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A β -Cyclodextrin-Albumin Conjugate for Enhancing Therapeutic Efficacy of Cytotoxic Drugs.

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Keywords

Cancer – drug delivery systems – *in situ* bioconjugation - albumin – β -cyclodextrin

Abstract. Enhancing the selectivity of anticancer drugs currently used in the clinic is of great interest in order to propose patients more efficient chemotherapies with less side effects. In this context, we developed a β -cyclodextrin trimer that binds to circulating albumin to form the corresponding bioconjugate in the bloodstream. This later can then entrap doxorubicin following its *i.v.* administration via the formation of a host-guest inclusion complex and deliver the drug in tumors. In this study, we demonstrate that the β -cyclodextrin trimer improves the therapeutic efficacy of doxorubicin for the treatment of a subcutaneous murine Lewis lung carcinoma (LLC) implanted in C57BL/6 mice. This outcome is associated to an increased deposition of doxorubicin in malignant tissues when used in combination with the β -cyclodextrin trimer compared to the administration of the drug alone.

Introduction

The selective delivery of potent anticancer drugs in tumors remains a major challenge in cancer chemotherapy. Advances in this field led recently to the approval of ten antibody-drug conjugates (ADCs) for various applications in oncology.¹⁻³ In parallel to antibodies, albumin was also intensively investigated as a tumor targeting unit for enhancing the selectivity of cancer chemotherapy.⁴⁻¹² Indeed, albumin accumulates within malignant tissues through the enhanced permeability and retention (EPR) effect as well as by receptor-mediated active transport. This property conducted to the development of the Abraxane®, an albumin paclitaxel nanoparticle that is currently used in the clinic for the therapy of breast, lung and pancreatic cancer.¹³⁻¹⁵ In another

approach, endogenous albumin was exploited to transport anticancer drugs toward tumor sites by mean of prodrugs possessing the ability to bind *in situ* to the thiol at the cysteine-34 position of the circulating protein through Michael addition. This strategy was pioneered by Kratz and co-workers^{16–20} who developed Aldoxorubicin, an albumin-binding prodrug of doxorubicin that is currently being evaluated in human for the therapy of soft tissue sarcoma.²¹ Taking advantage of both the accumulation of albumin in malignant tissues and the presence of high concentration of β -glucuronidase in the microenvironment of solid tumors,^{22–28} we and others designed enzyme-responsive albumin-binding prodrugs that demonstrated significant therapeutic efficacy in numerous animal models.^{29–37} Recently, Zelikin and co-workers developed glucuronide prodrugs that associated *in situ* to endogenous albumin through supramolecular interactions, thereby leading to superior antitumor activity than that of the free drug for the treatment of triple negative breast xenograft implanted in mice.³⁸

Herein, we present a novel approach that exploit the tumor targeting properties of albumin for enhancing the therapeutic efficacy of anticancer drugs (**Fig. 1**). Within this framework, we designed the β -cyclodextrin (β -CD) trimer **1** (**Scheme 1**) that can bind covalently to the free thiol of circulating albumin once in the blood stream, hence leading to the corresponding bioconjugate **2** (step 1). We hypothesized that the subsequent administration of an anticancer drug should result in the formation of the host-guest inclusion complex **3**^{39,40} (step 2). The bioconjugate **3** will then accumulate in the tumor microenvironment (step 3) to deliver the anticancer drug via the dissociation of the host-guest interactions, therefore enabling the drug to penetrate within cancer cells to exert its cytotoxic activity (step 4).

