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Transforming ENERGY

β-ketoadipic acid production in P. putida for performance-advantaged nylons

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Acknowledgements

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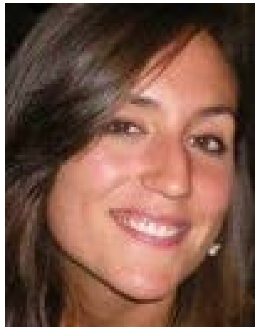
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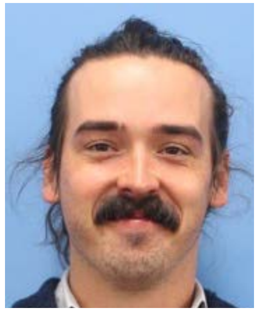
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Today's Papers

Joule

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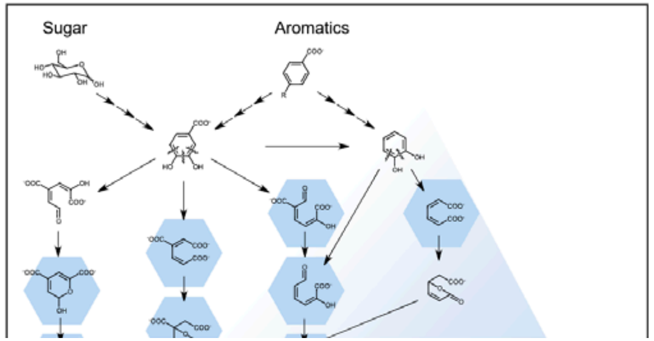
Article

Innovative Chemicals and Materials from Bacterial Aromatic Catabolic Pathways



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HIGHLIGHTS

Pseudomonas putida w
engineered to produce
intermediates of aroma
catabolism

Cell Reports
Physical Science

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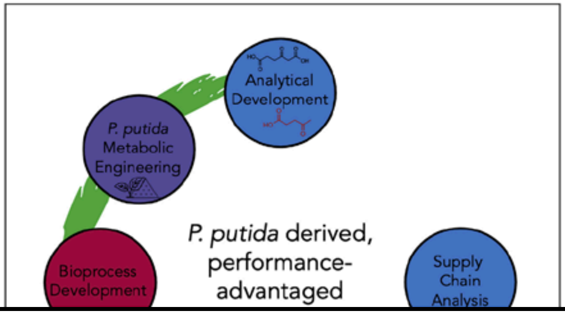
Article

Production of β-ketoadipic acid from glucose in *Pseudomonas putida* KT2440 for use in performance-advantaged nylons



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Highlights

Pseudomonas putida is engineered to produce β-ketoadipic acid (βKA) from glucose

βKA-nylon exhibits enhanced

PLASTICS

REVIEWS

Check for updates

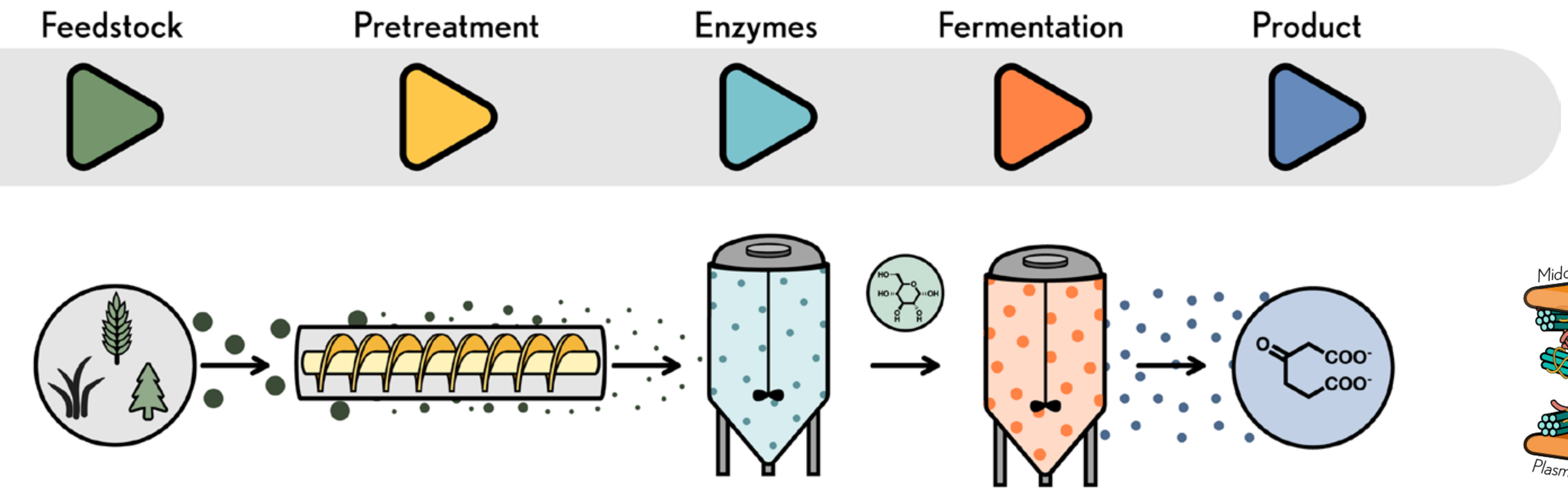
Bio-based polymers with performance-advantaged properties

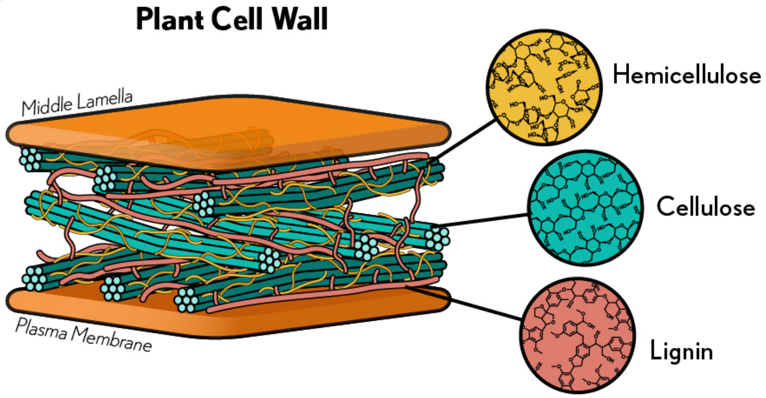
Robin M. Cywar[1,2], *Nicholas A. Rorrer*[2], *Caroline B. Hoyt*[2], *Gregg T. Beckham*[2] and *Eugene Y.-X. Chen*[1]

Abstract | Bio-based compounds with unique chemical functionality can be obtained through selective transformations of plant and other non-fossil, biogenic feedstocks for the development of new polymers to displace those produced from fossil carbon feedstocks. Although substantial efforts have been invested to produce bio-based polymers that are chemically identical to and directly replace those from petroleum, a long-pursued goal is to synthesize new, sustainable, bio-based polymers that either functionally replace or exhibit performance advantages relative to incumbent polymers. Owing to anthropogenic climate change and the environmental consequences of global plastics pollution, the need to realize a bio-based materials economy at scale is critical. To that end, in this Review we describe the concept of performance-advantaged, bio-based polymers (PBPs), highlighting examples wherein superior performance is facilitated by the inherent chemical functionality of bio-based feedstocks. We focus on PBPs with C–O and C–N inter-unit chemical bonds, as these are often readily accessible from bio-based feedstocks, which are heteroatom-rich relative to petroleum-derived feedstocks. Finally, we outline guiding principles and challenges to aid progress in the development of PBPs.

Most of Today's Information Will Be Taken From Three Publications

NREL's Approach to New Polymers





Images courtesy of Rita Clare, Formerly NREL

NREL Takes a Holistic Approach to Biomass Conversion

Our work attempts to enable the biorefinery by utilizing the entirety of biomass

- Recent work has also included the conversion of 'waste' plastics (e.g. PET) into the same monomers

Early work focused on direct replacements (e.g. Adipic and Terephthalic acid) however, as our worked evolved we started to target "*Performance Advantaged Bioproducts" (PABPs)*

- We classify performance advantages in three areas: **Manufacturing, Performance, End-of-Life**

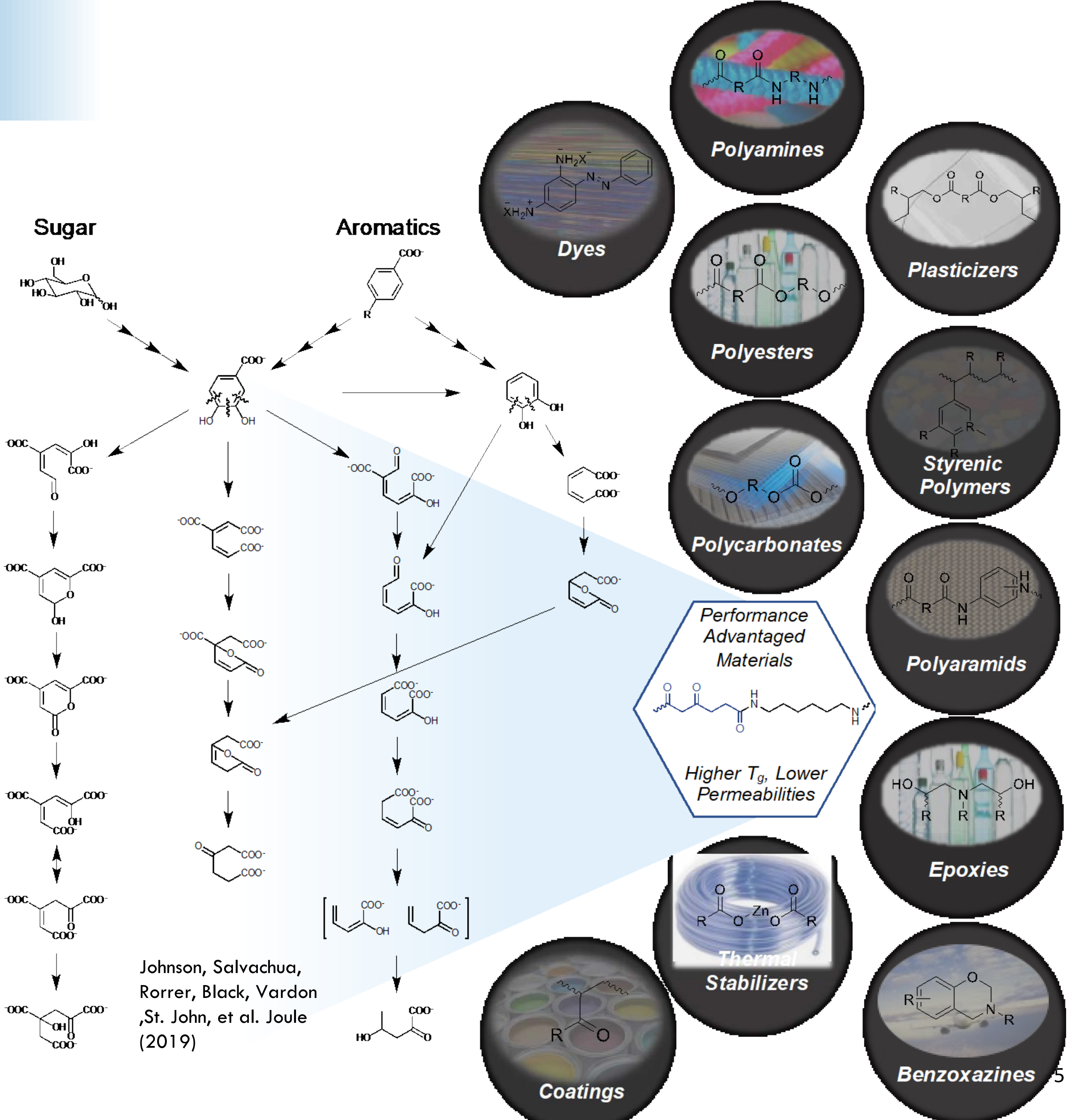
A Large Design Space

Chemical, biological, and hybrid transformation offer plenty of unique monomers

The transformation of biomass provides access to a wide variety of functionality such as:

