The 9th International Electronic Conference on Medicinal Chemistry (ECMC 2023)
01–30 November 2023 | Online

Aptamer-based molecular probes: a multidisciplinary pipeline

Chaired by Dr. Alfredo Berzal-Herranz and Prof. Dr. Maria Emília Sousa

pharmaceuticals MDPI

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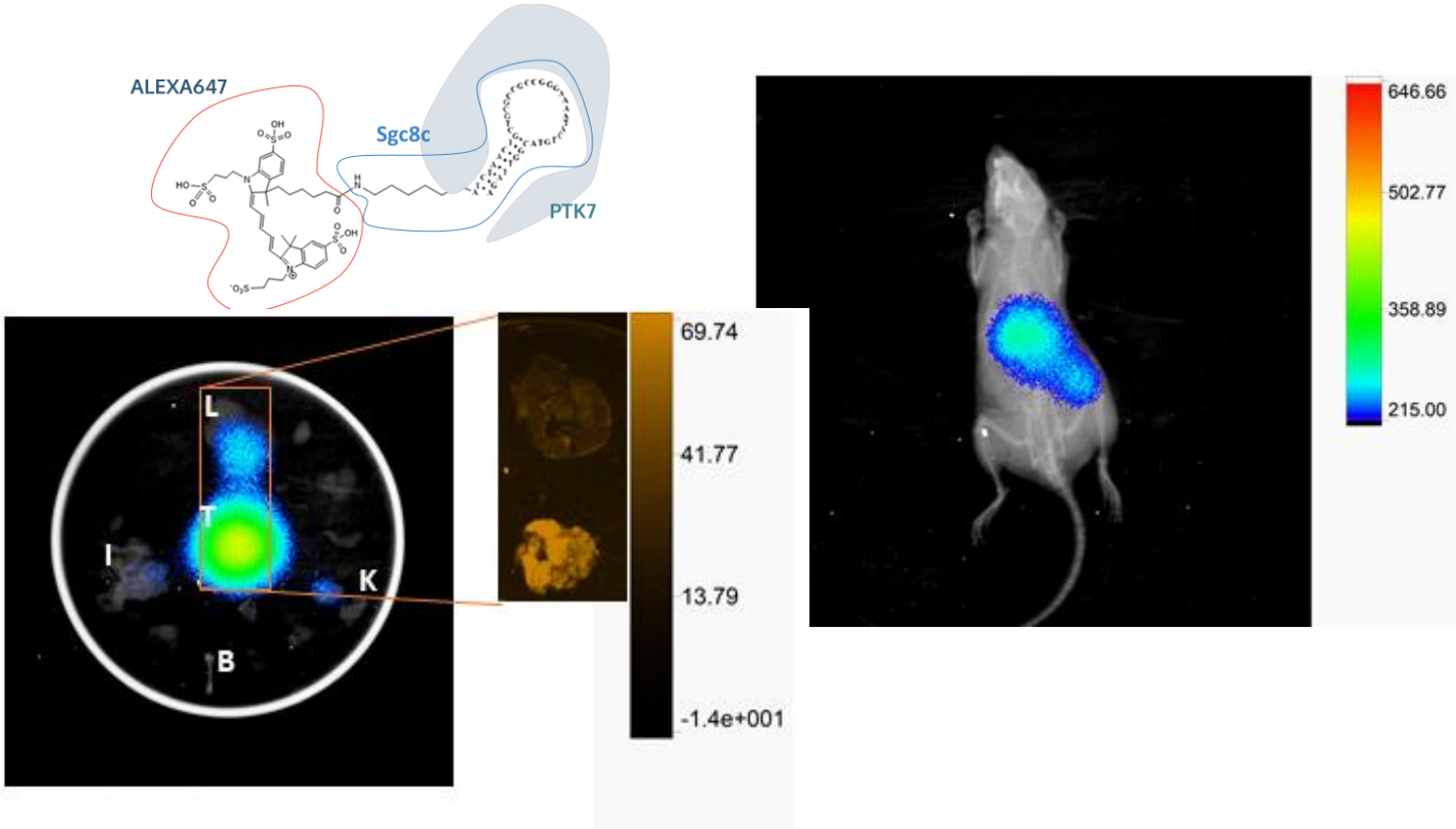
Aptamer-based molecular probes: a multidisciplinary pipeline



Abstract:

Aptamers are small oligonucleotides that specifically recognize a molecular target with high affinity and specificity. Based on their versatility and physicochemical properties, several techniques were developed to exploit the advantages of aptamers as probes for molecular imaging. In our previous work, a DNA aptamer which exhibits specific binding for PTK7 receptor, was used as a molecular imaging probe. This aptamer was modified at 5'-position with several chelator agents and radiolabeled with technetium-99m or gallium-67. An Alexa647 derivative was also used as near infrared dye. All probes were physicochemical and biologically evaluated in BALB/c mice bearing lymphoma-tumor. In order to increase the specificity, the RP-HPLC purification procedure of probes was very strict. The chemical modifications were confirmed by mass spectrometry and RP-HPLC-gamma detection. Confocal microscopy and flow cytometry were performed in several tumor cells with the fluorescent probe. *In vivo* images were obtained in mice using X-ray, gamma and optical imaging strategies. After 24 h of injection, the probes were retained in the tumor and ratios tumor/non-target organ with values over 20 were obtained. In addition, *in vivo* specificity was evaluated in competition assays. The retention over time of the probe in the tumor and its quick excretion support the utility of labeled aptamers as molecular probes for diverse image strategies.

