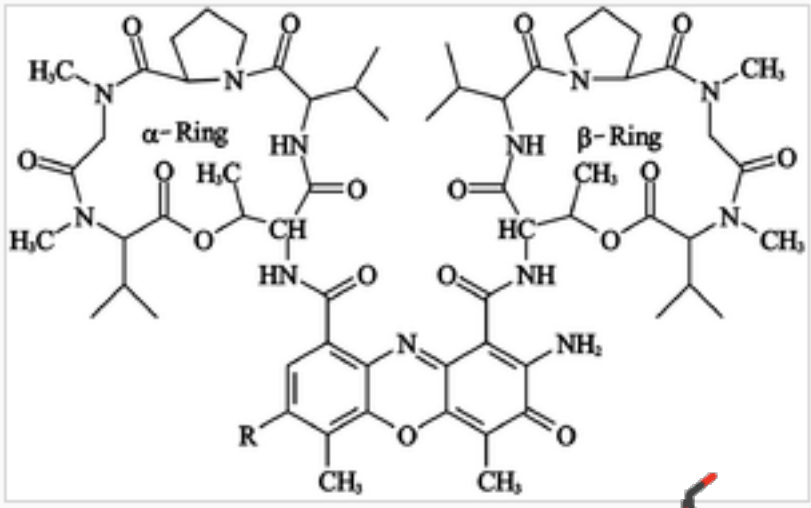
Timecourse study of *ATXN2* promoter expression

Hypothesis

- CAG length alters the increase of *ATXN2* expression over time
- CAG length alters the turnover of *ATXN2* over time

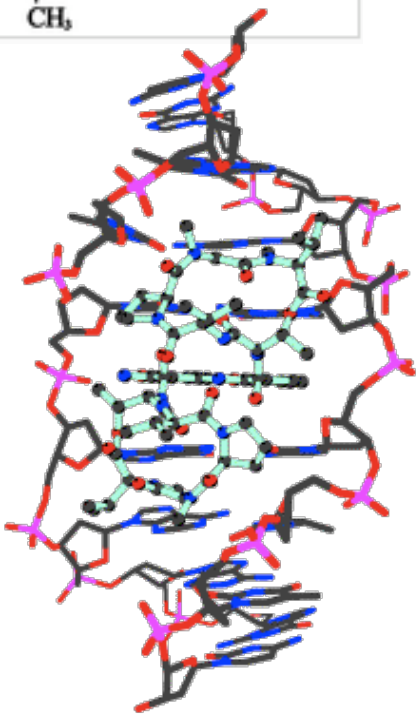
Actinomycin D

Isolated from soil streptomyces in 1940
Peptide antibiotic.



Actinomycin D is used as an investigative tool in cell biology to inhibit transcription. It does this by binding DNA at the transcription initiation complex and preventing elongation by RNA polymerase.

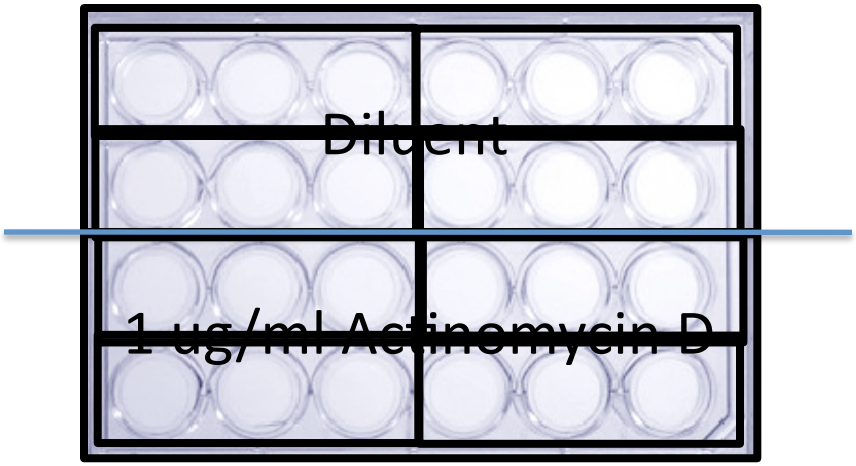
Because it can bind DNA duplexes, it can also interfere with DNA replication, but not as efficiently as other investigative compounds like hydroxyurea.



