Minutes-timescale *in situ* assembly of DNA origamis with living cells

Laura Bourdon,[1] Gerrit David Wilkens,[2] Samy Dehissi,[1] Salammbô Hotte,[1] Sergii Rudiuk,[1] Mathieu Morel,[1] Ayako Yamada,[1] Gaëtan Bellot,[2] Damien Baigl[1,*]

[1]CPCV, Department of Chemistry, École Normale Supérieure, PSL University, Sorbonne Université, CNRS, 75005 Paris, France

[2]Centre de Biologie Structurale, Université Montpellier, CNRS, Inserm, Montpellier, France

*correspondence to: damien.baigl@ens.psl.eu

Abstract

Synthetic DNA strand is a highly accessible and biocompatible building block that can be synthesized in virtually any length and sequence, chemically modified, and combined with high specificity *via* hybridization.[1] Applied within biological environments, *in situ* hybridization is a powerful biotechnological tool but limited to the use of small numbers of DNA strands. *Ex vivo*, this principle is the basis of structural DNA nanotechnology, in which hundreds of synthetic DNA strands are designed to self-assemble into highly programmable and versatile platforms,[2] with applications ranging from drug delivery to immunobiology.[3,4] However, the formation of such structures requires conditions such as temperature and ionic environments that are not only incompatible with living cells but also very long, imposing an *ex situ* fabrication step before any application with living matter. Here, we report that complex cocktails of DNA strands, coding for so-called DNA origamis,[5–7] can faithfully self-assemble *in situ* at 37 °C, in a variety of common cell culture media and directly in the presence of living cells, in just a few minutes. We apply this approach for the *in situ* assembly of two- and three-dimensional DNA origamis in the presence of human cell lines in 2D culture as well as with 3D assemblies of human induced pluripotent stem cells successfully evolving into cerebral organoids. This life-compatible and highly programmable self-assembly method, requiring only a few minutes of incubation at 37 °C, drastically simplifies the preparation of user-defined complex DNA nanostructures and enables the creation of *in situ* self-assembling nanomachines for direct and adaptive interactions with living cells.

Main

In situ hybridization of synthetic nucleic acids within living cell environments has proved to be a powerful strategy to selectively detect and/or interact with biological targets,[8] with applications ranging from biosensing[9–11] to therapeutic actions.[12–14] In a very different context, this ability of nucleic acids to self-assemble through complementary base pairing has been exploited by researchers to build elaborate nanostructures in a user-friendly and highly programmable manner, leading to the development of structural DNA nanotechnology.[15] Within the different methods, DNA origami self-assembly has emerged as one of the most widely studied and applied technologies.[5–7] Based on the guided folding of a circular single-stranded DNA scaffold through the addition of a designed cocktail of hundreds of short oligonucleotides acting as staples, DNA origamis can be produced at a high yield, with virtually any desired two- (2D)[5] or three-dimensional (3D)[16,17] morphology.[6] With typical size around 100 nm, these structures offer precise site-specific functionalization at subnanometric resolution,[18] making them powerful platforms for nanoscale organization of matter with exceptional degree of programmability and versatility. Among various application fields, DNA origamis have been particularly recognized as valuable tools to interact with living organisms, leading to emerging applications including virus recognition and capture,[19,20] mechanobiology,[21–23] synthetic immunology,[24,25] or drug delivery.[26,27] However, their conventional preparation is slow, taking hours to days, and relies on a constraining thermal annealing process (heating to around 80 °C followed by a slow cooling ramp over several hours to days) that is incompatible with living cells. Additionally, the self-assembly of DNA origamis typically requires magnesium concentrations ranging from 10 mM to 25 mM,[28] far exceeding physiological levels and posing further challenges for direct applications in biological environments. As a result, and as of today, origamis have always been first fabricated before being added to cells for further studies. There is thus a critical scientific and methodological gap: while *in situ* hybridization enables nucleic acids to interact with biological targets, it currently lacks the capacity for elaborate structural organization, whereas DNA origami assembly offers exquisite programmability but requires *ex situ* preparation under conditions unsuitable for live cells. To bridge this gap, we thus looked for a method allowing *in situ* assembly of synthetic nucleic acid cocktails into user-defined DNA origamis directly in the presence of live cells. We recently reported that DNA origamis can be isothermally self-assembled in a magnesium-free, monovalent salt buffer (e.g., 100 mM or 150 mM NaCl) at room temperature,[29] but the assembly remained long (typically 24 h) and buffer conditions were not compatible with cell culture and manipulation. In this work, our strategy was thus to look for existing cell culture buffers containing a sufficiently high amount of monovalent salts (typically around 150 mM), along with essential divalent cations (e.g., Mg^{2+}, Ca^{2+}) required for cell culture but at a concentration low enough, to ensure isothermal assembly conditions at a temperature favourable to live cell growth. In fact, several commercially available and widely used buffers satisfy these conditions. We discovered that directly mixing DNA origami scaffold and staples in such buffers resulted in the isothermal self-assembly of well-formed DNA origamis at 37 °C in a few minutes only. This is not only one to two orders of magnitude faster

than existing methods whether they require thermal annealing or not, but also constitutes the first origami assembly conditions fully compatible with live cell maintenance, culture and growth. We thus applied and characterized this method for the *in situ* programmable isothermal self-assembly of various 2D and 3D origami morphologies directly in the presence of live cells, ranging from conventional 2D culture to more complex 3D tissue-like systems (here, cerebral organoids).

