Author/s:
Reyhani, A;Nothling, MD;Ranji-Burachaloo, H;McKenzie, TG;Fu, Q;Tan, S;Bryant, G;Qiao, GG

Title:
Blood-Catalyzed RAFT Polymerization

Date:
2018-08-06

Citation:
Reyhani, A., Nothling, M. D., Ranji-Burachaloo, H., McKenzie, T. G., Fu, Q., Tan, S., Bryant, G. & Qiao, G. G. (2018). Blood-Catalyzed RAFT Polymerization. Angewandte Chemie International Edition, 57 (32), pp.10288-10292. https://doi.org/10.1002/anie.201802544.

Persistent Link:
https://hdl.handle.net/11343/284134

**Title:** Blood-Catalyzed RAFT Polymerization

**Authors:** Amin Reyhani; Mitchell D. Nothling; Hadi Ranji-Burachaloo; Thomas G. McKenzie; Qiang Fu; Shereen Tan; Gary Bryant; Greg G. Qiao, Prof.



**To be cited as:** 10.1002/anie.201802544

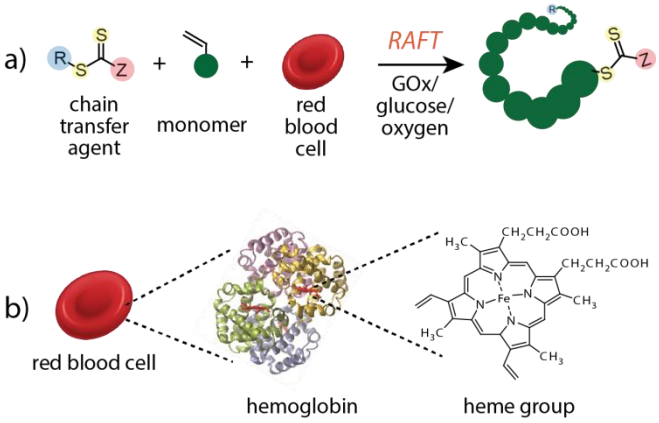
**Link to VoR:** https://doi.org/10.1002/anie.201802544

# Blood-Catalyzed RAFT Polymerization

Amin Reyhani,[a] Mitchell D. Nothling,[a] Hadi Ranji-Burachaloo,[a] Thomas G. McKenzie,[a] Qiang Fu,[a] Shereen Tan,[a] Gary Bryant,[b] and Greg G. Qiao*[a]

**Abstract:** The use of hemoglobin (Hb) contained within red blood cells to drive a controlled radical polymerization via a reversible addition-fragmentation chain transfer (RAFT) process is reported for the first time. No pre-treatment of the Hb or cells was required prior to their use as polymerization catalysts, indicating the potential for synthetic engineering in complex biological microenvironments without the need for *ex vivo* techniques. Due to the naturally-occurring prevalence of the reagents employed in the catalytic system (Hb and hydrogen peroxide), this approach may facilitate the development of new strategies for *in vivo* cell engineering with synthetic macromolecules.

Biocatalytic reversible deactivation radical polymerization (RDRP)[1] techniques are of great interest for both polymer chemists and biologists.[2] Novel approaches to bioengineering are becoming increasingly valued, with significant growth forecast in cell-related technologies such as cell separation and cell devices, or 'biological machines'.[3] The engineering of cells to perform specific functions including sensing, transport, or actuation *in situ* (i.e. in a biological microenvironment) may offer intriguing new therapies or applications.[4] These "active cells" can be engineered using chemical or biological means, or more likely through a combination of both. Recently, Hawker and colleagues have reported a method for functionalizing live cell surfaces with synthetic polymers, achieving a high grafting density, and allowing for the directed manipulation of cellular phenotype.[5] Despite the synthesis being cytocompatible for the cell-types investigated, the addition of small-molecule reagents – including the dye/photo-catalyst Eosin Y – may preclude the practicality of this technique in a clinical setting. In this regard, we envisioned the design of a system in which the cells themselves are the polymerization catalysts. Here, we present an initial proof-of-concept for the use of red blood cells to act in such a way for the synthesis of linear, water-soluble poly(acrylates) and poly(acrylamides). The polymerization system described is shown to be highly tolerant to biological conditions, while also being extremely robust as a synthetic method. This may open avenues towards more advanced cell-polymer therapies or applications.