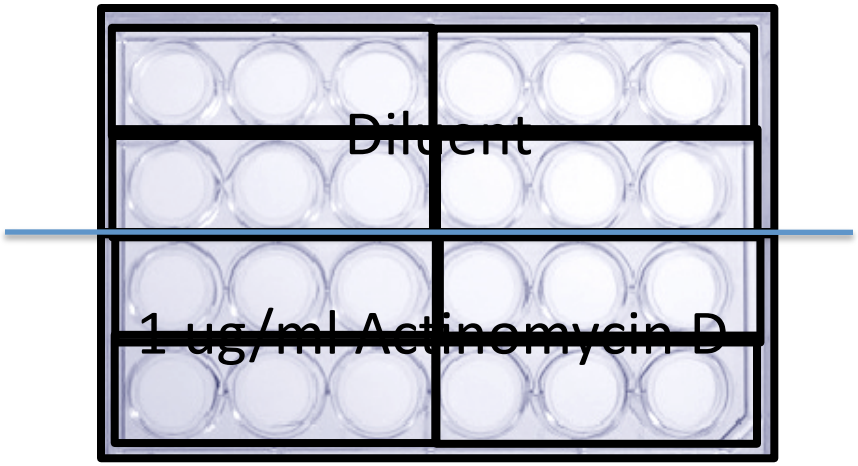
Actinomycin D is also used as an anticancer agent.

Four plates transfected identically with indicated plasmids and pRL-SV40 and harvested and assayed at the indicated times

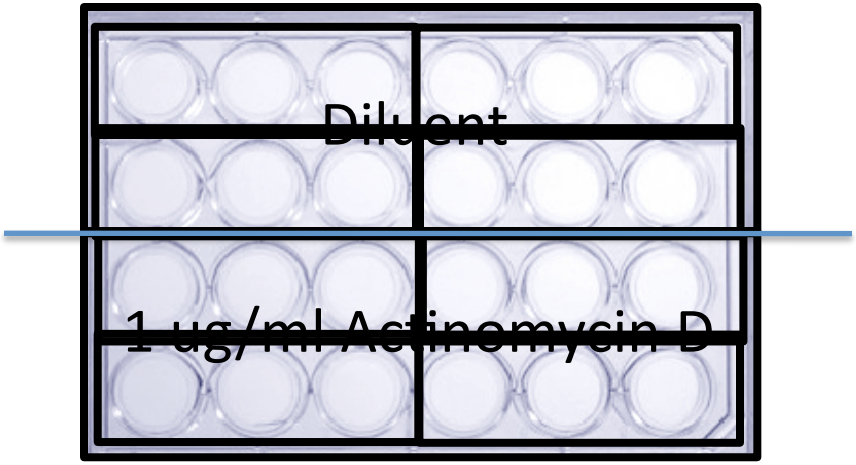
These [illegible] hours



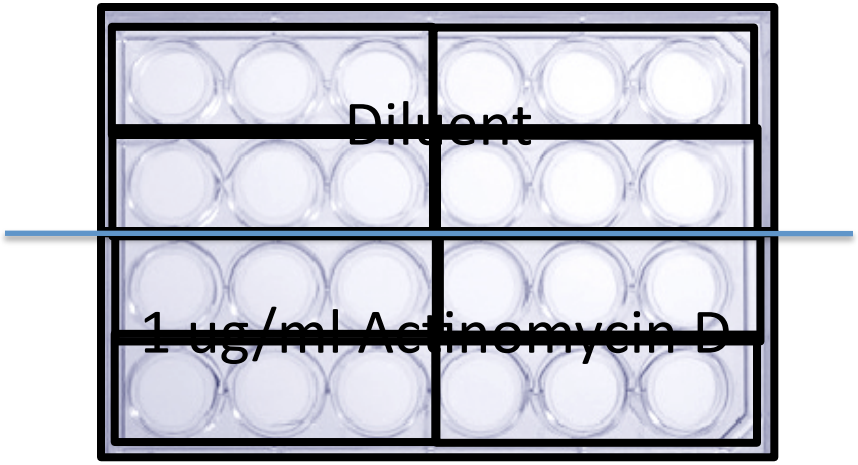
pGL2c.5A3c (CAG1)



