Here, we describe a simple, efficient formulation of a novel library of β-cyclodextrin-poly (β-amo
β-cyclodextrin-poly (β-amino ester) nanoparticles are a generalizable strategy for high loading and sustained release of HDAC inhibitors

Sauradip Chaudhuri¹, Martha J. Fowler¹, Afroz S. Mohammad², Wenqui Zhang², Cassandra Baker¹, Colton W. Broughton¹, Brandon E. Knight¹, William F. Elmquist², Sarah E. Stabenfeldt³, Rachael W. Sirianni¹

¹ Vivian L. Smith Department of Neurosurgery, University of Texas Health Science Center at Houston, Houston TX, USA

² Department of Pharmaceutics, University of Minnesota, Minneapolis MN, USA

³ School of Biological and Health Systems Engineering, Arizona State University, Tempe AZ, USA

*Author to whom correspondence should be addressed:

Rachael W. Sirianni

Dr. Rachael W. Sirianni

Medical School Building MSB 7.134

6431 Fannin Street

Houston, TX 77030

713-500-7492

Rachael.w.sirianni@uth.tmc.edu
ABSTRACT

Therapeutic development of histone deacetylase inhibitors (HDACi) has been hampered by a number of barriers to drug delivery, including poor solubility and inadequate tissue penetration. Nanoparticle encapsulation could be one approach to improve delivery of HDACi to target tissues; however, effective and generalizable loading of HDACi within nanoparticle systems remains a long-term challenge. We hypothesized that the common ionizable moiety on many HDACi molecules could be capitalized upon as a generalizable strategy for loading in polymeric nanoparticles. Here, we describe a simple, efficient formulation of a novel library of β-cyclodextrin-poly (β-amino ester) networks (CDN) to achieve this goal. We observed that network architecture was a critical determinant of CDN encapsulation of candidate molecules, with a more hydrophobic core enabling effective self-assembly and a PEGylated surface enabling high loading (up to ~30% w/w), effective self assembly of the nanoparticle, and slow release of drug into aqueous media (24 days) for the model HDACi panobinostat. Optimized CDN nanoparticles were taken up by GL261 cells in culture, and released panobinostat was confirmed to be bioactive. Pharmacokinetic analyses demonstrated that panobinostat was delivered to the brainstem, cerebellum, and upper spinal cord following intrathecal administration via cisterna magna injection in healthy mice. We next constructed a library of CDNs to encapsulate various small, hydrophobic, ionizable molecules (panobinostat, quisinostat, dacinostat, givinostat, and bortezomib, camptothecin, nile red, and cytarabine), which yielded important insights into the structural requirements for effective drug loading and CDN self-assembly. Taken in sum, these studies present a novel nanocarrier platform for encapsulation of HDACi via both ionic and hydrophobic interactions, which is an important step toward better treatment of disease via HDACi therapy.

Keywords: Drug delivery, nanomedicine, nanoparticle, HDACi, pharmacokinetics
INTRODUCTION

Histone Deacetylase inhibitors (HDACi) are a class of small molecules that promote hyper-acetylation of core histones, leading to relaxation of chromatin and therapeutic effects in a multitude of disease models.\textsuperscript{1-10} Unfortunately, utility of HDACi is plagued by delivery problems, including rapid clearance and poor tissue distribution when molecules are administered in free form.\textsuperscript{11-13} We and others have developed nanoparticle encapsulation strategies to improve drug tolerability, pharmacokinetics, and site-specific delivery of HDAC inhibitors.\textsuperscript{14-17} However, each of these reports has developed approaches for encapsulation of individual HDAC inhibitors, requiring development of unique loading strategies for individual drugs. These intensive efforts have thus far yielded only modest loading. HDACi loading in and controlled release from nanocarrier systems remains unoptimized, and a generalizable strategy for drug delivery for this class of molecules is lacking.

The majority of HDAC inhibitors bear a characteristic hydroxamic acid (zinc ion chelating domain), which is linked to the capping moiety by a spacer of appropriate chain length.\textsuperscript{18} This hydroxamic acid enables manipulation of charge and thus drug solubility through changes in solution pH. We previously loaded the HDAC inhibitor quisinostat (JNJ-26481585) onto polymeric nanoparticles composed of poly(lactic acid)-poly(ethylene glycol) (PLA-PEG) through a pH manipulation and ionization strategy. Although these quisinostat loaded PLA-PEG nanoparticles were highly loaded and useful for treating intracranial glioblastoma by intravenous injection, \textit{in vitro} studies demonstrated that drug was released rapidly once nanoparticles were exposed to an aqueous environment.\textsuperscript{14} Presumably this rapid release occurred because quisinostat was only associated with the surface of the nanoparticle, instead of being embedded within a particle core.

Here, we sought to develop β-cyclodextrin-poly (β-amino ester) (cyclodextrin networks, or CDNs) for delivery of HDAC inhibitors. We hypothesized that the common ionizable moiety in HDACi molecules could be leveraged for loading this class of agents into nanoparticles and enabling sustained release. To test this hypothesis, we describe the development of a small library of CDN materials that
self-assemble into drug loaded nanoparticles. We predicted that dual loading via both ionic and hydrophobic interactions would confer favorable characteristics for sustained release. Two uniquely surface functionalized (Aminated and PEGylated) versions of CDNs were generated via simple three component Michael addition reactions. Three subtypes of the Amine-CDN were developed, each with a successively increasing and flexible hydrophobic core, to obtain a total of four unique CDN structures. HDAC inhibitors (panobinostat, quisinostat, dacinostat, givinostat) and other small, hydrophobic molecules (bortezomib, camptothecin, nile red) were passively doped into the resultant particles. Some of these particles possess biophysical properties favorable for drug delivery applications, such as small size, close to neutral surface charge, and high loading (up to ~30%). Detailed structure-property investigation revealed that the key structural aspects of CDNs enabling self-assembly and effective loading include cross linker length, surface charge density, and the accessibility of the hydrophobic cyclodextrin core. Importantly, we observed that the encapsulated molecule needed to be ionizable with a flexible core to enable effective loading in CDNs. We expect that the high HDACi loading within CDNs could be generalized to other molecules bearing similar ionizable structural moieties. Thus, the nanoparticle platform described here offers new opportunities for nanomedicine development to deliver HDACi and other ionizable agents.

RESULTS AND DISCUSSION

Cyclodextrin network (CDN) architectures

Cyclodextrins are a class of cyclic oligosaccharides that have been used widely for solubilization of hydrophobic molecules to enable delivery to aqueous environments, including for the purpose of delivering HDAC inhibitors. Cyclodextrins are useful for enabling delivery of lipophilic molecules from aqueous media but do not, in unmodified form, facilitate controlled release. Synthetically modified amphiphilic cyclodextrins have been shown to not only form drug loaded nanoparticles, but also to outperform traditional polyester particles for certain drugs in terms of drug-loading and controlled
In addition, advanced cyclodextrin-based cross-linked and linear polymeric architectures have been reported in the literature for their potential application in therapeutics.

