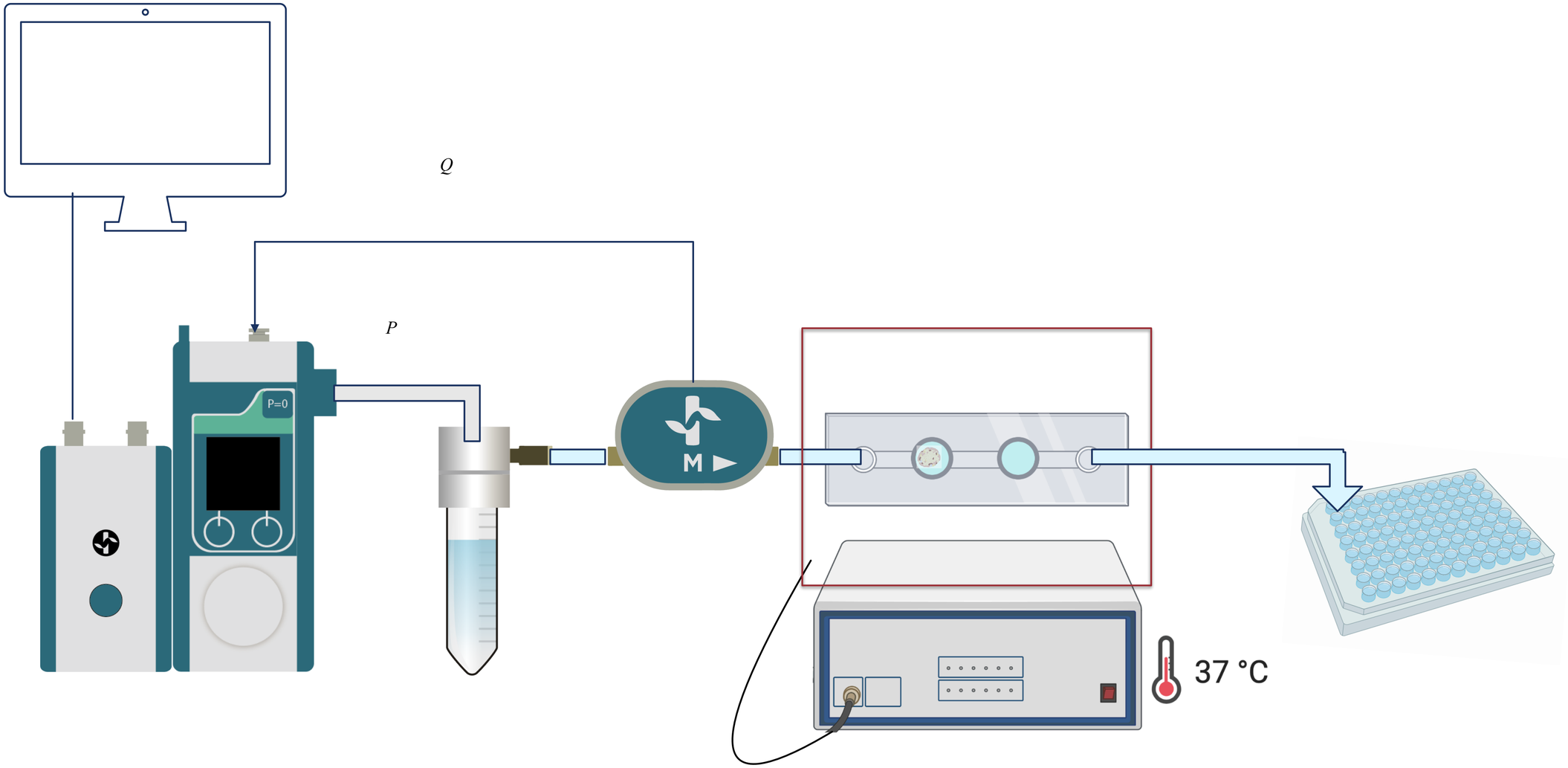
GSIS with perfusion

10/06/2021

by Leonid Pliner

Set-up



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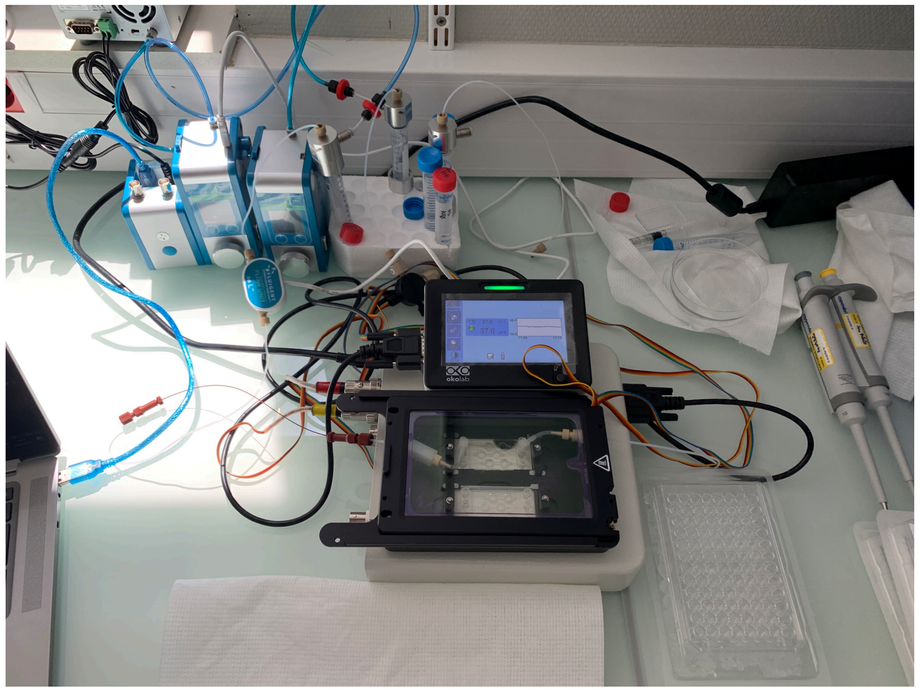
GSIS Procedure

1. Islet isolation, n=50
2. Resuspended in KRB LG (2.8 mM) and left for 30 min to attach to the bottom of the chip
3. 30 min in KRB LG with perfusion - sample collection start
4. 60 min in KRB HG (16.7 mM)
5. 30 min in KRB LG
6. Lysis

- Perfusion at flow rate 10 µl/min
- Temperature 37 degrees C

Notes on the experiment

- During the reattachment of the input and output tubing to the seeded chip, bubbles were introduced into the cell culture chambers.
- The 96 well plate was kept on ice.