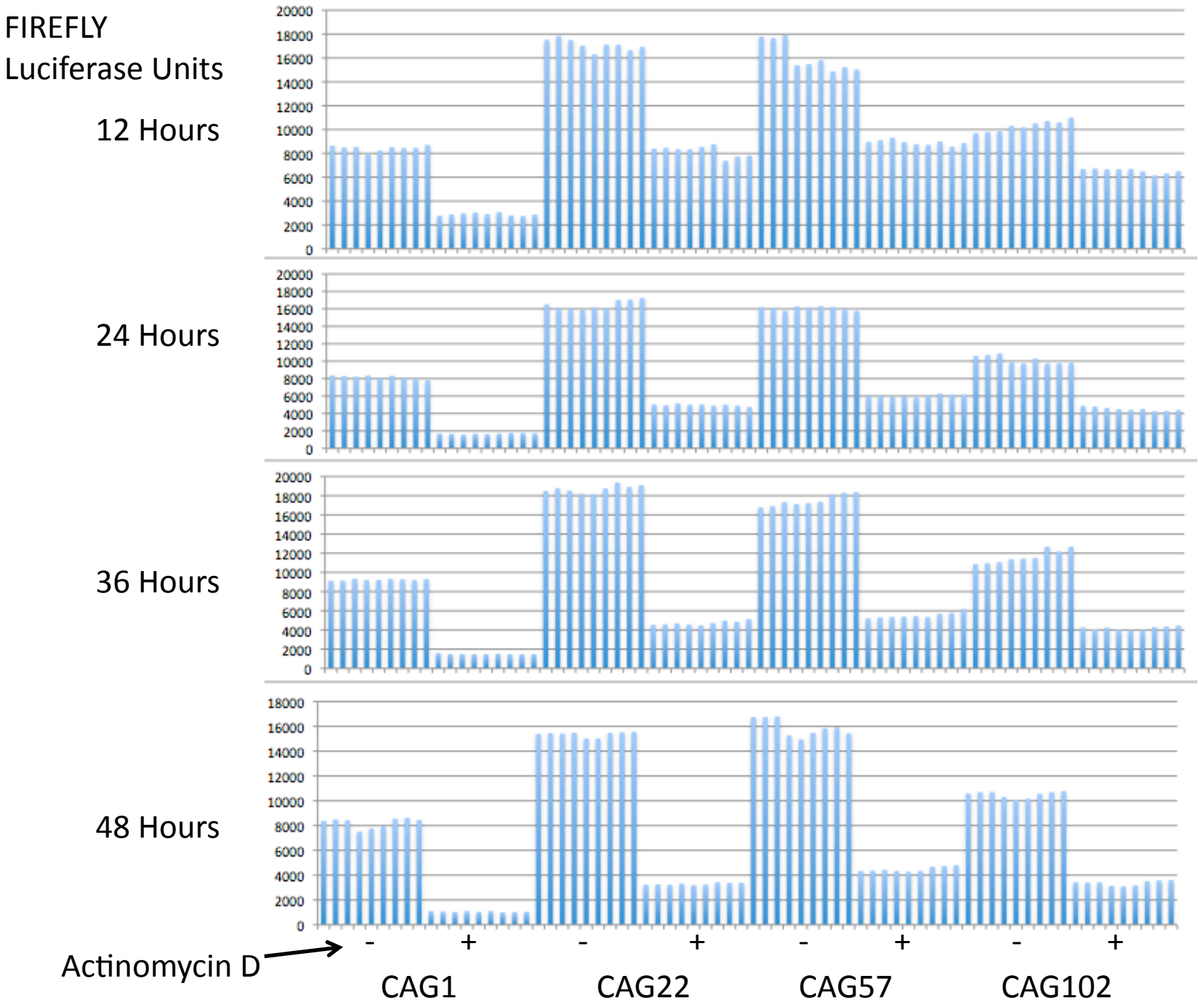
pGL2c.5B3c (CAG22)

