EARTH-LIFE
SCIENCE
INSTITUTE

Shawn McGlynn

Linking Enzyme Derived Isotope Fractionation Values to Cellular States

1
00:00:00,720 --> 00:00:10,780
[Music]

2
00:00:16,609 --> 00:00:14,000
thanks Yuichiro and I want to extend my

3
00:00:17,900 --> 00:00:16,619
thanks again to Eric in the scientific

4
00:00:21,170 --> 00:00:17,910
organizing committee for putting

5
00:00:23,120 --> 00:00:21,180
together a great series of talks and I

6
00:00:26,390 --> 00:00:23,130
I'm gonna try to hook hook my talk in

7
00:00:30,410 --> 00:00:26,400
here from from a perspective of trying

8
00:00:32,210 --> 00:00:30,420
to understand ways that we can observe

9
00:00:35,990 --> 00:00:32,220
the historical record of catalytic

10
00:00:39,200 --> 00:00:36,000
evolution through time and this is a

11
00:00:41,479 --> 00:00:39,210
very broad question and I'm going to use

12
00:00:46,270 --> 00:00:41,489
a case study of looking at microbial

13
00:00:48,770 --> 00:00:46,280

sulfate reduction as a system to try to

14
00:00:52,810 --> 00:00:48,780
see if we can do this if we can start to

15
00:00:55,099 --> 00:00:52,820
link enzyme sequence evolution to

16
00:00:57,349 --> 00:00:55,109
fractionation of stable isotopes and be

17
00:00:59,209 --> 00:00:57,359
able to monitor the historical record of

18
00:01:01,880 --> 00:00:59,219
that those isotope fractionation through

19
00:01:03,770 --> 00:01:01,890
time but I'm also interested in doing

20
00:01:05,719 --> 00:01:03,780
this from the perspective of

21
00:01:08,480 --> 00:01:05,729
understanding cells today from a

22
00:01:10,790 --> 00:01:08,490
microbial ecology perspective where we

23
00:01:12,289 --> 00:01:10,800
might be able to use stable isotope

24
00:01:14,480 --> 00:01:12,299
fractionation as a way of gaining

25
00:01:16,280 --> 00:01:14,490
insight onto the physiological state of

26
00:01:20,289 --> 00:01:16,290
a cell that we might have in a bottle or

27
00:01:25,100 --> 00:01:20,299
have in a lake or some other environment

28
00:01:27,710 --> 00:01:25,110
so this is a I'm presenting today but a

29
00:01:31,100 --> 00:01:27,720
lot of the work was done by Minh sub-sub

30
00:01:33,890 --> 00:01:31,110
it was a postdoc previously in my

31
00:01:36,789 --> 00:01:33,900
previous affiliation at Cal Tech he's

32
00:01:40,130 --> 00:01:36,799
now at a Seoul National University and

33
00:01:41,929 --> 00:01:40,140
here at Elsi Chris butch and Mackenzie

34
00:01:47,569 --> 00:01:41,939
Smith are also I'm working towards some

35
00:01:50,060 --> 00:01:47,579
of the goals of this project and so yeah

36
00:01:51,469 --> 00:01:50,070
I titled my talk and and I'm really

37
00:01:53,719 --> 00:01:51,479
going to Center the talk about this idea

38
00:01:57,859 --> 00:01:53,729

of using stable isotopes to understand

39
00:02:00,410 --> 00:01:57,869
cellular physiology but I but I want to

40
00:02:03,230 --> 00:02:00,420
make it a little bit more general than

41
00:02:05,209 --> 00:02:03,240
that and hopefully be able to get some

42
00:02:06,920 --> 00:02:05,219
criticism and comments and other ideas

43
00:02:09,259 --> 00:02:06,930
about how we might be able to use this

44
00:02:12,550 --> 00:02:09,269
approach of looking at stable isotope

45
00:02:15,319 --> 00:02:12,560
fractionation coupled with knowledge of

46
00:02:19,009 --> 00:02:15,329
catalytic kinetics to be able to follow

47
00:02:21,890 --> 00:02:19,019
enzyme evolution of time and so broadly

48
00:02:22,610 --> 00:02:21,900
speaking we have two main repositories

49
00:02:27,199 --> 00:02:22,620
of

50
00:02:30,140 --> 00:02:27,209
one isn't stuff that we can go out and

51
00:02:32,570 --> 00:02:30,150
see like these piles of cells that we

52
00:02:35,990 --> 00:02:32,580
call stromatolites which appear fairly

53
00:02:39,770 --> 00:02:36,000
early on the earth and we also have

54
00:02:43,850 --> 00:02:39,780
other material repositories these stable

55
00:02:46,220 --> 00:02:43,860
isotopes and so light carbon like as

56
00:02:48,619 --> 00:02:46,230
shown here this is a reference to one of

57
00:02:50,990 --> 00:02:48,629
you you each rose papers that there's a

58
00:02:53,059 --> 00:02:51,000
sign of metabolism deep in the past

59
00:02:55,729 --> 00:02:53,069
that's apparently recorded by the

60
00:02:57,890 --> 00:02:55,739
distribution or fractionation of light

61
00:03:00,559 --> 00:02:57,900
and heavy carbon the different isotopes

62
00:03:02,870 --> 00:03:00,569
so other than these material phases that

63
00:03:06,740 --> 00:03:02,880

we can go out and find an old rocks we

64
00:03:09,380 --> 00:03:06,750
also have molecular biology and we've

65
00:03:10,970 --> 00:03:09,390
seen a couple of phylogenetic trees come

66
00:03:13,400 --> 00:03:10,980
up in the last couple days and a couple

67
00:03:15,979 --> 00:03:13,410
mentions of the concept of using

68
00:03:19,550 --> 00:03:15,989
molecular clocks to try to put a

69
00:03:21,680 --> 00:03:19,560
timeline on how DNA and protein

70
00:03:24,440 --> 00:03:21,690
sequences evolve through time so these

71
00:03:27,020 --> 00:03:24,450
are kind of two main repositories of

72
00:03:29,360 --> 00:03:27,030
biological knowledge and what I'm going

73
00:03:32,059 --> 00:03:29,370
to propose is a pathway to be able to

74
00:03:35,240 --> 00:03:32,069
link these two to linked sequence

75
00:03:36,680 --> 00:03:35,250
evolution of enzyme catalysis to actual

76
00:03:39,229 --> 00:03:36,690
repositories and I think that might help

77
00:03:43,250 --> 00:03:39,239
us calibrate things this is a long-term

78
00:03:45,680 --> 00:03:43,260
goal that I have here so the model

79
00:03:48,559 --> 00:03:45,690
system that I'm going to discuss today

80
00:03:50,420 --> 00:03:48,569
is microbial sulfate reduction these

81
00:03:52,220 --> 00:03:50,430
this way of thinking about things it's

82
00:03:54,259 --> 00:03:52,230
not limited at all to microbial sulfate

83
00:03:56,839 --> 00:03:54,269
reduction but microbial sulfate

84
00:04:00,289 --> 00:03:56,849
reduction is a as a convenient starter

85
00:04:02,420 --> 00:04:00,299
system for this way of thinking because

86
00:04:05,120 --> 00:04:02,430
there is a long history of measuring

87
00:04:07,490 --> 00:04:05,130
stable sulfur isotopes and their

88
00:04:11,390 --> 00:04:07,500

patterns of fractionation throughout

89
00:04:14,000 --> 00:04:11,400
geological history microbial sulfur

90
00:04:17,199 --> 00:04:14,010
sulfate reduction is a eight electron

91
00:04:19,879 --> 00:04:17,209
addition on the sulfate to make sulfide

92
00:04:22,339 --> 00:04:19,889
sulfate is pretty oxidizing molecule and

93
00:04:24,589 --> 00:04:22,349
so what these organisms are doing are

94
00:04:27,050 --> 00:04:24,599
taking electrons and using the energy

95
00:04:29,029 --> 00:04:27,060
released when those electrons move on to

96
00:04:32,330 --> 00:04:29,039
sulfates to power their growth

97
00:04:35,149 --> 00:04:32,340
metabolism and and it's and they're

98
00:04:36,439 --> 00:04:35,159
fairly diverse - I should say this is

99
00:04:38,390 --> 00:04:36,449
the net equation

100
00:04:40,219 --> 00:04:38,400
that we could write for this process we

101
00:04:41,869 --> 00:04:40,229
could calculate the energy of it think

102
00:04:43,730 --> 00:04:41,879
about the the cellular yields but

103
00:04:46,070 --> 00:04:43,740
they're they're quite diverse group of

104
00:04:49,610 --> 00:04:46,080
organisms that are all breathing or