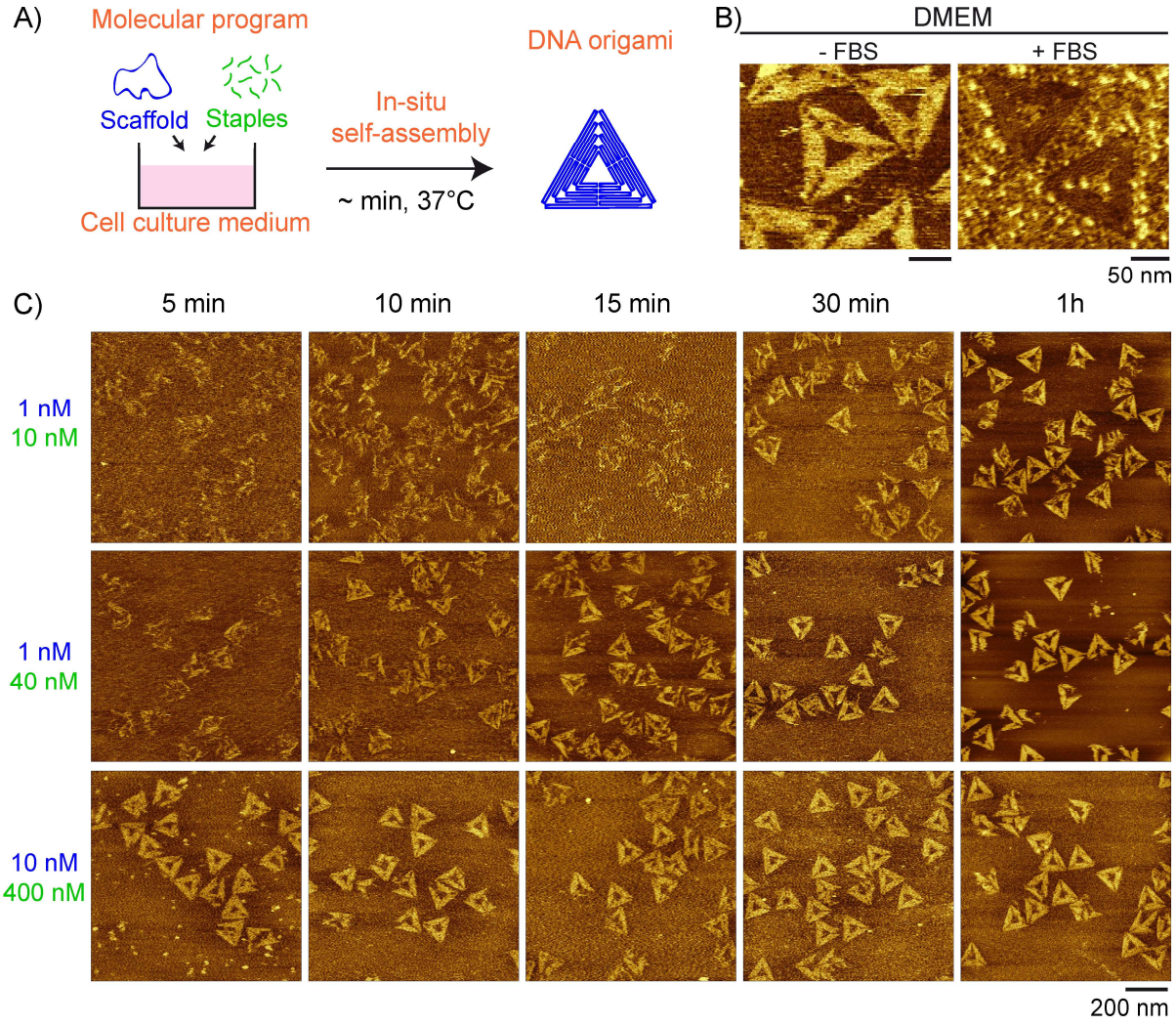

Figure 1. DNA origamis self-assemble at 37 °C in only a few minutes in the widely used Dulbecco's Modified Eagle Medium (DMEM) cell-culture medium. A) The experiment simply consists in the direct mixing of the origami molecular program (circular single-stranded DNA scaffold + staple cocktail) in a proper cell culture medium and let the DNA origamis self-assemble isothermally at 37 °C, without any pre- or post-thermal treatment. B) Atomic force microscopy (AFM) images of triangular DNA origamis obtained 6 h after self-assembly at 37 °C in DMEM, without (left) and with (right) 10% Fetal Bovin Serum (FBS). C) AFM observation of the self-assembly of triangle origamis over time. Each line corresponds to a given concentration in scaffold (top value indicated in blue) and staples (bottom value indicated in green).

Ultra-fast DNA origami self-assembly in cell culture media at 37 °C.

Ensuring flawless formation of DNA origamis is a challenge as hundreds of like-charged DNA strands have to associate while ensuring the formation of around 10 000 to 20 000 hydrogen bonds through specific base complementarity. A typical approach to achieve this involves a thermal annealing process in the presence of a high concentration of stabilizing dications, typically magnesium (usually at a concentration ranging from 10 mM to 25 mM, depending on the object).[28] Isothermal assembly at room temperature has been demonstrated as an alternative by replacing dications with a higher concentration of monovalent salts (typically 100 mM or 150 mM), ensuring both sufficient stability and reconfigurability for nearly flawless DNA assembly.[29] However, these conditions remain incompatible with cell culture and growth. With the aim to achieve isothermal assembly of DNA origamis in the presence of live cells at 37 °C, we first selected the widely used Dulbecco's Modified Eagle Medium (DMEM) for its high concentration in monovalent cations (around 160 mM, **Table S1**) and millimolar amounts of dications (mainly Ca^{2+}, Mg^{2+}, **Table S1**). We simply mixed a DNA origami-coding molecular program (scaffold and staple cocktail) in DMEM and incubated it at 37 °C for *in situ* self-assembly (**Fig. 1A**). With a program coding for triangle origamis, atomic force microscopy (AFM) revealed successful origami formation after a few hours of incubation (**Fig. 1B *left***, **Fig. S1 *top***). The experiment was repeated in the presence of 10 vol% of foetal bovin serum (FBS), a commonly required supplement for cell culture. Although the presence of proteins rendered less accurate the imaging of the DNA origamis, the distinction of well-formed triangles (**Fig. 1B *right***, **Fig. S1 *bottom***) indicated proper origami folding in these conditions as well. Isothermal self-assembly at 37 °C also led to well-formed DNA origamis in i) RPMI (Roswell Park Memorial Institute medium), another commonly used cell culture medium; ii) Essential 8™, a medium for induced pluripotent stem cells (iPSCs) culture; and iii) PBS (Phosphate Buffered Saline), a usual buffer for biological research (**Fig. S2**). All these buffers share a concentration of monovalent cations between 140 and 160 mM and divalent ones below 3 mM (**Table S1**), ensuring robust isothermal self-assembly at 37 °C. To investigate the kinetics of this isothermal self-assembly, we used AFM to analyse the morphology evolution after adding the molecular program into FBS-free DMEM as a function of incubation time at 37 °C. Using conventional concentrations[5] of scaffold (1 nM) and staples (40 nM each), we observed the formation of ill-shaped assemblies within 5 min, which progressively evolved into distinguishable triangles after 15 min and into particularly well folded structures after 1 h (**Fig. 1C *middle***). Decreasing the staple concentration to a 10-fold staple excess slowed the assembly but still resulted in complete folding within 1 h (**Fig. 1C *top***). Strikingly, increasing the scaffold concentration to 10 nM while maintaining a 40-fold staple excess led to the formation of well-folded triangles after 5 min only (**Fig. 1C *bottom***), which represents a considerably shorter time than the hours to days needed for known methods with[5] or without[29] thermal annealing.

