
AbSciCon
2019

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

2
00:00:11,560 --> 00:00:09,180
[Applause]

3
00:00:13,869 --> 00:00:11,570
hi everyone thank you guys for being

4
00:00:15,369 --> 00:00:13,879
here my name is Tia maka and today I'm

5
00:00:17,679 --> 00:00:15,379
going to talk to you about some of the

6
00:00:19,630 --> 00:00:17,689
work we've done concerning the role of

7
00:00:22,120 --> 00:00:19,640
cyclic phosphates in non enzymatic

8
00:00:26,019 --> 00:00:22,130
ligation and just studying some general

9
00:00:28,540 --> 00:00:26,029
principles of non-enzymatic ligation so

10
00:00:30,730 --> 00:00:28,550
not to belabor the point but we all know

11
00:00:33,250 --> 00:00:30,740
that non enzymatic nucleic acid

12
00:00:34,689 --> 00:00:33,260
replication is a complex process and so

13
00:00:37,509 --> 00:00:34,699

there have been a lot of studies to try

14
00:00:40,060 --> 00:00:37,519
and understand this and from our point

15
00:00:42,490 --> 00:00:40,070
of view they're basically two ways in

16
00:00:44,910 --> 00:00:42,500
which people generally approach this so

17
00:00:46,900 --> 00:00:44,920
in one case you can look at

18
00:00:48,130 --> 00:00:46,910
polymerization of nucleotides so you

19
00:00:50,139 --> 00:00:48,140
have a template and you try and

20
00:00:52,090 --> 00:00:50,149
polymerize your different nucleotides on

21
00:00:53,860 --> 00:00:52,100
it but on the second hand if you can

22
00:00:55,779 --> 00:00:53,870
imagine that there were there was a

23
00:00:57,850 --> 00:00:55,789
template on the early earth then it's

24
00:01:00,099 --> 00:00:57,860
not too far to assume that it would also

25
00:01:01,930 --> 00:01:00,109
be shorter the good nucleotides and so

26
00:01:04,329 --> 00:01:01,940
the questions we're trying to answer is

27
00:01:06,130 --> 00:01:04,339
how do you like get the shorter leg of

28
00:01:09,940 --> 00:01:06,140
nucleotides once you've hybridized them

29
00:01:11,499 --> 00:01:09,950
together and so we before we started

30
00:01:12,850 --> 00:01:11,509
this work we looked into literature to

31
00:01:15,040 --> 00:01:12,860
see some of the things that people had

32
00:01:18,490 --> 00:01:15,050
been doing because this problem dates

33
00:01:20,230 --> 00:01:18,500
back to decade old problems and so in

34
00:01:22,419 --> 00:01:20,240
general like I said earlier there would

35
00:01:24,910 --> 00:01:22,429
be a template and the idea is you're

36
00:01:29,230 --> 00:01:24,920
trying to like it won't be a sharp

37
00:01:31,059 --> 00:01:29,240
primer to your short strands one of the

38
00:01:32,499 --> 00:01:31,069

features of your ligo's of your of your

39
00:01:34,540 --> 00:01:32,509
primer is that you would have a

40
00:01:36,910 --> 00:01:34,550
phosphate and you would need to activate

41
00:01:39,400 --> 00:01:36,920
this phosphate so people have done this

42
00:01:42,010 --> 00:01:39,410
by you can have an activated phosphate

43
00:01:44,139 --> 00:01:42,020
that you then include in your study in

44
00:01:46,690 --> 00:01:44,149
your application but you can also

45
00:01:48,729 --> 00:01:46,700
include condensing agents which can then

46
00:01:50,919 --> 00:01:48,739
activate your phosphate and you can get

47
00:01:53,290 --> 00:01:50,929
your new strands and some of the things

48
00:01:55,419 --> 00:01:53,300
we found about condensing agents in

49
00:01:57,669 --> 00:01:55,429
