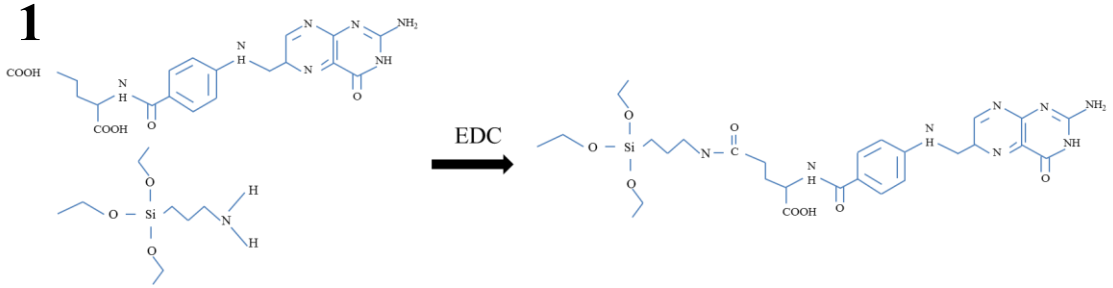
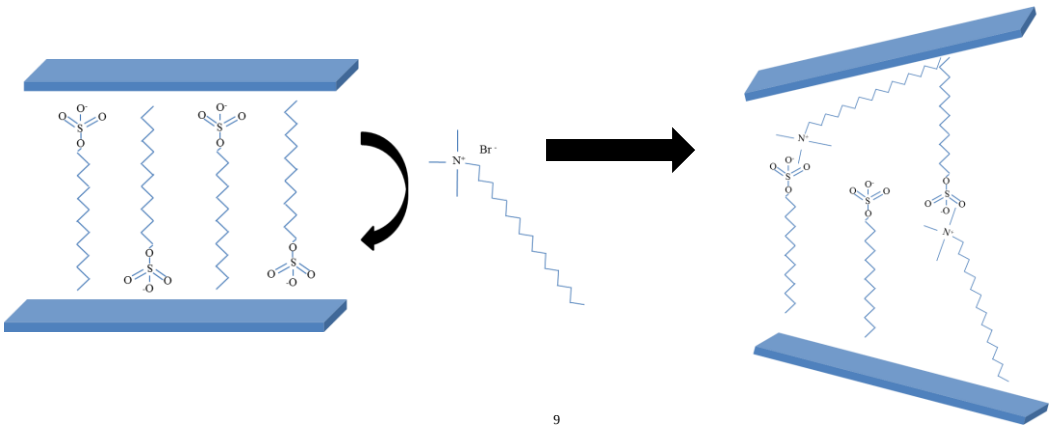
1