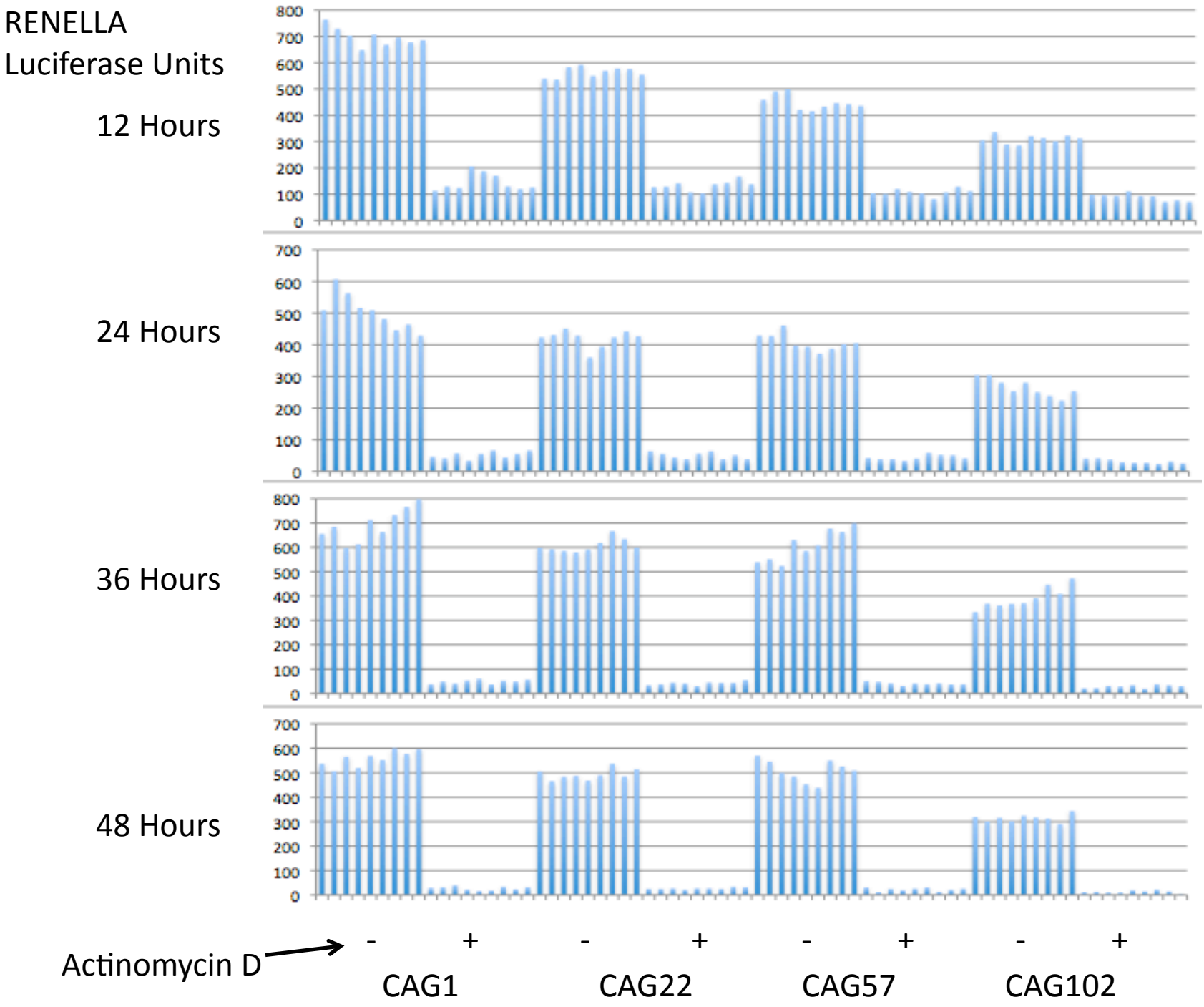


