counting_molecular_complexes

March 12, 2020

1 Counting Molecular Complexes

[28]:
```
import numpy as np
import pandas as pd
import panel as pn
import altair as alt
import tqdm
import prot.viz
prot.viz.altair_theme()
pn.extension('vega')
_ = alt.data_transformers.enable('default')
```

In this notebook, we provide a series of estimates for the copy numbers of various protein complexes that would be needed for a bacterium to double in the typical doubling time of $\approx$ 3000 seconds. Everything presented here is provisional and may be entirely wrong.

All plots in this notebook are interactive – you can zoom, pan, and hover over points to get more information about their identity.

1.1 Summary of Data

Nathan and I have spent the past few months assembling, curating, and evaluating an array of proteomic measurements of *E. coli* across a wide variety of growth conditions. After examining ~10 unique data sets, 4 met our criterion for being "valid" data sets. This means that a) the numbers reported by the authors are in "absolute units" meaning actual approximate number of proteins per cell and b) their supplemental information thoroughly described their quantitation procedure such that values from one data set to another could be manipulated into a form that is directly comparable. Nathan and I are working on an auxiliary document that throughly describes our curation procedure and briefly summarizes each data set. The four data sets we settled on using are described in the table below:

Title	**Author**	**Year**	**Number of unique proteins measured**	**Experimental Method**
The quantitative and condition-dependent *E. coli* proteome	Alexander Schmidt, (...), Matthias Heinemann	2016	2,054	Mass Spectrometry
Proteome reallocation in *E. coli* with increasing specific growth rate	Karl Peebo, (...), Raivo Vilu	2015	1,525	Mass Spectrometry
Quantifying absolute protein synthesis rates reveals principles underlying allocation of cellular resources	Gene Wei Li, (...), Jonathan Weissman	2014	3,041	Ribosomal Profiling
E. coli achieves faster growth by increasing catalytic translation rates of proteins	Kaspar Valgepea, (...), Raivo Vilu	2013	1,185	Mass Spectrometry

With this collection of data, we scraped the EcoCyc database to assign annotation to each gene along with a variety of identifiers and classifications of function. After all is said-and-done, our compiled data set includes measurements of 3442 unique proteins across 36 unique growth conditions. To our knowledge, this is the most comprehensive quantitative assembly of proteomic measurements of *E. coli* across growth rates which vary by one order of magnitude.

In addition to individual gene annotations, we compiled a list of all known protein and protein-RNA complexes in *E. coli* whose subunit composition has been experimentally verified. Given our proteomic measurements, we calculated the total number of unique *units* that could be formed for each protein. For example, consider the F1 ATP Synthase Complex. The stoichiometry of this complex (in terms of protein subunits) can be written as follows:

$$[\text{AtpC}]_1[\text{AtpH}]_1[\text{AtpA}]_3[\text{AtpG}]_1[\text{AtpD}]_3$$

where the subscript denotes the copy number of each protein in the complex. We will examine this complex in depth further on in this notebook, but lets assume that in our measurements, we observe n copies of each protein listed in the stoichiometric equation above. The total number of functional units that can be assembled can then be calculated as

$$N_\text{units} = \frac{n_\text{observed}}{n_\text{annotated}}. \tag{1}$$

Thus, the *maximum* number of assembled complexes corresponds to the *minimum* number of functional units possible given measurements of the individual constituents.

This would be an easy calculation to make assuming we had infinite precision in the measurements made by mass spectrometry. However, the extraction efficiency of proteins can be highly variable depending on their localization and the technique used by the researchers. This means that occasionally, some subunits may not be extracted as efficiently as other subunits in the same complex. Rather than relying on the minimum number of functional units observed for each subunit, we compute the *average* number of functional subunits across all subunits in the complex,

$$N_\text{complexes} = \frac{1}{N_\text{subunits in complex}} \sum_i \frac{n^{(i)}_\text{observed}}{n^{(i)}_\text{annotated}}. \tag{2}$$

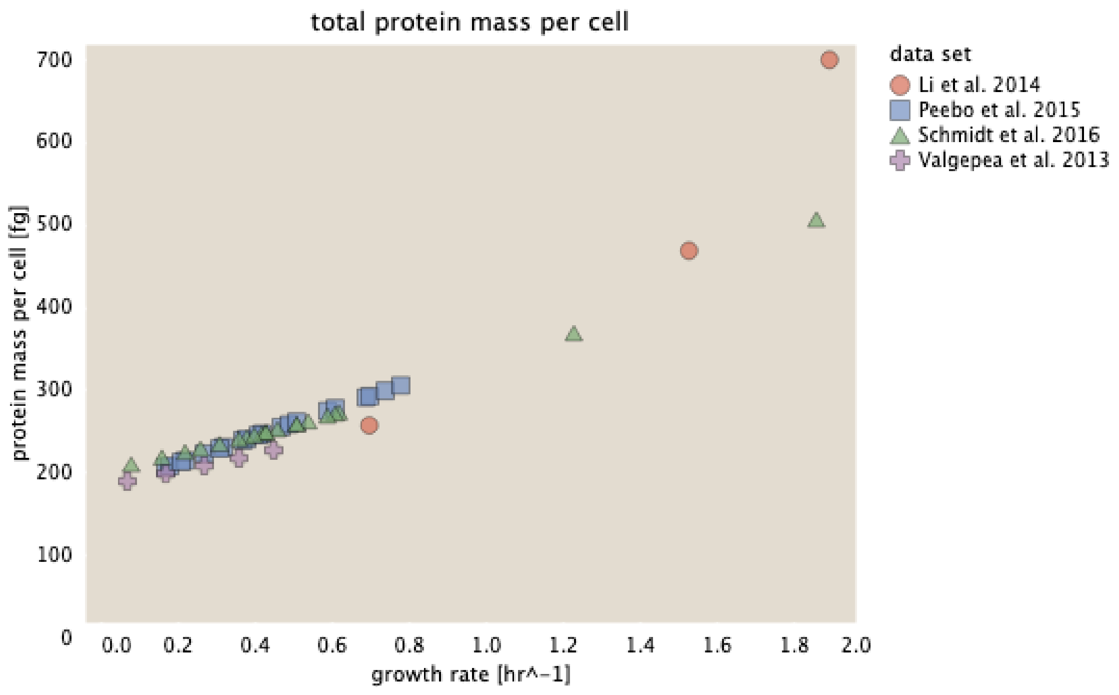
We believe that this averaging presents a realistic view of the total number of assembled complexes present per cell.

1.2 Introducing the Data Sets

Where are my manners? Lets first meet the data before we explore the protein complex copy numbers. While the data sets described in the table above only cover at best ∼ 60% of the total number of annotated genes in *E. coli*, they represent far-and-away the vast majority of the mass of the proteome across all conditions. This can be seen below where the total mass of the proteome (in fg / cell) is plotted as a function of the growth rate for each data set.

