2017 Astrobiology Graduate Conference

Charlottesville, VA

1
00:00:00,790 --> 00:00:07,150
[Music]

2
00:00:12,589 --> 00:00:10,610
hello my name is Victoria Lai and I'm

3
00:00:13,759 --> 00:00:12,599
here to talk about a potential model for

4
00:00:17,109 --> 00:00:13,769
life on Mars and how we're using

5
00:00:19,310 --> 00:00:17,119
combination of genomic and mutagenic

6
00:00:23,330 --> 00:00:19,320
analysis to look more closely at that

7
00:00:26,029 --> 00:00:23,340
model so first of all what do aliens

8
00:00:29,570 --> 00:00:26,039
look like pop culture depicts aliens and

9
00:00:31,040 --> 00:00:29,580
extraterrestrial life like this but we

10
00:00:35,090 --> 00:00:31,050
suspect it will look more like this

11
00:00:37,940 --> 00:00:35,100
so this is hallo bacterium NRC one

12
00:00:40,459 --> 00:00:37,950
isolated from the San Francisco Bay salt

13
00:00:42,979 --> 00:00:40,469

urns and then this is hallo rubrum lacus

14
00:00:46,040 --> 00:00:42,989
profund II and it was isolated from deep

15
00:00:48,740 --> 00:00:46,050
lake Antarctica so these are hallo

16
00:00:50,779 --> 00:00:48,750
archaea or salt-loving members of

17
00:00:52,939 --> 00:00:50,789
archaea that their domain of life and

18
00:00:54,410 --> 00:00:52,949
hailer archaea make good models for

19
00:00:55,790 --> 00:00:54,420
extraterrestrial life because they're

20
00:00:58,220 --> 00:00:55,800
thought to be a very ancient form of

21
00:01:02,180 --> 00:00:58,230
life and are found in extreme

22
00:01:05,179 --> 00:01:02,190
environments so NRC one and h-blocks

23
00:01:07,039 --> 00:01:05,189
profundity or HLA have been subjected to

24
00:01:08,570 --> 00:01:07,049
stratosphere because conditions such as

25
00:01:10,910 --> 00:01:08,580
pressure change freeze thaw and

26
00:01:13,670 --> 00:01:10,920
radiation both in the laboratory setting

27
00:01:15,410 --> 00:01:13,680
and with weather balloons and have been

28
00:01:16,910 --> 00:01:15,420
shown to survive relatively well and

29
00:01:18,580 --> 00:01:16,920
this was recently published in the

30
00:01:20,660 --> 00:01:18,590
International Journal of astrobiology

31
00:01:22,850 --> 00:01:20,670
they are also able to survive within

32
00:01:24,649 --> 00:01:22,860
Brien inclusions in salt crystals as you

33
00:01:30,850 --> 00:01:24,659
can see here in the colored portions

34
00:01:33,319 --> 00:01:30,860
that is those are living halo archaea so

35
00:01:34,550 --> 00:01:33,329
these stressors are similar to the kind

36
00:01:36,289 --> 00:01:34,560
of stresses that would be experienced on

37
00:01:39,410 --> 00:01:36,299
Mars specifically the extreme

38
00:01:41,420 --> 00:01:39,420

environment in which HLA is found is

39
00:01:43,039 --> 00:01:41,430
analogous to the conditions on Mars

40
00:01:45,469 --> 00:01:43,049
where there's thought to be periods of

41
00:01:47,840 --> 00:01:45,479
flowing liquid water shown in the

42
00:01:49,370 --> 00:01:47,850
occurring slope lineae at noon crater so

43
00:01:51,050 --> 00:01:49,380
this liquid would most likely be brine

44
00:01:52,819 --> 00:01:51,060
considering that the flows can be

45
00:01:54,830 --> 00:01:52,829
observed even at sub-zero temperatures

46
00:01:56,929 --> 00:01:54,840
so there's a number of sodium chloride

47
00:01:58,249 --> 00:01:56,939
magnesium perchlorates and other salts

48
00:02:00,709 --> 00:01:58,259
that have been reported to be present on

49
00:02:03,340 --> 00:02:00,719
Mars that could allow the water to

50
00:02:06,620 --> 00:02:03,350
remain liquid at such low temperatures

51
00:02:08,510 --> 00:02:06,630
so hla is not only able to survive

52
00:02:10,760 --> 00:02:08,520
extremely high salt concentrations but

53
00:02:12,500 --> 00:02:10,770
also low temperatures it's even been

54
00:02:13,640 --> 00:02:12,510
recorded to grow as low as negative 1

55
00:02:15,649 --> 00:02:13,650
degree Celsius

56
00:02:18,350 --> 00:02:15,659
so because HLA is a cold-adapted pelo

57
00:02:20,750 --> 00:02:18,360
rakia it's very promising as a potential

58
00:02:22,610 --> 00:02:20,760
model for life on Mars and by studying

59
00:02:25,130 --> 00:02:22,620
its evolutionary genetic adaptations

60
00:02:27,050 --> 00:02:25,140
we're looking for specific ways in which

