Electronic Supplementary Material (ESI) for ChemComm.
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Supplementary Information

A rapid-response fluorescent probe for sensitive and selective detection of human albumin in plasma and cell culture supernatants†

Yi-Ru Wang,[a,b,e] Lei Feng,[c,e] Liang Xu,[d,e] Yan Li,[a] Dan-Dan Wang,[a] Jie Hou,[b] Kun Zhou,[a] Qiang Jin,[b] Guang-Bo Ge,*[a,c] Jing-Nan Cui*[c] and Ling Yang*[a]

[a] *Lab of Pharmaceutical Resource Discovery, Dalian Institute of Chemical Physics, Chinese Academy of Sciences, Dalian, China. E-mail: geguangbo@dicp.ac.cn & ylingdicp@gmail.com*

[b] *Dalian Medical University, Dalian 116044, China.*

[c] *State Key Laboratory of Fine Chemicals, Dalian University of Technology, Dalian, 116024, China. E-mail: jncui@dlut.edu.cn*

[d] *School of Chemistry, Dalian University of Technology, Dalian, China*

[e] YRW, LF and LX contributed equally to this work.

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Chemicals and Reagents

Human serum albumin (HA), fibrinogen, globulins, hemoglobin, haptoglobin, transferrin, myoglobin, pepsin, α-chymotrypsin, trypsin, carbonic anhydrase, α-macroglobulin, c-reactive protein, acetylcholinesterase, butyrylcholinesterase, lysozyme, lipase protease k, α- acid glycoprotein, immunoglobulin G (IgG), Dithionthreitol (DTT), iodoacetamide (IAA), formic acid (FA) were obtained from Sigma-Aldrich (St. Louis, MO, USA). cDNA-expressed recombinant human carboxylesterase 1 (hCE1) were obtained from BD Biosciences (MA, USA). Bis-p-nitrophenyl phosphate (BNPP), fluorescein diacetate (FD) and fluorescein (F) were purchased from TCI (Tokyo, Japan). HEPES was purchased from Beijing solarbio science&technology co., ltd. (Beijing, China). HepG2 cells were provided by Cell bank (Shanghai, China). Minimum essential medium was obtained from Hyclone Company. Human plasma was obtained from 28 healthy volunteers. All human proteins were stored at -20 ºC until use. A stock solution of **ACDM** (2 mM) was prepared in DMSO and stored at -80 °C for future using. Millipore water (Millipore, USA) was used throughout. All other reagents, fine chemicals and LC solvents with the highest grade commercially available were obtained from J&K Chemical Ltd. (China) and Tedia (USA).

Ethical approval and live subject statement

All experiments were performed in compliance with the relevant laws and institutional guidelines. Ethical approval was given by the medical ethics committee of The First Affiliated Hospital of Dalian Medical University, Dalian, China, with the reference number of LCKY2015-12 which was approved on 2nd June, 2015. Collection of human plasma from both healthy and sepsis patients for quantification of serum proteins including human serum albumin has been approved by this committee. Furthermore, the informed consent was obtained from everyone participated in this project.

Instruments

^{1}H NMR, ^{13}C NMR and FTIR spectra were recorded using Bruker Avance II (400 MHz) spectrometer with chemical shifts reported as ppm (in DMSO-d_6; TMS as internal standard). Accurate mass detection was measured on Hybrid Ion Trap-Orbitrap Mass Spectrometer

(LTQ Orbitrap XL, Thermo). Absorption spectra and fluorescence emission/excitation spectra were measured on Synergy H1 Hybrid Multi-Mode Microplate Reader (BioTek) (hereinafter we call this instrument H1). The trypsin digest was purified with a SPE-C18 solid-phase extraction column (F&H new material Technology Corp., Guangzhou, China). Chromatographic separation was performed on an ACCHROM C18HC column (150 mm× 2.1 mm, 5 μm, Acchrom Technology Corp., Beijing, China) using an ACQUITY™ UPLC system equipped with a binary pump, a vacuum degasser and an auto-sampler (Waters Corp., Milford, MA, USA). The mass spectrometry detection was performed on an AB Sciex QTrap® 5500 system with a Turbo VTM source (Framingham, MA, USA)

Synthesis and structural characterization of ACDM

121 mg (1 mmol) of 4-aminobenzaldehyde, 219 mg TCF (1.1 mmol, 1.1 equiv) and 85 mg ammonium acetate (1.1 mmol, 1.1 equiv.) were dissolved in 10 ml THF/EtOH = 4:1 mixture solvents, then the mixture was stirred at room temperature and the reaction was monitored by TLC. The solvent was removed in vacuo, and the residual solid was purified by chromatography (silica gel, EtOAc–hexane as eluent, 1: 1, v/v) to afford 196 mg (65%) of ACDM as a purple solid. The chemical structure of ACDM was confirmed by ^{1}H NMR, ^{13}C NMR and HRMS spectroscopy (Fig. S1-S3, ESI†), and the data are as follows: ^{1}H NMR (400 MHz, DMSO-d6) δ 7.88 (1 H, d, J = 15.8), 7.67 (2 H, d, J = 8.6), 6.82 (1 H, d, J = 15.8), 6.66 (2 H, d, J = 8.7), 1.74 (6 H, s). ^{13}C NMR (100 MHz, DMSO-d6) δ 177.88, 176.13, 155.79, 150.37, 134.10, 122.49, 114.69, 114.01, 113.18, 112.56, 108.16, 98.57, 92.01, 51.19, 26.13. HRMS (ESI negative) calcd for [M-H] - 301.1095, found 301.1097.

Properties of ACDM

Solution in PBS analysis

Different final concentrations of **ACDM** (2.5 μM, 5 μM, 10 μM, 20 μM, 40 μM, 80 μM) dissolved in PBS with total volume 200 μL. Took 150 μL of supernatant in 96-well plate and measured ($\lambda_{ex}/\lambda_{em}$ =560/612 nm) by Microplate Reader.

Proteins binding kinetics analysis

Upon total volume 200 μL mixed with different concentrations of **ACDM** (1 μM, 2 μM, 5

μM, 10 μM, 15 μM, 20 μM, 30 μM, 40 μM) while HA maintained 5 μM. Took 150 μL of supernatant in 96-well plate and measured ($\lambda_{ex}/\lambda_{em}$ =560/612 nm) by Microplate Reader. To derive the binding parameters, data were analyzed using the non-linear Langmuir isotherm:

$$\Delta r = \Delta r_{max} * P/(K_d + P)$$

where Δr is the difference in fluorescence anisotropy in the absence and presence of the protein at concentration [P], Δr_{max} is the maximum possible change in the fluorescence anisotropy, K_d is the binding dissociation constant. The non-linear equation was fitted to the data using Graphpad Prism 5.