[37]:
```
# Load the compiled data
data = pd.read_csv('../../data/compiled_absolute_measurements.csv')

# Shrink the data set before encoding to save some space.
tot_prot_mass = data.groupby(['dataset_name', 'condition',␣
 ↪'growth_rate_hr'])['fg_per_cell'].sum().reset_index()

# Set up the base plot
total_base = alt.Chart(tot_prot_mass)

# Encode the data
total_plot = total_base.mark_point(size=80).encode(
                x=alt.X('growth_rate_hr:Q', axis={'title':'growth rate␣
 ↪[hr^-1]'}),
                y=alt.Y('fg_per_cell:Q', axis={'title':'protein mass per cell␣
 ↪[fg]'}),
                fill=alt.Color('dataset_name:N', legend={'title':'data set'}),
                shape='dataset_name:N',
                tooltip=['condition', 'dataset_name', 'growth_rate_hr',␣
↪'fg_per_cell:Q', 'tot_per_cell:Q'],
                strokeWidth=alt.value(0.5)
            ).interactive(
            ).properties(
                title='total protein mass per cell'
            )

# Display
total_plot
```

[37]:

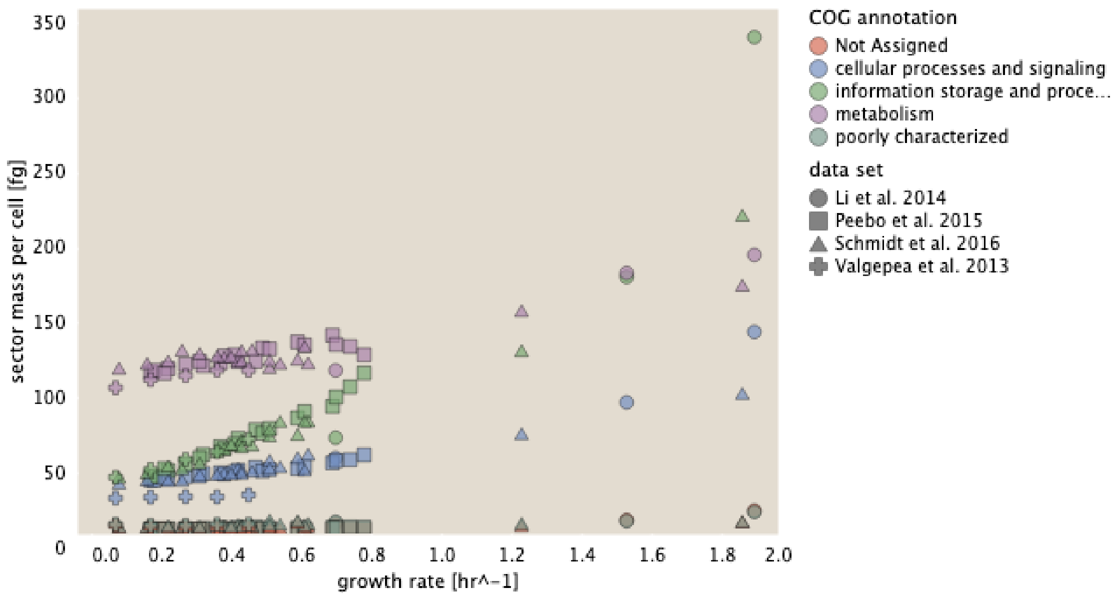


This plot illustrates that all of the data sets combined fall on approximately the same line. This illustrates that, between the different data sets, the majority of the proteome mass is measured in all cases. We can break this down further and examine the total mass of different segments of the proteome, broken down by their Cluster of Orthologous Group (COG) annotation.

[36]:
```
# Compute the sum total mass per sector
cog_mass_data = data.groupby(['dataset_name', 'condition', 'cog_class',␣
↪'growth_rate_hr'])['fg_per_cell'].sum().reset_index()

# Set up the base plot.
base = alt.Chart(cog_mass_data)

# Encode the data.
```

```
plot = base.mark_point(size=60).encode(
            x = alt.X('growth_rate_hr:Q', axis={'title':'growth rate [hr^-1]'}),
            y = alt.Y('fg_per_cell:Q', axis={'title':'sector mass per cell␣
 ↪[fg]'}),
            fill=alt.Color('cog_class:N', legend={'title':'COG annotation'}),
            shape=alt.Shape('dataset_name:N', legend={'title':'data set'}),
            strokeWidth=alt.value(0.5)
        ).interactive()
plot
```

[36]:

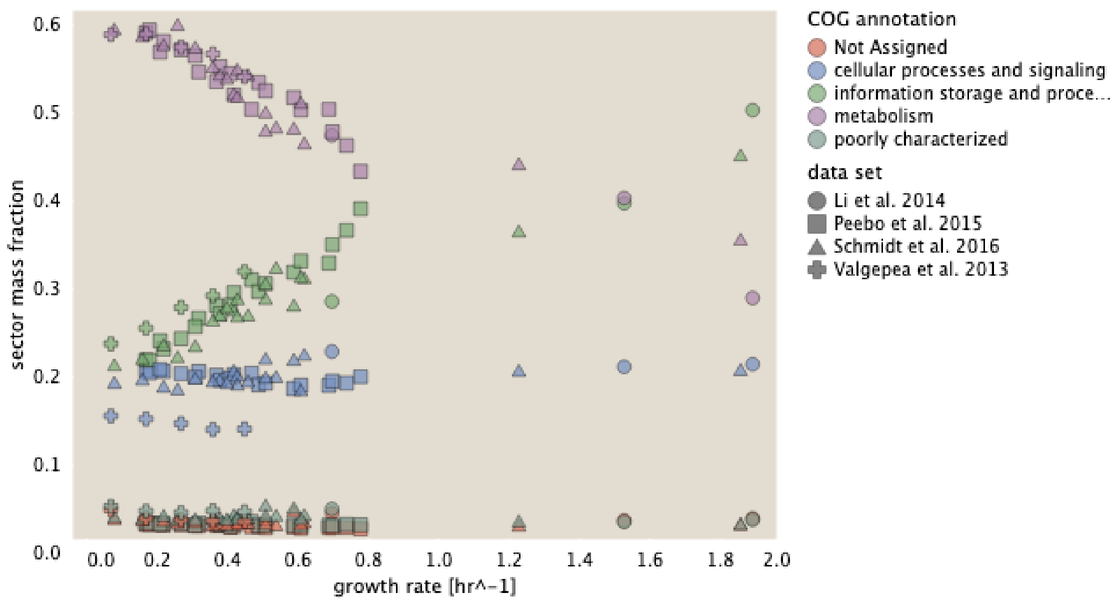


Even across different breakdowns of the proteome, we can see that the various data sets fall in close agreement with the total mass of each sector. Rather than considering only the total mass per cell of each sector, we can easily compute the *fraction* of the proteome devoted to each COG class.

