TOTAL SYNTHESIS OF LIPOPHILICALLY MASKED AND *CAGED* NAADP DERIVATIVES

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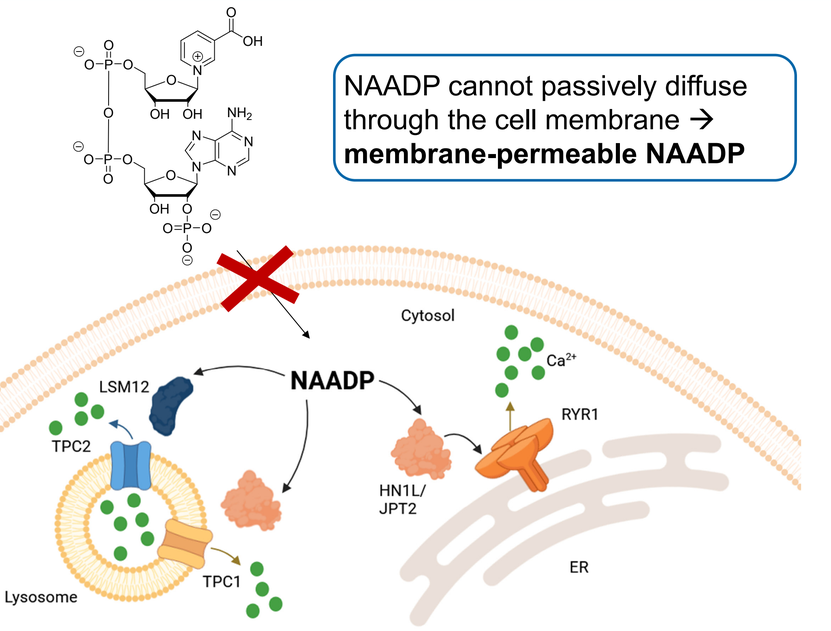
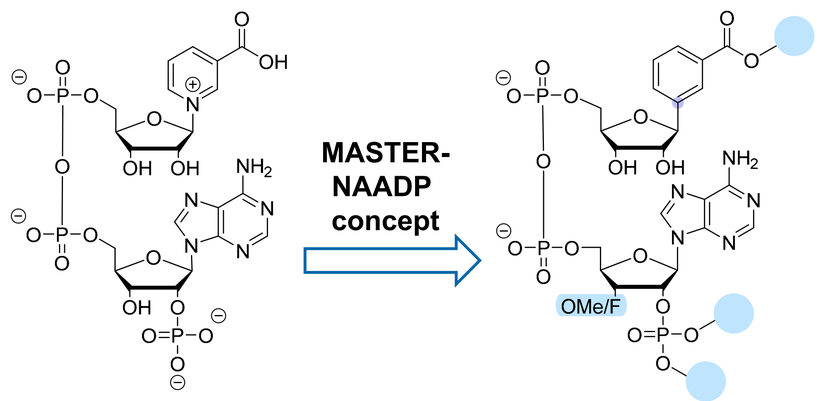
Introduction

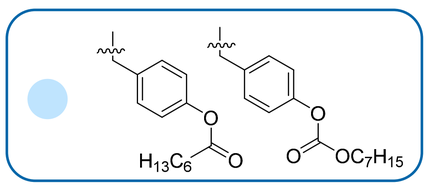
Nicotinic acid adenine dinucleotide phosphate (NAADP) is the most potent Ca^{2+}-releasing second messenger.[1] The intracellular release of Ca^{2+} by NAADP was verified in different eukaryotic cell types, e.g. in human T lymphocytes.[2] NAADP is a highly polar compound unable to penetrate cell membranes. In our group, a new **masked compound** was developed: **Membrane permeAble STabilized bio-rEversible pRotected NAADP** (MASTER-NAADP).[3] The MASTER concept includes several modifications leading to a **higher stability** compared to previously applied masking concept, e.g. the AM-approach.[4] The application of MASTER-NAADP leads to Ca^{2+} release in Jurkat T cells, confirming the **cell permeability** as well as the **release of the active compound**.[3] The aim of the current work is to further enhance the MASTER concept by incorporating **photo-cleavable** groups, which would allow precise spatial and temporal control of Ca^{2+} release.[5]



