Rapid, Heterogeneous Biocatalytic Hydrogenation and Deuteration in a Continuous Flow Reactor

Lisa A. Thompson,[a] Jack S. Rowbotham,[a] Jake H. Nicholson,[a] Miguel A. Ramirez,[a] Ceren Zor,[b] Holly A. Reeve,[a] Nicole Grobert,[b] and Kylie A. Vincent*[a]

The high selectivity of biocatalysis offers a valuable method for greener, more efficient production of enantiopure molecules. Operating immobilised enzymes in flow reactors can improve the productivity and handling of biocatalysts, and using H₂ gas to drive redox enzymes bridges the gap to more traditional metal-catalysed hydrogenation chemistry. Herein, we describe examples of H₂-driven heterogeneous biocatalysis in flow employing enzymes immobilised on a carbon nanotube column, achieving near-quantitative conversion in <5 min residence time. Cofactor recycling is carried out in-situ using H₂ gas as a clean reductant, in a completely atom-efficient process. The flow system is demonstrated for cofactor conversion, reductive amination and ketone reduction, and then extended to biocatalytic deuteration for the selective production of isotopically labelled chemicals.

Introduction

Typical chemical approaches for the reduction of unsaturated bonds in organic molecules rely on hydrogenation using molecular or supported nanoparticle metal catalysts, or hydride transfer from a donor such as NaBH₄. Direct hydrogenations with H₂ benefit from atom-efficiency, but often suffer from harsh reaction conditions, poor selectivity and contamination of the organic product with toxic metals. Biocatalysis is increasingly considered as a greener alternative for sustainable C=X bond reductions due to the high chemoselectivity and stereo-selectivity of enzymes under mild reaction conditions.[1] However, challenges still remain which prevent the widespread adoption of redox biocatalysis. Key barriers include the (often) high production cost and limited stability of biocatalysts, as well as the requirement of many redox enzymes for expensive cofactors. A large number of biocatalytic C=X bond reductions occur via delivery of hydride from the reduced form of a nicotinamide cofactor (NADH or NADPH), generating the oxidised cofactor, NAD(P)⁺. The high cost of these cofactors means that it is not feasible to add them in stoichiometric quantity, and so cofactor recycling methods have been developed and are widely used. These methods employ additional enzymes and sacrificial substrates, such as glucose dehydrogenase and glucose or alcohol dehydrogenase and isopropanol, generating significant quantities of carbon-based waste. Despite a rapidly growing interest in continuous processing in chemical production, the complexity of biocatalytic systems, together with difficulties in enzyme immobilisation, have hindered translation of biocatalysis into flow operation.

Continuous flow reactors have been widely used for organic synthesis over the past few decades,[2,3] and are increasingly being explored for biocatalysis.[4,5] Flow offers a number of advantages over batch,[6] including increased productivity, improved safety when handling toxic reagents or explosive gases, such as H₂,[7–10] and ease of automation and in-line reaction monitoring.[11–14] Another advantage of flow chemistry is the ability to retain catalysts within a column-type reactor, whilst pumping the reaction solution through the solid, allowing for easy separation, recovery and potential re-use of the catalyst, as well as increased contact between the solution and solid catalyst.[15–17] Immobilisation of biocatalysts often improves stability and tolerance to organic solvents and elevated temperatures.[18,19] The constant removal of reaction solution in flow can also prevent or minimise any product inhibition that may be experienced by the biocatalyst. As a result, there is an increasing number of examples of immobilised biocatalysts operated in flow,[20] with cofactor recycling provided by traditional methods.[21–23] However, there is currently no standard method for handling immobilised redox biocatalysts in flow, and so wider adoption outside the biotechnology community is limited. Therefore, a biocatalytic approach that more closely resembles the heterogeneous chemical methods already in widespread use in flow reactors could facilitate uptake of redox biocatalysis in flow.

To capture the advantages of heterogeneous hydrogenations and biocatalysis for selective C=X bond reductions, we have developed a biocatalytic NADH recycling system that uses only H₂ gas as a clean reductant (Figure 1).[24,25] The enzymes are immobilised on a carbon support by simple adsorption,
Results and Discussion

Continuous H\textsubscript{2}-driven Biocatalytic Cofactor Reduction

Experiments in this study employed the multi-wall carbon nanotube lined quartz tubes that we have previously used for biocatalysis in flow.\textsuperscript{[26]} To improve H\textsubscript{2} availability within the reactor, the set-up was modified to include a direct H\textsubscript{2} gas feed (Figure 2A). The separate liquid and gas feeds were combined at a Y-shaped connector prior to entering the column reactor, and the flow rates adjusted to give a gas:liquid feed ratio of approximately 3:1.