Chemical stability. Effect of serial pH on the fluorescence intensity at 612 nm of ACDM (20 μM) and HA (200 $mg \cdot L^{-1}$). The measurements were performed in different pH (2.2, 3.0, 4.0, 5.0, 6.0, 7.0, 7.4, 8.0, 9.2, 10.0, 10.8) of buffer. Effect of DMSO volume percent on the fluorescence intensity of **ACDM** (20 μM) and binding with HA (75 $mg \cdot L^{-1}$). The measurements were performed in PBS buffer with different DMSO (1.0%, 2.0%, 2.5%, 5.0%, 10%) volume percent. Took 150 μL of supernatant in 96-well plate and measured ($\lambda_{ex}/\lambda_{em}$=560/612 nm) by Microplate Reader.

Physical stability. Effect of serial temperature on the fluorescence intensity of ACDM (20 μM) and binding with HA (250 $mg \cdot L^{-1}$). The measurements were performed in different temperature (RT = room temperature, 30 ^{o}C, 37 ^{o}C, 40 ^{o}C, 50 ^{o}C, 60 ^{o}C) environment. Took 150 μL of supernatant in 96-well plate and measured ($\lambda_{ex}/\lambda_{em}$=560/612 nm) by Microplate Reader.

Anti-interference stability. The effects of endogenous compounds, ions and drugs on the fluorescence response of ACDM (20 μM) co-mixed with HA (5 μM). The concentrations of these endogenous compounds, ions were 0.5 μM except drugs were 10 μM. Took 150 μL of supernatant in 96-well plate and measured ($\lambda_{ex}/\lambda_{em}$=560/612 nm) by Microplate Reader.

Determination of the quantum efficiency of fluorescence

For determination of the quantum efficiency of fluorescence (Φ_{fl}), Fluorescein in water (Φ_{fl}=0.65) was used as a standard. Values were calculated according to the following equation. The quantum efficiencies of fluorescence were summarized in **Table S2**.

$$\Phi_x/\Phi_s = [A_s/A_x][n_x^2/n_s^2][D_x/D_s]$$

s: standard

x: sample

A: absorbance at the excitation wavelength

n: refractive index

D: area under the fluorescence spectra on an energy scale

Determination of the limit of detection

The detection limit was calculated based on the fluorescence titration. The fluorescence emission spectrum of **ACDM** was measured by five times and the standard deviation of blank measurement was achieved. To gain the slope, the fluorescence intensity at 612 nm was plotted as a concentration of HA. So the detection limit was calculated with the following equation:

Detection limit = $3\sigma/k$

Where σ is the standard deviation of blank measurement, k is the slope between the fluorescence intensity at 612 nm versus HA concentration.

Incubation conditions

The mixture system, with a total volume of 200 μL, consisted of 100 mM potassium phosphate buffer (pH = 8.0), and human albumin or other mentioned proteins. In all experiments, **ACDM** (2 mM dissolved in DMSO previously) was serially diluted to the required concentrations and the final concentration of DMSO did not exceed 2 % (v/v) in the incubation mixture. After pre-mixture at room temperature instantly, then aliquots of supernatant were taken for further analysis by Microplate Reader. Control mixture without HA was carried out to ensure that complex formation was concentration dependent. All complex throughout the study were performed in duplicate with S.E.M. values generally below 10%.

Job's Plot analysis.

The Job's Plot was constructed according to several previously published literatures [1]. In brief, **ACDM** was mixed with HA at different ratios in PBS buffer (pH = 8.0), while

maintaining the total concentrations (**ACDM** plus **HA**) at 10 μM.

Assignment of binding site(s) of ACDM on HA

ACDM was dissolved in DMSO to obtain a 2 mM stock solution. Site-specific drugs were dissolved in DMSO to obtain a 5 mM stock solutions. Displacement of ACDM from the HA-ACDM complex was performed by addition of one of site-specific drugs (50 μM), including warfarin (domain IIA), propofol (domain IIIA & IIIB), ibuprofen (domain IIIA), salicylic acid (domain IIA & IB), indomethacin (domain IIA) and digitoxin (domain IIIA). At room temperature,196 μL of albumin (5 μM) solution mixed with 2 μL site-specific drugs, and then incubated at for 3 min. After incubation, 150 μL supernatant was transferred into 96-well plate and measured ($\lambda_{ex}/\lambda_{em}$=560/612 nm) by Microplate Reader

Cell cultivation

HepG2 cells were plated in 6-well plate and grown in MEM (**Minimum essential medium**) culture medium supplemented with 10% fetal bovine serum (both from Invitrogen, Carlsbad, CA). Cell cultures were incubated in a humidified atmosphere of 95% air and 5% CO_2 at a constant temperature of 37℃. The medium was refreshed every other day. Until cultured HepG2 cells reached 80% confluence, the culture medium was discard and washed with PBS for three times. After that 2 mL of culture medium without fetal bovine serum was added into each well. At indicated time points, 0.2 mL of culture medium was taken from each well and kept at 4℃ until analysis.

HA detection by ACDM assays

HA detection in Plasma. ACDM was dissolved in DMSO to obtain a 2 mM stock solutions. Plasma samples were collected from healthy donors. Fluorescence response of **ACDM** (20 μM) in a PBS diluted plasma sample (1000 fold dilution) upon addition of various concentrations of HA (5.0 $mg \cdot L^{-1}$ to 300 $mg \cdot L^{-1}$). Took 150 μL of supernatant in 96-well plate and measured ($\lambda_{ex}/\lambda_{em}$ = 560/612 nm) by Microplate Reader. The linear parameter was expressed by the linear regression coefficient (R^2).

HA detection in cell culture supernatant. ACDM was dissolved in DMSO to obtain a 2 mM stock solutions. Fluorescence response of **ACDM** (20 μM) in PBS diluted with 2-fold Minimum essential medium (MEM) system upon addition of various concentrations of HA (2.5 $mg \cdot L^{-1}$ to 200 $mg \cdot L^{-1}$). Took 150 μL of supernatant in 96-well plate and measured ($\lambda_{ex}/_{em}$=560/612 nm) by Microplate Reader. The linear parameter was expressed by the linear regression coefficient (R^2).

HA detection by BCG method

We quantified serum albumin in plasma from 28 health volunteers by both BCG and **ACDM** method. **ACDM** (20 μM) in a 200 μL PBS diluted 1000-fold plasma system. Took 150 μL of supernatant in 96-well plate and measured ($\lambda_{ex}/\lambda_{em}$=560/612 nm) by a Microplate Reader. While the 198 μL BCG work solution added 2μL plasma. After Blending, took 150 μL supernatant in 96-well plate and measured by a Microplate Reader using ultraviolet spectroscopy at 630 nm. The concentrations of albumin were calculated by calibration in PBS.

