Photochemical Charge Control in High Vacuum

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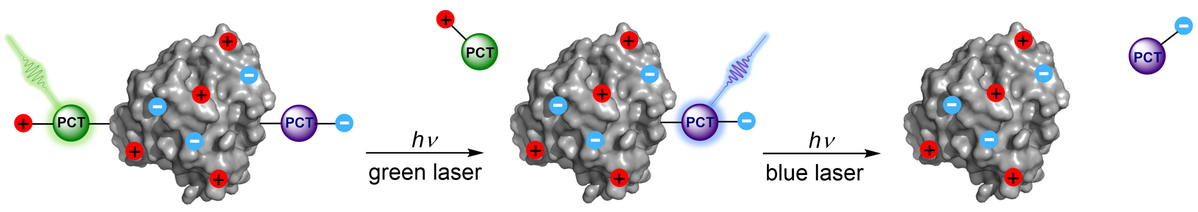
Objective

i) Selective removal of the last charge on directed protein ions by photocleavage (532 nm)
ii) Reionization of neutral protein molecules in the beam by photocleavage (355 nm)



singly charged protein ions launched by ESI or MALDI

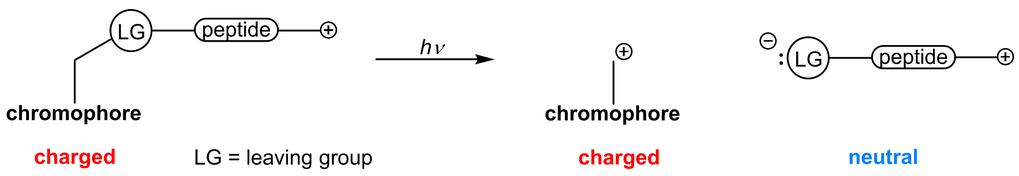
charge-neutral molecular beam

reionized protein ions for MS detection

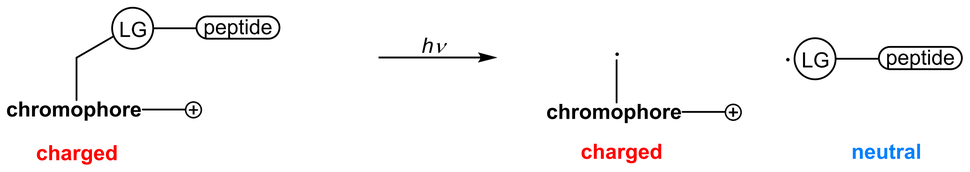
Approach and general considerations

i) Reported solution-phase photocages[1,2] are examined for efficient cleavage under irradiation in high vacuum.
ii) Observations inform the subsequent design and modification of identified candidates.
iii) Gas-phase-cleavage-behaviour can differ considerably from solution-phase-behaviour.
iv) Depending on the design either leaving group or chromophore remain covalently linked to the peptide after cleavage.
v) The cleavage behaviour determines if the leaving group (LG) or the chromophore need to carry permanent charges (e.g., tetraalkyl ammonium or sulfonate groups) to result in an altered charge state of the peptide upon cleavage.

Heterolysis – no charge-decoration of the chromophore required for charge removal



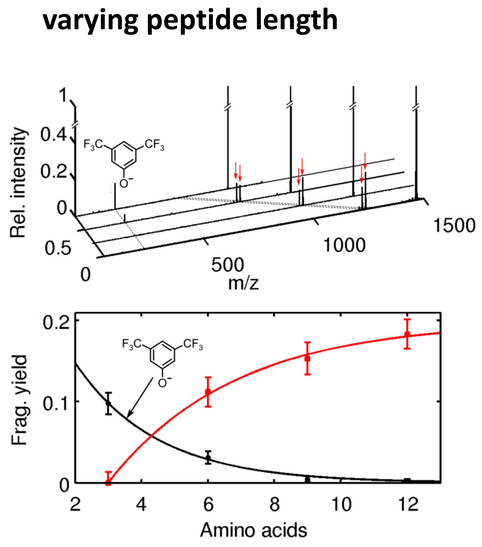
Homolysis (or H-transfer) – charge-decoration required

