Supplementary information

What can a kinetic modelling approach reveal about the intricacies of ball milling reaction kinetics?

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1 Experimenta setup of ball milling reactions used for kinetic studies

All milling experiments were performed using a 14.5 mL snap-closure homemade stainless steel milling jar containing two stainless steel balls (7 mm diameter). The jars were mounted in a Retsch MM400 ball mill and operated at 30 Hz. The jars consisted of two hemispherical caps (internal diameter: 18 mm) connected by a cylindrical section, giving an overall internal length of 50.5 mm.[1]

The studied reaction involves an equimolar mixture of 2-nitrophenyl disulfide (1–1) and 4-chlorophenyl disulfide (2–2) in the presence of the base catalyst 1,8-diazabicyclo[5.4.0]undec-7-ene (DBU, 2 mol%), yielding the asymmetric disulfide heterodimer (1–2). DBU is a highly viscous liquid.

Kinetic experiments were conducted under both neat grinding (NG, no added solvent) and liquid-assisted grinding (LAG) conditions. For LAG experiments, 50 μL of acetonitrile (MeCN) was added. This amount is sufficient to wet the powder surface and promote formation of the LAG polymorph, (1–2)B. The jars were milled at 30 Hz for varying durations to obtain well-resolved kinetic profiles.

The persistent polymorph (PP) and short-lived polymorph (SLP), used as seeds in seeded kinetic experiments, were prepared via disulfide exchange under "no-seed" conditions in the presence of DBU; consequently, these seed materials contain residual DBU.

For "**no seeds**", both for NG and LAG, an equimolar mixture of **1-1** (0.34 mmol, 104.8 mg) and **2-2** (0.34 mmol, 97.6 mg) were added to the grinding jar (total powder ~200 mg) and carefully mixed with a microspatula. Two 7 mm balls were carefully incorporated in the jar. 2μL DBU were dispensed on top of the balls and milling experiments started. For LAG, 50 μL of MeCN was added to the powder.

For "**3% SLP**", both for NG and LAG, an equimolar mixture of 48.5 mol% of **1-1** (0.34 mmol, 104.8 mg) and 48.5 mol% of **2-2** (0.34 mmol, 97.6 mg) and 3mol% of **SLP seeds** (0.02 mmol, 6.03 mg) were added to grinding jar (total powder ~200 mg) and carefully mixed with a microspatula. Two 7 mm balls were deposited in the jar. 2μL DBU was dispensed on top of the balls and milling experiments started at 30 Hz.

For "**23% SLP**", both for NG and LAG, an equimolar mixture of 38.5 mol% of **1-1** (0.262 mmol, 80.72mg) and 38.5 mol% of **2-2** (0.262 mmol, 75.20 mg) and 23mol% of **SLP seeds** (0.156 mmol, 46.45mg) were added to grinding jar (total powder ~200 mg) and carefully mixed with a microspatula. Two 7 mm balls were deposited in the jar. 2μL DBU was dispensed on top of the balls and milling experiments started.

2 Design of kinetic ball milling studies

Four sets of kinetic milling experiments (i to iv) were performed using an equimolar mixture of homodimers in the presence of DBU and an additional set of experiments (v) in the absence of dbu. The experiments were conducted under different seeding conditions, under NG and LAG.[1] The labels for i to iv are consistent with those used in the manuscript:

(i) "**no seeds**" (**Section 4**): no added seeds of the heterodimer 1–2 to 0.34 mmol of the equimolar mixture of homodimers (approximately 200 mg total powder)[1]

(ii) "**3% PP**" (**Section 6**): 3 mol% seeds (0.02 mmol) of PP polymorphs of 1–2, added to 0.34 mmol of the equimolar mixture of homodimers (approximately 200 mg total powder)[1]

(iii) "**3% SLP**": (**Section 7**): 3 mol% seeds (0.02 mmol) of SLP polymorphs of 1–2, added to 0.34 mmol of the equimolar mixture of homodimers (approximately 200 mg total powder)[1]

(iv) "**23% SLP**" (**Section 7**): 23 mol% seeds (0.156 mmol) of SLP added to 0.262 mmol of the equimolar mixture of homodimers (approximately 200 mg total powder)[1]

Additionally, one extra set of seeded experiment performed in the absence of DBU.

(v) "**23% SLP/no DBU**" (**Section 8**): 23 mol% seeds (0.156 mmol, absence of DBU) of SLP added to 0.262 mmol of the equimolar mixture of homodimers (approximately 200 mg total powder).

3 Monitoring of ball milling kinetic studies by PXRD & HPLC

All milling experiments were analysed using HPLC and PXRD.

PXRD profiles, when subjected to Rietveld refinement, provide the phase composition of all components present in the ball-milling reaction. However, PXRD has a relatively high limit of detection (LOD) of approximately 3 mol%.

In contrast, HPLC quantifies each component by integrating a single peak per species and offers a much lower LOD (ca. 0.1 mol%). As HPLC measurements are performed in solution, the technique cannot distinguish between different polymorphs of a given compound; instead, it reports the total composition of that component, corresponding to the combined contribution of (1-2)A and (1-2)B.[1]

HPLC data for the early experimental time points in the kinetic studies (peak for 1-2 eluting ~ 0.9 min, with composition expressed as mol% relative to all components, i.e., (1-1 + 2-2 + 1-2) are presented as insets in Figures S1–S10. HPLC analysis is particularly valuable during the initial stages of milling (induction stage), where the product concentration may fall well below 3 mol%.