Quantification of trace HA using LC-MS/MS

The HepG2 cell samples (50 μL) collected from each time point were diluted with 48 μL of denaturing buffer containing 8 M urea in MEM buffer (50 mM, pH 8.0). After then, 20 mM DTT (2 μL) was added into the mixture at 60℃ for 1 h and then alkylated by adding 4 μL of 40 mM IAA in the darkness at room temperature for 40 min. After that, the reduced and alkylated protein mixtures were diluted with 700 μL of 1 M urea in MEM buffer (50 mM, pH 8.0). For trypsin digestion, the digestion was performed at 37℃ overnight with an enzyme-to-protein ratio of 1/25 (w/w). Then, the trypsin digest was purified with a C18 solid-phase column and exchanged by 0.1% formic acid water before LC-MS/MS analysis [2, 3]. Chromatographic separation was performed on an ACCHROM C18HC column (150 mm× 2.1 mm, 5 μm, Acchrom Technology Corp., Beijing, China) using an ACQUITY™ UPLC system equipped with a binary pump, a vacuum degasser and an auto-sampler (Waters Corp., Milford, MA, USA). The mobile phase consisted of solvent A (0.1% formic acid in water)

and solvent B (0.1% formic acid in methanol), at a flow rate of 0.25 mL·min^{-1}. The gradient system started with 95% A for 2 min, programmed linearly to 5% A in 22 min, and held for 2 min, returned to 95% A in 0.5 min, and held there for 3.5 min to re-equilibrated the column. The mass spectrometry detection was performed on an AB Sciex QTrap® 5500 system with a Turbo V™ source (Framingham, MA, USA). Ionization was operated using an electrospray ionization (ESI) source in the positive ion mode. Optimized instrument settings specific for the peptides LVNEVTEFAK and QTALVELVK were as follows: Curtain Gas was 40 psi, Ion Source Gas 1 was 20 psi, Ion Source Gas 2 was 25 psi, IonSpray Voltage was 5500 V, Turbo Heater Temperature was 600℃. The doubly-charged precursor ion for LVNEVTEFAK at *m/z* 575.5 was selected in Q1 (DP: 90), and 2 single charged transitions were monitored in Q3: the y_8-ion NEVTEFAK at *m/z* 937.5 (CE: 25 eV, CXP: 37 V), the y7-ion EVTEFAK at *m/z* 823.5 (CE: 24 eV, CXP: 13 V). For QTALVELVK the doubly charged precursor ion at *m/z* 500.5 was selected in Q1 (DP: 100), and 2 single charged transitions were monitored in Q3: the y_5-ion VELVK at *m/z* 587.4 (CE: 25 eV, CXP: 35 V), the y_4-ion ELVK at *m/z* 488.4 (CE: 23 eV, CXP: 13 V). All MS data were acquired and processed using AB Sciex Analyst Ver. 1.6.1 software [4].

The calibration curve for quantification of human albumin was depicted according to the above mentioned procedure. Seven different HA concentrations (1, 2, 10, 50, 250, 500, 1000 mg·L^{-1} of HA in 50 mM MEM, pH 8.0) were used and the calibration curve was constructed based on the peak area versus the spiked concentrations by least square linear regression analysis with a weighting factor of $1/x^2$.

Molecular Docking and Molecular Dynamics Simulations

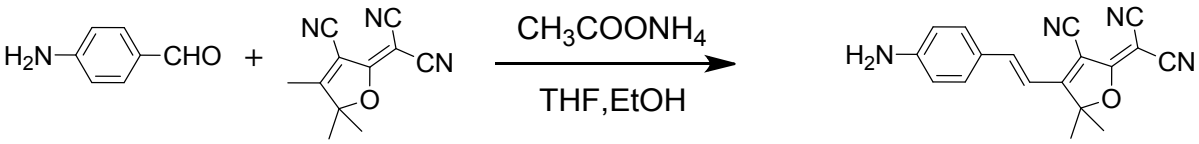
The protein structure of HA was taken from Protein Data Bank (PDB ID: 2I2Z).[5] AutodockTools[6] were used to prepare the input protein structure for docking. The CHARMM 27 force filed was used to represent the protein structure instead of adding Kollman or Gasteiger charges in order to well characterize 17 disulfide bonds in HA. The input docking file for the ligand ACDM was obtained using the PRODRG Server (http://davapc1.bioch.dundee.ac.uk/cgi-bin/prodrg).[7] The searching grid box was sufficiently

large to wrap the whole protein, and ACDM was blindly docked into the protein using the Lamarckian generic algorithm (GA). A total of 100 GA runs were performed, resulting in 100 docking conformations. The default docking parameters for other settings were applied. Autodock4[6] program was used to performing docking. The distribution of 100 conformations was shown in **Fig. S18**.

The protein-ligand complex with the lowest binding energy was taken from the above docking results and used as the initial conformation in the following molecular dynamics (MD) simulations. The protein of HA was represented with CHARMM27 parameters with CMAP corrections.[8] To obtain accurate force field parameter used in MD simulations, quantum chemistry calculations were performed. The structure of ACDM was optimized at the HF/6-31+G (d,p) level. The electrostatic potential was calculated at the same level using the MK scheme. The restrained electrostatic potential (RESP) method was used to fit the electrostatic potential to get atomic partial charges. All quantum chemical calculations were carried out with the Gaussian 03 software package.[9] The final charges employed in the MD simulations are presented in **Fig. S19**. The lacking parameters for ACDM (including force constants for bonds, angles, and dihedrals) were determined using the program PARATOOL, a plug-in for the molecular viewer VMD.[10]

The structure of HA was solvated in an 74×115×101 Å box filled with TIP3P water molecules. The distance between any atom of protein and the border of water box is at least 12 Å. Fourteen Na^+ ions were randomly added to neutralize the system. The total number of atoms is 79665, including 23476 water molecules. Periodic boundary conditions were applied in three dimensions. The long-range electrostatic interactions were treated using the PME method. The integration time step is 2 fs. The system was first energy minimized for 5000 steps with the protein, ACDM, and ions fixed in their initial positions. Subsequently, the system was heated from 0 to 310 K in seven stages of 20 ps each, with Cα atoms of protein and ACDM restrained with 20 $kcal•mol^{-1}•Å^{-2}$ spring constant and switched to 10, 5, 2.5, 1.2, 0.6, and 0.1 $kcal•mol^{-1}•Å^{-2}$ for each stage. Then the system was equilibrated at 310 K for 100 ps without any restraint. Langevin dynamics and Langevin piston methods were used to maintain the temperature at 310 K and pressure at 1 bar. The production MD simulations

were run for 15 ns. NAMD2[11] software was used to performed all MD simulations. It was found that the potential energy of the system does not fluctuate significantly throughout MD simulations (**Fig. S20**). And the protein seems to reach equilibrium after 10 ns, as the root-mean-square deviations (RMSD) of Cα atom of protein fluctuate within 1 Å (**Fig. S21**). It was observed that ACDM stably binds to HA in 15-ns MD simulations. In particular, ACDM form stable π-π interactions with Y161 (**Fig. S22**).



