



## Press Release

### Developing a new strategy to selectively deliver therapies to the brain

*Turning the problem into the solution: exploiting the impermeability of the brain vasculature to target nano-vehicles to the brain*

#### Summary:

Therapy-loaded nanoparticles may be directed to the brain by functionalization with ligands targeting BBB-associated proteins. However, such targeting strategies have inherent brain-specificity limitations, as the target proteins are also significantly expressed in peripheral organs, thereby limiting the clinical application of such strategies. We have developed a counterintuitive targeting strategy which exploits the high impermeability of the BBB itself to selectively retain molecular labels (*i.e.* targets) on the surface of brain endothelium. Nanoparticles capable of binding the displayed targets are consequently directed specifically to the brain microvasculature with minimal targeting to peripheral organs. This two-step targeting strategy therefore paves the way to overcome the peripheral ‘off-target’ nanoparticle accumulation, increasing the clinical translation of nanoparticle-based therapies. The results have been published in the July 23 issue of Proceedings of the National Academy of Science (Impact Factor = 9.5804).

**July 27, 2020 – Kawasaki / Japan:** The Innovation Center of NanoMedicine (Director: Prof. Kazunori Kataoka, Location: Kawasaki-City, Abbreviation: iCONM) announced that a new strategy to specifically target to the brain was discovered in collaboration with the Department of Bioengineering, Graduate School of Engineering, University of Tokyo. The details are published in the Proceedings of the National Academy of Science (Impact factor = 9.350 in 2019) issued on July 23. (Note 1)

Treatment of neurological diseases is severely hindered by the poor delivery of therapies to the brain due to the presence of the blood-brain barrier (BBB), a highly impermeable cellular barrier composed primarily by the specialized endothelial cells lining the brain microvasculature. Nanotechnology-based strategies have achieved modest success in

delivering therapeutics to the brain by loading them onto nanomachines (Note 2) decorated with ligands which bind to proteins associated with the BBB (Note 3). However, such targeting strategies have inherent brain-specificity limitations, as the target proteins are also significantly expressed in peripheral organs, leading to increased accumulation of nanomachines for instance in the lung and heart. Therefore, the clinical translation of current strategies is hampered by detrimental peripheral side-effects and reduced effective therapeutic doses reaching the brain. Hence, new strategies which exploit alternative features of the BBB need to be developed to overcome 'off-target' accumulation of nanomachines.

The group of Prof. Kataoka have developed a simple, yet counterintuitive strategy which turns the problem of therapy delivery to the brain, that is, the high impermeability of brain endothelial cells, into the solution to achieve specific brain targeting of nanomachines with minimal accumulation increase in peripheral organs.

The high impermeability of brain endothelial cells is in large part due to a markedly reduced level of endocytosis compared to peripheral endothelial cells. This feature may therefore be exploited to promote free, unconjugated molecular labels to be selectively retained on the surface of brain endothelial cells while being quickly removed (endocytosed) from the surface of endothelial cells of other organs in the body. In this way, nanomachines capable of efficiently recognizing the displayed molecular labels are specifically targeted to the brain with minimal targeting into other organs.

The feasibility of such an approach has been demonstrated by employing biotin-containing antibodies against the protein Platelet Endothelial Cell Adhesion Molecule (PECAM)-1, which is expressed in the vasculature of most organs. The authors demonstrated that if nanomachines decorated with the protein avidin (capable of very strongly binding to biotin) are injected into mice a short time-period after injection of biotin-PECAM-1 antibodies, the nanomachines accumulate preferentially in the lung, with accumulation also seen in the brain, heart and pancreas (note 4). However, if the time-interval between antibody and nanomachine injection is increased to allow removal of the antibody from the surface of peripheral endothelial cells, the ability of the nanomachines to accumulate in the lung, heart and pancreas steadily decreases, while accumulation in the brain remains constant. Hence, after an 8 hr time-interval, the nanomachines were only targeted to the brain, with no increase in accumulation seen in any peripheral organ.

