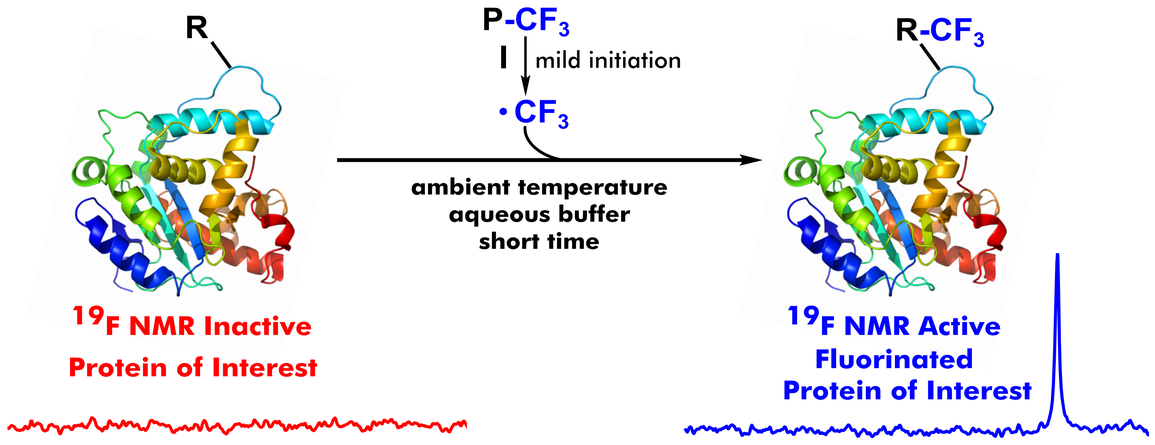
Tryptophan selective trifluoromethylation of native residues in proteins

Mateusz Imiołek, Gogulan Karunanithy, Wai-Lung Ng, Andrew J. Baldwin, Véronique Gouverneur and Benjamin G. Davis*

Chemistry Research Laboratory, Department of Chemistry, University of Oxford, 12 Mansfield Road, Oxford OX1 3TA, United Kingdom

Protein fluorination

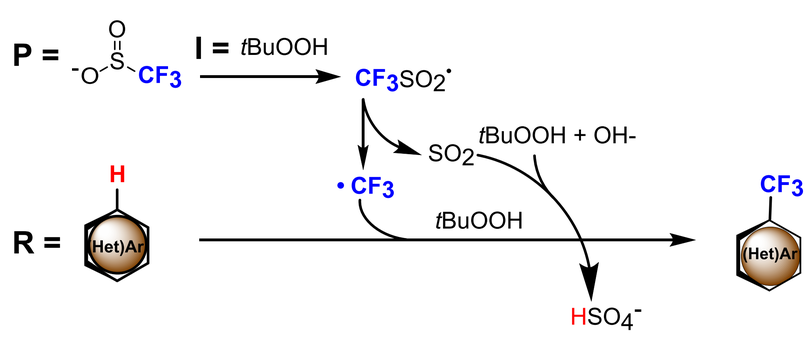
Precise chemical modification of biomolecules allows introduction of new unnatural moieties that can serve as chemical probes. Among them, fluorination offers a unique opportunity to study biomolecules and decipher the underlying biology with 'zero-background' ^{19}F-NMR. **Radical-based** approaches well suit this purpose as they proceed under ambient conditions with great operational simplicity. We wondered therefore, if we can use **native side chains reactivity** for introduction of minimal size trifluoromethyl radical generated under mild conditions.



