
AbSciCon
2019

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

2
00:00:11,850 --> 00:00:09,169
[Applause]

3
00:00:14,820 --> 00:00:11,860
so Bree the speaker set up very nicely

4
00:00:17,339 --> 00:00:14,830
how the proton gradient is related to

5
00:00:18,779 --> 00:00:17,349
ATP synthesis so but I also have a

6
00:00:21,089 --> 00:00:18,789
little bit of an introduction on that

7
00:00:23,609 --> 00:00:21,099
which I'll go through faster now thanks

8
00:00:25,890 --> 00:00:23,619
to his his presentation but just to get

9
00:00:27,060 --> 00:00:25,900
us all on the same page I had these

10
00:00:30,779 --> 00:00:27,070
first two slides

11
00:00:33,450 --> 00:00:30,789
so basically metabolism consists of two

12
00:00:36,900 --> 00:00:33,460
parts the part where you build up

13
00:00:37,710 --> 00:00:36,910

complex organic molecules from simpler

14
00:00:39,240 --> 00:00:37,720
ones

15
00:00:41,399 --> 00:00:39,250
that's called anabolism and that

16
00:00:45,539 --> 00:00:41,409
requires energy which is obtained by

17
00:00:48,450 --> 00:00:45,549
converting ATP to ADP and oxidizing NADH

18
00:00:51,479 --> 00:00:48,460
to NAD+ so that's shown in this arm of

19
00:00:53,369 --> 00:00:51,489
the schematic diagram here and the other

20
00:00:55,740 --> 00:00:53,379
part of metabolism is the breakdown of

21
00:00:57,869 --> 00:00:55,750
the complex molecules which releases

22
00:01:00,030 --> 00:00:57,879
energy and that's called catabolism and

23
00:01:03,450 --> 00:01:00,040
that released energy is then stored in

24
00:01:05,670 --> 00:01:03,460
the formation of ATP and NADH and that's

25
00:01:09,300 --> 00:01:05,680
shown in this arm of the cycle on the

26
00:01:13,950 --> 00:01:09,310
schematic the abbreviations are shown

27
00:01:16,530 --> 00:01:13,960
over here so how is this ATP synthesize

28
00:01:19,590 --> 00:01:16,540
ATP then is a very key molecule for all

29
00:01:24,720 --> 00:01:19,600
metabolism and and so are these

30
00:01:26,970 --> 00:01:24,730
so-called cofactors such as nad so in

31
00:01:30,450 --> 00:01:26,980
there are various ways of synthesizing

32
00:01:33,480 --> 00:01:30,460
ATP but in most meta depending on the

33
00:01:37,740 --> 00:01:33,490
metabolism of the organism so in most

34
00:01:39,870 --> 00:01:37,750
organisms except for the fermenters ATP

35
00:01:42,000 --> 00:01:39,880
synthesis depends on the formation of a

36
00:01:44,670 --> 00:01:42,010
pH gradient that's called a chemiosmotic

37
00:01:47,100 --> 00:01:44,680
potential across the cell membrane and

38
00:01:51,660 --> 00:01:47,110

coupled to the generation of this pH

39
00:01:55,370 --> 00:01:51,670
gradient is the generation of NADH from

40
00:01:58,020 --> 00:01:55,380
nad and this this entire process is

41
00:01:59,910 --> 00:01:58,030
achieved by transferring electrons down

42
00:02:02,789 --> 00:01:59,920
an electron transport chain that

43
00:02:04,410 --> 00:02:02,799
involves enzymes I'll go through the

44
00:02:07,680 --> 00:02:04,420
schematic in a minute but let me just

45
00:02:09,210 --> 00:02:07,690
finish reading these bullet points the

46
00:02:12,539 --> 00:02:09,220
movement of protons down the

47
00:02:16,020 --> 00:02:12,549
chemiosmotic potential drives the

48
00:02:17,910 --> 00:02:16,030
synthesis of ATP by this ATP synthase

49
00:02:20,970 --> 00:02:17,920
molecule that was presented in the

50
00:02:22,310 --> 00:02:20,980
previous presentation as well the

51
00:02:24,780 --> 00:02:22,320
enzymes that are involved

52
00:02:28,050 --> 00:02:24,790
