Supporting Information

for

Single-Molecule Force Spectroscopy of a Tetraaryl Succinonitrile Mechanophore

by

Martijn van Galen,†‡Jeya Prathap Kaniraj,**††Bauke Albada,**and Joris Sprakel*†

†Physical Chemistry and Soft Matter, Wageningen University Research, Stippeneng 4,6708 WE, Wageningen, the Netherlands
‡Laboratory of Organic Chemistry, Wageningen University Research, Stippeneng 4,6708WE, Wageningen, the Netherlands
*†These authors contributed equally

E-mail: joris.sprakel@wur.nl
# Table of Contents

## Contents

Table of Contents ........................................................................................................................................... 2

1. List of Abbreviations .................................................................................................................................... 3

2. Materials and methods ............................................................................................................................... 3

3. Experimental Procedures ............................................................................................................................ 4

3.1 Synthesis of Organic Compounds ........................................................................................................ 4

3.1.1 Synthesis of bisaldehyde (S1).......................................................................................................... 5

3.1.2 Synthesis of bis(2-hydroxyacetonitrile) (S2) .................................................................................. 7

3.1.3 Synthesis bis(2-(4-hydroxyphenyl)acetonitrile) (S3) ................................................................. 9

3.1.4 Synthesis of bis(2-(4-(prop-2-yn-1-yloxy)phenyl)acetonitrile) (1) ..................................... 11

3.1.5 Cyclization of bis(2-(4-(prop-2-yn-1-yloxy)phenyl)acetonitrile) to form cyclic TASN dialkyne (2) ................................................................................................................................. 14

3.1.6 Synthesis of alkyne-functionalized lipoic amide (S4) .............................................................. 17

3.1.6 Polymerization of bridged TASN (3) ....................................................................................... 19

3.2 Single molecule Force spectroscopy .................................................................................................... 21

3.2.1 Sample preparation and calibration .......................................................................................... 21

3.2.2 Force spectroscopy measurements ....................................................................................... 21

3.2.3 Force-distance curve analysis .............................................................................................. 21

3.2.4 Further analysis of the force-distance curves ................................................................... 22

3.3 Optical spectroscopy ............................................................................................................................. 23

4. Supporting data ........................................................................................................................................... 24

4.1 Raw single molecule force ramp spectroscopy data .................................................................. 24

4.2 Randomly selected overview of force-distance curves and fits after data processing ..... 26
4.3 Probability histograms of the enthalpic compliance $K$ and contour length $L$ obtained in the extensible worm-like chain fits .......................................................... 29
4.4 Extension length statistics at the point of dissociation .......................................................... 30
4.5 Analysis of the dissociation force as a function of the peak order ........................................ 31
4.6 Gel Permeation Chromatography ..................................................................................... 33
4.7 Mechanical activation of bridged mechanophore 2 through grinding .................................... 34
5. References ............................................................................................................................. 35

1. List of Abbreviations

- bis-azide TEG = 1,11-diazido-3,6,9-trioxaundecane
- DCC = dicyclohexylcarbodiimide
- DCM = dichloromethane
- DMF = dimethyl formamide
- DMSO = dimethyl sulfoxide
- ESI = Electron Spray Ionisation
- EtOAc = ethyl acetate
- PMDTA = $N,N,N',N''$-pentamethyldiethylenetriamine
- TEG = triethyleneglycole
- TASN = tetraarylsuccinonitrile

2. Materials and methods

All chemicals were purchased from Sigma-Aldrich, Fischer Scientific or TCI Chemicals and used as received unless indicated otherwise. All oxygen-sensitive reactions were performed under a nitrogen atmosphere. Column chromatography was performed on silica gel (particle size: 40–63 μm). Where the reaction solvent or eluent consisted of mixture of solvents, the ratios are reported by volume. $^1$H and $^{13}$C NMR spectra were recorded on a Bruker Avance III 400 MHz spectrometer. Mass spectra were recorded using ElectroSpray Ionization (ESI) on a Thermo Scientific Exactive mass spectrometer. IR analyses were performed on a Bruker Tensor 27 spectrometer with platinum ATR accessory. GPC was performed on a Agilent Technologies 1200 series GPC using a Plgel 5 um MIXED-D, 300 x 7.5 mm column.
3. Experimental Procedures

3.1 Synthesis of Organic Compounds

Scheme S1. Synthesis of TEG-bridged TSPN monomer 2.
3.1.1 Synthesis of bisaldehyde (S1)[1-3]

