# Journal of Visualized Experiments

## Synthesis of Information-bearing Peptoids and their Sequence-directed Dynamic Covalent Self-assembly

--Manuscript Draft--

| | |
|---|---|
| Article Type: | Invited Methods Article - JoVE Produced Video |
| Manuscript Number: | JoVE60442R1 |
| Full Title: | Synthesis of Information-bearing Peptoids and their Sequence-directed Dynamic Covalent Self-assembly |
| Section/Category: | JoVE Chemistry |
| Keywords: | self-assembly; dynamic covalent chemistry; peptoid; sequence-specific; molecular ladder; kinetic trapping; Lewis acid; supramolecular structures |
| Corresponding Author: | Timothy Scott<br>University of Michigan<br>Ann Arbor, MI UNITED STATES |
| Corresponding Author's Institution: | University of Michigan |
| Corresponding Author E-Mail: | tfscott@umich.edu |
| Order of Authors: | Samuel C Leguizamon |
| | Abdulla F. Alqubati |
| | Timothy F. Scott |
| Additional Information: | |

| Question | Response |
|---|---|
| Please indicate whether this article will be Standard Access or Open Access. | Open Access (US$4,200) |
| Please indicate the **city, state/province, and country** where this article will be **filmed**. Please do not use abbreviations. | Melbourne, Victoria, Australia |

1   **TITLE:**
2   Synthesis of Information-bearing Peptoids and their Sequence-directed Dynamic Covalent Self-
3   assembly
4
5   **AUTHORS AND AFFILIATIONS:**

6   Samuel C. Leguizamon[1], Abdulla F. Alqubati[1], Timothy F. Scott[1,2]
7   [1]Department of Chemical Engineering, University of Michigan, Ann Arbor, MI, USA
8   [2]Macromolecular Science and Engineering Program, University of Michigan, Ann Arbor, MI, USA
9
10  **Corresponding author:**
11  Timothy F. Scott          (tfscott@umich.edu)
12

13  **KEYWORDS:**
14  self-assembly, dynamic covalent chemistry, peptoid, sequence-specific, molecular ladder, kinetic
15  trapping, Lewis acid, supramolecular structures
16

17  **SUMMARY:**
18  A protocol is presented for the synthesis of information-encoded peptoid oligomers and for the
19  sequence-directed self-assembly of these peptoids into molecular ladders using amines and
20  aldehydes as dynamic covalent reactant pairs and Lewis acidic rare-earth metal triflates as multi-
21  role reagents.
22

23  **ABSTRACT:**
24  This protocol presents the use of Lewis acidic multi-role reagents to circumvent kinetic trapping
25  observed during the self-assembly of information-encoded oligomeric strands mediated by
26  paired dynamic covalent interactions in a manner mimicking the thermal cycling commonly
27  employed for the self-assembly of complementary nucleic acid sequences. Primary amine
28  monomers bearing aldehyde and amine pendant moieties are functionalized with orthogonal
29  protecting groups for use as dynamic covalent reactant pairs. Using a modified automated
30  peptide synthesizer, the primary amine monomers are encoded into oligo(peptoid) strands
31  through solid-phase submonomer synthesis. Upon purification by high-performance liquid
32  chromatography (HPLC) and characterization by electrospray ionization mass spectrometry (ESI-
33  MS), sequence-specific oligomers are subjected to high-loading of a Lewis acidic rare-earth metal
34  triflate which both deprotects the aldehyde moieties and affects the reactant pair equilibrium
35  such that strands completely dissociate. Subsequently, a fraction of the Lewis acid is extracted,
36  enabling annealing of complementary sequence-specific strands to form information-encoded
37  molecular ladders characterized by matrix assisted laser desorption/ionization mass
38  spectrometry (MALDI-MS). The simple procedure outlined in this report circumvents kinetic traps
39  commonly experienced in the field of dynamic covalent assembly and serves as a platform for
40  the future design of robust, complex architectures.
41

42  **INTRODUCTION:**
43  Progress in self-assembly, the process by which small sub-units generate larger architectures
44  through thermodynamically-driven pathways, has afforded improved control over macro- and

45 supra-molecular nanostructures typically by exploiting intermolecular interactions such as π-
46 stacking and hydrogen bonding[1–4]. In particular, nucleic acids (i.e., polynucleotides) have
47 emerged as remarkably versatile nano-construction media as the high information density
48 provided by Watson-Crick base pairing permits the assembly of complex, sequence-selective
49 structures[4,5]. Whereas the inherently low strength of these transient intermolecular bonds
50 enables sub-unit rearrangement and error-correction, the resultant structures are often
51 susceptible to thermal and mechanical degradation[6]. In contrast, dynamic covalent interactions[7–
52 9], a class of covalent bond-forming reactions that are reversible or rearrangeable under mild
53 conditions and have recently been employed to yield intricate macromolecules such as ladders[10–
54 13], cages[14–16], and stacks[17], offer increased bond strengths and robust structures. Unfortunately,
55 the capacity for rearrangement and error-checking is diminished by the relatively low
56 rearrangement rates of these covalent species, curtailing their capacity for self-assembly into
57 desired products[18]. To address this kinetic trapping, catalysts or harsh reaction conditions are
58 often utilized in conjunction with simple building blocks. Here, we report a process which
59 circumvents kinetic trapping to enable the self-assembly of molecular ladders from sequence-
60 specific oligomers where the hybridization is directed by the information encoded in the oligomer
61 residue sequences.

63 Given their synthetic accessibility, poly(N-substituted glycine)s (i.e., peptoids) are employed as
64 the oligomeric precursors from which the molecular ladders are assembled[19]. Peptoids are
65 structural isomers of peptides in which pendant groups are affixed to the backbone-borne
66 nitrogen instead of being coupled with the α-carbon[20]. Using solid-phase synthesis, exact
67 placement of dynamic covalent pendant groups along the peptoid chain is readily achieved,
68 allowing for the design of precursor oligomers that can assemble into complex supramolecular
69 structures[21].

71 The dynamic covalent rearrangement of imine connectivity is employed in this procedure as the
72 imine-generating condensation reaction provides a convenient means to characterize the self-
73 assembly by mass spectrometry as each bond formed results in a mass reduction of 18 g/mol[22].
74 Furthermore, the equilibrium between the amine and aldehyde reactants and imine product can
75 be varied by altering the acid concentration. Specifically, rare-earth metal triflates are used to
76 affect equilibrium, and additionally deprotect ethylene acetal-protected aldehydes[23–25]. To note,
77 scandium triflate is already commonly used in the field of dynamic covalent self-assembly,
78 including its recent success in aiding the synthesis of covalent organic frameworks (COFs) at room
79 temperature[26,27]. Additionally, the contrasting solubility of the oligo(peptoid) sequences and the
80 rare-earth metal triflate enables equilibrium control through liquid-liquid extraction. The process
81 reported utilizes this control to circumvent the kinetic barriers preventing information-directed
82 self-assembly.

84 **PROTOCOL:**

86 CAUTION: Several chemicals used in this protocol are corrosive, flammable or toxic and should
87 only be used under a chemical fume hood. Please use appropriate personal protective equipment
88 and consult all relevant safety data sheets (SDS) before use.

89

## 1. Monomer synthesis

90

91

NOTE: Primary amines were synthesized according to published approaches.

92

93

### 1.1. Synthesis of 4-(2-aminoethyl)-N-(allylcarbonyloxy)phenylamine (Npam)[25,28]

94

95

1.1.1. Add 5.0 g (36.7 mmol) of 4-(2-aminoethyl)aniline to 150 mL of 10% acetic acid (aqueous solution, v/v).

96
97

98

NOTE: The use of weak acid enables selective protection of the aromatic amine without affecting the aliphatic amine owing to the large difference in $pK_a$ value for between the two groups.

99
100

101

1.1.2. Prepare a solution of 4.9 g (40.4 mmol; 1.1 equiv.) allyl chloroformate in 150 mL of 1,4-dioxane.

102
103

104

1.1.3. Combine the solutions in a 500 mL round bottom flask equipped with a magnetic stir bar and stir the reaction mixture at room temperature overnight.

105
106

107

1.1.4. To work up the reaction, dilute with 500 mL of deionized (DI) water and wash with diethyl ether ($Et_2O$, 300 mL × 3). Discard the organic fractions.

108
109

110

1.1.5. Adjust the aqueous phase to pH 14 by adding 2 M NaOH (aqueous solution), and extract with $Et_2O$ (150 mL × 3).

111
112

113

1.1.6. Combine the organic fractions and wash with DI water (150 mL × 3).

114

115

1.1.7. Dry over $Na_2SO_4$, then filter.

116

117

1.1.8. Evaporate to dryness under reduced pressure.

118

119

1.1.9. Confirm the identity of the isolated product, **Npam**, by nuclear magnetic resonance (NMR) spectroscopy. Expect the following results: ^{1}H NMR (500 MHz, CdCl$_3$) $\delta$: 7.31 (d, $J$ = 8.0 Hz, 2H, Ar), 7.14 (d, $J$ = 8.5 Hz, 2H, Ar), 6.65 (s, 1H, -N$H$-), 6.04 – 5.89 (m, 1H, -C$H$=CH$_2$), 5.36 (dq, $J$ = 17.1, 1.6 Hz, 1H, -CH=CH$H$), 5.26 (dq, $J$ = 10.5, 1.4 Hz, 1H, -CH=CH$H$), 4.66 (dt, $J$ = 5.8, 1.5 Hz, 2H, -C$H_2$-CH=CH$_2$), 2.94 (t, $J$ = 6.8 Hz, 2H, -C$H_2$-NH$_2$), 2.70 (t, $J$ = 6.8 Hz, 2H, -C$H_2$-Ar), 1.04 (s, 2H, -CH$_2$-N$H_2$). ^{13}C NMR (125 MHz, CD$_3$OD) $\delta$: 154.85, 137.00, 134.98, 133.51, 129.36, 119.41, 116.92, 65.62, 59.89, 43.47, 38.72.

120
121
122
123
124
125
126

127

NOTE: The product is a light yellow solid and has an overall yield of 69%. Use the product without further purification.

128
129

130

### 1.2. Synthesis of 4-(1,3-dioxacyclopent-2-yl)benzonitrile[29,30]

131

132

133    1.2.1. Dissolve 25 g (0.19 mol) of 4-cyanobenzaldehyde in 200 mL of toluene.

134

135    1.2.2. Add 42.2 mL (0.768 mmol; 4 equiv.) of ethylene glycol and 0.02 g (0.1 mmol; 0.05 mol%)
136    of toluene-*p*-sulfonic acid to the reaction mixture.

137

138    1.2.3. Stir and reflux overnight at 120 °C using a Dean-Stark trap (i.e., azeotropic distillation) to
139    remove water generated during the reaction.

140

141    1.2.4. After the reaction is complete and cooled to room temperature, add 40 mL of 5% $NaHCO_3$
142    (w/v) aqueous solution.

143

144    1.2.5. Extract the organic layer, and wash with DI water three times.

145

146    1.2.6. Dry over $Na_2SO_4$, then filter.

147

148    1.2.7. Evaporate to dryness under reduced pressure.

149

150    1.2.8. Confirm the identity of the isolated product, by NMR spectroscopy. Expect the following
151    results: $^1$H NMR (400 MHz, $CDCl_3$) $\delta$: 7.67 (d, *J* = 8.0, 2H, Ar), 7.59 (d, *J* = 8.4, 2H, Ar), 5.84 (s, 1H,
152    CH), 4.12 - 4.03 (AA'BB', 4H, $(CH_2O)_2$). $^{13}$C NMR (100 MHz, $CDCl_3$) $\delta$: 143.20, 132.34, 127.30,
153    118.72, 113.02, 102.56, 65.57.