establishing the chemiosmotic potential

53
00:02:30,630 --> 00:02:28,060
contain these iron sulfur clusters in

54
00:02:33,330 --> 00:02:30,640
the subscript and simply means that this

55
00:02:35,820 --> 00:02:33,340
is the the stoichiometry of the iron and

56
00:02:37,830 --> 00:02:35,830
sulfur so they contain these iron sulfur

57
00:02:39,480 --> 00:02:37,840
clusters at their active sites and it's

58
00:02:41,760 --> 00:02:39,490
been therefore proposed for a long time

59
00:02:43,830 --> 00:02:41,770
in the literature that iron sulfur

60
00:02:46,320 --> 00:02:43,840
minerals might have played the roles of

61
00:02:47,880 --> 00:02:46,330
these enzymes and protocells so what are

62
00:02:50,040 --> 00:02:47,890
we talking about over here here's the

63
00:02:52,650 --> 00:02:50,050

membrane the phospholipid membrane shown

64
00:02:55,860 --> 00:02:52,660
here in the background this represents

65
00:02:58,290 --> 00:02:55,870
the interior of the bacterial cytoplasm

66
00:03:01,050 --> 00:02:58,300
and that's the outside or the the

67
00:03:02,550 --> 00:03:01,060
periplasm and in the case of this

68
00:03:05,180 --> 00:03:02,560
picture is taken for photosynthetic

69
00:03:08,580 --> 00:03:05,190
metabolism the electron transport chain

70
00:03:11,250 --> 00:03:08,590
so you have photosystem ii where the

71
00:03:13,170 --> 00:03:11,260
photolysis of water produces sorry not

72
00:03:17,010 --> 00:03:13,180
photolysis of the water but where water

73
00:03:19,020 --> 00:03:17,020
is broken down to form oxygen and two

74
00:03:21,300 --> 00:03:19,030
protons so this generates protons out

75
00:03:24,410 --> 00:03:21,310
here and the electrons in the process

76
00:03:28,880 --> 00:03:24,420
are picked up and transferred across to

77
00:03:31,680 --> 00:03:28,890
the cytochrome complex here by a quinone

78
00:03:33,570 --> 00:03:31,690
plastoquinone and the plastoquinone and

79
00:03:37,220 --> 00:03:33,580
the cytochrome complex spit out more

80
00:03:40,260 --> 00:03:37,230
protons out here for the transfer the

81
00:03:42,540 --> 00:03:40,270
electron using cluster sign on to first

82
00:03:45,750 --> 00:03:42,550
photosystem one for the system one

83
00:03:49,650 --> 00:03:45,760
transfers it using ferredoxin to nab 10

84
00:03:53,850 --> 00:03:49,660
ADP reductase and at NADP reductase a

85
00:03:57,750 --> 00:03:53,860
proton is combined with nad to convert

86
00:04:00,420 --> 00:03:57,760
it to NADH and so a proton is consumed

87
00:04:02,520 --> 00:04:00,430
over here so protons are produced on

88
00:04:04,710 --> 00:04:02,530

this side and consumed on this side and

89
00:04:06,540 --> 00:04:04,720
this generates a net enrichment of

90
00:04:09,210 --> 00:04:06,550
protons on this side of the membrane a

91
00:04:11,130 --> 00:04:09,220
net depletion of protons on this side of

92
00:04:13,110 --> 00:04:11,140
the membrane so this creates this kemiya

93
00:04:14,610 --> 00:04:13,120
osmotic potential and the fact that

94
00:04:16,740 --> 00:04:14,620
these electrons are being transferred

95
00:04:18,810 --> 00:04:16,750
from one enzyme to another this is the

96
00:04:21,360 --> 00:04:18,820
electron transport chain and here are

97
00:04:23,420 --> 00:04:21,370
the iron sulfide clusters that are in

98
00:04:26,820 --> 00:04:23,430
the active sites of all these different

99
00:04:30,270 --> 00:04:26,830
of all these different electron

100
00:04:33,360 --> 00:04:30,280
transport enzymes once it here's another

101
00:04:35,519 --> 00:04:33,370
picture now continuing on once you've

102
00:04:37,319 --> 00:04:35,529