The initial time point (t = 0) could only be measured after milling unseeded experiments in the absence of DBU for a few minutes to ensure a homogeneous mixture while avoiding disulfide exchange. Since seeds of both the persistent and short-lived polymorphs must be prepared via disulfide exchange and therefore contain DBU, t = 0 measurements could only be obtained using 3 mol% seed loading. At this concentration, the amount of DBU present does not immediately induce disulfide exchange, provided milling is conducted only briefly to achieve homogeneity.[1]

4 Unseeded kinetic studies in presence of DBU

The HPLC data presented in Figures S1 and S2 support the statement made in the manuscript that: "mechanochemical transformation occurs as soon as mechanical stress is applied, beginning with the

very first impact and without requiring a distinct chemical induction stage." Consistently, the detection of small amounts of product by HPLC at very short milling times supports this interpretation as shown in the inserts of Figures S1 and S2.

The induction stage is typically defined as the period during which no apparent reaction is observed. During this stage, the starting material crystals undergo mechanical activation: particles fracture, defects accumulate, and the solid becomes increasingly reactive. This does not preclude the possibility that some degree of reaction—albeit not yet significant—may occur from the very first impact. In practice, however, the earliest experimentally accessible time point in our unseeded milling experiments is 5 minutes, at 30 Hz.[1]

4.1 Unseeded kinetic studies under LAG conditions in presence of DBU

The reliability of phase quantification by Rietveld refinement of PXRD data at early milling time points (5 and 10 min) is limited due to its relatively high limit of detection (~3 mol%). In this regime, HPLC provides a valuable complementary technique owing to its significantly lower LOD. For example, at t = 5 min, HPLC analysis (see inset, Figure S1) already detects 1-2 at 1.1 mol%, a concentration below the PXRD detection threshold. Under LAG conditions, this product is believed to form exclusively as polymorph (1-2)B, the persistent polymorph.

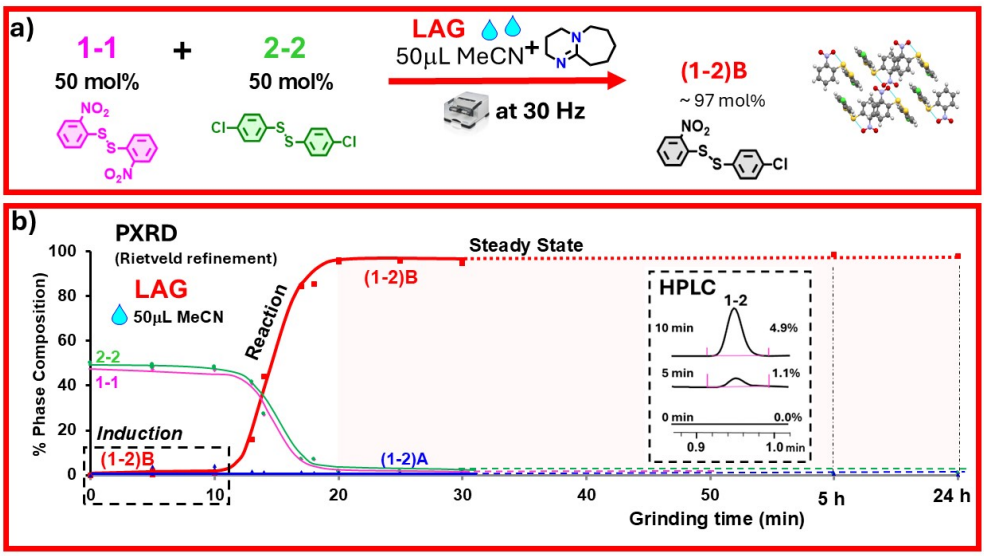

Figure S 1 Ball milling of equimolar homodimers 1–1 (pink) and 2–2 (green) under LAG conditions with 50 µL MeCN in the presence of DBU leading to formation of heterodimer (1-2)B (red). (a) Reaction scheme. (b) Reaction kinetics showing phase composition (mol%) as a function of grinding time, determined by Rietveld refinement of PXRD patterns (limit of detection > 3 mol%). Insert: HPLC analysis (limit of detection ≈ 0.1 mol%) of the reaction mixture at early time points (5 and 10 min), showing only the heterodimer peak eluting at ~0.95 min. The initial time point (0 min) was determined in the absence of DBU to prevent disulfide exchange and formation of the 1–2 heterodimer.[1] Adapted from Ref 1 with permission from JACS, 2014, 136, 16156-16166, Copyright {2014} American Chemical Society.