154

155    NOTE: The product is a white crystalline solid and has an overall yield of 86%. Use the product
156    without further purification.

157

158    **1.3. Synthesis of 4-(1,3-dioxacyclopent-2-yl)benzylamine (Npal)[29]**

159

160    1.3.1. Prepare a solution of 10 g (0.057 mol) of 4-(1,3-dioxacyclopent-2-yl)benzonitrile in 100 mL
161    of anhydrous $Et_2O$.

162

163    1.3.2. Carefully add 4.3 g (0.11 mol; 2 equiv.) of $LiAlH_4$ to 100 mL of anhydrous $Et_2O$ in a round
164    bottom flask at 0 °C. Stir to create a well-mixed suspension and seal the system under an inert
165    atmosphere using an argon-filled balloon. Carefully quench with ethanol any residual $LiAlH_4$ on
166    equipment used for weighing.

167

168    CAUTION: Lithium aluminum hydride ($LiAlH_4$) is a mild pyrophore; handle under inert gas and
169    protect from moisture.

170

171    1.3.3. Add the 4-(1,3-dioxacyclopent-2-yl)benzonitrile solution slowly using an addition funnel or
172    a syringe pump while maintaining the reaction mixture at a temperature of 0 °C.

173

174    1.3.4. Stir the reaction mixture for 4 h at 0 °C, followed with 12 h at room temperature.

175

176    1.3.5. After the reaction is complete and cooled to 0 °C, slowly add 95% ethanol (30 mL). Further

177     quench by adding 50% ethanol in water (v/v, 20 mL). A bubbler can be used to monitor the
178     quenching process.

180     NOTE: Add additional anhydrous $Et_2O$ as needed to maintain an adequate stirring rate.

182     1.3.6. Separate the ether supernatant and evaporate to dryness under reduced pressure.

184     1.3.7. Filter the resultant oil through a 0.45 µm syringe filter.

186     1.3.8. Confirm the identity of the isolated product, **Npal**, by NMR spectroscopy. Expect the
187     following results: $^1$H NMR (400 MHz, $CDCl_3$) $\delta$: 7.44 (d, $J$ = 8, 2H, Ar), 7.32 (d, $J$ = 8, 2H, Ar), 5.80
188     (s, 1H, CH), 4.14 - 4.0 (AA'BB', 4H, $(CH_2O)_2$), 3.87 (s, 2H, $-CH_2-NH_2$). $^{13}$C NMR (100 MHz, $CDCl_3$) $\delta$:
189     144.53, 136.53, 127.16, 126.77, 103.72, 65.39, 46.35.

191     NOTE: The product is a yellow oil and has an overall yield of 70%. Use the product without further
192     purification.

194     **1.4. Synthesis of 2-(2-ethoxyethoxy)ethyl tosylate**[29,31]

196     1.4.1. Add 20 g (0.15 mol) of diethylene glycol monoethyl ether and 50 mL of tetrahydrofuran
197     (THF) to a 250 mL round bottom flask with a magnetic stirrer.

199     1.4.2. Cool to 0 °C and seal the system under an inert atmosphere using an argon-filled balloon.

201     1.4.3.  Add 50 mL of 6 M aqueous NaOH (2 equiv.).

203     1.4.4. Dissolve 54 g (0.28 mol; 2 equiv.) of tosyl chloride in 80 mL of THF and add the solution to
204     the reaction mixture dropwise. Stir for 1 h at 0 °C.

206     1.4.5. Allow the reaction mixture to reach room temperature and stir for another hour.

208     1.4.6. Extract the reaction mixture with $Et_2O$ (400 mL).

210     1.4.7. Wash the organic layer with 1 M NaOH, then with DI water.

212     1.4.8. Dry over $Na_2SO_4$, then filter.

214     1.4.9. Evaporate to dryness under reduced pressure.

216     1.4.10. Confirm the identity of the isolated product by NMR spectroscopy. Expect the following
217     results: $^1$H NMR (400 MHz, $CDCl_3$) $\delta$: 7.78 (d, $J$ = 8.0, 2H, -S-C=CH-C$H$), 7.33 (d, $J$ = 8.5, 2H, -S-C=CH-
218     C$H$), 4.15 (t, $J$ = 5.0, 2H, $-CH_2-CH_2-O-Ts$), 3.68 (t, $J$ = 5.0, 2H, $CH_2-CH_2-O-Ts$), 3.60-3.42 (m, 6H, O-
219     $CH_2-CH_2-O-CH_2-CH_3$), 2.43 (s, 3H, $C-CH_3$), 1.17 (t, $J$ = 7.0, 3H, $O-CH_2-CH_3$). $^{13}$C NMR (100 MHz,
220     $CDCl_3$) $\delta$: 144.79, 132.95, 130.26, 129.80, 127.90, 126.95, 70.75, 69.68, 69.29, 68.61, 66.57, 21.56,

221 15.11.

222

223 NOTE: The product is a colorless liquid and has an overall yield of 98%. Use the product without
224 further purification.

225

226 **1.5. Synthesis of 2-(2-ethoxyethoxy)ethyl azide[29,31]**

227

228 1.5.1. Dissolve 40 g (0.14 mol) of 2-(2-ethoxyethoxy)ethyl tosylate in 250 mL of
229 dimethylformamide (DMF) in a round bottom flask with a magnetic stirrer. Seal the system under
230 an inert atmosphere using an argon-filled balloon.

231

232 1.5.2. Add 32 g (0.49 mol; 3.5 equiv.) of $NaN_3$ to the reaction mixture.

233

234 CAUTION: Do not use a metal spatula when weighing $NaN_3$. $NaN_3$ may react with lead and copper
235 which results in the formation of highly explosive metal azides. It is acutely toxic and fatal if
236 swallowed or in contact with skin.

237

238 1.5.3. Heat the reaction mixture to 60 °C and let it run for 36 h. Then cool to room temperature.

239

240 1.5.4. Dilute with large amount of water (500 mL) and extract with $Et_2O$ (150 mL × 3).

241

242 1.5.5. Isolate the organic layer and perform water washes.

243

244 1.5.6. Dry over $Na_2SO_4$, then filter.

245

246 1.5.7. Evaporate to dryness under reduced pressure.

247

248 1.5.8. Confirm the identity of the isolated product by NMR spectroscopy. Expect the following
249 results: ^{1}H NMR (400 MHz, $CDCl_3$) $\delta$: 3.64 (m, 4H, O-C$H_2$-C$H_2$-O), 3.58 (m, 2H, $N_3$-CH$_2$-C$H_2$-O), 3.51
250 (q, $J$ = 7.5, 2H, O-C$H_2$-CH$_3$), 3.38 (t, $J$ = 5.0, 2H, $N_3$-C$H_2$-CH$_2$-O), 1.19 (t, $J$ = 7.5, 3H, O-CH$_2$-C$H_3$). [13]C
251 NMR (100 MHz, $CDCl_3$) $\delta$: 70.70, 69.97, 69.80, 66.63, 50.60, 15.08.

252

253 NOTE: The product is a yellow liquid and has an overall yield of 85%. Use the product without
254 further purification.

255

256 **1.6. Synthesis of 2-(2-ethoxyethoxy)ethylamine (Neee)[29,31]**

257

258 1.6.1. Dissolve 20 g (0.13 mol) of 2-(2-ethoxyethoxy)ethyl azide in 160 mL of THF in a 500 mL
259 round bottom flask with a magnetic stirrer.

260

261 1.6.2. Add 40 g (0.15 mol, 1.1 equiv.) of triphenylphosphine and stir overnight at room
262 temperature under argon.

263

264 1.6.3. Quench the reaction mixture with water (220 mL) and allow it to stir for another day.

265

266  1.6.4. Wash the resulting solution with toluene, followed by dichloromethane (DCM).

267

268  1.6.5. Evaporate the aqueous layer under vacuum.

269

270  1.6.6. Confirm the identity of the isolated product, **Neee**, by NMR spectroscopy. Expect the
271  following results: $^1$H NMR (400 MHz, CDCl$_3$) $\delta$: 3.62-3.42 (m, 8H, NH$_2$-CH$_2$-C$H_2$-O-C$H_2$-CH$_2$-O-C$H_2$-
272  CH$_3$), 2.82 (m, 2H, NH$_2$-C$H_2$-CH$_2$-O), 1.48 (s, 2H, N$H_2$), 1.16 (t, $J$ = 7.5, 3H, O-CH$_2$-C$H_3$). $^{13}$C NMR
273  (100 MHz, CDCl$_3$) $\delta$: 73.14, 70.72, 69.64, 66.45, 41.35, 15.00.

274

275  NOTE: The product is a yellow liquid and has an overall yield of 58%. Use the product without
276  further purification.

277

278  **2. Solid-phase submonomer synthesis of oligo(peptoids)**

279

280  NOTE: The submonomer approach to solid-phase synthesis (SPS) was employed as it enables the
281  production of sequence-specific oligomers with high coupling efficiency. An automated peptide
282  synthesizer was adapted to rapidly generate oligo(peptoids). Settings may require modification
283  for different instrumentation.

284

285  **2.1. Preparation**

286

287  2.1.1. Weigh 0.125 g of Fmoc-Photolabile SS resin (0.8 mmol/g typical loading, 0.1 mmol scale,
288  100-200 mesh, 1% DVB) and add to a fritted automated synthesizer reaction vessel. Insert the
289  vessel into the microwave portion of the synthesizer.

290

291  2.1.2. Fill the main solvent bottle with DMF and the deprotection bottle with 20% 4-
292  methylpiperidine in DMF (v/v). Empty waste.

293

294  2.1.3. Prepare 1 M solutions of bromoacetic acid and N,N'-diisopropylcarbodiimide (DIC) in DMF
295  with total volumes of 1.5 mL × (number of residues in sequence) + 5 mL. The extra 5 mL ensures
296  that no air enters the machine. Add 0.47 mL of acetic anhydride to DMF to make a 5 mL capping
297  solution.

298

299  CAUTION: DIC can cause serious eye damage, skin irritation and sensitization, and respiratory
300  irritation and sensitization.

301

302  2.1.4. Prepare 0.5 M solutions of each primary amine (Npam, Npal, Neee, and Nma (2-
303  methoxyethylamine)) in N-methyl-2-pyrrolidone (NMP) used for the displacement step. Total
304  volumes of the primary amine solutions should be 2.5 mL × (number of residues of the
305  appropriate primary amine) + 2.5 mL.

306

307  2.1.5. Add all solutions to the automated synthesizer manifold.

308

**2.2. Synthesis**

NOTE: Perform using an automated peptide synthesizer.

2.2.1. Swell the resin at room temperature for 5 min with 10 mL of DMF. Drain the reaction vessel.

2.2.2. Cleave the Fmoc group with 3 mL of the 20% 4-methylpiperidine solution for 30 s at 75 °C and 90 s at 90 °C. Drain the vessel. Repeat. Wash with DMF (2 mL × 2).

2.2.3. Add to the vessel 1.5 mL of the bromoacetic acid solution and 1.5 mL of the DIC solution. Heat the reaction at 75 °C for 4.5 min to perform the bromoacetylation reaction. Wash the resin (5 mL of DMF × 3).