generated the select this chemiosmotic

103
00:04:40,439 --> 00:04:37,329
potential of more protons on one side

104
00:04:44,339 --> 00:04:40,449
than on the other side if they flow back

105
00:04:46,049 --> 00:04:44,349
through the ATP synthase where ADP

106
00:04:49,499 --> 00:04:46,059
combines with phosphorus and is

107
00:04:51,479 --> 00:04:49,509
converted into ATP so the question was

108
00:04:53,219 --> 00:04:51,489
posed in the previous talk and we will

109
00:04:55,549 --> 00:04:53,229
talk about it here as well as to how did

110
00:05:01,619 --> 00:04:55,559
this chemiosmotic potential first arise

111
00:05:03,749 --> 00:05:01,629
in order for ATP synthesis to occur so

112
00:05:06,329 --> 00:05:03,759
the hypotheses that we have built in our

113
00:05:08,059 --> 00:05:06,339

in our model in our experiment here are

114
00:05:10,259 --> 00:05:08,069
based on a couple I mean the the

115
00:05:12,449 --> 00:05:10,269
experimental system we've built here are

116
00:05:14,369 --> 00:05:12,459
based on a couple of hypotheses the

117
00:05:16,579 --> 00:05:14,379
first hypothesis is the earliest

118
00:05:18,959 --> 00:05:16,589
protocell metabolism was fermentative

119
00:05:21,119 --> 00:05:18,969
this was proposed by Las Carnot and

120
00:05:23,339 --> 00:05:21,129
Miller in the 90s and the idea is that

121
00:05:26,519 --> 00:05:23,349
those organisms didn't have this proton

122
00:05:28,199 --> 00:05:26,529
gradient to drive ATP synthesis but at

123
00:05:31,259 --> 00:05:28,209
some point there was some evolutionary

124
00:05:32,549 --> 00:05:31,269
transition to metabolisms that did have

125
00:05:35,069 --> 00:05:32,559
the electron transport chain and

126
00:05:38,129 --> 00:05:35,079
chemiosmotic potential in order to drive

127
00:05:40,439 --> 00:05:38,139
ATP synthesis so the hypothesis we have

128
00:05:42,749 --> 00:05:40,449
in our work here is that photocatalytic

129
00:05:44,909 --> 00:05:42,759
minerals may have played the roles of

130
00:05:48,059 --> 00:05:44,919
enzymes in this evolutionary transition

131
00:05:51,319 --> 00:05:48,069
including iron sulfur minerals which are

132
00:05:54,329 --> 00:05:51,329
such as pyrite which are photocatalytic

133
00:05:56,569 --> 00:05:54,339
so we're going to build an experimental

134
00:05:58,889 --> 00:05:56,579
system to test this hypothesis whether

135
00:06:03,059 --> 00:05:58,899
photocatalytic minerals can actually

136
00:06:04,819 --> 00:06:03,069
drive this nad to NADH reduction

137
00:06:07,469 --> 00:06:04,829
reaction as well as create and

138
00:06:10,529 --> 00:06:07,479

transmembrane chemiosmotic proton

139
00:06:13,019 --> 00:06:10,539
gradient so we've devised a model

140
00:06:16,649 --> 00:06:13,029
protocell consisting of a lipid membrane

141
00:06:19,409 --> 00:06:16,659
the lipid vesicles bilayer we're going

142
00:06:21,629 --> 00:06:19,419
to react we're going to use a

143
00:06:24,659 --> 00:06:21,639
photochemical mineral for the catalytic

144
00:06:26,819 --> 00:06:24,669
mineral and shine UV light on it which

145
00:06:29,339 --> 00:06:26,829
generates a hole and an electron since

146
00:06:31,319 --> 00:06:29,349
it is a photocatalytic mineral on the

147
00:06:33,599 --> 00:06:31,329
outside of this membrane we have a

148
00:06:34,889 --> 00:06:33,609
reductant we are choosing any common

149
00:06:36,659 --> 00:06:34,899
reductant that might have been present

150
00:06:39,899 --> 00:06:36,669
in the environment such as an amino acid

151
00:06:42,809 --> 00:06:39,909
we just picked serine it can be any

152
