1 **Advances in sulfur–fluoride exchange for chemical biology**

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13

14 **Abstract**

15 Since their introduction as a new strategy for synthesizing diverse chemotypes, sulfur(VI)–

16 fluoride exchange (SuFEx) transformations have found applications ranging from polymer

17 chemistry and covalent probe development to bioconjugation tools and chemistries for the

18 synthesis of compound libraries. The collection of SuFEx reactions has expanded

19 significantly since their introduction as a concept, comprising functionalities with varying

20 reactivities towards different nucleophiles; thus, enabling the generation of a wide array of

21 sulfur-containing functional groups for the linkage of structural elements in diverse

22 chemotypes. In this review, we focus on the most recent developments in the use of SuFEx

23 chemistry as a means for the preparation of compound libraries for biological screening as

24 well as the introduction of SuFEx hubs into various biomolecules.

Main text

26 **Sulfur(VI)–fluoride exchange chemistry as a new addition to the chemist's tool box**

27 The number of different chemical reactions that are broadly applied to synthesize compound

28 libraries for biological testing is still limited with most such efforts involving highly reliable

29 reactions such as amide bond formation, transition-metal-catalyzed reactions, reductive

30 amination, nucleophilic substitution, and sulfonamide formation reactions [1-3]. Over the

31 past 20 years, only a limited number of novel reactions have become routinely implemented

32 for the synthesis of small molecule compound libraries, including photoredox cross-

33 couplings [4] and copper(I)-catalyzed azide–alkyne cycloaddition (CuAAC) [5,6]. Most of

34 these reactions lead to molecules that are rich in $C(sp^2)$-bonds with an overall flat structure

35 that lack three-dimensional ("skeletal") diversity, which has been argued to furnish

36 compound collections with a lower chance of targeting protein binding pockets than more

37 diverse and natural product like ones [7-10]. Sulfur(VI)–fluoride exchange (SuFEx)

38 chemistry, identified as a new "click" chemistry reaction by Sharpless and co-workers [11],

39 has the potential to become a powerful addition to the arsenal of reactions broadly used for

40 library synthesis. SuFEx chemistry covers a range of reactions where compounds containing

41 S–F bonds with varying reactivity can be functionalized by a broad range of nucleophiles,

42 potentially providing access to compound libraries with increased chemical diversity of

43 interest in medicinal chemistry. Moreover, SuFEx hubs have received interest in the

44 discovery of covalent probes, because their latent electrophilic nature enables fluoride

45 exchange reactions to occur with specificity within protein binding sites, while the

46 functionalities are hydrolytically stable under assay and physiological conditions [12].

47 Besides SuFEx-based chemistry, the closely related sulfur(VI)–triazole exchange (SuTEx)

48 reactions have emerged (along with sulfur(VI)–azole exchange [13]) as promising options

49 with applications for the discovery of covalent inhibitors and activity-based protein profiling

50 (ABPP) targeting tyrosine [14-18].

51 In this review, we focus on the latest trends and developments in SuFEx chemistry for the

52 synthesis of compound libraries for medicinal chemistry investigations as well as the use of

53 SuFEx hubs in covalent probe discovery and ABPP. Finally, we discuss the incorporation of

54 SuFEx hubs into biomolecules as well as evolving technologies related to SuFEx chemistry.

55

56 **Sulfur(VI)–fluoride exchange linkage chemistry**

57 Since the discovery of the azide−alkyne click reaction [5,6,19], chemists have been seeking

58 other reliable, operationally simple, and chemoselective methods for forming chemical

59 bonds. SuFEx chemistry has gained significant attention due to the unique properties of

60 SO_2F groups, which have varying reactivities that can be selectively activated to form a

61 plethora of S^{VI} linkages [20].

62 Aryl fluorosulfates are readily accessible from the corresponding sulfonyl chlorides through

63 treatment with F^- or FHF^- [21]. Notably, in the presence of many common functional groups,

64 SO_2F_2 reacts preferentially with phenols to give aryl fluorosulfates [11] that can further react

65 with alcohols [22,23] or amines [24] to produce sulfates and sulfamates, respectively (Figure

66 1A). Furthermore, Sharpless and co-workers showed that two consecutive SuFEx reactions

67 between secondary amines and SO_2F_2 can also yield unsymmetrical sulfamides [11].

68 Sulfonyl fluorides can be accessed from the corresponding sulfonyl chloride or by radical

69 fluorosulfonylation of unsaturated bonds under photocatalytic activation [25-29]. They

70 readily react with alcohols [22], amines [22], $TMSCF_3$ [24], and organometallic agents, such

71 as boronic acids [30] (Figure 1B).

72 Conversely, SOF_4 exhibits chemoselective reactivity toward amine or aniline nucleophiles to

73 form iminosulfur oxydifluorides [31]. Subsequent reaction of these species with primary

