

Supporting Information

Artesunate Drug-loaded 2D Nano-shuttle Landing on RBCs Infected with Malaria Parasites

Ji-Yeong Kim,^a Hyun-Il Shin,^b Sang-Eun Lee,^b Huiyan Piao,^c N. Sanoj Rejinold,^c Goeun Choi,^{**c,d,e} Jin-Ho Choy^{*c,f,g}

- a. *Department of Chemistry and Nanoscience, Ewha Womans University, Seoul 03760, Republic of Korea*
- b. *Division of Vectors and Parasitic Diseases, Center for Laboratory Control of Infectious Disease, Korea Centers for Disease Control and Prevention, Chungbuk 28159, South Korea*
- c. *Intelligent Nanohybrid Materials Laboratory (INML), Institute of Tissue Regeneration Engineering (ITREN), Dankook University, Cheonan 31116, Republic of Korea*
- d. *College of Science and Technology, Dankook University, Cheonan 31116, Republic of Korea*
- e. *Department of Nanobiomedical Science and BK21 PLUS NBM Global Research Center for Regenerative Medicine, Dankook University, Cheonan 31116, Republic of Korea*
- f. *Department of Pre-medical Course, College of Medicine, Dankook University, Cheonan 31116, Republic of Korea*
- g. *International Research Frontier Initiative (IRFI), Institute of Innovative Research, Tokyo Institute of Technology, Yokohama 226-8503, Japan*

* corresponding author(s) E-mail: jhchoy@dankook.ac.kr and goeun.choi@dankook.ac.kr

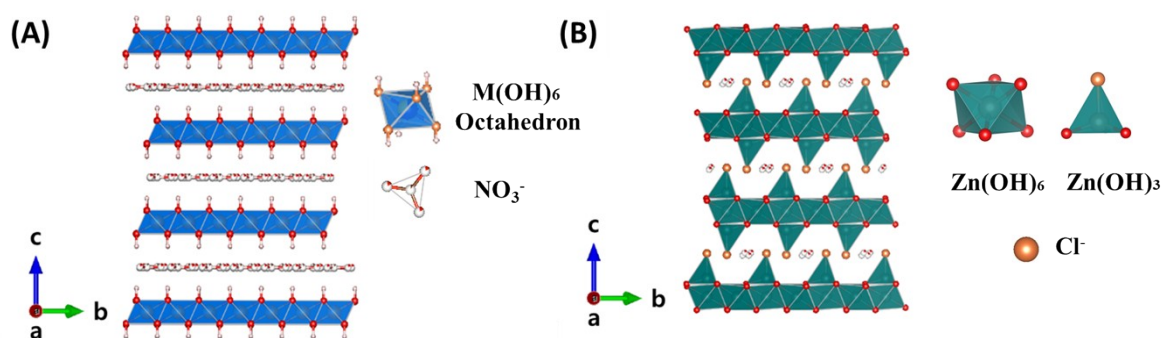


Fig. S1. Structures of **(A)** LDH and **(B)** ZBS.

The LDH consists of positively charged hydroxide layers and exchangeable interlayer anions with lamellar structure, and its general formula can be represented as $[M^{2+}_{1-x}M^{3+}_x(OH)_2]^{x+}(A^{n-})_{x/n} \cdot mH_2O$, where M^{2+} , M^{3+} and A^{n-} are divalent metal cations, trivalent ones, and interlayer anions ($M^{2+} = Mg^{2+}$, Zn^{2+} , Ni^{2+} , Ca^{2+} ..; $M^{3+} = Al^{3+}$, Fe^{3+} ..; $A^{n-} = CO_3^{2-}$, NO_3^- , Cl^- , SO_4^{2-} ..) respectively. The structure of LDH can be derived from that of brucite, $Mg(OH)_2$, in which $Mg(OH)_6$ octahedrons are bound one another by sharing their edges to form an infinite magnesium hydroxide layer, and the resulting layers are self-assembled along the crystallographic c-axis to build multi-layers by hydrogen bonding. In the LDH structure, however, divalent Mg ions are partially substituted by trivalent cations, i.e., Al^{3+} , to induce positive layer charge, as shown in the chemical formula above. To compensate for the layer charge generated, interlayer anions could be stabilized in the gallery space of LDH, so that the charge neutrality condition of the LDH lattice could be satisfied.

