Supporting Information

Accelerated Mechanochemistry in Helical Polymers

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Supporting Information

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I. General Experimental Details

All chemicals and reagents were purchased from commercial sources and used without further purification, unless specified. d-Glutamic acid γ-benzyl ester, γ-benzyl -l-glutamate NCA (l-type NCA), and γ-benzyl-δ-glutamate NCA (δ-type NCA) were prepared according to the literature.[1]

GPC analyses were performed in DMF containing 0.02M of LiBr (50°C, at a flow rate of 0.5 mL/min) using a Thermo LC system equipped with a TSKgel G4000HHR and a Eprogen CCS203-25 column in sequence. Detection was carried out with a “triple-detector” system which includes a Dionex DAD-3000 PDI UV-Vis Detector, Wyatt OPTILAB T-rEX refractometer, and a Wyatt MALS DAWN HELEOS II 8+TR. Wyatt’s Astra 7.1.4 software was used for GPC data analysis and polymer properties calculation (molecular weights, polydispersities, etc).

Ultrasonication experiments were performed in Suslick cells screwed onto a SONICS Vibra-cell ultrasonic processor (500 Watt), and tuned to 9.57 W·cm⁻². All NMR spectra were recorded using Bruker AVANCE III 400 MHz spectrometers at the Technion NMR facilities. UV-Vis was measured in a Thermo Evolution 220 Spectrophotometer. Circular dichroism measurements were performed using an Applied Photophysics chirascan circular dichroism spectrometer. High-resolution mass spectrometry was performed in a Waters LCT Premier Mass Spectrometer (ESI) and a Bruker maxis impact with APCI solid probe.
II. Experimental Procedures

Preparation of di-amine functionalized rhodamine (DAR)

Rhodamine 6G derivatives, including Rhodamine-OH and DHR, were prepared by following previously published procedures.\(^2\)

**DAR-BOC.** A solution of DHR (0.45 g, 0.9 mmol) in anhydrous dichloromethane (12 ml) under \(\text{N}_2\) was cooled in an ice-water bath. To this solution, boc-glycine (0.41g, 2.34 mmol) and dimethylaminopyridine (29 mg, 0.234 mmol) were added. After the mixture became homogenerous, \(\text{N}, \text{N}'\text{-Dicyclohexylcarbodiimide (0.48 g, 2.34 mmol)}\) was added in small portions and the reaction was stirred at room temperature for 24 h. The mixture was then cooled to -20 °C for 3 h and filtered to remove the white precipitate. The filtrate was further washed with aqueous HCl (0.5 M, 10 ml×3), saturated NaHCO\(_3\) (10 ml×3), DI water (10 ml×2), and brine (10 ml). The collected organic phase was dried over anhydrous Na\(_2\)SO\(_4\), filtered, and concentrated to give the crude product. The final product was obtained after purification by silica gel column chromatography using DCM/ethanol (v/v, 50/1) as eluent to afford a white solid: 0.60 g (82% yield). \(^1\)H NMR (400 MHz, CDCl\(_3\)): \(\delta\) 7.95 (m, 1H), 7.47 (m, 2H), 7.03 (m, 1H), 6.86 (s, 1H), 6.38 (s, 1H), 6.34 (s, 1H), 6.23 (s, 1H), 5.07-5.0 (br, 2H), 4.19 (t, 2H), 3.84-3.72 (m,
6H), 3.39 (t, 2H), 3.27 (t, 2H), 3.2 (m, 2H), 3.05 (q, 2H), 2.02 (s, 3H), 1.91 (s, 3H), 1.43(s, 9H), 1.41(s, 9H), 1.33(t, 3H), 1.03(t, 3H). \(^{13}\)C NMR (400 MHz, CDCl\(_3\)): \(\delta\) 170.4, 169.9, 168.7, 155.7, 153.4, 151.4, 150.4, 147.7, 132.7, 129.9, 129.7, 128.4, 128.3, 123.9, 123.1, 118.4, 113.8, 109.8, 105.3, 96.5, 80.7, 79.8, 64.8, 62.8, 62.2, 50.5, 48.0, 42.3, 38.8, 38.4, 28.3, 17.7, 16.8, 14.7, 12.3. APCI HR-MS: \(m/z\) found [M-H\(^+\)] for C\(_{44}\)H\(_{58}\)N\(_5\)O\(_{10}\)\(^+\) 816.4176 (calcd. 816.4184).

