



1
00:00:04,230 --> 00:00:10,930

[Music]

2
00:00:16,430 --> 00:00:14,089

hi everyone so I'm Lauren and I'm from

3
00:00:18,109 --> 00:00:16,440

Anna Lang's Lab at University of Sydney

4
00:00:20,210 --> 00:00:18,119

in Australia and today I'm going to tell

5
00:00:24,769 --> 00:00:20,220

you a bit about the work I do on model

6
00:00:28,609 --> 00:00:26,750

so I'm sure many of us here are trying

7
00:00:31,370 --> 00:00:28,619

to answer the question of how did life

8
00:00:34,069 --> 00:00:31,380

on earth begin now I'm focusing on a

9
00:00:36,470 --> 00:00:34,079

very very specific point in time when

10
00:00:38,690 --> 00:00:36,480

life had maybe begun to transition from

11
00:00:40,549 --> 00:00:38,700

super simple primitive cells perhaps

12
00:00:42,709 --> 00:00:40,559

composed of a fatty acid bilayer

13
00:00:44,630 --> 00:00:42,719

membrane it's a more complex protocells

14

00:00:47,090 --> 00:00:44,640

or primitive cells more akin to Modern

15

00:00:48,970 --> 00:00:47,100

Biology at this point in time we'd

16

00:00:51,410 --> 00:00:48,980

started to see the emergence of

17

00:00:54,650 --> 00:00:51,420

phospholipids and some simple enzymes

18

00:00:56,630 --> 00:00:54,660

but we hadn't yet thanks but we hadn't

19

00:00:58,850 --> 00:00:56,640

yet seen the emergence of all the

20

00:01:01,010 --> 00:00:58,860

complex cellular cellular Machinery that

21

00:01:02,810 --> 00:01:01,020

exists in our cells so things like

22

00:01:04,549 --> 00:01:02,820

membrane transport mechanisms for

23

00:01:06,289 --> 00:01:04,559

example that transport nutrients and

24

00:01:08,990 --> 00:01:06,299

waste molecules out of the cell maybe

25

00:01:11,090 --> 00:01:09,000

they hadn't come around yet so that begs

26

00:01:12,770 --> 00:01:11,100

the question how are these protocols

27

00:01:14,390 --> 00:01:12,780

actually able to feed themselves how

28

00:01:15,890 --> 00:01:14,400

were they able to grow how are they able

29

00:01:18,830 --> 00:01:15,900

to divide

30

00:01:20,749 --> 00:01:18,840

so I'm trying to answer this question by

31

00:01:22,910 --> 00:01:20,759

building a propagating synthetic cell

32

00:01:24,649 --> 00:01:22,920

what that basically means is it's an

33

00:01:26,810 --> 00:01:24,659

artificial cell that is able to feed

34

00:01:28,130 --> 00:01:26,820

itself can grow it can divide and I'm

35

00:01:30,050 --> 00:01:28,140

trying to achieve this using the

36

00:01:32,390 --> 00:01:30,060

simplest components possible to try and

37

00:01:34,490 --> 00:01:32,400

understand how life transitioned into

38

00:01:36,109 --> 00:01:34,500

what we know it as today

39

00:01:39,170 --> 00:01:36,119

so this involves a pretty large

40

00:01:41,630 --> 00:01:39,180

collaboration and with my collaborator

41

00:01:44,270 --> 00:01:41,640

in Japan yutatsu he's been able to build

42

00:01:46,969 --> 00:01:44,280

a preliminary synthetic cell composed of

43

00:01:49,910 --> 00:01:46,979

a phospholipid bilayer membrane also

44

00:01:52,609 --> 00:01:49,920

known as a vesicle and this vesicle is

45

00:01:55,310 --> 00:01:52,619

composed of a mixture of popc and prpg

46

00:01:57,469 --> 00:01:55,320

two types of phospholipids and within

47

00:01:59,690 --> 00:01:57,479

this vesicle we have a system that can

48

00:02:02,350 --> 00:01:59,700

be used to actually synthesize fatty

49

00:02:04,130 --> 00:02:02,360

acids and eventually synthesize

50

00:02:06,289 --> 00:02:04,140

phospholipids that can then be

51
00:02:08,449 --> 00:02:06,299
incorporated into the bilayer membrane

52
00:02:10,430 --> 00:02:08,459
actually allowing it to grow

53
00:02:13,309 --> 00:02:10,440
now one of the big problems with this

54
00:02:15,229 --> 00:02:13,319
system is that you can only encapsulate

55
00:02:16,850 --> 00:02:15,239
