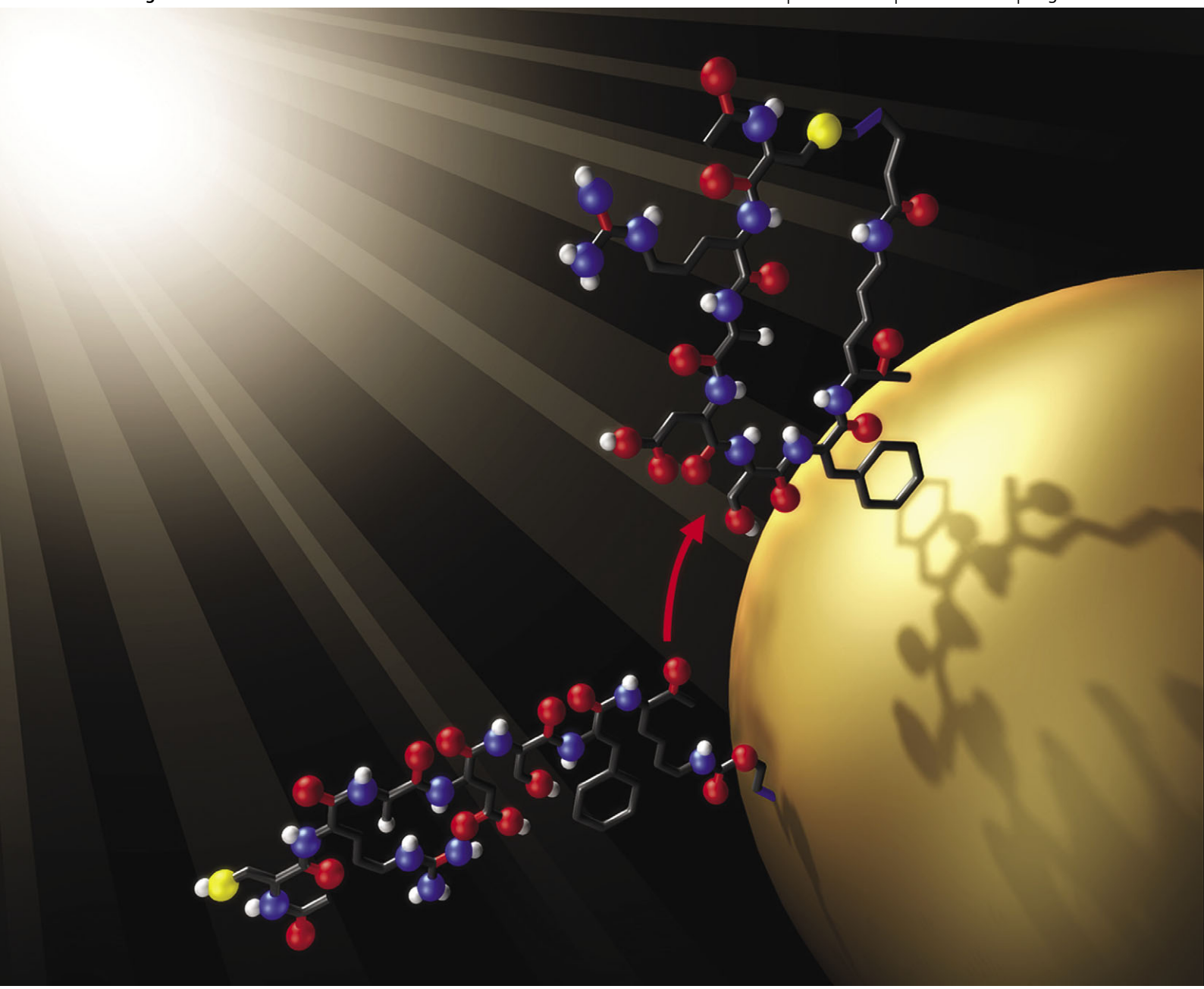
View Article Online / Journal Homepage / Table of Contents for this issue

ChemComm

Chemical Communications

www.rsc.org/chemcomm

Volume 46 | Number 23 | 21 June 2010 | Pages 4001–4204



ISSN 1359-7345

RSC Publishing

View Article Online

On-resin peptide macrocyclization using thiol–ene click chemistry†

Alex A. Aimetti,[a] Richard K. Shoemaker,[b] Chien-Chi Lin[c] and Kristi S. Anseth*[ac]

Received 20th January 2010, Accepted 23rd March 2010
First published as an Advance Article on the web 8th April 2010
DOI: 10.1039/c001375g

