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Albumin–Polymer–Drug Conjugates: Long Circulating, High Payload Drug Delivery Vehicles

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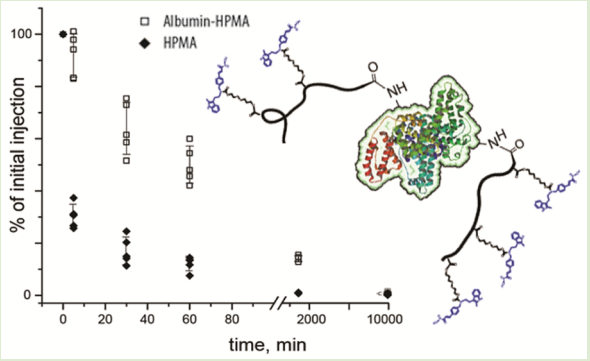
Supporting Information

ABSTRACT: Albumin is an exquisite tool of nature used in biomedicine to achieve long blood residence time for drugs, but the payload it can carry is typically limited to one molecule per protein. In contrast, synthetic macromolecular prodrugs contain multiple copies of drugs per polymer chain but offer only a marginal increase in the circulation lifetime of the drugs. We combine the benefits of the two platforms and at the same time overcome their respective limitations. Specifically, we develop the synthesis of albumin–polymer–drug conjugates to obtain long circulating, high payload drug delivery vehicles. In vivo data validate that albumin endows the conjugate with a blood residence time similar to that of the protein and well exceeding that of the polymer. Therapeutic activity of the conjugates is validated using prodrugs of panobinostat, an HIV latency reversal agent, in which case the conjugates matched the drug in terms of efficacy of treatment.



Extending the half-life of drugs has become a top priority of current medicinal chemistry, nanomedicine, and pharmaceutical sciences.[1,2] For injectable formulations and specifically for biological drugs, association of therapeutics with albumin has proven to be highly advantageous in that this protein is characterized with a phenomenal circulation lifetime—achieved through physiological mechanisms of protein recycling.[3] Conjugation of peptides and proteins to albumin results in a tremendous increase in the half-life of therapeutics,[4,5] and this technology has already brought products to the market.[6] However, the shortcoming of this platform for drug delivery is a restricted cargo loading—just one drug molecule per albumin.[4,7] This means that only highly potent drugs are suitable for this mode of drug delivery, whereas the overall majority of drugs on the market is not.

In contrast, macromolecular prodrugs (MPs) are highly beneficial in that each conjugate contains multiple copies of drug(s) creating a higher deliverable payload.[8] MPs facilitate drug delivery to tumors,[8] to the inflamed tissues,[9,10] and in antiviral efforts.[11−13] MPs enhance blood residence time of the drug by virtue of having an increased molar mass compared to the parent drug and thus being less susceptible to renal filtration.[8] However, MPs only extend circulation lifetime of small drugs from minutes to a few hours—compared to the 3 weeks long circulation times for albumin conjugates.[3] Further increase in blood residence time necessitates molar masses of MPs increased well above the renal filtration threshold (ca. 30 kDa)—up to the MDa range[14]—and this also aggravates the associated toxicity and the off-target effects.[15] The translation from lab to clinic for MPs has been nonyielding, and to date there is not a single product based on synthetic MPs on the market.

In this work, we put forward a hypothesis that conjugates of albumin and MP—herein termed albumin–polymer–drug conjugates (APD)—would overcome the respective limitations and combine the benefits of MPs and albumin for drug delivery. We envision that from albumin APD would inherit long blood circulation lifetime, whereas from MP it would inherit multiplicity of drug copies per carrier.

Albumin is the most abundant protein in human plasma and is understandably highly popular in drug delivery and imaging applications, specifically in the form of albumin-based aggregates, i.e., nanoparticles.[16] In these applications, albumin is often denatured,[17] cross-linked,[18] or else modified taking no care to preserve physiological characteristics of albumin such as the receptor recognition events. These nanoparticles are developed for anticancer treatment and/or imaging with the aim to facilitate tumor localization of the payload through the enhanced permeation–retention effect. This effect is due to the particles size (∼100 nm) and is observed for nanoparticles composed of virtually any organic or inorganic material, i.e., not unique to albumin.[19] Furthermore, to achieve long circulation