**Scheme 1.** a) BioRAFT polymerization scheme; and b) Heme group structure from hemoglobin inside red blood cells.

Our previous investigations into iron catalysis in radical initiation systems for controlled polymerization[6] spurred an interest in the use of alternative iron sources.[7] Iron is, by mass, the most abundant element on Earth, making up much of Earth's inner and outer cores. Additionally, its importance in biology is fundamental, as its ability to exist in multiple oxidation states can facilitate redox reactions, making it a key component of many enzymes and proteins. Of these, hemoglobin (Hb) is perhaps the most well-known, due to its role as an oxygen transporter in the vascular system of animals. Hb contains four "heme" groups, each with a complexed iron species at its core. Red blood cells are extremely rich in Hb, with around 270 million Hb molecules per cell. In humans, almost half of blood's volume is from red blood cells, while approximately a quarter of all cells in the human body are red blood cells.[8] Therefore, blood offers an extremely important supply of iron that can potentially be employed for catalysis in a biological environment. Hb has been used in iron-mediated controlled polymerizations via atom transfer radical polymerization (ATRP),[2d, 9] however pretreatment of the Hb was typically required for the iron to become an effective activator of the reaction. We therefore aimed to employ *native* Hb contained within red blood cells to drive a controlled polymerization, with the view of eventually conducting synthetic polymerization reactions in biological environments *in situ*, where no modification of the biological components is required.

Given our previous research interests,[10] we selected reversible addition-fragmentation chain transfer (RAFT) radical polymerization[11] as our synthetic method of choice; its high degree of monomer versatility, excellent tolerance towards aqueous systems, and advanced implementation in a number of bio-related applications make it an ideal candidate.[12] In addition to the use of Hb as the source of iron, we also employed glucose oxidase (GOx) to enzymatically produce hydrogen peroxide ($H_2O_2$) *in situ*[13] in order to establish a Fenton initiation system.[14] The commercial bovine Hb employed in this study is

[a] A, Reyhani, M.D. Nothling, H. Ranji-Burachaloo, Dr. T.G. McKenzie, Dr. Q. Fu, Dr. S. Tan, Prof. G.G. Qiao
Chemical & Biomolecular Engineering
University of Melbourne
Parkville, VIC 3010, Australia
E-mail: qregghq@unimelb.edu.au
[b] Prof. G. Bryant
Department of Physics
RMIT
Melbourne, VIC 3000, Australia

Supporting information for this article is given via a link at the end of the document.

likely in the oxidized $Fe^{3+}$ form (i.e. methemoglobin), and this was confirmed via UV-vis spectroscopic analysis (ESI, Figure S1). To facilitate the production of $H_2O_2$, glucose and oxygen are the only other required reagents. Although GOx is a non-mammalian enzyme (it is produced by certain species of fungi and insects), all other reagents may be reasonably expected to be present in the vasculature system. This system therefore serves as the platform for our investigation into red blood cell activation of a controlled polymerization (**Scheme 1**).

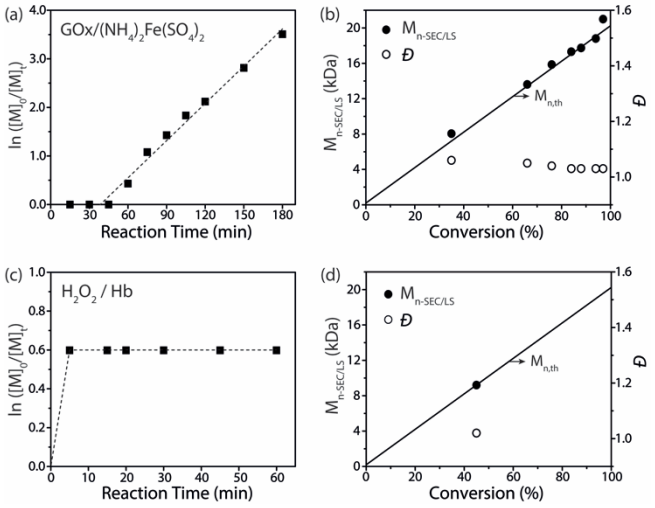



**Figure 1.** Kinetic data and molecular weight analysis of DMA polymerization employing (a-b) glucose oxidase (GOx) and an $Fe^{2+}$ salt (ESI, see Figure S2 for SEC peaks), and (c-d) hydrogen peroxide ($H_2O_2$) and hemoglobin (Hb).

