JACS
JOURNAL OF THE AMERICAN CHEMICAL SOCIETY
pubs.acs.org/JACS
Communication

Fluorosulfate as a Latent Sulfate in Peptides and Proteins

Chao Liu, Xueyi Liu, Mi Zhou, Chaoshuang Xia, Yuhan Lyu, Qianni Peng, Chintan Soni, Zefeng Zhou, Qiwen Su, Yujia Wu, Eranthie Weerapana, Jianmin Gao, Abhishek Chatterjee, Cheng Lin, and Jia Niu*

Cite This: J. Am. Chem. Soc. 2023, 145, 20189–20195
Read Online

ABSTRACT: Sulfation widely exists in the eukaryotic proteome. However, understanding the biological functions of sulfation in peptides and proteins has been hampered by the lack of methods to control its spatial or temporal distribution in the proteome. Herein, we report that fluorosulfate can serve as a latent precursor of sulfate in peptides and proteins, which can be efficiently converted to sulfate by hydroxamic acid reagents under physiologically relevant conditions. Photocaging the hydroxamic acid reagents further allowed for the light-controlled activation of functional sulfopeptides. This work provides a valuable tool for probing the functional roles of sulfation in peptides and proteins.

O-Sulfation of the tyrosine residue is a post-translational modification (PTM) that widely exists in eukaryotic peptides and proteins (Figure 1a), and has been implicated to regulate a variety of biological functions such as immune response, hemostasis, and pathogen evasion.[1,2] However, only a small fraction of the sulfoproteome has been annotated.[3,4] A long-standing challenge for studying the sulfoproteome is that sulfation is highly heterogeneous, with various sulfopeptides and sulfoproteins existing in different sulfoforms.[5] The seminal works of Schultz,[6] Liu,[7] Chatterjee,[8] Niu,[9] and Xiao[10] that incorporate sulfotyrosine (sY) into proteins as a noncanonical amino acid (ncAA) represent notable examples to address this challenge. Expanding upon these advances, methods that allow researchers to spatiotemporally control sulfation in the proteomic context would be highly valuable for studying their functional roles in biology.[11] Caging strategies have been developed for various protein PTMs to probe how these PTMs regulate dynamic cellular events. Although a broad collection of caging groups are available for a variety of PTMs, a caging group that stably protects sulfotyrosine (sY) residues in peptides and proteins and can be efficiently removed under physiological conditions remains elusive.[12] The reasons for such a knowledge gap includes the high energy barrier for chemically activating the sulfate group for coupling chemistries, the lability of sY to acid, heat, and high-energy ionization, and the strong electron-withdrawing propensity of sulfate that renders commonly used benzylic ester caging groups unstable.[13−15] On the other hand, while multiple alkyl and aryl esters have been successfully used as protecting groups of sY in solid-phase peptide synthesis,[17,18] such as 2,2,2-trichloroethyl (TCE),[18,19] 2,2-dichlorovinyl (DCV),[20,21] 2,2,2-trifluoroethyl (TFE),[22] neopentyl,[23,24] and phenyl[25] sulfate diesters, their deprotection conditions (e.g., hydrogenolysis,[18−21,25] strong base,[22] heating,[23] high salt concentraton,[24] etc.) are incompatible with living systems.

a) Examples of human sulfopeptides & sulfoproteins

Protein	Sulfation Sites
α-2-Antiplasmin	PPMEEDsYPQFGSP
CCR5	H_2N-MDsYQVSSPIsYDINsYsYTSEPCQ
PSGL-1	H_2N-QATEsYEsYLDsYDFLPET
Complement C4	MEANEDsYEDsYEsYDELPAK
HCII	DDsYLDLEKIFSEDDDsYID

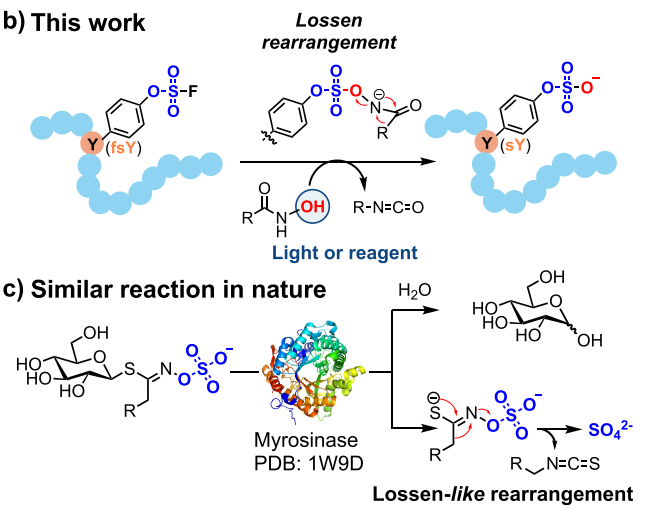

Figure 1. Background and our approach. (a) Sulfation widely exists in diverse bioactive peptides and proteins. (b) In this work, fluorosulfate is incorporated in peptides and proteins as a latent sulfate and can be efficiently converted to sulfate by hydroxamic acid reagents under physiologically relevant conditions. (c) Our approach mirrors the myrosinase-catalyzed Lossen-*like* rearrangement of glucosinolates in nature.