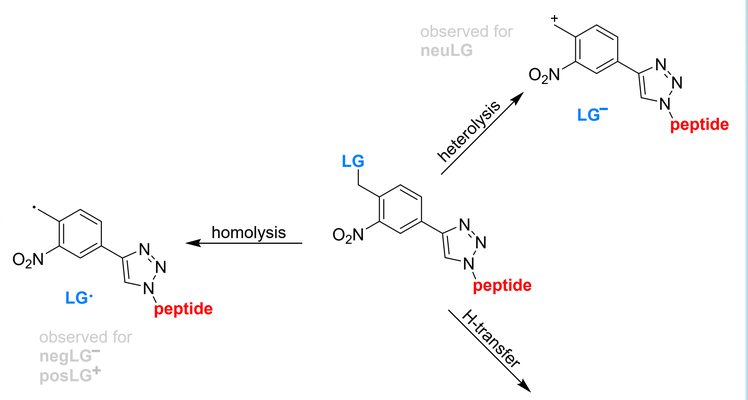


Previous studies at 266 nm[3,4,5]

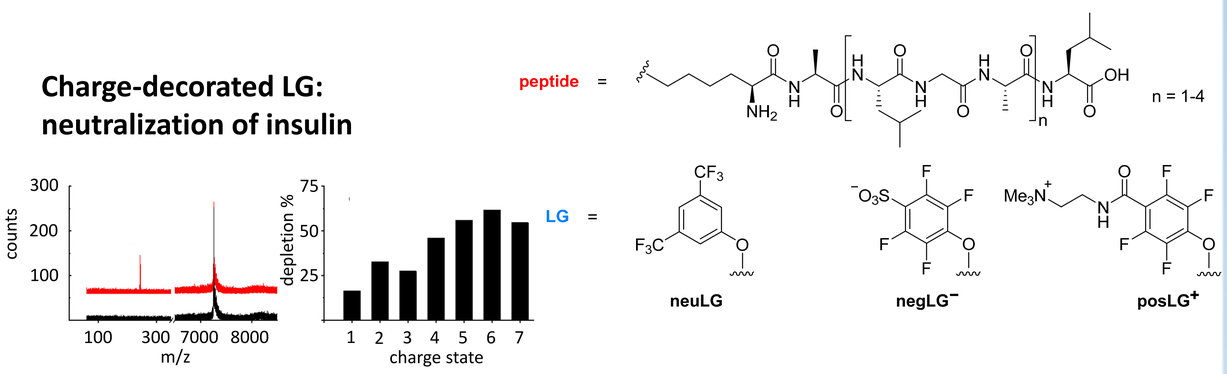
i) Nitrobenzylethers undergo cleavage at 266 nm in high vacuum.[2]
ii) The observed cleavage channel (heterolysis, homolysis, H-transfer) depends on the leaving group (charge-decorated vs. neutral) and peptide length.
iii) Tagged short-chain-peptides with non-charge-decorated leaving groups (**neuLG**) undergo heterolysis upon irradiation, as also observed by CID. Tagged long-chain-peptides with **neuLG** undergo H-transfer, resulting in an unchanged charge state.
iv) Tagged peptide with charge-decorated leaving groups (**negLG**$^-$ and **posLG**$^+$) cleave under H-transfer or homolysis, both resulting in charge removal.
v) Charge-decorated leaving groups enable the neutralization of insulin.
vi) The charge state of the parent ion affects the efficiency of the process. The higher the initial charge the more efficient is the cleavage.

Non-charge-decorated LG: varying peptide length



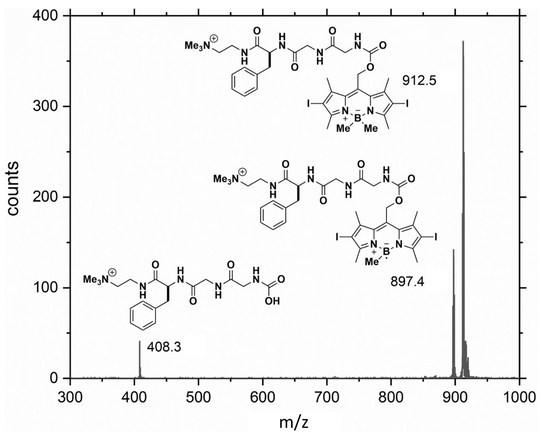


Charge-decorated LG: neutralization of insulin



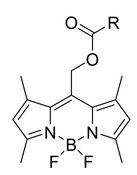
Tags for cleavage at 532 nm

i) Klán, Winter, Weinstain and coworkers reported a highly optimized bodipy-based photocage for cleavage in solution at 532 nm.[6]
ii) In the gas phase, photocleavage of a bodipy-tagged peptide occured under H-transfer, resulting in an unchanged charge state of the peptide. For the main cleavage channel the undesired loss of a methylgroup was observed.



