nature portfolio

# Peer Review File

# SO2F2 Mediated Click Chemistry Enables Modular Disulfide Formation in Diverse Reaction Media

REVIEWER COMMENTS

Reviewer #1 (Remarks to the Author):

Dynamic disulfide bond is one of the most used reversible linkages with broad utility, and their controlled construction with high selectivity is of importance. In this work the authors developed SO2F2 promoted oxidation of thiols to create disulfides. The reactions have wide substrate scope, high yield, and mild conditions in both organic and water media, matching the features of click reactions. The mechanistic studies, including computation of barriers, comparison of the reactivity of different oxidants, and control experiments, were conducted. The application in flow-chemistry and disulfide polymers was also demonstrated. The studies were performed with vigor, and the methodology enriches the toolbox of disulfide chemistry. However, there are several major concerns:

1. The method works well for symmetric sulfides, but not really for unsymmetrical ones. Only disulfides from one aromatic thiol and one bulky aliphatic thiol were shown (Figure 3B). Unsymmetrical disulfides from two aromatic thiols or two aliphatic thiols were not feasible. Unsymmetrical disulfides were also not attempted in other conditions, including water phase.

2. The authors claim “exceptional chemoselectivity”, but only compared thiols with triphenylphosphine. How about other interferences?

These issues must be addressed before further consideration for a high impact journal, such as Nature Communications.

Reviewer #2 (Remarks to the Author):

Professor An and colleagues reported a novel disulfide synthesis enabled by RSO2F reagents. This method exhibited robust performance in diverse reaction medias, showcasing its applicability even in water and physiological conditions. The elucidation of the kinetic and thermodynamic profiles of the reaction was achieved through a combination of DFT calculations and experimental evidence. Notably, the authors extended the utility of this chemistry in flow chemistry, polymer synthesis, and in-situ gelation. Upon initial review, my impression of this work is that both the manuscript and supplementary information are well-crafted, thoughtfully organized, and easy to follow.

RSO2F has been widely used for chemical ligation of phenols, amines, and alcohols, through the fluoride exchange reaction. It is an interesting discovery that thiol could not form a stable linkage at SVI hub, instead underwent self-condensation (resembling a formal oxidation process) to afford a disulfide linker. From an alternate perspective, however, the synthesis of thiosulfonates RSO2SR' from RSO2X and its conversion to disulfide under the treatment of thiols are known (Eur. J. Org. Chem. 2021, 5359-5366). The chemistry described in this manuscript is useful but may not reach the level of significance required for publication in a high-impact journal like Nat. Commun., at least in its current forms.

In summary, my recommendation is not suitable for publication at Nat. Commun.

Questions/concerns to be addressed:

1, Please revise if it is appropriate to use the word "oxidant" in describing SO2F2 and other RSO2F reagents in this reaction. It might be characterized as a formal "oxidation" process, considering the transformation of thiols to disulfide. However, this is realized through a nucleophilic substitution cascade at the SVI center of SO2F2 or RSO2F, rather than electron transfer from one species to another. For many other metal or metal salts that are usually cited as oxidant, the oxidation state of the corresponding central atom gets lower after reaction. However, their chemical formulas would not change.

2, Given the array of existing methods for synthesizing disulfides, a newly developed method should ideally address real challenges in the field. For instance, the preparation of

unsymmetric alkyl disulfides presents a notable challenge. It is nice to see that the aryl alkyl disulfide Ar-SS-Alkyl can be prepared through the reaction with SO2F2, by taking advantage of their intrinsic reactivity difference. However, the more challenging and useful unsymmetric alkyl disulfides Alkyl-SS-Alkyl is not demonstrated. could this group compounds be accessed using this method?

3, The gases SO2F2 regent is often problematic to operate, especially for a reaction that necessitates precise control of mole ratios. For the preparation of symmetric disulfide that do not contain other reactive sites towards SO2F2, it is ok to use excess amounts of gas (through balloon or in stock solution) or by using the flow technology. However, for substrates carrying amines, phenols, or alcohols, the amount of SO2F2 must be precisely controlled for the preparation of disulfide. Otherwise, these functional groups could undergo further fluoride exchange reaction with this gas. The use of mild inorganic oxidant would be more convenient in these cases.

4, can the RSSO2F species be isolated and characterized with specific substrate?

Reviewer #3 (Remarks to the Author):

An et al. reported a click chemistry method mediated by SO2F2 for the formation of sulfur–sulfur bonds via thiols. In this study, SO2F2 and its surrogates exhibited exceptional reactivity and selectivity across diverse reaction media, including water. It can be utilized for the assembly of symmetrical and unsymmetrical disulfides, extending to the synthesis of polydisulfides. This manuscript presents an advancement in disulfide linkage chemistry and is qualified for Nature Communication after these issues addressed.

1. When the authors presented protocols for the oxidation of thiols leading to the disulfides through S–S bond formation, a notable gap exists in terms of disulfide reagents capable of synthesizing disulfides via S–C bond linkage (e.g., Nat. Commun. 2018, 9, 2191; Nat. Commun. 2020, 11, 4170; Angew. Chem. Int. Ed. 2023, 62, e202314379). Emphasizing the

advantages of the SO2F2-mediated strategy would be beneficial, especially in the context of unsymmetrial disulfides in comparison to traditional disulfide reagents.

2. SuFEx chemistry not only is employed for the S–O or S–N linkage of SuFEx linkers, but also has been extended to the formation of S–C bonds (e.g., Nat. Synth. 2022, 1, 455; Angew. Chem. Int. Ed. 2022, 61, e202207100).

3. The authors used SO2F2 and its surrogates (II-V) to synthesize the corresponding disulfides and outlined the order of reactivity. However, this approach may lack rigor, as the reagent II may not fully represent the reactivity of FSO2N-containing compounds. Thus, the synthesis of PhNHSO2F or BnNHSO2F is recommended to assess reactivity. Additionally, consideration should be given to the aza-analog of sulfonyl fluoride, sulfonimidoyl fluoride, in evaluating reactivity and selectivity (e.g., SulfoxFluor, Org. Process Res. Dev. 2022, 26, 380).

4. The one-step assembly of unsymmetric disulfides is significant, but it is currently limited to aryl-alkyl disulfides. Have the authors explored a step-wise synthesis approach for unsymmetric aryl-aryl/alkyl-alkyl disulfides via diverse RSO2F reagents?

5. The title in Figure 1 is misaligned, and the color representation in Figures 2 and 3 appears faint. It is recommended that adjustments need to be made to the figures and tables in the manuscript to enhance clarity for the reviewer's understanding.

6. These identified issues require correction and examination in the manuscript.

1) '5 mmoL/h' should be revised as '5 mmol/h' in Page 7, Line 11.

2) The oxdiative polymerization of 5b is reported instead of 6a in Table 2.

3) The arrow of the catalytic cycle is oriented in the opposite direction in Figure S2.

# Point-by-Point Response to Referees

## Responses to the comments of reviewer 1.

Dynamic disulfide bond is one of the most used reversible linkages with broad utility, and their controlled construction with high selectivity is of importance. In this work the authors developed $SO_2F_2$ promoted oxidation of thiols to create disulfides. The reactions have wide substrate scope, high yield, and mild conditions in both organic and water media, matching the features of click reactions. The mechanistic studies, including computation of barriers, comparison of the reactivity of different oxidants, and control experiments, were conducted. The application in flow-chemistry and disulfide polymers was also demonstrated. The studies were performed with vigor, and the methodology enriches the toolbox of disulfide chemistry. However, there are several major concerns:

Response:

We sincerely thank the reviewer for the positive feedback and valuable suggestions. We have revised our manuscript based on your recommendations. Please find our detailed responses to your comments below.

