FAST FLUORALKYLATION OF PROTEINS (FFAP) UNCOVERS STRUCTURE AND DYNAMICS OF BIOLOGICAL MACROMOLECULES

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INTRODUCTION

Covalent labeling of proteins in combination with mass spectrometry has been established as a complementary technique to classical structural methods, such as X-Ray, NMR, or Cryo-EM used for protein structure determination. Although the current covalent labeling techniques enable to monitor protein solvent accessible areas with sufficient spatial resolution, there is still high demand for alternative, less complicated and inexpensive approaches. Here, we introduce a new covalent labeling method based on Fast Fluoroalkylation of Proteins (FFAP). The FFAP uses fluoroalkyl radicals formed by reductive decomposition of Togni reagents with ascorbic acid for labeling of proteins on a time scale of seconds [2]. The feasibility of FFAP for effective labeling of proteins was demonstrated on formation of human haptoglobin-hemoglobin complex. As the model of monoclonal antibody the drug Trastuzumab was used.

The obtained data confirmed the Togni reagent-mediated FFAP as an advantageous alternative method for covalent labeling in applications, such as protein footprinting and epitope mapping of proteins (and their complexes) in general.

Togni Reagents

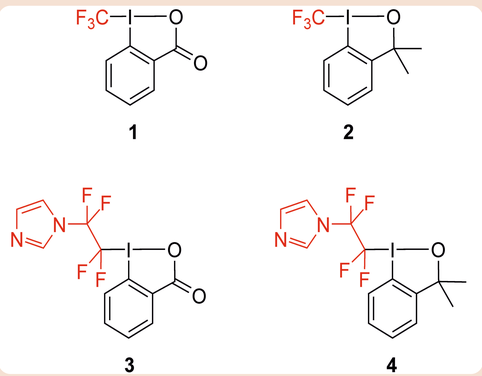

Figure 1: Structures of cyclic hypervalent iodine-fluoroalkyl reagents used in this study
1-acid type Togni-CF_3 reagent *1-(trifluoromethyl)-1λ³-benzo[d][1,2]iodaoxol-3(1H)-one*
2-alcohol type Togni-CF_3 reagent *3,3-dimethyl-1-(trifluoromethyl)-1,3-dihydro-1λ³-benzo[d][1,2]iodaoxole*
3-acid type Togni-CF_2F_2Im reagent *1-(1,1,2,2-tetrafluoro-2-(1H-imidazol-1-yl)ethyl)-1λ³-benzo[d][1,2]iodaoxol-3(1H)-one*
4-alcohol type Togni-CF_2CF_2Im reagent *1-(2-(3,3-dimethyl-1λ³-benzo[d][1,2]iodaoxol-1(3H)-yl)-1,1,2,2-tetrafluoroethyl)-1H-imidazole*
The resulting transferable fluoroalkyl moieties are highlighted in red.

METHODS

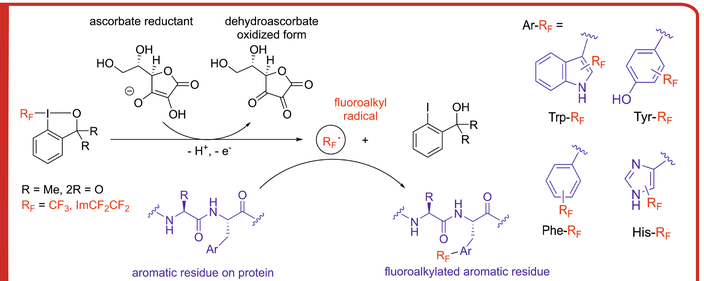

Figure 2: Top - The reaction scheme of Fast Fluoralkylation with all aromatics amino acids
Ascorbic acid induced the generation of radical from Togni reagent containing fluoroalkyl group, which reacted with aromatics aminoacids [1].

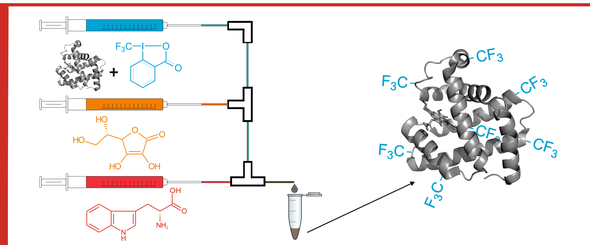

Figure 3: The simplified scheme of FFAP flow setup
FFAP platform contains three syringes, first with the sample and Togni reagent, second with the Ascorbic acid as the inducer of the reaction and the third one with the qeuncher - Tryptophan. The lenght and internal diameter of the capilary (Green one in scheme) together with flow rate are setting the time of reaction pulse. In our case for three second. The studied antibodies and their complexes were analyzed by bottom up approach using high resolution mass spectrometry (solariX XR 15T, Bruker Daltonics) where samples were digested by trypsin/Lys-C, separated on reverse phase column (Luna Omega Polar C18, 100Å) online coupled to mass spectrometer [2].