This modified reactor set-up was first applied to develop a single-pass flow reaction for H\textsubscript{2}-driven NADH production using SH immobilised in the carbon nanotube column reactor (Figure 2A). In this reaction, the liquid feed was comprised of 2 mM NAD\textsuperscript{+} in Tris-HCl buffer (50 mM, pH 8.0). The liquid flow rate was 32 \mu L min\textsuperscript{-1} and the gas flow rate was 100 \mu L min\textsuperscript{-1}. The reaction solution was collected as 2 \times reactor volume (RV) fractions, at the reactor outlet and analysed by UV-Vis spectroscopy. Quantitative conversion of NAD\textsuperscript{+} to NADH was achieved in only a 2.2 min t\textsubscript{Res} and was maintained for 40 RV (Figure 2B). This corresponded to 1544 enzyme turnovers, with a turnover frequency (TOF) of 18 min\textsuperscript{-1}, and a space-time yield (STY) of 38.7 g\textsubscript{product} L\textsuperscript{-1} h\textsuperscript{-1}. Good reproducibility was also achieved in the flow reactor, see SI.
The high conversion and short tRes in a single-pass reaction demonstrates a large improvement over the previous biocatalytic reactor set-up and enzyme system we described, owing to the increased H₂ availability.

The reaction was continued up to 200 RV (7.3 h), during which the SH remained active, however, the conversion decreased to 60%. Over this whole 200 RV operating time the total enzyme turnover number (TTN) was 6077 and the TOF was 11 min⁻¹ by the end of the reaction. Enzyme turnover numbers and frequencies were calculated assuming all of the SH added to the flow column had adsorbed onto the carbon nanotubes, therefore it is likely that these values are an under-estimate of the activity of the immobilised enzyme sample. The loss of SH activity in the flow reactor was probed further through repeated flow experiments with different tRes (Figure 3), and it was observed that the decrease in conversion occurred after 1200—1500 SH turnovers, regardless of the total time-on-stream.

This demonstrated the improved reactor set-up as a viable method for rapid H₂-driven turnover of cofactor, with the potential to provide efficient cofactor recycling when coupled to other NADH-dependent enzymes.

Continuous H₂-driven Biocatalytic Reductive Amination

The flow reactor was next tested for the biocatalytic reductive amination of pyruvate 1 with in-situ H₂-driven cofactor recycling (Figure 4A). For this step, SH and NADH-dependent alanine dehydrogenase (AlaDH) were co-immobilised within the column reactor. The liquid feed contained 10 mM sodium pyruvate, 100 mM NH₄Cl as the amine donor, and different amounts of NAD⁺, in Tris-HCl buffer (50 mM, pH 8.0). Flow rates were adjusted to achieve the desired tRes through the reactor, whilst maintaining the 3:1 gas:liquid ratio. The reaction solution was collected as 8–10 combined RV samples at the reactor outlet and the conversion of pyruvate 1 to alanine 2 was monitored by ¹H NMR spectroscopy (Table 1). Using 10 mol% cofactor, high conversions were achieved for 15, 5 and 2.2 min tRes, respectively (Entries 1–3). Only 23% conversion was achieved on decreasing the cofactor to 1 mol%, for a 5 min tRes (Entry 4). For Entry 2, high conversion was maintained across 24 RV, providing a STY of 13.3 g_product L⁻¹ h⁻¹. After this a decrease in conversion was observed that was consistent with the observed drop in activity of the SH over time (Figure 4B). The SH TOF was 79 min⁻¹ in the high activity region, which was higher than the TOF observed for the cofactor conversion experiments described in the previous section. In the SH-only cofactor reduction experiments, 2 mM NAD⁺ was used, whereas 10 mM substrate 1 was used in these reductive amination studies, with 1 mM NAD⁺. Therefore, the SH had to turn over the cofactor molecules more times to be able to supply sufficient NADH (10 mM) for the production of 2. Whilst Entry 3 generated a slightly lower conversion (90%), the shorter tRes provided a higher STY of 27.3 g_product L⁻¹ h⁻¹ as more material was processed per unit time. These results represent a vast improvement on previous results, in which overnight re-circulation generated just 40% conversion for the same reaction.²⁶