Scheme S1 The synthesis procedure of **ACDM**.

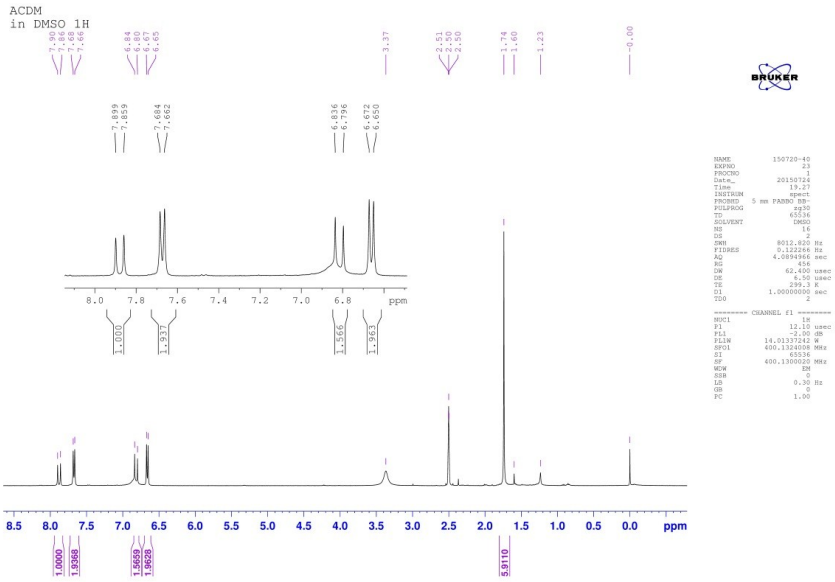


Fig. S1 ^{1}H NMR (400 MHz, DMSO) spectrum of **ACDM**.

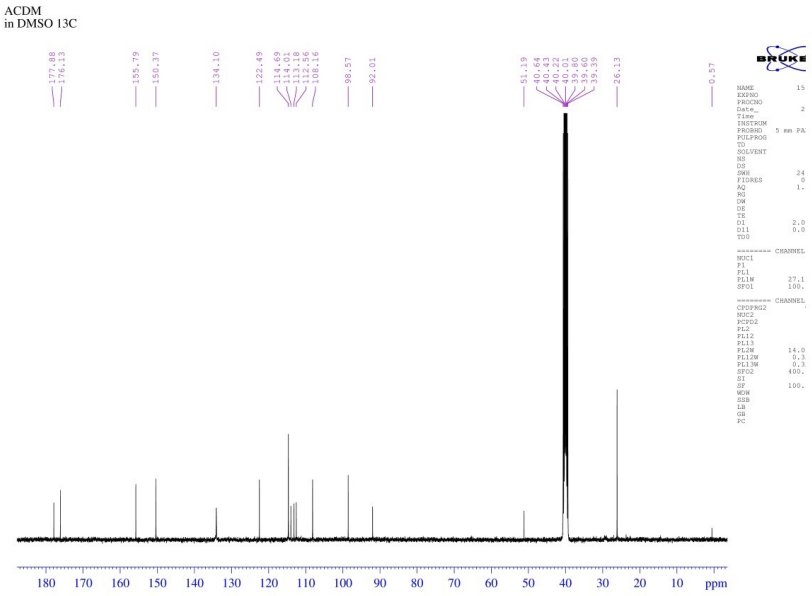


Fig.S2 ^{13}C NMR (100 MHz, DMSO) spectrum of **ACDM**.

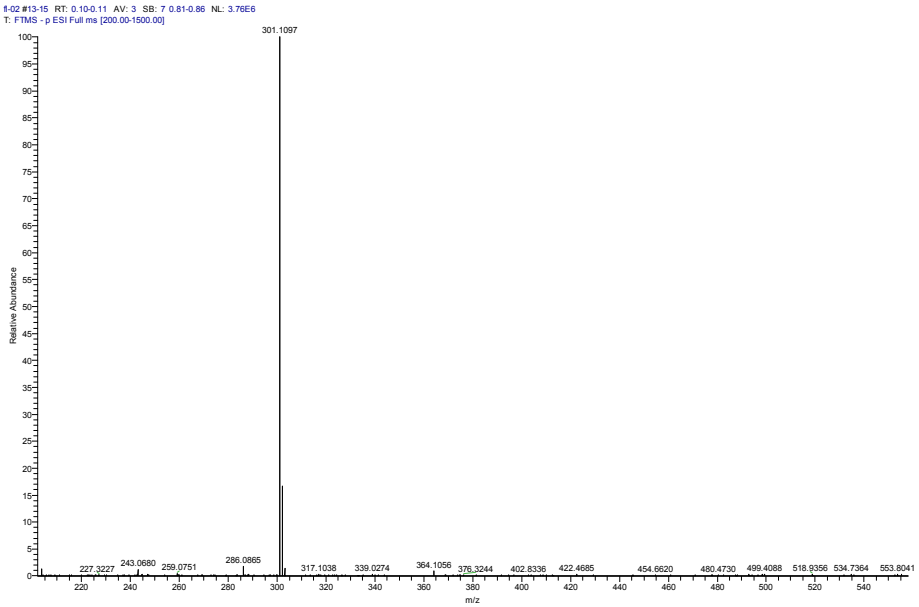


Fig. S3 HRMS spectrum of **ACDM**.

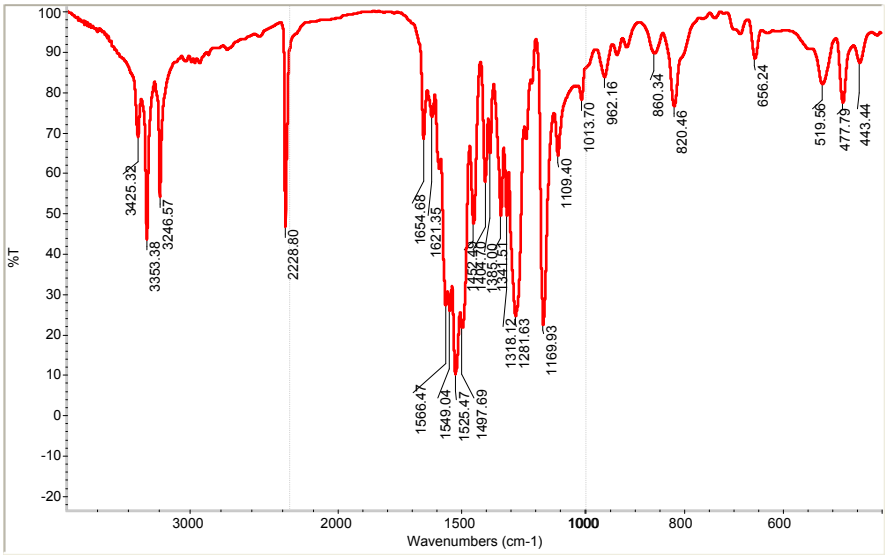


Fig. S4 FTIR spectrum of **ACDM**.