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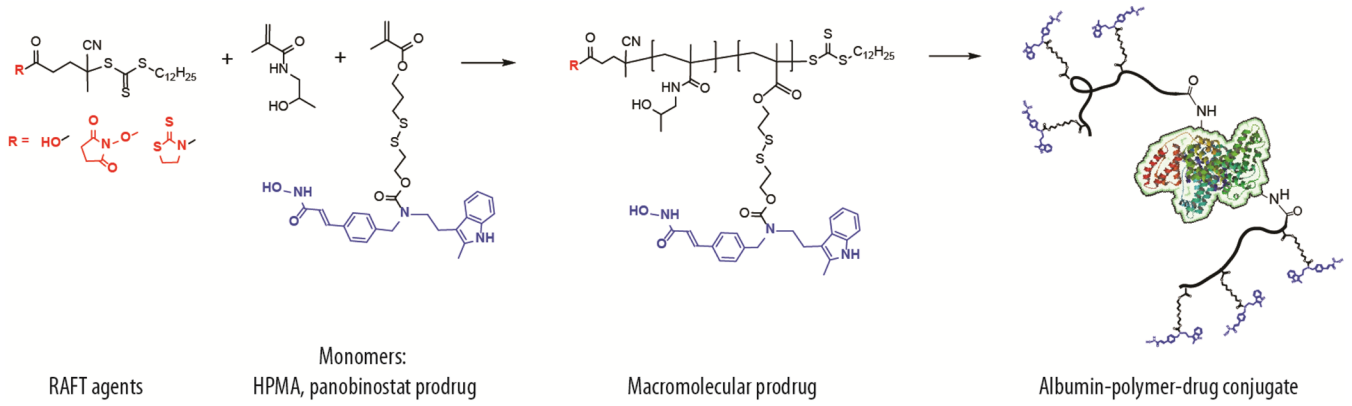

Figure 1. Schematic illustration of the synthesis of albumin−polymer−drug conjugates through copolymerization of the drug-containing monomer (drug: panobinostat, depicted dark blue) with HPMA via the RAFT mechanism whereby the RAFT agent is chosen to accommodate one-step conjugation with albumin via the polymer terminal group.

times, nanoparticles have to be PEGylated to avoid rapid clearance.[19] Powerful in their own right, albumin-based aggregates currently offer little to no clinical evidence for enhanced pharmaceutical performance of the associated drugs.

By contrast, the nature-inspired approach to use albumin as a drug carrier relies on the intact, nondenatured, pristine albumin. In this case, extended circulation lifetime for the associated drugs is achieved due to the recognition of albumin by the neonatal FcRn receptor.[20] This recognition serves as a basis for the physiological mechanism of recycling for albumin and is responsible for the 19 days long average half-life of albumin in humans[3] (~24 h in mice). The long circulation time is inherited by the albumin−drug conjugates. This approach is highly successful, and specifically for delivery of biological drugs, there are several products on the market exploiting this technique.[1,20] However, increasing the deliverable payload with the use of conjugated MP—that is, the synthesis of molecularly defined APD—has proven to be challenging and to our knowledge has not been accomplished to date.

In this work, we specifically address this challenge. We develop albumin conjugation for a polymer with a successful history of applications as a drug carrier, poly(*N*-2-hydroxypropylacrylamide), PHPMA.[21] As a drug, we use panobinostat, an FDA-approved anticancer agent[22] undergoing clinical trials as a human immunodeficiency virus (HIV) latency reversing agent.[23] We present in vivo quantification of the blood residence time for the albumin conjugates and quantify the therapeutic effect of APD. Our data present APD as long circulating, high payload bearing drug carriers.

Conjugation of polymers to proteins[24−26] and albumin in particular[27,28] can be performed via a divergent[28,29] or convergent[26] routes. In this work, the convergent route was chosen to avoid limitations on polymer synthesis imposed by protein stability in organic solvents. Design criteria for the conjugation were set such that the reaction proceeds via a one-step ligation at physiological conditions and is orthogonal with the chemistry linking the drug to the polymer (disulfide chemistry, *vide infra*). Polymer synthesis was accomplished through the reversible addition−fragmentation chain transfer (RAFT) polymerization,[30] a technique which allows synthesizing polymers with defined molar mass and low dispersity and also offers the choice of polymer terminal groups to suit subsequent conjugation reactions. RAFT agents were synthesized to contain two functionalities typically employed in bioconjugation, namely, the thiazolidine-2-thione group (TA) and *N*-hydroxysuccinimide (NHS). The carboxylic acid RAFT agent was used as a conjugation control. These RAFT agents were used to obtain polymers based on HPMA (Figure 1).