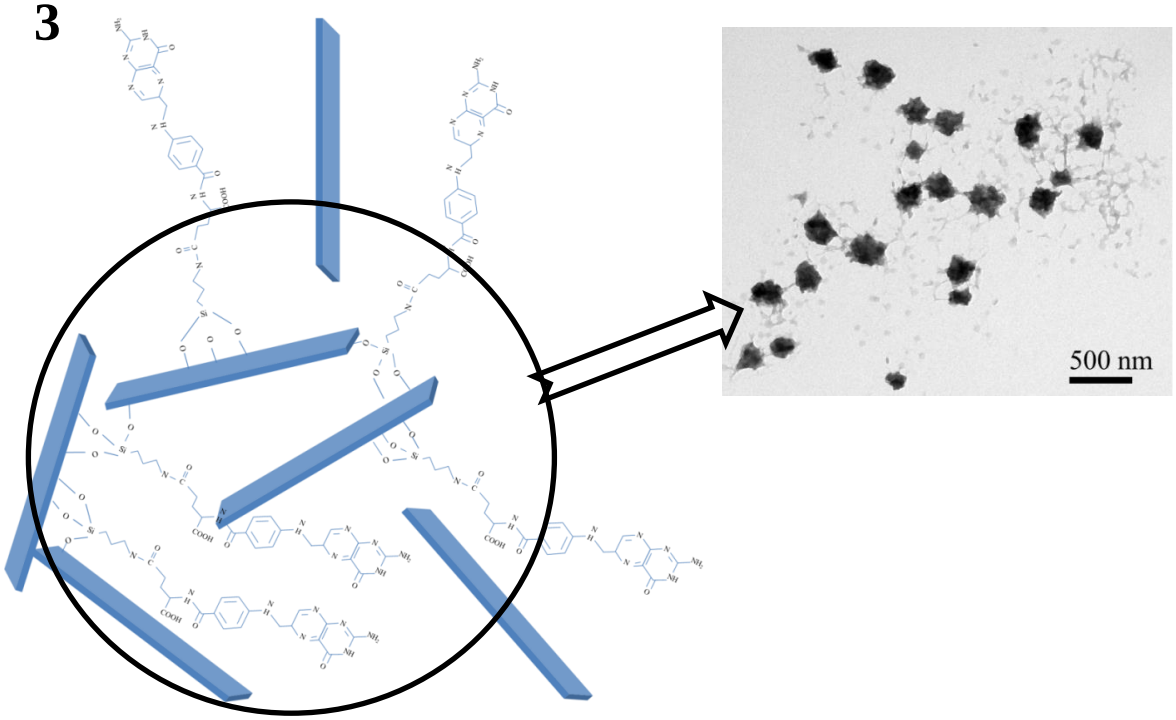
Folic acid and 3-APTES are covalently bonded (APTES-FA) in presence of EDC as a catalyst. (Although EDC was used as a catalyst to facilitate the reaction between the carboxyl group of FA and the amine group of APTES to form peptide bond, it was not possible to rule out the possibility of exchange between protic group in folate and the silyl ethers in APTES. However, with this happening, FA can also be bonded to LDH surface through APTES.)

2



CTAB extraction of SDS from LDH gallery: CTAB has high affinity towards SDS to form salt. The entrance of CTAB can further expand the gallery of LDH nanoparticles,

3

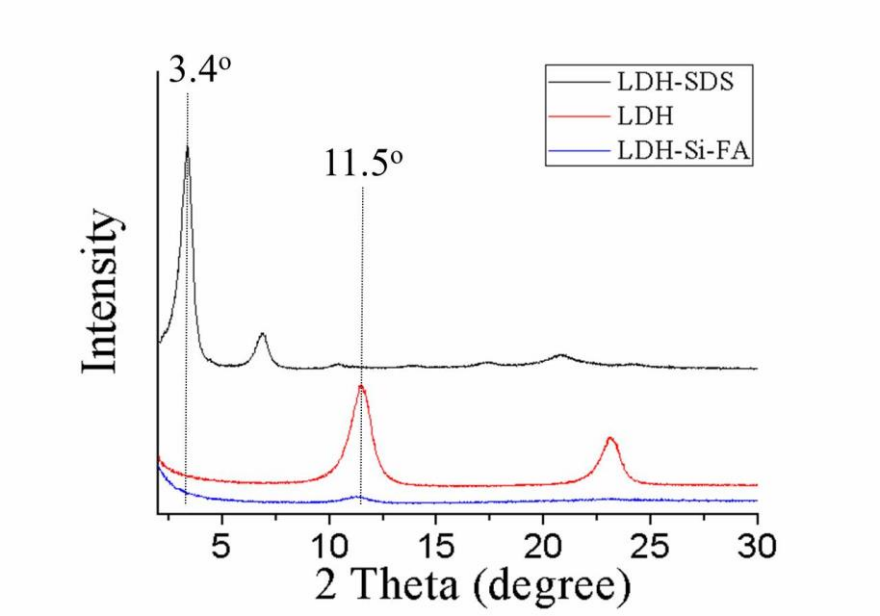


With strong ultrasonication, LDH-SDS nanoparticles defoliated and APTES-FA connected many LDH layers together and assembled to amorphous nanoparticles. The LDH nanoparticles which were not functionalized with APTES-FA restacked to layered structure as appeared in conventional LDH nanoparticles after drying.