Keywords: Aptamer, Molecular Imaging, probes, drug-delivery

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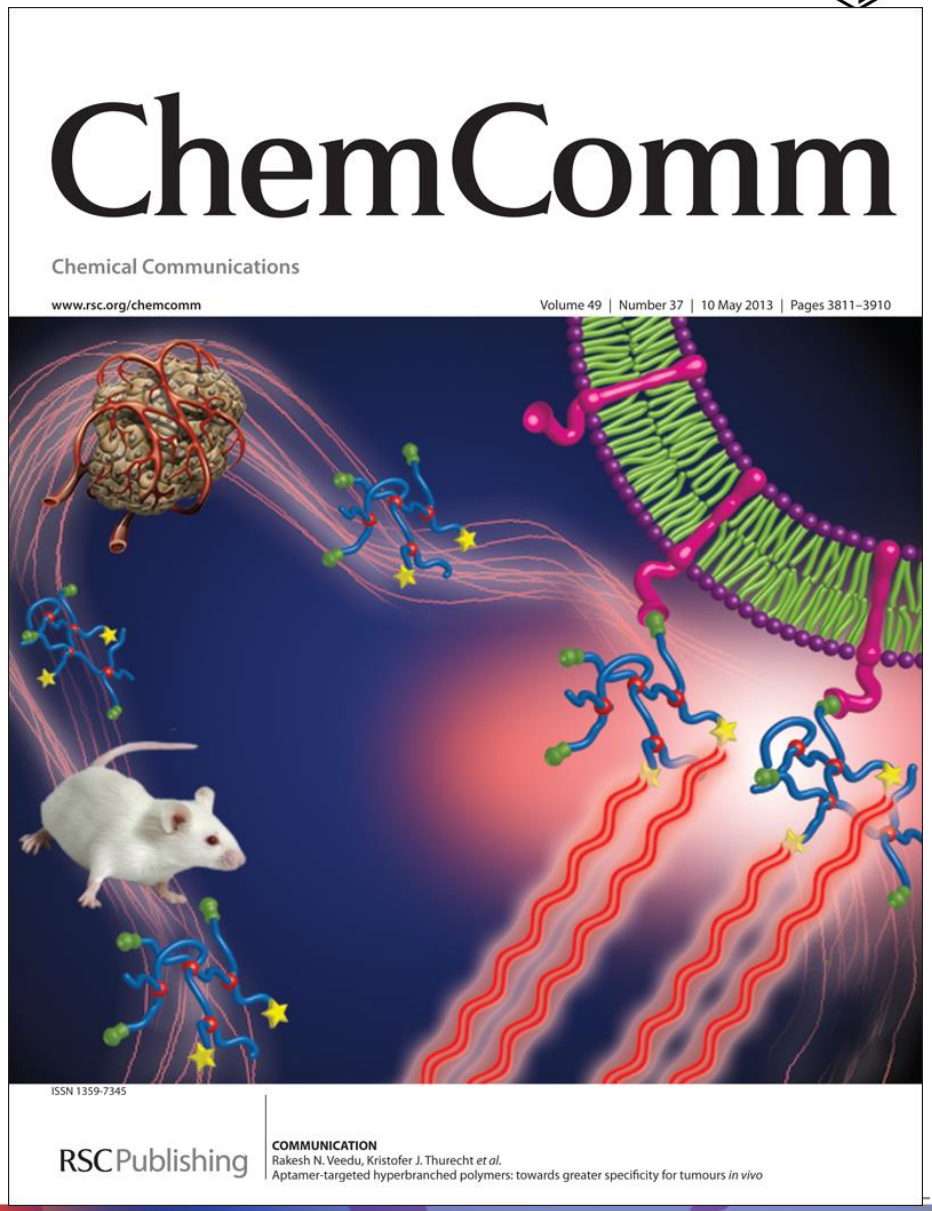
Calzada, V.; Zhang, X.; Fernandez, M.; Diaz-Miqueli, A.; Iznaga-Escobar, N.; Deutscher, S.; Balter, H.; Quin, T.; Cabral, P. A potencial theranostic agent for EGF-R expression tumors: (177)Lu-DOTA-nimotuzumab. Curr. Radiopharm. 2012 Oct;5(4):318-24.

