
AbSciCon
2019

1
00:00:00,790 --> 00:00:07,320
[Music]

2
00:00:12,209 --> 00:00:09,080
[Applause]

3
00:00:14,750 --> 00:00:12,219
my name is Jack smelly and I'm at st.

4
00:00:17,370 --> 00:00:14,760
Louis University with the bomb lab and

5
00:00:19,650 --> 00:00:17,380
we have been working in collaboration

6
00:00:23,070 --> 00:00:19,660
with the Burke lab for some time on

7
00:00:25,669 --> 00:00:23,080
these fa be binding app tumors are f80

8
00:00:27,870 --> 00:00:25,679
binding RNA aptamers that increase

9
00:00:32,190 --> 00:00:27,880
potentially can increase the the redox

10
00:00:33,690 --> 00:00:32,200
potential of the bound flea oven so I

11
00:00:35,370 --> 00:00:33,700
guess for starters just give a little

12
00:00:37,140 --> 00:00:35,380
bit of background although not so much

13
00:00:39,240 --> 00:00:37,150

because I feel like a lot of people are

14
00:00:41,970 --> 00:00:39,250
adequate with this out what we're

15
00:00:45,479 --> 00:00:41,980
following here is RNA world hypothesis

16
00:00:48,660 --> 00:00:45,489
in that before president day cells with

17
00:00:50,700 --> 00:00:48,670
RNA DNA and proteins there was simpler

18
00:00:53,430 --> 00:00:50,710
system in which RNA could have

19
00:00:55,169 --> 00:00:53,440
contributed to being both the genetic

20
00:00:58,080 --> 00:00:55,179
material and also have catalytic

21
00:01:00,360 --> 00:00:58,090
function and also I also put the the

22
00:01:01,799 --> 00:01:00,370
peptides in that little protocell there

23
00:01:04,079 --> 00:01:01,809
just show that there might have been

24
00:01:08,400 --> 00:01:04,089
peptides around but not necessarily a

25
00:01:11,640 --> 00:01:08,410
formed from the RNA at that time some

26
00:01:14,219 --> 00:01:11,650
evidence of this is the ribonucleotide

27
00:01:18,210 --> 00:01:14,229
cofactors that we see today so all these

28
00:01:20,819 --> 00:01:18,220
cofactors fa d na d and then coenzyme a

29
00:01:22,560 --> 00:01:20,829
they all have p adenosine portion in it

30
00:01:25,859 --> 00:01:22,570
which is right with nucleotide co-factor

31
00:01:30,690 --> 00:01:25,869
is kind of like a prehistoric fossil if

32
00:01:33,420 --> 00:01:30,700
you will of the RNA world current cells

33
00:01:37,280 --> 00:01:33,430
and proteins use these cofactors in

34
00:01:39,749 --> 00:01:37,290
order to metabolize reactions so they

35
00:01:41,880 --> 00:01:39,759
essentially what they do is they have

36
00:01:43,649 --> 00:01:41,890
differential binding towards either the

37
00:01:46,950 --> 00:01:43,659
oxidized or reduced formed of these

38
00:01:50,249 --> 00:01:46,960

cofactors and in doing so they can vary

39
00:01:52,289 --> 00:01:50,259
the redox potential of that cofactor in

40
00:01:55,920 --> 00:01:52,299
order to carry out a wide variety of

41
00:01:58,080 --> 00:01:55,930
metabolic reactions and so and so the

42
00:02:00,569 --> 00:01:58,090
the idea behind this project is that if

43
00:02:03,330 --> 00:02:00,579
proteins can do it and really utilize

44
00:02:06,630 --> 00:02:03,340
the variability that comes with changing

45
00:02:07,620 --> 00:02:06,640
the redox potential can RNA also do the

46
00:02:09,840 --> 00:02:07,630
same thing because that would be

47
00:02:12,780 --> 00:02:09,850
extremely beneficial it's really kind of

48
00:02:15,780 --> 00:02:12,790
pushing forward with the RNA and and how

49
00:02:17,309 --> 00:02:15,790
it evolves from just you know simply

50
00:02:18,580 --> 00:02:17,319