61
00:02:29,660 --> 00:02:27,060
it has diverged from its temperate or

62
00:02:31,069 --> 00:02:29,670
mesophilic counterparts therefore giving

63
00:02:33,140 --> 00:02:31,079

an indication of what kind of

64
00:02:36,319 --> 00:02:33,150
evolutionary change results in the

65
00:02:38,059 --> 00:02:36,329
ability to survive these extremes so HLA

66
00:02:39,860 --> 00:02:38,069
has a fully sequenced genome that's been

67
00:02:41,569 --> 00:02:39,870
compared to more mesophilic helú archaea

68
00:02:43,970 --> 00:02:41,579
in order to find residues that are

69
00:02:45,949 --> 00:02:43,980
conserved within the mesophyll meso

70
00:02:47,240 --> 00:02:45,959
files but then diverged in HLA

71
00:02:49,550 --> 00:02:47,250
suggesting that there's some

72
00:02:52,849 --> 00:02:49,560
evolutionary advantage to the residues

73
00:02:54,650 --> 00:02:52,859
in the cold adapted organism so we can

74
00:02:56,599 --> 00:02:54,660
look more deeply at a single enzyme by

75
00:02:58,759 --> 00:02:56,609
comparing the protein sequences from HLA

76
00:03:01,339 --> 00:02:58,769
to homologs in other mesophilic

77
00:03:03,349 --> 00:03:01,349
halophiles specifically in this study

78
00:03:12,530 --> 00:03:03,359
were looking at beta galactosidase just

79
00:03:14,479 --> 00:03:12,540
that one um and and we found six

80
00:03:17,180 --> 00:03:14,489
residues that were diverged in the cold

81
00:03:20,180 --> 00:03:17,190
adaptive beta galactosidase so you can

82
00:03:22,220 --> 00:03:20,190
see there's one in the beta sheets of

83
00:03:24,500 --> 00:03:22,230
the tim barrel here there's a couple on

84
00:03:28,220 --> 00:03:24,510
the Alpha helix ease of domain a and

85
00:03:30,259 --> 00:03:28,230
then two in domain B so they're found

86
00:03:34,369 --> 00:03:30,269
all throughout there's no real pattern

87
00:03:36,979 --> 00:03:34,379
there but beta galactosidase is a

88
00:03:39,080 --> 00:03:36,989

catalyst that facilitates hydrolysis by

89
00:03:41,360 --> 00:03:39,090
breaking a glycosidic bond in sugars

90
00:03:42,920 --> 00:03:41,370
such as lactose and that catalytic

91
00:03:44,599 --> 00:03:42,930
activity at low temperatures is

92
00:03:47,379 --> 00:03:44,609
generally even less effective than other

93
00:03:50,330 --> 00:03:47,389
enzymatic activities just in general

94
00:03:51,680 --> 00:03:50,340
because of the thermodynamic effect so

95
00:03:53,930 --> 00:03:51,690
we perform mutagenesis on beta

96
00:03:55,670 --> 00:03:53,940
galactosidase Express eats mutation in a

97
00:03:57,589 --> 00:03:55,680
halo archaeal expression system and

98
00:03:59,300 --> 00:03:57,599
purified it to test the effect of

99
00:04:01,640 --> 00:03:59,310
changing those single divergent residues

100
00:04:03,409 --> 00:04:01,650
to the ones found in the meso file

101
00:04:05,659 --> 00:04:03,419
through comparison to the wild-type and

102
00:04:06,860 --> 00:04:05,669
we use classical biochemical analysis

103
00:04:09,800 --> 00:04:06,870
through steady-state kinetics to

104
00:04:11,930 --> 00:04:09,810
quantify those effects so for those of

105
00:04:13,729 --> 00:04:11,940
you who may not be familiar steady-state

106
00:04:15,949 --> 00:04:13,739
kinetics uses this formula under

107
00:04:18,110 --> 00:04:15,959
conditions of substrate excess so you

108
00:04:22,250 --> 00:04:18,120
use the enzyme s is the substrate and

109
00:04:23,810 --> 00:04:22,260
then P is the product and es is what is

110
00:04:25,170 --> 00:04:23,820
these is what the steady-state is

111
00:04:29,760 --> 00:04:25,180
referring to

112
00:04:31,110 --> 00:04:29,770
so @k K minus 1 and K 1 you're measuring

113
00:04:32,999 --> 00:04:31,120

the rate of dissociation of the

114
00:04:36,629 --> 00:04:33,009
substrate from the enzyme and at K 2

115
00:04:37,680 --> 00:04:36,639
you're measuring the rate of reaction so

116
00:04:39,689 --> 00:04:37,690
we can think of this as like a

117
00:04:41,520 --> 00:04:39,699
lock-and-key situation where you want

118
00:04:43,620 --> 00:04:41,530
the key or the substrate to fit well

119
00:04:45,180 --> 00:04:43,630
with the lock the enzyme but still be

120
00:04:46,740 --> 00:04:45,190
able to remove the key or the product

121
