Electronic Supplementary Information (ESI)

A bimodal MRI and NIR liposome nanoprobe for tumor targeted molecular imaging

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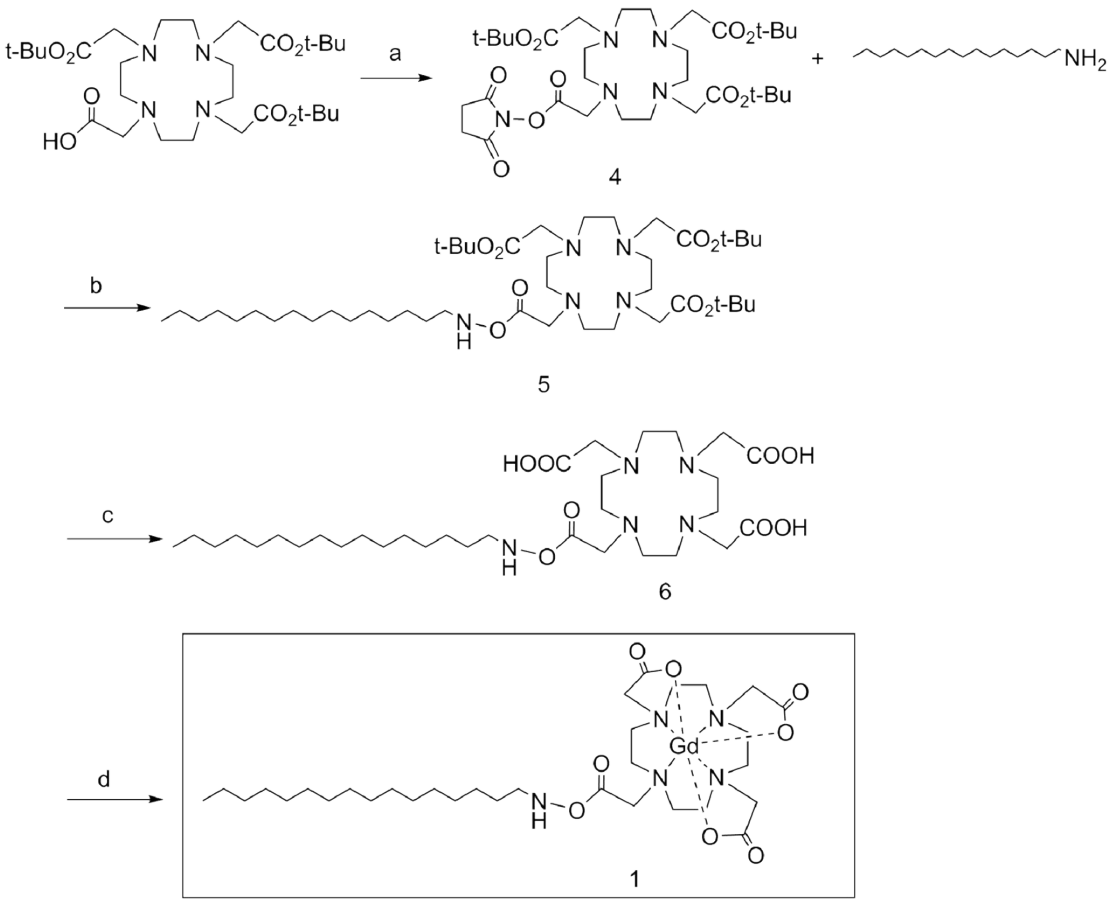


Scheme S1. a) DCC, NHS, CH_2Cl_2, 2 h; b) Et_3N, CH_2Cl_2, 12 h; c) TFA-CH_2Cl_2 1:1 v/v,2h; d) $Gd(CH_3COO)_3 \cdot H_2O$, 90 °C, 12 h.

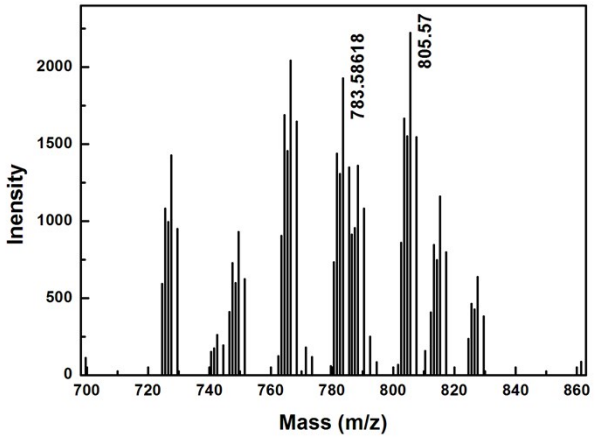


Fig. S1. MALDI-TOF mass spectrum of Gd-DOTA-HDA.