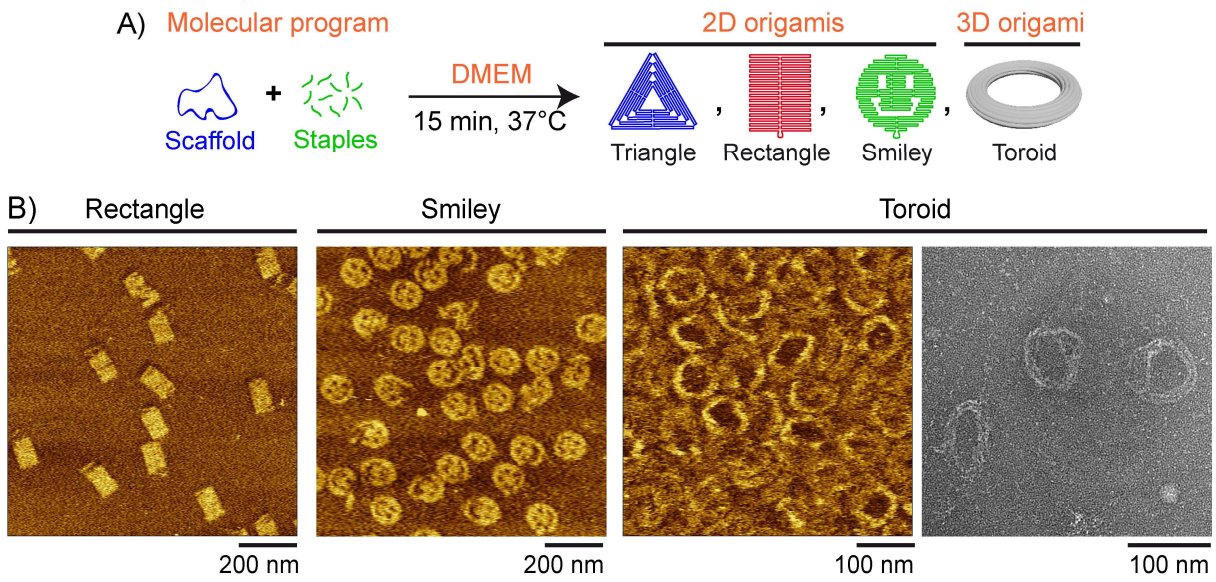

Figure 2. Versatile self-assembly into user-programmed two-dimensional (2D) and three-dimensional (3D) shapes. A) Each experiment consists in mixing a molecular program in DMEM and let it self-assemble at 37°C for 15 min. B) AFM (left and middle) and transmission electron microscopy (TEM, right) images of origamis obtained with molecular programs coding for rectangle, smiley or toroid shapes. [scaffold] = 10 nM; [staple] = 400 nM (rectangle, smiley) or 100 nM (toroid).

With a fixed scaffold concentration (10 nM), we assembled molecular programs coding for different morphologies in DMEM and let the self-assembly occur at 37 °C for 15 min (**Fig. 2A**). Using M13 scaffold, 2D structures including compact (rectangles) and circular (smileys) ones formed well (**Fig. 2B *left***). We also explored the challenge of a more complex design, consisting of a hollow 3D structure forming a toroid shape with a single layer of adjacent double helices. Using a p7560 scaffold and a 10-fold excess of staples, a high number of toroidal structures were detected by both AFM and TEM (**Fig. 2B *right*, Figs. S3–S4**), with a diameter 78 ± 3 nm (mean ± SD, n = 18) consistent with the design (**Fig. S5**). All these results show that mixing all DNA strands coding for DNA origami in a monovalent cation-rich mammalian cell culture medium, characteristic of most commonly used media, results in ultra-fast and isothermal self-assembly into user-programmed 2D or 3D morphology at 37 °C. The successful formation of origamis across various culture media led us to explore their *in situ* self-assembly in the presence of different living cell systems including 2D cell culture and organoid formation.