00:06:45,179 --> 00:06:42,819
amino acid or any simpler organic

153
00:06:48,420 --> 00:06:45,189
compound the serine takes up the whole

154
00:06:50,460 --> 00:06:48,430
and combines with water to produce GOx

155
00:06:52,890 --> 00:06:50,470
like a sadhana and it also produces

156
00:06:56,760 --> 00:06:52,900
protons in the process meanwhile the

157
00:06:59,730 --> 00:06:56,770
electron is picked up by a molecule

158
00:07:01,920 --> 00:06:59,740
called a poly aromatic hydrocarbon which

159
00:07:04,500 --> 00:07:01,930
was included in the membrane during the

160
00:07:07,170 --> 00:07:04,510
synthesis of this protocell vesicle so

161
00:07:09,150 --> 00:07:07,180
the poly aromatic hydrocarbons have been

162
00:07:10,410 --> 00:07:09,160
shown previously to be electron shuttles

163
00:07:12,120 --> 00:07:10,420

and their structure is actually very

164
00:07:14,640 --> 00:07:12,130
similar to the Queen knowns that we saw

165
00:07:17,550 --> 00:07:14,650
that act as electron shuttles in the

166
00:07:20,460 --> 00:07:17,560
actual biological system so in the step

167
00:07:21,720 --> 00:07:20,470
one of this electron transport chain the

168
00:07:23,460 --> 00:07:21,730
electron is picked up from the

169
00:07:26,220 --> 00:07:23,470
photocatalytic mineral transferred to

170
00:07:28,590 --> 00:07:26,230
the poly aromatic hydrocarbon in step

171
00:07:30,990 --> 00:07:28,600
two the poly aromatic hydrocarbon picks

172
00:07:33,840 --> 00:07:31,000
up the electron passes it through the

173
00:07:35,970 --> 00:07:33,850
membrane to an electron mediator in our

174
00:07:38,730 --> 00:07:35,980
case this is a model compound called

175
00:07:40,680 --> 00:07:38,740
rhodium by pyridinium which the

176
00:07:42,630 --> 00:07:40,690
structure is shown over here now this is

177
00:07:45,270 --> 00:07:42,640
not very pre-buy otic rhodium is not

178
00:07:48,240 --> 00:07:45,280
very common in the environment but we

179
00:07:49,950 --> 00:07:48,250
use this because it was it's capable of

180
00:07:52,650 --> 00:07:49,960
a two electron transfer which is what

181
00:07:55,320 --> 00:07:52,660
you need for nad to NADH reduction we're

182
00:07:57,420 --> 00:07:55,330
currently in our lab trying to also work

183
00:07:59,270 --> 00:07:57,430
now with the iron analog of this which

184
00:08:01,920 --> 00:07:59,280
will be more probiotics applause about

185
00:08:04,560 --> 00:08:01,930
and finally in step three of this

186
00:08:06,540 --> 00:08:04,570
process so when this when this rhodium

187
00:08:11,190 --> 00:08:06,550
by pyridinium picks up an electron from

188
00:08:13,470 --> 00:08:11,200

from PAH it's reduced to the plus one

189
00:08:17,640 --> 00:08:13,480
form from a plus three starting out form

190
00:08:20,220 --> 00:08:17,650
and this reduced molecule then reduces

191
00:08:23,460 --> 00:08:20,230
any D the cofactor which is included in

192
00:08:26,040 --> 00:08:23,470
this vesicle and reduces the nad to NADH

193
00:08:29,400 --> 00:08:26,050
in the process it itself gets oxidized

194
00:08:31,500 --> 00:08:29,410
back to the 3 plus form any D is

195
00:08:33,450 --> 00:08:31,510
prebiotic it has been synthesized by

196
00:08:37,620 --> 00:08:33,460
Steve Boehner's group in a prebiotic

197
00:08:39,900 --> 00:08:37,630
chemistry recently last year and the

198
00:08:41,490 --> 00:08:39,910
structure of nad and NADH again are

199
00:08:44,880 --> 00:08:41,500
shown over here so this is where the H

200
00:08:46,620 --> 00:08:44,890
is added to make it an NADH compound so

201
00:08:48,480 --> 00:08:46,630