Xylenol orange test

The sample of Gd-DOTA-HDA was dialysed (molecule weight cutoff 500 Da)

against PBS for 7 days. The outer PBS solution (dialyzate) was subjected to the Xylenol orange test. The leak and presence of free Gd^{3+} ions in the Gd-incorporated compounds was determined by measuring the absorbance at 573 nm of a mixture of Xylenol orange solution (0.5 mM) in 990 μL sodium acetate buffer (0.1 M, pH 5.2) and test solution (Gd compound in10 μL water).[1]

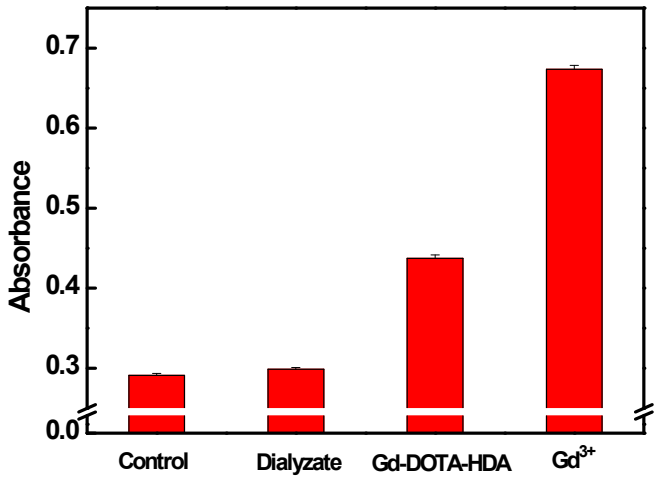
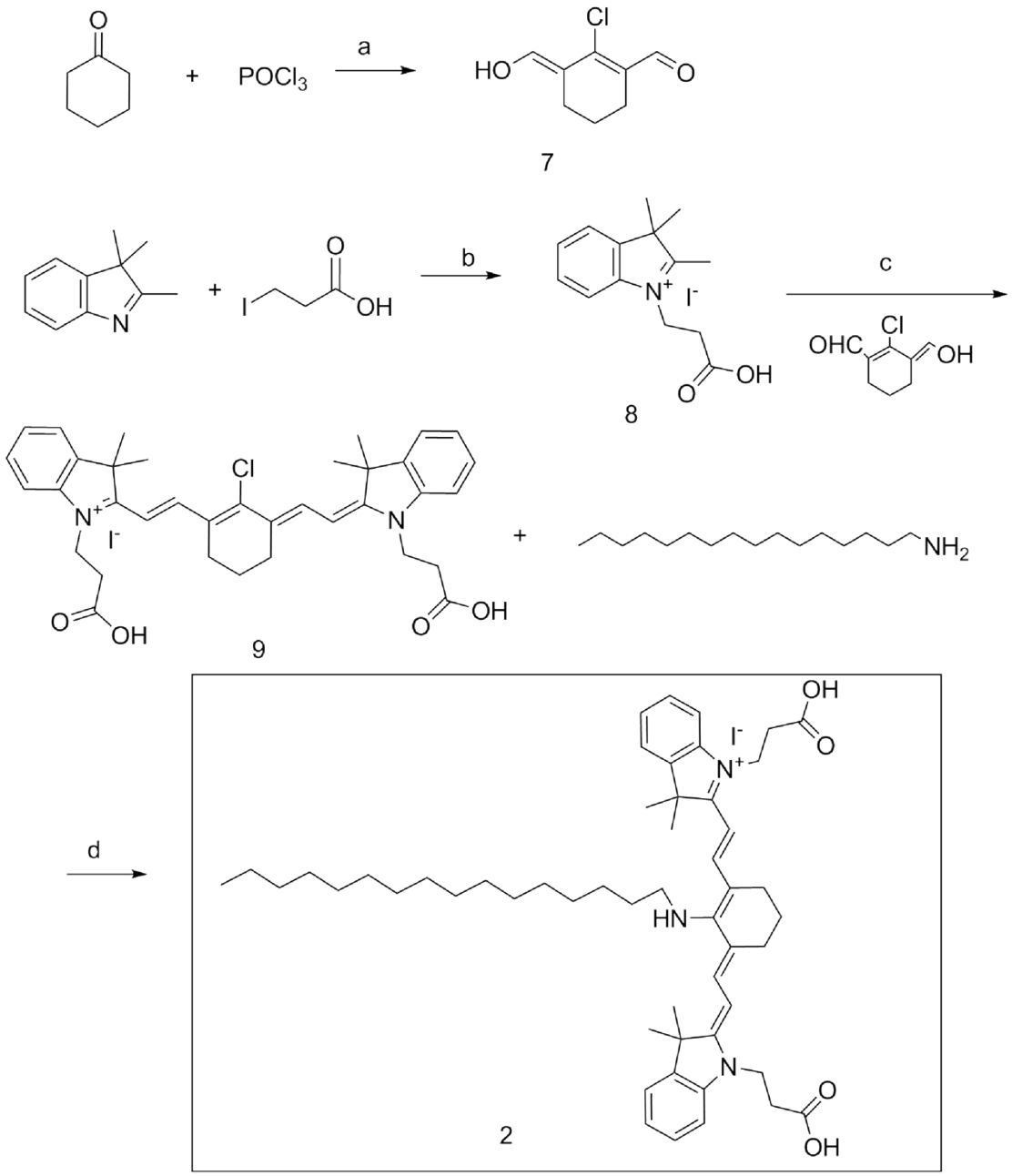