[39]:
```
# Compute the mass fraction
mass_frac = []
for g, d in data.groupby(['dataset', 'condition', 'growth_rate_hr']):
    tot_mass = d['fg_per_cell'].sum()
    sector_mass = d.groupby(['dataset', 'dataset_name', 'condition',␣
 ↪'growth_rate_hr', 'cog_class'])['fg_per_cell'].sum().reset_index()
    frac = sector_mass['fg_per_cell'].values / tot_mass
    sector_mass['frac'] = frac
    mass_frac.append(sector_mass)
mass_frac = pd.concat(mass_frac, sort=False)
```

[40]:
```
# Set up the base plot.
base = alt.Chart(mass_frac)

# Encode the data.
plot = base.mark_point(size=60).encode(
            x = alt.X('growth_rate_hr:Q', axis={'title':'growth rate [hr^-1]'}),
            y = alt.Y('frac:Q', axis={'title':'sector mass fraction'}),
            fill=alt.Color('cog_class:N', legend={'title':'COG annotation'}),
            shape=alt.Shape('dataset_name:N', legend={'title':'data set'}),
            strokeWidth=alt.value(0.5)
        ).interactive()
plot
```

[40]:

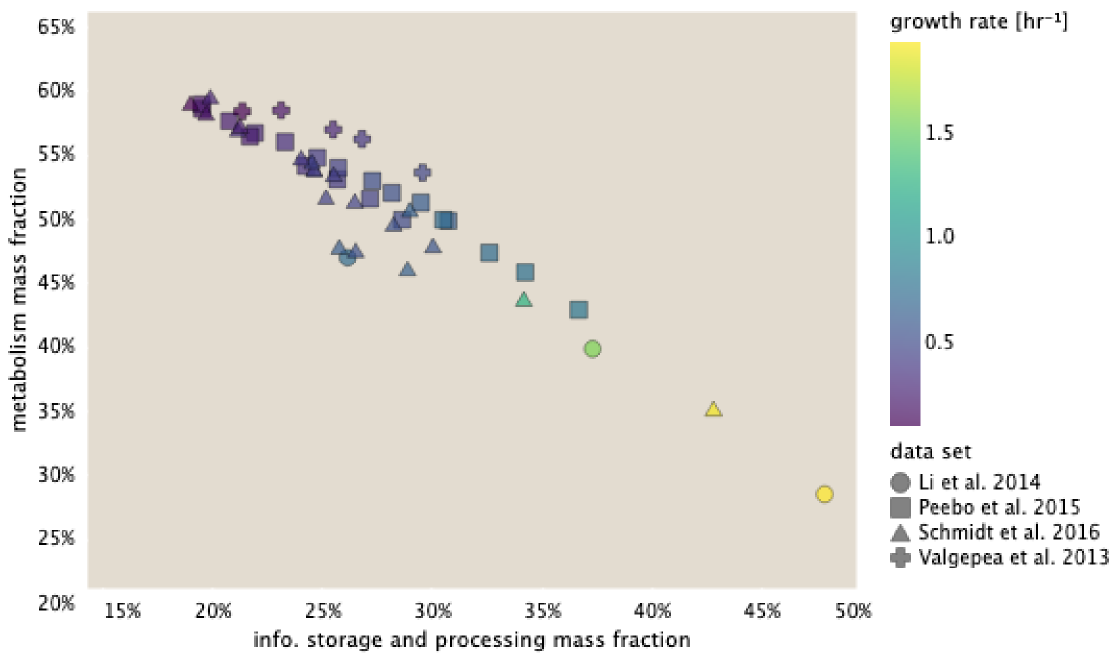


The plot above reveals that the sectors devoted to metabolism (purple) and information storage & processing (green) have the strongest dependence on the grwoth rate and are anti-correlated with one another. The other sectors appear more-or-less independent of growth rate. To quantify the relationship between the information storage & processing and metabolism sectors, we can plot the mass fraction of one against the other, and color the points by growth rate.

[43]:
```
# Compute the correlation dataframe.
info_metab_corr = pd.DataFrame([])
for g, d in mass_frac.groupby(['dataset_name', 'condition', 'growth_rate_hr']):
    metab = d[d['cog_class']=='metabolism']['frac'].values[0]
    info = d[d['cog_class'].str.contains('information')]['frac'].values[0]
    info_metab_corr = info_metab_corr.append({'dataset_name':g[0],
                                              'condition':g[1],
                                              'growth_rate_hr':g[2],
                                              'info_frac':info,
                                              'metab_frac':metab},
                                            ignore_index=True)
```

[50]:
```
# Define the defaults
defaults =  {'fill':alt.Color('growth_rate_hr:Q', scale=alt.
 ↪Scale(scheme='viridis'), legend=alt.Legend(title='growth rate␣
 ↪[hr\u207b\u00b9]'))}
base = alt.Chart(info_metab_corr)
base.mark_point(size=75).encode(
            x=alt.X('info_frac:Q',
                    axis=alt.Axis(format='%', title='info. storage and␣
 ↪processing mass fraction'),
                   scale=alt.Scale(domain=[0.15, 0.5])),
            y=alt.Y('metab_frac:Q',
                    axis=alt.Axis(format='%', title='metabolism mass fraction'),
                   scale=alt.Scale(domain=[0.2, 0.65])),
            shape=alt.Shape('dataset_name:O', legend={'title':'data set'}),
            strokeWidth=alt.value(0.5),
            **defaults
        ).interactive()
```

[50]:



[41]:
```
# Load the complex subunit counts.
subunits = pd.read_csv('../../data/compiled_annotated_complexes.csv')
```

```
# Load the compiled data
data = pd.read_csv('../../data/compiled_absolute_measurements.csv')
data['gene_name'] = data['gene_name'].str.lower()

# Compute the minimum number of complexes.
complex_count = subunits.groupby(['dataset', 'dataset_name', 'condition',␣
↪'growth_rate_hr', 'complex_annotation', 'complex'])['n_units'].mean().
↪reset_index()
```

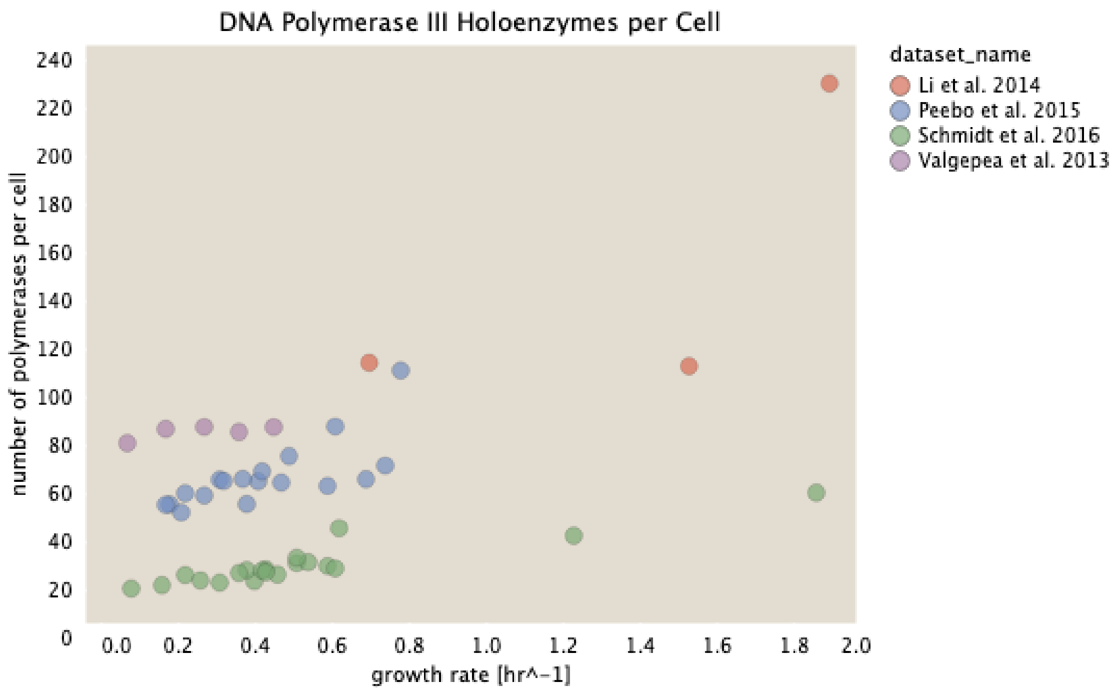
[15]:
```
complex_count.sort_values(by='complex_annotation', inplace=True)
selector = pn.widgets.Select(name='annotated complex',␣
↪options=list(complex_count['complex_annotation'].unique()), value='1-PFK')
@pn.depends(cplx=selector.param.value)
def plot_cplx(cplx):
    sel_cplx = complex_count[complex_count['complex_annotation']==cplx]
    chart = alt.Chart(sel_cplx, width=500, height=250).mark_point(size=40).
↪encode(
        x=alt.X('growth_rate_hr:Q', axis={'title':'growth rate [hr^-1]'}),
        y=alt.Y('n_units:Q', axis={'title':'minimimum copy number'}),
        fill='dataset_name:N',
        stroke=alt.value('black'),
        strokeWidth=alt.value(0.2),
        tooltip=['condition']
    ).properties(title=cplx.replace('&alpha;', ' ').replace('&beta;', ' ')).
↪interactive()
    return chart

pn.Column(selector, plot_cplx)
```