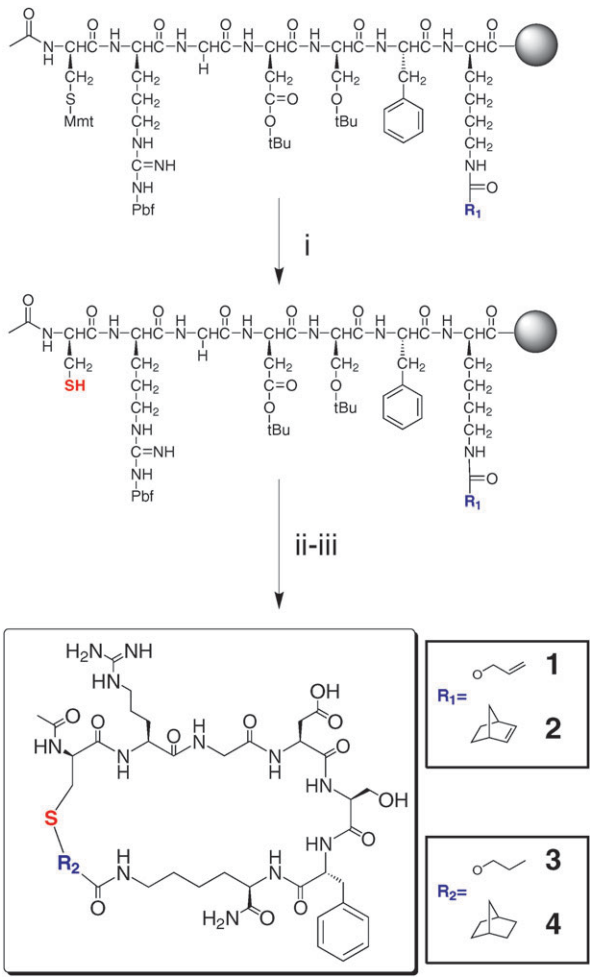
Published on 08 April 2010. Downloaded by Universitat Politècnica de València on 23/10/2014 18:33:00.

A versatile and rapid synthetic strategy has been developed for the on-resin cyclization of peptides using thiol–ene photochemistry. This unique method exploits the thiol group of natural cysteine amino acids and allows for various alkenes to be incorporated orthogonal to the peptide backbone.

Peptides have gained interest in the fields of chemical biology and drug discovery[1] due to their ability to bind to highly selective receptors of therapeutic interest. Additionally, peptides can be synthetically produced and optimized allowing for large-scale production. However, the drawbacks of peptide therapeutics are their short half-lives *in vivo*, poor bioavailability, and decreased cell permeability. Peptide macrocyclization, including peptide stapling,[2] has been shown to address these limitations and result in a molecule with increased potency. Cyclic peptides show increased stability to proteolytic degradation[3] relative to their linear precursors and are able to bind to their intended target at decreased effective concentrations, mainly attributed to the constrained conformation lowering the entropic cost of binding.[4]

The synthesis of cyclic peptides has been traditionally achieved, both on resin or in solution, by the formation of disulfide,[5] amide,[6] ester,[7] olefin,[8] and C–C bonds.[9] While these strategies result in peptide cyclization, the process can be extensive with reaction times ranging from hours to days. Due to the potential clinical advantages of cyclic peptides, there remains an unmet need to develop rapid and efficient synthetic strategies for their formation. As a result, researchers have become increasingly interested in using alternative synthetic strategies based on "click" reactions, termed by Sharpless, to achieve site-specific conjugation between two functional groups in very high yields. For example, the classic copper(I)-catalyzed azide–alkyne cycloaddition click reaction has been recently exploited for the on-resin formation of peptide macrocycles.[10] Alternatively, the thiol–ene click reaction is a radical mediated addition of a thiol to an alkene and has been utilized for the formation of dendrimers,[11] polymeric networks,[12,13] and disaccharides.[14] This contribution demonstrates the thiol–ene click reaction as an efficient and unique method for rapid formation of cyclic peptides on-resin.

Our strategy utilizes the natural amino acid, cysteine, as the thiol source for the reaction. However, peptides can be designed to contain cysteine residues that do not participate in the cyclization reaction by exploiting highly selective orthogonal Cys protecting groups. Various alkenes and their effect on the cyclization were studied. Commercially available Fmoc-Lys(Alloc)-OH was used as a building block to incorporate an allyl ester within the peptide sequence. The allyloxycarbonyl (Alloc) functional group is traditionally used as an orthogonal protecting group for lysine amino acids during peptide synthesis. However, recently the Lys(Alloc) monomer has been included



Scheme 1 Synthetic route to on-resin peptide macrocyclization using thiol–ene photochemistry. Conditions: **1**. (i) 2% TFA/CH_2Cl_2. **2**. (ii) DMPA (1 equiv.), *hν* (365 nm, 20 mW cm^{-2}), 20–60 min; (iii) TFA/TIPS/H_2O. Alkenes were incorporated using Fmoc-Lys(Alloc)-OH monomer (**1**, **3**) or on-resin modification using 5-norbornene-2-carboxylic acid (**2**, **4**).