Fig. S2 Gd^{3+} leaking and stability experiment of Gd-DOTA-HDA by a Xylenol orange test. PBS solution was used for control experiment. Dialyzate was the outer PBS solution after 7 days dialysis. Gd-DOTA-HDA was put into the dialysis bag with molecule weight cutoff 500 Da against PBS for 7 days. The free Gd3+ concentration in PBS was 5mM.



Scheme S2. a) DMF, DCM, N_2, 80 ^oC, 3 h, 33%; b)toluene, 100 ^oC, N_2, 3 h, 65%; c) DMF, toluene, N_2, 160 ^oC, 10 h, 31% ; d) Et_3N, DMF, N_2, 70 ^oC, 10 h, 23%.

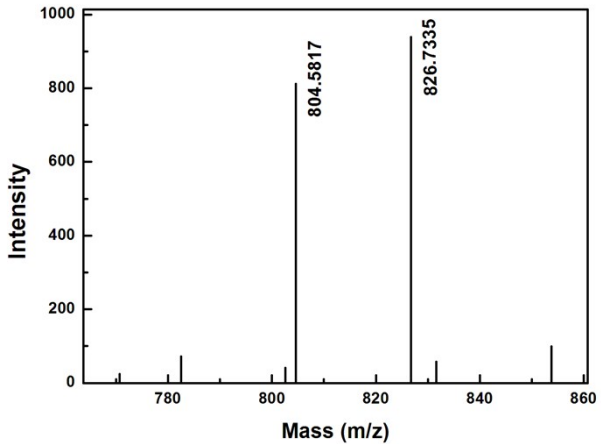
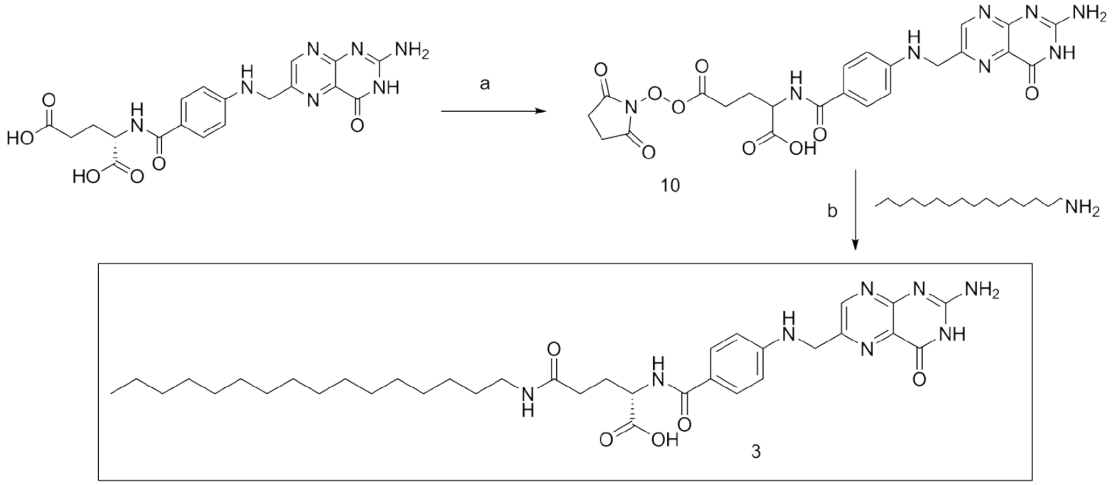


Fig. S3. MALDI-TOF mass spectrum of Cy7-HDA.



Scheme S3. a) Et_3N, DCC, NHS, DMSO, 12 h; b) Et_3N, DMSO-ethyl acetate 3:2 v/v, 24 h, 37%.