1. The method works well for symmetric sulfides, but not really for unsymmetrical ones. Only disulfides from one aromatic thiol and one bulky aliphatic thiol were shown (Figure 3B). Unsymmetrical disulfides from two aromatic thiols or two aliphatic thiols were not feasible. Unsymmetrical disulfides were also not attempted in other conditions, including water phase.

Response:

We sincerely thank the reviewer for this insightful comment. Your suggestion prompted us to further optimize the conditions for the synthesis of unsymmetrical disulfides. The optimized reaction conditions have now been successfully applied to the synthesis of 14 unsymmetrical disulfides, including those from two aromatic thiols and two aliphatic thiols, with yields ranging from 60% to 98%. In this revised manuscript, we have also demonstrated that the unsymmetrical disulfides can be synthesized smoothly under aqueous conditions, such as **3a** and **3c**. Those results have been summarized in the Figure below (Fig. 3B in the revised manuscript):

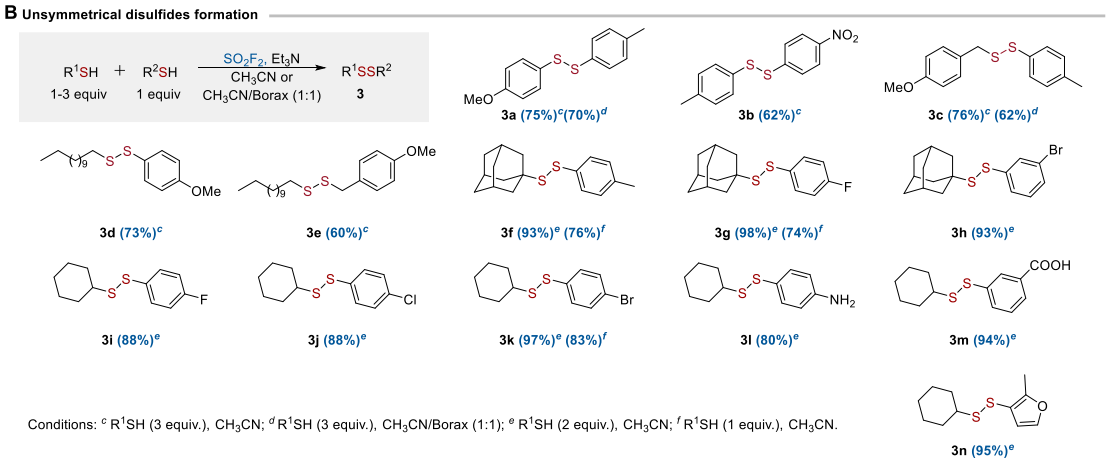



Fig. 3B Synthesis of unsymmetrical disulfides by click chemistry.

We have revised the manuscript and supporting information based on these experimental results. In the revised manuscript, "Figure 3B" has been updated and we have added the following statement: "*The efficiency of this click reaction in linking two different modules was evaluated through the direct synthesis of unsymmetrical disulfides (Fig. 3B). Using $SO_2F_2$ as the reagent, unsymmetrical disulfides were synthesized from the cross-coupling of one aromatic thiol and one aliphatic thiol, two aromatic thiols, and two aliphatic thiols, achieving yields of 60−98%, which were challenging to obtain with other known coupling reagents. In the coupling reactions involving one aromatic thiol and one aliphatic thiol (**3c - 3d** and **3f - 3n**), high yields >80% could even be achieved using equimolar amounts of the thiols (**3k**). This is likely due to the relatively higher acidity of aromatic thiols, which were more easily deprotonated under basic conditions and therefore react more rapidly with $SO_2F_2$ compared to aliphatic thiols, resulting in the formation of $ArSSO_2F$ as the predominant intermediate. The reaction of the intermediate $ArSSO_2F$ with aliphatic thiol subsequently produces the desired unsymmetrical disulfides. Despite the similar reactivity of the two starting materials, this method is also effective for the unsymmetrical coupling of two aromatic thiols (**3a-3b**) or two aliphatic thiols (**3e**), although 2.0 – 3.0 equiv. of one reactant was required to achieve high yields.*" and "*The unsymmetrical disulfides **3a** and **3c** can also be synthesized under this aqueous condition (Fig. 3B).*". The revised supporting information now includes these results, along with other relevant data, in Section 12 "The Synthesis of Unsymmetrical Disulfide using $SO_2F_2$" (SI, Page 42-49).

2. The authors claim “exceptional chemoselectivity”, but only compared thiols with triphenylphosphine. How about other interferences?

Response:

We sincerely thank the reviewer for highlighting this limitation in our previous manuscript. To address this, we have conducted additional experiments to test other potential interferences. In addition to triphenylphosphine, we tested several readily oxidizable organic compounds, including 1,2-bis(diphenylphosphino)ethane, phenylpropyl aldehyde, and 4-methylbenzaldehyde. The results demonstrated that all of these compounds exhibited stability in the presence of excess $SO_2F_2$. Furthermore, we tested ULTRANOX™ 626, a commercial antioxidant, which also resisted oxidation by $SO_2F_2$. These findings underscore $SO_2F_2$'s excellent resistance to most organic reductants, making it highly chemoselective for the thiol-to-disulfide conversion.

We have revised the manuscript and supporting information based on these experimental results. In the revised manuscript, we have added the following statement: “*Additionally, we have established that other readily oxidizable organic compounds, including 1,2-bis(diphenylphosphino)ethane, phenylpropyl aldehyde and 4-methylbenzaldehyde, exhibit stability in the presence of an excess of $SO_2F_2$. Furthermore, our experiments demonstrate that ULTRANOX™ 626, a commercial antioxidant, also resists oxidation by $SO_2F_2$, underscoring the remarkable reductive stability of this reagent (see SI 15 for details).*”. The revised supporting information now includes these results, along with other relevant data, in Section 15 “Resistance of $SO_2F_2$ toward Reduction” (SI, Page 53-56).

These issues must be addressed before further consideration for a high impact journal, such as Nature Communications.

## **Responses to the comments of reviewer 2**.

Professor An and colleagues reported a novel disulfide synthesis enabled by $RSO_2F$ reagents. This method exhibited robust performance in diverse reaction medias, showcasing its applicability even in water and physiological conditions. The elucidation of the kinetic and thermodynamic profiles of the reaction was achieved through a combination of DFT calculations and experimental evidence. Notably, the authors extended the utility of this chemistry in flow chemistry, polymer synthesis, and in-situ gelation. Upon initial review, my impression of this work is that both the

manuscript and supplementary information are well-crafted, thoughtfully organized, and easy to follow.

Response:

We sincerely thank the reviewer for the positive feedback and encouraging comments.