[a] *Department of Chemical & Biological Engineering, University of Colorado, Boulder, CO 80309, USA. E-mail: Kristi.Anseth@colorado.edu; Fax: +1 303-492-4341; Tel: +1 303-492-7471*
[b] *Department of Chemistry & Biochemistry, University of Colorado, Boulder, CO 80309, USA*
[c] *Howard Hughes Medical Institute, University of Colorado, Boulder, CO 80309, USA*

† Electronic supplementary information (ESI) available: Experimental details. See DOI: 10.1039/c001375g

View Article Online

Published on 08 April 2010. Downloaded by Universitat Politècnica de València on 23/10/2014 18:33:00.

within a peptide sequence to participate in a thiol–ene photoreaction to pattern within a hydrogel.[15] We have exploited this commercially available building block as a facile method to incorporate an alkene within the peptide sequence. Alternatively, a strained, bicyclic alkene (norbornene) was incorporated orthogonally to the peptide backbone, as this alkene exhibits higher reactivity.[12] Arg-Gly-Asp (RGD), a peptide ligand of the $\alpha_v\beta_3$ integrin,[16] was synthesized as a model peptide to demonstrate proof of concept. Scheme 1 illustrates the general procedure for cyclic peptide formation. Linear Ac-C(Mmt)RGDSfK(alkene)-NH_2 (**1–2**) was built on the solid phase using Fmoc chemistry. The monomethoxytrityl (Mmt) sulfhydryl protecting group was selectively removed on-resin using 2% TFA/CH_2Cl_2. A type I photoinitiator, 2,2-dimethoxy-2-phenylacetophenone (DMPA), was added to the peptidyl resin and exposed to 365 nm light. The reaction was well mixed to minimize the impact of light attenuation. An on-resin Ellman's test (2.5 mM 5,5′-dithiobis-(2-nitrobenzoic acid) and diisopropylethylamine in methanol) was utilized to qualitatively monitor the extent of thiol conversion.[17] The reaction with the allyl ester reached completion (based on the Ellman's test) at 1 h. However, the reaction with the strained norbornene reached completion after just 20 min. On-resin photocyclization reactions to achieve **4** and **3** were recovered at 37% and 24% yield, respectively (calculated based on initial resin substitution).

Further, cyclic products were also obtained using a thermal initiation scheme as well as a solution phase photoreaction using unprotected linear peptides (**1** and **2**). Isolated yield calculations for each synthetic route can be found in Supporting Information Table S1.† Thermal reactions performed at 65 °C using azobisisobutyronitrile (AIBN; $t_{1/2,\ 65\ ^\circ C} \sim 10$ h) required longer reaction times (~48 h) and resulted in decreased yields relative to the on-resin photoreaction. Additionally, linear peptide precursors were purified and solubilized in dilute concentrations (2 mM) with DMPA and exposed to 365 nm light to form their cyclic products. Although the yields for the cyclization reaction were comparable to the on-resin photoreaction, the need for two purification steps and additional work-up led to decreased overall yield of the final product.

Cyclized products were characterized using various NMR techniques. Fig. 1b illustrates unambiguous evidence of cyclization in regard to **4**. The HMBC spectrum shows proton shifts at the #4 position (1H δ 2.97–2.85 ppm, diastereotopic protons influenced by the *endo*/*exo* conformation of the norbornane ring) that correlate with the carbon assignment at position #62 (13C δ 47.87, 47.08 ppm) as shown using HSQC data. Further, Fig. S14 (Supporting Information†) confirms the presence of NOEs between protons 4, 2, 51 (Fig. 1a) and protons associated with the norbornane ring. As expected, cyclic peptides eluted earlier than their linear counterpart using RP-HPLC (Fig. 1c). Spectral characterization for **1–3** can be found in Supporting Information.†

