One-shot K-region-selective annulative $\pi$-extension for nanographene synthesis and functionalization

Kyohei Ozaki, Katsuaki Kawasumi, Mari Shibata, Hideto Ito & Kenichiro Itami

The optoelectronic nature of two-dimensional sheets of $sp^2$-hydridized carbons (for example, graphenes and nanographenes) can be dramatically altered and tuned by altering the degree of $\pi$-extension, shape, width and edge topology. Among various approaches to synthesize nanographenes with atom-by-atom precision, one-shot annulative $\pi$-extension (APEX) reactions of polycyclic aromatic hydrocarbons hold significant potential not only to achieve a ‘growth from template’ synthesis of nanographenes, but also to fine-tune the properties of nanographenes. Here we describe one-shot APEX reactions that occur at the K-region (convex armchair edge) of polycyclic aromatic hydrocarbons by the Pd(CH$_3$CN)$_4$(SbF$_6$)$_2$/o-chloranil catalytic system with silicon-bridged aromatics as $\pi$-extending agents. Density functional theory calculations suggest that the complete K-region selectivity stems from the olefinic (decreased aromatic) character of the K-region. The protocol is applicable to multiple APEX and sequential APEX reactions, to construct various nanographene structures in a rapid and programmable manner.

1 Institute of Transformative Bio-Molecules (WPI-ITbM) and Graduate School of Science, Nagoya University, Chikusa, Nagoya 464-8602, Japan.
2 JST-ERATO, Itami Molecular Nanocarbon Project, Nagoya University, Chikusa, Nagoya 464-8602, Japan. Correspondence and requests for materials should be addressed to K.I. (email: itami@chem.nagoya-u.ac.jp).
**Nanographenes**, which are nanometre-size subunits of graphenes (single-layer two-dimensional $sp^2$-hybridized carbon sheets)\textsuperscript{1–4} with a tunable bandgap, have become hot molecular entities in the field of nanocarbon materials science\textsuperscript{6}. As the properties of nanographenes depend heavily on the degree of $\pi$-extension, shape, width and edge topology, a novel bottom-up methodology for the precisely controlled synthesis of structurally uniform nanographenes is highly desirable\textsuperscript{6}. Among various approaches to synthesize nanographenes with atom-by-atom precision\textsuperscript{6}, one-shot annulative $\pi$-extension (APEX) reactions of polycyclic aromatic hydrocarbons (PAHs) hold significant potential not only to achieve a ‘growth from template’ synthesis of nanographenes, but also to fine-tune the properties of nanographenes.

In the last two decades, various bottom-up organic synthesis methods have been established for the controlled synthesis of large $\pi$-extended PAHs and nanographenes, as exemplified by the groundbreaking achievements of Mullen and colleagues\textsuperscript{7–10}, Scott and colleagues\textsuperscript{11,12}, Fasel and colleagues\textsuperscript{13,14} and others\textsuperscript{15} (Fig. 1a). In essence, most of the reported nanographene syntheses rely on a two-step sequence of (i) component assembly of small $\pi$-components, using reactions such as Diels–Alder reactions, Suzuki–Miyaura couplings and C–H activation reactions, to synthesize soluble nanographene precursors. Then, late-stage, fine tuning of nanographene properties is achieved through ‘growth from template’ synthesis (Fig. 1b–d).

**Figure 1** | **Organic synthesis approaches for structurally uniform nanographenes.** (a) The well-established two-step synthesis of nanographenes through $\pi$-component-assembling reaction and stitching (graphenization). (b) One-shot, region-selective APEX as alternative synthesis and functionalization of nanographenes through ‘growth from template’. (c) One-shot, bay-region-selective APEX by Diels–Alder reaction. (d) One-shot, K-region-selective APEX by double C–H activation (this work).
precursors, followed by (ii) stitching (graphenization) of soluble polyphenylene precursors by cyclodehydrogenation, flash-vacuum pyrolysis or photocyclization, to yield the target nanographenes (Fig. 1a)\(^6\),\(^{16}\). Although this state-of-the-art methodology has contributed significantly to the rapid progress of nanographene materials science, it has been well documented that the final and vital step of stitching (graphenization) is usually problematic.\(^{16}\) For example, intramolecular oxidative cyclodehydrogenation by Lewis acids and oxidants (Scholl-type reactions) often suffer from problems such as incomplete reaction, this can be categorized as the two-step method having a serious problem at the stitching (cyclodehydrogenation) stage.\(^{28}\).