To a solution of 4-hydroxybenzaldehyde potassium salt (3.2 g, 20.0 mmol, 2 eq) in dry DMF (15 ml) at 90 ºC, tri(ethyleneglycol) dichloride (1.9 g, 10.0 mmol, 1 eq) in dry DMF (5 mL) was added dropwise over the course of 30 minutes. The resulting mixture was stirred at 100 ºC for 4 h after which a yellow–brownish mixture was cooled and poured onto ice–water (200 mL). The oily product was left standing overnight, and the obtained solid was filtered, washed with water, air dried, and recrystallized from aqueous methanol. This yielded the target compound as brown crystals in 90% yield (3.2 g, 9 mmol). 1H NMR (400 MHz, CDCl3), δ (ppm): 9.88 (s, 2H), 7.83–7.81 (d, J = 8 Hz, 4H), 7.02–7.00 (d, J = 8 Hz, 4H), 4.22–4.20 (t, 4H), 3.91–3.88 (t, 4H). 13C NMR (100 MHz, CDCl3), δ (ppm): 190.8, 163.8, 132.0, 130.1, 114.9, 70.7, 69.6, 67.7. IR (ATR): 3075 (s), 2952 (w), 2888 (w), 2835 (w), 2762 (w), 1693 (s), 1598 (s), 1507 (m), 1456 (m), 1354 (m), 1250 (s), 1215 (m), 1105 (s), 1061 (m), 955 (m), 922 (m), 846 (s), 830 (m), 793 (w), 652 (m), 637 (m), 618 (s), 519 (m), 409 (w) cm⁻¹.

Figure S1. 1H NMR spectrum of bisaldehyde S1.
Figure S2. $^{13}$C NMR spectrum of bisaldehyde S1.

Figure S3. ATR spectrum of bisaldehyde S1.
3.1.2 Synthesis of bis(2-hydroxyacetonitrile) (S2)

Potassium cyanide (10.3 g, 157.5 mmol, 7.5 eq) was dissolved in a biphasic system composed of water:ethyl acetate (1:1 (v/v), 150 mL). While stirring at room temperature NaHSO$_3$ (16.4 g, 157.5 mmol, 7.5 eq) was added. Note: the order of addition is important; addition of KCN to a solution of NaHSO$_3$ results in a violent reaction. To this biphasic system, bisaldehyde S1 (7.5 g, 21 mmol, 1 eq) was added in one portion, after which the flask was sealed with an outlet, and the mixture was stirred vigorously at r.t. for 24 h. Organic material was extracted from the biphasic system using EtOAc (3 × 200 mL) and the combined organic phases were washed with brine (100 mL). The organic layer was dried over MgSO$_4$ and concentrated under reduced pressure yielding the product as a white solid in 75% yield (6.5 g, 15.8 mmol). Note: the cyanohydrin product was unstable, therefore crude materials (92% pure) were used without purification. $^1$H NMR (400 MHz, DMSO), $\delta$ (ppm): 7.40–7.38 (d, $J = 8$ Hz, 4H), 7.00–6.98 (d, $J = 8$ Hz, 4H), 6.5–6.4 (d, $J = 8$ Hz, 2H), 4.10–4.08 (t, $J = 4$ Hz, 4H), 3.75–3.73 (t, $J = 4$ Hz, 4H), 3.60 (s, 4H). $^{13}$C NMR (100 MHz, DMSO), $\delta$ (ppm): 191.8, 163.9, 159.3, 132.3, 130.1, 129.9, 128.4, 121.2, 115.4, 115.1, 70.4, 69.2, 67.7, 61.8, 44.0. IR (ATR): 3440 (w), 2912 (w), 2877 (w), 2747 (vw), 2240 (vw), 1692 (w), 1600 (m), 1511 (w), 1502 (w), 1452 (w), 1348 (w), 1305 (w), 1252 (s), 1166 (m), 1101 (s), 1056 (s), 953 (m), 923 (m), 834 (m), 803 (m), 770 (m), 700 (w), 628 (m), 523 (m), 494 (m), 408 (m) cm$^{-1}$.

Figure S4. $^1$H NMR spectrum of bis(2-hydroxyacetonitrile) S2.
Figure S5. $^{13}$C NMR spectrum of bis(2-hydroxyacetonitrile) S2.