Molecular Imaging

SPECT, γ emitters, ^{99m}Tc

PET, β^+ emitters, ^{18}F

FMT, fluorophores, > 640 nm



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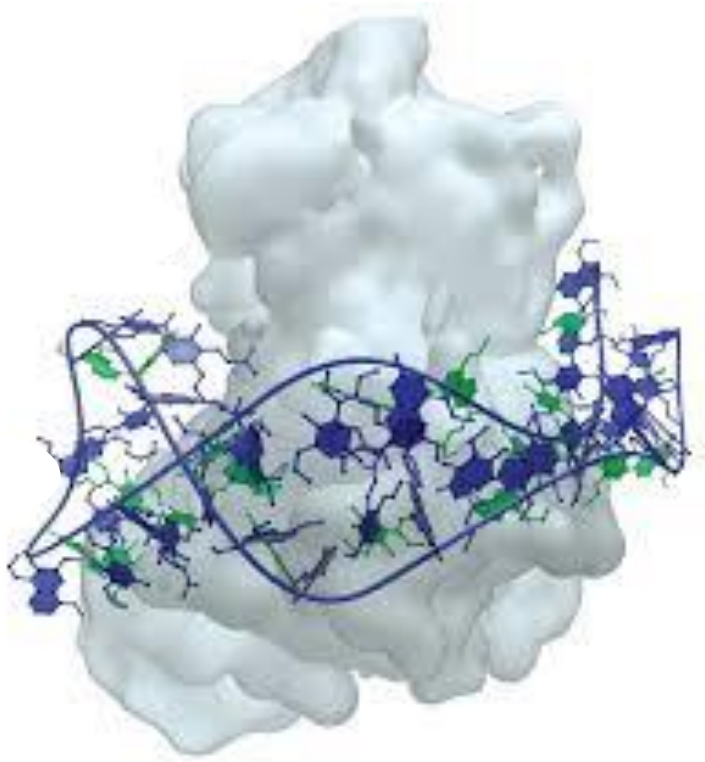
http://pubs.rsc.org/en/Content/ArticleLanding/2013/CC/C3CC90110F#!divAbstract

Applications

- Tumour stratification,
- Metastasis detection,
- Guided surgery,
- Quantification of an injury,
- THERANOSTIC