00:04:49,350 --> 00:04:46,750
after the lock is unlocked and the

122
00:04:50,640 --> 00:04:49,360
reaction is completed since I'm looking

123
00:04:52,620 --> 00:04:50,650
at steady-state kinetics with high

124
00:04:56,670 --> 00:04:52,630
substrate concentrations I can calculate

125
00:04:58,170 --> 00:04:56,680
the km and kitkat that's here so the km

126
00:05:00,990 --> 00:04:58,180
is similar to the dissociation constant

127
00:05:03,870 --> 00:05:01,000
of the substrate from the enzyme and a

128
00:05:07,860 --> 00:05:03,880
lower km is better as it indicates lost

129
00:05:09,330 --> 00:05:07,870
association so k NK cat represents the

130
00:05:12,990 --> 00:05:09,340
rate of the reaction under steady state

131
00:05:14,730 --> 00:05:13,000
conditions and then k cat over km is the

132
00:05:16,890 --> 00:05:14,740
ratio of the two and represents the

133
00:05:21,689 --> 00:05:16,900
efficiency of the enzyme to perform the

134
00:05:23,219 --> 00:05:21,699
reaction so the higher the k cat over km

135
00:05:25,350 --> 00:05:23,229
the more efficient the enzyme is is a

136
00:05:26,820 --> 00:05:25,360
catalyst and then we can look at these

137
00:05:28,860 --> 00:05:26,830
three constants compared to the wild

138
00:05:31,710 --> 00:05:28,870

type comparing the wild type to the

139
00:05:33,839 --> 00:05:31,720
mutated enzyme at temperatures from 0 to

140
00:05:37,969 --> 00:05:33,849
25 degrees Celsius in two molar KCl

141
00:05:40,320 --> 00:05:37,979
solution so this graph is showing the

142
00:05:42,899 --> 00:05:40,330
kms of the mutated enzymes in red

143
00:05:44,700 --> 00:05:42,909
compared to the wild type in blue so a

144
00:05:46,649 --> 00:05:44,710
brief overview of our results showed

145
00:05:48,060 --> 00:05:46,659
that all of the mutated enzymes affected

146
00:05:51,839 --> 00:05:48,070
temperature activity compared to the

147
00:05:54,629 --> 00:05:51,849
wild type with all of them in exhibiting

148
00:05:57,089 --> 00:05:54,639
an increased km at zero degree Celsius

149
00:05:59,939 --> 00:05:57,099
the degree of difference varying as you

150
00:06:01,800 --> 00:05:59,949
can see and then the wild type exhibited

151
00:06:03,450 --> 00:06:01,810
a direct relationship between the km and

152
00:06:05,399 --> 00:06:03,460
the temperature which would be

153
00:06:07,890 --> 00:06:05,409
characteristic of a cold adapted enzyme

154
00:06:09,600 --> 00:06:07,900
where it has the lowest km at the lowest

155
00:06:18,020 --> 00:06:09,610
temperature and then a higher km at

156
00:06:22,490 --> 00:06:20,840
where the six mutated enzymes showed

157
00:06:23,870 --> 00:06:22,500
inverse character compared to the wild

158
00:06:27,050 --> 00:06:23,880
type with a higher km at low

159
00:06:28,820 --> 00:06:27,060
temperatures so we that was exactly what

160
00:06:31,760 --> 00:06:28,830
we were hoping to see in changing it

161
00:06:33,820 --> 00:06:31,770
from the divergent cold-adapted residue

162
00:06:36,770 --> 00:06:33,830
to the one found in the Meza file and

163
00:06:38,510 --> 00:06:36,780

these results confirm the evolutionary

164
00:06:41,090 --> 00:06:38,520
importance of at least four of the six

165
00:06:42,170 --> 00:06:41,100
divergent amino acids in the improved

166
00:06:45,230 --> 00:06:42,180
function of the beta galactosidase

167
00:06:47,000 --> 00:06:45,240
enzyme at colder temperatures so I'm

168
00:06:49,180 --> 00:06:47,010
going to go into more detail about one

169
00:06:52,360 --> 00:06:49,190
of my mutants

170
00:06:55,610 --> 00:06:52,370
so one mutation in particular was at

171
00:07:00,380 --> 00:06:55,620
residue 482 which is located in a beta

172
00:07:02,000 --> 00:07:00,390
sheet in domain B and in all these

173
00:07:04,520 --> 00:07:02,010
graphs the mutated enzyme will be shown

174
00:07:06,260 --> 00:07:04,530
in red and the wild-type in blue so this

175
00:07:08,600 --> 00:07:06,270
mutation showed the highest increase in

176
00:07:10,280 --> 00:07:08,610
the km at lower temperatures suggesting

177
00:07:12,200 --> 00:07:10,290
that the mutated enzyme was less

178
00:07:14,180 --> 00:07:12,210
affected than the wild less effective

179
00:07:16,610 --> 00:07:14,190
than the wild-type at lower temperatures