4.2 Unseeded kinetic studies under LAG conditions in presence of DBU

Although under NG conditions, kinetics present a slower rate, the composition at 5 min is 2.9 mol%, a concentration below the PXRD detection threshold. Under NG conditions, this product is believed to form exclusively as polymorph (1-2)A, the persistent polymorph under these conditions.[1]

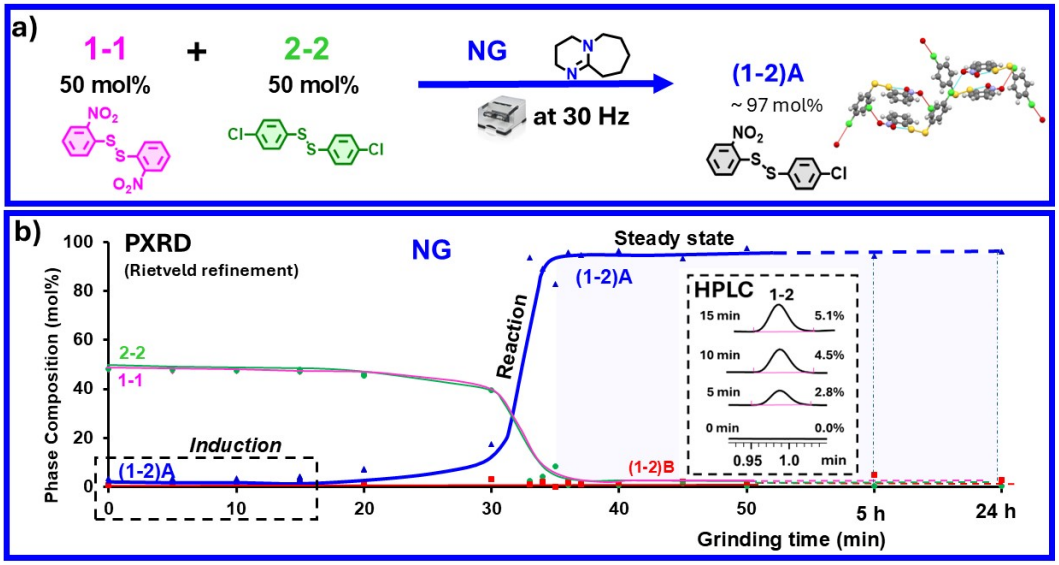

Figure S 2 Ball milling of equimolar homodimers 1–1 (pink) and 2–2 (green) under NG conditions in the presence of DBU leading to formation of heterodimer (1-2) A (blue). (a) Reaction scheme. (b) Reaction kinetics showing phase composition (mol%) as a function of grinding time, determined by Rietveld refinement of PXRD patterns (limit of detection > 3 mol%). Insert: HPLC analysis (limit of detection ≈ 0.1 mol%) of the reaction mixture at early time points (5, 10 and 15 min), showing only the heterodimer peak eluting at ~0.95 min. The initial time point (0 min) was determined in the absence of DBU to prevent disulfide exchange and formation of the 1–2 heterodimer.[1] Adapted from Ref 1 with permission from JACS, 2014, 136, 16156-16166, Copyright {2014} American Chemical Society.

5 Seeded kinetic studies with persistent polymorph in presence of DBU

When persistent polymorph seeds of 1-2, the disulfide heterodimer, are added to an equimolar mixture of the disulfide homodimer (1-1 adn 2-2) in the presence of DBU, the formation of the persistent polymorph during ball milling reaction accelerates. This is quite dramatic increase of this persistent polymorph under LAG (Section S5.1, Figure S3), while slow and moderate than for NG (Section S5.2, Figure S4).

5.1 Under LAG conditions with 3 mol% of persistent polymorph seeds

Under LAG conditions and in the presence of DBU, adding just 3 mol% of (1–2)B (the persistent polymorph) to 97 mol% of homodimers (1-1 and 2-2) causes a rapid increase in (1–2)B, as observed by PXRD. At the same time, the composition of the homodimers (1–1 and 2–2) decrease. See Figure S3.

HPLC confirms this change in composition, showing a fast rise in 1–2 within the first 4 minutes (see inset). In this kinetic study, the 1–2 formed is expected to consist entirely of (1–2)B, since (1–2)A does not form under LAG conditions.