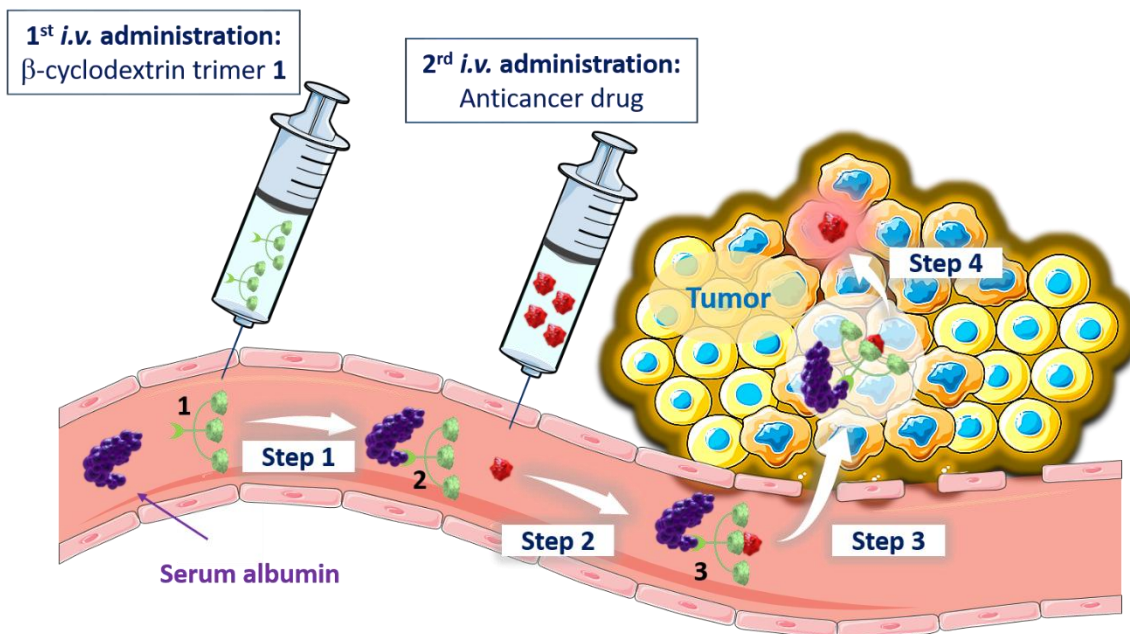


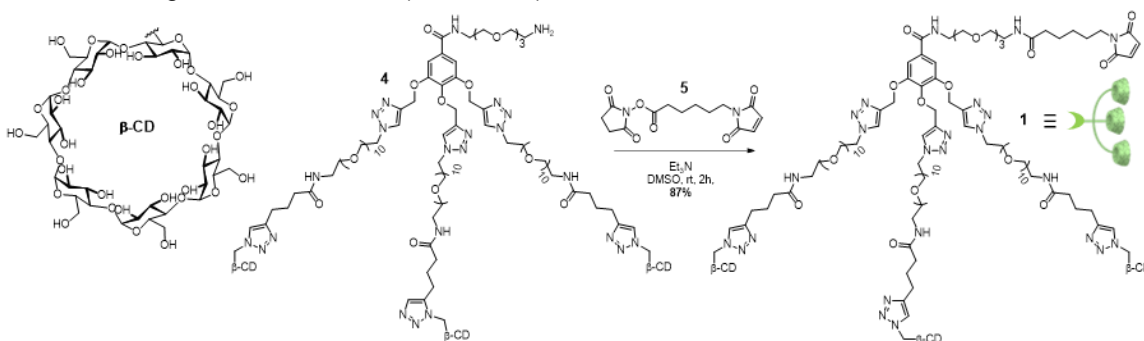
Figure 1. The principle of tumor targeting based on the use of the β -cyclodextrin trimer 1. In the blood, the β -cyclodextrin trimer **1** binds covalently to circulating albumin to generate the bioconjugate **2** (step 1). The *i.v.* administration of the anticancer drug leads to the formation of the supramolecular complex **3** (step 2). The complex **3** accumulates selectively in the tumor

microenvironment (step 3). The dissociation of **3** conducts to the release of the drug that penetrates within tumor cells for inducing the anticancer activity.

In this study, we demonstrated the validity of this strategy with doxorubicin (Dox). Indeed, the combination of the β -CD trimer **1** and the anthracycline led to superior therapeutic efficacy *in vivo* compared to the use of Dox alone. This enhanced antitumor activity was correlated with the increased deposition of Dox at the tumor site, therefore showing the benefit brought by the β -CD derivative **1** to improve the therapeutic index of this clinically used cytotoxic agent.

Results and discussion

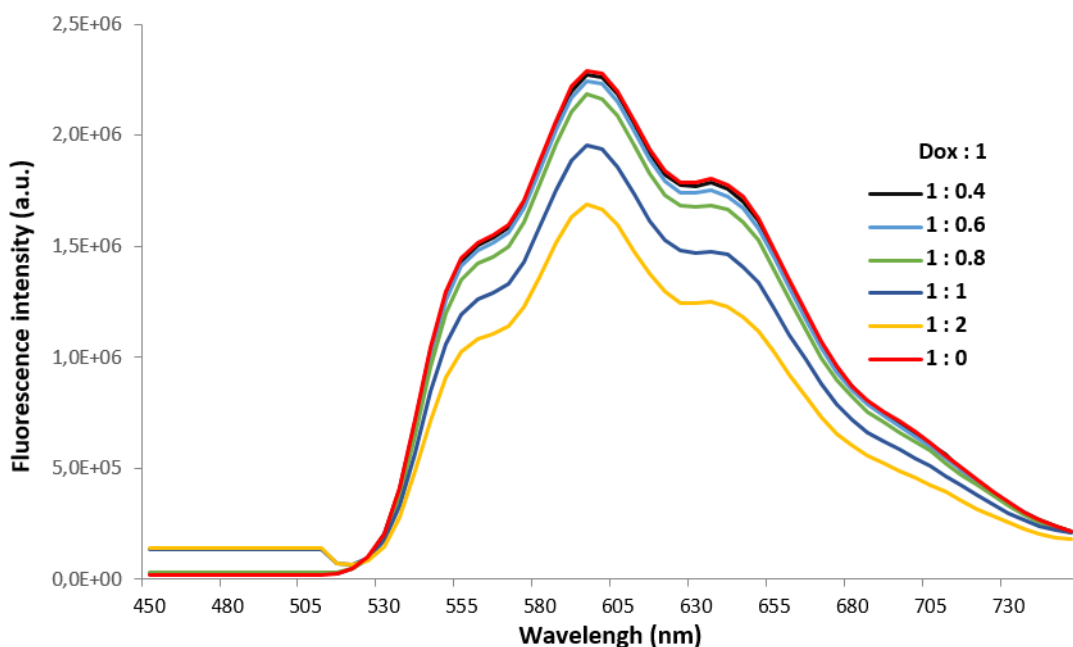
Our first aim was to prepare the bifunctional trimer **1** that comprises both β -CDs for enabling the encapsulation of doxorubicin and a maleimide allowing its binding to the free thiol of circulating albumin through Michael addition (**Scheme 1**).