[15]:
```
Column
    [0] Select(name='annotated complex', options=['&alpha;-D-xyloside xyloh…],
value='&alpha;-D-xyloside x…)
    [1] ParamFunction(function)
```

1.3 DNA Polymerase

[5]:
```
complexes = ['CPLX0-3803']
dna_pol = complex_count[complex_count['complex'].isin(complexes)]
base = alt.Chart(dna_pol)
individuals = base.mark_point(size=80).encode(
            x=alt.X('growth_rate_hr:Q', axis={'title':'growth rate [hr^-1]'}),
            y=alt.Y('n_units:Q', axis={'title':'number of polymerases per␣
↪cell'}),
            fill='dataset_name:N',
            strokeWidth=alt.value(0.2)
).interactive().properties(title='DNA Polymerase III Holoenzymes per Cell')
individuals.save('../../figures/dna_pol.html')
individuals
```

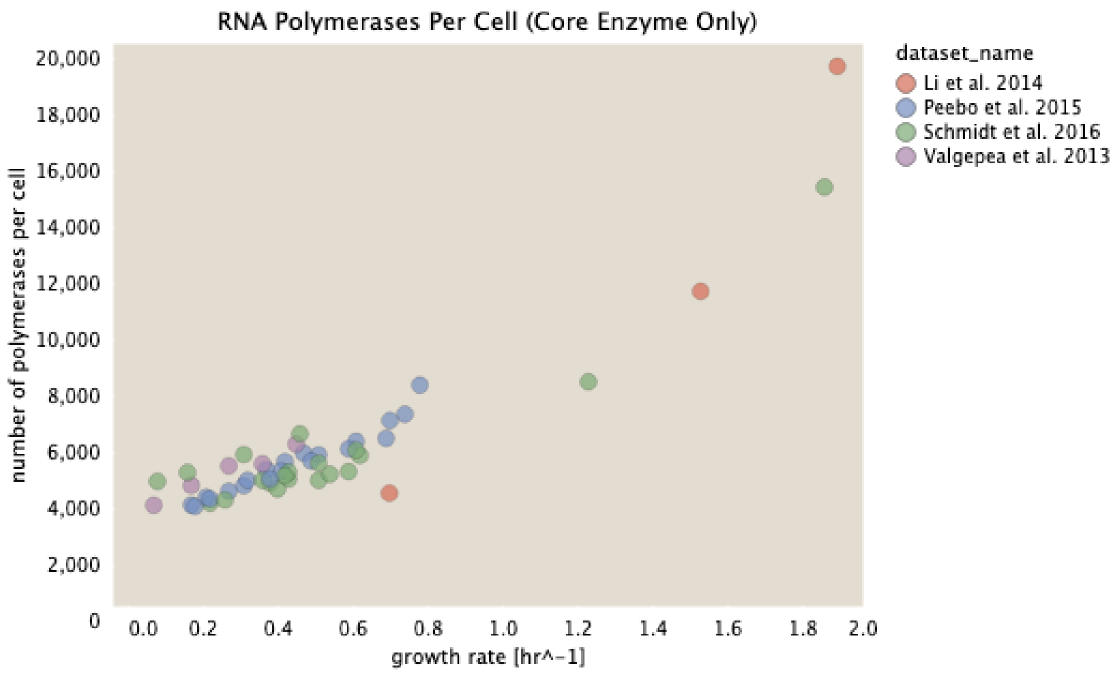
[5]:



1.4 RNA Polymerase

[6]:
```
complexes = ['APORNAP-CPLX']
rna_pol = complex_count[complex_count['complex'].isin(complexes)]
base = alt.Chart(rna_pol)
individuals = base.mark_point(size=80).encode(
            x=alt.X('growth_rate_hr:Q', axis={'title':'growth rate [hr^-1]'}),
            y=alt.Y('n_units:Q', axis={'title':'number of polymerases per␣
 ↪cell'}),
            fill='dataset_name:N',
            strokeWidth=alt.value(0.2)
).interactive().properties(title='RNA Polymerases Per Cell (Core Enzyme Only)')
individuals.save('../../figures/rnap.html')
individuals
```

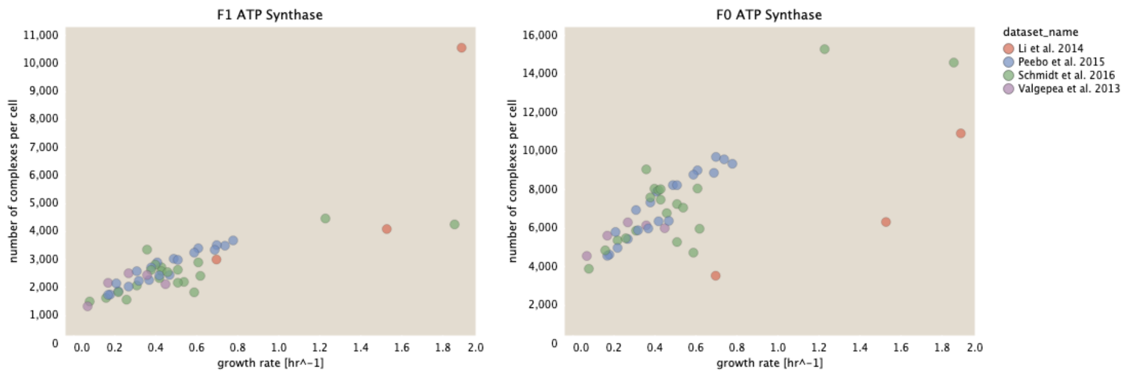
[6]:



1.5 ATP Synthesis

[8]:
```
complexes = ['F-1-CPLX', 'F-O-CPLX']
f1 = complex_count[complex_count['complex']=='F-O-CPLX']
f0 = complex_count[complex_count['complex']=='F-1-CPLX']
f1_base = alt.Chart(f1)
f0_base = alt.Chart(f0)
f1_individuals = f1_base.mark_point(size=80).encode(
            x=alt.X('growth_rate_hr:Q', axis={'title':'growth rate [hr^-1]'}),
            y=alt.Y('n_units:Q', axis={'title':'number of complexes per cell'}),
            fill='dataset_name:N',
            strokeWidth=alt.value(0.2)
).interactive().properties(title='F1 ATP Synthase')
f0_individuals = f0_base.mark_point(size=80).encode(
            x=alt.X('growth_rate_hr:Q', axis={'title':'growth rate [hr^-1]'}),
            y=alt.Y('n_units:Q', axis={'title':'number of complexes per cell'}),
            fill='dataset_name:N',
            strokeWidth=alt.value(0.2)
).interactive().properties(title='F0 ATP Synthase')
fig = f1_individuals | f0_individuals
fig.save('../../figures/f1f0_atp_synthase.html')
fig
```

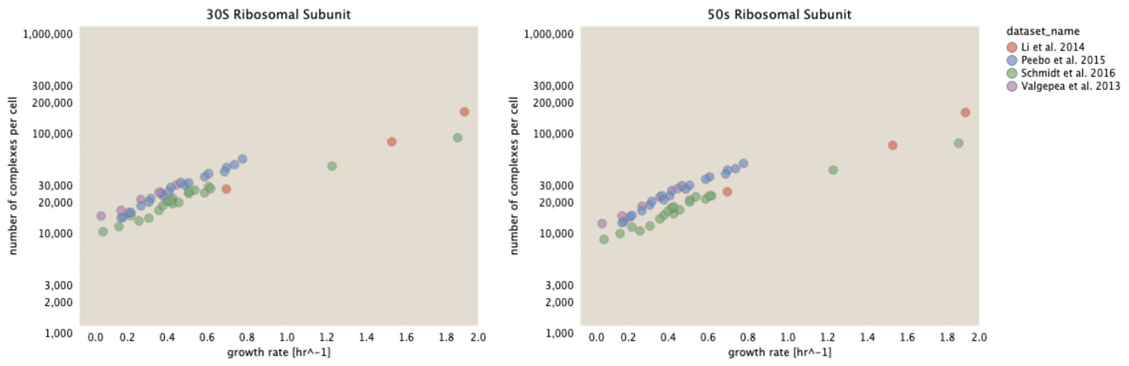
[8]:



1.6 Ribosomes

[9]:
```
small_sub = complex_count[complex_count['complex']=='CPLX0-3953']
large_sub = complex_count[complex_count['complex']=='CPLX0-3962']
small_base = alt.Chart(small_sub)
large_base = alt.Chart(large_sub)

small_individuals = small_base.mark_point(size=80).encode(
            x=alt.X('growth_rate_hr:Q', axis={'title':'growth rate [hr^-1]'}),
            y=alt.Y('n_units:Q', axis={'title':'number of complexes per cell'},␣
 ↪scale={'type':'log'}),
            fill='dataset_name:N',
            strokeWidth=alt.value(0.2)
).interactive().properties(title='30S Ribosomal Subunit')
large_individuals = large_base.mark_point(size=80).encode(
            x=alt.X('growth_rate_hr:Q', axis={'title':'growth rate [hr^-1]'}),
            y=alt.Y('n_units:Q', axis={'title':'number of complexes per cell'},␣
 ↪scale={'type':'log'}),
            fill='dataset_name:N',
            strokeWidth=alt.value(0.2)
).interactive().properties(title='50s Ribosomal Subunit')
fig = small_individuals | large_individuals
# fig.save('../../figures/f1f0_atp_synthase.html')
fig
```

[9]:



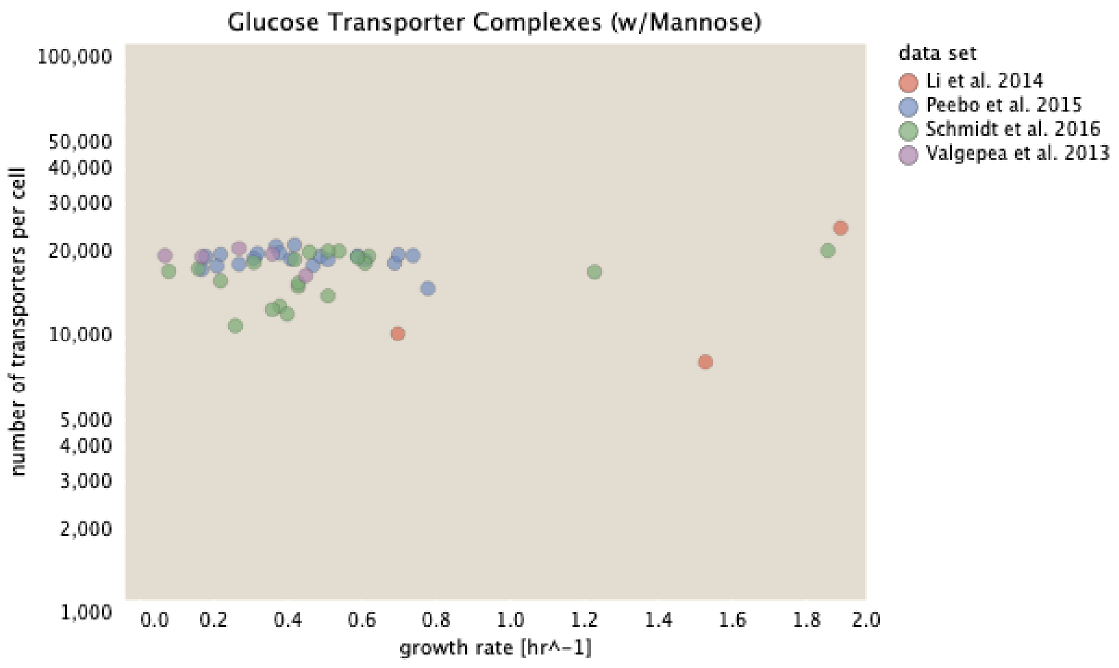
1.7 Sugar transporters

Using the GO term GO:0046323 which is assigned to proteins with putative Glucose import function

[53]:
```
pts_system_complexes = ['CPLX-157', 'ASCF-MONOMER', 'CPLX-165']
tporters = complex_count[complex_count['complex'].isin(pts_system_complexes)].
 ↪groupby(
    ['dataset_name', 'condition', 'growth_rate_hr', 'complex']).mean().
 ↪reset_index()
tporters = tporters[tporters['complex'].isin(pts_system_complexes)].groupby(
    ['dataset_name', 'condition', 'growth_rate_hr']).sum().reset_index()
tporters['n_units'] = np.round(tporters['n_units'].values)
```

[55]:
```
base = alt.Chart(tporters).mark_point(size=60)
min_complex_plot = alt.Chart(tporters).mark_point(size=60).encode(
            x=alt.X('growth_rate_hr:Q', axis={'title':'growth rate [hr^-1]'}),
            y=alt.Y('n_units:Q', axis={'title':'number of transporters per␣
 ↪cell'}, scale={'type':'log'}),
            fill=alt.Color('dataset_name:N',legend={'title':'data set'}),
            tooltip=['condition', 'dataset_name', 'growth_rate_hr', 'n_units'],
            strokeWidth=alt.value(0.2)).properties(title='Glucose Transporter␣
 ↪Complexes (w/Mannose)')

min_complex_plot.save('../../figures/glucose_transporters.html')
min_complex_plot
```