180
00:07:18,380 --> 00:07:16,620
which is in line with the hypothesis

181
00:07:20,360 --> 00:07:18,390
that changing that single residue will

182
00:07:23,870 --> 00:07:20,370
negatively impact the cold adaptiveness

183
00:07:26,210 --> 00:07:23,880
of the enzyme the change in the k-kat

184
00:07:28,850 --> 00:07:26,220
however is much more subtle than the km

185
00:07:30,680 --> 00:07:28,860
though you are still seeing a change at

186
00:07:34,940 --> 00:07:30,690
the higher temperatures where the

187
00:07:36,140 --> 00:07:34,950
mutated enzyme is more is has a better

188
00:07:37,580 --> 00:07:36,150

rate of reaction at those higher

189
00:07:40,010 --> 00:07:37,590
temperatures compared to the wild type

190
00:07:42,440 --> 00:07:40,020
which again fits with our hypothesis as

191
00:07:44,000 --> 00:07:42,450
we're changing it from the residue found

192
00:07:45,890 --> 00:07:44,010
in the cold adaptive enzyme to the one

193
00:07:48,260 --> 00:07:45,900
found in the Meza file and an increased

194
00:07:50,270 --> 00:07:48,270
rate of Zac reaction when you're losing

195
00:07:54,440 --> 00:07:50,280
that cold adaptiveness at higher

196
00:07:57,080 --> 00:07:54,450
temperatures make sense and then finally

197
00:07:58,550 --> 00:07:57,090
the enzyme efficiency was decreased for

198
00:08:00,310 --> 00:07:58,560
the mutated enzyme when compared to the

199
00:08:04,220 --> 00:08:00,320
wild type over the lower temperatures

200
00:08:05,900 --> 00:08:04,230
concei and increased at the higher

201
00:08:08,540 --> 00:08:05,910
temperatures and this trend was mainly

202
00:08:10,370 --> 00:08:08,550
influenced by the km as you can see

203
00:08:12,740 --> 00:08:10,380
before it had it was a much more drastic

204
00:08:14,660 --> 00:08:12,750
difference between the two but the k-kat

205
00:08:16,070 --> 00:08:14,670
is still taken into account and most

206
00:08:19,310 --> 00:08:16,080
likely shows its effects at those higher

207
00:08:21,680 --> 00:08:19,320
temperatures so these three constants

208
00:08:23,830 --> 00:08:21,690
when compared to the wild-type paint a

209
00:08:25,790 --> 00:08:23,840
picture of a less cold adapted enzyme

210
00:08:28,250 --> 00:08:25,800
supporting idea that these diverged

211
00:08:30,410 --> 00:08:28,260
residues are diverged to increase the

212
00:08:32,240 --> 00:08:30,420
ability of the beta galactosidase in HLA

213
00:08:35,180 --> 00:08:32,250

to function in the

214
00:08:38,839 --> 00:08:35,190
the next step is to examine the enzyme

215
00:08:40,430 --> 00:08:38,849
metal the enzyme model structurally are

216
00:08:42,230 --> 00:08:40,440
there patterns that can show us why

217
00:08:44,329 --> 00:08:42,240
certain residues in certain positions

218
00:08:46,670 --> 00:08:44,339
can affect the temperature range of the

219
00:08:48,829 --> 00:08:46,680
enzyme so if you think of proteins or

220
00:08:50,990 --> 00:08:48,839
enzymes like molecular machines which

221
00:08:53,210 --> 00:08:51,000
need to flex are moved to be

222
00:08:54,530 --> 00:08:53,220
catalytically active they need space to

223
00:08:56,060 --> 00:08:54,540
work effectively and at high

224
00:08:58,220 --> 00:08:56,070
temperatures the surrounding chemical

225
00:09:00,350 --> 00:08:58,230
groups and atoms are moving enough that

226
00:09:02,060 --> 00:09:00,360
there's plenty of space to work with but

227
00:09:05,840 --> 00:09:02,070
at lower temperatures this as we've

228
00:09:07,100 --> 00:09:05,850
talked about in the opening talk at

229
00:09:08,420 --> 00:09:07,110
lower temperatures the surrounding

230
00:09:11,000 --> 00:09:08,430
molecules are more densely packed and

231
00:09:12,860 --> 00:09:11,010
frozen like water in a liquid compared

232
00:09:14,240 --> 00:09:12,870
to in an ice state where the molecules

233
00:09:15,769 --> 00:09:14,250
are more structured so it stands to

234
00:09:17,990 --> 00:09:15,779
reason that the enzyme would need to be

235
00:09:20,329 --> 00:09:18,000
more flexible to function at those lower

236
00:09:22,370 --> 00:09:20,339
temperatures to counteract those effects

237
00:09:26,930 --> 00:09:22,380
so we look at the homology model

238
00:09:30,050 --> 00:09:26,940

comparing valine at position 482 here to