105
00:04:51,950 --> 00:04:49,620
respiring sulfate and as I said there's

106
00:04:55,700 --> 00:04:51,960
a long tradition in geochemistry of

107
00:04:57,469 --> 00:04:55,710
measuring stable isotopes of sulfur

108
00:04:59,629 --> 00:04:57,479
through time and also in different

109
00:05:02,899 --> 00:04:59,639
environments and so what I'm showing

110
00:05:07,100 --> 00:05:02,909
here is a compilation of sulfur isotopes

111
00:05:09,709 --> 00:05:07,110
between sulfates and sulfide and we're

112
00:05:14,929 --> 00:05:09,719
looking at the two isotopes of sulfate

113
00:05:18,189 --> 00:05:14,939

of sulfur 32 and sulfur 34 and what is

114
00:05:21,829 --> 00:05:18,199
being shown here is in this gray area

115
00:05:26,209 --> 00:05:21,839
the equilibrium fractionation values of

116
00:05:28,010 --> 00:05:26,219
sulfate 2 sulfide for zero degrees

117
00:05:30,409 --> 00:05:28,020
Celsius all the way up to 40 degrees

118
00:05:32,719 --> 00:05:30,419
Celsius so the temperature at which

119
00:05:35,570 --> 00:05:32,729
sulfur isotopes will équilibre between

120
00:05:37,999 --> 00:05:35,580
sulfate and sulfide will affect the

121
00:05:40,249 --> 00:05:38,009
final value of the system and then we

122
00:05:42,529 --> 00:05:40,259
have all of this other stuff out here

123
00:05:45,290 --> 00:05:42,539
that I labeled kinetic this is the stuff

124
00:05:48,230 --> 00:05:45,300
that life does and so life is able to

125
00:05:51,260 --> 00:05:48,240
operate its metabolism in a way that's

126
00:05:53,600 --> 00:05:51,270
fast enough that sulfide is produced so

127
00:05:55,610 --> 00:05:53,610
fast that the isotopes aren't able to

128
00:05:58,519 --> 00:05:55,620
equilibrating so we get these deviations

129
00:06:01,610 --> 00:05:58,529
from equilibrated sulfur isotopes and

130
00:06:04,399 --> 00:06:01,620
these kinetic fractionation factors have

131
00:06:06,499 --> 00:06:04,409
been thought to be related to all sorts

132
00:06:08,989 --> 00:06:06,509
of stuff the rate of the organisms

133
00:06:11,360 --> 00:06:08,999
growth the place that they grow are they

134
00:06:12,860 --> 00:06:11,370
marine are they terrestrial are they

135
00:06:15,379 --> 00:06:12,870
pure culture are they are they mixed

136
00:06:17,540 --> 00:06:15,389
culture and it still remains an issue to

137
00:06:19,689 --> 00:06:17,550
define exactly which parameters are

138
00:06:24,379 --> 00:06:19,699

affecting the kinetic isotope

139
00:06:27,619 --> 00:06:24,389
fractionation values that life the

140
00:06:30,379 --> 00:06:27,629
result of biologic biology and why and

141
00:06:33,429 --> 00:06:30,389
which magnitude these isotopes are

142
00:06:36,949 --> 00:06:33,439
deviating away from the equilibrium if

143
00:06:39,829 --> 00:06:36,959
we spread those data along axis of time

144
00:06:43,239 --> 00:06:39,839
and we think about long ago here and

145
00:06:46,480 --> 00:06:43,249
today here and we look at sulfide

146
00:06:48,649 --> 00:06:46,490
isotopes compared to sulfate isotopes

147
00:06:50,430 --> 00:06:48,659
sulfide in the form of pyrite that's

148
00:06:51,870 --> 00:06:50,440
preserved we can see that

149
00:06:56,360 --> 00:06:51,880
in modern-day environments there's a

150
00:06:59,670 --> 00:06:56,370
huge spread of sulphate sulfide isotope

151
00:07:00,990 --> 00:06:59,680
fractionation and then in the past we

152
00:07:02,550 --> 00:07:01,000
have this problem that there's not a lot

153
00:07:05,940 --> 00:07:02,560
of data because there's not a lot of old

154
00:07:08,640 --> 00:07:05,950
material on the earth but the kind of

155
00:07:11,210 --> 00:07:08,650
variance here collapses and what I've

156
00:07:13,560 --> 00:07:11,220
marked on here is this 20 per mil

157
00:07:15,960 --> 00:07:13,570
fractionation value between sulfate and

158
00:07:17,460 --> 00:07:15,970
sulfide and so I'm going to ask the I'm

159
00:07:21,210 --> 00:07:17,470
going to try to address the question or

160
00:07:23,910 --> 00:07:21,220
speculate on why this 20% difference

161
00:07:25,650 --> 00:07:23,920
might be in the Archaean but in the in

162
00:07:28,260 --> 00:07:25,660
the present-day environment we get these

163
00:07:33,150 --> 00:07:28,270

very very large fracture nations in

164
00:07:36,450 --> 00:07:33,160
biology so what's going on in this

165
00:07:38,760 --> 00:07:36,460
process of sulfate reduction sulfate I

166
00:07:41,070 --> 00:07:38,770
mentioned before it's fairly oxidizing

167
00:07:43,860 --> 00:07:41,080
molecule you can put electrons on to it

168
00:07:45,810 --> 00:07:43,870
and when energy is released as electrons

169
00:07:47,670 --> 00:07:45,820
go on to sulfate gained energy from that

170
00:07:50,580 --> 00:07:47,680
process but it's actually chemically

171
00:07:53,370 --> 00:07:50,590
inert and what organisms have to do is

172
00:07:57,000 --> 00:07:53,380
they have to activate sulfate by using

173
00:07:59,700 --> 00:07:57,010
an ATP and in this activated form they

174
00:08:02,460 --> 00:07:59,710
are able to put first two electrons onto

175
00:08:06,060 --> 00:08:02,470
sulfate to make sulfite and then there's

176
00:08:08,790 --> 00:08:06,070
a six electron reduction of sulfite to

177
00:08:12,210 --> 00:08:08,800
make sulfide so this is the pathway or a

178
00:08:14,310 --> 00:08:12,220
very simplified version of microbial

179
00:08:16,470 --> 00:08:14,320
sulfate reduction the process of

180
00:08:18,690 --> 00:08:16,480
breathing on sulfate step one is to

181
00:08:20,640 --> 00:08:18,700
overcome this kinetic barrier and kind

182
00:08:23,100 --> 00:08:20,650
of power through that by using an ATP

183
00:08:25,230 --> 00:08:23,110
and the second step is a first two

184
00:08:27,810 --> 00:08:25,240
electron reduction and the third step is

185
00:08:31,710 --> 00:08:27,820
this pretty awesome I would I think six

186
00:08:34,980 --> 00:08:31,720
electron reduction of sulfite and for a

187
00:08:37,380 --> 00:08:34,990
long time people have recognized that

188
00:08:40,650 --> 00:08:37,390

there's a a pretty neat relationship

189
00:08:43,589 --> 00:08:40,660
between the ability or the extent of

190
00:08:46,950 --> 00:08:43,599
fractionation of sulfate sulfide

191
00:08:48,990 --> 00:08:46,960
isotopes proportional to the rate that

192
00:08:50,760 --> 00:08:49,000
these cells are growing so let's think

193
00:08:52,230 --> 00:08:50,770
let's explore this diagram a little bit

194
00:08:54,540 --> 00:08:52,240
it's a simple curve but it's a little

195
00:08:57,480 --> 00:08:54,550
bit tricky to think about I said before

196
00:09:00,870 --> 00:08:57,490
let me go back that the equilibrium

197
00:09:04,199 --> 00:09:00,880
value is essentially set by temperature

198
00:09:06,480 --> 00:09:04,209
over here and C's high values over

199
00:09:09,299 --> 00:09:06,490
here this is around 60 per mill 80 per

200
00:09:10,859 --> 00:09:09,309
mill here and what biology is doing is

201
00:09:14,309 --> 00:09:10,869
spreading the data away from that

202
00:09:16,379 --> 00:09:14,319
equilibrium fractionation this is

203
00:09:18,239 --> 00:09:16,389
another way of drawing that but it's a

204
00:09:21,239 --> 00:09:18,249
way of drying it that emphasizes a rate

205
00:09:23,639 --> 00:09:21,249
dependence on the process here we're

206
00:09:24,989 --> 00:09:23,649
talking about fractionation factors in

207
00:09:26,850 --> 00:09:24,999