we have built this entire system in the

202
00:08:50,640 --> 00:08:48,490
lab and we followed the reaction whether

203
00:08:53,700 --> 00:08:50,650
it's actually happening by monitoring

204
00:08:57,240 --> 00:08:53,710
the production of NADH over time by

205
00:09:00,120 --> 00:08:57,250
using fluorescence spectroscopy how much

206
00:09:02,680 --> 00:09:00,130
time do I okay

207
00:09:05,290 --> 00:09:02,690
so what we're going to do now is going

208
00:09:06,850 --> 00:09:05,300
to look at a lipid vesicle with various

209
00:09:09,700 --> 00:09:06,860
different minerals we tested various

210
00:09:13,390 --> 00:09:09,710
photocatalytic minerals using a serene

211
00:09:15,670 --> 00:09:13,400
reductant and we are going to look at

212
00:09:19,300 --> 00:09:15,680
the production of NADH when UV light is

213
00:09:22,690 --> 00:09:19,310

shown on the system so here are the

214
00:09:24,700 --> 00:09:22,700
results here is the fluorescence as a as

215
00:09:26,890 --> 00:09:24,710
a function of at the scanning wavelength

216
00:09:28,480 --> 00:09:26,900
and you can see that at a certain

217
00:09:30,670 --> 00:09:28,490
wavelength there is a maximum in the

218
00:09:34,390 --> 00:09:30,680
peak which tells us that NADH is being

219
00:09:37,060 --> 00:09:34,400
being produced so after at various time

220
00:09:40,330 --> 00:09:37,070
points of one hour two hours and three

221
00:09:41,830 --> 00:09:40,340
hours of irradiation of UV light you can

222
00:09:44,080 --> 00:09:41,840
see that the peak is increasing that

223
00:09:46,660 --> 00:09:44,090
means NADH production is increasing in

224
00:09:49,750 --> 00:09:46,670
our protocell system so we achieve this

225
00:09:51,430 --> 00:09:49,760
using pyrite and green archite and we

226
00:09:53,740 --> 00:09:51,440
also use cadmium selenide again this is

227
00:09:55,870 --> 00:09:53,750
not prebiotic but this is just as a case

228
00:09:57,370 --> 00:09:55,880
study cadmium selenide or quantum dots

229
00:10:01,240 --> 00:09:57,380
and they're known to be half auto

230
00:10:04,090 --> 00:10:01,250
catalytic activity titania and is also

231
00:10:07,120 --> 00:10:04,100
photo catalytic and produces this NADH

232
00:10:09,880 --> 00:10:07,130
signal beautifully as well as zinc kite

233
00:10:11,950 --> 00:10:09,890
we also tried sphalerite and chalcocite

234
00:10:14,020 --> 00:10:11,960
but they didn't work and it might be

235
00:10:15,340 --> 00:10:14,030
that these were these were all synthetic

236
00:10:17,230 --> 00:10:15,350
minerals that we bought so their

237
00:10:17,920 --> 00:10:17,240
particle size is really small and it's

238
00:10:20,950 --> 00:10:17,930

in the right

239
00:10:22,390 --> 00:10:20,960
particle size to be catalytic these ones

240
00:10:24,400 --> 00:10:22,400
were natural minerals and we had to

241
00:10:27,010 --> 00:10:24,410
crush them up and we probably didn't get

242
00:10:28,960 --> 00:10:27,020
down to a you can't crush things down to

243
00:10:32,680 --> 00:10:28,970
the nanometer size you can only get like

244
00:10:35,020 --> 00:10:32,690
100 200 micron sized particles which may

245
00:10:36,730 --> 00:10:35,030
not be in the right size range to be

246
00:10:39,780 --> 00:10:36,740
photo catalytic so we didn't get the

247
00:10:42,250 --> 00:10:39,790
reaction with these sulfide minerals now

248
00:10:45,700 --> 00:10:42,260
so anyway there's a range of different

249
00:10:49,300 --> 00:10:45,710
minerals which are showing this property

250
00:10:52,810 --> 00:10:49,310
of NADH production the next thing we