The continuing evolution of nanographene science is heavily dependent on the discovery of new reactions and strategies that allow rapid and predictable synthesis, and functionalization of nanographenes. Here we illustrate the significant potential of one-shot APEX reactions to construct various nanographene structures in a rapid and versatile manner. Here, in we describe a one-shot APEX reaction that occurs selectively at the K-region of PAHs via double C–H activation\(^{36–39}\) (Fig. 1d). This reaction can be categorized as the two-step method having a serious problem at the stitching (cyclodehydrogenation) stage.\(^{28}\). The reactions at K-regions (convex armchair edges) are considered to be particularly difficult, as K-region bonds tend to have relatively high bond orders, and most aromatic substitution reactions occur preferentially at other aromatic C–H bonds.\(^{17}\)

Our APEX campaign\(^{29–34}\) began when we serendipitously discovered Pd(OAc)\(_2\)/o-chloranil as the first-generation C–H activation catalyst for PAHs in 2011 (ref. 35). This catalyst uniquely and effectively promotes the C–H arylation of non-functionalized PAHs with arylboroxines, with complete K-region selectivity. When coupled with the Scholl-type cyclodehydrogenation of the thus-formed arylated PAHs, a number of structurally intriguing nanographenes such as warped nanographenes\(^{34}\) (Fig. 1a) have been synthesized. Although this was a two-step nanographene synthesis at the time, we felt that the observed C–H activation reactivity and selectivity might be translated into an APEX reaction when the K-region is activated and annulated with properly arranged selectivity might be translated into an APEX reaction when the K-region is activated and annulated with properly arranged

---

**Figure 2 | One-shot K-region-selective C–H activation APEX.** (a) The established reaction conditions. (b) Deviation from standard conditions (effect of reaction parameters) in the reaction of 1b and 2a. (c) Scope of one-shot, K-region-selective, C–H activation APEX. Reaction conditions: polycyclic aromatic compounds 1 (0.2 mmol), silicon-bridged aromatics 2 (1.5 equiv), Pd(CH\(_3\)CN)\(_4\)(SbF\(_6\))\(_2\) (5 mol%), o-chloranil (2.0 equiv), 1,2-dichloroethane, 80 °C, 2 h. Bpin, pinacolobutyl.
respectively, while silicon-bridged aromatics 2 are optimal π-extending agents (Fig. 2a). For example, when 2,7-di-tert-butyl-phenanthrene (1a: 1.0 equiv) was treated with dimethyldibenzosilole (2a: 1.5 equiv) in 1,2-dichloroethane at 80 °C for 2 h in the presence of Pd(CH3CN)4(SbF6)2 (5 mol%) and o-chloranil (2.0 equiv), the corresponding K-region-annulated APEX product 3aa was obtained in 88% isolated yield, representing our standard APEX conditions. Unfunctionalized phenanthrene (1b) also underwent an APEX reaction with 2a to yield dibeno[g,p]chrysene (3ba). It should be noted that under these conditions, we observed the annulation exclusively at the K-region of phenanthrene 1a or 1b.

Listed in Fig. 2b are the effects of variations from the standard APEX conditions (1b + 2a → 3ba: 48% yield). For full lists of the effects of reaction parameters, see Supplementary Tables 2–4. As for the π-extension agents, we found that other dimetallocpHENyl derivatives such as 2,2'-bis(trimethylsilyl)-1,1'-biphenyl and dimethylidibenzogermole also reacted but led to the desired product in much lower yield. Dimethylidibenzostannole did not yield the product but generated a considerable amount of tetraphenylene and quaterphenyl by homodimerization. Changing the methyl groups of 2a only had detrimental effect on reaction efficiency. The commercially available cationic palladium complex Pd(CH3CN)4(BF4)2 showed APEX activity comparable to Pd(CH3CN)4(SbF6)2. Similar catalytic activities were also observed with the combined use of PdCl2 and silver salts such as AgOSO2CF3, AgBF4 and AgSbF6. On the other hand, neutral Pd(OAc)2 (our previous palladium pre-catalyst) did not promote the APEX reaction at all. These results clearly indicate the importance of a cationic palladium species for the APEX reaction.