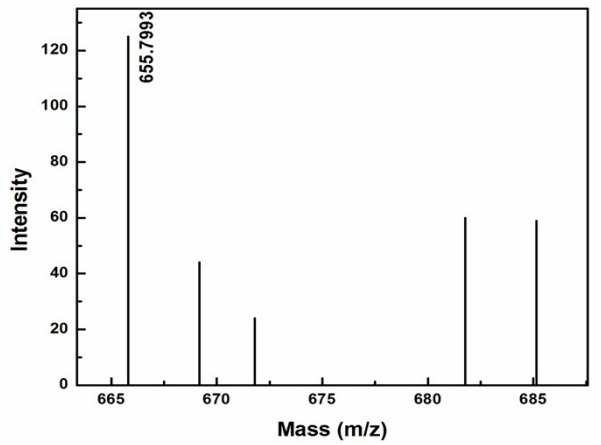


Fig. S4. MALDI-TOF mass spectrum of FA-HDA.

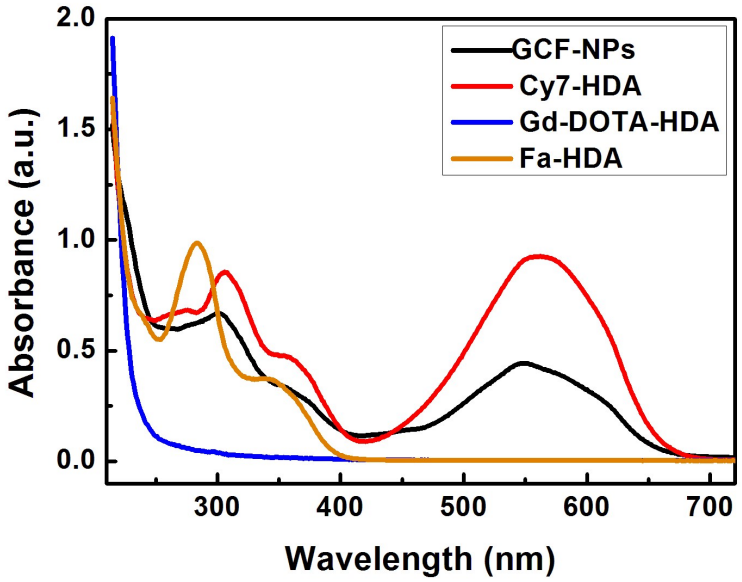


Fig. S5. Absorption spectra of GCF-HDA NPs, Cy7-HDA, Gd-DOTA-HDA, FA-HDA in water. GCF-HDA NPs: Gd-DOTA-HDA, Cy7-HDA and FA-HDA nanoparticles.

With increasing computational power for molecular modeling, it has become popular to use molecular dynamics (MD) simulation to model biomaterials because it provides an insightful understanding of the functions of biomaterials at a molecular level [2, 3]. However, due to large degrees of freedom and a small integration step, the accessibility of a traditional MD simulation is limited to some biological processes occurring at the timescales faster than microsecond. To enhance the capability of a MD simulation, a simplified model, namely Coarse-Grained (CG) model, is a good choice as the atomistic resolution of a biomolecular system is properly reduced [4-6]. To develop an effective CG model, it is critical to acquire an effective potential describing accurately the interactions between coarse-grained particles. Gay-Berne potential[7-9], which is a well-known anisotropic potential, has been wildly used for

describing the CG particles being considered to be elliptic. ACG model, adopting the framework by combining Gay-Berne with point electric multipole (EMP) potentials, has successfully been applied to model liquid waters, organic liquids and proteins.[10-12]

GBEMP mapping for self-assembly units

GBEMP mapping for three self-assembly units is depicted in Figure S6. The CG model of Cy7-HDA (C) is composed of one elliptic rigid body and three disk-like rigid bodies, that of Gd-DOTA-HDA (D) is made up of four elliptic rigid bodies and that of FA-HDA (F) consists of one elliptic rigid body and one disk-like rigid body. The hydrophobic tail of each model is represented by one elliptic rigid body that contains one Gay-Berne site and one non-interacting EMP site. The non-interacting EMP site (denoted by orange filled circle) would not involve in electrostaticinteraction but serve just as the connection purpose. In each model, the corresponding CG particles of hydrophilichead group contain one Gay-Berne site and one EMP site which both share a same location shown by a red filled circle. In addition, one or two non-interacting EMP sites are included into each CG particle in order to connect two different rigid bodies.