Reactions between the polymers and albumin were carried out in phosphate-buffered saline (PBS), HEPES, and carbonate buffers (pH 7.3, 8.3, and 8.3). Conjugates were analyzed using size exclusion chromatography (SEC) equipped with a refractive index detector, an 8-angle light-scattering detector, and a full-spectrum UV−vis detector (Figure 2). For polymers with molar mass over 15 kDa (degree of polymerization over ca. 100) conjugation efficiency was low and hardly detectable through SEC, regardless of the terminal group or buffer conditions (data not shown). This result is most likely due to steric hindrance and low accessibility of terminal groups. For carboxylate-containing polymers, protein revealed a negligible

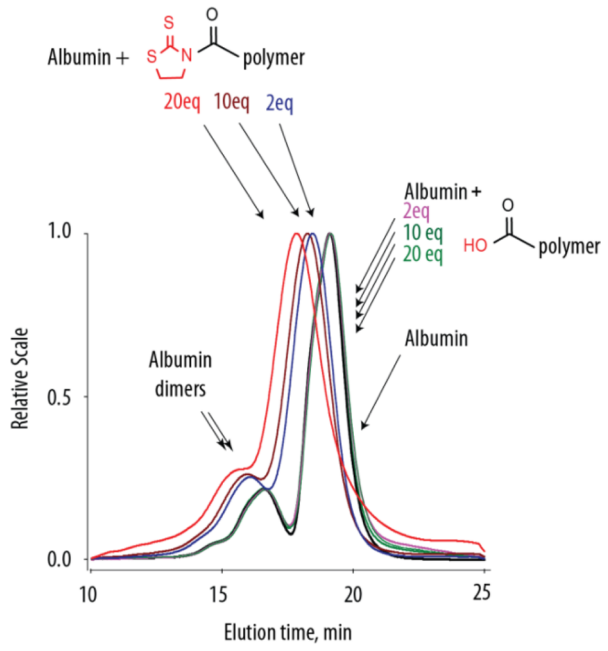

Figure 2. Size exclusion chromatography elution profiles for pristine albumin and results of conjugation of albumin with HPMA, the latter obtained using the RAFT agent with a carboxylic acid or thiazolidine-2-thione functionality, at polymer to protein content of 2, 10, and 20 mol equiv, carried out in phosphate-buffered saline.

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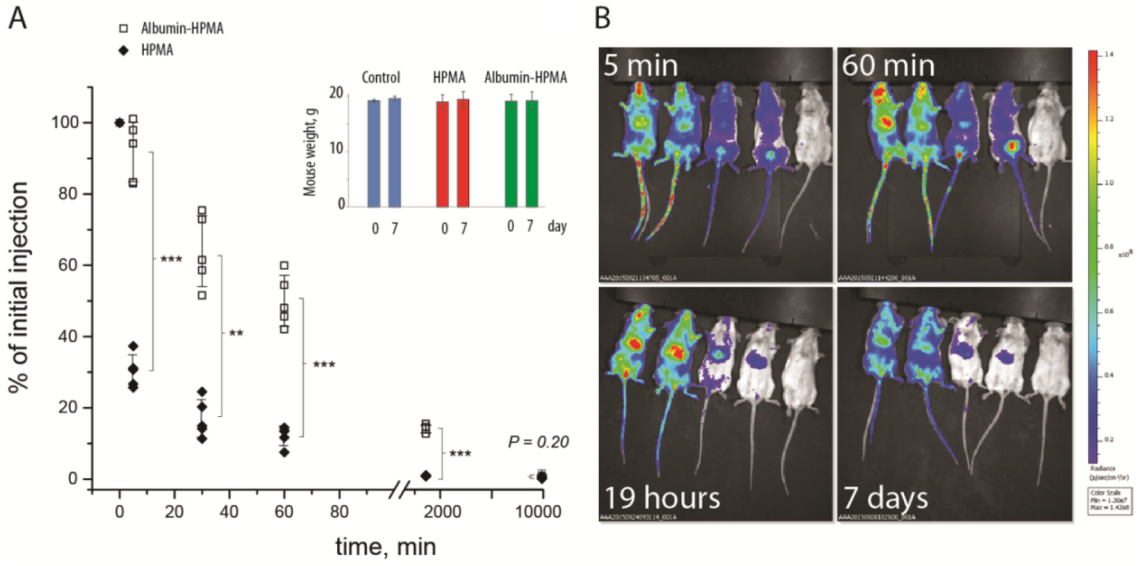

