
AbSciCon
2019

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

2
00:00:11,410 --> 00:00:09,120
[Applause]

3
00:00:15,010 --> 00:00:11,420
thank you thanks very much for the

4
00:00:16,900 --> 00:00:15,020
opportunity to speak so today I'd like

5
00:00:20,980 --> 00:00:16,910
to tell you about our work and looking

6
00:00:23,140 --> 00:00:20,990
at ribozyme catalysis inside of complex

7
00:00:26,620 --> 00:00:23,150
Kosar baits and I'm going to describe

8
00:00:28,720 --> 00:00:26,630
how this is assisted by anions and so we

9
00:00:31,210 --> 00:00:28,730
just heard a little bit about protocells

10
00:00:33,850 --> 00:00:31,220
and how those could come about from

11
00:00:35,860 --> 00:00:33,860
complex coacervate and we've been

12
00:00:38,260 --> 00:00:35,870
studying this in the lab for for several

13
00:00:40,690 --> 00:00:38,270

years now this is a collaboration with

14
00:00:42,690 --> 00:00:40,700
the Keating lab and some of the first

15
00:00:47,470 --> 00:00:42,700
work that we published looks at the

16
00:00:50,710 --> 00:00:47,480
coacervate in a poly cation poly a lil

17
00:00:55,630 --> 00:00:50,720
amine and a oligo anion ATP and when

18
00:00:57,730 --> 00:00:55,640
these come together by associative face

19
00:01:03,520 --> 00:00:57,740
operation to make a liquid liquid phase

20
00:01:05,620 --> 00:01:03,530
separation and these form just got cut

21
00:01:07,649 --> 00:01:05,630
off a little bit here there should be a

22
00:01:10,690 --> 00:01:07,659
picture here of coacervate sand then

23
00:01:13,300 --> 00:01:10,700
these are enriched with certain species

24
00:01:14,620 --> 00:01:13,310
that are favorable for for ribosome

25
00:01:16,680 --> 00:01:14,630
reactions such as enhanced

26
00:01:19,600 --> 00:01:16,690
concentrations of magnesium ions

27
00:01:21,460 --> 00:01:19,610
nucleotides and RNA oligomers and I'm

28
00:01:23,620 --> 00:01:21,470
going to show you a story today which we

29
00:01:26,830 --> 00:01:23,630
recently published just a few weeks ago

30
00:01:29,230 --> 00:01:26,840
came out an ACS chemical biology in

31
00:01:32,080 --> 00:01:29,240
which we have coacervate sand and in

32
00:01:33,820 --> 00:01:32,090
these are ribosomes our RNA enzymes

33
00:01:35,530 --> 00:01:33,830
which are shown in green and they go

34
00:01:38,440 --> 00:01:35,540
from this Mis folded state where they're

35
00:01:40,899 --> 00:01:38,450
associated with the poly cation and that

36
00:01:43,360 --> 00:01:40,909
we can rescue their activity by adding

37
00:01:46,210 --> 00:01:43,370
oligo anions that can displace these and

38
00:01:50,980 --> 00:01:49,420

so condensates are formed by liquid

39
00:01:53,800 --> 00:01:50,990
liquid phase separation in they're

40
00:01:57,340 --> 00:01:53,810
common in modern cells and so here's an

41
00:02:00,010 --> 00:01:57,350
image from cliff brain wines lab showing

42
00:02:02,950 --> 00:02:00,020
phase separation and this is with the

43
00:02:05,469 --> 00:02:02,960
the unstructured region of the laughs

44
00:02:07,840 --> 00:02:05,479
helicase and these come together to form

45
00:02:11,470 --> 00:02:07,850
these these condensates which are on the

46
00:02:13,410 --> 00:02:11,480
the micron size scale and we can make

47
00:02:17,370 --> 00:02:13,420
these in the lab as well so these are

48
00:02:18,930 --> 00:02:17,380
association in this case this is between

49
00:02:21,840 --> 00:02:18,940
spermine

50
00:02:25,670 --> 00:02:21,850
and a poly you as well as we can have

51
00:02:29,540 --> 00:02:25,680
them in which they're in which the poly

52
00:02:31,980 --> 00:02:29,550
cation is is the polymer and these give