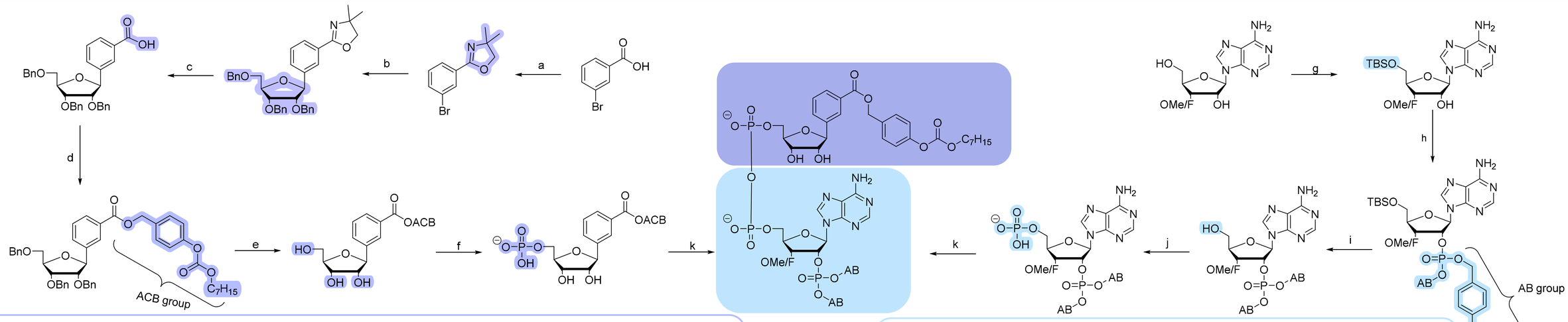
Created in BioRender.com based on GUSE[6]