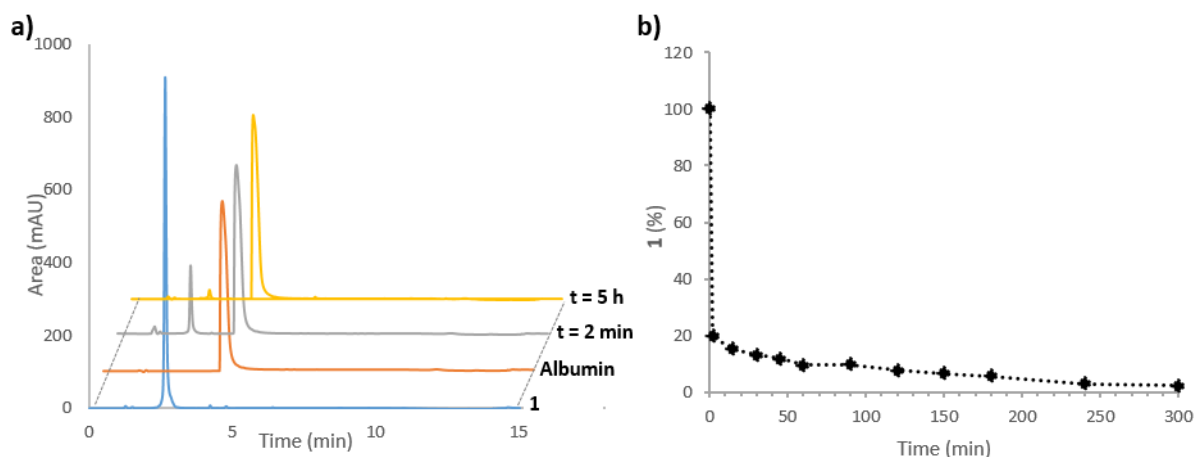


Scheme 1. Structure and synthesis of the β -CD trimer **1**.

The ability of β -CDs to entrap Dox by the formation host-guest inclusion complexes was already exploited for the design of several drug delivery systems.^{41–44} However, in our approach the formation of such an inclusion complex has to occur *in vivo*, which stays highly challenging. Under these circumstances, we designed the compound **1** that permits the attachment of three β -CD units per albumin molecule, thereby enhancing the potential of the circulating protein to bind doxorubicin once functionalized by **1** in the blood stream. Thus, the derivative **1** was synthesized from the primary amine **4**⁴⁵ already described in the literature via nucleophile substitution with the *N*-hydroxysuccinimide ester **5** (for the full synthesis of **4** see the supporting information).

Next, we determined the stability constant of the Dox/**1** complex in phosphate buffer (0.02 M, pH





7) by analyzing the changes in fluorescence intensity of Dox when incubated with various concentration of the β -CD trimer **1**. As shown in **Fig. 2**, the fluorescence intensity of DOX decreased as the concentration of **1** increased, indicating the supramolecular interaction between both compounds. The K_c value calculated using the Scott's equation was $6.9 \times 10^5 \text{ M}^{-1}$ which was three orders of magnitude higher than that of the complex between Dox and β -CD ($K_c 1.8 \times 10^2 \text{ M}^{-1}$).⁴⁶ This result indicated that the host-guest interactions between **1** and Dox should be strong enough to enable the formation of a relatively stable inclusion complex in the bloodstream.⁴⁷

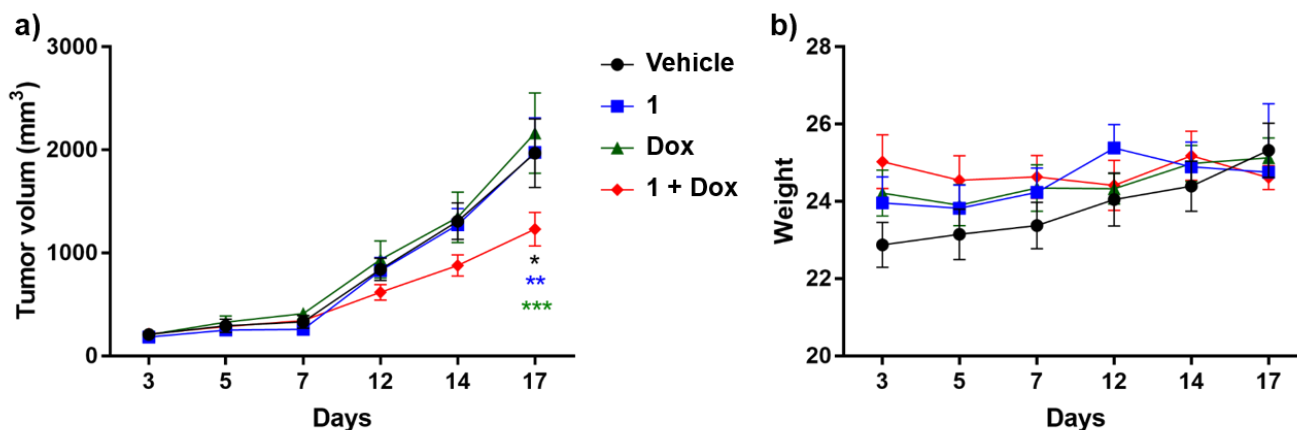
Figure 2. Effects of the β -CD trimer **1** on fluorescence spectrum of DOX in 0.02 M phosphate buffer at pH 7 (λ_{ex} 500 nm). [Dox] = 10^{-5} M; [**1**] = 4×10^{-6} or 6×10^{-6} or 8×10^{-6} or 10^{-5} or 2×10^{-5} M.