Figure 3. In vivo analysis of blood and full-body residence of HPMA (6.5 kDa) or albumin−HPMA conjugate monitored via fluorescence of polymer remaining in blood (A) and full body fluorescence (B). Inset in panel A shows mice body weight for each treatment performed toward monitoring toxicity effects. Each group consisted of 5 mice. In panel B: the two mice to the left were injected with the albumin−HPMA conjugate and the following two mice with HPMA, and the mouse to the right was a control injected with PBS.

increase in the molar mass, thus suggesting that conjugation was negligible, regardless of the polymer molar mass. In contrast, PHPMA with TA terminal groups and an average molar mass of 6 kDa (**P5**) underwent efficient conjugation to albumin (Figure 2). As low as 2 mol equiv of **P5** over albumin in PBS was sufficient to produce conjugates with well-defined increase in molar mass: 76 vs 68 kDa for the conjugate and albumin, respectively, which corresponds to the attachment of 1 polymer chain per albumin. Increasing polymer content led to progressively higher molar masses of the conjugates, up to 87 kDa at 20 mol equiv of **P5**, i.e., 3 polymer chains per albumin, signifying a higher conjugation density. SEC elution profiles also provide important information on colloidal stability of albumin and the formed conjugates. Pristine protein has a natural tendency to form dimers, which is readily illustrated by the SEC data. Conjugation to PHPMA does not change this tendency and, more importantly, does not lead to further aggregation of the conjugate into nanoparticles.

With regards to other conjugation conditions, we found that the reaction was also efficient but not superior in HEPES and carbonate buffers. NHS-containing polymers afforded lower increments in molar mass for the conjugates over albumin as compared to the TA-containing counterparts suggesting lower conjugation yields (see Supporting Information, Table S3). We note that albumin has at least 30 lysines available for conjugation. However, attached polymer chains create a steric shield and may interfere with the recognition between albumin and FcRn.[31] This may lead to an inefficient albumin recycling and will defeat the purpose of the proposed design of APD. For this reason, no effort was made to increase the polymer content per albumin.

The blood residence time for pristine PHPMA and albumin-conjugated polymer was tested in vivo in BALB/c mice (5 mice per group) using a 6.5 kDa polymer. Samples were labeled with a fluorophore emitting in the near-infrared part of the spectrum (**P6**, see Table S4). The dye was attached in a one-pot reaction to the albumin-conjugated polymer through the azide−alkyne copper-assisted cycloaddition. The dye is conjugated to the polymer via a spacer with only nonbiodegradable linkages, and localization of the fluorophore therefore serves as an unequivocal indicator of the fate of the test substrate upon administration into mice. The polymer−protein conjugate was prepared to contain on average 2 polymer chains per protein globule. No effort was made to increase the polymer content per protein such as not to compromise the affinity of albumin to FcRn. The parent polymer and the polymer−protein conjugate were administered as PBS solutions via tail vein injection following which mice were being observed for 7 days. During this time, blood samples were drawn and analyzed for fluorescence. At each time point, the detection of fluorophores in mice was also performed using an in vivo bioimaging instrument. The parent polymer was excreted from mice rapidly, as evidenced by a low level of the polymer-related fluorescence in the blood (Figure 3A) and accumulation of fluorescence signal in the mouse bladder (Figure 3B). Within 60 min of observation, nearly 90% of the administered PHPMA dose was eliminated. In contrast, the albumin conjugate was retained in circulation, as illustrated by a high fluorescence signal in the blood and as confirmed by the whole body images. Over half of the dose remained in circulation at the 60 min observation time point. By 19 h after injection, 80% of the albumin conjugate had been cleared from the blood—in agreement with the reported values of albumin blood half-life in mice[7] (cf. half-life in humans of nearly 3 weeks[3]—resulting in significantly higher values of half-lives of albumin-associated drugs in humans[4]). However, whole body images reveal that the conjugate was not eliminated but was extensively redistributed throughout the body in tissues (Figure 3B). Indeed, skin and muscles have been reported to be the dominant end points of albumin circulation,[32] and localization of the polymer in these tissues is not unexpected. By the 7 day time point, much of the fluorescence had disappeared from tissues and excreted, as suggested by drastically decreased levels of whole body fluorescence. No adverse effects of the administration of the polymer/polymer−conjugate were observed either directly after injection or during the 7 days of