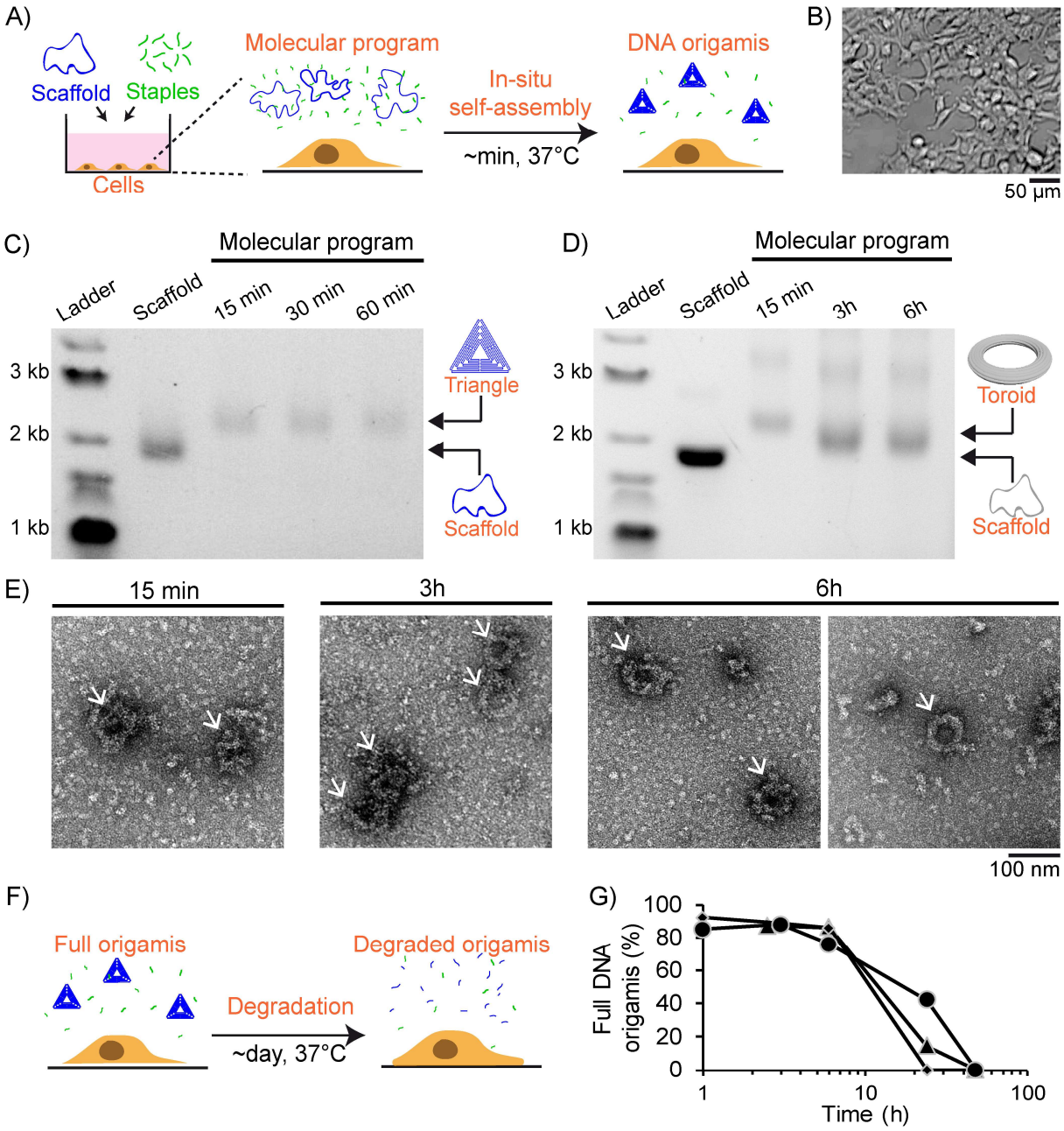

Figure 3. Fast self-assembly of stable 2D and 3D origamis in the presence of live cells (human embryonic kidney (HEK) 293T). A) The experiment consists in adding a molecular program to the surrounding medium of HEK cells in culture (DMEM, 10% FBS, Penicillin-Streptomycin) and incubating the system at 37 °C. B) Phase-contrast microscopy image of HEK cells cultured for 2 days with the DNA molecular program. C-D) Electrophoresis agarose gel of the medium with the molecular program coding for triangles (C) or toroids (D) in the presence of cells at 37 °C, as a function of incubation time. The two first lanes are the ladder and the scaffold alone, respectively. E) TEM images of toroid origamis (white arrows) obtained after 15 min, 3h, and 6 h of incubation at 37 °C in the presence of cells. Samples were purified by band extraction from the gel shown in D. F) Schematic of the long-term degradation of origamis and G) percentage of intact origamis, determined by gel electrophoresis,

as a function of incubation time in the presence of cells at 37 °C. Each symbol represents an independent experiment. [scaffold] = 1 nM (triangle), 10 nM (toroid); [staples] = 40 nM (triangle), 100 nM (toroid).

***In situ* origami assembly with live cells in 2D culture.**

We started with human embryonic kidney (HEK) 293T cells, a widely used immortalized cell line, in a conventional 2D culture with DMEM supplemented by 10 vol% FBS and the two antibiotics penicillin and streptomycin. The cells were cultured at 37 °C with 5 vol% CO_2 and the origami-coding molecular program (scaffold + staples) was directly added to the outer medium of the cultured cells (**Figs. 3A–B**). The cells were maintained at 37 °C and we characterized the evolution of the DNA assembly as a function of time using agarose gel electrophoresis (**Figs. 3C–D**). For both 2D-triangle and 3D-toroid programs, a band significantly shifted from that of the scaffold alone, which we attribute to the formation of self-assembled structures, was observed after 15 min of incubation and without much further evolution over time. For the 3D-toroids, this band was extracted for TEM observations, which confirmed the presence of toroidal shapes (**Fig. 3E**). The origamis appeared with a rough surface attributed to the non-specific adsorption of proteins[30] present in the medium, and dimensions (77 ± 6 nm, n = 14) in agreement with the bare design (**Fig. S5**). To our knowledge, this constitutes the first evidence that a molecular program of hundreds of synthetic DNA strands can faithfully self-assemble in the presence of live cells to produce user-defined DNA origamis. As observed in the absence of cells, the self-assembly process remained remarkably fast as it was completed in minutes timescale. We next investigated the stability of *in situ* self-assembled origamis over prolonged incubation with cells at 37 °C (**Fig. 3F**). Using agarose gel electrophoresis (**Fig. S6**), we established the fraction of intact DNA origamis over time and found that the self-assembled DNA origamis remained stable for the first 6 hours without detectable degradation (**Fig. 3G**). Beyond this time, the fraction of intact origamis progressively decreased, eventually disappearing after 1 to 2 days (**Fig. 3G**). With a formation completed in a few minutes, *in situ* self-assembled origamis thus remained structurally well-formed for several hours in the presence of cells. Moreover, during the 2-day incubation with the molecular program, the HEK cells proliferated (**Fig. S7**) and showed similar viability as in the absence of DNA (**Fig. S8**), demonstrating that neither the presence of cocktail of hundreds of staples (400 nM), nor *in situ* self-assembly into origamis interfered with normal cell activity.