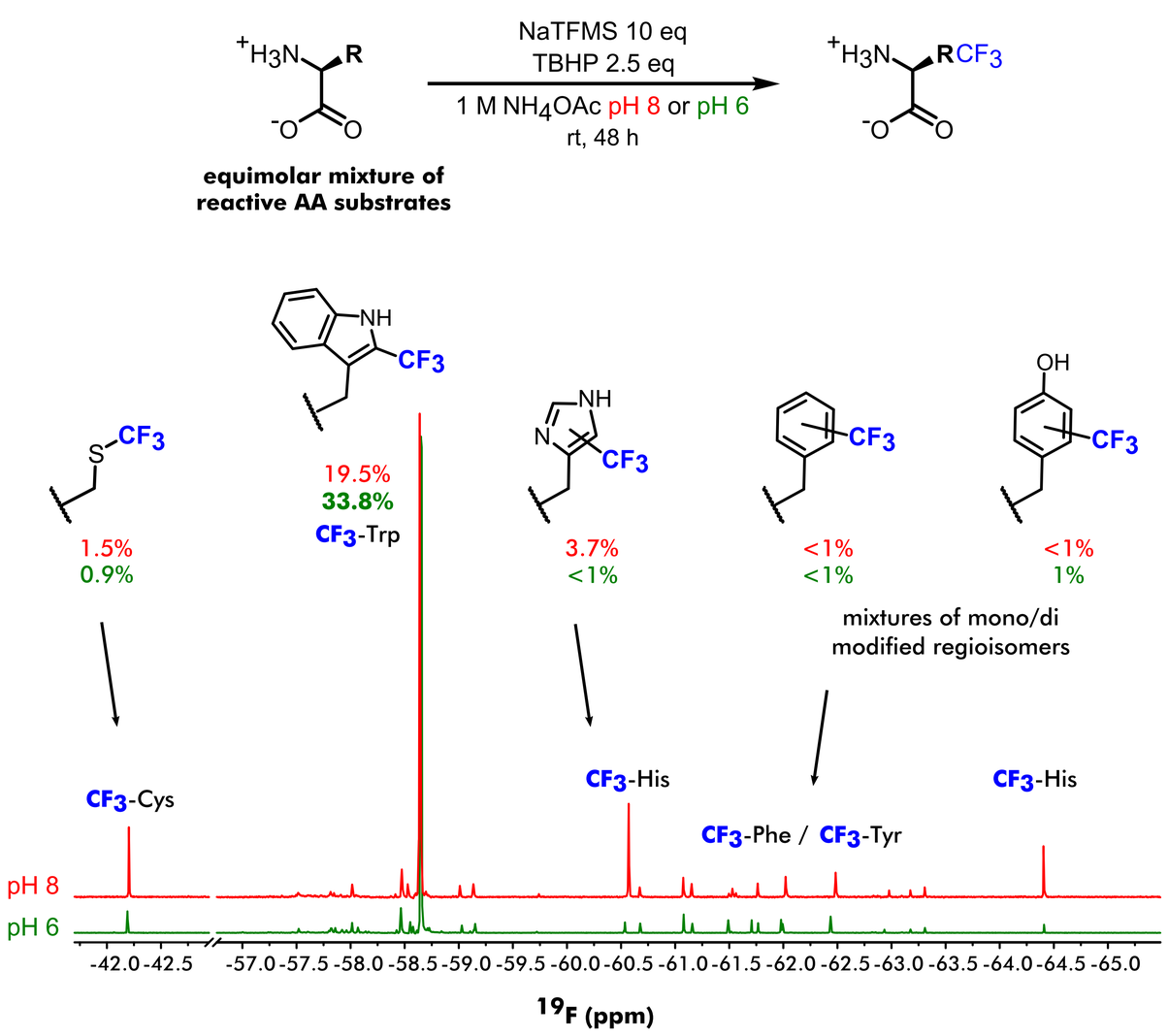
Small molecule model suggest tryptophan selectivity

We chose well known pair: sodium triflinate (NaTFMS)/*tert*-butyl hydroperoxide (TBHP) as a suitable radical generating system which competitive reactivity was investigated on a small molecule model – free amino acids.

Proposed trifluoromethylation system with the mechanism



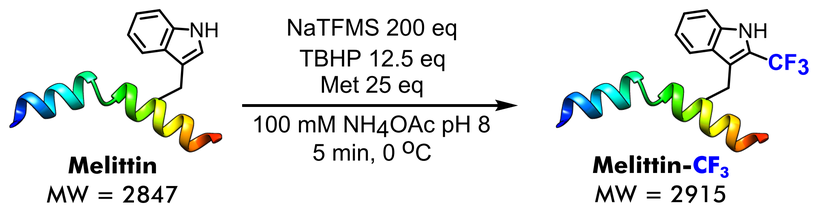
^{19}F NMR screen of competitive reactivity of •CF$_3$ with amino acids



- Higher selectivity for Trp in lower pH 6 (k_{rel} >~30-fold) potentially due to reduced reactivity of protonated His residues
- Main side reaction was oxidative dimerization of Cys without trifluoromethylation
- **Conclusion**: in the absence of free Cys, strongly chemo- and regio- Trp-selective trifluoromethylation might be possible in proteins

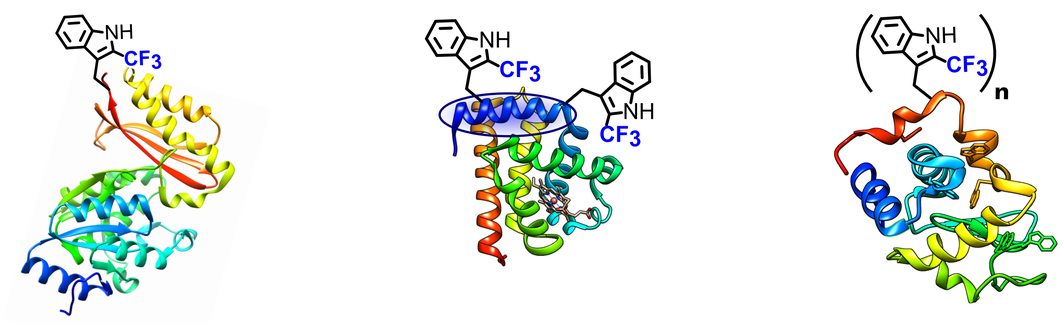
Translation to biomolecules

Use of excess of NaTFMS/TBHP allowed short reaction times at low concentration (~μM). Optimization of conditions (reactants loading, time, temperature etc.) enabled preparation of constructs at desired fluorination level.



