A modular flow platform enables selective and fast SuFEx ligation of small molecules, peptides, and proteins

Miguel Bernús,[1,2+] Daniele Mazzarella,[1,3+] Jelena Stanić,[1] Ziran Zhai,[4,5] Alejandro Yeste Vazquez,[6] Omar Boutureira,[2] Andrea F. G. Gargano,[4] Tom N. Grossmann,[6] Timothy Noël[1,*]

[1] Flow Chemistry Group, Van 't Hoff Institute for Molecular Sciences (HIMS), University of Amsterdam, Amsterdam, The Netherlands.

[2] Departament de Química Analítica i Química Orgànica, Universitat Rovira i Virgili, Tarragona, Spain.

[3] Department of Chemical Sciences, University of Padova Institution, Padova, Italy

[4] Analytical Chemistry Group, Van't Hoff Institute for Molecular Sciences (HIMS), University of Amsterdam, Amsterdam, The Netherlands.

[5] Centre for Analytical Sciences Amsterdam, Amsterdam, The Netherlands.

[6] Department of Chemistry and Pharmaceutical Sciences, Vrije Universiteit Amsterdam, Amsterdam, The Netherlands.

[+] These authors contributed equally to this work.

*Correspondence to: t.noel@uva.nl.

SuFEx click chemistry has established itself as a formidable tool to rapidly and effectively link chemical structures. Despite tremendous advancements in the field in recent years, the installment of the crucial -SO_2F handle still requires the use of purposely designed, expensive, and non-atom economical reagents. However, the use of the most obvious reagent, SO_2F_2, has been twarthed by the difficulties associated with the manipulation and dosage of this toxic gas, as well as its apparent low reactivity with amino functionalities. Herein, we disclose a modular flow platform, which is able to generate on

demand, and safely dose, gaseous SO_2F_2. Due to the use of flow technology, many lingering limitations of this transformation could be overcome, resulting in significantly reduced reaction times, high reactivity and exceptional reaction scope. The effectiveness of the process was demonstrated by the successful synthesis of a diverse set of fluorosulfates and sulfamoyl fluorides, including those derived from biorelevant compounds, peptides, and proteins.

Click chemistry is a powerful and efficient method for rapidly connecting chemical fragments, enabling the modification of biologically active molecules (*1*,*2*). Among the various types of click chemistry, SuFEx (Sulfur(VI) Fluoride Exchange) reactions have emerged as a reliable resource for drug discovery (*3*,*4*,5), chemical biology (*6*,*7*,*8*), polymer chemistry (*9*,*10*), and surface modifications (*11*,*12*). In particular, the unique properties of the sulfur(VI)-fluorine bond make SuFEx reactions highly versatile, allowing for the formation of covalent bonds under mild conditions (*13*). The S(VI)–F bond is highly stable, allowing it to withstand harsh conditions, yet readily cleavable in the presence of a suitable activator or reaction partner (*14*,*15*). Consequently, the versatility and efficiency of SuFEx reactions make them a valuable tool for researchers in a wide range of fields, including synthetic chemistry, drug discovery, and materials science (*16*).

Among the various SuFEx hubs, the -SO_2F moiety has received significant attention due to its unique biophysical properties (17) and its potential as a versatile connector between nucleophilic entities (*18*). One method for installing this moiety is the use of gaseous sulfuryl fluoride (SO_2F_2), which has been demonstrated for various organic molecules by the group of Sharpless (*13*). However, despite its potential as an economic and traceless compound, its mild toxicity and difficulty to handle have motivated researchers to seek for more practical alternatives. While in-situ generation of SO_2F_2 from 1,1'-sulfonyldiimidazole (SDI, Figure 1A) (*19*) or the use of solid reagents such as [4-(acetylamino)phenyl]imidodisulfuryl difluoride

(AISF) (*20*) or 1-(fluorosulfonyl)-2,3-dimethyl-1*H*-imidazol-3-ium trifluoromethanesulfonate (FDIT) (*21*) have been explored, these alternatives still require the use of SO_2F_2 in their preparation and produce unnecessary by-products. Hence, it is clear that these methods go against the principles of click chemistry and represent a challenge to date (*2*). Addressing these challenges by developing a new strategy for producing and dosing SO_2F_2 in a safe and controlled manner from simple chemicals would represent a significant advance in the field of SuFEx chemistry. Furthermore, such a process would significantly reduce the number of synthetic steps needed for SuFEx handle installation and therefore streamline the overall process.

In order to address the challenges associated with using sulfuryl fluoride (SO_2F_2) as a reagent, we considered using cheap commodity chemicals such as sulfuryl chloride (SO_2Cl_2) and potassium fluoride (KF) to generate SO_2F_2 in-situ. This approach was motivated by the greater thermodynamic stability of the S(VI)–F bond (~90 kcal/mol) compared to the S(VI)–Cl bond (~46 kcal/mol), which suggests that this exchange should be achievable (*22*,*13*). Our initial batch experiments using SO_2Cl_2 and KF in CH_3CN confirmed the feasibility of this approach, demonstrating succesful conversion of first SO_2Cl_2 to SO_2FCl and later to SO_2F_2 was achieved within two hours (see Supplementary Materials).