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amines yields unsymmetrical sulfamides, while reaction with secondary amines provides sulfuramidimidoyl fluorides [32] (Figure 1C).
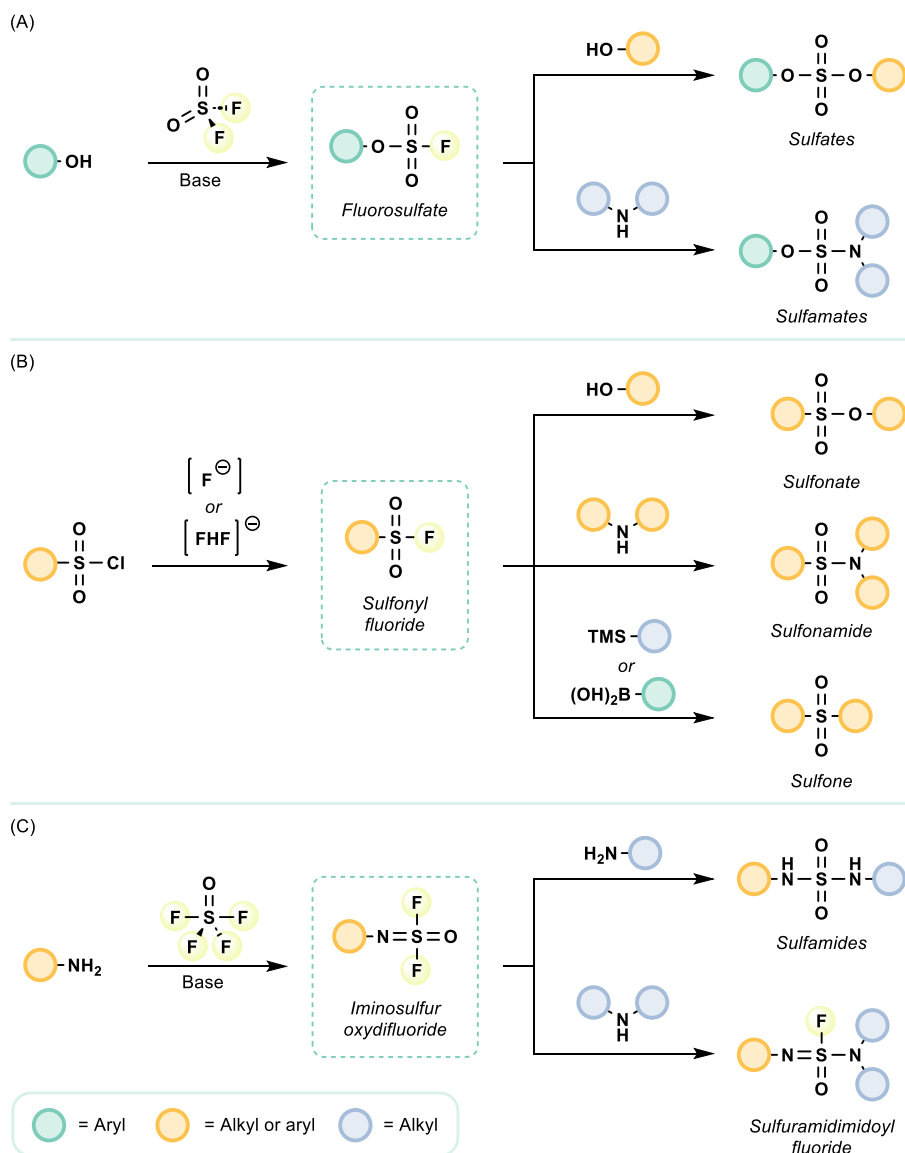
Figure 1. Examples of SuFEx hubs and resulting sulfur-containing linkages upon fluoride substitution. (A) Formation of sulfates and sulfamates. (B) Formation of sulfonates, sulfonamides, and sulfones. (C) Formation of sulfamides and sulfuramidimidoyl fluorides.

Covalent drug discovery and activity-based protein profiling

The introduction of SuFEx chemistry has helped widen the selection of amino acid residues, which can be covalently targeted, beyond cysteine; including, histidine [33], tyrosine [34,35], serine [36,37], and threonine [38]. Thus, the use of SuFEx hubs for the discovery of covalent

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inhibitors [36,39-41], small molecule probes for activity-based protein profiling (ABPP) studies [35,42-45], and inverse drug discovery efforts [42,46,47] have already been established as powerful applications of this chemistry. Recent advances have appeared in the literature [48-51], including the utilization of sulfonyl fluorides in DNA encoded libraries (DELs). Herein, a mass spectrometry-based workflow enabled the discovery of proteins which could potentially be targeted covalently with sulfonyl fluorides. Subsequently, selected target proteins were recombinantly expressed followed by selections with a sulfonyl fluoride based DEL. This effort allowed for the tyrosine based covalent inhibitors of multiple enzymes including PGAM1 and GSTP1 [48].

However, this area of applications of SuFEx chemistry will not be the major focus of the present account, because it has been excellent reviewed previously [12,20,52-57].

[18]F radiolabeling

The efficiency of SuFEx transformations have also found use in the preparation of radio-labeled tracers for positron emission tomography (PET) imaging, which is a powerful non-invasive technology for *in vivo* imaging. The PET technology relies primarily on the incorporation of fluorine-18 into bioactive molecules, as this radionuclide has sufficient half-life ($t_{1/2}$ = 110 min) and good imaging characteristics. Most PET tracers rely on the generation of a C–[18]F bond, utilizing a fluorination reagent as the final step of the synthesis, which imposes limitations in the variety of chemotypes that can be generated efficiently. Where [^{18}F]-sulfonyl fluorides have been prepared previously, they come with the limitation that they are unstable in a cellular environment (recently reviewed [58]), aryl fluorosulfates on the other hand have been shown to be inert under a wide array of reaction conditions and to be stable in cellular environments. These properties make them potential functionalities for PET-imaging. Seminal work by Wu and co-workers demonstrated that aryl fluorosulfates