The HDS is a family of layered hydroxides represented by the general formula of $[M^{2+}(OH)_2]_x(A^{n-})_{x/n} \cdot mH_2O$, where M^{2+} represents divalent metal cations ($M^{2+} = Mg^{2+}$, Zn^{2+} , Ni^{2+} , Cu^{2+} ..) and A^{n-} represents interlayer anions ($A^{n-} = CO_3^{2-}$, NO_3^- , Cl^- , SO_4^{2-} ..), respectively. ZBS with a chemical formula of $[Zn_5(OH)_8Cl_2 \cdot 2H_2O]$ is an example of HDS. In contrast to LDH, metal vacancies in the brucite-like layer are present in the ZBS structure, where coordinatively unsaturated $Zn(OH)_3$ units are formed up and down sides of octahedral vacant sites. Therefore, its basal plane is not flat but protruded with those units to have uneven structures up and down sides of brucite-like ZBS layers and spontaneously coordinated with interlayer water molecules solvated. The presence of coordinatively unsaturated $Zn(OH)_3$ units directed to interlayer space allows ZBS layers to strongly interact with negatively charged guest

molecules as ligands.

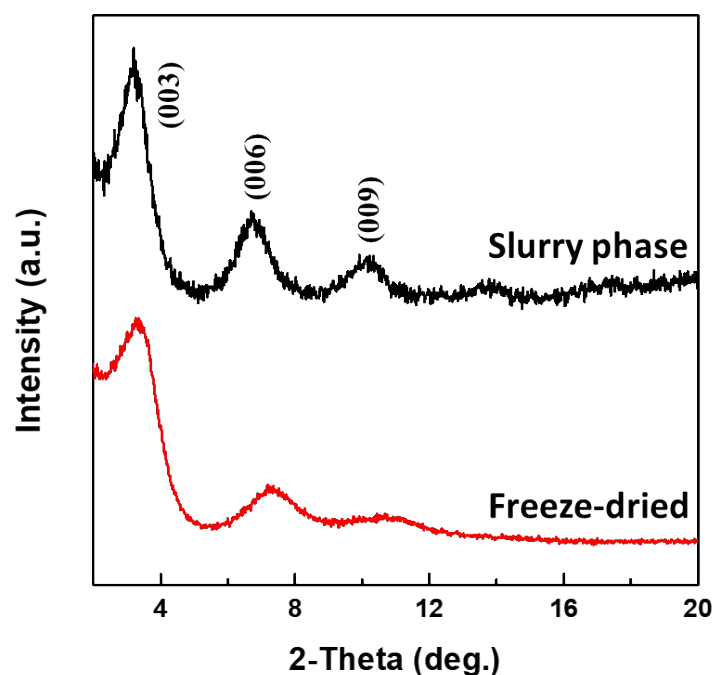


Fig. S2. X-ray diffraction patterns of LDH-AS⁻ freeze-dried and slurry phase.

As shown in Fig. S2, the d-spacing of the freeze-dried phase (24.2 Å) was determined to be slightly smaller than that of the slurry phase (26.7 Å) due to the deintercalation of interlayer water molecules after the drying process.

Considering the layer thickness of the ZBS layer (7.4 Å) and the LDH layer (4.8 Å), the gallery heights of ZBS-AS⁻ and LDH-AS⁻ were determined to be 20.1 Å and 19.4 Å, respectively, which were larger than the longitudinal and lateral molecular dimensions of AS⁰ (14.4 Å X 8.1 Å X 7.3 Å, calculated from Chem draw[®] ultra). Therefore, it was estimated that AS⁰ molecules were stabilized in a tilted double layer arrangement. To explain the interlayer structure, the steric limitations between the intercalated AS⁰ and charged sites of LDH and ZBS layers were examined. The equivalent area (A_e) of LDH and ZBS lattice and the area demand (A_c) of intercalated molecules, where A_e represents the area required for unit charge on the ZBS sheet and A_c represents the area demand for a single molecule with unit charge. In general, A_e for layered materials can be calculated from the equation of $A_e = ab \cdot \sin\gamma / (2\xi)$, where a , b , and γ are lattice parameters and ξ is the layer charge per formula unit. A_e for the ZBS phase is