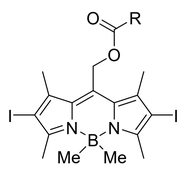
Enabling heterolysis at 532 nm

not tested



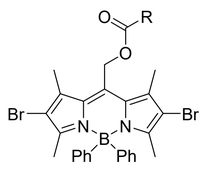
low ϕ_{cleave} in solution

cleaves under H-transfer
charge-decoration required



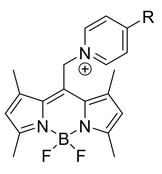
highly optimized photocage for solution-phase cleavage reported by Klan et al.

no gas-phase cleavage

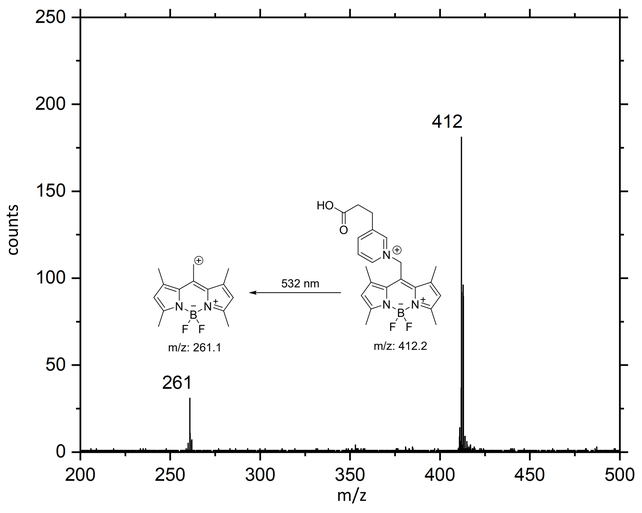


improved stability of B-C bond

cleaves under heterolysis
no charge-decoration required



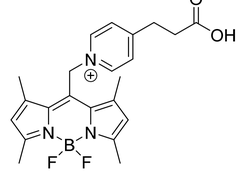
poorly photocleavable in solution



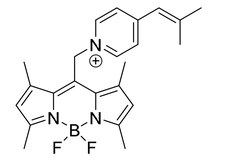
A pyridinium bodipy model compound cleaves efficiently under heterolysis in the gas-phase under irradiation at 532 nm.

Modification of peptides with bodipy-pyridinium tags

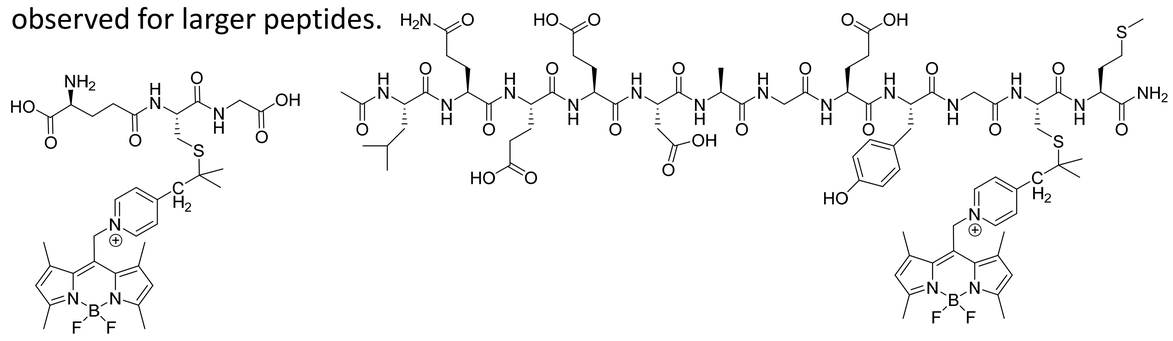
i) Pyridinium-bodipy tags are not stable in DMF and related solvents.
ii) Vinyl-pyridinium[7] derivatives can be successfully coupled to cysteine in aqueous buffer.
iii) The copmpounds remain cleavable also for larger peptide cargos (AA = 12).
iv) Competitive cleavage of the C-S bond is observed for larger peptides.



low stability in DMF
peptide coupling unsuccessful



cysteine-modification in aqueous buffer



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Acknowledgements

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