GBEMP energy function

The effective energy function of GBEMP model is given by a sum of bonded and non-bonded energy terms:

$$U_{GBEMP} = U_{bond} + U_{angle} + U_{torsion} + U_{GB} + U_{EMP} \quad (1)$$

where U_{bond}, U_{angle} and $U_{torsion}$represent the bond stretching, angle bending and torsional potentials respectively. The non-bonded energy terms are described by U_{GB}

and U_{EMP} that correspond to Gay-Berne anisotropic potential and electric multipole potential respectively.

In the GBEMP model, a fourth-order Taylor expansion of the Morse potential is used for the bond stretching energy term whenthe angle bending energy term adopts a sixth-order potential, and a three-term Fourier series expansion is employed to calculate torsional energies:

$$U_{bond} = K_b(b - b_0)^2\left[1 - 2.55(b - b_0) + \left(\frac{7}{12}\right)2.55(b - b_0)^2\right] \quad (2)$$

$$U_{angle} = K_\theta(\theta - \theta_0)^2[1 - 0.014(\theta - \theta_0) + 5.6 \times 10^{-5}(\theta - \theta_0)^2 - 7.0 \times 10^{-7}(\theta - \theta_0)^3 + 2.2 \times 10^{-8}(\theta - \theta_0)^4 \quad (3)$$

$$U_{torsion} = \sum_n K_{n\phi}[1 + cos(n\phi \pm \delta)] \quad (4)$$

In this work, the bond stretching, angle bending and torsional potentials were parametrized by fitting to the atomistic profiles of the potentials of mean force (PMFs) constructed from atomistic conformations of self-assembly units (Figure S7).

The Gay-Berne anisotropic potential energy function U_{GB} is given as the form:

$$U_{GB}(\hat{u}_i,\hat{u}_j,\hat{r}_{ij}) = 4\varepsilon(\hat{u}_i,\hat{u}_j,\hat{r}_{ij})\left[\left(\frac{d_w\sigma_0}{r_{ij} - \sigma(\hat{u}_i,\hat{u}_j,\hat{r}_{ij}) + d_w\sigma_0}\right)^{12} - \left(\frac{d_w\sigma_0}{r_{ij} - \sigma(\hat{u}_i,\hat{u}_j,\hat{r}_{ij}) + d_w\sigma_0}\right)^{6}\right] \quad (5)$$

The range parameter $\sigma(\hat{u}_i,\hat{u}_j,\hat{r}_{ij})$and the strength parameter $\varepsilon(\hat{u}_i,\hat{u}_j,\hat{r}_{ij})$for pair-wise interactions are functions of the relative orientationof the Gay-Berne particles*i* and *j*. Each uniaxial molecule is associated with a set of Gay-Berne parameters that describe its shape (such as ellipsoid, sphere or disk) and the orientation of its principal axis in

the inertial frame, defined according to its all-atom model. The term d_w is used to control the "softness" of the potential and σ_0 is defined as the square root of the sum of squared breadth of each particle. To parametrizethe Gay-Bernepotentialsfor nucleic acid models (shown in Figure S6), the atomistic energy profiles (using OPLS atomistic force field in this study) were constructed for the intermolecular van der Waals (VDW) interactions between two identical molecular fragments (homodimer), illustrated in Figure S8. In each case, diverse configurations were generated with different orientations (such as, cross, end-to-end, face-to-face, etc.) as well as at various separations. The initialGay-Berne parameters for CG particles were derived through fitting to the corresponding atomistic energy profiles in gas phase using a generic algorithm.

The interaction energy between two electric multipole sites can be expressed as its polytensor form:

$$U_{EMP} = \sum_{ij} U_{ij} = \sum_{ij} V_i^T M_{ij} V_j \tag{6}$$

or its expanded form

$$U_{ij} = \begin{bmatrix} q_i \\ d_{ix} \\ d_{iy} \\ d_{iz} \\ Q_{ixx} \\ \vdots \end{bmatrix}^T \begin{bmatrix} 1 & \frac{\partial}{\partial x_j} & \frac{\partial}{\partial y_j} & \cdots \\ \frac{\partial}{\partial x_i} & \frac{\partial^2}{\partial x_i \partial x_j} & \frac{\partial^2}{\partial x_i \partial y_j} & \cdots \\ \frac{\partial}{\partial y_i} & \frac{\partial^2}{\partial y_i \partial x_j} & \frac{\partial^2}{\partial y_i \partial y_j} & \cdots \\ \vdots & \vdots & \vdots & \ddots \end{bmatrix} \left(\frac{1}{r_{ji}}\right) \begin{bmatrix} q_j \\ d_{jx} \\ d_{jy} \\ d_{jz} \\ Q_{jxx} \\ \vdots \end{bmatrix} \tag{7}$$

Where q, d and Q are charge, dipole and quadrupole moment, respectively. When the EMP sites are determined in a rigid body, it should be straightforward to obtain the EMP parameters for the CG particle through the electric multipole expansion at the

specific locations based on an atomistic model.