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can undergo isotopic exchange to rapidly incorporate ^{18}F (< 5 minutes) with minimal purification to yield stable ^{18}F-labeled aryl fluorosulfates (Figure 2A) [59]. Similar isotopic exchange reaction has been reported for other S^{VI}–F hubs, but their properties have yet to be explored in vivo [58,60]. Furthermore advances includes the introduction of [^{18}F]-SuFEx into tetrazines, which could be valuable tools for pre-targeted imaging [61] and the development of a reagent, which can introduce [^{18}F]-SuFEx on phenols and amies as a final synthetic step (Figure 2B) [62].
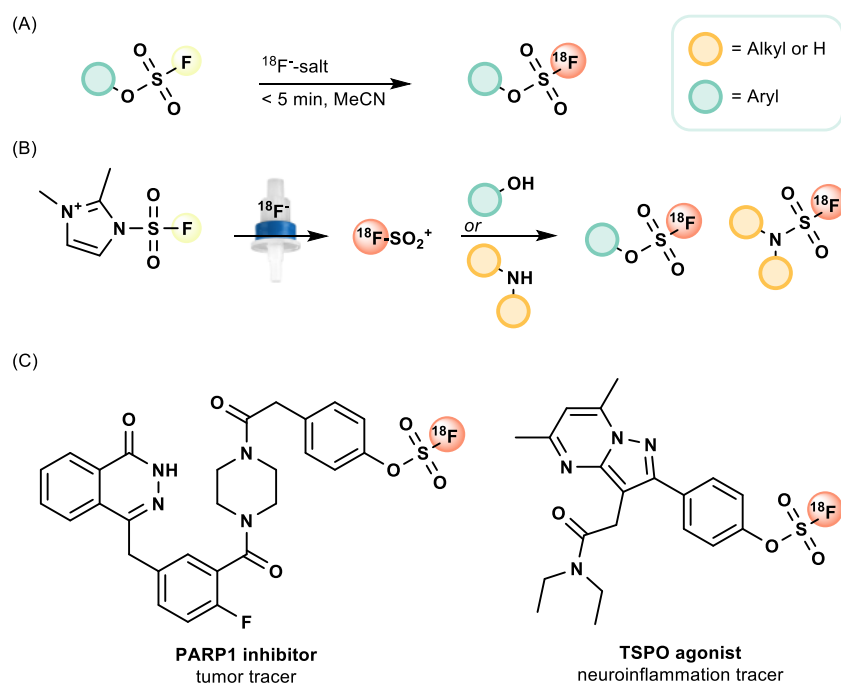
Figure 2. ^{18}F-labeling using SuFEx chemistry. (A) An isotopic exchange reaction. (B) An isotopic exchange reaction by SuFEx as the final synthetic step. (C) Structures of selected PET tracers utilizing [^{18}F]-aryl fluorosulfates.

Incorporation of a [^{18}F]-aryl fluorosulfate into the solvent exposed area of a poly ADP-ribose polymerase 1 (PARP1) inhibitor, provided a PET-tracer that was successfully used for tumor visualization in a mouse tumor (Figure 2C) [59]. Other studies have examined the limits of isotopic exchange within aryl fluorosulfates and found that electron deficient aryl groups

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127 were unstable under the applied reaction conditions and therefore required shorter reaction

128 times [63]. Nevertheless, a [^{18}F]-aryl fluorosulfate was introduced into a TSPO agonist, to

129 enable visualization of neuroinflammation in a rat stroke model (Figure 2C) [63]. The [^{18}F]-

130 aryl fluorosulfate should be carefully positioned in the molecule, as introduction of this in

131 electron deficient aryl leads to lowered stability of the PET-tracer [63,64].

132

Compound libraries generated using SuFEx chemistry

134 The easy and versatile functionalization of molecules using SuFEx chemistry has further

135 allowed for the synthesis of compound libraries for the discovery of biologically active

136 chemotypes [27-31]. In an early example, the focused diversification of a hit compound into

137 460 diverse analogues, furnished second-generation lead compounds with a 500-fold

138 increase in potency against the bacterial protease target [65]. In other studies, SuFEx

139 diversification allowed for the high-throughput discovery of PROTACs and molecular glues

140 [66,67].

141 Thus, hit scaffolds can be decorated with a SuFEx hub, which can then be diversified with

142 phenols or amines in multi-well microtiter plates to yield large libraries of compounds, which

143 upon simple evaporation of excess solvent and reagents, can undergo direct biochemical

144 screening. In a recent example of this approach, sulfonyl fluoride fragments were identified

145 that target various choline esterase enzymes [68]. By diversification using SuFEx chemistry,

146 the team synthesized >100 structurally diverse sulfonamides at picomolar scale in solvents

147 compatible with in vitro screening (Figure 3A). Optimization provided a protocol with close

148 to full conversion, and the plate-based in vitro screening gave results that were in agreement

149 with those obtained using purified inhibitors. These efforts enabled a 70-fold increase in

150 potency for selected compounds, which were further evaluated in Alzheimer's disease

151 models [69].

7

152   A similar strategy was applied to functionalize aniline scaffolds of F0045(S), an inhibitor of

153   the influenza antigen hemagglutinin, with SOF_4 to form iminosulfur oxydifluorides [70].

