Advances in sulfur fluoride exchange for chemical biology

Tobias N. Hansen,[a] Marcos San Segundo,[a,b] Annika M. Mergel,[a] and Christian A. Olsen [a,*]

[a]*Center for Biopharmaceuticals & Department of Drug Design and Pharmacology, Faculty of Health and Medical Sciences, University of Copenhagen, Jagtvej 160, DK-2100 Copenhagen, Denmark.*

[b]*Department of Plant and Environmental Sciences, Faculty of Science, University of Copenhagen, Thorvaldsensvej 40, DK-1871 Frederiksberg C, Denmark.*

*Correspondence: cao@sund.ku.dk (C. A. Olsen)

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Abstract

Since their introduction as a new strategy for synthesizing diverse chemotypes, sulfur fluoride exchange (SuFEx) transformations have found applications ranging from polymer chemistry and covalent probe development to bioconjugation tools and chemistries for the synthesis of compound libraries. The collection of SuFEx reactions has expanded significantly since their introduction as a concept, comprising functionalities with varying reactivities towards different nucleophiles; thus, enabling the generation of a wide array of sulfur-containing functional groups for the linkage of structural elements in diverse chemotypes. In this review, we focus on the most recent developments in the use of SuFEx chemistry as a means for the preparation of compound libraries for biological screening as well as the introduction of SuFEx hubs into various biomolecules.

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25 **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 Cu(I)-catalyzed azide–alkyne cycloaddition (CuAAC) [5,6]. Most of these
34 reactions lead to molecules that are rich in C(sp^2)-bonds with an overall flat structure that
35 lack three-dimensional ("skeletal") diversity, which has been argued to furnish compound
36 collections with a lower chance of targeting protein binding pockets than more diverse and
37 natural product like ones [7-10]. Sulfur–fluoride exchange (SuFEx) chemistry, identified as
38 a new "click" chemistry reaction by Sharpless and co-workers [11], has the potential to
39 become a powerful addition to the arsenal of reactions broadly used for library synthesis.
40 SuFEx chemistry covers a range of reactions where compounds containing S–F bonds with
41 varying reactivity can be functionalized by a broad range of nucleophiles, potentially
42 providing access to compound libraries with increased chemical diversity of interest in
43 medicinal chemistry. Moreover, SuFEx hubs have received interest in the discovery of
44 covalent probes, because their latent electrophilic nature enables fluoride exchange
45 reactions to occur with specificity within protein binding sites, while the functionalities are
46 hydrolytically stable under assay and physiological conditions [12]. Besides SuFEx-based
47 chemistry, the closely related sulfur–triazole exchange (SuTEx) reactions have emerged
48 (along with sulfur–azole exchange [13]) as promising options with applications for the

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49 discovery of covalent inhibitors and activity-based protein profiling (ABPP) targeting tyrosine
50 [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 fluoride exchange linkage chemistry

57 Since the discovery of the CuAAC reaction [5,6], which is a Cu(I)-catalyzed version of the
58 Huisgen 3+2 azide–alkyne cycloaddition [19] that provides regioselectivity and high
59 conversion of the triazole formation at room temperature, alternative click reactions have
60 been desired. SuFEx chemistry has gained significant attention due to the unique properties
61 of high oxidation state S–F-containing groups, which have varying reactivities that can be
62 selectively activated to form a plethora of sulfur-linkages [20,21].

63 Notably, in the presence of many common functional groups (including esters, alcohols,
64 acids, and azides), sulfuryl fluoride (SO_2F_2) reacts preferentially with phenols in the
65 presence of base to give aryl fluorosulfates ($-OSO_2F$) [11] that can further react with alcohols
66 [22,23] or amines [24] to produce sulfates and sulfamates, respectively (Figure 1A).
67 Furthermore, Sharpless and co-workers showed that two consecutive SuFEx reactions
68 between secondary amines and SO_2F_2 can also yield unsymmetrical sulfamides [11].

69 Sulfonyl fluorides ($-SO_2F$) have traditionally been accessed from the corresponding sulfonyl
70 chlorides by reaction with F- or FHF- (Figure 1B) [25] but in later examples, this moiety has
71 been introduced by radical fluorosulfonylation of unsaturated bonds under photocatalytic
72 activation, giving rise to new modalities [26-30]. Sulfonyl fluorides readily react with alcohols

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73 [22], amines [22], $TMSCF_3$ [24], and organometallic agents, such as boronic acids [31], to

