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## PEPTIDE- AND BIOTIN- OLIGONUCLEOTIDE-PYRROLE CONJUGATES FOR ELECTROCHEMICAL ADDRESSING ON SILICON CHIP

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**ABSTRACT:** The syntheses of pyrrole-oligonucleotide-peptide conjugates and pyrrole-oligonucleotide-biotin conjugates were described. The oligonucleotide moiety acted as an « active linker » which allowed the easy purification and quantitation of the conjugates and in turn controlled the grafting. The peptide conjugates were immobilised on a silicon array and their immunoreactivity was tested using biotinylated antibodies and a phycoerythrin-streptavidin staining. The biotin conjugate provided a fluorescence scale.

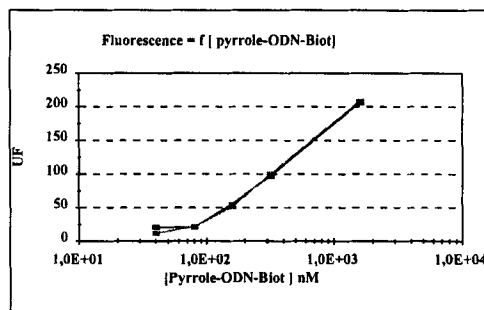
**Pyrrole-ODN conjugates:** Pyrrole-propionic acid N-hydroxysuccinimide ester was reacted with 4-N-(6-aminohexyl)-5-methyl-5'-O-DMT-2'-deoxycytidine<sup>1</sup> **1** and the resulting intermediate **2** (86% yield) was converted to the phosphoramidite **3** (80% purified yield)  $R_f=0.24$  &  $0.35$  in  $\text{CH}_2\text{Cl}_2/\text{AcOEt}/\text{TEA}(50:45:5)$ . The  $^1\text{H-NMR}$  (acetone- $d_6$ ) displayed characteristic signals at 6,8 ( $\alpha$ -pyrrole) and 6,06ppm ( $\beta$ -pyrrole). For the ODN synthesis, either a 4-N-(6-biotinamidoheptyl)- or a 4-N-(6-trifluoramidoheptyl)-5-methyl-2'-deoxycytidine unit<sup>1</sup> was introduced at the 3'end, after synthesis of a poly-T chain, the pyrrole-phosphoramidite **3** (0.1M in  $\text{CH}_3\text{CN}$ ) was coupled at the 5' position using the trityl-off option. After standard ammonia deprotection, the resulting 5'(<sup>Pyr</sup>dC-T<sub>13</sub>-dC<sup>bio</sup>) **4** was isolated by HPLC<sup>2</sup> ( $R_t=25\text{mn}$ ), the lipophilic character of the pyrrole residue allowed an easy separation from the failed sequences ( $R_t\sim 18.5\text{mn}$ ). UV( $\text{H}_2\text{O}$ ):  $\lambda_{\text{max}} = 265 \text{ nm}$  ( $\epsilon_{265}\sim 130\,000$  taking  $\epsilon_{265} = 9\,100$  for <sup>Pyr</sup>dC and <sup>bio</sup>dC,  $8\,400$  for T). MS (ES<sup>-</sup>):  $\text{M-H}^- = 5043.8$  (calc. :  $5044.79$ ). Similarly the aminoheptyl-ODN 5'(<sup>Pyr</sup>dC-T<sub>11</sub>-dC<sup>AH</sup>) **5** was isolated ( $R_t= 23.6 \text{ mn}$ )<sup>2</sup>. MS (ES<sup>-</sup>):  $\text{M-H}^- = 4209.4$  (calc. :  $4210.1$ ).

**Pyrrole-ODN-peptides conjugates:** The aminoethyl-ODN **5** (24 nmol) in 12  $\mu$ l of MOPS (50mM, pH 7.6) was treated with disuccinimidyl suberate (2.5  $\mu$ mol in 25 $\mu$ l DMF) overnight at 4°C, desalted (NAP 10) and concentrated by n-Butanol extraction.

The unmodified ACTH fragment 11-24 (65 nmol in 100 $\mu$ l MOPS) was added to the ODN. The pyrrole-ODN-ACTH(11-24) conjugate **6** was isolated ( $R_t$  = 26.4 mn)<sup>3</sup>. MS (ES<sup>-</sup>) : M-H<sup>-</sup> = 5999.4 (calc. 6000.33). Similarly an ACTH(18-39) conjugate **7** was made.

**Addressing of Pyrrole-ODN conjugates on silicon chip:** The conjugate **6** and **7** (1.4 $\mu$ M in 20 mM pyrrole), were electropolymerised on electrodes 5/7 and 4/6/8/9 respectively of a 48 x 50 $\mu$ m<sup>2</sup> electrodes silicon chip as described earlier<sup>4</sup>. The chip was incubated either with biotinyl-MAB(18-24) or biotinyl-MAB(34-39) (10 $\mu$ g/ml PBS, RT, 45mn) and then with SA-PE<sup>4</sup>. Epifluorescence<sup>4</sup> showed that MAB(18-24) gave positive signal on dots 4 to 9 and MAB(34-39) gave positive signals only on dots 5/7 as expected.

Conjugate **5** was electropolymerised as above (duplicates) using the respective concentrations 1.6 $\mu$ M, 320, 65, 32 and 16 nM. After incubation with SA-PE, fluorescence intensities measured on spots correlated well to the initial conjugates concentration (See graph).



**CONCLUSIONS:** These results show that the conjugation of biomolecules to a pyrrole-ODN scaffold, allow simple gel exclusion purification (increased M.W), HPLC purification at neutral pH and easy quantitation by UV absorption. Electropolymerized peptides conjugates retain their immunoreactivity. Control of the pyrrole/biotin-conjugate ratio during the polymerization give rise to a fluorescent scale on the chip (quantitation).

## REFERENCES

1. Roget, A., Bazin, H. and Teoule, R. *Nucleic Acids Res.*, **1989**, *17*, 7643-7651.
2. RP-18E (10 $\mu$ m) 250X10 ; 10% to 16% CH<sub>3</sub>CN in 50mM TEAA in 20mn, 5ml/mn.
3. RP-18E (5 $\mu$ m) 125X4 ; 10% to 18% CH<sub>3</sub>CN in 20mn, then to 27% in 15mn, 1ml/mn.
4. Livache T., Fouque B., Roget A., Marchand J., Bidan G., Teoule R. and Mathis G. *Anal. Biochem.*, **1998**, *255*, 188-194.