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Synthesis and Characterization of Ultra-Small Gold Nanoparticles: Midatech Pharma Midacore™ Platform

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About Midatech Pharma

Midatech Pharma is a nanomedicine company focused on the research and development of improved chemotherapeutics or new immunotherapeutics. Midatech is advancing a pipeline of clinical and pre-clinical product candidates based on its drug delivery technologies. Among its portfolio, Midatech owns the **Midacore™** platform: a proprietary technology based on ultra-small GNP.

About Nanocarb

Nanocarb is a Marie Sklodowska Curie European Training Network that brings together over a dozen leading European partners providing multidisciplinary training in biomedical glycoscience, nanotechnology and its industrial applications to a new generation of young scientists. It focuses on the development of carbohydrate-functionalized nanoparticles for a wide range of medical applications.

Introduction: Multifunctional Ultra-Small Gold Nanoparticle Platform

Gold nanoparticles (GNP) are a platform of interest with a broad range of applications in curative and preventive medicine (e.g.: cancer: tumor active or passive targeting, vaccine: antigen carrier) [1]. The system allows the presentation in high quantity of virtually any type of organic molecule on the surface of a gold core (Figure 1). The multivalent presentation of ligands, such as carbohydrates or proteins, can trigger a **cluster effect**, which allows to overcome the low affinity of the individual ligands towards their receptors [2].

