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1
00:00:06,389 --> 00:00:03,870
welcome to this presentation as part of

2
00:00:09,830 --> 00:00:06,399
astrobiology graduate conference

3
00:00:11,990 --> 00:00:09,840
organized virtually by elsi in tokyo

4
00:00:13,830 --> 00:00:12,000
i am thomas lostoler

5
00:00:16,230 --> 00:00:13,840
a phd candidate

6
00:00:17,510 --> 00:00:16,240
focused on probiotic chemistry in solid

7
00:00:19,750 --> 00:00:17,520
state

8
00:00:23,590 --> 00:00:19,760
and working at roger boschkovic

9
00:00:26,550 --> 00:00:25,429
first i want to highlight the key

10
00:00:28,390 --> 00:00:26,560
message

11
00:00:30,710 --> 00:00:28,400
i want you to take away from this

12
00:00:33,190 --> 00:00:30,720
presentation and this is that amino

13
00:00:34,950 --> 00:00:33,200

acids oligomerized to peptides on

14
00:00:37,190 --> 00:00:34,960
mineral surfaces

15
00:00:40,470 --> 00:00:37,200
without the need to interact with water

16
00:00:42,709 --> 00:00:40,480
or aqueous environment

17
00:00:44,869 --> 00:00:42,719
i want to acknowledge the amazing team

18
00:00:46,389 --> 00:00:44,879
of scientists with whom i worked on this

19
00:00:48,630 --> 00:00:46,399
project

20
00:00:51,350 --> 00:00:48,640
jose gregorio hernandez

21
00:00:54,790 --> 00:00:51,360
a scientist with world-class talent and

22
00:00:57,430 --> 00:00:54,800
the originator of this project idea

23
00:01:01,110 --> 00:00:57,440
kronos lavojarvic who is my phd

24
00:01:03,270 --> 00:01:01,120
supervisor and my biggest supporter

25
00:01:06,070 --> 00:01:03,280
the scientist with the right tools and

26
00:01:08,230 --> 00:01:06,080
solutions who has an incredible passion

27
00:01:11,510 --> 00:01:08,240
for doing science

28
00:01:14,550 --> 00:01:11,520
nicola ernest and sasha for offering us

29
00:01:16,950 --> 00:01:14,560
tremendous support and access

30
00:01:19,429 --> 00:01:16,960
to analytical techniques that we needed

31
00:01:21,510 --> 00:01:19,439
to carry out this project

32
00:01:24,870 --> 00:01:21,520
many thanks to croatian science

33
00:01:27,350 --> 00:01:24,880
foundation for financing

34
00:01:29,749 --> 00:01:27,360
to start with the presence of amino

35
00:01:32,149 --> 00:01:29,759
acids on early earth is consensually

36
00:01:34,390 --> 00:01:32,159
accepted by scientists in the field

37
00:01:37,350 --> 00:01:34,400
either stemming from endogenous chemical

38
00:01:39,590 --> 00:01:37,360

routes or delivered extraterrestrially

39
00:01:42,149 --> 00:01:39,600
by meteorites

40
00:01:44,789 --> 00:01:42,159
on the other hand peptides are important

41
00:01:45,990 --> 00:01:44,799
biological polymers with many catalytic

42
00:01:48,069 --> 00:01:46,000
functions

43
00:01:50,789 --> 00:01:48,079
and are regarded to be included in

44
00:01:53,190 --> 00:01:50,799
ancient molecular symbiosis with nucleic

45
00:01:55,030 --> 00:01:53,200
acids

46
00:01:58,230 --> 00:01:55,040
here we have a molecular structure of

47
00:02:00,950 --> 00:01:58,240
the simplest amino acid called glycine

48
00:02:03,270 --> 00:02:00,960
generally amino acids have an n-terminus

49
00:02:05,830 --> 00:02:03,280
with an amino group and a c-terminus

50
00:02:08,790 --> 00:02:05,840
with a carboxyl group

51
00:02:10,150 --> 00:02:08,800
peptides on the other hand are chains of

52
00:02:12,869 --> 00:02:10,160
amino acids

53
00:02:15,270 --> 00:02:12,879