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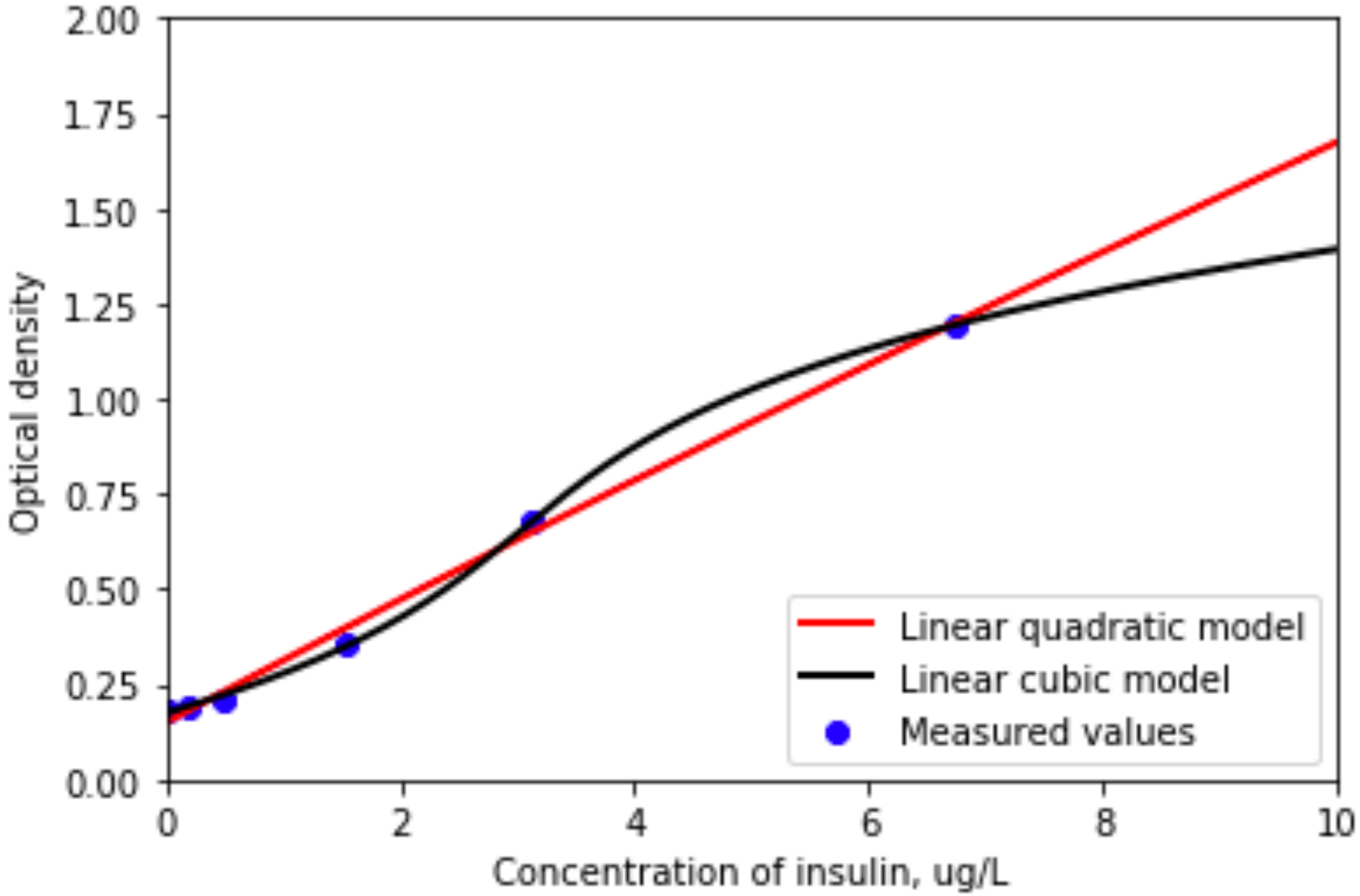
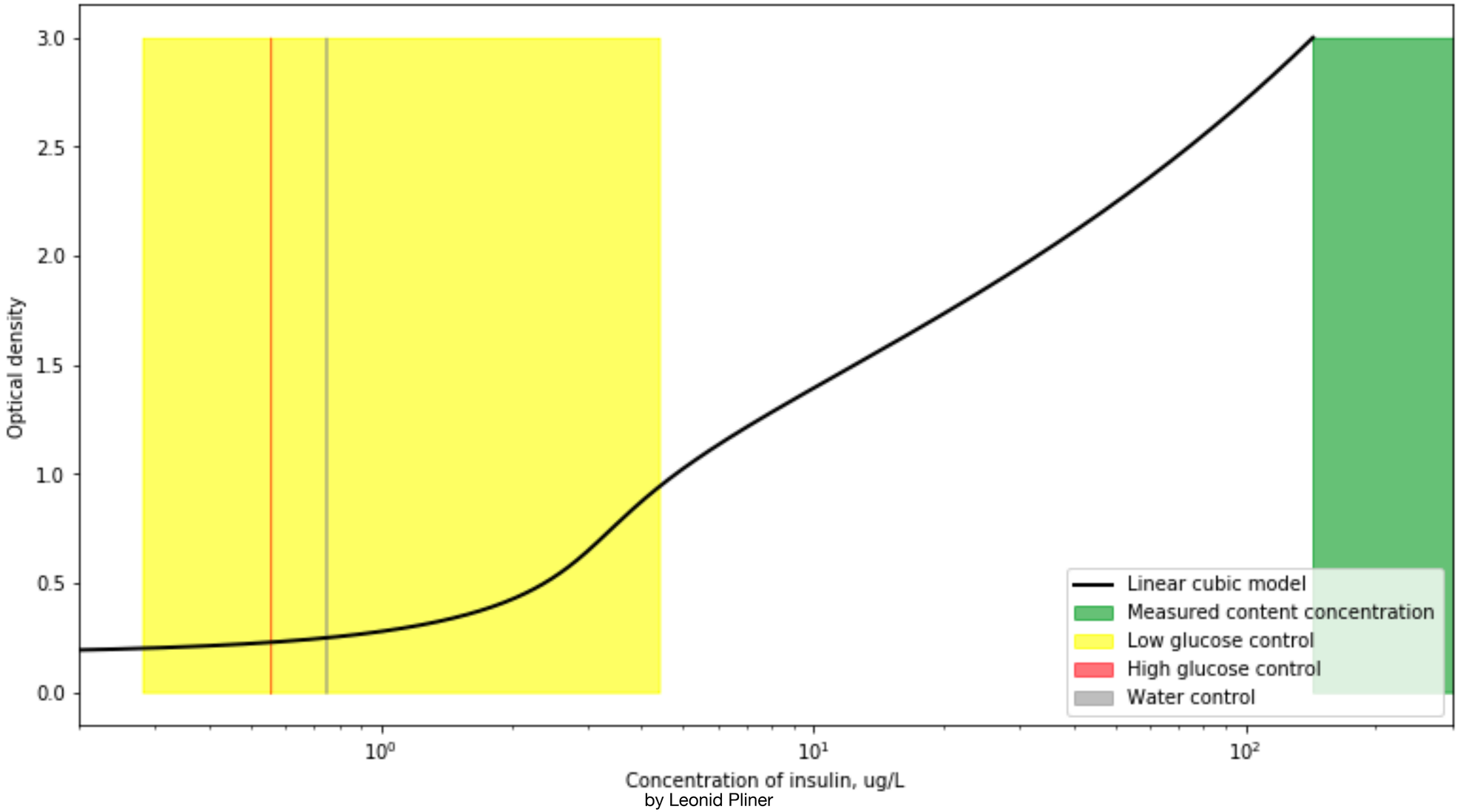
ELISA