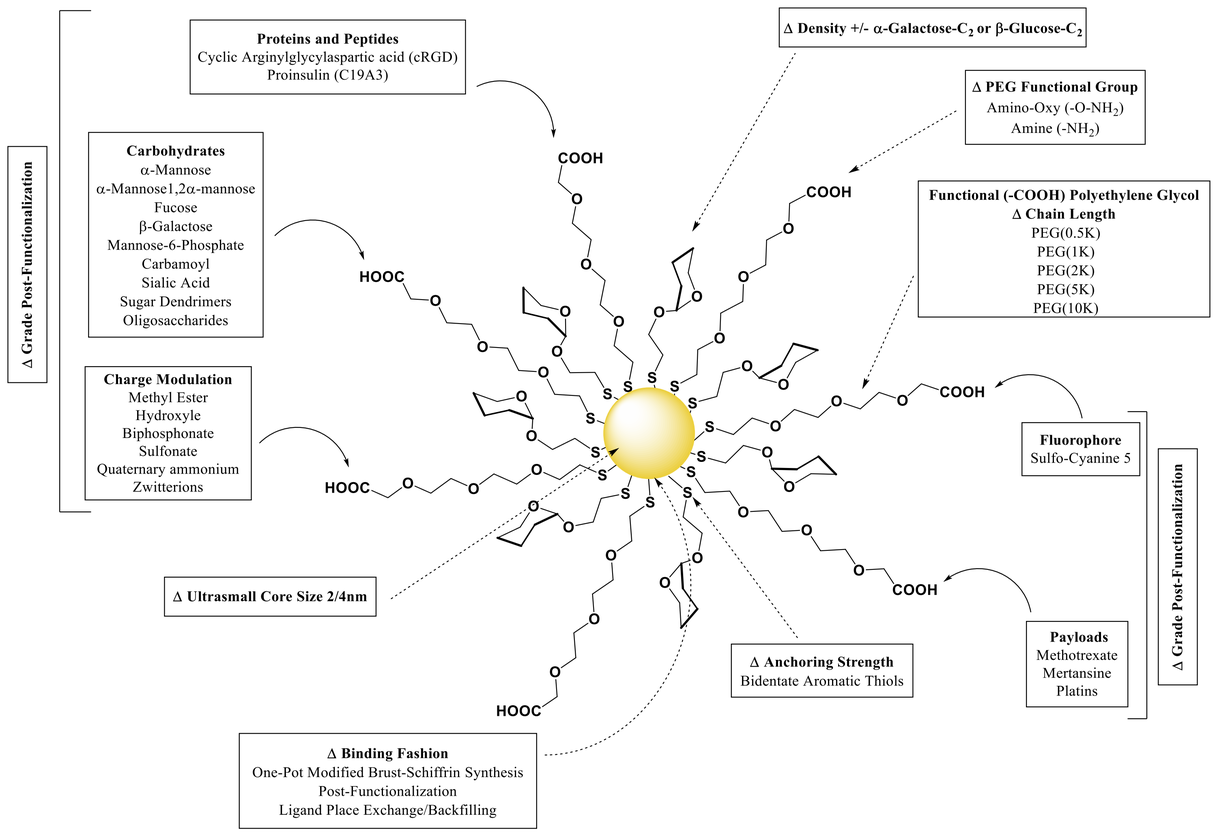


Figure 1: Overview of Midatech Pharma Multifunctional Ultra-Small Gold Nanoparticle Platform

Synthesis and Purification

Ultra-small GNPs are synthetized at medium scale using a modified **Brust-Schiffrin** method in a Syrris Atlas benchtop reactor. A gold salt is reduced by $NaBH_4$ in the presence of thiol or disulfide ligands in a "one pot synthesis" [3]. Resulting GNPs are purified by ultrafiltration using Repligen KR2i **Tangential Filtration Flow (TFF)** system [4]. Both synthesis and purification are performed in an automated fashion. The GNP surface can then be modified using biorthogonal chemistry: **Post-Functionalization** or **Ligand Place Exchange** (LPE).

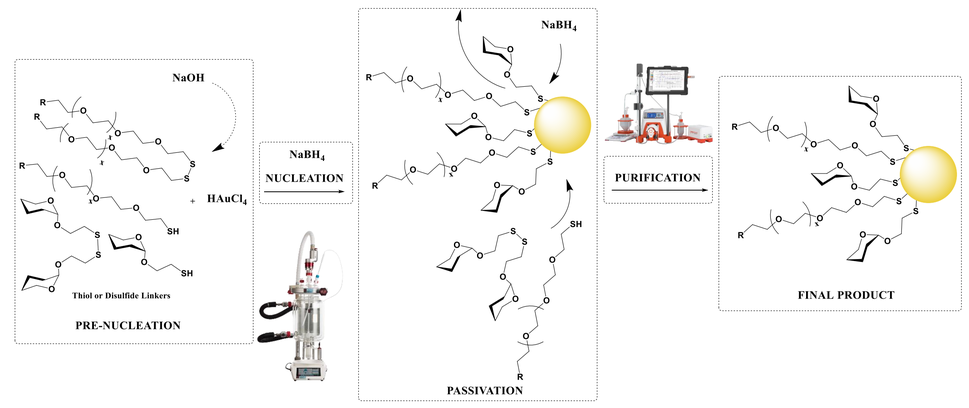


Figure 2: Overview of the automated one pot **Brust-Schiffrin** synthesis and purification

