Supporting Information

# Materials and instrumentation

## Materials

### Enzymes

### Substrates

## Instrumentation

### Spectroscopy

### High-performance liquid chromatography

### Flow experiments

## Software

# Production and characterisation of polyacrylamide-enzyme beads

# General pipeline for experiments in flow

# Overview of experiments

Demonstration of simple table syntax

| Right | Left | Center | Default |
| --- | --- | --- | --- |
| 12 | 12 | 12 | 12 |
| 123 | 123 | 123 | 123 |
| 1 | 1 | 1 | 1 |

Overview of single-enzyme experiments

| Code | flowrate | enzymes | E volume | E batch | input substrates | observed substrates | observation techniques |
| --- | --- | --- | --- | --- | --- | --- | --- |
| SNCA14 | 750 | GDH | 10 | 1 | G,NAD | NADH | offline absorbance |
| SNCA15 | 750 | GDH | 10 | 1 | G,NAD | NADH | offline absorbance |
| SNCA17 | 750 | HK | 10 | 1 | G,ATP | G6P | G6P assay |
| SNCA18 | 750 | HK | 1 | 1 | G,ATP | G6P | G6P assay |
| SNKS03 | 750 | HK | 1 | 1 | G,ATP | G6P | G6P assay |
| SNKS04 | 750 | HK | 1 | 1 | G,ATP | G6P,ATP | G6P assay,HPLC |
| SNKS08 | 750 | G6PDH | 10 | 1 | G6P,NAD | NADH | offline absorbance |
| SNKS11 | 750 | GDH | 0.5 | 2 | G,NAD | NADH | offline absorbance |
| SNKS12 | 750 | GDH | 0.5 | 2 | G,NAD | NADH | offline absorbance |
| SNKS18 | 750 | GDH | 2.0 | 3 | G,NAD | NADH | offline absorbance |
| SNKS19 | 750 | GDH | 5.0 | 3 | G,NAD | NADH | offline absorbance |
| SNKS20 | 750 | G6PDH | 2.0 | 1 | G6P,NAD | NADH | online absorbance |

Overview of multi-enzyme experiments

| Code | flowrate | enzymes | E volume | E batch | input substrates | observed substrates | observation techniques |
| --- | --- | --- | --- | --- | --- | --- | --- |
| SNKS06 | 750 | GDH,HK | 10.0,1.0 | 1,1 | G,NAD,ATP | NADH,ADP | offline absorbance,HPLC |
| SNNS002 | 750 | GDH,HK | 0.05,1.0 | 2,2 | G,NAD,ATP | NADH | offline absorbance |
| SNNS003 | 750 | GDH,HK | 0.025,1.5 | 2,1 | G,NAD,ATP | NADH | offline absorbance |
| SNNS004 | 750 | GDH,HK | 0.0333,1.33 | 2,2 | G,NAD,ATP | NADH | offline absorbance |
| SNNS005 | 750 | GDH,HK | 0.0666,0.666 | 2,1 | G,NAD,ATP | NADH | offline absorbance |
| SNNS006 | 750 | GDH,HK | 0.075,0.5 | 2,1 | G,NAD,ATP | NADH | offline absorbance |
| SNNS007 | 750 | GDH,HK | 0.05,1.0 | 2,1 | G,NAD,ATP | NADH | offline absorbance |

# Overview of computational methods

# References