

Specific nanotargeting of bovine Y-chromosome bearing sperm

R. Mancini^{1*}, L. Gamrad², D. Werner², D. Tiedemann¹, U. Taylor¹, C. Rehbock², S. Klein¹,
W. Kues¹, S. Barchikowsky² and D. Rath¹

¹. Institute for Farm Animal Genetics, Friedrich-Loeffler-Institut, Höltystr. 10, 31535 Neustadt, Germany

². Technical Chemistry I and Center for Nanointegration Duisburg-Essen (CENIDE), Universitaetstr. 7, 45141 Essen, Germany

*corresponding author: mancini@tzv.fal.de

Abstract. The current method available to separate populations of X- and Y-chromosome bearing sperm in bovine is based on relative DNA differentiation by flow cytometry. Within the research of a new technique for bovine sperm sexing, we analyzed a new approach based on laser generated gold nanoparticles (AuNPs), functionalized with triplex forming oligonucleotide probes (TFO) to target enriched sequences on the bovine Y-chromosome. The bio-conjugate was made by 6nm AuNPs labeled to TFO hybridizes via triplex formation to bovine XY genomic DNA. These gold nanoparticles agglomerate in situ in a specific qualitative manner. When DNA is condensed in chromatin structure like in sperm nuclei, the probe does not hybridize on Y-chromosome bearing sperm nuclei in a significant manner respect to X-ones. More studies are needed to improve signaling of AuNPs in sperm nuclei.

Keywords: Gold nanoparticles, sperm sexing, triplex forming oligonucleotides

Introduction

Sperm sexing by flow cytometry allows the separation of Y-chromosome bearing sperm from X-ones in order to facilitate a sex pre-selection of the offspring in mammals [1]. The approach is currently commercially available for dairy cows. It is based on relative DNA differentiation between X- and Y-chromosome bearing sperm by flow cytometry and the use of fluorescent DNA dyes [2]. A new approach is needed to avoid toxic DNA dyes and gold nanoparticles may represent a novel fluorophore-alternative. Our study analyzes the possible use of AuNPs as signaling devices to detect the Y-chromosome in bovine sperm. For sperm sex sorting solving the problem of DNA toxic dyes. AuNPs conjugated to oligonucleotides have been the topic of intensive research as for therapeutic drugs or marker devices [3-9]. AuNPs functionalized with oligonucleotides may allow the detection of specific sequences in vital cells or selecting haploid gametes that trigger desirable genetic traits [3, 5, 10].

Triplex forming oligonucleotides (TFOs) are short ssRNA or ssDNA probes of about 20-25bp, which hybridize to double stranded target DNA avoiding its denaturation [11, 12]. TFOs hybridize poly-purine sequences on the DNA target called triplex target sites (TTS) through Hoogsteen hydrogen bonds [11, 13]. Chemical modifications of DNA nucleotides such as locked nucleic acids (LNA) and 5-methyl-cytosine are necessary to increase TFOs hybridization efficiency to dsDNA under physiological condition [14-16].

TFO probes have been used to trigger AuNPs onto specific genomic sequences leading to local aggregations and therefore transforming nanoparticles into signaling devices for DNA sequences [9, 17]. AuNPs change in surface plasmon resonance (SPR) when they are pulled closer to each other on a triplex hybridization, this phenomenon is called bathochromic effect. Y-chromosome specific hybridization results in a AuNPs agglomeration and therefore in a change in absorbance.

Materials and Methods

In this study we identified a 20bp triplex target site characterized by a cytosine content of 35% and enriched on the bovine Y-chromosome. The target sequence was 5'-AGAAAGGAAGAAGAGGAAAG and the TFO probe sequence was 5'-(thiol)-TTTTTTTTTXYXYXXYTXYYTXXTTYX. Deoxycytidine residues (X) and locked nucleic acids (Y) were used to improve probe stability and enhance the triplex hybridization at a