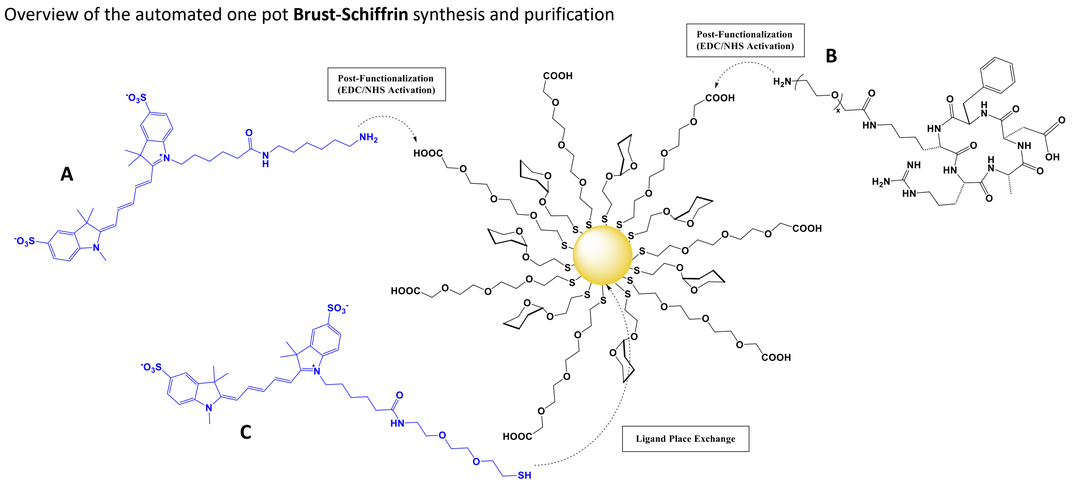


Figure 3: Examples of Post-Functionalization with Sulfo-Cy5 amine **(A)** and cRGD amine **(B)**. Ligand Place Exchange (LPE) with Sulfo-Cy5 PEG-SH **(C)**

Characterization

LC-CAD

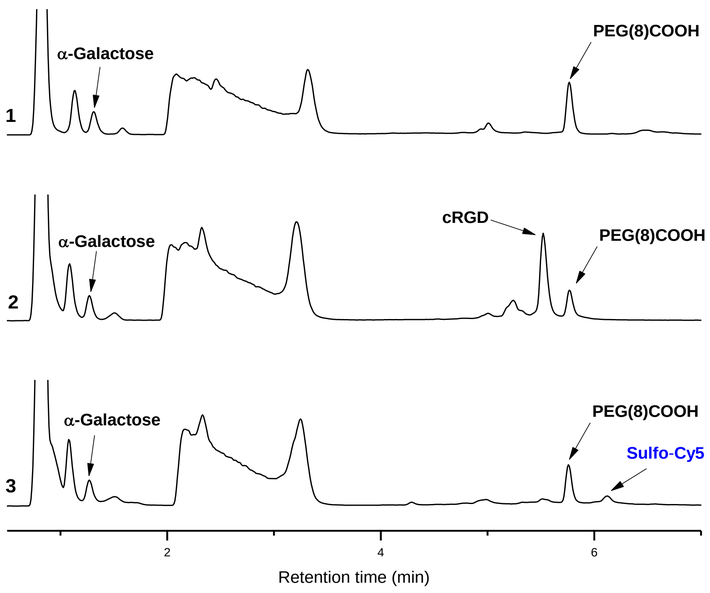


Figure 4: **LC-CAD** chromatograms after TCEP/KCN treatment of $(PEG(8)COOH)_{145}(\alpha\text{-Galactose-}C_2)_{145}@Au_{2000}$ GNP **(1)** with cRGD **(2)** or Sulfo-Cy5 GNP **(3)**