Figure S6. ATR spectrum of bis(2-hydroxyacetonitrile) S2.
3.1.3 Synthesis bis(2-(4-hydroxyphenyl)acetonitrile) (S3)

Bis(2-hydroxyacetonitrile) S2 (5.3 g, 13.0 mmol, 1 eq) and phenol (5.8 g, 62.4 mmol, 4.8 eq) were dissolved in 35% H$_2$SO$_4$ (aq) (25 mL) and stirred at 50 °C for 21 h. After this, the solvent was decanted, the residue product was extracted with EtOAc (2 × 100 mL). The combined organic phase was washed with water (2 × 100 mL) and brine (100 mL), after which it was dried over MgSO$_4$. After filtration, the solvent removed under reduced pressure and the obtained residue was purified via flash column chromatography (SiO$_2$, eluent: 4% acetone in DCM), yielding a pale-yellow solid in 40% yield (2.9 g, 5.3 mmol). $^1$H NMR (400 MHz, DMSO), $\delta$ (ppm): 9.58 (s, 2H), 7.24–7.22 (d, $J$ = 8 Hz, 4H), 7.14–7.12 (d, $J$ = 8 Hz, 4H), 6.95–6.92 (d, $J$ = 8 Hz, 4H), 6.76–6.74 (d, $J$ = 8 Hz, 4H), 5.54 (s, 2H), 4.05–4.03 (t, $J$ = 8 Hz, 4H), 3.72–3.69 (t, $J$ = 4 Hz, 4H), 3.57 (s, 4H). $^{13}$C NMR (100 MHz, CDCl$_3$), $\delta$ (ppm): 158.5, 157.5, 129.7, 129.0, 127.6, 121.4, 116.2, 115.4, 70.4, 69.3, 67.7, 31.1. IR (ATR): 3355 (w), 2924 (w), 2877 (w), 2243 (w), 1609 (m), 1508 (s), 1450 (m), 1353 (w), 1303 (w), 1245 (s), 1174 (m), 1104 (m), 1059 (m), 945 (m), 926 (m), 828 (s), 772 (m), 640 (w), 623 (m), 588 (m), 546 (m), 509 (m), 428 (w) cm$^{-1}$.

Figure S7. $^1$H NMR spectrum of bis(2-(4-hydroxyphenyl)acetonitrile) S3.
Figure S8. $^{13}$C NMR spectrum of bis(2-(4-hydroxyphenyl)acetonitrile) S3.

Figure S9. ATR spectrum of bis(2-(4-hydroxyphenyl)acetonitrile) S3.
3.1.4 Synthesis of bis(2-(4-(prop-2-yn-1-yloxy)phenyl)acetonitrile) (1)

Bis(2-(4-hydroxyphenyl)acetonitrile) S3 (564 mg, 1 mmol, 1 eq) was dissolved in dry DMF (5 mL), K$_2$CO$_3$ (360 mg, 2.6 mmol, 1.3 eq) was added and the resulting mixture was stirred at 70 °C for 1 h. After this, a propargyl bromide (0.26 mL, 2.6 mmol) was added in a dropwise fashion over the course of 30 minutes and the resulting mixture was stirred at 70 °C for 16 h. The resulting yellow–brownish mixture was cooled and poured onto ice–water (20 mL). The product was extracted with EtOAc (3 × 20 mL) and the combined organic layers were washed with brine. The organic layer was dried over MgSO$_4$ and after filtration the filtrate was concentrated under reduced pressure. The obtained residue was purified via flash column chromatography (SiO$_2$, eluent: 2 % acetone in DCM), yielding a solid in 70% yield (450 mg, 0.71 mmol). $^1$H NMR (400 MHz, DMSO), δ (ppm): 7.33–7.25 (m, 8H), 7.00–6.94 (m, 8H), 5.63 (s, 2H), 4.79 (s, 4H), 4.05 (t, $J$ = 8 Hz, 4H), 3.71 (t, $J$ = 8 Hz, 4H), 3.58–3.54 (s, 2H). $^{13}$C NMR (100 MHz, CDCl$_3$), δ (ppm): 158.5, 157.2, 130.2, 129.3, 129.0, 128.4, 121.2, 115.9, 115.4, 115.1, 79.5, 78.9, 70.4, 69.3, 67.7, 55.9, 50.3, 29.8. IR (ATR): 3286 (w), 3038 (w), 2923 (w), 2874 (w), 2242 (w), 2120 (w), 1608 (m), 1585 (w), 1500 (vs), 1453 (s), 1353 (m), 1303 (m), 1222 (s), 1178 (s), 1114 (m), 1024 (s), 925 (m), 827 (s), 678 (m), 641 (m), 624 (m), 547 (m), 510 (m) cm$^{-1}$.

Figure S10. $^1$H NMR spectrum of bis(2-(4-(prop-2-yn-1-yloxy)phenyl)acetonitrile) 1.
Figure S11. COSY NMR spectrum of bis(2-(4-(prop-2-yn-1-yloxy)phenyl)acetonitrile) I.