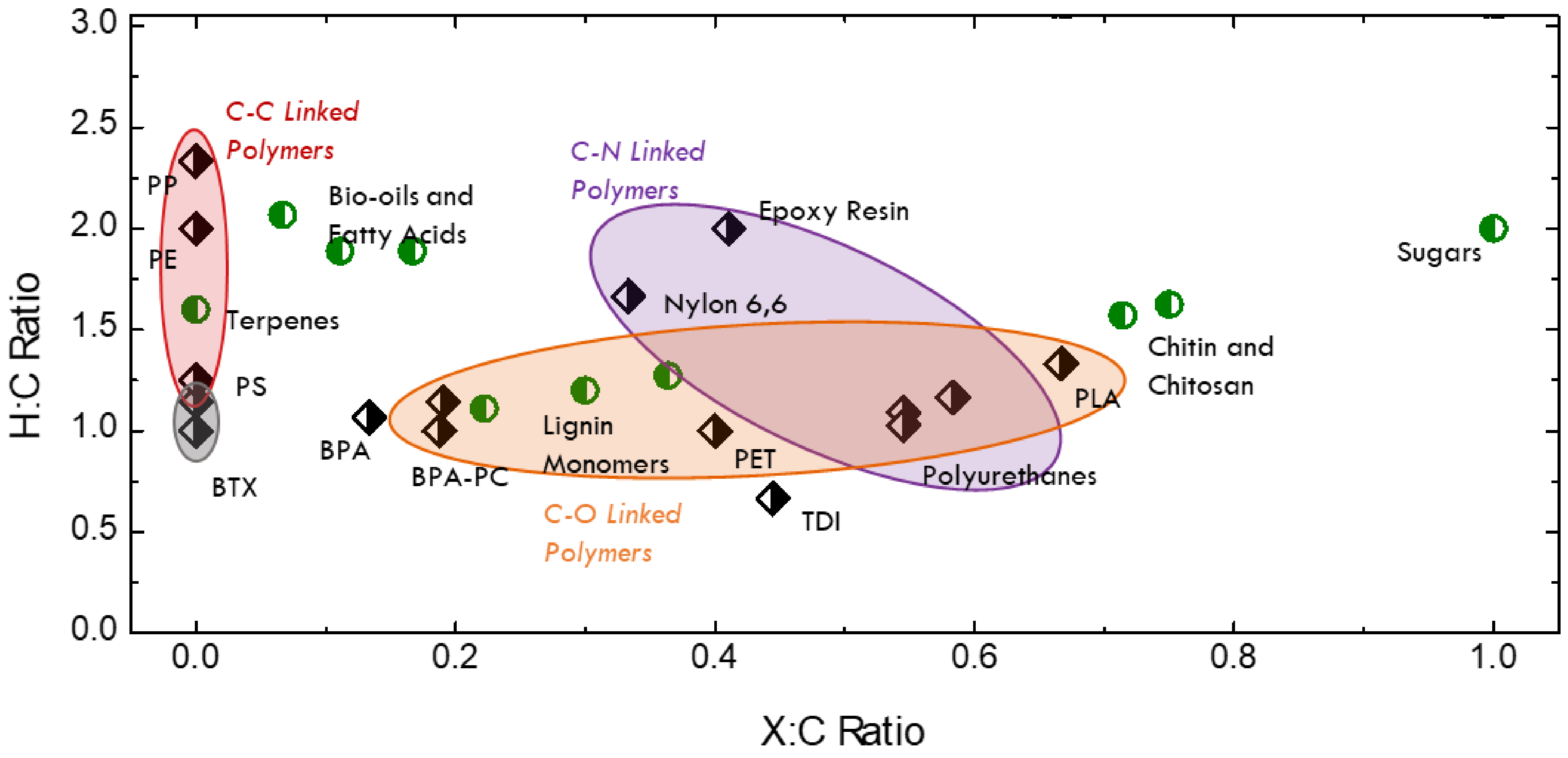
- Carboxylic Acids, including Diacids
- Anhydrides
- Alcohols, including diols and polyols
- Amines, including diamines and multifunctional amines
- Epoxies
- Styrenic Monomers
- Olefinc or Unsaturated Structures

These chemicals provide access to a wide variety of material classes and narrowing the chemical design space is a constant challenge



Johnson, Salvachua, Rorrer, Black, Vardon ,St. John, et al. Joule (2019)

Maintaining Biomass' Functionality to Target Engineering Plastics

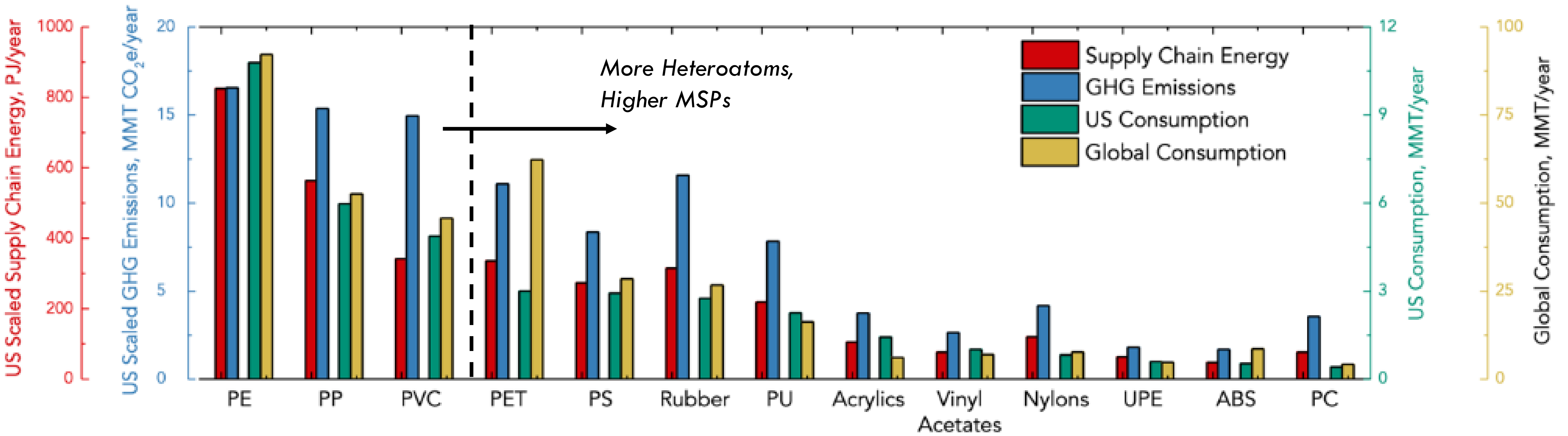


Van Krevelen diagram showing chemical distances of feedstocks to polymers (X is a heteroatom, typically oxygen or nitrogen)

Maintaining Biomass' Functionality

- Adding or removing functionality, especially heteroatom functionality, from chemicals (biobased or petrochemical) requires energy and emits GHG
- Thus, the heteroatom functionality of biomass makes it ideal for PABPs, notably performance polymers

Maintaining Biomass' Functionality to Target Engineering Plastics

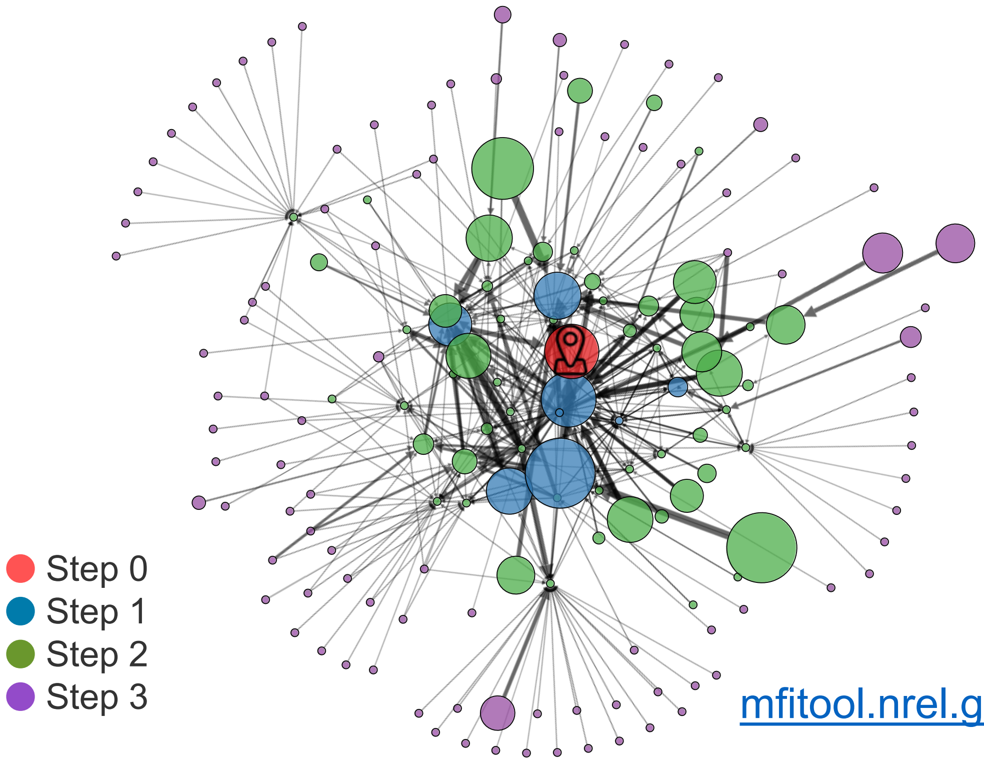


From Nicholson, Rorrer, Joule 2021

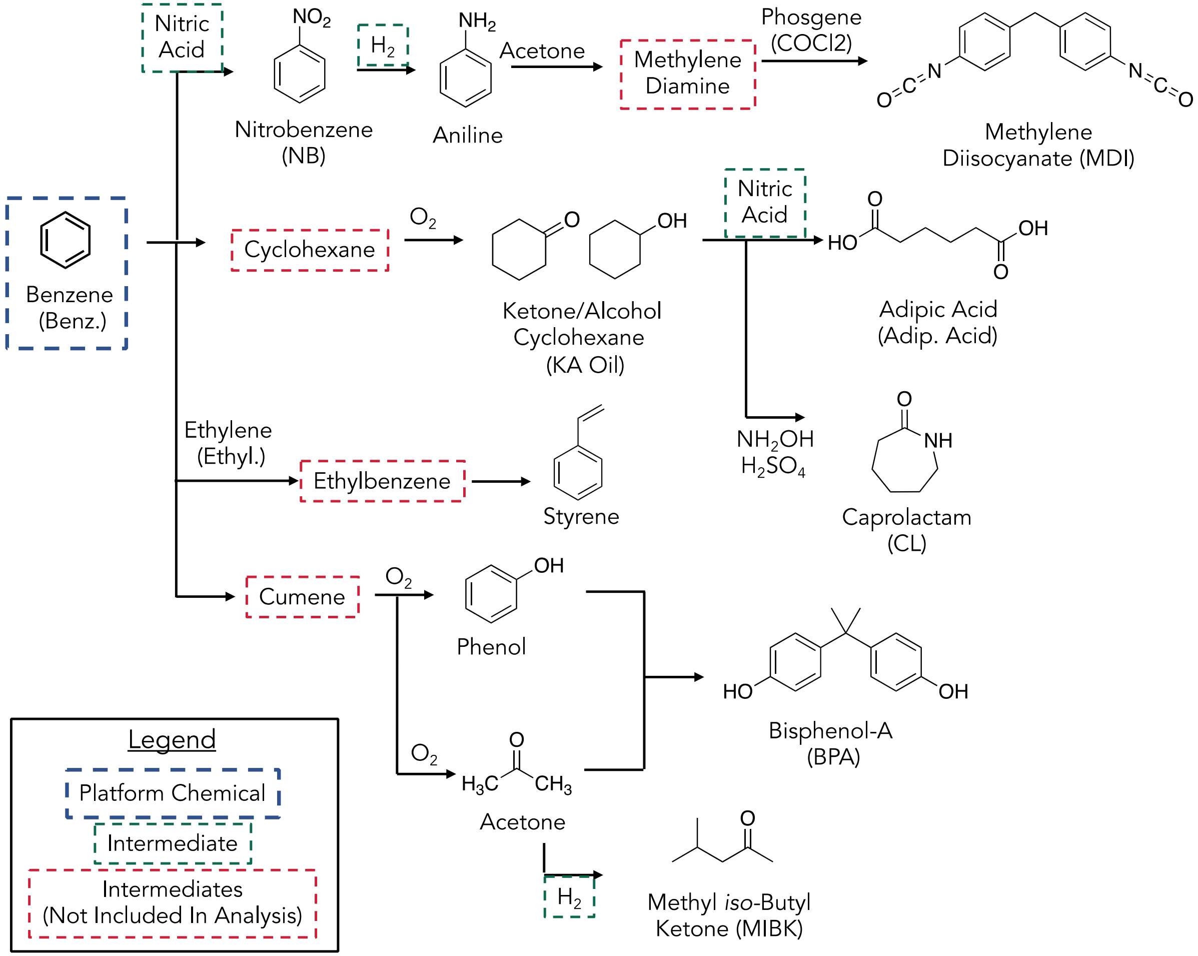
Heteroatom Containing Polymers Are Formulated For Specific Applications