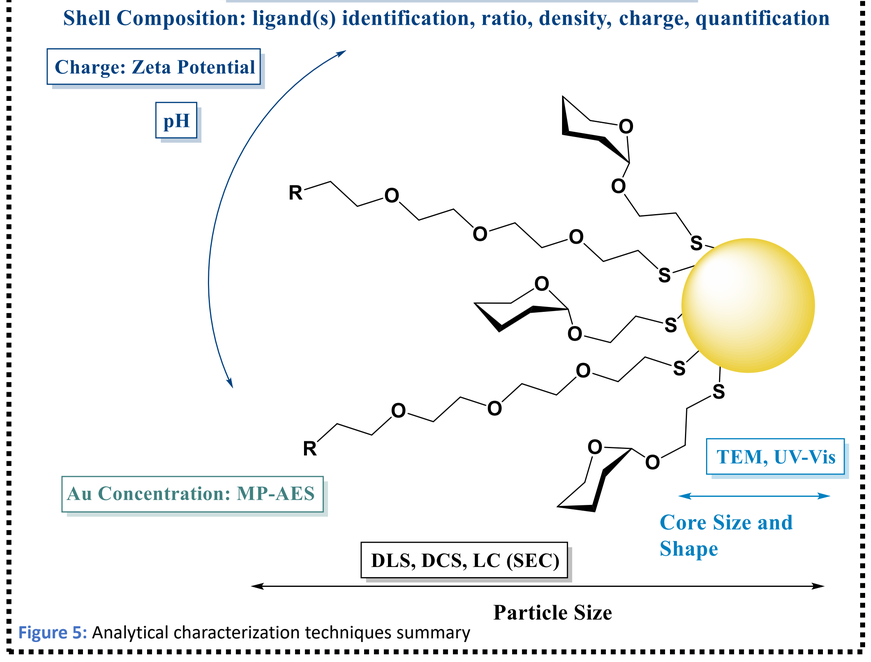


Figure 5: Analytical characterization techniques summary

^{1}H NMR

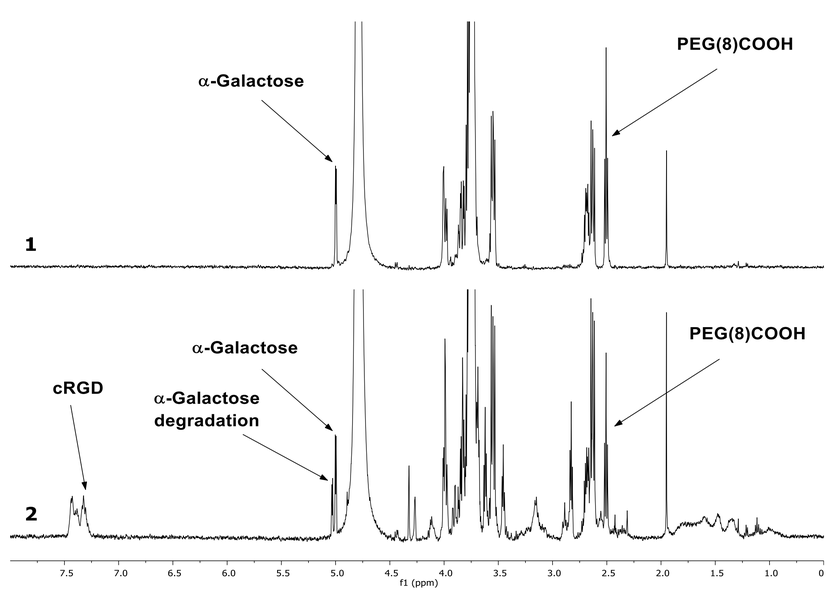
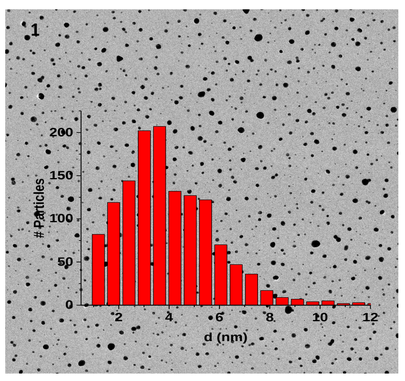


Figure 6: **^{1}H NMR** after KCN treatment of $(PEG(8)COOH)_{145}(\alpha\text{-Galactose-}C_2)_{145}@Au_{2000}$ GNP **(1)** with cRGD **(2)**