Figure S12. $^{13}$C NMR spectrum of bis(2-(4-(prop-2-yn-1-yloxy)phenyl)acetonitrile) I.
Figure S13. ATR spectrum of bis(2-(4-(prop-2-yn-1-yl)oxy)phenyl)acetonitrile 1.
3.1.5 Cyclization of bis(2-(4-(prop-2-yn-1-yloxy)phenyl)acetonitrile) to form cyclic TASN dialkyne (2)

Bis(2-(4-(prop-2-yn-1-yloxy)phenyl)acetonitrile) (1) (179 mg, 0.28 mmol) was dissolved in a mixture of solvents CHCl₃:MeOH (15:20 = 35 mL) and whilst stirring, potassium ferricyanide K₃[Fe(CN)₆] (272.2 mg, 0.84 mmol, 1.5 eq), as a solution in 5 M NaOH (5 mL), was added dropwise over the course of 5 minutes. The resulting mixture was stirred at r.t. for 0.5 h after which the reaction mixture (i.e. yellow precipitate) was washed with MeOH (100 mL) and water (100 mL), left alone overnight for air dry, resulting a colorless solid obtained in 84% yield (150 mg, 0.24 mmol). HRMS (ESI), found: 639.2490, calculated for C₄₀H₃₄N₂O₆ [M+H]+ 639.2490.

¹H NMR (400 MHz, CDCl₃), δ (ppm): 7.29–7.25 (m, 2H), 7.20–7.11 (m, 6H), 6.94–6.92 (d, J = 8 Hz, 2H), 6.88–6.86 (d, J = 8 Hz, 2H), 6.81–6.79 (d, J = 8 Hz, 2H), 6.75–6.73 (d, J = 8 Hz, 2H), 4.66–4.63 (m, 4H), 4.11–4.06 (m, 4H), 3.83–3.82 (m, 4H), 3.72–3.71 (m, 4H), 2.52–2.49 (m, 2H). ¹³C NMR (100 MHz, CDCl₃), δ (ppm): 158.6, 157.4, 131.3, 131.3, 130.1, 129.3, 128.9, 128.3, 128.3, 121.3, 115.5, 115.2, 115.1, 114.8, 114.2, 113.9, 78.1, 76.0, 70.9, 69.7, 58.4, 55.9. IR (ATR): 3287 (w, s), 2923 (w, s), 2873 (w, s), 1607 (m), 1500 (s), 1454 (w), 1300 (m), 1186 (s), 1124 (m), 1059 (m), 1024 (s), 926 (m), 825 (m), 678 (w), 639 (m), 538 (m) cm⁻¹.

Figure S14. ¹H NMR spectrum of cyclic TASN dialkyne 2.
Figure S15. COSY NMR spectrum of cyclic TASN dialkyne 2.

Figure S16. $^{13}$C NMR spectrum of cyclic TASN dialkyne 2.
Figure S17. ATR spectrum of cyclic TASN dialkyne 2.

Figure S18. HRMS of cyclic TASN dialkyne 2.
3.1.6 Synthesis of alkyne-functionalized lipoic amide (S4)

Lipoic acid (0.5 g, 2 mmol) and N-hydroxysuccinimide (0.23 g, 2 mmol) were dissolved in DCM (4 mL) and cooled to 0 °C. Then, a solution of DCC (0.5 g, 2.5 mmol) in 3 mL DCM was added dropwise, and the mixture was stirred for 1 h. After this, the mixture was kept in the refrigerator overnight to fully precipitate the formed dicyclohexylurea (DCU). The precipitate was filtered and the filtrate was evaporated in vacuo. Next, the product (0.5 g, 1.56 mmol) was dissolved in 10 mL DCM and added to an ice-cold solution of propargylamine (100 μL, 1.56 mmol) in DCM (10 mL). The mixture was stirred at r.t. for 3 h after which it was diluted with CH₂Cl₂ (30 mL) and washed with saturated Na₂CO₃. The organic layer was washed with brine, and dried with anhydrous Na₂SO₄. After filtration, the filtrate was concentrated in vacuo and the residue was purified by silica gel column chromatography (eluent: 2 % acetone in DCM), to afford 2 (0.33 g, 89%) as yellow solid. ¹H NMR (400 MHz, CDCl₃), δ (ppm): 5.63 (brs, 1H), 4.07–4.05 (dd, J = 4, 2H), 3.61–3.54 (m, 1H), 3.22–3.09 (m, 2H), 2.51–2.43 (m, 1H), 2.25–2.18 (m, 3H), 1.96–1.88 (m, 2H), 1.75–1.61 (m, 5H), 1.54–1.44 (m, 2H). ¹³C NMR (100 MHz, DMSO), δ (ppm): 172.2, 170.7, 169.4, 81.8, 73.2, 56.6, 56.4, 38.6, 35.3, 34.6, 34.3, 30.5, 28.7, 28.2, 25.9, 25.4, 24.5. IR (ATR): 3266 (w), 3227 (w), 3070 (w), 2938 (w), 2854 (s), 1810 (m), 1781 (m), 1730 (s), 1544 (s), 1459 (m), 1425 (m), 1374 (w), 1248 (m), 1205 (s), 1075 (s), 880 (m), 809 (w), 704 (s), 573 (m), 412 (m), 358 (w) cm⁻¹.