a small number of nutrients actually

56
00:02:18,770 --> 00:02:16,860
within the synthetic cell within the

57
00:02:21,170 --> 00:02:18,780
vesicle there's only room for so many

58
00:02:23,210 --> 00:02:21,180
molecules and if you encapsulate as many

59
00:02:25,070 --> 00:02:23,220
as you can you only really get one to

60
00:02:27,050 --> 00:02:25,080
two percent membrane growth which isn't

61
00:02:28,790 --> 00:02:27,060
really sustainable for a cell that we

62
00:02:31,490 --> 00:02:28,800
want to make grow and divide

63
00:02:33,770 --> 00:02:31,500

so what we really need is some sort of

64

00:02:36,290 --> 00:02:33,780

regular nutrient Supply so we need an

65

00:02:38,750 --> 00:02:36,300

external feedstock of nutrients that can

66

00:02:40,250 --> 00:02:38,760

actually permeate the lipid bilayer

67

00:02:42,229 --> 00:02:40,260

membrane to make its way from the

68

00:02:43,869 --> 00:02:42,239

outside of the cell into to the inside

69

00:02:47,330 --> 00:02:43,879

of the cell that way we can actually

70

00:02:49,250 --> 00:02:47,340

achieve continual membrane growth

71

00:02:50,690 --> 00:02:49,260

so that's my role in this project I'm

72

00:02:52,130 --> 00:02:50,700

trying to figure out a way that we can

73

00:02:54,290 --> 00:02:52,140

actually get the nutrients that we need

74

00:02:56,210 --> 00:02:54,300

for the synthetic cell to function to

75

00:02:59,630 --> 00:02:56,220

make its way across the lipid bilayer

76

00:03:01,550 --> 00:02:59,640

membranes the interior synthetic cell

77

00:03:03,110 --> 00:03:01,560

so in order to do that I've needed a

78

00:03:05,570 --> 00:03:03,120

technique that can be used to monitor

79

00:03:07,490 --> 00:03:05,580

solute permeability or the permeability

80

00:03:09,290 --> 00:03:07,500

of different nutrients and to do that

81

00:03:11,690 --> 00:03:09,300

I'm using what's known as a shrink soil

82

00:03:14,030 --> 00:03:11,700

assay basically that involves preparing

83

00:03:15,830 --> 00:03:14,040

vesicles encapsulating a fluorescent dye

84

00:03:17,990 --> 00:03:15,840

known as calcine

85

00:03:20,750 --> 00:03:18,000

when you mix these vesicles with your

86

00:03:22,850 --> 00:03:20,760

solute or your nutrient interests the

87

00:03:25,149 --> 00:03:22,860

difference in osmolarity actually causes

88

00:03:27,710 --> 00:03:25,159

the vesicles to shrink because water

89

00:03:29,570 --> 00:03:27,720

rapidly exits the vesicle and we kind of

90

00:03:31,369 --> 00:03:29,580

see this these shriveled up sort of

91

00:03:33,649 --> 00:03:31,379

shrunken structures

92

00:03:35,270 --> 00:03:33,659

and because we've had water exit the

93

00:03:37,430 --> 00:03:35,280

vesicles we've actually had an increase

94

00:03:39,350 --> 00:03:37,440

in concentration of the diet that's on

95

00:03:40,970 --> 00:03:39,360

the inside of these vesicles and that

96

00:03:43,550 --> 00:03:40,980

actually causes the fluorescent signal

97

00:03:44,750 --> 00:03:43,560

to decrease because calcium is a

98

00:03:47,210 --> 00:03:44,760

self-quenching diet about certain

99

00:03:49,130 --> 00:03:47,220

concentrations so at high concentrations

100

00:03:50,570 --> 00:03:49,140

of fluorescence actually goes down

101
00:03:52,369 --> 00:03:50,580
rather than going up so it's a little

102
00:03:56,270 --> 00:03:52,379
bit counter-intuitive

103
00:03:57,830 --> 00:03:56,280
but over time if we have a permeable

104
00:03:59,869 --> 00:03:57,840
solute so a solute that does actually

105
00:04:01,850 --> 00:03:59,879
enter the vesicle you'll start to see

106
00:04:03,170 --> 00:04:01,860
the vesicles slowly swell up again and

107
00:04:05,690 --> 00:04:03,180
it'll roughly get back to its original

108
00:04:07,490 --> 00:04:05,700
size and with that we have a decrease in

109
00:04:09,050 --> 00:04:07,500