2.2.4. Perform the displacement reaction by addition of 2.5 mL primary amine monomer solution to the reaction vessel. Heat at 75 °C for 4.5 min. Wash resin (5 mL of DMF× 3).

2.2.5. Repeat steps 2.2.3. and 2.2.4. while sequentially substituting the primary amine monomer used in step 2.2.4. to grow oligo(peptoid) chain in a sequence-specific manner.

2.2.6. After the final displacement step, cap the sequence by adding 2.5 mL of the acetic anhydride solution and 2 mL of the DIC solution. Heat at 50 °C for 2 min. Wash the resin (5 mL of DMF × 6).

2.2.7. Transfer the resin to a fritted glass reaction vessel equipped with a 3-way stopcock. The glass reaction vessel should be previously siliconized to prevent beads from adhering to the walls. Silanize the walls by filling the vessel with a 5% dichlorodimethylsilane in dichloroethane (DCE) (v/v) solution to the top and letting it sit for 30 min. Drain the vessel and wash with DCE and methanol. Dry glass vessel before use.

2.2.8. Wash the resin with DCM (5 mL × 3), bubbling with $N_2$ through one arm and pulling vacuum with another.

2.2.9. Dry and store resin and attached oligo(peptoid) until deprotection and cleavage.

**2.3. Alloc-amine deprotection and cleavage from resin**

2.3.1. If the resin has been stored for more than a day, reswell the resin by bubbling with 5 mL of DMF for 10 min. Then drain the vessel and add a small magnetic stir bar.

2.3.2. Add 3 mL of dry DCM to the glass peptide vessel.

2.3.3. Weigh 0.1 equivalents of tetrakis(triphenylphosphine)palladium(0) and 25 equivalents of phenylsilane per Alloc-group. Use a clamp to position the reaction vessel at an angle above a stir

plate such that the resin undergoes gentle agitation while remaining suspended in the solvent. To prevent the DCM from evaporating, cap the reaction vessel.

2.3.4. After 1 h, filter off the solution and wash the resin with DCM (3 × 5 mL).

2.3.5. Repeat steps 2.3.2. and 2.3.3.

2.3.6. Rinse the resin sequentially with methanol and DCM twice.

2.3.7. Transfer the resin and magnetic stir bar to a 20 mL vial.

2.3.8. Submerge the resin in DMF, stir, and cleave under irradiation for 36 h at approximately 25 mW.cm$^{-2}$ with 405 nm. A small portion of resin can be cleaved and characterized in ESI-MS before this step to ensure complete Alloc deprotection of amine. If any Alloc groups remain, repeat steps 2.3.2 and 2.3.3.

2.3.9. Separate liberated oligo(peptoid) from resin via a syringe filter. Remove solvent under vacuum.

**2.4. Purification and characterization of oligo(peptoids)**

2.4.1. Reconstitute the peptoids in a 50/50 mixture of water/acetonitrile.

2.4.2. Purify with reverse-phase preparative HPLC (C18). Combine purified fractions, freeze, and lyophilize to yield off-white powder. The powder can be stored for further use.

2.4.3. Analyze with ESI-MS after purification.

2.4.4. Perform MALDI mass spectrometry in reflectron positive ion mode. Mix 2 μL of a solution of the sample (1 mM) with 6 μL of a mixture of 10 mg of matrix [2-(4-hydroxyphenylazo)benzoic acid (HABA)] in 200 μL of acetonitrile. Spot on a MALDI sample plate and allow to air dry.

2.4.5. For purity, perform analytical HPLC of purified oligo(peptoids).

**3. Sequence-selective ladder self-assembly**

**3.1. Self-assembly through dissociation/extraction/annealing**

3.1.1. Prepare 10 mM stock solutions of each oligo(peptoid) sequence used for self-assembly and a 10 mM stock solution of scandium triflate (Sc(OTf)$_3$) in anhydrous acetonitrile.

3.1.2. To a 3 mL vial equipped with a magnetic stir bar, add 20 μL of each peptoid stock solution. Add 1.5 eq of Sc(OTf)$_3$ per potential imine bond from the stock solution. Add enough water and acetonitrile to form a 200 μL 2% (v/v) of water/acetonitrile solution total.

397

398   3.1.3. Stir gently at 70 °C for 2 h for acetal-deprotection of the aldehyde and dissociation of all
399   strands.

401   3.1.4. Charge the vial with 200 μL of chloroform and 2 mL of water. Shake gently.

403   3.1.5. Allow the mixture to stand (at least 15 min) and, upon complete phase separation, extract
404   the organic layer with a microliter syringe.

406   3.1.6. Stir in a new vial at 70 °C for oligomer annealing, typically 6 h. Ladder hybridization can
407   also be performed at room temperature but over a longer period.

409 **3.2. Characterization of self-assembled species**

411 3.2.1. Perform MALDI-TOF mass spectrometry on the reaction mixture solutions after steps
412 3.1.3., 3.1.5., and 3.1.6. to monitor the reaction. If hybridization is incomplete, add 1.5 eq of
413 Sc(OTf)$_3$ per potential imine bond from the stock solution and repeat steps 3.1.3-3.1.6. until
414 complete.

416 3.2.2. Dry the sample under a steady stream of nitrogen and reconstitute in 1 mL of 2% nitric acid
417 (aqueous solution, v/v). Dilute $4 \times 10^6$-fold with HPLC water. Determine post-extraction scandium
418 concentration with inductively coupled plasma mass spectrometry (ICP-MS).

420 **REPRESENTATIVE RESULTS:**
421 To demonstrate the ability of information-encoded peptoids to undergo sequence-selective
422 dynamic covalent self-assembly into molecular ladders, a representative strand, H$_2$N-[Npam-
423 Neee-Npal-Neee]$_2$-Npam-Nma, was synthesized and hybridized with its complementary peptoid
424 sequence. The monomers Npam and Npal (characterized by $^1$H NMR (500 MHz), **Figure 1**) were
425 employed as dynamic covalent reactant pairs with Neee aiding solubility of final self-assembled
426 products. Additionally, the incorporation of the commercially available Nma monomer enables a
427 mass differentiation between the two complementary sequences. Upon completion of the solid-
428 phase submonomer synthesis, the Alloc-group was removed with Pd(PPh$_3$)$_4$. Prior to and after
429 deprotection, portions of the resin were cleaved under 405 nm light and characterized by ESI-MS
430 (**Figure 2**). The sequence was purified by prep HPLC, lyophilized to achieve an off-white powder,
431 and purity confirmed with analytical HPLC (**Figure 3**). The oligo(peptoid) was subsequently
432 hybridized with its complementary sequence, H$_2$N-[Npal-Neee-Nam-Neee]$_2$-Npal, to afford an in-
433 registry ladder confirmed by MALDI-MS (**Figure 4**).

435 **FIGURE AND TABLE LEGENDS:**
436 **Figure 1. Monomer synthetic schemes and $^1$H-NMR spectra. (A)** Monomer synthetic schemes
437 with reagents and conditions: **(i)** allyl chloroformate, 10% aqueous acetic acid, 1,4-dioxane, room
438 temperature, overnight; **(ii)** ethylene glycol, toluene-p-sulfonic acid, toluene, reflux, overnight;
439 **(iii)** LiAlH$_4$, anhydrous Et$_2$O, 0 °C for 4 h then room temperature for 12 h; **(iv)** tosyl chloride, THF,
440 0 °C; **(v)** NaN$_3$, DMF, 60 °C, 36 h; **(vi)** triphenylphosphine, THF, overnight. **(B)** Monomer $^1$H-NMR

441  spectra (500 MHz, CDCl₃): **(i)** 4-(2-aminoethyl)-N-(allylcarbonyloxy)phenylamine (Npam); **(ii)** 4-
442  (1,3-dioxacyclopent-2-yl)benzylamine (Npal); **(iii)** 2-(2-ethoxyethoxy)ethylamine (Neee).

444  **Figure 2. Synthesis and deprotection of a sequence-specific oligo(peptoid). (A)** Structures of
445  H₂N-[Npam-Neee-Npal-Neee]₂-Npam-Nma before and after Alloc-protecting group removal with
446  accompanying **(B)** ESI mass spectrum.

448  **Figure 3. Purification and characterization of an information-encoded peptoid. (A)** HPLC
449  chromatogram of the strand purification by preparative HPLC with a linear gradient of
450  acetonitrile (MeCN) and water: (1) 30% MeCN, 0.1-2.1 min; (2) 30-95% MeCN, 2.1-16.1 min; (3)
451  95% MeCN, 16.1-23.1 min; (4) 95% MeCN, 23.1-26.1 min. Peaks *i* and *ii* correspond to low
452  molecular weight reaction by-products, primarily DIC-urea, and oligomeric species including the
453  desired product, respectively. **(B)** Analytical HPLC chromatogram and **(C)** ESI mass spectrum of
454  H₂N-[Npam-Neee-Npal-Neee]₂-Npam-Nma after lyophilization.

456  **Figure 4. Self-assembly of H₂N-[Npam-Neee-Npal-Neee]₂-Npam-Nma and its complementary**
457  **sequence, H₂N-[Npal-Neee-Nam-Neee]₂-Npal. (A)** Structures of the two sequences and the
458  resulting sequence-driven assembly. **(B)** MALDI mass spectrum of the molecular ladder following
459  annealing at room temperature overnight. Masses: expected $[M+Na]^+$ = 3306.7, found 3306.0;
460  expected $[M_{-1\,imine}+Na]^+$ = 3324.7, found 3323.9; expected $[M_{-2\,imine}+Na]^+$ = 3342.7, found 3342.8;
461  expected $[M_{-2\,imine}+CH_3OH+H]^+$ = 3352.8, found 3352.0.

463  **DISCUSSION:**
464  The technique herein describes the dynamic covalent assembly of information-bearing peptoid
465  oligomers, where information is encoded in the sequence of their pendant groups. The use of an
466  Alloc-protected amine monomer in conjunction with an ethylene acetal-protected aldehyde
467  monomer allows for orthogonal deprotection, enabling Alloc deprotection on bead and acetal
468  deprotection in situ during the self-assembly reaction, thereby ensuring the synthesized
469  sequences do not prematurely react prior to oligomer purification and characterization.
470  Importantly, the solid-phase synthesis is performed using a photolabile resin to enable oligomer
471  cleavage from the bead under UV or violet light irradiation, precluding premature deprotection
472  of the acid-labile, ethylene acetal-based protecting group. Several alternative deprotection
473  schemes might be considered. For example, we initially employed dual acid-labile protecting
474  groups (Boc-amine and ethylene acetal-aldehyde) with the intention of in situ deprotection by a
475  strong acid followed with neutralization to allow the self-assembly reaction to proceed; however,
476  this approach resulted in the immediate generation of precipitate upon addition of base.
477  Alternatively, protection of the amine with a photolabile protecting group, 2-(2-
478  nitrophenyl)propoxycarbonyl (NPPOC), was envisioned as the aldehyde could be selectively
479  deprotected upon treatment with trifluoroacetic acid (TFA) prior to purification. Unfortunately,
480  in situ photolysis of the protecting group with UV light did not afford quantitative deprotection,
481  even in the presence of photosensitizers and after extended irradiation periods[25].
482  Trimethylsilylethoxycarbonyl (i.e., Teoc) can be employed as an amine protecting group and is
483  subject to cleavage upon treatment with rare-earth metal triflates; however, quantitative Teoc
484  deprotection requires much higher rare-earth metal triflate loadings than that necessary for

485 ethylene acetal deprotection. For this protocol, Teoc-amines may be used, but the Lewis acid
486 concentration must be adjusted accordingly as sub-quantitative amine deprotection could prove
487 problematic for larger self-assembled structures. Aliphatic functional groups were briefly
488 considered, but deprotection of aliphatic aldehydes requires harsh conditions that truncate
489 peptoid sequences[32, 33].