Figure S19. ¹H NMR spectrum of alkyne-functionalized lipoic amide S4.
Figure S20. $^{13}$C NMR spectrum of alkyne-functionalized lipoic amide $S_4$.

Figure S21. ATR spectrum of alkyne-functionalized lipoic amide $S_4$. 
3.1.6 Polymerization of bridged TASN (3)

Scheme S2. Synthesis of TASN (1)-linked polymer 3.

Bridged TASN dialkyne 1 (21.1 mg, 0.03 mmol, 1 eq) and bis-azide TEG (8 mg, 0.33 mmol, 1 eq) and CuBr (0.5 mg, 3 µmol, 0.1 eq) were dissolved in anhydrous DMSO (1 mL) and stirred at r.t. for 5 minutes. Then, PMDTA (1.14 mg, 66 µmol, 2 eq) was added and continuously stirred for 2 h. After this, and additional amount of bis-azide TEG (1.6 mg, 66 µmol, 0.2 eq) was added and the mixture was continued to be stirred for an hour in order to ensure that all alkyne functionalities of TASN dialkyne 1 reacted with bis-azide TEG. Finally, alkyne-functionalized lipoic amide S4 (4.14 mg, 17 µmol, 0.5 eq) was added to the reaction mixture which was, again, stirred for 2–3 h. After this, the pale green colored reaction mixture was passed through a plug of basic alumina to remove Cu-precipitate and the filtrate was precipitated in water. The precipitate was washed with MeOH (20 mL) and air dried, yielding a light yellow solid (12 mg, 50% over two steps). $^1$H NMR (400 MHz, CDCl$_3$), δ (ppm): 7.81 (br m, 3H), 7.14–6.93 (br m, 8H), 6.82–6.78 (br m, 8H), 5.13 (br m, 4H), 4.50 (br m, 4H), 4.09 (br m, 4H), 3.83–3.72 (br m, 12H), 3.52 (br m, 8H), 3.15 (br s, 1H). IR (ATR): 3136 (w, s), 2871 (w, s), 1606 (m), 1500 (s), 1458 (w), 1298 (s), 1252 (m), 1186 (m), 1119 (m), 1051 (w), 1011 (w), 928 (w), 826 (m), 772 (w), 638 (w), 595 (w), 539 (m) cm$^{-1}$. For GPC-analysis, see section 4.4.
Figure S22. $^1$H NMR spectrum of polymer 3.

Figure S23. ATR spectrum of polymer 3.
3.2 Single molecule Force spectroscopy

3.2.1 Sample preparation and calibration

Sample treatment for single molecule force spectroscopy experiments was based on previously reported procedures [4]. Force spectroscopy experiments were performed on a JPK ForceRobot® 300 automated force spectroscope, using Bruker's Sharp Microlever probe (MSNL-10, triangular cantilever D). As a substrate, we used P/Boron doped silicon wafers ordered from Si-mat with a resistivity of 1-30 µm and crystal orientation 100. These silicon wafers were cut into 1x1 cm square pieces, rinsed in isopropanol, sonicated for 10 minutes in isopropanol in a sonicator bath at room temperature, dried with compressed nitrogen, and plasma-cleaned for 15 minutes. Next, the surfaces were coated with an Au-layer by sputter deposition, rinsed with isopropanol and left to dry. In a similar fashion, the MSNL-10 AFM tip was rinsed with isopropanol, left to dry, plasma-cleaned for 15 minutes, and coated with an Au-layer using sputter deposition. The tip was then rinsed another time in isopropanol and left to dry. The AFM tip was calibrated using the thermal vibration method, and its spring constant was found to be $k_{\text{cantilever}} = 0.046$ N/m.

3.2.2 Force spectroscopy measurements

Prior to the measurements, the Au-coated silicon wafers were incubated with a 1 mg/ml solution of the lipoic acid-capped bridged TASN-bearing polymer 3 in dimethyl formamide (DMF). After 1 minute of incubation, the slides were rinsed thoroughly in DMF. AFM force ramp measurements were carried out immediately afterwards in a sealed liquid cell filled with DMF. During each approach-retract cycle, the tip was pressed against the surface until a compression force of 1.43 nN was reached. The tip was kept at this force for 500 ms, after which it was retracted at a constant retract velocity of 0.35, 0.71 or 1.44 µm/s up to a surface-separation distance of 1 µm. The data acquisition rate during the retraction step was set to ensure that at least 1000 datapoints were collected per force-distance curve. The x-y position at which the tip contacts the substrate was changed roughly every 100 approach-retract cycles in order to sample varying positions on the surface.