- Standard curve constructed from 6 points by quadratic and cubic regression model
- Cubic was selected as a more accurate approximation
- All samples were diluted as 1/30



ELISA



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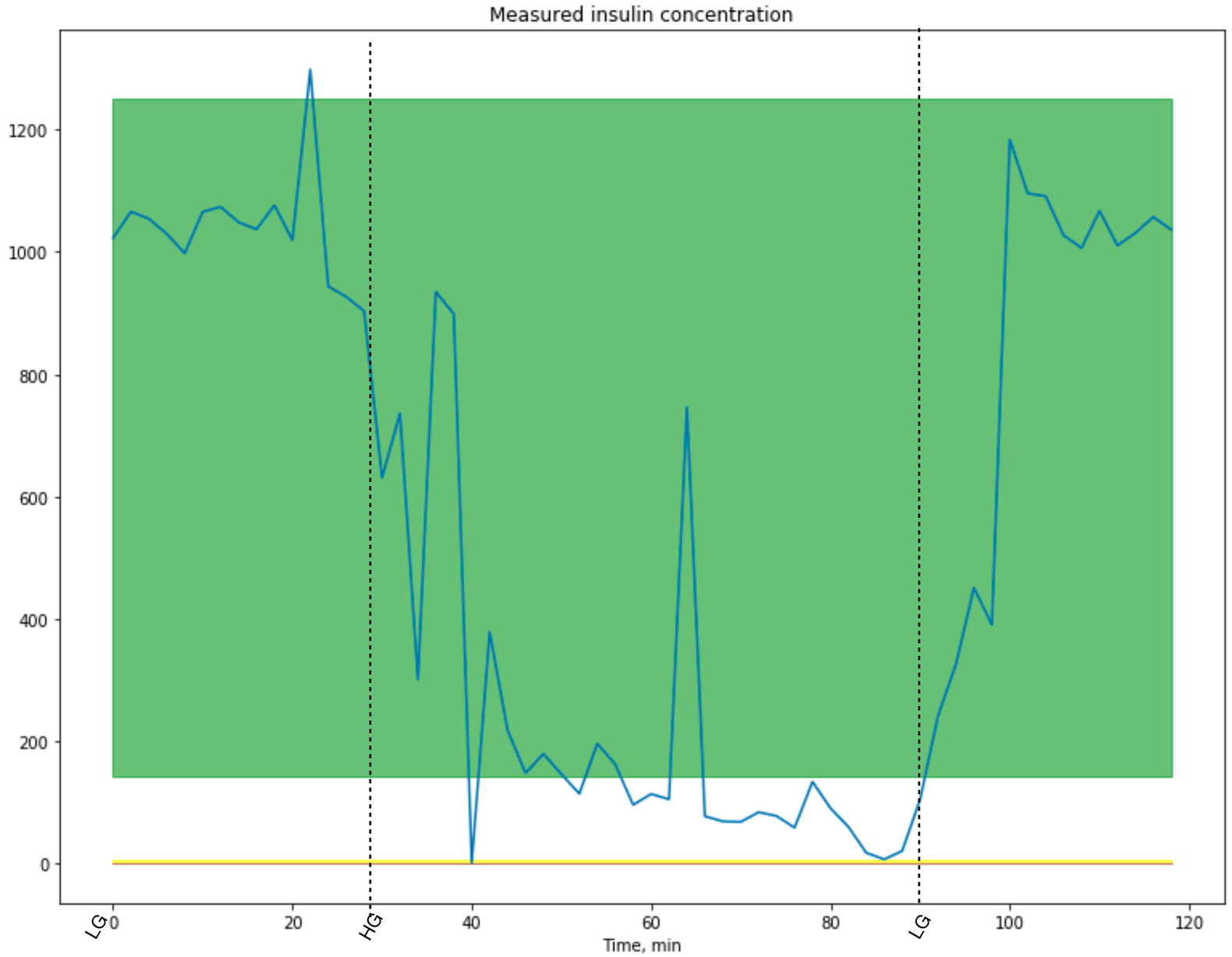
ELISA

