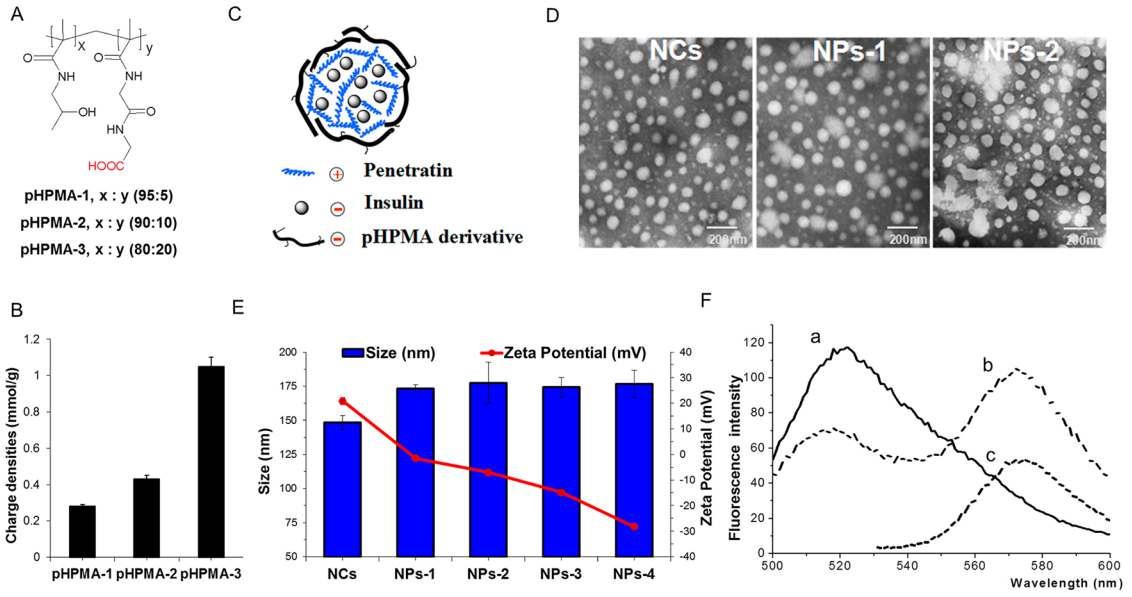

Figure 1. (A) Chemical structure of HPMA polymer derivatives with different ratio of MA-GG-OH monomer. (B) Charge densities of the three pHPMAs as measured by titration method. Data are means ± SD (n = 3). (C) Schematic diagram of NP, which possessed a penetratin/insulin nanocomplex core with sheddable pHPMA coating. (D) TEM images of NCs, NPs-1 and NPs-2. (E) Size and zeta potential of NCs and different NPs. Data are means ± SD (n = 3). (F) Emission spectrum of free F-insulin (a), NPs (b), and T-pHPMA (c) with excitation at 440 nm.

TABLE 1. Characterizations of NCs and NPs[a]

sample	pHPMA	concentration mg/mL	size (nm)	PDI	EE (%)	DL (%)
NCs	-	-	148.3 ± 5.1	0.31 ± 0.09	95.3 ± 1.6	65.6 ± 4.0
NPs-1	pHPMA-1	2	173.4 ± 2.7	0.22 ± 0.02	94.4 ± 1.7	48.5 ± 6.7
NPs-2	pHPMA-2	1	177.3 ± 15.2	0.24 ± 0.03	94.9 ± 1.1	52.6 ± 1.5
NPs-3	pHPMA-3	1	174.4 ± 7.2	0.26 ± 0.03	89.1 ± 3.5	49.9 ± 7.6
NPs-4	pHPMA-3	2	176.7 ± 9.8	0.24 ± 0.02	83.8 ± 4.4	44.4 ± 5.6

[a] Data are means ± SD (n = 3).

After the pHPMA coating, the all NPs possessed a negative zeta potential ranging from −1.47 to −28.2 mV. Lower surface charge was observed with NPs prepared with pHPMA of higher charge density (NPs-2 and NPs-3) or with higher amount of pHPMA in the formulation (NPs-4). The reversal of surface charge suggested the successful coating of anionic pHPMA on the outer surface of the NPs. The coating of pHPMA was further validated using a fluorescence resonance energy transfer (FRET) analysis.[27] The NPs were prepared with TRITC labeled HPMA polymers (T-pHPMA) and FITC labeled insulin (F-insulin), which formed a FRET pair. The emission intensity of F-insulin decreased at 520 nm and that of TRITC increased at 573 nm, which implied the energy transfer from donor to acceptor (Figure 1F). The FRET efficiency between two interacting partners was 38.8% and the FRET distance was calculated to be 5.7 nm, which again suggested the formation of pHPMA coated NPs. Moreover, the enzymatic stability of the insulin encapsulated in the particles was investigated using trypsin and α-chymotrypsin. All NPs exhibited better protection for loaded insulin against digestive enzyme compared with NCs or free insulin (Supporting Information Figures S7 and S8).

Mucus Permeation Ability of NPs. The mucus layer has evolved to protect the body with excellent ability to immobilize and remove cationic and hydrophobic molecules and particles.[10,28] The negative charges of carboxyl or sulfate groups on the mucin proteoglycans and the periodic hydrophobic globular regions along mucin strands allow efficient formation of multiple low-affinity adhesive interactions with the cationic and hydrophobic regions on the surfaces of foreign substances.[10] Since major types of proteins have both hydrophilic and hydrophobic region in their structures, and CPPs also possess strong polycationic character, the CPP−protein complex would exhibit high affinity with the mucin. We hypothesize that by concealing the nanocomplex beneath the negatively charged hydrophilic HPMA polymer, the particle could exhibit reduced interaction with the mucus layer. To test our hypothesis, we first evaluated the interaction of the particles with mucin by measuring the amount of particles−mucin aggregates formed in different concentration of mucin. As shown in Figure 2A, significant