Continuous H₂-driven Biocatalytic Ketone Reduction

An NADH-dependent alcohol dehydrogenase (ADH105, Johnson Matthey) was also implemented in the flow reactor for the selective reduction of 4'-chloroacetophenone 3 to (S)-1-(4-chlorophenyl)ethanol (S)-4 (Scheme 1A), in combination with SH for H₂-driven cofactor recycling. Using the carbon nanotube column reactor this provided 90% conversion of 10 mM 3 to (S)-4 in 2.2 min tRes, with 10 mol% cofactor. However, a

![Figure 3. Comparison of multiple flow experiments showing SH activity loss during NAD⁺ conversion to NADH for: 10 min tRes (△), 2.2 min tRes (●), and 1 min tRes (■). Plotted in terms of (A) Reactor volumes and (B) Reaction time. Reaction conditions: SH (10 U), 2 mM NAD⁺, 50 mM Tris–HCl buffer, pH 8.0, H₂, gas:liquid ratio = 3:1. TN = enzyme turnover number.](Image)

| Entry | tRes [min] | Cofactor [mol%] | Conversion [%]²⁶[a] |
|-------|------------|----------------|------------------|
| 1     | 15         | 10             | >99              |
| 2     | 5          | 10             | >99              |
| 3     | 2.2        | 10             | 90               |
| 4     | 5          | 1              | 23               |

[a] Reaction conditions: SH (10 U), AlaDH (10 U), 10 mM sodium pyruvate, 100 mM NH₄Cl, 50 mM Tris–HCl (pH 8.0), gas:liquid ratio = 3:1, RV = 288 μL.
[b] Determined by ¹H NMR spectroscopy.
A decrease in conversion was observed after only 8 RV (to 1% after 100 RV, SI). This may have been due to poorer adsorption of the ADH on to the carbon nanotubes, leading to leaching of the enzyme from the reactor. Further development is underway to improve the stability of this biocatalytic system, including the use of different carbon supports for ADH immobilisation in flow.

An additional ketone substrate was next explored using the quinuclidinone reductase from *Agrobacterium tumefaciens* (AtQR) for the reduction of 3-quinuclidinone to (R)-3-quinuclidinol (Scheme 1B). The product, 6, is an important component of a number of pharmaceutical compounds, and so methods to selectively produce the enantiomerically pure alcohol are a valuable synthetic tool.[28,29] For this substrate, the flow system was again tested with both AtQR and SH co-immobilised within the carbon nanotube column reactor, which gave quantitative conversion of 5 mM substrate to (R)-6 in a 5 min tRes. This provided an SH TOF of 60 min⁻¹ and STY of 9.8 g_product L⁻¹ h⁻¹, and demonstrated the potential of H₂-driven...
biocatalysis in flow for the production of chiral pharmaceutical molecules. The manufacture of (R)-3-quinuclidinol at a suitable purity has been a key challenge for process chemists in recent years,\textsuperscript{[20-22]} and this is the first demonstration of a flow-based system to generate a product with exceptionally high enantio-purity.

**Continuous H\textsubscript{2}-driven Biocatalytic Deuteration**

The biocatalytic flow system was next applied to the selective deuteration of NAD\textsuperscript{+} (Scheme 2A). Molecules bearing asymmetric deuterium (\textsuperscript{2}H) centers are desirable for medicinal and analytical applications,\textsuperscript{[23,24]} but are synthetically difficult to achieve. We have identified H\textsubscript{2}-driven biocatalysis in heavy water, \textsuperscript{2}H\textsubscript{2}O, as a new route to these targets.\textsuperscript{[25]} The H\textsubscript{2}-driven system presented here allows for biocatalytic deuteration without the need to co-feed expensive pre-labelled compounds such as D-\{\textsuperscript{1}-H\}\textsuperscript{+}-glucose and \textsuperscript{2}H\textsubscript{3}-isopropanol, and without the need for H\textsubscript{2} gas. Instead, by simply exchanging the buffer from H\textsubscript{2}O to \textsuperscript{2}H\textsubscript{2}O (> 99% conversion) of 2 m\textsuperscript{M} NAD\textsuperscript{+} \textsuperscript{7} to the deuterated reduced form [45-\textsuperscript{2}H]-NADH \textsuperscript{8} was achieved in 2.2 min in the flow reactor, and with >99% isotopic selectivity. This was consistent with the conversion achieved for the production of non-deuterated NADH in the flow reactor, described above, demonstrating straightforward transferal of the flow protocol to generate isotopically labelled products.