calcium concentration so we have an

110
00:04:11,149 --> 00:04:09,060
increase in the fluorescence roughly to

111
00:04:13,130 --> 00:04:11,159
what it was to begin with

112
00:04:15,649 --> 00:04:13,140
and we can basically monitor that whole

113
00:04:16,969 --> 00:04:15,659

process using some sort of fluorimeter or

114

00:04:19,009 --> 00:04:16,979

something that can measure fluorescence

115

00:04:21,170 --> 00:04:19,019

and we can monitor the changes intensity

116

00:04:23,090 --> 00:04:21,180

over time so we can see initially the

117

00:04:24,530 --> 00:04:23,100

intensity is somewhere up here and then

118

00:04:27,110 --> 00:04:24,540

with the addition of the solute the

119

00:04:29,090 --> 00:04:27,120

intensity drops down and then if we have

120

00:04:31,310 --> 00:04:29,100

a permeable solute that signal will

121

00:04:33,650 --> 00:04:31,320

start to recover and we start to see the

122

00:04:35,510 --> 00:04:33,660

original intensity kind of values there

123

00:04:37,249 --> 00:04:35,520

now because these intensities are kind

124

00:04:39,290 --> 00:04:37,259

of a direct measure of the calcium

125

00:04:41,510 --> 00:04:39,300

concentration we can convert

126
00:04:43,790 --> 00:04:41,520
concentration to volume so the plots

127
00:04:46,909 --> 00:04:43,800
I'll be showing you today show the

128
00:04:48,650 --> 00:04:46,919
changes in volume over time

129
00:04:50,390 --> 00:04:48,660
and it's just worth noting that the

130
00:04:52,490 --> 00:04:50,400
initial stage where the vesicles shrink

131
00:04:54,290 --> 00:04:52,500
and swivel up shrivel up actually

132
00:04:56,150 --> 00:04:54,300
happens very quickly and the

133
00:04:57,230 --> 00:04:56,160
experimental setup I currently have I

134
00:04:59,270 --> 00:04:57,240
can't actually see that because it just

135
00:05:01,070 --> 00:04:59,280
happens too fast so the data I'll be

136
00:05:03,469 --> 00:05:01,080
showing you is just the recovery of that

137
00:05:07,010 --> 00:05:05,330
so the basic workflow for a lot of my

138
00:05:08,990 --> 00:05:07,020

experiments is to First prepare the

139

00:05:12,290 --> 00:05:09,000

vesicles composed of an equal mixture of

140

00:05:14,629 --> 00:05:12,300

popc popg in 50 millimolar happy so

141

00:05:17,450 --> 00:05:14,639

that's just the buffer it's roughly

142

00:05:18,890 --> 00:05:17,460

buffering around physiological pH and

143

00:05:20,689 --> 00:05:18,900

these vesicles all encounter all

144

00:05:22,969 --> 00:05:20,699

encapsulate that fluorescent iron is

145

00:05:25,310 --> 00:05:22,979

calcium and then make sure all the

146

00:05:27,409 --> 00:05:25,320

unencapsulated calcium is removed and I

147

00:05:29,450 --> 00:05:27,419

mix the vesicles with the solute all the

148

00:05:31,850 --> 00:05:29,460

nutrient of interest and monitor the

149

00:05:33,170 --> 00:05:31,860

changes in fluorescence over time and

150

00:05:35,930 --> 00:05:33,180

then determine the changes in volume

151

00:05:39,529 --> 00:05:37,790

so this is what some of the data looks

152

00:05:42,469 --> 00:05:39,539

like on the left we can see some

153

00:05:44,510 --> 00:05:42,479

examples of some permeable solutes so

154

00:05:47,090 --> 00:05:44,520

our control here was the 50 millimolar

155

00:05:48,890 --> 00:05:47,100

happyes in the blue and all of this data

156

00:05:51,290 --> 00:05:48,900

is normalized to the volume of that

157

00:05:53,330 --> 00:05:51,300

control at time zero so we see in the

158

00:05:55,189 --> 00:05:53,340

blue 50mm Heavies it's just a flat line

159

00:05:56,990 --> 00:05:55,199

there not much is changing and that's

160

00:05:58,670 --> 00:05:57,000

what we'd expect it's our control it's

161

00:06:01,189 --> 00:05:58,680

what we made our vesicles in so we don't

162