53
00:02:34,230 --> 00:02:31,990
these give compartments that are similar

54
00:02:37,080 --> 00:02:34,240
on their size scale and the microns

55
00:02:39,090 --> 00:02:37,090
scale to the ones that are found in vivo

56
00:02:41,190 --> 00:02:39,100
and in order to form these condensates

57
00:02:43,560 --> 00:02:41,200
some of the features that are important

58
00:02:46,170 --> 00:02:43,570
are polyelectrolytes unstructured

59
00:02:48,900 --> 00:02:46,180
peptide domains Crowder's and co solutes

60
00:02:53,430 --> 00:02:48,910
as well as it depends on the pH ions and

61
00:02:56,130 --> 00:02:53,440
temperature and recently we published a

62
00:02:58,770 --> 00:02:56,140
review on this in biochemistry in which

63
00:03:01,290 --> 00:02:58,780

we see to see a number of processes that

64
00:03:05,430 --> 00:03:01,300
are important that could emerge for RNA

65
00:03:07,110 --> 00:03:05,440
function in an RNA world scenario and

66
00:03:09,270 --> 00:03:07,120
those are the concentration of

67
00:03:13,380 --> 00:03:09,280
nucleotides that can happen and then

68
00:03:16,800 --> 00:03:13,390
function in terms of non enzymatic these

69
00:03:19,650 --> 00:03:16,810
are templated polymerization reactions

70
00:03:21,930 --> 00:03:19,660
as well as ribosome reactions and then

71
00:03:23,760 --> 00:03:21,940
we also get the concentration not only

72
00:03:27,120 --> 00:03:23,770
are the nucleotides but also of the

73
00:03:29,040 --> 00:03:27,130
longer rnas which are enriched and

74
00:03:32,280 --> 00:03:29,050
encapsulated within the proto cell and

75
00:03:35,160 --> 00:03:32,290
these shorter ones which are less less

76
00:03:37,590 --> 00:03:35,170
so in that in that sense and I just want

77
00:03:38,729 --> 00:03:37,600
to point out that a lot of the work much

78
00:03:41,250 --> 00:03:38,739
of the work I'm going to talk about

79
00:03:44,400 --> 00:03:41,260
today is to work from a postdoc in our

80
00:03:47,190 --> 00:03:44,410
lab dr. Raghava Dial and he will be

81
00:03:49,080 --> 00:03:47,200
speaking on Thursday on template

82
00:03:51,690 --> 00:03:49,090
directed RNA polymerize ation and

83
00:03:54,330 --> 00:03:51,700
enhanced ribozyme catalysis inside of

84
00:03:57,259 --> 00:03:54,340
membrane compartments formed by

85
00:03:59,490 --> 00:03:57,269
coacervate so we're going to use

86
00:04:02,070 --> 00:03:59,500
ribosomes in order to look at function

87
00:04:06,060 --> 00:04:02,080
inside of side of complex class debates

88
00:04:07,890 --> 00:04:06,070

as models for proto cells and one of the

89
00:04:09,930 --> 00:04:07,900
reasons to look at ribozymes is we're

90
00:04:12,420 --> 00:04:09,940
interested in whether or not RNA can

91
00:04:15,300 --> 00:04:12,430
fold into a functional form inside of

92
00:04:18,330 --> 00:04:15,310
these coacervate sand ribosomes are

93
00:04:21,210 --> 00:04:18,340
ideal for that because they reveal their

94
00:04:24,270 --> 00:04:21,220
folding through the self cleavage and

95
00:04:27,600 --> 00:04:24,280
their enzymatic activity plus RNA

96
00:04:31,640 --> 00:04:27,610
activity and reactions are of an

97
00:04:35,730 --> 00:04:31,650
intrinsic interest to the RNA world

98
00:04:38,820 --> 00:04:35,740
scenario so this begins to show the

99
00:04:40,740 --> 00:04:38,830
process of an in a mechanistic way in

100
00:04:42,900 --> 00:04:40,750
the absence of coacervate that are going

101
00:04:44,790 --> 00:04:42,910
to help us understand in some way about

102
00:04:46,560 --> 00:04:44,800
how coacervate s-- themselves promote

103
00:04:48,600 --> 00:04:46,570
the reaction and it's a fairly simple

104