3.2.3 Force-distance curve analysis

We analyzed the raw force-distance curves, obtained from the approach-retract cycles described above, using a custom-written Python script. Data-analysis was performed in the following steps: First, we convert the Piezo height to true height of the tip above the surface. This is done by subtracting the deflection of the cantilever, $(F/k_{\text{cantilever}})$ from the piezo height.

Next, we performed a baseline correction by fitting the final 300 datapoints - which did not contain any dissociation events - with the sum of a sine wave and a linear function. We then
subtracted this fit from the force-distance curve to remove any off-sets and intensity fluctuations caused by interference[5]. Next, we defined the contact point between the tip and the surface as the first datapoint in our force-extension curve where the force exceeds this baseline value. This intersection point was set to height $h = 0 \, \mu m$, defining contact between tip apex and substrate, so that all other heights are defined relative to this contact point. The script then determines the location of potential dissociation events by finding points where the force drops at least 5 pN over a height distance of 0.4 nm. A fit of the extensible worm-like-chain model was then fitted through the 30 datapoints leading up to the suggested rupture location[6]. This method also finds many points that are not real dissociation events. For this reason, the script only suggests dissociation events, which the user then accepts or rejects by hand with a graphic user interface. We only accepted those events that show a clearly nonlinear stretching behavior based on the extensible worm-like chain fit, in order to distinguish between polymer stretch events and events due to aspecific adhesion, such as those resulting from van der Waals interactions. The peaks are sorted based on the order in which they appear in the force-distance curves, for further analysis discussed in section 4.5. In this way, the final event of each force-distance curve was rejected, as this event can only be caused by dissociation of the lipoic acid end group from the surface, rather than mechanophore activation. For each accepted event we stored the dissociation force and dissociation height, as well as the persistence length and contour length obtained from the WLC fit for further analysis.

3.2.4 Further analysis of the force-distance curves

Bin sizes of the rupture force histograms were chosen using the Freedman-Diaconis rule. We determined the loading rates $r_F \, (N/s)$ of each accepted event by multiplying the tip retract velocity $v$ by the derivative of the worm-like chain fit $dF(h)/dh$ at the dissociation height:

$$r_F = \frac{dF(h)}{dt} = \frac{dF(h)}{dh} \frac{dh}{dt} = \left[ \frac{1}{2L} \left( 1 - \frac{h}{L} \right)^{-3} - \frac{1.72}{L} \left( \frac{h}{L} \right)^{1.15} + \frac{1}{L} \right] \frac{k_B T}{L_p} \cdot v$$

Here, $L$ represents the contour length and $L_p$ denotes the persistence length. Dissociation histograms were transformed into force-lifetime curves with the Dudko-Hummer-Szabo (DHS) method[7]. This method transforms each bin in the dissociation force histogram to a datapoint in the force-lifetime curve, provided that the loading rate $r_F$ is known. For a precise description of this method, the reader is referred to reference[7]. Here, we apply their method in the following way: We consider that our dissociation histograms consist of $N$ bins with a bin width $\Delta F$. We then define the minimum force of the first bin as $F_0$, resulting in a maximum force of the final bin: $F_N = F_0 + N \Delta F$. If we write the number of counts in the $i$th bin as $C_i$, we can calculate the height of the bins as $h_i = C_i / (N_{tot} \Delta F)$, with $N_{tot}$ the total number of counts. Then, for $k=1, 2, \ldots$ we calculate the lifetime $\tau(F_0 + (k-1/2) \Delta F)$ at the center of each force bin as follows:
Because the loading rate $r_F$ varies between events, we use the mean loading rate of the events included in each bin for the DHS transformation. The resulting DHS force-life time curves were fitted using a modified Bell-Evans equation\cite{7}:

$$\tau(F_0+(k-1/2)\Delta F) = \frac{\left(h_k/2 + \sum_{i=k+1}^{N} h_i\right) \Delta F}{h_k \ r_F \ (F_0 + (k - 1/2)\Delta F)}$$

Here, $\tau$ denotes the life time, $\tau_0$ is the life time in absence of force, $x^\dagger$ is the activation length and $\beta=1/k_B T$. This equation also takes into account the height of the energy barrier $\Delta G^\dagger$ for mechanophore activation, as well as the shape of the energy barrier, denoted by the parameter $\alpha$. For a harmonic potential well with a barrier that is cusp-like, $\alpha = 1/2$. For a square barrier, $\alpha = 1$ and the equation reverts back to the Bell-Evans equation\cite{7}. We performed three fits of the force-lifetime curves with three fixed values of $\alpha$ of $1/2$, $2/3$ and $1$.