APTAMERS

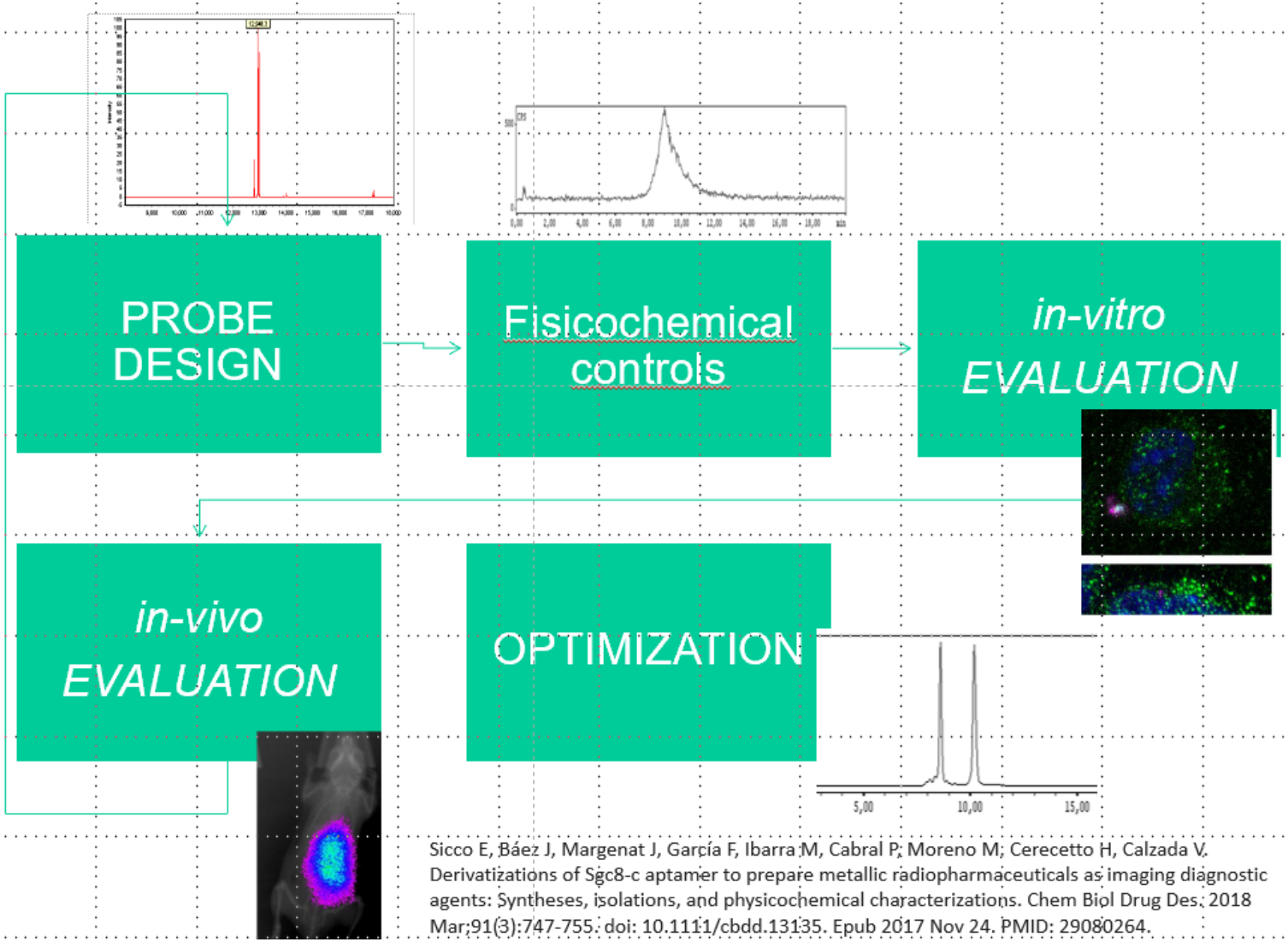


Aptamers are small oligonucleotides that specifically recognize a molecular target with high affinity and specificity.

Probe Design

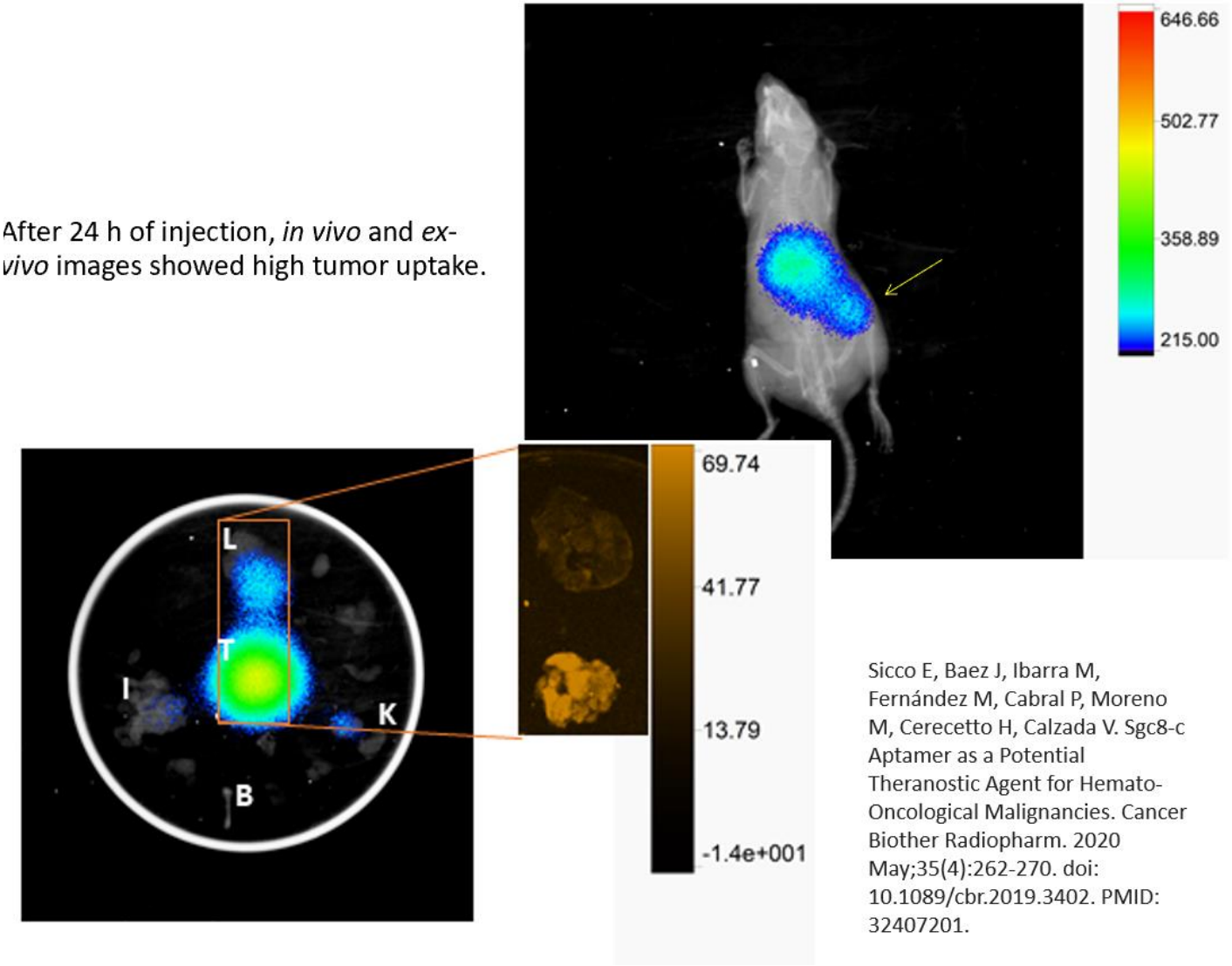
Ref.: Shangguan, D.; Tang, Z.; Mallikaratchy, P.; Xiao, Z.; Tan, W. Optimization and modifications of aptamers selected from live cancer cell lines. Chembiochem 2007 Apr 16;8(6):603-6.