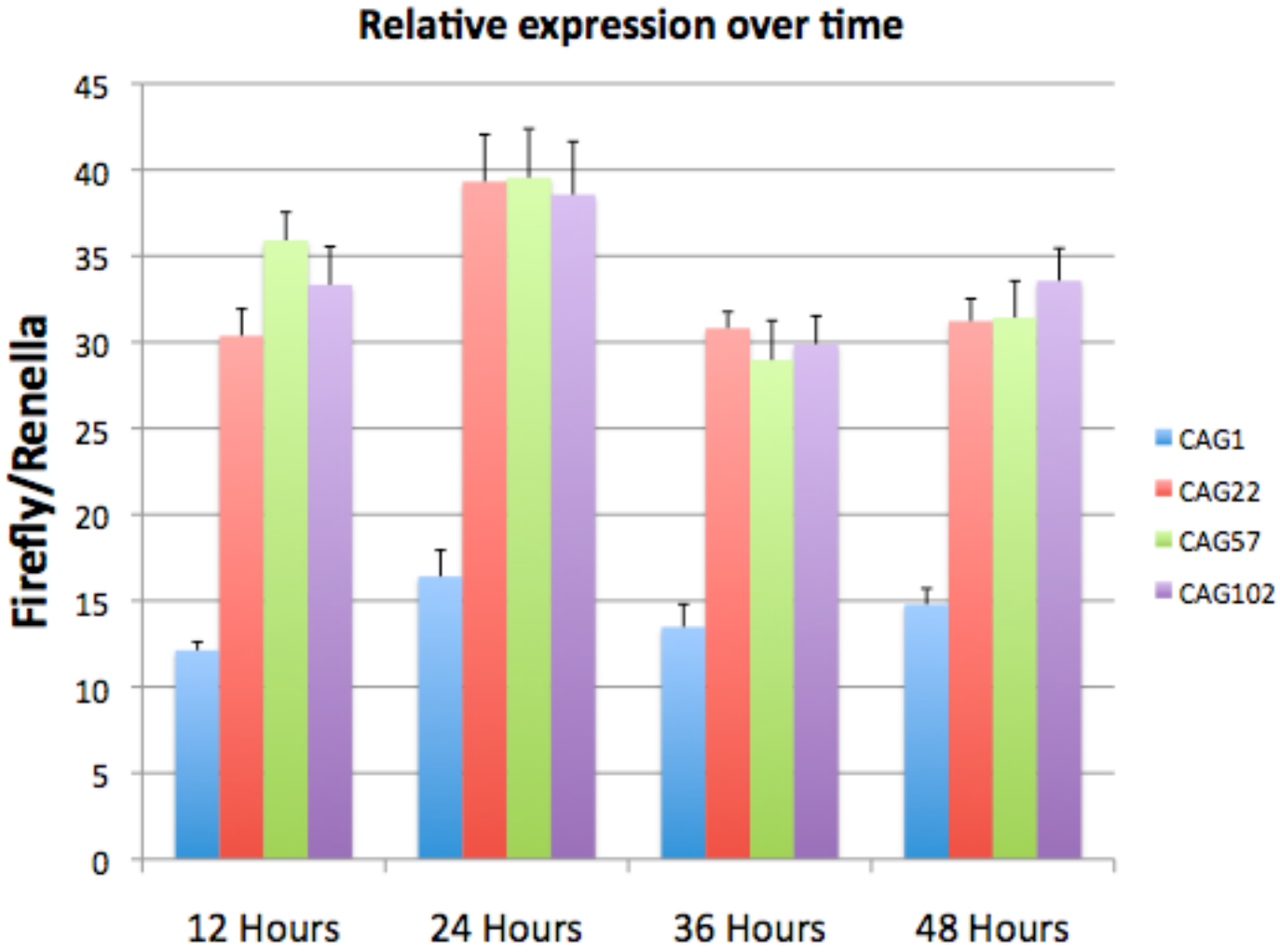
pGL2c.5C3c (CAG57)