Due to the slow kinetics observed in batch reactions and the mixture of SO_2FCl and SO_2F_2 obtained, we turned to flow technology as a tool for more effectively generating and controlling the delivery of this reactive gas (*23*,*24*). Hereto, we designed a modular system (Figure 1B) using microfluidic technology to greatly enhance the safety and scalability of the overall process (*25*). Our modular system consists of two interconnected flow reactors. The first reactor is a packed-bed reactor filled with KF that generates SO_2F_2 on demand via chlorine-fluorine exchange. The second reactor is where the generated gaseous SO_2F_2 is mixed with the nucleophilic partner, ultimately yielding the desired SuFEx product. Since the first reactor

generates SO_2F_2 on demand, the reagent remains contained and is subsequently mixed with the nucleophilic partner in the second reactor. By immediately reacting away the toxic SO_2F_2 in the SuFEx module, our modular system effectively eliminates the safety and practical concerns associated with the handling of this reagent, while generating only the required quantities. In addition, we anticipated that our use of flow technology would also reduce the time required for halogen substitution reactions, thanks to enhanced liquid-to-solid contact in the first Cl-F exchange module and excellent gas-to-liquid mass transfer in the SuFEx module (*26*). Our experiments confirmed the effectiveness of our modular system: when we directed a solution of sulfuryl chloride over the packed-bed reactor filled with a mixture of KF and glass beads (Figure 1C, see Supplementary Materials for further details), we observed a rapid and selective formation of SO_2F_2. We found that varying the flow rate was crucial for the selectivity of the transformation, and that 0.5 mL/min was the optimal flow rate in terms of conversion, selectivity, and time (7 min reaction time), effectively avoiding the presence of undesired SO_2FCl. Under optimized conditions, the packed-bed reactor was able to produce ~18 mmol of SO_2F_2 starting from a ~80 mmol KF bed (see Supplementary Materials for further details).

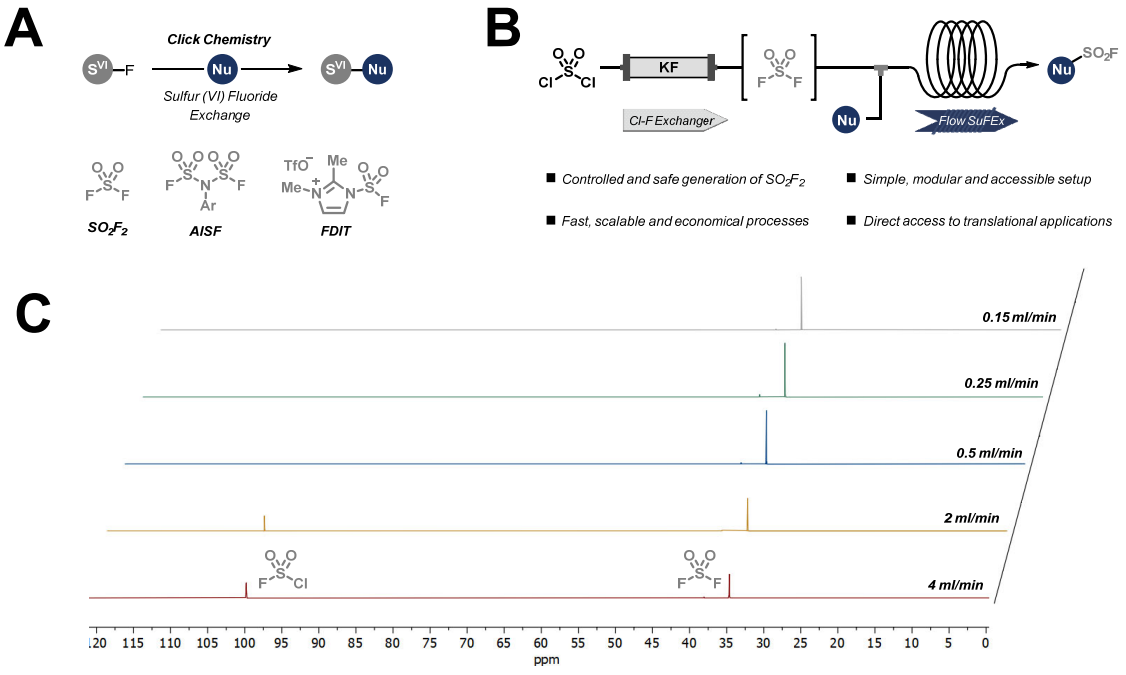

Figure 1. Sulfur (VI) fluoride exchange (SuFEx) click chemistry. (A) While stable under various conditions (hydrolysis, reduction/oxidation), S(VI) fluorides are susceptible for nucleophilic attack enabling efficient click-type reactions. However, the synthesis of S(VI) fluorides can be cumbersome requiring challenging reagents, such as gaseous SO_2F_2 or atom-inefficient solid reagents. (B) In flow-generated gaseous SO_2F_2 enables a practical, fast and selective preparation of S(VI) fluoride reagents. (C) Flow experiments showing the feasibility of the outlined strategy to produce the coveted SO_2F_2 in high selectivity (results obtained by ^{19}F-NMR).