We then ascertained whether the bioconjugate **2** could be formed spontaneously under physiological conditions. For this purpose, the β -CD trimer **1** and human serum albumin (HSA) were incubated together in phosphate buffer (0.02 M, pH 7) at 37°C and the evolution of the mixture over the time was monitored by HPLC. Under these conditions, 80 % of **1** was converted in two minutes as the result of its rapid covalent binding with the protein (**Fig. 3**). Furthermore, the peak corresponding to the compound **1** disappeared almost completely after five hours of incubation. Trypsin digestion followed by HPLC/HRMS analysis confirmed the formation of the coupling product **2** with the detection of the HSA peptide fragment including the cysteine-34 linked to **1** (see the supporting information). These results suggested that the kinetics of formation of the albumin conjugate **2** is compatible with the use of the β -CD trimer **1** for the targeting of doxorubicin *in vivo*.^{29,30,35}

Figure 3. The β -CD trimer **1 binds covalently with HSA under physiological conditions (phosphate buffer, pH 7, 37°C).** a) Chromatograms of **1** (blue) incubated with HSA (orange) after 2 min (grey) and 5 h (yellow); b) Disappearance of **1** over the time when placed in the presence of HSA.

The efficacy of the combined therapy involving both the β -CD trimer **1** and Dox was assessed in a subcutaneous murine Lewis lung carcinoma (LLC) implanted in C57BL/6 mice. The animals

received two *i.v.* administrations of the 1/Dox combination, 1 or Dox alone at days 4 and 11 after tumor transplantation. In the case of the combined treatment, the Dox was injected 5 hours after



the trimer 1. It is worth mentioning that neither the 1/Dox ratio nor the delay between the administrations of these two compounds were optimized here. In this pilot study, the dose of 1 (38.8 mg.kg^{-1} , $6.5 \mu\text{mol.kg}^{-1}$, $19.5 \mu\text{mol.kg}^{-1}$ equivalent β -CD) was chosen in order to administer an excess of β -CDs compared to Dox (5 mg.kg^{-1} , $8.6 \mu\text{mol.kg}^{-1}$) for favoring the formation of the inclusion complex in the bloodstream. Furthermore, the five hour separating the injections of 1 and Dox was selected based on our binding kinetics studies (Fig. 3) with the aim to limit the concentration of the free β -CD trimer in the circulation, prior to administer the drug. As shown in Fig 4, the Dox did not exhibit any antitumor activity at the tested dose. In contrast, the combined treatment produced a significant therapeutic effect with a slowdown in tumor growth compared to the control group. At the end of this experiment (day 17), the tumor volumes measured in the group of mice that received the 1/Dox combination were 40% lower than those recorded in the animals treated with Dox. This superior therapeutic efficacy can be attributed to a potential interaction between 1 and Dox in the bloodstream, since the β -CD trimer did not induce antitumor activity when injected alone. Moreover, the combined treatment was well tolerated without any body weight loss or sign of overt toxicity.

Figure 4. Antitumor activity of the 1/Dox combination on LLC xenograft. a) LLC tumor growth inhibition under therapy with vehicle, 1, Dox and 1/Dox combination *i.v.* administered at days 4 and 11. Each point shows the mean \pm s.e.m. from 6 tumor volumes. * $P < 0.1$, ** $P < 0.01$ and *** $P < 0.001$. b) Mean body weight of each group bearing LLC xenografts.

To verify that this enhanced efficacy was the result of a higher deposition of the drug within malignant tissues, mice were euthanized at day 17 and the relative concentration of Dox in tumors was quantified by HPLC/HRMS (Fig. 5). These analyses revealed that the amount of drug delivered at the tumor site was 1.4-fold higher following the administration of the combined 1/Dox therapy than that of Dox alone.

Overall, these results demonstrates that the β -CD trimer 1 potentiates the therapeutic efficacy of Dox by increasing its concentration within tumor mass without inducing side effects. However,

while this study represents a proof of principle, the potential of this targeting strategy could be further improved. Indeed, as the bifunctional derivative **1** ensures the interface between the anticancer drug and circulating albumin, the administered dose of the β -CD trimer could be optimized in order to increase the deposition of Dox in the tumor microenvironment. In the same way, the delay between the administrations of the two partners of the combined treatment could be adjusted for injecting the Dox when the plasma concentration of the bioconjugate **2** will be optimal.