Name: Sgc8-c (DNA)
Size = 41 nb,
MW = 12634 Da
Target: PTK7 receptor



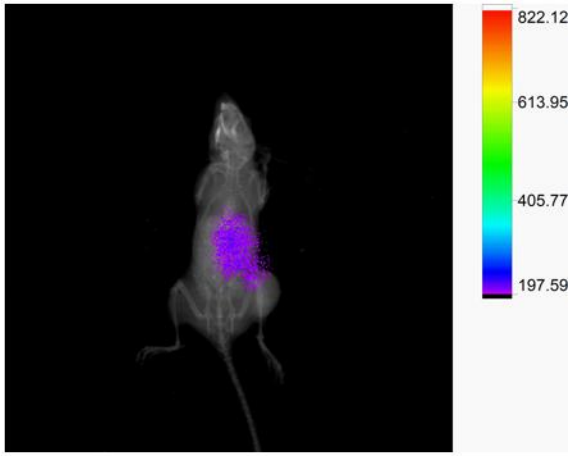
Sicco E, Báez J, Margenat J, García F, Ibarra M, Cabral P, Moreno M, Cerecetto H, Calzada V. Derivatizations of Sgc8-c aptamer to prepare metallic radiopharmaceuticals as imaging diagnostic agents: Syntheses, isolations, and physicochemical characterizations. Chem Biol Drug Des. 2018 Mar;91(3):747-755. doi: 10.1111/cbdd.13135. Epub 2017 Nov 24. PMID: 29080264.

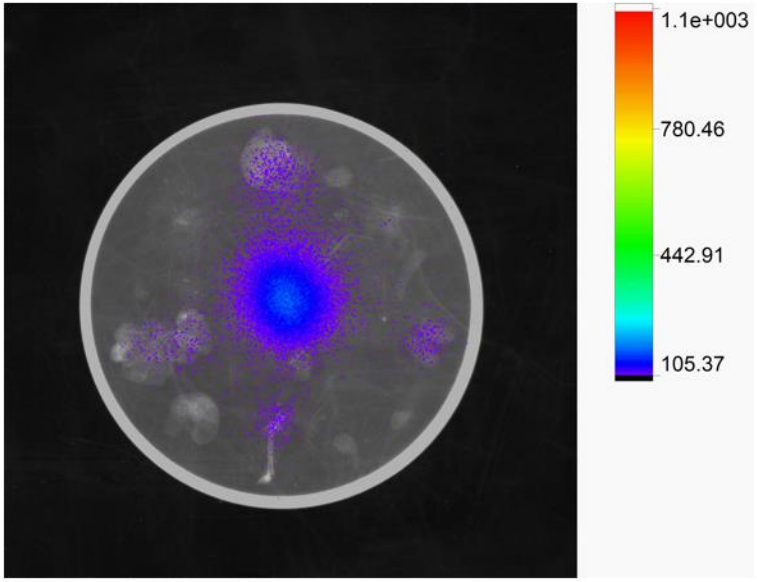
After 24 h of injection, *in vivo* and *ex-vivo* images showed high tumor uptake.



Sicco E, Baez J, Ibarra M, Fernández M, Cabral P, Moreno M, Cerecetto H, Calzada V. Sgc8-c Aptamer as a Potential Theranostic Agent for Hemato-Oncological Malignancies. Cancer Biother Radiopharm. 2020 May;35(4):262-270. doi: 10.1089/cbr.2019.3402. PMID: 32407201.

