Background:

The submitted proposal had two major aims: i) To systematically explore the repertoire of enzymes that allow E. coli to grow on two non-native carbon sources when acquired horizontally from metagenomic libraries and ii) Use this information to test the hypothesis that horizontal transfer of enzyme side activities can contribute to growth on a new carbon source (as opposed to the classical view where enzyme main activities play a role).

In line with our original research plan, we made progress in developing the functional metagenomic pipeline to discover genes that allow growth in a new condition (e.g. carbon source). This involved growing E. coli transformed with metagenomic fragments on two non-native carbon sources (D-lyxose and D-2-deoxyribose), sequencing and bioinformatics analysis of growth-conferring fragments. However, our screens failed to discover any new enzyme that allowed growth on these carbon sources. Specifically, we could recover several homologs of *yihS* and *rbsK*, which were expected based on our prior work and which validated the method itself, but no interesting new hits were found. Although larger metagenomic libraries representing more diverse environmental sources might have yielded interesting hits, this would have required an enourmous amount of extra work with no guarantee to yield better results.

Results 1:

Therefore we decided to modify our original aims as follows. In Aim 1, we focused on using our metagenomic pipeline to compare the transferability of genes conferring resistance to conventional antibiotics vs antimicrobial peptides (AMPs) from the gut microbiome into E. coli. AMPs represent an important class of promising antimicrobial agents that are also part of the innate immune response of virtually all species, including human. These analyses yielded several fundamental new insights into the differences between the mobilization of antibiotic vs AMP resistance genes:

(i) Short genomic fragments from the gut microbiota rarely confer AMP resistance
(ii) This pattern cannot be explained by the lack of AMP resistant bacteria or AMP resistance genes in the human gut
(iii) Rather, we revealed that phylogenetic barriers limit the transferability of AMP resistance phenotypes from distant bacteria to E. coli.

These results were recently published in Nature Microbiology (Kintses et al. 2019), with Balint Kintses, Csaba Pal and me as leading authors. Adam Gyorkei, who participates in the present grant, is also a co-author on the paper.

Results 2:

Aim 2 was modified as follows. Instead of studying the role of enzyme side activities in new carbon source utilization phenotypes, we focused on exploring their role in new biosynthetic pathways towards industrially important metabolites. This research aim addresses a fundamental open question in bioengineering: Can weak and physiologically silent enzyme side activities be tapped for the building of

new biosynthetic pathways? If so, this would represent an important alternative to heterologous expression of enzymes from foreign species.

Our study uses computational approaches and relies on our previously reconstructed network of enzyme side activities in E. coli (Notebaart et al. PNAS 2014). With this network in hand, we used the computational method of flux balance analysis to systematically probe the biosynthetic producibility of ~280 metabolites, all which are industrially relevant (as compiled from the literature). We found several dozen candidate metabolites whose production is either enabled (i.e. no synthesis was possible without it) or enhanced by enzyme side activities. Furthermore, we showed that, on average, side reactions of endogenous enzymes have as much potential to contribute to new biosynthetic pathways as the reactions encoded by heterologous enzymes. Together, these computational results show that enzyme side activities are a rich, but mostly overlooked, reservoir of useful chemical reactions and therefore could be a promising addition to the toolbox of industrial strain development. Our results are now being prepared for publication.