- Low and high regions seem to be ‘inverted’
- Mixing has to be considered



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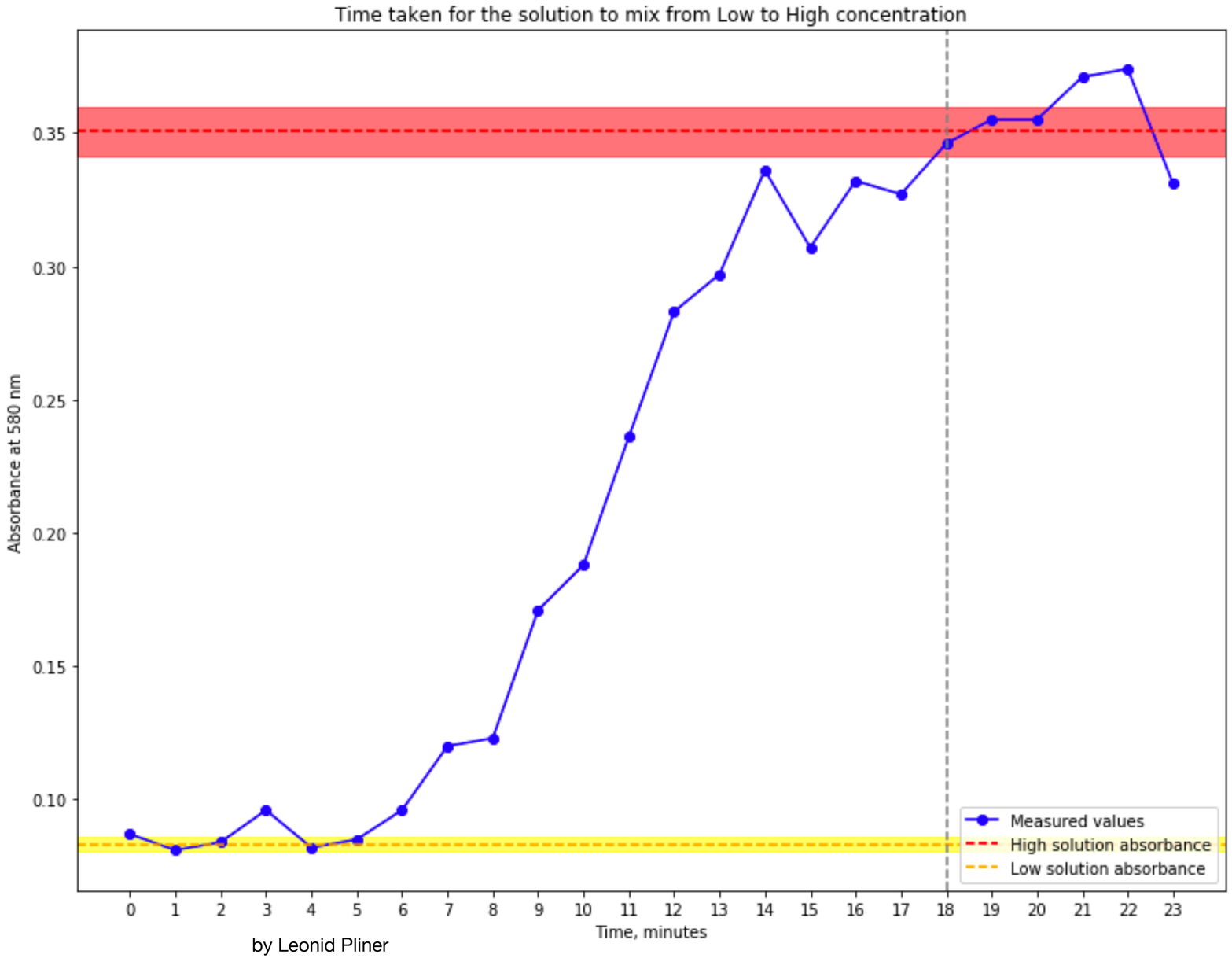
Mixing

- Investigated using trypan blue with a similar ratio between L and H concentrations
- Collected 24 samples over 24 mins at flow rate 50 ul/min