linked together via peptide bonds which

54
00:02:17,990 --> 00:02:15,280
is a covalent bond between carbon from a

55
00:02:19,670 --> 00:02:18,000
carbonyl group and a nitrogen from an nh

56
00:02:22,949 --> 00:02:19,680
group

57
00:02:25,430 --> 00:02:22,959
how did nature go from amino acid to

58
00:02:27,190 --> 00:02:25,440
peptides is a major question in

59
00:02:30,309 --> 00:02:27,200
probiotic chemistry

60
00:02:32,710 --> 00:02:30,319
which is chemistry that led to life

61
00:02:35,670 --> 00:02:32,720
the formation of peptide bonds is an

62
00:02:38,710 --> 00:02:35,680
example of a condensation reaction where

63
00:02:41,350 --> 00:02:38,720

two amino acids such as glycine are

64
00:02:43,830 --> 00:02:41,360
joined together or condensed

65
00:02:47,350 --> 00:02:43,840
via peptide bond together with the

66
00:02:49,430 --> 00:02:47,360
elimination of a water molecule

67
00:02:50,390 --> 00:02:49,440
looking at the basic le chatelier

68
00:02:52,710 --> 00:02:50,400
principle

69
00:02:54,309 --> 00:02:52,720
if this reaction is carried out in

70
00:02:56,150 --> 00:02:54,319
aqueous media

71
00:02:58,470 --> 00:02:56,160
it is highly thermodynamically

72
00:03:00,229 --> 00:02:58,480
unfavorable

73
00:03:02,949 --> 00:03:00,239
because in order for this reaction to

74
00:03:05,430 --> 00:03:02,959
proceed water molecules need to be

75
00:03:09,110 --> 00:03:05,440
eliminated

76
00:03:12,710 --> 00:03:09,120
so it seems that we have a water paradox

77
00:03:15,110 --> 00:03:12,720
on one hand water is essential for life

78
00:03:16,710 --> 00:03:15,120
and for biological evolution

79
00:03:19,110 --> 00:03:16,720
but on the other hand

80
00:03:22,390 --> 00:03:19,120
it seems that water is not desired for

81
00:03:24,309 --> 00:03:22,400
the synthesis of life's building blocks

82
00:03:26,149 --> 00:03:24,319
this is not just related for the

83
00:03:28,070 --> 00:03:26,159
synthesis of peptides

84
00:03:29,589 --> 00:03:28,080
but is a broader issue in probiotic

85
00:03:31,589 --> 00:03:29,599
chemistry

86
00:03:32,630 --> 00:03:31,599
because there are many condensation

87
00:03:35,350 --> 00:03:32,640
reactions

88
00:03:38,630 --> 00:03:35,360

such as the formation of glycosidic

89
00:03:40,229 --> 00:03:38,640
or phosphodiester bonds

90
00:03:41,030 --> 00:03:40,239
this has been recognized by more and

91
00:03:42,949 --> 00:03:41,040
more

92
00:03:45,589 --> 00:03:42,959
scientists in the field

93
00:03:49,190 --> 00:03:45,599
and there is a growing realization that

94
00:03:51,190 --> 00:03:49,200
dry land was critical for concentrating

95
00:03:55,270 --> 00:03:51,200
organic building blocks

96
00:03:56,710 --> 00:03:55,280
and enabling their chemical evolution

97
00:03:58,949 --> 00:03:56,720
there are different literature

98
00:04:00,710 --> 00:03:58,959
approaches for prebiotic peptide

99
00:04:03,190 --> 00:04:00,720
synthesis

100
00:04:05,670 --> 00:04:03,200
firstly thermal polymerization which

101
00:04:07,110 --> 00:04:05,680
requires high temperatures going back to

102
00:04:08,630 --> 00:04:07,120
late 50s

103
00:04:10,869 --> 00:04:08,640
soon after the

104
00:04:13,190 --> 00:04:10,879
discovery of famous

105
00:04:16,069 --> 00:04:13,200
military experiment which served as a

106
00:04:19,110 --> 00:04:16,079
birth of probiotic chemistry

107
00:04:21,349 --> 00:04:19,120
secondly hypervelocity impact studies

108
00:04:23,430 --> 00:04:21,359