00:04:50,970 --> 00:04:48,610
reaction which we can understand through

105
00:04:53,220 --> 00:04:50,980
kind of a Michaelis Menten types of a

106
00:04:55,680 --> 00:04:53,230
formula that's shown here these are

107
00:04:57,210 --> 00:04:55,690
under single turnover conditions meaning

108
00:04:59,130 --> 00:04:57,220
that the substrate is in limiting

109
00:05:01,140 --> 00:04:59,140
amounts in this particular example it's

110
00:05:03,690 --> 00:05:01,150
radio labeled and it can associate with

111
00:05:05,850 --> 00:05:03,700
the enzyme to make a complex that we can

112
00:05:07,770 --> 00:05:05,860
look at on a native page this is a

113
00:05:10,080 --> 00:05:07,780

particular case where the reaction is

114
00:05:11,700 --> 00:05:10,090
prevented from advancing because at the

115
00:05:14,070 --> 00:05:11,710
cleavage site here for the hammerhead

116
00:05:16,350 --> 00:05:14,080
ribozyme there's actually a deoxy which

117
00:05:18,240 --> 00:05:16,360
removes the nucleophile in the reaction

118
00:05:20,640 --> 00:05:18,250
and we can see that as we increase the

119
00:05:23,370 --> 00:05:20,650
enzyme concentration that it shifts the

120
00:05:26,250 --> 00:05:23,380
limiting amount of the limiting reagent

121
00:05:28,530 --> 00:05:26,260
of the substrate up to this higher

122
00:05:31,560 --> 00:05:28,540
mobility species which is the enzyme

123
00:05:33,540 --> 00:05:31,570
substrate complex and we can plot this

124
00:05:35,250 --> 00:05:33,550
the fraction of the substrates that's

125
00:05:37,320 --> 00:05:35,260
bound as a function of enzyme

126
00:05:39,000 --> 00:05:37,330
concentration and we can see that in

127
00:05:41,790 --> 00:05:39,010
order to get most of the substrate bound

128
00:05:44,220 --> 00:05:41,800
it takes an increasing amount of enzyme

129
00:05:46,650 --> 00:05:44,230
concentration as its we're all familiar

130
00:05:48,780 --> 00:05:46,660
with and the question is then how does a

131
00:05:50,850 --> 00:05:48,790
similar type of reaction happen inside

132
00:05:53,610 --> 00:05:50,860
of coacervate so these are coacervate

133
00:05:57,180 --> 00:05:53,620
that are made from a quaternary amine

134
00:05:59,910 --> 00:05:57,190
polymer poly daya will dimethyl ammonium

135
00:06:01,200 --> 00:05:59,920
chloride which is a 53 mer and we're

136
00:06:03,330 --> 00:06:01,210
going to bring this together with some

137
00:06:06,380 --> 00:06:03,340
algo aspartic acids there are varying

138
00:06:09,420 --> 00:06:06,390

lengths from 10 out to a hundred

139
00:06:12,810 --> 00:06:09,430
monomeric units and here you can see in

140
00:06:14,490 --> 00:06:12,820
the ddic image that these coacervate s--

141
00:06:16,770 --> 00:06:14,500
are forming and that they're on the

142
00:06:18,870 --> 00:06:16,780
micron scale and that these also

143
00:06:20,340 --> 00:06:18,880
encapsulate the ribosome and so if the

144
00:06:22,140 --> 00:06:20,350
enzyme strand is labeled with a

145
00:06:25,080 --> 00:06:22,150
fluorescing you can see that it's

146
00:06:27,480 --> 00:06:25,090
enriched in the same droplets here again

147
00:06:29,580 --> 00:06:27,490
on the micron scale and what's important

148
00:06:32,190 --> 00:06:29,590
is that if we take this and we make a

149
00:06:34,290 --> 00:06:32,200
calibration curve and solution we can

150
00:06:36,570 --> 00:06:34,300
see that the concentration from the

151
00:06:38,880 --> 00:06:36,580
dilute or bulk phase into the coacervate

152
00:06:42,410 --> 00:06:38,890
s-- is quite large it's increased about

153
00:06:44,990 --> 00:06:42,420
five thousand times so if we add only 10

154
00:06:50,300 --> 00:06:45,000