Having demonstrated the formation of [45-\textsuperscript{2}H]-NADH in the flow reaction setup, the system could then be extended to produce labelled [3\textsuperscript{R}-\textsuperscript{2}H]-3-quinuclidinol \textsuperscript{9} from \textsuperscript{5} by including the A1OR (Scheme 2B). Operating under buffered \textsuperscript{2}H\textsubscript{2}O, > 99% conversion from \textsuperscript{5} to \textsuperscript{9} was achieved, with \geq 95% deuterium incorporation at C3, in a 5 min tRes. Additional \textsuperscript{2}H incorporation was also observed on C2 of the product, owing to non-enzymatic exchange of the enolisable protons on the substrate with the solvent (see SI). After an initial period of high conversion, a subsequent decrease in the production of the alcohol was observed from 2687 SH turnovers onwards, consistent with the loss of activity of the SH discussed earlier. However an SH TOF of 77 min\textsuperscript{-1} was achieved in the initial period, and a TTN of 10206 was achieved over the whole operating time, indicating that the SH activity was not negatively impacted by switching to the deuterated buffer system. Upon further development, the process described here could prove to be a valuable route to selectively deuterated pharmaceutical building blocks in continuous flow, made accessible by the straight-forward translation of reactions into \textsuperscript{2}H\textsubscript{2}O operating conditions.

**Conclusions**

We have demonstrated an improved multi-phase flow reactor for rapid heterogeneous biocatalysis with in-situ H\textsubscript{2}-driven cofactor recycling. Using only H\textsubscript{2} gas as the reductant more closely resembles traditional chemo-catalysed routes, whilst overcoming the high expense of using cofactors, and the waste generated by traditional biocatalytic cofactor recycling methods. The main challenge within this work was the stability of the SH activity within the flow reactor, and so further development is required to overcome this limitation. Through development of the reactor set-up, overnight re-circulating reactions (>12 h) were improved to 2–5 min tRes in a single-pass for >99% conversion. The flow reactor was demonstrated as a simple method for the production of important pharmaceutical fragments with exceptionally high ee. It was also shown that simple modifications to the reaction set-up allowed the flow-apparatus to be employed as a route to isotopically labelled compounds.

**Experimental Section**

**General information:** NAD\textsuperscript{+} and NADH were purchased from Prozomix. Sodium pyruvate, ammonium chloride, 4’-chloroaceto-phenone and Trizma-base were purchased from Sigma Aldrich. All reagents were used as received, without any additional purification. All solutions were prepared with MilliQ water (Millipore, 18 M\textsuperscript{Ω}cm) or \textsuperscript{2}H\textsubscript{2}O (99.98%, Sigma Aldrich). Deuterated buffer solutions were prepared by suspending Trizma-base in \textsuperscript{2}H\textsubscript{2}O and evaporating to dryness several times to exchange labile protons. The solutions of [\textsuperscript{1}H\textsubscript{3}]-Tris were then adjusted to the required p\textsubscript{H} with the addition of small quantities of HCl. UV-Vis spectra were obtained using an Agilent Cary 60 spectrophotometer. \textsuperscript{1}H NMR spectra were obtained using either a Bruker Avance III HD nanobay (400 MHz) or a Bruker Avance III HD (500 MHz) instrument. For samples in H\textsubscript{2}O, 10% v/v \textsuperscript{2}H\textsubscript{2}O was added for field locking purposes. GC-FID data was recorded using a Thermo Scientific TRACE 1310 instrument, equipped with a CP-Chirasil-Dex CB (Agilent), 25 m length, 0.25 mm diameter, 0.25 μm (film thickness), fitted with a guard of 10 m deactivated fused silica of the same diameter.

**Enzymes used:** Soluble hydrogenase (SH) from Ralstoniaeutropha, alamine dehydrogenase (AlaDH) and quinuclidinone reductase from Agrobacterium tumefaciens (A1OR) were prepared in-house.\textsuperscript{[25]} Alcohol dehydrogenase (ADH105, Johnson Matthey, Cambridge, UK) was used as supplied.