estimated to be 8.6 \AA^2 , with lattice parameters of $a= 6.23 \text{ \AA}$, $b=5.51 \text{ \AA}$ and $\gamma = 90^\circ$. A_e for the LDH phase is determined to be 12.3 \AA^2 , with the lattice parameters of $a= b=3.07 \text{ \AA}$, $\gamma = 120^\circ$ and $1/3 e^-$ per unit cell. The area demand (A_c) of AS^- is 59.2 \AA^2 ($8.1 \text{ \AA} \times 7.3 \text{ \AA}$). Because A_c is larger than $2A_e$, the intercalated AS^- molecules have a tilted bilayer arrangement in both ZBS and LDH layers to avoid steric hindrance.

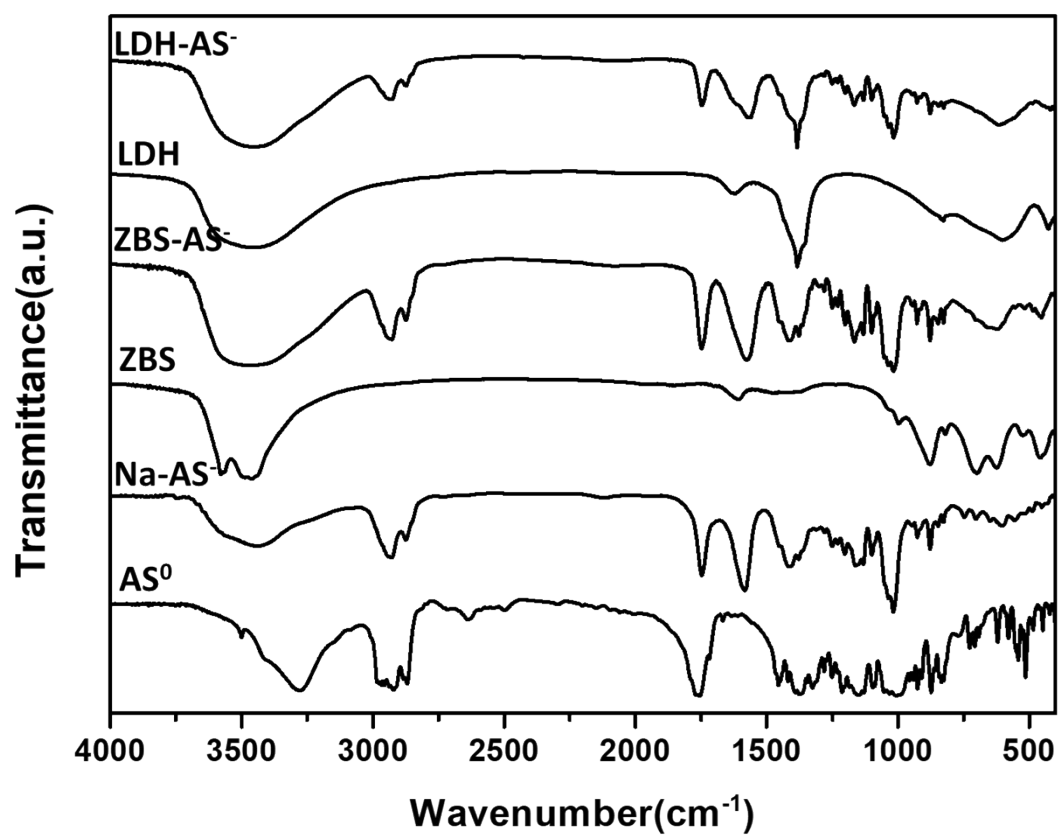


Fig. S3. FT-IR spectra of intact AS⁰, Na-AS⁻, pristine ZBS, ZBS-AS⁻, pristine LDH and LDH-AS⁻.

Table S1. FT-IR peaks and corresponding functional groups.