491 Incorporation of Neee and Nma as inert spacer residues serve to improve oligomer solubility and
492 enable facile mass-tagging of the precursor oligomers to afford ready identification of the
493 generated species by mass spectroscopy. Furthermore, given the 'Σ-strand' conformation of
494 peptoids where adjacent backbone segments adopt opposing rotational states to form a linear,
495 twist-free oligomer[34,35], sequences incorporating alternating dynamic covalent and inert spacer
496 residues facilitates a structure in which reactive pendant groups are oriented in the same
497 direction. Given the versatility of the submonomer method, a large and diverse library of primary
498 amines can be employed to further modify the peptoid oligomers but may require adjustments
499 to the protocol to maintain high coupling efficiency.

501 Whereas oligo(peptoids) can be synthesized manually in a glass reaction vessel[19], automation of
502 the process decreases the time for each residue addition from several hours to half an hour.
503 Additionally, automation diminishes the quantity of monomer and wash solvent waste,
504 particularly desirable when using primary amine monomers that are not commercially available.
505 Although Alloc cleavage from the protected-amine residues is an efficient reaction, palladium
506 oxidation can result in incomplete deprotection. Consequently, it is suggested to test cleave a
507 portion of the resin and characterize the extent of deprotection with ESI-MS. For test cleavages,
508 30 min under 405 nm irradiation releases sufficient peptoid for mass spectrometry. Partial
509 deprotection can be limited with the use of anaerobic conditions or repeating the deprotection
510 reaction.

512 Whereas this article focuses on Sc(OTf)$_3$ as a multi-role reagent, other rare-earth metal triflates,
513 such as ytterbium triflate, have been shown to successfully mediate the information-directed
514 assembly of molecular ladders. Notably, Sc(OTf)$_3$ is the most Lewis acidic of the rare-earth metal
515 triflates; thus, owing to the reduced catalytic ability afforded by other rare-earth metal
516 triflates[24,36], greater equivalents may be required to effect complete ethylene acetal
517 deprotection and strand dissociation. The number of equivalents required can be determined
518 with MALDI mass spectrometry by observing point at which strands completely dissociate.
519 Dissociation is critical in the self-assembly process and is analogous to the melting of nucleic acid
520 strands at raised temperature. The subsequent extraction of catalyst enables the formation and
521 disruption of dynamic covalent pairings propelling the assembly of sequence-specific duplexes.
522 This gradual annealing of the oligomeric strands circumvents the kinetic trapping (which, for
523 molecular ladders, can yield out-of-registry species or incorrectly pair sequences) experienced by
524 other methods.

526 Chloroform is an excellent solvent as phase separation in the chloroform/acetonitrile/water
527 ternary system used here promotes the partial extraction of Lewis acid without resulting in
528 precipitation of self-assembled structures[37]. Additionally, chloroform is one of the few solvents

529 that promotes imine formation while maintaining molecular ladder solubility. Trace amounts of
530 out-of-registry and incorrectly paired duplexes can often be observed owing to the dynamic
531 nature of the system. Although this system is largely unaffected by small variation in rare-earth
532 metal triflate concentrations upon extraction, on occasion, insufficient catalyst extraction
533 generates a significant portion of incomplete hybridization and non-specific oligomer couplings.
534 In this case, it is generally preferable to first re-dissociate with a further 1.5 equivalents of catalyst
535 and then extract a second time rather than to re-extract immediately, as the complete
536 dissociation of single strands is vital to the process. To simultaneously assemble several unique
537 information-encoded molecular ladders, it may be necessary to increase the concentration of the
538 rare-earth metal triflate stock solution used to maintain equivalents and total reaction volume.
539
540 While these self-assemblies are primarily characterized by mass spectrometry, other techniques
541 including fluorescence resonance energy transfer (FRET) are possible. Limitations include
542 quantity of material necessary, affordability of monomers, and signal-to-noise ratio. Techniques
543 requiring solvents, such as $^1$H NMR, can additionally suffer from insolubility of self-assembled
544 structures. Furthermore, rare-earth metal triflate concentrations post-extraction can be
545 determined through such methods as ICP-MS or $^{19}$F NMR with an internal standard.
546
547 As progress towards improved control over macro- and supra-molecular nanostructures and
548 materials proceeds, the challenge of designing and fabricating regular, but modifiable,
549 assemblies arises. The protocol described in this report provides a pathway to achieve such
550 nanostructures through sequence-selective assemblies via dynamic covalent interactions.
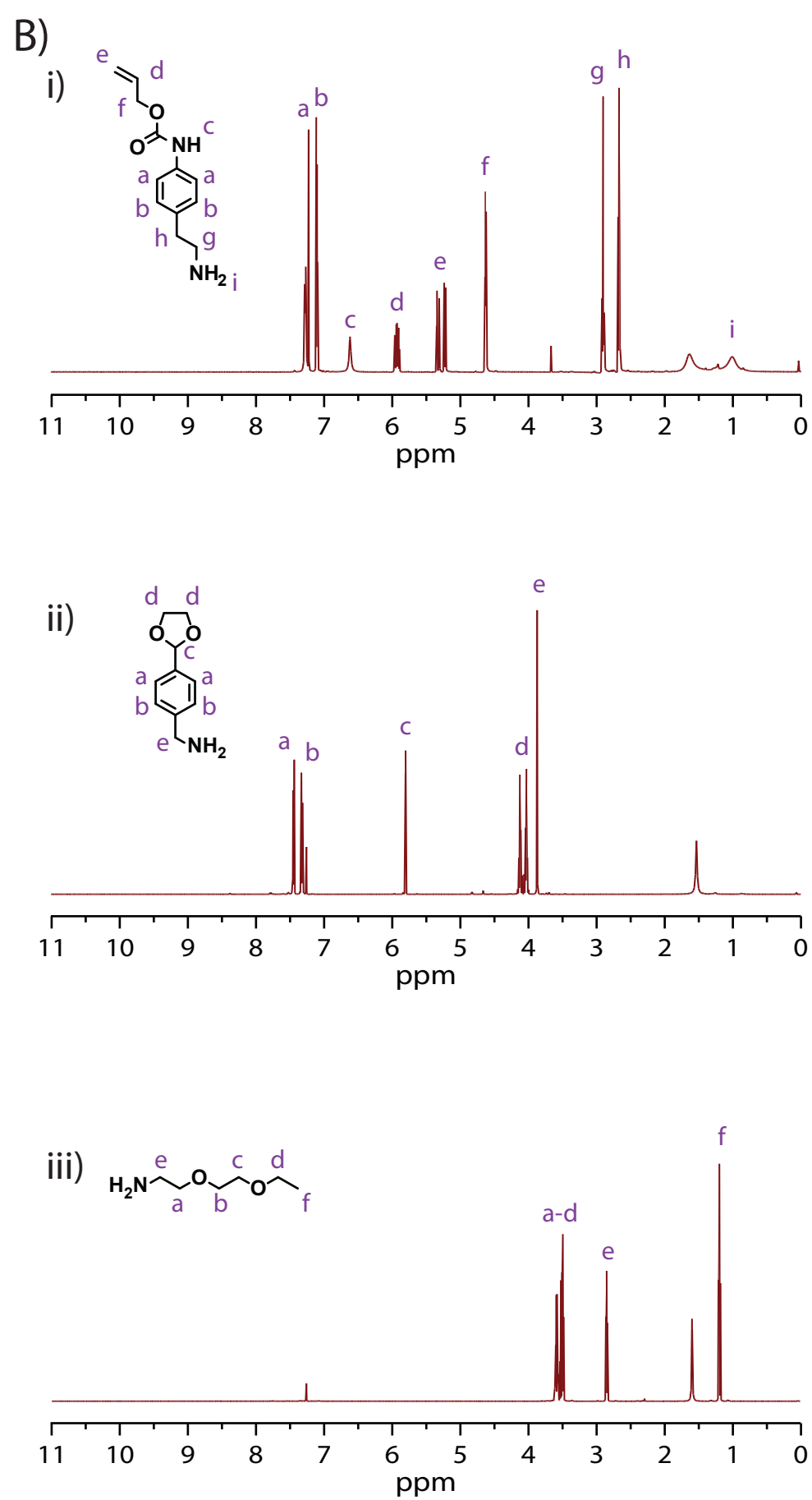551

557

558 **DISCLOSURES:**
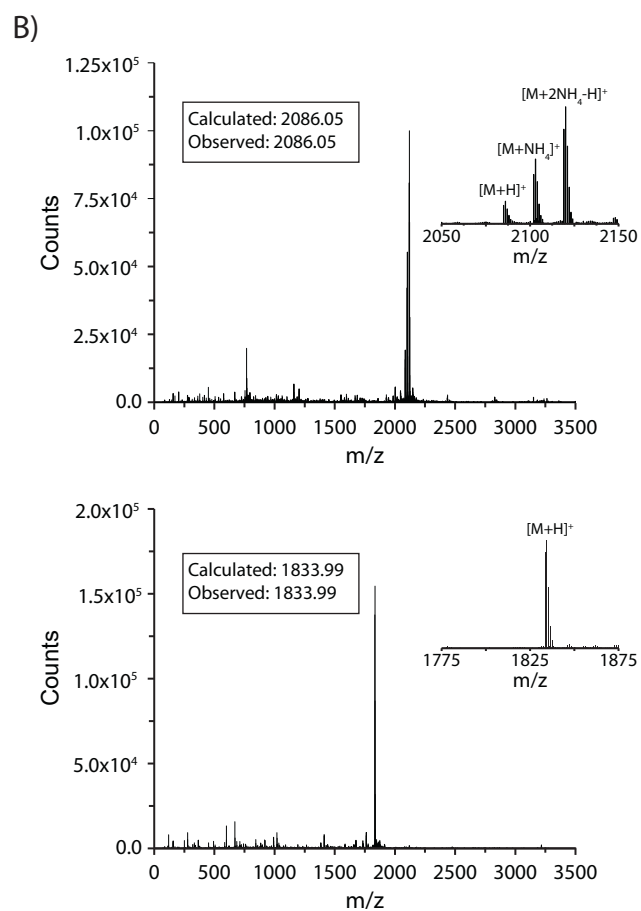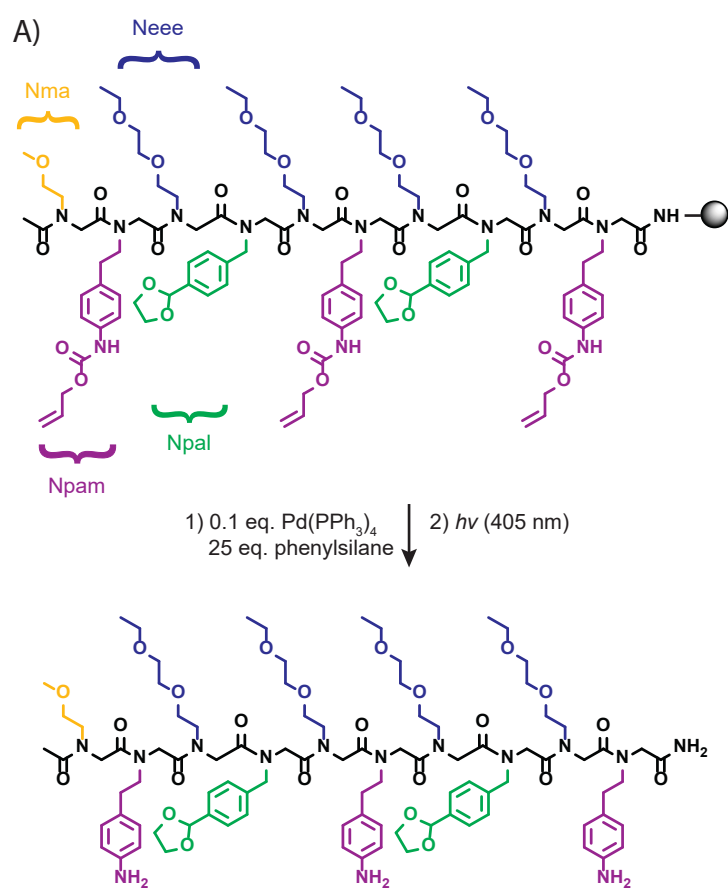559 The authors have nothing to disclose.
560