00:06:02,810 --> 00:06:01,199

expect any volume change there but when

163

00:06:05,930 --> 00:06:02,820

we mix our vesicles with something like

164

00:06:08,029 --> 00:06:05,940

glucose or glycine for example so the

165

00:06:10,129 --> 00:06:08,039

red and the purple we see that

166

00:06:11,870 --> 00:06:10,139

characteristic curve telling us that the

167

00:06:13,850 --> 00:06:11,880

volume is actually increasing after the

168

00:06:15,830 --> 00:06:13,860

vesicles initially kind of shriveled up

169

00:06:17,629 --> 00:06:15,840

so that tells us that these two solutes

170

00:06:19,850 --> 00:06:17,639

are both permeable across these lipid

171

00:06:21,770 --> 00:06:19,860

bilayer membranes and glycine is

172

00:06:24,650 --> 00:06:21,780

actually more permeable than the glucose

173

00:06:27,650 --> 00:06:24,660

because it increases at a faster rate

174

00:06:28,670 --> 00:06:27,660

on that plot I also have glycerol at

175

00:06:30,890 --> 00:06:28,680

first glance it kind of looks like

176

00:06:32,870 --> 00:06:30,900

nothing happens there but glycerol is

177

00:06:34,850 --> 00:06:32,880

known to be super permeable across lipid

178

00:06:36,830 --> 00:06:34,860

bilayers so it's likely that I just

179

00:06:38,689 --> 00:06:36,840

actually missed that initial shrinkage

180

00:06:40,070 --> 00:06:38,699

and swelling stage so it just looks like

181

00:06:41,330 --> 00:06:40,080

nothing's happened right now because I

182

00:06:43,010 --> 00:06:41,340

missed the first kind of couple minutes

183

00:06:45,050 --> 00:06:43,020

of this process

184

00:06:47,270 --> 00:06:45,060

now on the other plot we have some

185

00:06:49,189 --> 00:06:47,280

examples of some impermeable solutes and

186

00:06:50,629 --> 00:06:49,199

you can see there's quite a few there so

187

00:06:52,730 --> 00:06:50,639

a lot of the solids I've looked at so

188

00:06:54,469 --> 00:06:52,740

far are quite impermeable and that's we

189

00:06:56,150 --> 00:06:54,479

can see that there's like those flat

190

00:06:58,189 --> 00:06:56,160

lines at the bottom there there's no

191

00:06:59,809 --> 00:06:58,199

increase in volume over time so over

192

00:07:02,029 --> 00:06:59,819

these this time frame none of these

193

00:07:03,770 --> 00:07:02,039

Solutions are permeable which is kind of

194

00:07:05,570 --> 00:07:03,780

disappointing because a lot of these

195

00:07:08,990 --> 00:07:05,580

solutes would be useful nutrients for a

196

00:07:10,730 --> 00:07:09,000

synthetic cell things like amp ATP for

197

00:07:13,070 --> 00:07:10,740

energy and then something like sodium

198

00:07:14,930 --> 00:07:13,080

acetate I was hoping to use as a carbon

199

00:07:18,050 --> 00:07:14,940

source to actually synthesize fatty

200

00:07:19,969 --> 00:07:18,060

acids and then the phospholipids

201
00:07:21,469 --> 00:07:19,979
but it's not particularly surprising

202
00:07:23,510 --> 00:07:21,479
that a lot of these solutes were

203
00:07:24,950 --> 00:07:23,520
impermeable It's relatively well

204
00:07:27,050 --> 00:07:24,960
established in literature child that

205
00:07:28,850 --> 00:07:27,060
phospholipid bilayers aren't the most

206
00:07:31,070 --> 00:07:28,860
permeable things especially when

207
00:07:33,409 --> 00:07:31,080
comparing them to a fatty acid bilayer

208
00:07:34,670 --> 00:07:33,419
for example so fatty acid bilayers are

209
00:07:37,189 --> 00:07:34,680
kind of useful to think about because

210
00:07:39,350 --> 00:07:37,199
they have been proposed to have made up

211
00:07:41,629 --> 00:07:39,360
the first protocell membranes before we

212
00:07:43,430 --> 00:07:41,639
saw the emergence of phospholipids and

213
00:07:45,950 --> 00:07:43,440

we can see in this plot here for

214

00:07:48,469 --> 00:07:45,960

something like glucose it's a bit less

215