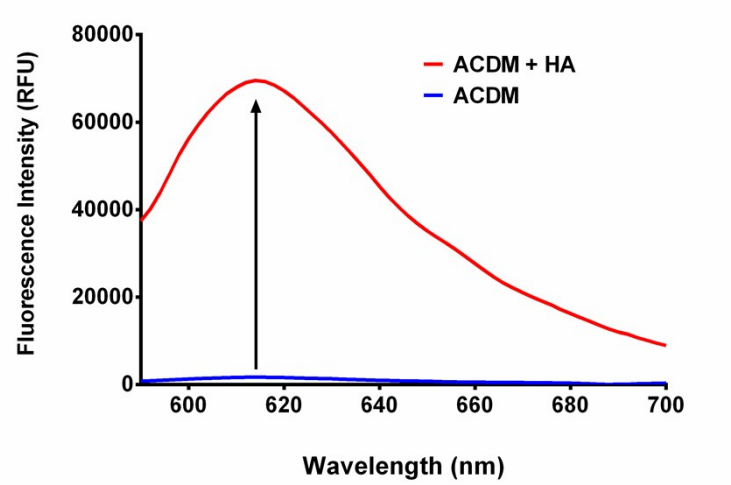


Fig. S5 Fluorescence spectra of **ACDM** (20 μM) upon addition of HA (300 mg•L^{-1}) in PBS (pH 8.0 at room temperature)

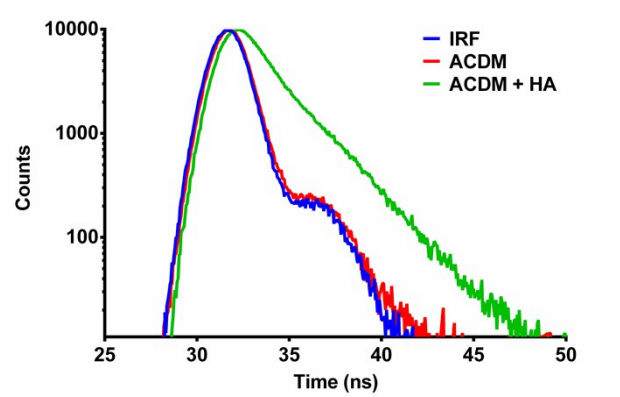


Fig. S6 Fluorescence lifetime decay profiles of **ACDM** in HA solution (300 mg•L^{-1} at pH 7.0). Red line represents free **ACDM** and green line represents **ACDM-HA** complex, while blue line represents instrument response function (IRF). The free **ACDM** exhibits a single exponential decay with a lifetime of 0.14 ns. Upon the addition of HA, **ACDM** exhibited a double exponential decay with lifetimes of 0.57 ns (40.11%), 2.05 ns (58.09%). $\lambda_{ex}/\lambda_{em}$ = 560 nm/612 nm

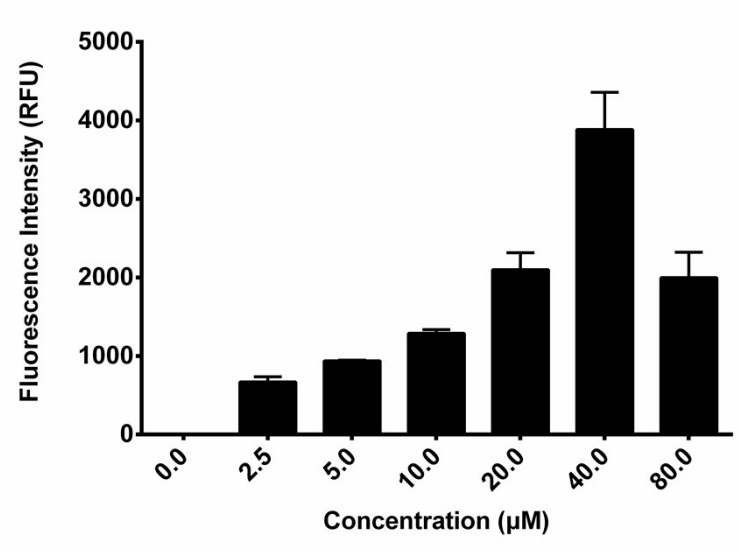


Fig. S7 Fluorescence of different concentration of **ACDM** dissolved in PBS (pH = 8.0). The results demonstrated that **ACDM** is water-soluble and does not form aggregates in aqueous buffer at concentrations lower than 40 μM

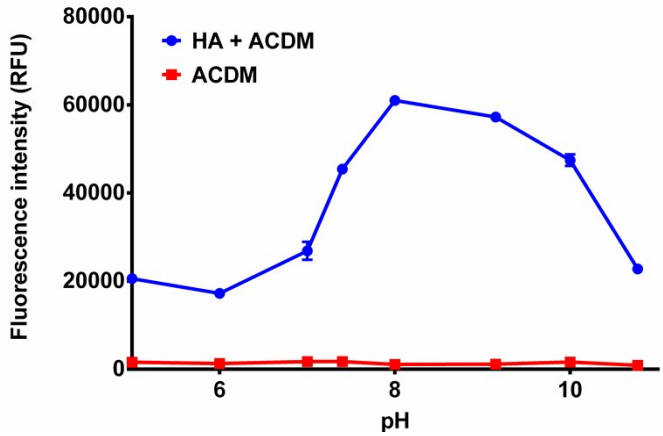


Fig. S8 Stability of **ACDM** in different pH (5.0-10.77) buffer

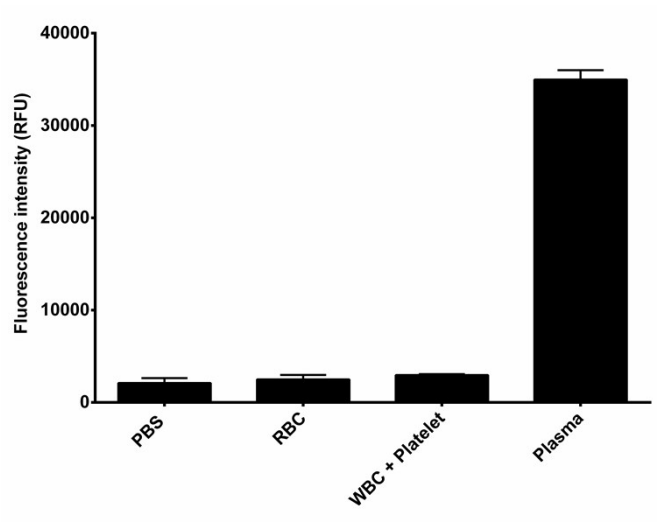


Fig. S9 The fluorescence intensity of ACDM (20 μM) co-incubated with plasma, red blood cell (RBC), white blood cell (WBC) and platelet (all were measured in PBS diluted 200-fold, pH8.0).