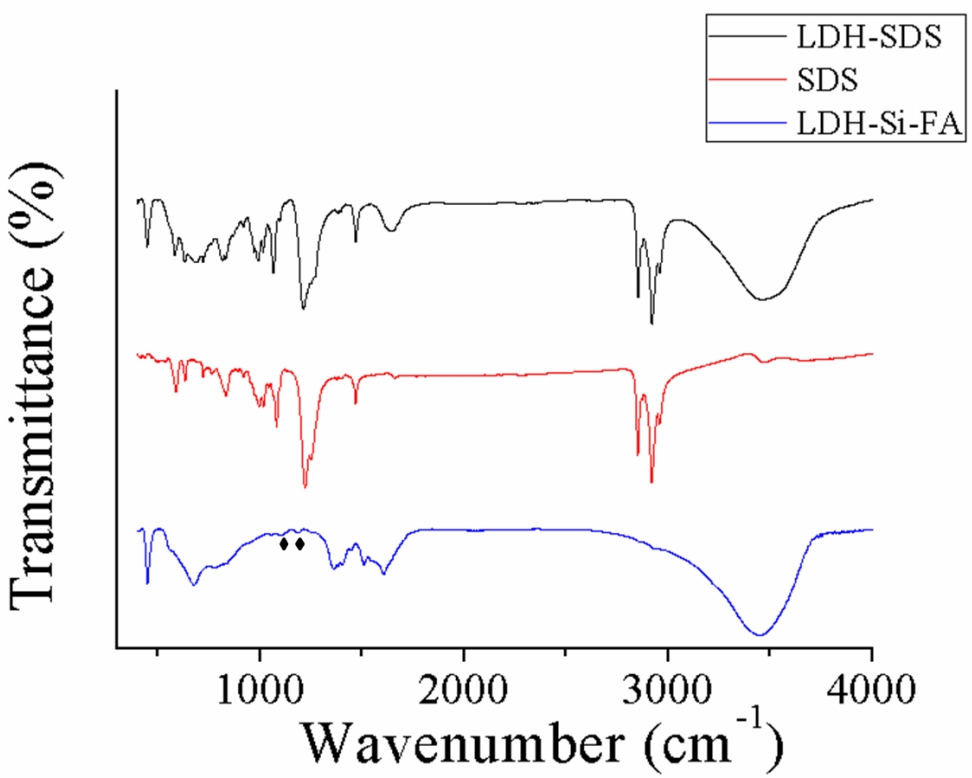
Supporting Scheme 1. Schematic illustration of the preparation of FA conjugated self-assembled LDH nanoparticles.

Supporting Table 1. Atomic compositions (%) of FA-Conjugated self-assembled LDH nanoparticles.

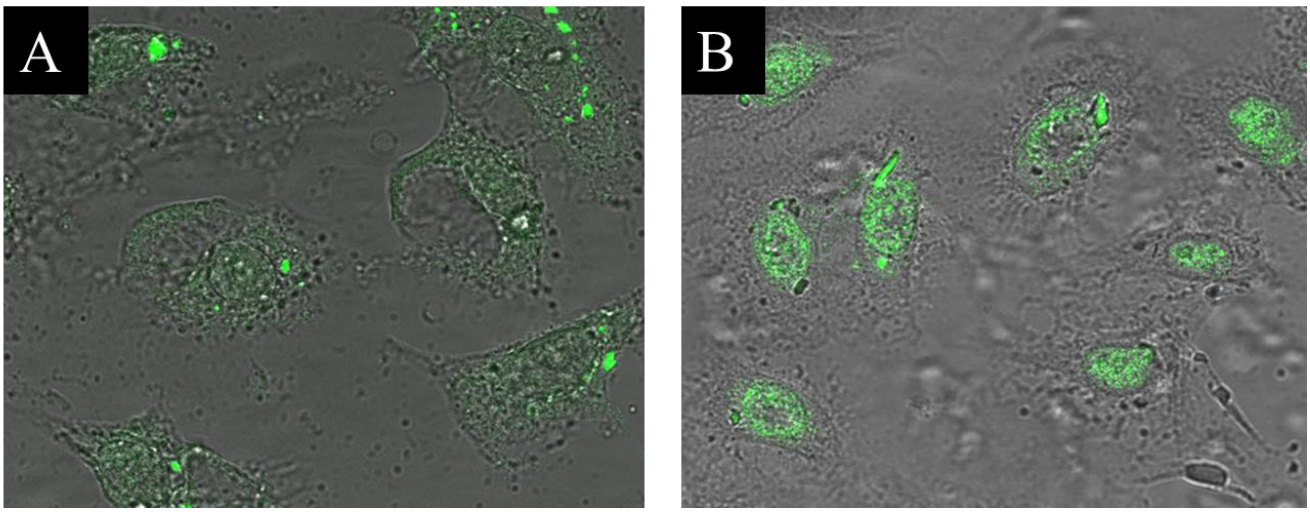
	Al	Mg	Si
Atomic Percentage (%)	**62.61**	**35.12**	**2.27**
Standard Deviation (±%)	**0.20**	**0.45**	**0.50**