Having obtained promising results from our SO_2F_2 generator, we proceeded to integrate it with the SuFEx module to enable the reaction with nucleophilic partners. By introducing the appropriate nucleophiles with an excess of a base, we were able to obtain a diverse range of SuFExed products with excellent isolated yields in just two minutes of residence time (Figure 2). This short residence time can be attributed to the intimate contact between gas and liquid phase in flow (i.e., enhanced gas-liquid mass transfer) (*27*,*28*), which should allow for the generation of large libraries of SuFExed compounds with minimal effort and time. Notably, a variety of phenols could be cleanly converted to their corresponding fluorosulfates regardless of the position or electronic nature of the substituents (Figure 2, compounds **1**-**6**). While using large quantities of gaseous SO_2F_2 in batch reactions can be challenging, our flow protocol overcomes this issue, allowing for a gram-scale synthesis of fluorosulfate **1** by simply increasing the amount of starting materials pumped through the reactor assembly (*29*). Importantly, no reoptimization of reaction conditions was required, and no loss of chemical efficiency was observed during the scaled-up experiment.

We further found that our flow protocol was not limited to the synthesis of simple phenol-based fluorosulfates. In fact, we observed that a wide variety of natural products, drugs, and fluorescent tracers could be successfully reacted using this approach. For instance, *N*-Boc-protected tyrosine, broxyquinoline, fluorescein, α-tocopherol, estrone, and unprotected amoxicillin were all cleanly converted in just 2 minutes of reaction time (Figure 2, compounds

7-**12**), demonstrating the excellent functional group tolerance of our method. Similarly, the challenging class of nitrogen-based nucleophiles could also be subjected to our protocol delivering the corresponding sulfamoyl fluorides in excellent isolated yields. For example, 4- to 7-membered ring secondary amines (Figure 2, compounds **13**-**17**), heterocyclic derivatives (Figure 2, compounds **18**-**20**), and an aniline compound (Figure 2, compound **21**) were effectively reacted with SO_2F_2. Our flow protocol was also effective in synthesizing analogs of several pharmaceutically relevant molecules, such as stanozolol, paroxetine, desloratadine, amoxapine, and olanzapine, which rapidly yielded the desired SuFExed products (Figure 2, compounds **22**-**26**). Additionally, nucleosides such as adenosine and cytidine derivatives (Figure 2, compounds **27**-**29**) could be used as competent reaction partners. Finally, we were able to use bidentate nucleophiles, such as BINOL, catechol, and salicylamide (Figure 2, compounds **30**-**32**), to produce the corresponding sulfates and sulfamates.