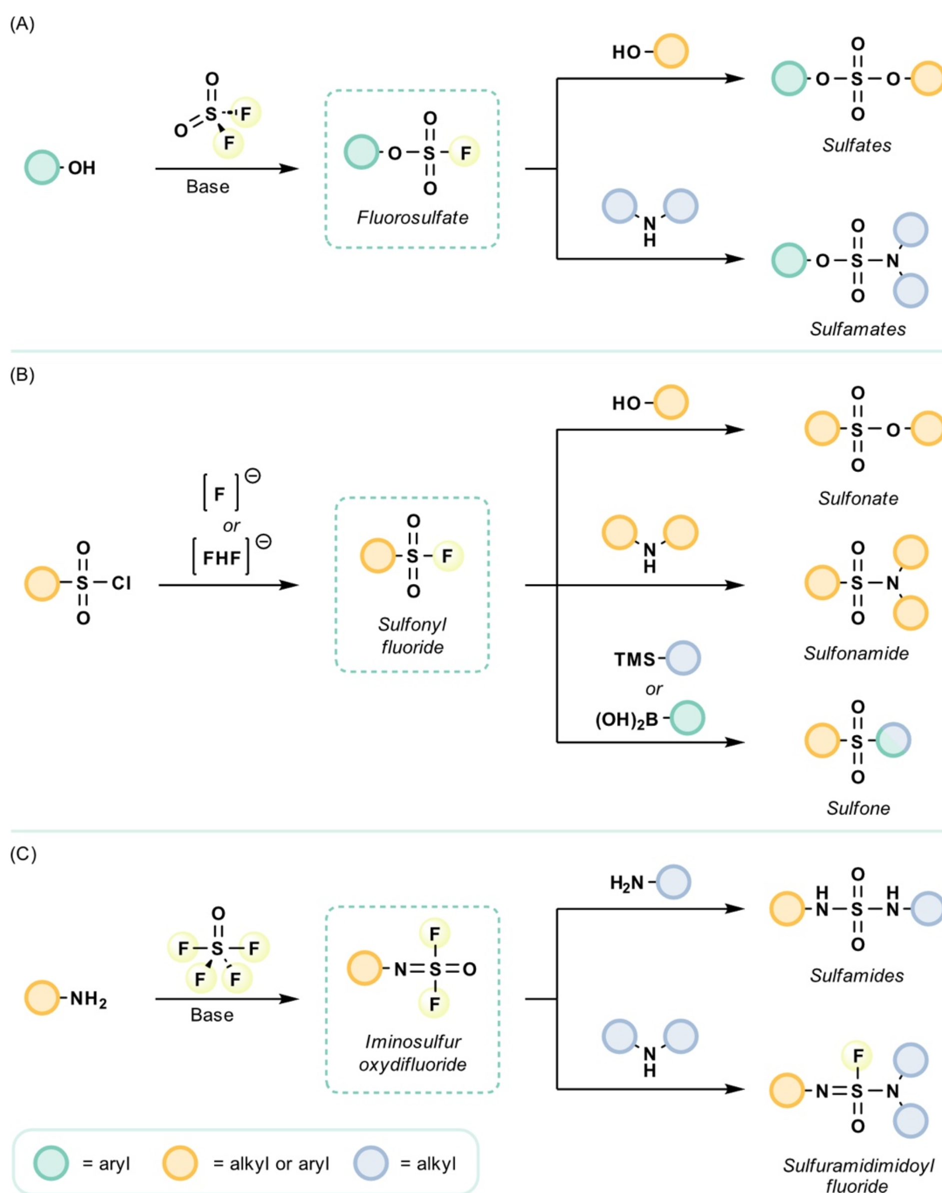
74 give sulfonates, sulfonamides, and sulfones, respectively (Figure 1B).

75 Conversely, thionyl tetrafluoride (SOF_4) exhibits chemoselective reactivity toward amine or

76 aniline nucleophiles to form iminosulfur oxydifluorides (-N=S(O)F_2) [32]. Subsequent

77 reaction of these species with primary amines yields unsymmetrical sulfamides, while

78 reaction with secondary amines provides sulfuramidimidoyl fluorides [33] (Figure 1C).



79

80 **Figure 1.** Examples of SuFEx hubs and resulting sulfur-containing linkages upon fluoride

81 substitution. (A) Formation of sulfates and sulfamates. (B) Formation of sulfonates,

82 sulfonamides, and sulfones. (C) Formation of sulfamides and sulfuramidimidoyl fluorides.

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83

84 **Covalent drug discovery and activity-based protein profiling**

85 The introduction of SuFEx chemistry has helped widen the selection of amino acid residues,
86 which can be covalently targeted, beyond cysteine; including, histidine [34], tyrosine [35,36],
87 serine [37,38], and threonine [39]. Thus, the use of SuFEx hubs for the discovery of covalent
88 inhibitors [37,40-42], small molecule probes for activity-based protein profiling (ABPP)
89 studies [36,43-46], and inverse drug discovery efforts [43,47,48] have already been
90 established as powerful applications of this chemistry. Recent advances have appeared in
91 the literature [49-52], including the utilization of sulfonyl fluorides in DNA encoded libraries
92 (DELs) [49]. Herein, a mass spectrometry-based workflow enabled the discovery of proteins
93 which could potentially be targeted covalently with sulfonyl fluorides. Subsequently, selected
94 target proteins were recombinantly expressed followed by selections with a sulfonyl fluoride-
95 based DEL. This effort allowed for the tyrosine based covalent inhibitors of multiple enzymes
96 including the phosphoglycerate mutase 1 (PGAM1) and glutathione *S*-transferase P
97 (GSTP1) [49].

98 However, this area of applications of SuFEx chemistry will not be the major focus of the
99 present account, because it has been excellent reviewed previously [12,20,53-58].

100

101 **^{18}F radiolabeling**

102 The efficiency of SuFEx transformations have also found use in the preparation of radio-
103 labeled tracers for positron emission tomography (PET) imaging, which is a powerful non-
104 invasive technology for *in vivo* imaging. The PET technology relies primarily on the
105 incorporation of fluorine-18 into bioactive molecules, as this radionuclide has sufficient half-
106 life ($t_{½}$ = 110 min) and good imaging characteristics. Most PET tracers rely on the generation
107 of a C–^{18}F bond, utilizing a fluorination reagent as the final step of the synthesis, which