finding things to it to actually

51
00:02:22,900 --> 00:02:18,590
catalyzing reactions

52
00:02:24,610 --> 00:02:22,910
such so the selections I'm just gonna go

53
00:02:26,940 --> 00:02:24,620
through really briefly because this was

54
00:02:30,220 --> 00:02:26,950
done well before I was even an undergrad

55
00:02:31,869 --> 00:02:30,230
but the Berk group identified a handful

56
00:02:35,170 --> 00:02:31,879
of RNA aptamers that preferentially

57
00:02:39,430 --> 00:02:35,180
binds to the oxidized fe D over the

58
00:02:41,830 --> 00:02:39,440
reduced fadh2 as you can see here so

59
00:02:45,430 --> 00:02:41,840
this is just an inline probing gel if

60
00:02:48,369 --> 00:02:45,440
you don't know what it is essentially if

61
00:02:50,050 --> 00:02:48,379
the band is dark here that indicates

62
00:02:52,900 --> 00:02:50,060
that the RNA took on a different

63
00:02:56,440 --> 00:02:52,910

structure in that conformation and

64
00:02:58,870 --> 00:02:56,450
whenever it was cleaved atom so in this

65
00:03:01,600 --> 00:02:58,880
Lane right here is the oxidized FA D and

66
00:03:03,580 --> 00:03:01,610
then here is reduced fadh2 and you can

67
00:03:05,289 --> 00:03:03,590
see that at similar concentrations we

68
00:03:07,360 --> 00:03:05,299
have different cleaving patterns which

69
00:03:10,630 --> 00:03:07,370
indicates different conformation the RNA

70
00:03:12,640 --> 00:03:10,640
that shows that the this RNA aptamer is

71
00:03:15,759 --> 00:03:12,650
preferentially binding to the oxidized

72
00:03:18,309 --> 00:03:15,769
fe D over at the reduced fadh2 and and

73
00:03:22,270 --> 00:03:18,319
just through inline probing they got

74
00:03:24,759 --> 00:03:22,280
rough dissociation constants of about 30

75
00:03:27,340 --> 00:03:24,769
micro molar for the oxidized FA D and

76
00:03:29,979 --> 00:03:27,350
greater than 500 micro molar for the

77
00:03:31,479 --> 00:03:29,989
reduced form now they're not you know

78
00:03:33,430 --> 00:03:31,489
super precise numbers but there's a

79
00:03:35,710 --> 00:03:33,440
there's a clear distinction that the

80
00:03:39,089 --> 00:03:35,720
these after MERS preferentially binds

81
00:03:42,670 --> 00:03:39,099
the oxidized form over the reduced form

82
00:03:45,819 --> 00:03:42,680
so now this is where I took over we

83
00:03:48,370 --> 00:03:45,829
decided to first look at UV vis of this

84
00:03:53,500 --> 00:03:48,380
so what I have appear structures of FA B

85
00:03:56,379 --> 00:03:53,510
and then 3 3 RNA aptamers that bind to

86
00:03:58,030 --> 00:03:56,389
FA D this X to be 2 right here was the

87
00:04:00,039 --> 00:03:58,040
one from the previous slide so this is

88
00:04:02,920 --> 00:04:00,049

the one that preferentially binds to the

89
00:04:05,409 --> 00:04:02,930
oxidized flavin over the reduced this F

90
00:04:07,509 --> 00:04:05,419
test one down here is a another one from

91
00:04:09,580 --> 00:04:07,519
the Burke lab they found that there was

92
00:04:13,390 --> 00:04:09,590
no difference between the oxidized and

93
00:04:16,029 --> 00:04:13,400
reduced binding and then this 27 F 81 up

94
00:04:17,710 --> 00:04:16,039
here the Burke lab originally it was

95
00:04:19,180 --> 00:04:17,720
proposed to be an Fe D Optima but they

96
00:04:21,009 --> 00:04:19,190
found that it actually is more than

97
00:04:24,279 --> 00:04:21,019
likely actually binding to the adenine a

98
00:04:26,310 --> 00:04:24,289
portion of FA D so we had three RNA

99
00:04:29,830 --> 00:04:26,320