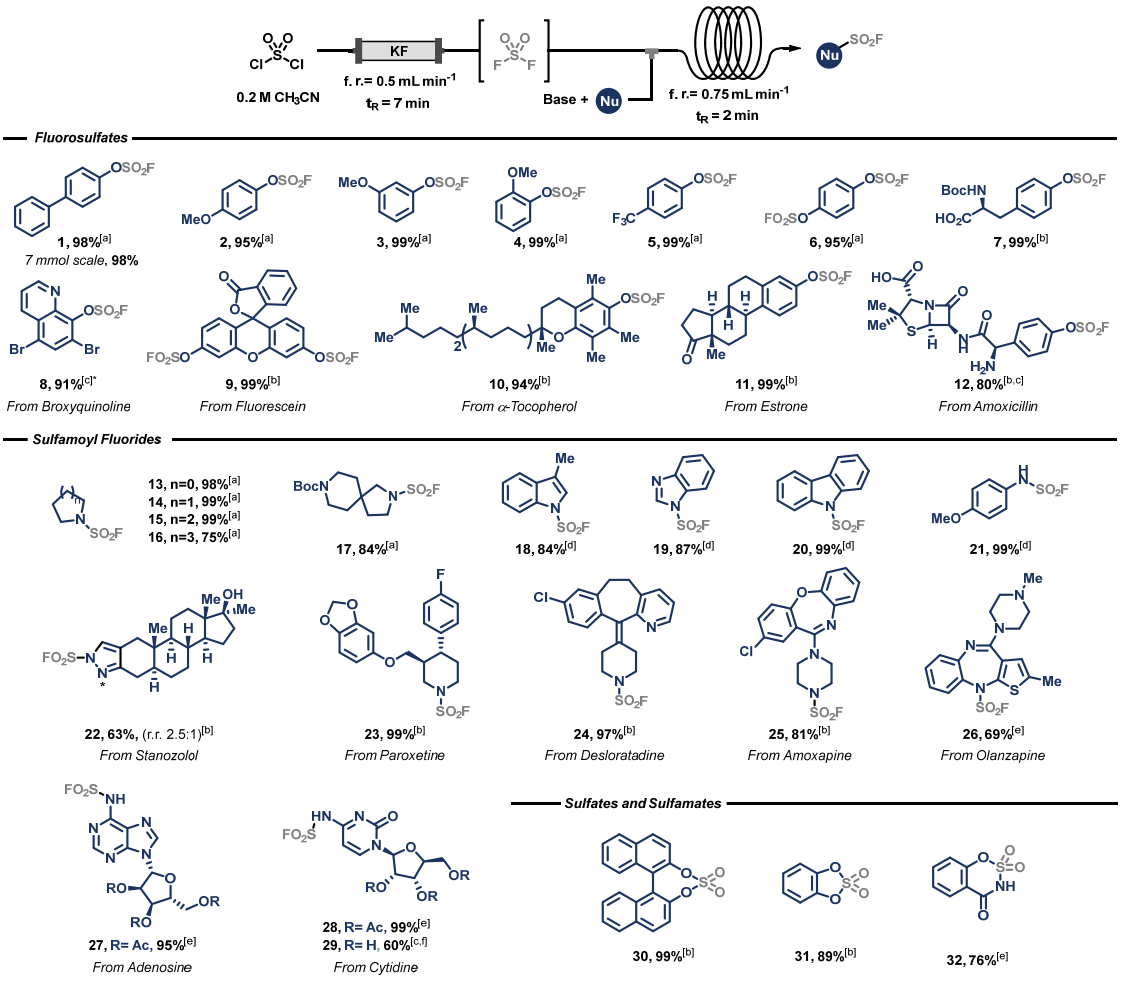

Figure 2. Rapid SuFEx ligation of small molecules in flow, yielding fluorosulfates, sulfamoyl fluorides and sulfates and sulfamates. All yields are those of isolated compounds. Standard conditions for the SO_2F_2 generation: SO_2Cl_2 (2 equiv, 0.2 M in CH_3CN) passed through a 3.8 mL cartridge filled with a 1:1 mixture of KF and glass beads. Standard conditions for the second step: [a] Nucleophile (1 equiv, 0.2 M in CH_3CN), Et_3N (2.5 equiv). [b] Nucleophile (1 equiv, 0.2 M in DMF), Et_3N (2.5 equiv). [c] The compound has been isolated after an acetylation step. [d] Nucleophile (1 equiv, 0.2 M in CH_3CN), DBU (4.0 equiv). [e] Nucleophile (1 equiv, 0.2 M in DMF), DBU (4.0 equiv). [f] Nucleophile (1 equiv, 0.1 M in DMSO), K_2CO_3 (4.0 equiv). *5 equiv of DBU were used. DBU: 2,3,4,6,7,8,9,10-Octahydropyrimido[1,2-a]azepine. DMF: *N*,*N*-Dimethylformamide. DMSO: dimethylsulfoxide.

Next, we capitalized on the modular nature of our setup and developed a multistep process that orchestrates several reactions in a sequential fashion (Figure 3). For instance, we streamlined

a three-module flow setup to synthesize sulfate derivative **33** (67%) uninterrupted. After generating sulfuryl fluoride in the packed-bed reactor and trapping it with estrone in the SuFEx capillary reactor, the resulting fluorosulfate was reacted with (4-iodophenoxy)trimethylsilane in a second SuFEx capillary. Our flow approach carefully balances the stoichiometry of the gaseous reagent, preventing any remaining SO_2F_2 from unproductively consuming the silyl ether in the second SuFEx step (*13*). Furthermore, after a solvent switch, the reaction crude can also immediately be used to carry out a base-promoted hydrolysis of ethylenacetal-protected estrone to obtain bisulfate derivative **34** (80% yield) or a palladium-catalyzed Suzuki-Miyaura-type cross coupling to yield product **35** in 55% yield.