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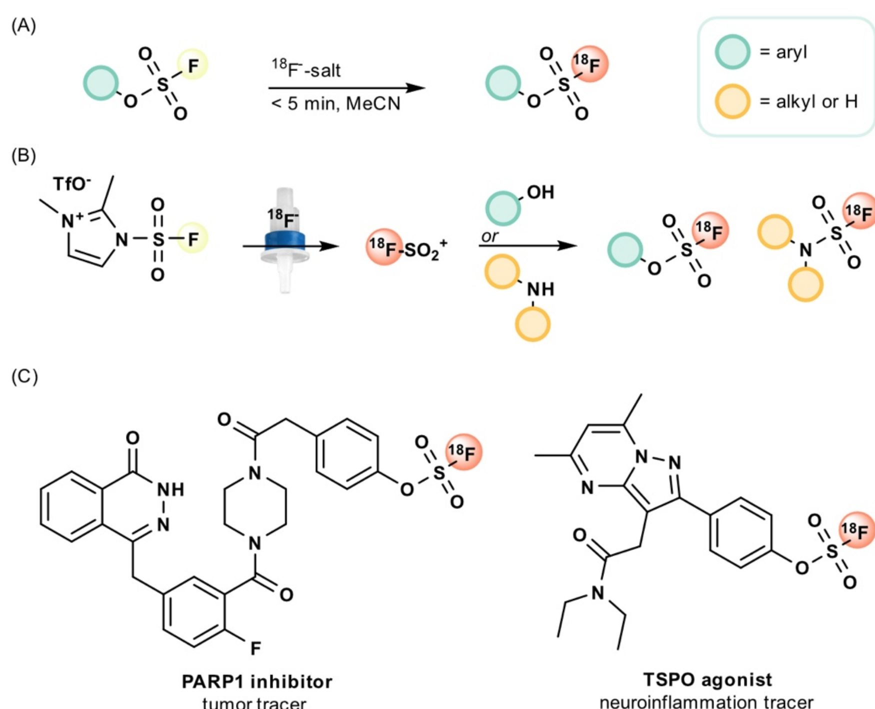
108 imposes limitations in the variety of chemotypes that can be generated efficiently. Where
109 [^{18}F]-sulfonyl fluorides have been prepared previously, they come with the limitation that
110 they are unstable in a cellular environment (recently reviewed [59]), aryl fluorosulfates on
111 the other hand have been shown to be inert under a wide array of reaction conditions and
112 to be stable in cellular environments. These properties make them potential functionalities
113 for PET-imaging. Seminal work by Wu and co-workers demonstrated that aryl fluorosulfates
114 can undergo isotopic exchange to rapidly incorporate ^{18}F (< 5 minutes) with minimal
115 purification to yield stable ^{18}F-labeled aryl fluorosulfates (Figure 2A) [60]. Similar isotopic
116 exchange reaction has been reported for other S^{VI}–F hubs, but their properties have yet to
117 be explored in vivo [59,61]. Furthermore advances includes the introduction of [^{18}F]-SuFEx
118 into tetrazines, which could be valuable tools for pre-targeted imaging [62] and the
119 development of a reagent, which can introduce [^{18}F]-SuFEx on phenols and amies as a final
120 synthetic step (Figure 2B) [63].
121



122

123 **Figure 2.** ^{18}F-labeling using SuFEx chemistry. (A) Isotopic exchange reaction as final
124 synthetic step [60]. (B) Introduction of -OSO$_2$[^{18}F] and -NSO$_2$[^{18}F] as final synthetic step from

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125 phenol or amine [63]. (C) Structures of selected PET tracers utilizing [^{18}F]-aryl fluorosulfates
126 [60,64].
127
128 Incorporation of a [^{18}F]-aryl fluorosulfate into the solvent exposed area of a poly ADP-ribose
129 polymerase 1 (PARP1) inhibitor, provided a PET-tracer that was successfully used for tumor
130 visualization in a mouse tumor (Figure 2C) [60]. Other studies have examined the limits of
131 isotopic exchange within aryl fluorosulfates and found that electron deficient aryl groups
132 were unstable under the applied reaction conditions and therefore required shorter reaction
133 times [64]. Nevertheless, a [^{18}F]-aryl fluorosulfate was introduced into a translocator protein
134 (TSPO) agonist, to enable visualization of neuroinflammation in a rat stroke model (Figure
135 2C) [64]. The [^{18}F]-aryl fluorosulfate should be carefully positioned in the molecule, as
136 introduction of this in electron deficient aryl leads to lowered stability of the PET-tracer
137 [64,65].
138
139 **Compound libraries generated using SuFEx chemistry**
140 The easy and versatile functionalization of molecules using SuFEx chemistry has further
141 allowed for the synthesis of compound libraries for the discovery of biologically active
142 chemotypes [27-31]. In an early example, the focused diversification of a hit compound into
143 460 diverse analogues, furnished second-generation lead compounds with a 500-fold
144 increase in potency against the bacterial cysteine protease SpeB [66]. In other studies,
145 SuFEx diversification allowed for the high-throughput discovery of PROTACs for the
146 selective degradation of the transcriptional coactivator ENL [67] and molecular glues
147 targeting the G-to-S phase transition 1 (GSPT1) protein [68]. Both studies produced new
148 modalities, which could be of interest for the development of novel leukaemia treatments.

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Thus, hit scaffolds can be decorated with a SuFEx hub, which can then be diversified with phenols or amines in multi-well microtiter plates to yield large libraries of compounds, which upon simple evaporation of excess solvent and reagents, can undergo direct biochemical screening. In a recent example of this approach, sulfonyl fluoride fragments were identified that target various choline esterase enzymes [69]. By diversification using SuFEx chemistry, the team synthesized >100 structurally diverse sulfonamides at picomolar scale in solvents compatible with in vitro screening (Figure 3A). Optimization provided a protocol with close to full conversion, and the plate-based in vitro screening gave results that were in agreement with those obtained using purified inhibitors. These efforts enabled a 70-fold increase in potency for selected compounds, which were further evaluated in Alzheimer's disease models [69].

A similar strategy was applied to functionalize aniline scaffolds of F0045(S), an inhibitor of the influenza antigen hemagglutinin, with SOF_4 to form iminosulfur oxydifluorides [70]. These were subsequently reacted with primary and secondary amines in 384-well plates yielding ~690 compounds that were screened directly (Figure 3B).