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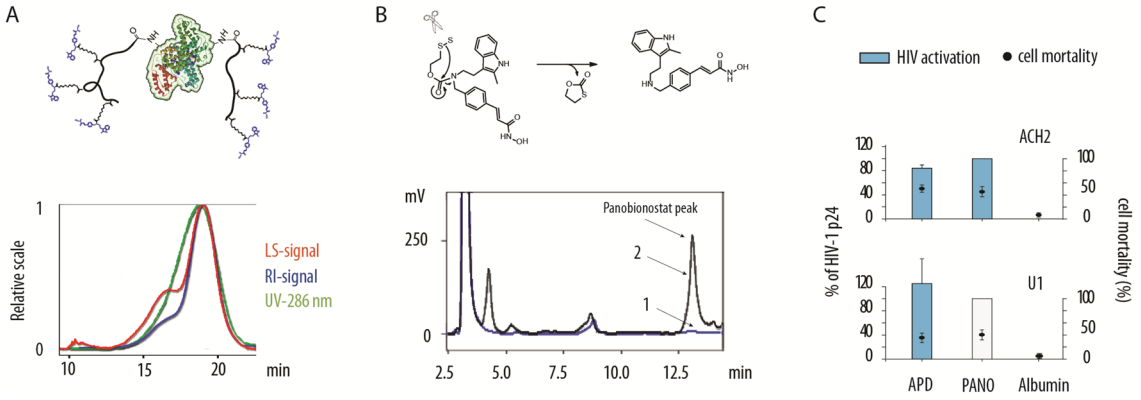

Figure 4. (A) Size exclusion chromatography elution profile for the albumin−polymer−drug conjugate illustrating coelution of albumin (light scattering and refractive index detectors) and panobinostat (UV detector). (B) Drug release was monitored via HPLC to show that in phosphate buffer saline spontaneous drug release was negligible (trace 1), whereas addition of reducing agents (5 mM DTT) resulted in release of panobinostat (trace 2). (C) Utility of the albumin−polymer−panobinostat conjugate as a latency reversing agent for HIV as studied in ACH2 and U1 cells latently infected with HIV using APD (50 mg/L), panobinostat (100 nM), and albumin.

observation, and further there was no difference in the body weight of treated mice compared to untreated controls (Figure 3A, inset). Taken together, the results in Figure 3 illustrate that we successfully endowed short polymers with molar mass well below the renal secretion threshold with a highly sought after characteristic of having a long blood residence time—by conjugation to albumin, the most abundant protein in plasma.

The envisioned utility of the APD pertains to delivery of drugs to abnormal tissues susceptible to accumulation of macromolecular solutes, namely, tumors[8] and inflamed tissues[9] as well as organs and tissues which are naturally exposed to high concentrations of albumin, such as blood[20] and lymph.[33] The latter two are important anatomical reservoirs of latent HIV—harboring persistent pools of the virus which remain refractory to standard antiretroviral treatment and hence cannot be cured. HIV antilatency approaches are highly warranted and are under intensive investigation,[34,35] specifically with the use of histone deacetylase inhibitors such as panobinostat. A clinical trial of panobinostat as an antilatency agent has yielded promising results,[23] and at higher concentrations this drug is approved by the FDA for the treatment of multiple myeloma.[34] Panobinostat is a relatively hydrophobic drug, exhibits a pronounced albumin binding, and has a plasma half-life in humans of ∼16 h (ref 36). In this form, panobinostat is subject to uncontrollable release into plasma as a result of drug−drug interactions such as displacement from albumin by competing solutes, i.e., aspirin. Pharmacokinetics of this drug is also complex, and the use of this drug is associated with diverse side effects.[37] We used panobinostat as a model drug to develop APD—with an ultimate aim to generate safer formulations to achieve reversal of latency of HIV.