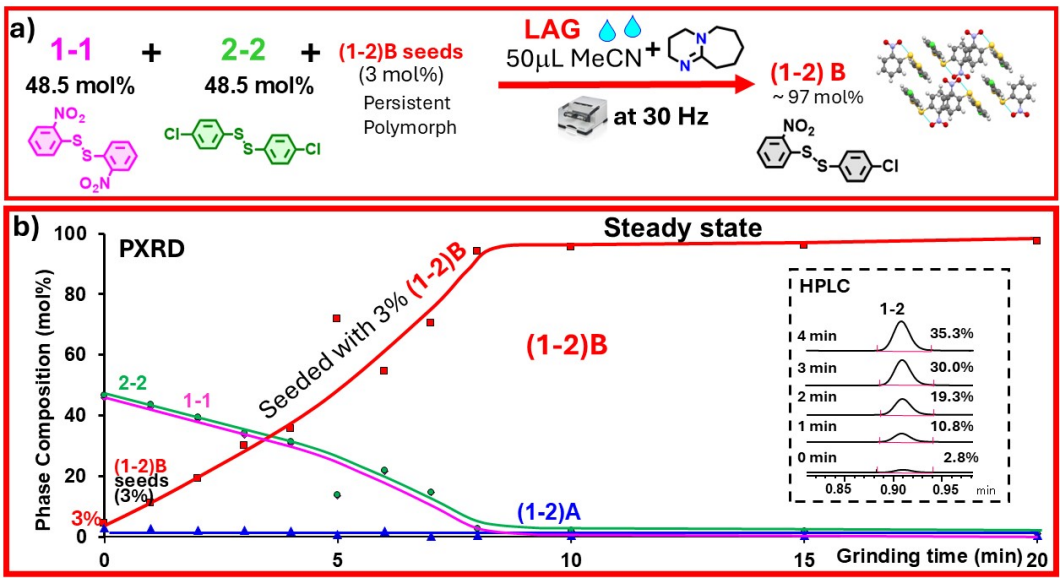

Figure S 3 Ball milling of equimolar homodimers 1–1 (pink) and 2–2 (green) with the addition of 3 mol% of (1-2)B (persistent polymorph) under LAG conditions (50 µL MeCN) in the presence of DBU, leading to the formation of heterodimer (1-2)B (red). (a) Reaction scheme. (b) Reaction kinetics showing phase composition (mol%) as a function of grinding time, as determined by Rietveld refinement of PXRD patterns (limit of detection > 3 mol%). Inset HPLC analysis of the reaction mixture at early time points (1–4 min), showing only the heterodimer1-2 peak eluting at ~0.9 min. The initial time point (t = 0 min) was determined in the absence of added DBU to prevent disulfide exchange and formation of the 1–2 heterodimer.[1] Adapted from Ref 1 with permission from JACS, 2014, 136, 16156-16166, Copyright {2014} American Chemical Society.

5.2 Under NG conditions with 3 mol% of persistent polymorph seeds

Under NG conditions and in the presence of DBU, adding just 3 mol% of (1–2)A (the stable polymorph) to 97 mol% of homodimers (1-1 and 2-2) causes a slow and moderate increase over milling time in the composition of (1–2)A, as observed by PXRD. At the same time, the composition of the homodimers (1–1 and 2–2) decrease correspondingly. See Figure S4.

HPLC confirms this change in composition, showing a slow and moderate rise in 1–2 within the first 15 minutes (see inset). In this kinetic study, the 1–2 formed is expected to consist entirely of (1–2)A, since (1–2)B does not form under NG conditions.

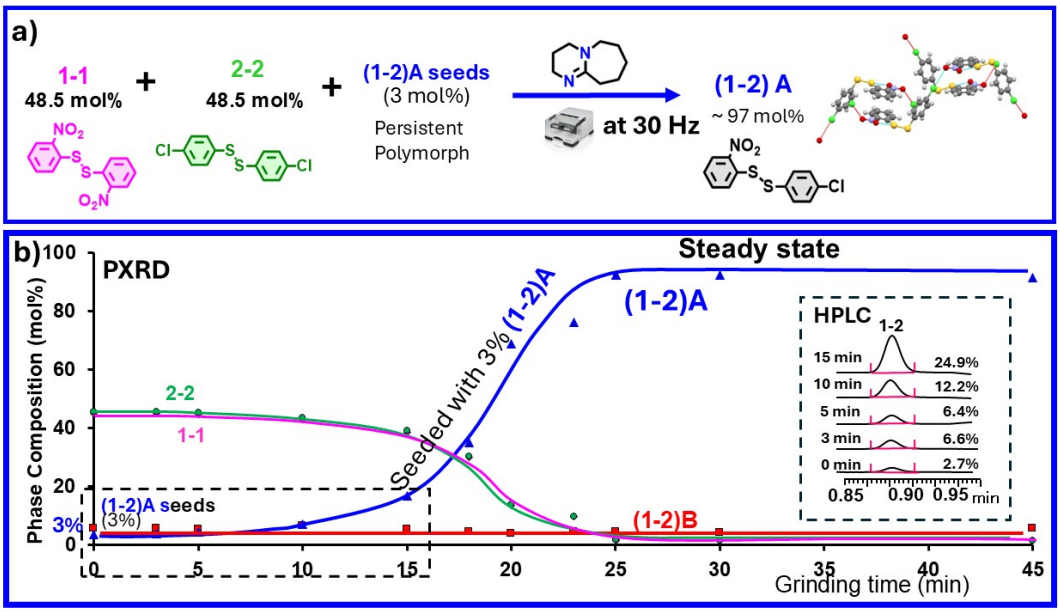

Figure S 4 Ball milling of equimolar homodimers 1–1 (pink) and 2–2 (green) with the addition of 3 mol% of (1-2)A (persistent polymorph) under NG conditions in the presence of DBU, leading to the formation of heterodimer (1-2)A (blue). (a) Reaction scheme. (b) Reaction kinetics showing phase composition (mol%) as a function of grinding time, as determined by Rietveld refinement of PXRD patterns (limit of detection > 3 mol%). Inset HPLC analysis of the reaction mixture at early time points (3–15 min), showing only the heterodimer1-2 peak eluting at ~0.9 min. The initial time point (t = 0 min) was determined in the absence of added DBU to prevent disulfide exchange and formation of the 1–2 heterodimer.[1] Adapted from Ref 1 with permission from JACS, 2014, 136, 16156-16166, Copyright {2014} American Chemical Society.

6 Seeded kinetic studies with short-lived polymorph in presence of DBU

Two distinct mechanisms govern the fate of the added short-lived polymorph of (1–2) to an equimolar mixture of homodimers (1-1 and 2-2) in the presence of DBU, depending on whether milling is performed under LAG (Section 6.1, Figure S-5) or NG (Section 6.2, Figure S-6) conditions.