Initially, the ability of GOx to generate $H_2O_2$ that can subsequently be utilized in a Fenton redox initiation system[15] for RAFT polymerization was assessed using an inorganic ferrous salt (ammonium ferrous sulphate) as the iron source. A model reaction system of *N,N'*-dimethylacrylamide (DMA), and a water-soluble trithiocarbonate RAFT agent (TTC), was employed. As oxygen is required for the enzymatic reaction, the samples were not degassed but simply combined in a sealed vial (together with glucose) at ambient temperature, with samples extracted periodically for analysis via $^1$H NMR and SEC. A smooth polymerization was observed following a short induction period (ca. 30 – 45 min), with linear trends observed for both the semi-logarithmic monomer conversion kinetics, and the growth of polymer molecular weight with conversion (Figure 1a-b). Moreover, observed molecular weights were in close agreement with theoretical values, and the polymers displayed narrow dispersities throughout. Next, we wanted to determine whether Hb could indeed be employed as a "biological Fenton reagent".[16] Although the subject of much investigation,[17] there remains some controversy over the exact mechanism of iron-assisted oxidative damage in biological systems.[17f] Using Hb together with added $H_2O_2$ in a reaction mixture degassed via sparging with $N_2$ (as there is no GOx present for this purpose), we observed significant polymerization after only 5 minutes. However, the conversion did not increase further with reaction time (Figure 1c). This agrees well with our previous studies,[6] and is thought to be due to the rapid depletion of the added $H_2O_2$ via both radical forming, and non-radical-forming 'wasting' reactions. Nevertheless, the synthesized polymer showed excellent structural characteristics (molecular weight and dispersity) as analyzed via SEC (Figure 1d), indicating that Hb was an effective iron-based catalyst for RAFT polymerization. It is known that in the presence of $H_2O_2$ the heme group of Hb can degrade, releasing free iron ions into the solution.[18] Therefore, we propose that in our system the released $Fe^{3+}$ ions can react with $H_2O_2$ via a Fenton-type reduction,[6] generating the more reactive $Fe^{2+}$ ions that participate in the main Fenton reaction to produce initiating hydroxyl radicals (ESI, Figure S3).

Next, the utilization of GOx together with Hb in an entirely biological polymerization system (i.e. "BioRAFT") was attempted. Pleasingly, the polymerization proceeded smoothly following an induction period similar to the previously-attempted GOx system (Figure 2a). The reaction was noticeably slower than the analogous system employing the inorganic iron salt, which may be due to the gradual release of iron from the heme group. To investigate this, we monitored Soret band ($\lambda_{max}$ = 410 nm) of the commercial bovine Hb[2d] by UV-vis spectroscopy (ESI, Figure S4). During polymerization the Soret band intensity decreases and ultimately disappears after 6 hours. This indicates the gradual degradation of the protein heme unit, altering the native state of Hb as the reaction progress and releasing iron into the reaction mixture during the BioRAFT process. However, near-quantitative monomer conversions were still observed within a reasonable timescale (<8 h), with the observed molecular weights were in close agreement with theoretical values, and the polymer maintained a narrow dispersity throughout (Figure 2b-c). Importantly, no evidence of chain transfer to cysteine residues in the Hb was seen, with a pure white polymer product obtained and a monomodal peak observed via SEC indicating no protein-polymer conjugate formation (ESI, Figure S5).