### 3.3 Optical spectroscopy

Fluorescence spectrometry experiments were performed using a Cary Eclipse fluorescence spectrophotometer (Agilent) with a temperature-controlled cuvette holder. UV-vis spectroscopy experiments were run in an Evolution 220 spectrophotometer (Thermo Scientific). For each spectrophotometry experiment, the bridged TASN mechanophore 2 was dissolved in dimethyl acetamide (DMAc) at a concentration of 1 mg/mL. Temperature-dependent fluorescence emission and UV-vis spectra were recorded in incremental heating steps from 20 °C to 100 °C. After reaching each temperature, the system was equilibrated for two minutes before the spectra were recorded. The decay of the fluorescence over time was measured by heating the sample to 80 °C, after which the intensity at 560 nm was recorded every two minutes.
4. Supporting data

4.1 Raw single molecule force ramp spectroscopy data

Figure S24 Overview of twenty randomly selected raw force-distance curves collected during the force ramp spectroscopy measurements. No data processing has been performed on these plots, except for the subtraction of the cantilever deflection from the height obtained by the piezo path, as explained in section 3.2.3. Note the sinusoidal shape in the baseline of some of the raw datasets, which is typically caused by interference of the laser. We correct for this interference by fitting our baseline with a sinusoid, as explained in section 3.2.3.
Figure S25 Zoomed-out versions of the force-distance curves displayed in figure S24, showing that the force-distance curves are vertical in the indentation regime of the curves after subtracting the cantilever deflection from the height obtained by the piezo path.
4.2 Randomly selected overview of force-distance curves and fits after data processing

Figure S26 Overview of twenty randomly selected force-distance curves obtained at retract velocity 0.35 µm/s, after performing the data processing steps explained in section 3.2.3. Extensible worm-like chain fits of the accepted events are shown in black.
Figure S27 Overview of twenty randomly selected force-distance curves obtained at retract velocity 0.71 µm/s, after performing the data processing steps explained in section 3.2.3. Extensible worm-like chain fits of the accepted events are shown in black.
Figure S28 Overview of twenty randomly selected force-distance curves obtained at retract velocity 1.44 µm/s, after performing the data processing steps explained in section 3.2.3. Extensible worm-like chain fits of the accepted events are shown in black.
4.3 Probability histograms of the enthalpic compliance \( K \) and contour length \( L \) obtained in the extensible worm-like chain fits

![Probability histograms of (a) the enthalpic compliance \( K \) and (b) the contour length \( L \) found for the accepted extensible worm-like chain fits for each retract velocity. Each of these histograms contains at least 97 per cent of the data. The remaining small percentage of the fits contained contour lengths or spring constants greater than the limits of the plotted horizontal axes. As we vary the retract velocity, we find that the typically observed enthalpic compliances and contour lengths change little. This is to be expected, as these properties depend on the polymer rather than the experimental design of the approach-retract cycles. The contour lengths show an optimum in the range of \( \sim 20 – 40 \) nm, which is similar to the polymer lengths found by Gel Permeation Chromatography (Fig. S32)
4.4 Extension length statistics at the point of dissociation

Figure S30 Probability histograms showing the extension length at the point of dissociation $h_D$ observed for the recorded dissociation events at each retract velocity. We find an optimum in the dissociation lengths in the regime from ~20 to ~40 nm, which is similar to the contour lengths obtained in both the gel permeation chromatography analysis (Fig. S32) and the contour lengths obtained in the extensible worm-like chain fits (Fig. S29b). We can therefore conclude that the polymers are typically stretched to a length close to their contour length at the point where dissociation occurs. When polymers are stretched to this extent, enthalpic contributions to the force-extension curves play a major role. These extension length distributions therefore provide further evidence of the necessity to fit the force-distance curve with a model that considers enthalpic as well as entropic distributions, such as the extensible worm-like chain model we use in our data analysis.
4.5 Analysis of the dissociation force as a function of the peak order

