Supporting Information

Blue shift of fluorescence emission reveals dsRNA-loading capacity of cationic nanocarriers

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Materials and characterisation

DAPI (2-(4-amidinophenyl)-1H-indole-6-carboxamidine) and polyethyleneimine (PEI, branched, 408727) were purchased from Sigma-Aldrich. Magnesium chloride hexahydrate, aluminium chloride hexahydrate, and sodium hydroxide were purchased from Chem-Supply Australia. 2b dsRNA was obtained from Genolution Inc. while DNA was obtained from GeneWorks.

All fluorescence measurements were conducted on an RF-5301PC Spectro fluorophotometer (SHIMADZU, Japan) using TrUView cuvette (Bio-Rad Laboratories, U.S.A) with excitation at 358 nm and emission between 400 and 600 nm. The excitation and emission slits were both set as 15 nm. Zeta potentials of PEI and LDH were tested by Nano ZS (Malvern Panalytical, U. K.). XRD pattern of LDH was obtained using a Bruker D8 Advance powder XRD with Cu *Kα* radiation and DIFFRAC. measurement centre v.6.5.0. The LDH particles were imaged by Hitachi HT-7700 (Hitachi TEM system v.02.29.15.54). Gel electrophoresis was conducted under 80 V for 30 min (PowerPac Universal Power Supply, Bio-Rad Laboratories, U.S.A) and imaged using ChemiDoc™ MP Imaging Systems (Bio-Rad Laboratories, U.S.A).

Methods

Sample preparation for fluorescence measurements

DAPI and dsRNA were mixed at a DAPI/dsRNA molar ratio of 0.05 and the calculation of agents' dosages was based on keeping the DAPI concentrations in all samples at 2.5 ng/μL, which was determined according to Fig. S1. The complete loading of dsRNA by PEI and LDH were determined as N/P ratio of 2 and LDH/RNA mass ratio of 3, respectively, by gel electrophoresis. The samples for fluorescence measurements were then prepared with the nanocarrier/dsRNA ratios at the values of 25%, 50%, 75%, and 100% of the complete loading capacities, i.e., N/P = 0.5, 1, 1.5, and 2 for PEI and LDH/dsRNA = 0.75, 1.5, 2.25, and 3 for LDH. In addition, the stepwise additions of cationic nanocarriers had extra sampling points with the doubled nanocarrier/dsRNA ratios, i.e., N/P = 4 for PEI and LDH/dsRNA = 6 for LDH. All samples were kept in lightproof condition. All tests were conducted with three replicas to confirm the consistency of the results.

LDH synthesis

Typically, a 20-mL solution containing 0.05 M $AlCl_3$ and 0.15 M $MgCl_2$ and a 20-mL solution containing 0.4 M NaOH were added simultaneously into a 70 mL bottle and stirred at 300 rpm for 60 min. The suspension was centrifuged with 5000 RCF for 5 min. The slurry was washed twice by MillQ water with same volume as the previous supernatants. The final slurry was mixed with MillQ water with same volume as the previous supernatants. The

mixture was shaken till homogeneous without visible aggregate and aged for 5 days under normal condition to obtain translucent LDH suspension. The concentration of LDH was determined by evaporating the suspension at 100 ˚C overnight and weighing the dry matter. Characterisation results in Fig. S3 confirmed that our product was LDH.

Stepwise addition of PEI/LDH into dsRNA-DAPI solution

Stepwise addition of cationic nanocarriers was assessed to confirm the re-use of the samples. PEI+(dsRNA-DAPI) at N/P ratio of 0.5 was prepared and tested by the fluorometer. Then, PEI stock suspension with high PEI concentration was added into PEI+(dsRNA-DAPI) to obtain PEI+(dsRNA-DAPI) suspension at N/P ratio of 1, which was hand-shaken and soon tested by the fluorometer. The addition of PEI stock solution to the same cuvette was repeated to increase the N/P ratio to 1.5, 2, and 4, which were all tested by the fluorometer. The PEI concentration in the stock suspension was much higher than the PEI suspension used for plotting Fig. 1 to minimise the volume change. The LDH-series of samples were prepared and tested in the same way. With stepwise addition, the consumption of dsRNA, which is the most expensive content in the formulation, can be kept at the minimal level to save the cost, no matter how many sampling points are in the protocol, favouring the industry need of quality administration.