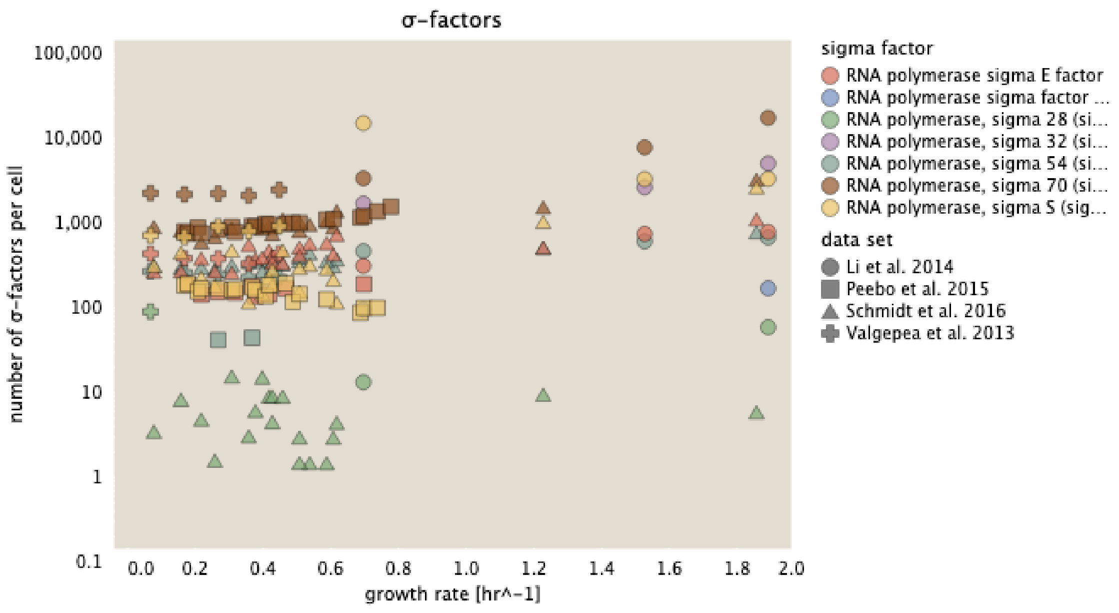
[55]:



1.8 Sigma Factors

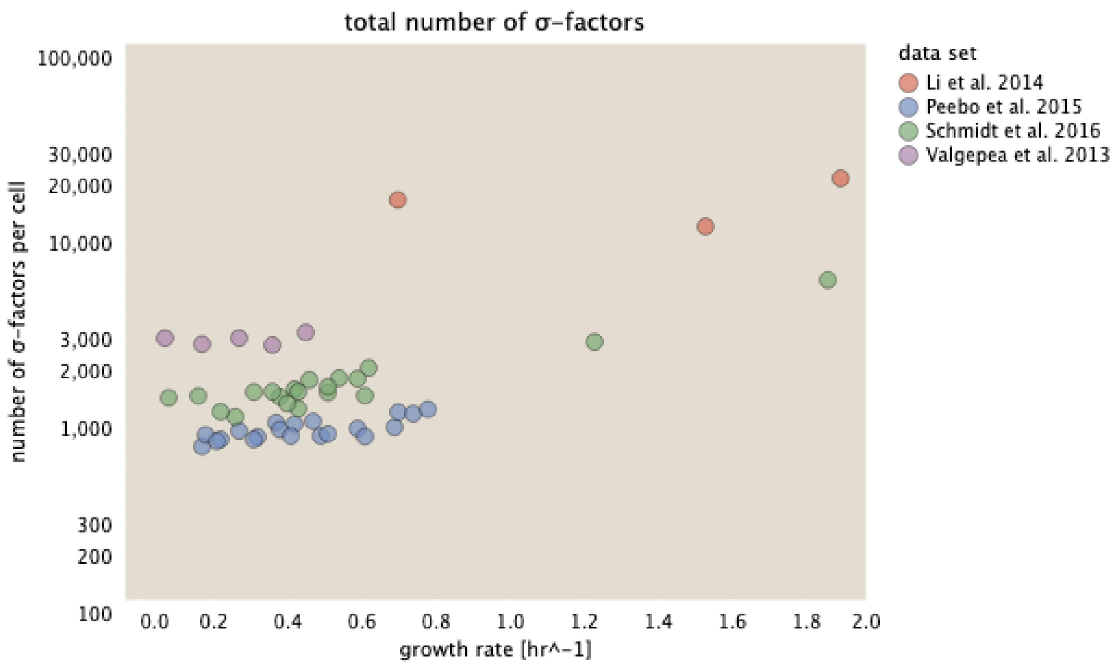
[77]:
```
sigma_factor_complexes = ['rpoe', 'feci', 'flia','rpoh','rpon', 'rpod', 'rpos']
sigma_factors = subunits[subunits['gene_name'].str.lower().
 ↪isin(sigma_factor_complexes)]
sigma_factors_sum = subunits[subunits['gene_name'].str.lower().isin(
                            sigma_factor_complexes)
                            ].groupby(['dataset_name',
                                       'condition',
                                       'growth_rate_hr']).sum().reset_index()
indiv_plot = alt.Chart(sigma_factors).mark_point(size=80).encode(
            x=alt.X('growth_rate_hr:Q', axis={'title':'growth rate [hr^-1]'}),
            y=alt.Y('n_units:Q', axis={'title':'number of  -factors per cell'},␣
 ↪scale={'type':'log'}),
            shape=alt.Shape('dataset_name:N',legend={'title':'data set'}),
            fill=alt.Color('gene_product:N', legend={'title':'sigma factor'}),
            tooltip=['condition', 'dataset_name', 'growth_rate_hr', 'n_units'],
            strokeWidth=alt.value(0.5)).properties(title=' -factors')
indiv_plot
```

[77]:



[94]:
```
tot_plot = alt.Chart(sigma_factors_sum).mark_point(size=80).encode(
            x=alt.X('growth_rate_hr:Q', axis={'title':'growth rate [hr^-1]'}),
            y=alt.Y('n_units:Q', axis={'title':'number of  -factors per cell'},␣
 ↪scale={'type':'log'}),
            fill=alt.Color('dataset_name:N',legend={'title':'data set'}),
            tooltip=['condition', 'dataset_name', 'growth_rate_hr', 'n_units'],
            strokeWidth=alt.value(0.5)).properties(title='total number of␣
 ↪ -factors')
tot_plot
```