$RSO_2F$ has been widely used for chemical ligation of phenols, amines, and alcohols, through the fluoride exchange reaction. It is an interesting discovery that thiol could not form a stable linkage at $S^{VI}$ hub, instead underwent self-condensation (resembling a formal oxidation process) to afford a disulfide linker. From an alternate perspective, however, the synthesis of thiosulfonates $RSO_2SR'$ from $RSO_2X$ and its conversion to disulfide under the treatment of thiols are known (Eur. J. Org. Chem. 2021, 5359-5366). The chemistry described in this manuscript is useful but may not reach the level of significance required for publication in a high-impact journal like Nat. Commun., at least in its current forms.
In summary, my recommendation is not suitable for publication at Nat. Commun.

Response:

We sincerely thank the reviewer for the constructive feedback and valuable comments. We understand that our manuscript, in its original form, did not meet the high standards required for publication in Nature Communications. Based on your insightful suggestions, we have conducted additional experiments and made substantial revisions to the manuscript. We hope these improvements address your concerns and enhance the significance of our work.

We began working on this reaction in 2019, inspired and encouraged by discussions with Prof. Barry Sharpless. Originally, we aimed to synthesize thiosulfonates from the reaction of $SO_2F_2$ and thiols for the discovery of novel agrichemicals. We were also surprised to discover that thiols could not form a stable linkage at the $S^{VI}$ center. As the reviewer noted, the synthesis of thiosulfonates $RSO_2SR'$ from $RSO_2Cl$ and their conversion to disulfides under the treatment of thiols are known, and $SO_2Cl_2$-mediated thiol-to-disulfide conversion also follows this pathway. However, the chemoselectivity and efficiency of these reactions were not perfect. We decided to pursue this reaction because initial results suggested that the $SO_2F_2$-mediated thiol-to-disulfide conversion could be a nearly perfect "spring-loaded" reaction.

In our subsequent studies, we found that the high chemoselectivity and efficiency of this reaction enabled the synthesis of challenging sterically hindered disulfides, unsymmetrical and cyclic disulfides, and even polysulfides directly from thiols. We acknowledge the limitations in our original manuscript and believe that the quality has significantly improved after careful revision based on your comments and suggestions. We hope you find the revised manuscript satisfactory.

1. Please revise if it is appropriate to use the word "oxidant" in describing $SO_2F_2$ and other $RSO_2F$ reagents in this reaction. It might be characterized as a formal "oxidation" process, considering the transformation of thiols to disulfide. However, this is realized through a nucleophilic substitution cascade at the $S^{VI}$ center of $SO_2F_2$ or $RSO_2F$, rather than electron transfer from one species to another. For many other metal or metal salts that are usually cited as oxidant, the oxidation state of the corresponding central atom gets lower after reaction. However, their chemical formulas would not change.

Response:

We sincerely thank the reviewer for pointing out the inappropriate description of the reaction. After careful consideration of your suggestion, we understand that the $SO_2F_2$-mediated thiol-to-disulfide conversion is indeed a nucleophilic substitution cascade process rather than a traditional oxidation. It was not accurate to describe $SO_2F_2$ as an oxidant.

Additionally, we conducted further experiments involving $SO_2F_2$ and ULTRANOX™ 626, a commercial antioxidant. The results demonstrated that even a strong organic reductant such as ULTRANOX™ 626 cannot be oxidized by $SO_2F_2$. These findings further support that $SO_2F_2$ should not be described as an oxidant. This also explains the exceptional chemoselectivity of the $SO_2F_2$-mediated thiol-to-disulfide conversion compared to conversions mediated by traditional oxidants.

In the revised manuscript, we have corrected the descriptions regarding $SO_2F_2$ and added the results of the reaction between $SO_2F_2$ and ULTRANOX™ 626 in both the manuscript and the supporting information.

In the revised manuscript, we have made the following correction:

Page 1, line 13: we changed "*The oxidation of thiols to their corresponding disulfides*" to "*The conversion of thiols to their corresponding disulfides*".

Page 1, line 16: we changed "*which display exceptional oxidative selectivity toward thiol oxidations*" to "*which display exceptional selectivity toward disulfide formation through an effective nucleophilic substitution cascade*".

Page 2, line 16: we changed "*the oxidation of thiols is the most straightforward route*" to "*using thiols as the precursors is the most straightforward route*".

Page 2, line 17: we changed "*Thiol oxidation can be accomplished by many oxidants*" to "*Converting thiols into disulfides can be accomplished by many oxidants*".

Page 2, line 32: we changed "*we present a click reaction that enable modular synthesis of disulfides from thiols*" to "*we present a click reaction that enable modular synthesis of disulfides from thiols via a nucleophilic substitution cascade*".

Page 3, line 8: we changed "*Development of thiol oxidation click reaction*" to "*Development of $SO_2F_2$ mediated click chemistry for efficient thiol-to-disulfide conversion*".

Page 3, line 9: we changed "*The key to developing the click chemistry for disulfide formation is a highly reactive and selective oxidant, which should be a potent oxidant with a low kinetic barrier specifically for thiol oxidation*" to "*The key to developing the click chemistry for disulfide formation is a highly reactive and selective reagent, which effectively converts thiols into disulfides without oxidizing the products and non-target functional groups*".

Page 3, line 11: we changed "*As a high valent sulfur oxidant, $SO_2Cl_2$ is effective for the oxidation of primary and secondary thiols*" to "*As a high valent sulfur reagent, $SO_2Cl_2$ is effective for converting primary and secondary thiols into disulfides*".

Page 4, line 11: we changed "*we observed that $SO_2F_2$ can be quickly reduced by a thiol through a two-step process*" to "*we observed that $SO_2F_2$ can quickly reacted with a thiol through a nucleophilic substitution cascade*".

Page 4, line 12: we changed "*In this thiol oxidation using $SO_2F_2$*" to "*In the reaction between thiol and $SO_2F_2$*".

Page 4, line 14: we changed "*During the first step*" to "*During the first nucleophilic substitution step*".

Page 4, line 16: we changed "*the highly reactive oxidant 1' was reduced by an additional molecule of thiol*" to "*the highly reactive intermediate 1' was attacked by an additional molecule of thiol*".

Page 4, line 17: we changed "*We also validated both experimentally and computationally that the oxidation of thiol by $SO_2F_2$ possesses a considerably lower activation energy in comparison to the oxidation of other species*" to "*We also validated both experimentally and computationally that the reaction between thiol and $SO_2F_2$ possesses a considerably lower activation energy in comparison to the reaction between $SO_2F_2$ and other reductant*".

Page 4, line 20: we changed "*Owing to the distinctive mechanism of thiol oxidation by $SO_2F_2$*" to "*Owing to the distinctive mechanism of the thiol-to-disulfide conversion by $SO_2F_2$*".

Page 4, line 23: we changed "*After recognizing the potential of $SO_2F_2$ as a suitable oxidant for the thiol oxidation click reaction*" to "*After recognizing the potential of $SO_2F_2$ as a suitable reagent for the thiol-to-disulfide conversion*".

Page 4, line 24: we changed "*Our results indicated that thiol oxidation by $SO_2F_2$ required only simple conditions with weak base and exhibited insensitivity towards the reaction medium and oxygen*" to "*Our results indicated that the reaction between thiols and $SO_2F_2$ required only simple conditions with weak base and exhibited insensitivity towards the reaction medium and oxygen*".

Page 5, line 24: we changed "*Captopril (**2ae**), a medicine, was also smoothly oxidized*" to "*Captopril (**2ae**), a medicine, was also smoothly converted into the corresponding disulfide*".