00:07:50,029 --> 00:07:48,479

permeable in phospholipids than in fatty

216

00:07:51,770 --> 00:07:50,039

acid bilayers

217

00:07:54,110 --> 00:07:51,780

there's something like sodium potassium

218

00:07:55,969 --> 00:07:54,120

it's significantly less permeable and

219

00:07:58,070 --> 00:07:55,979

like over this scale that that's a

220

00:08:00,050 --> 00:07:58,080

really significant margin there

221

00:08:02,930 --> 00:08:00,060

and then for something like amp and

222

00:08:04,730 --> 00:08:02,940

magnesium that's not actually on the

223

00:08:06,589 --> 00:08:04,740

scale shown here for phospholipids so

224

00:08:08,450 --> 00:08:06,599

it's way less permeable in phospholipid

225

00:08:09,890 --> 00:08:08,460

membranes than it is in fatty acid

226

00:08:11,089 --> 00:08:09,900

membranes

227

00:08:13,129 --> 00:08:11,099

so again these results aren't

228

00:08:15,230 --> 00:08:13,139

particularly surprising but I still

229

00:08:17,210 --> 00:08:15,240

wanted to try and find a way to actually

230

00:08:19,969 --> 00:08:17,220

get these nutrients to permeate the

231

00:08:21,589 --> 00:08:19,979

bilayer so today I'm going to talk about

232

00:08:22,969 --> 00:08:21,599

a couple of different strategies I've

233

00:08:25,369 --> 00:08:22,979

tried to use to actually improve

234

00:08:28,309 --> 00:08:25,379

permeability the first of which is

235

00:08:30,290 --> 00:08:28,319

modifying the membrane composition

236

00:08:32,810 --> 00:08:30,300

so as I said all the vesicles I've been

237

00:08:35,630 --> 00:08:32,820

working with so far have been composed

238

00:08:37,070 --> 00:08:35,640

of pobc and popg now these lipids are

239

00:08:38,570 --> 00:08:37,080

quite nice to work with they're

240

00:08:40,430 --> 00:08:38,580

cylindrical in shape so they pack

241

00:08:42,469 --> 00:08:40,440

together in these really nice bilayers

242

00:08:44,990 --> 00:08:42,479

they're very uniform but what would

243

00:08:46,550 --> 00:08:45,000

happen if I were to say disrupt the way

244

00:08:49,370 --> 00:08:46,560

the lit lipid molecules actually pack

245

00:08:51,590 --> 00:08:49,380

together what if I introduced a leopard

246

00:08:54,769 --> 00:08:51,600

with a different shape something like

247

00:08:56,810 --> 00:08:54,779

laser PC which has a single carbon chain

248

00:08:58,790 --> 00:08:56,820

rather than two but it still has a

249

00:09:01,009 --> 00:08:58,800

relatively bulky head group so it has

250

00:09:02,930 --> 00:09:01,019

this more conical like shape and when

251

00:09:05,210 --> 00:09:02,940

that gets inserted into bilayers it can

252

00:09:07,009 --> 00:09:05,220

cause defects to form and pores which

253

00:09:08,990 --> 00:09:07,019

would potentially allow nutrients to

254

00:09:10,610 --> 00:09:09,000

pass through the membrane so I wanted to

255

00:09:12,949 --> 00:09:10,620

explore what effect adding small

256

00:09:14,509 --> 00:09:12,959

quantities of lyso PC would have on

257

00:09:17,030 --> 00:09:14,519

permeability

258

00:09:19,550 --> 00:09:17,040

and the other lipid I looked at is oleic

259

00:09:21,829 --> 00:09:19,560

acid which is a fatty acid has a single

260

00:09:23,810 --> 00:09:21,839

carbon chain and a relatively small head

261

00:09:25,430 --> 00:09:23,820

group but because it's a fatty acid it

262

00:09:27,829 --> 00:09:25,440

has very different properties to what

263

00:09:29,630 --> 00:09:27,839

phospholipids do so I wanted to explore

264

00:09:33,949 --> 00:09:29,640

how those different properties might

265

00:09:33,959 --> 00:09:37,930

membrane only I found

266

00:09:43,310 --> 00:09:40,910

I prepared all the vesicles the same way

267

00:09:46,550 --> 00:09:43,320

I did before but added about 10 either

268

00:09:48,710 --> 00:09:46,560