an inverse way so this is a little bit

208
00:09:28,679 --> 00:09:26,860
confusing but we could translate this

209
00:09:30,389 --> 00:09:28,689
number here to about a sixty per mil

210
00:09:33,720 --> 00:09:30,399
fractionation that's that equilibrium

211
00:09:36,509 --> 00:09:33,730
value that would be met as the rate goes

212
00:09:38,579 --> 00:09:36,519
to zero we would encounter the y-axis

213
00:09:40,470 --> 00:09:38,589

here and that would happen depending on

214
00:09:43,290 --> 00:09:40,480
the temperature right around 60 or 280

215
00:09:46,400 --> 00:09:43,300
per ml fractionation but as cells grow

216
00:09:49,109 --> 00:09:46,410
faster and faster the fractionation

217
00:09:51,329 --> 00:09:49,119
changes it's kind of muted away from

218
00:09:54,059 --> 00:09:51,339
that equilibrium value this is an old

219
00:09:56,189 --> 00:09:54,069
observation that has been reproduced

220
00:09:58,739 --> 00:09:56,199
again and again historically about every

221
00:10:01,319 --> 00:09:58,749
10 years actually another group is able

222
00:10:02,790 --> 00:10:01,329
to reproduce this curve and it leads to

223
00:10:06,840 --> 00:10:02,800
this question of like why does why does

224
00:10:07,650 --> 00:10:06,850
this happen and a couple years ago I

225
00:10:10,530 --> 00:10:07,660
think there was a pretty nice

226
00:10:12,059 --> 00:10:10,540
breakthrough in my mind with trying to

227
00:10:14,929 --> 00:10:12,069
understand the shape of this curve and

228
00:10:17,579 --> 00:10:14,939
that breakthrough was accomplished in a

229
00:10:19,730 --> 00:10:17,589
model that related the rate of the

230
00:10:22,109 --> 00:10:19,740
process the energy of the process and

231
00:10:23,549 --> 00:10:22,119
knowledge of the fractionation factors

232
00:10:25,590 --> 00:10:23,559
of these enzymes or the inferred

233
00:10:28,230 --> 00:10:25,600
fractionation factors of these enzymes

234
00:10:30,509 --> 00:10:28,240
and really this is coming out of some

235
00:10:32,429 --> 00:10:30,519
work a while ago by Clinton stoner

236
00:10:35,340 --> 00:10:32,439
and which was later on followed up by

237
00:10:37,699 --> 00:10:35,350
Dan beard and honking I don't know how

238
00:10:42,799 --> 00:10:37,709

to say that name I shouldn't try sorry

239
00:10:45,689 --> 00:10:42,809
these folks of trying to relate

240
00:10:49,410 --> 00:10:45,699
reversibility of a chemical process to

241
00:10:51,359 --> 00:10:49,420
isotope BAC flux and the the force or

242
00:10:56,009 --> 00:10:51,369
the chemical potential of a given

243
00:10:58,289 --> 00:10:56,019
reaction and so this knowledge of being

244
00:11:00,660 --> 00:10:58,299
able to relate the chemical potential of

245
00:11:02,999 --> 00:11:00,670
a reaction to the BAC flux which is

246
00:11:06,720 --> 00:11:03,009
related to isotope exchange was employed

247
00:11:09,509 --> 00:11:06,730
by Baz weighing and eat I have Lee in a

248
00:11:11,249 --> 00:11:09,519
2014 paper were they yours

249
00:11:13,499 --> 00:11:11,259
they were able to put a line on this

250
00:11:15,539 --> 00:11:13,509
data by building a model and so they

251
00:11:17,669 --> 00:11:15,549
could say okay now we have a model that

252
00:11:17,940 --> 00:11:17,679
describes the state of a cell we know

253
00:11:19,920 --> 00:11:17,950
that

254
00:11:21,810 --> 00:11:19,930
parameters of the model and we can we

255
00:11:24,350 --> 00:11:21,820
can make a line that actually fits the

256
00:11:26,960 --> 00:11:24,360
data and so this is very very satisfying

257
00:11:29,970 --> 00:11:26,970
because what they were able to do is

258
00:11:32,160 --> 00:11:29,980
infer the processes that are occurring

259
00:11:34,860 --> 00:11:32,170
inside of a cell here's a cell that's

260
00:11:37,110 --> 00:11:34,870
doing microbial sulfate reduction and

261
00:11:39,270 --> 00:11:37,120
they broke the process apart and put

262
00:11:41,430 --> 00:11:39,280
those components into their model which

263
00:11:44,610 --> 00:11:41,440

is relying on knowledge of rate and

264
00:11:47,310 --> 00:11:44,620
isotope fractionation to say when

265
00:11:48,990 --> 00:11:47,320
sulfate is imported into the cell there

266
00:11:51,510 --> 00:11:49,000
might be a fractionation that occurs and

267
00:11:54,390 --> 00:11:51,520
then here's our activation step this is

268
00:11:57,330 --> 00:11:54,400
to get get rid of this kinetic problem

269
00:12:00,870 --> 00:11:57,340
here's the this third step the first two

270
00:12:03,060 --> 00:12:00,880
electron reduction of of the sulfur atom

271
00:12:05,130 --> 00:12:03,070
and then there's this final step there's

272
00:12:09,720 --> 00:12:05,140
six electron reduction so this seems

273
00:12:11,070 --> 00:12:09,730
like a satisfying way of looking at

274
00:12:15,750 --> 00:12:11,080
things to be able to understand the

275
00:12:17,430 --> 00:12:15,760
shape of that curve but it left us still

276
00:12:19,740 --> 00:12:17,440
with a little bit of a missing some

277
00:12:20,760 --> 00:12:19,750
missing data we could still take the

278
00:12:23,580 --> 00:12:20,770
model and come up with different

279
00:12:26,040 --> 00:12:23,590
scenarios to accomplish the same isotope

280
00:12:29,000 --> 00:12:26,050
fractionation another this is a common

281
00:12:31,140 --> 00:12:29,010
process in science many hypotheses can

282
00:12:33,330 --> 00:12:31,150
accomplice aim data set so there's a

283
00:12:36,900 --> 00:12:33,340
degeneracy built into this model and

284
00:12:39,930 --> 00:12:36,910
that degeneracy I'm seeking to get rid

285
00:12:42,780 --> 00:12:39,940
of by trying to find out two pieces of

286
00:12:45,270 --> 00:12:42,790
knowledge that have not really been

287
00:12:47,280 --> 00:12:45,280
described one what are the actual

288
00:12:48,720 --> 00:12:47,290

fractionation factors that are happening

289
00:12:50,490 --> 00:12:48,730
at the different steps in the metabolism

290
00:12:53,070 --> 00:12:50,500
these have never been determined

291
00:12:57,300 --> 00:12:53,080
empirically and instead they've just

292
00:12:58,680 --> 00:12:57,310
been estimated numerically and also what

293
00:13:00,450 --> 00:12:58,690
is the rate determining step of the

294
00:13:02,280 --> 00:13:00,460
metabolism knowledge of the rate

295
00:13:03,930 --> 00:13:02,290
determining step of metabolism is very

296
00:13:05,190 --> 00:13:03,940
very important in this case because

297
00:13:07,620 --> 00:13:05,200
that's going to be the bottleneck that

298
00:13:09,780 --> 00:13:07,630
will be expressed that'll be the

299
00:13:12,420 --> 00:13:09,790
fractionation factor that we actually

300
00:13:14,750 --> 00:13:12,430
measure later on it will be dependent on

301
00:13:18,060 --> 00:13:14,760
that rate determining determining step

302
00:13:22,050 --> 00:13:18,070
so to try to determine what that rate

303
00:13:24,780 --> 00:13:22,060
limiting step is or in part to do that

304
00:13:26,220 --> 00:13:24,790
min sub was actually able to make some

305
00:13:28,710 --> 00:13:26,230
measurements of intracellular

306
00:13:31,079 --> 00:13:28,720
concentrations of these sulfur and

307
00:13:33,179 --> 00:13:31,089
metabolites in the cell and

308
00:13:35,309 --> 00:13:33,189
here's a complicated graph and min sub

309
00:13:37,829 --> 00:13:35,319
uses kind of funny symbols so be careful

310
00:13:39,689 --> 00:13:37,839
here here is cell growth here we're

311
00:13:41,669 --> 00:13:39,699