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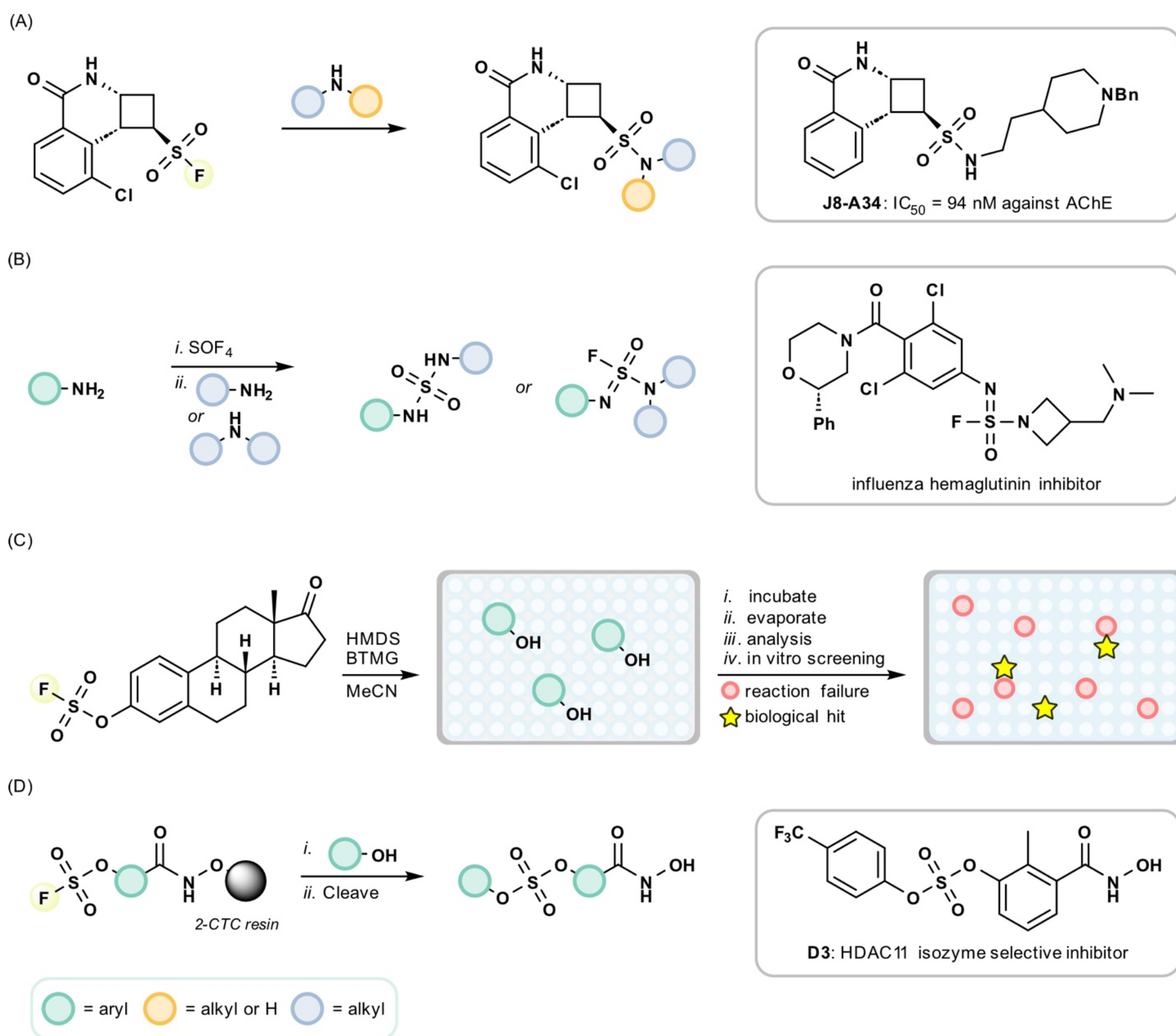

164

165 **Figure 3.** SuFEx -enabled libraries for discovery of biologically active small molecules. (A)
166 Library of sulfonamides by SuFEx chemistry [69]. (B) Library of sulfuramidimidoyl fluoride-
167 containing compounds [70]. (C) Multi-well microtiter plate-based SuFEx library synthesis
168 and screening [71]. (D) Solid-phase SuFEx synthesis of histone deacetylase inhibitors [72].

169

170 Further SAR studies furnished hit compounds with nanomolar EC_{50} values against a panel
171 of influenza viruses.

172 The easy accessibility of aryl fluorosulfates (Ar-OSO_2F) from phenols has also enabled the
173 development of numerous protocols for the synthesis of chemical libraries. Recently, Moses
174 and co-workers reported the rapid and efficient synthesis of libraries estrone analogues,

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175 containing sulfate-based appendages, using SuFEx chemistry in multi-well plates (Figure
176 3C) [71]. The protocol involved the addition of phenols to individual wells of a 96-well plate,
177 followed by solvent removal and addition of a stock solution containing estrone fluorosulfate
178 **1**, BTMG, and HMDS. In this study, LC-MS analysis revealed products purities that were
179 deemed suitable for biological evaluation for 41% of the compound, while 21% exceeded
180 90% conversion.

181 In a recent study, arrays of histone deacetylase (HDAC) inhibitors were prepared by SuFEx
182 chemistry, combined with solid-phase synthesis [72]. Here, the aryl fluorosulfate SuFEx
183 hubs were synthesized in a two-chamber reactor; generating the SO_2F_2 in one chamber in
184 situ and consuming the gas in the other chamber by different phenol derivatives. The SuFEx
185 hubs were then coupled via a carboxylic acid to solid supported hydroxylamine, furnishing
186 aryl fluorosulfate-containing, resin-bound hydroxamic acids (Figure 3D). Subsequent
187 treatment of each resin with collections of phenols and cleavage from the resin, then
188 provided biaryl sulfates for biochemical testing. In this study, each compound was purified
189 by preparative HPLC to secure reliable assay results, and novel isozyme-selective inhibitors
190 of HDACs 6 and 11 were discovered.