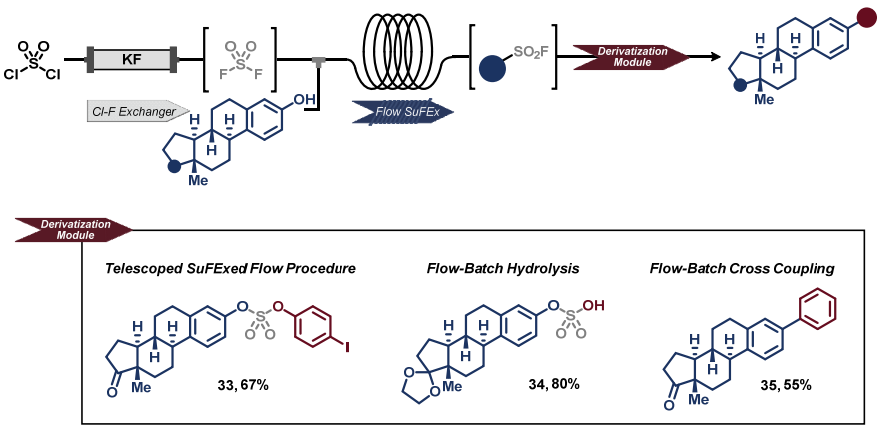

Figure 3. A three-step protocol that combines the SO_2F_2 generator module, the SuFEx module, and the derivatization module, allowing for seamless SuFEx click chemistry, fluorosulfate hydrolysis, and late-stage cross-coupling chemistry. All yields are those of isolated compounds. Full description of the experimental details can be found in the Supplementary Materials.

Our flow approach has demonstrated excellent functional group tolerance and selectivity in producing SuFExed products from various small molecules. This is due to the favorable reaction conditions, including high mass transfer and excellent dosing of gaseous reagents, provided by the flow protocol that allows for reduced reaction times of just two minutes, thereby avoiding the formation of deleterious byproducts. We next sought to explore the potential of our microfluidic strategy for the late-stage modification of unprotected peptides

(*30*). Our aim was to directly install the sulfur-centered electrophilic handle within the peptide core, allowing for later SuFEx-enabled derivatization opportunities (*31*). Based on our previous experience with small molecules, we anticipated that the reaction would be kinetically favored for tyrosine residues, which should enable site-selective modification within the complex peptidic framework. After a minimal re-optimization of the reaction conditions (see Supplementary Materials), we investigated the reactivity of different nucleophilic amino acid residues within different pentapeptides. We found that tyrosine-containing peptides were successfully converted in a site-selective fashion in just two minutes (Figure 4, compounds **36** and **37**). Our evaluation also showed limited modification at lysine and histidine (Figure 4, compounds **38** and **39**), while other nucleophilic residues, such as tryptophan and cysteine, were not reactive under our reaction conditions (see Supplementary Materials). Encouraged by these results, we subjected various complex and therapeutically valuable peptides to our flow protocol. Cyclic peptides (Figure 4, compounds **40** and **41**) and therapeutic drugs such as Angiotensin II and Bivalirudin (Figure 4, compounds **42** and **43**) were selectively modified at the tyrosine residue in good to excellent results. Even natural peptides, such as α-Endorphin and β-Amyloid (1-28), were effectively converted into the corresponding fluorosulfates with good to excellent conversions (Figure 4, compounds **44** and **45**).

As the ultimate test for our SuFEx ligation protocol, we focused on the direct modification of proteins in flow. By minimizing lysine competition (see Supplementary Information), we managed to exclusively install the electrophilic SO_2F handle on tyrosine residues in just 1.5 minutes. To the best of our knowledge, this is one of the fastest methods for direct protein modifications reported to date (*32*). For instance, we merged a solution of β-Casein with the SO_2F_2-containing stream and observed predominantly single, chemoselective functionalization at different tyrosine residues with a Y180/Y193/Y114 = 10.7/2.4/1 regioselectivity ratio (Figure 4, compound **46**). Notably, Myoglobin was obtained as a single Y103-modified adduct

(Figure 4, compound **47**), without observing denaturation or loss of the heme group, demonstrating the mild nature of the protocol. Remarkably, when we attempted to perform the same experiments in fed-batch mode, only a 16% conversion was obtained, while using a batch H-type reactor only yielded a complex mixture of products (Figure 4, bottom right). These results demonstrate that the enhanced mass transfer and confined access to the gaseous and hydrophobic SO_2F_2 observed in capillary flow reactors are critical to enable efficient SuFEx hub installation into complex macromolecular systems.