monitoring the production of sulfide

312
00:13:45,289 --> 00:13:41,679
it's the respiratory product of the cell

313
00:13:47,399 --> 00:13:45,299

so the cells grow sulfate Goes Down and

314
00:13:49,349 --> 00:13:47,409
sulfates inside of the cell kind of

315
00:13:51,359 --> 00:13:49,359
bounces around in concentration it's not

316
00:13:52,739 --> 00:13:51,369
clear really what it's related to up

317
00:13:55,529 --> 00:13:52,749
here but it's pretty high in the cell

318
00:13:56,729 --> 00:13:55,539
then here's the concentration of ApS in

319
00:13:58,589 --> 00:13:56,739
the cell this is the kinetically

320
00:14:00,479 --> 00:13:58,599
activated sulfur compound that those

321
00:14:02,549 --> 00:14:00,489
first two electrons will be put onto and

322
00:14:05,399 --> 00:14:02,559
then here's the sulfite this is the

323
00:14:07,799 --> 00:14:05,409
compound that has six electrons added on

324
00:14:09,779 --> 00:14:07,809
to it and what min sub found was that

325
00:14:13,919 --> 00:14:09,789
basically that ApS is always at a higher

326
00:14:15,649 --> 00:14:13,929
concentration than sulfite and so one

327
00:14:18,659 --> 00:14:15,659
thing this this implies is that

328
00:14:20,729 --> 00:14:18,669
kinetically this this aps reduction step

329
00:14:22,859 --> 00:14:20,739
is a little bit slower than the sulfite

330
00:14:24,419 --> 00:14:22,869
step and once sulphide is produced the

331
00:14:27,299 --> 00:14:24,429
final six electrons go right onto

332
00:14:28,979 --> 00:14:27,309
sulfite making sulfide and this this

333
00:14:31,979 --> 00:14:28,989
seems fairly comfortable chemically

334
00:14:34,739 --> 00:14:31,989
sulfites pretty pretty reactive and so

335
00:14:36,599 --> 00:14:34,749
it kind of goes to this the suggestion

336
00:14:38,969 --> 00:14:36,609
that it may be this ApS step is rate

337
00:14:40,919 --> 00:14:38,979
limiting if that's the case this ApS

338
00:14:42,659 --> 00:14:40,929

step will be very important in

339
00:14:44,609 --> 00:14:42,669
controlling the observed sulphur isotope

340
00:14:47,669 --> 00:14:44,619
fraction fractionation factor of the

341
00:14:50,909 --> 00:14:47,679
metabolism and so we could say that

342
00:14:52,349 --> 00:14:50,919
possibly this rate determining step or

343
00:14:54,359 --> 00:14:52,359
at least in some cellular conditions

344
00:14:57,119 --> 00:14:54,369
would be that first two electron

345
00:15:00,059 --> 00:14:57,129
reduction of ApS and that leaves us with

346
00:15:02,129 --> 00:15:00,069
a second part missing part of the data

347
00:15:06,209 --> 00:15:02,139
here is the actual numbers that are

348
00:15:08,369 --> 00:15:06,219
associated with these enzymes as an

349
00:15:10,169 --> 00:15:08,379
individual catalyst what is the isotopes

350
00:15:13,109 --> 00:15:10,179
that stable isotope fractionation of

351
00:15:17,069 --> 00:15:13,119
this catalyst so we we sought to address

352
00:15:21,479 --> 00:15:17,079
that question by purifying the enzyme

353
00:15:23,159 --> 00:15:21,489
and a say in it using isotope ratio a

354
00:15:24,809 --> 00:15:23,169
mass spectrometry of the substrates and

355
00:15:27,089 --> 00:15:24,819
products to determine that value so

356
00:15:28,979 --> 00:15:27,099
here's the enzyme remember it takes this

357
00:15:30,899 --> 00:15:28,989
kinetically activated form of sulfate

358
00:15:34,139 --> 00:15:30,909
and adds two electrons on to it to make

359
00:15:36,389 --> 00:15:34,149
sulfite here are some other iron sulfur

360
00:15:38,099 --> 00:15:36,399
clusters that are found in biology this

361
00:15:40,199 --> 00:15:38,109
time these iron sulfur clusters are

362
00:15:43,039 --> 00:15:40,209
relatively high potential they're not

363
00:15:44,730 --> 00:15:43,049

sitting at minus 500 millivolts

364
00:15:48,210 --> 00:15:44,740
ferredoxin or more

365
00:15:49,980 --> 00:15:48,220
than that and that's because sulfate is

366
00:15:51,720 --> 00:15:49,990
more oxidizing than that you could

367
00:15:53,579 --> 00:15:51,730
imagine electrons coming in to these

368
00:15:55,560 --> 00:15:53,589
four iron four sulfur Q Bane's and then

369
00:15:58,079 --> 00:15:55,570
being transferred on to this fa D which

370
00:16:01,380 --> 00:15:58,089
is hiding up here in yellow and then at

371
00:16:02,460 --> 00:16:01,390
that stage sulfite is going to dock

372
00:16:04,199 --> 00:16:02,470
somewhere around here

373
00:16:06,210 --> 00:16:04,209
we're sorry the APS is going to dock

374
00:16:12,930 --> 00:16:06,220
around here and sulfite will be produced

375
00:16:16,070 --> 00:16:12,940
so he D akio gotta who was previously at

376
00:16:20,639 --> 00:16:16,080
the NPI for chemical energy conversion

377
00:16:23,190 --> 00:16:20,649
he grows you know 200 liter vats of this

378
00:16:25,440 --> 00:16:23,200
organism dissolve of Vibrio Voges and so

379
00:16:27,660 --> 00:16:25,450
he was able to donate some of this

380
00:16:29,340 --> 00:16:27,670
protein material to the laboratory and

381
00:16:32,670 --> 00:16:29,350
save us a lot of work he did a partial

382
00:16:36,510 --> 00:16:32,680
purification of the APR a and B sub

383
00:16:39,600 --> 00:16:36,520
units and sent them to us at which time

384
00:16:43,170 --> 00:16:39,610
min sub was able to conduct protein

385
00:16:46,860 --> 00:16:43,180
assays and what he did was he put the

386
00:16:49,170 --> 00:16:46,870
substrate of the enzyme the enzyme and

387
00:16:51,930 --> 00:16:49,180
an artificial electron donor into a tube

388
00:16:53,940 --> 00:16:51,940

in an anaerobic environment and then he

389
00:16:56,760 --> 00:16:53,950
was able to use ion chromatography to

390
00:16:58,500 --> 00:16:56,770
separate out the products and the

391
00:17:01,079 --> 00:16:58,510
substrate of this reaction so in this

392
00:17:03,060 --> 00:17:01,089
case he's going to collect a PS the

393
00:17:05,069 --> 00:17:03,070
substrate of the reaction and sulfite

394
00:17:09,540 --> 00:17:05,079
the product of the reaction and then

395
00:17:11,850 --> 00:17:09,550
later on take those molecules and launch

396
00:17:14,689 --> 00:17:11,860
them into the Neptune instrument and

397
00:17:18,329 --> 00:17:14,699
we'll be able to determine the 3234

398
00:17:20,579 --> 00:17:18,339
isotope values in a compound specific

399
00:17:23,699 --> 00:17:20,589
way and be able to understand isotope

400
00:17:25,910 --> 00:17:23,709
fractionation value of the enzyme here

401
00:17:29,010 --> 00:17:25,920
are the enzyme kinetics at two different

402
00:17:31,560 --> 00:17:29,020
temperatures and so what min sub was

403
00:17:34,200 --> 00:17:31,570
able to show was that cold slows the

404
00:17:37,040 --> 00:17:34,210
enzyme down and heat speeds it up so

405
00:17:39,930 --> 00:17:37,050
change of 12 degrees changed the rate of

406
00:17:42,450 --> 00:17:39,940
sulfite production by around five times

407
00:17:45,419 --> 00:17:42,460
a pretty dramatic change in the rate of

408
00:17:51,270 --> 00:17:45,429
the enzyme and he was able to monitor

409
00:17:54,000 --> 00:17:51,280
the sulfur isotope compound value for

410
00:17:55,770 --> 00:17:54,010
aps the substrate of the reaction and

411
00:17:57,900 --> 00:17:55,780
the product of the reaction and then

412
00:17:58,649 --> 00:17:57,910
make these basically Rayleigh

413
00:18:01,200 --> 00:17:58,659

fractionation

414
00:18:06,089 --> 00:18:01,210
and curves where the slope of this line