Benchmarking chemicals production to identify PABP targets

MFI models chemicals production as steps of supply chain inputs to the final product



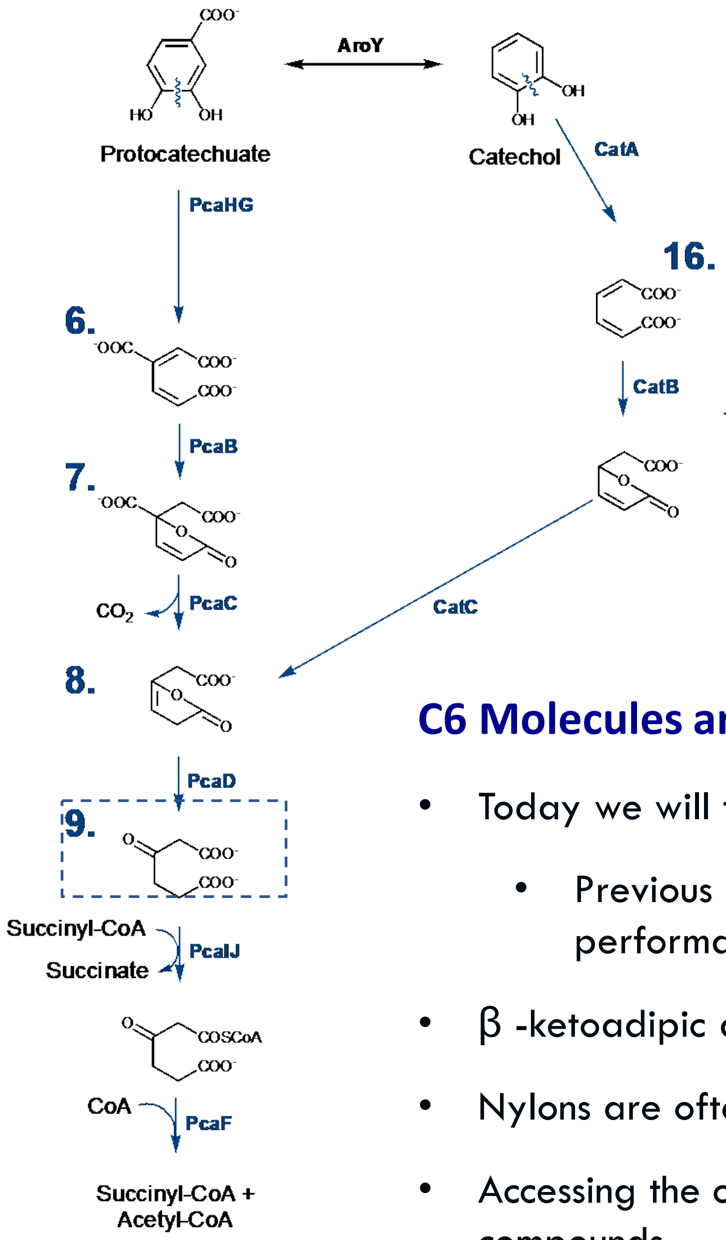
mfitool.nrel.gov

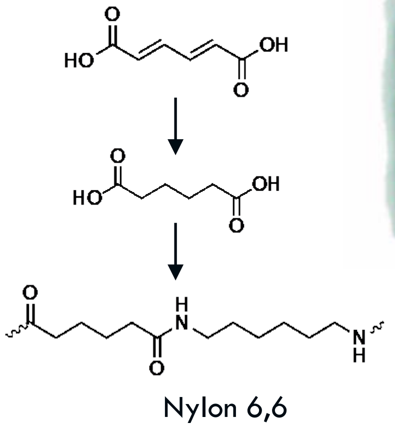


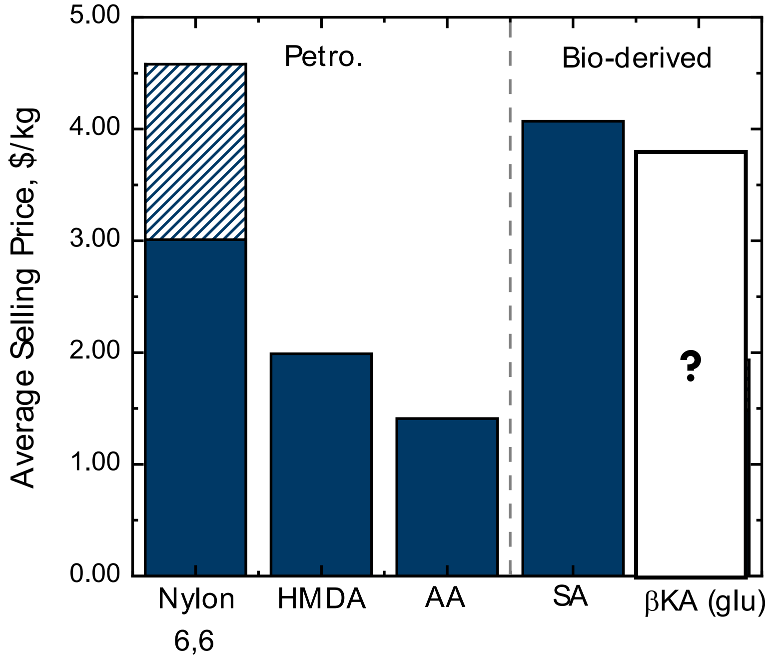
Using MFI to estimate supply chain energy & GHG emissions of today's commodity chemicals

- Analogous to plastics benchmarking analysis in BOTTLE (Nicholson *et al. Joule* 2021)
- PABP targets can be benchmarked against these data for supply chain energy and GHG emissions

C6 Dicarboxylic Acids are Natural Polymer Targets



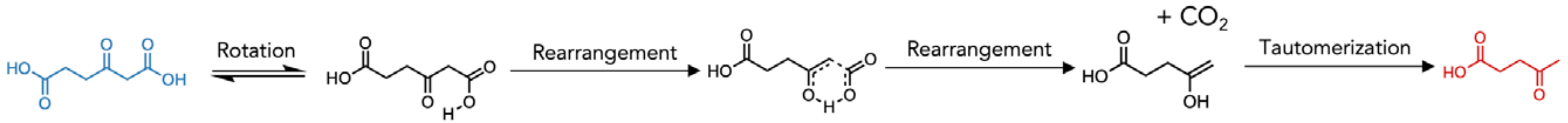


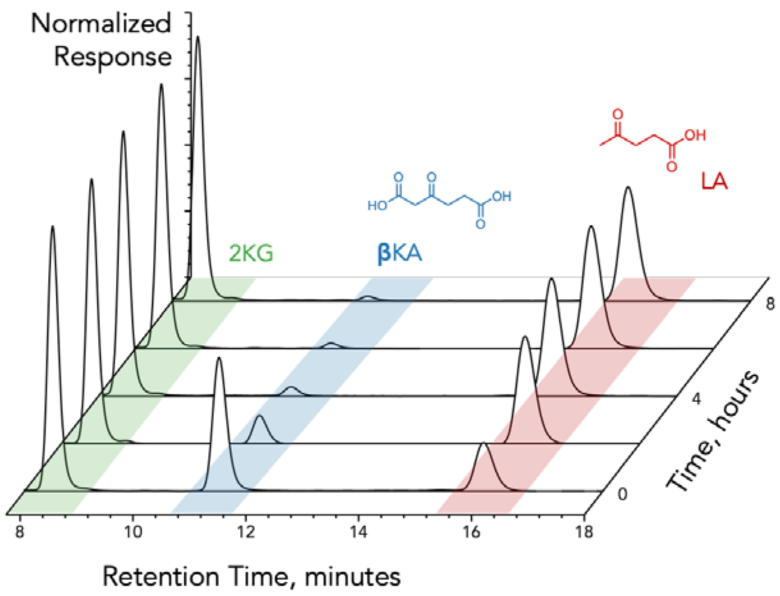


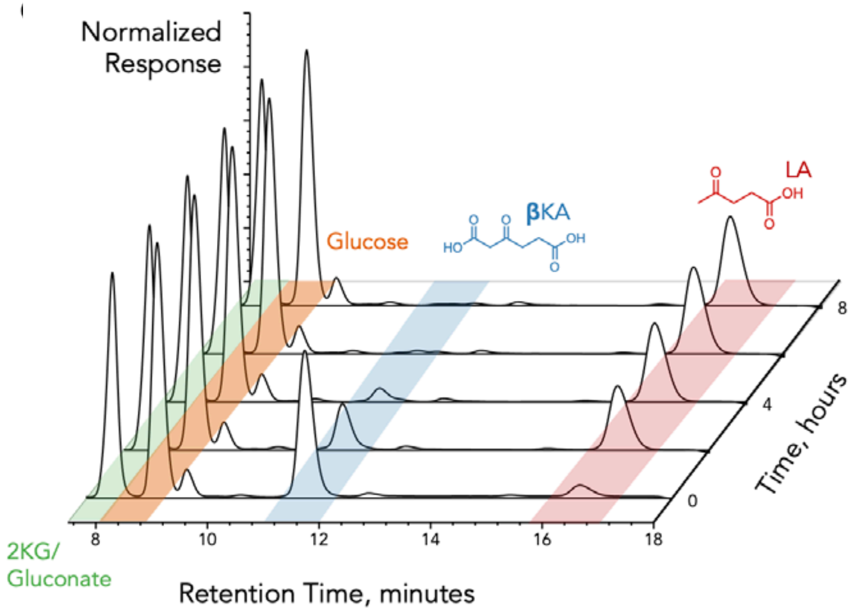
C6 Molecules are Ideal for Multiple Polymers Including Nylons

- Today we will focus on β-ketoadipic acid (βKA) as
  - Previous work from our group and others has focused on muconic acid and its diene structure as a direct and performance advantaged replacement
- β -ketoadipic acid has a ketone that may enable greater polymer rigidity
- Nylons are often highly formulated and can demand price premiums for performance
- Accessing the compounds through petrochemical routes is not easy, so thus we must develop strategies to produces these compounds.

Analytical Development for βKA



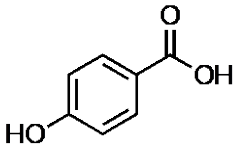




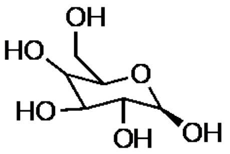
βKA Quantification is Not Straightforward

- βKA can decarboxylate abiotically to levulinic acid
    - This will require care in polymerizations and separations
- In order to enable quantification, we forced βKA to levulinic acid and ensured that it did not overlap with other metabolites