191

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

193 Nucleosides and nucleic acids can be functionalized and targeted with SuFEx chemistry.
194 Sharpless and co-workers included an example of a functionalized nucleoside in their
195 studies of functionalization of primary amino groups with SOF_4 gas, to give reactive
196 iminosulfur oxydifluoride derivatives for further diversification [33]. The group of Jemielity
197 and co-workers expanded the number of nucleoside modifications by introducing sulfamoyl-
198 fluoride functionalized nucleosides ("SuFNucs"), obtained by reaction of NH_2 groups of
199 nucleosides with ex situ generated sulfonyl fluoride. The sulfamoyl fluoride moieties can

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200 then undergo SuFEx reaction with amines resulting in derivatives termed sulfamide
201 nucleosides (“SulfamNucs”) [73]. These findings provide new avenues for nucleoside-based
202 bioconjugates and libraries of modified nucleosides. The application of SuFEx-modified
203 DNA as a tool for bioconjugation is further supported by work from Sharpless and co-
204 workers, demonstrating the suitability of SOF_4-based SuFEx conditions for the labeling of
205 single-stranded DNA (ssDNA) [74], which may find use for the synthesis of DELs. Further,
206 SuFEx chemistry has been incorporated into oligonucleotide libraries by reversible linkage
207 to a phosphorothioate compatible with DNA synthesis by polymerase chain reaction (PCR).
208 Utilizing this technique, covalent oligonucleotide-based inhibitors of protein–protein
209 interactions were discovered by in vitro selection [75]. Instead of adding the SuFEx handle
210 to the nucleobase, sulfonyl fluorides were attached to the backbone of an aptamer, which
211 furnished a covalent aptamers, targeting the spike protein of SARS-CoV2 [76].
212 Carbohydrates have been sparsely modified by SuFEx chemistry thus far; however, an
213 elegant aryl fluorosulfate-based strategy for *O*-sulfation in carbohydrates has been
214 developed [23].
215
216 **SuFEx hubs in peptides**
217 Incorporation of SuFEx hubs into peptides and proteins has also been applied for the
218 investigation of protein–substrate and protein–protein interactions (PPIs) as well as the
219 design of covalent inhibitors of PPIs. Incorporation of SuFEx handles into peptides has
220 mostly relied on amino acid building blocks containing a sulfonyl fluoride or an aryl
221 fluorosulfate group. As such, protected fluorosulfate-containing tyrosine building blocks
222 have been incorporated during solid-phase peptide synthesis (SPPS) [23,77-79] or tyrosine
223 residues have been functionalized post-SPPS [80]. Examples of the incorporation of SuFEx

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224 hubs into peptides post-SPPS also include functionalization of the ε-*N*-amino group of lysine
225 residues [81-83] and functionalization of cysteine [82,84].
226 Introduction of SuFEx handles into medium-sized cyclic peptides was achieved by Bogyo
227 and co-workers using SPPS. The aim of their study was to identify an electrophile that could
228 be incorporated into libraries of alkyne-labeled macrocycles, compatible with
229 chemoproteomic workflows (Figure 4A). When assessing selectivity and reactivity profiles
230 of fluorosulfate and sulfonyl fluoride electrophiles, the fluorosulfate was chosen due to its
231 lower reactivity towards unwanted nucleophiles and stability during SPPS. The generated
232 compound library was screened for covalent targeting of proteins in HEK293 cell lysate, to
233 demonstrate the potential of this strategy for discovery of covalent ligands [77]. Most
234 recently, they have similarly introduced aryl fluorosulfates on bis-electrophiles which were
235 used for the cyclization of peptides in a phage library [85]. In another study of covalent
236 targeting of proteins, aryl fluorosulfate-containing dipeptides were decorated with different
237 aryl fluorosulfate building blocks at a lysine residue to mimic lysine succinylation or
238 glutarylation. These efforts provided small aryl fluorosulfate-based peptides that selectively
239 targeted the HDAC enzyme, sirtuin 5 (SIRT5) (Figure 4B). Incorporation of an alkyne handle
240 for CuAAC chemistry, furnished probes that enabled fluorescence labeling and pulldown of
241 SIRT5 from both cell lysate and cultured cells, upon functionalization with biotin [81].
242 Fujimori and co-workers developed covalent inhibitors of the ε-*N*-trimethyllysine chromatin
243 "reader" domain, plant homeodomain 3 (PHD3), by adding sulfonyl fluoride or aryl
244 fluorosulfate functionalities into cyclic peptides that targeted this domain (Figure 4B).
245 Further, the introduction of a biotin residue, enabled pulldown of recombinant His_6-MBP-
246 PHD3 spiked into HEK293T cell lysate, to demonstrate the applicability of the probes in a
247 more complex environment [82]. Pentelute and co-workers included SuFEx hubs in a
248 strategy that they termed "electrophile scanning", which is a tool to determine hotspots for