Page 5, line 27: we changed "*The results revealed that all those reagents were as effective as $SO_2F_2$ for the oxidation of benzyl thiols and thiophenols*" to "*The results revealed that all those reagents were as effective as $SO_2F_2$ for the reaction with benzyl thiols and thiophenols*".

Page 7, line 14: we changed "*the oxidation of unsymmetrical 1,3- and 1,4-dithiols (dihydrolipoic acid methyl ester and dithiothreitol) was also examined*" to "*the reaction of 1,3- and 1,4-dithiols (dihydrolipoic acid methyl ester and dithiothreitol) was also examined*".

Page 7, line 17: we changed "*Thiol oxidation click chemistry in water*" to "*Thiol-to-disulfide click chemistry in water*".

Page 8, line 1: we changed "*thereby triggering the oxidation of thiols*" to "*thereby triggering the thiol-to-disulfide conversion*".

Page 8, line 9: we changed "*The reactor shown in Fig. 5 has been used in the oxidation of captopril and glutathione which gave >95% yields and a production rate of 5 mmol/h*" to "*The reactor shown in Fig. 5 has been used in the thiol-to-disulfide conversion of captopril and glutathione which gave >95% yields and a production rate of 5 mmol/h*".

Page 8, line 20: we changed "*the oxidative polymerization was investigated using a hydrophobic monomer **5a** and a hydrophilic monomer **5b** as models*" to "*the polymerization was investigated using a hydrophobic monomer **5a** and a hydrophilic monomer **5b** as models*".

Page 8, line 30: we changed "*The rapid and controllable oxidative polymerization of dithiols*" to "*The rapid and controllable polymerization of dithiols*".

Page 9, line 3: we changed "*In situ gelation by thiol oxidation click reaction*" to "*In situ gelation by thiol-to-disulfide click reaction*".

Page 9, line 8: we changed "*This thiol oxidation click reaction was applied as a new cross-linking method for the in-situ gelation of hydrogels*" to "*This thiol-to-disulfide click reaction was applied as a new cross-linking method for the in-situ gelation of hydrogels*".

Page 10, line 1: we changed "*Subsequent histological analysis conducted after seven days revealed favorable biocompatibility for the hydrogel cross-linked by thiol oxidation click reaction*" to "*Subsequent histological analysis conducted after seven days revealed favorable biocompatibility for the hydrogel cross-linked by thiol-to-disulfide click reaction*"

Page 10, line 6: we changed "*These findings indicated the potential for future applications of this thiol oxidation click chemistry in the field of biomaterial*" to "*These findings indicated the potential for future applications of this thiol-to-disulfide click chemistry in the field of biomaterial*"

Page 10, line 10: we changed "*This thiol oxidation click reaction is poised to enable the development of novel drugs*" to "*This thiol-to-disulfide click reaction is poised to enable the development of novel drugs*"

2. Given the array of existing methods for synthesizing disulfides, a newly developed method should ideally address real challenges in the field. For instance, the preparation of unsymmetric alkyl disulfides presents a notable challenge. It is nice to see that the aryl alkyl disulfide Ar-SS-Alkyl can be prepared through the reaction with $SO_2F_2$, by taking advantage of their intrinsic reactivity difference. However, the more challenging and useful unsymmetric alkyl disulfides Alkyl-SS-Alkyl is not demonstrated. could this group compounds be accessed using this method?

Response:

We sincerely thank the reviewer for this insightful comment. Your suggestion prompted us to further optimize the conditions for the synthesis of unsymmetrical disulfides. The optimized reaction conditions have now been successfully applied to the synthesis of 14 unsymmetrical disulfides, including those from two aromatic thiols and two aliphatic thiols, with yields ranging from 60% to 98%. Please see the summarized results below (Fig. 3B in this revised manuscript):

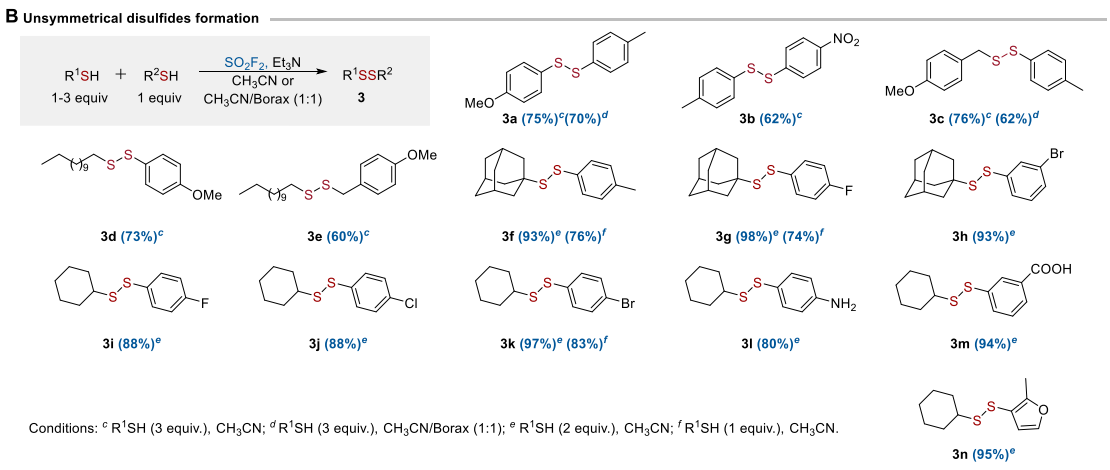



Fig. 3B Synthesis of unsymmetrical disulfides by click chemistry.

We have revised the manuscript and supporting information based on these experimental results. In the revised manuscript, "Figure 3B" has been updated and we have added the following statement: "*The efficiency of this click reaction in linking two different modules was evaluated through the direct synthesis of unsymmetrical disulfides (Fig. 3B). Using $SO_2F_2$ as the reagent, unsymmetrical disulfides were*