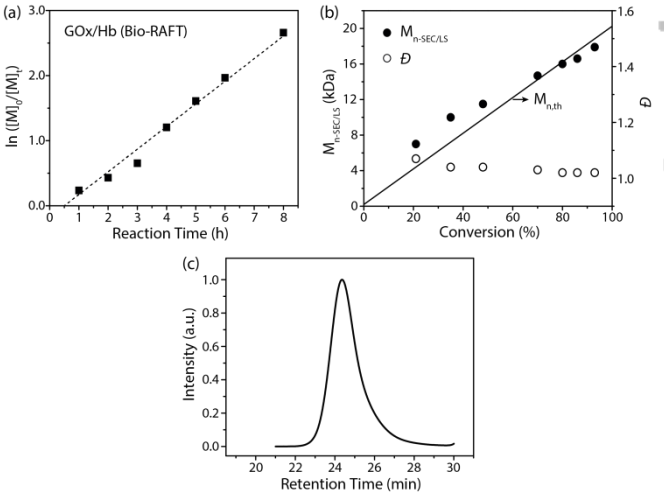



**Figure 2.** (a) Kinetic data for DMA polymerization system employing GOx and Hb (BioRAFT); (b) Molecular weight and dispersity data as determined via SEC-LS analysis; (c) SEC chromatogram of the final polymer showing monomodal and relatively symmetrical distribution.

A wide range of reagent concentrations were then screened to ascertain the optimum working conditions for the polymerization reaction, as well as the effect that the loading level of each component in the catalyst system has on the reaction kinetics and structural integrity of the final polymer product (ESI, Tables S1-S4, Figure S6). Control experiments in which glucose or Hb were selectively omitted resulted in no polymer formation (ESI, Table S5). Further control experiments performed in the absence of the RAFT agent resulted in the physical gelation of the reaction mixture, indicating an uncontrolled polymerization process as per free radical initiation (ESI, Figure S7).

Chain extension experiments conducted at near-complete monomer conversion demonstrated exceptionally high chain-end fidelity, or "livingness", of the synthesized polymers (ESI, Figure S8), allowing facile access to (multi)block copolymer structures. Additionally, the synthesis of a wide variety of polymer chain lengths was achieved by tuning the initial ratio of monomer to RAFT agent, while keeping the catalyst system (i.e. Hb/GOx/glucose) concentrations constant (ESI, Table S6). A number of different acrylate and acrylamide monomers were successfully synthesized under the same conditions, with several different trithiocarbonate RAFT agents also successfully employed (ESI, Table S7). Taken together, the above-mentioned investigations underscore the robust nature of the Hb-mediated polymerization system, as well as highlighting the versatility of the RAFT technique.[19]

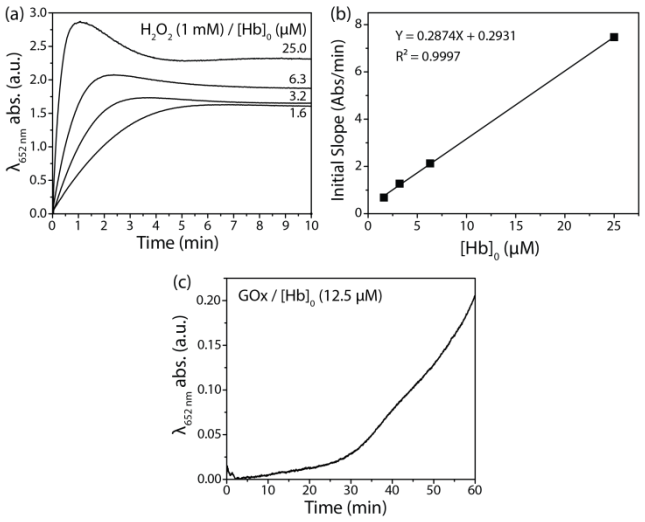



**Figure 3.** UV-vis spectroscopic analysis of 3,3',5,5'-tetramethyl benzidine (TMB) oxidation by hydroxyl radicals generated via Hb-catalysed Fenton reaction at $\lambda_{max}$ = 652 nm: (a) Fixed amount of $H_2O_2$ with increasing Hb loading concentrations; (b) Plot of initial slop of curves (Abs/min) against initial Hb concentration; and (c) GOx for in situ generation of $H_2O_2$ in the presence of Hb.