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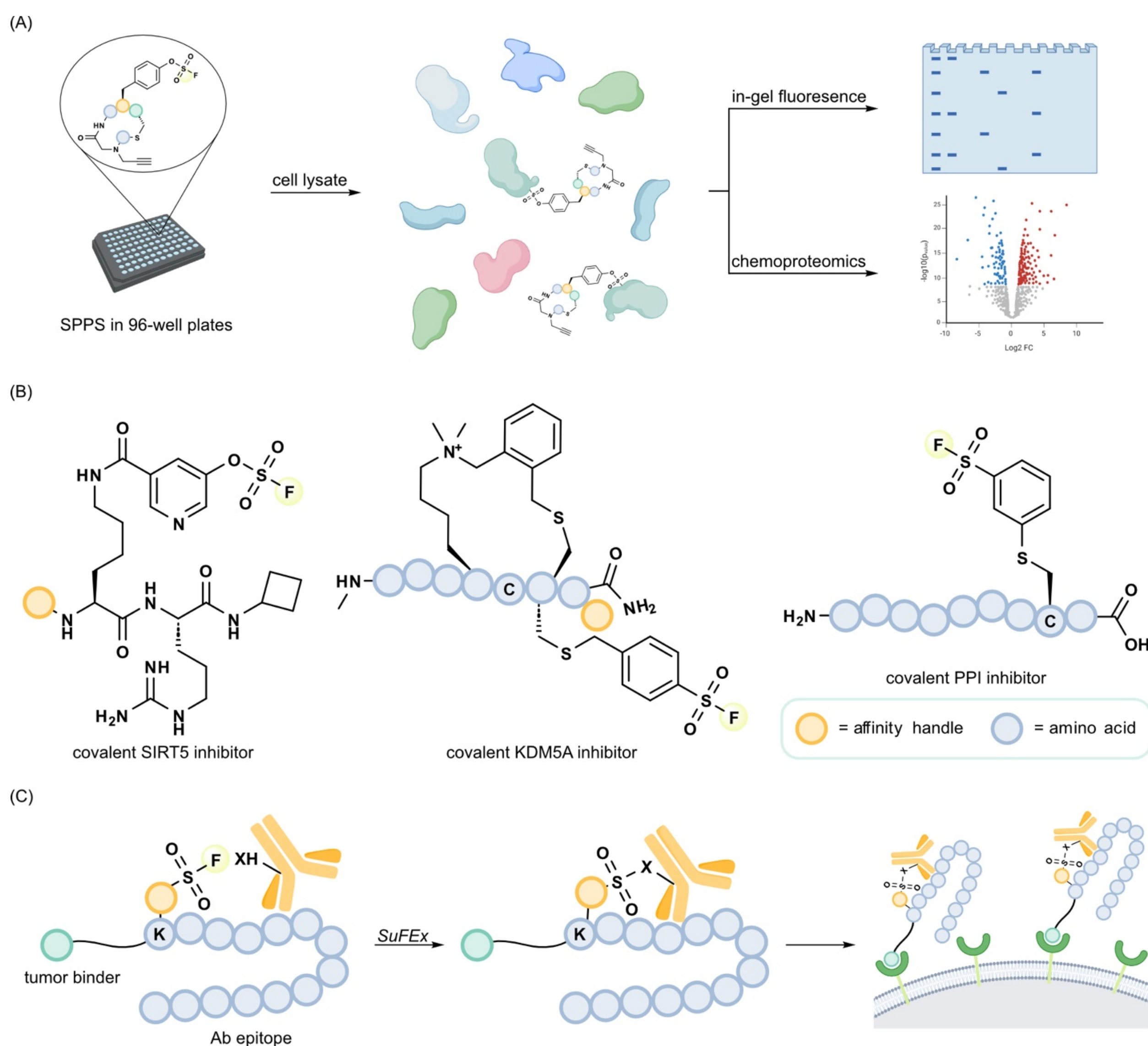
249 covalent reactivity in peptide ligands (Figure 4B). The reactivity hotspots were identified by
250 proximity-driven crosslinking between the sulfonyl fluoride-containing peptides and the
251 target protein, to develop potent covalent inhibitors disrupting the PPI of HLA-E and CD94-
252 NKG2A, which is associated with resistance to immune activation [84].
253



254
255 **Figure 4.** SuFEx hubs in peptides. (A) Medium-sized peptides in proteomic study, enabled
256 by SuFEx reactivity [77]. (B) Examples of peptides containing SuFEx hubs at cysteine or
257 lysine [81,82,84]. (C) Covalent immune-proximity induction [80].
258

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259 SuFEx-engineered bifunctional peptides have been applied in a workflow termed “covalent
260 immune-proximity induction”, which has potential applications in antitumoral
261 immunotherapy. Here, the designed peptides contain naturally occurring viral immunogenic
262 epitopes, functionalized with a SuFEx hub, combined with a tumor antigen-binding molecule.
263 The binding of the epitopes to the desired anti-viral antibodies in the human blood suffers
264 from poor binding affinity, resulting in dissociation of the antibody and thereby loss of activity,
265 which is overcome by covalent conjugation to the antibody in this new strategy (Figure 4C)
266 [80].
267 In another study taking advantage of proximity-induced chemical crosslinking, DeGrado and
268 co-workers used SuFEx chemistry to covalently stabilize the CsgG–CsgF complex, which is
269 part of the pore-forming membrane-bound bacterial curli system [86]. To improve the
270 investigation of curli complexes, one of the binding partners, the CsgF, was equipped with
271 sulfonyl fluoride functionality, to enable covalently binding to CsgG subunits by proximity-
272 enhanced crosslinking. The strategy provided stabilized membrane complexes with
273 significantly improved homogeneity [86].
274

275 **SuFEx hubs in proteins**

276 Early attempts at including SuFEx handles into proteins relied on the modification of fully
277 translated proteins, either by reaction with SO_2F_2 gas [87] or succinimide ester-activated
278 compounds that contained SuFEx hubs [88]. While both early approaches lack selectivity,
279 the site selective introduction of SuFEx hubs into proteins has since been achieved by
280 genetic code expansion methods [89]. Here, aryl fluorosulfates were introduced either as
281 tyrosine mimetics (called **FSY**; Figure 5A) [90-92] or functionalized lysine derivatives (called
282 **FSK**) [93] by amber suppression technology. Further, aryl sulfonyl fluorides have recently
283 been incorporated by genetic code expansion; though, the sulfonyl fluoride required

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

285 [94,95].