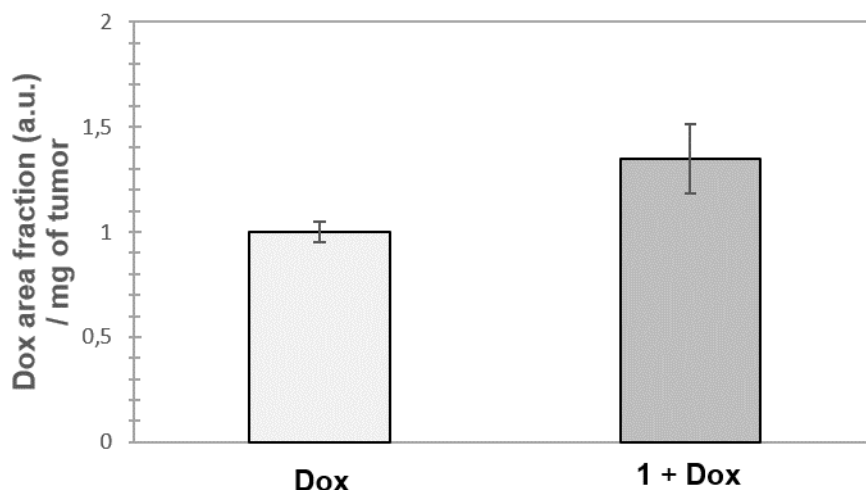


Figure 5. Relative concentration of Dox delivered in tumors when mice were treated with Dox or 1/Dox combination.

Conclusion

In summary, we developed a novel targeting approach based on the use of a β -CD trimer that binds *in situ* to serum albumin via a covalent linkage. The resulting albumin conjugate possesses the ability to entrap the Dox in the bloodstream through the formation of a host-guest inclusion complex, thereby allowing the transport and delivery of the anticancer drug in malignant tissues. Thus, when employed in combination with the β -CD trimer, the Dox exhibits a higher antitumor activity than when used alone as the consequence of its increased deposition in the tumor. As several other anticancer drugs such as paclitaxel, camptothecin or cisplatin have been successfully incorporated in β -CD, this bifunctional trimer could serve as a versatile drug carrier to enhance the efficacy of cancer chemotherapy. Under such circumstances, this targeting strategy could be very advantageous since the therapeutic index of various cytotoxics could be potentiated thanks to a unique drug delivery system.