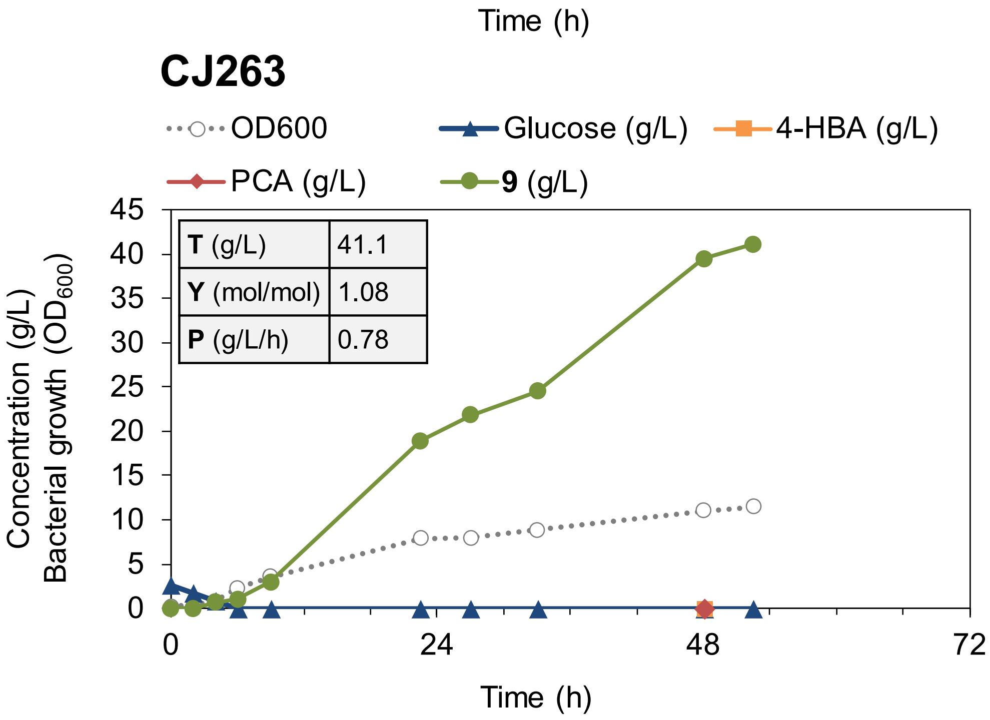
βKA Production from Aromatic Compounds

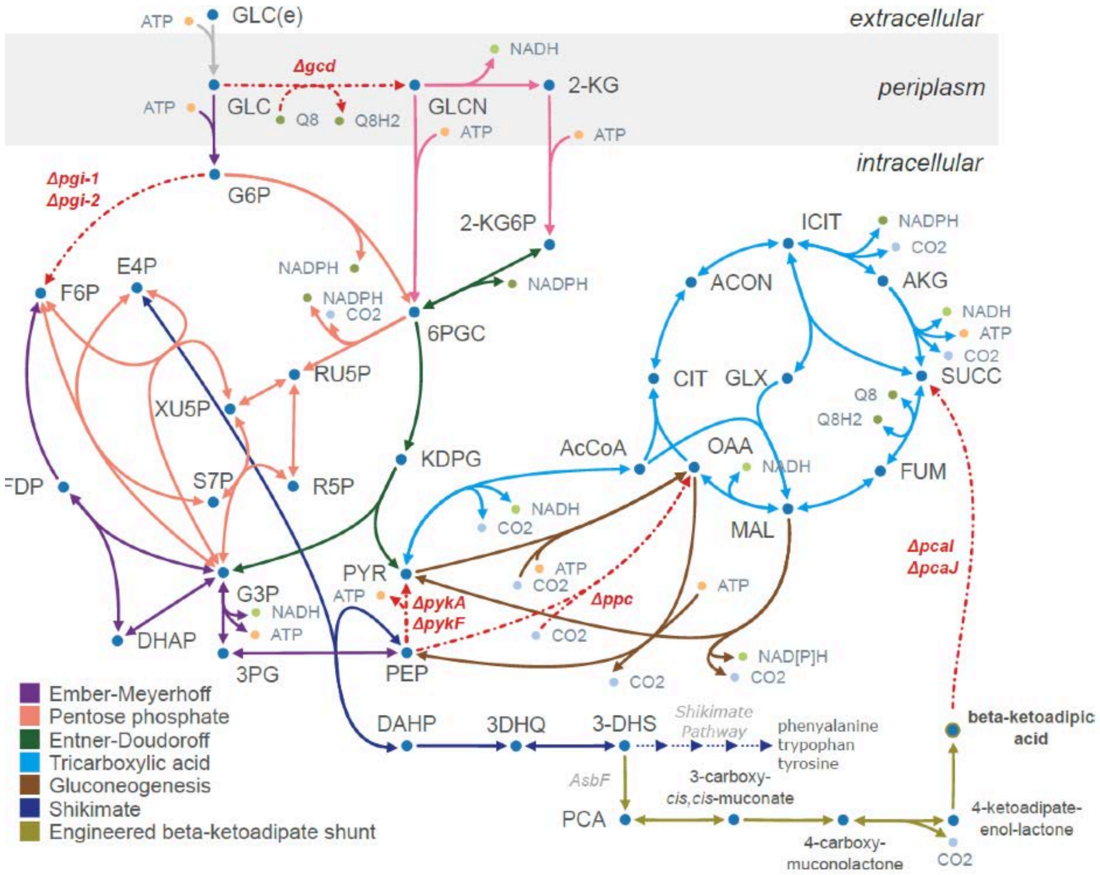


For Production



For Growth

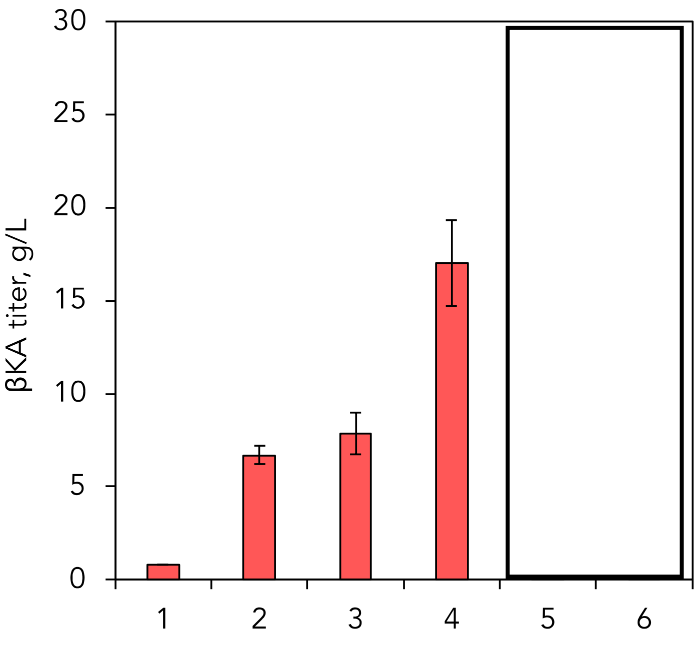


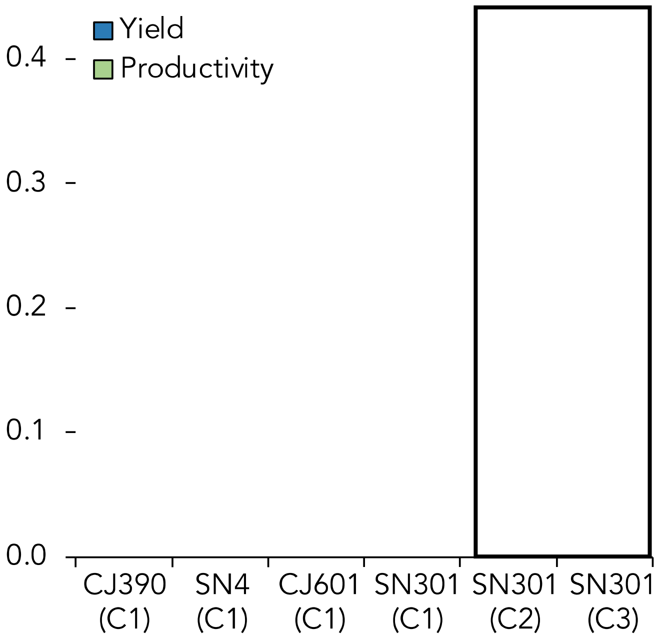


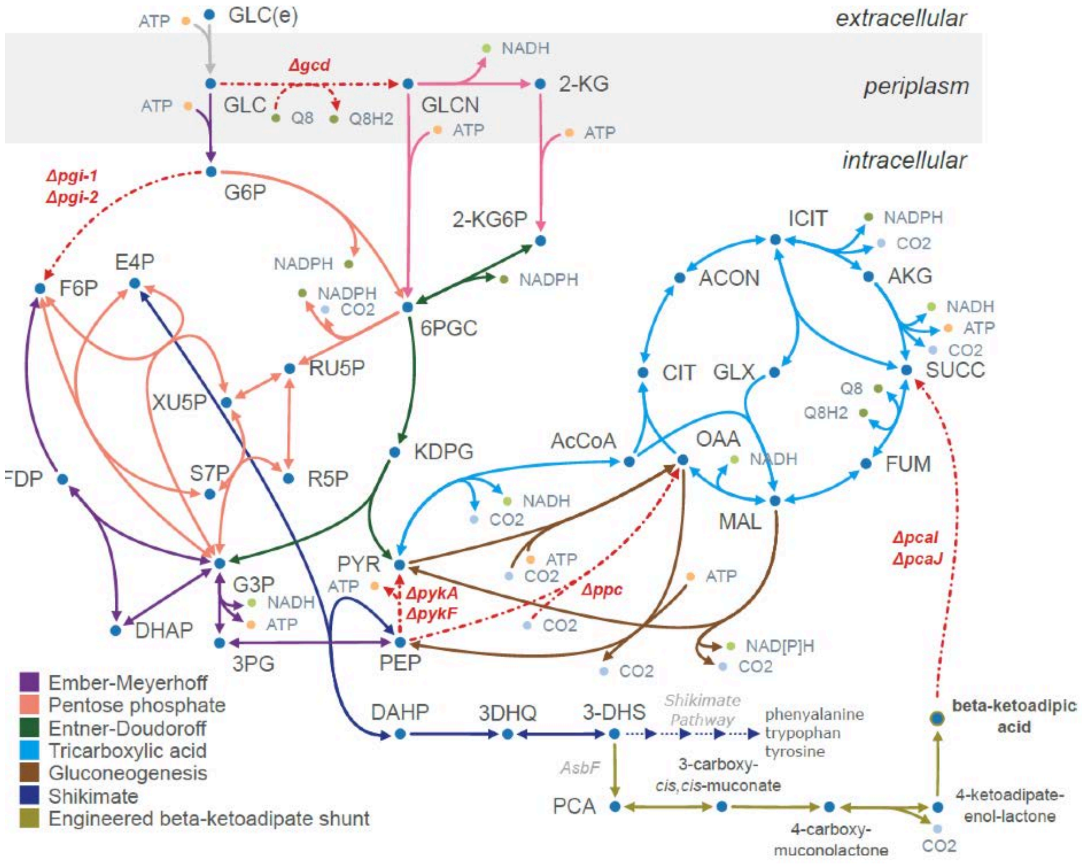
βKA Was Initially Demonstrated from Aromatic Compounds

- Strains were initially engineered to produce βKA in *Johnson et al.* in which high yields, titers, and moderate productivities were achieved
- However, there are advantages to demonstrating molecule production from sugars (e.g. single carbon source, higher TRL, etc.)

βKA Production from Glucose



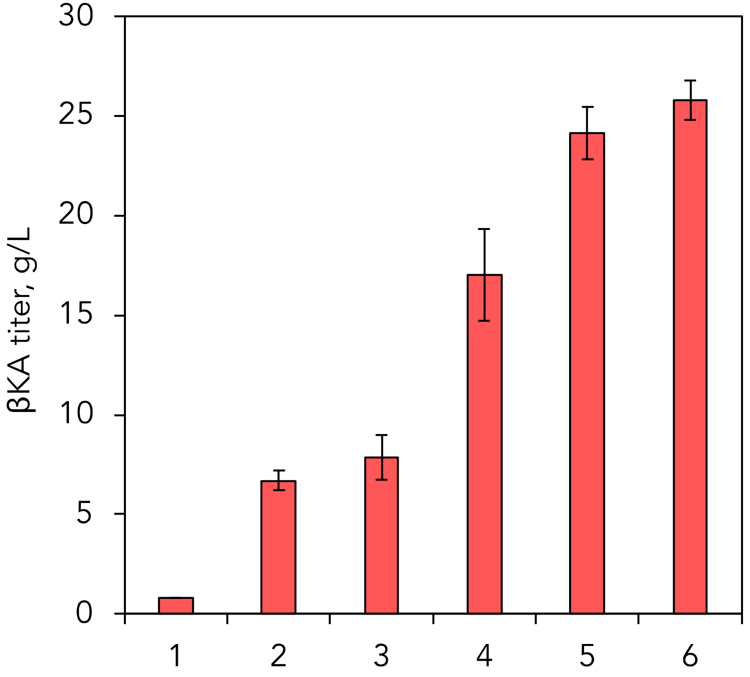


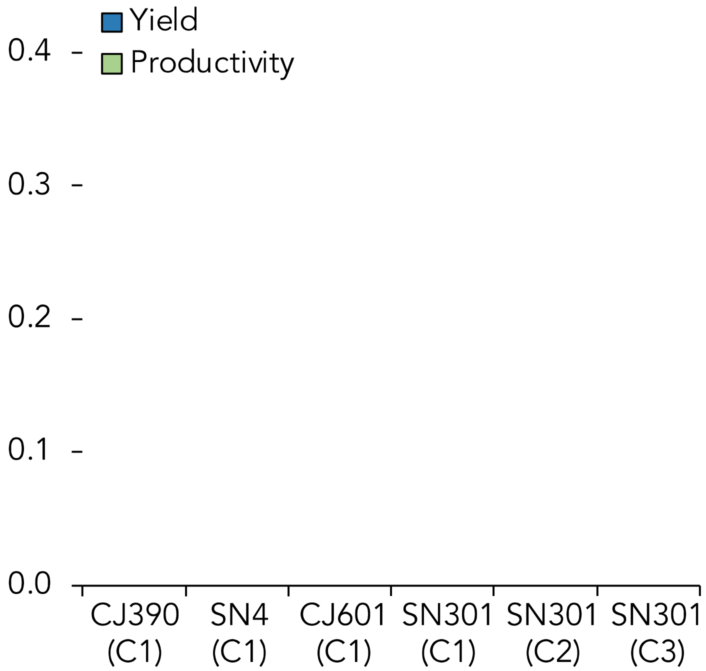


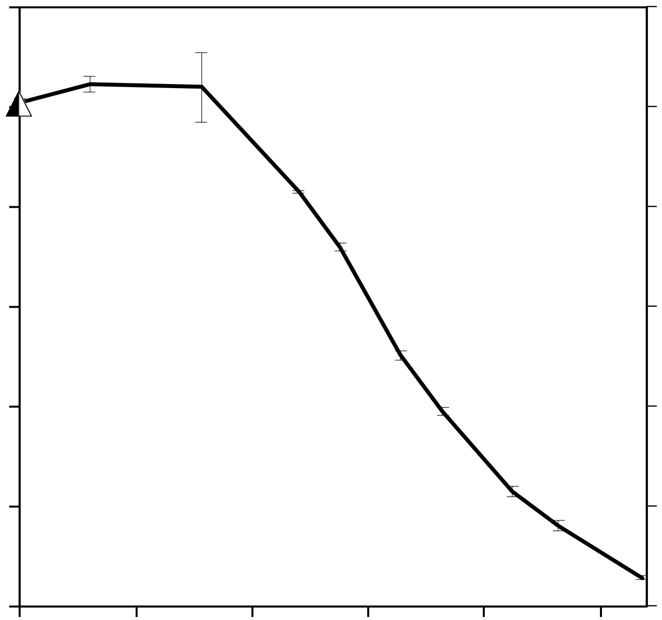
βKA Metabolic Engineering Required Multiple Evolutions