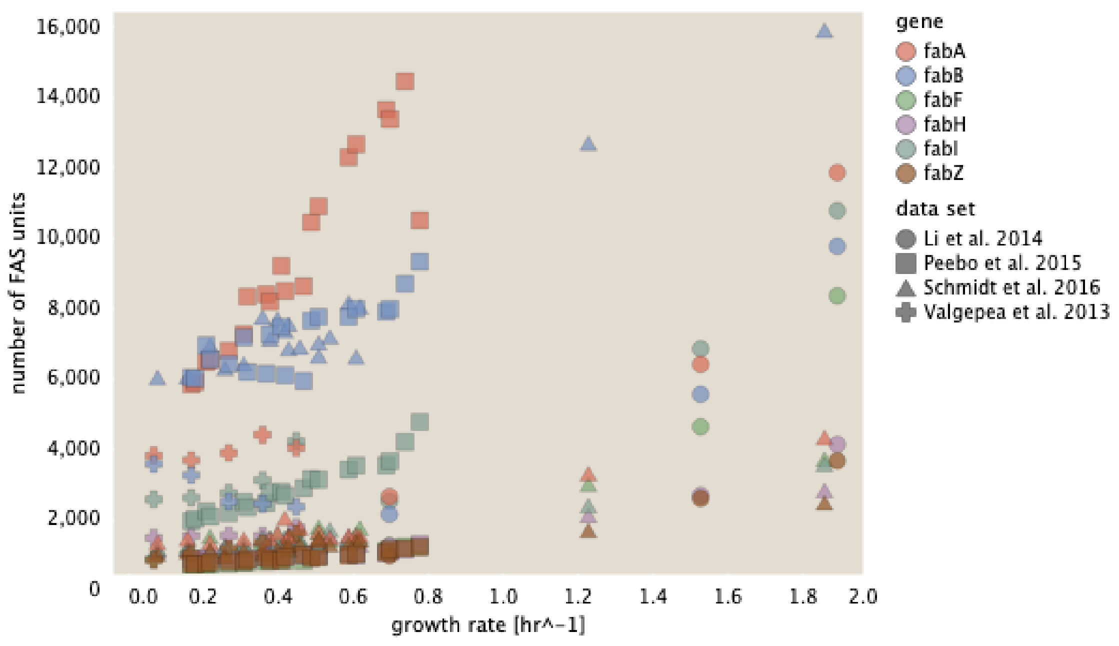
[94]:



1.9 Fatty Acid Synthesis

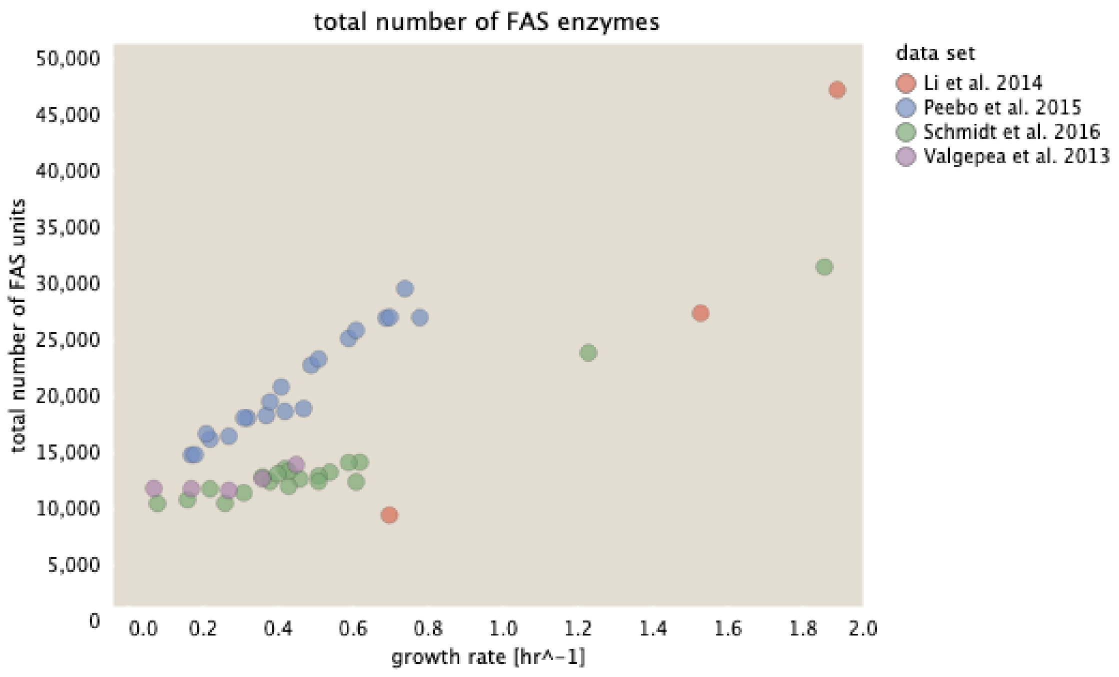
[95]:
```
fabs = subunits[subunits['gene_name'].str.contains('fab')]
alt.Chart(fabs).mark_point(size=80).encode(
        x=alt.X('growth_rate_hr:Q', axis={'title':'growth rate [hr^-1]'}),
        y=alt.Y('n_units:Q', axis={'title':'number of FAS units'}),
        fill=alt.Color('gene_name', legend={'title':'gene'}),
        shape=alt.Shape('dataset_name', legend={'title':'data set'}),
        strokeWidth=alt.value(0.2)
).interactive()
```

[95]:



```
[96]: fabs_tot = subunits[subunits['gene_name'].str.contains('fab')].
       ↪groupby(['dataset_name', 'condition', 'growth_rate_hr']).sum().reset_index()
      alt.Chart(fabs_tot).mark_point(size=80).encode(
              x=alt.X('growth_rate_hr:Q', axis={'title':'growth rate [hr^-1]'}),
              y=alt.Y('n_units:Q', axis={'title':'total number of FAS units'}),
              fill=alt.Color('dataset_name', legend={'title':'data set'}),
              strokeWidth=alt.value(0.2)
      ).interactive().properties(title='total number of FAS enzymes')
```

