Lab Book

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Preface

This is a Laboratory Notebook for experiments conducted from March 2022 to June 2023. The results obtained during this time were used to write the thesis " " to obtain the title "Dr. med. univ.".

The notebook is sorted by experiments and not in chronological order. After each individual repitition has been desccribed, a summary of all the data is show.

1 Competitive inhibition of transcription using STYX

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['3-T1', 'Figure4', 'Flourometry', 'WB']
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1.1 Flourometry

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['Results.csv', '1', '2', '3', '4']
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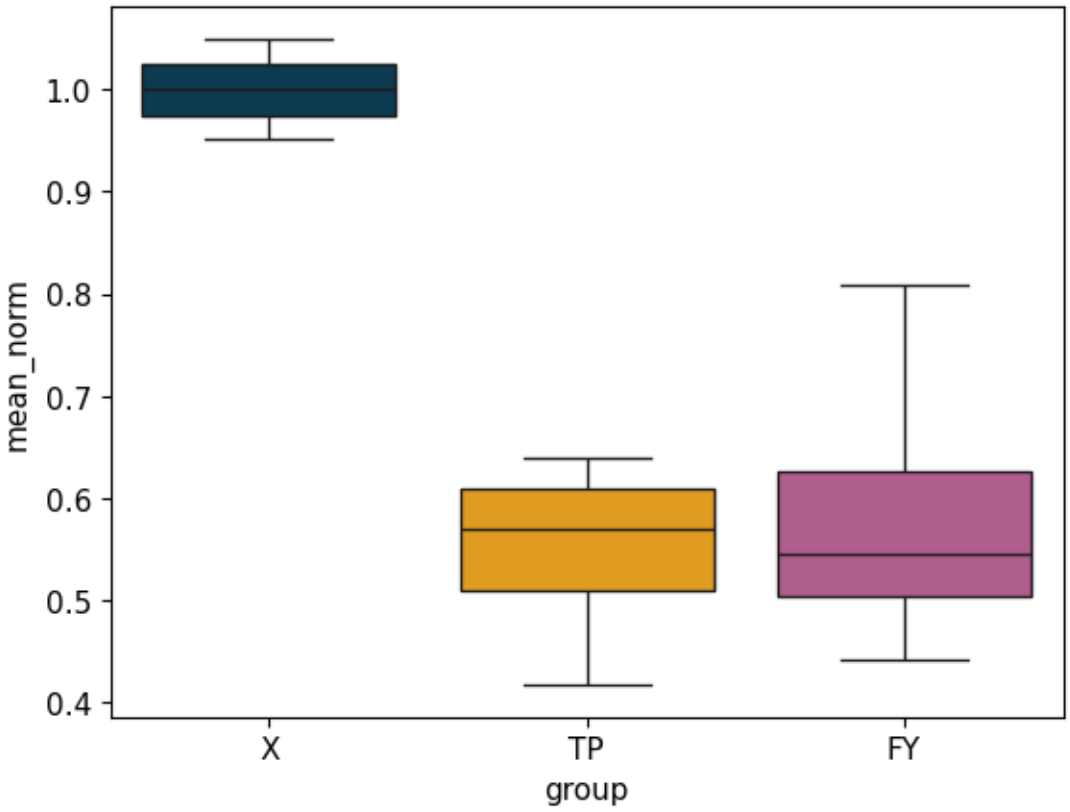
1.1.1 Attempt 1

1.1.1.1 Seeding (28.11.2022)

- Seed 15.000 HeLa cells per well in 96 Well (diluted in 100µL DMEM)
- Layout:
  - 4 Wells reserved for Blank measurement (termed NT)
  - 4 Wells per row per condition: Conditions: X, TP, FY #### Transfection (29.11.2022)
- Change Media beforehand
- Transfect in a 1:2 manner (Plasmid1:Plasmid2)
- 0.1µg total amount of Plasmid per Well
- Conditions:
  - X: P1 = GFP, P2 = STYX
  - TP: P1 = GFP, P2 = TP-Linker-Protein
  - FY: P1 = GFP, P2 = FY-Linker-Protein
- Change Media 4h after Transfection #### Measurement (30.11.2022)
- Change Media to 100µL PBS
- Measure with Spark @ 488nm
- Incubate on ice for 20mins
- Replace PBS with 10µL RIPA(+PI)
- Incubate for 20mins
- Take out 10µL of cell lysate into new 96-Well