- Initially, *AsbF* was introduced to convert 3-dehydoshikamate into protocatechuate to enable conversion into βKA (CJ390). Further engineering applied previous learnings from muconic acid production to increase production (SN4)
- Two other strains, SN4 and CJ601, accumulate metabolic intermediates (e.g. 2-ketogluconate) and *HexR*, a transcriptional repressor, was deleted to yield our final strain SN301

βKA Production from Glucose



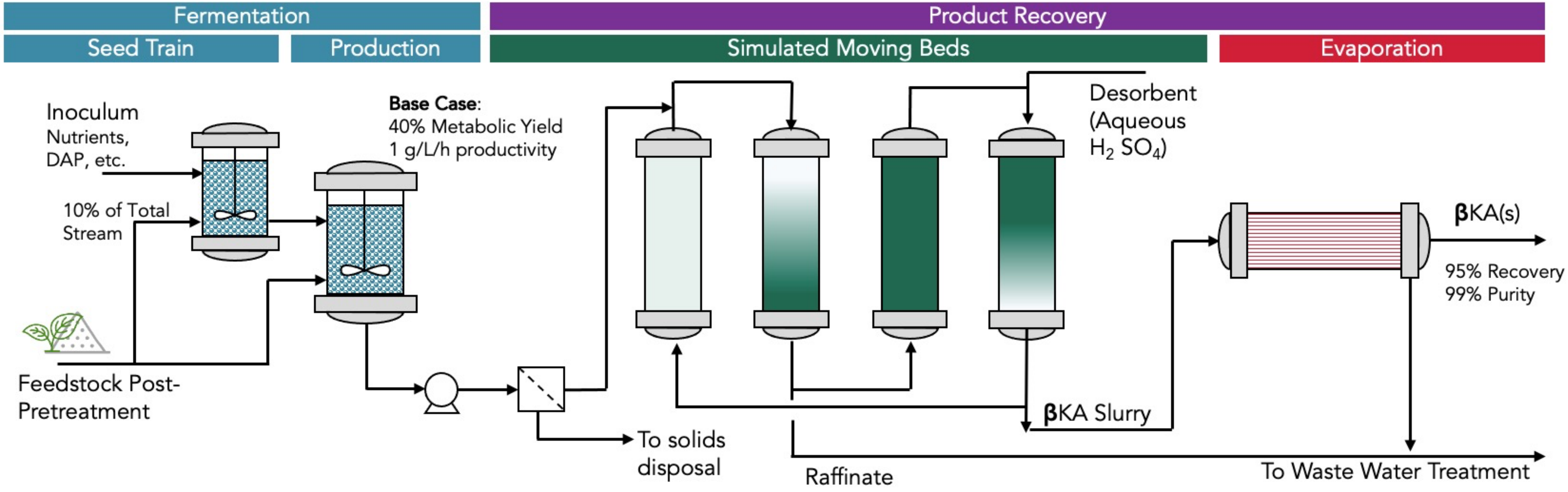




Bioreactor Feed Strategy Can Also Yield Improvements in Titer

- Multiple feed strategies were implemented to resemble industry relevant strategies
  - C1 – Fed Batch, 1L Volume, C2 – Fed Batch, 1.5L Volume, C3 – Batch with Non-washed cells
- Optimum conditions resulted in a 26 g/L titer, 0.21 g/L/h productivity, a 36% yield, and near complete glucose consumption

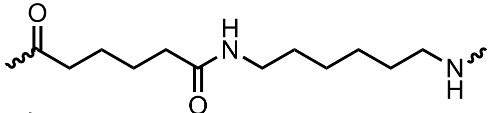
Scale up and Separation of βKA



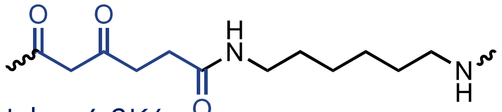
βKA was recovered from fermentations for all subsequent polymer work

- On a lab scale this was accomplished by acidification, liquid-liquid extraction, and rotovap
- This process will be modeled later in this presentations where the implemented separation method is simulated moving beds

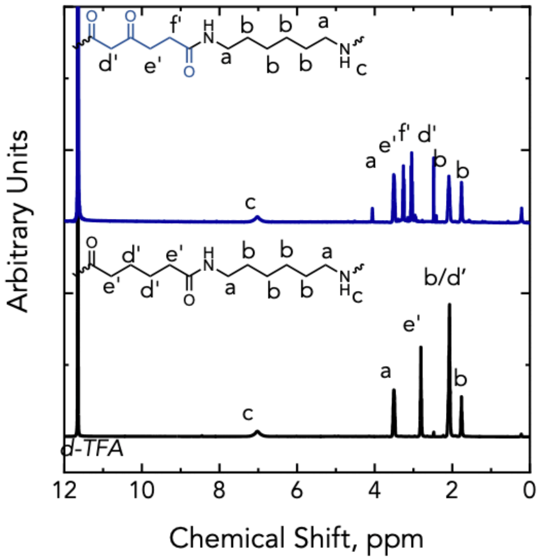
Performance-advantaged nylons enabled by β-keto diacids

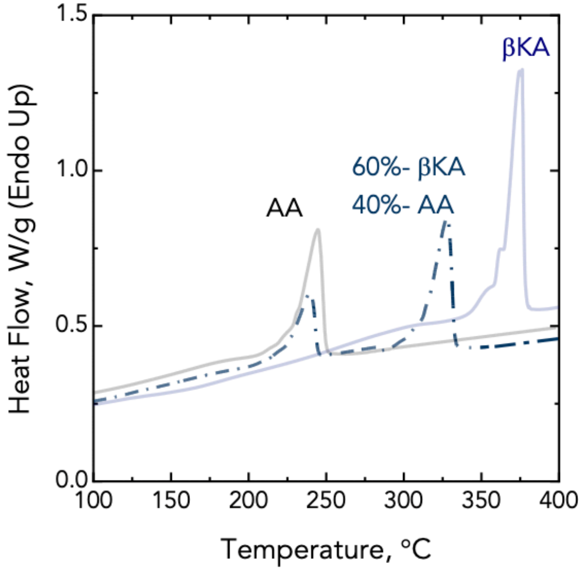


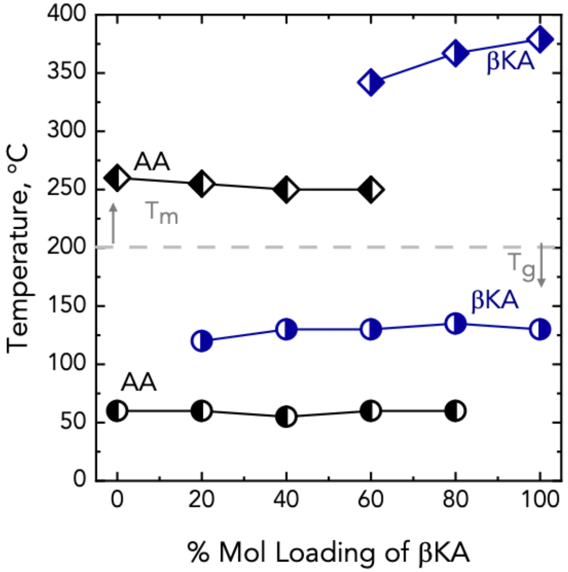
Nylon 6,6 1.01 g*mm/m²*day, T_g = 60°C



Nylon 6,βK6 0.8 g*mm/m²*day, T_g = 130°C

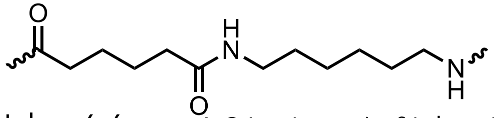






C6 dicarboxylic acids with β-ketone enable enhanced nylon performance

- Nylon 6,6, analogues were synthesized by multiple methods including an industry relevant salt polymerization, the use of acyl chlorides, and other couplings to avoid decarboxylation
- NMR indicates no imine formation while DSC reveals blocky behavior in co-polymers
- The use of βKA reduced water permeability by 20% while simultaneously increasing the T_g by 216% in the homopolymer



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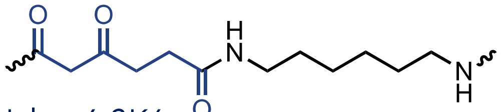
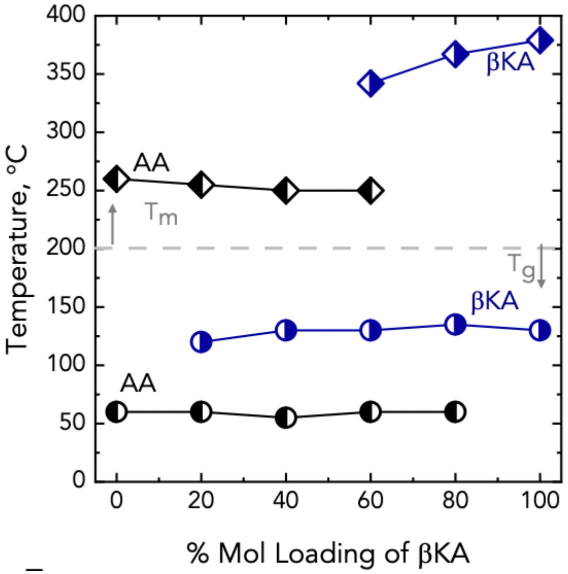
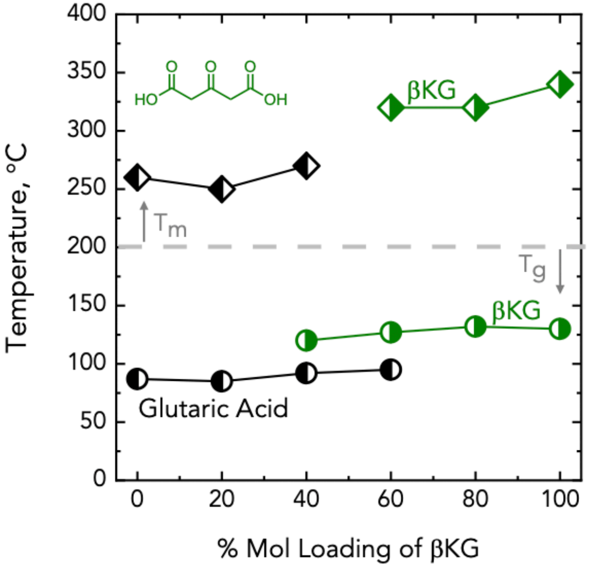
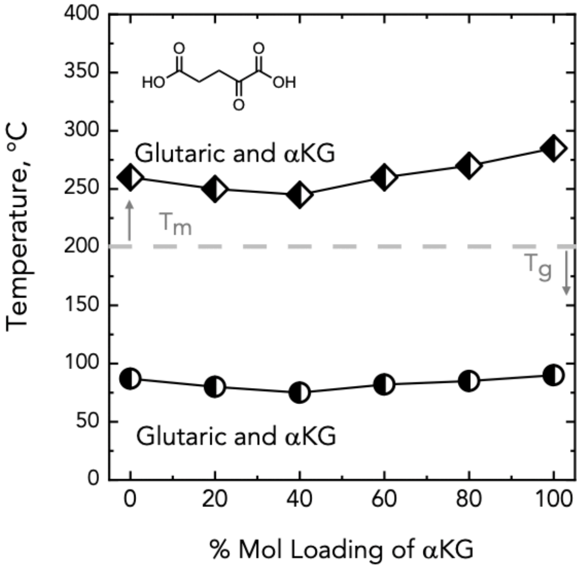