6.1 Under LAG conditions with 3 mol% and 23% mol of short-lived polymorph seeds

Exposing (1–2)A, the short-lived polymorph of (1–2), to an equimolar mixture of homodimers (1–1 and 2–2) in the presence of DBU results in rapid conversion to the corresponding homodimers (1–1 and 2–2). The concentrations of both homodimers increase concurrently, with this transformation occurring almost immediately, within the first minute of grinding. Thereafter, the system follows the typical induction, reaction, and steady-state regimes. Accordingly, this pathway is described as a "sequential pathway": (1–2)A → 1–1 + 2–2 → (1–2)B.

When 3 mol% of (1–2)A is added, the initial stages of the sequential pathway cannot be monitored by PXRD due to its relatively high limit of detection (~3 mol%). However, the transformation of (1–2) into the homodimers can be readily followed by HPLC, as shown in the inset of Figure S5.

This limitation is mitigated when a higher seed loading is used. Upon addition of 23 mol% of (1–2)A, the fate of the seeds can be clearly monitored by both PXRD and HPLC, as shown in Figure S6.

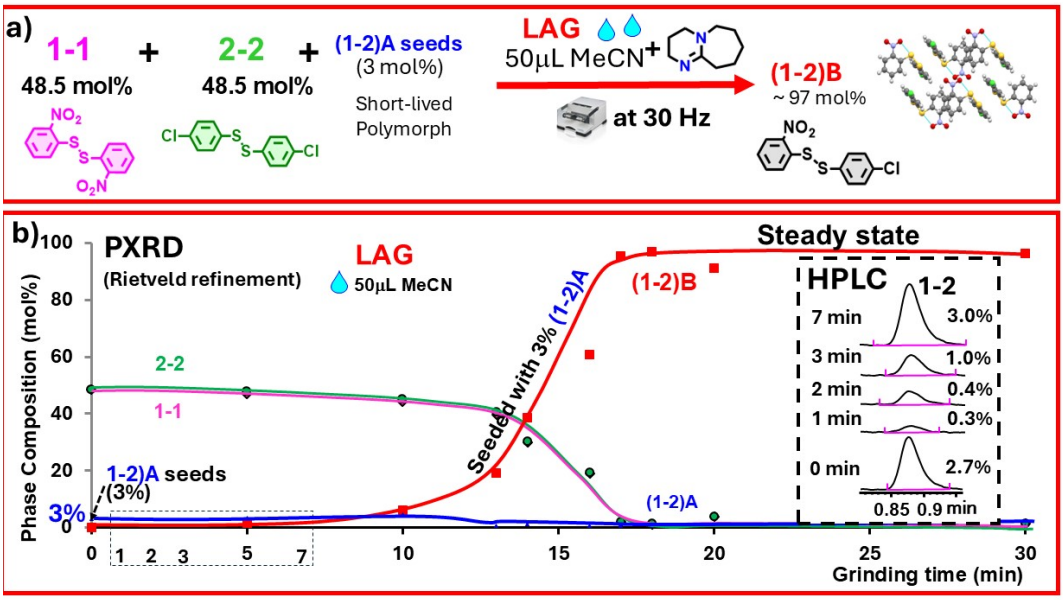

Figure S 5 Ball milling of equimolar homodimers 1–1 (pink) and 2–2 (green) with the addition of 3 mol% of (1-2)A (short-lived polymorph) under LAG conditions (50 µL MeCN) in the presence of DBU, leading to the formation of heterodimer (1-2)B (red). (a) Reaction scheme. (b) Reaction kinetics showing phase composition (mol%) as a function of grinding time, as determined by Rietveld refinement of PXRD patterns (limit of detection > 3 mol%). Inset: HPLC analysis (limit of detection ≈ 0.1 mol%) of the reaction mixture at early time points (1–7 min), showing only the heterodimer peak eluting at ~0.9 min. The initial time point (t = 0 min) was determined in the absence of added DBU to prevent disulfide exchange and formation of additional 1–2 heterodimer.[1] Adapted from Ref 1 with permission from JACS, 2014, 136, 16156-16166, Copyright {2014} American Chemical Society.