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

287 5A) was incorporated into sialic acid-binding immunoglobulin-like lectin 7 (siglec-7), which

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

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

290 evolved pyrrolysyl-tRNA synthetase (PylRS) mutant with specificity for **SFY** [94]. These

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

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

293

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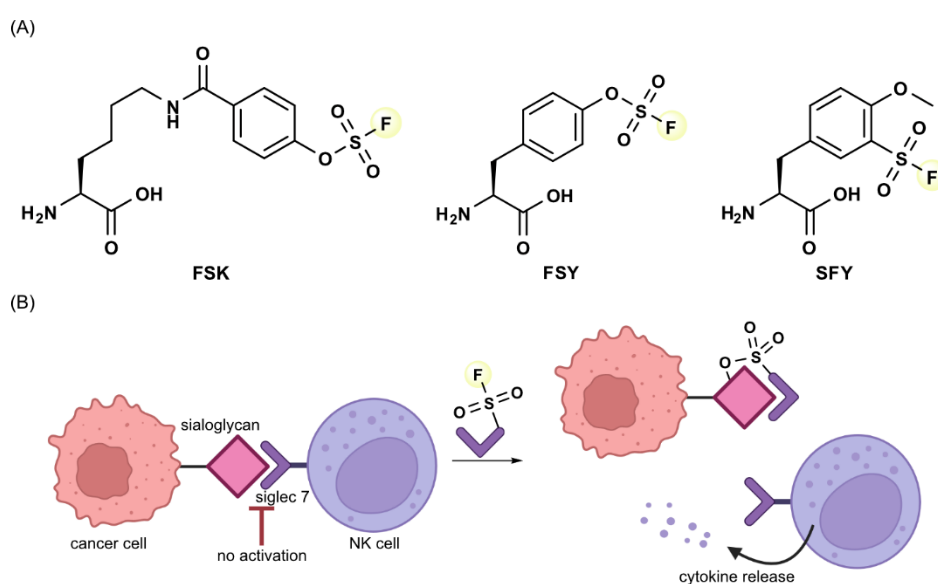

295 **Figure 5**. SuFEx hubs in proteins. (A) Non-canonical amino acid residues amenable to

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

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

298 NK cell [94].

299

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

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

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302 enzyme in early biotin biosynthesis in *Escherichia coli*, which enabled detailed investigation
303 of the AcpP–BioF interface by X-ray crystallography. This work demonstrated selectivity of
304 the sulfonyl fluorides for amino acid residues located in the protein binding pocket, which
305 may enable expansion of utility of the developed probes for additional applications, such as
306 pulldown assays to identify other AcpP partner enzymes [96].
307
308 **Expanding the scope to phosphorous**
309 P–F bonds have been explored in multiple biological settings as they are inert under
310 physiological conditions [97-100]; however, they had not found interest as a means of click
311 chemistry until recent reports by Moses and co-workers [101]. Thus, the P^{V}–F bond can
312 undergo transformations like the ones described for SuFEx, which, in turn, has been termed
313 phosphorus(V) fluoride exchange (PFEx) chemistry. Utilizing Lewis base catalysis, multiple
314 P–O and P–N linked products have been formed with amines and aryl and alkyl alcohols.
315 Stepwise addition of nucleophiles to P–F hubs allow for the generation of complex three-
316 dimensional structures (Figure 6A) [101,102]. Further, PFEx warheads into proteins have
317 bene incorporated into proteins by genetic code expansion, allowing for covalent protein
318 modification. By synthesis and incorporation of the Tyr analogue **PFY** (Figure 6B) into
319 proteins they show that PFEx warheads are highly reactive towards His and Tyr and to a
320 lesser extent Lys and Cys, albeit the reactivity can be altered with pH [103]. This they utilized
321 to covalently link the plasma protein Z with an affibody (Afb) to form a covalently linked
322 complex (Figure 6C). Besides incorporation of **PFY** in *E. coli*, it was also shown to function
323 in mammalian cells and ecGST was shown to be crosslinked to form a covalent dimer. By
324 synthesis and incorporation of **PFK** (Figure 6B), they allowed for a more flexible system,
325 which was capable of crosslinking with a higher degree of flexibility. This study showcases
326 the capabilities in PFEx chemistry, which could be another tool for protein engineering [103].

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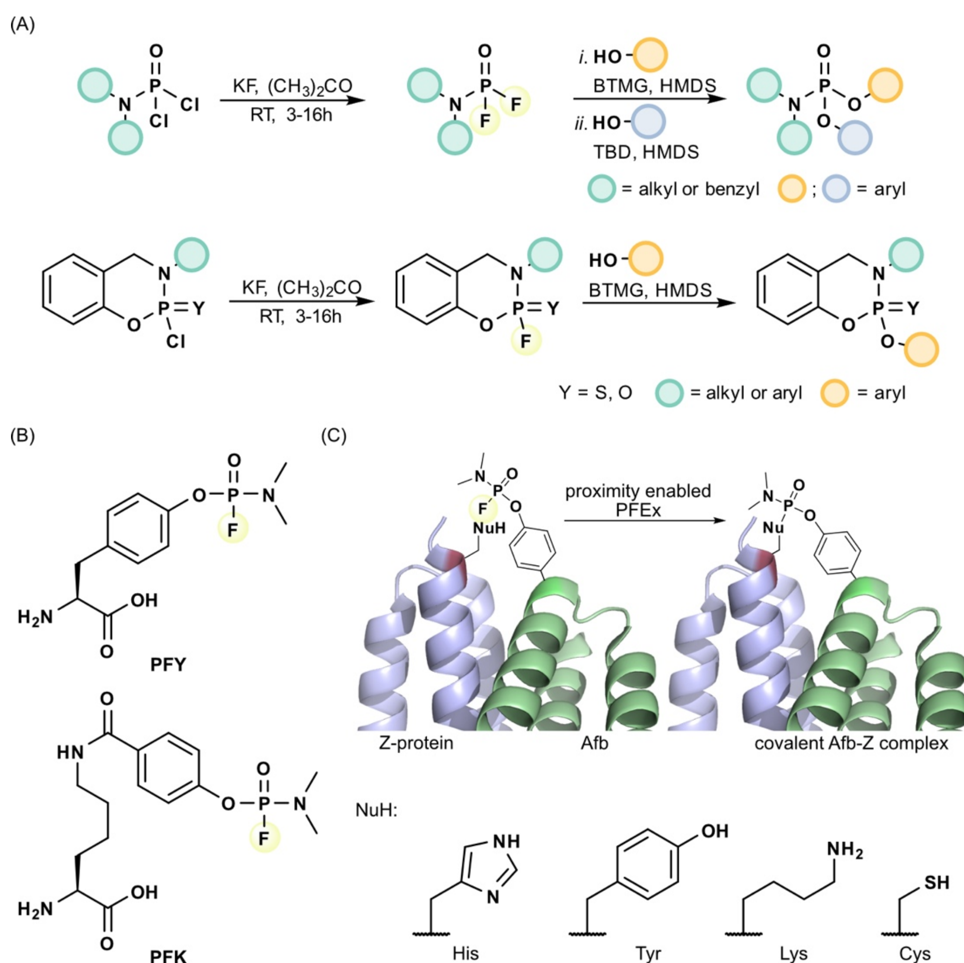
327 Optimization of the area around the PFEx reaction further, by e.g. incorporation of Arg
328 residues, has been shown to increase the rate of cross-linking [92].
329 While the potential toxicity of PFEx and SuFEx hubs, such as phosphoramidic difluorides
330 and phosphoramidofluoridates [102,104], warrants careful consideration, it is essential to
331 contextualize these concerns within the broader scope of chemical safety. Similarly, safety
332 perceptions in click chemistry reagents, like azides, have evolved over time [105]. Another
333 example of handling the toxicity of reagents, is the production of equimolar gaseous
334 reagents in closed two-chamber systems as for SO_2F_2 [106].
335