Received: July 24, 2023
Published: August 30, 2023

https://doi.org/10.1021/jacs.3c07937
J. Am. Chem. Soc. 2023, 145, 20189−20195

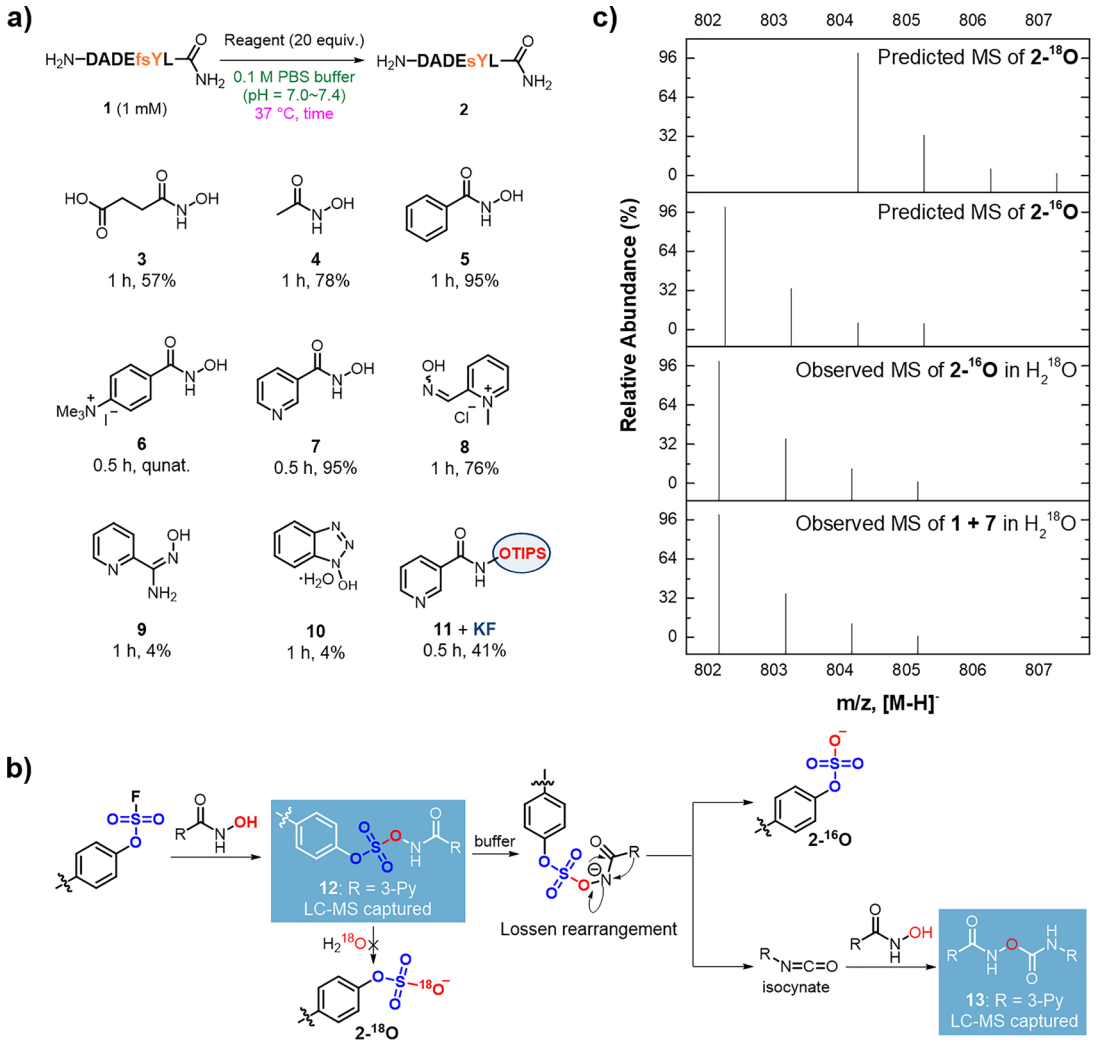

Figure 2. Reagent screen and mechanistic investigation. (a) A variety of HA reagents were investigated for their ability to activate fluorosulfate in model peptide **1**. Yields were determined by HPLC. (b) Real-time LC-MS reaction monitoring identified two adducts of **7**, **12**, and **13**, suggesting a Lossen rearrangement mechanism. (c) No ^{18}O-labeled products were found from the reaction in $H_2{}^{18}O$ buffer, suggesting that the sulfate product was not generated from direct hydrolysis.

In 2014, Sharpless et al. reported the reactivity of fluorosulfate in Sulfur(VI) Fluoride exchange (SuFEx) reaction.[26−29] Compared to other halogen-substituted sulfate derivatives, fluorosulfate not only has a size closest to that of sulfate, but is also far less electrophilic due to the π-donation from fluorine to sulfur.[30] As a result, fluorosulfate has demonstrated excellent metabolic stability *in vivo*.[31,32] The chemical inertness of fluorosulfate has allowed its tyrosine derivative, L-fluorosulfotyrosine (fsY), to be incorporated into peptides and proteins via solid-phase peptide synthesis[12,33] and ncAA mutagenesis.[13,32] Herein, we demonstrate that fluorosulfate can serve as a latent sulfate in sulfopeptides and sulfoproteins and can be efficiently converted to sulfate (hereafter denoted as "decaging") by hydroxamic acid (HA) reagents under physiologically relevant conditions. Mechanistic studies revealed an unusual Lossen rearrangement pathway of fluorosulfate activation and decaging (Figure 1b) that is analogous to the myrosinase-mediated Lossen-*like* rearrangement of glucosinolate in nature (Figure 1c).[34]

Our initial investigation confirmed that fluorosulfate is stable in various aqueous physiologically relevant conditions, such as buffer solution, cell lysate, and serum at neutral pH. Specifically, negligible (<5%) hydrolysis of fluorosulfate could be detected in aqueous buffer at neutral pH after 24 h (Table S1, entries 1−2). Fluorosulfate also remained mostly intact after 12 h in serum and after 48 h in cell lysate (Table S2). Even tetramethylguanidine, a reagent previously reported to promote SuFEx reaction in aqueous solution,[35] showed no reactivity against fluorosulfate alone (Table S1, Entry 3). Interestingly, we found the hydrolysis product of *N*-hydroxylsuccimide (Table S3 and Figure S2), *N*-hydroxylsuccinic acid monoamide (**3**), converted a fsY-containing hexapeptide **1** into the corresponding sulfopepitde **2** in 57% yield in 1 h (Figure 2a). Encouraged by this finding, we examined other HA derivatives (Figures 2a, S4). Acetohydroxamic acid (**4**) promoted the reaction to 78% over 1 h. Good yield (95%) of **2** was obtained using aromatic benzohydroxamic acid (**5**) under the same conditions. The highest efficiency was observed when the cationic HA **6** and heteroaromatic HA **7** were used, achieving quantitative conversion in 30 min. Other non-HA α-nucleophile reagents such as oxime **8**,[36] 2-aminoxime **9**, and 1-hydroxybenzotriazole (**10**)[37] resulted in lower reaction efficiency. In contrast, triisopropylsilyl ether (TIPS)-masked HA **11** showed no reactivity until potassium fluoride (KF) was added to remove the TIPS protecting group (Table S4 and