that uses impacts in the range of

109
00:04:26,390 --> 00:04:23,440
kilometers per second

110
00:04:29,110 --> 00:04:26,400
and results in short peptides such as

111
00:04:31,510 --> 00:04:29,120
dye and tripeptides

112
00:04:33,350 --> 00:04:31,520
thirdly wet dry cycling which uses

113
00:04:36,550 --> 00:04:33,360

different approaches

114
00:04:40,070 --> 00:04:36,560
but always uses water for mixing of the

115
00:04:42,950 --> 00:04:40,080
reactants and its subsequent evaporation

116
00:04:45,510 --> 00:04:42,960
in order for peptide bond to form during

117
00:04:48,629 --> 00:04:45,520
the dry phase

118
00:04:50,870 --> 00:04:48,639
so we hypothesized what if we avoid the

119
00:04:53,110 --> 00:04:50,880
use of water and instead turn to

120
00:04:56,710 --> 00:04:53,120
solid-state reaction environment

121
00:04:58,790 --> 00:04:56,720
and use only solid mineral surfaces

122
00:05:00,950 --> 00:04:58,800
our approach was to use mechanical

123
00:05:04,870 --> 00:05:00,960
energy that could have been provided

124
00:05:07,670 --> 00:05:04,880
probiotically by impacts erosion

125
00:05:11,029 --> 00:05:07,680
earthquakes and weathering

126
00:05:13,270 --> 00:05:11,039
and to use thermal energy

127
00:05:15,909 --> 00:05:13,280
the availability of dry land is often

128
00:05:18,629 --> 00:05:15,919
discussed in the field and although its

129
00:05:20,390 --> 00:05:18,639
availability was only a few percentage

130
00:05:22,390 --> 00:05:20,400
of today's land mass

131
00:05:23,830 --> 00:05:22,400
it is considered that there was some

132
00:05:26,390 --> 00:05:23,840
land available

133
00:05:28,550 --> 00:05:26,400
near volcanoes and on the emerging

134
00:05:31,590 --> 00:05:28,560
continents

135
00:05:33,590 --> 00:05:31,600
we used mechanochemistry as a tool to

136
00:05:35,830 --> 00:05:33,600
carry out chemical reactions in the

137
00:05:37,749 --> 00:05:35,840
absence of solvents

138
00:05:41,270 --> 00:05:37,759

mechanochemistry has been previously

139
00:05:43,749 --> 00:05:41,280
used in prebiotic chemistry contexts

140
00:05:46,150 --> 00:05:43,759
photosynthesis of alpha amino nitriles

141
00:05:48,710 --> 00:05:46,160
and sugars

142
00:05:51,430 --> 00:05:48,720
we also used temperature controlled ball

143
00:05:53,749 --> 00:05:51,440
milling setup developed recently by our

144
00:05:55,350 --> 00:05:53,759
group

145
00:05:58,150 --> 00:05:55,360
we decided to use

146
00:06:01,189 --> 00:05:58,160
the simplest amino acid glycine

147
00:06:03,749 --> 00:06:01,199
due to its probiotic availability

148
00:06:05,270 --> 00:06:03,759
the presence in meteorites and the

149
00:06:07,029 --> 00:06:05,280
availability of

150
00:06:10,870 --> 00:06:07,039
analytical techniques

151
00:06:13,590 --> 00:06:10,880
needed to characterize reaction products

152
00:06:16,070 --> 00:06:13,600
reaction mixture was

153
00:06:19,749 --> 00:06:16,080
closed in a milling jar and was bold

154
00:06:21,830 --> 00:06:19,759
milled via three stainless steel balls

155
00:06:23,510 --> 00:06:21,840
the milling jar together with the

156
00:06:26,950 --> 00:06:23,520
reaction mixture

157
00:06:27,749 --> 00:06:26,960
was oscillated horizontally

158
00:06:30,870 --> 00:06:27,759
and

159
00:06:33,110 --> 00:06:30,880
attached to it was a thermal

160
00:06:36,230 --> 00:06:33,120
ring element that was connected to a

161
00:06:39,110 --> 00:06:36,240