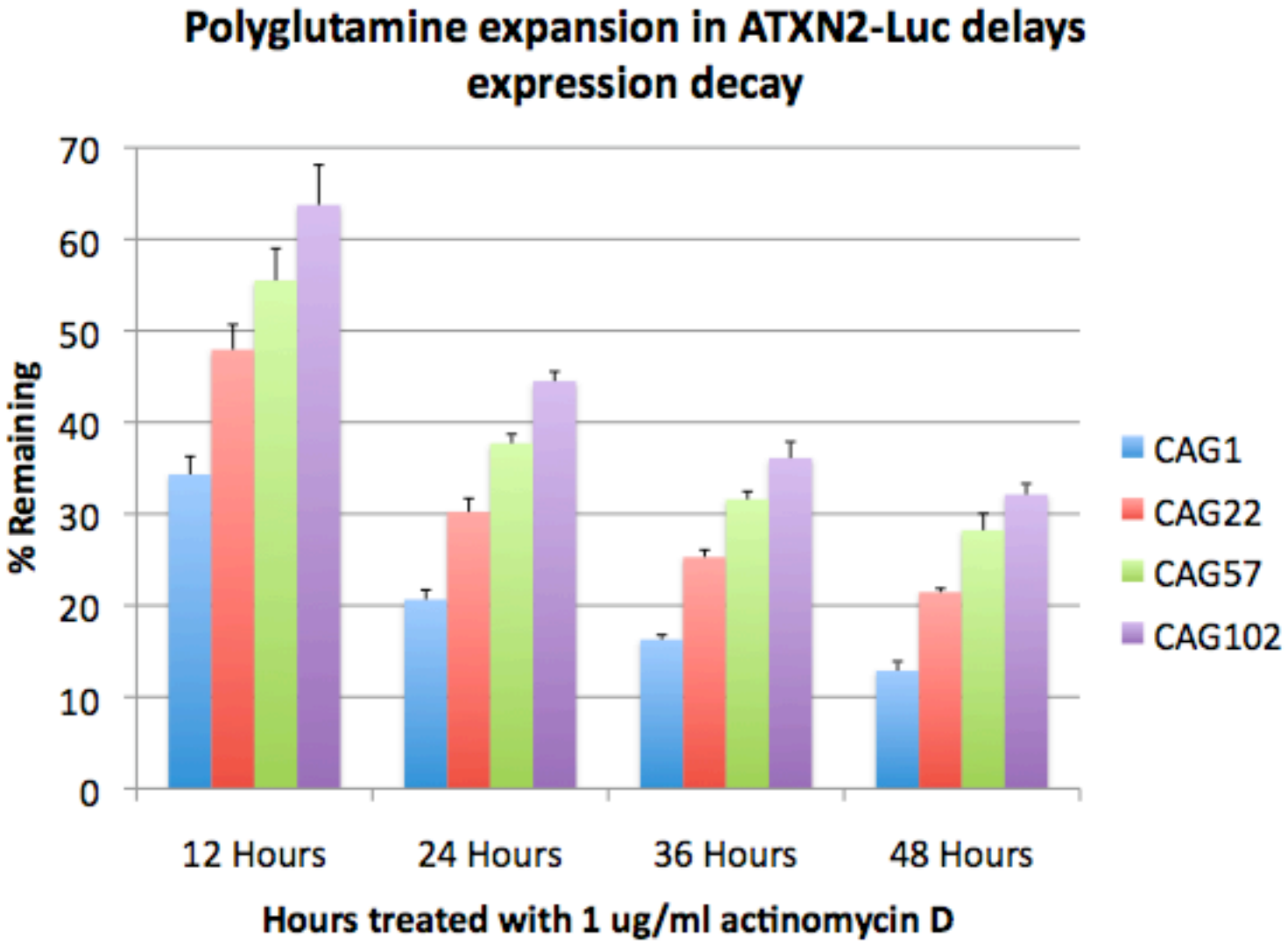
pGL2c.5D$_k$3c (CAG102)