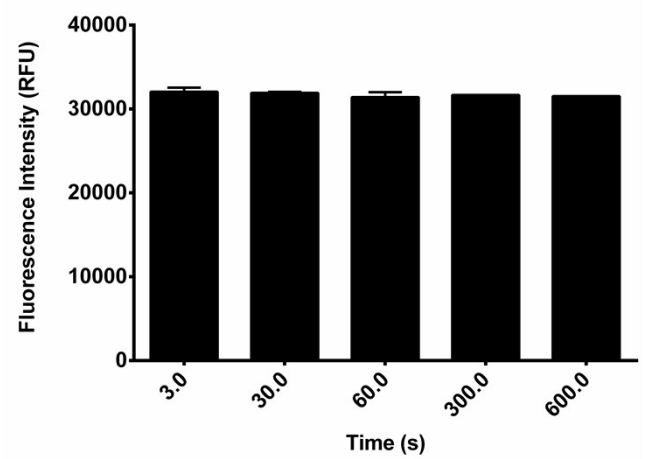


Fig. S10 The fluorescence intensity of ACDM (20 μM) binding with HA (150 mg•L^{-1}) in different time periods.

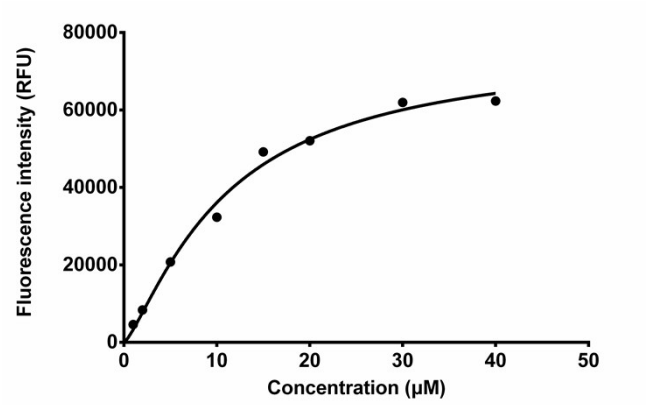


Fig. S11 Binding Affinity and Kinetic Analysis of **ACDM** on HA.

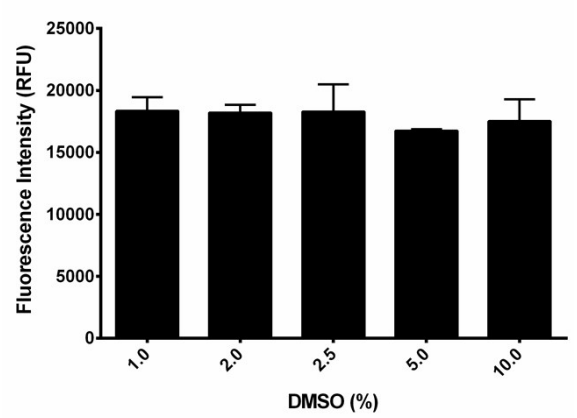


Fig. S12 The effects of DMSO volume percent on the fluorescence intensity at 612 nm

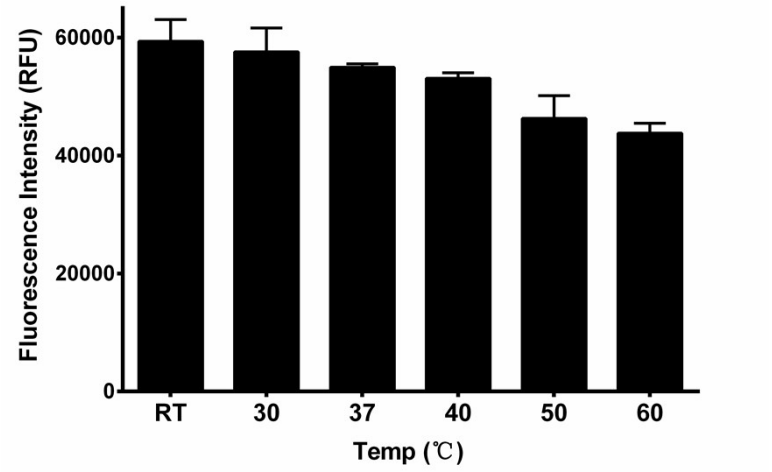


Fig. S13 The effects of temperature on the fluorescence intensity at 612 nm

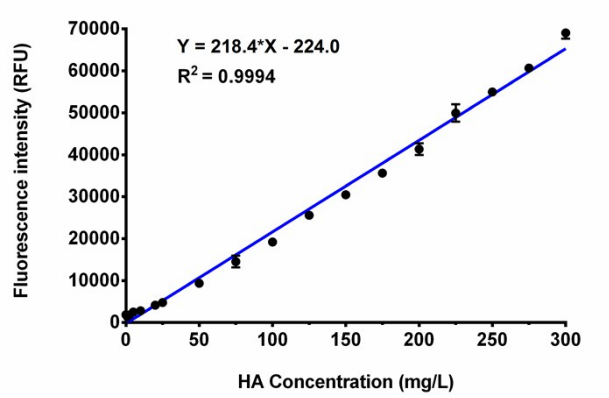


Fig. S14 The quantitative calibration for human albumin in PBS (pH 7.4).

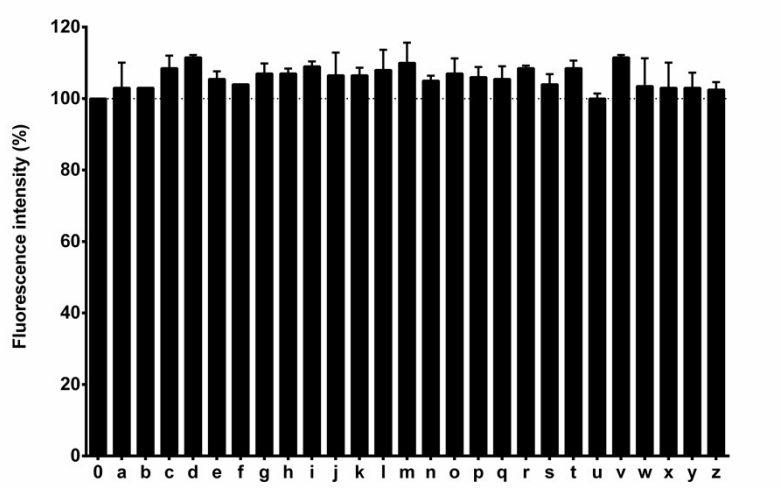


Fig. S15. The effects of endogenous compounds and ions on the fluorescence response of ACDM (20 μM) co-mixed with HA (5 μM). a-v were relative fluorescence intensity of added ACDM after endogenous compounds and ions (0.5 μM) co-mixed with HA (5 μM). 0 = HA, a = Arg, b = Glu, c = Gln, d = Cys, e = Tyr, f = Trp, g = Lys, h = Gly, i = Ser, j = Vitamin C, k = GSH, l = Bilirubin, m = Glucose, n = Ca^{2+}, o = Mg^{2+}, p = K^{+}, q = Al^{3+}, r = Fe^{3+}, s = Mn^{2+}, t = Cu^{2+}, u = Zn^{2+}, v = Co^{+}, w = Stearic acid, x = Palmic acid, y = Lauric acid, z = Myristic acid.