ten animal or of the enzyme its enriched

155
00:06:51,650 --> 00:06:50,310
up to 44 micromolar and so so now we'll

156
00:06:54,470 --> 00:06:51,660
go in and we'll look at the effect of

157
00:06:57,470 --> 00:06:54,480
different lengths of the Ala go aspartic

158
00:06:59,480 --> 00:06:57,480
acid from ten to thirty fifty and a

159
00:07:02,330 --> 00:06:59,490
hundred MERS and in this particular

160
00:07:03,530 --> 00:07:02,340
assay with the poly cation apollyon i

161
00:07:06,290 --> 00:07:03,540
and are allowed to come together a

162
00:07:09,470 --> 00:07:06,300
charge matched conditions of one to one

163
00:07:10,880 --> 00:07:09,480

and then the first the enzyme is

164
00:07:12,380 --> 00:07:10,890
introduced and then the substrate is

165
00:07:14,630 --> 00:07:12,390
introduced and we'll look at the

166
00:07:17,360 --> 00:07:14,640
reaction that happens and this is again

167
00:07:19,100 --> 00:07:17,370
with a radio labeled substrate and so

168
00:07:21,530 --> 00:07:19,110
when reaction happens it goes from this

169
00:07:23,900 --> 00:07:21,540
higher mobility species down to the

170
00:07:25,850 --> 00:07:23,910
product here from the unreacted to the

171
00:07:27,890 --> 00:07:25,860
cleaves so these are now denaturing page

172
00:07:29,960 --> 00:07:27,900
gels and what we can see that is if the

173
00:07:31,580 --> 00:07:29,970
reaction is done in buffer and these are

174
00:07:34,640 --> 00:07:31,590
done under conditions under so-called

175
00:07:37,160 --> 00:07:34,650
k-kat over km conditions though so that

176
00:07:39,650 --> 00:07:37,170
even though the enzyme is in excess it's

177
00:07:41,630 --> 00:07:39,660
far below the the the KD for the

178
00:07:44,570 --> 00:07:41,640
reaction so there's very little reaction

179
00:07:48,170 --> 00:07:44,580
out to 30 minutes but that as we bring

180
00:07:50,870 --> 00:07:48,180
in the the coacervate we begin to see

181
00:07:53,810 --> 00:07:50,880
more and more cleaved product form and

182
00:07:55,820 --> 00:07:53,820
then we can look at and plot the amount

183
00:07:58,850 --> 00:07:55,830
of product that's formed at 30 minutes

184
00:08:01,190 --> 00:07:58,860
as a function of the length of the anion

185
00:08:03,260 --> 00:08:01,200
in these coacervate sand we can see that

186
00:08:06,050 --> 00:08:03,270
we get an increase in that there there's

187
00:08:08,360 --> 00:08:06,060
a certain range of the length of the

188
00:08:11,000 --> 00:08:08,370

polygon ion that's optimal or what I'm

189
00:08:13,310 --> 00:08:11,010
calling a so-called Goldilocks effect so

190
00:08:15,470 --> 00:08:13,320
if it's too short the reactions not as

191
00:08:17,720 --> 00:08:15,480
good and if it's too long it's also not

192
00:08:19,550 --> 00:08:17,730
as good so we wanted to delve into what

193
00:08:21,260 --> 00:08:19,560
could be the mechanistic basis for this

194
00:08:24,290 --> 00:08:21,270
and so the first thing we did was to

195
00:08:25,850 --> 00:08:24,300
look at the encapsulation of the bribe

196
00:08:28,100 --> 00:08:25,860
design in this case we're looking at the

197
00:08:30,500 --> 00:08:28,110
encapsulation of the substrate into

198
00:08:32,240 --> 00:08:30,510
coacervate that come from the different

199
00:08:34,490 --> 00:08:32,250
lengths of the poly anions and you can

200
00:08:38,870 --> 00:08:34,500
see somewhat paradoxically that the

201
00:08:40,790 --> 00:08:38,880
shorter poly anion of the 10 mer has

202
00:08:42,320 --> 00:08:40,800
greater encapsulation than the longer

203
00:08:45,200 --> 00:08:42,330
one does even though this is one of the

204
00:08:47,090 --> 00:08:45,210