561 **REFERENCES:**
562 1.    Seeman, N.C. Nucleic acid junctions and lattices. *Journal of Theoretical Biology*. **99** (2),
563       237–247 (1982).
564 2.    Rothemund, P.W.K. Folding DNA to create nanoscale shapes and patterns. *Nature*. **440**
565       (March), 297–302 (2006).
566 3.    Watt, A.A.R., Bothma, J.P., Meredith, P. The supramolecular structure of melanin. *Soft*
567       *Matter*. **5** (19), 3754–3760 (2009).
568 4.    Tørring, T., Voigt, N. V., Nangreave, J., Yan, H., Gothelf, K. V. DNA origami: A quantum leap
569       for self-assembly of complex structures. *Chemical Society Reviews*. **40** (12), 5636–5646
570       (2011).
571 5.    Wei, B., Dai, M., Yin, P. Complex shapes self-assembled from single-stranded DNA tiles.
572       *Nature*. **485** (7400), 623–626 (2012).

573    6.    Clausen-Schaumann, H., Rief, M., Tolksdorf, C., Gaub, H.E. Mechanical stability of single
574         DNA molecules. *Biophysical Journal*. **78** (4), 1997–2007 (2000).

575    7.    Rowan, S.J., Cantrill, S.J., Cousins, G.R.L., Sanders, J.K.M., Stoddart, J.F. *Dynamic covalent*
576         *chemistry. Angewandte Chemie - International Edition*. **41** (6). (2002).

577    8.    Jin, Y., Yu, C., Denman, R.J., Zhang, W. Recent advances in dynamic covalent chemistry.
578         *Chemical Society Reviews*. **42** (16), 6634–6654 (2013).

579    9.    Furgal, J.C., Dunn, M., Wei, T., Scott, T.F. Emerging Applications of Dynamic Covalent
580         Chemistry from Macro- to Nanoscopic Length Scales. *Dynamic Covalent Chemistry:*
581         *Principles, Reactions, and Applications*. 389–434 (2017).

582   10.    Hartley, C.S., Elliott, E.L., Moore, J.S. Covalent assembly of molecular ladders. *Journal of*
583         *the American Chemical Society*. **129** (15), 4512–4513 (2007).

584   11.    Wei, T., Furgal, J.C., Jung, J.H., Scott, T.F. Long, self-assembled molecular ladders by
585         cooperative dynamic covalent reactions. *Polymer Chemistry*. **8** (3), 520–527 (2017).

586   12.    Dunn, M.F., Wei, T., Scott, T.F., Zuckermann, R.N. Aqueous dynamic covalent assembly of
587         molecular ladders and grids bearing boronate ester rungs. *Polymer Chemistry*. (18), 2337–
588         2343 (2019).

589   13.    Furgal, J.C., Dijck, J.M. Van, Leguizamon, S.C., Scott, T.F. Accessing sequence specific hybrid
590         peptoid oligomers with varied pendant group spacing. *European Polymer Journal*. (118),
591         306–311 (2019).

592   14.    Tozawa, T. *et al.* Porous organic cages. *Nature Materials*. **8** (12), 973–978 (2009).

593   15.    Tian, J., Thallapally, P.K., Dalgarno, S.J., McGrail, P.B., Atwood, J.L. Amorphous molecular
594         organic solids for gas adsorption. *Angewandte Chemie - International Edition*. **48** (30),
595         5492–5495 (2009).

596   16.    Jin, Y., Wang, Q., Taynton, P., Zhang, W. Dynamic covalent chemistry approaches toward
597         macrocycles, molecular cages, and polymers. *Accounts of Chemical Research*. **47** (5), 1575–
598         1586 (2014).

599   17.    Ren, F., Day, K.J., Hartley, C.S. Two- and three-tiered stacked architectures by covalent
600         assembly. *Angew. Chem. Int. Ed.* **55** (30), 8620–8623 (2016).

601   18.    Elliott, E.L., Hartley, C.S., Moore, J.S. Covalent ladder formation becomes kinetically
602         trapped beyond four rungs. *Chemical Communications*. **47** (17), 5028–5030 (2011).

603   19.    Tran, H., Gael, S.L., Connolly, M.D., Zuckermann, R.N. Solid-phase submonomer synthesis
604         of peptoid polymers and their self-assembly into highly-ordered nanosheets. *Journal of*
605         *Visualized Experiments*. (57), 1–6 (2011).

606   20.    Zuckermann, R.N. Peptoid origins. *Biopolymers*. **96** (5), 545–555 (2011).

607   21.    Sun, J., Zuckermann, R.N. Peptoid polymers: A highly designable bioinspired material. *ACS*
608         *Nano*. **7** (6), 4715–4732 (2013).

609   22.    Belowich, M.E., Stoddart, J.F. Dynamic imine chemistry. *Chemical Society Reviews*. **41** (6),
610         2003–2024 (2012).

611   23.    Giuseppone, N., Schmitt, J.L., Schwartz, E., Lehn, J.M. Scandium(III) catalysis of
612         transimination reactions. Independent and constitutionally coupled reversible processes.
613         *Journal of the American Chemical Society*. **127** (15), 5528–5539 (2005).

614   24.    Shū, K. Scandium triflate in organic synthesis. *European Journal of Organic Chemistry*. **1999**
615         (1), 15–27 (1999).

616   25.    Wei, T., Furgal, J.C., Scott, T.F. In situ deprotection and dynamic covalent assembly using a

617        dual role catalyst. *Chemical Communications*. **53** (27), 3874–3877 (2017).

618   26.   Matsumoto, M. *et al.* Rapid, low temperature formation of imine-linked covalent organic
619        frameworks catalyzed by metal triflates. *Journal of the American Chemical Society*. **139**
620        (14), 4999–5002 (2017).

621   27.   Ma, X., Scott, T.F. Approaches and challenges in the synthesis of three-dimensional
622        covalent-organic frameworks. *Communications Chemistry*. In Press (2018).

623   28.   Perron, V., Abbott, S., Moreau, N., Lee, D., Penney, C., Zacharie, B. A method for the
624        selective protection of aromatic amines in the presence of aliphatic amines. *Synthesis*. (2),
625        283–289 (2009).

626   29.   Wei, T., Jung, J.H., Scott, T.F. Dynamic covalent assembly of peptoid-based ladder
627        oligomers by vernier templating. *Journal of the American Chemical Society*. **137** (51),
628        16196–16202 (2015).

629   30.   Ouari, O., Chalier, F., Bonaly, R., Pucci, B., Tordo, P. Synthesis and spin-trapping behaviour
630        of glycosylated nitrones. *Journal of the Chemical Society, Perkin Transactions 2*. (10), 2299–
631        2308 (1998).

632   31.   Sun, J., Stone, G.M., Balsara, N.P., Zuckermann, R.N. Structure-conductivity relationship
633        for peptoid-based PEO-mimetic polymer electrolytes. *Macromolecules*. **45** (12), 5151–
634        5156 (2012).

635   32.   Sartori, G., Ballini, R., Bigi, F., Bosica, G., Maggi, R., Righi, P. Protection (and deprotection)
636        of functional groups in organic synthesis by heterogeneous catalysis. *Chemical Reviews*.
637        **104** (1), 199–250 (2004).

638   33.   Kim, S. *et al.* Unusual truncation of N-acylated peptoids under acidic conditions. *Organic*
639        *& biomolecular chemistry*. **12** (28), 5222–6 (2014).

640   34.   Mannige, R. V. *et al.* Peptoid nanosheets exhibit a new secondary-structure motif. *Nature*.
641        **526** (7573), 415–420 (2015).

642   35.   Edison, J.R. *et al.* Conformations of peptoids in nanosheets result from the interplay of
643        backbone energetics and intermolecular interactions. *Proceedings of the National*
644        *Academy of Sciences*. **115** (22), 5647–5651 (2018).

645   36.   Kobayashi, S., Sugiura, M., Kitagawa, H., Lam, W.W.L. Rare-earth metal triflates in organic
646        synthesis. *Chemical Reviews*. **102** (6), 2227–2302 (2002).