- Put in 150mL of Bradford-assay
- Measure @ 660nM #### Results The mean value of all 5 measurements in each well is calculated. The intensity of the blank measurement is subtracted. Then the resulting value is divided by the measured protein concentration and then further normalised to the mean of the control.

group	mean	std	count
X	1.000	0.043	4
TP	0.549	0.097	4
FY	0.585	0.157	4



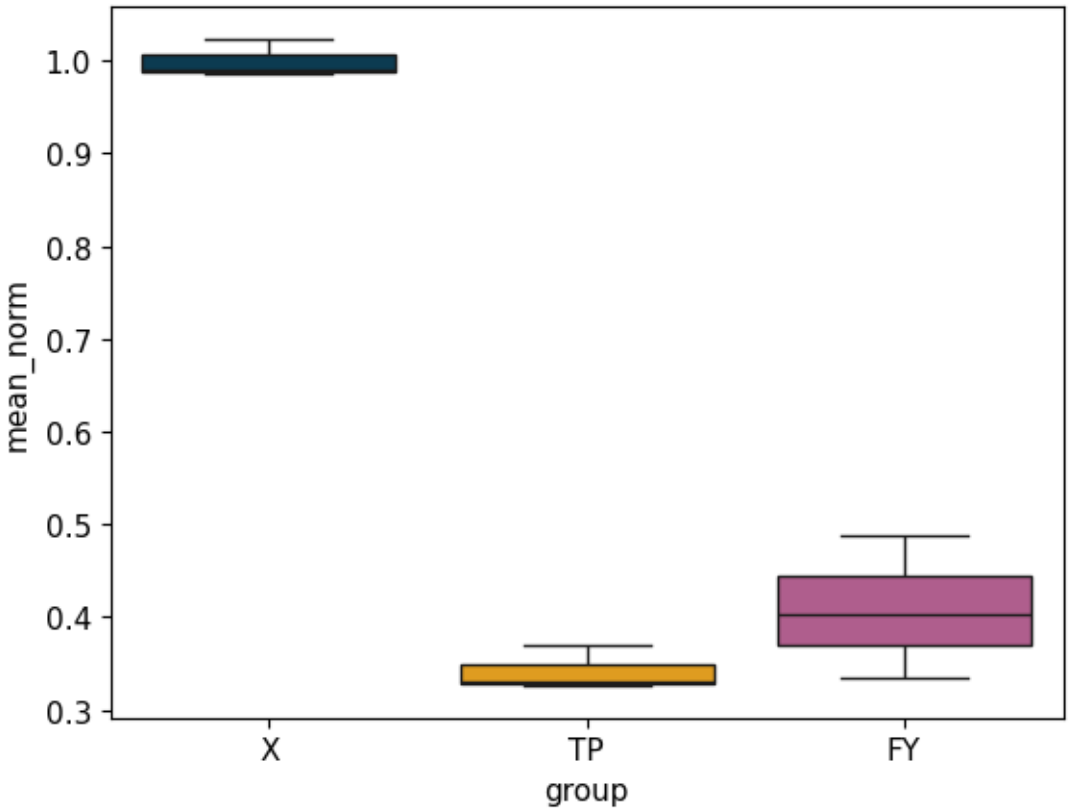
1.1.2 Attempt 2

1.1.2.1 Seeding (23.08.2022)

- Seed 15.000 HeLa cells per well in 96 Well (diluted in 100µL DMEM)