To investigate the initiation system in more detail, we employed a colorimetric assay to determine the rate of hydroxyl radical production in the Hb catalyst system. It should be noted that hydroxyl radicals are the sole source of initiating species in these systems, and hence are the determining factor for the rate of RAFT polymerization. To this end, 3,3',5,5'-tetramethyl benzidine (TMB) was employed as a chromogenic probe, as it has high specificity towards hydroxyl radicals. At a fixed concentration of $H_2O_2$, the production of hydroxyl radicals was observed to increase with increasing Hb concentration, with a saturation/stabilization effect seen within relatively short timescales (Figure 3a). Figure 3b illustrates the quantitative analysis for this kinetic assay data as a linear plot of the initial slopes as a function of Hb concentration. Conversely, when GOx with glucose was employed to generate $H_2O_2$ *in situ*, the hydroxyl radical generation in the system was seen to be dormant for a period of time, before increasing steadily (Figure 3c). These observations correlate strongly with the polymerization kinetics observed in Figures 1 and 2, and provide further evidence that Hb can act as an iron source for the catalytic generation of hydroxyl radicals, acting as the initiating species in a RAFT polymerization.

Finally, we wanted to assess the possibility of using the native Hb contained within red blood cells to drive the polymerization reaction, without requiring any isolation or pre-treatment. The robustness of the Hb-mediated polymerization was initially screened in a wide range of biological media to assess its tolerance towards bio-relevant conditions. Excellent results were obtained in almost all cases, with high monomer conversions and narrowly dispersed linear polymers obtained (ESI, Table S8). Moving on from isolated bovine Hb to the use of native Hb–containing red blood cells (erythrocytes), we employed ovine blood as the source of iron, which was obtained directly from a sheep. With RO water as solvent (Table 1, entry 1; Table S9, entries 1-3) (erythrocytes = 1 vol%), $H_2O_2$/erythrocyte–mediated BioRAFT showed 25% monomer conversion after only a few minutes (Figure 4a), consistent with our previous experiments (*vide supra*).[6] Next, a range of blood-catalyzed BioRAFT polymerizations were conducted at 1 vol% of erythrocytes (Table 1, entries 2 & 3; Table S9, entries 4-8). Figure 4b illustrates pseudo first-order kinetics of erythrocytes/whole blood–activated BioRAFT polymerization. Monodisperse linear polymers (*Đ* < 1.1) were observed throughout the polymerization, with experimental molecular weights in good agreement with theoretical values (Figure 4c-d). Selective omission of either GOx or D-glucose resulted in no monomer conversion after 24 h, while GOx/glucose alone (without erythrocytes) also showed no polymer (Table 1, entries 4-6; ESI, Table S9, entries 6-8). The reaction using whole blood was slightly faster than the isolated erythrocytes, which is ascribed to the lower concentration of red blood cells (which make up ca. 45 vol% of whole blood). This was also observed in the $H_2O_2$/erythrocyte–mediated BioRAFT, where increasing the erythrocyte concentration resulted in lower monomer conversions (Figure 4a). This effect is ascribed to increased hydroxyl radical 'wasting reactions' caused by excess iron, and agrees well with our previous studies on Fenton-mediated polymerizations.[6] Furthermore, real blood cells are comprised of a complex mixture of dissolved salts and proteins which may interfere with the polymerization (see Table S8). Therefore, the polymerization rate is dependent on the red blood cell loading level.

Interestingly, while polymerization in RO water was successful, we observed no monomer conversion in phosphate buffered saline (PBS) (ESI, Table S9, Entries 9-13). This is attributed to the lysis of the blood cells in unbuffered water due to osmotic

pressure leading to the release of hemoglobin into solution that increased its availability for subsequent reactions with $H_2O_2$. Cell lysis under these conditions was confirmed via optical microscopy (ESI, Figure S9). Taken together, these results indicate that red blood cells can be exploited as precursors for Fenton-mediated radical polymerization.