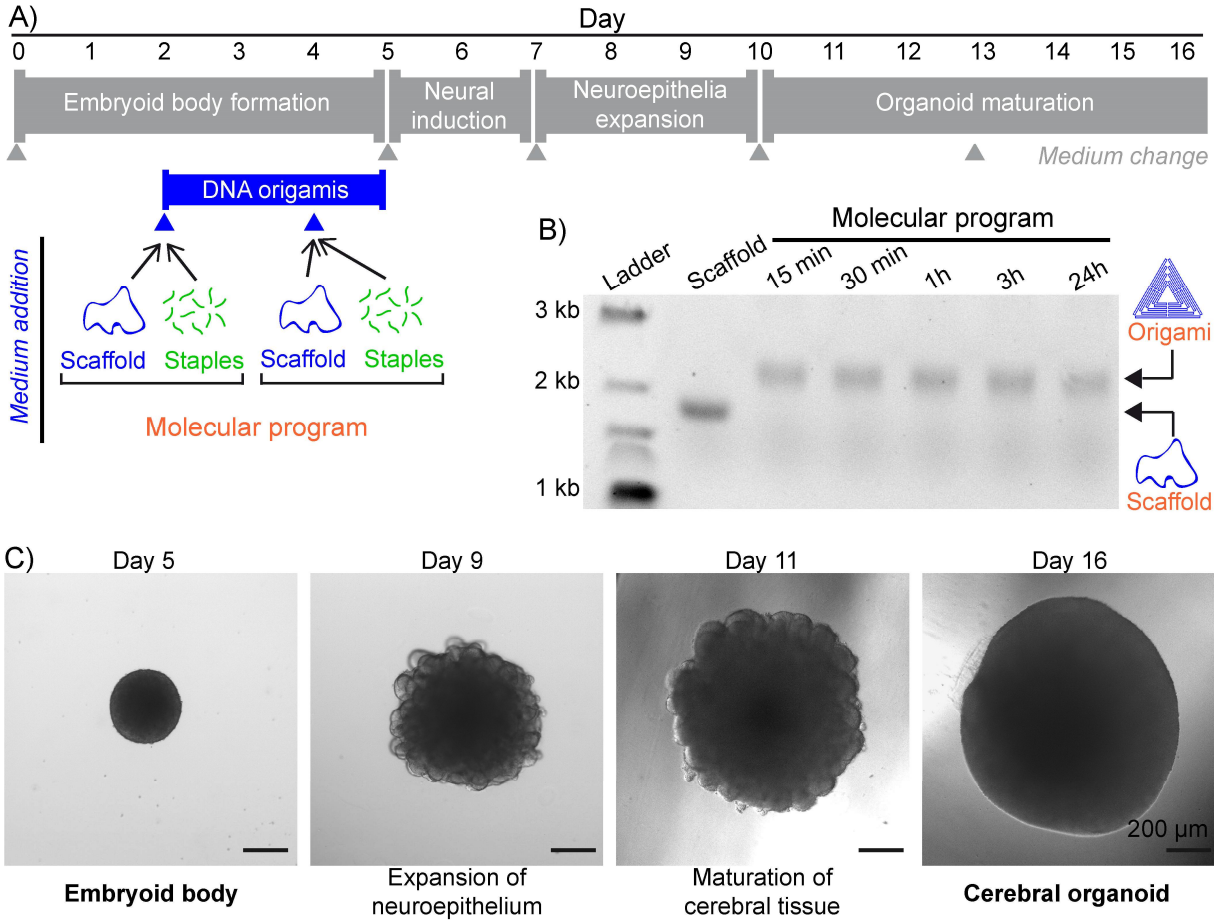

Figure 4. *In situ* self-assembly of DNA origamis during the formation of human cerebral organoids. A) *Top:* protocol for cerebral organoid formation, with the different stages indicated on a grey background. Grey triangles indicate a change of the medium. Day 0 corresponds to human induced pluripotent stem cells (hiPSCs) seeding. Bottom: The medium supplemented by a molecular program coding for triangle origamis is added on day 2 and 4 (blue triangles). Final concentrations in the culture medium are [scaffold] = 1 nM and [staples] = 40 nM. B) Electrophoresis agarose gel of the medium at day 2 as a function of time (15 min, 30 min, 1 h, 3 h, 24 h) after addition of the molecular program. The two first lanes are the ladder and the scaffold alone, respectively. C) Representative bright-field microscopy images showing the evolution of an embryoid body after *in situ* self-assembly of DNA origamis (day 5) into cerebral organoid (day 16). All scale bars are 200 µm.

In situ origami assembly during cerebral organoid formation

Next, we explored the feasibility of *in situ* origami self-assembly in the presence of a more complex 3D tissue-like system. As a proof of principle, it was implemented during the formation of cerebral organoids from human induced pluripotent stem cells (hiPSCs), which is a multi-step process involving different medium exchanges bringing the necessary factors for embryoid body (EB) formation (day 0–5), neural induction (day 5–7), neuroepithelium expansion (day 7–10) and organoid maturation (from day 10). A molecular program coding for triangle origami was implemented during EB formation by a

first addition of fresh medium containing all the DNA strands at day 2 (**Fig. 4A, Fig. S9**). Interestingly, we found that DNA origamis also successfully self-assembled in 15 min only in this complex system, and remained stable for at least a day (**Fig. 4B**). This process was repeated at day 4 of EB formation (**Fig. S9**). Notably, at day 5, well-formed EBs with smooth spherical shapes were obtained (**Fig. 4C *left*, Figs. S9–S10**), showing that neither the addition of DNA strands nor the subsequent *in situ* self-assembly of DNA origamis perturbed the EB formation. Following their formation subjected to *in situ* origami assembly, EBs were differentiated by neural induction and evolved to bigger and raspberry-shaped structures indicating successful neuroepithelium expansion (**Figs. 4C and S10**, day 9), prior to maturation into structures becoming progressively larger and smoother (**Figs. 4C and S10**, day 11). At day 16, particularly smooth and well-defined cerebral organoids were obtained (**Figs. 4C *right*, S10 *bottom***), showing that EBs subjected to *in situ* origami self-assembly maintained their full capability of differentiation.