Objective



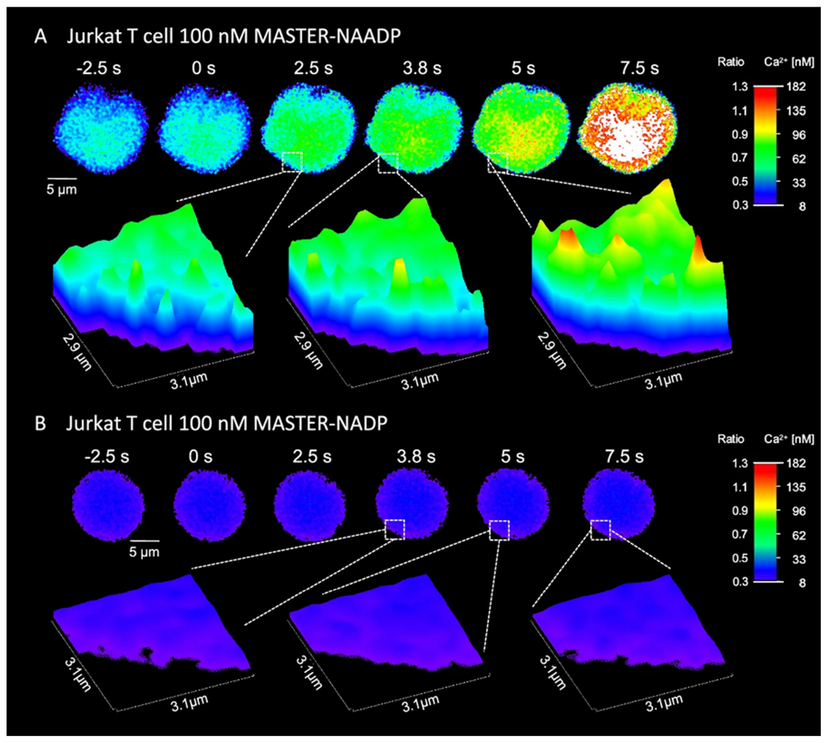


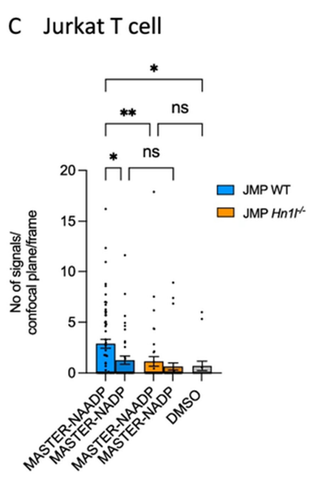
Results

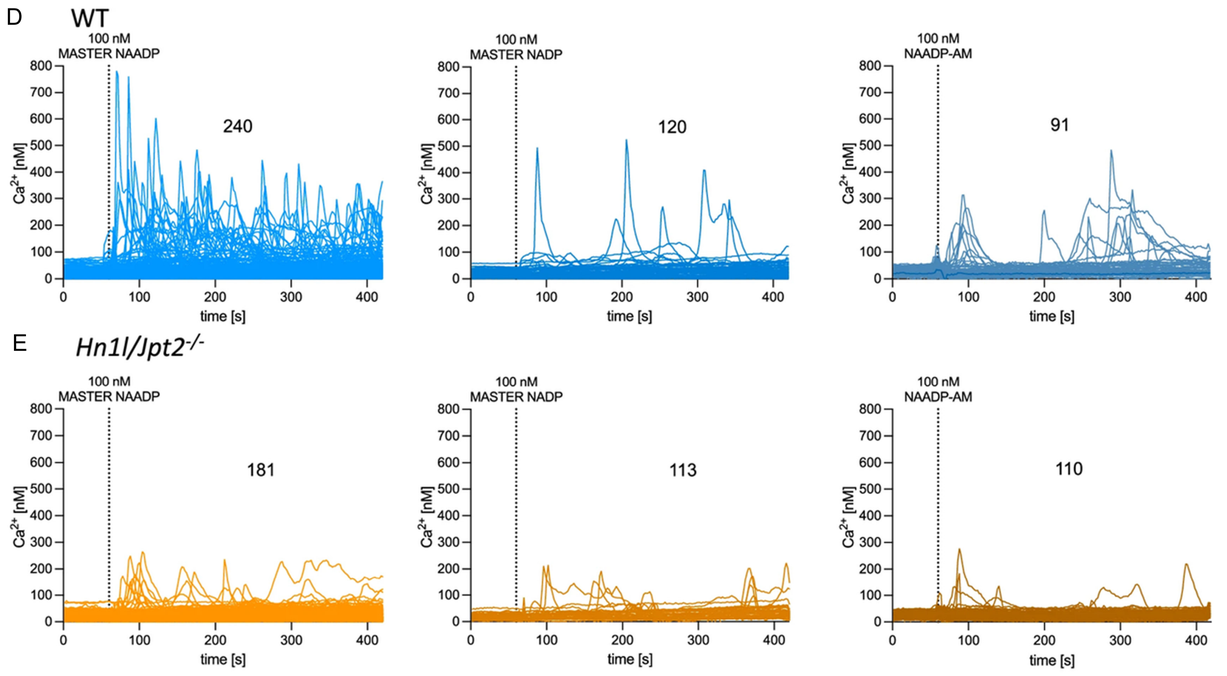


(i) $SOCl_2$, cat. DMF, 80 °C, 2 h, (ii) 2.0 eq. 2-amino-2-methyl propanol, CH_2Cl_2, rt, 2 h, (iii) $SOCl_2$, rt, 16 h, 83 %; b) (i) 2.3 eq. ^{t}BuLi, THF, - 78 °C, 45 min, (ii) 1.2 eq. $LaCl_3$·2LiCl, THF, - 78 °C, 0.5 h, (iii) 1.0 eq. 2,3,5-tri-*O*-benzyl-ribono-γ-lactone, THF, - 78 °C - rt, 18 h, (iv) 3.0 eq. Et_3SiH, 2.5 eq. BF_3·Et_2O, CH_2Cl_2, 0 °C - rt, 18 h, 49 %; c) (i) CH_3NO_2/MeI 2:1, reflux, 20 h, (ii) MeOH/KOH aq. (20 %) 1:1 v/v, reflux, 20 h, quant.; d) (i) 1.5 eq. TCBC, 1.5 eq. Et_3N, THF, rt, 0.5 h, (ii) 1.2 eq. ACB-mask, 0.30 eq. DMAP, THF, 70 °C, 3 h, rt, 16 h, 47 %; e) 3.5 eq. BCl_3 (1 M in CH_2Cl_2), CH_2Cl_2, - 78 °C - 0 °C, 2 h, 73 %; f) (i) 7.5 eq. $POCl_3$, 2.0 eq. proton sponge, TMP, 0 °C, 3.5 h, (ii) 26 eq. TEAB (1 M), (iii) RP-column, (iv) ion-exchange, (v) RP-column, 46 %.[4,7]