aptamers that we want to look at and

100
00:04:31,010 --> 00:04:29,840
looking at ub vis we see that once you

101
00:04:35,200 --> 00:04:31,020
mix together the

102
00:04:37,580 --> 00:04:35,210
be in the RNA the ones that bind to this

103
00:04:40,490 --> 00:04:37,590
isoh locks Azim ring system here

104
00:04:43,369 --> 00:04:40,500
actually shift the uv-vis spectra of

105
00:04:45,140 --> 00:04:43,379
this and you know this is indicative of

106
00:04:48,760 --> 00:04:45,150
you know hydrogen bonding or ionic

107
00:04:51,890 --> 00:04:48,770
effects on different phases of fa D

108
00:04:54,559 --> 00:04:51,900
really some important parts is that this

109
00:04:58,040 --> 00:04:54,569
the X to be two Optima shifted the

110
00:05:00,110 --> 00:04:58,050
Maxima from 448 to 456 nanometers and

111
00:05:03,920 --> 00:05:00,120
then there's this presence of a shoulder

112
00:05:06,469 --> 00:05:03,930
at 482 nanometers so we had a good kind

113
00:05:08,240 --> 00:05:06,479

of baseline is to gauge whether or not

114
00:05:10,010 --> 00:05:08,250
binding is occurring so we decided to

115
00:05:13,430 --> 00:05:10,020
look at some kind of characteristics of

116
00:05:15,620 --> 00:05:13,440
binding with this app tumor so a couple

117
00:05:18,580 --> 00:05:15,630
of things we looked at is the time that

118
00:05:21,170 --> 00:05:18,590
it takes to bind and it's extremely fast

119
00:05:24,740 --> 00:05:21,180
probably within the order of seconds

120
00:05:27,589 --> 00:05:24,750
this is me just quickly pipetting RNA

121
00:05:28,790 --> 00:05:27,599
aptamer into a qubit and then pressing

122
00:05:31,820 --> 00:05:28,800
measure as quickly as I can

123
00:05:34,219 --> 00:05:31,830
and within 20 seconds we see this full

124
00:05:36,320 --> 00:05:34,229
peak shifts over to the fully bound form

125
00:05:38,120 --> 00:05:36,330
so really this this experiment was

126
00:05:39,770 --> 00:05:38,130
probably just diffusion limited as to

127
00:05:42,499 --> 00:05:39,780
how quickly it can actually diffuse to

128
00:05:44,959 --> 00:05:42,509
the solution but we know that it is

129
00:05:46,850 --> 00:05:44,969
extremely quick at binding and we know

130
00:05:48,499 --> 00:05:46,860
that this peak shift is indicative of

131
00:05:50,029 --> 00:05:48,509
binding because under denaturing

132
00:05:52,219 --> 00:05:50,039
conditions the peak will shift back

133
00:05:54,529 --> 00:05:52,229
towards that of PFA T so if we heat it

134
00:05:56,629 --> 00:05:54,539
up and it will shift its free Fe D let

135
00:05:58,339 --> 00:05:56,639
it cool down it will go back to the

136
00:06:02,959 --> 00:05:58,349
bound states and then also eight molar

137
00:06:04,519 --> 00:06:02,969
urea cetera et cetera so another thing

138
00:06:06,649 --> 00:06:04,529

we looked at was the divalent metal

139
00:06:09,200 --> 00:06:06,659
dependence so these selections were done

140
00:06:11,420 --> 00:06:09,210
with magnesium so we wanted to see how

141
00:06:13,820 --> 00:06:11,430
dependent they were on the divalent

142
00:06:15,980 --> 00:06:13,830
metals and we saw obviously with the

143
00:06:18,050 --> 00:06:15,990
increasing diving lit metals we get more

144
00:06:20,180 --> 00:06:18,060
and more binding with that at about 10

145
00:06:23,689 --> 00:06:20,190
millimolar kind of maxed out there was

146
00:06:26,180 --> 00:06:23,699
no change in binding there so interested

147
00:06:27,529 --> 00:06:26,190
in e i titled this the IV metal

148
00:06:30,140 --> 00:06:27,539