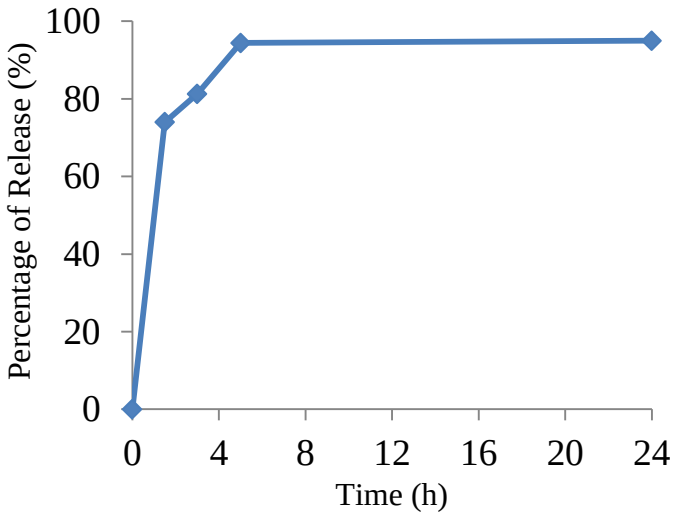
Supporting Figure 1. The powder X-ray diffraction patterns of LDH-SDS nanoparticles, pristine LDH nanoparticles and FA-Conjugated self-assembled LDH nanoparticles (LDH-Si-FA).



Supporting Figure 2. Fourier transform infrared spectroscopy of LDH-SDS nanoparticles, SDS molecules and FA-Conjugated self-assembled LDH nanoparticles (LDH-Si-FA).



Supporting Figure 3. Laser scanning confocal microscopy images of HeLa cells after incubating with fluorescein sodium salt cojugated LDH nanoparticles treatment for 14 hours



Supporting Figure 4. Release curve of MTX at pH 7.4 from FA conjugated self-assembled LDH nanoparticles. At pH 5, whole FA conjugated self-assembled LDH nanoparticles decomposed and all drug released.

Experimental Section

Preparation of LDH-SDS nanoparticles: 80 ml of 0.15 M NaOH (International Laboratory, USA) solution with 4 mmol of SDS (Acros) was mixed with 20 ml of solution containing 2.0 mmol of $MgCl_2$ (International Laboratory, USA) and 1.0 mmol of $AlCl_3$ (Sigma, USA) under vigorous stirring. The container was sealed and the solution was under stirring for 10 minutes. Next, the solution was centrifuged and washed once with water. The obtained slurry was dispersed in 80 ml of water and stirred at 80 ℃ for 24 hours in an airtight container with N_2 atmosphere. After heat treatment, the LDH-SDS particle was collected by centrifuge at 4000 rpm for 5 minutes and dried under vacuum.