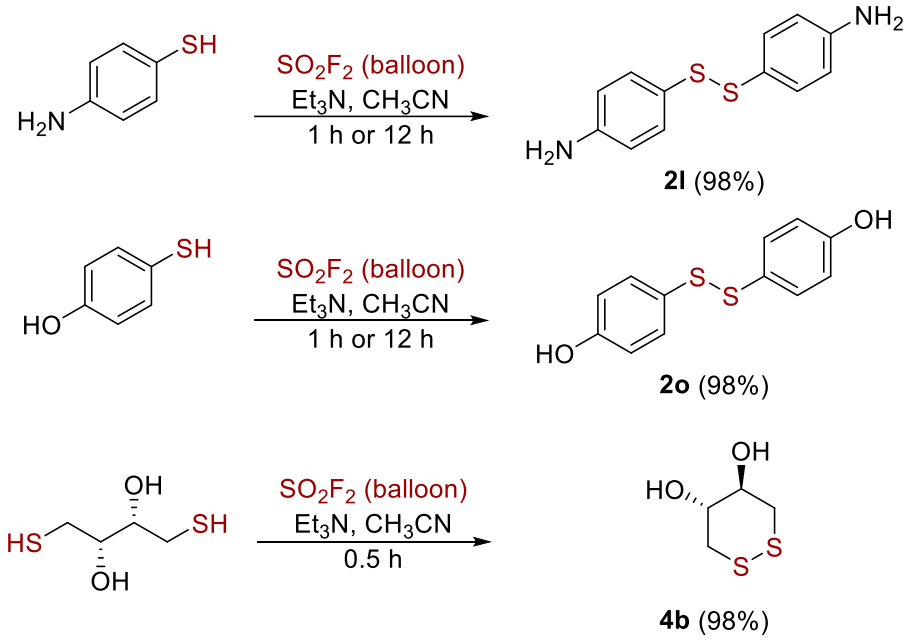
*synthesized from the cross-coupling of one aromatic thiol and one aliphatic thiol, two aromatic thiols, and two aliphatic thiols, achieving yields of 60−98%, which were challenging to obtain with other known coupling reagents. In the coupling reactions involving one aromatic thiol and one aliphatic thiol (**3c - 3d** and **3f - 3n**), high yields >80% could even be achieved using equimolar amounts of the thiols (**3k**). This is likely due to the relatively higher acidity of aromatic thiols, which are more easily deprotonated under basic conditions and therefore react more rapidly with $SO_2F_2$ compared to aliphatic thiols, resulting in the formation of $ArSSO_2F$ as the predominant intermediate. The reaction of the intermediate $ArSSO_2F$ with aliphatic thiol subsequently produces the desired unsymmetrical disulfides. Despite the similar reactivity of the two starting materials, this method is also effective for the unsymmetrical coupling of two aromatic thiols (**3a-3b**) or two aliphatic thiols (**3e**), although 2.0 – 3.0 equiv. of one reactant was required to achieve high yields.*". The revised supporting information now includes these results, along with other relevant data, in Section 12 "The Synthesis of Unsymmetrical Disulfide using $SO_2F_2$" (SI, Page 42-49).

3. The gases $SO_2F_2$ regent is often problematic to operate, especially for a reaction that necessitates precise control of mole ratios. For the preparation of symmetric disulfide that do not contain other reactive sites towards $SO_2F_2$, it is ok to use excess amounts of gas (through balloon or in stock solution) or by using the flow technology. However, for substrates carrying amines, phenols, or alcohols, the amount of $SO_2F_2$ must be precisely controlled for the preparation of disulfide. Otherwise, these functional groups could undergo further fluoride exchange reaction with this gas. The use of mild inorganic oxidant would be more convenient in these cases.

Response:

We thank the reviewer for highlighting the potential limitation of the $SO_2F_2$-mediated thiol-to-disulfide conversion. To evaluate the tolerance of this reaction with substrates carrying amines, phenols, or alcohols, we included thiol substrates with amine (**2l** and **2r**), phenol (**2o**), and alcohol (**4b**) in the revised manuscript. The results demonstrated that, even with an excess amount of $SO_2F_2$ present, the desired disulfides were formed in ≥ 98% yields. This is probably because the reaction between phenol, aniline, or alcohol and $SO_2F_2$ requires different reaction conditions. For example, the reaction between phenols and $SO_2F_2$ is initiated with 1.5 equiv. of $Et_3N$, whereas the reaction of

**2l** or **2o** with $SO_2F_2$ requires only 0.3 equiv. of $Et_3N$. Therefore, even when the reaction time of **2l** or **2o** with excess $SO_2F_2$ was lengthened from 1 h to 12 h, no by-products were observed (see equations below). Additionally, if precise control of mole ratios is necessary in certain cases, a range of solid and liquid surrogates to $SO_2F_2$, such as solid reagents II-VIII tested in the manuscript, can also be used instead.



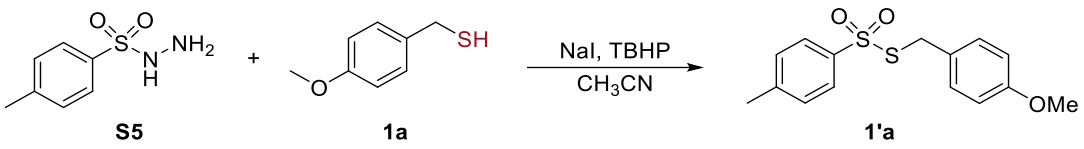

We have revised the manuscript and supporting information based on these experimental results. In the revised manuscript, we have added the following statement: "*Although phenol could react with $SO_2F_2$, **2o** underwent selective reaction on the thiol group while precluding any undesired interference from the phenol. This can be attributed to the fact that the reactions between phenol and $SO_2F_2$ generally necessitate distinct reaction conditions. For example, the reaction between phenols and $SO_2F_2$ was typically triggered by 1.5 equiv. of $Et_3N$[29]. In contrast, the reaction of **2l** or **2o** with $SO_2F_2$ requires 0.3 equiv. of $Et_3N$. Therefore, even when the reaction time of **2l** or **2o** with excess $SO_2F_2$ was lengthened from 1 h to 12 h, no by-products were observed.*". The revised supporting information now includes these results, along with other relevant data, in Section 4.3 "The synthesis of symmetrical disulfide using $SO_2F_2$" (SI, Page 15-17).

4. Can the $RSSO_2F$ species be isolated and characterized with specific substrate?

Response:

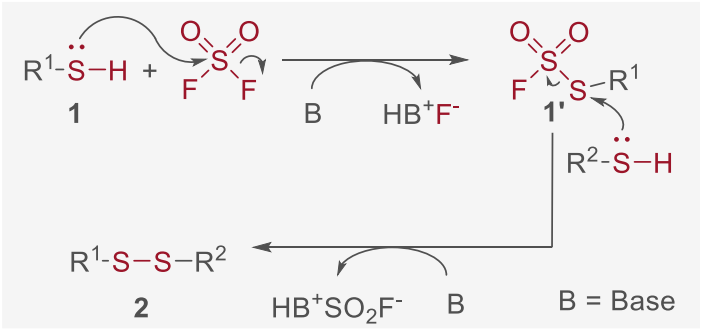
We sincerely thank the reviewer for this insightful suggestion. This prompted us to attempt the isolation of the intermediate and conduct further control experiments with the intermediate. Given that sulfonyl fluorides **VI** exhibit lower reactivity compared to $SO_2F_2$, we tried to isolate the intermediate **1'a** formed in the reaction between **VI** and **1a**. However, direct isolation was unsuccessful. Nevertheless, we synthesized the intermediate via an alternative reaction between **1a** and 4-methylbenzenesulfonohydrazide (**S5**). The reaction was shown below:

The synthesis of intermediate **1'a**:

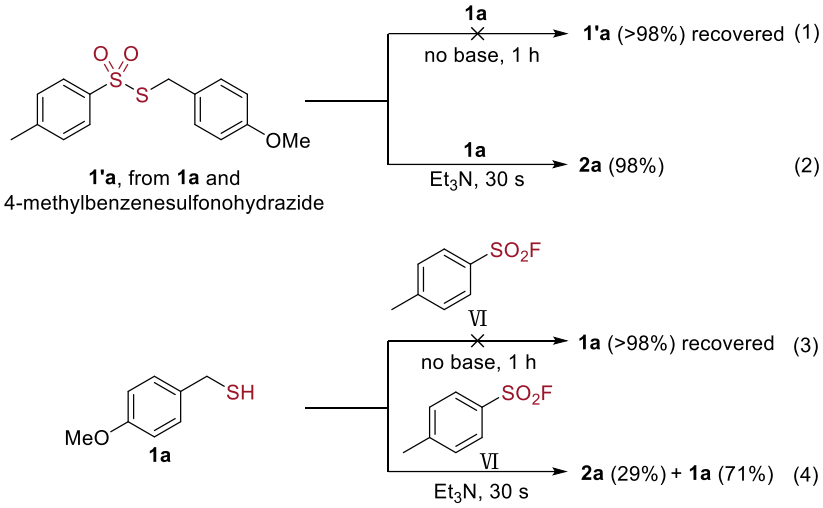



**S5** **1a** **1'a**

With the synthesized intermediate in hand, we conducted further control reactions. The results indicated that a base is essential for both steps of the reaction, with the first step being the rate-determining step. The proposed mechanism and the control experiments were shown below:




Control experiments:




These results have been thoroughly documented in the revised manuscript and supporting information. Specifically, the following statement was added in the revised manuscript (Page 4 line 36-46, Page 5 line 1-5 and eq 1-4): "*Given that sulfonyl fluoride **VI** exhibit lower reactivity compared to $SO_2F_2$, we attempted to isolate the intermediate **1'a** formed in the reaction between **VI** and **1a** to conduct control experiments. However, direct isolation of the intermediate from the reaction was unsuccessful. Nevertheless, we successfully synthesized the intermediate via the reaction between **1a** and 4-methylbenzenesulfonohydrazide. With the synthesized intermediate in hand, we tested the reaction between **1'a** and **1a** without a base (eq 1), and the results showed that only the starting materials were recovered, indicating that a base is essential for the second step of the reaction (**1'** to **2**, Fig 2C). Subsequently, we tested the reaction between **1a** and **VI** without a base (eq 3), and similarly, only the starting materials were recovered with no formation of intermediate **1'a**, suggesting that a base is also crucial for the first step (**1** to **1'**, Fig 2C). Next, in the presence of a base, we repeated both experiments, but the reaction was quenched after 30 seconds. The reaction between **1'a** and **1a** yielded >98% of **2a** within 30 seconds (eq 2), indicating that the second step proceeds very rapidly in the presence of a base. Conversely, the reaction between **1a** and **VI** in the presence of a base resulted in only 29% yield of **2a** (eq 4), with the major mass-balance being unreacted **1a**, indicating that the first step is slower than the second step and constitutes the rate-determining step of the reaction.*". In the revised supporting information, we have included these results, along with other relevant data, as "Synthesis of Intermediate and Control Reactions" section (SI, Page 56-58).

## **Responses to the comments of reviewer 3**.

An et al. reported a click chemistry method mediated by $SO_2F_2$ for the formation of sulfur–sulfur bonds via thiols. In this study, $SO_2F_2$ and its surrogates exhibited exceptional reactivity and selectivity across diverse reaction media, including water. It can be utilized for the assembly of symmetrical and unsymmetrical disulfides, extending to the synthesis of polydisulfides. This manuscript presents an advancement in disulfide linkage chemistry and is qualified for Nature Communication after these issues addressed.

Response:

We sincerely thank the reviewer for the positive feedback and encouraging comments. We are grateful for the recognition of our work and the valuable suggestions provided. We have revised the manuscript according to your recommendations. Detailed responses to your comments are provided below.

1. When the authors presented protocols for the oxidation of thiols leading to the disulfides through S–S bond formation, a notable gap exists in terms of disulfide reagents capable of synthesizing disulfides via S–C bond linkage (e.g., Nat. Commun. 2018, 9, 2191; Nat. Commun. 2020, 11, 4170; Angew. Chem. Int. Ed. 2023, 62, e202314379). Emphasizing the advantages of the $SO_2F_2$-mediated strategy would be beneficial, especially in the context of unsymmetrial disulfides in comparison to traditional disulfide reagents.

Response:

We sincerely thank the reviewer for the valuable suggestions. We appreciate your observation regarding the gap in our presentation of disulfide reagents capable of synthesizing disulfides. In this revised manuscript, we have added the following statement in the introduction (Page 2 line 22-24): "*Recently, Jiang's group reported robust polysulfurating and bilateral disulfurating reagents for the synthesis of unsymmetrical disulfides*[24-26]." We have also included the references mentioned by the reviewer (Nat. Commun. 2018, 9, 2191; Nat. Commun. 2020, 11, 4170; Angew. Chem. Int. Ed. 2023, 62, e202314379) as ref 24-26 in the reference section of our revised manuscript.

2. SuFEx chemistry not only is employed for the S–O or S–N linkage of SuFEx linkers, but also has been extended to the formation of S–C bonds (e.g., Nat. Synth. 2022, 1, 455; Angew. Chem. Int. Ed. 2022, 61, e202207100).

Response:

We deeply appreciate the reviewer's insightful comments and valuable suggestions. We recognize the importance of highlighting the application of SuFEx chemistry in the formation of S–C bonds. In this revised manuscript, we have included the following statement in the introduction (Page 3 line 2-5): "*Among these reactions are the sulfur(VI)- fluoride exchange (SuFEx) processes developed by Sharpless, Moses, Dong, Jiang and colleagues*[28-37]." Additionally, we have included the references mentioned by the reviewer (Nat. Synth. 2022, 1, 455; Angew. Chem. Int. Ed. 2022, 61, e202207100) in the reference section as ref 36 and 37 in our revised manuscript.

3. The authors used $SO_2F_2$ and its surrogates (II-V) to synthesize the corresponding disulfides and outlined the order of reactivity. However, this approach may lack rigor, as the reagent II may not fully represent the reactivity of $FSO_2N$-containing compounds. Thus, the synthesis of $PhNHSO_2F$ or $BnNHSO_2F$ is recommended to assess reactivity. Additionally, consideration should be given to the aza-analog of sulfonyl fluoride, sulfonimidoyl fluoride, in evaluating reactivity and selectivity (e.g., SulfoxFluor, Org. Process Res. Dev. 2022, 26, 380, https://pubs.acs.org/doi/10.1021/acs.oprd.1c00431?goto=supporting-info).

Response:

We sincerely thank the reviewer for the insightful suggestions and for providing valuable references for synthesis methods. We agree that reagent **II** may not fully represent the reactivity of $FSO_2N$-containing compounds. Therefore, we synthesized more representative sulfamoyl fluoride (**IV**) and tested their reactivity with the respective thiol substrates. As suggested by the reviewer, we further tested the reactivity of the aza-analog of sulfonyl fluoride (**III**) and sulfonimidoyl fluoride (**V**) using their respective thiol substrates. Please see the results below:

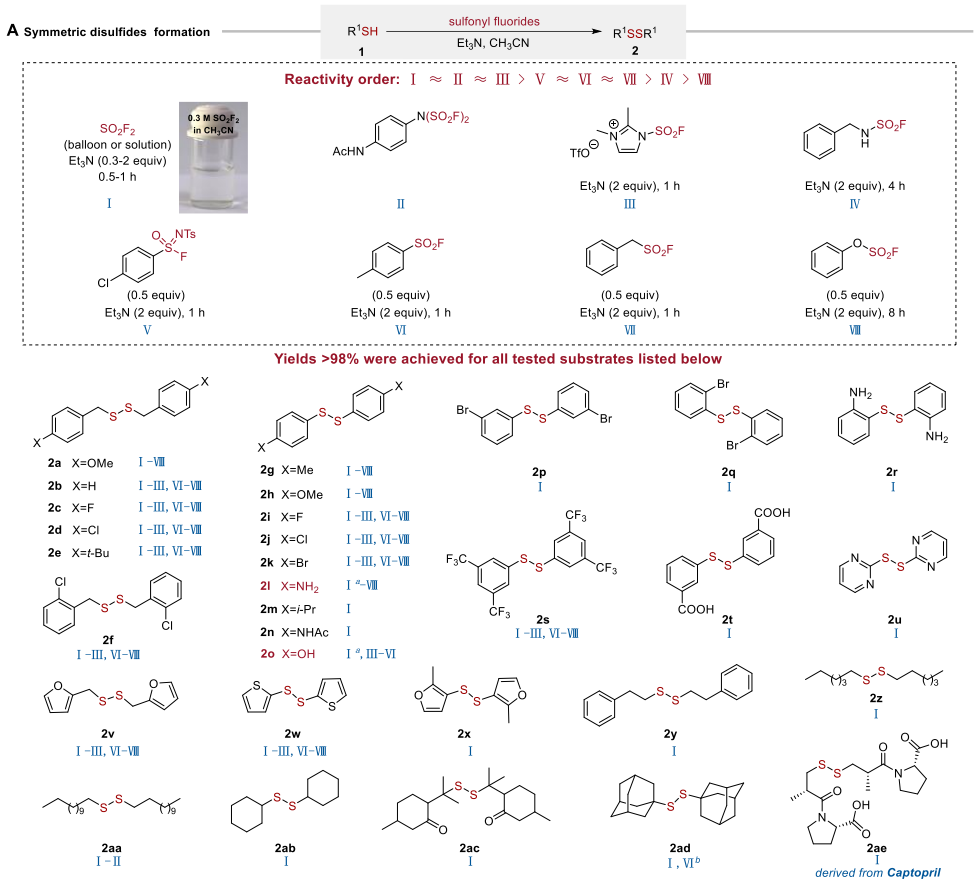



Fig. 3A Synthesis of symmetrical disulfides by click chemistry.

The results showed that all these reagents are effective for the thiol-to-disulfide conversion, albeit with different reactivity profiles. In the revised manuscript, we included the following statement (Page 4 line 30-35): "*Apart from $SO_2F_2$ (**I**) gas, a range of solid and liquid surrogates to $SO_2F_2$, namely, sulfamoyl fluorides **II, III** and **IV**, sulfonimidoyl fluoride **V**, sulfonyl fluorides **VI** and **VII**, and fluorosulfate **VIII** could all successfully convert thiols into the corresponding disulfides, with varied reactivity. Based on extensive experimentations, their order of reactivity was as follows: **I** ≈ **II** ≈ **III** > **V** ≈ **VI** ≈ **VII** > **IV** > **VIII**.*"

We have also revised Figure 3A and the supporting information based on these results. The revised supporting information now includes these results, along with other relevant data, in section 6-8 (SI, Page 26-31).

4. The one-step assembly of unsymmetric disulfides is significant, but it is currently limited to aryl-alkyl disulfides. Have the authors explored a step-wise synthesis approach for unsymmetric aryl-aryl/alkyl-alkyl disulfides via diverse $RSO_2F$ reagents?

Response:

We sincerely thank the reviewer for this insightful comment. Your suggestion prompted us to further optimize the conditions for the synthesis of unsymmetrical disulfides. The optimized reaction conditions have now been successfully applied to the synthesis of 14 unsymmetrical disulfides, including those from two aromatic thiols and two aliphatic thiols, with yields ranging from 60% to 98%. Please see the results below:

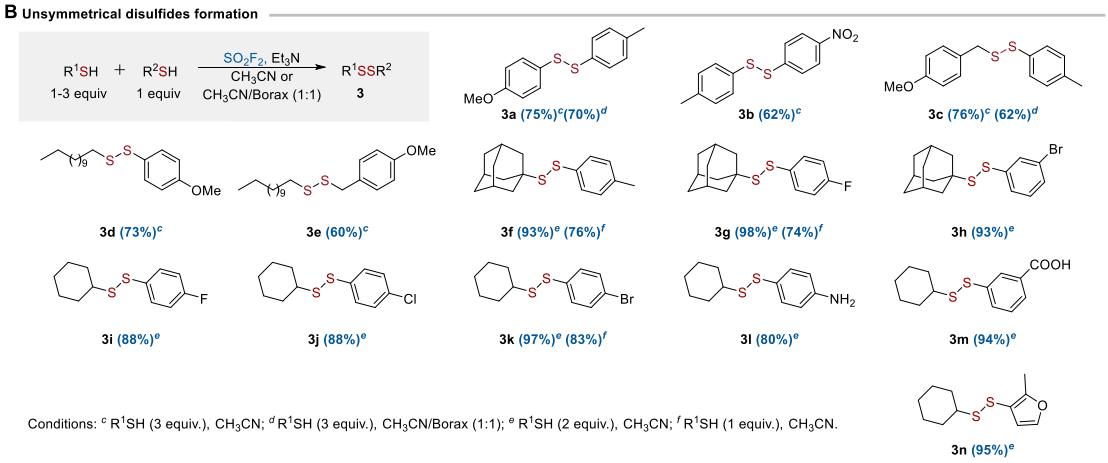



Fig. 3B Synthesis of unsymmetrical disulfides by click chemistry.

We have revised the manuscript and supporting information based on these experimental results. In the revised manuscript, "Figure 3B" has been updated and we have added the following statement: "*The efficiency of this click reaction in linking two different modules was evaluated through the direct synthesis of unsymmetrical disulfides (Fig. 3B). Using $SO_2F_2$ as the reagent, unsymmetrical disulfides were synthesized from the cross-coupling of one aromatic thiol and one aliphatic thiol, two aromatic thiols, and two aliphatic thiols, achieving yields of 60−98%, which were challenging to obtain with other known coupling reagents. In the coupling reactions involving one aromatic thiol and one aliphatic thiol (**3c - 3d** and **3f - 3n**), high yields >80% could even be achieved using equimolar amounts of the thiols (**3k**). This is likely due to the relatively higher acidity of aromatic thiols, which are more easily deprotonated under basic conditions and therefore react more rapidly with $SO_2F_2$ compared to aliphatic thiols, resulting in the formation of $ArSSO_2F$ as the predominant intermediate. The reaction of the intermediate $ArSSO_2F$ with aliphatic thiol subsequently produces the desired unsymmetrical disulfides. Despite the similar reactivity of the two starting materials, this method is also effective for the unsymmetrical coupling of two aromatic thiols (**3a-3b**) or two aliphatic thiols (**3e**), although 2.0 – 3.0 equiv. of one reactant was required to achieve high yields.*". The revised supporting information now includes these results, along with other relevant data, in Section 12 "The Synthesis of Unsymmetrical Disulfide using $SO_2F_2$" (SI, Page 42-49).

5. The title in Figure 1 is misaligned, and the color representation in Figures 2 and 3 appears faint. It is recommended that adjustments need to be made to the figures and tables in the manuscript to enhance clarity for the reviewer's understanding.

Response:

We sincerely thank the reviewer for pointing out these important issues. We have made the necessary adjustments to the figures and tables as recommended. Specifically, we have realigned the title in Figure 1 and enhanced the color representation in Figures 2 and 3 to improve clarity. These changes have been incorporated into the revised manuscript.