general was that even though this has

50
00:01:59,410 --> 00:01:57,679
been studied a lot in the past that did

51
00:02:02,380 --> 00:01:59,420
not seem to be a consensus on which

52
00:02:03,999 --> 00:02:02,390
condensing agents were superior so for

53
00:02:05,889 --> 00:02:04,009
instance we looked at a lot of studies

54
00:02:08,529 --> 00:02:05,899
with cyanogen bromide which has very

55
00:02:10,869 --> 00:02:08,539
fast ligation kinetics bots energy

56
00:02:12,580 --> 00:02:10,879
bromide is highly toxic and the fast

57
00:02:14,710 --> 00:02:12,590
reaction kinetics actually prevents you

58
00:02:16,000 --> 00:02:14,720
from understanding the mechanisms on the

59
00:02:18,220 --> 00:02:16,010
other hand we looked at what else would

60
00:02:19,990 --> 00:02:18,230
work how would I image and we found that

61
00:02:21,400 --> 00:02:20,000
it could take weeks and perhaps even

62
00:02:22,330 --> 00:02:21,410
months for you to get your maximum

63
00:02:24,220 --> 00:02:22,340

products

64
00:02:26,260 --> 00:02:24,230
which again is a bit hard to tease out

65
00:02:28,449 --> 00:02:26,270
the mechanisms involved with ligation

66
00:02:31,030 --> 00:02:28,459
and perhaps the most interesting thing

67
00:02:34,180 --> 00:02:31,040
that we found was that generally you had

68
00:02:36,520 --> 00:02:34,190
lower youth for RNA ligation compared to

69
00:02:38,280 --> 00:02:36,530
DNA ligation and this is interesting if

70
00:02:41,050 --> 00:02:38,290
you put it in a context of the RNA world

71
00:02:43,210 --> 00:02:41,060
and also and perhaps more interesting is

72
00:02:45,430 --> 00:02:43,220
that a lot of these so if you have an

73
00:02:47,410 --> 00:02:45,440
RNA or the connector oligonucleotide

74
00:02:49,270 --> 00:02:47,420
with the three prime phosphate it would

75
00:02:50,890 --> 00:02:49,280
form a cyclic phosphate which a lot of

76
00:02:52,750 --> 00:02:50,900
people believe to be an activated

77
00:02:54,910 --> 00:02:52,760
intermediate and so if you have this

78
00:02:56,410 --> 00:02:54,920
activated intermediate for the RNA or

79
00:02:58,180 --> 00:02:56,420
the GU nucleotide why don't you get

80
00:03:00,970 --> 00:02:58,190
higher yields compared to DNA Lagoon

81
00:03:02,590 --> 00:03:00,980
acute IDEs and so this brings us to the

82
00:03:05,410 --> 00:03:02,600
two questions are trying to answer in

83
00:03:08,979 --> 00:03:05,420
this study the first one is what

84
00:03:11,589 --> 00:03:08,989
parameters affect the efficacy of

85
00:03:16,690 --> 00:03:11,599
chemical ligation and the second one is

86
00:03:18,430 --> 00:03:16,700
how how relevant or how is the cyclic

87
00:03:20,229 --> 00:03:18,440
does your two prime three prime cyclic

88
00:03:23,710 --> 00:03:20,239

phosphate actually enhance the rate of a

89
00:03:25,809 --> 00:03:23,720
chemical equation and to do this this is

90
00:03:27,849 --> 00:03:25,819
our experimental design so we have a

91
00:03:30,069 --> 00:03:27,859
hairpin which we've optimized to be very

92
00:03:32,140 --> 00:03:30,079
stable and we're going to try and like

93
00:03:36,180 --> 00:03:32,150
get a shot a piece of illegal to the

94
00:03:38,470 --> 00:03:36,190
stem LIGO is a three prime phosphate and

95
00:03:40,840 --> 00:03:38,480
generally we mix in our hairpin and our

96
00:03:43,809 --> 00:03:40,850
leaders together I'm after hybridization

97
00:03:46,569 --> 00:03:43,819