β-cyclodextrin-poly (β-amino ester) nanoparticles were previously reported by Lowe and colleagues as a blood-brain barrier (BBB) permeable platform to support sustained release of doxorubicin (CDN-1). Here, we engineered the constituent polymer to develop a library of materials possessing different cross-linker concentration, length, and amine functionalities (CDN-2, CDN-3, and CDN-4, respectively). This library was designed to study the complex structure-function relationships between loading of small molecules and network compositions. The 1,4-butanediol diacrylate cross-linker was employed at two different concentrations (1.3mmol [1.0eqv] and 2.7mmol [2.0eqv] for CDN-1 and CDN-2, respectively), whereas CDN-3 was synthesized from 1,6-hexanediol diacrylate (2.7mmol, [2.0eqv]). CDN-4 was synthesized from mPEG_{550}–Amine with 1,4-butanediol diacrylate cross-linker (2.7mmol, [2.0eqv]). Synthesis of materials with expected chemical structure was confirmed by NMR (see supporting information), while the nanoparticle formation was determined by DLS and SEM images (see supporting information). The overall biophysical characterization of the CDNs without any encapsulated payload are elucidated in Figure 1(A-C). Comparing CDN-1 against CDN-2, the latter has more surface-amine functionalities than the former. CDN-2 also has an expanded and flexible hydrophobic core. CDN-3 possesses a greater cross-linked length but self-assembles into smaller sized nanoparticles than CDN-1&2. Lastly, CDN-4 possesses a PEG modified surface, that we expect will confer favorable properties for in vivo application.
Panobinostat Loaded CDNs

To assess drug loading capacity in CDNs, we first focused on the HDAC inhibitor panobinostat (LBH589). Panobinostat is a pan-HDACi that is of interest for treatment of a multitude of diseases, including solid and hematological cancers, neuroinflammation, and traumatic brain injury. Similar to other HDACi, panobinostat has several delivery challenges that are expected to reduce its therapeutic potential. First, panobinostat is poorly water soluble and difficult to administer in free form. Second, both peripheral and central pharmacokinetic analyses show that it is cleared rapidly from fluid compartments. Therefore, we focused on panobinostat as a candidate molecule for nanoparticle delivery based on the
expectation that solubilization within a carrier system capable of sustained release could be significant for its use to treat human disease. Panobinostat-loaded nanoparticles were formulated by doping a given concentration of the drug with CDN in 2.5 vol% DMSO-aqueous medium. Panobinostat was added at different theoretical loadings, and the amount of panobinostat incorporated into the network after thorough washing was quantified by absorbance.

Panobinostat incorporated effectively into all four CDNs. However, drug loading was found to vary starkly for the different structural variations of the networks, with both the diacrylate cross-linkers and the functionality of the amine affecting drug loading capacity (Figure 2). Drug loading increased across CDNs 1-3, which was associated with a larger and more flexible hydrophobic core of the nanoparticles imparted by the hexyl cross-linkers (CDN-3). Loading was significantly higher in CDN-4 compared to other nanoparticles. High loading of panobinostat in CDN-4 is consistent with our prior report of favorable interaction of ionized quisinostat with PEG present at the surface of polyester nanoparticles. To appreciate the mechanisms by which drug incorporates into the CDN network, we engaged in detailed biophysical characterization of each formulation.
Figure 2. Plot of measured panobinostat loading for CDN-1, 2, 3 & 4 as a function of different theoretical loadings. Error bars show standard deviations of at least three independently formulated batches.

The maximum panobinostat loading achieved for CDN-1 was approximately 5%, and the experimental drug loading did not increase with additional drug (Figure 3A). Nanoparticles produced from panobinostat loaded CDN-1 were micron-sized, as evident from the DLS size measurements. The zeta potential of the particles was found to increase linearly with increasing drug loading (Figure 3B). The larger size is likely due to the formation of particle or drug aggregates and eventual network collapse at increasing drug concentration (Figure 3C). The linear increase in zeta-potential can be explained based on an acid-base interaction between the drug (hydroxamic acid) and the surface-amine functionalities (Lewis base). Thus, CDN-1 could incorporate panobinostat in the micron range but did not self-assemble into stably condensed nanoparticles (Figure 3D).
**Figure 3.** Plot of average (A) experimental loading, (B) average zeta-potential, (C) mean hydrodynamic size of CDN-1 nanoparticles against theoretical loading of panobinostat. (D) Aggregate formation as a mechanism of drug stabilization by the CDN-1 nanoparticles. Error bars show the standard deviation of three separate batches.

Upon increasing the hydrophobic core of the network in CDN-2, experimental loading for panobinostat was found to increase up to approximately 8% (**Figure 4A**). This increase in drug loading was associated with a rise in zeta potential for up to 5% theoretical loading and a steady increase in particle size with increasing drug incorporation (**Figure 4B**). While the same acid-base interaction of the drug and surface amine functionalities holds true in this case, we speculate that a larger hydrophobic core assists in stabilizing the increasing amount of drug in the network as compared to CDN-1 as drug incorporation exceeds 5% (**Figure 4C**). Thus, CDN-2 was capable of self-assembling into relatively stable nanoparticles with modest, saturable loading capacity (**Figure 4D**).
**Figure 4.** Plot of average (A) experimental loading, (B) average zeta-potential, (C) mean hydrodynamic size of CDN-2 nanoparticles against theoretical loading of panobinostat. (D) Stable drug-nanoparticle interactions. Error bars show the standard deviation of three separate formulations.

CDN-3 employed a longer length cross-linker (1,6-hexanediol diacrylate) that enabled self-assembly of polymer and drug into smaller and more consistently sized nanoparticles than CDN-1 or CDN-2 (Figure 5A). Presumably, this increased stability is due to the more flexible network with a more hydrophobic core that can accept increasing quantities of drug. Zeta potential was observed to increase as loading increased (Figure 5B). Interestingly, aqueous diameter was observed to increase for nanoparticles formed from CDN-3 with a 5% theoretical loading compared to other loadings (Figure 5C). This observation of an outlying high diameter at 5% theoretical loading was reproducible in at least three independent experimental replicates. This could likely be ascribed to cyclodextrin-drug complexation at lower theoretical loadings owing to an expanded hydrophobic core, which facilitates easier access to the cyclodextrin units (Figure 5D).
Figure 5. Plot of (A) average experimental loading, (B) average zeta-potential, (C) mean hydrodynamic size of CDN-3 nanoparticles against theoretical loading of panobinostat. (D) Expanded hydrophobic core stabilizing drug complexation with cyclodextrin cavity. Error bars show the standard deviation of three separate formulations.

CDN-4, synthesized with mPEG\textsubscript{550}-amine, exhibited the highest extent of panobinostat incorporation, with measured loading of up to ~30% (Figure 6A). This high level of experimental loading was achieved at the cost of encapsulation efficiency. The particle size was found to decrease steadily with increased drug loading, while the near-neutral zeta-potential decreased slightly with increasing drug loading (Figure 6 (B & C)). This is likely due to the association of ionized panobinostat molecules with the mPEG\textsubscript{550} shell of the particles (Figure 6D).
Figure 6. Plot of average (A) experimental loading, (B) average zeta-potential, (C) mean hydrodynamic size of CDN-4 nanoparticles against theoretical loading of panobinostat. (D) mPEG_{550}-drug interaction stabilizing higher loaded CDN-4 nanoparticles. Error bars show the standard deviation of three separate formulations.

