nosa bacterial pulmonary cystic fibrosis infections, adhere to pulmonary mucus and do not effectively reach sites of infections after drug administration. Previously, for nanoparticle formulations, the ability to encapsulate soluble APIs and control release had been limited to liposomal formulations; and while some liposomes can be loaded by a mechanism of transfer across the liposome bilayer and precipitation inside the liposome, this technique is not available for highly ionic API's such as the antibiotics considered herein. Therefore, the ion pairing approach presented herein provides a powerful new tool for encapsulation of API's that was not previously available.

[0051] Pharmaceutical companies can apply the disclosed technology to enhance drug-based therapies that are already in the market. Pharmaceutical companies can also apply this technology to enable drug therapies to satisfy clinical trial objectives that would be otherwise not met.

[0052] The controlled delivery of active therapeutic ingredients from nanocarriers can result in improved bioavailability, reduced toxicity, sustained activity, simplified dosing regimens, improved patient adherence, and enhanced overall efficacy [Solaro, R., F. Chiellini, and A. Battisti, Targeted Delivery of Protein Drugs by Nanocarriers. *Materials,* 2010. 3(3): p. 1928-1980.]. NC formulations (e.g., nanoparticles) can be used to target delivery, and then to release cargo at the desired site. NC formation through direct precipitation methods is attractive because these methods form NCs, e.g., rapidly form active pharmaceutical ingredient (API) nanoparticles, with high mass loadings in a scalable and continuous fashion, as in the case of Flash NanoPrecipitation (FNP) [Immordino, M. L., F. Dosio, and L. Cattel, Stealth liposomes: review of the basic science, rationale, and clinical applications, existing and potential. *International Journal of Nanomedicine,* 2006. 1(3): p. 297-315; Steichen, S. D., M. Caldorera-Moore, and N. A. Peppas, A review of current nanoparticle and targeting moieties for the delivery of cancer therapeutics. *European Journal of Pharmaceutical Sciences,* 2013. 48(3): p. 416-427.; Galindo-Rodriguez, S. A., et al., *Polymeric nanoparticles for oral delivery of drugs and vaccines: a critical evaluation of in vivo studies. Critical Reviews in Therapeutic Drug Carrier Systems,* 2005. 22(5): p. 419-464.]. In nanoprecipitation processes, APIs, e.g., hydrophobic APIs, may be dissolved in organic solvents and mixed with water as an antisolvent to induce precipitation and NC self-assembly. However, such direct precipitation methods are only feasible for water insoluble compounds (Log P>4), and cannot be used to encapsulate hydrophilic peptides and biologics into NC form. Water soluble biologics may be encapsulated through water-in-oil-in-water emulsion (W/O/W) or liposomal processes that require multiple steps, suffer from poor encapsulation efficiencies, or exhibit low drug mass loadings [Li, S.-D. and L. Huang, Pharmacokinetics and Biodistribution of Nanoparticles. *Molecular Pharmaceutics,* 2008. 5(4): p. 496-504; Owens Iii, D. E. and N. A. Peppas, Opsonization, biodistribution, and pharmacokinetics of polymeric nanoparticles. *International Journal of Pharmaceutics,* 2006. 307(1): p. 93-102; Pattni, B. S., V. V. Chupin, and V. P. Torchilin, New Developments in Liposomal Drug Delivery. *Chemical Reviews,* 2015. 115(19): p. 10938-10966.]. The new methods set forth herein, which efficiently encapsulate hydrophilic APIs into nanocarriers using direct water precipitation methods, can expand the nanocarrier API pharmacokinetic (PK) and/or pharmacodynamics (PD) properties that can be achieved.

[0053] Engineering of API water solubilities can enable new processing methods for nanocarrier encapsulation. This can be performed by reversible covalent conjugation of APIs with hydrophobic molecules, which results in the production of a hydrophobic prodrug. After encapsulation and delivery, covalent linkages are cleaved, thereby producing the original API for therapeutic activity. However, covalent modification of APIs results in the creation of a new molecular entity, which requires additional comprehensive, costly, and time-consuming testing for Food & Drug Administration (FDA) approval. API salt form engineering is an alternative route to tune API water solubility. In this process, charged functional groups on the API are ion-paired with a counter ion to produce a API with transiently altered solubilities. An advantage of salt form engineering is that resultant products are not considered new molecular entities, and do not require full FDA reapproval. However, in most instances, API salt forms are screened and engineered for enhanced water solubility. The alternative use of hydrophobic ion-pairs to create hydrophobic salt forms is a non-conventional method to increase API hydrophobicity. This technique has been used for the precipitation of weakly hydrophobic small molecules (c Log P=2-5) into nanocarriers, but not for highly water soluble (c Log P>0) and positively charged biologics. In this text, hydrophobic ion pairing is demonstrated to encapsulate highly soluble, positively charged antibiotic biologics, such as gentamycin (c Log P=−4.21) and polymyxin B (c Log P=−5.6). The ion pairs (IPs) are processed into stable nanocarriers through Flash NanoPrecipitation, a continuous and scalable water precipitation process. A priori, it might have been expected that such biologics could not be precipitated in water with hydrophobic ion-pairs, because of their high API water solubilities. The chemical identities of ion pairs, rules governing precipitation, and processing methods that can give rise to API salts with the desired solubilities for encapsulation were previously unknown.

[0054] Previously, ion pairing has been used to formulate nanoparticles from hydrophobic compounds. These are defined by having log P values greater than 1, 2, 3, 4, or 5 at neutral pH [Pinkerton, N. M., et al., Formation of stable nanocarriers by in situ ion pairing during block-copolymer directed rapid precipitation. Molecular pharmaceutics, 2013. 10(1): p. 319-328]. For example, Song et. al [Song, Y. H., et al., A novel in situ hydrophobic ion pairing (HIP) formulation strategy for clinical product selection of a nanoparticle drug delivery system. Journal of Controlled Release, 2016. 229: p. 106-119.] used ion pairing for AZD281, which had a log P of 2 at a neutral pH of 7. Another group of researchers has complexed proteins and polymeric charged species with oppositely charged polymeric compounds with high solubility. High solubility could mean log P values less than −2 and/or solubilities in aqueous media of over 10 mg/ml at pH=7. Patel and Guadana [Gaudana, R., et al., Design and evaluation of a novel nanoparticulate-based formulation encapsulating a HIP complex of lysozyme. Pharmaceutical development and technology, 2013. 18(3): p. 752-759; Patel, A., R. Gaudana, and A. K. Mitra, A novel approach for antibody Nanocarriers development through hydrophobic ion-pairing complexation. Journal of Microencapsulation, 2014. 31(6): p. 542-550] showed that soluble antibody or