154   These were subsequently reacted with primary and secondary amines in 384-well plates

155   yielding ~690 compounds that were screened directly (Figure 3B).
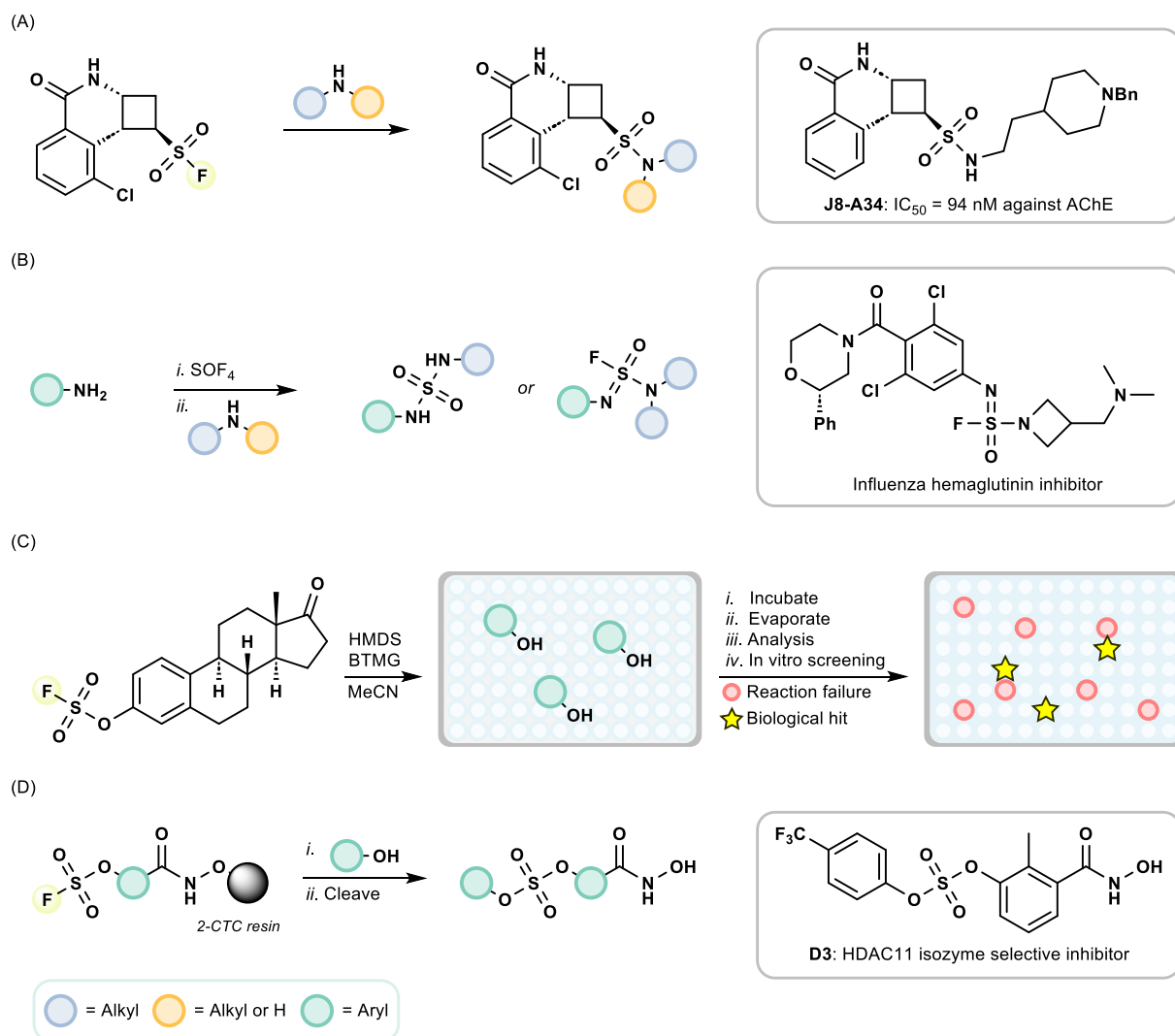
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157   **Figure 3.** SuFEx-chemistry-enabled libraries for discovery of biologically active small

158   molecules. (A) Library of sulfonamides by SuFEx chemistry. (B) Library of sulfuramidimidoyl

159   fluoride-containing compounds. (C) Multi-well microtiter plate-based SuFEx library synthesis

160   and screening. (D) Solid-phase SuFEx synthesis of histone deacetylase inhibitors.

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162   Further SAR studies furnished hit compounds with nanomolar EC_{50} values against a panel

163   of influenza viruses.

8

164 The easy accessibility of aryl fluorosulfates (Ar-OSO$_3$F) from phenols has also enabled the

165 development of numerous protocols for the synthesis of chemical libraries. Recently, Moses

166 and co-workers reported the rapid and efficient synthesis of libraries estrone analogues,

167 containing sulfate-based appendages, using SuFEx chemistry in multi-well plates (Figure

168 3C) [69]. The protocol involved the addition of phenols to individual wells of a 96-well plate,

169 followed by solvent removal and addition of a stock solution containing estrone fluorosulfate

170 **1**, BTMG, and HMDS. In this study, LC-MS analysis revealed products purities that were

171 deemed suitable for biological evaluation for 41% of the compound, while 21% exceeded

172 90% conversion.

173 In a recent study, arrays of histone deacetylase (HDAC) inhibitors were prepared by SuFEx

174 chemistry, combined with solid-phase synthesis [71]. Here, the aryl fluorosulfate SuFEx

175 hubs were synthesized in a two-chamber reactor; generating the SO$_2$F$_2$ in one chamber in

176 situ and consuming the gas in the other chamber by different phenol derivatives. The SuFEx

177 hubs were then coupled via a carboxylic acid to solid supported hydroxylamine, furnishing

178 aryl fluorosulfate-containing, resin-bound hydroxamic acids (Figure 3D). Subsequent

179 treatment of each resin with collections of phenols and cleavage from the resin, then

180 provided biaryl sulfates for biochemical testing. In this study, each compound was purified

181 by preparative HPLC to secure reliable assay results, and novel isozyme-selective inhibitors

182 of HDACs 6 and 11 were discovered.