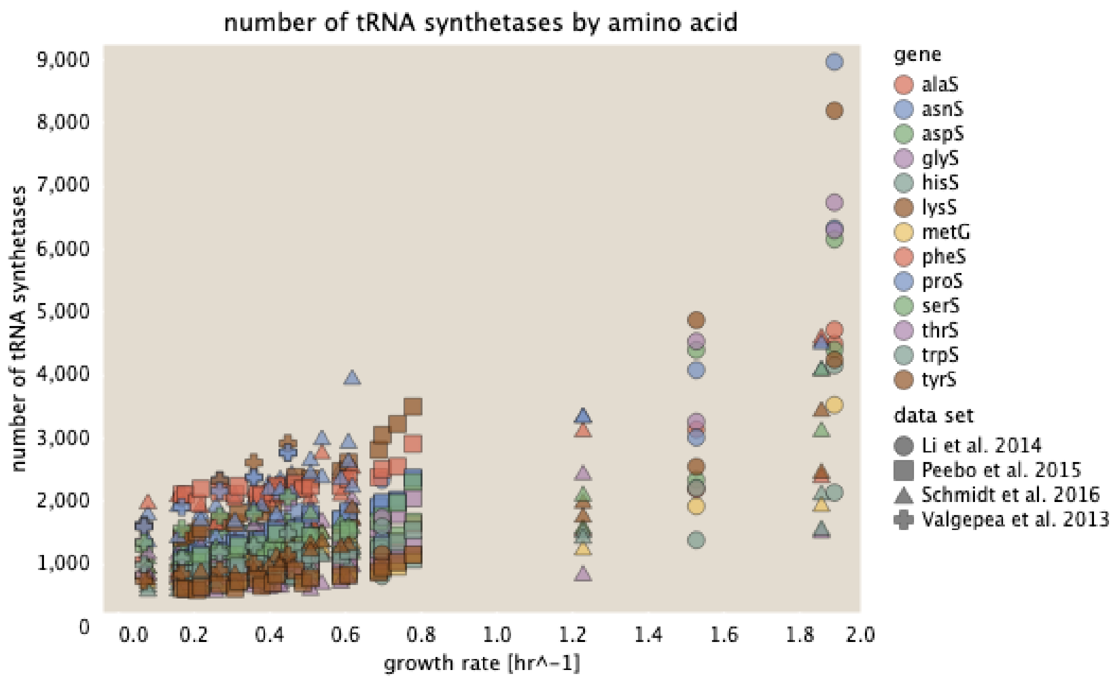
```
[96]:
```



1.10 tRNA Synthesis

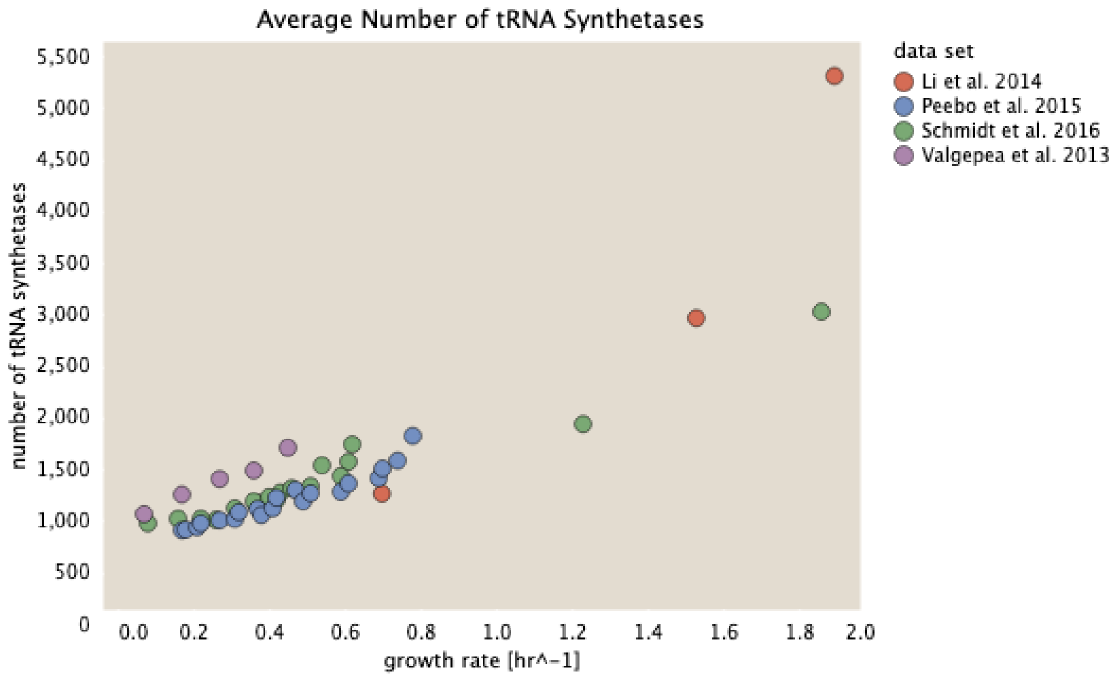
```
[97]: tRNA_prots = ['args', 'cyss', 'glns', 'gltx',
                   'iles', 'leus', 'vals', 'alas',
                   'asns', 'asps', 'tyrs', 'trps',
                   'thrs', 'sers', 'pros', 'phes',
                   'metg', 'lyss', 'hiss', 'glys']
      tRNA_syns = subunits[subunits['gene_name'].str.lower().isin(tRNA_prots)]
      alt.Chart(tRNA_syns).mark_point(size=80).encode(
              x=alt.X('growth_rate_hr:Q', axis={'title':'growth rate [hr^-1]'}),
              y=alt.Y('n_units:Q', axis={'title':'number of tRNA synthetases'}),
              fill=alt.Color('gene_name:N', legend={'title':'gene'}),
              shape=alt.Shape('dataset_name:N', legend={'title':'data set'}),
              strokeWidth=alt.value(0.5)
      ).interactive().properties(title='number of tRNA synthetases by amino acid')
```

[97]:



```
[98]: alt.Chart(tRNA_syns).mark_point(size=80).encode(
              x=alt.X('growth_rate_hr:Q', axis={'title':'growth rate [hr^-1]'}),
              y=alt.Y('mean(n_units):Q', axis={'title':'number of tRNA synthetases'}),
              fill=alt.Color('dataset_name:N', legend={'title':'data set'}),
              strokeWidth=alt.value(0.5)
      ).interactive().properties(title='Average Number of tRNA Synthetases')
```

[98]:



2 Stoichiometries

[99]:
```
subunits.head()
```

[99]:
```
  gene_name b_number     condition  \
0      fruK    b2168      lb_miller
1      fruK    b2168  glycerol_pAA
2      fruK    b2168        acetate
3      fruK    b2168       fumarate
4      fruK    b2168      galactose

                                            go_terms   cog_class  \
0  GO:0008443; GO:0042803; GO:0046835; GO:0005829…  metabolism
1  GO:0008443; GO:0042803; GO:0046835; GO:0005829…  metabolism
2  GO:0008443; GO:0042803; GO:0046835; GO:0005829…  metabolism
3  GO:0008443; GO:0042803; GO:0046835; GO:0005829…  metabolism
4  GO:0008443; GO:0042803; GO:0046835; GO:0005829…  metabolism

                          cog_category cog_letter  growth_rate_hr  \
0  carbohydrate transport and metabolism          G            1.90
1  carbohydrate transport and metabolism          G            1.27
2  carbohydrate transport and metabolism          G            0.30
3  carbohydrate transport and metabolism          G            0.42
4  carbohydrate transport and metabolism          G            0.26

             gene_product  tot_per_cell  fg_per_cell       dataset  \
0  1-phosphofructokinase    220.512437     0.012356  schmidt_2016
1  1-phosphofructokinase    118.506404     0.006641  schmidt_2016
2  1-phosphofructokinase     25.649312     0.001437  schmidt_2016
3  1-phosphofructokinase     56.611667     0.003172  schmidt_2016
4  1-phosphofructokinase     92.210367     0.005167  schmidt_2016

          dataset_name   strain complex complex_annotation  n_subunits  \
0  Schmidt et al. 2016  BW25113   1-PFK                NaN         2.0
1  Schmidt et al. 2016  BW25113   1-PFK                NaN         2.0
2  Schmidt et al. 2016  BW25113   1-PFK                NaN         2.0
3  Schmidt et al. 2016  BW25113   1-PFK                NaN         2.0
4  Schmidt et al. 2016  BW25113   1-PFK                NaN         2.0

      n_units
0  110.256218
1   59.253202
2   12.824656
3   28.305834
```

```
4   46.105183
```

```
[ ]:
```