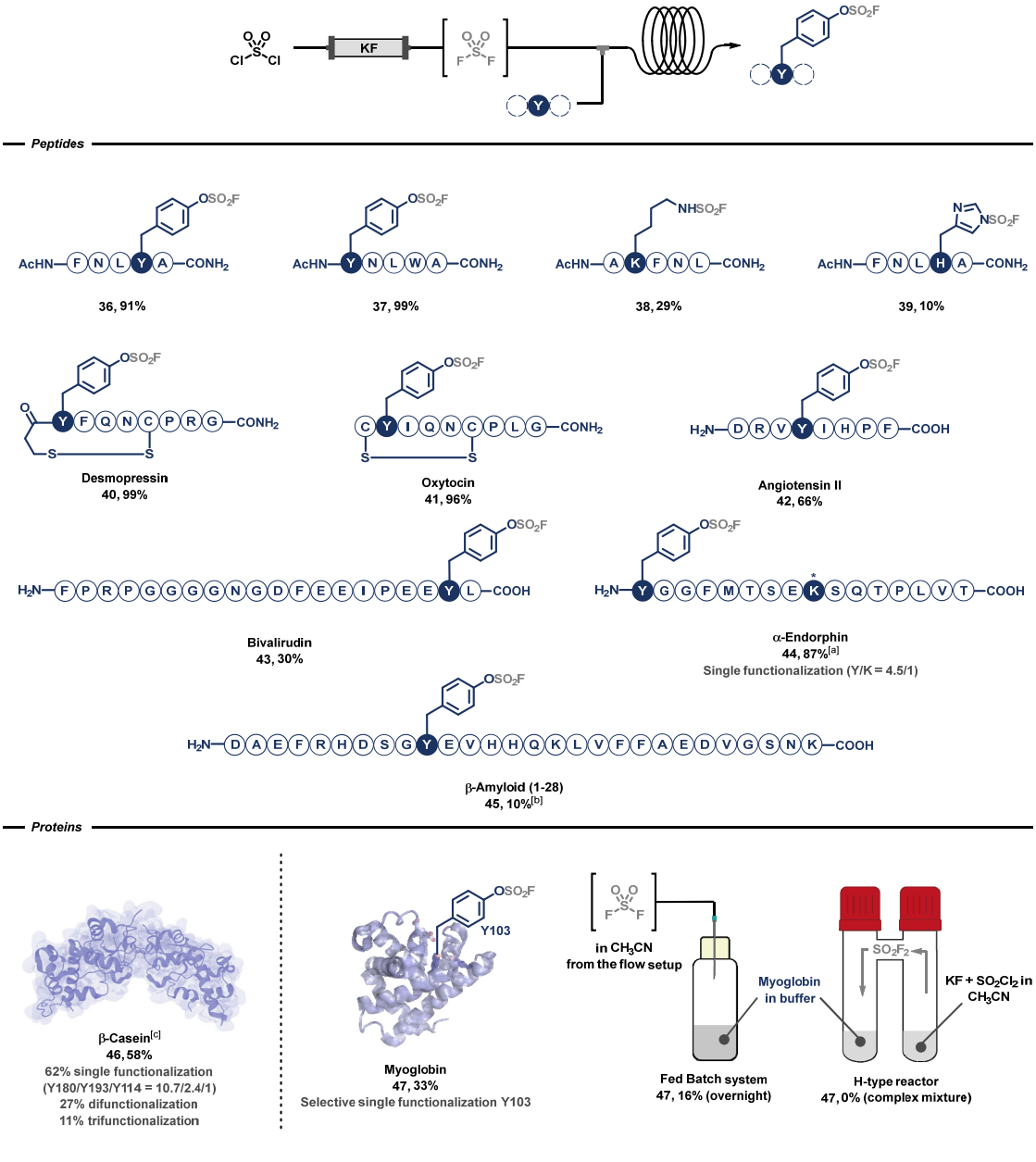

Figure 4. Application of the flow SuFEx ligation protocol to the direct modification of peptides and proteins. Conversions reported as ratios of areas under the peak of product and starting compound obtained by LC/MS analysis. Peptides: Standard conditions for the SO_2F_2 generation: SO_2Cl_2 (40 equiv, 0.2 M in CH_3CN) passed through a 3.8 mL cartridge filled with a 1:1 mixture of KF and glass beads at f.r. 0.5 mL/min. Peptide (1 equiv, 10 mM in CH_3CN:H_2O 1:1), Et_3N (6 equiv), f.r. 0.25 mL/min, res. time 2 min at room temperature. [a] α-Endorphin (6 mM), SO_2F_2 (67 equiv). [b] β-Amyloid (3 mM), SO_2F_2 (133 equiv). [c] A picture of a general protein was chosen to represent β-Casein as no crystal structure is reported. Proteins: Standard conditions for the SO_2F_2 generation: SO_2Cl_2 (0.1 M in CH_3CN) passed through a 3.8 mL cartridge filled with a 1:1 mixture of KF and glass beads at f.r. 0.1 mL/min. β-Casein (1 equiv, 5 mM in Tris buffer pH = 7.7), TMG (10 equiv), f.r. 0.9 mL/min, res. time 1.5 min at room temperature, SO_2F_2 (2.2 equiv). Myoglobin (1 equiv, 1 mM in 10 mM acetate buffer pH = 5), TMG (1 equiv), f.r. 0.9 mL/min, res. time 1.5 min at room temperature SO_2F_2 (11 equiv). TMG: 1,1,3,3-Tetramethylguanidine.