Comparison of the loading, size, and surface charge of particles formed by these different CDNs for a single encapsulated drug yields important insight into the mechanisms of loading and self-assembly that enables such a high degree of drug incorporation. For CDNs 1-3 (amine functionalized CDNs), the zeta potential was observed to increase with increased drug loading. This observation supports an ionic interaction of panobinostat with surface-exposed amine groups. Comparing CDN-1, CDN-2, and CDN-3, the experimental drug loading was found to increase with an expansion of the predicted size of hydrophobic core of the nanoparticles, supporting additional hydrophobic interactions as being significant for loading drug and promoting self-assembly. These hydrophobic interactions likely explain the aggregate formation in CDN-1, which can be contrasted with the stability of nanoparticles formed by CDN-2 & 3 that possess greater capacity to incorporate drug. The high loading of panobinostat in nanoparticles formed by CDN-4 (mPEG_{550} functionalized) suggests an interaction of panobinostat with the mPEG_{550} outer shell, which could be a similar loading mechanism as what we have previously reported for the HDAC inhibitor quisinostat in polyester nanoparticles.\textsuperscript{14} The near-neutral zeta potential of these particles precludes the possibility of an acid-base interaction between panobinostat and the particle surface. We therefore attribute the drug-loading induced negative zeta potential to the alignment of ionized drug molecules aligning along the mPEG_{550} exterior shell of the particle. This drug-polymer interaction would account for the substantially higher drug loading (and minimal saturation) observed for CDN-4.
Controlled release of panobinostat was studied for two different experimental loadings of formulations with CDN-2, 3 & 4 in PBS at 37°C. Controlled release from CDN-1 was not studied due to the large and highly variable size of resultant particles, which makes it unsuitable for *in vivo* application. All CDNs provided effective controlled release, with release times ranging between 5 and 24 days for different CDN structures. CDN-4 was found to have the slowest release kinetics in comparison to CDN-2 & 3, presumably due to slowed diffusion of panobinostat through the PEG layer. Experimental loading was observed to affect controlled release for CDN-2 & 4. For instance, highly loaded CDN-2 was observed to completely release panobinostat within 4 days (Figure 7B) as compared to 8 days (Figure 7A) for the less highly loaded formulation. Similarly, highly loaded CDN-4 was found to completely release panobinostat within 13 days (Figure 7F) as compared to 24 days for the less highly loaded formulation. The apparently slower release kinetics for less highly loaded particles for CDN-2 & 4 suggests that drug may exhibit a greater surface association when particles are highly loaded. In contrast, CDN-3 in contrast exhibits similar release kinetics for both higher and lower drug loaded samples, signifying the role of an expanded hydrophobic core in enabling stable incorporation of larger quantities of drug.
Figure 7. Controlled release of panobinostat loaded CDN samples for (A) 5% (w/w) & (B) 30% (w/w) theoretically loaded CDN-2 samples; (C) 5% (w/w) & (D) 20% (w/w) theoretically loaded CDN-3 samples; (E) 5% (w/w) & (F) 20% (w/w) theoretically loaded CDN-4 samples. Error bars are reported as the standard deviation of at least two separate measurements.

Application of panobinostat loaded CDNs in vitro and in vivo

The zeta potential of colloidal materials plays an essential role in how particles interact with tissues and cells, governing biodistribution, cellular uptake, and drug lifespan in vivo. Tumor cell membranes tend to be more negative in charge than healthy cells, which some investigators have utilized for the purposes of targeting nanoparticle delivery. However, less negative surface charges can promote nonspecific cellular uptake or be plagued by aggregation due to absorption of plasma proteins. As an
alternative strategy, PEGylation of nanoparticles can improve their plasma half-life and shield the encapsulated drug during circulation, which can increase the drug’s lifespan.\textsuperscript{32} Thus, to move towards assessment of nanoparticle CDNs for drug delivery, we focused on CDN-4, that bears a PEGylated surface as well as size and surface charge that are expected to be compatible for effective delivery \textit{in vivo}. Bioactivity of panobinostat loaded CDN-4 was evaluated in GL261 cells, a murine glioblastoma line. Following 72 hours of incubation, free panobinostat exhibited an IC\textsubscript{50} value of 0.17\textmu M, while panobinostat loaded CDN-4 resulted in IC\textsubscript{50} values of 0.56\textmu M, respectively (\textit{Figure 8A}). To test whether panobinostat loses activity during formulation, we subjected panobinostat without CDN materials to the complete formulation process and assessed IC\textsubscript{50}; non-CDN formulated panobinostat possessed an IC\textsubscript{50} value of 0.23\textmu M. When BIODIPY-labeled CDN-4 nanoparticles were incubated with cells for 48 hours, a perinuclear fluorescence signal was observed, confirming that nanoparticles are effectively internalized by cells (\textit{Figure 8B}). The drug loading studies described above provide evidence that panobinostat efficiently loads into the cyclodextrin-containing core of CDN-4 and is released slowly. Thus, the apparent increase in IC\textsubscript{50} observed for panobinostat loaded into CDN-4 likely represents a consequence of controlled release. Increased IC\textsubscript{50} following drug encapsulation due to reduced cellular availability of encapsulated drug has been reported for other nanoparticle preparations but do not necessarily represent a problem for \textit{in vivo} development of these systems, since prolonged release will confer advantages \textit{in vivo} that are not captured in cellular assays.\textsuperscript{33-36}
Figure 8. (A) *In-vitro* GL261 cell viability assay for panobinostat loaded CDN-4 formulation. IC$_{50}$ values after 72 hours of incubation: free Pb = 0.17μM; CDN(4)-Pb = 0.56μM, and non-CDN-formulated Pb = 0.23μM. Freely solubilized panobinostat is abbreviated as Pb.  
(B) Association of BODIPY dye labeled CDN-4 ($\lambda_{ex} = 503$nm; $\lambda_{em} = 509$nm) to GL261 cells confirmed via fluorescence microscopy experiments.  
(C) Bioavailability of panobinostat in CNS, confirmed by pharmacokinetic studies.

We next sought to test whether incorporation of panobinostat into the CDN network would enable administration to aqueous tissue compartments. Our laboratory is focused on the development of nanocarrier systems for the central nervous system (CNS) via direct administration to the cerebrospinal fluid (CSF) that bathes the brain and spinal cord.$^{37}$ This particular mode of administration offers a number of advantages for CNS drug delivery, including achievement of high concentrations of drug in the CNS compartment while minimizing off-target tissue toxicity.$^{38}$ We first assessed the tolerability of panobinostat loaded CDNs in healthy mice following administration through the cisterna magna,
identifying a maximum tolerated dose (MTD) of 960ng for panobinostat loaded CDN-4 nanoparticles. At this dosing level, mice exhibited neurological symptoms immediately following treatment administration that were reversible within 30 minutes and did not exceed 15% weight loss during the recovery period. This dose was therefore considered tolerable, but the dose was reduced for subsequent studies to ensure minimal side effects. Pharmacokinetic assessments confirmed that panobinostat was delivery to CNS tissues following administration of 560ng panobinostat loaded into CDN-4 nanoparticles (Figure 8C). Particle systems delivered directly to cerebrospinal fluid (CSF) are not expected to penetrate the parenchyma in large quantities, although drug is expected to be delivered passively from the subarachnoid space into CSF exposed tissues. Pharmacokinetic assessments were therefore performed on specific CNS tissue regions instead of bulk brain homogenate. One hour after administration, panobinostat levels reached 243 ng/g in the brainstem / cerebellum, clearing to reach a concentration of 3.6 ng/g after 48 hours. Panobinostat concentration was initially low in the upper spinal cord, reaching 183 ng/g 4 hours after administration and clearing thereafter. These data support movement of nanoparticles from the brain compartment into the spinal cord following cisternal administration, which we have previously reported. Panobinostat was not detectable in plasma at any time point after delivery up to 48 hours (data not shown). These data confirm that encapsulation within CDN-4 nanoparticles enables delivery in vivo. Taken in sum, in vitro and in vivo work demonstrate that nanoparticles formed by CDN-4 can deliver panobinostat to cells and tissues.