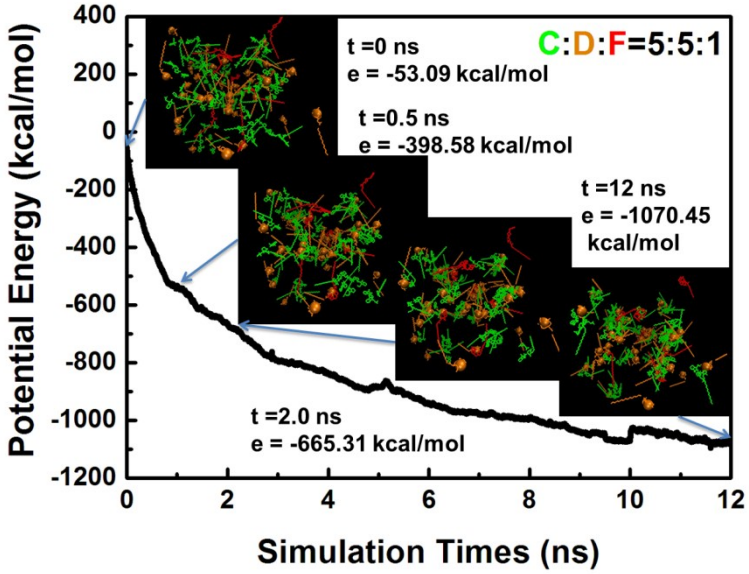


Figure S6. Potential energies of the system (the mixture of C, D and F was built in a ratio of 5:5:1) evolving with MD simulation time. The starting structure (t = 0 ns) of the system was constructed as a random form.