**Table 1.** BioRAFT polymerization of DMA catalyzed directly by ovine blood.

| Entry [a] | Peroxide Source | Iron source | Time (h) | Conv. (%) | $M_{n\text{-SEC/LS}}$ (Da) | $M_{n,the.}$ [b] (Da) | $Đ$ |
|---|---|---|---|---|---|---|---|
| 1 | $H_2O_2$ | Erythrocytes | 1 | 25 | - | - | - |
| 2 | GOx/ D-glucose | Erythrocytes | 24 | 78 | 16.9 | 15.8 | 1.04 |
| 3 | GOx/ D-glucose | Whole blood | 24 | 92 | 17.5 | 18.5 | 1.02 |
| 4 | no GOx/ D-glucose | Erythrocytes | 24 | 0 | - | - | - |
| 5 | GOx/no D-glucose | Erythrocytes | 24 | 0 | - | - | - |
| 6 | GOx/ D-glucose | None | 24 | 0 | - | - | - |

[a] In all reactions volume fraction of either erythrocytes or whole blood is 1 vol%, and solvent is RO water (b) Calculated from conversion values using the following formula: $M_{n,th}$ = [DMA]/[TTC] x conversion x $MW_{DMA}$ + $MW_{TTC}$.

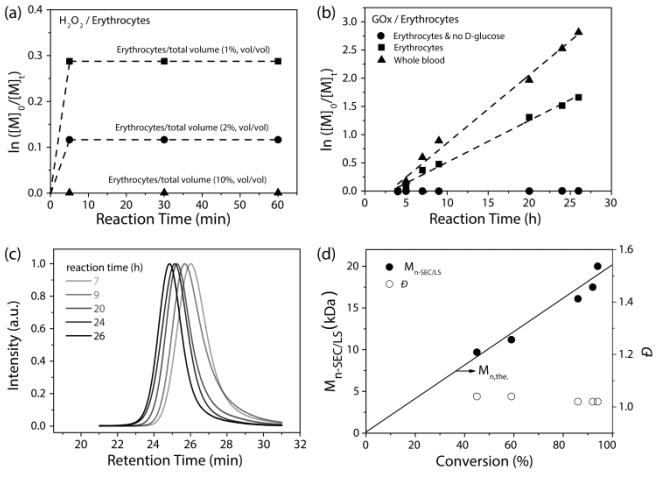



**Figure 4.** Kinetic studies of DMA polymerization ([DMA] = 4.85 M). (a) Plot of ln $([M]_0/[M]_t)$ against reaction time at different doses of erythrocytes (i.e., 1-10 vol%) via $H_2O_2$/erythrocyte-mediated BioRAFT process; (b) Plot of ln $([M]_0/[M]_t)$ against reaction time via erythrocyte (1 vol%) or whole blood (1 vol%)-mediated BioRAFT process; (c) SEC chromatograms of obtained PDMAs and (d) Molecular weight and dispersity values of PDMA synthesized via BioRAFT polymerization activated by whole blood.

We believe that the presented BioRAFT system could be potentially extended to cases with endogenously-produced $H_2O_2$[20] – for example, where it is often over-produced inside of cancer cells. This would allow for a fully autonomous initiation system for a controlled polymerization *in vivo*,[21] where the synthetic reagents may be shipped to the biological environments through advanced delivery techniques. This may in turn open new strategies to combat infections or diseases, and facilitate complicated cell engineering without the need for *ex vivo* manipulations. In addition, this polymerization system may be considered highly "green"[22] given that the human body is continuously producing the required catalyst (i.e. red blood cells) at a rate of 2 million per second.[8]

In conclusion, the use of red blood cells to catalyze a RAFT polymerization directly is reported. Through investigations into the behaviour of isolated hemoglobin as a Fenton reagent, the polymerization is thought to proceed via a redox initiation system capable of generating reactive hydroxyl radicals. The system is shown to be highly tolerant towards biological milieu, and may potentially be expanded to other biological environments where hydrogen peroxide is produced endogenously.

## Acknowledgements

A. R. and H. R. are supported by the Melbourne International Research Scholarship (MIRS) and the Melbourne International Fee Remission Scholarship (MIFRS). G.B. acknowledges the Australian Microscopy & Microanalysis Research Facility at RMIT University. G.G.Q. acknowledges the Future Fellowship (FT110100411) scheme and ARC Discovery Project (DP170104321). Authors are thankful to Professor Jean-Pierre Scheerlinck from the Centre for Animal Biotechnology, Faculty of Veterinary and Agricultural Sciences, University of Melbourne, for providing the ovine blood sample.