251
00:10:55,360 --> 00:10:52,820
wanted to test is this is just a bar

252
00:10:57,940 --> 00:10:55,370
chart that quantifies the amount of NADH

253
00:11:00,220 --> 00:10:57,950
produced as a function of time and you

254
00:11:02,860 --> 00:11:00,230
can see that titanium is the most

255
00:11:06,550 --> 00:11:02,870
efficient and then followed by cadmium

256
00:11:08,650 --> 00:11:06,560
sulfide zinc oxide cadmium selenide and

257
00:11:10,210 --> 00:11:08,660
the least efficient actually is pyrite

258
00:11:12,850 --> 00:11:10,220
but that doesn't matter there was plenty

259
00:11:13,600 --> 00:11:12,860
of pyrite around on early Earth and by

260
00:11:15,759 --> 00:11:13,610
three hours

261
00:11:17,560 --> 00:11:15,769
it's doing a pretty decent job of

262
00:11:20,710 --> 00:11:17,570
producing the NADH

263
00:11:22,420 --> 00:11:20,720

now that was using a lipid membrane

264
00:11:24,069 --> 00:11:22,430
which was based on phospholipids and

265
00:11:28,480 --> 00:11:24,079
phospholipids have actually been

266
00:11:31,720 --> 00:11:28,490
synthesized in prebiotic synthesis so

267
00:11:33,460 --> 00:11:31,730
it's not as prebiotic ly implausible to

268
00:11:35,860 --> 00:11:33,470
use a phospholipid membrane as some

269
00:11:37,960 --> 00:11:35,870
might think but just for our

270
00:11:41,949 --> 00:11:37,970
satisfaction we also tested this with a

271
00:11:44,079 --> 00:11:41,959
fatty acid membrane so we used oleic

272
00:11:45,550 --> 00:11:44,089
acid which is a commonly used fatty acid

273
00:11:48,069 --> 00:11:45,560
and is considered to be more pre-battle

274
00:11:50,350 --> 00:11:48,079
plausible than a phospholipid and we are

275
00:11:52,840 --> 00:11:50,360
able to produce NADH in exactly the same

276
00:11:54,910 --> 00:11:52,850
way using the same system set up with

277
00:11:57,670 --> 00:11:54,920
serene as the extra vesicular or

278
00:12:03,550 --> 00:11:57,680
extracellular reductant terminal

279
00:12:05,199 --> 00:12:03,560
electron donor we also so this compares

280
00:12:06,970 --> 00:12:05,209
the oleic acid system to the

281
00:12:09,220 --> 00:12:06,980
phospholipid system that we just looked

282
00:12:11,829 --> 00:12:09,230
at in the previous slide using serene

283
00:12:15,310 --> 00:12:11,839
but we also decided to change up the

284
00:12:16,960 --> 00:12:15,320
extracellular reductant and used three

285
00:12:19,389 --> 00:12:16,970
different ones we've used serine we've

286
00:12:20,860 --> 00:12:19,399
used glycine and we've used isopropanol

287
00:12:24,130 --> 00:12:20,870
and as you can see pretty much any

288
00:12:25,900 --> 00:12:24,140

simple organic reductant can work in

289
00:12:28,509 --> 00:12:25,910
this reaction scheme and we get the

290
00:12:30,370 --> 00:12:28,519
production of nadh ignored these for the

291
00:12:31,780 --> 00:12:30,380
moment I won't get into the details of

292
00:12:34,780 --> 00:12:31,790
this if anybody has questions about

293
00:12:36,939 --> 00:12:34,790
these I can come back to them basically

294
00:12:40,410 --> 00:12:36,949
these graphs show that the nadh being

295
00:12:43,329 --> 00:12:40,420
produced is the biologically active

296
00:12:45,579 --> 00:12:43,339
isomer which is the one six form of NADH

297
00:12:50,769 --> 00:12:45,589
and not the inactive form which is the

298
00:12:54,220 --> 00:12:50,779
2/3 version of NADH so we're able to

299
00:12:57,100 --> 00:12:54,230
generate this protocell with the drive

300
00:12:59,230 --> 00:12:57,110
the reduction of nad to NADH using a

301