dependence instead of magnesium

149
00:06:33,620 --> 00:06:30,150
dependence because it also works with

150
00:06:35,600 --> 00:06:33,630
manganese calcium zinc and nickel so all

151
00:06:37,670 --> 00:06:35,610
of those diability metals induced

152
00:06:39,490 --> 00:06:37,680
binding it really didn't matter which

153
00:06:42,110 --> 00:06:39,500
one we use that they all caused the same

154
00:06:43,430 --> 00:06:42,120
amount of binding there and this was you

155
00:06:44,879 --> 00:06:43,440
know proven where we just add in an

156
00:06:46,619 --> 00:06:44,889
excess of EDTA to

157
00:06:48,450 --> 00:06:46,629
Tukey laid out the divalent metals and

158
00:06:51,360 --> 00:06:48,460
then that would shift it back towards

159
00:06:53,459 --> 00:06:51,370
that of free fe d another interesting

160
00:06:56,550 --> 00:06:53,469
point is that the binding remained

161
00:06:59,010 --> 00:06:56,560
unchanged between pH is 4 and 10 so

162
00:07:00,350 --> 00:06:59,020
there was no effect on the on the on the

163
00:07:03,480 --> 00:07:00,360

peak shifts there between that and

164
00:07:06,899 --> 00:07:03,490
really that's that's the range that I

165
00:07:10,140 --> 00:07:06,909
put because experiments at pH 3 and

166
00:07:13,589 --> 00:07:10,150
lower or pH 11 and higher just degraded

167
00:07:15,450 --> 00:07:13,599
the RNA immediately so really at the pH

168
00:07:17,760 --> 00:07:15,460
ranges where the RNA was happy it was

169
00:07:19,709 --> 00:07:17,770
fully binding home with the flavin and

170
00:07:23,820 --> 00:07:19,719
and again that's divalent metal

171
00:07:25,559 --> 00:07:23,830
dependent at all those PHS so the next

172
00:07:29,309 --> 00:07:25,569
step we took was to do some mutations to

173
00:07:31,580 --> 00:07:29,319
kind of gauge where the which

174
00:07:34,200 --> 00:07:31,590
nucleotides were important for binding

175
00:07:36,659 --> 00:07:34,210
so this is the predicted secondary

176
00:07:38,760 --> 00:07:36,669
structure and essentially what we did is

177
00:07:41,369 --> 00:07:38,770
we just did some point mutations along

178
00:07:43,230 --> 00:07:41,379
these loop regions here and then some

179
00:07:44,309 --> 00:07:43,240
base swaps in the base pairing regions

180
00:07:47,850 --> 00:07:44,319
either constructive or destructive

181
00:07:51,059 --> 00:07:47,860
towards the secondary structure and in

182
00:07:53,490 --> 00:07:51,069
short this is what we saw so red means

183
00:07:55,290 --> 00:07:53,500
that that mutation effectively killed

184
00:07:57,990 --> 00:07:55,300
the binding there was no longer any peak

185
00:08:00,059 --> 00:07:58,000
shift whenever we introduced it blue

186
00:08:02,159 --> 00:08:00,069
indicates partial binding so there was a

187
00:08:05,189 --> 00:08:02,169
small peak shift but not significant to

188
00:08:07,159 --> 00:08:05,199

that of the parent Optima and then green

189
00:08:09,719 --> 00:08:07,169
indicates that there was full binding

190
00:08:13,079 --> 00:08:09,729
retain so there was still the full peak

191
00:08:15,269 --> 00:08:13,089
shift that was there and these aren't

192
00:08:17,519 --> 00:08:15,279
very interesting because the proposed

193
00:08:20,490 --> 00:08:17,529
binding site is up here in these loop

194
00:08:22,200 --> 00:08:20,500
regions so down here that's you know it

195
00:08:24,300 --> 00:08:22,210
doesn't really affect the secondary

196
00:08:25,740 --> 00:08:24,310
structure too much what was really

197
00:08:28,649 --> 00:08:25,750