As a result of our experimental design, we expect that the force-extension curves presented in this work contain a series of TASN mechanophore dissociation events followed by a final dissociation event of the Au-S interactions which attach the mechanophore-bearing polymers to the surface. Au-S dissociation forces have previously been reported to lie in the range of 0.5-1.5 nN [8], which is higher than the TASN dissociation force we typically observe in our data. Based on our hypothesis that the final dissociation event in each force-extension curve belongs to Au-S dissociation, we would therefore expect that the last dissociation event in a series occurs at greater forces than the preceding dissociation events. To test this hypothesis, we sorted the dissociation force distributions based on the order in which they occur in the force-extension curves (Fig. S31). Interestingly, we find that the last peak does not occur at substantially greater forces than the second last or third last peaks in the force-extension curves. One potential explanation is that some fraction of the mechanophores inside our polymer are not successfully bridged. In this case, the activation of such a mechanophore would break the connection between the AFM tip and the surface, meaning that the final dissociation event in the series could also be caused by mechanophore activation events. Another potential explanation is that the Au-S interactions in our experiments break at lower forces than previously measured by Xue et al. [8] and the dissociation force actually comparable to the dissociation force of our mechanophore. The measurements performed by Xue et al. were performed in aqueous solutions, and the forces were found to vary widely based on the surface modification, and solvent conditions such as pH. It is therefore possible that the dissociation force of Au-S interactions in the organic solvent conditions we used is of the same order of magnitude as the force required for mechanophore activation.

Regardless of which of these explanations is true, however, we still expect that all peaks in the force-extension curves with the exception of the final peak are caused by mechanophore activation rather than Au-S dissociation. This is because any Au-S dissociation event would break the connection between the AFM tip and the surface, automatically making this event the final event in the series.

Figure S31 Dissociation force probability histograms sorted on the order in which the peaks occurred in the force-extension curves, showing the last, second to last and third last peak of each series, for each of the three retract velocities we tested.
Another interesting observation is that we do not find a clearly increasing trend in the peak force over the course of the peaks approaching the final peak. Such an increasing trend is typically observed in studies on tandem protein unfolding, such as the sequential unfolding of protein domains in titin [9-11]. In these proteins, there is some variety in the strength between the various protein domains, which causes the weaker proteins to break first [9]. We do not observe such a trend, because the TASN mechanophores we probe are much simpler than the complex protein subunits in titin: The dissociation events of TASN rely on the breakage of a single covalent bond, rather than the unfolding of a protein domain comprised of multiple noncovalent interactions varying in strength. As a result, we there is much less variety in strength between the TASN mechanophores in our polymers than there is between the protein domains of titin. We can therefore expect the dissociation events to occur at much more similar forces.
4.6 Gel Permeation Chromatography

The molecular weight of the TASN-mechanophore bearing polymer 3 was evaluated with Gel Permeation Chromatography (GPC) in Chloroform at 36 °C. The results are shown in figure S32. Overall, we find the polymer to be relatively short and polydisperse.

Based on the GPC analysis, we observe values of $M_n = 4.5$ kg/mol and $M_p = 5.9$ kg/mol. Our mechanophore-bearing polymer consists of a two lipoic acid end-caps with a combined molecular mass of 731.22 g/mol, and a repeating TASN-bearing core with a molecular mass of $N*883.27$ g/mol, based on its molecular structure (Fig. 1c). Here, N is the number of TASN units present in the polymer. We calculate the average number of TASN mechanophores per polymer $<N>$ with the equation: $M = 731.22 + <N>*883.27$, and we find $<N> = 4.3$ based on $M_n$ and $<N>=5.9$ based on $M_p$. Using an average carbon-carbon bond length of 1.54 Å, we then calculate the contour length to range from 26 nm based on $M_n$ to 33 nm based on $M_p$. These values are comparable to the contour length optima found in the extensible worm-like chain fits (Fig. 29b).

| Peak | $M_p$ (g/mol) | $M_n$ (g/mol) | $M_w$ (g/mol) | $M_z$ (g/mol) | $M_{z+1}$ (g/mol) | $M_v$ (g/mol) | PD |
|------|--------------|--------------|--------------|--------------|-----------------|--------------|----|
| Peak 1 | 5940 | 4496 | 8785 | 14515 | 20050 | 13685 | 1.954 |

![Distribution Plot](image1.png)

Figure S32 Gel permeation chromatography results of the TASN mechanophore-bearing polymer 3.
4.7 Mechanical activation of bridged mechanophore 2 through grinding

Bridged mechanophore 2 can also be activated mechanically by grinding with a pestle and mortar, as shown in Fig. S33. Before grinding, the mechanophore is yellow ambient light (Fig. S33a) and not fluorescent when irradiated with a UV-lamp (Fig. S33b). After grinding, the mechanophore changes color from yellow to pink (Fig. S33c) and becomes brightly yellow fluorescent when irradiated with a UV-lamp (Fig. S33d) indicating activation of the mechanophore and formation of the fluorescent radical. The fluorescence remains for several minutes after grinding.

Figure S33 Photographs of a sample of solid bridged TASN mechanophore 2 before (a,b) and after (c,d) grinding with a pestle, under Ambient light (a,c) and under a UV lamp (b,d).
5. References

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