physiologic condition [4, 14, 15, 18]. A 10 thymine base spacer was added to separate the TFO from the AuNP. A thiol group was added on the 5' prime of the TFOs to immobilize the LNA probes onto the gold nanoparticles. Laser generated gold nanoparticles were synthesized as described by Barcikowski et al. [3, 19] with a size of 6.5 ± 1.1 nm in diameter. Ex situ conjugation was performed to label the TFO onto the nanoparticles [3]. Hybridization on free genomic DNA was performed incubating 22.6 $\mu\text{g/mL}$ of AuNP/TFO with 26 $\text{ng}/\mu\text{L}$ of genomic DNA respectively of male and female cattle. A second hybridization assay was performed incubating 22.6 $\mu\text{g/mL}$ of AuNP/TFO conjugate with 1×10^6 sorted sperm nuclei. Incubation took place at 19 °C in 1x PBS buffer pH 7. Hybridization was evaluated through the change in absorbance by a UV-VIS analysis after 1 hour of incubation, from a wavelength of 200 up to 700 nm. Conjugate synthesis and SPR analyses were performed by Center for Nanointegration of Duisburg-Essen (CENIDE).

Results

Fig. A shows the result of the absorbance peak of the sample AuNPs-TFO incubated with male and female gDNA. The conjugate agglomeration on XY genome (blue curve) results with a shift in absorbance of 10 nm respect the probe alone (black curve). The conjugate agglomeration on XX has an absorbance that shifts of 1 nm (red curve) respect the probe alone. The shift of the XY-peak is of 9 nm higher than the XX-one. Fig. B shows the result of the absorbance peak of the samples AuNPs-TFO incubated respectively with X- and Y-chromosome bearing spermatozoa. The absorbance peak of the sample AuNPs-TFO with Y-chromosome bearing sperm is of 534 nm (blue curve), results with a shift in absorbance of 10 nm respect the probe alone (black curve). The conjugate agglomeration on X-chromosome bearing sperm is of 533 nm (red curve), results in a absorbance that shifts of 9 nm (red curve) respect the probe alone. The difference between the Y- and X-chromosome bearing sperm hybridized with AuNPs-TFO is 1nm.

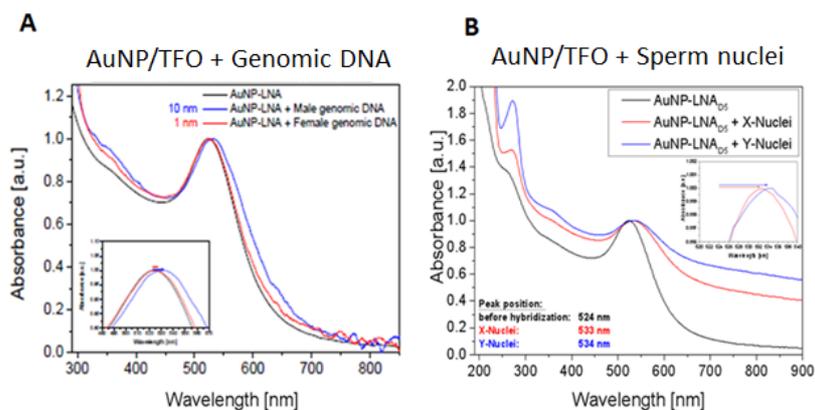


Fig. 1: UV-Vis analysis of AuNPs hybridization on genomic DNA and on sperm nuclear matrix. A) Hybridization of AuNPs/TFO to female and male bovine genomic DNA and. Blue curves indicate AuNPs-TFO hybridization to male gDNA, while the red curve indicates the female gDNA. Black curve shows the AuNPs-TFO alone. Small inner squares show the peak position at different wavelength of the different probes. B) Hybridization of AuNPs-TFO incubated with Y- and X-chromosome bearing sperm nuclear matrix. Blue curve indicates AuNPs-TFO wavelength when incubated with Y-chromosome sperm nuclear matrix, the red curve indicates the X-chromosome sperm sample. In black, the curve of the AuNPs-TFO alone. Small inner square represents the peak position at respective wavelength.