Conclusion

We have shown that direct mixing of complex DNA molecular programs (scaffold + staple cocktail) in a monovalent cation-rich cell culture medium at 37 °C enables the ultra-fast, *in situ* self-assembly into user-defined elaborate 2D and 3D DNA nanostructures. The method was demonstrated to be particularly versatile and robust, functioning across a range of widely used biological media, including DMEM and RPMI (highly common for mammalian cell culture), E8 (for hiPSCs culture) or PBS (involved in a variety of biological protocols). Unlike conventional DNA origami assembly methods, which require hours to days, this isothermal self-assembly processus was found to be extremely rapid, well-formed DNA origamis being typically obtained in 5–15 min. As such, it can be seen as a very convenient way to prepare DNA origamis requiring nothing more than brief incubation at physiological temperature. But its most striking feature is that such a complex yet faithful self-assembly is achieved in conditions compatible with live-cell environments. This has allowed us to achieve the *in situ* self-assembly of user-defined 2D and 3D DNA origamis directly in the presence of living mammalian cells, with nanostructures forming within minutes and remaining stable for several hours. The formation and presence of DNA origamis did not perturb the cell behaviour: HEK cells maintained normal growth and viability, while hiPSCs successfully formed embryoid bodies and differentiated into well-defined human cerebral organoids. While demonstrated here with DNA origamis, this approach could be extended to other DNA assemblies, such as single-stranded titles (SST),[31] DNA nanogrids[32] or DNA nanotubes.[33–35] However, adapting these structures may require sequence optimization to fine-tune their melting temperature (T_m). For instance, DNA nanotubes have a lower T_m than DNA origamis in similar buffer conditions. As the method was functional with common cells (HEK cell line, commercially available hiPSCs) and in different formats (conventional 2D culture, 3D organoid formation), we envisage that the *in situ* DNA origami assembly described here is readily applicable to other cell types and tissues.

Considering the hundreds of DNA bricks and thousands of base pairs to be properly combined, one may wonder how can such an intricate isothermal assembly process occur so efficiently in such a short time. A part of the answer may lie in the intrinsic properties of the DNA origami method and especially the scaffold staple excess strategy,[5] which facilitates high-yield folding even for complex 3D architectures.[16,17] Our study further highlights the crucial role of staple excess and scaffold concentration as key-factors driving rapid *in situ* self-assembly. Devised by Paul Rothemund, the concept of DNA origamis was published nearly 20 years ago in a breakthrough paper[5] which, by concretizing the seminal ideas of Nadrian Seeman,[36] revolutionized nanoscience and many fields of research. During two decades, DNA origami method has proved to be not only exquisitely programmable but also exceptionally robust. Our findings further underscore this robustness, revealing that DNA origami self-assembly naturally aligns with the ionic conditions optimized for biological function. The second part of the answer may lie in the fact that, although machine-made, synthetic DNA has the exact same chemistry as biological DNA. Knowing that biological systems have evolved to conditions optimizing their functioning, which includes proper ionic conditions compatible with dynamic DNA assembly/disassembly, it is perhaps unsurprising that the media developed for cell culture and maintenance are also fortuitously optimized for ultra-fast isothermal self-assembly of DNA origamis at 37 °C. In this study, we have focused on origamis made of structural DNA bricks only, but the method is readily compatible with the incorporation of modified staples to enrich their functionality, for instance toward the cellular interface. By enabling the formation of DNA origamis *in situ* within live-cell environments in minutes-timescale, this method is the ground for the creation of a new class of environmental DNA nanomachines self-assembling directly within biological systems to facilitate applications ranging from cell surface nanosensing to adaptive drug delivery and dynamic mechanobiological activation.

Methods

In situ self-assembly of DNA origamis without cells

The self-assembly medium, supplemented by water when concentrated, was first heated at 37°C using a ThermoMixer® (Eppendorf®) prior to direct introduction of the desired volume of a concentrated staple mix without thermal pretreatment. After briefly vortexing the solution, a desired volume of concentrated scaffold solution was added and gently mixed by pipetting and tapping. The solution was shortly centrifuged, and kept at 37°C to start the incubation. Incubation time for DNA self-assembly was stopped when the sample was fixed on a surface for imaging (AFM and TEM) or quickly cooled by introducing the solution into ice and liquid nitrogen for storage at -20°C until use. For all experiments in DMEM (except **Fig. 1B**), DMEM 5× was used as a concentrated medium and its final concentration was 1×. For other experiments, 89 µL of 1× medium was used and supplemented with 10 µL of concentrated staples and 1 µL of concentrated scaffold. The final DNA concentration in each sample is indicated in the figure legends.

Agarose gel electrophoresis

1 wt% agarose gel was prepared with GrGreen (0.8×) DNA gel stain in TBE 0.5× buffer containing 10 mM of $MgCl_2$. Each DNA solution (18 µL), such as the ladder (1kbp plus DNA), the scaffold in the DMEM HG (1 or 10 nM), and the molecular program in the cell media, was mixed with 3 µL of loading dye solution (Gel loading dye 6×) and kept on ice prior to their loading in the gel. The electrophoresis was run at 50 V (BioRad PowerPac™ HC) for 3 h in an ice bath. After observation of the gel (Syngene G:Box), the bands of interest were cut for DNA extraction using spin columns (Montage DNA gel extraction kit from Merck).

AFM observation of DNA origamis in liquid

High-resolution environmental AFM was used to characterize the DNA assemblies. DNA assemblies in DMEM at 37°C were fixed at room temperature on a freshly cleaved mica surfaces (Nano-Tec V-1 grade Muscovite, Micro to Nano innovative Microscopy Supplies) that was pre-treated with 20 µL of 0.1M spermine tetrahydrochloride solution for 10 min before being washed with deionized water and a buffered saline solution (Tris-Acetate pH 8, 100 mM NaCl). 15 µL of DNA solution was deposited on the treated mica and left for 10 min adsorption. The solution was blotted off and the mica surface was gently rinsed with the buffered saline solution, and kept moist in this solution. The samples were observed with a Cypher ES AFM Microscope (Oxford Instruments) in tapping mode with 17-45 kHz resonance frequency in liquid and 0.09 N/m force constant tip (BL-AC40TS, Olympus), using the blueDrive™ photothermal excitation mode. Raw images were subjected to polynomial background

subtraction, plane level correction, rows alignment using various methods and horizontal scars correction in Gwyddion.