- Layout:
  - 4 Wells reserved for Blank measurement (termed NT)
  - 4 Wells per row per condition: Conditions: X, TP, FY #### Transfection (24.08.2022)
- Change Media beforehand
- Transfect in a 1:2 manner (Plasmid1:Plasmid2)
- 0.1µg total amount of Plasmid per Well
- Conditions:
  - X: P1 = GFP, P2 = STYX
  - TP: P1 = GFP, P2 = TP-Linker-Protein
  - FY: P1 = GFP, P2 = FY-Linker-Protein
- Change Media 4h after Transfection #### Measurement (25.08.2022)
- Change Media to 100µL PBS
- Measure with Spark @ 488nm
- Incubate on ice for 20mins
- Replace PBS with 10µL RIPA(+PI)
- Incubate for 20mins
- Take out 10µL of cell lysate into new 96-Well
- Put in 150mL of Bradford-assay
- Measure @ 660nM #### Results The mean value of all 5 measurements in each well is calculated. The intensity of the blank measurement is subtracted. Then the resulting value is divided by the measured protein concentration and then further normalised to the mean of the control.

group	mean	std	count
X	1.000	0.021	3
TP	0.342	0.023	3
FY	0.409	0.076	3



1.1.3 Attempt 3

1.1.3.1 Seeding (05.12.2022)

- Seed 15.000 HeLa cells per well in 96 Well (diluted in 100µL DMEM)
- Layout:
  - 4 Wells reserved for Blank measurement (termed NT)
  - 4 Wells per row per condition: Conditions: X, TP, FY

1.1.3.2 Transfection (06.12.2022)

- Change Media beforehand
- Transfect in a 1:2 manner (Plasmid1:Plasmid2)
- 0.1µg total amount of Plasmid per Well
- Conditions:
  - X: P1 = GFP, P2 = STYX

  - TP: P1 = GFP, P2 = TP-Linker-Protein
  - FY: P1 = GFP, P2 = FY-Linker-Protein

- Change Media 4h after Transfection

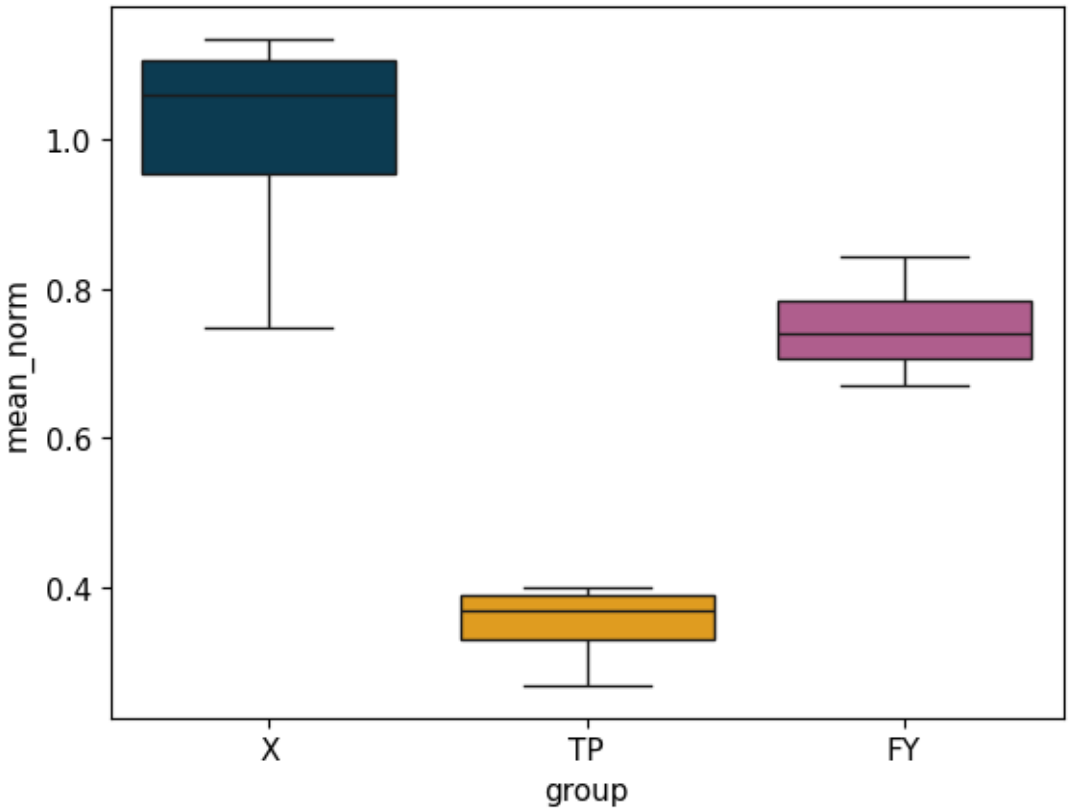
1.1.3.3 Measurement (07.12.2022)