**DAR**. A solution of DAR-BOC (0.15 g, 0.184 mmol) in anhydrous dichloromethane (1 ml) was cooled in an ice-water bath, before trifluoroacetic acid (1.41 ml, 18.4 mmol) was added to it. The mixture was allowed to return to room temperature, stirred for 7 h, and then evaporated under vacuum. The residues were dissolved in ethyl acetate (5 ml), and triethyl amine (1 ml) was added to neutralize the mixture. The mixture was concentrated again under vacuum and precipitated in diethyl ether to provide the crude product. The final product was obtained after purification by silica gel column chromatography (DCM/methanol/ammonia, v/v/v, 10/1/0.1) to afford a pale yellow solid: 85 mg (60% yield). \(^1\)H NMR (400 MHz, CDCl\(_3\)): \(\delta\) 7.87 (m, 1H), 7.46 (m, 2H), 7.06 (m, 1H), 6.84 (s, 1H), 6.36 (m, 1H), 6.33 (s, 1H), 6.12 (s, 1H), 4.16-3.94 (m, 4H), 3.58 (m, 2H), 3.40 (m, 2H), 3.30-3.17 (m, 6H), 3.01 (m, 2H), 1.95 (s, 3H), 1.87 (s, 3H), 1.28(t, 3H), 1.01(t, 3H). \(^{13}\)C NMR (400 MHz, CDCl\(_3\)): \(\delta\) 174.1, 170.1, 153.5, 151.5, 150.6, 150.5, 147.8, 133.0, 130.3, 130.0, 129.5, 128.5, 128.0, 123.9, 123.2, 118.5, 113.8, 110.4, 104.8, 96.5, 65.7, 62.4, 59.1, 53.76, 48.3, 44.8, 38.4, 29.4, 17.9, 16.8, 14.7, 11.7. APCI HR-MS: \(m/z\) found [M-H\(^+\)] for C\(_{34}\)H\(_{42}\)N\(_5\)O\(_6\)\(^+\) 616.3136 (calcd. 616.3135).
Ring opening polymerization of γ-benzyl-glutamate N-carboxyl anhydride (NCA).

In nitrogen purged glove box, the desired NCA (210 mg, 0.8 mmol) was dissolved in anhydrous THF in a vial containing a magnetic stir bar. To that a LiHMDS in THF solution was added. The polymerization was stirred for 2 h at room temperature, before a few drops of HCOOH were added to quench the polymerization. The vial was then transferred outside the glovebox and the solution was slowly poured into excess cold diethyl ether to precipitate the polymer. The polymer was collected by centrifugation and reprecipitated 3 times using THF and diethyl ether again. Finally, the polymer was dried under the vacuum for 24 h to provide a white solid.

PB(L)G$_{376}$: 1.2 ml of THF and 0.4 ml of a 0.02 M LiHMDS solution were used. Product: 166 mg, 95% yield.

PB(D)G$_{365}$: 1.2 ml of THF and 0.4 ml of a 0.02 M LiHMDS solution were used, polymerization time was extended to 10 h. Product: 163 mg, 93% yield.

PB(D$_{0.27}$L$_{0.73}$)G$_{388}$: 1.6 ml of THF, d-NCA (57.3 mg) and l-NCA (155.0 mg), and 0.04 ml of a 0.04 M LiHMDS THF solution were used, polymerization time was extended to 24 h. Product: 165 mg, 94% yield.
PB(D_{0.5}L_{0.5})G_{32}: 1.6 ml of THF, dL-racemic NCA (210 mg), and 0.04 ml of a 0.04 M LiHMDS THF solution were used, polymerization time was extended to 24 h. Product: 153 mg, 87% yield.

