Increasing the potency of biomaterials for tissue growth

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ABSTRACT

In recent years, it has been shown that synthetic biomaterials are not optimal for tissue repair due to their lack of many intrinsic biological properties. In order to improve the potency of biomaterials and increase the reach of tissue engineering (TE), tethered biomolecules such as proteins have been targeted to enhance the biochemical properties of the materials. However, when attached non-specifically (without control over which amino acids are modified) proteins suffer a drastic loss of activity [1,2]. Our goal is to be able to modify growth factors proteins in a highly specific manner, to provide biomaterials with the potent biological functionality required for cells to form functional new tissue.

OBJECTIVES

To target the growth factors FGF-2 and VEGF, a library of peptides will be used to perform ligand-directed chemistry at a single site on a protein (**Figure 1A**).[3,4] The peptide ligands will be modified with a catalyst that can attach a wide range of functional groups to the protein surface, including fluorophores, photocrosslinkable groups, or specific chemical groups for attachment to a biomaterial (**Figure 1B**). This chemistry should provide a more precise and unique way to modify each protein and allow their use in TE without a loss of activity.

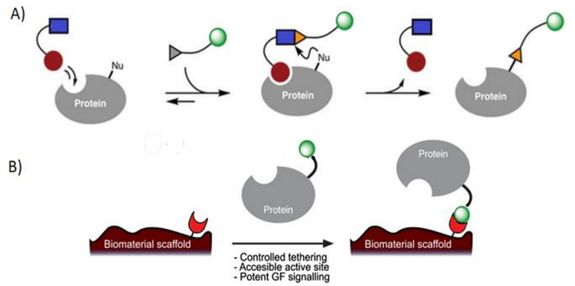

Figure 1: A) Scheme to modify selectively a protein through ligand-directed chemistry, B) Controlled tethering to a biomaterial, with in red a ligand, in blue the catalyst, in orange the ligand-directed target activated by the catalyst and in green the R-group being attached to Nu a nucleophilic amino acid nearby the active site

RESULTS

A. Synthesis of organic probes

In order to have specific targeted modification, two types of chemistry were developed. First thiol-maleimide reaction and second amidation reaction between an amine residue from the peptide with an activated carboxylic acid. The goal of the organic probes are to provide peptides with specific functionality such as diazirines (for photoaffinity labelling with UV light); fluorophores (for imaging and quantifying the binding strength of peptides to the protein). The different probes are presented in **Figure 2.**

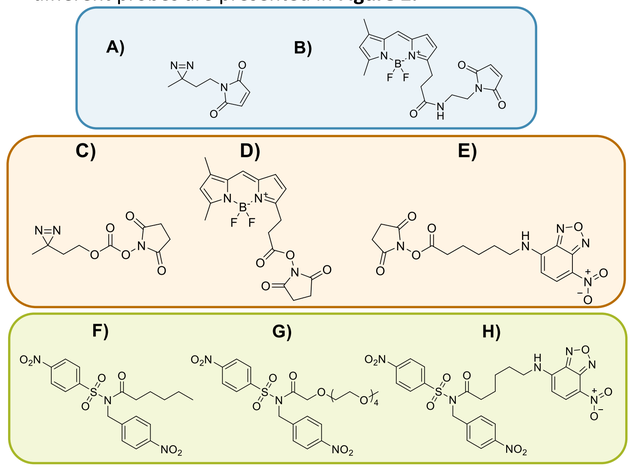

Figure 2: In blue, targets synthesized for thiol-chemistry with both A) diazirine and B) BODIPY chain groups; In orange, targets synthesized for amidation-chemistry with C) diazirine, D) BODIPY and E) NBD chain groups; In green, targets synthesized for ligand-directed chemistry with F) hexane chain, G) PEG and H) NBD chain groups

B. Synthesis of peptides

To develop the ligand-directed chemistry, peptides identified by phage display binding to the protein of interest have been synthesized in house on a solid-phase peptide synthesizer using Rink Amide MBHA to give C-amide terminus peptide on a 0.1 mmol scale. The different sequences have been synthesized in high purity with reasonable yield. Some biorthogonal groups were needed such as Alloc groups to ensure specific modification. Thus a deprotection step was conducted to obtain the amine needed for the reaction with the organic probes, **Figure 3.**

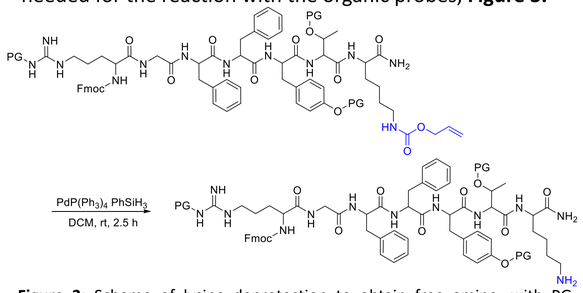

Figure 3: Scheme of lysine deprotection to obtain free amine, with PG protective group

Modification of amine residues was then performed on resin to introduce the groups presented in part A. or the oxime catalyst needed for ligand-directed chemistry, see **Figure 4.** [5]

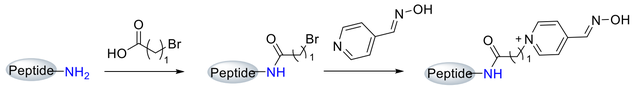

Figure 4: Scheme for pyridinium oxime modified peptide synthesis

C. Use of insertion groups: decomposition and imaging

After successful insertion of the groups onto the peptides of either diazirine or fluorophore, both were used for further analysis. For diazirine, the goal is to use them for photolabelling of the proteins, to study peptide binding. It appears that in half an hour the conversion of diazirine was complete with formation of several compounds whose ratio were depending on solvents, see **Figure 5**. For fluorophores, fluorescence and fluorescence polarization assays were done to show the efficiency of the probes when attached to a peptide and to quantify the binding strength of peptides to the protein, see **Figure 6**.

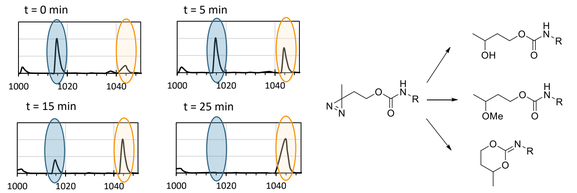

Figure 5: On left-hand side, LC-MS data showing disappearance of the diazirine specie (blue) to the new compounds presented on the the right-hand side (orange)

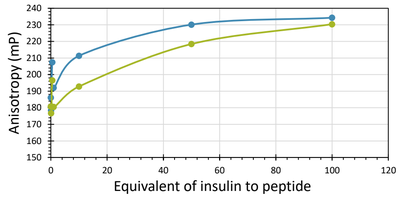

Figure 6: FP data obtained for CRGFFYT BODIPY peptide (in blue) and RGFFYTC BODIPY peptide (in green)

CONCLUSIONS

The chemistry has been developed showing encouraging result for further modification of protein using ligand-directed chemistry. Once the protein modified successful and obtained in high purity, cell study will be conducted in Biology using HEK-293 cell to see the influence of the modified protein.

ACKNOWLEDGMENTS

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