we add in a EDC so in this case we're

98
00:03:48,759 --> 00:03:46,579
using EDC what else in the book how

99
00:03:50,740 --> 00:03:48,769
would i omit as a condensing agent and

100
00:03:52,479 --> 00:03:50,750
after activation we incubate it for a

101
00:03:55,479 --> 00:03:52,489
period of time at different temperatures

102
00:03:57,750 --> 00:03:55,489
and reformed alligator products and we

103
00:04:00,160 --> 00:03:57,760
typically run our reactions on a gel

104
00:04:01,690 --> 00:04:00,170
because the hairpin is family bought and

105
00:04:06,819 --> 00:04:01,700
so that that allows us to see our

106
00:04:08,770 --> 00:04:06,829
products and so this is just an example

107
00:04:10,420 --> 00:04:08,780
of so in this study we're looking at the

108
00:04:14,530 --> 00:04:10,430
effect of the activated nucleotide on

109
00:04:17,229 --> 00:04:14,540
ligation so our hairpin is all DNA and

110
00:04:19,900 --> 00:04:17,239
most of the shot oligonucleotides so

111
00:04:22,690 --> 00:04:19,910
most of the nucleotides on this illegals

112
00:04:26,140 --> 00:04:22,700
are DNA except for the last one so the

113
00:04:28,840 --> 00:04:26,150

last nucleotide is either DNA RNA or a 2

114
00:04:32,290 --> 00:04:28,850
prime or methyl and the idea is that

115
00:04:34,690 --> 00:04:32,300
once we activate this with EDC the EDC

116
00:04:36,550 --> 00:04:34,700
attaches itself to the phosphate so

117
00:04:40,090 --> 00:04:36,560
creating a good leaving group for the

118
00:04:42,790 --> 00:04:40,100
legations to continue but in the case of

119
00:04:45,430 --> 00:04:42,800
the irony it forms a cyclic phosphate

120
00:04:47,200 --> 00:04:45,440
and so again we're looking at the effect

121
00:04:50,950 --> 00:04:47,210
of this cyclic phosphate on enhancing

122
00:04:54,070 --> 00:04:50,960
ligation rates and so in this first

123
00:04:55,720 --> 00:04:54,080
experiments what we do is study at

124
00:04:57,460 --> 00:04:55,730
different temperatures so this gel I'm

125
00:04:59,740 --> 00:04:57,470
showing you was taken after only two

126
00:05:01,240 --> 00:04:59,750
hours so at different temperatures what

127
00:05:04,150 --> 00:05:01,250
is the effect of having the different

128
00:05:07,000 --> 00:05:04,160
nucleotides on that terminal end and so

129
00:05:09,370 --> 00:05:07,010
we see very quickly that for your DNA

130
00:05:11,230 --> 00:05:09,380
Liga nucleotides as you increase the

131
00:05:13,300 --> 00:05:11,240
temperature you increase the rate of the

132
00:05:15,250 --> 00:05:13,310
chemical reaction so this is kind of

133
00:05:17,590 --> 00:05:15,260
what we expected but you can see that

134
00:05:21,010 --> 00:05:17,600
after only two hours you're almost at

135
00:05:24,280 --> 00:05:21,020
80% of chemical ligation products formed

136
00:05:26,110 --> 00:05:24,290
after at tight temperatures you see that

137
00:05:27,910 --> 00:05:26,120
for the area nado even though we're from

138
00:05:30,790 --> 00:05:27,920

in a cyclic phosphate and so we verified

139
00:05:32,650 --> 00:05:30,800
this with HB s and lc/ms you don't get

140
00:05:34,570 --> 00:05:32,660
any products formed after two hours

141
00:05:38,170 --> 00:05:34,580
whereas you get a lot of products formed

142
00:05:40,450 --> 00:05:38,180
for the DNA but once you block that the

143
00:05:42,580 --> 00:05:40,460
two prime hydroxyl with an met with a

144
00:05:44,530 --> 00:05:42,590