183

184 **SuFEx on nucleosides, nucleic acids, and carbohydrates**

185 Nucleosides and nucleic acids can be functionalized and targeted with SuFEx chemistry.

186 Sharpless and co-workers included an example of a functionalized nucleoside in their

187 studies of functionalization of primary amino groups with SOF$_4$ gas, to give reactive

188 iminosulfur oxydifluoride derivatives for further diversification [32]. The group of Jemielity

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and co-workers expanded the number of nucleoside modifications by introducing sulfamoyl-fluoride functionalized nucleosides ("SuFNucs"), obtained by reaction of NH_2 groups of nucleosides with ex situ generated sulfonyl fluoride. The sulfamoyl fluoride moieties can then undergo SuFEx reaction with amines resulting in derivatives termed sulfamide nucleosides ("SulfamNucs") [72]. These findings provide new avenues for nucleoside-based bioconjugates and libraries of modified nucleosides. The application of SuFEx-modified DNA as a tool for bioconjugation is further supported by work from Sharpless and co-workers, demonstrating the suitability of SOF_4-based SuFEx conditions for the labeling of ssDNA [73], which may find use for the synthesis of DNA-encoded libraries (DEL). Further, SuFEx chemistry has been incorporated into oligonucleotide libraries by reversible linkage to a phosphorothioate compatible with DNA synthesis by polymerase chain reaction (PCR). Utilizing this technique, covalent oligonucleotide-based inhibitors of protein–protein interactions were discovered by in vitro selection [74]. Instead of adding the SuFEx handle to the nucleobase, sulfonyl fluorides were attached to the backbone of an aptamer, which furnished a covalent aptamers, targeting the spike protein of SARS-CoV2 [75].

Carbohydrates have been sparsely modified by SuFEx chemistry thus far; however, an elegant aryl fluorosulfate-based strategy for *O*-sulfation in carbohydrates has been developed [23].

SuFEx hubs in peptides

Incorporation of SuFEx hubs into peptides and proteins has also been applied for the investigation of protein–substrate and protein–protein interactions (PPIs) as well as the design of covalent inhibitors of PPIs. Incorporation of SuFEx handles into peptides has mostly relied on amino acid building blocks containing a sulfonyl fluoride or an aryl fluorosulfate group. As such, protected fluorosulfate-containing tyrosine building blocks

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have been incorporated during solid-phase peptide synthesis (SPPS) [23,76-78] or tyrosine residues have been functionalized post-SPPS [79]. Examples of the incorporation of SuFEx hubs into peptides post-SPPS also include functionalization of the ε-*N*-amino group of lysine residues [80-82] and functionalization of cysteine [81,83].

Introduction of SuFEx handles into medium-sized cyclic peptides was achieved by Bogyo and co-workers using SPPS. The aim of their study was to identify an electrophile that could be incorporated into libraries of alkyne-labeled macrocycles, compatible with chemoproteomic workflows (Figure 4A). When assessing selectivity and reactivity profiles of fluorosulfate and sulfonyl fluoride electrophiles, the fluorosulfate was chosen due to its lower reactivity towards unwanted nucleophiles and stability during SPPS. The generated compound library was screened for covalent targeting of proteins in HEK293 cell lysate, to demonstrate the potential of this strategy for discovery of covalent ligands [76]. Most recently, they have similarly introduced aryl fluorosulfates on bis-electrophiles which were used for the cyclization of peptides in a phage library [84]. In another study of covalent targeting of proteins, aryl fluorosulfate-containing dipeptides were decorated with different aryl fluorosulfate building blocks at a lysine residue to mimic lysine succinylation or glutarylation. These efforts provided small aryl fluorosulfate-based peptides that selectively targeted the HDAC enzyme, sirtuin 5 (SIRT5) (Figure 4B). Incorporation of an alkyne handle for CuAAC chemistry, furnished probes that enabled fluorescence labeling and pulldown of SIRT5 from both cell lysate and cultured cells, upon functionalization with biotin [80]. Fujimori and co-workers developed covalent inhibitors of the ε-*N*-trimethyllysine chromatin "reader" domain, plant homeodomain 3 (PHD3), by adding sulfonyl fluoride or aryl fluorosulfate functionalities into cyclic peptides that targeted this domain (Figure 4B). Further, the introduction of a biotin residue, enabled pulldown recombinant His_6-MBP-PHD3 spiked into HEK293T cell lysate, to demonstrate applicability of the probes in a more