Conclusion. The practical flow protocol presented in this study enables the safe and efficient generation of the coveted gaseous SO_2F_2 reagent, as well as high reaction rates of the subsequent SuFEx ligation, with wide applicability to various substrates, including therapeutically relevant small molecules, peptides, and proteins. Based on these findings, we believe that this protocol opens up new opportunities in the field of SuFEx click chemistry. In particular, the use of this flow process makes sulfuryl fluoride a viable reagent for installing the -SO_2F handle on a variety of phenol and amino functionalities.

Acknowledgements. We acknowledge financial support from the European Union H2020 research and innovation program for an ERC CoG grant for T.N. (FlowHAT, No. 101044355) and a Marie S. Curie Grant fellowship for D. M. (ELECTRORGANO, No. 101022144). The Spanish Government-MCIN, the national agency of investigation-AEI/10.13039/501100011033, and the European Regional Development Fund-ERDF for project PID2020-120584RB-I00 to O.B., and FPU Fellowship (FPU19/01969 and EST22/00303) to M.B.

Competing interests. No competing interests need to be declared.

227

228 **References.**

[1] H. C. Kolb, M. G. Finn, K. B. Sharpless, Click chemistry: Diverse chemical function from a few good reactions. *Angew. Chem. Int. Ed*. **40**, 2004–2021 (2001).

[2] A. D. Moorhouse, J. A. Homer, J. E. Moses, The certainty of a few good reactions. *Chem*, DOI: 10.1016/j.chempr.2023.03.017 (2023).

[3] S. Kitamura, Q. Zheng, J. L. Woehl, A. Solania, E. Chen, N. Dillon, M. V. Hull, M. Kotaniguchi, J. R. Cappiello, S. Kitamura, V. Nizet, K. B. Sharpless, D. W. Wolan, Sulfur(VI) fluoride exchange (SuFEx)-enabled high-throughput medicinal chemistry. *J. Am. Chem. Soc.* **142**, 10899–10904 (2020).

[4] Z. Liu, J. Li, S. Li, G. Li, K. B. Sharpless, P. W. SuFEx Click Chemistry Enabled Late-Stage Drug Functionalization. *J. Am. Chem. Soc.* **140**, 2919–2925 (2018).

[5] Q. Zheng, J. L. Woehl, S. Kitamura, D. Santos-Martins, C. J. Smedley, G. Li, S. Forli, J. E. Moses, D. W. Wolan, K. B. Sharpless, Sufex-enabled, agnostic discovery of covalent inhibitors of human neutrophil elastase. *Proc. Natl. Acad. Sci. U.S.A.* **116**, 18808–18814 (2019).

[6] L. H. Jones, Emerging utility of fluorosulfate chemical probes. *ACS Med. Chem. Lett.* **9**, 584–586 (2018).

[7] H. M. McCann, B. P.M. Lake, K. S. Hoffman, M. E. Davola, K. L. Mossman, A. F. Rullo, Covalent immune proximity-induction strategy using SUFEX-engineered bifunctional viral peptides. ACS Chem. Biol. **17**, 1269–1281 (2022).

[8] K. E. Gilbert, A. Vuorinen, A. Aatkar, P. Pogány, J. Pettinger, E. K. Grant, J. M. Kirkpatrick, K. Rittinger, D. House, G. A. Burley, J. T. Bush, Profiling Sulfur(VI) Fluorides as Reactive Functionalities for Chemical Biology Tools and Expansion of the Ligandable Proteome. *ACS Chem. Biol.* **18**, 285-295 (2023).

[9] S. Li, G. Li, B. Gao, S. P. Pujari, X. Chen, H. Kim, F. Zhou, L. M. Klivansky, Y. Liu, H. Driss, D.-D. Liang, J. Lu, P. Wu, H. Zuilhof, J. Moses, K. B. Sharpless, SuFExable polymers with helical structures derived from thionyl tetrafluoride. *Nat. Chem.* **13**, 858–867 (2021).

[10] J. Dong, K. B. Sharpless, L. Kwisnek, J. S. Oakdale, V. V. Fokin, SuFEx-based synthesis of polysulfates. *Angew. Chem. Int. Ed.* **53**, 9466–9470 (2014).

[11] K. Durie, J. Yatvin, C. D. McNitt, R. A. Reese, C. Jung, V. V. Popik, J. Locklin, Multifunctional surface manipulation using orthogonal click chemistry. *Langmuir*. **32**, 6600–6605 (2016).

[12] J. D. Randall, D. J. Eyckens, F. Stojcevski, P. S. Francis, E. H. Doeven, A. J. Barlow, A. S. Barrow, C. L. Arnold, J. E. Moses and L. C. Henderson, Modification of carbon fibre surfaces by sulfur-fluoride exchange click chemistry. *ChemPhysChem*. **19**, 3176–3181 (2018).

[13] J. Dong, L. Krasnova, M. G. Finn, K. B. Sharpless, Sulfur(VI) fluoride exchange (SuFEx): Another good reaction for Click Chemistry. *Angew. Chem. Int. Ed.* **53**, 9430–9448 (2014).