interesting though was these two kind of

198
00:08:32,579 --> 00:08:28,659
outliers just kind of randomly placed in

199
00:08:34,409 --> 00:08:32,589
there and when looking at the uv-vis of

200
00:08:38,100 --> 00:08:34,419
each of these mutations we can see that

201
00:08:39,449 --> 00:08:38,110
the L 14 Cu mutation actually shifted

202
00:08:40,860 --> 00:08:39,459
the peak a little bit more and the

203
00:08:46,290 --> 00:08:40,870
shoulders a little bit more pronounced

204
00:08:48,000 --> 00:08:46,300
and then the L 24 you a mutation the

205
00:08:51,060 --> 00:08:48,010
Maxima is about the same but the

206
00:08:54,569 --> 00:08:51,070
shoulder here is not as prevalent as

207
00:08:56,550 --> 00:08:54,579
that in the mutation so now I'll quickly

208
00:08:58,560 --> 00:08:56,560
go into how we determine the redox

209
00:09:00,720 --> 00:08:58,570
potential Devon flavin

210
00:09:02,430 --> 00:09:00,730
so what we use was xanthine xanthine

211
00:09:04,590 --> 00:09:02,440
oxidase redox si

212
00:09:06,570 --> 00:09:04,600
it's uv-vis based assay but in short

213
00:09:07,320 --> 00:09:06,580

xanthine oxidase will catalyze this

214
00:09:09,990 --> 00:09:07,330
reaction here

215
00:09:12,660 --> 00:09:10,000
if you remove oxygen from the system and

216
00:09:14,790 --> 00:09:12,670
place in a high redox potential dye like

217
00:09:16,800 --> 00:09:14,800
methyl violet in here it can use the

218
00:09:18,480 --> 00:09:16,810
methyl violet in catalyzed reaction and

219
00:09:21,420 --> 00:09:18,490
then you have this reduced methyl violet

220
00:09:24,240 --> 00:09:21,430
in a species that's in solution if you

221
00:09:27,000 --> 00:09:24,250
have your flavin and then a redox active

222
00:09:29,280 --> 00:09:27,010
dye that you can monitor the methyl

223
00:09:32,970 --> 00:09:29,290
violet gene will then go and then react

224
00:09:36,420 --> 00:09:32,980
that the redox potential dye is known

225
00:09:39,060 --> 00:09:36,430
whereas the flavin is unknown we can do

226
00:09:40,980 --> 00:09:39,070
is then compared the two nurse equations

227
00:09:44,030 --> 00:09:40,990
with the dye and the flavin to each

228
00:09:46,890 --> 00:09:44,040
other whenever you plot these log terms

229
00:09:48,240 --> 00:09:46,900
against each other the y-intercept is

230
00:09:50,610 --> 00:09:48,250
getting equal the difference in redox

231
00:09:53,610 --> 00:09:50,620
potential between the dye and the and

232
00:09:55,050 --> 00:09:53,620
the flavin so so a couple things one

233
00:09:57,870 --> 00:09:55,060
this has been used on flavor proteins

234
00:09:59,160 --> 00:09:57,880
before but not on apt tumors it's

235
00:10:01,530 --> 00:09:59,170
important to pick a redox dye that's

236
00:10:04,200 --> 00:10:01,540
close in redox potential to that of your

237
00:10:07,740 --> 00:10:04,210
flavin otherwise then the dye can react

238
00:10:09,600 --> 00:10:07,750

with the flavin or vice versa and then

239
00:10:11,640 --> 00:10:09,610
essentially so the absorbance change is

240
00:10:14,790 --> 00:10:11,650
how we're good to see that ratio of

241
00:10:16,800 --> 00:10:14,800
oxidized to reduced so here's what a

242
00:10:20,490 --> 00:10:16,810
spectra just kind of looks like as time

243
00:10:22,860 --> 00:10:20,500
progresses the absorbance goes down so

244
00:10:25,980 --> 00:10:22,870
this is f ad with a redox dye

245
00:10:28,530 --> 00:10:25,990
anthraquinone - so folic acid which is

246
00:10:30,140 --> 00:10:28,540