https://doi.org/10.1021/jacs.3c07937
J. Am. Chem. Soc. 2023, 145, 20189−20195

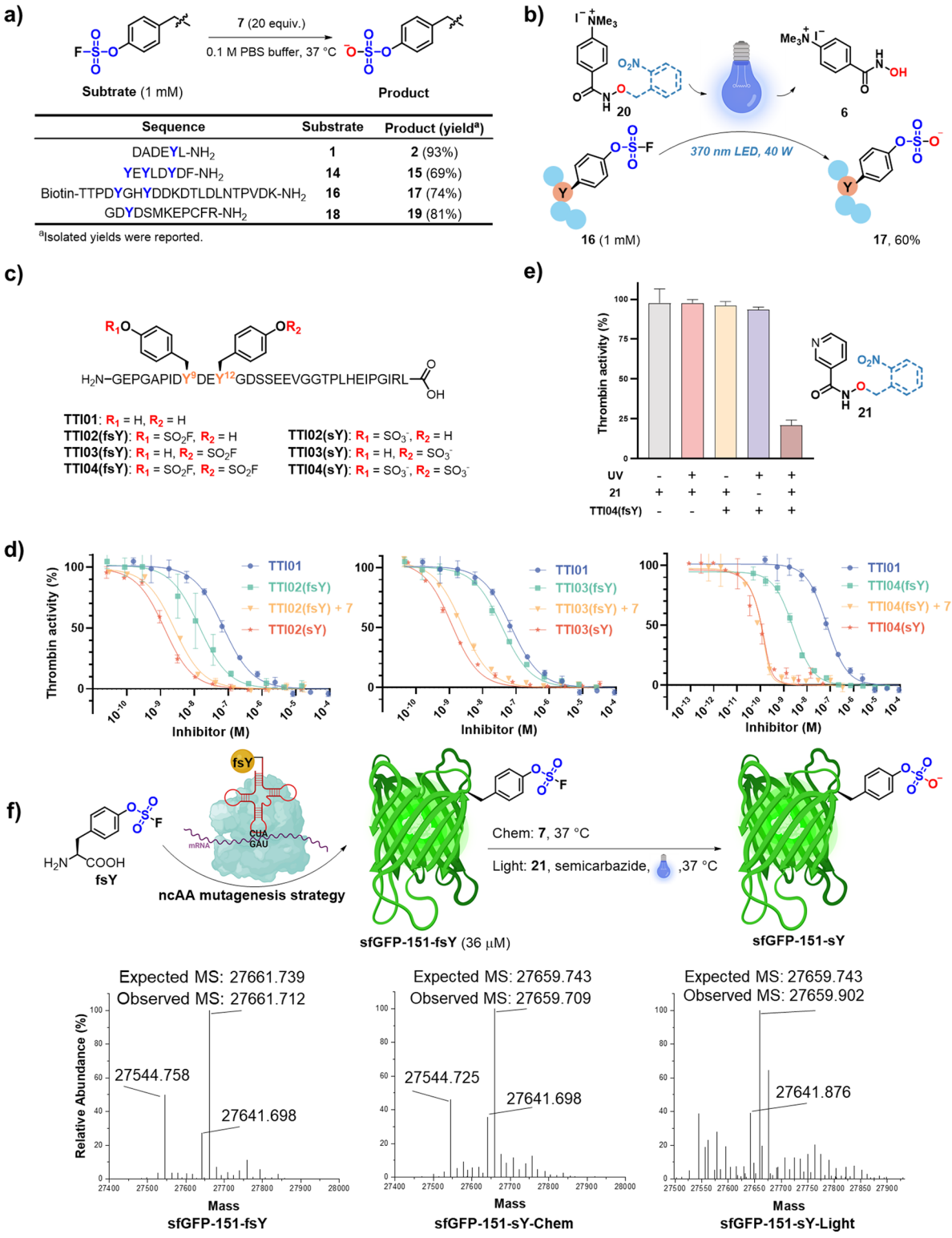

Figure 3. Decaging of fluorosulfate-containing peptides and proteins under physiologically relevant conditions. (a) Fluorosulfate decaging in fsY-containing synthetic peptides. (b) Light-mediated fluorosulfate decaging in C5aR1 22mer peptide using photocaged reagent **20**. (c) TTI peptide sequences and sulfation patterns. (d) Thrombin inhibition assay of TTI peptides. Data were fitted to the Morrison inhibition model, and error bars represent the standard deviation of three independent measurements. (e) Light-mediated activation and decaging of fluorosulfate-containing TTI peptide **TTI04(fsY)** regulate its sulfation-dependent thrombin inhibitory activity. (f) Fluorosulfate decaging in fsY-containing protein sfGFP-151 fsY and its corresponding high-resolution mass spectrometry.[50]

Figure S5), confirming that HA is the reactive center for fluorosulfate activation. It is also noteworthy that the decaging reaction mediated by 7 proceeded with no detectable side reaction in the presence of 20 equiv of amino acids including lysine, histidine, tyrosine, and cysteine (Table S5).