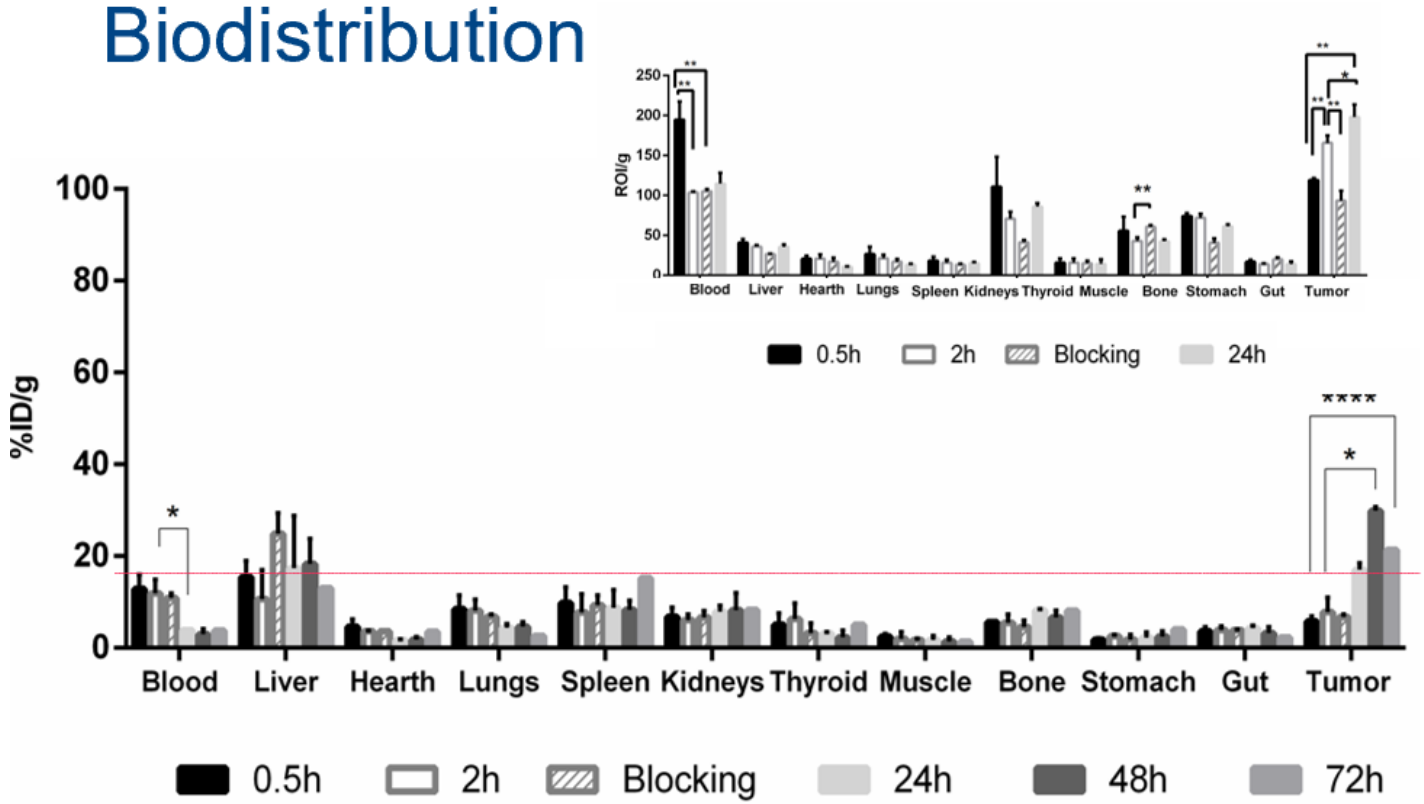
Melanoma Sgc8-c-NOTA-^{67}Ga 24h





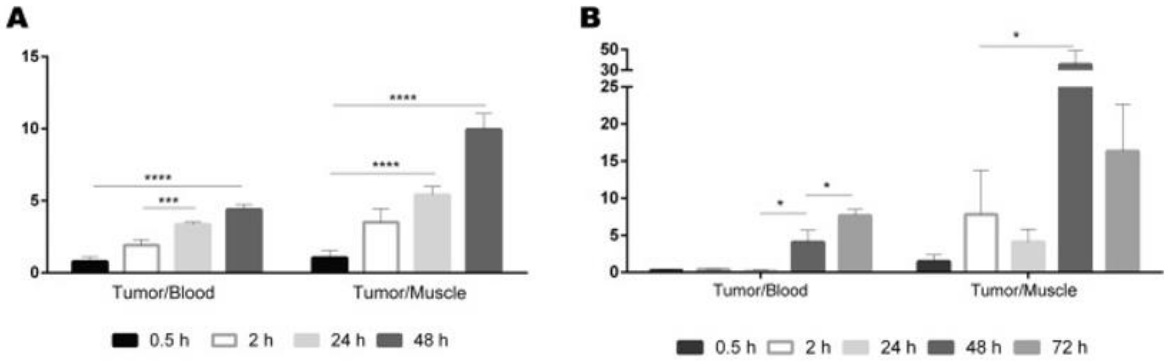
Sicco, Estefanía et al. "Metastatic and non-metastatic melanoma imaging using Sgc8-c aptamer PTK7-recognizer." *Scientific reports* vol. 11,1 19942. 7 Oct. 2021, doi:10.1038/s41598-021-98828-6

Biodistribution



Calzada V, Moreno M, Newton J, González J, Fernández M, Gambini JP, Ibarra M, Chabalgoity A, Deutscher S, Quinn T, Cabral P, Cerecetto H. Development of new PTK7-targeting aptamer-fluorescent and -radiolabelled probes for evaluation as molecular imaging agents: Lymphoma and melanoma in vivo proof of concept. Bioorg Med Chem. 2017 Feb 1;25(3):1163-1171. doi: 10.1016/j.bmc.2016.12.026. Epub 2016 Dec 24. PMID: 28089349.