Acknowledgments

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References

- (1) Baah, S.; Laws, M.; Rahman, K. M. Antibody–Drug Conjugates—A Tutorial Review. *Molecules* **2021**, *26* (10), 2943. <https://doi.org/10.3390/molecules26102943>.
- (2) Chau, C. H.; Steeg, P. S.; Figg, W. D. Antibody–Drug Conjugates for Cancer. *The Lancet* **2019**, *394* (10200), 793–804. [https://doi.org/10.1016/S0140-6736\(19\)31774-X](https://doi.org/10.1016/S0140-6736(19)31774-X).
- (3) Beck, A.; Goetsch, L.; Dumontet, C.; Corvaia, N. Strategies and Challenges for the next Generation of Antibody–Drug Conjugates. *Nat. Rev. Drug Discov.* **2017**, *16* (5), 315–337. <https://doi.org/10.1038/nrd.2016.268>.
- (4) Yu, L.; Hua, Z.; Luo, X.; Zhao, T.; Liu, Y. Systematic Interaction of Plasma Albumin with the Efficacy of Chemotherapeutic Drugs. *Biochim. Biophys. Acta Rev. Cancer* **2022**, *1877* (1), 188655. <https://doi.org/10.1016/j.bbcan.2021.188655>.
- (5) Spada, A.; Emami, J.; Tuszynski, J. A.; Lavasanifar, A. The Uniqueness of Albumin as a Carrier in Nanodrug Delivery. *Mol. Pharm.* **2021**, *18* (5), 1862–1894. <https://doi.org/10.1021/acs.molpharmaceut.1c00046>.
- (6) Rahimizadeh, P.; Yang, S.; Lim, S. I. Albumin: An Emerging Opportunity in Drug Delivery. *Biotechnol. Bioprocess Eng.* **2020**, *25* (6), 985–995. <https://doi.org/10.1007/s12257-019-0512-9>.
- (7) Zhao, P.; Wang, Y.; Wu, A.; Rao, Y.; Huang, Y. Roles of Albumin-Binding Proteins in Cancer Progression and Biomimetic Targeted Drug Delivery. *Chembiochem Eur. J. Chem. Biol.* **2018**, *19* (17), 1796–1805. <https://doi.org/10.1002/cbic.201800201>.
- (8) Tao, C.; Chuah, Y. J.; Xu, C.; Wang, D.-A. Albumin Conjugates and Assemblies as Versatile Bio-Functional Additives and Carriers for Biomedical Applications. *J. Mater. Chem. B* **2019**, *7* (3), 357–367. <https://doi.org/10.1039/C8TB02477D>.
- (9) Liu, Z.; Chen, X. Simple Bioconjugate Chemistry Serves Great Clinical Advances: Albumin as a Versatile Platform for Diagnosis and Precision Therapy. *Chem. Soc. Rev.* **2016**, *45* (5), 1432–1456. <https://doi.org/10.1039/C5CS00158G>.
- (10) Kratz, F. A Clinical Update of Using Albumin as a Drug Vehicle - a Commentary. *J. Control. Release Off. J. Control. Release Soc.* **2014**, *190*, 331–336. <https://doi.org/10.1016/j.jconrel.2014.03.013>.
- (11) Elsadek, B.; Kratz, F. Impact of Albumin on Drug Delivery--New Applications on the Horizon. *J. Control. Release Off. J. Control. Release Soc.* **2012**, *157* (1), 4–28. <https://doi.org/10.1016/j.jconrel.2011.09.069>.
- (12) Kratz, F. Albumin as a Drug Carrier: Design of Prodrugs, Drug Conjugates and Nanoparticles. *J. Control. Release Off. J. Control. Release Soc.* **2008**, *132* (3), 171–183. <https://doi.org/10.1016/j.jconrel.2008.05.010>.
- (13) Gradishar, W. J.; Tjulandin, S.; Davidson, N.; Shaw, H.; Desai, N.; Bhar, P.; Hawkins, M.; O’Shaughnessy, J. Phase III Trial of Nanoparticle Albumin-Bound Paclitaxel Compared with Polyethylated Castor Oil-Based Paclitaxel in Women with Breast Cancer. *J. Clin. Oncol. Off. J. Am. Soc. Clin. Oncol.* **2005**, *23* (31), 7794–7803. <https://doi.org/10.1200/JCO.2005.04.937>.
- (14) Chiorean, E. G.; Cheung, W. Y.; Giordano, G.; Kim, G.; Al-Batran, S.-E. Real-World Comparative Effectiveness of Nab-Paclitaxel plus Gemcitabine versus FOLFIRINOX in Advanced Pancreatic Cancer: A Systematic Review. *Ther. Adv. Med. Oncol.* **2019**, *11*, 1758835919850367. <https://doi.org/10.1177/1758835919850367>.
- (15) Zhang, Y.; Xu, J.; Hua, J.; Liu, J.; Liang, C.; Meng, Q.; Ni, Q.; Shi, S.; Yu, X. Nab-Paclitaxel plus Gemcitabine as First-Line Treatment for Advanced Pancreatic Cancer: A Systematic Review and Meta-Analysis. *J. Cancer* **2019**, *10* (18), 4420–4429. <https://doi.org/10.7150/jca.29898>.
- (16) Kratz, F.; Müller-Driver, R.; Hofmann, I.; Dreves, J.; Unger, C. A Novel Macromolecular Prodrug Concept Exploiting Endogenous Serum Albumin as a Drug Carrier for Cancer Chemotherapy. *J. Med. Chem.* **2000**, *43* (7), 1253–1256. <https://doi.org/10.1021/jm9905864>.
- (17) Kratz, F.; Warnecke, A.; Scheuermann, K.; Stockmar, C.; Schwab, J.; Lazar, P.; Drückes, P.; Esser, N.; Dreves, J.; Rognan, D.; Bissantz, C.; Hinderling, C.; Folkers, G.; Fichtner, I.;