https://doi.org/10.1021/jacs.3c07937
J. Am. Chem. Soc. 2023, 145, 20189−20195

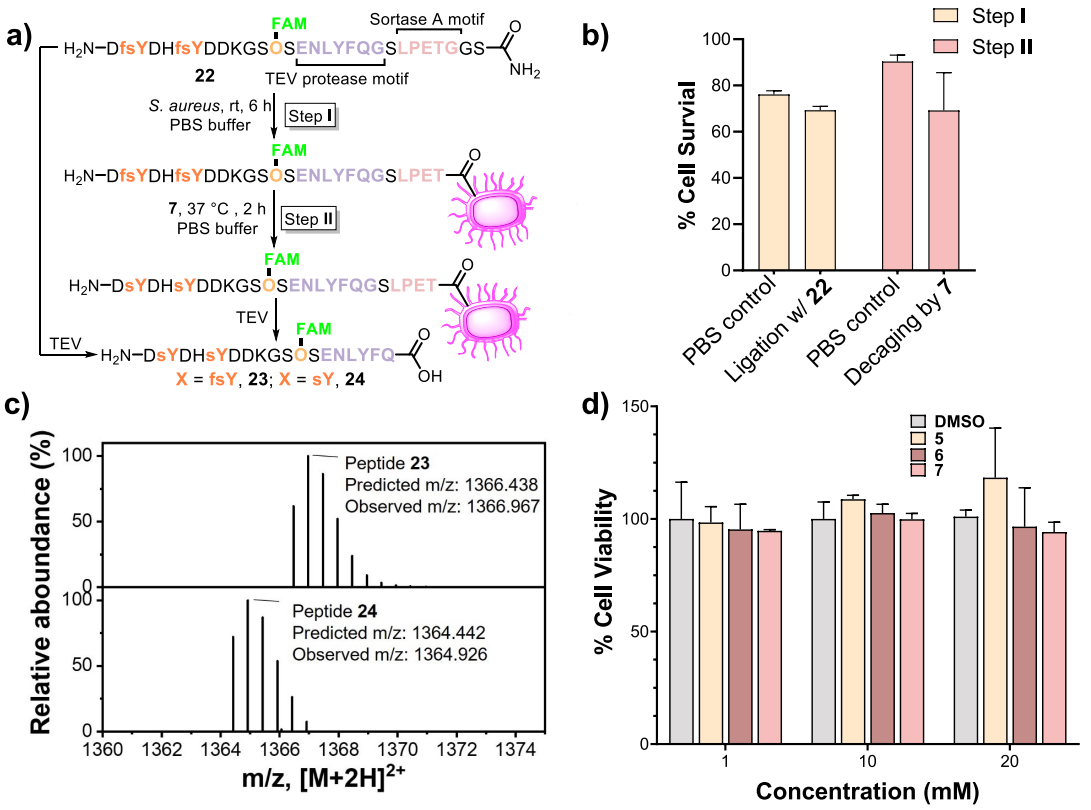

Figure 4. Cytocompatibility of the reagents. (a) Sortase A-mediated ligation of peptide **22** onto the *S. aureus* cell surface and its decaging followed by the TEV protease cleavage. (b) Percent of *S. aureus* cells survived after sortase A-mediated ligation of **22** (Step **I**) and after fluorosulfate decaging by **7** (Step **II**) compared to the cells treated with PBS. The average data of two trials were plotted. (c) LC-MS analysis of samples after the TEV cleavage identified the decaged peptide (**24**, bottom) compared to the cleaved peptide before decaging (**23**, top). (d) MTT assay of the mammalian HEK-293T cells after incubation with various concentrations of reagents **5**, **6**, and **7**. The average data of three trials were plotted.

To gain insight into the reaction mechanism, the reaction with **1** as the substrate and reagent **7** was monitored using liquid chromatography–mass spectrometry (LC-MS) to capture the reaction intermediates (Figure 2b and Figure S6). An adduct (**12**) of **7** and **1** was detected, confirming the nucleophilic coupling between the HA reagent and the substrate. Surprisingly, an isocyanate adduct **13** was also detected within 10 min at 37 °C, suggesting an uncommon intramolecular Lossen rearrangement pathway. To further probe this possibility, we performed the decaging reaction of **1** by **7** in the buffer prepared exclusively using $H_2{}^{18}O$. This reaction yielded **2** that contained no ^{18}O isotope (Figure 2c and Figure S7), suggesting that the conversion of fluorosulfate into sulfate is not through direct hydrolysis. These results further support a Lossen rearrangement mechanism.[38−40] Such a pathway is similar to the myrosinase-catalyzed Lossen-*like* rearrangement of glucosinolate in *Brassia* plants, in which an inorganic sulfate and isothiocyante are generated from a thiohydroximate-*O*-sulfate intermediate (Figure 1c).[34]

We then examined the decaging of various fluorosulfate-containing peptides mediated by **7** under physiological pH. Notably, peptides that contain multiple fsY residues or nucleophilic residues (e.g., lysine or cysteine) were successfully decaged in high yields (Figure 3a). In addition, decaging can be achieved in a light-mediated fashion. The fsY residues in **16** was efficiently decaged after a 2-nitrobenzyl-caged reagent **20** was exposed to 370 nm UV light irradiation (Figure 3b).[41] No conversion was observed in the dark or without **20** (Figure S31).

We used tsetse thrombin inhibitor (TTI)[42] peptides as a model system to probe the utility of the HA reagents in controlling the bioactivities associated with sulfation under physiologically relevant conditions. We used a standard human α-thrombin activity assay with Chromozym TH substrate to determine the inhibitory effects of the TTI peptides (latent) consisting of fsY residues at position 9 and 12: **TTI02(fsY)**, **TTI03(fsY)**, and **TTI04(fsY)**, and the corresponding sY-containing TTI peptides (active): **TTI02(sY)**, **TTI03(sY)**, and **TTI04(sY)** (Figure 3c).[43,44] Although the latent TTI peptides still exhibited minor inhibitory effects compared to the nonsulfated control **TTI01**, the active TTI peptides demonstrated significantly higher potencies (Figure 3d and Figure S9).[45] The latent TTI peptides that were decaged in situ by reagent **7** all showed inhibitory effects similar to those of the purified active TTI peptides (Figure 3d). These results confirmed that fluorosulfate can serve as an effective latent sulfate in peptides, and can be facilely decaged in aqueous solution at neutral pH. Light-controlled decaging is also possible. For example, while the latent **TTI04 (fsY)** remains inactive for thrombin inhibition at 3.7 nM in the presence of 2-nitrobenzyl protected reagent **21** in the dark, after irradiation, thrombin activity was reduced to 21% (Figure 3e).