- 4-hydroxy-TEMPO was used for termination of reaction (radical-mediated nature)
- Methionine was used as an oxidative buffer to limit side reactivity

Scope - model proteins with varying number of Trp residues

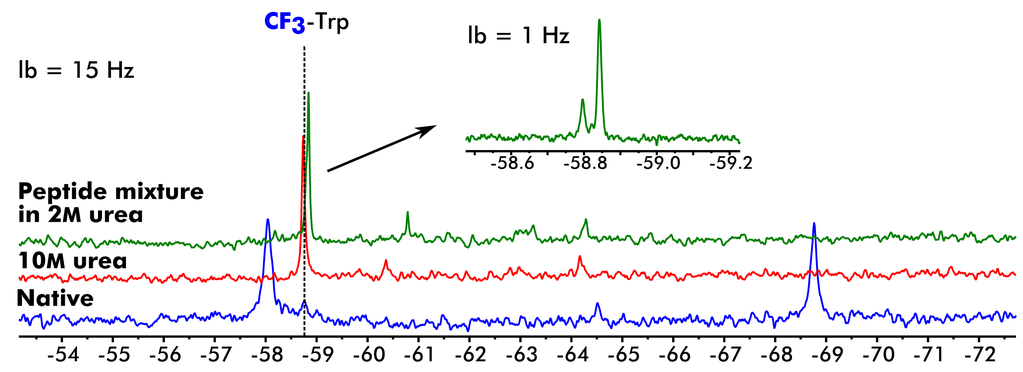


- Reaction worked on natively folded proteins with the structure (CD, UV-VIS spectroscopy) and the function (lysozyme *Micrococcus lysodeikticus* lysis assay) retained after the reaction

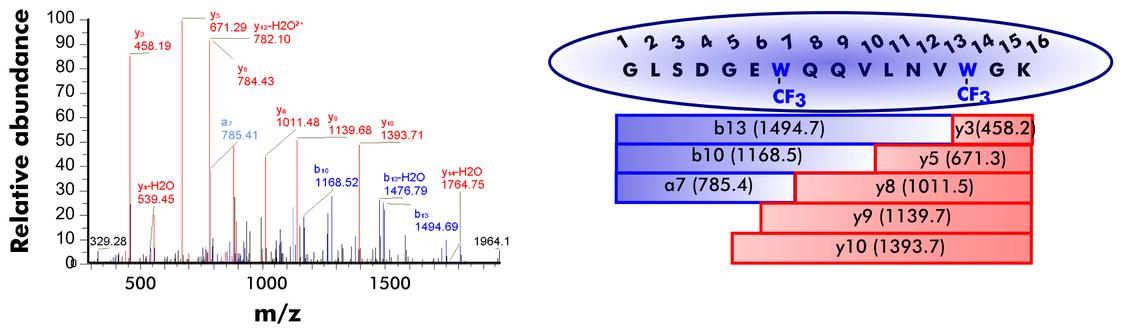
Characterization confirmed selectivity

MS and NMR experiments were used to elucidate modification sites. In all cases tryptophans were dominant modified residues as indicated by MS/MS fragmentation of tryptic peptides and chemical shift of collapsed peaks in ^{19}F NMR spectra to that of the small molecule model. Below data for trifluoromethylated myoglobin.

^{19}F NMR spectra of different forms of trifluoromethylated myoglobin



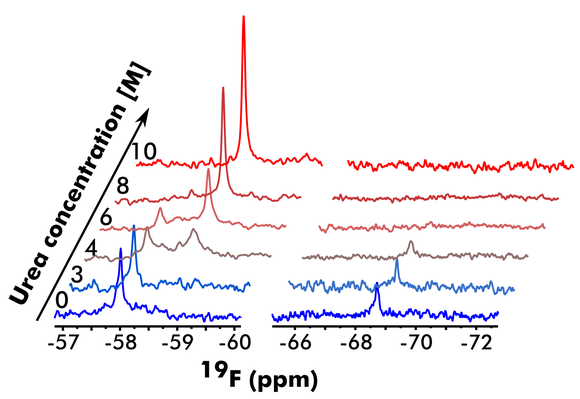
Tryptic digest LC-MS/MS



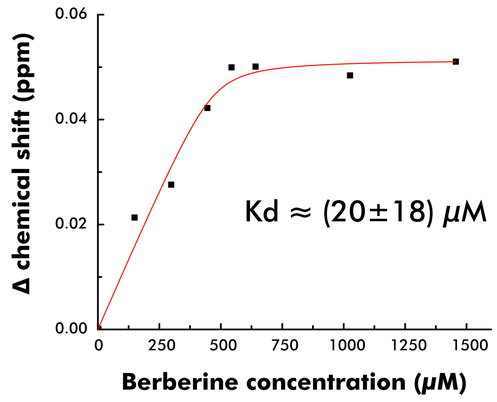
Proof-of-concept PrOF studies

Fluorinated proteins were studied in PrOF experiments: qualitatively - denaturation of myoglobin and quantitatively - determination of the binding constant of a ligand for lysozyme.

Changes in protein fold



Protein observed ligand binding



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