- Change Media to 100µL PBS
- Measure with Spark @ 488nm
- Incubate on ice for 20mins
- Replace PBS with 10µL RIPA(+PI)
- Incubate for 20mins
- Take out 10µL of cell lysate into new 96-Well
- Put in 150mL of Bradford-assay
- Measure @ 660nM

1.1.3.4 Results

The mean value of all 5 measurements in each well is calculated. The intensity of the blank measurement is subtracted. Then the resulting value is divided by the measured protein concentration and then further normalised to the mean of the control.

group	mean	std	count
X	1.000	0.175	4
TP	0.350	0.059	4
FY	0.749	0.073	4



1.1.4 Attempt 4

1.1.4.1 Seeding (06.12.2022)

- Seed 15.000 HeLa cells per well in 96 Well (diluted in 100µL DMEM)
- Layout:
  - 4 Wells reserved for Blank measurement (termed NT)
  - 4 Wells per row per condition: Conditions: X, TP, FY

1.1.4.2 Transfection (07.12.2022)

- Change Media beforehand
- Transfect in a 1:2 manner (Plasmid1:Plasmid2)
- 0.1µg total amount of Plasmid per Well
- Conditions:
  - X: P1 = GFP, P2 = STYX

  - TP: P1 = GFP, P2 = TP-Linker-Protein
  - FY: P1 = GFP, P2 = FY-Linker-Protein

- Change Media 4h after Transfection

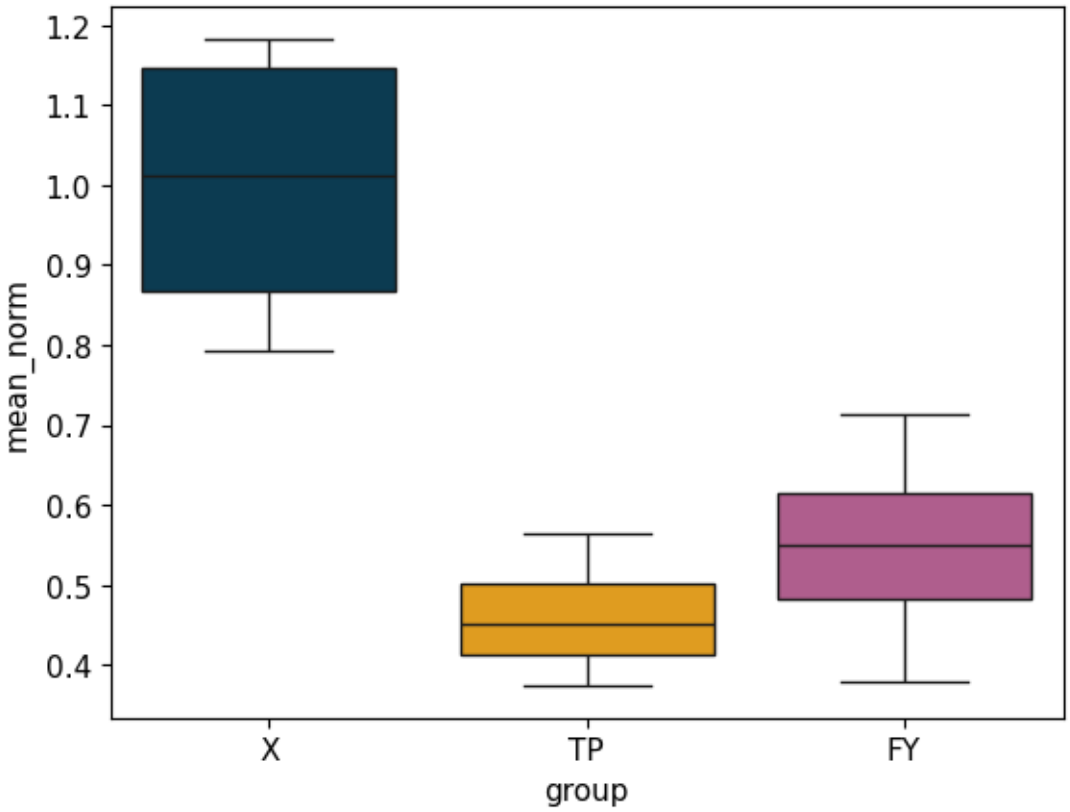
1.1.4.3 Measurement (08.12.2022)