The small size of fluorine atom allows fsY to be facilely incorporated into proteins as a ncAA.[32,46] Following the procedure established by Wang et al.,[32] we cloned the fsY-specific aminoacyl tRNA synthetase FsTyrRS and an optimal pyrrolysyl tRNA into plasmids for fsY incorporation into proteins. A sfGFP gene containing a TAG codon at position

https://doi.org/10.1021/jacs.3c07937
J. Am. Chem. Soc. 2023, 145, 20189−20195

151 was cotransformed along with the genes containing the FsTyrRA/tRNA pair into B95 *E. coli* cells.[47] The targeted sfGFP-151 fsY was successfully expressed in 12 mg/L yield. Tandem MS results verified the incorporation of fsY at the TAG-specified position-151 (Figure S10).[32,48] Next, to confirm the conversion from fluorosulfate to sulfate in sfGFP-151 fsY by **7**, as well as the integrity of the resulting sulfoprotein, we performed whole protein intact mass analyses of sfGFP-151 fsY before and after decaging using high-resolution Orbitrap mass spectrometry, which is capable of achieving sub-5 ppm mass accuracy[49] and can confidently resolve the 1.996 Da mass shift after decaging (Figure 3f and Figure S11).[50] Similarly, incorporation of fsY at position 3 of sfGFP and the subsequent decaging by **7** were also confirmed (Figures S12−S13). Furthermore, we showed the light-mediated decaging of sfGFP-151 fsY by the photocaged reagent **19** (Figure 3f), highlighting the potential of our approach for the spatiotemporal release of caged sulfoproteins.

Last, we tested the cytocompatibility of the fluorosuflate decaging reagents. Previously, cesium carbonate (Cs_2CO_3)/ethylene glycol[33] or 2 M ammonium acetate (NH_4OAc) aqueous solution[24,43] was used to remove the protecting groups for sulfate in peptides and small molecules. However, these conditions were found to be strongly denaturing to proteins (Figure S15) and highly toxic to live cells (Figures S16 and S17). In contrast, our reagents caused no protein denaturation and have low toxicity to cells at various concentrations. To mimic the cell membrane-bound sulfoproteins,[51] we examined *in situ* fluorosulfate decaging on the surface of live *Staphylococus aureus* (*S. aureus*) cells (Figure 4a−d). *S. aureus* cells were chosen because there are no known endogenous sulfopeptides expressed on their surface, and the endogenous sortase A on their surface can be used to ligate peptides.[52,53] A fluorescently labeled peptide **22** consisting of a *Tobacco Etch Virus* (TEV) protease cleavage sequence[54] and an LPETG sortase A-recognition motif was ligated to the cell surface of *S. aureus* (Figures 4a, S18, and S19). The cell-surface-ligated peptide was then decaged by reagent **7**. Compared to the phosphate-buffered saline (PBS) buffer control, neither the cell surface ligation nor the fsY decaging experiments caused a significant reduction of cell viability (Figure 4b). LC-MS analysis of the peptide residues cleaved after the decaging reaction (**24**) confirmed that the fsY was successfully converted into sY on the live cell surface (Figure 4c). Finally, reagents **5**−**7** also exhibited low toxicity to mammalian cells even at millimolar concentrations based on the MTT assay (Figure 4d).[55]

In conclusion, we demonstrated that fluorosulfate is a physiologically compatible latent sulfate in peptides and proteins. Fluorosulfate is stable in neutral aqueous buffers, cell lysates, and serum and can be efficiently converted into sulfate by easily modified and readily accessible HA reagents under physiologically relevant conditions via Lossen rearrangement. Leveraging the facile incorporation of fluorosulfate-containing amino acid fsY via solid-phase peptide synthesis and ncAA mutagenesis, our reported approach can be applied to studying a wide range of sulfopeptides and sulfoproteins in their physiological states. The excellent compatibility of our reagents with both bacterial and mammalian cells suggests that they are promising candidates for decaging fluorosulfate-containing peptides and proteins in experiments involving live systems.

■ ASSOCIATED CONTENT

Supporting Information

The Supporting Information is available free of charge at https://pubs.acs.org/doi/10.1021/jacs.3c07937.

Supplementary figures and tables, characterization data and $^{1}H/^{13}C$ NMR spectra, and detailed experimental protocols (PDF)

■ AUTHOR INFORMATION

Corresponding Author

Jia Niu − *Department of Chemistry, Boston College, Chestnut Hill, Massachusetts 02467, United States;* orcid.org/0000-0002-5622-6362; Email: jia.niu@bc.edu

Authors

Chao Liu − *Department of Chemistry, Boston College, Chestnut Hill, Massachusetts 02467, United States*

Xueyi Liu − *Department of Chemistry, Boston College, Chestnut Hill, Massachusetts 02467, United States*

Mi Zhou − *Department of Chemistry, Boston College, Chestnut Hill, Massachusetts 02467, United States*

Chaoshuang Xia − *Center for Biomedical Mass Spectrometry, Boston University School of Medicine, Boston, Massachusetts 02118, United States;* orcid.org/0000-0001-7076-4813

Yuhan Lyu − *Department of Chemistry, Boston College, Chestnut Hill, Massachusetts 02467, United States*

Qianni Peng − *Department of Chemistry, Boston College, Chestnut Hill, Massachusetts 02467, United States*

Chintan Soni − *Department of Chemistry, Boston College, Chestnut Hill, Massachusetts 02467, United States*

Zefeng Zhou − *Department of Chemistry, Boston College, Chestnut Hill, Massachusetts 02467, United States*

Qiwen Su − *Department of Chemistry, Boston College, Chestnut Hill, Massachusetts 02467, United States*

Yujia Wu − *Department of Chemistry, Boston College, Chestnut Hill, Massachusetts 02467, United States*

Eranthie Weerapana − *Department of Chemistry, Boston College, Chestnut Hill, Massachusetts 02467, United States;* orcid.org/0000-0002-0835-8301

Jianmin Gao − *Department of Chemistry, Boston College, Chestnut Hill, Massachusetts 02467, United States;* orcid.org/0000-0001-9341-1287

Abhishek Chatterjee − *Department of Chemistry, Boston College, Chestnut Hill, Massachusetts 02467, United States;* orcid.org/0000-0002-6231-5302

Cheng Lin − *Center for Biomedical Mass Spectrometry, Boston University School of Medicine, Boston, Massachusetts 02118, United States;* orcid.org/0000-0003-3653-9633

Complete contact information is available at: https://pubs.acs.org/10.1021/jacs.3c07937

Notes

The authors declare no competing financial interest.