methyl so not true prime or my field

145
00:05:46,060 --> 00:05:44,540
case you see that as you increase the

146
00:05:48,610 --> 00:05:46,070
temperature you get more products that

147
00:05:50,380 --> 00:05:48,620
are formed it's also interesting to note

148
00:05:53,110 --> 00:05:50,390
that at low temperatures so at four

149
00:05:55,180 --> 00:05:53,120
degrees Celsius you don't see a much of

150
00:05:57,130 --> 00:05:55,190
any products for mean and we think that

151
00:05:59,020 --> 00:05:57,140
but we think that this is because at

152
00:06:03,490 --> 00:05:59,030
this low temperatures the two prime or

153
00:06:05,620 --> 00:06:03,500
methyl is in it has steric hindrance

154
00:06:07,540 --> 00:06:05,630
that is preventing this ligation from

155
00:06:09,070 --> 00:06:07,550
going on and by his not the temperature

156
00:06:12,400 --> 00:06:09,080
you can reduce the effect of the

157
00:06:15,070 --> 00:06:12,410
conformation and so we do this via a

158
00:06:17,800 --> 00:06:15,080
widespread so we do higher temperatures

159
00:06:19,750 --> 00:06:17,810
but we also look at it for two hours and

160
00:06:22,210 --> 00:06:19,760
24 hours so the blocks represent two

161
00:06:24,820 --> 00:06:22,220
hours and the dash represent 24 hours

162
00:06:27,310 --> 00:06:24,830
and just generally you see that at high

163
00:06:29,470 --> 00:06:27,320

temperatures for the DNA and the two

164
00:06:31,750 --> 00:06:29,480
prime or methyl you get about the same

165
00:06:34,390 --> 00:06:31,760
rate of product or the same amount of

166
00:06:36,760 --> 00:06:34,400
products that are formed immediately but

167
00:06:39,190 --> 00:06:36,770
like I showed you earlier and you might

168
00:06:42,160 --> 00:06:39,200
have we shared it more here after two

169
00:06:44,620 --> 00:06:42,170
hours the DNA is almost at 60% at 25

170
00:06:47,290 --> 00:06:44,630
degrees Celsius but a 2 prime or methyl

171
00:06:48,140 --> 00:06:47,300
is only at 20% and so what this showed

172
00:06:49,820 --> 00:06:48,150
us was that

173
00:06:51,560 --> 00:06:49,830
it's important to make sure your

174
00:06:53,240 --> 00:06:51,570
reaction has gone into completion so if

175
00:06:55,670 --> 00:06:53,250
we had just looked at instantaneous

176
00:06:57,110 --> 00:06:55,680
rates you might think that DNA ligation

177
00:06:59,930 --> 00:06:57,120
was perhaps better than to promote

178
00:07:04,219 --> 00:06:59,940
methyl but that is very temperature and

179
00:07:05,930 --> 00:07:04,229
time dependence we also saw that again

180
00:07:07,610 --> 00:07:05,940
even after 24 hours of four degrees

181
00:07:11,060 --> 00:07:07,620
Celsius you don't get any products form

182
00:07:13,280 --> 00:07:11,070
for the to prime or methyl but even on

183
00:07:15,530 --> 00:07:13,290
the best condition so 25 degrees after

184
00:07:17,990 --> 00:07:15,540
24 hours you barely see any irony on

185
00:07:19,760 --> 00:07:18,000
ligation going on now we're not saying

186
00:07:21,740 --> 00:07:19,770
that you don't get you can get some

187
00:07:23,120 --> 00:07:21,750
products formed if you increase the

188
00:07:25,730 --> 00:07:23,130

amount of excess oligonucleotides that

189
00:07:27,680 --> 00:07:25,740
you have so in this experiment we had

190
00:07:29,570 --> 00:07:27,690
only 1.5 excess of Olli goes to the

191
00:07:31,370 --> 00:07:29,580
template but if you increase it to say

192
00:07:33,170 --> 00:07:31,380