[14] E. R. Falardeau, D. D. DesMarteau, Synthesis of pentafluorophenoxy derivatives of sulfur(IV) and -(VI) fluorides. *J. Chem. Eng. Data*. **21**, 386–387 (1976).

[15] E. Ciuffarin, L. Senatore, M. Isola, Nucleophilic substitution at four-co-ordinate sulphur. mobility of the leaving group. *J. Chem. Soc., Perkin Trans. 2*, 468–471 (1972).

[16] A. S. Barrow, C. J. Smedley, Q. Zheng, S. Li, J. Dong, J. E. Moses, The growing applications of Sufex Click Chemistry. *Chem. Soc. Rev.* **48**, 4731–4758 (2019).

[17] M. Gehringer, S. A. Laufer, Emerging and re-emerging warheads for targeted covalent inhibitors: Applications in Medicinal Chemistry and Chemical Biology. *J. Med. Chem.* **62**, 5673–5724 (2018).

[18] D. Zeng, W.-P. Deng, X. Jiang, Advances in the construction of diverse SuFEx Linkers. *Natl. Sci. Rev.*, nwad123 (2023).

[19] C. Veryser, J. Demaerel, Bieliūnas Vidmantas, P. Gilles, W. M. De Borggraeve, *Ex situ* generation of sulfuryl fluoride for the synthesis of Aryl fluorosulfates. *Org. Lett.* **19**, 5244–5247 (2017).

[20] H. Zhou, P. Mukherjee, R. Liu, E. Evrard, D. Wang, J. M. Humphrey, T. W. Butler, L. R. Hoth, J. B. Sperry, S. K. Sakata, C. J. Helal, C. W. am Ende, Introduction of a crystalline, shelf-stable reagent for the synthesis of sulfur(VI) fluorides. *Org. Lett.* **20**, 812–815 (2018).

[21] T. Guo, G. Meng, X. Zhan, Q. Yang, T. Ma, L. Xu, K. B. Sharpless, J. Dong, A new portal to SuFEx Click Chemistry: A stable fluorosulfuryl imidazolium salt emerging as an "F−SO2+" donor of unprecedented reactivity, selectivity, and Scope. *Angew. Chem. Int. Ed.* **57**, 2605–2610 (2018).

[22] T. Kiang, R. N. Zare, Stepwise bond dissociation energies for the removal of fluorine from thionyl fluoride and sulphuryl fluoride. *J. Chem. Soc., Chem. Commun.*, 1228 (1980).

[23] M. B. Plutschack, B. Pieber, K. Gilmore, P. H. Seeberger, The Hitchhiker's Guide to Flow Chemistry. *Chem. Rev.* **117**, 11796–11893 (2017).

[24] D. Dallinger, B. Gutmann, C. O. Kappe, The concept of chemical generators: On-site on-demand production of hazardous reagents in Continuous Flow. *Acc. Chem. Res.* **53**, 1330–1341 (2020).

[25] L. Capaldo, Z. Wen, T. Noël, A field guide to flow chemistry for Synthetic Organic Chemists. *Chemical Science*. **14**, 4230–4247 (2023).

[26] C. J. Mallia, I. R. Baxendale, The Use of Gases in Flow Synthesis. *Org. Process Res. Dev.* **20**, 327–360 (2016).

[27] A. Casnati, H. P. Gemoets, E. Motti, N. Della Ca', T. Noël, Homogeneous and gas–liquid catellani-type reaction enabled by continuous-flow chemistry. *Chem. Eur. J.* **24**, 14079–14083 (2018).

[28] N. J. Straathof, Y. Su, V. Hessel, T. Noël, Accelerated gas-liquid visible light photoredox catalysis with continuous-flow photochemical microreactors. *Nat. Protoc.* **11**, 10–21 (2015).

[29] Z. Dong, Z. Wen, F. Zhao, S. Kuhn, T. Noël, Scale-up of micro- and Milli-reactors: An overview of strategies, design principles and applications. *Chem. Eng. Sci. X*. **10**, 100097 (2021).

[30] P. Thirumurugan, D. Matosiuk, K. Jozwiak, Click chemistry for drug development and diverse chemical–biology applications. *Chem. Rev.* **113**, 4905–4979 (2013).

[31] N. Wang, B. Yang, C. Fu, H. Zhu, F. Zheng, T. Kobayashi, J. Liu, S. Li, C. Ma, P. G. Wang, Q. Wang, L. Wang, Genetically Encoding Fluorosulfate-L-tyrosine To React with Lysine, Histidine, and Tyrosine via SuFEx in Proteins in Vivo. *J. Am. Chem. Soc.* **140**, 4995–4999 (2018).

[32] O. Boutureira, G. J. Bernardes, Advances in Chemical Protein Modification. *Chem. Rev.* **115**, 2174–2195 (2015).