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complex environment [81]. Pentelute and co-workers included SuFEx hubs in a strategy that they termed "electrophile scanning", which is a tool to determine hotspots for covalent reactivity in peptide ligands (Figure 4B). The reactivity hotspots were identified by proximity-driven crosslinking between the sulfonyl fluoride-containing peptides and the target protein, to develop potent covalent inhibitors of this PPI [83].
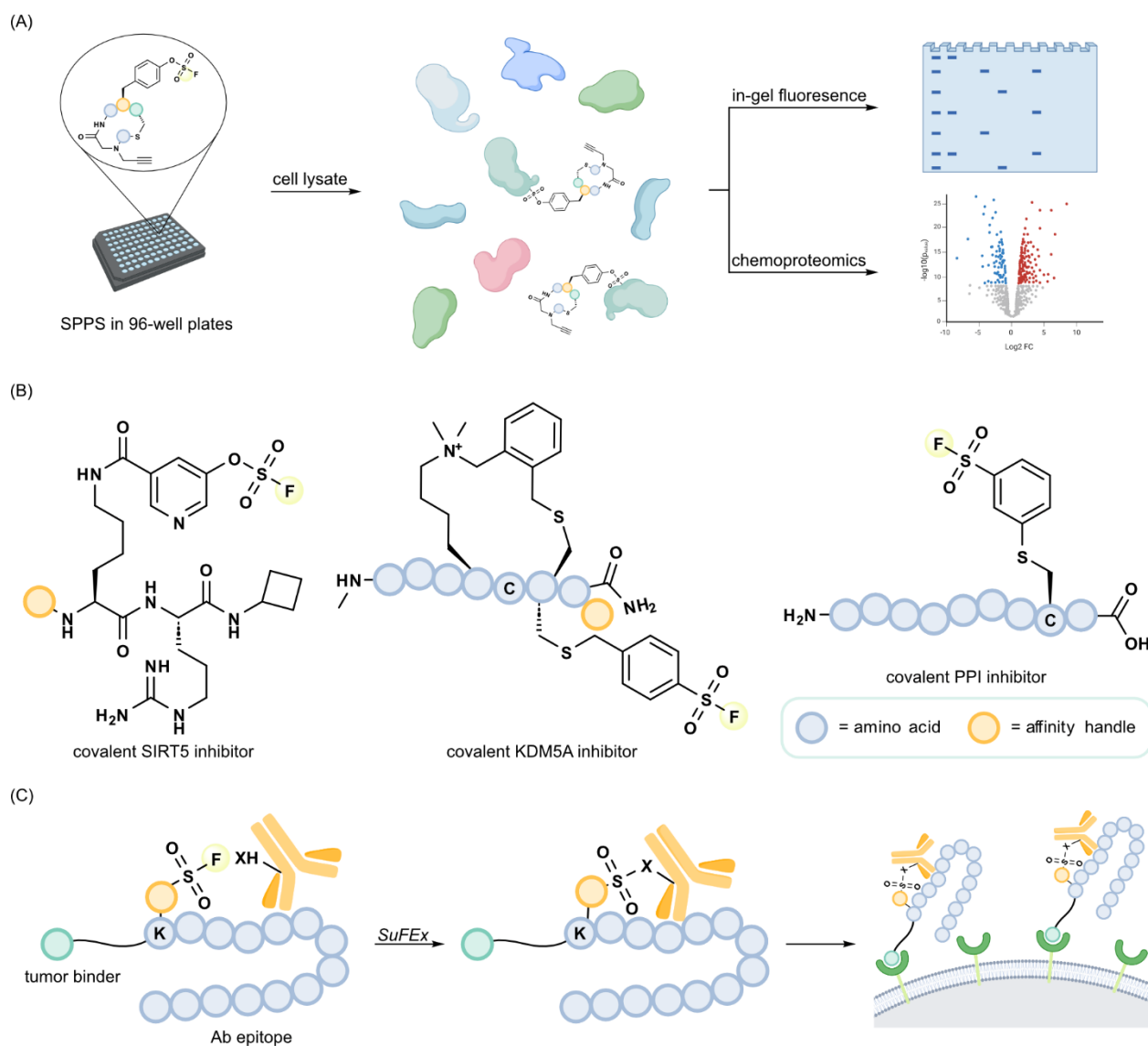
Figure 4. SuFEx hubs in peptides. (A) Medium-sized peptides in proteomic study, enabled by SuFEx reactivity. (B) Examples of peptides containing SuFEx hubs at cysteine or lysine. (C) Covalent immune-proximity induction.

250 SuFEx-engineered bifunctional peptides have been applied in a workflow termed "covalent

251 immune-proximity induction", which has potential applications in antitumoral

252 immunotherapy. Here, the designed peptides contain naturally occurring viral immunogenic

253 epitopes, functionalized with a SuFEx hub, combined with a tumor antigen-binding molecule.

254 The binding of the epitopes to the desired anti-viral antibodies in the human blood suffers

255 from poor binding affinity, resulting in dissociation of the antibody and thereby loss of activity,

256 which is overcome by covalent conjugation to the antibody in this new strategy (Figure 4C)

257 [79].

258 In another study taking advantage of proximity-induced chemical crosslinking, DeGrado and

259 co-workers used SuFEx chemistry to covalently stabilize the CsgG–CsgF complex, which is

260 part of the pore-forming membrane-bound bacterial curli system [85]. To improve the

261 investigation of curli complexes, one of the binding partners, the CsgF, was equipped with

262 sulfonyl fluoride functionality, to enable covalently binding to CsgG subunits by proximity-

263 enhanced crosslinking. The strategy provided stabilized membrane complexes with

264 significantly improved homogeneity [85].

265

266 **SuFEx hubs in proteins**

267 Early attempts at including SuFEx handles into proteins relied on the modification of fully

268 translated proteins, either by reaction with SO_2F_2 gas [86] or succinimide ester-activated

269 compounds that contained SuFEx hubs [87]. While both early approaches lack selectivity,

270 the site selective introduction of SuFEx hubs into proteins has since been achieved by

271 genetic code expansion methods [88]. Here, aryl fluorosulfates were introduced either as

272 tyrosine mimetics (called **FSY**; Figure 5A) [89-91] or functionalized lysine derivatives (called

273 **FSK**) [92] by amber suppression technology. Further, aryl sulfonyl fluorides have recently

274 been incorporated by genetic code expansion; though, the sulfonyl fluoride required

275   deactivation substitutions on the phenyl ring to stabilize the SuFEx hub during translation

276   [93,94].

277   In a prominent example, a sulfonyl fluoride-containing tyrosine derivative (called **SFY**; Figure

278   5A) was incorporated into sialic acid-binding immunoglobulin-like lectin 7 (siglec-7), which

279   is an inhibitory transmembrane receptor expressed on natural killer (NK) cells. Several lysine

280   residues were exchanged for **SFY** by non-canonical amino acid mutagenesis, using an

281   evolved pyrrolysyl-tRNA synthetase (PylRS) mutant with specificity for **SFY** [93]. These

282   efforts enabled the crosslinking of interacting carbohydrates to the siglec-7 protein in vitro

283   and on the surface of cultured cancer cells (Figure 5B).