FIREFLY
Luciferase Units
12 Hours
24 Hours
36 Hours
48 Hours
20000
18000
16000
14000
12000
10000
8000
6000
4000
2000
0
Actinomycin D
- + - + - + - +
CAG1
CAG22
CAG57
CAG102


RENELLA
Luciferase Units
12 Hours
24 Hours
36 Hours
48 Hours
800
700
600
500
400
300
200
100
0
Actinomycin D
- + - + - + - +
CAG1
CAG22
CAG57
CAG102



This is different than previously observed. Maybe differences have to do with the different experimental conditions of this actinomycin design. These in this chart were the controls and they were actually cultured for an additional 48 hours more than shown. At 48 hours an equal volume of media mixed with actinomycin diluent (water) was added and cultures were allowed to go for additional time as shown in the chart. 12 hours is actually 60 hours post transfection.



Two-way ANOVA $P<0.0001$ for CAG length

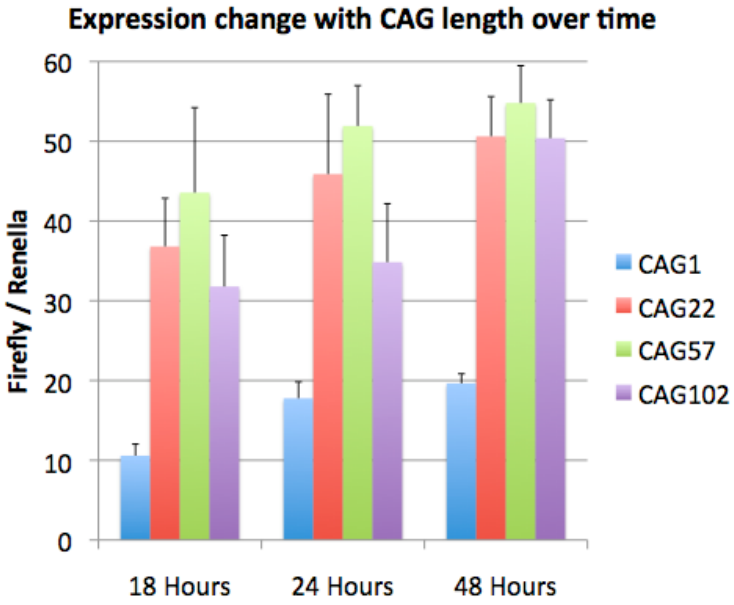
Bonferroni postest: $P<0.001$ for a test between any pair of CAGs

Conclusion

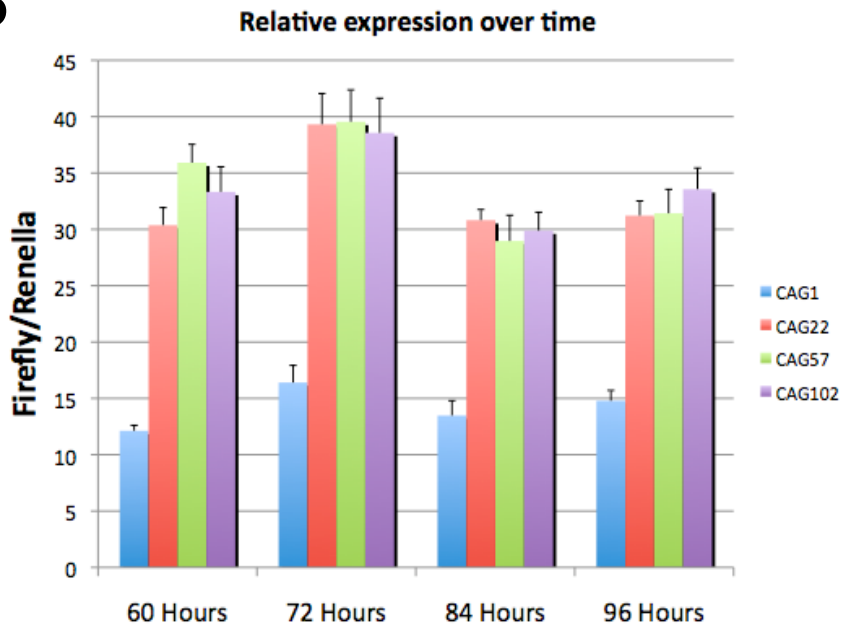
- *ATXN2*s with longer CAGs turn over at a reduced rate
- I think *ATXN2*s with longer CAGs take more time to reach maximal expression…but I have to test this with shorter times in the timecourse (24 hrs induction, harvest every 12 hrs).