Library of Drug Loaded CDNs

Initial studies with panobinostat suggested that the loading mechanism includes both hydrophobic (within the cyclodextrin/polymer core) and ionic (possibly within the amine or PEG layer) interactions. We predicted that these interactions would be useful for the encapsulation of other ionizable molecules. To test this prediction, we attempted to load other HDACi’s containing ionizable hydroxamic acids (quisinostat, dacinostat and givinostat), as well as non-polar molecules that are either non-ionizable (nile
red), possess reduced ionization capacity (camptothecin), or contain an alternative ionizable boronic acid group (bortezomib). As a control hydrophilic drug, we also attempted to encapsulate cytarabine with all the CDNs, but this yielded poor experimental loading (data not shown).

**Figure 9.** Library of drug loaded CDN nanoparticles. The average experimental drug loading values of various small molecules for CDN 1-4 are shown for a theoretical loading of 10% (w/w). Molecular structures of compounds illustrating the hydrophobic (red) and ionizable (green) moieties. Error bars are reported as the standard deviation of three separate formulations.

Drugs with ionizable moieties in their molecular structure (quisinostat, panobinostat, dacinostat, givinostat and bortezomib) loaded particularly well in CDNs 1-3, supporting the role of surface amine functionality enabling drug interaction via H-bonding (Figure 9). Drug-loading was found to increase from CDN-1 to CDN-3 for all the HDACi’s investigated except for givinostat (ITF2357). Given that
givinostat possess the bulkiest, inflexible hydrophobic end, these data support the role of a flexible hydrophobic core for effective encapsulation of drugs in CDNs. CDN-4 was found to load all the HDACi's and bortezomib except dacinostat. The molecular structures of quisinostat, panobinostat, givinostat, and bortezomib are relatively slender, flexible and polar. However, the structure of dacinostat (LAQ824) bears an ionizable (hydroxyl) offshoot midway throughout the molecule, which perturbs the hydrophobic end. We speculate that the flexible, slender structures of these other HDAC inhibitors facilitate efficient association with the exterior mPEG$_{550}$ layer of the particle, which is not possible in the case of dacinostat owing to its steric incompatibility. These results highlight ionizability as being the most significant factor for drug incorporation in particles formed from CDNs 1-3 and steric compatibility as being an essential requirement for drug loading into particles formed from CDN-4.

These studies present a novel approach for generating nanoparticles that are highly loaded with HDACi drugs or other molecules bearing ionizable moieties. To our knowledge, the maximum reported HDACi loading in polymeric system is around 9%, which in turn is higher than most other lipid derived systems (~2.5-5.0%). The described particle system herein exhibits a maximum observed loading of ~30% in comparison, with a sustained rate of drug release (13-24 days). Importantly, all the tested HDACi could be loaded at a relatively high capacity in the CDN systems. Electrolyte/co-solvent induced ionic drug loading and synthetic polymer constructs with ionizable backbone have been widely reported in the literature as reliable ionic drug loading strategies. Nevertheless, the general utility of these strategies is questionable, as the choice of the inorganic salt/electrolyte is highly drug-specific. Our work directly addresses the problem of finding a generalizable strategy for loading drugs bearing ionizable moieties into polymeric nanoparticles. The modular nature of the CDN platform described here provides the advantage of enabling easy modification to both the internal and external structures, thereby tuning the material properties for drug loading and sustained release, and enabling, in future work, further engineering to optimize delivery for specific disease models.
CONCLUSIONS

We have demonstrated the general utility of the β-cyclodextrin-poly (β-amino ester) network as an efficient drug-loading platform for hydrophobic drugs with ionizable moieties. The architectures of these networks are key to their drug loading capacities and biophysical characteristics, as revealed by structure-property relationships. While both the linker and the surface functionality determine the experimental loadings of the drug, the amine-CDNs (1, 2 & 3) displayed a positive surface charge and the mPEG<sub>550</sub>-CDN (CDN-4) exhibited a near-neutral surface charge. We discovered that a slender, ionizable drug molecular structure (e.g., panobinostat and quisinostat) maximizes drug encapsulation in these networks. While CDN-3 completely releases the drug over 7 days, CDN-4 reduces the release kinetics to affect the complete release within 13-24 days depending on the drug loading. CDN-4 nanoparticles are internalized by cells and deliver bioactive panobinostat in a GL261 model. Pharmacokinetic analyses confirm that CDN-4 nanoparticles can deliver panobinostat to aqueous tissue compartments, with intrathecal administration of panobinostat loaded CDN-4 yielding delivery to the brainstem/cerebellum and upper spinal cord. These studies describe the development of a generalizable strategy for nanoparticle encapsulation of ionizable, hydrophobic molecules, that will be an important step forward in developing HDAC<sub>i</sub> systems for the treatment of disease.
**i) Synthesis of Acrylated-CD**

\[
\beta-CD + \text{Acryloyl chloride} \rightarrow \text{Acrylated-CD}
\]

**ii) Synthesis of blank CDN nanoparticles**

\[
\text{Acrylated-CD} + \text{disacrylate cross linkers} + \text{R-Amine} \rightarrow \text{Amine-CDN}
\]

Michael addition:

\[
\text{EtOAceCHCl}_{3} (3:7) \quad 65^\circ \text{C}, 12 h, 660 \text{ rpm} \rightarrow \text{PEG-CDN}
\]

**iii) Synthesis of panobinostat loaded CDN nanoparticles**

\[
\text{Blank Amine-CDN nanoparticle (1 & 2 & 3)} \quad \rightarrow \quad \text{Panobinostat loaded Amine-CDN nanoparticle (1 & 2 & 3)}
\]

DMSO (2.5 vol% aq. soln) 18 h, agitation

---

**Figure 10.** Schematic representation of synthesis of i) acrylated β-cyclodextrin; ii) blank; and iii) panobinostat-loaded CDN nanoparticles.
METHODS

Materials

The following reagents were obtained from Alfa Aesar: β-cyclodextrin, Acrolyl chloride, Chloroform (99%, ACS reagent), Ethyl acetate (99%, ACS reagent), Dimethyl sulfoxide (99%, ACS reagent). 1-methyl-2-pyrrolidone (NMP) and Diethylether (99+%) were purchased from Acros Organics. 1,4-butandiol diacrylate and 1,6-hexandiol diacrylate were purchased from Sigma Aldrich. Methoxy terminated polyethylene glycol (MW 550, mPEG\textsubscript{550})-Amine was obtained from Creative Peg Works (Chapel Hill, NC). N,N-dimethylethlenediamine was obtained from Oakwood chemicals (Estill, SC, USA). Phosphate Buffer Saline (PBS, pH 7.4), Polyester Transwell inserts (96 well-plate, 0.4 μm pore size) were obtained from Costar (Cambridge, MA). Dialysis cassettes (MWCO 3,500 Dalton) were obtained from Thermo Fisher Scientific (Waltham, MA). CellTiter-Glo was purchased from Promega (Madison, WI, USA). Costar 96-well plates were purchased from VWR International (Radnor, PA, USA). Dulbecco’s modified Eagle medium (DMEM), fetal bovine serum (FBS), trypan blue, and 0.25%trypsin-EDTA were purchased from Gibco Invitrogen (Carlsbad, CA, USA). Quisinostat (JNJ-26481585) and Panobinostat (LBH589) were purchased from ApexBio (Houston, TX, USA). Camptothecin and Dacinostat (LAQ824) were purchased from Selleckchem (Houston, TX, USA). Nile red was purchased from TCI America. All these chemicals were used as received without further purification unless otherwise noted.