Figure 3 | Mechanistic considerations. (a) A possible mechanism of K-region-selective C–H activation APEX. (b) Theoretical calculations of π-complexation and insertion steps. Structures were optimized by the density functional theory (DFT) calculations using B3PW91 hybrid functional (hydrogen atoms are omitted for clarity). Reaction pathways were followed by intrinsic reaction coordinate (IRC) computations, and high-accuracy, single-point energy calculations of DFT-optimized structures were performed with Møller-Plesset perturbation theory (MP2) (ref. 49). Energies are relative to that of o-chloranil-bound cationic phenylpalladium species.
reaction to occur. In the investigation of oxidants, commonly used oxidants such as benzoquinone, p-chloranil, DDQ and CuCl₂ (ref. 35) displayed virtually no APEX-type activity. We assume that the high reactivity of o-chloranil stems not only from its high oxidation aptitude but also from its unique o-quinone structure, which can bind to palladium in a bidentate manner and modulate the redox property effectively⁴¹,⁴². Our preferred solvent is 1,2-dichloroethane, but aromatic solvents such as toluene, chlorobenzene, 1,2-dichlorobenzene, fluorobenzene and trifluoromethylbenzene can also be used for the present APEX reactions.

Scope of K-region-selective APEX. As shown in Fig. 2c, various structurally and electronically diverse silicon-bridged aromatics 2 were found to react with 2,7-di-tert-butylphenanthrene (1a), providing the corresponding dibenzo[ghi]chrysene (3ab–3aj) in good-to-excellent yield with virtually complete K-region selectivity. In particular, dibenzosiloles having electron-deficient substituents (2b–2e)⁴⁰,⁴³ showed excellent reactivity. The tribenzo[a,c,f]tetraphene framework (3aj) can be readily constructed by the APEX reaction of 1a and benzonaphthosilole 2j⁴³. Notably, dichloro- and diboryl-substituted dibenzosiloles underwent the APEX reaction smoothly, leaving C–Cl and C–B bonds intact (3ad, 3ae and 3af). The tolerance of the reaction for these bonds makes it attractive for further \( \pi \)-extension and functionalization, using well-established cross-coupling chemistry. Furthermore, methylene-bridged phenanthrene 1c reacted with 2a to afford benzoindenochrysene 3ca, which can potentially lead to soluble nanographenes by facile substitution at the methylene moiety. Ease of post-functionalization is particularly advantageous for controlled surface alignment of nanographenes for device applications.

Mechanistic considerations of K-region-selective APEX. Although the exact mechanism of the present APEX reaction remains unclear, our current assumption is shown in Fig. 3a. A palladium(II) species undergoes the first transmetalation with o-chloranil (53% yield) and palla-
dium(0), the latter of which is oxidized to the active palladium(II) species by the action of \( \pi \)-complexation at the K-region before the insertion step, as depicted in Fig. 3a. To prove this hypothesis, we conducted density functional

Figure 4 | Multiple APEX and sequential APEX. Multiple APEX (a–c). (a) 2:1 APEX. Reaction conditions: 4 (1 equiv), 1a (5 equiv), Pd(CH₃CN)₄(SbF₆)₂ (5 mol%), o-chloranil (4 equiv), 1,2-dichloroethane, 80 °C, 2 h. (b) 1:2 APEX. Reaction conditions: 6 (1 equiv), 2a (3 equiv), Pd(CH₃CN)₄(SbF₆)₂ (5 mol%), o-chloranil (4 equiv), 1,2-dichloroethane, 80 °C, 8 h. (c) Reaction conditions: 8 (1 equiv), 2a (10 equiv), Pd(CH₃CN)₄(SbF₆)₂ (15 mol%), o-chloranil (10 equiv), 1,2-dichloroethane, 80 °C, 1 h. (d) Sequential APEX. 2:1 APEX conditions (A): 6 (3 equiv), 10 (1 equiv), Pd(CH₃CN)₄(SbF₆)₂ (5 mol%), o-chloranil (4 equiv), 1,2-dichloroethane, 80 °C, 6 h. 1:2 APEX conditions (B): 11 (1 equiv), 2a (4 equiv), Pd(CH₃CN)₄(SbF₆)₂ (15 mol%), o-chloranil (5 equiv), 1,2-dichloroethane, 80 °C, 4 h.
theory calculations (using B3PW91 hybrid functional) for the π-complexation and insertion steps on a model reaction of o-chloranil-bound cationic phenylpalladium species with phenanthrene yielding the alkylpalladium species shown in Fig. 3b (a model reaction relevant to A→B→C in Fig. 3a). Reaction pathways were followed by intrinsic reaction coordinate computations, and high-accuracy, single-point energy calculations of density functional theory-optimized structures were performed with Moller–Plesset perturbation theory. Among possible π-coordination complexes at C1−C2, C2−C3, C3−C4 and C9−C10 bonds, the π-complex at C9−C10 (K-region) was found to be most stable (9,10Pd). This may be due to the tendency of this bond to have the most olefinic (less aromatic) character. We also calculated all possible transition states of insertion from these π-complexes to give alkylpalladium complexes (Fig. 3b). The formation of C9-Pd complex (9Pd-10Ph), which leads to APEX at the K-region, was found to be most favourable both kinetically and thermodynamically. Thus, the basis of K-region selectivity in our new APEX reaction have been supported by computational theory. Detailed computational studies of Pd/o-chloranil-catalysed C–H activation, including the present APEX reactions, will be reported in due course. Based on these calculations, we predict the most olefinic (least aromatic) K-region π-bond to be the first APEX reaction site in future functionalizations of related π-extended PAHs and nanographenes.