6. These identified issues require correction and examination in the manuscript.
   (1) '5 mmoL/h' should be revised as '5 mmol/h' in Page 7, Line 11.
   (2) The oxdiative polymerization of 5b is reported instead of 6a in Table 2.
   (3) The arrow of the catalytic cycle is oriented in the opposite direction in Figure S2.

Response:

We sincerely thank the reviewer for helping us identify these issues. We have made the necessary corrections as per your suggestions.

(1) We have revised '5 mmoL/h' to '5 mmol/h' in Page 8, Line 11.

(2) We have corrected the reported polymerization of **6a** to **5b** in Table 2.

(3) We have reoriented the arrow of the catalytic cycle to the correct direction in Figure S2.

Additionally, we have conducted a thorough review of the entire manuscript to ensure all identified issues have been addressed. These changes have been incorporated into the revised manuscript and supporting information.

Other Changes:

(1) Dr. Zhaonong Hu has made substantial contributions during the revision of this manuscript, including data analysis and visualization. Consequently, he is being designated as an author for this study in the revised version.

REVIEWERS' COMMENTS

Reviewer #1 (Remarks to the Author):

The manuscript is significantly improved after addressing reviewer's concerns. I now recommend the acceptance for Nature Communications.

Reviewer #2 (Remarks to the Author):

The revised manuscript demonstrated significant improvement. The authors have addressed most of my concerns and done excellent additional work in response to suggestions and comments from reviewers. My suggestion is publication as it is.

Reviewer #3 (Remarks to the Author):

Based on the peer review, the authors have addressed all the concerns raised in my previous review. Positive revisions have been incorporated into this version. For instance, the representative S–F-containing surrogates were synthesized to test the reactivity of thiol substrates, demonstrating a clear and adequate sequence. This reaction substrates were thoroughly explored, extending beyond the initial aryl-alkyl disulfides to include aryl-aryl and alkyl-alkyl disulfides. The manuscript now includes pertinent references on the synthesis of disulfides via S–C bond linkage. In conclusion, these revisions meet the requirements, and I recommend this manuscript for publication in the current version.

Reviewer #4 (Remarks to the Author):

In this manuscript, the authors tried to prepare a new cross-linking method for the in-situ gelation of hydrogel. The major concern about this is the utilization of CH3CN as a solvent to dissolve SO2F2 for gelation. It is obvious that CH3CN is not a biocompatible solvent for biomedical application. I also have the following comments for revision. In general, this work does not reach the level for publication in Nat Commun.

1. The thiol-to-disulfide click chemistry can also occur between polymers and skin, which would cause the cross-link between materials and tissues. This hampers my passion to consider it as a biocompatible strategy for tissue engineering.

2. There is no scale bar in Figure 6D, S12, and S13.

3. In cell culture experiment (page 67), adding 5 mg hydrogel to a well in 96-well plate. Cell viability was 98%. In general, 5 mg may be too little, although the author has calculated the concentration is 2500 ug/ml. Before adding to the cell, was the hydrogel freshly prepared? Was there any purification steps that wash away the soluble chemicals?

4. Adding hydrogel onto the top of cells is not a usual performance for biocompatibility test. Either culture cell on a hydrogel coated plate, or adding the extract from the hydrogel to the cells.

5. In vivo experiment only has 3 rats. The number is too small. Before adding the hydrogel to the wound, was the hydrogel freshly prepared? Were there any purification steps that wash away the soluble chemicals?

6. With small number of rats and very limited data, it is not appropriate to draw the following conclusion: “which demonstrated a significantly superior result compared to the cyanoacrylate treated group. In comparison to the CK group, the wounds treated with the hydrogel exhibited enhanced healing. These findings indicated the potential for future applications of this thiol-to-disulfide click chemistry in the field of biomaterials.”

# Point-by-Point Response to Referees

## Reviewer 1.

The manuscript is significantly improved after addressing reviewer's concerns. I now recommend the acceptance for Nature Communications.:

Response:

We sincerely thank the reviewer for the recommendation.

## Reviewer 2.

The revised manuscript demonstrated significant improvement. The authors have addressed most of my concerns and done excellent additional work in response to suggestions and comments from reviewers. My suggestion is publication as it is.

Response:

We sincerely thank the reviewer for the recommendation.

## Reviewer 3.

Based on the peer review, the authors have addressed all the concerns raised in my previous review. Positive revisions have been incorporated into this version. For instance, the representative S–F-containing surrogates were synthesized to test the reactivity of thiol substrates, demonstrating a clear and adequate sequence. This reaction substrates were thoroughly explored, extending beyond the initial aryl-alkyl disulfides to include aryl-aryl and alkyl-alkyl disulfides. The manuscript now includes pertinent references on the synthesis of disulfides via S–C bond linkage. In conclusion, these revisions meet the requirements, and I recommend this manuscript for publication in the current version.

Response:

We sincerely thank the reviewer for the recommendation.

## Reviewer 4.

In this manuscript, the authors tried to prepare a new cross-linking method for the in-situ gelation of hydrogel. The major concern about this is the utilization of $CH_3CN$ as a solvent to dissolve $SO_2F_2$ for gelation. It is obvious that $CH_3CN$ is not a biocompatible

solvent for biomedical application. I also have the following comments for revision. In general, this work does not reach the level for publication in Nat Commun.

1. The thiol-to-disulfide click chemistry can also occur between polymers and skin, which would cause the cross-link between materials and tissues. This hampers my passion to consider it as a biocompatible strategy for tissue engineering.

Response:

We sincerely thank the reviewer for the comments. We have removed the relevant cell culture and in vivo experiments from the manuscript and supporting information.

2. There is no scale bar in Figure 6D, S12, and S13.

Response:

We sincerely thank the reviewer for the comments. We have removed the relevant in vivo experiments from the manuscript and supporting information.

3. In cell culture experiment (page 67), adding 5 mg hydrogel to a well in 96-well plate. Cell viability was 98%. In general, 5 mg may be too little, although the author has calculated the concentration is 2500 ug/ml. Before adding to the cell, was the hydrogel freshly prepared? Was there any purification steps that wash away the soluble chemicals?

Response:

We sincerely thank the reviewer for the comments. We have removed the relevant cell culture experiments from the manuscript and supporting information.

4. Adding hydrogel onto the top of cells is not a usual performance for biocompatibility test. Either culture cell on a hydrogel coated plate, or adding the extract from the hydrogel to the cells.

Response:

We sincerely thank the reviewer for the comments. We have removed the relevant cell culture experiments from the manuscript and supporting information.

5. In vivo experiment only has 3 rats. The number is too small. Before adding the hydrogel to the wound, was the hydrogel freshly prepared? Were there any purification steps that wash away the soluble chemicals?

Response:

We sincerely thank the reviewer for the comments. We have removed the relevant in vivo experiments from the manuscript and supporting information.

6. With small number of rats and very limited data, it is not appropriate to draw the following conclusion: “which demonstrated a significantly superior result compared to the cyanoacrylate treated group. In comparison to the CK group, the wounds treated with the hydrogel exhibited enhanced healing. These findings indicated the potential for future applications of this thiol-to-disulfide click chemistry in the field of biomaterials.”

Response:

We sincerely thank the reviewer for the comments. We have removed the relevant in vivo experiments from the manuscript and supporting information.