shorter reaction rates and so if we plot

205
00:08:50,330 --> 00:08:47,100
this out these these colors and in all

206
00:08:53,270 --> 00:08:50,340
the slides are color matched and you can

207
00:08:56,090 --> 00:08:53,280
see indeed that there's less substrate

208
00:09:00,319 --> 00:08:56,100
less substrate encapsulated

209
00:09:03,199 --> 00:09:00,329
inside of the inside of the coacervate

210
00:09:05,119 --> 00:09:03,209
and so what seems to be happening is

211
00:09:06,769 --> 00:09:05,129
that the larger polygon ions are

212
00:09:09,499 --> 00:09:06,779
preventing uptake of the ribozyme

213
00:09:12,919 --> 00:09:09,509

because the larger polygon ions are

214
00:09:15,220 --> 00:09:12,929
strongly associated with the cations but

215
00:09:18,319 --> 00:09:15,230
on the other hand here in the shorter

216
00:09:20,809 --> 00:09:18,329
poly anions the ribosome goes in more

217
00:09:22,999 --> 00:09:20,819
more readily but it associates so

218
00:09:25,960 --> 00:09:23,009
strongly with with the poly cations that

219
00:09:28,939 --> 00:09:25,970
it's probably not in the correct fold so

220
00:09:30,590 --> 00:09:28,949
having made this observation we then

221
00:09:32,929 --> 00:09:30,600
came up with the idea that we perhaps

222
00:09:35,720 --> 00:09:32,939
could stimulate that reaction by adding

223
00:09:38,239 --> 00:09:35,730
even more poly on ions and so here's the

224
00:09:40,249 --> 00:09:38,249
effect of adding excess on poly anions

225
00:09:43,669 --> 00:09:40,259
and you'll see that excess short poly

226
00:09:46,309 --> 00:09:43,679
anions actually enhance catalysis and so

227
00:09:48,979 --> 00:09:46,319
so you can see here this is now in the

228
00:09:52,129 --> 00:09:48,989
case of the of the d-10 if we go away

229
00:09:55,129 --> 00:09:52,139
from charge match conditions to a 2 fold

230
00:09:56,239 --> 00:09:55,139
up to 5 fold excess of the all ago anion

231
00:09:59,299 --> 00:09:56,249
you can see that the rate of the

232
00:10:01,639 --> 00:09:59,309
reaction is enhanced up to 2 fold more

233
00:10:05,359 --> 00:10:01,649
and now this is a chart of the the

234
00:10:07,400 --> 00:10:05,369
various encapsulation of the enzyme

235
00:10:08,989 --> 00:10:07,410
under these various conditions and the

236
00:10:11,960 --> 00:10:08,999
two most optimal conditions for

237
00:10:15,289 --> 00:10:11,970
catalysis are with the excess charge on

238
00:10:17,659 --> 00:10:15,299

the d-10 as well as then with the charge

239
00:10:19,549 --> 00:10:17,669
matched on the d50 and you can see that

240
00:10:21,829 --> 00:10:19,559
they have similar uptake kind of modest

241
00:10:24,139 --> 00:10:21,839
uptake in which it kind of finds that

242
00:10:27,460 --> 00:10:24,149
sort of magic zone between being up

243
00:10:29,989 --> 00:10:27,470
takes some what the the RNA being up to

244
00:10:32,179 --> 00:10:29,999
taken up into the coacervate somewhat

245
00:10:34,340 --> 00:10:32,189
but not I'm so strongly that it's miss

246
00:10:35,989 --> 00:10:34,350
folded so then we wanted to see whether

247
00:10:38,059 --> 00:10:35,999
these effects are general which would

248
00:10:40,549 --> 00:10:38,069
make them kind of more robust in an

249
00:10:42,590 --> 00:10:40,559
early Earth scenario so the next thing

250
00:10:44,659 --> 00:10:42,600
we did was move away from biogenic to a

251
00:10:47,479 --> 00:10:44,669
biogenic on carboxylates

252
00:10:49,999 --> 00:10:47,489
at going from 30 to 45 and we can see

253
00:10:51,889 --> 00:10:50,009
that the reaction in 45 is also much

254
00:10:53,779 --> 00:10:51,899
enhanced over the buffer if we go a