lyso PC or oleic acid to the system and

269

00:09:51,470 --> 00:09:48,720

generally as I said didn't make much of

270

00:09:53,750 --> 00:09:51,480

a difference we have one of our example

271

00:09:56,210 --> 00:09:53,760

permeable solutes there so glucose which

272

00:09:57,829 --> 00:09:56,220

we saw was permeable earlier we can see

273

00:09:59,750 --> 00:09:57,839

in those three curves there isn't really

274

00:10:01,610 --> 00:09:59,760

much difference between them if anything

275

00:10:03,829 --> 00:10:01,620

the green curve the oleic acid is

276
00:10:05,449 --> 00:10:03,839
shifted down slightly but the shapes of

277
00:10:07,370 --> 00:10:05,459
the curves are all very similar which is

278
00:10:09,410 --> 00:10:07,380
telling us that the permeability hasn't

279
00:10:11,389 --> 00:10:09,420
really changed and we see something

280
00:10:13,670 --> 00:10:11,399
simple similar with one of the

281
00:10:15,590 --> 00:10:13,680
impermeable solutes that being lysine

282
00:10:17,269 --> 00:10:15,600
they still have all those flat lines so

283
00:10:19,610 --> 00:10:17,279
we saw no improvement in permeability

284
00:10:21,650 --> 00:10:19,620
there and this was generally the case

285
00:10:23,509 --> 00:10:21,660
across a lot of the different solutes or

286
00:10:25,070 --> 00:10:23,519
nutrients that I actually tried there

287
00:10:27,769 --> 00:10:25,080
weren't any marked improvements in

288
00:10:29,630 --> 00:10:27,779

permeability except for when I tried

289

00:10:32,509 --> 00:10:29,640

sodium acetate

290

00:10:35,090 --> 00:10:32,519

so when we had the presence of 10 oleic

291

00:10:36,949 --> 00:10:35,100

acid in the system we see a significant

292

00:10:39,350 --> 00:10:36,959

Improvement in permeability compared to

293

00:10:41,750 --> 00:10:39,360

what it was originally with the popcpg

294

00:10:44,569 --> 00:10:41,760

system and also compared to adding the

295

00:10:46,069 --> 00:10:44,579

10 lyso PC so we do see that curve

296

00:10:47,750 --> 00:10:46,079

that's telling us that the vesicle is

297

00:10:49,910 --> 00:10:47,760

swelling up again and we actually have

298

00:10:54,110 --> 00:10:49,920

the solute molecules permeating into the

299

00:10:55,910 --> 00:10:54,120

vesicle which is good news we saw one of

300

00:10:57,710 --> 00:10:55,920

these solutes actually

301
00:11:00,050 --> 00:10:57,720
kind of worked and actually were able to

302
00:11:01,790 --> 00:11:00,060
permeate the membrane and as I said um

303
00:11:03,530 --> 00:11:01,800
it was great to see that it did work

304
00:11:05,509 --> 00:11:03,540
with sodium acetate because we wanted to

305
00:11:07,850 --> 00:11:05,519
use that as our carbon source for fatty

306
00:11:09,350 --> 00:11:07,860
acid synthesis then phospholipid

307
00:11:11,690 --> 00:11:09,360
synthesis and that would actually help

308
00:11:13,430 --> 00:11:11,700
getting the membrane to grow

309
00:11:15,110 --> 00:11:13,440
but as I said overall it didn't work for

310
00:11:17,990 --> 00:11:15,120
a lot of the stuff I tried so I wanted

311
00:11:19,730 --> 00:11:18,000
to try it a second strategy to try and

312
00:11:21,790 --> 00:11:19,740
improve permeability and that was

313
00:11:23,930 --> 00:11:21,800

through the addition of divalent cations

314

00:11:25,970 --> 00:11:23,940

so in literature it's pretty well

315

00:11:28,009 --> 00:11:25,980

established that divalent cations also

316

00:11:29,750 --> 00:11:28,019

affect the packing of the way of the

317

00:11:31,850 --> 00:11:29,760

lipid molecules so the way the bilayers

318

00:11:32,990 --> 00:11:31,860

actually packed together so I wanted to

319

00:11:34,430 --> 00:11:33,000

see if that would make a difference to

320

00:11:36,769 --> 00:11:34,440

permeability

321

00:11:38,630 --> 00:11:36,779

and again generally I found it didn't