415
00:18:07,680 --> 00:18:06,099
is indicative of the 3432 sulphur

416
00:18:10,710 --> 00:18:07,690
isotope difference between the product

417
00:18:12,359 --> 00:18:10,720
and the reaction reactant here I said

418
00:18:16,379 --> 00:18:12,369
before that the rate of the enzyme here

419
00:18:19,229 --> 00:18:16,389
is 5 times faster than the cool 20

420
00:18:21,029 --> 00:18:19,239
degree assay version here but something

421
00:18:22,830 --> 00:18:21,039
interesting the isotope fractionation

422
00:18:27,080 --> 00:18:22,840
factor is basically the same between

423
00:18:30,109 --> 00:18:27,090
these two cases and so with this in hand

424
00:18:33,629 --> 00:18:30,119
we have a model that describes

425
00:18:35,849 --> 00:18:33,639
mathematically how isotopes will will be

426
00:18:39,299 --> 00:18:35,859
partitioned from sulfate to sulfide

427
00:18:40,499 --> 00:18:39,309
under various cellular states and now we

428
00:18:43,049 --> 00:18:40,509
have a real number

429
00:18:46,619 --> 00:18:43,059
now the first number of a purified

430
00:18:53,070 --> 00:18:46,629
enzyme I should qualify that statement

431
00:18:54,629 --> 00:18:53,080
by saying entirely pure non missing

432
00:18:56,729 --> 00:18:54,639
subunit version of the enzyme here

433
00:18:58,529 --> 00:18:56,739
previously about two years ago

434
00:18:59,909 --> 00:18:58,539
another group measured an enzyme but it

435
00:19:02,759 --> 00:18:59,919
was missing a subunit and so this was

436
00:19:04,859 --> 00:19:02,769
actually the first purified complete

437
00:19:07,619 --> 00:19:04,869
enzyme component for which sulfur

438
00:19:10,680 --> 00:19:07,629

isotope values have been measured and we

439
00:19:13,409 --> 00:19:10,690
also have this data before which is

440
00:19:16,469 --> 00:19:13,419
suggestive of this rate limiting step of

441
00:19:18,330 --> 00:19:16,479
this enzymes enzyme here and so what we

442
00:19:22,289 --> 00:19:18,340
did is we took this previously

443
00:19:24,629 --> 00:19:22,299
constructed model and we started

444
00:19:27,269 --> 00:19:24,639
plotting expected isotope fractionation

445
00:19:29,159 --> 00:19:27,279
values of the cells for different

446
00:19:30,839 --> 00:19:29,169
chemical conditions different chemical

447
00:19:33,930 --> 00:19:30,849
conditions in terms of the driving force

448
00:19:36,239 --> 00:19:33,940
of the metabolic reaction so if the

449
00:19:38,820 --> 00:19:36,249
cells have lots of energy that would be

450
00:19:40,889 --> 00:19:38,830
sitting over here at these more negative

451
00:19:43,229 --> 00:19:40,899
redox potentials and if the cells have

452
00:19:45,749 --> 00:19:43,239
less energy they would be using more

453
00:19:47,519 --> 00:19:45,759
oxidizing electron donors or higher

454
00:19:49,889 --> 00:19:47,529
potential electron donors to review

455
00:19:52,349 --> 00:19:49,899
sulfate and they would have less driving

456
00:19:53,969 --> 00:19:52,359
force behind their reaction and what we

457
00:19:56,399 --> 00:19:53,979
found when we started plot in the data

458
00:19:59,009 --> 00:19:56,409
like this in this model was that

459
00:20:01,739 --> 00:19:59,019
actually the observed isotope

460
00:20:03,989 --> 00:20:01,749
fractionation of the cell does not ever

461
00:20:07,829 --> 00:20:03,999
go over the isotope fractionation of

462
00:20:09,930 --> 00:20:07,839
this one single enzyme until that enzyme

463
00:20:11,430 --> 00:20:09,940

step becomes reversible and so this is

464
00:20:14,799 --> 00:20:11,440
this BAC flux

465
00:20:17,259 --> 00:20:14,809
a contribution here when there's not a

466
00:20:19,899 --> 00:20:17,269
sufficient amount of energy to push that

467
00:20:22,930 --> 00:20:19,909
reaction forward the isotopes can react

468
00:20:24,909 --> 00:20:22,940
Willa Breit across between APs and

469
00:20:27,729 --> 00:20:24,919
sulfite and that's what leads to these

470
00:20:30,279 --> 00:20:27,739
large close to equilibrium fraction

471
00:20:33,129 --> 00:20:30,289
nations and we're able to do that for a

472
00:20:35,379 --> 00:20:33,139
number of different rates of microbial

473
00:20:36,639 --> 00:20:35,389
sulfate reduction and you can see when

474
00:20:38,769 --> 00:20:36,649
cells are really cranking through

475
00:20:41,229 --> 00:20:38,779
sulfate and making sulfide that kind of

476
00:20:43,449 --> 00:20:41,239
mutes everything they're pulling the

477
00:20:45,909 --> 00:20:43,459
sulfate through their metabolism so fast

478
00:20:48,639 --> 00:20:45,919
that it doesn't ever have time to

479
00:20:51,669 --> 00:20:48,649
approach these equilibrium values but at

480
00:20:53,469 --> 00:20:51,679
these slower rates again the situation

481
00:20:56,829 --> 00:20:53,479
as long as the cells have sufficient

482
00:20:59,049 --> 00:20:56,839
electron driving force the sulfur

483
00:21:01,810 --> 00:20:59,059
isotopes can't really ever go above this

484
00:21:05,560 --> 00:21:01,820
value which is set kinetically on this

485
00:21:07,839 --> 00:21:05,570
one particular enzyme so that's just

486
00:21:11,259 --> 00:21:07,849
what I said it can't increase more than

487
00:21:14,680 --> 00:21:11,269
the actual aps enzyme step when there's

488
00:21:16,180 --> 00:21:14,690

sufficient energy so that's kind of the

489
00:21:17,919 --> 00:21:16,190
nuts and bolts of the data that I want

490
00:21:20,049 --> 00:21:17,929
to present and I want to kind of

491
00:21:23,349 --> 00:21:20,059
hypothesize about the possible

492
00:21:26,709 --> 00:21:23,359
implications of this purified enzyme

493
00:21:28,680 --> 00:21:26,719
sulfur isotope values as it might have

494
00:21:30,729 --> 00:21:28,690
something to do with our historical

495
00:21:32,949 --> 00:21:30,739
interpretation of these isotope values

496
00:21:35,499 --> 00:21:32,959
and so we can ask this question why in

497
00:21:36,819 --> 00:21:35,509
the Archaean or do we not see close to

498
00:21:38,769 --> 00:21:36,829
equilibrium sulphur isotope

499
00:21:41,739 --> 00:21:38,779
fractionation values here we see these

500
00:21:43,239 --> 00:21:41,749
really this really big spread some of

501
00:21:45,849 --> 00:21:43,249
them are narrow some of but there's a

502
00:21:49,269 --> 00:21:45,859
lot of variance here and one thing we

503
00:21:51,129 --> 00:21:49,279
might say is that in the modern

504
00:21:53,799 --> 00:21:51,139
environment there there's a huge

505
00:21:56,440 --> 00:21:53,809
diversity of environments and but in

506
00:21:58,239 --> 00:21:56,450
particular these organisms are under a

507
00:22:00,940 --> 00:21:58,249
lot of competition and they might be

508
00:22:03,249 --> 00:22:00,950
using very modest electron donors and

509
00:22:05,109 --> 00:22:03,259
there's very modest electron donors put

510
00:22:06,789 --> 00:22:05,119
us over over here on the right side of

511
00:22:10,049 --> 00:22:06,799
our graph where the cells are growing

512
00:22:12,279 --> 00:22:10,059
closer to equilibrium and therefore

513
00:22:15,729 --> 00:22:12,289

relating these larger isotope

514
00:22:19,449 --> 00:22:15,739
fractionation values it's very tempting

515
00:22:21,729 --> 00:22:19,459
to speculate that a lot using the same

516
00:22:25,180 --> 00:22:21,739
similar line of thinking that in the

517
00:22:27,460 --> 00:22:25,190
Archaean the fact that we don't see

518
00:22:29,830 --> 00:22:27,470
large sulphur isotope fractionation x'