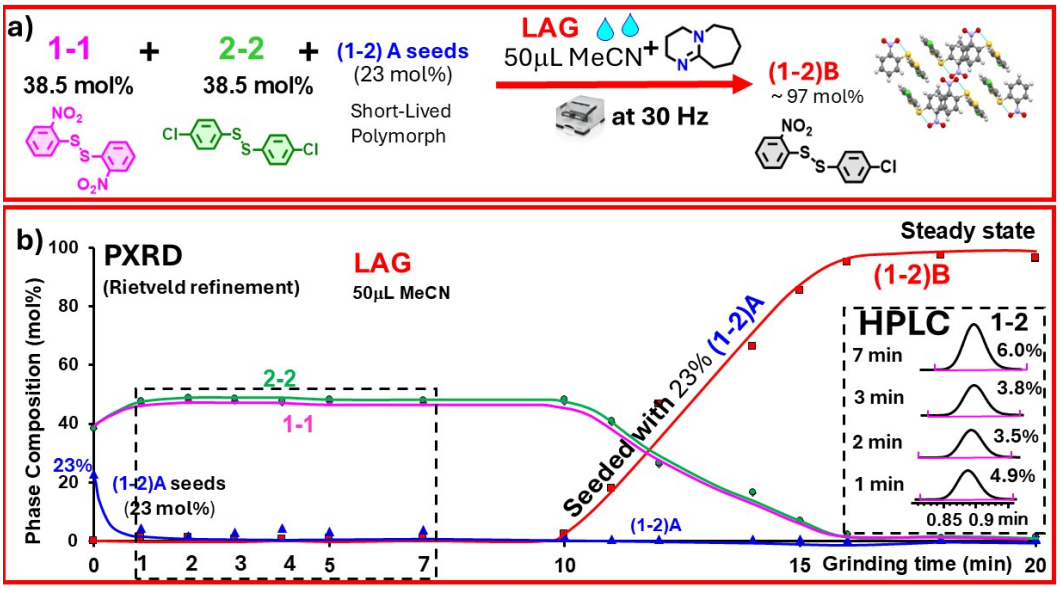

Figure S 6 Ball milling of 77 mol% equimolar homodimers 1–1 (pink) and 2–2 (green) with the addition of 23 mol% of (1-2)A (short-lived polymorp, blue) under LAG conditions (50 µL MeCN) in the presence of DBU, leading to the formation of heterodimer (1-2)B (red). (a) Reaction scheme. (b) Reaction kinetics showing phase composition (mol%) as a function of grinding time, as determined by Rietveld refinement of PXRD patterns. Inset: HPLC analysis of the reaction mixture [1-2=(1-2)A+(1-2)B] at early time points (1–7 min), showing only the heterodimer1-2 peak eluting at ~0.9 min.[1] Adapted from Ref 1 with permission from JACS, 2014, 136, 16156-16166, Copyright {2014} American Chemical Society.

6.2 Under NG conditions with 3 mol% and 23% mol of short-lived polymorph seeds

The fate of (1-2)B, the added short lived seeds of 1-2, under NG conditions in the presence of DBU, do not longer follow a bond breaking and bond reforming mechanism as would be expected from a sequential pathway. PXRD and HPLC in Figure S7 and S8 supports this observation. The composition of 1-2 from the initial 3 mol% does not go down, instead goes slightly up at 5 min while PXRD shows little increase of the composition of the homodimers 1-1 and 2-2 (Figure S7). In a similar way 23

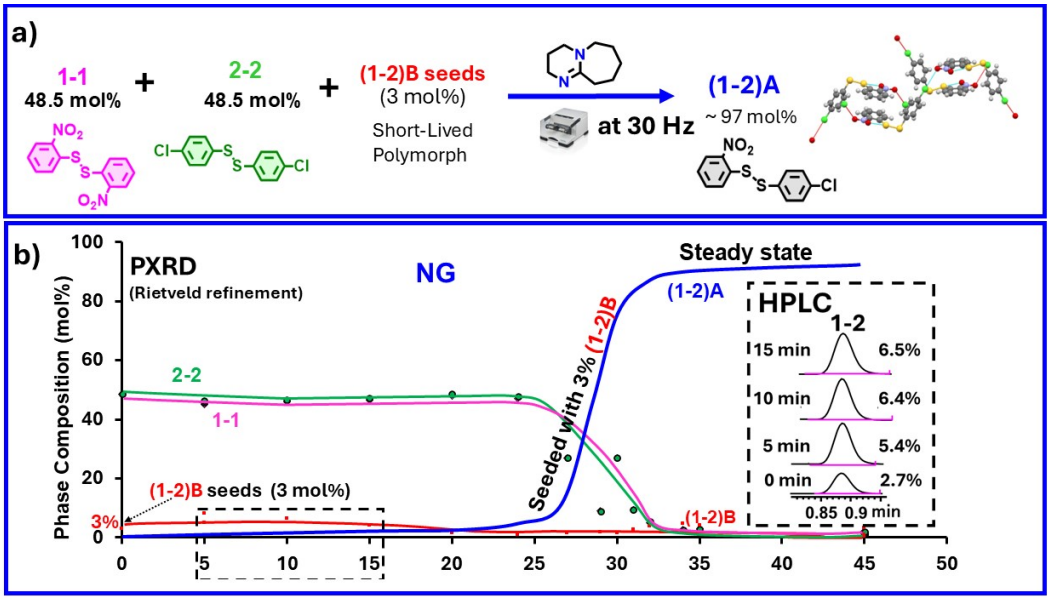

Figure S 7 Ball milling of 97 mol% mixture of equimolar homodimers 1–1 (pink) and 2–2 (green) seeded with 3 mol% of Form B of 1-2 (short lived polymorph) under NG conditions in the presence of DBU. This leads to formation of heterodimer (1-2)A (PP, blue). (a) Reaction scheme. (b) Reaction kinetics showing phase composition (mol%) as a function of grinding time, determined by Rietveld refinement of PXRD patterns. Inset: HPLC analysis of the reaction mixture at early time points (5, 10 and 15 min), showing only the heterodimer 1-2 peak eluting at ~0.95 min. The initial time point (0 min) was determined in the absence of DBU to prevent disulfide exchange and formation of the 1–2 heterodimer.[1] Adapted from Ref 1 with permission from JACS, 2014, 136, 16156-16166, Copyright {2014} American Chemical Society.