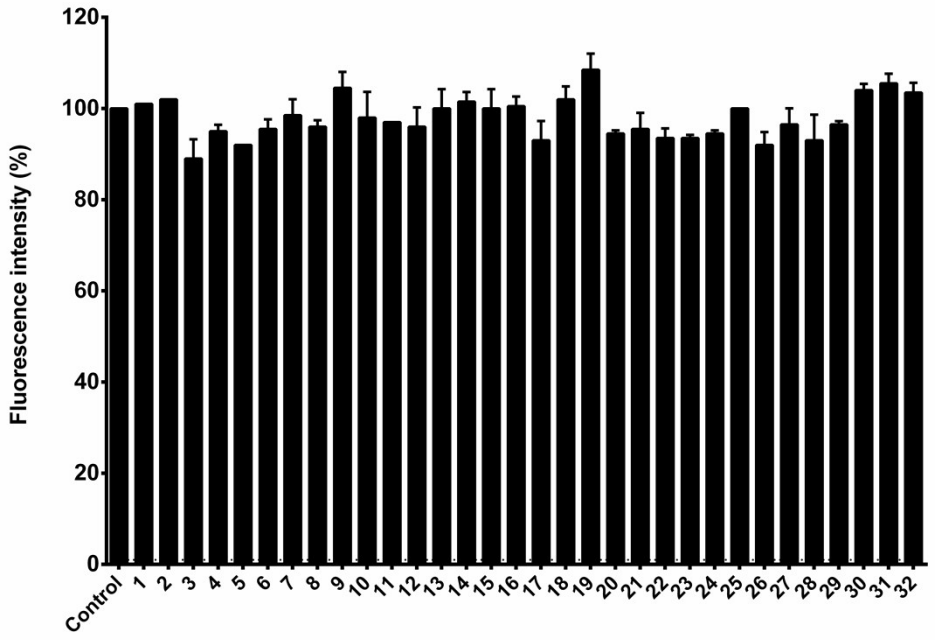


Fig. S16. The effects of commonly used therapeutic drugs and bioactive natural compound on the fluorescence response of **ACDM** (20 μM) co-mixed with HA (5 μM). 1-21 were relative fluorescence intensity of added ACDM after drugs (0.5 μM) co-mixed with HA (5 μM). Control = HA, 1 = paclitaxel, 2 = docetaxel, 3 = prasugrel, 4 = irinotecan, 5 = gemcitabine, 6 = icotinib, 7 = vandetanib, 8 = imatinib, 9 = gefitinib, 10 = amlodipine, 11 = nifedipine, 12 = cinildipine, 13 = metronidazole, 14 = sulbactam sodium, 15 = ampicillin, 16 = flurbiprofen, 17 = acetaminophen, 18 = naproxen, 19 = apigenin, 20 = luteolin, 21 = daphnetin, 22 = β-Methylcou marin, 23 = glycyrrhetinic acid, 24 = oleanolic acid, 25 = ursolic acid, 26 = betulinic acid, 27 = schisandrol, 28 = deoxyschizandrin, 29 = protocatechualdehyde, 30 = protopanaxatriol, 31 = protopanoxadiol, 32 = testosterone.

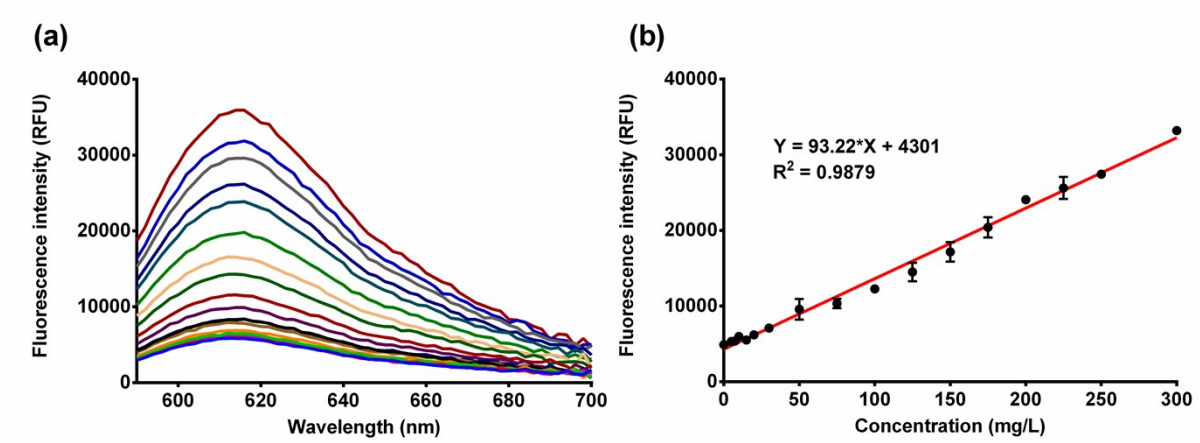


Fig. S17 (a) Fluorescence response of **ACDM** (20 μM) in the presence of increasing concentrations of HA. The spectra were measured in PBS diluted plasma (1000-fold, pH 8.0) at room temperature. λex = 560 nm. (b) The fluorescence intensity at 612 nm of **ACDM** (20 μM) was linearly related to the concentration of HA (0–300 mg•L^{-1}) in PBS diluted plasma (1000-fold, pH 8.0) at room temperature.

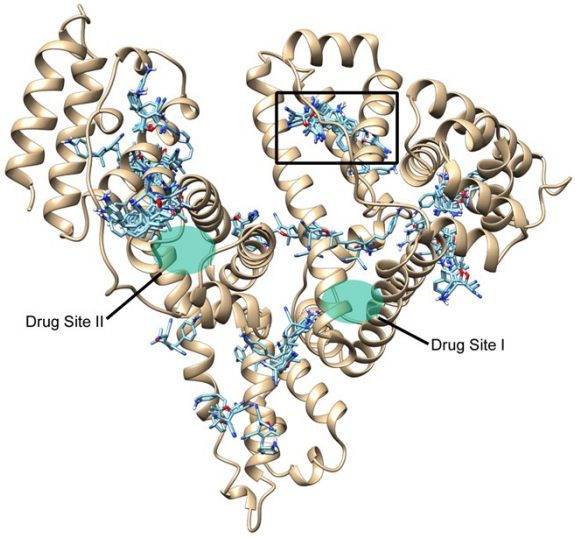


Fig. S18 The distribution of 100 binding conformations, with the most frequency and lowest binding energy highlighted with black rectangle.