**Preparation of four DAR-PBGs** (DAR-PB(L)G_{61}, DAR-PB(D_{0.5}L_{0.5})G_{104}, DAR-PB(L)G_{102}, and DAR-PB(D_{0.5}L_{0.5})G_{143}), using diamine-functionalized spirolactam rhodamine (DAR) as initiator.

![Chemical Structure](image)

The desired NCA (100 mg, 0.38 mmol) was dissolved in anhydrous DMF (1 ml) in a Schlenk flask containing a magnetic stir bar under N\textsubscript{2}. The flask was cooled to 0 °C in an isopropanol cooling bath. Then, a DAR solution in anhydrous DMF was added, and the reaction was stirred for 3 d at 0 °C. Finally, the solution was poured into excess cold diethyl ether, precipitating the polymer product, which was collected by centrifugation and reprecipitated 3 times using DMF and diethyl ether. Finally, the polymer was dried under the vacuum for 24 h to provide a white solid.

DAR-PB(L)G_{61}: 0.25 ml of 0.01 M of a DAR solution was used. Product: 40 mg, 48% yield.

DAR-PB(L)G_{102}: 0.20 ml of 0.01 M of a DAR solution was used. Product: 40.2 mg, 48% yield.

PB(D_{0.5}L_{0.5})G_{104}: DL-racemic NCA mixtures (d/L, 50 mg / 50 mg), and 0.20 ml of 0.01 M of a DAR solution was used. Product: 43.3 mg, 52% yield.

PB(D_{0.5}L_{0.5})G_{143}: DL-racemic NCA mixtures (d/L, 50 mg / 50 mg), and 0.16 ml of 0.01 M of a DAR solution was used. Product: 45.8 mg, 55% yield.

**Ultrasonication experiments**

All sonication experiments were carried out by the same protocol. Specifically, 17 ml of each polymer solution (1 mg/mL, in THF) was transferred into an oven-dried Suslick cell, which was subsequently connected to the sonicator collar and probe. Then, the solution was placed inside an
acetone cooling bath (-9 °C). N₂ was purged for 15 min prior to ultrasound irradiation and continued throughout the experiment. Pulsed sonication (1 s on, 2 s off) was carried out using 20% amplitude (9.57 W·cm⁻²) for 2-3 h. For determining of change in molecular weight, aliquots (0.5 ml) were taken every 15 min and analyzed by SEC to record the degradation trend of the polymer. For determining rhodamine mechanophore activation, samples (1.5 ml) were taken every hour and immediately analyzed by UV-Vis.

The recorded number average molecular weights (Mₙ) were directly used to determine the mechanochemistry scission rate constant (kₛ) by the method developed by Sato and Nalepa.[3] The corresponding equation was described as the following:

\[
\frac{1}{M_{n,t}} - \frac{1}{M_{n,0}} = k't \quad (1)
\]

\[
k' = \frac{k_s}{M_w} \quad (2)
\]

Where the \(M_{n,t}\) is the recorded number-average molecular weight at specific sonication time t, \(M_{n,0}\) is the original number-average molecular weight of the testing polymer, and \(M_w\) is the molar mass of the repeat unit in the polymer (for PBG, \(M_w = 219.1\) Da). Then, plotting \((1/M_{n,t} - 1/M_{n,0})\) versus t will give \(k'\). mechanochemistry scission rate constant \((k_s)\) is calculated by multiplying \(k'\) by \(M_w\).

Tables S3-7 summarize the number-average molecular weights (\(M_n\)) at the different sonication times for each PBG, including PB(L)G₃₇₆, PB(D)G₃₆₅, PB(D₀.₂₇L₀.₇₃)G₃₈₈, PB(D₀.₅L₀.₅)G₃₃₂. Table S7 summarizes all of the calculated \(k'\) of the above PBGs.
**UV-Vis analysis.** The activations of rhodamine mechanophore within all DAR-PBGs with the different sonication times were assessed by the corresponding UV-Vis spectra. In a typical experiment, 1.5 mL of aliquot taken from the sonication solution was diluted in another 1.5 ml of THF. After sufficient mixing, its UV-Vis spectrum was immediately recorded. Then, the changes in intensity of absorption at 538 nm were further used to evaluate the mechanochemical activation of rhodamine.