- Change Media to 100µL PBS
- Measure with Spark @ 488nm
- Incubate on ice for 20mins
- Replace PBS with 10µL RIPA(+PI)
- Incubate for 20mins
- Take out 10µL of cell lysate into new 96-Well
- Put in 150mL of Bradford-assay
- Measure @ 660nM

1.1.4.4 Results

The mean value of all 5 measurements in each well is calculated. The intensity of the blank measurement is subtracted. Then the resulting value is divided by the measured protein concentration and then further normalised to the mean of the control.

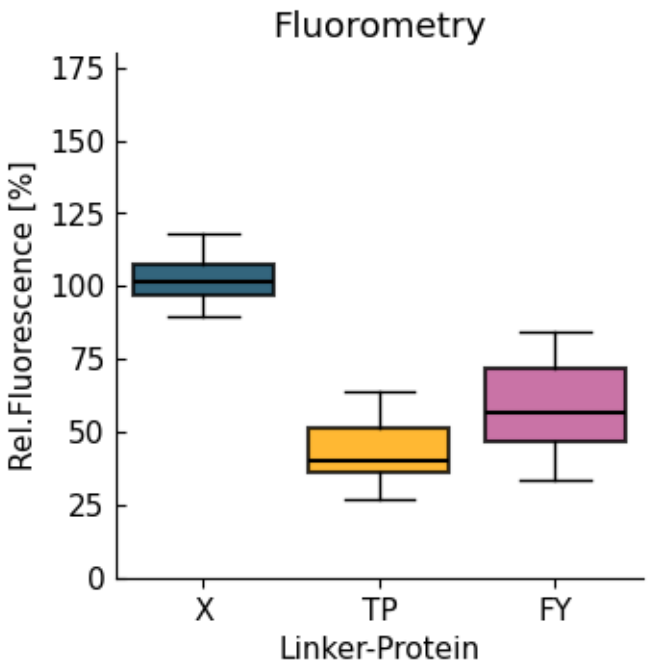
group	mean	std	count
X	1.000	0.188	4
TP	0.461	0.082	4
FY	0.548	0.139	4



1.1.5 Final Result

group	mean	sd	ci_lo	ci_hi	count
X	1.000	0.117	0.936	1.057	15
TP	0.431	0.106	0.377	0.485	15
FY	0.583	0.156	0.501	0.664	15

group	mean [CI]	value	star
X	1.0 [0.936, 1.057]	NaN	NaN
TP	0.431 [0.377, 0.485]	0.0	****
FY	0.583 [0.501, 0.664]	0.0	****


Fluorometry
175
150
125
100
75
50
25
0
Rel.Fluorescence [%]
X
TP
FY
Linker-Protein

2