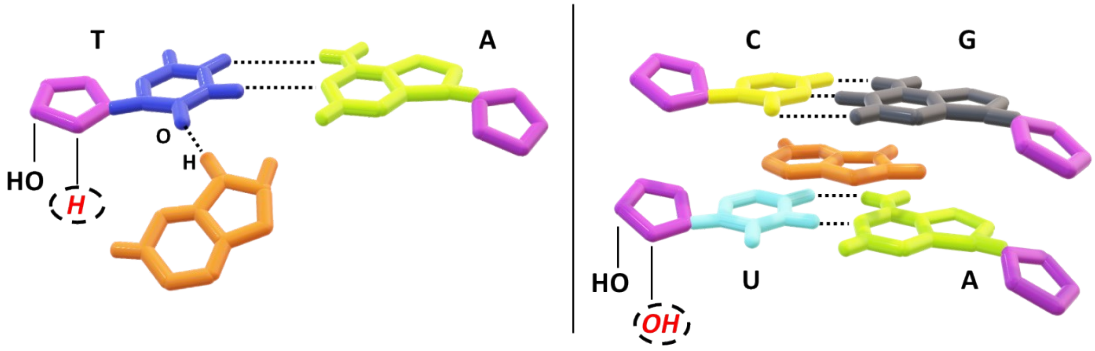

Fig. S1 Scheme of DAPI binding on (left) DNA and (right) RNA.

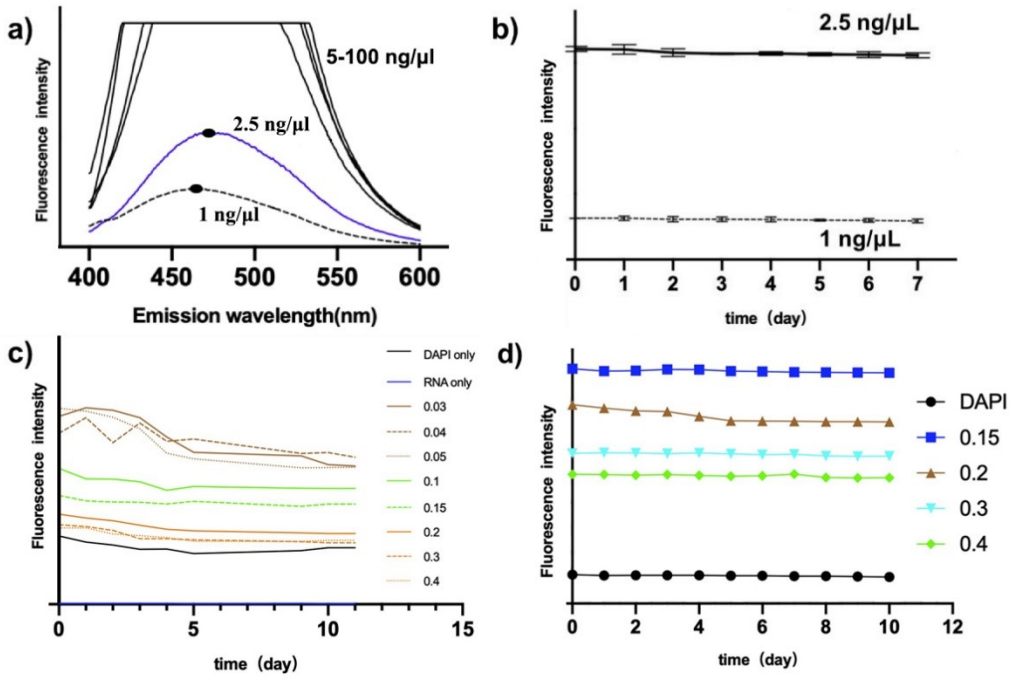

Fig. S2 Determination of a) working concentration of DAPI; b) stability of DAPI fluorescence; c) DAPI/dsRNA molar ratio; and d) DAPI/DNA molar ratio. The optimal working concentration of DAPI in the fluorescence samples was found to be 2.5 ng/µL as the emission fluorescence intensity from the higher ones exceeded the detection range of the facility. The fluorescence was stable for at least 7 days suggesting the results were reliable. Consequently, the DAPI/dsRNA and DAPI/DNA ratios were determined prior to the addition of cationic nanocarriers. DAPI/dsRNA = 0.03-0.05 and DAPI/DNA = 0.15 had the highest fluorescence intensity and good stability for at least 10 days. Thus DAPI/dsRNA = 0.05 and DAPI/DNA = 0.15 were chosen in our work.