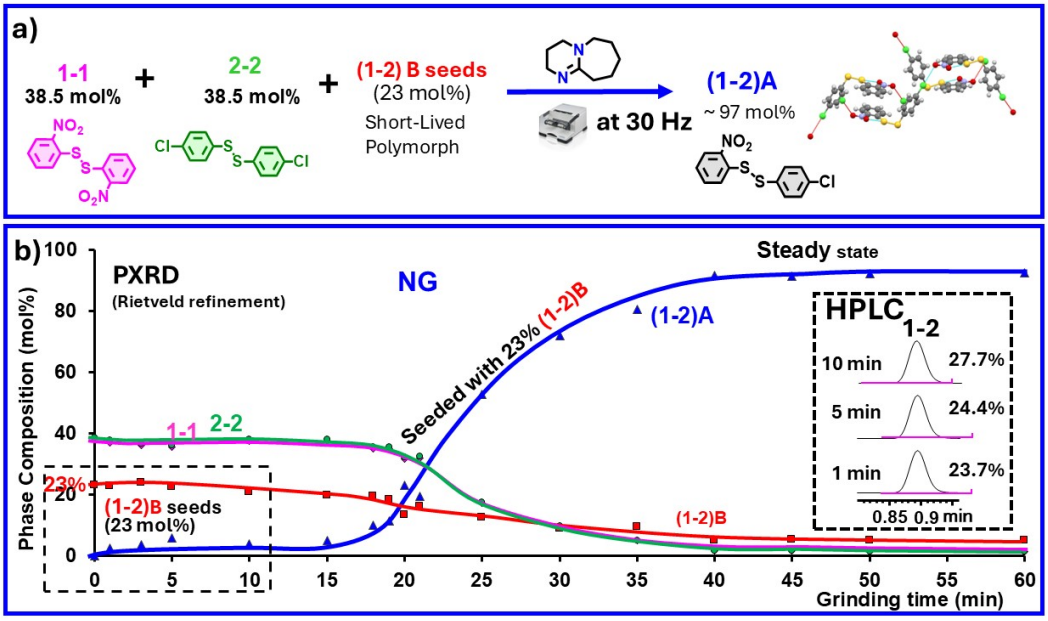

Figure S 8 Ball milling of 77 mol% equimolar homodimers 1–1 (pink) and 2–2 (green) with the addition of 23 mol% of (1-2)B (short-lived polymorp, red) under NG conditions in the presence of DBU, leading to the formation of heterodimer (1-2)A (blue). (a) Reaction scheme. (b) Reaction kinetics showing phase composition (mol%) as a function of grinding time, as determined by Rietveld refinement of PXRD patterns. Inset: HPLC analysis of the reaction mixture [1-2=(1-2)A+(1-2)B] at early time points (1–10 min), showing only the heterodimer1-2 peak eluting at ~0.9 min.[1] Adapted from Ref 1 with permission from JACS, 2014, 136, 16156-16166, Copyright {2014} American Chemical Society.

7 Seeded kinetic studies with short-lived polymorph in the absence of DBU

Without DBU, disulfide bonds can't reshuffle, so the molecules in the mixture (1-1, 2-2 and 1-2) stay the same. This becomes clear when a short-lived crystal form of the disulfide heterodimer 1-2 is added to a mixture of the two disulfide homodimers. No matter if milling is performed under LAG with MeCN or NG conditions, the short-lived form always converts completely into the persistent polymorph, this being the more stable one.

The direction of this change depends on the conditions: with LAG (Section 7.1, Figure S9) (1–2)A transforms to (1–2)B, while with NG (Section 7.2, Figure S10) (1–2)B transforms to (1–2)A.

HPLC shows that the proportion of the heterodimer (around 22–23%) stays constant during milling, confirming that no chemical reaction is happening. At the same time, PXRD shows that the crystal structure does change, meaning the same molecules are simply rearranging into a different solid form.

7.1 NG with 23 mol % short-lived polymorph seeds (No DBU)

Figure S9 shows how the system changes over time after adding 23 mol% of (1–2)A to 77 mol% of an equal mixture of the two homodimers (1–1 and 2–2). The experiment was carried out under LAG conditions using 50 µL of MeCN, and importantly, without DBU.

Under these conditions, (1–2)A is the short-lived crystal form, while (1–2)B is the more stable (persistent) form