Multiple APEX and sequential APEX. To showcase the utility of our APEX methodology in accessing a variety of nanographene (π-extended PAH) structures in a rapid and programmable manner, we examined several types of multiple APEX reactions (Fig. 4a–c). For example, a 2:1 APEX reaction occurs when treating ladder-type bis-silicon-bridged p-terphenyl 4 (ref. 51) with an excess of 2,7-di-tert-butylphenanthrene (1a) in the presence of Pd(CH3CN)4(SbF6)2/o-chloranil, to construct the dibenzodiphenanthracene framework 5 in 69% yield (Fig. 4a). Other isomers were not observed in the reaction, highlighting the fidelity of the present method to specific reaction sites. An alternative mode of the double APEX reaction (1:2 APEX) was also possible by the reaction of 2,7-di-tert-butylpyrene 6 (1.6 g, 1.0 equiv) and dibenzosiloene 2a (3.2 g, 3.0 equiv) in the presence of Pd(CH3CN)4(SbF6)2 (5 mol%) and o-chloranil (4.0 equiv) (Fig. 4b). It is noteworthy that the reaction could be conducted on a gram scale to yield di-tert-butylhexabenzozenotetracene 7 in 2.6 g (83% yield). The gram-scale synthesis clearly underscores the high capability of the present reaction conditions for multiple APEX reactions. Furthermore, the Pd(CH3CN)4(SbF6)2/o-chloranil system effectively promoted the one-shot fourfold APEX reaction of 7,7′-di-tert-butyl-2,2′-bipyrrene 8 (1.0 equiv) with dibenzosiloene 2a (10 equiv), to provide biphexabenzozenotetracene 9 in 31% yield (Fig. 4c).

To further examine the applicability of APEX technology for the construction of larger molecules, sequential APEX reactions were investigated (Fig. 4d). Pleasingly, the 2:1 APEX reaction of 2,7-di-tert-butylpyrene 6 (3.0 equiv) with bis-silicon bridged biphenyl 10 (ref. 51) (1.0 equiv) under Pd(CH3CN)4(SbF6)2/o-chloranil conditions afforded tetra-tert-butylhexabenzozenocene 11 in 53% yield. The follow-up 1:2 APEX reaction of 11 (1.0 equiv) with dibenzosiloene 2a (4.0 equiv) also took place to furnish the target decabenzoacenocene framework 12 in 17% yield. In this particular reaction, we recovered a considerable amount of starting material likely to be attributed to poor solubility in the reaction media. Despite being relatively small in molecular size, the electronic structures of these nanographenes can be systematically altered. With the increase in molecular length, the HOMO-LUMO gap becomes smaller. In line with this trend, we observed decent red shift in both absorption and fluorescence (see Supplementary Fig. 41 for details).

Discussion

It should also be mentioned that we observed the first sign of the possibility of employing APEX in an oligomerization/polymerization manifold when we detected oligomers by matrix-assisted laser desorption/ionization time-of-flight mass spectrometry analysis in the first 2:1 APEX reaction shown in Fig. 4d. Although we have not yet investigated this possible mode of reaction extensively, we envisage that the present APEX reaction could be applied to even larger molecules through judicious choice of solubilizing substrates on the substrates. Thus, the present result bodes well for the potential application of our APEX methodology to the bottom-up synthesis of graphene nanoribbons with controlled edge structures.