■ ACKNOWLEDGMENTS

The research is supported by a startup fund from Boston College to J.N., the NIH Director's New Innovator Award (1DP2HG011027-01) to J.N., and NIH R35GM136437 to A.C. The NSF MRI award CHE-2117246, and the NIH HEI-S10 award 1S10OD026910-01A1 are also acknowledged.

■ ABBREVIATIONS

CCR5, C−C chemokine receptor type 5; PGSL-1, P-selectin glycoprotein ligand-1; HCII, heparin cofactor II; C5aR1, complement component 5a receptor 1

■ REFERENCES

(1) Mehta, A. Y.; Heimburg-Molinaro, J.; Cummings, R. D.; Goth, C. K. Emerging patterns of tyrosine sulfation and *O*-glycosylation cross-talk and co-localization. *Curr. Opin. Struct. Biol.* **2020**, *62*, 102−111.

(2) Huttner, W. B. Sulphation of tyrosine residues—a widespread modification of proteins. *Nature* **1982**, *299*, 273−276.

(3) Moore, K. L. The biology and enzymology of protein tyrosine *O*-sulfation. *J. Biol. Chem.* **2003**, *278*, 24243−24246.

(4) Baeuerle, P. A.; Huttner, W. B. Tyrosine sulfation of yolk proteins 1, 2, and 3 in Drosophila melanogaster. *J. Biol. Chem.* **1985**, *260*, 6434−6439.

(5) Ludeman, J. P.; Stone, M. J. The structural role of receptor tyrosine sulfation in chemokine recognition. *Br. J. Pharmacol.* **2014**, *171*, 1167−1179.

(6) Liu, C. C.; Schultz, P. G. Recombinant expression of selectively sulfated proteins in *Escherichia coli*. *Nat. Biotechnol.* **2006**, *24*, 1436−1440.

(7) Li, X.; Liu, C. C. Site-Specific Incorporation of Sulfotyrosine Using an Expanded Genetic Code. *Methods Mol. Biol.* **2018**, *1728*, 191−200.

(8) Italia, J. S.; Peeler, J. C.; Hillenbrand, C. M.; Latour, C.; Weerapana, E.; Chatterjee, A. Genetically encoded protein sulfation in mammalian cells. *Nat. Chem. Biol.* **2020**, *16*, 379−382.

(9) He, X.; Chen, Y.; Beltran, D. G.; Kelly, M.; Ma, B.; Lawrie, J.; Wang, F.; Dodds, E.; Zhang, L.; Guo, J.; Niu, W. Functional genetic encoding of sulfotyrosine in mammalian cells. *Nat. Commun.* **2020**, *11*, 4820.

(10) Chen, Y.; Jin, S.; Zhang, M.; Hu, Y.; Wu, K.-L.; Chung, A.; Wang, S.; Tian, Z.; Wang, Y.; Wolynes, P. G.; Xiao, H. Unleashing the potential of noncanonical amino acid biosynthesis to create cells with precision tyrosine sulfation. *Nat. Commun.* **2022**, *13*, 5434.

(11) Kehoe, J. W.; Bertozzi, C. R. Tyrosine sulfation: a modulator of extracellular protein-protein interactions. *Chem. Biol.* **2000**, *7*, R57−R61.

(12) So, W. H.; Wong, C. T. T.; Xia, J. Peptide photocaging: A brief account of the chemistry and biological applications. *Chin. Chem. Lett.* **2018**, *29*, 1058−1062.

(13) Yang, B.; Wang, N.; Schnier, P. D.; Zheng, F.; Zhu, H.; Polizzi, N. F.; Ittuveetil, A.; Saikam, V.; DeGrado, W. F.; Wang, Q.; Wang, P. G.; Wang, L. Genetically introducing biochemically reactive amino acids dehydroalanine and dehydrobutyrine in proteins. *J. Am. Chem. Soc.* **2019**, *141*, 7698−7703.

(14) Yang, W.; Eken, Y.; Zhang, J.; Cole, L. E.; Ramadan, S.; Xu, Y.; Zhang, Z.; Liu, J.; Wilson, A. K.; Huang, X. Chemical synthesis of human syndecan-4 glycopeptide bearing O-, N-sulfation and multiple aspartic acids for probing impacts of the glycan chain and the core peptide on biological functions. *Chem. Sci.* **2020**, *11*, 6393−6404.

(15) Tiruchinapally, G.; Yin, Z.; El-Dakdouki, M.; Wang, Z.; Huang, X. Divergent heparin oligosaccharide synthesis with preinstalled sulfate esters. *Chem.—Eur. J.* **2011**, *17*, 10106−10112.

(16) Al-Horani, R. A.; Desai, U. R. Chemical sulfation of small molecules-advances and challenges. *Tetrahedron* **2010**, *66*, 2907−2918.

(17) Stone, M. J.; Payne, R. J. Homogeneous sulfopeptides and sulfoproteins: synthetic approaches and applications to characterize the effects of tyrosine sulfation on biochemical function. *Acc. Chem. Res.* **2015**, *48*, 2251−2261.

(18) Ingram, L. J.; Taylor, S. D. Introduction of 2, 2, 2-Trichloroethyl-Protected Sulfates into Monosaccharides with a Sulfuryl Imidazolium Salt and Application to the Synthesis of Sulfated Carbohydrates. *Angew. Chem., Int. Ed.* **2006**, *45*, 3503−3506.

(19) Bunschoten, A.; Kruijtzer, J. A.; Ippel, J. H.; de Haas, C. J.; van Strijp, J. A.; Kemmink, J.; Liskamp, R. M. J. A general sequence independent solid phase method for the site specific synthesis of multiple sulfated-tyrosine containing peptides. *Chem. Commun.* **2009**, 2999−3001.