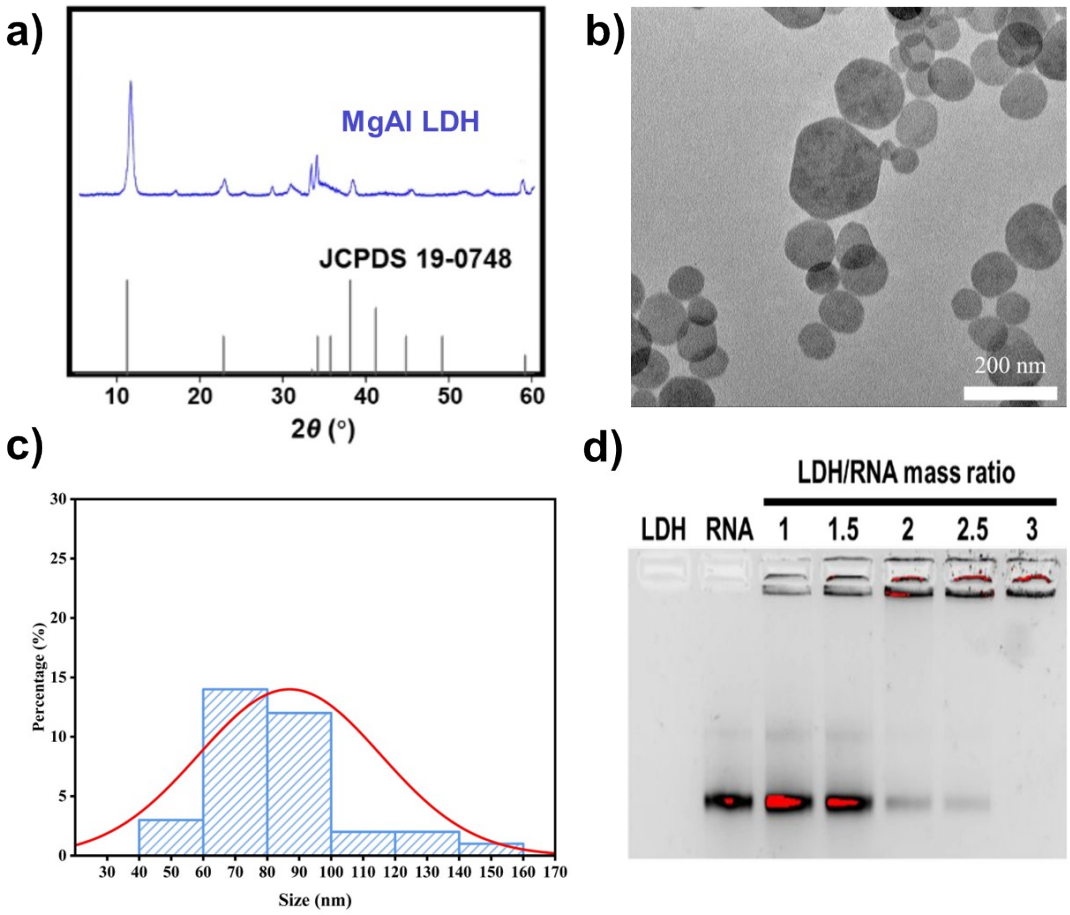

Fig. S3 Characterisation of layered double hydroxide (LDH): a) XRD pattern; b) TEM image; c) particle size distribution via DLS; and d) confirmation of dsRNA loading. The XRD pattern, TEM image, and particle size distribution were all aligned with the typical features of LDH. The dsRNA loading capacity was double checked with a different ratio interval to confirm that the loading at LDH/dsRNA mass ratio = 2.5 was still not complete.