Synthesis of Acrylated-CD

β-cyclodextrin was oven dried for 12 hours at 110°C. N-methyl-2-pyrrolidone (NMP) was stored with oven-parched molecular sieves (4A) for at least 24hrs. β-cyclodextrin (2.3g, 2.0mmol) was measured and stirred in NMP in a 25mL round-bottom flask. The solution was cooled with ice-cold water and acrolyl chloride (2.0mL, 24.7mmol) was added via a syringe. The resultant solution was stirred under ice-cold condition for 1hr, after which the solution was heated up to 21°C. Stirring was continued for 48hrs, after
which the reaction mixture was poured into approximately 200mL of distilled water. The precipitated solid was homogenously bath-sonicated, followed by filtration. The collected residue was washed with distilled water twice and then allowed to dry over 48 hrs. The dry white powder recovered was weighed out (3.59g), and subsequently analyzed by $^1$H-NMR and MALDI-TOF spectrometry (see supporting information).

**Synthesis of blank CDN nanoparticles**

*Amine-CDNs (CDN-1, 2 & 3)*

β-cyclodextrin-poly(β-amino ester) CDNs with various structures were synthesized employing Michael addition of a three component mixture. Acrylated β-cyclodextrin (cyclodextrin precursor; 0.06mmol), 1, 4-butanediol or 1, 6-hexanediol diacrylate (cross linking polyesters; 1.3mmol and 2.7mmol) and $N,N$-dimethylethylediamine (1.9mmol) were dissolved in a solvent mixture of EtOAc/CHCl$_3$ (3:7) and heated at 65°C under stirring at 600rpm for 12-14 hours. The reaction mixture was subsequently dried under reduced pressure, and the resultant crude was redissolved in chloroform (approx. 2mL). This solution was dispersed in 15mL (repeated twice with 5mL) of diethylether to precipitate the insoluble network. The supernatant was discarded, and the obtained precipitate was filtered, dried and re-dispersed into fresh DI water (40mL). The aqueous dispersion of the network was subsequently washed via ultrafiltration through two Amicon Ultra-15 centrifugal filters (10kDa MWCO, 15mL capacity) for 20 mins (x2) spins at 5000 RCF. Aliquots were frozen and lyophilized to determine recovery and biophysical characteristics (size and zeta potential).

*mPEG$_{550}$-CDN (CDN-4)*

Acrylated β-cyclodextrin (cyclodextrin precursor; 0.015mmol), 1, 4-butanediol (cross linking polyesters; 0.675mmol) and mPEG$_{550}$-Amine (0.475mmol) were dissolved in a solvent mixture of EtOAc/CHCl$_3$ (3:7) and heated at 65°C under stirring at 600rpm for 12 hours. The reaction mixture was subsequently
dried under reduced pressure, and the resultant crude was re-dispersed into fresh DI water (40mL). The aqueous dispersion thus obtained was subsequently washed via ultrafiltration through Amicon Ultra-15 centrifugal filters (10kDa MWCO, 15mL capacity) for 2, 20 mins spins at 5000 RCF. Aliquots were frozen and lyophilized to determine recovery and biophysical characteristics (size and zeta potential).

**Synthesis of BODIPY labeled CDN nanoparticles**

Acrylated β-cyclodextrin (cyclodextrin precursor; 0.015mmol), 1, 4-butanediol (cross linking polyesters; 0.675mmol), mPEG$_{550}$-Amine (0.354mmol) and Amino-PEG12-propionic acid (0.121mmol) were dissolved in a solvent mixture of EtOAc/CHCl$_3$ (3:7) and heated at 65°C under stirring at 600rpm for 12 hours. The reaction mixture was subsequently dried under reduced pressure, and the crude obtained was re-dispersed into fresh DI water (40mL). The aqueous dispersion thus obtained was subsequently washed via ultrafiltration through Amicon Ultra-15mL centrifugal filters (10kDa MWCO, 15mL capacity) for 2, 20 mins spins at 5000 RCF. Aliquots were frozen and lyophilized overnight. For labelling, 5.0 mg of the lyophilized sample was further treated with N-hydroxysuccinimide (6.2mg), EDC (9.5µL) and BDP-FL-amine (0.44µmol) in DMSO (500 µL) at room temperature for 24 hours. The reaction mixture was subsequently washed with DI water via ultrafiltration through Amicon Ultra-2mL centrifugal filters (3kDa MWCO, 2mL capacity) for 3, 20 mins spins at 5000 RCF. Aliquots were frozen and lyophilized overnight.

**MALDI-Tof spectrometry**

20mg/mL solutions were separately made for analyte (Acrylated-CD) and matrix (2,5-Dihydroxy benzoic acid) in acetonitrile/water (1/9) mixture. A mixture of 4µL analyte, 14µL matrix and 2µL of sodium acetate as cationization agent (1mg/mL aqueous solution) was homogenized by vortexing. 2µL of the mixture was transferred onto a MALDI target plate, followed by air drying to prepare a thin
matrix/analyte film. Mass spectra was obtained using MS Bruker Autoflex MALDI-ToF mass spectrometer equipped with a nitrogen laser delivering 2 ns laser pulses at 337 nm with positive ion ToF detection performed using an accelerating voltage of 25 kV.

**NMR spectroscopy**

Lyophilized CDN samples were dispersed in dimethyl sulfoxide-d6 (DMSO-d6). $^1$H-NMR spectra were obtained using 300MHz Bruker Avance NMR spectrometer. The spectra were compared to those of the individual components employed in the synthesis of CDNs.

**Scanning Electron Microscopy**

Lyophilized nanoparticles were suspended in an aqueous solution to a final concentration of 20 mg/ml. 5 µl droplets of the above solution were placed onto aluminum stubs with carbon adhesive. Samples were allowed to air dry prior to coating. Samples were sputter coated using a Denton Desk-V Sputter system with gold at 20 mAmps for 20 seconds and imaged using a FEI Quanta 400 environmental scanning electron microscope with an ETD detector at 20 kV and a 4 mm working distance.

**Dynamic Light Scattering (DLS)**

100µL of CDN (CDN-1, 2 & 3 or 50 µL of CDN-4) aqueous solutions (20mg/mL) is added to approximately 3mL of distilled water in a clean dry cuvette. The dispersion is homogenized by repeated agitation and then allowed to stabilize for 2 minutes. Mean hydrodynamic size of the particles were measured using NanoBrook 90Plus Zeta Particle Size Analyzer (Brookhaven Instruments, Holtsville, NY USA). Results were reported as the average of at least three separate readings.
**Zeta-potential**

60µL of CDN (CDN-1, 2, 3 & 4) aqueous solutions (20mg/mL) is added to approximately 2mL of KCl (1mM aq. solution) in a clean dry cuvette. The dispersion is homogenized by repeated agitation and then allowed to stabilize for 5 minutes. Zeta-potential measurements were carried out using NanoBrook 90Plus Zeta Particle Size Analyzer (Brookhaven Instruments, Holtsville, NY USA). Results were reported as the average of at least three separate readings.