TEM



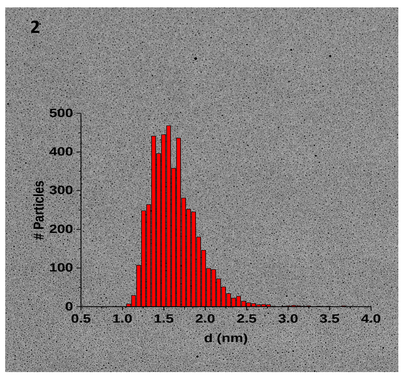


Figure 11: **TEM** of 4 nm **(1)** and 2 nm **(2)** core GNP

UV-Vis

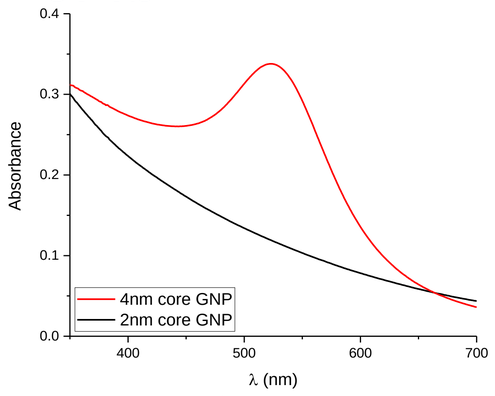


Figure 8: **UV-Vis spectra** of 4 and 2 nm core GNP

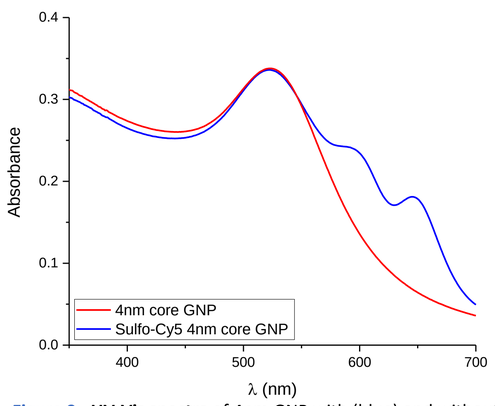


Figure 9: **UV-Vis spectra** of 4nm GNP with (blue) and without (red) Sulfo-Cy5

DLS

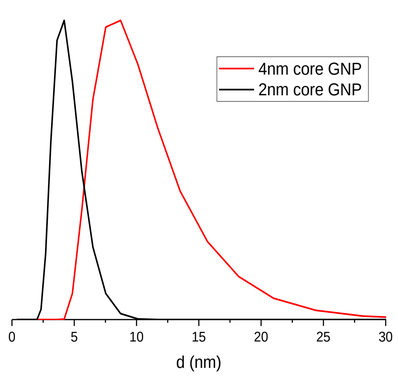


Figure 10: **DLS** size distribution of 4 and 2 nm core GNP

DCS

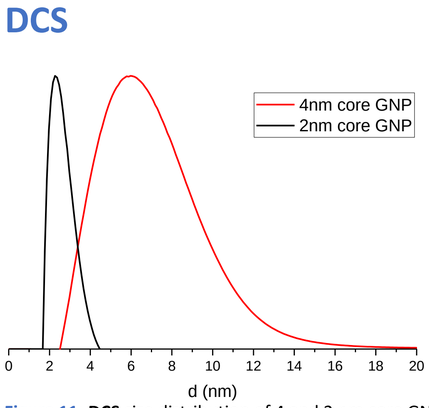
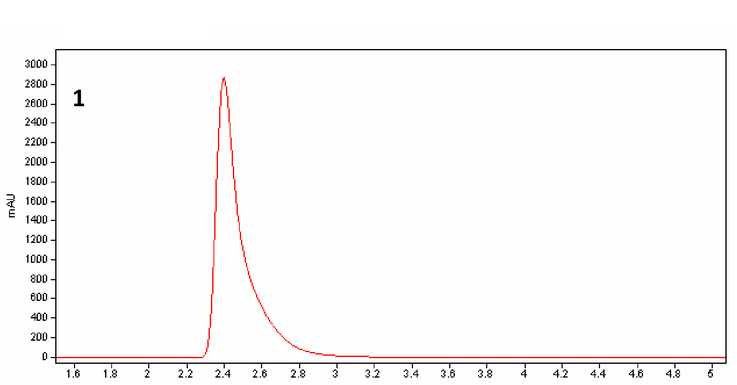