00:13:01,199 --> 00:12:59,240
photocatalytic mineral with different

302
00:13:05,189 --> 00:13:01,209
lipid membranes and with different

303
00:13:07,960 --> 00:13:05,199
reductants so the system is quite robust

304
00:13:11,259 --> 00:13:07,970
what about the generation of the extras

305
00:13:14,620 --> 00:13:11,269
the transmembrane pH gradient what we do

306
00:13:17,230 --> 00:13:14,630
is we enclose this fluorescent molecule

307
00:13:19,740 --> 00:13:17,240
inside the vesicle and this fluorescent

308
00:13:22,540 --> 00:13:19,750
molecule is sensitive to changes in pH

309
00:13:24,850 --> 00:13:22,550
over a period of three hours as we

310
00:13:29,140 --> 00:13:24,860
irradiate at our system and

311
00:13:31,060 --> 00:13:29,150
the NAD was being reduced to NADH we saw

312
00:13:33,130 --> 00:13:31,070
that the the fluorescence intensity

313
00:13:35,830 --> 00:13:33,140

inside these vesicles was changing and

314
00:13:38,080 --> 00:13:35,840
by calibrating it to a pH curve we were

315
00:13:41,920 --> 00:13:38,090
able to determine what was the pH inside

316
00:13:45,130 --> 00:13:41,930
the vessel we were able to determine

317
00:13:47,530 --> 00:13:45,140
what was the the pH inside the vesicle

318
00:13:49,630 --> 00:13:47,540
so at the starting point at time zero

319
00:13:51,520 --> 00:13:49,640
hours the pH is the same as eight point

320
00:13:53,560 --> 00:13:51,530
six which is the pH at which the

321
00:13:55,180 --> 00:13:53,570
vesicles were synthesized so the outside

322
00:13:58,150 --> 00:13:55,190
and the inside are both at pH eight

323
00:14:01,480 --> 00:13:58,160
point six when we use the phospholipid

324
00:14:03,900 --> 00:14:01,490
which is a very imperiled membrane the

325
00:14:05,800 --> 00:14:03,910
pH doesn't really change much over time

326
00:14:08,320 --> 00:14:05,810
thank you

327
00:14:10,660 --> 00:14:08,330
when we use the oleic acid phospholipid

328
00:14:12,520 --> 00:14:10,670
mixture in a ratio of five is to one the

329
00:14:14,680 --> 00:14:12,530
pH drops over a period of time and this

330
00:14:17,230 --> 00:14:14,690
is not surprising because oleic acid

331
00:14:19,210 --> 00:14:17,240
vesicles fatty acid vesicles are not as

332
00:14:21,340 --> 00:14:19,220
impermeable so what's happening is that

333
00:14:24,160 --> 00:14:21,350
the protons are getting carried in from

334
00:14:26,350 --> 00:14:24,170
the outside where the protons were

335
00:14:28,900 --> 00:14:26,360
generated by remember that serine when

336
00:14:31,540 --> 00:14:28,910
it is oxidized it produces the protons

337
00:14:34,690 --> 00:14:31,550
so those the outside of the vesicle is

338
00:14:36,640 --> 00:14:34,700

getting acidified and that pH gradient

339
00:14:39,370 --> 00:14:36,650
is being maintained in the case of the

340
00:14:41,980 --> 00:14:39,380
popc vesicles but it dissipates over a

341
00:14:44,710 --> 00:14:41,990
period of three hours because the fatty

342
00:14:48,430 --> 00:14:44,720
acids shown by these orange molecules

343
00:14:50,800 --> 00:14:48,440
here do this lipid flip-flop so they're

344
00:14:53,050 --> 00:14:50,810
initially deprotonated as the exterior

345
00:14:55,000 --> 00:14:53,060
becomes more and more acidic they pick

346
00:14:59,650 --> 00:14:55,010
up a proton and they do the lipid

347
00:15:01,240 --> 00:14:59,660
flip-flop and carry the the the proton

348
00:15:02,890 --> 00:15:01,250
from the outer leaflet into the inner

349
00:15:04,960 --> 00:15:02,900
leaflet and release it into the inside

350
00:15:08,020 --> 00:15:04,970
of the vesicle and so that's why the