**Drug-loaded CDN nanoparticles**

Aqueous dispersions (2.0 mL) of blank CDN nanoparticles (10mg/mL) were doped with various drugs dissolved in DMSO (50 µL). The aqueous dispersion was left to agitate for 18h and then subjected to concentration via ultrafiltration through Amicon Ultra-15 centrifugal filters (3kDa MWCO, 0.5mL capacity) for 4, 20 mins spins at 5000 RCF. Aliquots were frozen and lyophilized prior to characterization. Drug loading was quantified by absorbance (310nm for quisinostat, panobinostat and dacinostat; 285nm for givinostat; 365nm for camptothecin; 550nm for Nile Red) on a Tecan plate reader. Lyophilized nanoparticles were dissolved at 5 mg/ml in DMSO. Samples were plated in triplicate (40µL nanoparticles and 10µL DMSO per well) in a clear, flat bottom 96-well assay plate. A control curve was constructed in technical triplicate by adding 40µL blank nanoparticles per well and spiking with 10µL of known drug concentration in DMSO. Experimental drug loading was calculated as mass of drug/mass of nanoparticles (w/w %). 40µL of bortezomib loaded nanoparticles (5 mg/ml aqueous solution) were extracted with 100µL of ethylacetate. The ethylacetate layer was collected and dried under nitrogen. The contents were dissolved in 40µL of DMSO and plated in triplicate. A control curve was constructed in technical triplicate by spiking with 10µL of known drug concentration in DMSO. Absorbance was measured at 285nm. Experimental drug loading was calculated as mass of drug/mass of nanoparticles (w/w %).
Controlled release

Lyophilized nanoparticles were dispersed in aqueous solution to a final drug concentration of 1mg/ml (i.e. 20mg/mL panobinostat for a sample with 5% experimental loading) and 400μL was transferred to a 3.5k MWCO Slide-A-Lyzer Dialysis cassette (Thermo Fisher Scientific, Waltham, MA USA). The cassette was immersed in 4L PBS (pH 7, replaced at every 24hrs time point) at 37 °C with gentle stirring (75 rpm). At each time point, 30 μl nanoparticles was removed from the cassette and dissolved in 120μL DMSO. 50 μl dissolved nanoparticles was added in triplicate to a clear, flat bottom, 96-well plate, and the amount of drug remaining was quantified by absorbance.

In vitro experiments

For IC50 experiments, GL261 cells were seeded at 3,000 cells/100µl into 96 well plates containing Dulbecco’s Modified Eagle Medium (DMEM) and 10% Fetal Bovine Serum (FBS). Cells were given 4 hours to adhere prior to drug treatments. Free panobinostat, drug-loaded and blank CDN-4 (21% drug loaded) were dispersed in 2.5% DMSO in cell culture media at 10mg/mL drug concentration. Equivalent doses were added to the 96 well plates at 16 serial dilutions with concentrations from 0-70μM and incubated at 37°C with 5% CO2 for 72 hours. Cell viability was determined with an IC50 value obtained from a CellTiter-Glo Luminescence assay and calculated using GraphPad Prism (San Diego, CA USA). For uptake experiments, GL261 cells were seeded at 90,000 cells/1mL into 48 well plates containing DMEM and 10% FBS. Panobinostat loaded CDN-4 nanoparticles (250ug) were incubated with cells for 48 hours, after which cells were thoroughly washed, fixed with 4% paraformaldehyde, and counterstained with DAPI. Images were collected on an upright Nikon Ti2 microscope.
**In vivo experiments**

All animal procedures were approved by the Institutional Animal Care and Use Committee at University of Texas Health Science at Houston in accordance with all relevant guidelines. Healthy, 6 week old female C57 mice were used for in vivo experiments. CDN nanoparticles were administered by direct infusion into the cisternal magna. Briefly, mice were anesthetized with isoflurane (2%) and mounted on a stereotaxic frame (Kopf Instruments, Tujunga, CA, USA). With the head tilted forward, an incision was made across the back of the neck and muscles were gentle retracted to expose the atlanto-occipital membrane. A Hamilton syringe (29 gauge needle, 30o bevel tip) in a 2uL volume delivered over 1 minute at an injection dept of 1mm. The needle was left in place for an additional 2 minutes to prevent backflow. All animals received a subcutaneous injection of buprenorphine (1mg/kg) prior to surgery to control pain. For MTD studies, panobinostat loaded CDN-4 nanoparticles were administered at increasing concentrations (n=3 mice per dose level). Mice were observed for at least 2 hours after injection to identify any acute neurological reactions to the infusion, and they were weighed daily thereafter. For pharmacokinetic studies, mice received CDN-4 nanoparticles at a dose of 560ug panobinostat and euthanized at specified time points (1, 4, 8, 24, and 48 hours after nanoparticle administration, n=5 mice per time point). One mouse in the 24 hour time point died during surgery, leaving n=4 for that experimental group. Blood was collected by cardiac puncture, transferred to a heparinized tube, and centrifuged at 2000 RPM for 10 minutes at 4oC to obtain plasma. The brainstem and cerebellum were removed from whole brain. The spinal cord was obtained by hydraulic extrusion and divided in two to obtain the upper half. All tissues were flash frozen and stored for later pharmacokinetic analysis.

**Pharmacokinetic analysis**
Drug concentrations of panobinostat in plasma, brain, and spinal cord were determined using reverse-phase liquid chromatography (Micromass Quattro Ultima mass spectrometer, Waters, Milford, MA). Brain and spinal cord samples were homogenized with 3 tissue volumes of 5% bovine serum albumin solution using a homogenizer (PowerGen 125, Thermo Fisher Scientific, Waltham, MA). Liquid-liquid extractions were performed for plasma, brain and spinal cord homogenates by spiking panobinostat-d8 hydrochloride salt as internal standard, adding 5 volumes of ethyl acetate and an equal volume of pH 11 buffer. Samples were mixed by vortexing for 5 minutes and then centrifuged at 7500 rpm at 4deg for 5 minutes. The organic layer was dried under nitrogen and reconstituted in mobile phase (80:20 of distilled water with 0.1% formic acid: acetonitrile with 0.1% formic acid), and injected onto a YMC-pack ODS-AM column (35/2.0 mm, S-3 µm, 12 nm; YMC, Inc., Allentown, PA) for chromatographic separation. The m/z transitions were 350.04 – 157.91 for panobinostat and 357.92 – 165.04 for with panobinostat-d8 hydrochloride salt (positive-ionization mode). The limit of quantification was 1 ng/mL and the precision (expressed as the CV percentage) was less than 15%.

Acknowledgements

This project was supported by the National Institutes of Health (R21NS107985, R01HD099543) and the Ian’s Friends Foundation. We are thankful to the Nuclear Magnetic Resonance and Mass Spectrometry facility at M.D. Anderson Cancer Center and BioScience Research Collaborative (BRC) at Rice University respectively.

Supporting Information Available

MALDI-Tof spectra of acrylated β-cyclodextrin; 1H-NMR spectra; SEM images of CDN nanoparticles.

The Supporting Information is available free of charge via the Internet at http://pubs.acs.org
REFERENCES

1. Campos, B.; Bermejo, J. L.; Han, L.; Felsberg, J.; Ahmadi, R.; Grabe, N.; Reifenberger, G.; Unterberg, A.; Herold-Mende, C. Expression of nuclear receptor corepressors and class I histone deacetylases in astrocytic gliomas. *Cancer Sci.* 2011, 102, 387-392.

2. Ceccacci, E.; Minucci, S. Inhibition of histone deacetylases in cancer therapy: lessons from leukaemia *Br. J. Cancer* 2016, 114, 605-611.

3. Dvorakova, M.; Vanek, T. Histone deacetylase inhibitors for the treatment of cancer stem cells *Med. Chem. Commun.* 2016, 7, 2217-2231.

4. Giudice, F. S.; D.S.P Jr., Nör, J. E.; Squarize, C. H.; Castilho, R. M. Inhibition of histone deacetylase impacts cancer stem cells and induces epithelial-mesenchyme transition of head and neck cancer. *PLoS One* 2013, 8, 58672.

5. Cornago, M.; Garcia-Alberich, C.; Blasco-Angulo, N.; Vall-llaura, N.; Nager, M.; Herreros, J.; Comella, J. X.; Sanchis, D.; Llovera, M. Histone deacetylase inhibitors promote glioma cell death by G2 checkpoint abrogation leading to mitotic catastrophe. *Cell. Death. Dis.* 2014, 5, 1435.