Nylon 6,βK6 0.8 g*mm/m²*day, T_g = 130°C



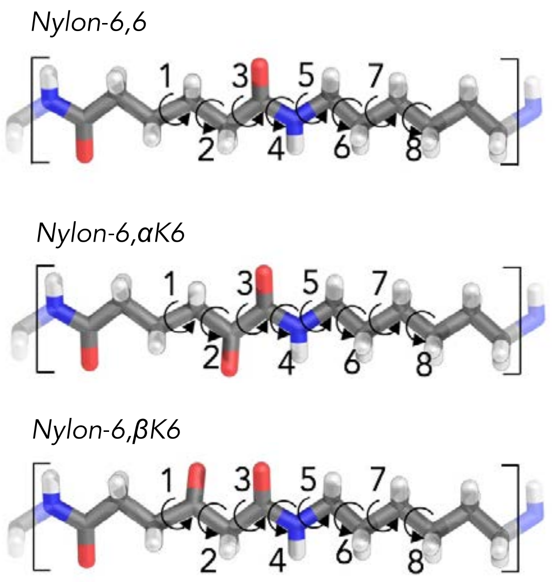


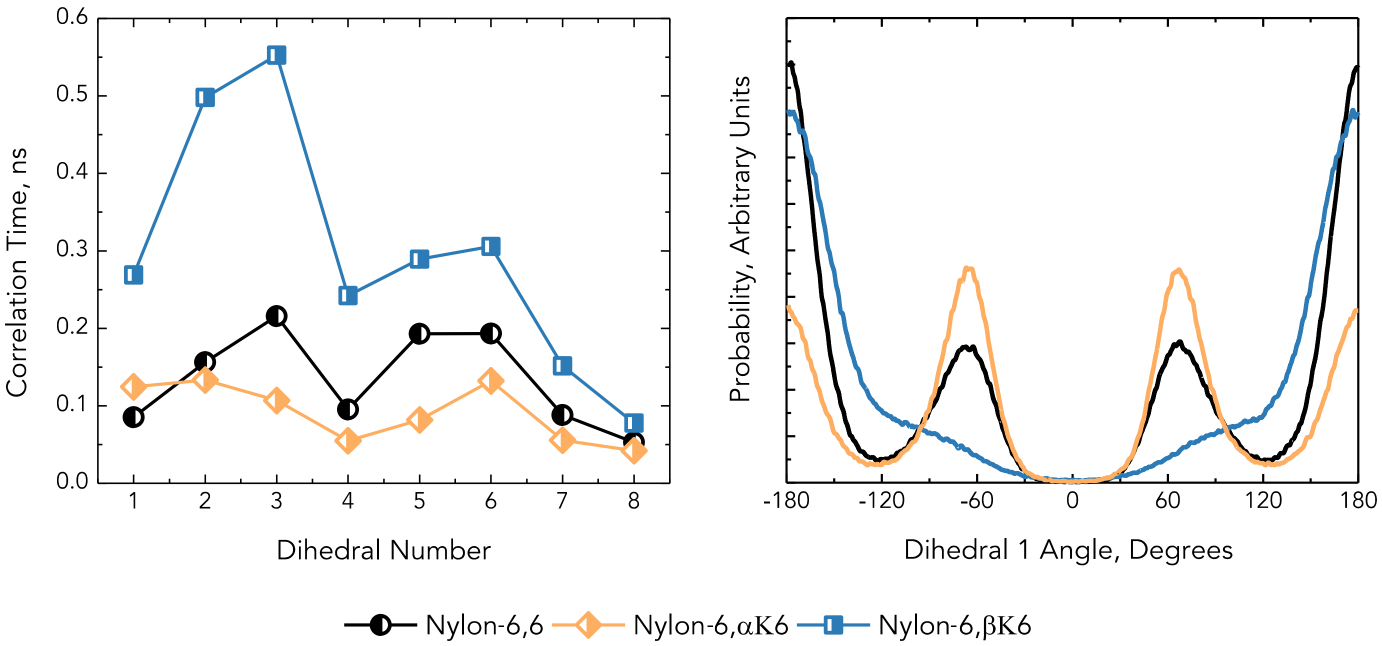


The β-ketone enables enhanced nylon performance over α-ketones

- We expanded our diacid suite to include other keto diacids to be used in polymerization
- β-ketoglutaric acid demonstrates similar thermal trends to βKA while α-ketoglutaric acid does not exhibit the same trends
- These results indicate that the β-ketone may induce further backbone rigidity

Performance-advantaged nylons enabled by β-keto diacids

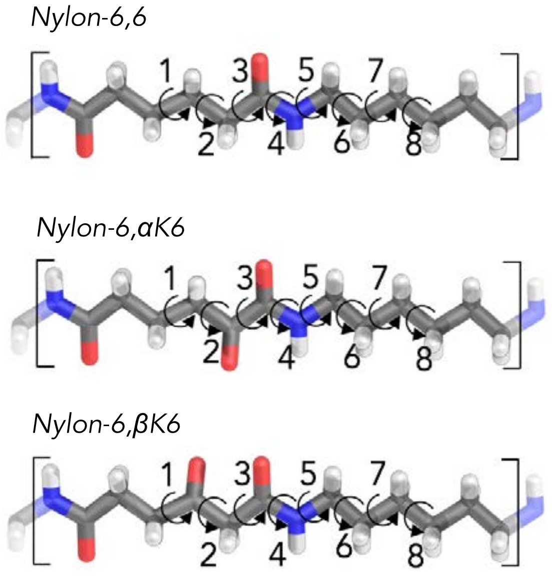


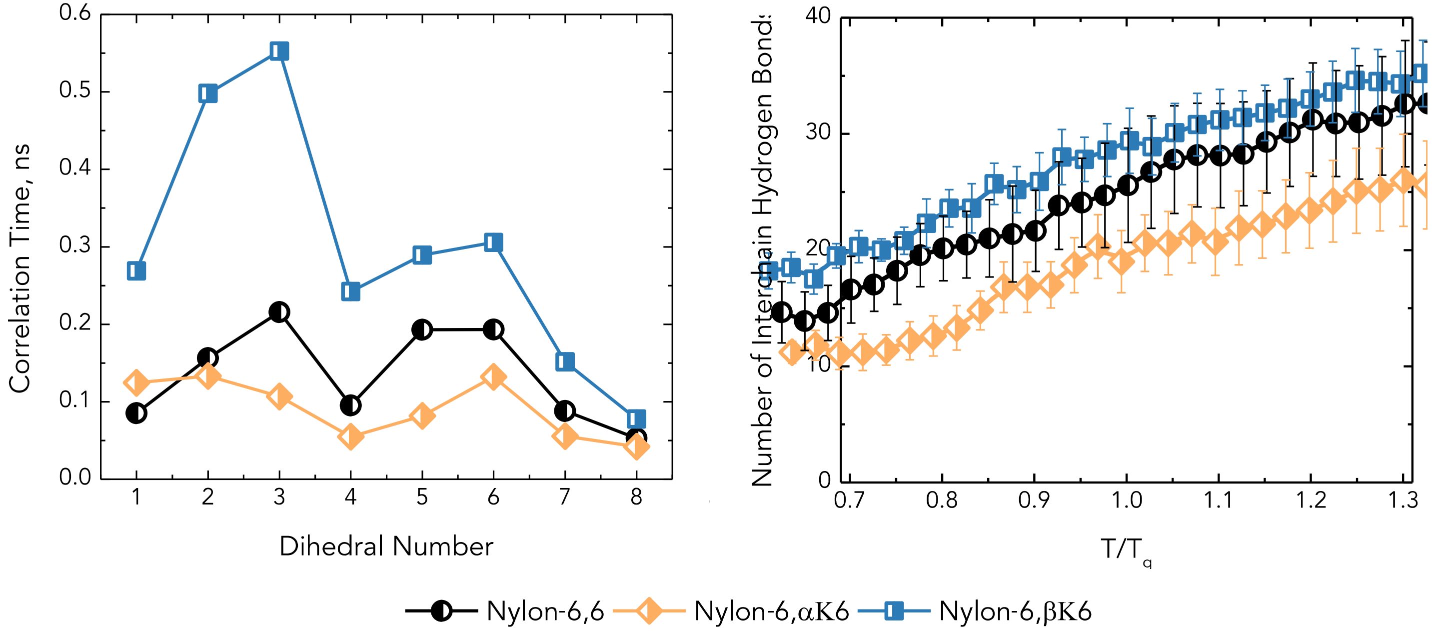


C6 dicarboxylic acids with β-ketones enforce backbone rigidity

- Nylon 6,6, forcefields were generated to ensure that the nylon crystal structure could be replicated
- To understand backbone rigidity, we examined the dihedrals along the polymer backbone centered on the amide bond
- The β-ketone results in longer correlations times and fewer configurations that the polymer backbone can exist in, confirming enhanced rigidity

Performance-advantaged nylons enabled by β-keto diacids

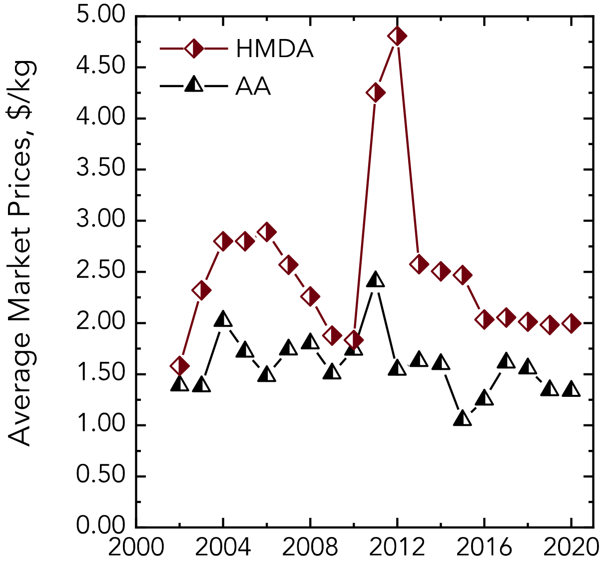


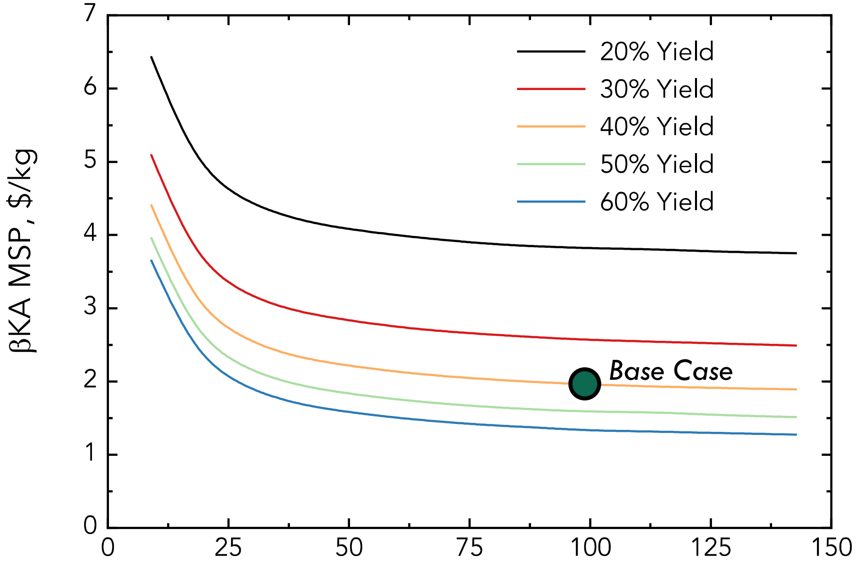


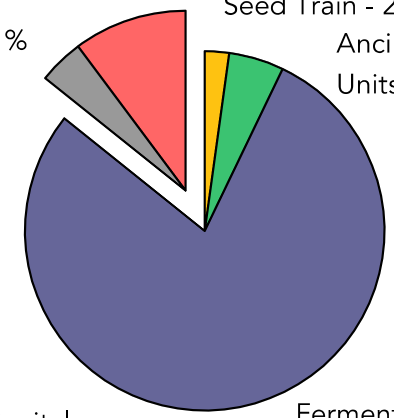
C6 dicarboxylic acids with β-ketones enable enhanced nylon performance