**General procedure for continuous flow reactions:** A detailed preparation of the carbon nanotube column reactors has been

![Scheme 2. (A) H\textsubscript{2}-driven biocatalytic deuteration of NAD\textsuperscript{+} \textsuperscript{7} to [45-\textsuperscript{2}H]-NADH \textsuperscript{8} in flow; (B) H\textsubscript{2}-driven biocatalytic production of [3\textsuperscript{R}-\textsuperscript{2}H]-3-quinuclidinol \textsuperscript{9} in flow. *Non-enzymatic \textsuperscript{2}H incorporation at C2 was also detected (see SI). (SH = soluble hydrogenase, A1OR = quinuclidinone reductase).](image-url)
described previously.\textsuperscript{24} The required mixture of enzymes was immobilised within the carbon nanotube column reactor by adsorption at 4 °C. After 1 h, the column was flushed with Tris-HCl buffer (50 mM, pH 8.0) to remove any un-adsorbed enzymes. For the reactions to be carried out, the column reactor (288 μL/RV) was connected to the required pumps (Vapourtec V-3 peristaltic pumps, Syrris Asia syringe pump or Harvard Apparatus Model 11 syringe pump) using PTFE tubing (1/16” OD, 1/32” ID) and TefzelTM or PEEK fittings (\(1/8\)-28, 0.02” thru-hole, IDEX). In a typical reaction, a liquid feed and a \(1\) atm gas feed were joined at a Y-piece (PEEK, \(1/8\)-28, 0.02” thru-hole, IDEX) before entering the column reactor. The flow rates of the pumps were adjusted according to the desired \(t\)Res through the reactor column, and with a gas:liquid flow rate ratio of approximately 3:1. All reactions were carried out at room temperature. The reaction solution was pumped through the reactor to waste for 2 x RV before samples were collected for analysis.

Further details of the enzyme preparation and immobilisation, and protocols for running continuous biocatalytic reactions are provided in the SI, together with \(^{1}H\) NMR, chiral GC and UV-Vis spectroscopic characterisation of the products.

**Acknowledgements**

L.T, J.R, J.N, M.R, H.R and K.V. are supported by Engineering and Physical Sciences Research Council (EPSRC) IB Catalyst award EP/N013514/1. N.G. acknowledges the Royal Society for financial support. The authors would like to thank Dr Beatriz Dominguez (Johnson Matthey) for providing ADH105. Dr Oliver Lenz (TU Berlin) is thanked for providing theRalstonia eutropha strain for \(SH\) production.

**Conflict of Interest**

The authors declare no conflict of interest.