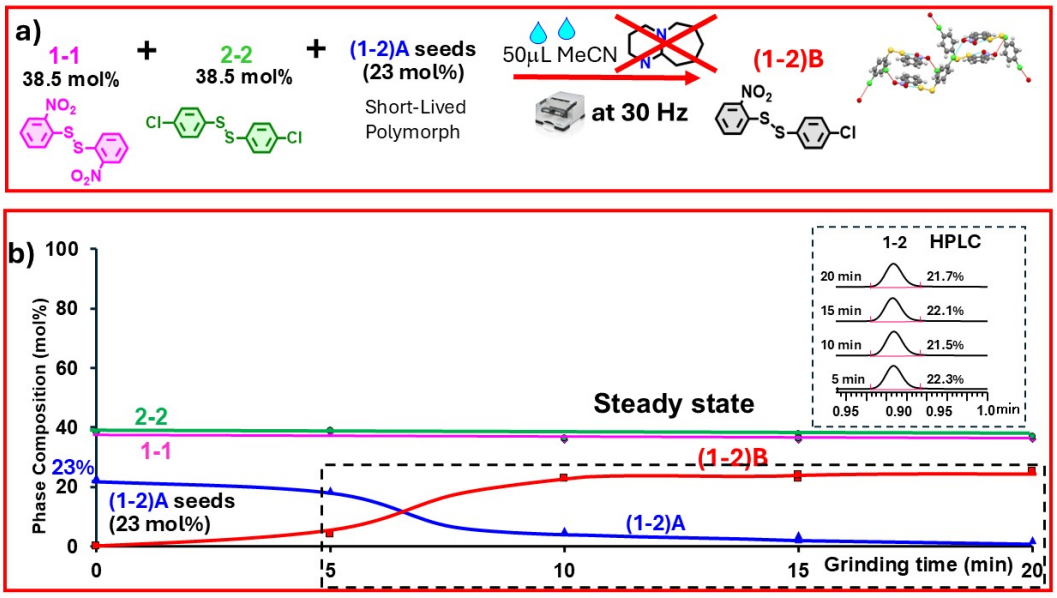

Figure S 9 Ball milling of a mixture containing 77 mol% equimolar homodimers 1–1 (pink) and 2–2 (green) with the addition of 23 mol% of (1-2)A (short-lived polymorph, with DBU neutralised by addition of TFA; blue) under LAG conditions (50 µL MeCN) in the absence of DBU. (a) Reaction scheme. (b) Reaction kinetics showing phase composition (mol%) as a function of grinding time, as determined by Rietveld refinement of PXRD patterns. Inset: HPLC analysis of the reaction mixture [1-2 = (1-2)A + (1-2)B], showing only the heterodimer 1-2 peak eluting at ~0.9 min. The overall composition of 1-2 remains constant throughout the milling experiment, while (1-2)A is directly transformed into (1-2)B under LAG conditions.[1] Adapted from Ref 1 with permission from JACS, 2014, 136, 16156-16166, Copyright {2014} American Chemical Society.

7.2 NG with 23 mol % short-lived polymorph seeds (No DBU)

Figure S10 shows how the system changes over time after adding 23 mol% of (1–2)B to 77 mol% of an equal mixture of the two homodimers (1–1 and 2–2). The experiment was carried out under NG conditions, and importantly, without DBU.

Under NG conditions, (1–2)B is the short-lived crystal form, while (1–2)A is the more stable (persistent) form.

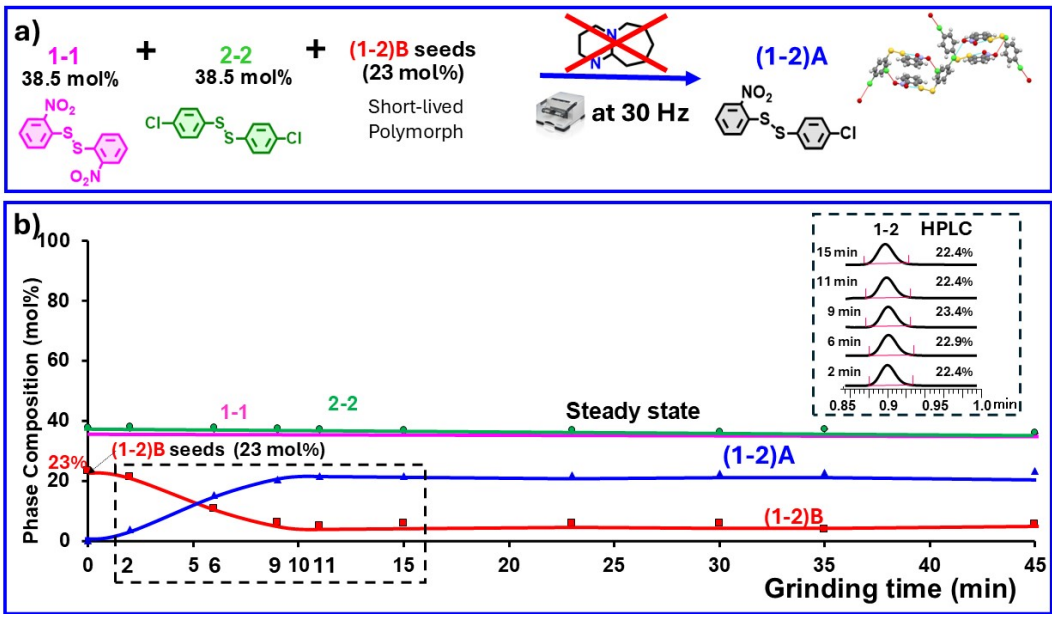

Figure S 10 Ball milling of a mixture containing 77 mol% equimolar homodimers 1–1 (pink) and 2–2 (green) with the addition of 23 mol% of (1-2)B (short-lived polymorph, recrystallised from EtOH and rinsed to remove traces of DBU) under NG conditions in the absence of DBU. (a) Reaction scheme. (b) Reaction kinetics showing phase composition (mol%) as a function of grinding time, as determined by Rietveld refinement of PXRD patterns. Inset: HPLC analysis of the reaction mixture [1-2 = (1-2)A + (1-2)B], showing only the heterodimer 1-2 peak eluting at ~0.9 min. The overall composition of 1-2 remains constant throughout the milling experiment, while (1-2)B is directly transformed into (1-2)A under NG conditions.[1] Adapted from Ref 1 with permission from JACS, 2014, 136, 16156-16166, Copyright {2014} American Chemical Society.

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1. A. M. Belenguer, G. I. Lampronti, D. J. Wales and J. K. M. Sanders, Direct Observation of Intermediates in a Thermodynamically Controlled Solid-State Dynamic Covalent Reaction, *Journal of the American Chemical Society*, 2014, **136**, 16156-16166.