temperature controller

162
00:06:40,070 --> 00:06:39,120
to characterize the reaction products we

163
00:06:42,390 --> 00:06:40,080

used

164
00:06:45,270 --> 00:06:42,400
iron pairing high performance liquid

165
00:06:48,070 --> 00:06:45,280
chromatography

166
00:06:50,230 --> 00:06:48,080
as we can see on the chromatograms

167
00:06:52,390 --> 00:06:50,240
raising the reaction temperature going

168
00:06:56,550 --> 00:06:52,400
from room

169
00:06:58,870 --> 00:06:56,560
40 degrees 70 100 and 130 degrees

170
00:07:02,230 --> 00:06:58,880
celsius

171
00:07:05,110 --> 00:07:02,240
we have observed the formation of longer

172
00:07:06,390 --> 00:07:05,120
glycine oligomers

173
00:07:09,270 --> 00:07:06,400
this is

174
00:07:11,189 --> 00:07:09,280
happening together with the formation of

175
00:07:14,870 --> 00:07:11,199
higher amounts of

176
00:07:17,430 --> 00:07:14,880
dicato piperazine a cyclic glycine dimer

177
00:07:21,110 --> 00:07:17,440
that is sometimes regarded as a dead end

178
00:07:24,550 --> 00:07:21,120
for probiotic peptide synthesis

179
00:07:26,150 --> 00:07:24,560
the total yield of all linear oligomers

180
00:07:28,870 --> 00:07:26,160
was highest when the reaction was

181
00:07:33,830 --> 00:07:28,880
carried out at 100 degrees celsius and

182
00:07:38,950 --> 00:07:35,990
in order to better understand the

183
00:07:40,070 --> 00:07:38,960
dynamics of mechanochemical peptide bone

184
00:07:43,589 --> 00:07:40,080
synthesis

185
00:07:49,270 --> 00:07:43,599
we carried out reactions starting from

186
00:07:51,830 --> 00:07:49,280
dipeptide tripeptide and dicatopirazine

187
00:07:54,230 --> 00:07:51,840
we can see on the chromatograms

188
00:07:57,430 --> 00:07:54,240

that for all these cases we observed the

189
00:07:59,350 --> 00:07:57,440
formation of odd and even number of

190
00:08:01,589 --> 00:07:59,360
oligomer lengths

191
00:08:04,390 --> 00:08:01,599
meaning that the mechanochemical peptide

192
00:08:05,830 --> 00:08:04,400
bond formation is a dynamic process with

193
00:08:09,589 --> 00:08:05,840
simultaneous

194
00:08:11,510 --> 00:08:09,599
forming and breaking of peptide bonds

195
00:08:13,749 --> 00:08:11,520
importantly

196
00:08:15,990 --> 00:08:13,759
when starting the reaction

197
00:08:18,869 --> 00:08:16,000
from dicato piperazine

198
00:08:20,869 --> 00:08:18,879
we have ascertained that it can be a

199
00:08:23,270 --> 00:08:20,879
productive intermediate

200
00:08:24,830 --> 00:08:23,280
for the synthesis of peptides as we

201
00:08:27,189 --> 00:08:24,840
observed the

202
00:08:33,029 --> 00:08:27,199
formation of

203
00:08:35,430 --> 00:08:33,039
peptides containing 10 glycine residues

204
00:08:37,110 --> 00:08:35,440
in order to unambiguously determine the

205
00:08:39,190 --> 00:08:37,120
presence of

206
00:08:41,670 --> 00:08:39,200
different oligomer length

207
00:08:43,670 --> 00:08:41,680
we have developed a method for ultra

208
00:08:46,389 --> 00:08:43,680
performance liquid chromatography

209
00:08:48,710 --> 00:08:46,399
coupled with mass spectrometer

210
00:08:51,350 --> 00:08:48,720
so first

211
00:08:54,790 --> 00:08:51,360
to separate different oligomers and then

212
00:08:56,310 --> 00:08:54,800
to detect them using a q-tof detector

213
00:08:58,470 --> 00:08:56,320

according to their

214
00:09:01,509 --> 00:08:58,480
molecular mass

215
00:09:04,389 --> 00:09:01,519
the longest oligomer detected by milling