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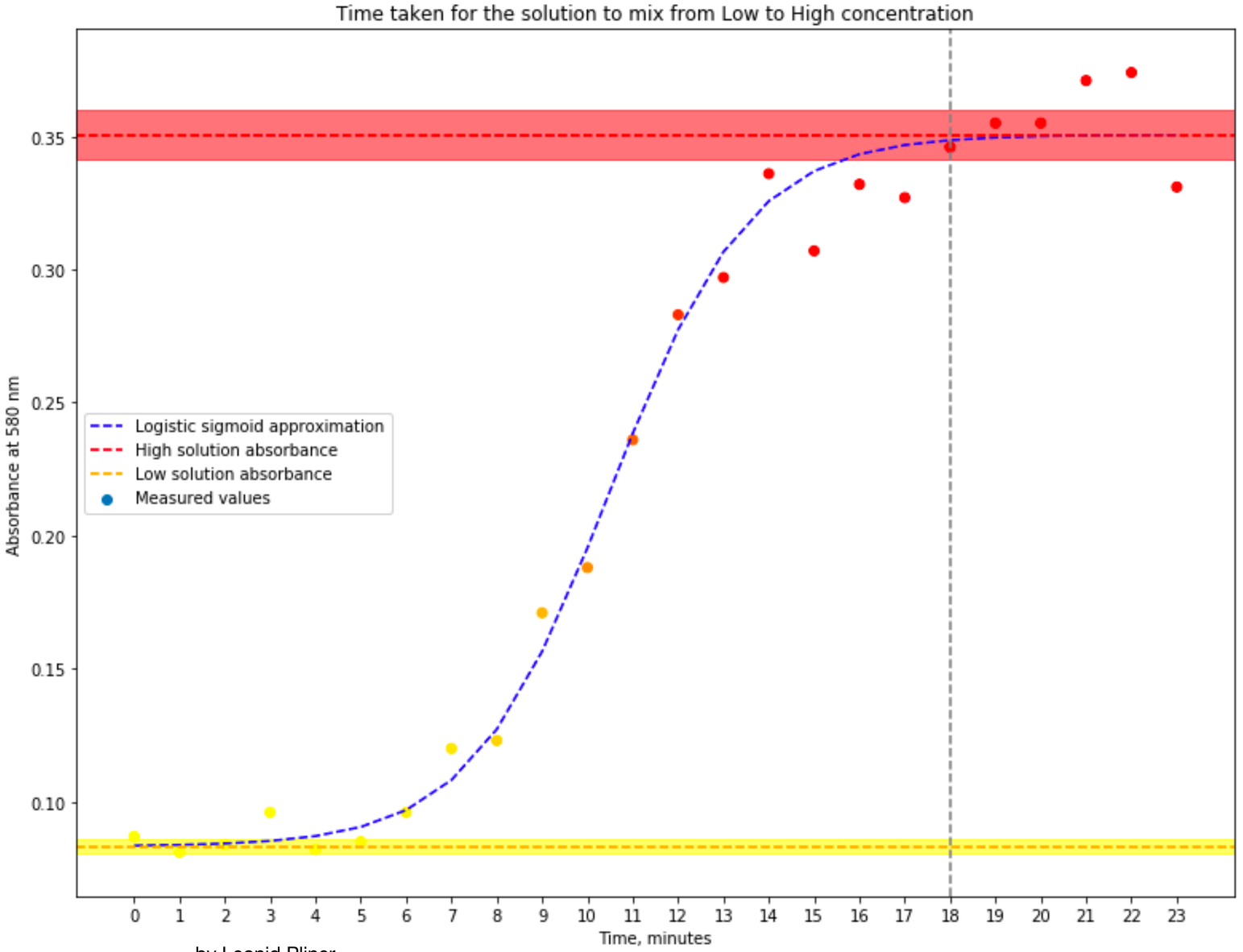
Mixing

- Mixing time 18 mins or 90 mins at the GSIS experiment flow rate
- Mixing dynamics can be approximated as logistic sigmoid