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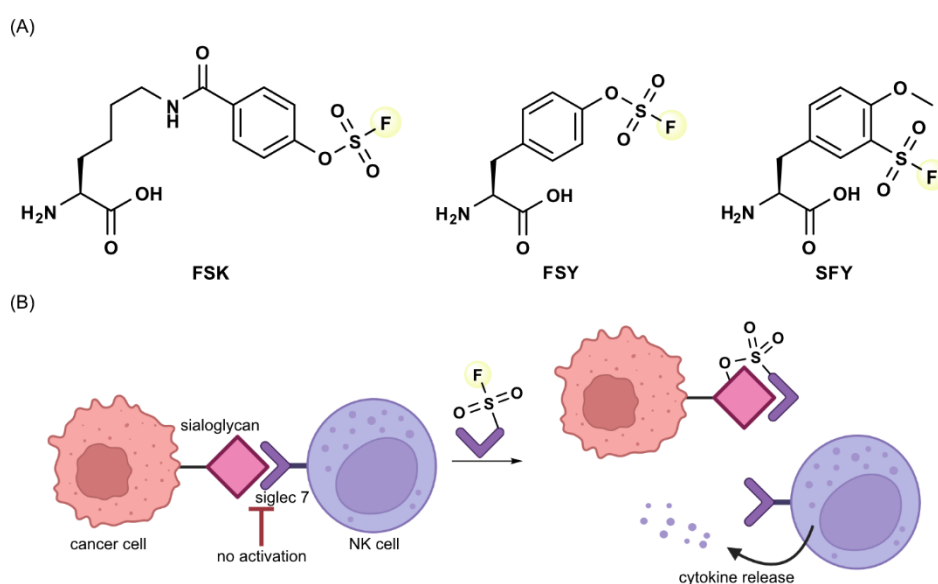
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286   **Figure 5**. SuFEx hubs in proteins. (A) Non-canonical amino acid residues amenable to

287   incorporation using genetic code expansion. (B) Covalent conjugation of SFY-containing

288   siglec-7 outcompetes sialoglycan–NK cell interaction to stimulate cytokine release from the

289   NK cell.

290

291   In a different approach to protein functionalization, SuFEx-hub-containing chemotypes were

292   applied to crosslink acyl carrier protein (AcpP) with its natural binding partner BioF, an

14

enzyme in early biotin biosynthesis in *Escherichia coli*, which enabled detailed investigation of the AcpP–BioF interface by X-ray crystallography. This work demonstrated selectivity of the sulfonyl fluorides for amino acid residues located in the protein binding pocket, which may enable expansion of utility of the developed probes for additional applications, such as pulldown assays to identify other AcpP partner enzymes [95].

Expanding the scope to phosphorous

P–F bonds have been explored in multiple biological settings as they are inert under physiological conditions [96-99]; however, they had not found interest as a means of click chemistry until recent reports by Moses and co-workers [100]. Thus, the P^V–F bond can undergo transformations like the ones described for SuFEx, which, in turn, has been termed phosphorus(V)–fluoride exchange (PFEx) chemistry. Utilizing Lewis base catalysis, multiple P–O and P–N linked products have been formed with amines and aryl and alkyl alcohols. Stepwise addition of nucleophiles to P–F hubs allow for the generation of complex three-dimensional structures (Figure 6A) [100,101]. Further, PFEx warheads into proteins have bene incorporated into proteins by genetic code expansion, allowing for covalent protein modification. By synthesis and incorporation of the Tyr analogue **PFY** (Figure 6B) into proteins they show that PFEx warheads are highly reactive towards His and Tyr and to a lesser extent Lys and Cys, albeit the reactivity can be altered with pH. This they utilized to covalently link the plasma protein Z with an affibody (Afb) to form a covalently linked complex (Figure 6C). Besides incorporation of **PFY** in *E. coli*, it was also shown to function in mammalian cells and ecGST was shown to be crosslinked to form a covalent dimer. By synthesis and incorporation of **PFK** (Figure 6B), they allowed for a more flexible system, which was capable of crosslinking with a higher degree of flexibility. This study showcases the capabilities in PFEx chemistry, which could be another tool for protein engineering.

15

318   Optimization of the area around the PFEx reaction further, by e.g. incorporation of Arg

319   residues, have been shown to increase the rate of cross-linking [91]. Although PFEx

320   chemistry is potentially appealing, it should be noted that the toxicity of PFEx hubs

321   (phosphoramidic difluorides and phosphoramidofluoridates) is considerable and should be

322   handled with care while low boiling intermediates should be avoided all together as they

323   closely resemble phosphonofluoridates such as sarin gas [101,102].