216
00:09:08,230 --> 00:09:04,399
glycine with titanium dioxide was a

217
00:09:10,070 --> 00:09:08,240
peptide with 11 residues of glycine

218
00:09:12,310 --> 00:09:10,080
this oligomer length

219
00:09:14,550 --> 00:09:12,320
is long enough for it to establish a

220
00:09:16,550 --> 00:09:14,560
three-dimensional structure

221
00:09:19,030 --> 00:09:16,560
which is associated

222
00:09:22,470 --> 00:09:19,040
with the catalytic properties of

223
00:09:25,110 --> 00:09:22,480
peptides and proteins

224
00:09:27,750 --> 00:09:25,120
to summarize our key results

225
00:09:29,670 --> 00:09:27,760
we have successfully formed peptides

226
00:09:30,870 --> 00:09:29,680
when also using different mineral

227
00:09:34,070 --> 00:09:30,880
surfaces

228
00:09:34,829 --> 00:09:34,080
such as silicon dioxide montmorillonite

229
00:09:37,670 --> 00:09:34,839
and

230
00:09:39,910 --> 00:09:37,680
mica mechanochemical peptide bond

231
00:09:41,110 --> 00:09:39,920
formation tolerates the presence of

232
00:09:43,509 --> 00:09:41,120
moisture

233
00:09:45,670 --> 00:09:43,519
as verified by performing

234
00:09:49,110 --> 00:09:45,680
liquid-assisted grinding experiments

235
00:09:51,269 --> 00:09:49,120
where we added small amounts of water

236
00:09:53,590 --> 00:09:51,279
this was to show the robustness of

237
00:09:55,750 --> 00:09:53,600
mechanochemical route

238
00:09:57,430 --> 00:09:55,760

that does not require glovebox

239
00:09:59,910 --> 00:09:57,440
conditions

240
00:10:02,310 --> 00:09:59,920
that were certainly not available on an

241
00:10:05,509 --> 00:10:02,320
earlier scenario

242
00:10:08,230 --> 00:10:05,519
we also tried to use different amino

243
00:10:11,030 --> 00:10:08,240
acids such as l-alanine and after

244
00:10:15,670 --> 00:10:11,040
milling it with titanium dioxide we have

245
00:10:20,790 --> 00:10:18,150
when milling together glycine and

246
00:10:23,110 --> 00:10:20,800
l-alanine with titanium dioxide

247
00:10:25,190 --> 00:10:23,120
it resulted in the formation of their

248
00:10:27,269 --> 00:10:25,200
heteropeptides

249
00:10:29,910 --> 00:10:27,279
and we have successfully shown

250
00:10:35,910 --> 00:10:29,920
that it is possible to incorporate other

251
00:10:39,350 --> 00:10:36,870
again

252
00:10:42,069 --> 00:10:39,360
i want to highlight a key message for

253
00:10:43,829 --> 00:10:42,079
you to take away from this presentation

254
00:10:46,230 --> 00:10:43,839
and this is that amino acids

255
00:10:48,710 --> 00:10:46,240
oligomerized to peptides on mineral

256
00:10:52,470 --> 00:10:48,720
surfaces without the need to interact

257
00:10:53,990 --> 00:10:52,480
with water or aqueous environment

258
00:10:57,110 --> 00:10:54,000
using only

259
00:11:00,069 --> 00:10:57,120
the input of mechanical and thermal

260
00:11:01,269 --> 00:11:00,079
energy

261
00:11:02,710 --> 00:11:01,279
finally

262
00:11:04,870 --> 00:11:02,720
what are the implications of

263
00:11:06,949 --> 00:11:04,880

mechanochemical route towards peptides

264
00:11:09,190 --> 00:11:06,959
for astrobiology

265
00:11:10,870 --> 00:11:09,200
can we expect to find peptides in

266
00:11:12,790 --> 00:11:10,880
asteroid samples

267
00:11:15,910 --> 00:11:12,800
these are some of the very exciting

268
00:11:18,470 --> 00:11:15,920
questions please do get in contact with

269
00:11:20,389 --> 00:11:18,480
us if you have any additional questions