

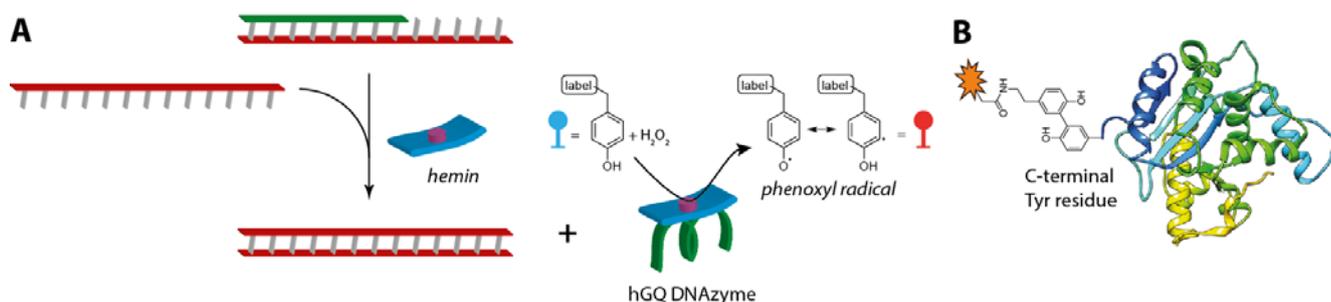
Group	: Nanochemical Biology
Project	: Tyrosine cross-coupling using trigger-activated DNAzymes
Supervisors	: Bauke Albada, Jorick Bruijns, and Floris van Delft

Keywords: artificial protein modification, DNA nanochemistry, tyrosine radicals

Horseradish peroxidase (HRP) is used to activate tyrosine residues in proteins, converting them into tyrosine radicals, which then cross-react with other amino acid residues (mostly tyrosine). A major drawback of the HRP is its high reactivity, leading to dirty products that are very difficult to purify; this drawback is circumvented using a HRP mimicking DNAzyme. Furthermore, the HRP enzyme is always ON, making it difficult to control the extent of protein reactivity. In this project we will tackle both of these problems using (i) proteins that have a uniquely reactive tyrosine residue at the C- or N-terminus, and (ii) a hidden HRP-mimicking hemin/G-quadruplex (hGQ) DNAzyme sequences that can be activated on demand. **The goal of this project is to selectively conjugate to the C- or N-terminus of a protein using activatable hGQ DNAzyme systems.**

Approach

Using various G-quadruplex forming DNA sequences, in combination with the commercially available biotin- and CF350-tyramine tags, we will study the effect of the activity of the hGQ DNAzyme on the labeling efficiency of the protein. For this, we will first use the laminarase A protein that is equipped with a C-terminal GGGGY tag (G₄Y). Using HPLC (and SDS-PAGE analysis), the efficiency of the labeling is quickly monitored, and the competing side-reaction that leads to homo-dimers of the tag and protein can be determined. Once the optimal hGQ DNAzyme sequence and labeling conditions are established, we apply a hidden hGQ DNAzyme that is only activated in the presence of an external trigger.



hGQ DNAzyme conjugation of tyramine-tags to C-terminally positioned exposed tyrosine residues. Panel A shows how the active HRP-mimicking hGQ DNAzyme is generated by a fuel-strand; panel B shows the product of the tyrosine-tyramine coupling.

Topics to be studied (including techniques to be applied)

- Study under what conditions and with which hGQ DNAzyme the protein is labeled most efficiently (HPLC, SDS-PAGE) (BSc/MSc).
- Study the site-selective modification of laminarase A using G₄Y-extended lamA and native lamA (LC-MS/MS) (BSc/MSc).
- Design a hidden hGQ DNAzyme sequence and study its trigger-dependent activation efficiency (UV-vis, fluorescence) (BSc/MSc).
- Design a switchable device that depends on various (biological) triggers (e.g. ATP, thrombin, cocaine, pesticides) (MSc).
- Apply this approach to antibodies in order to generate mono-functionalized antibody-tag conjugates (MSc).
- Evaluate the performance of the site-selectively anchored antibody on the surface with those that are randomly anchored (MSc).

Information

Bauke Albada, room Helix 8.057, tel. 0317-482365, e-mail: bauke.albada@wur.nl