	Functional group	Wavenumber (cm ⁻¹)
AS ⁰ Na-AS ⁻	O-H vibration	3300
	C-H vibration	2800-3000
	C-O vibration	1026
	-O-O-C stretching in boat/twist vibration	870
	C=O vibration of ester and carboxylic acid	1750
Pristine ZBS	O-H stretching	3400-3500
	H ₂ O bending	1640
	M-O, O-M-O lattice vibration	866, 628, 453
Pristine LDH	O-H stretching	3400-3500
	H ₂ O bending	1640
	M-O, O-M-O lattice vibration (LDH)	830, 601, 425
	NO ₃ ⁻ band	1380
ZBS-AS ⁰ LDH-AS ⁰ Na-AS ⁻	COO ⁻ asymmetric / symmetric stretching	1578, 1414

The FT-IR spectra of intact AS⁰, Na-AS⁻, pristine ZBS, ZBS-AS⁻, pristine LDH and LDH-AS⁻ are shown in Fig. S3. In the intact AS⁰ and Na-AS⁻ spectra, the characteristic bands of AS⁰ molecules at 3300 cm⁻¹, 2800-3000 cm⁻¹, 1026 cm⁻¹ and 870 cm⁻¹ were assigned as $\nu(\text{O-H})$, $\nu(\text{C-H})$, $\nu(\text{C-O})$ vibration and $\nu(-\text{O-O-C})$ stretching in boat/twist form vibration. The broad absorption band at 1750 cm⁻¹ corresponded to the $\nu(\text{C=O})$ vibration from ester and carboxylic acid.¹ Also, in the Na-AS⁻ spectra, the COO⁻ asymmetric and symmetric stretching bands at 1570 and 1420 cm⁻¹ appeared due to the deprotonated COOH of AS⁰ to Na-AS⁻. For the pristine ZBS, a broad peak at 3400-3500 cm⁻¹ was attributed to $\nu(\text{O-H})$ stretching vibration. H₂O bending was shown at 1640 cm⁻¹. The lattice vibrations of ZBS sheets were observed at 850 to 600 cm⁻¹ and 440 cm⁻¹ due to the $\nu(\text{M-O})$ and $\nu(\text{O-M-O})$ vibrations, respectively. In the pristine LDH, the characteristic bands at the 3500 cm⁻¹, 1640 cm⁻¹ and 1380 cm⁻¹ were assigned to the $\nu(\text{O-H})$ vibration of the lattice and H₂O bending and NO₃⁻ band.

Both the ZBS-AS⁻ and LDH-AS⁻ spectra showed that the COO⁻ asymmetric and symmetric stretching bands at 1570 and 1420 cm⁻¹ appeared due to the deprotonated COOH of AS⁰ in the ZBS lattice. In ZBS-AS⁻ and LDH-AS⁻, all the characteristic bands from Na-AS⁻ and ZBS and LDH lattice were observed due to the encapsulation of deprotonated AS⁰ molecules without any degradation.

Table S2. Chemical formulas and artesunate contents.

	Chemical formula ^{[a], [b]}	Contents of AS ^{-[a]}
Pristine ZBS	$Zn_5(OH)_8Cl_2 \cdot 0.9H_2O$	-
ZBS-AS ⁻	$Zn_5(OH)_8(C_{19}H_{27}O_8)_{1.1}Cl_{0.9} \cdot 1.9H_2O$	43.6 ± 0.6 wt. %
Pristine LDH	$Zn_{0.755}Al_{0.245}(OH)_2(NO_3)_{0.245} \cdot 0.1H_2O$	-
LDH-AS ⁻	$Zn_{0.755}Al_{0.245}(OH)_2(C_{19}H_{27}O_8)_{0.163}(NO_3)_{0.082} \cdot 0.6H_2O$	36.7 ± 0.2 wt. %

calculated from HPLC^[a], ICP and CHN^[b] analyses (Table S3 and Table S4)

Table S3. ICP results of pristine LDH.

	Zn	Al
1st	293.72	39.94
2nd	291.75	38.24
Ave.	292.73 ± 0.98	39.09 ± 0.85
Nominal value	292.00	39.00

Table S4. CHN result of AS⁻ nanohybrids.