MP was synthesized using copolymerization of HPMA and PANO-containing monomer and using a TA-containing RAFT agent (Figure 1). Panobinostat was conjugated to the polymer through a disulfide linkage which remains stable in blood but is rapidly degraded when exposed to reductive conditions such as found inside mammalian cells.[38] Panobinostat has no thiol functionality, and conjugation through disulfide was successfully accomplished using a self-immolative linker.[39] MP was characterized with M_n 7 kDa and drug content 5 mol % and thus contained ca. 3 drug molecules per polymer chain. MP was conjugated to albumin through the TA terminal group to generate APD with a composition identical to that analyzed in Figure 3, i.e., having 2 polymer chains per albumin, shown to have extended blood residence time. This composition corresponds to 6 panobinostat molecules per APD. This drug loading is 6-fold higher than that for loading of biological drugs and exceeds the typical level of loading for antibodies within antibody−drug conjugates (typically not exceeding 4 drug molecules per antibody). SEC profiles (Figure 4A) provide evidence for coelution of albumin and panobinostat, the latter having a UV signature different from the protein (A_{max} = 286 and 278 nm for panobinostat and albumin, respectively). There was no detectable free drug in the polymer sample used in bioconjugation, and therefore all detected drug is covalently linked to PHPMA and albumin. The SEC profile also reveals that despite the hydrophobicity of the drug APD synthesized in this work remained colloidally stable and nonaggregated (but contained a characteristic albumin dimer, as discussed above).

For drug release, we[38,40,41] and others[42,43] have previously investigated this disulfide trigger and this self-immolative linker for delivery of diverse drugs. Specifically, we showed that MPs are stable in cell culture media and in biological buffers but readily release their payload in the presence of glutathione and in cell lysates.[41] In the context of creating long circulating drug reservoirs, it is important to note that this disulfide trigger and the SIL have been shown to survive albumin recycling, i.e., cellular internalization and release.[44] However, once in a reducing environment such as found in cytosole, the prodrugs release their payload. In agreement with the prior studies, MP and APD synthesized in this work released panobinostat in reducing conditions (Figure 4B).

Synthesized APDs were highly active as HIV latency reversing agents, as studied in two model cell lines, a monocytic line, and a T-cell derived cell line (ACH2 and U1, respectively) (Figure 4C). APDs were less potent than the pristine drug, as is customary for prodrugs[45] and albumin-bound biological drugs,[4] and in cell culture, efficient HIV activation required concentration of APD of 50 mg/L. However, levels of HIV p24 protein following transcriptional activation of HIV-1 with APD were as high as those achieved using pristine panobinostat in both tested cell lines indicating that APDs were equally efficacious as the pristine drug.

Taken together, the results of this work present a novel approach to create long circulating, high payload bearing drug delivery systems—through the synthesis of macromolecular

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prodrugs and their conjugation to albumin. The benefits of APD can be summarized as follows. Compared to the polymer, designed APDs have markedly longer blood residence time. The same holds true when comparing APD to panobinostat: half-life of the drug in human plasma is 16 h (ref 36)—far less than for albumin and therefore less than for the covalently linked architecture of APD. Finally, compared to albumin, APD has a higher drug loading, at least 6-fold higher for the formulation characterized above. We anticipate that the existing arsenal of tools in polymer chemistry and bioconjugation makes it facile to adapt the proposed methodology to other drug candidates and drug targets. We are now applying this toolbox to the design of APD for anticancer and antiviral applications.

■ ASSOCIATED CONTENT

Supporting Information

The Supporting Information is available free of charge on the ACS Publications website at DOI: 10.1021/acsmacrolett.6b00544.

Details on the monomer and polymer synthesis and characterization, bioconjugation, protocols and methods for in vivo quantification of blood residence time, and HIV latency reactivation using albumin−polymer−drug conjugates (PDF)

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Author Contributions

The manuscript was written through contributions of all authors. All authors have given approval to the final version of the manuscript.

Notes

The authors declare no competing financial interest.

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■ REFERENCES

(1) Mitragotri, S.; Burke, P. A.; Langer, R. *Nat. Rev. Drug Discovery* **2014**, *13*, 655−672.

(2) Mitragotri, S.; Anderson, D. G.; Chen, X.; Chow, E. K.; Ho, D.; Kabanov, A. V.; Karp, J. M.; Kataoka, K.; Mirkin, C. A.; Petrosko, S. H.; Shi, J.; Stevens, M. M.; Sun, S.; Teoh, S.; Venkatraman, S. S.; Xia, Y.; Wang, S.; Gu, Z.; Xu, C. *ACS Nano* **2015**, *9*, 6644−6654.