TEM observation of negatively-stained DNA origamis

The DNA self-assemblies were fixed on a plasma-treated 200 mesh copper grid which supports a carbon film (Ted Pella, Inc.): 10 µL of solution was added on the grid and left for 3 min before blotting it with a filter paper. The sample was negatively-stained using uranyl acetate (UA): 5 µL of 2 wt% UA water solution was added and quickly blotted, then 15 µL UA solution was again added and left for 1 minute before a last blotting. The samples were observed using a JEOL microscope at 200 kV (toroid origamis self-assembled in DMEM), or a Tecnai Spirit microscope at 120 kV (toroid origamis self-assembled with HEK cells).

Cell culture and *in situ* DNA origami self-assembly

HEK cells were cultured in 1× DMEM HG supplemented with 10 vol% FBS and 100 $U.mL^{-1}$ Penicillin-Streptomycin, at 37°C and 5% CO_2. The cells were passaged every 3 to 4 days at ~80% confluence using 2× Trypsin solution and were seeded at 1.10^5 $cell.mL^{-1}$. For the *in situ* DNA origamis self-assembly experiments, we used HEK cells at 20% confluence obtained the day after their seeding in 100 µL of culture medium. A volume of medium corresponding to the total concentrated DNA volume to be added was first withdrawn, before addition of 10 µL (triangle, final concentration 40 nM) or 27.8 µL (toroid, final concentration 100 nM) concentrated staple solution and gentle mixing by pipetting. 1 µL (triangle, final concentration 1 nM) or 2.5 µL (toroid, final concentration 10 nM) of concentrated scaffold was rapidly introduced and gently mixed by pipetting and tapping the cell plate, before being re-incubated at 37°C.

hiPSCs were cultured at 37°C and 5% CO_2, on vitronectin-coated dishes in Essential 8™, or in mTeSR™ (from STEMdiff™ Cerebral organoid kit) for the differentiation into brain organoid. They were detached every 3 to 4 days at 80% confluence using 0.5 mM ethylenediaminetetraacetic acid (EDTA) solution, and seeded in their cell culture medium supplemented with 10 µM Y-27632 after 10 or 5-fold dilution. For the formation of embryoid bodies, 9 000 iPSCs in 100 µL medium were first seeded in an ultra-low attachment V-bottom 96 well plate (Greiner Bio-One) and kept in culture for 5 days, as indicated in the STEMdiff™ Cerebral Organoid kit protocol. At day 2 after the seeding, a mixture of 78 µL of fresh medium, 20 µL concentrated staple solution and 2 µL of concentrated scaffold solution was added. This was repeated at day 4 with a mixture of 89 µL of fresh medium, 10 µL of concentrated staple solution and 1 µL of concentrated scaffold solution. In both cases, the final concentration of scaffold and the staples in the resulting cell medium was 1 nM and 40 nM, respectively. The differentiation of the embryoid bodies was initiated at day 5 and carried out until day 16 in a cell-

repellent tissue culture dish (Greiner Bio-One), as indicated by the kit supplier. The formation of brain organoids was characterized by bright-field imaging using a Leica DMi1 microscope and Leica MC170 HD camera.

Gel electrophoresis analysis of DNA degradation by cells

Electrophoresis DNA agarose gels were used to quantify the degradation of *in situ* self-assembled DNA origamis, in the presence of HEK cells. We identified DNA bands corresponding to well-formed origamis and free staples in cell media, and assumed that the DNA detected between these two bands corresponded to degraded origamis (**Fig. S6**). Using Image J, we defined and plotted the lanes integrating the band of well-formed origami and the one attributed to degraded origamis, for each incubation time (**Fig. S6**). The percentage of full DNA origami in the cell medium was calculated from the amounts of well-formed origamis and degraded origamis determined by peak area integration of fluorescence intensity.

HEK MTT assay

The proliferation assay was performed using a MTT toxicology assay kit. The cell culture media were replaced by serum-free cell culture media containing 10 vol% MTT reagent and introduced to each cell culture well and to another one without cells (control), before maintaining the plate at 37°C for 3 h incubation. 100 µL of MTT solubilization solution was added in each well, and the plate was gently mixed at room temperature for 15 min, protected from light. A microplate reader (Biotek Synergy HT) was used to measure the absorption of the solutions at 570 nm, and at 690 nm for subtraction.

Acknowledgements

We thank F. El Fakih (ENS) for training in DNA origami manipulation and AFM imaging; X.Z. Xu (ESPCI) and D. Lévy (Institut Curie) for access to TEM facilities; A. Di Cicco (Institut Curie) for TEM imaging. We acknowledge the Cell and Tissue Imaging core facility (PICT IBiSA), Institut Curie, member of the French National Research Infrastructure France-BioImaging (ANR10-INBS-04). This project has received funding from the European Research council ERC under the European Unions "HORIZON EUROPE Research and Innovation Programme (Grant Agreement No 101096956)" (D.B.), the Institut Universitaire de France IUF (D.B.) and the Fondation pour la Recherche Médicale FRM No ARF202209015925 (L.B.).

Contributions

L.B. performed all experiments; G.D.W. designed the 3D toroid origamis; S.D. and S.H. contributed to hiPSCs culture; M.M. advised on HEK culture; A. Y. supervised the hiPSC culture and cerebral organoid formation; G.B. conceptualized and supervised the 3D origami design; all authors contributed to experimental design, data analysis and manuscript edition; L.B. and D.B. conceived the work and wrote the manuscript; D.B. supervised the entire work.

Competing interests

The authors declare no competing interests.