	LDH-AS ⁻				ZBS-AS ⁻			
	1st	2nd	3rd	AVE.	1st	2nd	3rd	AVE.
C	25.789	24.816	25.835	25.835±0.332	27.108	27.750	27.762	27.540±0.216
H	4.478	4.361	4.302	4.380±0.052	4.396	4.476	4.432	4.435±0.023
N	0.656	0.732	0.711	0.700±0.023	-	-	-	-

According to the HPLC and elemental analyses, the loading content of AS⁻ in ZBS-AS⁻ and LDH-AS⁻ was determined to be 43.6 ± 0.6 wt.% and 36.7 ± 0.2 wt.%, respectively. The chemical formulas estimated from the above results were as follows: $Zn_5(OH)_8(C_{19}H_{27}O_8)_{1.1}Cl_{0.9} \cdot 1.9H_2O$ and $Zn_{0.755}Al_{0.245}(OH)_2(C_{19}H_{27}O_8)_{0.163}(NO_3)_{0.082} \cdot 0.6H_2O$. The chemical compositions of the pristine ZBS and LDH were determined to be $Zn_5(OH)_8Cl_2 \cdot 0.9H_2O$ and $Zn_{0.755}Al_{0.245}(OH)_2(NO_3)_{0.245} \cdot 0.1H_2O$, respectively, as shown in Table S2.

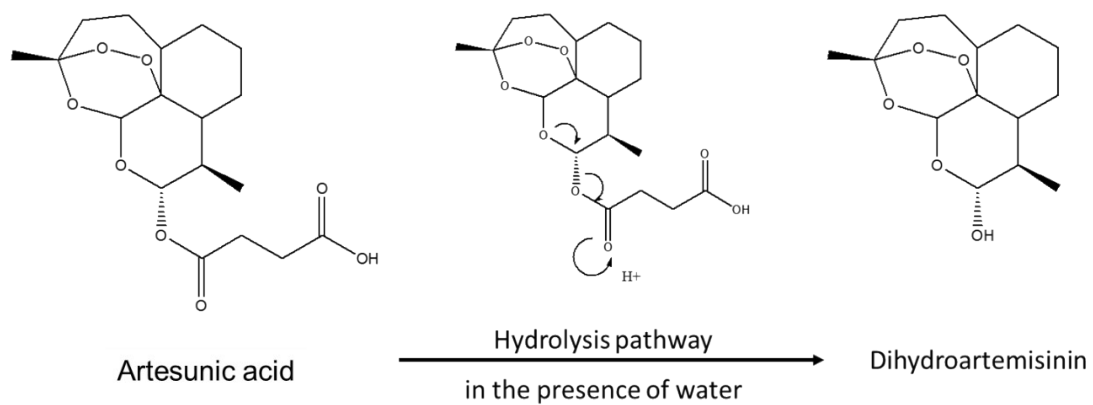


Fig. S4. Hydrolysis pathway of artesunic acid in the presence of water.

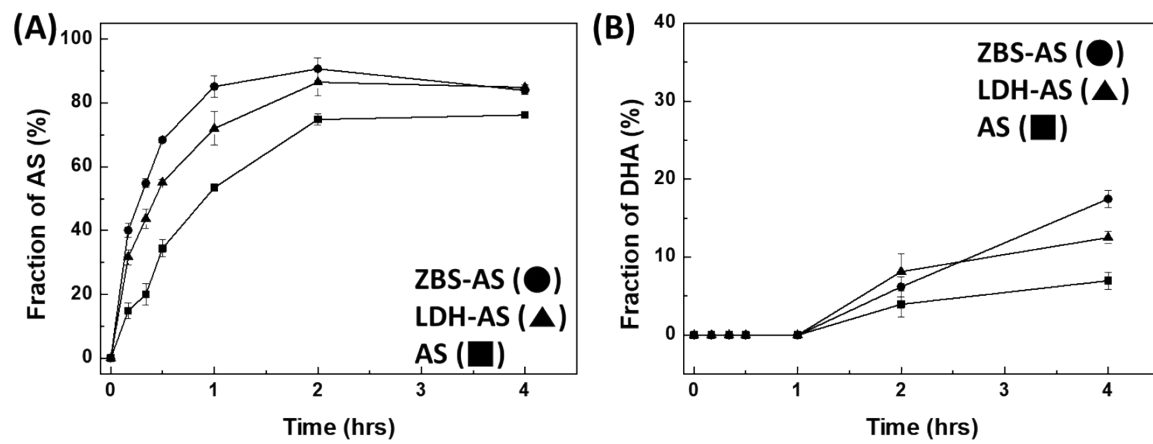


Fig. S5. (A) Release fraction and **(B)** its metabolite, DHA, from intact AS⁰ (■), LDH-AS⁻ (▲) and ZBS-AS⁻ (●).