(3) Sockolosky, J. T.; Szoka, F. C. *Adv. Drug Delivery Rev.* **2015**, *91*, 109−24.

(4) Lau, J.; Bloch, P.; Schäffer, L.; Pettersson, I.; Spetzler, J.; Kofoed, J.; Madsen, K.; Knudsen, L. B.; McGuire, J.; Steensgaard, D. B.; Strauss, H. M.; Gram, D. X.; Knudsen, S. M.; Nielsen, F. S.; Thygesen, P.; Reedtz-Runge, S.; Kruse, T. *J. Med. Chem.* **2015**, *58*, 7370−7380.

(5) Elsadek, B.; Kratz, F. *J. Controlled Release* **2012**, *157*, 4−28.

(6) Drucker, D. J.; Dritselis, A.; Kirkpatrick, P. *Nat. Rev. Drug Discovery* **2010**, *9*, 267−268.

(7) Kratz, F. *J. Controlled Release* **2008**, *132*, 171−83.

(8) Duncan, R.; Vicent, M. J. *Adv. Drug Delivery Rev.* **2013**, *65*, 60−70.

(9) Crielaard, B. J.; Lammers, T.; Schiffelers, R. M.; Storm, G. *J. Controlled Release* **2012**, *161*, 225−34.

(10) Liu, X. M.; Quan, L. D.; Tian, J.; Laquer, F. C.; Ciborowski, P.; Wang, D. *Biomacromolecules* **2010**, *11*, 2621−8.

(11) Smith, A. A. A.; Kryger, M. B. L.; Wohl, B. M.; Ruiz-Sanchis, P.; Zuwala, K.; Tolstrup, M.; Zelikin, A. N. *Polym. Chem.* **2014**, *5*, 6407−6425.

(12) Wohl, B. M.; Smith, A. A. A.; Jensen, B. E. B.; Zelikin, A. N. *J. Controlled Release* **2014**, *196*, 197−207.

(13) Smith, A. A. A.; Zuwala, K.; Kryger, M. B. L.; Wohl, B. M.; Guerrero-Sanchez, C.; Tolstrup, M.; Postma, A.; Zelikin, A. N. *Chem. Sci.* **2015**, *6*, 264−269.

(14) Shiah, J. G.; Dvořák, M.; Kopečková, P.; Sun, Y.; Peterson, C. M.; Kopeček, J. *Eur. J. Cancer* **2001**, *37*, 131−139.

(15) Kratz, F.; Müller, I. A.; Ryppa, C.; Warnecke, A. *ChemMedChem* **2008**, *3*, 20−53.

(16) Jiang, Y.; Stenzel, M. *Macromol. Biosci.* **2016**, *16*, 791−802.

(17) Wu, Y.; Ihme, S.; Feuring-Buske, M.; Kuan, S. L.; Eisele, K.; Lamla, M.; Wang, Y.; Buske, C.; Weil, T. *Adv. Healthcare Mater.* **2013**, *2*, 884−894.

(18) Chen, Q.; Liu, X.; Chen, J.; Zeng, J.; Cheng, Z.; Liu, Z. *Adv. Mater.* **2015**, *27*, 6820−6827.

(19) Kamaly, N.; Xiao, Z.; Valencia, P. M.; Radovic-Moreno, A. F.; Farokhzad, O. C. *Chem. Soc. Rev.* **2012**, *41*, 2971−3010.

(20) Sleep, D.; Cameron, J.; Evans, L. R. *Biochim. Biophys. Acta, Gen. Subj.* **2013**, *1830*, 5526−5534.

(21) Kopecek, J.; Kopeckova, P. *Adv. Drug Delivery Rev.* **2010**, *62*, 122−149.

(22) Greig, S. L. *Target. Oncol.* **2016**, *11*, 107−14.

(23) Rasmussen, T. A.; Tolstrup, M.; Brinkmann, C. R.; Olesen, R.; Erikstrup, C.; Solomon, A.; Winckelmann, A.; Palmer, S.; Dinarello, C.; Buzon, M.; Lichterfeld, M.; Lewin, S. R.; Østergaard, L.; Søgaard, O. S. *Lancet HIV 1* **2014**, *1*, 13−21.

(24) Le Droumaguet, B.; Nicolas, J. *Polym. Chem.* **2010**, *1*, 563−598.

(25) Dehn, S.; Chapman, R.; Jolliffe, K. A.; Perrier, S. *Polym. Rev.* **2011**, *51*, 214−234.