This novel two-step targeting strategy therefore paves the way to overcome the limitation of peripheral "off-target" nanomachine accumulation, thereby increasing the clinical translation of nanomachine-based therapies.

(Note 1) Daniel Gonzalez-Carter, Xueying Liu, Theofilus A. Tockary, Anjaneyulu Dirisala,

Kazuko Toh, Yasutaka Anraku and Kazunori Kataoka, "Targeting nanoparticles to the brain by exploiting the blood-brain barrier impermeability to selectively label the brain endothelium", Proc. Natl. Acad. Sci., 2020 in press. (doi.org/10.1073/pnas.2002016117)

(Note 2) Smart Nanomachine: Spherical or rod-shaped molecular assembly (nanomicelles) with a size of tens of nm formed by associating amphiphatic polymers with various functional molecules in water. Horacio Cabral, Keijiro Miyata, Kensuke Osada and Kazunori Kataoka, "Block copolymer micelles in nanomedicine applications" Chem. Rev.118 (14) 6844-6892 (2018) (doi.org/10.1021/acs.chemrev.8b00199)

(Note 3) Jinbing Xie, Daniel Gonzalez-Carter, Theofilus Tockary, Noriko Nakamura, Yonger Xue, Makoto Nakakido, Hiroki Akiba, Anjaneyulu Dirisala, Xuying Liu, Kazuko Toh, Tao Yang, Zengtao Wang, Shigeto Fukushima, Junjie Li, Sabina Quader, Kouhei Tsumoto, Takanori Yokota, Yasutaka Anraku and Kazunori Kataoka, "Dual-Sensitive Nanomicelles Enhancing Systemic Delivery of Therapeutically Active Antibodies Specifically into the Brain", ACS Nano, 2020, 14 (6), 6729-6742.

<https://pubs.acs.org/doi/abs/10.1021/acsnano.9b09991>

(Note 4) Biotin-conjugated anti-PECAM1 antibody (biotin- $\alpha$ -PECAM1) was injected through the tail vein of mice, and after a predetermined time (15 minutes, 2 hours, 4 hours, or 8 hours), avidin-decorated nanomachines (avidin-NM) were intravenously injected. After 16 hours, the mice were anesthetized and perfused with D-PBS before the lung, brain, heart, and pancreas were collected to quantify avidin-NM accumulation. The results shown in Fig. 1 were obtained by evaluating the targeting of the nanomicelles to each organ.

### **Innovation Center of NanoMedicine (iCONM):**

The Innovation Center of NanoMedicine (iCONM) is a leading facility of King Skyfront, that is a biotech and healthtech innovation cluster in Kawasaki City. iCONM started the operation in April 2015 with Kawasaki Institute of Industrial Promotion in order to drive "Center of Open Innovation Network for Smart Health (COINS)" as a part of Japanese governmental research program "Center of Innovation (COI) Stream". Designed for the purpose of promoting "open innovation" through industry-academia-government and medicine-engineering collaborations with state-of-the-art facilities and experimental equipment capable of conducting the R&D from organic synthesis and micro-processing to preclinical studies. This is a very unique research center that is hardly found in the world.

<https://iconm.kawasaki-net.ne.jp/en/index.html>

### **Department of Bioengineering, Graduate School of Engineering, University of Tokyo:**

In a society where the population ages and the birth rate declines with the sustainable development being longed for, the Department of Bioengineering aims to contribute to the promotion of health and well-being of the humanity. To achieve this goal, we promote the education and research of bioengineering, which is the multidisciplinary academic field

integrating the existing disciplines of engineering and those of life sciences at their interface. The key features of bioengineering are to establish its theoretical basis by understanding and clarifying the interactions of materials and systems with living bodies, and to develop fundamental technologies that control these interactions based on the theory. The control of the interactions with living bodies renders materials and systems far more useful and compatible, promising the birth of groundbreaking medical technologies. <http://www.bioeng.t.u-tokyo.ac.jp/en/overview/index.html>

