Non-Enzymatic Protein Catalyses Amide Bond Formation

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1 Principle Templated Amide Reaction

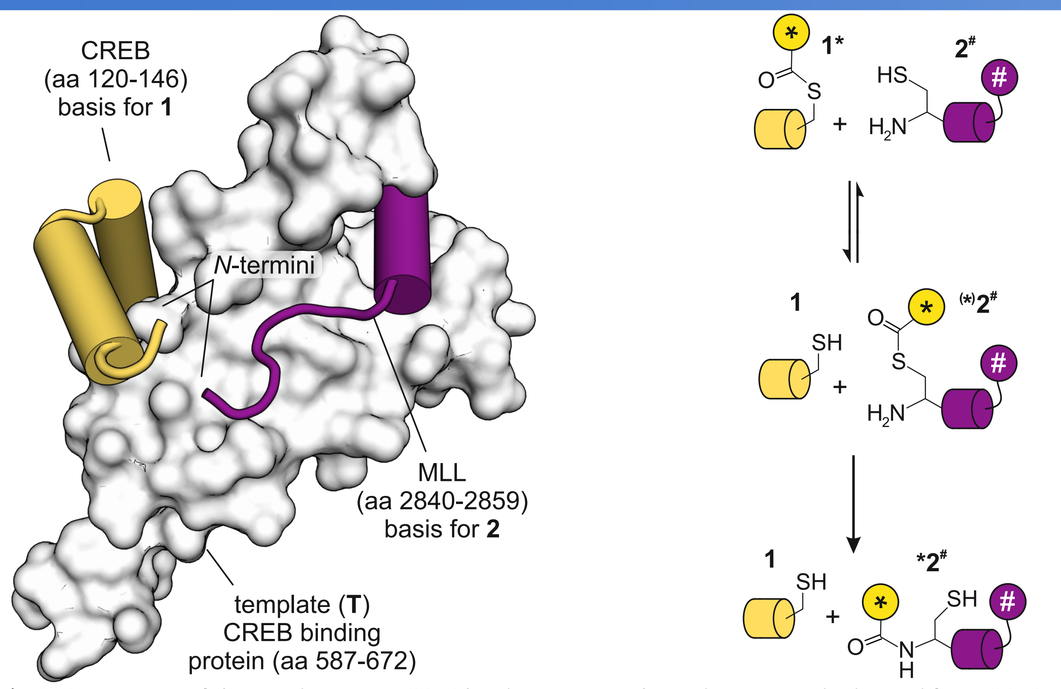

Figure 1: A) NMR-structure of the KIX domain or CREB binding proteins bound to a peptide derived from CREB and MLL (PDB ID 2lxt). N-termini of peptides are indicated. B) Principle of template-mediated transfer reaction based on native chemical ligation. * transferrable fluorescent group, # fluorescence quencher.

The spatial alignment of functional groups is a central aspect of most catalytic processes. Protein scaffolds with their exceptional molecular recognition properties have evolved into powerful biological catalysts. However, the rational design of artificial enzymes starting from noncatalytic protein domains proved challenging. Herein, we report the **use of a non-enzymatic protein as template for amide bond formation**. Starting from a protein adaptor domain capable of simultaneously binding to two peptide ligands, we designed **a catalytic transfer reaction based on the native chemical ligation**. This system was used for the **selective labelling of a target protein** validating its high chemoselectivity and potential as a novel tool for the **selective covalent modification of proteins**.

2 Peptide Design and Testing of Reaction

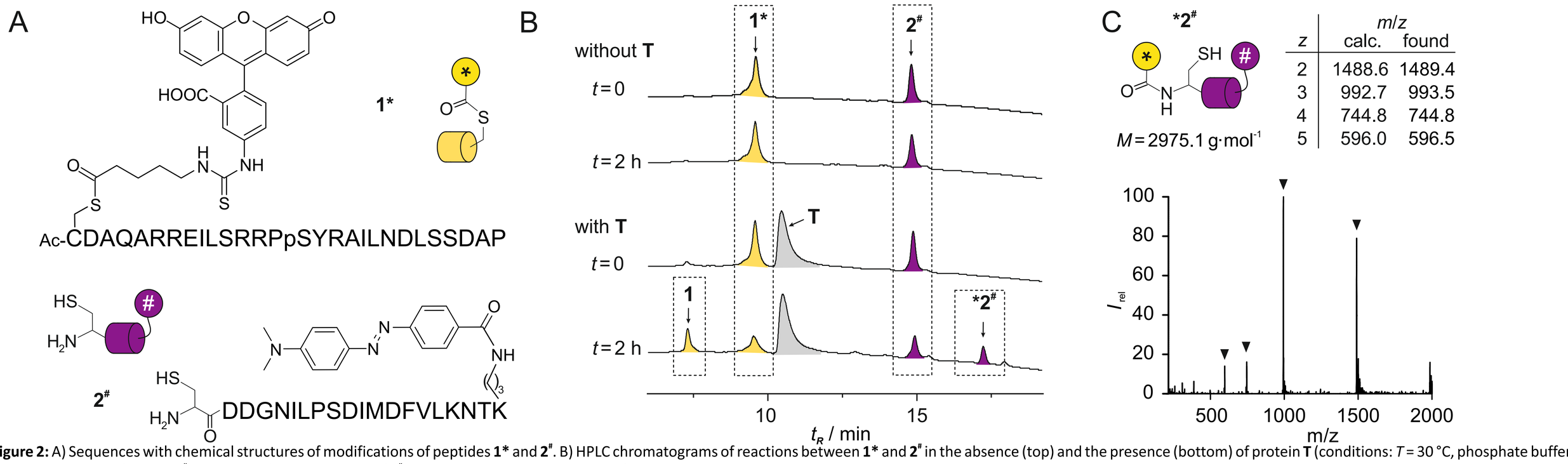

Figure 2: A) Sequences with chemical structures of modifications of peptides **1*** and **2**#. B) HPLC chromatograms of reactions between **1*** and **2**# in the absence (top) and the presence (bottom) of protein **T** (conditions: *T* = 30 °C, phosphate buffer, pH 7.4, *c* = 2.5 mM **1***, 5 mM **2**#, 2.5 mM **T**. C) MS spectrum of ***2**# including calculated and found m/z values.