322

00:11:41,990 --> 00:11:38,640

make that much of a difference except

323

00:11:44,870 --> 00:11:42,000

for the presence of sodium acetate so

324

00:11:46,910 --> 00:11:44,880

with the 10 Lake acid system and the

325

00:11:49,069 --> 00:11:46,920

sodium acetate when I added magnesium

326

00:11:51,110 --> 00:11:49,079

chloride which is what we see in the

327

00:11:52,850 --> 00:11:51,120

orange there was a Improvement in

328

00:11:54,650 --> 00:11:52,860

permeability if you see that curve kind

329

00:11:56,269 --> 00:11:54,660

of increases faster than what the the

330

00:11:58,550 --> 00:11:56,279

blue line which is just the pure sodium

331

00:12:00,710 --> 00:11:58,560

acetate and the green line which is just

332

00:12:02,509 --> 00:12:00,720

the magnesium chloride so for whatever

333

00:12:05,269 --> 00:12:02,519

reason the addition of magnesium

334

00:12:07,370 --> 00:12:05,279

chloride with the sodium acetate and in

335

00:12:09,889 --> 00:12:07,380

the temps in oleic acid system did

336

00:12:12,590 --> 00:12:09,899

improve permeability overall

337

00:12:14,150 --> 00:12:12,600

but again generally I found that this

338

00:12:16,130 --> 00:12:14,160

was quite difficult to achieve in a lot

339

00:12:17,870 --> 00:12:16,140

of the solutes I tried I've also tried

340

00:12:19,009 --> 00:12:17,880

other divalent cations which I haven't

341

00:12:22,370 --> 00:12:19,019

shown here

342

00:12:25,610 --> 00:12:22,380

um in general it was pretty tough

343

00:12:27,290 --> 00:12:25,620

so what does that mean overall so

344

00:12:29,569 --> 00:12:27,300

generally I've found that permeability

345

00:12:32,030 --> 00:12:29,579

can somewhat be modulated for specific

346

00:12:34,670 --> 00:12:32,040

specific sites by changing the membrane

347

00:12:36,889 --> 00:12:34,680

composition and divalent cations can

348

00:12:39,710 --> 00:12:36,899

somewhat improve the permeability of

349

00:12:41,569 --> 00:12:39,720

specific solutes but overall it was

350

00:12:43,670 --> 00:12:41,579

pretty difficult to actually see any

351
00:12:45,170 --> 00:12:43,680
marked improvements in permeability for

352
00:12:47,930 --> 00:12:45,180
a lot of the different things I studied

353
00:12:50,329 --> 00:12:47,940
so what does that mean for origins of

354
00:12:52,190 --> 00:12:50,339
life well perhaps we needed protein

355
00:12:54,410 --> 00:12:52,200
channels to actually co-evolve with

356
00:12:56,449 --> 00:12:54,420
phospholipid synthesis as I mentioned

357
00:12:58,129 --> 00:12:56,459
earlier fatty acid membranes are much

358
00:12:59,930 --> 00:12:58,139
more permeable than phospholipid

359
00:13:02,210 --> 00:12:59,940
membranes so if they were the first

360
00:13:04,550 --> 00:13:02,220
protocell membranes they were able to

361
00:13:06,170 --> 00:13:04,560
access nutrients a lot easier than

362
00:13:07,550 --> 00:13:06,180
phospholipid membranes on their own

363
00:13:09,410 --> 00:13:07,560

would be able to so we've probably

364

00:13:12,230 --> 00:13:09,420

needed some sort of mechanism to

365

00:13:15,650 --> 00:13:12,240

actually co-evolve with phospholipids as

366

00:13:17,449 --> 00:13:15,660

they started to emerge but this is a

367

00:13:19,129 --> 00:13:17,459

very much an ongoing project I'm trying

368

00:13:21,290 --> 00:13:19,139

a lot of different membrane compositions

369

00:13:23,389 --> 00:13:21,300

a lot of different divalent cations and

370

00:13:25,129 --> 00:13:23,399

I'm still working on other methods to

371

00:13:26,750 --> 00:13:25,139

kind of improve permeability so

372

00:13:29,389 --> 00:13:26,760

hopefully in the future I'll have some

373

00:13:30,949 --> 00:13:29,399

more definitive results with some

374

00:13:33,470 --> 00:13:30,959

improvements in permeability for a lot

375

