excess peptide/QD addition enhanced individual GDH and LDH on-QD k_{cat} a further 2- and 1.5×, respectively, above that seen just by QD display to a maximum of ~10-fold GDH enhancement.

J. Breger, E.R. Goldman, K. Susumu, E. Oh, C.M. Green, I.L. Medintz and G.A. Ellis; *Nanoscale*, **2023**,15, 10159-10175

Acknowledgements

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