g) 1.5 eq. TBSCl, dry pyridine, 24 h, rt, 71 %; h) (i) 1.3 eq. amidite, 1.2 eq. pyridinium trifluoracetate, CH_2Cl_2, 50 min, rt, (ii) 1.5 eq. tert-butyl-hydroxyperoxide (5.5 M in decane), 0 °C, 1 h, 87 %; i) 5.2 eq. TREAT HF, CH_2Cl_2, 19 h, rt, 81 %; j) 4.0 eq. $POCl_3$, TMP, 6.5 h, 0 °C, 31 %; k) (i) 16 eq. Et_3N, 20 eq. TFAA, CH_3CN, 0 °C, 15 min, (ii) 1.0 eq. **"northern" monophosphate**, CH_3CN, 0 °C, 15 min, (iii) 6 eq. NMI, 10 eq. Et_3N, CH_3CN, rt, 15 min, (iv) 0.9 eq. **diphosphate**, CH_3CN/DMF 5:1, rt, 3 h, 57 %.[4,5,7]





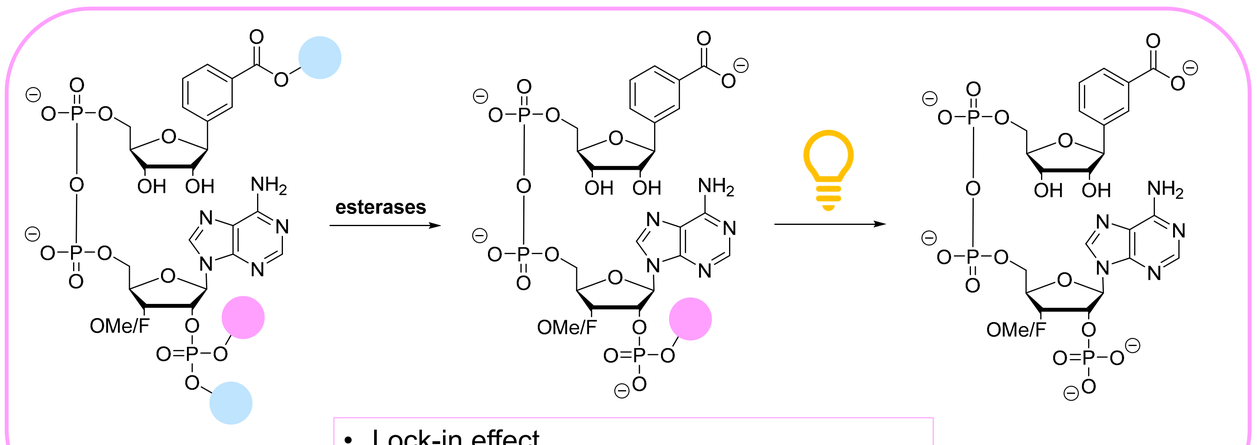


- Addition of 100 nM MASTER-NAADP to Jurkat T cells leads to formation of Ca^{2+} microdomains (A) and a global Ca^{2+} signal (D)
- MASTER-NADP as well as commercially available NAADP-AM do not release Ca^{2+} under the same conditions (B,E)
- Knockout of Hn1l/Jpt2 leads to a significant decrease in the Ca^{2+} signal (C, F)[3]

Conclusion

- Established total synthesis of a membrane permeable and stabilized NAADP derivative with high flexibility due to a convergent synthesis approach
- Active NAADP derivative is enzymatically released *in cellulo*
- Effects not due to cleaved masking groups as shown by controls
- Better performance compared to commercially available alternatives
- No spatial and temporal control
- Next step: introduction of a photo-cleavable protecting group (PPG)

Outlook



- Lock-in effect
- Better spatio-temporal control of release

Desired properties of the PPGs
- Absorption maximum > 400 nm
- Compatible with Ca^{2+} dyes
- Low cytotoxicity

→ Possible PPGs: coumarins, *o*-nitrobenzyl derivatives, BODIPY, xanthene derivatives…

References

[1] R. Aarhus *et al*. *Journal of Biological Chemistry* **1995**, *270*, 30327; [2] A. Gasser, S. Bruhn, A. H. Guse, *Journal of Biological Chemistry* **2006**, *281*, 16906; [3] S. Krukenberg, F. Möckl, *et al.*, *Nature Communications* **2024**, *15*, 8008; [4] T. F. Walseth, A. H. Guse, *Frontiers in Immunology* **2021**, 12, 703326; [5] M. Kock, master thesis, University of Hamburg, 2024; [6] A. H. Guse, *Cells* **2022**, *11*; [7] M. Yoshikawa, T. Kato, T. Takenishi, *Tetrahedron Letters* **1967**, *8*, 5065.

Acknowledgements

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