239
00:09:34,880 --> 00:09:30,060
leucine at that same position in these

240
00:09:37,069 --> 00:09:34,890
images what you're seeing is the the

241
00:09:39,380 --> 00:09:37,079
residue in question and then residues

242
00:09:41,900 --> 00:09:39,390
and side chains which within six

243
00:09:44,900 --> 00:09:41,910
angstroms of that and the white area

244
00:09:48,680 --> 00:09:44,910
represents the edges of the Vander Waal

245
00:09:50,480 --> 00:09:48,690
shells for each side chain each atom so

246
00:09:51,590 --> 00:09:50,490
you can see with valine you're getting

247
00:09:54,380 --> 00:09:51,600
this space here

248
00:09:56,569 --> 00:09:54,390
that's unoccupied and you're getting a

249
00:09:59,000 --> 00:09:56,579
similar space in leucine here maybe a

250
00:10:01,490 --> 00:09:59,010
little bit smaller you are losing that

251
00:10:03,740 --> 00:10:01,500
space here so there's more potential

252
00:10:06,110 --> 00:10:03,750
there's more potential flexibility here

253
00:10:07,850 --> 00:10:06,120
and fewer potential interactions with

254
00:10:09,139 --> 00:10:07,860
these residues up here whereas with the

255
00:10:11,180 --> 00:10:09,149
leucine there's more potential for

256
00:10:14,000 --> 00:10:11,190
interaction here a greater chance of

257
00:10:15,740 --> 00:10:14,010
reduced flexibility and leucine is the

258
00:10:17,509 --> 00:10:15,750
one that's in the meso file and the

259
00:10:19,880 --> 00:10:17,519
valine is the original one in the cold

260
00:10:22,130 --> 00:10:19,890
adapted enzyme and then if we look at it

261
00:10:24,860 --> 00:10:22,140
from another angle you can see even more

262
00:10:27,530 --> 00:10:24,870
clearly that this valine here there's

263
00:10:29,300 --> 00:10:27,540

this additional space that isn't present

264
00:10:32,180 --> 00:10:29,310
when you have that additional carbon

265
00:10:35,870 --> 00:10:32,190
when it's a leucine so you're losing

266
00:10:37,490 --> 00:10:35,880
that space and then you have more

267
00:10:42,889 --> 00:10:37,500
potential for interactions more

268
00:10:44,420 --> 00:10:42,899
potential less potential flexibility so

269
00:10:44,990 --> 00:10:44,430
the studies performed on these six

270
00:10:46,910 --> 00:10:45,000
different

271
00:10:50,120 --> 00:10:46,920
Haitians are providing deeper insights

272
00:10:52,400 --> 00:10:50,130
into how enzymes may function at lower

273
00:10:55,250 --> 00:10:52,410
limit of temperature in an extreme of

274
00:10:56,840 --> 00:10:55,260
bio living in Antarctica so in

275
00:10:58,940 --> 00:10:56,850
conclusion when thinking about the

276
00:11:00,770 --> 00:10:58,950
potential for life on other planets we

277
00:11:02,330 --> 00:11:00,780
can first look at life in the extremes

278
00:11:04,540 --> 00:11:02,340
on earth to give us a better framework

279
00:11:07,010 --> 00:11:04,550
for how this kind of life may survive in

280
00:11:08,570 --> 00:11:07,020
our approach we can first look at those

281
00:11:10,610 --> 00:11:08,580
divergent regions and the proteins

282
00:11:12,710 --> 00:11:10,620
encoded in the genomes of microorganisms

283
00:11:15,050 --> 00:11:12,720
adapted to extreme environments and then

284
00:11:16,490 --> 00:11:15,060
use experimental methods to confirm

285
00:11:19,160 --> 00:11:16,500
their importance in the ability to

286
00:11:21,590 --> 00:11:19,170
survive these extremes and in doing so

287
00:11:24,080 --> 00:11:21,600
we're able to use past evolution as a

288
00:11:25,760 --> 00:11:24,090

guide pointing us towards novel adaptive

289
00:11:27,860 --> 00:11:25,770
mechanisms leading to new discoveries

290
00:11:29,840 --> 00:11:27,870
and expanding our understood ranges of

291
00:11:32,510 --> 00:11:29,850
survivability thus giving us a better

292
00:11:35,170 --> 00:11:32,520
basis for how life may arise even

293
00:11:38,570 --> 00:11:35,180
outside what we consider life's limits

294
00:11:40,010 --> 00:11:38,580
so I'd like to thank my lab group for

295
00:11:41,510 --> 00:11:40,020
supporting me with this in the

296
00:11:44,660 --> 00:11:41,520
University of Maryland and NASA for

297
00:11:50,749 --> 00:11:44,670
providing funding

298
00:11:53,999 --> 00:11:50,759
[Applause]