**Keywords:** RAFT • hemoglobin • polymerization • red blood cells

[1] a) Y.-H. Ng, F. di Lena, C. L. Chai, Chem. Comm. 2011, 47, 6464-6466; b) S. J. Sigg, F. Seidi, K. Renggli, T. B. Silva, G. Kali, N. Bruns, Macromol. Rapid Comm. 2011, 32, 1710-1715.

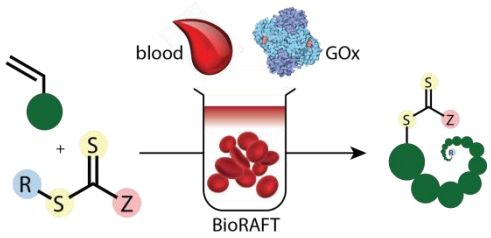
[2] a) E. P. Magennis, F. Fernandez-Trillo, C. Sui, S. G. Spain, D. Bradshaw, D. Churchley, G. Mantovani, K. Winzer, C. Alexander, Nat. Mater. 2014, 13, 748; b) S. J. Sigg, F. Seidi, K. Renggli, T. B. Silva, G. Kali, N. Bruns, Chimia 2012, 66, 66; c) K. Renggli, M. Spulber, J. Pollard, M. Rother, N. Bruns, in Green Polymer Chemistry: Biocatalysis and Materials II, ACS Publications, 2013, pp. 163-171; d) T. B. Silva, M. Spulber, M. K. Kocik, F. Seidi, H. Charan, M. Rother, S. J. Sigg, K. Renggli, G. Kali, N. Bruns, Biomacromolecules 2013, 14, 2703-2712; e) K. Renggli, N. Sauter, M. Rother, M. G. Nussbaumer, R. Urbani, T. Pfohl, N. Bruns, Polym. Chem. 2017, 8, 2133-2136; f) K. Matyjaszewski, L. F. Fu, A. R. Russell, A. E. Enciso, Angew. Chem. 2017; g) B. Zhang, X. Wang, A. Zhu, K. Ma, Y. Lv, X. Wang, Z. An, Macromolecules 2015, 48, 7792-7802; h) Y. Lv, Z. Liu, A. Zhu, Z. An, J. Polym. Sci. A: Polym. Chem. 2017, 55, 164-174; i) A. P. Danielson, D. B. Van Kuren, M. E. Lucius, K. Makaroff, C. Williams, R. C. Page, J. A. Berberich, D. Konkolewicz, Macromol. Rapid Comm. 2016, 37, 362-367; j) S. Shanmugam, J. Xu, C. Boyer, Angew. Chem. Int. Ed. 2016, 55, 1036-1040.

[3] http://www.sbwire.com/press-releases/biological-machines-market/release-741009.htm (retrieved: 741013-Feb-742018).

