Labeling of neuroregulin 1β extracellular domain with ^{89}Zr and stability studies.

V. Radchenko[1], D. Vugts[2], G. A. M. S. van Dongen[2], W Wozny[3], A. Schrattenholz[3], F. Rösch[1]

[1]Institute of Nuclear Chemistry, Johannes Gutenberg-University Mainz, D-55128 Mainz, Germany;
[2]VU University Medical Center, Nuclear Medicine & PET Research, Amsterdam, The Netherlands;
[3]Proteosys AG Mainz, D-5519 Mainz, Germany

Introduction: The neuregulins are a family of four structurally-related proteins of growth and differentiation factors with a wide range of functions in the nervous system [1]. Neuregulin 1 is one of four proteins in the neuregulin family produced in numerous isoforms by alternative splicing, which allows performing a wide variety of functions. Neuregulin 1 interactions are thought to play an important role in the pathological mechanism of diseases such as multiple sclerosis, schizophrenia and traumatic spinal cord and brain injuries.
Positron emission tomography (PET) is an attractive tool which can be applied for visualization of *in vivo* mechanisms and processes of biomolecules. Aim of this project was the development of a radiolabeling for neuroregulin 1β for future application in PET imaging.

Experimental: For this study, neuregulin 1β extracellular domain (NRG 1β ECD) (kindly provided by Proteosys AG) 1.58 mg/ml in PBS (pH 7.4) was used. NRG 1β ECD was modified through the N-SucDf method [1]. In short, the chelator deferoxamine (Df) was converted into N-succinyl-deferoxamine B (N-sucDf) and hydroxamate groups of N-sucDf were temporarily blocked with Fe(III). N-sucDf-Fe was esterified with TFP, and subsequently TFP-N-sucDf-Fe was coupled to the NRG 1β ECD (200 µg). Thereafter, Fe(III) was removed by trans-chelation to ethylene-diaminetetraacetic acid (EDTA) (formation of [Fe(III)EDTA]). Chelate-to-NRG ratio was monitored by HPLC via absorption of Fe^{3+} at UV line 430 nm. After modification of the NRG, labeling with ^{89}Zr was applied.
^{89}Zr ($T_{1/2}$=78.4 h, β^+=22.6%, in 1 M oxalic acid) was produced by BV Cyclotron VU (Amsterdam, The Netherlands) by (p,n)-reaction on natural yttrium-89 and isolated via a hydroxamate column.
^{89}Zr-labeling was performed at room temperature in a volume of 2 ml for 60 min; to 200 µl ^{89}Zr (15-17 MBq) solution 90 µl 2 M Na_2CO_3 solution was added to neutralize oxalic acid. After 3 min, 300 µl 0.5M HEPES buffer solution (pH 7.0), 710 µl NRG-N-sucDf (200 µg) and 700 µl 0.5M HEPES (pH 7.0) were added. At 60 min, the labeling mixture was analyzed by ITLC (strips "BIODEX" Model #150-771) using a solution of 450 µl 20mM citric acid and 50 µl of acetonitrile as mobile phase. HPLC was carried out with 0.005 M phosphate buffer, 0.15 M NaCl, 0.01 M NaN_3 as elution buffer, flow rate: 0.5 ml/min (Column: Phenomenex®, BioSep-SEC-S 2000, 300 x 7.8 mm).
The product was purified by size exclusion chromatography (SEC, PD-10 column) using 0.9% sodium chloride solution as the mobile phase. Stability studies were performed in buffer solution at 4°C for 4 days.

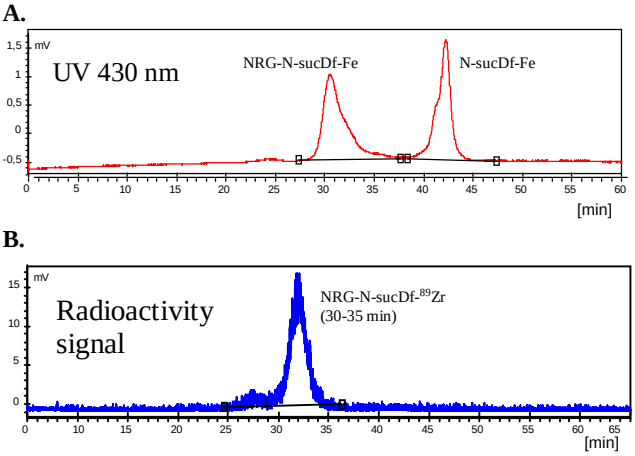

Figure 1. HPLC profiles **A.** Determination of chelate-to-NRG ratio via UV absorption of Fe^{3+} by line 430 nm. **B.** Labeling of NRG 1 β ECD with ^{89}Zr after 60 min (radioactivity signal).

Results: HPLC analysis showed a coupling yield of around one Df-molecule per molecule of neuregulin 1 β ECD (Fig. 1A).
The labeling yield at 30 min was 78% (ITLC). After 60 min, the ITLC analysis showed 94.6% of the labeled product while a HPLC profile showed more than 96% labeling (Fig. 1B). The purity of product after SEC purification was higher than 99% (HPLC, ITLC). Stability studies of the ^{89}Zr-labeled product performed at 4 °C in elution buffer (0.9% saline buffer) demonstrated more than 80% of unaltered parent compound.

Conclusions: The results indicate the availability of ^{89}Zr-labeled neuregulin 1 β ECD which enables its use in PET as suitable tool for visualization of biological proprieties of the neuregulin peptid family.
Furthermore, this approach is also suitable for other positron emitters (^{68}Ga, ^{90}Nb) and allows variations in the PET imaging protocols.

References

[1] Esper R.M. *et al., J Brain Res. Rev.*2006, 51, 161-175.
[2] Verel I. *et al., J. Nucl. Med.* 2003, 44, 1663–1670.

Acknowledgement

This collaborative project is kindly supported by the COST Action BM0607. NRG 1β ECD was kindly provided by Proteosys AG Mainz.