3 Catalytic Cycle and Activity

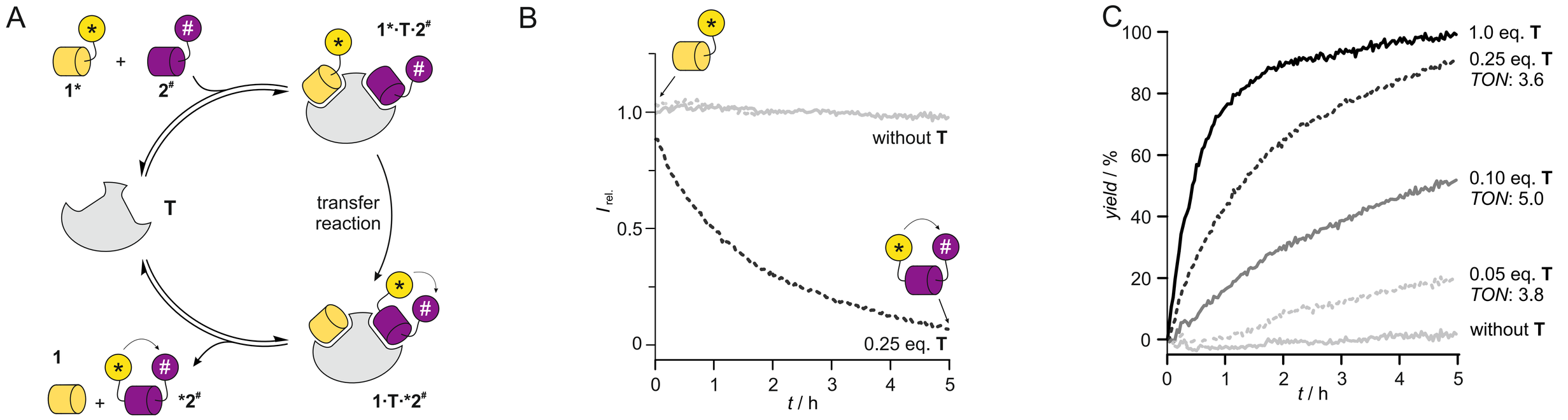

Figure 3: A) Scheme of catalytic cycle starting from **1*** and **2**# and providing reaction products **1** and ***2**#. B) Fluorescent readout of transfer reaction (conditions: *T* = 30 °C, phosphate buffer, pH 7.4, *c* = 5 mM **1***, 10 mM **2**#, 1.25 mM **T** (0.25 eq.). C) Reaction time course determined based on fluorescence intensity changes for different equivalents of **T** (conditions: *T* = 30 °C, phosphate buffer, pH 7.4, *c* = 5 mM **1***, 10 mM **2**#). Turnover numbers (TON) are provided.

4 Template-mediated Protein Labelling

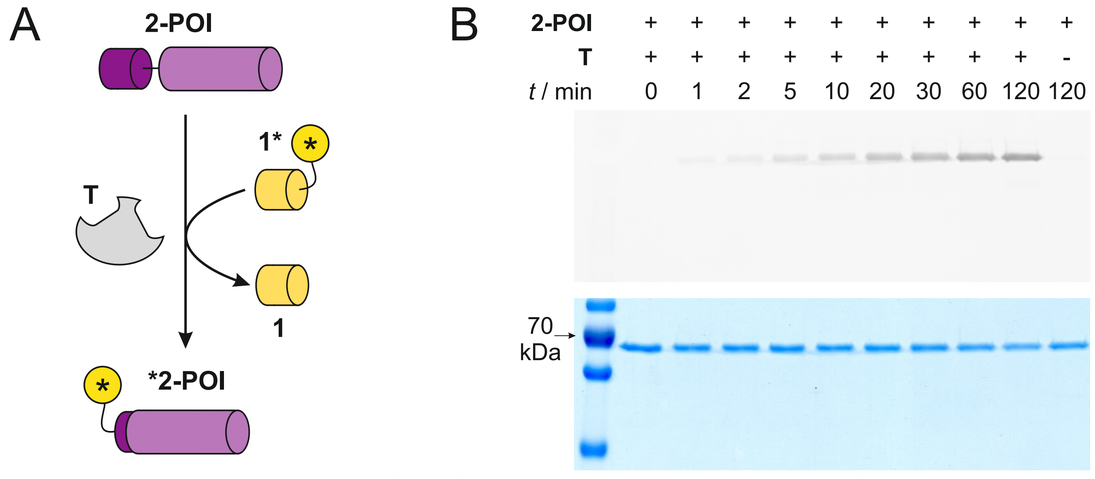

Figure 4: A) Scheme of T-mediated labelling of protein **2-POI**. B) SDS-PAGE analysis of time-dependence of labelling reaction with **1***, **2-POI** and **T** (each *c* = 1 mM, buffer: 20 mM sodium phosphate, pH 7.4, 500 mM TCEP, *T* = 30 °C). Top: Fluorescence imaging of gel indicating labelled protein ***2-POI**, Bottom: Coomassiestained gel indicative of total protein content **2-POI** and ***2-POI**.

Want to know more?

Support

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