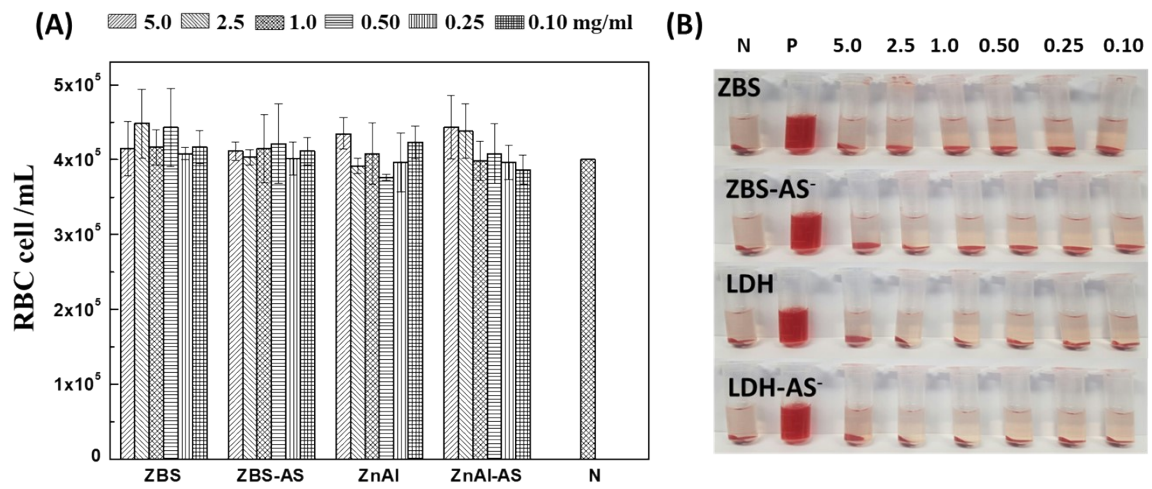


Fig. S6. (A) Cell counting assay of RBCs after haemolysis with 2D inorganic carrier and AS⁻ nanohybrids. **(B)** Photograph haemolysis samples for 2D inorganic carrier and AS⁻ nanohybrids. (The negative and positive controls are the same as indicated in the Fig.)