Preparation of fluorescein sodium salt conjugated nanoparticles: 2 mg of fluorescein sodium salt (International Laboratory, USA) was mixed with 2 ml of (3-Aminopropyl) triethoxysilane (International Laboratory, USA) for 48 hours in presence of EDC (Acros) as a catalyst. 100 mg of LDH-SDS nanoparticles were further dried in vacuum for 30 minutes before next reaction. 7.5 ml of methylene chloride (Acros) was mixed with 0.4625g *N*-cetyl-*N*,*N*,*N*-trimethylammonium (CTAB) (Acros) at 40 ^{o}C to make CTAB fully dissolvable. Then, the prepared fluorescein sodium salt conjugated APTES and methylene chloride with CTAB solution were simultaneously added into a glass vial containing 100 mg of LDH-SDS nanoparticles. Subsequently, the solution was ultrasonicated for 30 minutes at around 40-45 ^{o}C. After sonication, the solution was kept stirring for 24 hours at around 40-50 ^{o}C. Finally, the prepared nanoparticles were collected by centrifugation and washed with methylene chloride, Millipore water and ethanol.

Preparation of FA conjugated nanoparticles: Firstly, 20mg of FA (Acros) was dissolved in 2ml of dimethyl sulfoxide (DMSO) (Acros) solution, and mixed with 50 µl of (3-Aminopropyl) triethoxysilane (International Laboratory, USA) for 48 hours in presence of EDC (Acros) as a catalyst. 100 mg of LDH-SDS nanoparticles were further dried in vacuum for 30 minutes before next reaction. 7.5 ml of methylene chloride (Acros) was mixed with 0.4625g *N*-cetyl-*N*,*N*,*N*-trimethylammonium (CTAB) (Acros) at 40 ^{o}C. Then, 410 µl of FA-Conjugated APTES and methylene chloride with CTAB solution were simultaneously added into a glass vial containing 100 mg of LDH-SDS nanoparticles.

The rest procedures were the same as those for preparing fluorescein sodium salt conjugated nanoparticles.

Characterization of nanoparticles: Zeta potential and size distribution were measured by Zetasizer (Malvern). TEM images were taken on Philips, Technai 12.

Drug Loading: the same volume of 2mg/ml of free MTX and 2mg/ml of FA-conjugate self-assembled LDH nanoparticles were mixed for 24 hours. After mixing MTX and FA-LDH, free drug was removed by centrifuging at 14,000 rpm for 10 minutes. After centrifugation, the free drug was in solution while drug loaded FA-LDH nanoparticles were precipitated. The amount of drug loading was calculated from the absorbance difference (370 nm) of MTX in the solution before and after mixing with FA-LDH nanoparticles. The loading capacity was calculated by the weight of loaded drug amount divided by that of the FA-LDH nanoparticles.

Laser scanning confocal microscopy: HeLa cell suspension was seeded to a sterile glass coverslip in a 35-mm tissue culture dish for 24 h. Then the cell culture medium was removed and a fresh medium containing 50 μg/ml of fluorescein sodium salt conjugated nanoparticles was added following by post-culture for 14 hours. Finally, after washing with PBS, the coverslip was mounted onto slides for confocal microscopy. Imaging was performed on a confocal microscope (Leica TCS SPE) with an excitation wavelength at 490 nm.

Cell viability measurement: HeLa cells were washed twice with PBS. Hela Cells suspended in DMEM (Gibco) (with 10% FBS (Gibco), 1% penicillin/streptomycin (Gibco)) were plated into 96-well plates (Corning) (100 μl DMEM; 1,500-3,000 cells per well). The cells were incubated at 37 °C for 24 hours before further treatment. Then, another 100μl of DMEM containing various concentrations of MTX (Acros), nanoparticles and MTX-loaded nanoparticles was added into 96-well plates for additional 48 and 72 hours incubation. After certain periods of incubation, the original medium in each well was removed. Subsequently, 180 μL of DMEM (without FBS) and 20 μL of MTT (Invitrogen) stock solution (5 mg/mL in PBS) were added and incubated for 4 hours. Then the medium containing MTT was completely removed, followed by adding 200 μL of DMSO (Acros) to each well. Cell viabilities were determined by reading the absorbance of the plates at 540 nm using a BioTek Powerwave XS microplate reader. The same protocol was used for measuring the viabilities of KB cells but with three different media: DMEM (Gibco), 1640 FA free (Gibco) and DMEM (Gibco) with 1mM FA (Acros).