647   37.   Fujinaga, S., Hashimito, M., Tsukagoshi, K. Investigation of the composition for a ternary
648        solvent system in tube radial distribution chromatography. *Journal of Liquid*
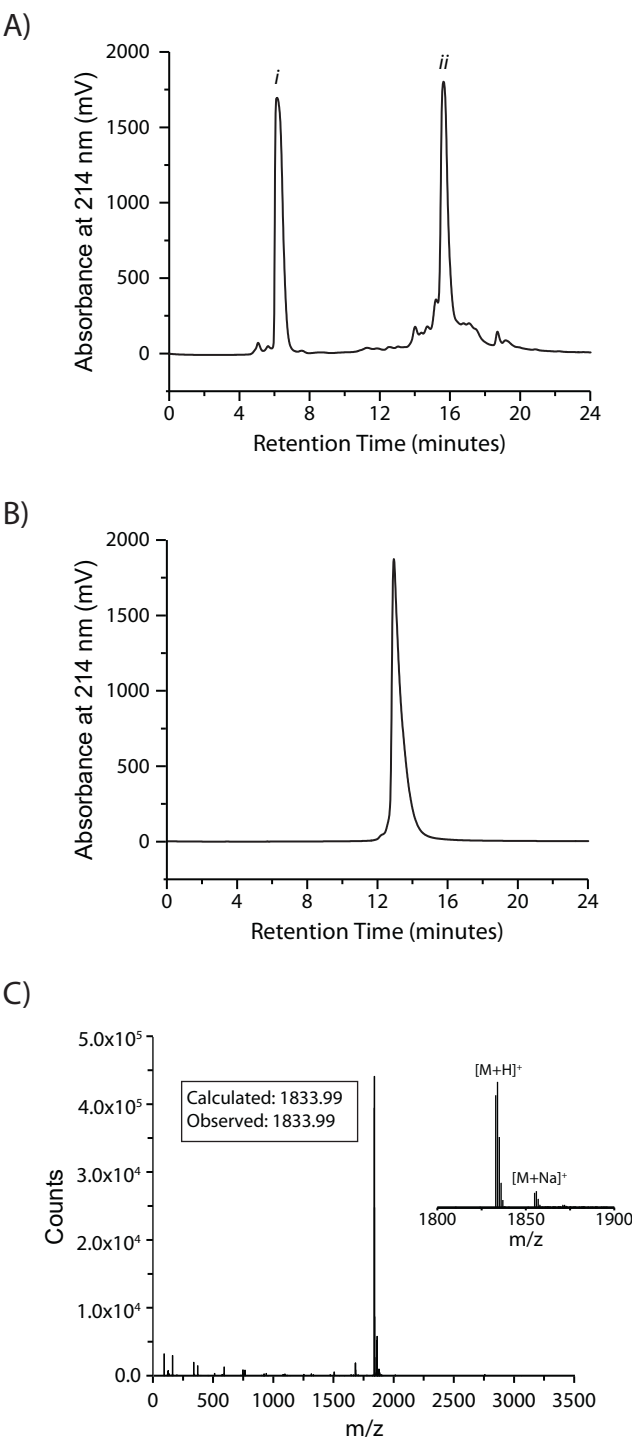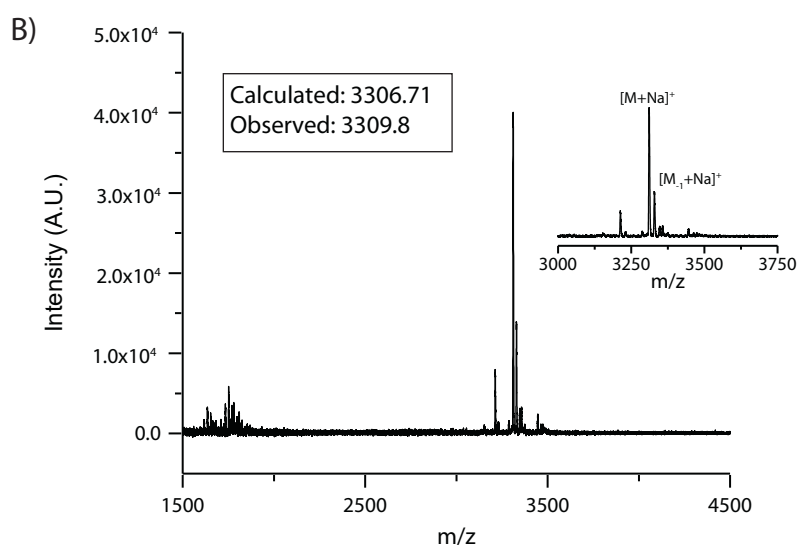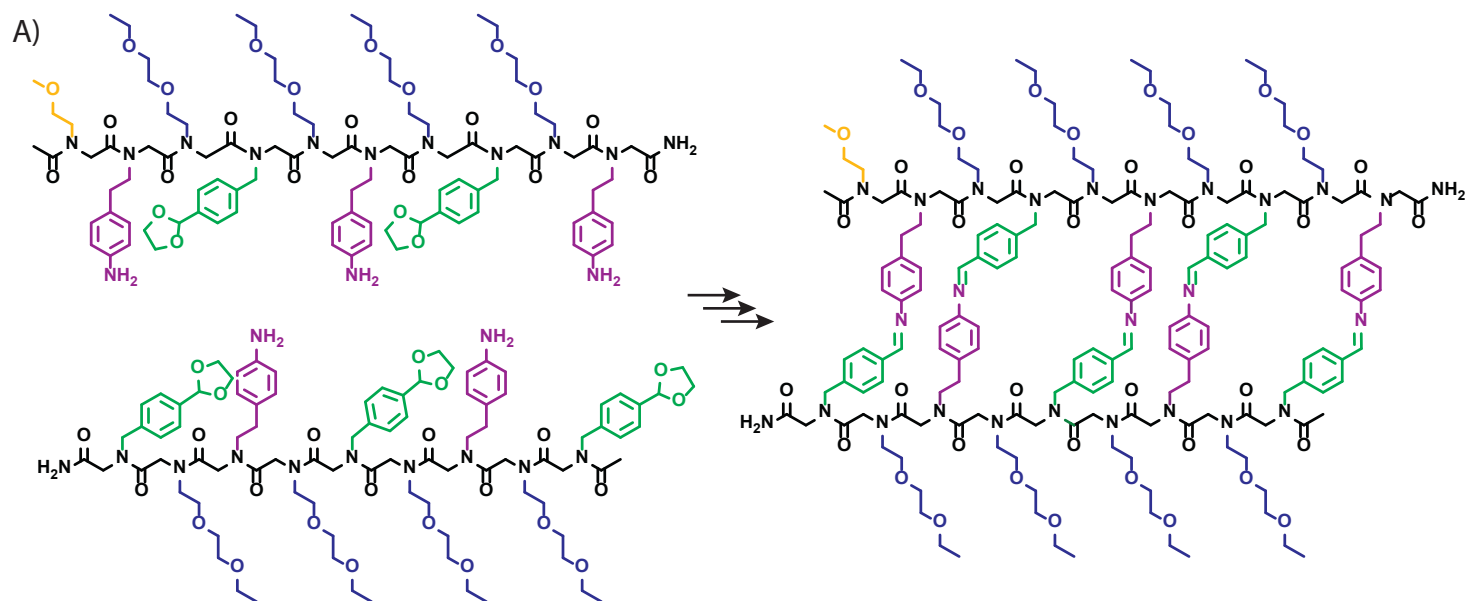649        *Chromatography and Related Technologies*. **38** (5), 600–606 (2015).

650

Figure 1

Click here to access/download;Figure;190730 - Figure 1.ai ±

Figure 2    Click here to access/download;Figure;190730 - Figure 2.ai



A)

B)

Figure 3　　　　　　　　　　　　　　　　　　　　　　　　Click here to access/download;Figure;190730 - Figure 3.ai ⬇

Figure 4    Click here to access/download;Figure;190730 - Figure 4.ai ⤓

A)



B)



Calculated: 3306.71
Observed: 3309.8

[M+Na]⁺

[M₋₁+Na]⁺

Table of Materials

| Name of Material/ Equipment | Company | Catalog Number |
|---|---|---|
| 1,4-Dioxane | Fisher Scientific | D1114 |
| 2-(4-Hydroxyphenylazo)benzoic acid (HABA) | Millipore-Sigma | 54793 |
| 4-(2-Aminoethyl)aniline | Ontario Chemicals | A2076 |
| 4-Cyanobenzaldehyde | Oakwood Chemical | 049317 |
| 4-Methylpiperidine | TCI America | P0445 |
| 4-Toluenesulfonyl chloride | Oakwood Chemical | BR1703 |
| 50 mL High Performance Centrifuge Tubes | VWR International | 21008-240 |
| Acetic acid | Fisher Scientific | A38-212 |
| Acetic anhydride | Fisher Scientific | A10 |
| Acetonitrile | Millipore-Sigma | 34851 |
| All-plastic Norm-Ject syringes | Thermo Fisher Scientific | S7510-10 |
| Allyl chloroformate | Acros Organics | 221741000 |
| Bromoacetic acid | Alfa Aesar | A14403 |
| Chloroform | Millipore-Sigma | 288306 |
| Chloroform-d | Acros Organics | AC320690075 |
| Dichlorodimethylsilane | Acros Organics | 1133100 |
| Dichloroethane | Fisher Scientific | E175 |
| Dichloromethane | Fisher Scientific | D37-4 |
| Diethyl ether | Acros Organics | 615080010 |
| Diethylene glycol monoethyl ether | TCI America | E0048 |
| Ethanol | Decon Labs | 2701 |
| Ethylene glycol | Fisher Scientific | E178 |
| Fmoc-Photolabile SS resin | CreoSalus | SA50785 |
| Glass Peptide Vessel | Chemglass | CG-1866-02 |
| LC-6AD HPLC pumps | Shimadzu Corporation | |
| LED 405nm | ThorLabs | M405L2-C1 |
| LED Driver | ThorLabs | LEDD1B |
| Liberty Blue Automated Peptide Synthesizer | CEM Corporation | |
| Lithium aluminum hydride | Millipore-Sigma | 199877 |
| Luna C18 analytical RP-HPLC column | Phenomenex | 00G-4252-E0 |
| Luna C18 prepatory RP-HPLC column | Phenomenex | 00G-4253-P0-AX |

| | | |
|---|---|---|
| Methanol | Fisher Scientific | A412 |
| Microliter Syringe | Hamilton Company | 80700 |
| N,N'-Diisopropylcarbodiimide (DIC) | Oakwood Chemical | M02889 |
| N,N-Dimethylformamide | Millipore-Sigma | 319937 |
| Nitric acid | Fisher Scientific | A200-212 |
| Nitrogen gas | Cryogenic Gases | |
| Phenylsilane | Oakwood Chemical | S13600 |
| Prominence SPD-10A UV/vis Detector | Shimadzu Corporation | |
| p-Toluenesulfonic acid monohydrate | Millipore-Sigma | 402885 |
| Scandium(III) triflate | Oakwood Chemical | 009343 |
| Single-use Needle | Exel International | 26420 |
| Sodium azide | Oakwood Chemical | 094448 |
| Sodium bicarbonate | Fisher Scientific | S233 |
| Sodium hydroxide | Fisher Scientific | S318-100 |
| Sodium sulfate | Fisher Scientific | S421-500 |
| Syringe Filter 0.45 µm | VWR International | 28145-497 |
| Tetrahydrofuran | Fisher Scientific | T397 |
| Tetrakis(triphenylphosphine) palladium(0) | Oakwood Chemical | 034279 |
| Toluene | Fisher Scientific | T324 |
| Triphenylphosphine | Oakwood Chemical | 037818 |

## Comments/Description

Certified ACS

Matrix substance for MALDI-MS; ≥99.5%

98%

99%

≥98.0%

99%

Centrifuge Tubes used for automated synthesizer

Glacial

Certified ACS

For HPLC; Gradient grade; ≥99.9%

Luer-Slip Syringe

97%

≥98.0%

Anhydrous; ≥99%; Contains 0.5-1.0% ethanol as stabilizer

For NMR; 99.8 atom % D; Packaged in 0.75 ml ampoules

≥99.0%

Certified ACS

Stabalized; Certified ACS

Anhydrous; ACS reagent

≥99.0%

200 Proof; Anhydrous

Certified

100-200 mesh; 1% DVB

Solid Phase, T-Bore PTFE Stpk, Vacuum, Medium Frit, GL 25 Thread

Equipment

405 nm LED used for photocleavage of peptoid

Driver for LED light used in photocleavage of peptoid

Equipment

Powder; Reagent grade; 95%; CAUTION: Mildly pyrophoric, handle under
inert gas and protect from moisture

Equipment

Equipment

Certified ACS

Cemented Needle (N)

≥99.0%; CAUTION: DIC is hazardous to eyes, skin, via respiratory inhalation, and may cause skin sensitization

ACS reagent; ≥99.8%

Certified ACS Plus

Contents under pressure, may explode if heated

97%

Equipment

ACS reagent; ≥98.5%

99%

18G x 1 1/2"

99%; CAUTION: $NaN_3$ may react with lead and copper which results in the

formation of highly explosive metal azides. It is acutely toxic and fatal if

swallowed or in contact with skin.