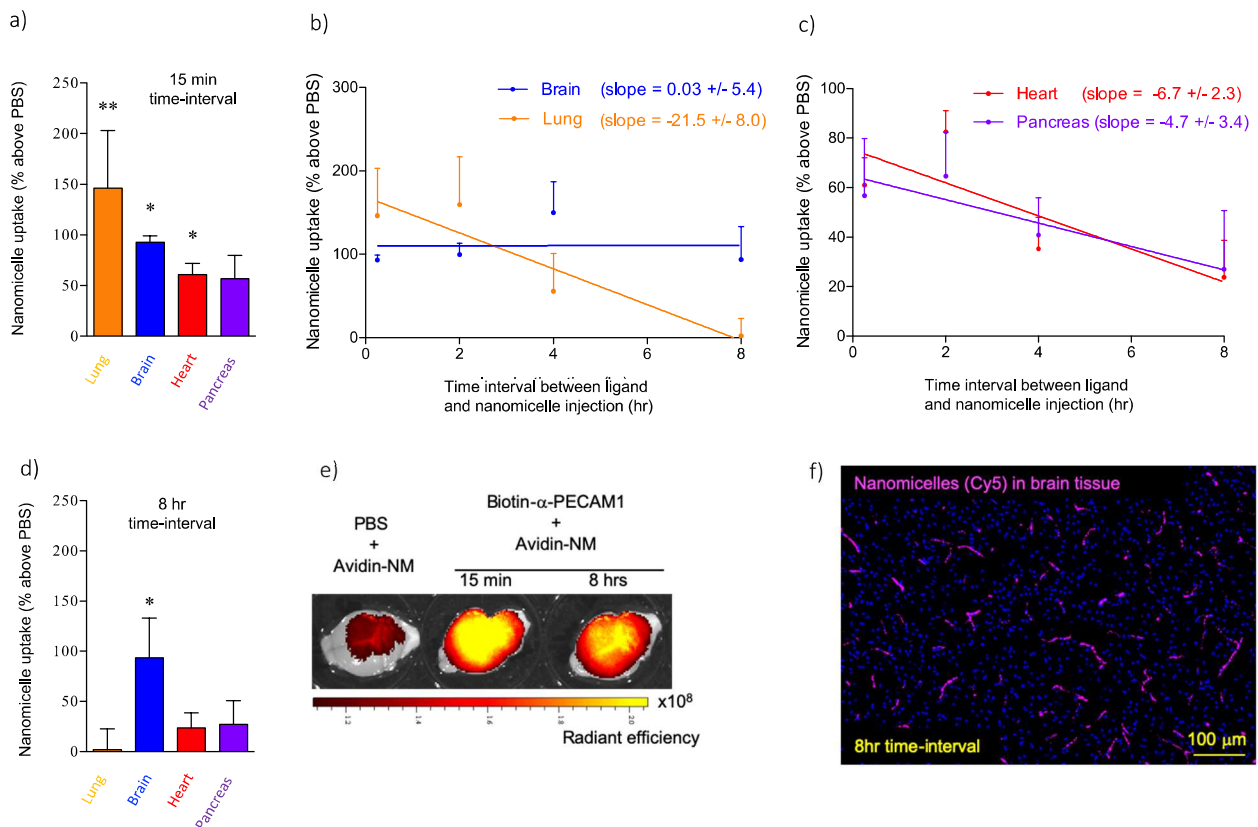


Fig. 1. Short time-intervals (15 mins) between injection of biotin-PECAM1 antibody and injection of avidin-decorated nanomachines (avidin-NM) results in avidin-NM targeting to the lung (yellow), brain (blue), heart (red), and pancreas (purple) (a). However, targeting to peripheral organs decreases as a function of time-interval length, while targeting to the brain remains constant (b, c). Consequently, an 8h time-interval results in specific targeting of avidin-NM to the brain, with no targeting seen in peripheral organs (d). Avidin-NM accumulation was visualized in freshly excised brains (e) and fixed brain tissue (f).