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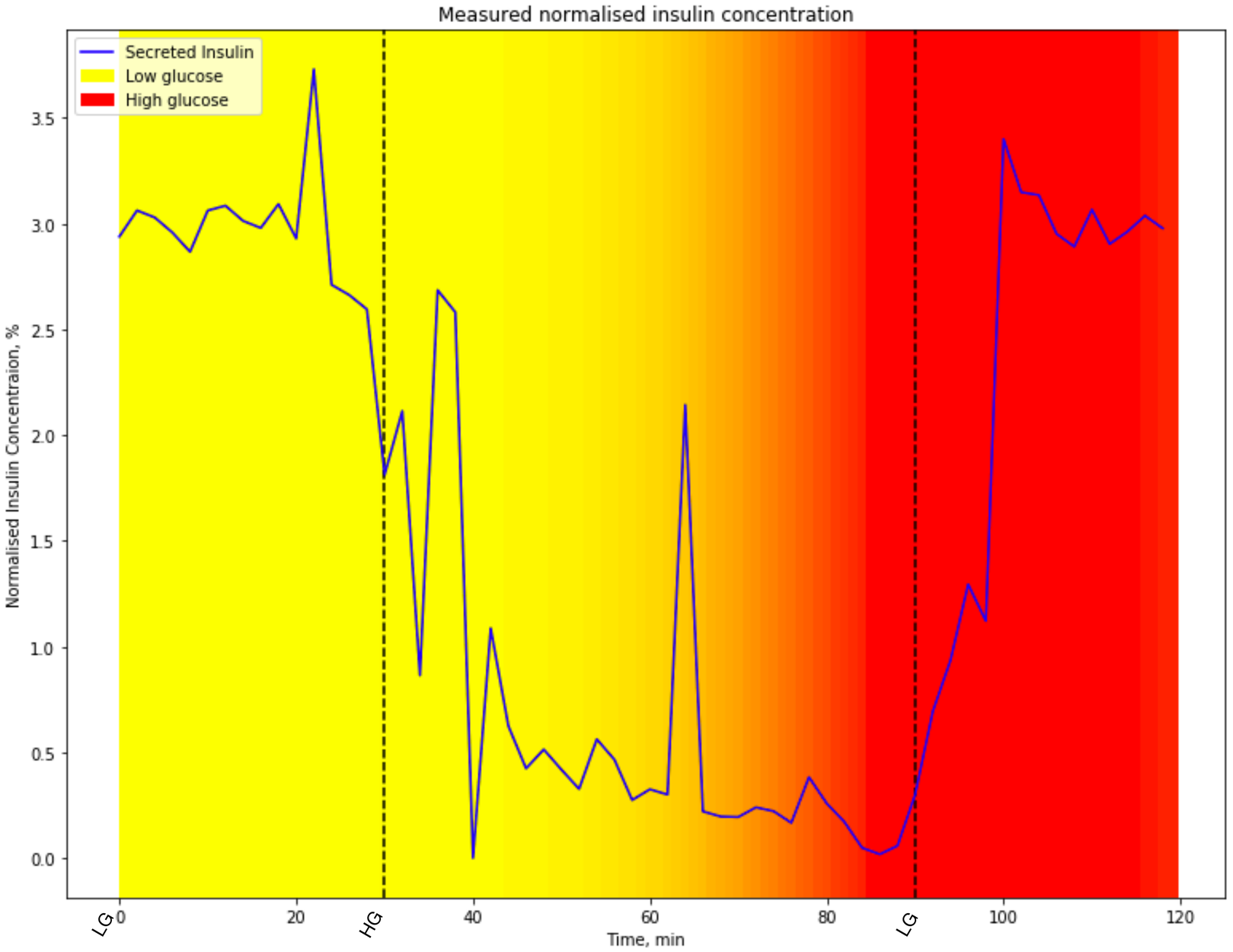
ELISA

- With mixing information the insulin secretion can be understood better
- Time of LG was 60 min < mixing time 90 min



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Caveats

- Because of BSA there were bubbles in the seeded chambers - can avoid using it.
- Bubble trap needs to be used to avoid bubbles.
- ELISA dilution was not enough for the lab standard, so the concentrations were extrapolated from the regression and are rather estimative then accurate.
- Mixing dynamics is approximated, because other solution was perfused at a higher flow rate, so also estimative.
- KCl solution can be used at the end for max insulin release.
- Perfusion introduces extra stress after the 0 flow seeding stage, so it has to be started earlier.
- A switch valve is needed for more controlled glucose concentration.