Tumor/no tumor RATIOS



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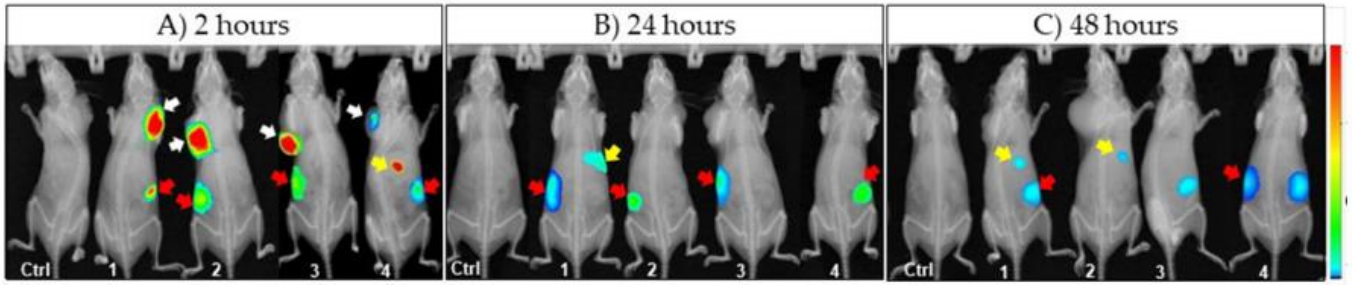
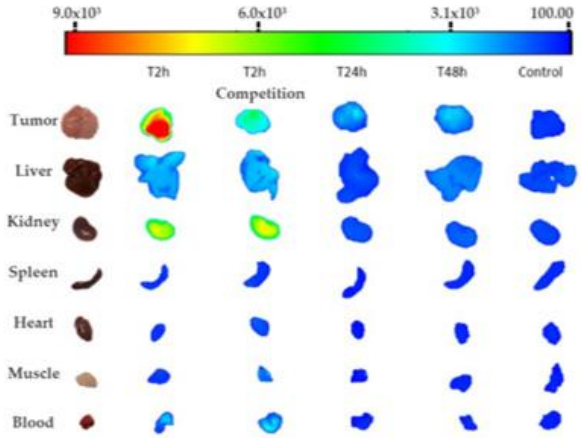
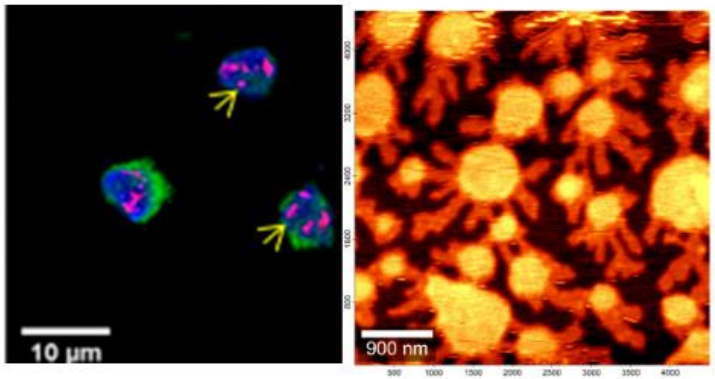
Chemistry modifications effect

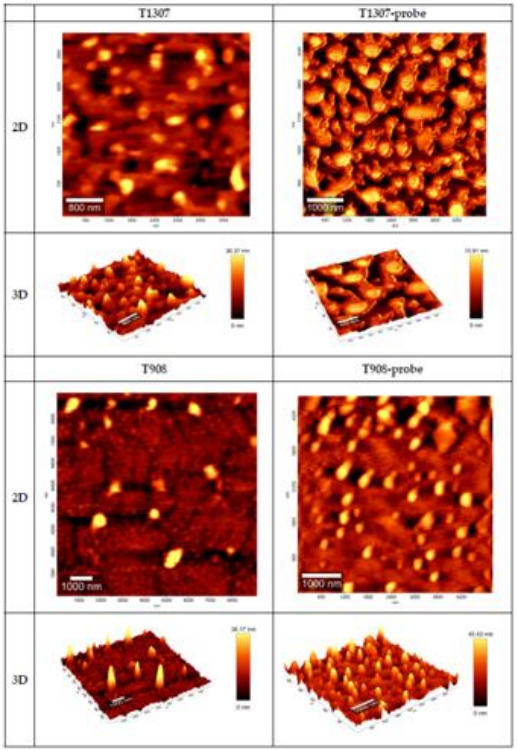




Arévalo AP, Castelli R, Ibarra M, Crispo M, Calzada V. In Vivo Evaluation of Sgc8-c Aptamer as a Molecular Imaging Probe for Colon Cancer in a Mouse Xenograft Model. Int J Mol Sci. 2022 Feb 23;23(5):2466. doi: 10.3390/ijms23052466. PMID: 35269608; PMCID: PMC8910571.