00:13:35,990 --> 00:13:33,480

of other solutes too

376

00:13:37,970 --> 00:13:36,000

so with that I'd like to acknowledge the

377

00:13:39,949 --> 00:13:37,980

entire Land Group and my supervisor Anna

378

00:13:41,569 --> 00:13:39,959

for all her support thank you to my

379

00:13:49,329 --> 00:13:41,579

sources of funding and thank you to

380

00:13:49,339 --> 00:13:57,230

okay we have time for a few questions

381

00:14:03,050 --> 00:14:00,590

thank you I am from Ohio University I

382

00:14:06,470 --> 00:14:03,060

study multiplayer biology I'm just

383

00:14:09,889 --> 00:14:06,480

interested in the method that you use to

384

00:14:12,470 --> 00:14:09,899

measure your membrane permeability is it

385

00:14:20,389 --> 00:14:12,480

um Arch Clump electrophysiology or what

386

00:14:25,129 --> 00:14:22,370

actually a measure of the changes in

387

00:14:27,170 --> 00:14:25,139

volume but it's reliant on the fact that

388

00:14:28,850 --> 00:14:27,180

with those changes in volume they can

389

00:14:31,430 --> 00:14:28,860

only occur because the solute is

390

00:14:33,110 --> 00:14:31,440

actually permeating the membrane so it's

391

00:14:35,389 --> 00:14:33,120

actually fluorescence-based technique so

392

00:14:37,129 --> 00:14:35,399

I use a floor a fluimeter or plate radar

393

00:14:38,810 --> 00:14:37,139

depending on the experimental setup I

394

00:14:41,150 --> 00:14:38,820

have going to actually measure those

395

00:14:43,069 --> 00:14:41,160

changes in fluorescence over time and

396

00:14:45,470 --> 00:14:43,079

from that you can calculate the changes

397

00:14:47,449 --> 00:14:45,480

in volume which you can actually then go

398

00:14:48,829 --> 00:14:47,459

on to work out permeability coefficients

399

00:14:50,269 --> 00:14:48,839

from those curves which is something I

400

00:14:52,550 --> 00:14:50,279

haven't done yet but you can actually

401
00:14:54,769 --> 00:14:52,560
fit a model to that curve and extract

402
00:14:58,310 --> 00:14:54,779
the permeability coefficients as well

403
00:15:00,970 --> 00:14:58,320
okay I'm I'm just actually thinking that

404
00:15:03,290 --> 00:15:00,980
um if you can employ the patch Club

405
00:15:06,189 --> 00:15:03,300
electrophysiology method it can give

406
00:15:08,689 --> 00:15:06,199
insight into like membrane polarity

407
00:15:11,210 --> 00:15:08,699
hyperpolarization yeah which can also

408
00:15:12,170 --> 00:15:11,220
give more insight on this yeah 100 in

409
00:15:19,550 --> 00:15:12,180
this

410
00:15:19,560 --> 00:15:22,310
makes it interested

411
00:15:25,370 --> 00:15:23,930
hi I'm Tim from University of

412
00:15:27,530 --> 00:15:25,380
Wisconsin-Madison

413
00:15:29,750 --> 00:15:27,540

um I was wondering uh do you think there

414

00:15:32,150 --> 00:15:29,760

could be an effect of physical size or

415

00:15:34,009 --> 00:15:32,160

specifically surface area uh on the

416

00:15:35,629 --> 00:15:34,019

degree of permeability and if so did you

417

00:15:38,329 --> 00:15:35,639

like control for size did you extrude

418

00:15:40,730 --> 00:15:38,339

your vesicles I sure did good question

419

00:15:42,590 --> 00:15:40,740

yeah so these vesicles for this system

420

00:15:44,810 --> 00:15:42,600

I'm working with have all been extruded

421

00:15:46,910 --> 00:15:44,820

through 50 nanometer pause

422

00:15:48,949 --> 00:15:46,920

um they're roughly

423

00:15:52,310 --> 00:15:48,959

they're probably about 40 nanometers in

424

00:15:54,949 --> 00:15:52,320

diameter for the most part it's a

425

00:15:57,590 --> 00:15:54,959

relatively even distribution added to

426

00:15:59,870 --> 00:15:57,600

DLS on pretty similar systems

427

00:16:01,850 --> 00:15:59,880

um yeah but that's why I did extrude

428

00:16:05,689 --> 00:16:01,860

because I would expect family ability to