The present APEX methodology is complementary to the state-of-the-art two-step nanographene synthetic methods, thereby finding significant use in the ‘growth from template’ nanographene synthesis and in the late-stage fine-tuning of nanographene properties. Moreover, our APEX technology is not limited to the synthesis and functionalization of π-extended PAHs and nanographenes. One of the most significant features of the present APEX reaction is that unfunctionalized PAHs can be directly used for π-component assembly and π-extension with any pre-functionalization. Thus, various π-conjugated molecules made by many research groups (for many different purposes) will be suitable substrates for our APEX reaction, furnishing even more exciting classes of π-conjugated systems. The realization of APEX polymerization, the development of new APEX reactions for other PAH regions and acquisition of the first structure–property relationships for various nanographene structures are now ongoing in our laboratory.

Methods

Materials and characterization data. For the synthesis of all starting materials, siloles and π-extended PAHs, and their characterization, see Supplementary Methods. 1H, 13C and 19F NMR spectra were obtained for all compounds, see Supplementary Fig. 1–40. Ultraviolet–visible absorption and fluorescence spectra of 6, 11 and 12 are provided in Supplementary Fig. 41. Calculated energy surface of π-complexation and insertion are provided in Supplementary Fig. 42. Calculated energies of stationary points and their Cartesian coordinates are summarized in Supplementary Table 1. Effects of reaction parameters are provided in Supplementary Tables 2–4.

Typical procedure of K-region-selective APEX reaction. 2,7-Di-tert-butylphenanthrene (1a: 58 mg, 0.2 mmol, 1.0 equiv), dimethyldibenzosiloene (2a: 63 mg, 0.3 mmol, 1.5 equiv), Pd(CH3CN)4(SbF6)2 (7.4 mg, 10 μmol, 5 mol%), o-chloranil (98 mg, 0.4 mmol, 2.0 equiv) and a stirring bar were placed in a screw cap test tube. The tube was sealed with a perforated plastic cap and air was removed through a short pad of silica gel (eluent: CH2Cl2). After 2 h, the reaction mixture was cooled to room temperature and then passed through a short pad of silica gel (eluent: CH2Cl2). After the organic solvents were removed under reduced pressure, the residue was purified by silica-gel chromatography (eluent: hexane) to afford 3,14-di-tert-butylbenzo[6,7]chrysene (3aa) in 88% yield (77.0 mg) as a white powder.