To confirm the thiol–ene reaction did not exhibit deleterious effects on the cyclic peptides' activity, a competitive binding ELISA was performed. Glycoprotein IIb-IIIa (GPIIb/IIIa) is present on platelets and its binding to fibrinogen has been associated with platelet aggregation. RGD has been shown to inhibit the binding of GPIIb/IIIa to fibrinogen.[18] Briefly, RGD peptide derivatives and fibrinogen were added to a Maxisorp 96-well plate coated with GPIIb/IIIa and incubated for 3 h. A goat polyclonal to fibrinogen antibody labelled with HRP was added (1 h) and later detected using ABTS substrate and the absorbance measured at 405 nm. Fig. 2 shows the inhibition of fibrinogen binding to GPIIb/IIIa in response to **3**

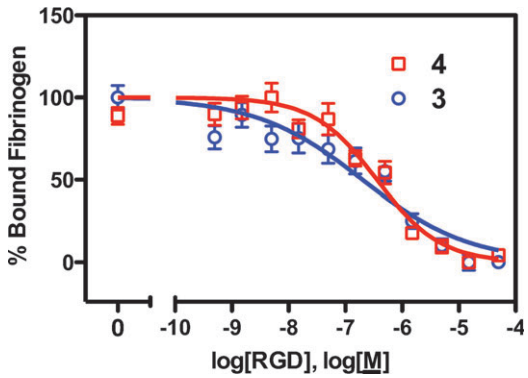


Fig. 2 Inhibition of fibrinogen binding to GPIIb/IIIa in the presence of cyclic RGD derivatives formed *via* thiol–ene click chemistry.

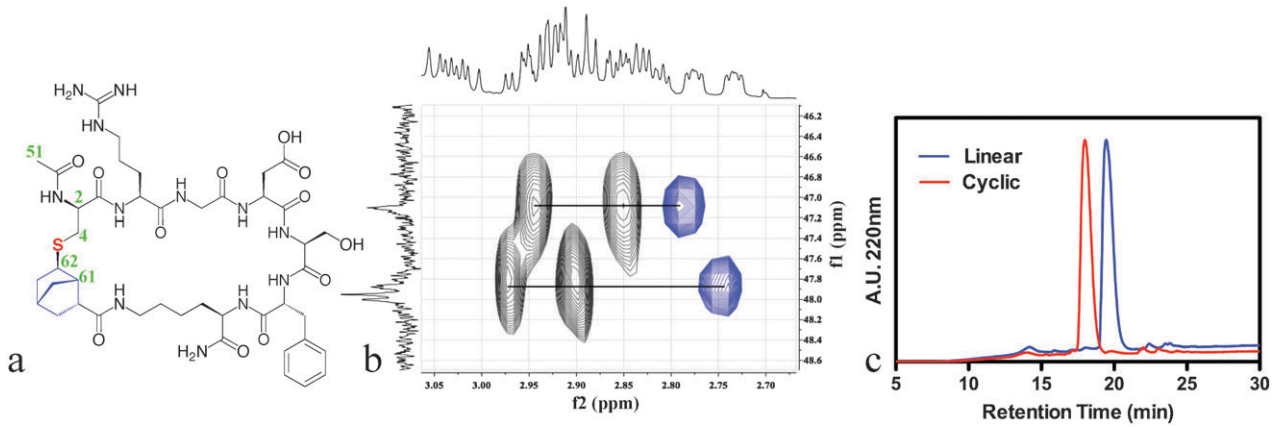


Fig. 1 (a) Chemical structure of **4** containing numbered atoms. (b) NMR spectrum (HMBC (black)/HSQC (blue) overlay) confirming bond correlations across the thioether bond. (c) RP-HPLC chromatogram highlighting varying elution times between cyclic (**4**) and linear (**2**) purified peptides.

 This journal is © The Royal Society of Chemistry 2010

View Article Online

Published on 08 April 2010. Downloaded by Universitat Politècnica de València on 23/10/2014 18:33:00.

and **4** exhibiting IC_{50} values of 0.20 ± 0.09 and 0.36 ± 0.09 μM, respectively, which are comparable to reported literature values.[18] The linear peptide (**1**) was less potent in inhibiting fibrinogen binding ($IC_{50} = 1.41 \pm 0.28$ μM).

In summary, we report the use of the thiol–ene click reaction for the on-resin macrocyclization of peptides. Peptides can be formed using commercially available amino acids without any post-synthetic modification (**1**, **3**). Alternatively a strained alkene (norbornene; **2**, **4**) can be utilized to achieve enhanced reaction kinetics and cyclization within 20 min. This Communication demonstrates the thiol–ene click photoreaction as a facile method for the rapid synthesis (~20 min) of cyclic peptides with improved yields relative to other on-resin reactions.