255
00:10:55,639 --> 00:10:53,789
little bit shorter in the 25 mirror we

256
00:10:57,559 --> 00:10:55,649
can see the rate it's not as high and

257
00:10:59,599 --> 00:10:57,569
the charge matched but again if we add

258
00:11:01,789 --> 00:10:59,609
excess poly an i and just like we saw in

259
00:11:03,109 --> 00:11:01,799
the other case that the rate is enhanced

260
00:11:05,869 --> 00:11:03,119
but if we add too much that the rate

261
00:11:08,690 --> 00:11:05,879
starts to come back down to further test

262
00:11:13,020 --> 00:11:11,400
generality of this mechanism we then

263
00:11:15,510 --> 00:11:13,030

looked at these Pollyanna and assisted

264
00:11:18,510 --> 00:11:15,520
catalysis and asked whether other poly

265
00:11:20,280 --> 00:11:18,520
anions that that might be present and to

266
00:11:22,230 --> 00:11:20,290
test the robustness of this without

267
00:11:23,970 --> 00:11:22,240
whether these could also stimulate the

268
00:11:26,130 --> 00:11:23,980
reactions so we test this with sulfates

269
00:11:28,080 --> 00:11:26,140
and phosphates and the first thing is if

270
00:11:31,500 --> 00:11:28,090
we look at phosphates by adding the

271
00:11:33,180 --> 00:11:31,510
excess anion as RNA and the phosphate

272
00:11:35,580 --> 00:11:33,190
you can see that the rate is stimulated

273
00:11:38,100 --> 00:11:35,590
this is by adding half of an equivalent

274
00:11:40,140 --> 00:11:38,110
excess of the of the poly an i and it

275
00:11:42,330 --> 00:11:40,150
also works with sulfate and here this is

276
00:11:45,300 --> 00:11:42,340
heparin and this also enhances the

277
00:11:47,460 --> 00:11:45,310
reaction so then in the last data slide

278
00:11:50,790 --> 00:11:47,470
that I have we're going to test whether

279
00:11:54,000 --> 00:11:50,800
excess pollyannas poly anions can rescue

280
00:11:56,760 --> 00:11:54,010
RNA catalysis in otherwise incompatible

281
00:11:58,500 --> 00:11:56,770
complex coacervate and so we start here

282
00:12:01,050 --> 00:11:58,510
and rather rather than starting with the

283
00:12:03,900 --> 00:12:01,060
the allgäu aspartic acid interacting

284
00:12:06,750 --> 00:12:03,910
weakly with a quaternary amine we let it

285
00:12:08,340 --> 00:12:06,760
interact strongly with with al ago

286
00:12:11,270 --> 00:12:08,350
arginine in which there's very little

287
00:12:13,650 --> 00:12:11,280
reaction and you can see that as you add

288
00:12:16,320 --> 00:12:13,660

increasing amounts of the ala go

289
00:12:18,720 --> 00:12:16,330
arginine that the reaction increases and

290
00:12:22,410 --> 00:12:18,730
so the first thing again is as we move

291
00:12:24,990 --> 00:12:22,420
from the quaternary amine to the illegal

292
00:12:27,450 --> 00:12:25,000
arginine the rate drops tenfold but then

293
00:12:29,460 --> 00:12:27,460
as we add in up to threefold excess of

294
00:12:32,370 --> 00:12:29,470
the ala goal arginine the rate is r is

295
00:12:35,820 --> 00:12:32,380
rescued and it increases back up 12 fold

296
00:12:38,970 --> 00:12:35,830
so so quite strikingly these that this

297
00:12:40,320 --> 00:12:38,980
mechanism is general and works in in

298
00:12:43,860 --> 00:12:40,330
cases where there's otherwise

299
00:12:47,100 --> 00:12:43,870
incompatible coacervate so just to wrap

300
00:12:50,040 --> 00:12:47,110
up and what i just told you so up here

301
00:12:51,870 --> 00:12:50,050
is is the mechanistic diagram for what's

302
00:12:53,550 --> 00:12:51,880
happening and here's a little reaction

303
00:12:55,829 --> 00:12:53,560
i'm a chemist so i'd like to think about

304
00:12:57,060 --> 00:12:55,839
things in terms of simple reactions so