[4] R. F. Fakhrullin, A. I. Zamaleeva, R. T. Minullina, S. A. Konnova, V. N. Paunov, Chem. Soc. Rev. 2012, 41, 4189-4206.
[5] J. Niu, D. J. Lunn, A. Pusuluri, J. I. Yoo, M. A. O'Malley, S. Mitragotri, H. T. Soh, C. J. Hawker, Nat. Chem. 2017, 9, 537-545.
[6] A. Reyhani, T. G. McKenzie, H. Ranji‐Burachaloo, Q. Fu, G. G. Qiao, Chem. Eur. J. 2017, 23, 7221-7226.
[7] S. Enthaler, K. Junge, M. Beller, Angew. Chem. Int. Ed. 2008, 47, 3317-3321.
[8] F. Pierigè, S. Serafini, L. Rossi, M. Magnani, Adv. Drug Deliv. Rev. 2008, 60, 286-295.
[9] a) G. Kali, T. B. Silva, S. J. Sigg, F. Seidi, K. Renggli, N. Bruns, in Progress in Controlled Radical Polymerization: Mechanisms and Techniques, Vol. 1100, American Chemical Society, 2012, pp. 171-181; b) A. Simakova, M. Mackenzie, S. E. Averick, S. Park, K. Matyjaszewski, Angew. Chem. Int. Ed. 2013, 52, 12148-12151.
[10] T. G. McKenzie, Q. Fu, M. Uchiyama, K. Satoh, J. Xu, C. Boyer, M. Kamigaito, G. G. Qiao, Adv. Sci. 2016, 3, 1500394.
[11] a) J. Chiefari, Y. Chong, F. Ercole, J. Krstina, J. Jeffery, T. P. Le, R. T. Mayadunne, G. F. Meijs, C. L. Moad, G. Moad, E. Rizzardo, S. H. Thang, Macromolecules 1998, 31, 5559-5562; b) G. Moad, E. Rizzardo, S. H. Thang, Aust. J. Chem. 2012, 65, 985-1076.
[12] C. Boyer, V. Bulmus, T. P. Davis, V. Ladmiral, J. Liu, S. b. Perrier, Chem. Rev. 2009, 109, 5402-5436.
[13] a) H. Iwata, Y. Hata, T. Matsuda, Y. Ikada, Journal of Polymer Science Part A: Polym. Chem. 1991, 29, 1217-1218; b) F. Oytun, M. U. Kahveci, Y. Yagci, J. Polym. Sci. A: Polym. Chem. 2013, 51, 1685-1689; c) R. Chapman, A. J. Gormley, K.-L. Herpoldt, M. M. Stevens, Macromolecules 2014, 47, 8541-8547; d) R. Chapman, A. J. Gormley, M. H. Stenzel, M. M. Stevens, Angew. Chem. 2016, 128, 4576-4579.
[14] H. J. H. Fenton, J. Chem. Soc., Trans. 1894, 65, 899-910.
[15] J. Woodward, M. Wagner, K. W. Lennon, G. Zanin, M. A. Scott, Enzyme Microb. Technol. 1985, 7, 449-453.
[16] S. Sadrzadeh, E. Graf, S. S. Panter, P. Hallaway, J. Eaton, J. Biol. Chem. 1984, 259, 14354-14356.
[17] a) H. B. Dunford, Free Radical Biol. Med. 1987, 3, 405-421; b) J. Imlay, S. Chin, S. Linn, Science 1988, 240, 640-642; c) B. R. Van Dyke, P. Saltman, Free Radical Biol. Med. 1996, 20, 985-989; d) J. Prousek, Pure Appl. Chem. 2007, 79, 2325-2338; e) J. M. Rifkind, J. G. Mohanty, E. Nagababu, Front. Physiol. 2014, 5; f) B. Wang, J. Lu, K. D. Dubey, G. Dong, W. Lai, S. Shaik, J. Am. Chem. Soc. 2016, 138, 8489-8496.
[18] a) J. M. Gutteridge, FEBS letters 1986, 201, 291-295; b) J. Morris, K. Wong, C. Penaherrera, C. Payne, Biomater. Sci. 2016, 4, 331-337; c) J. Flores, C. Payne, J. Morris, RSC Adv. 2017, 7, 12017-12021; d) E. Nagababu, J. M. Rifkind, Biochemistry 2000, 39, 12503-12511; e) E. Nagababu, J. M. Rifkind, Biochem. Biophys. Res. Commun. 1998, 247, 592-596; f) A. Puppo, B. Halliwell, Biochem. J. 1988, 249, 185-190.
[19] M. R. Hill, R. N. Carmean, B. S. Sumerlin, Macromolecules 2015, 48, 5459-5469.
[20] J. A. Imlay, Annu. Rev. Biochem. 2008, 77, 755-776.
[21] A. Singh, D. L. Kaplan, in Enzyme-Catalyzed Synthesis of Polymers, Springer, 2006, pp. 211-224.
[22] M. Semsarilar, S. Perrier, Nat. Chem. 2010, 2, 811-820.

**Entry for the Table of Contents**

## COMMUNICATION




The use of hemoglobin (Hb) contained within red blood cells to drive a controlled radical polymerization via a RAFT process is reported for the first time. No pre-treatment of the Hb or cells was required prior to their use as polymerization catalysts, indicating the potential for synthetic engineering in complex biological microenvironments without the need for *ex vivo* techniques.

*Amin Reyhani, Mitchell D. Nothling, Hadi Ranji-Burachaloo, Thomas G. McKenzie, Qiang Fu, Shereen Tan, Gary Bryant, Greg G. Qiao**

***Page No. – Page No.***

**Blood-Catalyzed RAFT Polymerization**