299
00:11:56,430 --> 00:11:54,009
uh fans for the nice talk I have a

300
00:11:58,259 --> 00:11:56,440
question about your ki measurements I'm

301
00:12:01,170 --> 00:11:58,269
wondering what is your reporter essay

302
00:12:04,439 --> 00:12:01,180
for studying the km that's my first

303
00:12:08,220 --> 00:12:04,449
question and also are you limiting

304
00:12:11,879 --> 00:12:08,230
yourself to just a single residue

305
00:12:14,910 --> 00:12:11,889
mutagenesis at 482 based on your bowing

306
00:12:16,980 --> 00:12:14,920
4matic analysis sorry I may not have you

307
00:12:18,150 --> 00:12:16,990
may have covered already but didn't

308
00:12:20,699 --> 00:12:18,160
capture the answer to the first question

309
00:12:24,480 --> 00:12:20,709
I'm using a colorimetric essay using

310
00:12:28,050 --> 00:12:24,490
onp g at absorbance 420 so I'm doing

311
00:12:30,600 --> 00:12:28,060
like measuring the change in color there

312
00:12:33,660 --> 00:12:30,610
and then using the V zero to calculate

313
00:12:38,429 --> 00:12:33,670

the km using lineweaver-burk graphing

314
00:12:40,949 --> 00:12:38,439
and the second question I have all of

315
00:12:42,269 --> 00:12:40,959
those mutations that I talked about the

316
00:12:44,610 --> 00:12:42,279
six different ones there at six

317
00:12:47,100 --> 00:12:44,620
different residues I just talked about

318
00:12:49,170 --> 00:12:47,110
482 for the sake of time just going into

319
00:12:50,730 --> 00:12:49,180
one in specific I do have a poster that

320
00:12:58,530 --> 00:12:50,740
I'll put up later if you want to see the

321
00:13:02,280 --> 00:12:58,540
other six or the other five questions hi

322
00:13:05,009 --> 00:13:02,290
I have two questions like so um what are

323
00:13:09,300 --> 00:13:05,019
the uncertainties and measuring the cake

324
00:13:11,220 --> 00:13:09,310
hat and km values are there any was the

325
00:13:12,300 --> 00:13:11,230
problem with like putting standard error

326
00:13:15,210 --> 00:13:12,310
bars and things like that on these

327
00:13:18,449 --> 00:13:15,220
graphs is that these values are based

328
00:13:20,970 --> 00:13:18,459
off of so the way that they're

329
00:13:23,189 --> 00:13:20,980
calculated is you do those color metric

330
00:13:25,379 --> 00:13:23,199
assays with different substrate

331
00:13:27,780 --> 00:13:25,389
concentrations and then based off of the

332
00:13:29,639 --> 00:13:27,790
v-0 that you calculate from that you

333
00:13:31,740 --> 00:13:29,649
create a lineweaver-burk graph and then

334
00:13:33,329 --> 00:13:31,750
based off of the x and y-intercepts of

335
00:13:36,059 --> 00:13:33,339
that lineweaver-burk graph that's where

336
00:13:37,439 --> 00:13:36,069
you get the km and k-kat so it's a

337
00:13:40,139 --> 00:13:37,449
little bit difficult to get the

338
00:13:41,879 --> 00:13:40,149

uncertainties there okay so as an

339
00:13:47,280 --> 00:13:41,889
astronomer I'll ask you later about what

340
00:13:50,759 --> 00:13:47,290
half of those words mean okay um are

341
00:13:53,910 --> 00:13:50,769
were there any mutations in the timbrel

342
00:13:58,600 --> 00:13:53,920
region there was one so the whole blue

343
00:14:04,310 --> 00:14:02,630
so the Tim barrel is these beta sheets

344
00:14:07,760 --> 00:14:04,320
and then the surrounding alpha helixes

345
00:14:09,110 --> 00:14:07,770
with them and the reason why a mutation

346
00:14:11,570 --> 00:14:09,120
in the Tim barrel would be of interest

347
00:14:13,310 --> 00:14:11,580
would be because it's that's where you

348
00:14:18,590 --> 00:14:13,320
would get closest to the active site and

349
00:14:21,290 --> 00:14:18,600
there was one here a 263 s that was in

350
00:14:24,050 --> 00:14:21,300
the beta sheets of the Tim barrel that

351
00:14:26,210 --> 00:14:24,060
one didn't have a very strong

352
00:14:29,210 --> 00:14:26,220
temperature dependent effect it just had

353
00:14:30,380 --> 00:14:29,220