essentially once it gets reduced it this

247
00:10:32,700 --> 00:10:30,150
starts to come up right here and and

248
00:10:35,490 --> 00:10:32,710
basically what we do is we pick the I

249
00:10:38,520 --> 00:10:35,500
suspect this points for each the redox

250
00:10:40,230 --> 00:10:38,530
dye and the F ad and measure the other

251
00:10:43,320 --> 00:10:40,240
one at that point so essentially an I

252
00:10:45,600 --> 00:10:43,330
suspect this point is 4f ad no matter

253
00:10:47,430 --> 00:10:45,610
what the ratio of oxidized to reduced is

254
00:10:49,080 --> 00:10:47,440
it's the absorbance is get a remain

255
00:10:52,560 --> 00:10:49,090
exactly the same so we measured the dye

256
00:10:54,450 --> 00:10:52,570
at that location so that's just a

257
00:10:56,190 --> 00:10:54,460
spectra for that one and then this is

258
00:10:58,260 --> 00:10:56,200
for the optimal with F ad I guess just

259
00:11:00,570 --> 00:10:58,270
one thing to note is that we still see

260
00:11:04,500 --> 00:11:00,580
the characteristic binding peak shifts

261
00:11:06,720 --> 00:11:04,510
there even though throughout the

262
00:11:08,480 --> 00:11:06,730
entirety of the of the reaction going on

263
00:11:12,200 --> 00:11:08,490

so we know that it's still binding with

264
00:11:13,490 --> 00:11:12,210
though this is just a comparison of pots

265
00:11:15,110 --> 00:11:13,500
just to see kind of how the data is

266
00:11:16,460 --> 00:11:15,120
spread and to see how much of a

267
00:11:19,160 --> 00:11:16,470
difference there actually was between

268
00:11:23,030 --> 00:11:19,170
free f ad here and then the app de Mer

269
00:11:25,430 --> 00:11:23,040
with F ad and then this is just the

270
00:11:27,530 --> 00:11:25,440
results with the Optima and then the

271
00:11:29,540 --> 00:11:27,540
mutations here so what we can see is

272
00:11:32,540 --> 00:11:29,550
this F test one which was the Optima

273
00:11:35,300 --> 00:11:32,550
that didn't had no preference between

274
00:11:37,190 --> 00:11:35,310
the oxidized and reduced Flavin's we can

275
00:11:39,020 --> 00:11:37,200
see that there was little to no change

276
00:11:42,340 --> 00:11:39,030
in the redox potential that was measured

277
00:11:44,900 --> 00:11:42,350
of F ad the X to be to mutant cause

278
00:11:49,100 --> 00:11:44,910
negative 12 millivolt shift in redox

279
00:11:51,380 --> 00:11:49,110
potential and then the the l14 CU mutant

280
00:11:53,510 --> 00:11:51,390
which was this one right here actually

281
00:11:56,140 --> 00:11:53,520
doubled that redox shift so there was an

282
00:11:59,240 --> 00:11:56,150
minus 24 millivolt shift in potential

283
00:12:02,780 --> 00:11:59,250
interestingly those this 24 you a

284
00:12:04,940 --> 00:12:02,790
mutation actually killed the redox shift

285
00:12:08,180 --> 00:12:04,950
so this one while it still was binding

286
00:12:11,630 --> 00:12:08,190
to F ad this mutation essentially lost

287
00:12:14,180 --> 00:12:11,640
that that distinction between the

288
00:12:16,550 --> 00:12:14,190

oxidized and reduced forms of F ad and

289
00:12:19,700 --> 00:12:16,560
so like just really to kind of point out

290
00:12:22,160 --> 00:12:19,710
that the the Optima here and then this

291
00:12:24,650 --> 00:12:22,170
mutation caused us enhance electron

292
00:12:26,390 --> 00:12:24,660
transfer of this so we essentially you

293
00:12:29,000 --> 00:12:26,400
know it just shows that the binding of

294
00:12:32,360 --> 00:12:29,010
this AB temir changed redox potential of