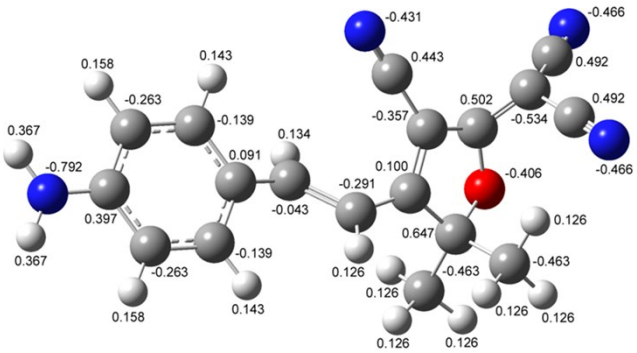


Fig. S19 RESP charges of ACDM used in MD simulations.

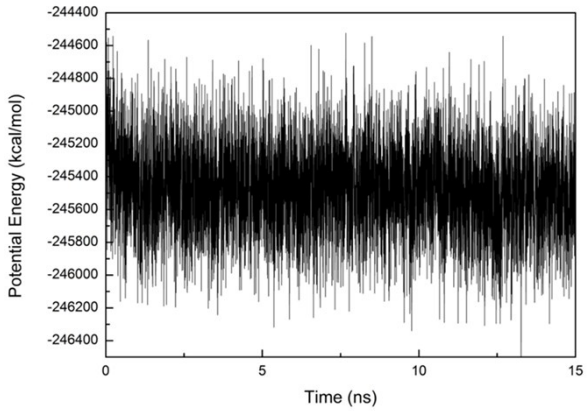


Fig. S 20 Potential energy of the system during MD simulations.

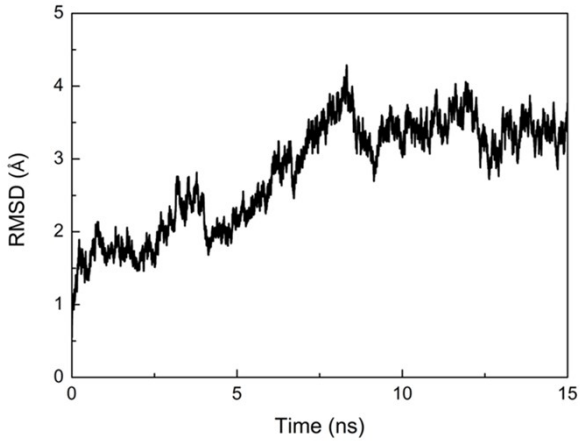


Fig. S21 Time evolution of Cα RMSD of the protein structure of HA.

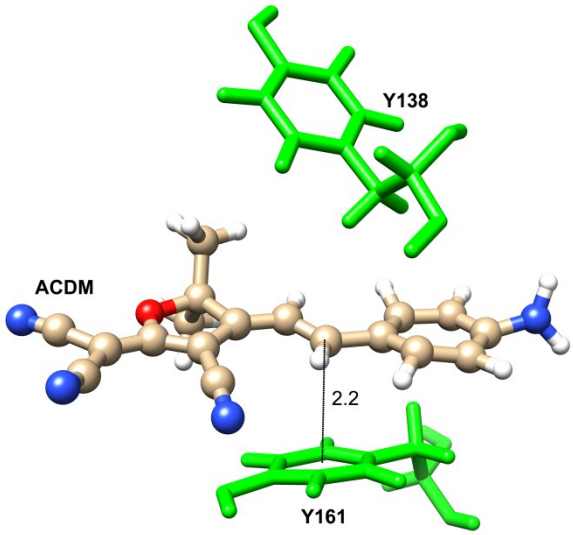


Fig. S22 Representative stable π-π interactions between ACDM and Y161. The average distance between the olefinic bond of ACDM and phenyl ring of Y161 is about 2.2 Å.

Table. S1 HA quantification for plasma from 28 health volunteers by both BCG method and **ACDM** method

NO.	Gender	Age	BCG ($g \cdot L^{-1}$)	ACDM ($g \cdot L^{-1}$)	Ratio (BCG/ACDM)
1	Male	22	41.90 ± 1.24	40.85 ± 2.45	1.02
2	Female	27	49.19 ± 2.05	48.46 ± 1.97	1.02
3	Male	21	44.60 ± 1.84	42.38 ± 2.34	1.05
4	Female	51	51.51 ± 0.97	46.08 ± 3.74	1.12
5	Male	21	48.65 ± 2.30	50.85 ± 1.84	0.96
6	Male	27	39.19 ± 3.54	38.97 ± 2.40	1.01
7	Male	25	45.95 ± 1.58	44.22 ± 0.79	1.04
8	Female	19	58.79 ± 2.41	55.18 ± 1.64	1.07
9	Male	31	53.26 ± 1.25	52.42 ± 2.30	1.02
10	Female	29	36.49 ± 2.97	31.95 ± 3.41	1.14
11	Male	34	53.38 ± 1.74	47.61 ± 3.55	1.12
12	Female	46	54.73 ± 0.54	54.40 ± 1.38	1.01
13	Female	44	42.11 ± 4.21	43.57 ± 2.41	0.97
14	Female	30	56.76 ± 3.12	55.32 ± 0.70	1.03
15	Male	23	47.54 ± 2.85	49.91 ± 0.09	0.95
16	Female	40	41.46 ± 3.10	37.53 ± 0.86	1.10
17	Female	44	38.41 ± 3.44	34.57 ± 3.21	1.11
18	Female	41	36.13 ± 3.76	35.88 ± 2.85	1.01
19	Female	47	49.06 ± 2.84	48.47 ± 1.76	1.01
20	Female	29	46.78 ± 0.74	43.19 ± 2.87	1.08
21	Female	21	55.58 ± 2.84	52.96 ± 1.35	1.05
22	Female	22	58.30 ± 3.45	59.76 ± 0.64	0.98
23	Male	37	45.26 ± 2.18	40.17 ± 1.81	1.13
24	Male	26	49.58 ± 2.00	51.47 ± 3.10	0.96
25	Male	20	48.30 ± 3.11	45.20 ± 2.91	1.07
26	Male	45	58.01 ± 0.84	51.57 ± 1.34	1.13
27	Male	30	49.82 ± 3.45	48.63 ± 1.00	1.02
28	Male	25	45.26 ± 2.00	41.48 ± 1.00	1.09

Table S2 The fluorescence quantum efficiencies of **ACDM** and **ACDM**-HA in PBS (pH 8.0)

Compound	λ_{ex} (nm)	λ_{em} (nm)	Φ_{fl}[a]
ACDM	560	612	0.006
ACDM-HA	560	612	0.159

[a] Quantum efficiencies were determined by using Fluorescein in water (Φ_{fl} = 0.65) as a

standard

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