Powder; Certified ACS

Pellets; Certified ACS

Anhydrous; Granular; Certified ACS

PTFE, Syringe Filters with Polypropylene Housing

Certified

98%

Certified ACS

99%

**jove**
JOURNAL OF VISUALIZED EXPERIMENTS

1 Alewife Center #200
Cambridge, MA 02140
tel. 617.945.9051
www.jove.com

# ARTICLE AND VIDEO LICENSE AGREEMENT

| Title of Article: | Synthesis of Information-bearing Peptoids and their Sequence-directed Dynamic Covalent Self-assembly |
|---|---|
| Author(s): | Samuel C. Leguizamon, Abdulla F. Alqubati, Timothy F. Scott |

**Item 1:** The Author elects to have the Materials be made available (as described at http://www.jove.com/publish) via:

☐ Standard Access          ☒ Open Access

**Item 2:** Please select one of the following items:

☒ The Author is **NOT** a United States government employee.

☐ The Author is a United States government employee and the Materials were prepared in the course of his or her duties as a United States government employee.

☐ The Author is a United States government employee but the Materials were NOT prepared in the course of his or her duties as a United States government employee.

## ARTICLE AND VIDEO LICENSE AGREEMENT

1.      **Defined Terms.** As used in this Article and Video License Agreement, the following terms shall have the following meanings: "**Agreement**" means this Article and Video License Agreement; "**Article**" means the article specified on the last page of this Agreement, including any associated materials such as texts, figures, tables, artwork, abstracts, or summaries contained therein; "**Author**" means the author who is a signatory to this Agreement; "**Collective Work**" means a work, such as a periodical issue, anthology or encyclopedia, in which the Materials in their entirety in unmodified form, along with a number of other contributions, constituting separate and independent works in themselves, are assembled into a collective whole; "**CRC License**" means the Creative Commons Attribution-Non Commercial-No Derivs 3.0 Unported Agreement, the terms and conditions of which can be found at: http://creativecommons.org/licenses/by-nc-nd/3.0/legalcode; "**Derivative Work**" means a work based upon the Materials or upon the Materials and other pre-existing works, such as a translation, musical arrangement, dramatization, fictionalization, motion picture version, sound recording, art reproduction, abridgment, condensation, or any other form in which the Materials may be recast, transformed, or adapted; "**Institution**" means the institution, listed on the last page of this Agreement, by which the Author was employed at the time of the creation of the Materials; "**JoVE**" means MyJove Corporation, a Massachusetts corporation and the publisher of The Journal of Visualized Experiments; "**Materials**" means the Article and / or the Video; "**Parties**" means the Author and JoVE; "**Video**" means any video(s) made by the Author, alone or in conjunction with any other parties, or by JoVE or its affiliates or agents, individually or in collaboration with the Author or any other parties, incorporating all or any portion of the Article, and in which the Author may or may not appear.

2.      **Background.** The Author, who is the author of the Article, in order to ensure the dissemination and protection of the Article, desires to have the JoVE publish the Article and create and transmit videos based on the Article. In furtherance of such goals, the Parties desire to memorialize in this Agreement the respective rights of each Party in and to the Article and the Video.

3.      **Grant of Rights in Article.** In consideration of JoVE agreeing to publish the Article, the Author hereby grants to JoVE, subject to **Sections 4** and **7** below, the exclusive, royalty-free, perpetual (for the full term of copyright in the Article, including any extensions thereto) license (a) to publish, reproduce, distribute, display and store the Article in all forms, formats and media whether now known or hereafter developed (including without limitation in print, digital and electronic form) throughout the world, (b) to translate the Article into other languages, create adaptations, summaries or extracts of the Article or other Derivative Works (including, without limitation, the Video) or Collective Works based on all or any portion of the Article and exercise all of the rights set forth in (a) above in such translations, adaptations, summaries, extracts, Derivative Works or Collective Works and(c) to license others to do any or all of the above. The foregoing rights may be exercised in all media and formats, whether now known or hereafter devised, and include the right to make such modifications as are technically necessary to exercise the rights in other media and formats. If the "Open Access" box has been checked in **Item 1** above, JoVE and the Author hereby grant to the public all such rights in the Article as provided in, but subject to all limitations and requirements set forth in, the CRC License.

612542.6    For questions, please contact us at submissions@jove.com or +1.617.945.9051.

![jove logo] JOURNAL OF VISUALIZED EXPERIMENTS

1 Alewife Center #200
Cambridge, MA 02140
tel. 617.945.9051
www.jove.com

ARTICLE AND VIDEO LICENSE AGREEMENT

**4.** **Retention of Rights in Article.** Notwithstanding the exclusive license granted to JoVE in **Section 3** above, the Author shall, with respect to the Article, retain the non-exclusive right to use all or part of the Article for the non-commercial purpose of giving lectures, presentations or teaching classes, and to post a copy of the Article on the Institution's website or the Author's personal website, in each case provided that a link to the Article on the JoVE website is provided and notice of JoVE's copyright in the Article is included. All non-copyright intellectual property rights in and to the Article, such as patent rights, shall remain with the Author.

**5.** **Grant of Rights in Video – Standard Access.** This **Section 5** applies if the "Standard Access" box has been checked in **Item 1** above or if no box has been checked in **Item 1** above. In consideration of JoVE agreeing to produce, display or otherwise assist with the Video, the Author hereby acknowledges and agrees that, Subject to **Section 7** below, JoVE is and shall be the sole and exclusive owner of all rights of any nature, including, without limitation, all copyrights, in and to the Video. To the extent that, by law, the Author is deemed, now or at any time in the future, to have any rights of any nature in or to the Video, the Author hereby disclaims all such rights and transfers all such rights to JoVE.

**6.** **Grant of Rights in Video – Open Access.** This **Section 6** applies only if the "Open Access" box has been checked in **Item 1** above. In consideration of JoVE agreeing to produce, display or otherwise assist with the Video, the Author hereby grants to JoVE, subject to **Section 7** below, the exclusive, royalty-free, perpetual (for the full term of copyright in the Article, including any extensions thereto) license (a) to publish, reproduce, distribute, display and store the Video in all forms, formats and media whether now known or hereafter developed (including without limitation in print, digital and electronic form) throughout the world, (b) to translate the Video into other languages, create adaptations, summaries or extracts of the Video or other Derivative Works or Collective Works based on all or any portion of the Video and exercise all of the rights set forth in (a) above in such translations, adaptations, summaries, extracts, Derivative Works or Collective Works and (c) to license others to do any or all of the above. The foregoing rights may be exercised in all media and formats, whether now known or hereafter devised, and include the right to make such modifications as are technically necessary to exercise the rights in other media and formats. For any Video to which this **Section 6** is applicable, JoVE and the Author hereby grant to the public all such rights in the Video as provided in, but subject to all limitations and requirements set forth in, the CRC License.

**7.** **Government Employees.** If the Author is a United States government employee and the Article was prepared in the course of his or her duties as a United States government employee, as indicated in **Item 2** above, and any of the licenses or grants granted by the Author hereunder exceed the scope of the 17 U.S.C. 403, then the rights granted hereunder shall be limited to the maximum rights permitted under such statute. In such case, all provisions contained herein that are not in conflict with such statute shall remain in full force and effect, and all provisions contained herein that do so conflict shall be deemed to be amended so as to provide to JoVE the maximum rights permissible within such statute.

**8.** **Protection of the Work.** The Author(s) authorize JoVE to take steps in the Author(s) name and on their behalf if JoVE believes some third party could be infringing or might infringe the copyright of either the Author's Article and/or Video.

**9.** **Likeness, Privacy, Personality.** The Author hereby grants JoVE the right to use the Author's name, voice, likeness, picture, photograph, image, biography and performance in any way, commercial or otherwise, in connection with the Materials and the sale, promotion and distribution thereof. The Author hereby waives any and all rights he or she may have, relating to his or her appearance in the Video or otherwise relating to the Materials, under all applicable privacy, likeness, personality or similar laws.

**10.** **Author Warranties.** The Author represents and warrants that the Article is original, that it has not been published, that the copyright interest is owned by the Author (or, if more than one author is listed at the beginning of this Agreement, by such authors collectively) and has not been assigned, licensed, or otherwise transferred to any other party. The Author represents and warrants that the author(s) listed at the top of this Agreement are the only authors of the Materials. If more than one author is listed at the top of this Agreement and if any such author has not entered into a separate Article and Video License Agreement with JoVE relating to the Materials, the Author represents and warrants that the Author has been authorized by each of the other such authors to execute this Agreement on his or her behalf and to bind him or her with respect to the terms of this Agreement as if each of them had been a party hereto as an Author. The Author warrants that the use, reproduction, distribution, public or private performance or display, and/or modification of all or any portion of the Materials does not and will not violate, infringe and/or misappropriate the patent, trademark, intellectual property or other rights of any third party. The Author represents and warrants that it has and will continue to comply with all government, institutional and other regulations, including, without limitation all institutional, laboratory, hospital, ethical, human and animal treatment, privacy, and all other rules, regulations, laws, procedures or guidelines, applicable to the Materials, and that all research involving human and animal subjects has been approved by the Author's relevant institutional review board.

**11.** **JoVE Discretion.** If the Author requests the assistance of JoVE in producing the Video in the Author's facility, the Author shall ensure that the presence of JoVE employees, agents or independent contractors is in accordance with the relevant regulations of the Author's institution. If more than one author is listed at the beginning of this Agreement, JoVE may, in its sole

![jove logo] 1 Alewife Center #200
Cambridge, MA 02140
tel. 617.945.9051
www.jove.com

## ARTICLE AND VIDEO LICENSE AGREEMENT

discretion, elect not take any action with respect to the Article until such time as it has received complete, executed Article and Video License Agreements from each such author. JoVE reserves the right, in its absolute and sole discretion and without giving any reason therefore, to accept or decline any work submitted to JoVE. JoVE and its employees, agents and independent contractors shall have full, unfettered access to the facilities of the Author or of the Author's institution as necessary to make the Video, whether actually published or not. JoVE has sole discretion as to the method of making and publishing the Materials, including, without limitation, to all decisions regarding editing, lighting, filming, timing of publication, if any, length, quality, content and the like.

12. **Indemnification.** The Author agrees to indemnify JoVE and/or its successors and assigns from and against any and all claims, costs, and expenses, including attorney's fees, arising out of any breach of any warranty or other representations contained herein. The Author further agrees to indemnify and hold harmless JoVE from and against any and all claims, costs, and expenses, including attorney's fees, resulting from the breach by the Author of any representation or warranty contained herein or from allegations or instances of violation of intellectual property rights, damage to the Author's or the Author's institution's facilities, fraud, libel, defamation, research, equipment, experiments, property damage, personal injury, violations of institutional, laboratory, hospital, ethical, human and animal treatment, privacy or other rules, regulations, laws, procedures or guidelines, liabilities and other losses or damages related in any way to the submission of work to JoVE, making of videos by JoVE, or publication in JoVE or elsewhere by JoVE. The Author shall be responsible for, and shall hold JoVE harmless from, damages caused by lack of sterilization, lack of cleanliness or by contamination due to

the making of a video by JoVE its employees, agents or independent contractors. All sterilization, cleanliness or decontamination procedures shall be solely the responsibility of the Author and shall be undertaken at the Author's expense. All indemnifications provided herein shall include JoVE's attorney's fees and costs related to said losses or damages. Such indemnification and holding harmless shall include such losses or damages incurred by, or in connection with, acts or omissions of JoVE, its employees, agents or independent contractors.

13. **Fees.** To cover the cost incurred for publication, JoVE must receive payment before production and publication of the Materials. Payment is due in 21 days of invoice. Should the Materials not be published due to an editorial or production decision, these funds will be returned to the Author. Withdrawal by the Author of any submitted Materials after final peer review approval will result in a US$1,200 fee to cover pre-production expenses incurred by JoVE. If payment is not received by the completion of filming, production and publication of the Materials will be suspended until payment is received.