519
00:22:33,010 --> 00:22:29,840
might be coincident with the idea that

520
00:22:35,140 --> 00:22:33,020
these organisms were electron replete

521
00:22:37,300 --> 00:22:35,150
they had a lot of power to run their

522
00:22:42,940 --> 00:22:37,310
metabolism and they essentially weren't

523
00:22:44,740 --> 00:22:42,950
starving for electrons and to help help

524
00:22:47,260 --> 00:22:44,750
us kind of probe around this idea and

525
00:22:49,710 --> 00:22:47,270
interpret this idea we can think of what

526
00:22:52,300 --> 00:22:49,720
sulfate reduction might have meant

527
00:22:54,940 --> 00:22:52,310
evolutionarily if you were a microbe

528
00:22:57,580 --> 00:22:54,950
swimming in the ocean in the Archaean

529
00:22:59,680 --> 00:22:57,590
and you were using hydrogen if you were

530
00:23:01,840 --> 00:22:59,690
a sulfate reducer or methanogens or an

531
00:23:04,990 --> 00:23:01,850
acid again we could use the concept of

532
00:23:08,500 --> 00:23:05,000
the threshold concentration of hydrogen

533
00:23:11,110 --> 00:23:08,510
and the expected energy yield for the

534
00:23:14,080 --> 00:23:11,120
same concentration of hydrogen to either

535
00:23:15,700 --> 00:23:14,090
take electrons from hydrogen and reduce

536
00:23:17,710 --> 00:23:15,710
sulfate or take electrons from hydrogen

537
00:23:20,110 --> 00:23:17,720
and reduce co2 in either of these two

538
00:23:22,030 --> 00:23:20,120

different ways and because sulfate is

539
00:23:24,310 --> 00:23:22,040
such a good electron acceptor because as

540
00:23:27,130 --> 00:23:24,320
a more positive midpoint potential it's

541
00:23:28,990 --> 00:23:27,140
easier to reduce than co2 what sulphur

542
00:23:30,670 --> 00:23:29,000
sulfate reducers are able to do and

543
00:23:32,530 --> 00:23:30,680
people have known about this for a long

544
00:23:34,330 --> 00:23:32,540
time they're able to suck hydrogen

545
00:23:37,240 --> 00:23:34,340
concentrations down to a much lower

546
00:23:39,100 --> 00:23:37,250
level than either of these organisms I

547
00:23:41,350 --> 00:23:39,110
think a good way to interpret this is

548
00:23:43,690 --> 00:23:41,360
just to look at these Gibbs free energy

549
00:23:47,250 --> 00:23:43,700
of reactions for the electron addition

550
00:23:49,570 --> 00:23:47,260
on to either of these acceptors and so

551
00:23:52,270 --> 00:23:49,580
probably the evolution of sulfate

552
00:23:54,130 --> 00:23:52,280
reduction was a major bioenergetic

553
00:23:56,410 --> 00:23:54,140
innovation and what that allowed these

554
00:23:58,180 --> 00:23:56,420
organisms to do is in the case of using

555
00:24:00,670 --> 00:23:58,190
a common electron donor or was they were

556
00:24:02,350 --> 00:24:00,680
able to out-compete anything else that

557
00:24:05,140 --> 00:24:02,360
was living around they're putting

558
00:24:07,780 --> 00:24:05,150
electrons onto very meager electron

559
00:24:09,550 --> 00:24:07,790
acceptors like co2 and that would be

560
00:24:11,850 --> 00:24:09,560
kind of coincident coincident with this

561
00:24:15,310 --> 00:24:11,860
idea that they were running electron

562
00:24:17,470 --> 00:24:15,320
replete metabolisms after you know today

563
00:24:19,750 --> 00:24:17,480

these organisms are shoved into all

564
00:24:22,360 --> 00:24:19,760
sorts of different energy environments

565
00:24:25,660 --> 00:24:22,370
and they're living off of a lot of

566
00:24:27,430 --> 00:24:25,670
scraps where they they don't have a lot

567
00:24:29,710 --> 00:24:27,440
of driving force in their reaction and

568
00:24:31,750 --> 00:24:29,720
that might be one way to interpret this

569
00:24:33,760 --> 00:24:31,760
historical distribution of stable

570
00:24:35,350 --> 00:24:33,770
isotopes through time very replete

571
00:24:37,990 --> 00:24:35,360
conditions lots of electron donor

572
00:24:38,690 --> 00:24:38,000
they're competing against things with

573
00:24:40,730 --> 00:24:38,700
very

574
00:24:42,830 --> 00:24:40,740
artists electron acceptors but today

575
00:24:44,270 --> 00:24:42,840
there's a lot more competition there's a

576
00:24:50,060 --> 00:24:44,280
lot more high potential metabolisms

577
00:24:51,860 --> 00:24:50,070
available and I want to take this

578
00:24:53,690 --> 00:24:51,870
concept a little bit further and spit

579
00:24:56,540 --> 00:24:53,700
and use use our knowledge of isotopes

580
00:24:58,370 --> 00:24:56,550
here to speculate on processes of energy

581
00:25:01,280 --> 00:24:58,380
conservation that might be occurring in

582
00:25:03,560 --> 00:25:01,290
these cells I said earlier that these

583
00:25:06,460 --> 00:25:03,570
microbes are quite metabolically diverse

584
00:25:10,130 --> 00:25:06,470
and they are and in fact we don't have a

585
00:25:11,960 --> 00:25:10,140
unified understanding of how electron

586
00:25:13,550 --> 00:25:11,970
transfer processes are coupled to energy

587
00:25:16,340 --> 00:25:13,560
conservation in these cells or energy

588
00:25:18,200 --> 00:25:16,350

conversion if you will to say that

589
00:25:19,460 --> 00:25:18,210
simply we don't know which steps in

590
00:25:22,450 --> 00:25:19,470
these metabolisms are coupled to

591
00:25:25,400 --> 00:25:22,460
chemiosmotic potential generation and

592
00:25:27,020 --> 00:25:25,410
that will probably be variable for

593
00:25:29,000 --> 00:25:27,030
different types of cells but it's

594
00:25:30,800 --> 00:25:29,010
interesting for me to consider whether

595
00:25:33,440 --> 00:25:30,810
or not we could use isotopes as a way of

596
00:25:35,870 --> 00:25:33,450
rule of making hypotheses that are

597
00:25:37,490 --> 00:25:35,880
testable here so here's a picture of a

598
00:25:40,400 --> 00:25:37,500
cell that might be putting electrons

599
00:25:42,140 --> 00:25:40,410
onto sulfate pumping ions out in that

600
00:25:43,730 --> 00:25:42,150
process and then making ATP as those

601
00:25:46,610 --> 00:25:43,740
ions come back in across the potential

602
00:25:49,790 --> 00:25:46,620
and we could think about growing a

603
00:25:51,560 --> 00:25:49,800
sulphate reducer in a laboratory

604
00:25:53,570 --> 00:25:51,570
environment this is something that I

605
00:25:56,270 --> 00:25:53,580
would like to do or if you want to do it

606
00:25:58,190 --> 00:25:56,280
please talk to me and we can do it

607
00:25:59,780 --> 00:25:58,200
together you have some sulfate reducers

608
00:26:02,090 --> 00:25:59,790
growing at home but you could imagine

609
00:26:05,090 --> 00:26:02,100
growing sulfate reducers with a chemical

610
00:26:08,630 --> 00:26:05,100
potential for their metabolism and you

611
00:26:10,670 --> 00:26:08,640
could set them by modulating the

612
00:26:11,870 --> 00:26:10,680
concentrations and the temperature and

613
00:26:14,150 --> 00:26:11,880

you could grow them at this potential

614
00:26:17,000 --> 00:26:14,160
and you could say well if this organism

615
00:26:18,050 --> 00:26:17,010
was pushing against a membrane bound ion

616
00:26:19,430 --> 00:26:18,060
potential of a hundred and eighty

617
00:26:21,800 --> 00:26:19,440
millivolts that would translate into

618
00:26:23,800 --> 00:26:21,810
about 20 kilojoules per mole and then

619
00:26:27,320 --> 00:26:23,810
you would expect the cells to be able to

620
00:26:28,880 --> 00:26:27,330
gain or to have a free or remaining

621
00:26:30,920 --> 00:26:28,890
amount of energy of about 10 kilojoules

622
00:26:33,230 --> 00:26:30,930
per mole of reaction this would mean

623
00:26:35,120 --> 00:26:33,240
more reversible and more fractionation

624
00:26:37,310 --> 00:26:35,130
if it was coupled but if you took those

625
00:26:38,780 --> 00:26:37,320
same cells and they weren't coupled they

626
00:26:40,070 --> 00:26:38,790
weren't pushing against the ion

627
00:26:42,170 --> 00:26:40,080
potential they weren't using this to

628
00:26:44,150 --> 00:26:42,180
direct directly conserve energy you

629
00:26:47,420 --> 00:26:44,160
would get zero minus 30 so minus minus

630
00:26:50,420 --> 00:26:47,430
thirty kilovolts for a reaction and from

631
00:26:52,250 --> 00:26:50,430
a sulphur isotope perspective what this

632
00:26:54,020 --> 00:26:52,260
would mean would it be a

633
00:26:55,640 --> 00:26:54,030
where it's less reversible and less

634
00:26:57,470 --> 00:26:55,650
fractionation this is a testable

635
00:27:00,020 --> 00:26:57,480
hypothesis that we might be able to use

636
00:27:01,580 --> 00:27:00,030
isotope fractionation from whole cell

637
00:27:03,310 --> 00:27:01,590
environments to be able to find

638
00:27:09,200 --> 00:27:03,320

something out about their biophysical an

639
00:27:13,130 --> 00:27:09,210
energy transduction system so the next

640
00:27:15,860 --> 00:27:13,140
steps that were pretty excited to do now

641
00:27:18,020 --> 00:27:15,870
that we have one data point on one of

642
00:27:19,850 --> 00:27:18,030
these enzymes is to try to entertain