For the numerical evaluation of the activation extent of rhodamine in DAR-PBGs polymers after 3 hours sonication, we assumed the molar extinction coefficient (ε) of activated zwitterionic rhodamine is the same as rhodamine 6G. A calibration curve was prepared using a series of rhodamine 6G at different concentrations (0.08-1.95 × 10^{-5} M, THF/H_2O, v/v, 9/1) presenting ε (95000 M^{-1}cm^{-1}) as slope (Figure S11). The maximal theoretical absorbance (Abs_{theoretical}, 538 nm) of each DAR-PBG solution was calculated using the Beer-Lambert law: Abs_{theoretical} = ε×c×l, where ε = 95000 M^{-1} cm^{-1}, l = 1 cm, c = 0.5/M_n,DAR-PBG (0.5 mg/ml is the testing concentration of DAR-PBG solution). The content of activated rhodamine was derived from the ratio of the Abs_{measured} and Abs_{theoretical}. (Table S2).

For testing the thermal reversion of mechanically activated rhodamine mechanophores, two DAR-PBGs solution (DAR-PB(L)G_{102}, DAR-PB(D_{0.5}L_{0.5})G_{143} in THF, 1 mg/ml) after 3 hours sonication were stored at -9 °C under the N_2 protection. Their absorbance (538 nm) was recorded over storage time (0, 10, 30, 60, 120, 180 min). (Figures S12-13)
**Circular Dichroism (CD).** The solution of each PBG (0.1mg/ml in THF) was prepared at least 12 h before CD measurements. Scans were performed over the 300 to 200 nm region at a step of 1 nm at room temperature. The spectra were obtained by averaging three scans per sample in a fused quartz cell (path length = 0.1 cm). The helix content of each PBG was calculated from the intensity of absorption at 222 nm in its CD using the following equation: \( \% \text{helix} = \frac{-[\theta_{222}] + 3000}{39000} \).\[^4\]

**Calculation of persistence length of PBGs**

The persistence length \( (l_p) \) values were calculated from radius of gyration \( (R_g) \) data determined by multi-angle light scattering (MALS) using the worm-like model:\[^5\]

\[
R_g^2 = \frac{L \times l_p}{3} - l_p^2 + \frac{2l_p^3}{L} \left[ 1 - \frac{l_p}{L} (1 - e^{\frac{-L}{l_p}}) \right]
\]

\( L \) is the contour length, which can be calculated by multiplying the degree of polymerization (DP) by the length of the monomer unit, 0.28 nm, which was estimated from ChemBio3D.
III. Characterization & Results

NMR spectra of initiator and polymers

Figure S1. $^1$H (a) and $^{13}$C (b) NMR spectra of DAR-BOC, in CDCl$_3$. 
Figure S2. $^1$H (a) and $^{13}$C (b) NMR spectra of DAR, in CDCl$_3$. 
Figure S3. $^1$H-NMR spectra of four PBGs, in CDCl$_3$/CF$_3$COOD (v/v, 8/2).
Figure S4. $^1$H-NMR spectra of DAR-PB(L)G$_{61}$ (a) and DAR-PB(L)G$_{102}$ (b), in CDCl$_3$/CF$_3$COOD (v/v, 8/2).
Figure S5. $^1$H-NMR spectra of PB(D$_{0.5}$L$_{0.5}$)G$_{104}$ (a) and PB(D$_{0.5}$L$_{0.5}$)G$_{143}$ (b), in CDCl$_3$/CF$_3$COOD (v/v, 8/2).
**Figure S6.** DMF-SEC curves of PBGs used in this work.