(20) Ali, A. M.; Taylor, S. D. Efficient solid-phase synthesis of sulfotyrosine peptides using a sulfate protecting-group strategy. *Angew. Chem., Int. Ed.* **2009**, *48*, 2024−2026.

(21) Ali, A. M.; Taylor, S. D. Synthesis of disulfated peptides corresponding to the N-terminus of chemokines receptors CXCR6 (CXCR6 1−20) and DARC (DARC 8−42) using a sulfate-protecting group strategy. *J. Pept. Sci.* **2010**, *16*, 190−199.

(22) Desoky, A. Y.; Hendel, J.; Ingram, L.; Taylor, S. D. Preparation of trifluoroethyl-and phenyl-protected sulfates using sulfuryl imidazolium salts. *Tetrahedron* **2011**, *67*, 1281−1287.

(23) Simpson, L. S.; Widlanski, T. S. A comprehensive approach to the synthesis of sulfate esters. *J. Am. Chem. Soc.* **2006**, *128*, 1605−1610.

(24) Simpson, L. S.; Zhu, J. Z.; Widlanski, T. S.; Stone, M. J. Regulation of chemokine recognition by site-specific tyrosine sulfation of receptor peptides. *Chem. Biol.* **2009**, *16*, 153−161.

(25) Liu, C.; Yang, C.; Hwang, S.; Ferraro, S. L.; Flynn, J. P.; Niu, J. A General Approach to *O*-Sulfation by a Sulfur(VI) Fluoride Exchange Reaction. *Angew. Chem., Int. Ed.* **2020**, *59*, 18435−18441.

(26) Dong, J.; Krasnova, L.; Finn, M.; Sharpless, K. B. Sulfur(VI) fluoride exchange (SuFEx): another good reaction for click chemistry. *Angew. Chem., Int. Ed.* **2014**, *53*, 9430−9448.

(27) Barrow, A. S.; Smedley, C. J.; Zheng, Q.; Li, S.; Dong, J.; Moses, J. E. The growing applications of SuFEx click chemistry. *Chem. Soc. Rev.* **2019**, *48*, 4731−4758.

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

(29) Miloserdov, F.; Zuilhof, H. Binding S(VI) to alkynes. *Nat. Synth.* **2022**, *1*, 415−416.

(30) Lee, C.; Cook, A. J.; Elisabeth, J. E.; Friede, N. C.; Sammis, G. M.; Ball, N. D. The Emerging Applications of Sulfur(VI) Fluorides in Catalysis. *ACS Catal.* **2021**, *11*, 6578−6589.

(31) Mukherjee, H.; Debreczeni, J.; Breed, J.; Tentarelli, S.; Aquila, B.; Dowling, J. E.; Whitty, A.; Grimster, N. P. A study of the reactivity of S(VI)-F containing warheads with nucleophilic amino-acid side chains under physiological conditions. *Org. Biomol. Chem.* **2017**, *15*, 9685−9695.

(32) While reactivities of fluorosulfate with cellular nucleophiles have been reported, these examples all require the close spatial proximity through ligand−receptor binding. For more details, please see references: (a) Wang, N.; Yang, B.; Fu, C.; Zhu, H.; Zheng, F.; Kobayashi, T.; Liu, J.; Li, S.; Ma, C.; Wang, P. G.; Wang, Q.; Wang, L. Genetically Encoding Fluorosulfate-L-tyrosine To React with Lysine, Histidine, and Tyrosine via SuFEx in Proteins *in Vivo*. *J. Am. Chem. Soc.* **2018**, *140*, 4995−4999. (b) Chen, W.; Dong, J.; Plate, L.; Mortenson, D. E.; Brighty, G. J.; Li, S.; Liu, Y.; Galmozzi, A.; Lee, P. S.; Hulce, J. J.; Cravatt, B. F.; Saez, E.; Powers, E. T.; Wilson, I. A.; Sharpless, K. B.; Kelly, J. W. Arylfluorosulfates Inactivate Intracellular Lipid Binding Protein(s) through Chemoselective SuFEx Reaction with a Binding Site Tyr Residue. *J. Am. Chem. Soc.* **2016**, *138*, 7353−7364. (c) Han, Y.; Yang, Z.; Hu, H.; Zhang, H.; Chen, L.; Li, K.; Kong, L.; Wang, Q.; Liu, B.; Wang, M.; Lin, J.; Chen, P. R. Covalently engineered protein minibinders with enhanced neutralization efficacy against escaping SARS-CoV-2 variants. *J. Am. Chem. Soc.* **2022**, *144*, 5702−5707.

(33) Chen, W.; Dong, J.; Li, S.; Liu, Y.; Wang, Y.; Yoon, L.; Wu, P.; Sharpless, K. B.; Kelly, J. W. Synthesis of Sulfotyrosine-Containing Peptides by Incorporating Fluorosulfated Tyrosine Using an Fmoc-Based Solid-Phase Strategy. *Angew. Chem., Int. Ed.* **2016**, *55*, 1835−1838.

(34) Bones, A. M.; Rossiter, J. T. The myrosinase-glucosinolate system, its organisation and biochemistry. *Physiol. Plant.* **1996**, *97*, 194−208.