References

1. Hughes, R. A. & Ellington, A. D. Synthetic DNA Synthesis and Assembly: Putting the Synthetic in Synthetic Biology. *Cold Spring Harb Perspect Biol* **9**, a023812 (2017).
2. Jones, M. R., Seeman, N. C. & Mirkin, C. A. Programmable materials and the nature of the DNA bond. *Science (1979)* **347**, 1260901–1260901 (2015).
3. Seeman, N. C. & Sleiman, H. F. DNA nanotechnology. *Nat Rev Mater* **3**, 17068 (2018).
4. Li, G. *et al.* DNA-Origami-Based Precise Molecule Assembly and Their Biological Applications. *Nano Lett* **24**, 11335–11348 (2024).
5. Rothemund, P. W. K. Folding DNA to create nanoscale shapes and patterns. *Nature* **440**, 297–302 (2006).
6. Hong, F., Zhang, F., Liu, Y. & Yan, H. DNA Origami: Scaffolds for Creating Higher Order Structures. *Chem Rev* **117**, 12584–12640 (2017).
7. Dey, S. *et al.* DNA origami. *Nature Reviews Methods Primers* **1**, 13 (2021).
8. Liu, J., Tang, J., Tong, Z., Teng, G. & Yang, D. DNA-guided self-assembly in living cells. *iScience* **26**, 106620 (2023).
9. Femino, A. M., Fay, F. S., Fogarty, K. & Singer, R. H. Visualization of Single RNA Transcripts in Situ. *Science (1979)* **280**, 585–590 (1998).
10. Chai, H., Cheng, W., Jin, D. & Miao, P. Recent Progress in DNA Hybridization Chain Reaction Strategies for Amplified Biosensing. *ACS Appl Mater Interfaces* **13**, 38931–38946 (2021).
11. Jensen, E. Technical Review: *In Situ* Hybridization. *Anat Rec* **297**, 1349–1353 (2014).
12. Beals, N., Model, M. A., Worden, M., Hegmann, T. & Basu, S. Intermolecular G-Quadruplex Induces Hyaluronic Acid–DNA Superpolymers Causing Cancer Cell Swelling, Blebbing, and Death. *ACS Appl Mater Interfaces* **10**, 6869–6878 (2018).
13. Li, F. *et al.* Dynamic Assembly of DNA Nanostructures in Living Cells for Mitochondrial Interference. *J Am Chem Soc* **144**, 4667–4677 (2022).
14. Guo, X. *et al.* Construction of Organelle-Like Architecture by Dynamic DNA Assembly in Living Cells. *Angewandte Chemie International Edition* **59**, 20651–20658 (2020).
15. Pinheiro, A. V., Han, D., Shih, W. M. & Yan, H. Challenges and opportunities for structural DNA nanotechnology. *Nat Nanotechnol* **6**, 763–772 (2011).
16. Andersen, E. S. *et al.* Self-assembly of a nanoscale DNA box with a controllable lid. *Nature* **459**, 73–76 (2009).
17. Wagenbauer, K. F., Sigl, C. & Dietz, H. Gigadalton-scale shape-programmable DNA assemblies. *Nature* **552**, 78–83 (2017).
18. Knappe, G. A., Wamhoff, E.-C. & Bathe, M. Functionalizing DNA origami to investigate and interact with biological systems. *Nat Rev Mater* **8**, 123–138 (2023).
19. Monferrer, A. *et al.* DNA origami traps for large viruses. *Cell Rep Phys Sci* **4**, 101237 (2023).
20. Chauhan, N. *et al.* Net-Shaped DNA Nanostructures Designed for Rapid/Sensitive Detection and Potential Inhibition of the SARS-CoV-2 Virus. *J Am Chem Soc* **145**, 20214–20228 (2023).

21. Dutta, P. K. *et al.* Programmable Multivalent DNA-Origami Tension Probes for Reporting Cellular Traction Forces. *Nano Lett* **18**, 4803–4811 (2018).

22. Mills, A. *et al.* A modular spring-loaded actuator for mechanical activation of membrane proteins. *Nat Commun* **13**, 3182 (2022).

23. Mills, A., Aissaoui, N., Finkel, J., Elezgaray, J. & Bellot, G. Mechanical DNA Origami to Investigate Biological Systems. *Adv Biol* **7**, (2023).

24. Paloja, K. *et al.* Balancing the Nanoscale Organization in Multivalent Materials for Functional Inhibition of the Programmed Death-1 Immune Checkpoint. *ACS Nano* **18**, 1381–1395 (2024).

25. Veneziano, R. *et al.* Role of nanoscale antigen organization on B-cell activation probed using DNA origami. *Nat Nanotechnol* **15**, 716–723 (2020).

26. Zhang, Q. *et al.* DNA Origami as an *In Vivo* Drug Delivery Vehicle for Cancer Therapy. *ACS Nano* **8**, 6633–6643 (2014).

27. Weiden, J. & Bastings, M. M. C. DNA origami nanostructures for controlled therapeutic drug delivery. *Curr Opin Colloid Interface Sci* **52**, 101411 (2021).

28. Wagenbauer, K. F. *et al.* How We Make DNA Origami. doi:10.1002/cbic.201700377:t.

29. Rossi-Gendron, C. *et al.* Isothermal self-assembly of multicomponent and evolutive DNA nanostructures. *Nat Nanotechnol* **18**, 1311–1318 (2023).

30. Luo, J. *et al.* Nanocarrier imaging at single-cell resolution across entire mouse bodies with deep learning. *Nat Biotechnol* (2025) doi:10.1038/s41587-024-02528-1.

31. Wei, B., Dai, M. & Yin, P. Complex shapes self-assembled from single-stranded DNA tiles. *Nature* **485**, 623–626 (2012).

32. Yan, H., Park, S. H., Finkelstein, G., Reif, J. H. & LaBean, T. H. DNA-Templated Self-Assembly of Protein Arrays and Highly Conductive Nanowires. *Science (1979)* **301**, 1882–1884 (2003).

33. Rothemund, P. W. K. *et al.* Design and Characterization of Programmable DNA Nanotubes. *J Am Chem Soc* **126**, 16344–16352 (2004).

34. Green, L. N. *et al.* Autonomous dynamic control of DNA nanostructure self-assembly. *Nat Chem* **11**, 510–520 (2019).

35. Agarwal, S., Klocke, M. A., Pungchai, P. E. & Franco, E. Dynamic self-assembly of compartmentalized DNA nanotubes. *Nat Commun* **12**, 3557 (2021).

36. Seeman, N. C. Nucleic acid junctions and lattices. *J Theor Biol* **99**, 237–247 (1982).