- The enhanced rigidity explains the enhanced $T_{g,}$ but does not *fully* explain the lower permeability
- Thus, we examined the intermolecular interactions between the polymers, namely hydrogen bonding
  - The β-ketone does posses enhanced interactions across a wide thermal range while the α-ketone may interfere with hydrogen bonding

Production of βKA – Base Case



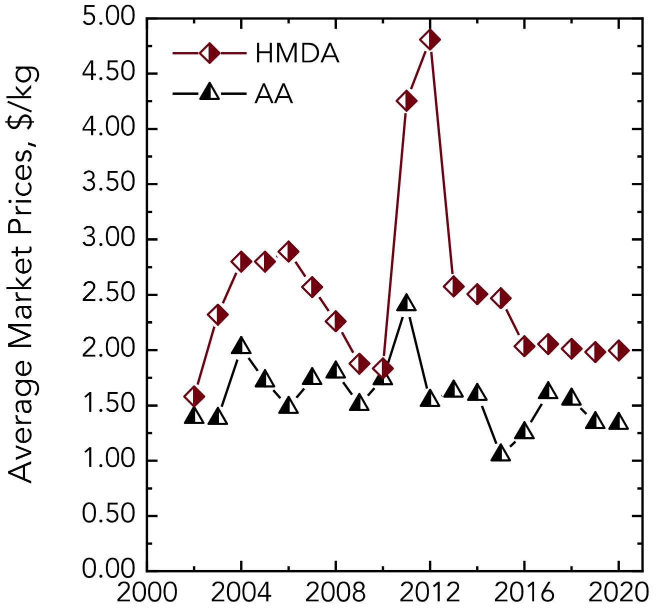


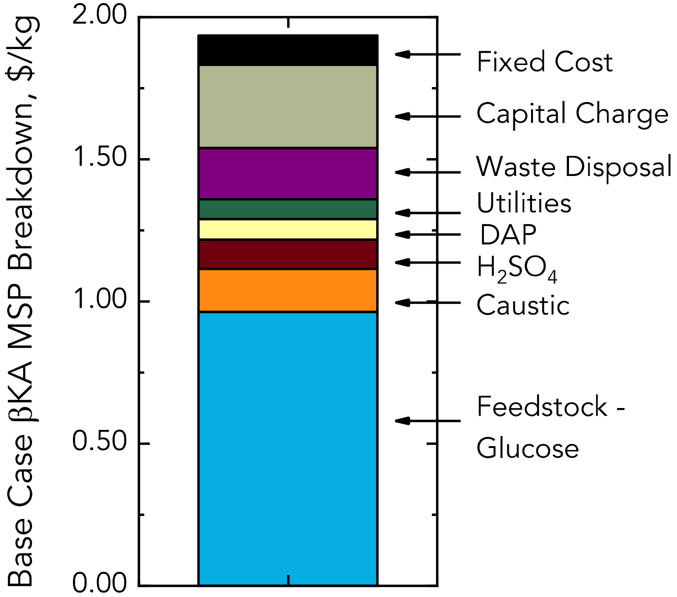


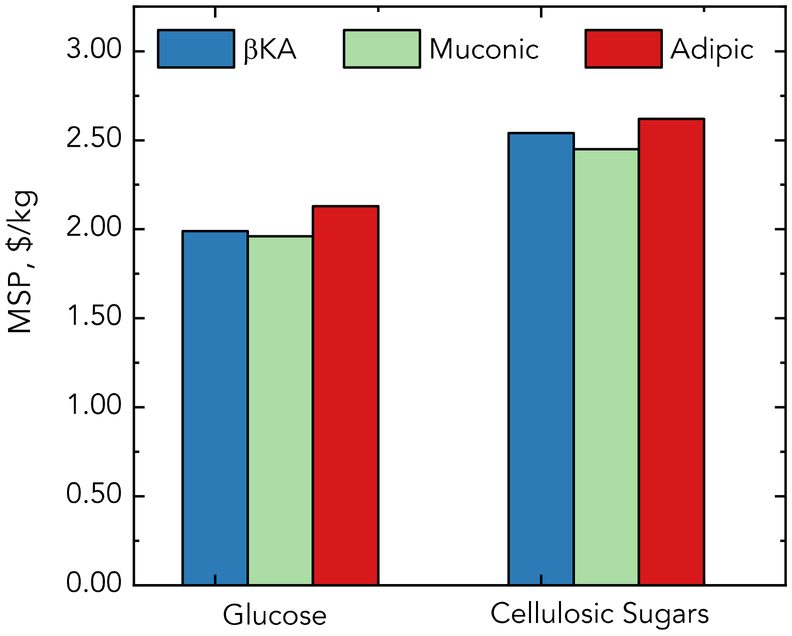
βKA analysis is affected by titer, yield, feedstock and production plant size

- βKA production has baselined relative to adipic acid production
- Most analysis was compared to a base case, which also corresponds to a titer of 1 g/L/h
- Capital expenditures are driven by fermentations which is the result of the size of the fermenters needed

Production of βKA – Minimum Selling Price



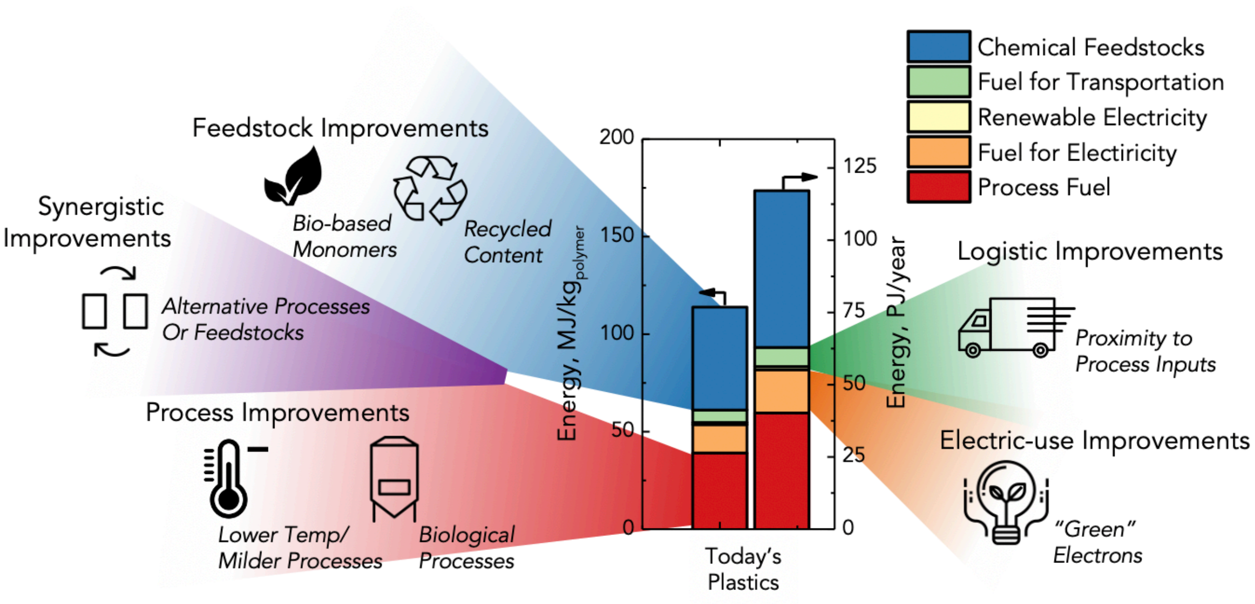


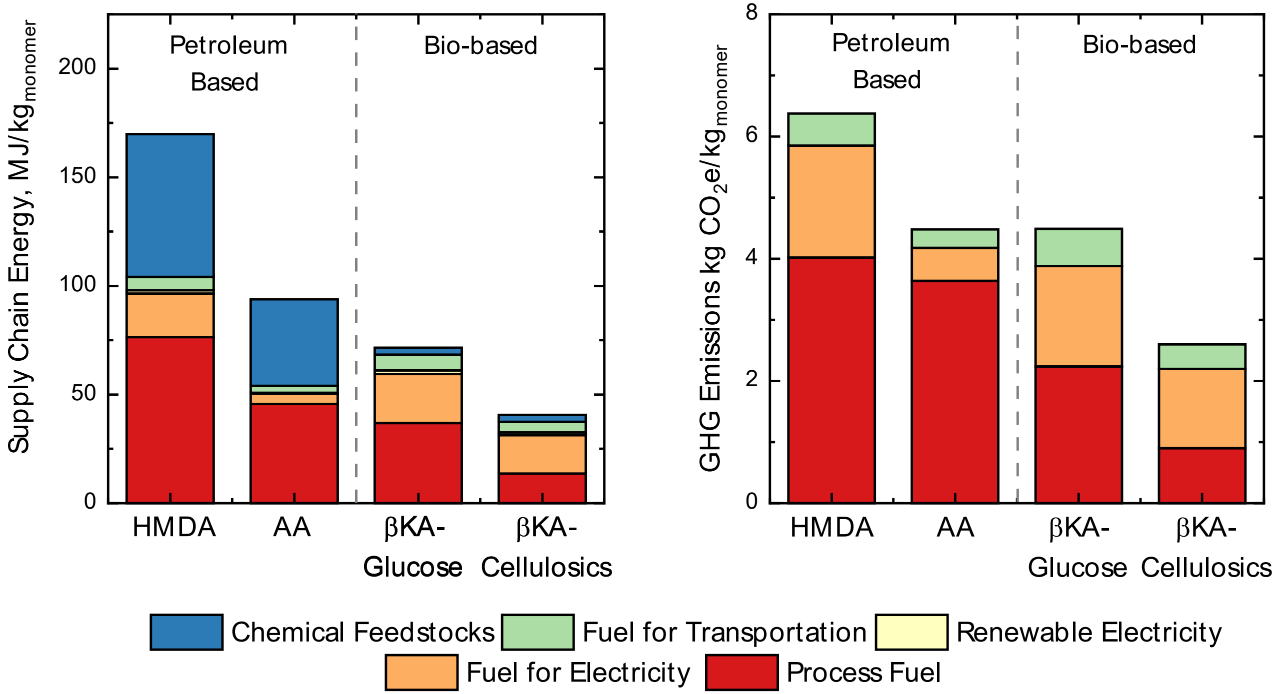


βKA costs more than adipic acid and more from cellulosic sugars

- Feedstock is the largest contributor to cost
- The use of acid and base contribute to multiple other factors
- Different diacids from biological conversion have similar MSPs despite processing alternative separation strategies