(35) Choi, E. J.; Jung, D.; Kim, J. S.; Lee, Y.; Kim, B. M. Chemoselective Tyrosine Bioconjugation through Sulfate Click Reaction. *Chem.—Eur. J.* **2018**, *24*, 10948−10952.

https://doi.org/10.1021/jacs.3c07937
J. Am. Chem. Soc. 2023, 145, 20189−20195

(36) Eddleston, M.; Buckley, N. A.; Eyer, P.; Dawson, A. H. Management of acute organophosphorus pesticide poisoning. *Lancet* **2008**, *371*, 597−607.
(37) Wei, M.; Liang, D.; Cao, X.; Luo, W.; Ma, G.; Liu, Z.; Li, L. A Broad-Spectrum Catalytic Amidation of Sulfonyl Fluorides and Fluorosulfates. *Angew. Chem., Int. Ed.* **2021**, *60*, 7397−7404.
(38) Hurd, C. D.; Bauer, L. A novel rearrangement of hydroxamic acids using sulfonyl chlorides. *J. Am. Chem. Soc.* **1954**, *76*, 2791−2792.
(39) Hackley, B. E., Jr; Plapinger, R.; Stolberg, M.; Wagner-Jauregg, T. Acceleration of the hydrolysis of organic fluorophosphates and fluorophosphonates with hydroxamic acids. *J. Am. Chem. Soc.* **1955**, *77*, 3651−3653.
(40) Zhang, G.; Cui, Y.; Zhao, Y.; Cui, Y.; Bao, S.; Ding, C. A Practical Approach to Ureas and Thiocarbamates: SO_2F_2-Promoted Lossen Rearrangement of Hydroxamic Acid. *ChemistrySelect* **2020**, *5*, 7817−7821.
(41) Semicarbazide was added to scavenge the nitrosobenzaldehyde produced in the light-mediated reaction (Figure S8). For more detailed information, please see reference: Hansen, M. J.; Velema, W. A.; Lerch, M. M.; Szymanski, W.; Feringa, B. L. Wavelength-selective cleavage of photoprotecting groups: strategies and applications in dynamic systems. *Chem. Soc. Rev.* **2015**, *44*, 3358−3377.
(42) Cappello, M.; Bergum, P. W.; Vlasuk, G. P.; Furmidge, B. A.; Pritchard, D. I.; Aksoy, S. Isolation and characterization of the tsetse thrombin inhibitor: a potent antithrombotic peptide from the saliva of Glossina morsitans morsitans. *Am. J. Trop. Med. Hyg.* **1996**, *54*, 475−480.
(43) Calisto, B. M.; Ripoll-Rozada, J.; Dowman, L. J.; Franck, C.; Agten, S. M.; Parker, B. L.; Veloso, R. C.; Vale, N.; Gomes, P.; de Sanctis, D.; Payne, R. J.; Pereira, P. J. B. Sulfotyrosine-mediated recognition of human thrombin by a tsetse fly anticoagulant mimics physiological substrates. *Cell Chem. Biol.* **2021**, *28*, 26−33.
(44) Spannagl, M.; Bichler, J.; Birg, A.; Lill, H.; Schramm, W. Development of a chromogenic substrate assay for the determination of hirudin in plasma. *Blood Coagul. Fibrinolysis* **1991**, *2*, 121−128.
(45) Williams, J. W.; Morrison, J. F. The kinetics of reversible tight-binding inhibition. *Methods Enzymol.* **1979**, *63*, 437−467.
(46) Chatterjee, A.; Sun, S. B.; Furman, J. L.; Xiao, H.; Schultz, P. G. A versatile platform for single- and multiple-unnatural amino acid mutagenesis in *Escherichia coli*. *Biochemistry* **2013**, *52*, 1828−1837.
(47) Xiao, H.; Nasertorabi, F.; Choi, S.-h.; Han, G. W.; Reed, S. A.; Stevens, R. C.; Schultz, P. G. Exploring the potential impact of an expanded genetic code on protein function. *Proc. Natl. Acad. Sci. U.S.A.* **2015**, *112*, 6961−6966.
(48) Weerapana, E.; Wang, C.; Simon, G. M.; Richter, F.; Khare, S.; Dillon, M. B.; Bachovchin, D. A.; Mowen, K.; Baker, D.; Cravatt, B. F. Quantitative reactivity profiling predicts functional cysteines in proteomes. *Nature* **2010**, *468*, 790−795.
(49) Scheffler, K.; Viner, R.; Damoc, E. High resolution top-down experimental strategies on the Orbitrap platform. *J. Proteomics* **2018**, *175*, 42−55.
(50) The 27544.758 and 27544.725 Da mass peaks correspond to the misincorporation of glutamine into sfGFP at position of 151 (expected exact mass: 27544.804 Da), a known byproduct of the ncAA mutagenesis method. The observed 27641.698 and 27641.876 Da mass peaks correspond to an intramolecular reaction of fluorosulfotyrosine with a nucleophilic residue (expected exact mass: 27641.732 Da). For more information, please see references: (a) Italia, J. S.; Addy, P. S.; Wrobel, C. J.; Crawford, L. A.; Lajoie, M. J.; Zheng, Y.; Chatterjee, A. An orthogonalized platform for genetic code expansion in both bacteria and eukaryotes. *Nat. Chem. Biol.* **2017**, *13*, 446−450. (b) Italia, J. S.; Latour, C.; Wrobel, C. J. J.; Chatterjee, A. Resurrecting the bacterial tyrosyl-tRNA Synthetase/tRNA pair for expanding the genetic code of both *E. coli* and eukaryotes. *Cell Chem. Biol.* **2018**, *25*, 1304−1312.
(51) Hille, A.; Huttner, W. B. Occurrence of tyrosine sulfate in proteins-a balance sheet: 2. Membrane proteins. *Eur. J. Biochem.* **1990**, *188*, 587−596.
(52) Reja, R. M.; Wang, W.; Lyu, Y.; Haeffner, F.; Gao, J. Lysine-targeting reversible covalent inhibitors with long residence time. *J. Am. Chem. Soc.* **2022**, *144*, 1152−1157.
(53) Rentero Rebollo, I.; McCallin, S.; Bertoldo, D.; Entenza, J. M.; Moreillon, P.; Heinis, C. Development of potent and selective *S. aureus* sortase A inhibitors based on peptide macrocycles. *ACS Med. Chem. Lett.* **2016**, *7*, 606−611.
(54) Kapust, R. B.; Tözsér, J.; Copeland, T. D.; Waugh, D. S. The P1′ specificity of tobacco etch virus protease. *Biochem. Biophys. Res. Commun.* **2002**, *294*, 949−955.
(55) Mosmann, T. Rapid colorimetric assay for cellular growth and survival: application to proliferation and cytotoxicity assays. *J. Immunol. Methods* **1983**, *65*, 55−63.

https://doi.org/10.1021/jacs.3c07937
J. Am. Chem. Soc. 2023, 145, 20189−20195