Figure 11: **DCS** size distribution of 4 and 2 nm core GNP

LC-SEC



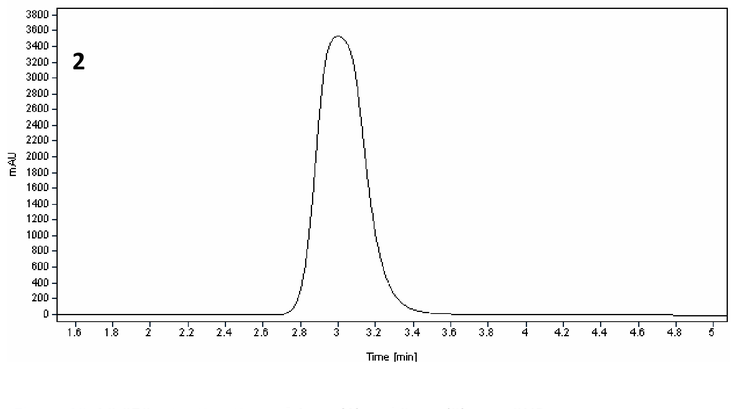


Figure 12: **LC-SEC** retention time of 4 nm **(1)** and 2 nm **(2)** core GNP

Results and Conclusion

Midatech Pharma has focused on being able to produce and purify ultrasmall GNPs through **automated and user-friendly systems**. That allows the delivery of material in quantities to **supply clinical trials** with methods translatable to **GMP (Good Manufacturing Practice)** production.

Moreover, an extensive and comprehensive analytical array has been developed to characterize the constructs using state of art methods with over a dozen routine techniques [6]. ^{1}H NMR and chromatographic techniques such as LC-CAD-MS (Charged Aerosol Detection coupled with Mass Spectrometry) or HPLC-DAD allow ligands identification, ratio determination and API quantification. Elemental analyses such as MP-AES, ICP-AES and XPS give information about the [Au] but also the ligand density through the S/Au ratio (data not shown). TEM, DLS, DCS and LC-SEC offer information regarding either the size of the core or the whole construct. UV-Vis spectroscopy allows to differentiate between plasmonic and non-plasmonic ultrasmall GNP, but also gives information about the GNP shell. These **analytical techniques** have been integrated into Midatech Pharma **QC (Quality Control)** system and allow the delivery of material matching regulatory criteria for clinical use.

References

[1] Dykman LA, Khlebtsov NG. Gold Nanoparticles in Biology and Medicine: Recent Advances and Prospects. Acta Naturae. 2011;3(2):34–55.
[2] Lundquist, J. J. & Toone, E. J. The Cluster Glycoside Effect. *Chem. Rev.* **102**, 555–578 (2002).
[3] Zhao, P., Li, N. & Astruc, D. State of the art in gold nanoparticle synthesis. *Coord. Chem. Rev.* **257**, 638–665 (2013).
[4] Sweeney, S. F., Woehrle, G. H. & Hutchison, J. E. Rapid Purification and Size Separation of Gold Nanoparticles via Diafiltration. J. Am. Chem. Soc. 128, 3190–3197 (2006).
[5a] Sperling RA, Parak WJ. Surface modification, functionalization and bioconjugation of colloidal inorganic nanoparticles. Philos Trans R Soc Lond Math Phys Eng Sci. 2010 Mar 28;368(1915):1333–83. [5b] Yeh, Y.-C., Creran, B. & Rotello, V. M. Gold nanoparticles: preparation, properties, and applications in bionanotechnology. *Nanoscale* **4**, 1871–1880 (2012).
[6a] Mourdikoudis, S., Pallares, R. M. & Thanh, N. T. K. Characterization techniques for nanoparticles: comparison and complementarity upon studying nanoparticle properties. *Nanoscale* **10**, 12871–12934 (2018). [6b] Lin, P.-C., Lin, S., Wang, P. C. & Sridhar, R. Techniques for physicochemical characterization of nanomaterials. *Biotechnol. Adv.* **32**, 711–726 (2014).

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