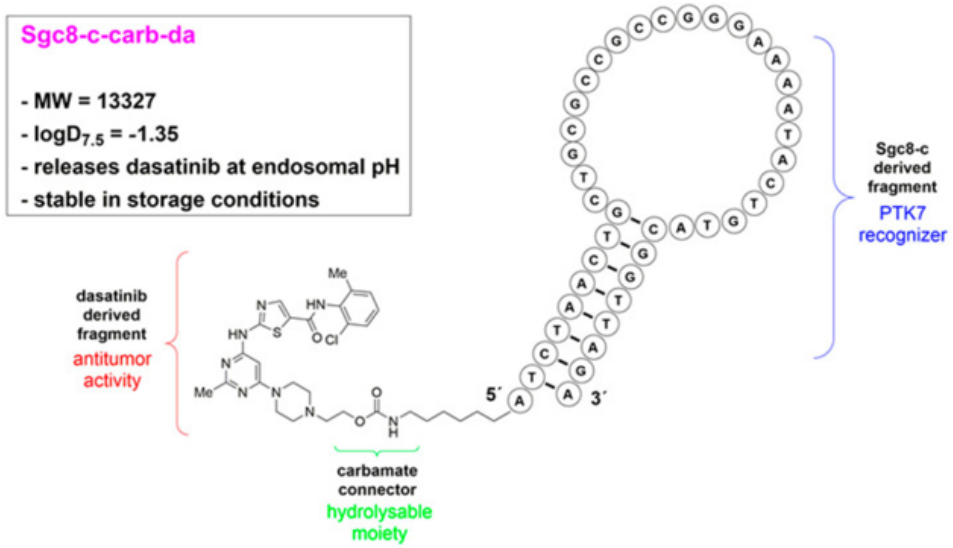
Polymeric micelles are good candidates to improve the pharmacokinetics and the tumor uptake of aptamer-based probes





Castelli R, Ibarra M, Faccio R, Miraballes I, Fernández M, Moglioni A, Cabral P, Cerecetto H, Glisoni RJ, Calzada V. T908 Polymeric Micelles Improved the Uptake of Sgc8-c Aptamer Probe in Tumor-Bearing Mice: A Co-Association Study between the Probe and Preformed Nanostructures. Pharmaceuticals (Basel). 2021 Dec 23;15(1):15. doi: 10.3390/ph15010015. PMID: 35056072; PMCID: PMC8780797.

Aptamers as drug-delivery system



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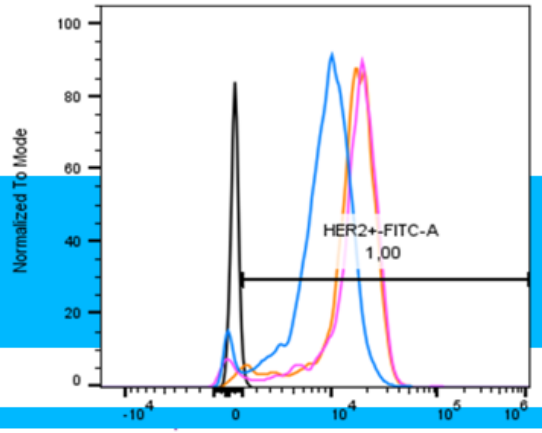
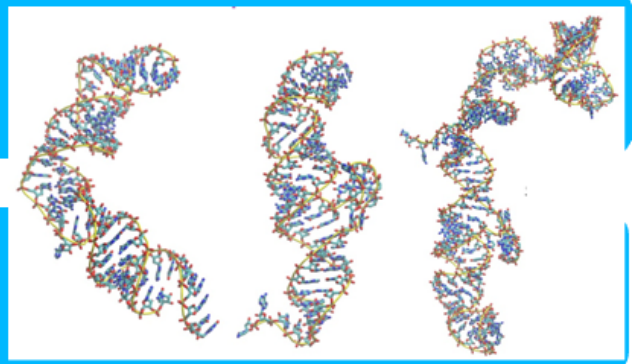
Aptamer_uy group



- SELEX→ more information
- Sequencing and data analyses



- Screening
- Characterization and optimization

Versatility

THANKS

FOR WOMEN IN SCIENCE
L'ORÉAL
UNESCO
United Nations Educational, Scientific and Cultural Organization
ANII AGENCIA NACIONAL DE INVESTIGACIÓN E INNOVACIÓN
UNIVERSIDAD DE LA REPÚBLICA URUGUAY
PEDECIBA MEC-UDELAR
Ministerio de Educación y Cultura