643
00:27:23,840 --> 00:27:19,860
this possibility of looking across the

644
00:27:25,220 --> 00:27:23,850
sequence phylogeny of these see these

645
00:27:26,840 --> 00:27:25,230
sequences are found in different

646
00:27:28,160 --> 00:27:26,850
organisms these different homologues and

647
00:27:30,440 --> 00:27:28,170
see if there's any evolutionary

648
00:27:32,480 --> 00:27:30,450
variation on this catalyst through time

649
00:27:34,490 --> 00:27:32,490
and I think that will be very very

650
00:27:36,820 --> 00:27:34,500
important for us to understand whether

651
00:27:39,590 --> 00:27:36,830
or not this hypothesis that I set up it

652
00:27:41,870 --> 00:27:39,600
could be true at all if there's a lot of

653
00:27:45,380 --> 00:27:41,880
isotope variability certainly we can't

654
00:27:49,220 --> 00:27:45,390
go and make claims about how this - 20

655
00:27:51,080 --> 00:27:49,230
per mil number has meaning in deep time

656
00:27:54,860 --> 00:27:51,090
instead we'll be left with a question of

657
00:27:57,350 --> 00:27:54,870
what are the ancestral state variants of

658
00:27:58,670 --> 00:27:57,360
these enzymes operating out in terms of

659
00:27:59,990 --> 00:27:58,680
their sulfur isotopes and so that's

660
00:28:02,720 --> 00:28:00,000
something that we'll do in the future is

661
00:28:04,880 --> 00:28:02,730
try to gain some evolutionary insight

662
00:28:06,500 --> 00:28:04,890
onto the history of sulfur isotope

663
00:28:09,350 --> 00:28:06,510

fractionation from the perspective of

664
00:28:11,210 --> 00:28:09,360
individual protein sequences and I think

665
00:28:12,620 --> 00:28:11,220
this will be a really nice way of us to

666
00:28:15,050 --> 00:28:12,630
try to bridge these two biological

667
00:28:16,970 --> 00:28:15,060
records that we have on the planet we

668
00:28:18,560 --> 00:28:16,980
have these material records such as the

669
00:28:21,260 --> 00:28:18,570
distribution of isotopes through time

670
00:28:23,030 --> 00:28:21,270
and we also have this molecular biology

671
00:28:24,290 --> 00:28:23,040
record and I think these isotopes might

672
00:28:28,250 --> 00:28:24,300
be a nice way to connect these two

673
00:28:30,050 --> 00:28:28,260
pieces of information together and the

674
00:28:31,760 --> 00:28:30,060
next place we're going with this in

675
00:28:34,130 --> 00:28:31,770
addition to that is to try to understand

676
00:28:36,470 --> 00:28:34,140
enzyme mechanism from the from these

677
00:28:38,090 --> 00:28:36,480
stable isotope fractionation x' this

678
00:28:42,080 --> 00:28:38,100
enzyme has a pretty cool proposed

679
00:28:43,820 --> 00:28:42,090
intermediate this is this is one of

680
00:28:45,200 --> 00:28:43,830
those intermediates in the proposed

681
00:28:49,370 --> 00:28:45,210
catalytic cycle and what you can see

682
00:28:51,260 --> 00:28:49,380
here is a fa d up here those four iron

683
00:28:53,510 --> 00:28:51,270
four sulfur clusters would be kind of

684
00:28:57,080 --> 00:28:53,520
off the stage floating up here electrons

685
00:28:58,970 --> 00:28:57,090
come down into the fa D and ApS is

686
00:29:02,030 --> 00:28:58,980
reduced leaving a covalently linked

687
00:29:03,560 --> 00:29:02,040
sulfite addict here so this is a pretty

688
00:29:04,480 --> 00:29:03,570

interesting proposed mechanism that

689
00:29:07,670 --> 00:29:04,490
hasn't received

690
00:29:09,650 --> 00:29:07,680
sufficient testing I don't think but one

691
00:29:12,620 --> 00:29:09,660
thing we're trying to do is use this

692
00:29:15,050 --> 00:29:12,630
proposed I this measured isotope value

693
00:29:17,240 --> 00:29:15,060
to test this proposed mechanism and I

694
00:29:21,470 --> 00:29:17,250
think that might be possible because the

695
00:29:23,060 --> 00:29:21,480
these ki es are kind of reports of the

696
00:29:24,440 --> 00:29:23,070
bond vibrations that can be expected in

697
00:29:25,430 --> 00:29:24,450
this type of environment so we might be

698
00:29:30,710 --> 00:29:25,440
able to approach that from a

699
00:29:32,210 --> 00:29:30,720
computational perspective in the

700
00:29:34,310 --> 00:29:32,220
beginning of the talk I said that this

701
00:29:38,210 --> 00:29:34,320
type of approach even though I'm going

702
00:29:42,230 --> 00:29:38,220
to talk about sulfate reduction it's

703
00:29:45,170 --> 00:29:42,240
it's very applicable to other microbial

704
00:29:48,260 --> 00:29:45,180
metabolisms we could look at some of

705
00:29:49,850 --> 00:29:48,270
Khmer Hasan's enzymes with a similar

706
00:29:51,650 --> 00:29:49,860
approach to look at the isotope

707
00:29:53,900 --> 00:29:51,660
fractionation of carboxylation or

708
00:29:59,150 --> 00:29:53,910
decarboxylation reactions we could also

709
00:30:00,860 --> 00:29:59,160
find interesting knowledge from testing

710
00:30:02,450 --> 00:30:00,870
other metabolisms like these apparently

711
00:30:04,340 --> 00:30:02,460
reverse metabolisms that I've put on

712
00:30:06,230 --> 00:30:04,350
here where one organism is putting

713
00:30:08,330 --> 00:30:06,240

electrons onto co2 to make methane and

714
00:30:11,990 --> 00:30:08,340
another one is taking electrons off off

715
00:30:13,730 --> 00:30:12,000
of methane to make co2 so I hope that

716
00:30:16,070 --> 00:30:13,740
this this way of determining enzyme

717
00:30:17,750 --> 00:30:16,080
specific apparent kinetic isotope

718
00:30:20,620 --> 00:30:17,760
fractionation factors will help us

719
00:30:22,280 --> 00:30:20,630
understand a lot of metabolisms and how

720
00:30:26,000 --> 00:30:22,290
sequences evolved through time

721
00:30:28,100 --> 00:30:26,010
catalytically and then when I was

722
00:30:31,040 --> 00:30:28,110
watching George's talk it occurred to me

723
00:30:31,430 --> 00:30:31,050
you know really enforced that if we're

724
00:30:33,380 --> 00:30:31,440
careful

725
00:30:35,690 --> 00:30:33,390
we will also be able to look at kind of

726
00:30:39,200 --> 00:30:35,700
these proto metabolic networks and if we

727
00:30:41,270 --> 00:30:39,210
can start measuring catalytic or rates

728
00:30:43,640 --> 00:30:41,280
rates of these types of reactions by

729
00:30:45,590 --> 00:30:43,650
different catalysts we'll be able to

730
00:30:48,260 --> 00:30:45,600
start to use these types types of

731
00:30:50,770 --> 00:30:48,270
isotope measurements to understand in

732
00:30:55,610 --> 00:30:50,780
more detail these non-biological

733
00:30:56,990 --> 00:30:55,620
reaction networks and so that's that's

734
00:30:58,540 --> 00:30:57,000
where I'm gonna stop with my talk and

735
00:31:04,010 --> 00:30:58,550
I'm gonna open it up for questions there

736
00:31:07,730 --> 00:31:04,020
and I just want to mention that this

737
00:31:13,090 --> 00:31:07,740
last year me batula Kochhar Daniel C

738
00:31:16,160 --> 00:31:13,100

gray Vaz wing Chris Bush and Chris house

739
00:31:17,980 --> 00:31:16,170
we were we started a proposal to look at

740
00:31:19,480 --> 00:31:17,990
ancient thio ester

741
00:31:22,450 --> 00:31:19,490
chemistry from a number of different

742
00:31:26,370 --> 00:31:22,460
perspectives one of them involves

743
00:31:30,610 --> 00:31:26,380
looking at promiscuity of thio ester

744
00:31:34,810 --> 00:31:33,430
how are you ki Tomi gave a really really

745
00:31:36,430 --> 00:31:34,820
fascinating talk a couple of days ago

746
00:31:38,830 --> 00:31:36,440
when he was talking and he mentioned

747
00:31:46,090 --> 00:31:38,840
that some enzymes are able to use an

748
00:31:49,600 --> 00:31:46,100
acetyl 2 amino ethyle I got it

749
00:31:51,010 --> 00:31:49,610
instead of acetyl co a as a cofactor and

750
00:31:53,140 --> 00:31:51,020
I think that is pretty interesting from

751
00:31:55,270 --> 00:31:53,150
an evolutionary perspective but we're

752
00:31:57,669 --> 00:31:55,280
also interested in making carbon and

753
00:31:59,410 --> 00:31:57,679
sulfur isotope measurements of these

754
00:32:01,000 --> 00:31:59,420
enzymes and trying to find out

755
00:32:03,970 --> 00:32:01,010
in a similar vein as what I presented

756
00:32:06,640 --> 00:32:03,980
here today how these isotope values may

757
00:32:09,400 --> 00:32:06,650
change through time or not and we're

758
00:32:13,060 --> 00:32:09,410
also interested in in trying to

759
00:32:14,860 --> 00:32:13,070
constrain how much thioester metabolism

760
00:32:19,090 --> 00:32:14,870
might be possible in a metabolic Network

761
00:32:21,190 --> 00:32:19,100
today or in the past so with that I'd

762
00:32:24,940 --> 00:32:21,200
like to thanks thank everybody for your

763
00:32:26,440 --> 00:32:24,950

attention and give a special extra

764
00:32:28,810 --> 00:32:26,450
acknowledgement to the funding that was

765
00:32:32,440 --> 00:32:28,820
supporting this process this progress

766
00:32:34,930 --> 00:32:32,450
and which was provided by NASA and also

767
00:32:41,639 --> 00:32:34,940