20 times if you saturate the amount of

193
00:07:35,300 --> 00:07:33,180
illegals that are present then you can

194
00:07:37,100 --> 00:07:35,310
see some ligation happening if you also

195
00:07:38,990 --> 00:07:37,110
increase the pH of the reaction you can

196
00:07:40,730 --> 00:07:39,000
get some products formed but in both

197
00:07:43,760 --> 00:07:40,740
those cases the maximum product that you

198
00:07:45,500 --> 00:07:43,770
get is only about 20% whereas are not

199
00:07:48,230 --> 00:07:45,510
best conditions for DNA are to promote

200
00:07:52,129 --> 00:07:48,240
methyl you get up to 80 90 percent on

201
00:07:54,290 --> 00:07:52,139
the product formation and so haven't

202
00:07:56,990 --> 00:07:54,300
picked the best conformation so we know

203
00:07:59,960 --> 00:07:57,000
we want to use the DNA oligonucleotide

204
00:08:01,490 --> 00:07:59,970
primer to do a ligation we decided to

205
00:08:04,159 --> 00:08:01,500
look at the effect of sequences so this

206
00:08:05,750 --> 00:08:04,169
has been done before but in our case we

207
00:08:08,330 --> 00:08:05,760
believe that we had optimized all the

208
00:08:09,770 --> 00:08:08,340
possible and problems and so we wanted

209
00:08:12,110 --> 00:08:09,780
to see if they would have any effect on

210
00:08:14,629 --> 00:08:12,120
our ligation reactions and the things we

211
00:08:16,670 --> 00:08:14,639
pay attention to here is that this - or

212
00:08:19,730 --> 00:08:16,680
this line represents the nick of the

213
00:08:21,529 --> 00:08:19,740

ligation and so in this first gel again

214
00:08:23,570 --> 00:08:21,539
this experiment was done after only two

215
00:08:26,719 --> 00:08:23,580
hours but in this first job we're

216
00:08:28,969 --> 00:08:26,729
looking at the GG being opposite the

217
00:08:31,339 --> 00:08:28,979
ligation Nick versus being at the

218
00:08:33,589 --> 00:08:31,349
ligation Nick and again we look at it at

219
00:08:35,899 --> 00:08:33,599
different temperatures and you see that

220
00:08:39,170 --> 00:08:35,909
when you're GG so when you have periods

221
00:08:41,269 --> 00:08:39,180
at opposite the ligation Nick you see a

222
00:08:43,070 --> 00:08:41,279
really fast increase in the amount of

223
00:08:46,160 --> 00:08:43,080
products that you get when you increase

224
00:08:47,980 --> 00:08:46,170
the temperature but when you have the GG

225
00:08:50,540 --> 00:08:47,990
stocking at the nick of the ligation

226
00:08:53,470 --> 00:08:50,550
then you see a decrease in the amount of

227
00:08:55,490 --> 00:08:53,480
products that are formed as you increase

228
00:08:57,680 --> 00:08:55,500
initially so at 4 degrees Celsius

229
00:08:58,880 --> 00:08:57,690
there's barely any product forming but

230
00:09:00,809 --> 00:08:58,890
as you increase the temperature then you

231
00:09:02,309 --> 00:09:00,819
can increase the reaction rate

232
00:09:05,039 --> 00:09:02,319
and so again like we signed the case of

233
00:09:07,949 --> 00:09:05,049
the two primal metal there seems to be a

234
00:09:09,900 --> 00:09:07,959
difference that there seems to be some

235
00:09:13,289 --> 00:09:09,910
effect of stacking on our ligation rates

236
00:09:15,719 --> 00:09:13,299
and so we do this some experiments at

237
00:09:18,299 --> 00:09:15,729
different times so two hours and 24

238
00:09:20,549 --> 00:09:18,309

hours studies and we also do we also

239
00:09:23,369 --> 00:09:20,559