6. Johnstone, R. W. Histone-deacetylase inhibitors: novel drugs for the treatment of cancer. *Nature reviews Drug discovery* 2002, 1, 287-299.

7. Cote, S.; Rosenauer, A.; Bianchini, A.; Seiter, K.; Vandewiele, J.; Nervi, C.; Miller, W. H. Jr. Response to histone deacetylase inhibition of novel PML/RARalpha mutants detected in retinoic acid-resistant APL cells. *Blood* 2002, 100, 2586–2596.

8. Pandolfi, PP. Transcription therapy for cancer. *Oncogene.* 2001, 20, 3116–3127.

9. Fischer, A.; Sananbenesi, F.; Wang, X.; Dobbin, M.; Tsai, L. H. Recovery of learning and memory is associated with chromatin remodelling. *Nature.* 2007, 447, 178–182.

10. Dash, P. K.; Orsi, S. A.; Moore, A. N. HDAC inhibition combined with behavioral therapy enhances learning and memory following traumatic brain injury. *Neuroscience* 2009, 163, 1-8.

11. Arts, J.; King, P.; Mariën, A.; Floren, W.; Beliën, A.; Janssen, L.; Pilatte, I.; Roux, B.; Decrane, L.; Gilissen, R.; Hickson, I.; Vreys, V.; Cox, E.; Bol, K.; Talloen, W.; Goris, I.; Andries, L.; Jardin, M. D.; Janicot, M.; Page, M.; van Emelen, K.; Angibaud, P. JNJ-26481585, a novel second-generation oral histone deacetylase inhibitor shows broad-spectrum preclinical antitumoral activity. *Am. Assoc. Cancer Res.* 2009, 15, 6841–6851.

12. Carol, H.; Gorlick, R.; Kolb, E. A.; Morton, C. L.; Manesh, D. M.; Keir, S. T.; Reynolds, C. P.; Kang, M. H.; Maris, J. M.; Wozniak, A.; Hickson, I.; Lyalin, D.; Kurmasheva, R. T.; Houghton, P.J.; Smith, M. A.; Lock, R. Initial testing (Stage 1) of the histone deacetylase inhibitor, quisinostat (JNJ-26481585), by the pediatric preclinical testing program. *Pediatr Blood Cancer* 2014, 61, 245–252.
13. Venugopal, B.; Baird, R.; Kristeleit, R. S.; Plummer, R.; Cowan, R.; Stewart, A.; Fournneau, N.; Hellemons, P.; Elsayed, Y.; Mcclue, S.; Smit, J. W.; Forslund, A.; Phelps, C.; Camm, J.; Evans, T. R. J.; de Bono, J. S.; Banerji, U. A phase I study of quisinostat (JNJ-26481585), an oral hydroxamate histone deacetylase inhibitor with evidence of target modulation and antitumor activity, in patients with advanced solid tumors. *Clin. Cancer Res.* **2013**, 19, 4262–4272.

14. Householder, K. T.; DiPerna, D. M.; Chung, E. P.; Luning, A. R.; Nguyen, D. T.; Stabenfeldt, S. E.; Mehta, S.; Sirianni, R. W. pH driven precipitation of quisinostat onto PLA-PEG nanoparticles enables treatment of intracranial glioblastoma. *Colloids and Surfaces B: Biointerfaces* **2018**, **166**, 37-44.

15. Wang, E. C.; Min, Y.; Palm, R. C.; Fiordalisi, J. J.; Wagner, K. T.; Hyder, N.; Cox, A. D.; Caster, J.; Tian, X.; Wang, A. Z. Nanoparticle formulations of histone deacetylase inhibitors for effective chemoradiotherapy in solid tumors. *Biomaterials* **2015**, **51**, 208–215.

16. Mohamed, E. A.; Zhao, Y.; Meshali, M. M.; Remsberg, C. M.; Borg, T. M.; Foda, A. M. M.; Takemoto, J. K.; Sayre, C. L.; Martinez, S. E.; Davies, N. M.; Forrest, M. L. Vorinostat with sustained exposure with high solubility in poly(ethylene glycol)-b-poly(d, l-lactic acid) micelle nanocarriers: characterization and effects on pharmacokinetics in rat serum and urine. *Journal of Pharmaceutical Sciences* **2012**, **101**, 3787-3798.

17. Tang, X.; Liang, Y.; Liu, X.; Zhou, S.; Liu, L.; Zhang, F.; Xie, C.; Cai, S.; Wei, J.; Zhu, Y.; Hou, W. PLGA-PEG nanoparticles coated with Anti-CD45RO and loaded with HDAC plus Protease inhibitors activate latent HIV and inhibit viral spread. *Nanoscale Research Letters* **2015**, **10**, 413.

18. Somoza, J. R.; Skene, R. J.; Katz, B. A.; Mol, C.; Ho, J. D.; Jennings, A. J.; Luong, C.; Arvai, A.; Buggy, J. J.; Chi, E.; Tang, J.; Sang, B. C.; Verner, E.; Wynands, R.; Leahy, E. M.; Dougan, D. R.; Snell, G.; Navre, M.; Knuth, M. W.; Swanson, R. V.; McRee, D. E.; Tari, L. W. Structural Snapshots of Human HDAC8 Provide Insights into the Class I Histone Deacetylases. *Structure* **2004**, **12**, 1325.

19. Davis, M. E.; Brewster, M. E. Cyclodextrin-based pharmaceutics: past, present and future. *Nat. Rev. Drug Discovery* **2004**, **3**, 1023−1035.

20. Coulter, T.; Damment, S.; Pace, A.; Palmer, D. Cyclodextrin-panbinostat adduct. *Int. Pat. Appl.* PCT/EP2017/057472, March 29, 2017.

21. Bonnet, V.; Gervaise, C.; Djedai`ni-Pilard, F.; Furlan, A.; Sarazin, C. Cyclodextrin nanoassemblies: a promising tool for drug delivery. *Drug Discovery Today* **2015**, **20**, 1120-1126.

22. Cirpanli, Y.; Bilensoy, E; Dog˘an, A. L.; Calis, S. Comparative evaluation of polymeric and amphiphilic cyclodextrin nanoparticles for effective camptothecin delivery. *European Journal of Pharmaceutics and Biopharmaceutics* **2009**, **73**, 82–89.

23. Caldera, F.; Tannous, M.; Cavalli, R.; Zanetti, M.; Trotta, F. Evolution of cyclodextrin nanosponges. *International Journal of Pharmaceutics* **2017**, **531**, 470-479.
24. Clark, A. J.; Wiley, D. T.; Zuckerman, J. E.; Webster, P.; Chao, J.; Lin, J.; Yen, Y.; Davis, M. E. CRLX101 nanoparticles localize in human tumors and not in adjacent, nonneoplastic tissue after intravenous dosing. *PNAS* 2016, 113, 3850-3854.

25. Gil, E. S.; Wu, L.; Xu, L.; Lowe, T. L. β-Cyclodextrin-poly(β-Amino Ester) Nanoparticles for Sustained Drug Delivery across the Blood–Brain Barrier. *Biomacromolecules* 2012, 13, 3533-3541.

26. Nicoletta, F.; Francesca, M.; Andrea, B.; Roberto, F.; Maria C. G.; Giuseppe, B. The pan-histone deacetylase inhibitor LBH589 (panobinostat) alters the invasive breast cancer cell phenotype. *Int J. Oncol.* 2014, 44, 700.

27. Evangelos, T. The synergistic effect of panobinostat (LBH589) with melphalan or doxorubicin on multiple myeloma cells; rationale for the use of combination regimens in myeloma patients. *Leuk. Res.* 2011, 35, 295-296.