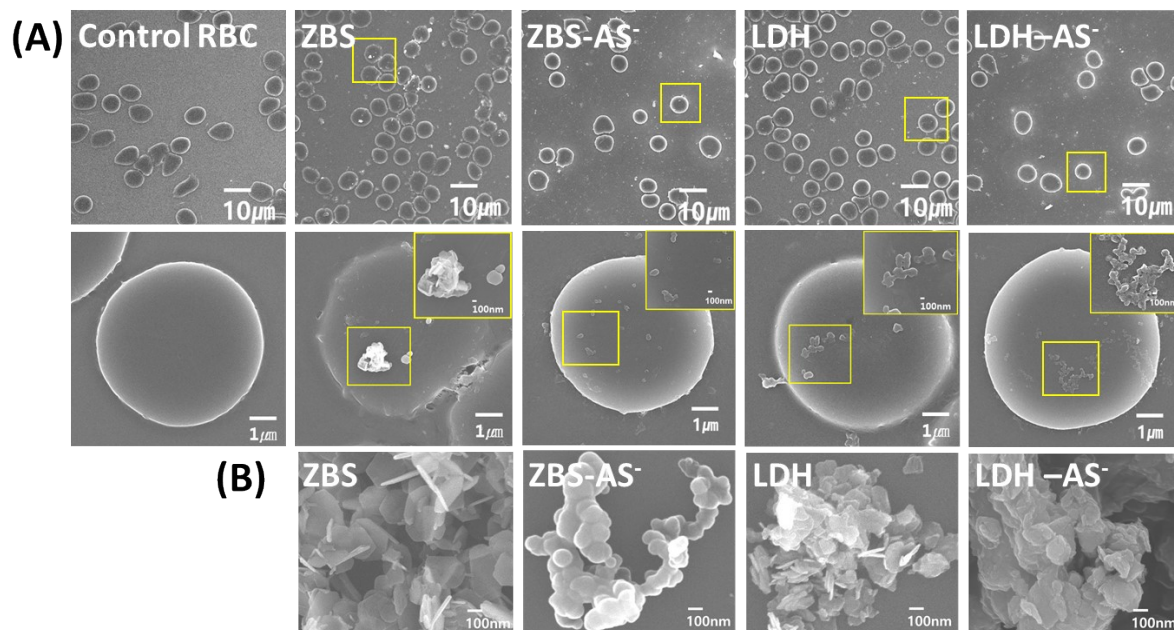


Fig. S7. FE-SEM images of **(A)** RBCs only, 2D inorganic carriers and AS⁻ nanohybrids on RBCs after haemolysis assay, and **(B)** 2D nanocarriers and AS⁻ nanohybrid drugs only.

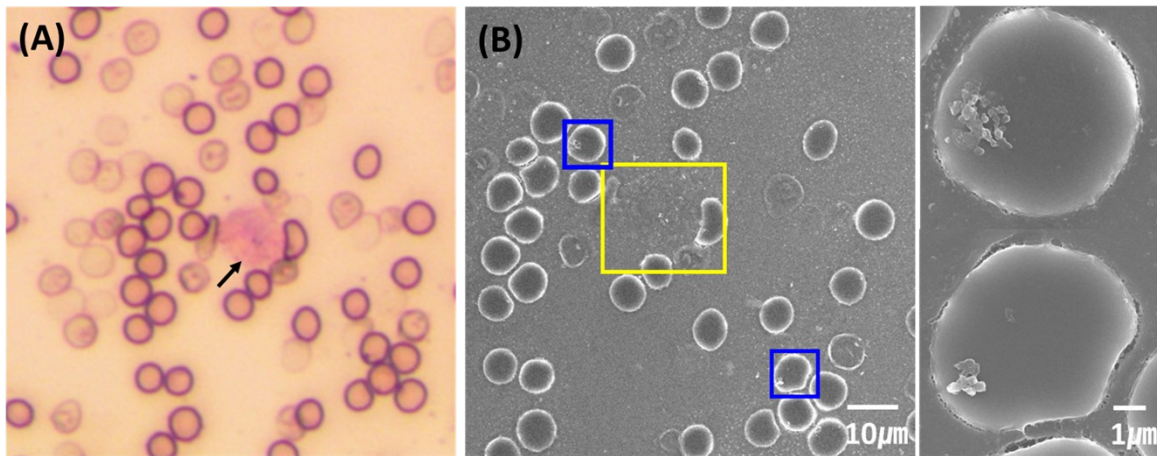


Fig. S8. (A) optical microscopic image of whole blood with nanohybrids and (B) SEM images of the same sample. ((A) The arrow is white blood cell (WBC) and the others are red blood cell (RBC). (B) yellow box is WBC and blue boxes are enlarged in the right section)

Table S5. zeta-potentials and size of 2D inorganic carrier and AS⁻nanohybrids.

	Zeta-potential (mV)	Size (nm)
Pristine ZBS	+ 27.4 ± 0.8	228.7 ± 12.9
ZBS-AS ⁻	+ 23.4 ± 0.3	214.6 ± 13.3
Pristine LDH	+ 45.0 ± 0.2	175.1 ± 14.7
LDH-AS ⁻	+ 25.5 ± 0.4	164.2 ± 12.5

For the LDH-AS⁻ system, the zeta-potential of LDH-AS⁻ (25.5 mV) was significantly reduced after the intercalation of negatively charged AS⁻ molecules into LDH (45.0 mV), due to the ionic bonding interaction between host LDH and guest AS⁻. In the case of ZBS-AS⁻, however, anionic AS⁻ ligand molecules were conjugated on the coordinatively unsaturated Zn(OH)₃ units in the interlayer space of ZBS as well demonstrated in Fig. S1. And therefore, the zeta-potential of ZBS-AS⁻ (23.4 mV) was changed little upon intercalation of AS⁰ into ZBS (27.4 mV) surely due to the coordinative bonding interaction.

Reference

- 1 J. Y. Kim, J. H. Yang, J. H. Lee, G. Choi, D. H. Park, M. R. Jo, S. J. Choi and J. H. Choy, *Chem. - An Asian J.*, 2015, **10**, 2264–2271.