**Figure S7.** (a) Representative DMF-SEC curves of DAR-PBGs, including DAR-PB(L)G_{102} and DAR-PB(D_{0.5}L_{0.5})_{143}, before and after 3 h sonication. (b) The number-average molecular weight ($M_n$) of the above-DAR-PBGs before and after 3 h sonication.
CD spectra

**Figure S8.** CD spectra of THF solutions: (a) four PBGs (PB(L)G\(_{376}\), PB(D)G\(_{365}\), PB(D\(_{0.27}L_{0.73})G_{388}\), PB(D\(_{0.5}L_{0.5})G_{332}\) before sonication; (b) the above four PBGs after sonication; (c) four DAR-PBGs (DAR-PB(L)G\(_{61}\), DAR-PB(L)G\(_{102}\), DAR-PB(D\(_{0.5}L_{0.5})G_{104}\), DAR-PB(D\(_{0.5}L_{0.5})G_{143}\)).
| Polymer                  | %Helix |       |       |
|--------------------------|--------|-------|-------|
| PB(L)G376                | Original | 71.5 | After sonication | 67.6 |
| PB(D)G365                | 63.7   |       | 61.0 |
| PB(DL0.27L0.73)G388      | 47.3   |       | 51.5 |
| PB(DL0.5L0.5)G332        | 0.0    |       | 0.0  |

The helicity of each of the synthesized PBGs was determined from circular dichroism (CD) spectroscopy, using tetrahydrofuran (THF) as solvent.
UV-Vis spectra

Figure S9. UV-Vis spectra of DAR-PB(L)G_{61} (a) and DAR-PB(L)G_{102} (b) in THF as a function of sonication time.

Figure S10. UV-Vis spectra of DAR-PB(D_{0.5}L_{0.5})G_{104} (a) and DAR-PB(D_{0.5}L_{0.5})G_{143} (b) in THF as a function of sonication time.
Figure S11. (a) UV-Vis spectra of rhodamine 6G at different concentrations in mixed THF/H₂O (v/v, 9/1). (b) Calibration curves using absorbance at 534 nm.
UV-Vis spectra of sonicated DAR-PBG and thermal reversion

**Figure S12.** (a) The normalized UV-Vis spectra of sonicated DAR-PB(L)G\textsubscript{102} solution (1 mg/ml) over different storage time. (b) The relative absorbance (538 nm) of sonicated DAR-PB(L)G\textsubscript{102} solution as function of storage time.

**Figure S13.** (a) The normalized UV-Vis spectra of sonicated DAR-PB(D\textsubscript{0.5}L\textsubscript{0.5})G\textsubscript{143} solution (1 mg/ml) over different storage time. (b) The relative absorbance (538 nm) of sonicated DAR-PB(D\textsubscript{0.5}L\textsubscript{0.5})G\textsubscript{143} solution as function of storage time.
Table S2. Rhodamine mechanophore activation in DAR-PBGs

| Polymer                  | $M_c$ (kDa) | $c$ (mM) | $\text{Abs}_{\text{theoretical}}^{[a]}$ | $\text{Abs}_{\text{measured}}^{[b]}$ | Activated content (%) |
|--------------------------|-------------|----------|----------------------------------------|--------------------------------------|-----------------------|
| DAR-PB(L)G$_{63}$       | 13.9        | 0.036    | 3.417                                  | 0.00525                              | 0.15                  |
| DAR-PB(D$_{0.5}$L$_{0.5}$)G$_{106}$ | 23.4       | 0.021    | 2.030                                  | 0.00214                              | 0.10                  |
| DAR-PB(L)G$_{102}$      | 23          | 0.022    | 2.065                                  | 0.00849                              | 0.41                  |
| DAR-PB(D$_{0.5}$L$_{0.5}$)G$_{145}$ | 32.1       | 0.016    | 1.480                                  | 0.00673                              | 0.45                  |

$^{[a]} \text{Abs}_{\text{theoretical}} = \varepsilon \times c \times l$, where $\varepsilon = 95000$ M$^{-1}$ cm$^{-1}$, $l = 1$ cm.$^{[b]}$ The absorbance of each DAR-PBG solution (0.5 mg/ml) was measured after 3 hours sonication.
Sonication results