14. **Transfer, Governing Law.** This Agreement may be assigned by JoVE and shall inure to the benefits of any of JoVE's successors and assignees. This Agreement shall be governed and construed by the internal laws of the Commonwealth of Massachusetts without giving effect to any conflict of law provision thereunder. This Agreement may be executed in counterparts, each of which shall be deemed an original, but all of which together shall be deemed to me one and the same agreement. A signed copy of this Agreement delivered by facsimile, e-mail or other means of electronic transmission shall be deemed to have the same legal effect as delivery of an original signed copy of this Agreement.

A signed copy of this document must be sent with all new submissions. Only one Agreement is required per submission.

## CORRESPONDING AUTHOR

Name: Timothy F. Scott

Department: Department of Chemical Engineering

Institution: University of Michigan

Title: Associate Professor

Signature: _[signature]_   Date: June 17, 2019

Please submit a **signed** and **dated** copy of this license by one of the following three methods:
1. Upload an electronic version on the JoVE submission site
2. Fax the document to +1.866.381.2236
3. Mail the document to JoVE / Attn: JoVE Editorial / 1 Alewife Center #200 / Cambridge, MA 02140

**MICHIGAN ENGINEERING**

UNIVERSITY of MICHIGAN ■ COLLEGE of ENGINEERING

TIMOTHY F. SCOTT
ASSOCIATE PROFESSOR OF CHEMICAL ENGINEERING
ASSOCIATE PROFESSOR OF MACROMOLECULAR SCIENCE AND ENGINEERING
2800 PLYMOUTH ROAD
NCRC BUILDING 28, ROOM 1044W
ANN ARBOR, MICHIGAN 48109-2800
734 763-3493
tfscott@umich.edu

30 July, 2019

Dear Dr. Wu,

Thank you very much for the review of our manuscript as we believe that the reviews and our responses to them have improved the paper. In this context, we have examined the comments and suggestions made by the reviewers of our paper "Synthesis of Information-bearing Peptoids and their Sequence-directed Dynamic Covalent Self-assembly". To accompany our resubmitted manuscript, the following represents our detailed responses to the reviewers' points.

## Reviewer 1

Reviewer 1 comment: *This paper clearly describes a straightforward synthetic protocol for peptoid with dynamic covalent chemistry, which allows access to interesting structures like ladders. This protocol makes use of rare-earth metal triflates as Lewis acids to circumvent kinetic trapping often observed during the self-assembly of complex molecules with multiple interaction sites.*

We appreciate the reviewer's comments and address their points as described below.

Reviewer 1 comment: *I recommend adding ChemDraws for the full synthetic schemes of the Fmoc-monomers (or at least ChemDraws of the final monomers used in the peptoid synthesis). The structures can be found in papers cited by this manuscript but having the structures more clearly in this protocol would be helpful.*

To accommodate the reviewer's request, we have updated Figure 1 to include the full synthetic schemes of the monomers.

## Reviewer 2

Reviewer 2 comment: *The authors describe the methodology required to develop peptoids that align to form dynamic covelant bonds. The synthesis of the required monomers, automated peptoid synthesis, and assembly methods are described. The descriptions are thorough and easy to follow.*

We thank the reviewer for their comments and address their points as described below.

Reviewer 2 comment: *The relevance to information storage was unclear - this could be expanded on to motivate the dynamic peptoid ladders.*

We note that the utilization of self-assembled molecular ladders or their oligomeric precursors as information storage media is not addressed in this manuscript; rather, as is indicated in the Discussion section, the information lies in the pedant group sequence incorporated in peptoid

oligomers. To clarify the utility of this information, we have modified the last sentence of the first paragraph of the Introduction section to read:

> Here, we report a process which circumvents kinetic trapping to enable the self-assembly of molecular ladders from sequence-specific oligomers where the hybridization is directed by the information encoded in the oligomer residue sequences.

Reviewer 2 comment: *Including the number of equivalents throughout the methods (monomer synthesis, peptoid synthesis, etc.) would increase the ease of translating these methods.*

We very much appreciate the reviewer's close examination of our manuscript and, as requested, have incorporated the number of equivalents throughout the synthetic protocols.

Reviewer 2 comment: *A justification for the selection of the photocleavable linker would be valuable for those trying to modify this strategy. Additionally, more details on the instrumentation used for the cleavage would be valuable for replication.*

We appreciate the reviewer's suggestion and agree that identifying the necessity for the photocleavable linker would be invaluable. Thus, to accommodate the reviewer's request, we have included the following sentence:

> Importantly, the solid-phase synthesis is performed using a photolabile resin to enable oligomer cleavage from the bead under UV or violet light irradiation, preventing premature deprotection of the acid-labile, ethylene acetal-based protecting group.

Additionally, we have included the LED and LED driver in the Table of Materials.

Reviewer 2 comment: *In 2.3.3., how was a stir plate used for agitation?*

To address the reviewer's question, we have expanded on the use of a stir plate for agitation.

Reviewer 2 comment: *Figure 3 - labeling the peaks could make this figure more clear.*

As requested, we have labeled the peaks in Figure 3.

## Reviewer 3

Reviewer 3 comment: *The authors report a procedure for the solid-phase synthesis of imine-based molecular ladders which utilizes a modified automated peptide synthesizer and circumvents the formation of kinetic traps that would be detrimental to the yield of a single discrete product. The ladder backbone is a poly(N-substituted glycine) which is constructed in a stepwise, and therefore sequence-defined, manner. The pendant groups on the backbone contain alloc-protected aromatic amines, acetal-protected aromatic aldehydes, or solubilizing groups. Orthogonal protecting groups allow for the selective deprotection of the amines. After alloc deprotected and cleavage from the resin, complimentary strands are ready for oligomer annealing (ladder formation).*

*The acetal deprotection is performed on two complimentary strands in the same pot to prevent the self-condensation of two identical strands. The deprotection is performed with excess Sc(OTf)$_3$*

*(1.5 equiv per potential imine bond formed) under aqueous conditions. In this way, the acetals are deprotected and premature imine condensation is prevented. After deprotection, the reaction mixture is extracted with CHCl₃ and the organic layer is transferred to a new reaction vessel. In addition to removing H2O/MeCN from the reaction mixture, the extraction also removes an indeterminate amount of Sc(OTf)₃. Upon heating to 70 °C, oligomer annealing occurs. The major product after the reaction (determined by MALDI) is the correctly aligned ladder with complimentary strands.*

*The reported procedure is an efficient automated synthesis of information-encoded oligomers which form duplexes (ladders) with complimentary strands. The automated synthesis allows for shortened reaction times, more efficient transitions between reactions, and conservation of material. The in situdeprotection of the acetal groups prevents the self-condensation of identical strands. Ladders are formed in the presence of Sc(OTf)₃ in a reaction manifold that does not suffer from the detrimental effect of off-target kinetic traps.*

We thank the reviewer for their assessment of our manuscript and have endeavored to address their comments and suggestions as described below.

Reviewer 3 comment: *The manuscript has good discussion of synthetic logic and possible pitfalls; however, the discussion of possible kinetic traps could be expanded. What specific kinetic traps are possible (misaligned oligomers that don't self-correct?), and what features of the current protocol are most critical for avoiding kinetic traps (full dissociation of oligomers with excess acid, acid concentration during annealing, etc.)? Have the authors done studies to determine what percentage of Sc(OTf)₃ is extracted during the CHCl₃ extraction? What is the concentration of Sc(OTf)₃ during annealing? How sensitive is the reaction outcome to perturbations of this concentration? What is the simplest method of determining Sc(OTf)₃ concentration under the reaction conditions (19F NMR of an aliquot with an internal standard?)?*

We appreciate the reviewer's suggestions and have specified the kinetic trapped species that can arise during the dynamic covalent assembly of molecular ladders. We note that, as this manuscript focuses on the protocol, we have omitted studies on the significance of each phase and rare-earth metal triflate final concentrations. Nevertheless, we have included several methods for determining Sc(OTf)₃ concentrations, including the welcome suggestion of $^{19}$F NMR, along with an affirmation of the robustness of the method to perturbations of the final catalyst concentrations.

Reviewer 3 comment: *Characterization of reported compounds is incomplete. In the 1H NMR data, coupling constants should be given for all peaks where multiplicity is determined. Additionally, peaks should not be listed as multiplets when coupling constants can be determined. For example, in the synthesis of Npam, the peak in the NMR ranging from 5.95 to 6.04 ppm is likely not a multiplet but instead a dddd(a diagnostic signal for monosubstituted terminal olefins). It's difficult to be certain without access to the fid, but the authors should revisit this characterization data and provide more information where applicable. 13C NMR data should also be provided.*

To accommodate the reviewer's request, we have updated the NMR section to include the coupling constants for $^1$H NMR spectra and have added $^{13}$C NMR data.

Reviewer 3 comment: *The authors claim that out-of-registry products are formed only in trace amounts, but otherwise make few comments on the presence of higher-molecular weight species evident in the MALDI mass spectrum (Figure 4b). Here, a list of the masses apparent in the spectrum with corresponding molecular ion assignments would be beneficial to distinguish out-of-registry products from incompletely condensed in-registry kinetic intermediates. Furthermore, analysis of the product distribution via GPC or HPLC might give better insight to the degree to which sequence specificity and complementarity of strands circumvents kinetic trapping. Without such a quantitative evaluation, the efficacy of sequence specificity as a means of circumventing kinetic trapping is ambiguous.*

To address the reviewer's comments, we have listed the masses apparent in the mass spectrum presented in Figure 4b in the figure caption. Notably, out-of-registry products are indistinguishable from incompletely condensed in-registry species by mass spectrometry. Moreover, as the masses of misaligned and incorrectly coupled sequences do not significantly deviate from the in-registry species, characterization of the reaction mixtures by GPC and HPLC yields uniform elution times, precluding their use to provide further insights into the degree of sequence specificity or the circumvention of kinetic trapping.

**Reviewer 4**

Reviewer 4 comment: *This work reports a detailed protocol for synthesis of peptoid-based assemblies through sequence-directed dynamic imine chemistry. The "Dissociation/Extraction/Annealing" approach developed by the authors successfully overcomes the commonly encountered challenges of kinetic trapping, thus allowing thermodynamically most favored assembly product predominantly formed. Given the great importance and rapidly growing interest in dynamic covalent assembly study, and the method reported in this work could guide future synthesis of more complex molecular/macromolecular architectures through sequence-directed self-assembly process, publication of this work on JoVE is recommended. The procedures are provided with great details and easy to follow. The authors also gave a nice discussion on possible alternatives for orthogonal deprotection.*

We thank the reviewer for their comments and have addressed their suggestions as detailed below.

Reviewer 4 comment: *First paragraph, the following review articles on dynamic covalent chemistry should be cited, probably after "...dynamic covalent interactions", which would give readers a more comprehensive view of the available tools in this field and their application potential: Angew. Chem. Int. Ed. 2002, 41, 898; Chem. Soc. Rev. 2013, 42, 6634; "Dynamic Covalent Chemistry: Principles, Reactions, and Applications." Weinheim:Wiley-VCH, 2017. Print. ISBN: 978-1-119-07563-9.*

*The following account article on cage design and synthesis through dynamic covalent chemistry should be added to ref 11-12: Acc. Chem. Res. 2014, 47, 1575.*

We appreciate the reviewer's suggestions for additional references and have included them in the manuscript.

Sincerely,

Timothy F. Scott