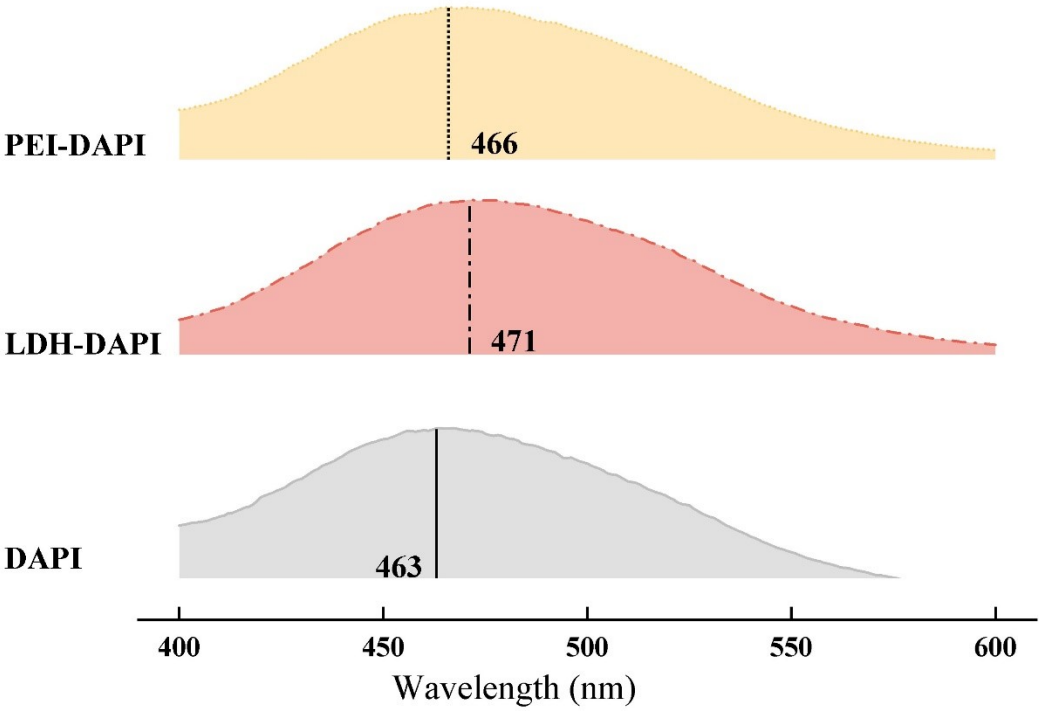

Fig. S4 Variation of emission wavelength after directly adding cationic nanocarriers to DAPI

Table S1 estimated time and cost of current and proposed method

Method	Equipment		Reagents and consumables		Time[b] (hour)
	Type	Cost[a] (kUSD$)	Type	Cost[b] (USD$)	
Gel electrophoresis	Power supply Imaging	~17	Agarose, TAE buffer, stain	5	2.5
HPLC	System Column Freeze dryer	~70	Mobile phase Trypsin	10	13
UV absorbance	UV-Vis Centrifuge	~24	RNase PBS	<1	2.5
Quant-iT	Plate reader	~13	Assay kit Plate	15	2
Fluorescence correction	Plate reader	~13	Stain plate	10	2
Fluorescence emission (stepwise)	Fluorophotometer	~10	DAPI	<1	1.5

[a] The costs of equipment were estimated according to those available in the authors' institutes. In detail, the power supply and imaging system were Mini-Sub Cell, PowerPac Basic and ChemiDoc™ MP, respectively, for gel electrophoresis; the HPLC system and column were Agilent 1260 and Discovery C18, respectively while the freeze dryer was LyoQuest; the facilities of UV absorbence were NanoDrop 1000 and Sorvall ST 8 centrifuge; the plate reader was Tecan M1000.

[b] The costs and time were estimated using a 6-sample test, since one typical test of electrophoresis commonly uses a 8-well gel with 2 wells for pure RNA and pure nanocarrier as the controls and 6 wells for samples. Only the specific reagents and consumables were counted. If there are more sampling points, the advantage on the cost and operational time of our fluorescence emission method will be more significant.

The consumption of dsRNA and nanocarriers of our proposed stepwise method was the lowest among the listed ones. All other methods need to consume isolated sample for each sampling point. Taking a 6-sample test again as an example, the methods using isolated samples would consume 7 parts of dsRNA and sum of nanocarriers. By contrast, the method of stepwise fluorescence emission would consume 1 part of dsRNA and the nanocarrier only at the highest carrier/dsRNA ratio.

Table S2. zeta potentials of PEI at different pH and LDH

pH	PEI	LDH
4	41.3 ± 3.2 mV	--
7	33.2 ± 3.8 mV	23.3 ± 0.8 mV
10	3.5 ± 0.5 mV	--