336 

337 **Figure 6**. Current state-of-the-art in PFEx chemistry. (A) Synthesis of PFEx hubs and
338 sequential PFEx reactions to achieve diverse molecules [101]. (B) Structure of **PFY** and

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339 **PFX**, which can be genetically encoded into proteins [103]. (C) Crosslinking of Z-protein and
340 an affibody (Afb) utilizing PFEx chemistry [103].
341

342 **Concluding remarks**

343 In summary, SuFEx chemistry has been continuously developing in diverse directions since
344 its introduction a decade ago. The various chemical transformations, enabled by the
345 availability of gasses and reagents to generate SuFEx hubs, have led to the development
346 of reactions yielding a range of sulfur-containing functionalities that were previously more
347 difficult to access. In turn, these developments have facilitated the application of SuFEx
348 reactions in a wide variety of contexts, including covalent medicinal chemistry and ABPP,
349 enabled by the latent electrophilic nature of SuFEx hubs, like the sulfonyl fluorides and the
350 aryl fluorosulfates. Further, the repertoire of reactions that can be performed and the
351 diversity of functional groups that can be accessed have been substantially expanded. Thus,
352 SuFEx chemistry has emerged as an attractive collection of chemical transformations, which
353 have been demonstrated to have potential in the generation of diverse compound libraries
354 for biological screening. However, it remains to be seen whether SuFEx chemistry will
355 become a prevalent choice in the future syntheses of large compound libraries. Further, we
356 have focused substantially on applications of SuFEx hubs introduced into biomolecules,
357 including peptides, proteins, and nucleic acids, which have developed extensively in recent
358 years. The latent electrophilicity of SuFEx warheads have enabled the discovery of a broad
359 variety of covalent research tool compounds. However, just a few ligands have been applied
360 in *in vivo* studies and it therefore remains to be answered whether compounds containing
361 SuFEx warheads may find applications *in vivo* or if they may even progress to clinical
362 development.

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363 Finally, the fluoride exchange chemistry concept has been expanded by the introduction of
364 PFEx chemistry, the initial account of which we have also discussed in this review article.
365 With this review article, we wish to shed light on the creative recent applications of SuFEx
366 chemistry beyond the discovery of covalent small molecule inhibitors, which is already a
367 well-established and powerful application of this chemistry. It is our hope that this focus will
368 help provide inspiration for new applications of SuFEx chemistry in chemical biology.
369

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376

377 **Declaration of interest**

378 The authors declare no conflict of interest.
379

380 **Glossary**

381 **Activity-based protein profiling (ABPP):** a chemical proteomic technology that relies on
382 reactive chemotypes, targeting certain sub-sets of amino acid residues or protein binding
383 sites within the proteome.
384 **Copper(I)-catalyzed azide–alkyne cycloaddition (CuAAC):** known as the original "click"
385 reaction, in which the Huisgen azide–alkyne cycloaddition proceeds with high efficiency and
386 regioselectivity to give 1,4-disubstituted-1,2,3-trizazoles in the presence of catalytic amounts
387 of Cu(I).

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388 **Positron emission tomography (PET):** technique used for in vivo imaging, utilizing
389 radioactive isotopes incorporated into molecules of interest. One example is
390 [^{18}F]fluorodeoxyglucose, which is used for the imaging of tumors.
391 **Solid-Phase Peptide Synthesis (SPPS):** synthetic technique developed for the rapid
392 synthesis of peptides on an insoluble polymer. The peptide is attached at the C-terminus
393 and subsequent rounds of addition of protected and activated amino acid building blocks to
394 the N-terminus, followed by protecting group removal to liberate the N-terminus of the last
395 added residue, enabling another round of peptide extension. Finally, the peptides are
396 obtained by cleavage from resin and global removal of side chain protecting groups.
397 **Sulfur fluoride exchange (SuFEx):** chemistry that relies on the varying reactivity of
398 structurally diverse S–F species towards different nucleophiles.
399 **Phosphorous(V) fluoride exchange (PFEx):** this chemistry much like SuFEx relies on the
400 latent reactivity of P^V-F species towards primarily phenols.
401

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