RESULTS

Complex of Haptoglobin and Hemoglobin

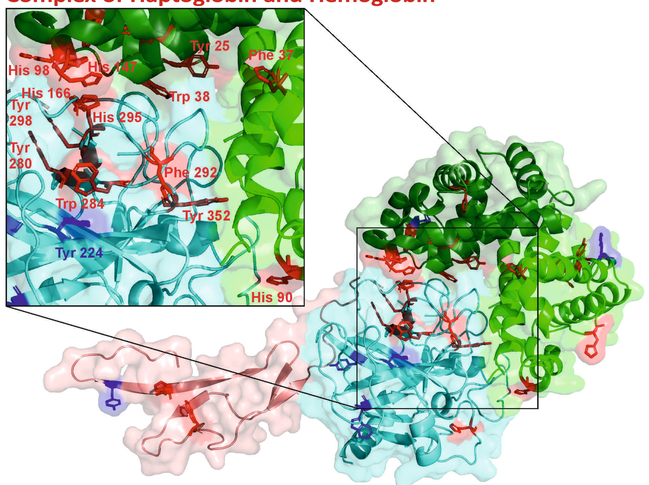

Figure 4: Structure of Hp-Hb complex with highlighted modified residues after reaction with Togni reagent 3.
Red residues are less modified in Hp-Hb complex and blue are modified to higher levels in Hp-Hb complex. Subunits of complex are highlighted as follows: alpha subunit of Hp- pink, beta subunit of Hp-light blue, alpha subunit of Hb - light green and beta subunit of Hb - dark green. Zoom – Interaction interface of Hp-Hb complex [2].

Model antibody - Trastuzumab

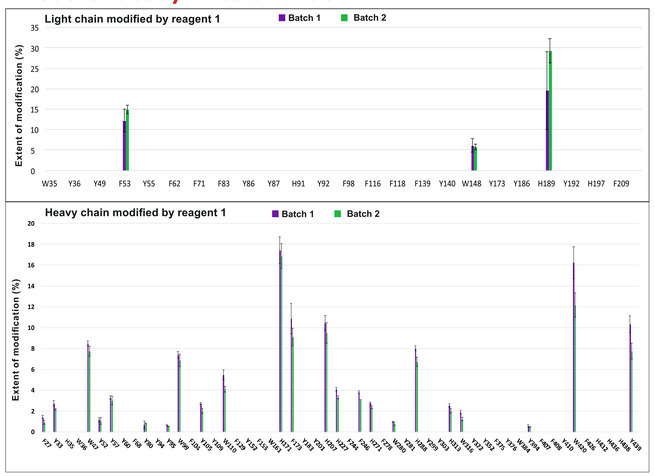

Figure 5: On the right side - Quantification of modification for aromatic residues of Trastuzumab for reagent **1**. **On the left side** - The structure of FAB fragment with modified aromatic amino acids, Blue - Light chain, Purple - Heavy chain, Gray - Her2 (Her2 was not part of the experiment) . The upper graphs are the quantification of light chain modification, the bottom graphs are quantification of heavy chain modification. Purple columns show the extent of modification for tested batch 1 and green columns show tested batch2. Data were performed in biological triplicate and were statistically processed. P-value was calculated for each residue.

Conclusion

We successfully introduced the new method of radical labeling with alkylfluor radicals which is capable for the structure studies of proteins and their complexes.
Radicals are created from Togni reagent by ascorbic acid and the labeling is done in aquatic environment.
Labeling is done by three second pulse and only surface accessible and reactive aromatic amino acids are modified by alkylfluorine radicals generated from Togni reagents.
The capibility of the Togni reagents for epitope mapping of monoclonal antibodies has been verified on drug Trastuzumab.

References

[1]Rahimidashaghoul K. et al. Chem. – A Eur. J. 2019, 25 (69), 15779–15785.
[2] Fojtik L. et al. J. Am. Chem. Soc. 2021 Dec 15;143(49),20670-20679.

LABORATORY OF STRUCTURAL BIOLOGY AND CELL SIGNALING

This work was mainly supported by the Czech Science Foundation (grant number 19-16084S), Charles University Grant Agency (Grant number 359221), European Commission H2020 (EPIC-XS - grant agreement ID: 823839 and EU FT-ICR MS - grant agreement ID: 731077).