scramble the positions of the GS and the

240
00:09:25,139 --> 00:09:23,379
C's and you see that generally at high

241
00:09:28,619 --> 00:09:25,149
temperatures that doesn't seem to be any

242
00:09:31,109 --> 00:09:28,629
effect on the sequences location but

243
00:09:33,809 --> 00:09:31,119
after 24 hours when you have your stack

244
00:09:35,969 --> 00:09:33,819
in at the nick of ligation there's it

245
00:09:37,619 --> 00:09:35,979
really decreases the amount of products

246
00:09:40,919 --> 00:09:37,629
that you get compared to any have you're

247
00:09:42,539 --> 00:09:40,929
stacking opposite the ligation Nick and

248
00:09:46,019 --> 00:09:42,549
so we decided to look at whether this

249
00:09:47,759 --> 00:09:46,029
was just a feature of the GG base

250
00:09:49,979 --> 00:09:47,769
pairing system where this could also be

251
00:09:52,529 --> 00:09:49,989
applied if we had an 80 base pairing

252
00:09:55,229 --> 00:09:52,539
system so we kept almost everything the

253
00:09:57,359 --> 00:09:55,239
same but now instead of CG closing base

254
00:09:59,669 --> 00:09:57,369
pairs we looked at 80 closing base pairs

255
00:10:02,699 --> 00:09:59,679
and you see about the same effect that

256
00:10:04,109 --> 00:10:02,709
we observed so in this first bar when

257
00:10:06,509 --> 00:10:04,119
you have your purines so you're a a

258
00:10:08,129 --> 00:10:06,519
stock opposite ligation Nick you say

259
00:10:10,679 --> 00:10:08,139
really fast increase in the

260
00:10:12,629 --> 00:10:10,689
instantaneous rate and it approaches

261
00:10:15,119 --> 00:10:12,639
maximum but when you have your puing

262
00:10:16,739 --> 00:10:15,129
stocking at the nick of ligation then

263
00:10:18,899 --> 00:10:16,749

you see a decrease in the amount of

264
00:10:20,729 --> 00:10:18,909
products that are formed and again as

265
00:10:22,409 --> 00:10:20,739
you increase the temperature you allow

266
00:10:24,439 --> 00:10:22,419
it attained more confirmation and you

267
00:10:26,909 --> 00:10:24,449
can get more products that are formed

268
00:10:29,579 --> 00:10:26,919
answering conclusion from just a general

269
00:10:31,199 --> 00:10:29,589
nucleic acid standpoint we see that

270
00:10:33,869 --> 00:10:31,209
their multiple factors that can affect

271
00:10:35,609 --> 00:10:33,879
non-enzymatic ligation one of the first

272
00:10:37,439 --> 00:10:35,619
ones we looked at is just the effect of

273
00:10:39,899 --> 00:10:37,449
the sugar so having a 2 prime hydroxyl

274
00:10:42,840 --> 00:10:39,909
versus an O methyl on your

275
00:10:45,799 --> 00:10:42,850
oligonucleotides we also saw that base

276
00:10:48,569 --> 00:10:45,809
flanking so the position of the purines

277
00:10:50,159 --> 00:10:48,579
really affected how fast your reaction

278
00:10:52,559 --> 00:10:50,169
weren't especially at low temperatures

279
00:10:55,409 --> 00:10:52,569
and from just an irony award for

280
00:10:57,210 --> 00:10:55,419
spective we saw our data showed that the

281
00:10:59,909 --> 00:10:57,220
super imperium cyclic phosphate is

282
00:11:01,919 --> 00:10:59,919
perhaps not an activated intermediate in

283
00:11:04,710 --> 00:11:01,929
template director type ligation

284
00:11:06,299 --> 00:11:04,720
reactions and with this I'd like to

285
00:11:08,219 --> 00:11:06,309
thank NASA and a cell phone in the

286
00:11:11,280 --> 00:11:08,229
center my group members are my