- Unger, C. Probing the Cysteine-34 Position of Endogenous Serum Albumin with Thiol-Binding Doxorubicin Derivatives. Improved Efficacy of an Acid-Sensitive Doxorubicin Derivative with Specific Albumin-Binding Properties Compared to That of the Parent Compound. *J. Med. Chem.* **2002**, *45* (25), 5523–5533. <https://doi.org/10.1021/jm020276c>.
- (18) Schmid, B.; Chung, D.-E.; Warnecke, A.; Fichtner, I.; Kratz, F. Albumin-Binding Prodrugs of Camptothecin and Doxorubicin with an Ala-Leu-Ala-Leu-Linker That Are Cleaved by Cathepsin B: Synthesis and Antitumor Efficacy. *Bioconjug. Chem.* **2007**, *18* (3), 702–716. <https://doi.org/10.1021/bc0602735>.
- (19) Abu Ajaj, K.; Graeser, R.; Fichtner, I.; Kratz, F. In Vitro and in Vivo Study of an Albumin-Binding Prodrug of Doxorubicin That Is Cleaved by Cathepsin B. *Cancer Chemother. Pharmacol.* **2009**, *64* (2), 413–418. <https://doi.org/10.1007/s00280-009-0942-8>.
- (20) Pes, L.; Koester, S. D.; Magnusson, J. P.; Chercheja, S.; Medda, F.; Abu Ajaj, K.; Rognan, D.; Daum, S.; Nollmann, F. I.; Garcia Fernandez, J.; Perez Galan, P.; Walter, H.-K.; Warnecke, A.; Kratz, F. Novel Auristatin E-Based Albumin-Binding Prodrugs with Superior Anticancer Efficacy in Vivo Compared to the Parent Compound. *J. Control. Release Off. J. Control. Release Soc.* **2019**, *296*, 81–92. <https://doi.org/10.1016/j.jconrel.2019.01.010>.
- (21) Cranmer, L. D. Spotlight on Aldoxorubicin (INNO-206) and Its Potential in the Treatment of Soft Tissue Sarcomas: Evidence to Date. *Oncotargets Ther.* **2019**, *12*, 2047–2062. <https://doi.org/10.2147/OTT.S145539>.
- (22) Fishman, W. H.; Anlyan, A. J. Comparison of the Beta-Glucuronidase Activity of Normal, Tumor, and Lymph Node Tissues of Surgical Patients. *Science* **1947**, *106* (2742), 66–67. <https://doi.org/10.1126/science.106.2742.66>.
- (23) Boyland, E.; Wallace, D. M.; Williams, D. C. The Activity of the Enzymes Sulphatase and Beta-Glucuronidase in the Urine, Serum and Bladder Tissue. *Br. J. Cancer* **1955**, *9* (1), 62–79. <https://doi.org/10.1038/bjc.1955.6>.
- (24) Albin, N.; Massaad, L.; Toussaint, C.; Morizet, J.; Parise, O.; Gouyette, A.; Chabot, G. G. Main Drug-Metabolizing Enzyme Systems in Human Breast Tumors and Peritumoral Tissues. *6*.
- (25) Sperker, B.; Werner, U.; Mürdter, T. E.; Tekkaya, C.; Fritz, P.; Wacke, R.; Adam, U.; Gerken, M.; Drewelow, B.; Kroemer, H. K. Expression and Function of Beta-Glucuronidase in Pancreatic Cancer: Potential Role in Drug Targeting. *Naunyn. Schmiedebergs Arch. Pharmacol.* **2000**, *362* (2), 110–115. <https://doi.org/10.1007/s002100000260>.
- (26) Nygren, C.; von Holst, H.; Månsson, J.-E.; Fredman, P. Increased Activity of Lysosomal Glycohydrolases in Glioma Tissue and Surrounding Areas from Human Brain. *Acta Neurochir. (Wien)* **1997**, *139* (2), 146–150. <https://doi.org/10.1007/BF02747195>.
- (27) Bosslet, K.; Czech, J.; Hoffmann, D. A Novel One-Step Tumor-Selective Prodrug Activation System. *Tumor Target.* **1995**, *1*, 45–50.
- (28) Bosslet, K.; Straub, R.; Blumrich, M.; Czech, J.; Gerken, M.; Sperker, B.; Kroemer, H. K.; Gesson, J. P.; Koch, M.; Monneret, C. Elucidation of the Mechanism Enabling Tumor Selective Prodrug Monotherapy. *Cancer Res.* **1998**, *58* (6), 1195–1201.
- (29) Legigan, T.; Clarhaut, J.; Renoux, B.; Tranoy-Opalinski, I.; Monvoisin, A.; Berjeaud, J.-M.; Guilhot, F.; Papot, S. Synthesis and Antitumor Efficacy of a β -Glucuronidase-Responsive Albumin-Binding Prodrug of Doxorubicin. *J. Med. Chem.* **2012**, *55* (9), 4516–4520. <https://doi.org/10.1021/jm300348r>.
- (30) Renoux, B.; Raes, F.; Legigan, T.; Péraudeau, E.; Eddhif, B.; Poinot, P.; Tranoy-Opalinski, I.; Alsarraf, J.; Koniev, O.; Kolodych, S.; Lerondel, S.; Le Pape, A.; Clarhaut, J.; Papot, S. Targeting the Tumour Microenvironment with an Enzyme-Responsive Drug Delivery System for the Efficient Therapy of Breast and Pancreatic Cancers †Electronic Supplementary Information (ESI) Available. See DOI: 10.1039/C7sc00472a Click Here for Additional Data File. *Chem. Sci.* **2017**, *8* (5), 3427–3433. <https://doi.org/10.1039/c7sc00472a>.
- (31) Compain, G.; Oumata, N.; Clarhaut, J.; Péraudeau, E.; Renoux, B.; Galons, H.; Papot, S. A β -Glucuronidase-Responsive Albumin-Binding Prodrug for Potential Selective Kinase