a in general D latias effect so that's

354
00:14:34,220 --> 00:14:30,390
why I didn't go into that one in detail

355
00:14:37,940 --> 00:14:34,230
here but it was interesting okay thank

356
00:14:41,540 --> 00:14:37,950
you hi can you go to the slide with the

357
00:14:47,000 --> 00:14:41,550
to comparative structures real quick Oh

358
00:14:48,940 --> 00:14:47,010
which one oh yeah one of these so yeah

359
00:14:51,800 --> 00:14:48,950
you guys did a valine to leucine

360
00:14:54,500 --> 00:14:51,810
mutation what if you did like a valine

361
00:14:56,780 --> 00:14:54,510
to something smaller like an alanine or

362
00:14:59,000 --> 00:14:56,790
something do you think that it would

363
00:15:02,120 --> 00:14:59,010

show kind of an opposite that's what

364
00:15:04,040 --> 00:15:02,130
we're hoping would happen and that would

365
00:15:05,420 --> 00:15:04,050
that would be a possible next step

366
00:15:06,920 --> 00:15:05,430
because all of these mutations that we

367
00:15:09,380 --> 00:15:06,930
did were based on that bioinformatic

368
00:15:11,600 --> 00:15:09,390
analysis going from the cold-adapted to

369
00:15:13,310 --> 00:15:11,610
him as a philic but now that we're

370
00:15:16,460 --> 00:15:13,320
seeing that that does have the effect

371
00:15:19,300 --> 00:15:16,470
that we were expecting the possibility

372
00:15:22,580 --> 00:15:19,310
of creating a more cold-adapted enzyme

373
00:15:24,680 --> 00:15:22,590
exists potentially so that that would be

374
00:15:26,360 --> 00:15:24,690
a possible next step would be to then

375
00:15:27,620 --> 00:15:26,370
reverse that go in the opposite

376
00:15:30,130 --> 00:15:27,630
direction of the patterns that we're

377
00:15:32,990 --> 00:15:30,140
seeing take it smaller and see if that

378
00:15:35,240 --> 00:15:33,000
increase in space causes an increase in

379
00:15:37,190 --> 00:15:35,250
flexibility that could then result in an

380
00:15:49,629 --> 00:15:37,200
enzyme that works better at those colder

381
00:15:54,740 --> 00:15:52,430
thank you for that talk continuing on

382
00:15:57,550 --> 00:15:54,750
that last question I was wondering is

383
00:16:01,730 --> 00:15:57,560
there any advantages to having a less

384
00:16:03,860 --> 00:16:01,740
flexible enzyme at higher temperatures

385
00:16:06,019 --> 00:16:03,870
it seems like it would always be better

386
00:16:08,960 --> 00:16:06,029
to have less kind of steric hindrance

387
00:16:11,319 --> 00:16:08,970
going on no I mean um like was talked

388
00:16:15,050 --> 00:16:11,329

about in the initial talk having that

389
00:16:17,449 --> 00:16:15,060
increased structural aspect in at a

390
00:16:20,180 --> 00:16:17,459
higher temperature you do get that

391
00:16:22,250 --> 00:16:20,190
there's the potential for more stability

392
00:16:24,019 --> 00:16:22,260
and less denaturation at those higher

393
00:16:32,980 --> 00:16:24,029
temperatures so it would potentially

394
00:16:37,600 --> 00:16:36,010
I have a question for you um why did you

395
00:16:39,700 --> 00:16:37,610
guys pick this enzyme and are there

396
00:16:43,510 --> 00:16:39,710
other enzymes that you're thinking about

397
00:16:45,130 --> 00:16:43,520
Joseph enzyme because our hailer Akil

398
00:16:48,280 --> 00:16:45,140
expression system doesn't have a beta

399
00:16:50,320 --> 00:16:48,290
galactosidase and there's a very easy

400
00:16:52,870 --> 00:16:50,330
colorimetric assay as a sort of proof of

401
00:16:59,200 --> 00:16:52,880
principle but we could do it with other

402
00:17:00,610 --> 00:16:59,210
ones it's definitely possible okay we

403
00:17:09,020 --> 00:17:00,620
have time for more questions if there

404
00:17:15,650 --> 00:17:13,340
have you used any control enzyme to for

405
00:17:17,360 --> 00:17:15,660
your experiment related to enzyme

406
00:17:21,620 --> 00:17:17,370
activity means you compare your results

407
00:17:24,199 --> 00:17:21,630
with mesophilic enzymes and cyclic

408