28. Shao, W.; Growney, J. D.; Feng, Y.; O’Connor, G.; Pu, M.; Zhu, W.; Yao, Y-M.; Kwon, P.; Fawell, S.; Atadja, P. Activity of deacetylase inhibitor panobinostat (LBH589) in cutaneous T-cell lymphoma models: defining molecular mechanisms of resistance. *Int. J. Cancer* 2010, 127, 2199–2208.

29. Pei, Y.; Liu, K-W.; Wang, J.; Garancher, A.; Tao, R.; Esparza, L. A.; Maier, D. L.; Udaka, Y. T.; Murad, N.; Morrissy, S.; Seker-Cin, H.; Brabetz, S.; Qi, L.; Kogiso, M.; Schubert, S.; Olson, J. M.; Cho, Y-J.; Li, X-N.; Crawford, J. R.; Levy, M. L.; Kool, M.; Pfister, S. M.; Taylor, M. D.; Wechsler-Reya, R. J. HDAC and PI3K Antagonists Cooperate to Inhibit Growth of MYC-driven Medulloblastoma *Cancer Cell.* 2016, 29, 311–323.

30. Chopra, V.; Quinti, L.; Khanna, P.; Paganetti, P.; Kuhn, R.; Young, A. B.; Kazantsev, A. G.; Hersch, S. LBH589, A Hydroxamic Acid-Derived HDAC Inhibitor, is Neuroprotective in Mouse Models of Huntington’s Disease. *J Huntingtons Dis.* 2016, 5, 347-355.

31. Honary, S.; Zahir, F. Effect of Zeta Potential on the Properties of Nano-Drug Delivery Systems - A Review (Part 1). *Tropical Journal of Pharmaceutical Research* 2012, 12, 255-264.

32. Hamidi, M.; Azadi, A.; Rafiei, P. Pharmacokinetic Consequences of Pegylation. *Drug Delivery* 2006, 13, 399-409.

33. Lundberg, B. B.; Griffiths, G.; Hansen, H. J. Cellular Association and Cytotoxicity of Doxorubicin-Loaded Immunoliposomes Targeted via Fab’ Fragments of an Anti-CD74 Antibody. *Drug Delivery* 2008, 14, 171-175.

34. Duan, J.; Zhang, Y.; Han, S.; Chen, Y.; Li, B.; Liao, M.; Chen, W.; Deng, X.; Zhao, J.; Huang, B. Synthesis and in vitro/in vivo anti-cancer evaluation of curcumin-loaded chitosan/poly (butyl cyanoacrylate) nanoparticles. *Int. J. Pharmaceutics* 2010, 400, 211-220.

35. Li, Q.; Sun, Y.; Sun, Y.; Wen, J.; Zhou, Y.; Bing, Q.; Isaacs, L. D.; Jin, Y.; Gao, H.; Yang, Y. Mesoporous Silica Nanoparticles Coated by Layer-by-Layer Self-assembly Using Cucurbit[7]uril for in Vitro and in Vivo Anticancer Drug Release. *Chem. Mater.* 2014, 26, 6418-6431.
36. Xin, H.; Jiang, X.; Gu, J.; Sha, X.; Chen, L.; Law, K.; Chen, Y.; Wang, X.; Jiang, Y.; Fang, X. Angiopep-conjugated poly(ethylene glycol)-co-poly(e-caprolactone) nanoparticles as dual-targeting drug delivery system for brain glioma. *Biomaterials* **2011**, *32*, 4293-4305.

37. Householder, K. T.; Dharmaraj, S.; Sandberg, D. I.; Wechsler-Reya, R. J.; Sirianni, R. W. Fate of nanoparticles in the central nervous system after intrathecal injection in healthy mice. *Sci. Rep.* **2019**, *9*, 12587.

38. Fowler, M.; Cotter, J.; Knight, B. E.; Sevick-Muraca, E. M.; Sandberg, D. I.; Sirianni, R. W. Intrathecal drug delivery in the era of nanomedicine. *Adv. Drug Deliv. Rev.* **2020**, *In press*.

39. Urbinati, G.; Marsaud, V.; Plassat, V.; Fattal, E.; Lesieur, S.; Renoir, J-M. Liposomes loaded with histone deacetylase inhibitors for breast cancer therapy. *Int. J. Pharm.* **2010**, *397*, 184-193.

40. Tran, T. H.; Chu, D. T.; Truong, D. H.; Tak, J. W.; Jeong, J.; Hoang, V. L.; Yong, C. S.; Kim, J. O. Development of lipid nanoparticles for a histone deacetylases inhibitor as a promising anticancer therapeutic. *Drug Deliv.* **2016**, *23*, 1335-1343.

41. Wang, Y.; Tu, S.; Steffen, D.; Xiong, M. P. Iron Complexation to Histone Deacetylase Inhibitors SAHA and LAQ824 in PEGylated Liposomes Can Considerably Improve Pharmacokinetics in Rats. *J Pharm Pharm Sci.* **2014**, *17*, 583–602.

42. Al-Maaieh, A.; Flanagan, D. R. Salt and cosolvent effects on ionic drug loading into microspheres using an O/W method. *Journal of Controlled Release* **2001**, *70*, 169-181.

43. Neugebauer, D.; Mielanczyk, A.; Bielas, R.; Odrobinska, J.; Kupczak, M.; Niesyto, K. Ionic polymethacrylate based delivery systems: effect of carrier topology and drug loading. *Pharmaceutics* **2019**, *11*, 337.
β-cyclodextrin-poly (β-amino ester) nanoparticles are a generalizable strategy for high loading and sustained release of HDAC inhibitors

Sauradip Chaudhuri¹, Martha J. Fowler¹, Afroz S. Mohammad², Wenqui Zhang², Cassandra Baker¹, Colton W. Broughton¹, Brandon E. Knight¹, William F. Elmquist², Sarah E. Stabenfeldt³, Rachael W. Sirianni¹*
Table of Contents

Figure S1........................................................................................................................................S3

Figure S2........................................................................................................................................S4

Figure S3........................................................................................................................................S5

Figure S4........................................................................................................................................S6

Figure S5........................................................................................................................................S7

Figure S6........................................................................................................................................S8

Figure S7........................................................................................................................................S9
Figure S1 MALDI-Tof mass spectra of Acrylated-CD recorded in MS Bruker Autoflex MALDI-Tof mass spectrometer. Individual peaks for different degrees (n) of acrylation are identified.
Figure S2 $^1$H-NMR spectra of Acrylated-CD in DMSO-d$_6$ recorded in Bruker Avance 300 MHz NMR spectrometer.
Figure S3 $^1$H-NMR spectra of CDN-1 in DMSO-$d_6$ recorded in Bruker Avance 300 MHz NMR spectrometer. Stoichiometric ratios of constituent units are recorded.
Figure S4  $^1$H-NMR spectra of CDN-2 in DMSO-d$_6$ recorded in Bruker Avance 300 MHz NMR spectrometer. Stoichiometric ratios of constituent units are recorded.
Figure S5 $^{1}$H-NMR spectra of CDN-3 in DMSO-$d_6$ recorded in Bruker Avance 300 MHz NMR spectrometer. Stoichiometric ratios of constituent units are recorded.
Figure S6 $^1$H-NMR spectra of CDN-4 in DMSO-d$_6$ recorded in Bruker Avance 300 MHz NMR spectrometer. Stoichiometric ratios of constituent units are recorded.
**Figure S7** Scanning electron microscope images of (A) CDN-1; (B) CDN-2; (C) CDN-3 & (D) CDN-4 recorded using FEI Quanta 400 environmental scanning electron microscope. Scale bar = 1μm.