**Keywords:** Biocatalysis · Cofactors · Continuous flow · Hydrogenation · Isotopic labelling

---

1. R. A. Sheldon, J. M. Woodley, *Chem. Rev.* 2018, 118, 801–838.
2. M. Baumann, I. R. Baxendale, *Beilstein J. Org. Chem.* 2015, 11, 1194–1219.
3. A. R. Bogdan, A. W. Dombrowski, *J. Med. Chem.* 2019, 62, 6422–6468.
4. J. Britton, S. Majumdar, G. A. Weiss, *Chem. Soc. Rev.* 2018, 47, 5891–5918.
5. L. Tamborini, P. Fernandes, F. Paradisi, F. Molinari, *Trends Biotechnol.* 2018, 36, 73–88.
6. M. B. Plutschack, B. Pieker, C. Gilmore, P. H. Seeberger, *Chem. Rev.* 2017, 117, 11796–11813.
7. B. Gutmann, D. Cantillo, C. O. Kappe, *Angew. Chem. Int. Ed.* 2015, 54, 6688–6728; *Angew. Chem.* 2015, 127, 6788–6832.
8. B. Gutmann, C. O. Kappe, *J. Flow Chem. 2017*, 7, 65–71.
9. M. Ifan, T. N. Glason, C. O. Kappe, *ChemSusChem* 2011, 4, 300–316.
10. P. J. Coisar, L. Huizestedi, M. I. Simone, A. McCluskey, K. C. Gordon, *Organ. Biomol. Chem.* 2015, 13, 7119–7130.
11. V. Sams, L. Cronin, *Chem. Soc. Rev.* 2016, 45, 2032–2043.
12. D. C. Fabry, E. Sugiono, M. Rueping, *React. Chem. Eng.* 2016, 1, 129–133.
13. G. A. Price, D. Malik, M. G. Organ, *J. Flow Chem. 2017*, 7, 82–86.
14. P. Sagmeister, J. D. Williams, C. A. Hone, C. O. Kappe, *React. Chem. Eng. 2019*, 4, 1571–1578.
15. R. Munirathinam, J. Huskens, W. Verboom, *Adv. Synth. Catal.* 2015, 357, 1093–1123.
16. K. Masuda, T. Ichiotsuka, N. Koumura, K. Sato, S. Kobayashi, *Tetrahedron* 2018, 74, 1705–1730.
17. M. Colella, C. Carlucci, R. Luisi, *Top. Curr. Chem.* 2018, 376, DOI 10.1007/s41061-018-0225-0.
18. R. A. Sheldon, S. van Pelt, *Chem. Soc. Rev.* 2013, 42, 6223–6235.
19. R. C. Rodrigues, C. Ortiz, Á. Berenguer-Murcia, R. Torres, R. Fernández-Lafuente, *Chem. Soc. Rev.* 2013, 42, 6290–6307.
20. M. P. Thompson, I. Peñañuel, S. C. Cosgrove, N. J. Turner, *Org. Process Res. Dev.* 2019, 23, 9–18.
21. F. Dall’Oglio, M. L. Contente, P. Conti, F. Molinari, D. Monfredi, A. Pinto, D. Romano, D. Ubiali, L. Tamborini, I. Serra, *Catol. Commun.* 2017, 92, 29–32.
22. V. De Vitis, F. Dall’Oglio, A. Pinto, C. De Micheli, F. Molinari, P. Conti, D. Romano, L. Tamborini, *ChemistryOpen* 2017, 6, 668–673.
23. M. L. Contente, F. Paradisi, *Nat. Commun.* 2018, 9, 452–459.
24. H. A. Reeve, L. Lauterbach, P. A. Ash, O. Lenz, K. A. Vincent, *Chem. Commun.* 2012, 48, 1589–1591.
25. H. A. Reeve, L. Lauterbach, O. Lenz, K. A. Vincent, *ChemCatChem* 2015, 7, 3480–3487.
26. A. Por, H. A. Reeve, J. Quinson, L. A. Thompson, T. H. Lonsdale, F. Dillon, N. Grobert, K. A. Vincent, *Chem. Commun.* 2017, 53, 9839–9841.
27. L. Lauterbach, O. Lenz, K. A. Vincent, *FEBS J.* 2013, 280, 3058–3068.
28. R. Chavakula, J. S. Chakradhar Saladi, N. G. B. Shankar, D. Subba Reddy, K. Raghu Babu, *Org. Chem. Ind.* 2018, 74, 127–131.
29. A. Radman Kastelic, R. Odžak, I. Pezdirc, K. Sović, T. Hrenar, A. Čipak Gašparović, M. Skočibušič, I. Primozič, *Molecules* 2019, 24, 2675.
30. K. Tsutsui, T. Katayama, N. Usutmi, K. Murata, N. Arai, N. Kuroto, T. Ohkuma, *Org. Process Res. Dev.* 2009, 13, 625–628.
31. R. Chavakula, N. R. Mutyal, S. R. Chennupati, *Org. Prep. Proced. Int.* 2013, 45, 507–509.
32. Q. Chen, B. Xie, L. Zhou, L. Sun, S. Li, Y. Chen, S. Shi, Y. Li, M. Yu, W. Li, *Org. Process Res. Dev.* 2019, 23, 34.
33. T. Pirali, M. Serafini, S. Cargnin, A. A. Genazzani, *J. Med. Chem.* 2019, 62, 5276–5279.
34. J. Altrutz, V. S. J. Kerr, M. Reid, *Angew. Chem. Int. Ed.* 2018, 57, 1758–1784; *Angew. Chem.* 2018, 130, 1774–1802.
35. J. S. Rowbotham, M. A. Ramirez, O. Lenz, H. A. Reeve, K. A. Vincent, *Nat. Commun.* 2020, 11, 1454.

Manuscript received: January 29, 2020
Revised manuscript received: March 20, 2020
Accepted manuscript online: April 3, 2020
Version of record online: June 12, 2020