00:17:28,850 --> 00:17:24,209
enzymes oh the problem is there aren't a

409
00:17:31,900 --> 00:17:28,860
lot of enzymes that have been looked at

410
00:17:34,760 --> 00:17:31,910
enough to be a control at least in these

411
00:17:37,160 --> 00:17:34,770
in these specific conditions because

412
00:17:39,230 --> 00:17:37,170
like I said I had to use a 2 molar

413
00:17:40,880 --> 00:17:39,240

potassium chloride solution in order to

414
00:17:43,090 --> 00:17:40,890
do these reactions and generally you

415
00:17:46,850 --> 00:17:43,100
wouldn't use that high of a salt for

416
00:17:48,020 --> 00:17:46,860
enzymatic activity assays and I wouldn't

417
00:17:49,340 --> 00:17:48,030
want to necessarily compare it to

418
00:17:50,750 --> 00:17:49,350
something that doesn't have that high of

419
00:17:52,280 --> 00:17:50,760
salt because you don't know what kind of

420
00:17:54,799 --> 00:17:52,290
confounding variables you're adding in

421
00:17:58,310 --> 00:17:54,809
at that point and which method you use

422
00:18:00,830 --> 00:17:58,320
for to study the data to change in

423
00:18:03,590 --> 00:18:00,840
protein conformation as well as amino

424
00:18:06,560 --> 00:18:03,600
acid sequence so this is homology

425
00:18:07,880 --> 00:18:06,570
modeling using Swiss PDB viewer so

426
00:18:10,760 --> 00:18:07,890
that's a free software that's available

427
00:18:12,890 --> 00:18:10,770
online and so we made a homology model

428
00:18:15,230 --> 00:18:12,900
of our enzyme using thermus thermophilus

429
00:18:18,049 --> 00:18:15,240
because it has a characterized and

430
00:18:20,840 --> 00:18:18,059
crystallized structure of its beta

431
00:18:22,790 --> 00:18:20,850
galactosidase so we use that to create

432
00:18:25,370 --> 00:18:22,800
apology model and like so this is all

433
00:18:29,540 --> 00:18:25,380
very theoretical we we haven't been able

434
00:18:33,350 --> 00:18:29,550
to get our enzyme crystallized so this

435
00:18:35,510 --> 00:18:33,360
is all super theoretical this is just

436
00:18:39,410 --> 00:18:35,520
the most likely one given the sequence

437
00:18:41,419 --> 00:18:39,420
that we've put into the system and like

438
00:18:42,890 --> 00:18:41,429

obviously that would be a really good

439
00:18:43,790 --> 00:18:42,900
next step is to get it crystallized so

440
00:18:45,169 --> 00:18:43,800
if anyone knows any good

441
00:18:47,390 --> 00:18:45,179
crystallographers that they want to

442
00:18:52,910 --> 00:18:47,400
refer to me i'd be happy to take the

443
00:18:58,550 --> 00:18:55,700
what about cryo-em have you done create

444
00:19:01,970 --> 00:18:58,560
cryo Yemen I have tried to but nobody

445
00:19:03,680 --> 00:19:01,980
will respond to my emails I really want

446
00:19:14,060 --> 00:19:03,690
to get this crystallized you guys trust

447
00:19:15,890 --> 00:19:14,070
me hi just a super quick question so I'm

448
00:19:17,270 --> 00:19:15,900
a geologist at work in XRD what are the

449
00:19:21,020 --> 00:19:17,280
obstacles of crystallizing your amino

450
00:19:22,400 --> 00:19:21,030
acids I think it's the salt I got talked

451
00:19:23,510 --> 00:19:22,410
to a lot of crystallographers and

452
00:19:25,910 --> 00:19:23,520
whenever I bring up the fact that

453
00:19:28,130 --> 00:19:25,920
there's a lot of salt in it they see

454
00:19:31,550 --> 00:19:28,140
they get kind of excited they think that

455
00:19:33,110 --> 00:19:31,560
it's good but then in practice there

456
00:19:34,610 --> 00:19:33,120
seems to be some sort of hindrance I

457
00:19:36,770 --> 00:19:34,620
don't know a ton about coastal ography

458
00:19:39,530 --> 00:19:36,780
so I don't know what's holding it back

459
00:19:41,000 --> 00:19:39,540
maybe it's the size I'm not sure but I

460
00:19:43,670 --> 00:19:41,010
mean we've crystallized things that are

461
00:19:44,600 --> 00:19:43,680
smaller and bigger than this so I the

462
00:19:53,730 --> 00:19:44,610
only thing that I could think of would