The authors would like to thank Cole DeForest for initial conceptual discussions and Kristen Feaver for assistance with the binding ELISA. This work was funded by the NIH (Grant RO1 DK076084), HHMI, and Graduate Assistance in Areas of National Need fellowship (A.A.A.).

Notes and references

1 R. E. Moellering, M. Cornejo, T. N. Davis, C. Del Bianco, J. C. Aster, S. C. Blacklow, A. L. Kung, D. G. Gilliland, G. L. Verdine and J. E. Bradner, *Nature*, 2009, **462**, 182.
2 (*a*) L. D. Walensky, A. L. Kung, I. Escher, T. J. Malia, S. Barbuto, R. D. Wright, G. Wagner, G. L. Verdine and S. J. Korsmeyer, *Science*, 2004, **305**, 1466; (*b*) M. M. Madden, C. I. R. Vera, W. J. Song and Q. Lin, *Chem. Commun.*, 2009, 5588.
3 D. Besser, B. Muller, P. Kleinwachter, G. Greiner, L. Seyfarth, T. Steinmetzer, O. Arad and S. Reissmann, *J. Prakt. Chem.*, 2000, **342**, 537.
4 C. Gilon, D. Halle, M. Chorev, Z. Selinger and G. Byk, *Biopolymers*, 1991, **31**, 745.
5 D. Ranganathan, V. Haridas, S. Kurur, R. Nagaraj, E. Bikshapathy, A. C. Kunwar, A. V. S. Sarma and M. Vairamani, *J. Org. Chem.*, 2000, **65**, 365.
6 Y. Shao, W. Y. Lu and S. B. H. Kent, *Tetrahedron Lett.*, 1998, **39**, 3911.
7 W. D. F. Meutermans, S. W. Golding, G. T. Bourne, L. P. Miranda, M. J. Dooley, P. F. Alewood and M. L. Smythe, *J. Am. Chem. Soc.*, 1999, **121**, 9790.
8 (*a*) S. J. Miller and R. H. Grubbs, *J. Am. Chem. Soc.*, 1995, **117**, 5855; (*b*) C. E. Schafmeister, J. Po and G. L. Verdine, *J. Am. Chem. Soc.*, 2000, **122**, 5891.
9 M. Hiroshige, J. R. Hauske and P. Zhou, *J. Am. Chem. Soc.*, 1995, **117**, 11590.
10 (*a*) V. D. Bock, R. Perciaccante, T. P. Jansen, H. Hiemstra and J. H. van Maarseveen, *Org. Lett.*, 2006, **8**, 919; (*b*) S. Punna, J. Kuzelka, Q. Wang and M. G. Finn, *Angew. Chem., Int. Ed.*, 2005, **44**, 2215; (*c*) R. A. Turner, A. G. Oliver and R. S. Lokey, *Org. Lett.*, 2007, **9**, 5011.
11 K. L. Killops, L. M. Campos and C. J. Hawker, *J. Am. Chem. Soc.*, 2008, **130**, 5062.
12 B. D. Fairbanks, M. P. Schwartz, A. E. Halevi, C. R. Nuttelman, C. N. Bowman and K. S. Anseth, *Adv. Mater.*, 2009, **21**, 5005.
13 T. Y. Lee, T. M. Roper, E. S. Jonsson, C. A. Guymon and C. E. Hoyle, *Macromolecules*, 2004, **37**, 3606.
14 M. Fiore, A. Marra and A. Dondoni, *J. Org. Chem.*, 2009, **74**, 4422.
15 (*a*) B. D. Polizzotti, B. D. Fairbanks and K. S. Anseth, *Biomacromolecules*, 2008, **9**, 1084; (*b*) C. A. DeForest, B. D. Polizzotti and K. S. Anseth, *Nat. Mater.*, 2009, **8**, 659.
16 R. Haubner, R. Gratias, B. Diefenbach, S. L. Goodman, A. Jonczyk and H. Kessler, *J. Am. Chem. Soc.*, 1996, **118**, 7461.
17 J. P. Badyal, A. M. Cameron, N. R. Cameron, D. M. Coe, R. Cox, B. G. Davis, L. J. Oates, G. Oye and P. G. Steel, *Tetrahedron Lett.*, 2001, **42**, 8531.
18 P. L. Barker, S. Bullens, S. Bunting, D. J. Burdick, K. S. Chan, T. Deisher, C. Eigenbrot, T. R. Gadek, R. Gantzos, M. T. Lipari, C. D. Muir, M. A. Napier, R. M. Pitti, A. Padua, C. Quan, M. Stanley, M. Struble, J. Y. K. Tom and J. P. Burnier, *J. Med. Chem.*, 1992, **35**, 2040.