Conclusions

Six nanometer laser generated AuNPs do not interfere with the hybridization on genomic DNA free in solution at pH7. LNA probes conjugated to $\sim 6\text{nm}$ AuNPs selectively hybridize on XY genome and consequently trigger AuNPs on a specific genomic loci. Y-bearing sperm nuclei do not show a significant surface plasmon resonance shift compared to X-chromosomes. More studies are required to confirm and enhance AuNPs/TFO hybridization on genomic DNA in sperm chromatin status.

References

- [1] Gledhill, B.L., Selection and separation of X- and Y- chromosome-bearing mammalian sperm. *Gamete Res*, 1988. 20(3): p. 377-95.
- [2] Durand, R.E. and P.L. Olive, Cytotoxicity, Mutagenicity and DNA damage by Hoechst 33342. *J Histochem Cytochem*, 1982. 30(2): p. 111-6.
- [3] Barchanski, A., et al., Golden perspective: application of laser-generated gold nanoparticle conjugates in reproductive biology. *Reprod Domest Anim*, 2011. 46 Suppl 3: p. 42-52.
- [4] McKenzie, F., K. Faulds, and D. Graham, LNA functionalized gold nanoparticles as probes for double stranded DNA through triplex formation. *Chem Commun (Camb)*, 2008(20): p. 2367-9.
- [5] McKenzie, F., K. Faulds, and D. Graham, Sequence-specific DNA detection using high-affinity LNA-functionalized gold nanoparticles. *Small*, 2007. 3(11): p. 1866-8.
- [6] Anstaett, P. and G. Gasser, Peptide nucleic acid - an opportunity for bio-nanotechnology. *Chimia (Aarau)*, 2014. 68(4): p. 264-8.
- [7] Torabi, S.F. and Y. Lu, Functional DNA nanomaterials for sensing and imaging in living cells. *Curr Opin Biotechnol*, 2014. 28: p. 88-95.
- [8] Veigas, B., A.R. Fernandes, and P.V. Baptista, AuNPs for identification of molecular signatures of resistance. *Front Microbiol*, 2014. 5: p. 455.
- [9] Giljohann, D.A., et al., Gold nanoparticles for biology and medicine. *Angew Chem Int Ed Engl*, 2010. 49(19): p. 3280-94.
- [10] Brown, S.D., et al., Gold nanoparticles for the improved anticancer drug delivery of the active component of oxaliplatin. *J Am Chem Soc*, 2010. 132(13): p. 4678-84.
- [11] Besch, R., et al., Characterization and quantification of triple helix formation in chromosomal DNA. *J Mol Biol*, 2004. 341(4): p. 979-89.
- [12] Boutorine, A.S., et al., Fluorescent probes for nucleic Acid visualization in fixed and live cells. *Molecules*, 2013. 18(12): p. 15357-97.
- [13] Boutorine, A.S. and C. Escude, Biophysical analysis of triple-helix formation. *Curr Protoc Nucleic Acid Chem*, 2007. Chapter 7: p. Unit 7.12.
- [14] Brunet, E., et al., Intercalator conjugates of pyrimidine locked nucleic acid-modified triplex-forming oligonucleotides: improving DNA binding properties and reaching cellular activities. *Nucleic Acids Res*, 2005. 33(13): p. 4223-34.
- [15] Jepsen, J.S. and J. Wengel, LNA-antisense rivals siRNA for gene silencing. *Curr Opin Drug Discov Devel*, 2004. 7(2): p. 188-94.
- [16] Petersen, M. and J. Wengel, LNA: a versatile tool for therapeutics and genomics. *Trends Biotechnol*, 2003. 21(2): p. 74-81.
- [17] Boisselier, E. and D. Astruc, Gold nanoparticles in nanomedicine: preparations, imaging, diagnostics, therapies and toxicity. *Chemical Society Reviews*, 2009. 38(6): p. 1759-1782.
- [18] Kaur, H., et al., Thermodynamic, counterion and hydration effects for the incorporation of locked nucleic acid (LNA) nucleotides in duplex. *Nucleic Acids Symp Ser (Oxf)*, 2008(52): p. 425-6.
- [19] Barcikowski, S. and G. Compagnini, Advanced nanoparticle generation and excitation by lasers in liquids. *Phys Chem Chem Phys*, 2013. 15(9): p. 3022-6.