305
00:12:59,250 --> 00:12:57,070
in some ways you can think of this as a

306
00:13:01,320 --> 00:12:59,260
single displacement reaction and with

307
00:13:03,780 --> 00:13:01,330
little version so little a minus little

308
00:13:06,450 --> 00:13:03,790
algo anions come in and displace the

309
00:13:09,030 --> 00:13:06,460
larger pollyana and the RNA and allow it

310
00:13:11,040 --> 00:13:09,040
to go from a miss folded to a folded

311
00:13:13,470 --> 00:13:11,050
state as shown here and there's certain

312
00:13:15,510 --> 00:13:13,480
conditions that are special and they

313
00:13:18,670 --> 00:13:15,520

have little gold stars to indicate that

314
00:13:21,370 --> 00:13:18,680
they work really well and those can be

315
00:13:23,260 --> 00:13:21,380
short all ago anions where we add large

316
00:13:25,240 --> 00:13:23,270
amounts of them or for the longer ones

317
00:13:26,769 --> 00:13:25,250
where they're charged matched and so

318
00:13:29,380 --> 00:13:26,779
there's three conclusions or three

319
00:13:31,450 --> 00:13:29,390
takeaways first is that the complex

320
00:13:34,810 --> 00:13:31,460
coacervate spar tition ribozymes very

321
00:13:36,519 --> 00:13:34,820
strongly about 5,000 fold the maximal

322
00:13:38,710 --> 00:13:36,529
rate of catalysis at the charge match

323
00:13:40,420 --> 00:13:38,720
conditions occur at an intermediate poly

324
00:13:42,670 --> 00:13:40,430
anion length which is a type of

325
00:13:44,470 --> 00:13:42,680
Goldilocks effect and the mechanistic

326
00:13:47,470 --> 00:13:44,480
basis for this seems to be a balance of

327
00:13:49,210 --> 00:13:47,480
strong RNA sequestration which in the

328
00:13:51,490 --> 00:13:49,220
sense that when you have short poly on

329
00:13:53,230 --> 00:13:51,500
ions that are any a strongly sequestered

330
00:13:54,130 --> 00:13:53,240
in a way that's good because it's inside

331
00:13:56,230 --> 00:13:54,140
the bioreactor

332
00:13:58,510 --> 00:13:56,240
but it's kind of bad in the sense that

333
00:14:00,490 --> 00:13:58,520
the interactions so strong that the RNA

334
00:14:02,800 --> 00:14:00,500
might not be able to fold very well and

335
00:14:04,900 --> 00:14:02,810
the final conclusion is that if you add

336
00:14:07,480 --> 00:14:04,910
excess polygon ions you can enhance

337
00:14:10,030 --> 00:14:07,490
catalysis and complex coacervate and

338
00:14:12,760 --> 00:14:10,040

it's very general so short poly anions

339
00:14:15,040 --> 00:14:12,770
work the best they can be any kind of

340
00:14:17,350 --> 00:14:15,050
poly anion carboxylates phosphates

341
00:14:20,079 --> 00:14:17,360
sulfates they work well can be any kind

342
00:14:22,570 --> 00:14:20,089
of poly cation P DAC and I'll go

343
00:14:24,850 --> 00:14:22,580
arginine work perfectly fine the the

344
00:14:27,280 --> 00:14:24,860
anions and cations can be biogenic or a

345
00:14:28,930 --> 00:14:27,290
biogenic and it works in any ribozyme a

346
00:14:30,790 --> 00:14:28,940
release in two ribozymes i showed you

347
00:14:32,620 --> 00:14:30,800
the data on the Hammerhead and we also

348
00:14:34,600 --> 00:14:32,630
have data on the hairpin ribozyme that

349
00:14:37,240 --> 00:14:34,610
he didn't have time to to show you and

350
00:14:39,730 --> 00:14:37,250
then finally the excess short poly

351
00:14:41,650 --> 00:14:39,740
anions can rescue RNA catalysis and

352
00:14:43,269 --> 00:14:41,660
otherwise incompatible protocells

353
00:14:45,699 --> 00:14:43,279
and so this comes from a collaboration

354
00:14:48,100 --> 00:14:45,709
of my lab and these are the people have

355
00:14:50,500 --> 00:14:48,110