- Inhibitor-Based Cancer Chemotherapy. *Eur. J. Med. Chem.* **2018**, *158*, 1–6.
<https://doi.org/10.1016/j.ejmech.2018.08.100>.
- (32) Renoux, B.; Fangous, L.; Hötten, C.; Péraudeau, E.; Eddhif, B.; Poinot, P.; Clarhaut, J.; Papot, S. A β -Glucuronidase-Responsive Albumin-Binding Prodrug Programmed for the Double Release of Monomethyl Auristatin E. *MedChemComm* **2018**, *9* (12), 2068–2071.
<https://doi.org/10.1039/c8md00466h>.
- (33) Lange, J.; Eddhif, B.; Tarighi, M.; Garandeau, T.; Péraudeau, E.; Clarhaut, J.; Renoux, B.; Papot, S.; Poinot, P. Volatile Organic Compound Based Probe for Induced Volatolomics of Cancers. *Angew. Chem. Int. Ed.* **2019**, *58* (49), 17563–17566.
<https://doi.org/10.1002/anie.201906261>.
- (34) Raes, F.; Badiane, S. M.; Renoux, B.; Papot, S.; Lerondel, S.; Le Pape, A. Development of an Embedded Multimodality Imaging Platform for Onco-Pharmacology Using a Smart Anticancer Prodrug as an Example. *Sci. Rep.* **2020**, *10* (1), 2661.
<https://doi.org/10.1038/s41598-020-59561-8>.
- (35) Châtre, R.; Lange, J.; Péraudeau, E.; Poinot, P.; Lerondel, S.; Le Pape, A.; Clarhaut, J.; Renoux, B.; Papot, S. In Vivo Synthesis of Triple-Loaded Albumin Conjugate for Efficient Targeted Cancer Chemotherapy. *J. Controlled Release* **2020**, *327*, 19–25.
<https://doi.org/10.1016/j.jconrel.2020.08.008>.
- (36) Huang, Y.; Wang, L.; Cheng, Z.; Yang, B.; Yu, J.; Chen, Y.; Lu, W. SN38-Based Albumin-Binding Prodrug for Efficient Targeted Cancer Chemotherapy. *J. Control. Release Off. J. Control. Release Soc.* **2021**, *339*, 297–306. <https://doi.org/10.1016/j.jconrel.2021.09.040>.
- (37) Cheng, Z.; Huang, Y.; Shen, Q.; Zhao, Y.; Wang, L.; Yu, J.; Lu, W. A Camptothecin-Based, Albumin-Binding Prodrug Enhances Efficacy and Safety in Vivo. *Eur. J. Med. Chem.* **2021**, *226*, 113851. <https://doi.org/10.1016/j.ejmech.2021.113851>.
- (38) Jarlstad Olesen, M. T.; Walther, R.; Poier, P. P.; Dagnæs-Hansen, F.; Zelikin, A. N. Molecular, Macromolecular, and Supramolecular Glucuronide Prodrugs: Lead Identified for Anticancer Prodrug Monotherapy. *Angew. Chem. Int. Ed.* **2020**, *59* (19), 7390–7396.
<https://doi.org/10.1002/anie.201916124>.
- (39) Tian, B.; Hua, S.; Liu, J. Cyclodextrin-Based Delivery Systems for Chemotherapeutic Anticancer Drugs: A Review. *Carbohydr. Polym.* **2020**, *232*, 115805.
<https://doi.org/10.1016/j.carbpol.2019.115805>.
- (40) Bonnet, V.; Gervaise, C.; Djedaïni-Pilard, F.; Furlan, A.; Sarazin, C. Cyclodextrin Nanoassemblies: A Promising Tool for Drug Delivery. *Drug Discov. Today* **2015**, *20* (9), 1120–1126. <https://doi.org/10.1016/j.drudis.2015.05.008>.
- (41) Mohammad, N.; Singh, S. V.; Malvi, P.; Chaube, B.; Athavale, D.; Vanuopadath, M.; Nair, S. S.; Nair, B.; Bhat, M. K. Strategy to Enhance Efficacy of Doxorubicin in Solid Tumor Cells by Methyl- β -Cyclodextrin: Involvement of P53 and Fas Receptor Ligand Complex. *Sci. Rep.* **2015**, *5*, 11853. <https://doi.org/10.1038/srep11853>.
- (42) Okamatsu, A.; Motoyama, K.; Onodera, R.; Higashi, T.; Koshigoe, T.; Shimada, Y.; Hattori, K.; Takeuchi, T.; Arima, H. Folate-Appended β -Cyclodextrin as a Promising Tumor Targeting Carrier for Antitumor Drugs in Vitro and in Vivo. *Bioconjug. Chem.* **2013**, *24* (4), 724–733. <https://doi.org/10.1021/bc400015r>.
- (43) Fiorica, C.; Palumbo, F. S.; Pitarresi, G.; Puleio, R.; Condorelli, L.; Collura, G.; Giammona, G. A Hyaluronic Acid/Cyclodextrin Based Injectable Hydrogel for Local Doxorubicin Delivery to Solid Tumors. *Int. J. Pharm.* **2020**, *589*, 119879.
<https://doi.org/10.1016/j.ijpharm.2020.119879>.
- (44) Santos, A. C.; Costa, D.; Ferreira, L.; Guerra, C.; Pereira-Silva, M.; Pereira, I.; Peixoto, D.; Ferreira, N. R.; Veiga, F. Cyclodextrin-Based Delivery Systems for in Vivo-Tested Anticancer Therapies. *Drug Deliv. Transl. Res.* **2021**, *11* (1), 49–71.
<https://doi.org/10.1007/s13346-020-00778-5>.
- (45) Plumet, C.; Mohamed, A. S.; Vendevre, T.; Renoux, B.; Clarhaut, J.; Papot, S. Cell–Cell Interactions via Non-Covalent Click Chemistry. *Chem. Sci.* **2021**, *12* (26), 9017–9021.
<https://doi.org/10.1039/D1SC01637G>.

- (46) Bakkour, Y.; Vermeersch, G.; Morcellet, M.; Boschin, F.; Martel, B.; Azaroual, N. Formation of Cyclodextrin Inclusion Complexes with Doxycyclin-Hyclate: NMR Investigation of Their Characterisation and Stability. *J. Incl. Phenom.* **2006**, *54*, 109–114. <https://doi.org/10.1007/s10847-005-5108-7>.
- (47) Stella, V. J.; He, Q. Cyclodextrins. *Toxicol. Pathol.* **2008**, *36* (1), 30–42. <https://doi.org/10.1177/0192623307310945>.