References

1. Novoselov, K. S. et al. Electric field effect in atomically thin carbon films. Science 306, 666–669 (2004).
2. Allen, M. J., Tung, V. C. & Kaner, R. B. Honeycomb carbon: a review of graphene. Chem. Rev. 110, 132–145 (2010).
3. Geim, A. K. Graphene: status and prospects. Science 324, 1530–1534 (2009).
4. Wu, J., Paula, W. & Mullen, K. Graphenes as potential material for electronics. Chem. Rev. 107, 718–747 (2007).
5. Dreyer, D. R., Ruoff, R. S. & Bialkowski, C. W. From conception to realization: an historical account of graphene and some perspectives for its future. Angew. Chem. Int. Ed. 49, 9336–9344 (2010).
6. Chen, L., Hernandez, Y., Feng, X. & Muller, K. From nanographene and graphene nanoribbons to graphene sheets: chemical synthesis. Angew. Chem. Int. Ed. 54, 7640–7654 (2012).
7. Muller, K. Evolution of graphene molecules: structural and functional complexity as driving forces behind nanoscience. ACS Nano 8, 6531–6541 (2014).
8. Watson, M. D., Fechtenkoetter, A. & Muller, K. Big is beautiful – “aromaticity” revisited from the viewpoint of macromolecular and supramolecular benzene chemistry. Chem. Rev. 101, 1267–1309 (2001).
9. Narita, A. et al. Synthesis of structurally well-defined and liquid-phase-processable graphene nanoribbons. Nat. Chem. 6, 126–132 (2014).
10. Schwab, M. G. et al. Structurally defined graphene nanoribbons with high lateral extension. J. Am. Chem. Soc. 134, 18169–18172 (2012).
11. Scott, L. T. et al. Geodesic polyaranes with exposed concave surfaces. Pure Appl. Chem. 71, 2099–2119 (1999).
12. Tesevikas, V. M. & Scott, L. T. Geodesic polyaranes by flash vacuum pyrolysis. Chem. Rev. 106, 4868–4884 (2006).
13. Cai, J. M. et al. Atonymically precise bottom-up fabrication of graphene nanoribbons. Nature 466, 470–473 (2010).
14. Treier, M. et al. Surface-assisted cyclodehydrogenation provides a synthetic route towards easily processable and chemically tailored nanographenes. Nat. Chem. 3, 61–67 (2011).
15. Vo, T. H. et al. Large-scale solution synthesis of narrow graphene nanoribbons. Nat. Commun. 5, 3189 (2014).
16. Feng, X., Psula, W. & Muller, K. Large polycyclic aromatic hydrocarbons: synthesis and dicotic organization. Pure Appl. Chem. 81, 2203–2224 (2009).
17. Harvey, R. G. Polycyclic Aromatic Hydrocarbons (Wiley-VCH, 1997).
18. Sanchez-Valencia, J. R. et al. Controlled synthesis of single-chirality carbon nanotubes. Nature 512, 61–64 (2014).
19. Omachi, H., Nakayama, T., Takahashi, E., Segawa, Y. & Itami, K. Initiation of carbon nanotube growth by well-defined carbon nanoribons. Nat. Chem. 5, 572–576 (2013).
20. Fort, E. H., Donovan, P. M. & Scott, L. T. Diels–Alder reactivity of polycyclic aromatic hydrocarbon bay regions: implications for metal-free growth of single-chirality carbon nanotubes. J. Am. Chem. Soc. 131, 16006–16007 (2009).
21. Fort, E. H. & Scott, L. T. One-step conversion of aromatic hydrocarbon bay regions into new unsubstituted benzene rings. A reagent for the low-temperature, metal-free growth of single-chirality carbon nanotubes. Angew. Chem. Int. Ed. 49, 6626–6628 (2010).
22. Fort, E. H. & Scott, L. T. Gas-phase Diels–Alder cycloaddition of benzene to an aromatic hydrocarbon bay region. Groundwork for the selective solvent-free growth of armchair carbon nanotubes. Tetrahedron Lett. 52, 2031–2033 (2011).
23. Li, J., Jiao, C., Hsiao, K. W. & Wu, J. Lateral extension of π conjugation along the bay regions of bisanthenes through a Diels–Alder cycloaddition reaction. Chem. Eur. J. 17, 14672–14680 (2011).
24. Konishi, A., Hirano, Y., Matsumoto, K., Kurata, H. & Kubo, T. Facile synthesis and lateral π-expansion of bisanthenes. Chem. Lett. 42, 592–594 (2013).
25. Schuler, R. et al. From perylene to a 22-rings aromatic hydrocarbon in one-pot. Angew. Chem. Int. Ed. 53, 9004–9006 (2014).
26. Fort, E. H. & Scott, L. T. Carbon nanotubes from short hydrocarbon templates. Nature 466, 470–473 (2010).
27. Clar, E. & Zander, M. Syntheses of coronene and 1:2-7:8-dibenzocoronene. Angew. Chem. Int. Ed. 51, 8960–9009 (2012).
28. Tesevikas, V. M. & Scott, L. T. Geodesic polynes by flash vacuum pyrolysis. Chem. Rev. 106, 4888–4884 (2006).
29. Ossmat, K., Zhang, H., Ito, H., Lei, A. & Itami, K. One-shot indole-to-carbazole π-extension by Pd-Cu-Ag trinuclear system. Chem. Sci. 4, 3416–3420 (2013).
Reprints and permission information is available online at http://npg.nature.com/reprintsandpermissions/

How to cite this article: Ozaki, K. et al. One-shot K-region-selective annulative \(\pi\)-extension for nanographene synthesis and functionalization. Nat. Commun. 6:6251 doi: 10.1038/ncomms7251 (2015).

This work is licensed under a Creative Commons Attribution 4.0 International License. The images or other third party material in this article are included in the article’s Creative Commons license, unless indicated otherwise in the credit line; if the material is not included under the Creative Commons license, users will need to obtain permission from the license holder to reproduce the material. To view a copy of this license, visit http://creativecommons.org/licenses/by/4.0/