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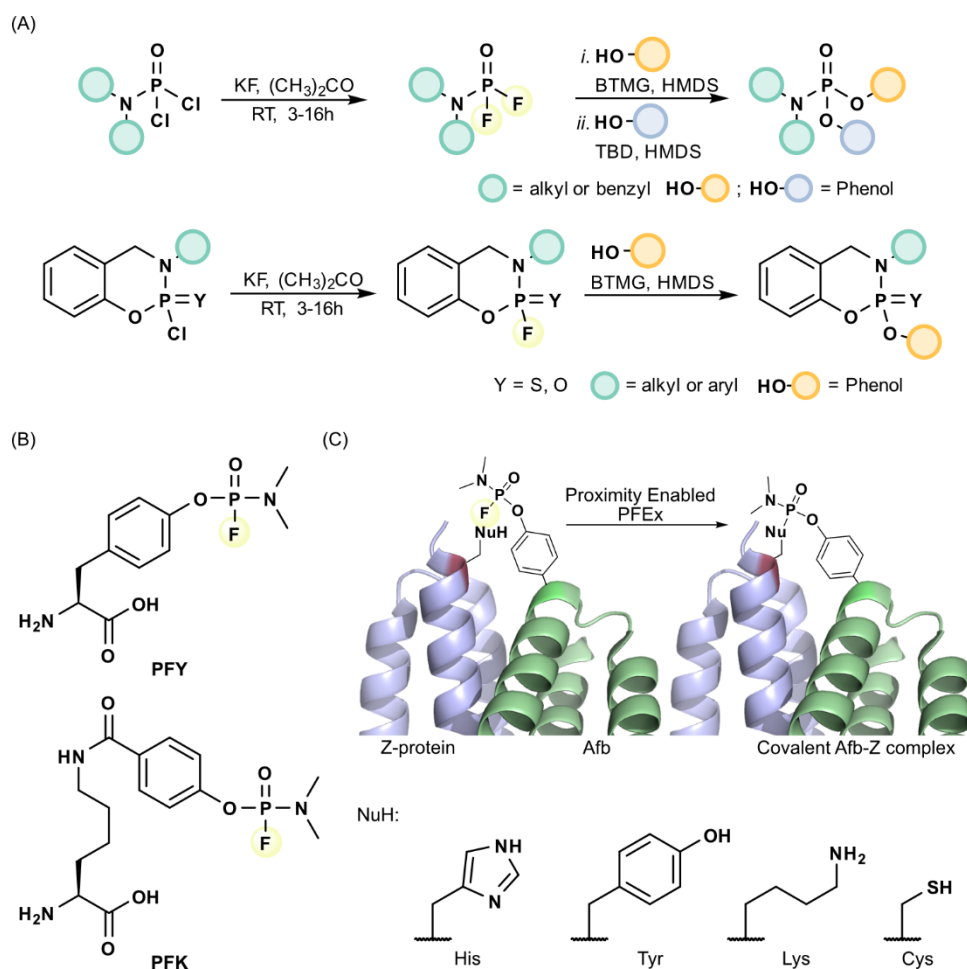
326   **Figure 7**. Current state-of-the-art in PFEx chemistry. (A) Synthesis of PFEx hubs and

327   sequential PFEx reactions to achieve diverse molecules. (B) Structure of **PFY** and **PFX**,

328   which can be genetically encoded into proteins. (C) Crosslinking of Z-protein and an affibody

329   (Afb) utilizing PFEx chemistry.

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Concluding remarks

In summary, SuFEx chemistry has been continuously developing in diverse directions since its introduction a decade ago. The repertoire of reactions that can be performed and, in turn, the diversity of functional groups that can be accessed have been substantially expanded, enabling efficient generation of structurally diverse compound libraries for the discovery of biologically active ligands. Further, we have focused substantially on applications of SuFEx hubs introduced into biomolecules, including peptides, proteins, and nucleic acids, which have developed extensively in recent years. Finally, the fluoride exchange chemistry concept has been expanded by the introduction of PFEx chemistry, the initial account of which we have also discussed in this review article.

With this review article, we wish to shed light on the creative recent applications of SuFEx chemistry beyond the discovery of covalent small molecule inhibitors, which is already a well-established and powerful application of this chemistry. It is our hope that this focus will help provide inspiration for new applications of SuFEx chemistry in chemical biology.

Highlights

The application of SuFEx chemistry has developed tremendously since its introduction a decade ago. The various chemical transformations, enabled by the availability of gasses and reagents to generate SuFEx hubs, have led to the development of reactions yielding a range of sulfur-containing functionalities that were previously more difficult to access. In turn, these developments have facilitated the application of SuFEx reactions in a wide variety of contexts, including covalent medicinal chemistry and ABPP, enabled by the latent electrophilic nature of SuFEx hubs, like the sulfonyl fluoride and the aryl fluorosulfates.

Several examples have appeared in the literature, where the SuFEx chemistries have found application in the generation of diverse compound libraries for the discovery of biologically

active ligands. Thus, SuFEx could become a new addition to the still today rather limited selection of reliable reaction types used in small molecule library synthesis.

Recently, the use of SuFEx chemistry has found applications in the functionalization of biomolecules, such as nucleic acids, peptides, and proteins. We envision that these types of molecules will serve as a space for diverse applications of SuFEx chemistry in the future.

Outstanding questions

Few examples of the use of SuFEx chemistry in nucleic acids research have been published so far. It will be interesting to follow whether new innovative applications may still be developed within this area.

Few studies have developed SuFEx-hub-containing ligands that target other biomacromolecules than proteins; however, the potential of covalent targeting of oligonucleotides or carbohydrates remains to be explored further.

The latent electrophilicity of SuFEx warheads have enabled the discovery of a broad variety of covalent research tool compounds. However, just a few ligands have been applied in *in vivo* studies and it therefore remains to be answered whether compounds containing SuFEx warheads may find applications *in vivo* or if they may even progress to clinical development?

SuFEx chemistry has emerged as an attractive collection of chemical transformations, which have been demonstrated to have potential in the generation of diverse compound libraries for biological screening. It remains an outstanding question whether SuFEx chemistry will become a prevalent choice in the future syntheses of large compound libraries.

387

388 **Declaration of interest**

389 The authors declare no conflict of interest.

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391

392 **Glossary**

393 **Activity-based protein profiling (ABPP):** a chemical proteomic technology that relies on

394 reactive chemotypes, targeting certain sub-sets of amino acid residues or protein binding

395 sites within the proteome.

396 **Copper(I)-catalyzed azide–alkyne cycloaddition (CuAAC):** known as the original "click"

397 reaction, in which the Huisgen azide–alkyne cycloaddition proceeds with high efficiency and

398 regioselectivity to give 1,4-disubstituted-1,2,3-trizazoles in the presence of catalytic amounts

399 of Cu(I).

400 **Sulfur(VI)–fluoride exchange (SuFEx):** chemistry that relies on the varying reactivity of

401 structurally diverse S^{VI}–F species towards different nucleophiles.

402 **Phosphorous(V)–fluoride exchange (PFEx):** this chemistry much like SuFEx relies on the

403 latent reactivity of P^{V}-F species towards primarily phenols.

404

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