### Table S3. $M_n$ values during sonication of PB(L)G$_{376}$

| Time/min | Sonication1 $M_n$ / kDa | Sonication2 $M_n$ / kDa | Sonication3 $M_n$ / kDa |
|----------|-------------------------|--------------------------|--------------------------|
| 0        | 82.3                    | 82.3                     | 82.3                     |
| 15       | 79.2                    | 79.9                     | 78.3                     |
| 30       | 73.8                    | 74.4                     | 74.6                     |
| 45       | 70.2                    | 68.9                     | 70.9                     |
| 60       | 67.4                    | 66.1                     | 67.5                     |
| 75       | 66.6                    | 65.3                     | 65.4                     |
| 90       | 63.7                    | 63.1                     | 63.5                     |
| 105      | 60.8                    | 59.8                     | 59.6                     |
| 120      | 59.8                    | 57.5                     | 58.0                     |

### Table S4. $M_n$ values during sonication of PB(D)G$_{365}$

| Time/min | Sonication1 $M_n$ / kDa | Sonication2 $M_n$ / kDa | Sonication3 $M_n$ / kDa |
|----------|-------------------------|--------------------------|--------------------------|
| 0        | 80.0                    | 80.0                     | 80.0                     |
| 15       | 76.8                    | 78.9                     | 77.5                     |
| 30       | 74.1                    | 75.4                     | 73.8                     |
| 45       | 71.8                    | 72.3                     | 71.8                     |
| 60       | 69.1                    | 69.1                     | 70.3                     |
| 75       | 68.2                    | 69.6                     | 68.8                     |
| 90       | 66.5                    | 66.7                     | 66.3                     |
| 105      | 65.3                    | 65.4                     | 64.8                     |
| 120      | 63.3                    | 64.0                     | 63.4                     |
### Table S5. $M_n$ values during sonication of PB(D$_{0.27}$L$_{0.73}$)G$_{388}$

| Time/min | Sonication 1 $M_n$ / kDa | Sonication 2 $M_n$ / kDa | Sonication 3 $M_n$ / kDa |
|----------|--------------------------|--------------------------|--------------------------|
| 0        | 85.0                     | 85.0                     | 85.0                     |
| 15       | 82.3                     | 82.5                     | 81.6                     |
| 30       | 81.4                     | 80.3                     | 80.0                     |
| 45       | 79.1                     | 79.1                     | 78.4                     |
| 60       | 76.7                     | 76.4                     | 77.3                     |
| 75       | 75.8                     | 75.7                     | 75.5                     |
| 90       | 74.2                     | 74.3                     | 74.3                     |
| 105      | 74.0                     | 73.9                     | 72.4                     |
| 120      | 72.9                     | 72.3                     | 71.8                     |

### Table S6. $M_n$ values during sonication of PB(D$_{0.5}$L$_{0.5}$)G$_{332}$

| Time/min | Sonication 1 $M_n$ / kDa | Sonication 2 $M_n$ / kDa | Sonication 3 $M_n$ / kDa |
|----------|--------------------------|--------------------------|--------------------------|
| 0        | 72.9                     | 72.9                     | 72.9                     |
| 15       | 72.3                     | 72.3                     | 72.2                     |
| 30       | 71.4                     | 70.6                     | 71.2                     |
| 45       | 71.2                     | 69.9                     | 70.6                     |
| 60       | 69.6                     | 69.4                     | 69.9                     |
| 75       | 69.1                     | 68.7                     | 69.0                     |
| 90       | 68.7                     | 68.6                     | 68.5                     |
| 105      | 68.5                     | 67.9                     | 68.1                     |
| 120      | 68.0                     | 67.0                     | 67.7                     |
Table S7. Slope $k'$ of the linear fit.

| Polymer                  | $k'$ \(\text{g mol}^{-1} \cdot \text{min}^{-1}\) | $R^2$ |
|--------------------------|-----------------------------------------------|-------|
| PB(L)\(_{376}\)         | 4.13 ± 0.07                                   | 0.998 |
| PB(D)\(_{365}\)         | 2.80 ± 0.04                                   | 0.998 |
| PB(D\(_{0.27}\):L\(_{0.73}\))\(_{188}\) | 1.89 ± 0.01                                   | 0.999 |
| PB(D\(_{0.5}\):L\(_{0.5}\))\(_{332}\)     | 0.96 ± 0.02                                   | 0.996 |
V. References

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