295
00:12:35,120 --> 00:12:32,370
the other bound flavin compared to you

296
00:12:36,490 --> 00:12:35,130
know that in solution and and that you

297
00:12:40,960 --> 00:12:36,500
know this can kind of really just be

298
00:12:43,280 --> 00:12:40,970
there this is more just beginning

299
00:12:45,110 --> 00:12:43,290
experiments and whatnot but you can

300
00:12:48,170 --> 00:12:45,120
imagine if this is like a components on

301
00:12:50,120 --> 00:12:48,180
a ribozyme that can bring in this F ad

302
00:12:52,180 --> 00:12:50,130
with with a different redox potential in

303
00:12:55,250 --> 00:12:52,190
order to catalyze some kind of reactions

304
00:12:57,590 --> 00:12:55,260
all right and then just to finish off

305
00:12:58,790 --> 00:12:57,600
thank st. Louis University bomb lab

306
00:13:01,430 --> 00:12:58,800
Burke lab University of

307
00:13:03,590 --> 00:13:01,440
missouri-columbia and then on NASA as

308
00:13:08,970 --> 00:13:03,600
well for the funding and with that I

309
00:13:19,710 --> 00:13:11,050
Thank You jack we have time for maybe

310
00:13:29,320 --> 00:13:21,760
so the rate and the rate of electrons

311
00:13:37,750 --> 00:13:29,330
like how how long that yeah exactly so

312
00:13:39,190 --> 00:13:37,760
actually so right so the in you're

313
00:13:42,460 --> 00:13:39,200

talking about like the assay essentially

314
00:13:45,490 --> 00:13:42,470
in that that I was showing so the assay

315
00:13:47,620 --> 00:13:45,500
the actually idea in order to get good

316
00:13:50,230 --> 00:13:47,630
results is actually to keep that as slow

317
00:13:53,019 --> 00:13:50,240
as possible and if there is a difference

318
00:13:55,870 --> 00:13:53,029
it will show up and the reason why is

319
00:13:58,030 --> 00:13:55,880
because we want the flavin and the dye

320
00:13:59,320 --> 00:13:58,040
to be kind of reducing at the same rate

321
00:14:00,550 --> 00:13:59,330
what's gonna happen though is but

322
00:14:02,290 --> 00:14:00,560
they're not an equilibrium right at the

323
00:14:15,970 --> 00:14:02,300
beginning that's when one is going to

324
00:14:23,320 --> 00:14:20,830
I mean I'm not entirely sure like if if

325
00:14:24,700 --> 00:14:23,330
I were just to like I mean it should be

326
00:14:26,590 --> 00:14:24,710
pretty quickly if I were just to put

327
00:14:46,259 --> 00:14:26,600
like a highly reduced species in there

328
00:14:50,460 --> 00:14:48,660
right so actually you can actually kind

329
00:14:53,669 --> 00:14:50,470
of gauge that as to how quickly each

330
00:14:56,040 --> 00:14:53,679
species is being reduced by in those

331
00:14:57,900 --> 00:14:56,050
plots whenever you plot them if it's a

332
00:14:59,790 --> 00:14:57,910
slope of one that means everything is

333
00:15:01,769 --> 00:14:59,800
reducing at the same rate which means

334
00:15:03,329 --> 00:15:01,779
that if there's not a slope of one that

335
00:15:05,129 --> 00:15:03,339
means something is reducing quicker than

336
00:15:07,559 --> 00:15:05,139
the other thing and it's not a very good

337
00:15:09,720 --> 00:15:07,569
test and it's not really showing what's

338
00:15:11,400 --> 00:15:09,730

actually happening and all these that we

339
00:15:13,859 --> 00:15:11,410
did all the mutants and everything they

340
00:15:15,689 --> 00:15:13,869
also a slope of one on that indicating

341
00:15:20,329 --> 00:15:15,699
that everything is is reducing at the

342
00:15:23,220 --> 00:15:20,339
same rate throughout the reaction so