(26) Alconcel, S. N. S.; Baas, A. S.; Maynard, H. D. *Polym. Chem.* **2011**, *2*, 1442−1448.

(27) Boyer, C.; Bulmus, V.; Liu, J.; Davis, T. P.; Stenzel, M. H.; Barner-Kowollik, C. *J. Am. Chem. Soc.* **2007**, *129*, 7145−7154.

(28) Averick, S.; Simakova, A.; Park, S.; Konkolewicz, D.; Magenau, A. J. D.; Mehl, R. A.; Matyjaszewski, K. *ACS Macro Lett.* **2012**, *1*, 6−10.

(29) Sumerlin, B. S. *ACS Macro Lett.* **2012**, *1*, 141−145.

(30) Moad, G.; Rizzardo, E.; Thang, S. H. *Aust. J. Chem.* **2012**, *65*, 985−1076.

(31) Petersen, S. S.; Kläning, E.; Ebbesen, M. F.; Andersen, B.; Cameron, J.; Sørensen, E. S.; Howard, K. A. *Mol. Pharmaceutics* **2016**, *13*, 677−682.

(32) Gitlin, D.; Klinenberg, J. R.; Hughes, W. L. *Nature* **1958**, *181*, 1064−1065.

(33) Liu, H.; Moynihan, K. D.; Zheng, Y.; Szeto, G. L.; Li, A. V.; Huang, B.; Van Egeren, D. S.; Park, C.; Irvine, D. J. *Nature* **2014**, *507*, 519−522.

(34) Ruelas, D. S.; Greene, W. C. *Cell* **2013**, *155*, 519−529.

(35) Dahabieh, M. S.; Battivelli, E.; Verdin, E. *Annu. Rev. Med.* **2015**, *66*, 407−421.

(36) Ellis, L.; Pan, Y.; Smyth, G. K.; George, D. J.; McCormack, C.; Williams-Truax, R.; Mita, M.; Beck, J.; Burris, H.; Ryan, G.; Atadja, P.; Butterfoss, D.; Dugan, M.; Culver, K.; Johnstone, R. W.; Prince, H. M. *Clin. Cancer Res.* **2008**, *14*, 4500−4510.

(37) FDA evaluation of Pharmacology of panobinostat. http://www.accessdata.fda.gov/drugsatfda_docs/nda/2015/205353Orig1s000PharmR.pdf (accessed September 15, 2016).

(38) Zuwala, K.; Smith, A. A. A.; Tolstrup, M.; Zelikin, A. N. *Chem. Sci.* **2016**, *7*, 2353−2358.

(39) Riber, C. F.; Smith, A. A. A.; Zelikin, A. N. *Adv. Healthcare Mater.* **2015**, *4*, 1887−1890.

(40) Kock, A.; Zuwala, K.; Smith, A. A. A.; Ruiz-Sanchis, P.; Wohl, B. M.; Tolstrup, M.; Zelikin, A. N. *Chem. Commun.* **2014**, *50*, 14498−14500.

DOI: 10.1021/acsmacrolett.6b00544
ACS Macro Lett. 2016, 5, 1089−1094

(41) Ruiz-Sanchis, P.; Wohl, B. M.; Smith, A. A. A.; Zuwala, K.; Melchjorsen, J.; Tolstrup, M.; Zelikin, A. N. *Adv. Healthcare Mater.* **2015**, *4*, 65−68.
(42) Khan, A. R.; Magnusson, J. P.; Watson, S.; Grabowska, A. M.; Wilkinson, R. W.; Alexander, C.; Pritchard, D. *Polym. Chem.* **2014**, *5*, 5320−5329.
(43) Yang, X.; Dogan, I.; Pannala, V. R.; Kootala, S.; Hilborn, J.; Ossipov, D. *Polym. Chem.* **2013**, *4*, 4621−4630.
(44) Brulisauer, L.; Valentino, G.; Morinaga, S.; Cam, K.; Bukrinski, J. T.; Gauthier, M. A.; Leroux, J. C. *Angew. Chem., Int. Ed.* **2014**, *53*, 8392−8396.
(45) Lu, Z. R.; Kopeckova, P.; Kopecek, J. *Nat. Biotechnol.* **1999**, *17*, 1101−1104.

DOI: 10.1021/acsmacrolett.6b00544
ACS Macro Lett. 2016, 5, 1089−1094