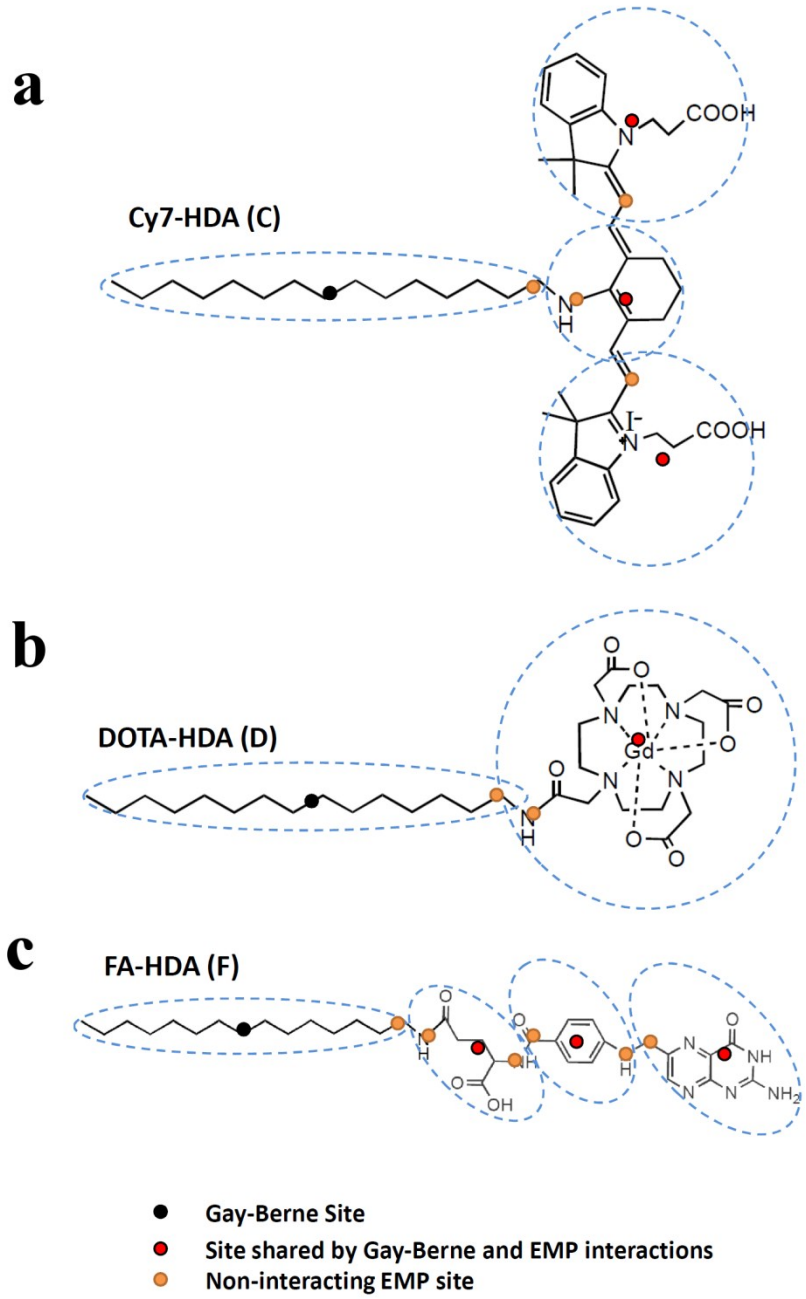


Figure S7. GBEMP mapping for (a) Cy7-HDA, (b) Gd-DOTA-HDA and (c) FA-HDA. Each rigid body, being enclosed by a dash line, is composed of a Gay-Berne interactingsite and one or more electric multipole (EMP) sites. Gay-Berne interactingsites are indicated black filled circles while the interacting EMP sites and non-interaction EMP sites are indicated by red and orange filled circles respectively. Red filled circles represent the sites shared by Gay-Berne and EMP interactions.

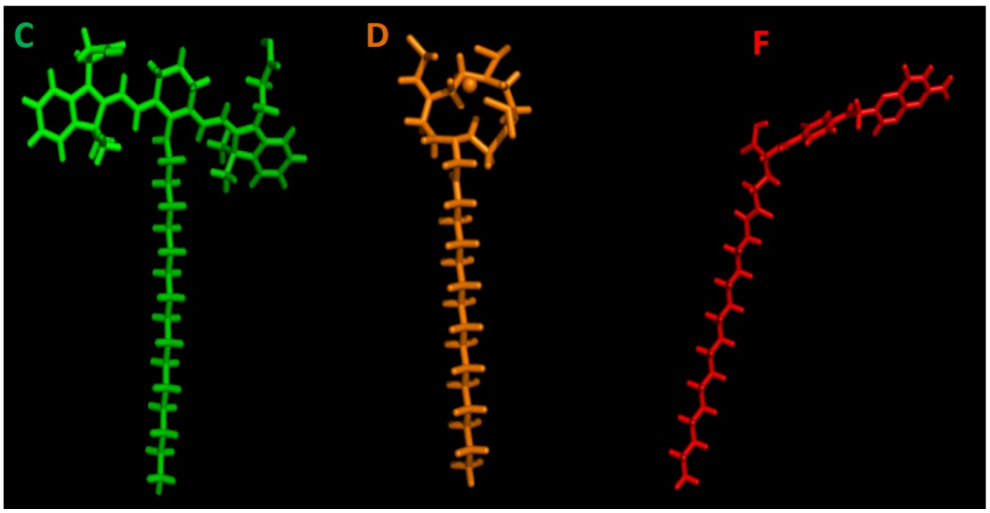


Figure S8. Atomistic representation for Cy7-HDA (C), Gd-DOTA-HDA (D) and FA-HDA (F) are in green, orange and red colors, respectively.