A is a follow-up experiment to B. When comparing to previous work that I did testing expression at ~40 hours, B suggested to me that ATXN2-LUC with 102 CAGs take longer time to gain expression equal to ATXN2-Luc with CAG22. (This is because the previous experiments always showed CAG102 lower than CAG22 while in B CAG102 was equal, and I had never before taken the experiment out to 60 hours like in B.) So I set up the experiment shown in A. The data are still suggestive but CAG102 appears to lag.

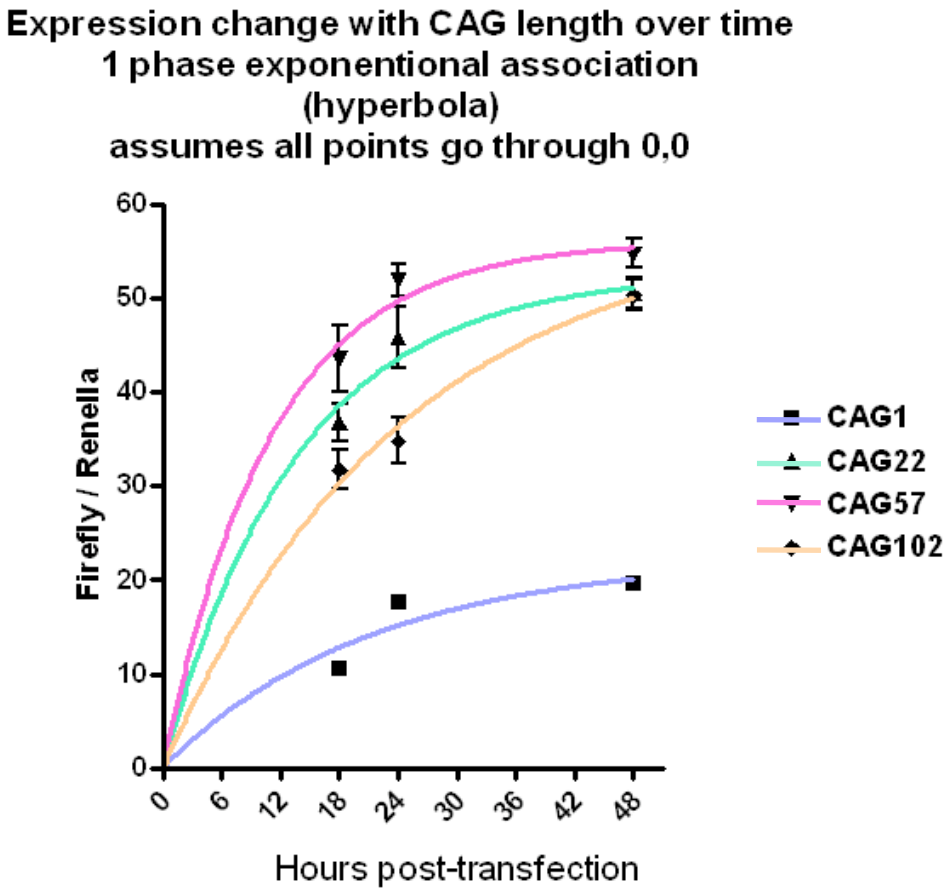
A



B



Note also that CAG57 appears to have strong expression. After reviewing my notebook of other trials with CAG57, the only time it was lower than CAG22 was when Patrick Gordon tested it for me.


Expression change with CAG length over time
1 phase exponentional association
(hyperbola)
assumes all points go through 0,0
Firefly / Renella
60
50
40
30
20
10
0
0
6
12
18
24
30
36
42
48
Hours post-transfection
CAG1
CAG22
CAG57
CAG102