351
00:15:11,260 --> 00:15:08,030
interior starts getting acidifying but

352
00:15:14,350 --> 00:15:11,270
basically when by the time the evolution

353
00:15:15,520 --> 00:15:14,360
of phospholipid membranes had come about

354
00:15:16,930 --> 00:15:15,530
in these protocells

355
00:15:18,340 --> 00:15:16,940
you would have had a pretty tight

356
00:15:21,270 --> 00:15:18,350
membrane which was capable of

357
00:15:24,700 --> 00:15:21,280
maintaining this chemiosmotic potential

358
00:15:26,590 --> 00:15:24,710
so in conclusion various photocatalytic

359
00:15:28,830 --> 00:15:26,600
minerals are capable of promoting a

360
00:15:32,740 --> 00:15:28,840
transmembrane electron transfer chain

361
00:15:34,600 --> 00:15:32,750
with reduction of NAD+ to NADH and the

362
00:15:37,360 --> 00:15:34,610
generation of the transmembrane pH

363
00:15:39,379 --> 00:15:37,370

gradient these reactions utilize an

364
00:15:41,360 --> 00:15:39,389
extra

365
00:15:43,310 --> 00:15:41,370
organic compound as the terminal

366
00:15:46,370 --> 00:15:43,320
electron donor such as serine and

367
00:15:48,860 --> 00:15:46,380
glycine thus the model represents a

368
00:15:51,019 --> 00:15:48,870
proto photo heterotrophic metabolism

369
00:15:55,460 --> 00:15:51,029
because it's utilizing this reduced

370
00:15:57,259 --> 00:15:55,470
organic compound for the metabolism the

371
00:15:58,699 --> 00:15:57,269
minerals we are showing actually for the

372
00:16:00,410 --> 00:15:58,709
you know there there are lots of

373
00:16:02,210 --> 00:16:00,420
hypotheses that minerals may have played

374
00:16:04,009 --> 00:16:02,220
the roles of enzymes and proteomic tabal

375
00:16:05,600 --> 00:16:04,019
ISM but this is one of the first cases

376
00:16:07,610 --> 00:16:05,610
where there's a demonstration that

377
00:16:09,220 --> 00:16:07,620
that's actually working and it's

378
00:16:12,170 --> 00:16:09,230
happening in a photo heterotrophic

379
00:16:14,720 --> 00:16:12,180
metabolism as membranes evolve towards

380
00:16:16,759 --> 00:16:14,730
more phospholipid rich compositions this

381
00:16:19,639 --> 00:16:16,769
new this developed chemiosmotic

382
00:16:21,710 --> 00:16:19,649
potential would have occurred and as

383
00:16:25,340 --> 00:16:21,720
evolution proceeded this might have

384
00:16:27,620 --> 00:16:25,350
eventually been used for the synthesis

385
00:16:31,819 --> 00:16:27,630
of ATP as the evolution eventually

386
00:16:34,490 --> 00:16:31,829
produced an ATP synthase so I'd like to

387
00:16:37,250 --> 00:16:34,500
conclude with thanking my postdoc Poonam

388
00:16:39,560 --> 00:16:37,260

the ly who did all of this work and was

389
00:16:42,139 --> 00:16:39,570
assisted with a really amazing graduate

390
00:16:44,540 --> 00:16:42,149
student putos Triana and funding from

391
00:16:46,579 --> 00:16:44,550
the Simons Foundation the National

392
00:16:48,620 --> 00:16:46,589
Science Foundation grant University of

393
00:16:51,769 --> 00:16:48,630
Akron start-up funds and very generous

394
00:16:53,329 --> 00:16:51,779
gift funds from a a person who's just

395
00:16:54,710 --> 00:16:53,339
interested in origins of life research

396
00:16:56,160 --> 00:16:54,720
and thank you for your attention

397
00:16:59,190 --> 00:16:56,170
thank you very much

398
00:17:01,120 --> 00:16:59,200
[Applause]

399
00:17:03,280 --> 00:17:01,130
unfortunately we don't have time for

400
00:17:05,650 --> 00:17:03,290
questions as the session has concluded

401
00:17:07,090 --> 00:17:05,660
I'd like you to let let's all join