Performance-advantaged nylons – advantages in manufacturing

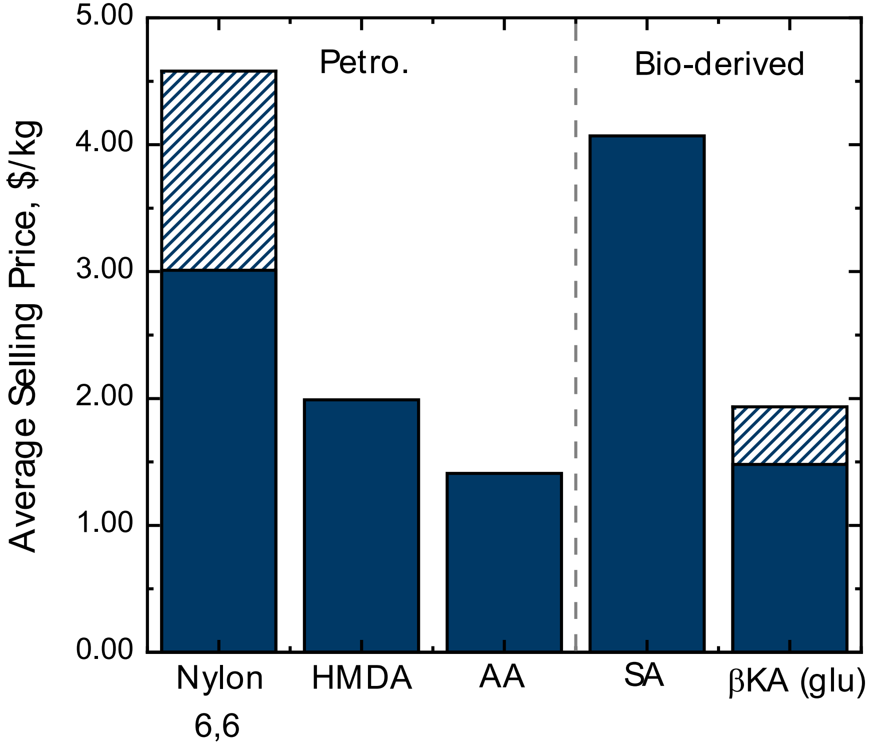


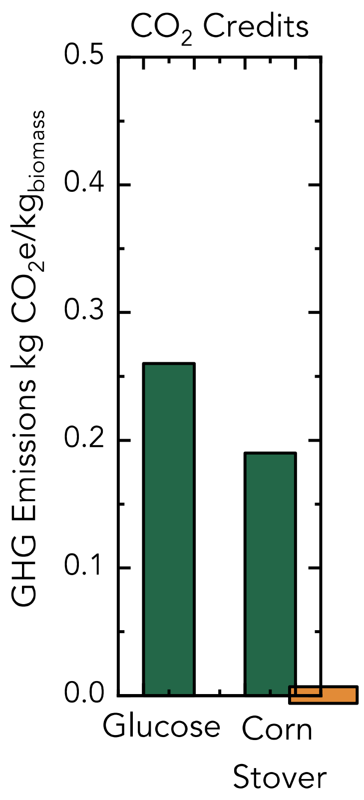


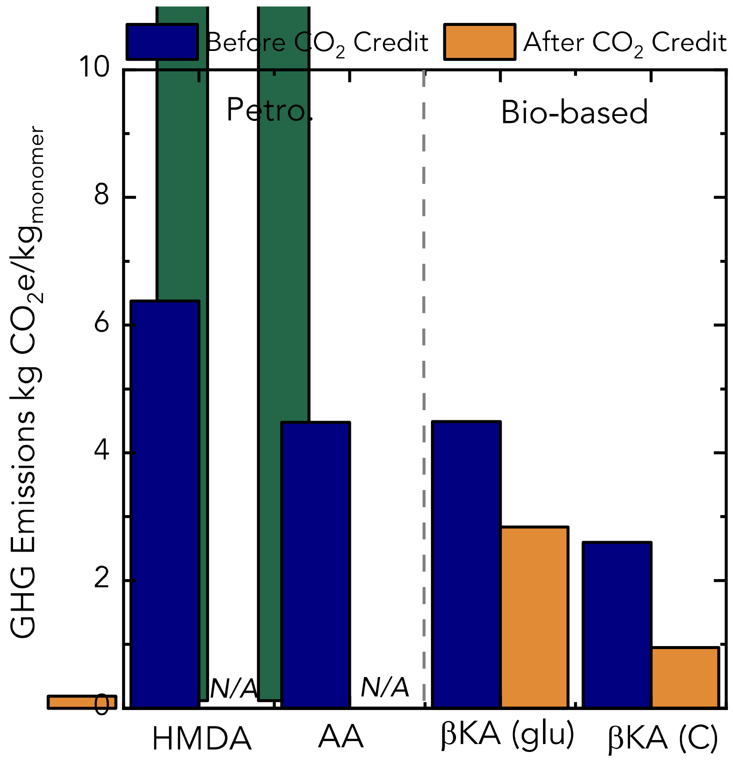
The use of βKA can reduce supply chain energies and GHG emissions

- Supply chain energies and GHG emissions depend on feedstock: in all cases, they are lower for bio-based monomers
- Trends are similar for the produced polymers
- Significant potential to target N-containing monomers in future work (HMDA)

Engineering plastics – An ideal first market







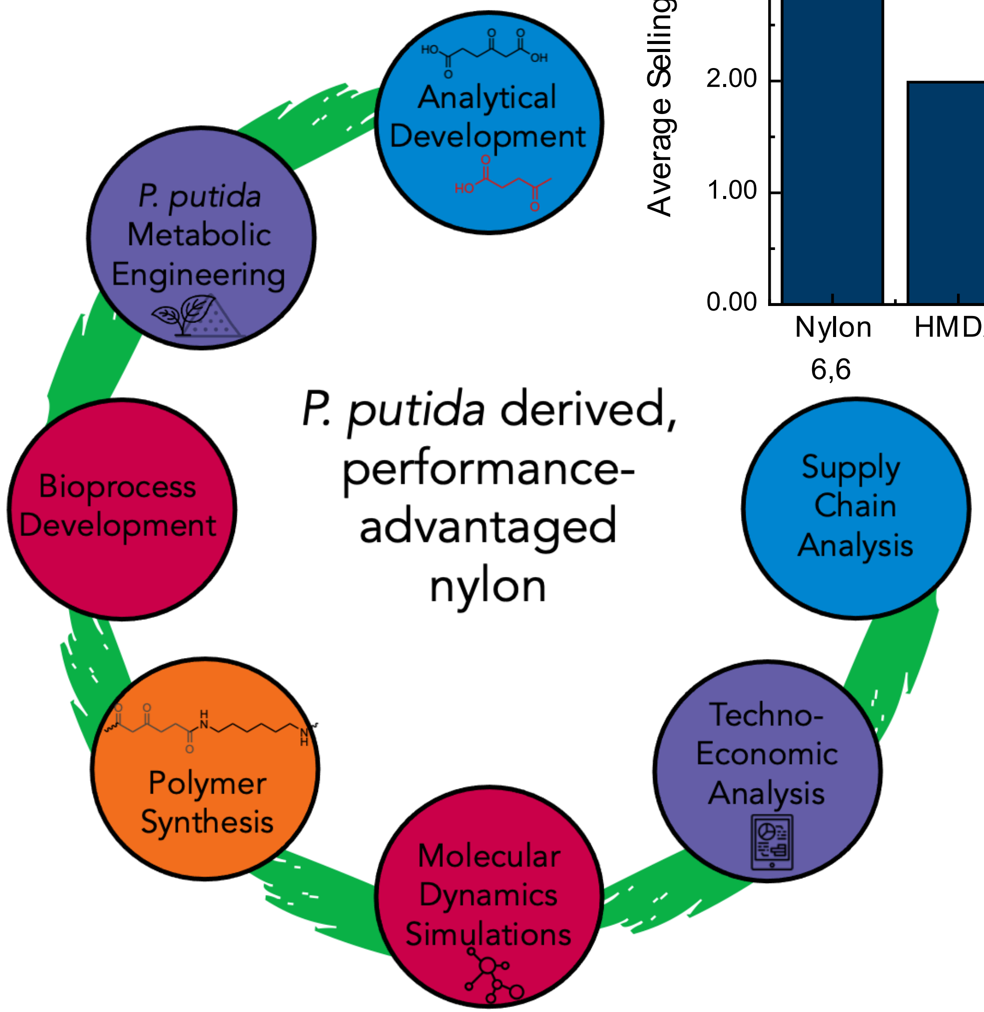
Extend analysis reveals further benefits

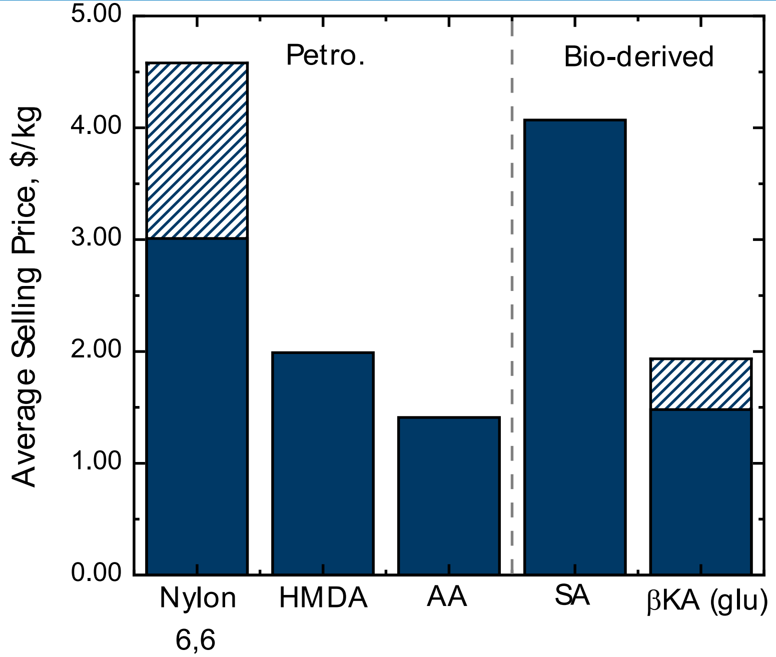
- Nylons are often heavily formulated and can demand a price premium
- βKA could sell for less than sebacic acid currently demands on the market. Sebacic acid has already experienced market penetration in nylon-6,10
- When CO_2 credits for biomass cultivation are accounted for there is a great GHG reduction potential

Conclusions – An Ideal First Market, A New Platform Chemical?

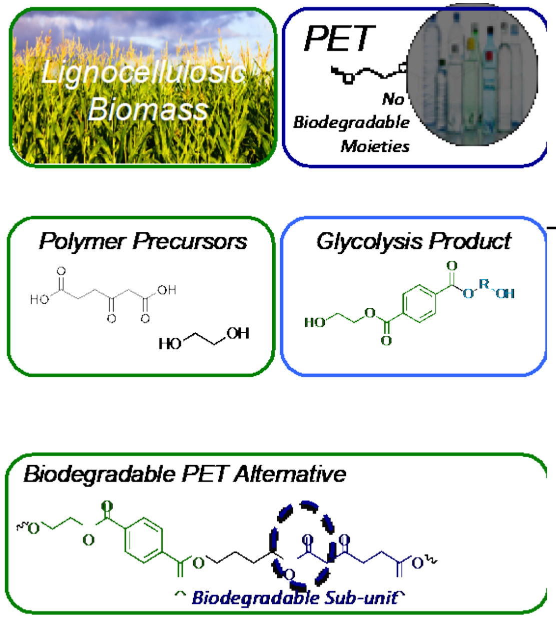
βka can enable robust properties for engineering plastics

- An integrated approach to performance advantaged materials elucidates key challenges in new platform chemicals
- Improvements in metabolic engineering are still possible; however, multiple pathways to βKA are now achievable
- β-ketones provide rigidity across multiple backbone carbons and further interactions across polymer chains
- β-ketone diacids may be used in other applications to enhance T_g relative to adipic acid (e.g. PBAT)
- Engineering Plastics are an ideal market for performance advantaged bioproducts from both economic and emissions perspectives



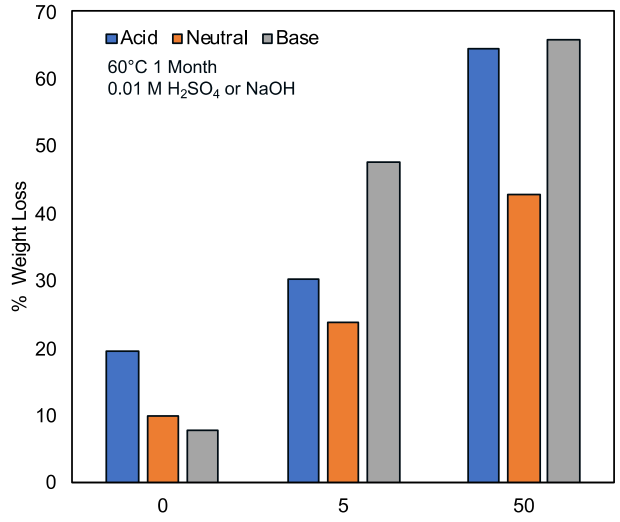


Future Work? - Leveraging the β-Ketone in Polyesters



Poly(butylene β-ketoadipate) [PBKAT]

BKA Loading	Virgin				Reclaimed			
	T_g	T_m	Permeability (g 25µ/m^2/day)	E' (0.1 Hz,35°C)	T_g	T_m	Permeability (g 25µ/m^2/day)	E' (0.1 Hz,35°C)
PET	69	262	20	4.1 GPa	67	257	22	3.7 GPa
PBKAT – 1%	72	265	18	4.3 GPa	69	261	19	4.1 GPa
PBKAT – 5%	73	257	22	4.5 GPa	70	259	21	3.9 GPa
PBKAT – 10 %	71	261	21	4.1 GPa	66	252	23	4.0 GPa
PBKAT – 50 %	68	254	--	--	70	260	--	--
PBKAT	70	--	--	--	--	--	--	--



β-Ketones Can Maintain Polyester Properties, Yet Enable Degradation

When Adipic Acid is placed into PET, the polymer is extremely plasticized. When βKA is put in place of TPA in PET, it can maintain properties while enabling facile degradation

Thank You!

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