contributed the most and in particular

356
00:14:53,019 --> 00:14:50,510
the the work that I show today is

357
00:14:59,250 --> 00:14:53,029
primarily that of raghava dial and drew

358
00:15:03,670 --> 00:14:59,260
Venus who this is done lab that's mine

359
00:15:06,069 --> 00:15:03,680
okay I don't know how to stop in lab

360
00:15:08,130 --> 00:15:06,079
retreat photo and here's raga hiding

361
00:15:12,460 --> 00:15:08,140
right there and there's drew and then

362
00:15:13,750 --> 00:15:12,470
collaboration with with with the Keating

363
00:15:16,449 --> 00:15:13,760

lab and this work has been funded by

364
00:15:18,430 --> 00:15:16,459
NASA as well as rogue ops supporters

365
00:15:23,980 --> 00:15:18,440
come from the Simons Foundation I'd be

366
00:15:29,000 --> 00:15:27,350
we have time for one short question so

367
00:15:35,000 --> 00:15:29,010
if your question is short please keep

368
00:15:37,280 --> 00:15:35,010
your hand up hey fascinating work bill

369
00:15:39,610 --> 00:15:37,290
but my question is about the coercive

370
00:15:43,250 --> 00:15:39,620
eighths themselves is there a minimum

371
00:15:45,110 --> 00:15:43,260
length for the the polymers that make

372
00:15:47,210 --> 00:15:45,120
the coacervate in order to get a

373
00:15:50,210 --> 00:15:47,220
coercive in other words prebiotic ly how

374
00:15:52,610 --> 00:15:50,220
long could would you need these polymers

375
00:15:55,130 --> 00:15:52,620
to get in order to make a coacervate yes

376
00:15:56,780 --> 00:15:55,140
so that's ongoing work and and see human

377
00:15:59,210 --> 00:15:56,790
sitting right next to you it's been

378
00:16:01,010 --> 00:15:59,220
investigating that and and I think you

379
00:16:04,160 --> 00:16:01,020
can get a little bit shorter than than

380
00:16:07,910 --> 00:16:04,170
ten but certainly with with the ala go

381
00:16:10,519 --> 00:16:07,920
on D 10 and you get the coacervate but

382
00:16:12,590 --> 00:16:10,529
as I said the ribosome is inactive but

383
00:16:15,110 --> 00:16:12,600
then you can rescue that activity by

384
00:16:17,150 --> 00:16:15,120
adding more and more of that and so so I

385
00:16:18,380 --> 00:16:17,160
don't mean to sort of stand up here and

386
00:16:20,240 --> 00:16:18,390
say that this is the way that it

387
00:16:22,610 --> 00:16:20,250
happened that the coacervate were

388
00:16:24,590 --> 00:16:22,620

necessarily made out of this anion or

389
00:16:26,600 --> 00:16:24,600
this cation in some ways it doesn't

390
00:16:28,850 --> 00:16:26,610
matter right so you can see lots of

391
00:16:33,440 --> 00:16:28,860
different an ion cation combinations can

392
00:16:35,920 --> 00:16:33,450
work and basically if the if the RNA in

393
00:16:38,510 --> 00:16:35,930
this case but I'm sure could be any any

394
00:16:40,190 --> 00:16:38,520
informational polymer goes into there if

395
00:16:42,110 --> 00:16:40,200
it goes in too strongly and interacts

396
00:16:43,460 --> 00:16:42,120
too strongly it gets unfolded and so

397
00:16:45,860 --> 00:16:43,470
then you kind of need to loosen that up

398
00:16:48,170 --> 00:16:45,870
so so I think you see here a specific

399
00:16:49,760 --> 00:16:48,180
example but really the take-home is that

400
00:16:51,470 --> 00:16:49,770
sort of the general principles for

401
00:16:53,750 --> 00:16:51,480
drawing it in but not drawing it in too

402
00:16:55,940 --> 00:16:53,760
strongly to allow it to fold and

403
00:16:59,500 --> 00:16:55,950
hopefully and robustly and I would I

404
00:17:02,990 --> 00:16:59,510
think many many different systems

405
00:17:04,270 --> 00:17:03,000
alright lets thankful again