more recently from the WPI program here

768
00:32:59,169 --> 00:32:57,759
okay now the time for question so coming

769
00:33:01,810 --> 00:32:59,179
back to one of the themes of the meeting

770
00:33:03,610 --> 00:33:01,820
that there's always a lot of chemical

771
00:33:05,649 --> 00:33:03,620
stuff going on but maybe some of it

772
00:33:10,990 --> 00:33:05,659
matters and some of it is just kind of

773
00:33:12,159 --> 00:33:11,000
noise along the side the statement this

774
00:33:13,869 --> 00:33:12,169
is a question about whether this is a

775
00:33:16,090 --> 00:33:13,879
correct way to look at things your

776
00:33:19,180 --> 00:33:16,100
statement that sulfate is relatively

777
00:33:21,269 --> 00:33:19,190
unreactive species and so you have to do

778
00:33:23,769 --> 00:33:21,279
something unusual to it to activate it

779
00:33:26,499 --> 00:33:23,779
reminds me of what is the case also with

780
00:33:28,659 --> 00:33:26,509
nitrate which has tremendous capacity to

781
00:33:31,090 --> 00:33:28,669
reach oxidize but it's difficult to get

782
00:33:33,159 --> 00:33:31,100
at and it's a little bit like the

783
00:33:34,930 --> 00:33:33,169
Rubisco problem that co2 is not

784
00:33:36,399 --> 00:33:34,940
particularly reactive and it's not all

785
00:33:38,649 --> 00:33:36,409
that different from oxygens so

786
00:33:41,950 --> 00:33:38,659
activating it and distinguishing it

787
00:33:44,169 --> 00:33:41,960
discriminating it is hard all of the

788
00:33:46,720 --> 00:33:44,179

enzymes that do this seem to have big

789
00:33:49,720 --> 00:33:46,730
isotope signatures and the signatures

790
00:33:52,480 --> 00:33:49,730
seem to be related to how hard you can

791
00:33:56,379 --> 00:33:52,490
drive the reaction so we see things like

792
00:33:58,419 --> 00:33:56,389
a regular relation between isotope

793
00:34:00,249 --> 00:33:58,429
fractionation and the discriminating

794
00:34:03,129 --> 00:34:00,259
capacity and Rubisco x' and things like

795
00:34:06,039 --> 00:34:03,139
that but the great thing about these non

796
00:34:08,079 --> 00:34:06,049
reactive species is that vase they

797
00:34:10,569 --> 00:34:08,089
should be the chemical bottlenecks that

798
00:34:12,159 --> 00:34:10,579
wind up trapping free energy in one

799
00:34:14,440 --> 00:34:12,169
domain without a lot of reaction

800
00:34:16,270 --> 00:34:14,450
partners for it so that it can transport

801
00:34:19,180 --> 00:34:16,280
globally and become sort of a major

802
00:34:22,899 --> 00:34:19,190
carrier of disequilibrium should we

803
00:34:25,149 --> 00:34:22,909
expect that the major isotope signatures

804
00:34:27,579 --> 00:34:25,159
are the way they are because the major

805
00:34:29,859 --> 00:34:27,589
opportunities for biochemistry to take

806
00:34:31,510 --> 00:34:29,869
advantage of kinetic traps are these

807
00:34:33,099 --> 00:34:31,520
molecular like you know following

808
00:34:35,589 --> 00:34:33,109
avarice point are these molecular

809
00:34:37,000 --> 00:34:35,599
species that are hard to activate so the

810
00:34:38,319 --> 00:34:37,010
enzymes that can figure out how to do

811
00:34:43,270 --> 00:34:38,329
that are frequently going to wind up

812
00:34:45,730 --> 00:34:43,280
showing these kind of signatures I'm not

813
00:34:47,899 --> 00:34:45,740

sure I think that's I think that's a

814
00:34:52,460 --> 00:34:47,909
really great question

815
00:34:55,250 --> 00:34:52,470
I yeah I think there are they're

816
00:34:59,120 --> 00:34:55,260
probably examples of enzymes that are

817
00:35:00,950 --> 00:34:59,130
doing over overcoming well nitrogenase

818
00:35:02,089 --> 00:35:00,960
is one it's overcoming a huge kinetic

819
00:35:05,210 --> 00:35:02,099
barrier but it's isotope fractionation

820
00:35:07,819 --> 00:35:05,220
is really small and so it's it's hard

821
00:35:18,380 --> 00:35:07,829
for me right now to understand exactly

822
00:35:19,730 --> 00:35:18,390
how yeah what's general there yeah so

823
00:35:21,620 --> 00:35:19,740
that was actually the question I was

824
00:35:24,740 --> 00:35:21,630
just gonna ask you about nitrogenase and

825
00:35:27,920 --> 00:35:24,750
but I've got a second one he showed this

826
00:35:30,289 --> 00:35:27,930
huge spread of it wasn't your data but a

827
00:35:32,150 --> 00:35:30,299
huge spread of isotopic fractionation

828
00:35:34,730 --> 00:35:32,160
Xand people have tried to separate them

829
00:35:36,260 --> 00:35:34,740
out based on environment type and and so

830
00:35:39,380 --> 00:35:36,270
on and so forth but it has somebody I

831
00:35:41,329 --> 00:35:39,390
mean maybe this is obvious to people

832
00:35:43,490 --> 00:35:41,339
that do this work more but complete

833
00:35:45,109 --> 00:35:43,500
versus incomplete oxidizers whether or

834
00:35:48,230 --> 00:35:45,119
not they use cytochromes versus the

835
00:35:52,250 --> 00:35:48,240
sulfa vihren different major

836
00:35:53,839 --> 00:35:52,260
physiological variations and sulfate

837
00:35:56,240 --> 00:35:53,849
reduction that I believe are pretty well

838
00:35:59,180 --> 00:35:56,250

prescribed on to the evolution of like

839
00:36:00,920 --> 00:35:59,190
the the sulfate reducers themselves can

840
00:36:02,359 --> 00:36:00,930
you start backing out some of these

841
00:36:03,769 --> 00:36:02,369
patterns of fractionation based on

842
00:36:06,559 --> 00:36:03,779
cellular physiology and whether or not

843
00:36:09,200 --> 00:36:06,569
that tracks molecular in the evolution

844
00:36:10,880 --> 00:36:09,210
of these taxa I think that's a really

845
00:36:13,510 --> 00:36:10,890
great idea I'm not aware of anybody

846
00:36:16,640 --> 00:36:13,520
doing that in a comprehensive way that

847
00:36:19,010 --> 00:36:16,650
that Harrison and thawed paper from 1957

848
00:36:22,190 --> 00:36:19,020
has guided the field really strongly to

849
00:36:24,260 --> 00:36:22,200
try to really cell specific sulfate

850
00:36:26,539 --> 00:36:24,270
reduction rates to the observed

851
00:36:28,789 --> 00:36:26,549
fractionation and so people have gone

852
00:36:31,700 --> 00:36:28,799
out and measured many many types of SRB

853
00:36:36,170 --> 00:36:31,710
and only parameterised it against that

854
00:36:38,210 --> 00:36:36,180
rate but I think from what we know now

855
00:36:42,740 --> 00:36:38,220
especially from that model that Boz and

856
00:36:44,539 --> 00:36:42,750
etai constructed and then are and

857
00:36:46,279 --> 00:36:44,549
they're also recent permit permutation

858
00:36:47,990 --> 00:36:46,289
of that of being able to spread the data

859
00:36:50,210 --> 00:36:48,000
along an axis that is the amount of

860
00:36:52,609 --> 00:36:50,220
energy released and the reaction we

861
00:37:06,559 --> 00:36:52,619
should be able to do just that I think

862
00:37:10,109 --> 00:37:08,370
thank you for your talk

863
00:37:12,809 --> 00:37:10,119

he's very interesting so you have

864
00:37:15,120 --> 00:37:12,819
planned to apply the similar strategy to

865
00:37:18,329 --> 00:37:15,130
study the carbon isotope fractionation

866
00:37:21,390 --> 00:37:18,339
so in your case you is that a very pure

867
00:37:24,059 --> 00:37:21,400
system like like one and then or one

868
00:37:26,249 --> 00:37:24,069
organism and you what the molecule was

869
00:37:30,660 --> 00:37:26,259
the molecules that you hear try to

870
00:37:32,130 --> 00:37:30,670
target like carbon dioxide methane or or

871
00:37:37,319 --> 00:37:32,140
something like this it's a mineral

872
00:37:38,969 --> 00:37:37,329
record which data you a to write yes

873
00:37:41,269 --> 00:37:38,979
there's a couple yeah there's a lot of

874
00:37:43,169 --> 00:37:41,279
different it depends on the question

875
00:37:45,539 --> 00:37:43,179
wants to be addressed I think the

876
00:37:47,929 --> 00:37:45,549
autotrophic ones will be really

877
00:37:50,459 --> 00:37:47,939
interesting for example acetyl co a

878
00:37:53,849 --> 00:37:50,469
synthase synthetase would be a really

879
00:37:56,039 --> 00:37:53,859
interesting one to look at but in the in

880
00:37:59,039 --> 00:37:56,049
the cell even for non autotrophic

881
00:38:00,329 --> 00:37:59,049
pathways i think it we have to know what

882
00:38:02,669 --> 00:38:00,339
the rate limiting step

883
00:38:04,289 --> 00:38:02,679
is in a pathway and then we that helps

884
00:38:05,009 --> 00:38:04,299
us understand which one is the important

885
00:38:06,959 --> 00:38:05,019
one to look at

886
00:38:10,169 --> 00:38:06,969
and then for that question getting that

887
00:38:12,479 --> 00:38:10,179
enzyme specific number okay so the

888
00:38:15,449 --> 00:38:12,489

question will not be to explain the

889
00:38:32,120 --> 00:38:15,459
mineral record but to understand a

890
00:38:38,050 --> 00:38:36,440
right so if known so thank you very much

891
00:38:58,770 --> 00:38:38,060
[Applause]