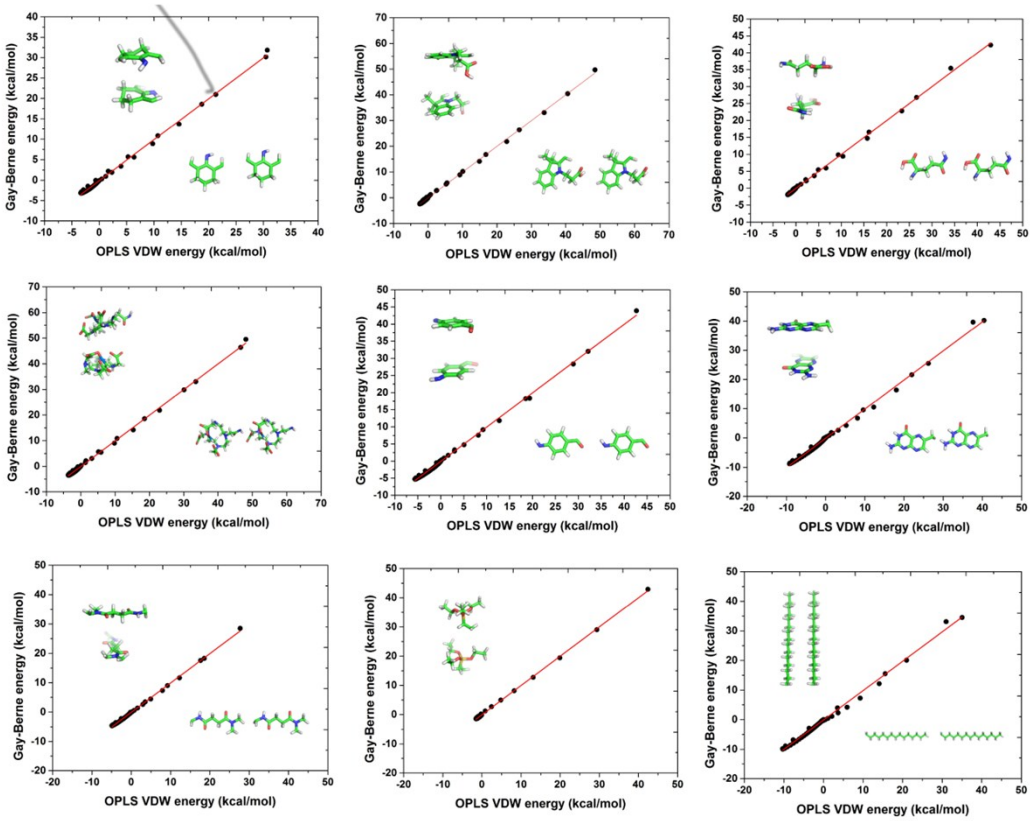


Figure S9. Correlations between the GBEMP and atomistic (OPLS) results in the calculations of van der Waals (VDW) interactions between the homodimers respectively. In each figure, insets display the homodimers adopting different orientations.

GBEMP force field for self-assembly units

##################### Mu Nu

munudw 2.0 1.0

##

Tail rgbtype 11

##

crslm 11 "Tail1" 211.0 92.0 1070.0 1070.0

gblst 11 112

mplst 11 111

GB Amide site:Center of Mass

gbcrs 112 15.171 2.468 0.389 0.067 1.264 10.436

gbsite 112 0.000 0.000 0.000 1.0 0.0 0.0

EMP site (C)

charge 111 0.000 1.000

mpsite 111 -9.040274 0.394360 -0.008524

##

Tail rgbtype 12

##

crslm 12 "Tail21" 211.0 92.0 1070.0 1070.0

gblst 12 122

mplst 12 121

GB Amide site:Center of Mass

gbcrs 122 14.171 2.768 0.389 0.067 1.264 10.436

gbsite 122 0.000 0.000 0.000 1.0 0.0 0.0

EMP site (C)

charge 121 0.000 1.000

mpsite 121 8.914423 0.046827 0.197846

##

Tail rgbtype 13

##

crslm 13 "Tail22" 211.0 92.0 1070.0 1070.0

gblst 13 132

mplst 13 131

GB Amide site:Center of Mass

gbcrs 132 15.171 2.468 0.389 0.067 1.264 10.436

gbsite 132 0.000 0.000 0.000 1.0 0.0 0.0

EMP site (C)

charge 131 0.000 1.000

mpsite 131 -8.761357 0.417558 -0.365652

##

Folic (F21) rgbtype is 21

##

Mass Ix IyIz

```
crslm     21    "F21"         144.0      182.200      785.693    940.864
gblst     21    212
mplst     21    211 213 214
############## GB C site: Center of mass
gbcrs     212    6.654      3.128      1.776      0.022      0.826    0.062
gbsite    212    0.000      0.000      0.000      1.0    0.0    0.0
############### EMP site1
charge    211      0.000      1.315
mpsite    211      3.395736       1.156645       0.083495
############### EMP site2
charge    213      0.000      1.315
dipole    213    -1.629    -0.249    -0.758
mpsite    213      0.000      0.000      0.000
############### EMP site3
charge    214      0.000      1.315
mpsite    214    -2.008247      -1.853397       0.007080
#########################################################################
### Folic (F22) rgbtype is 22
#########################################################################
###                              Mass          Ix           IyIz
crslm    22    "F22"       119.000       98.060    488.708    585.853
gblst    22    222
mplst    22    221 223    224
################ GB site: Center of mass
gbcrs     222       7.499      2.100      5.600      0.001      1.164    17.260
gbsite    222       0.000      0.000      0.000       1.0    0.0    0.0
############### EMP site 1
charge    221       0.000      1.315
mpsite    221       2.441284       0.485587       0.095184
############### EMP site 2
charge    223       0.000      1.315
dipole    223       1.352      0.562    -0.029
mpsite    223       0.000      0.000      0.000
############### EMP site 3
charge    224       0.000      1.315
mpsite    224       -3.196645      -0.353496      -0.015172
#############################################################################
### Folic (F23) rgbtype is 23
#############################################################################
###                              Mass      Ix             IyIz
crslm     23    "F23"       176.0    307.161    806.362    1111.526
gblst     23      232
mplst     23      231 233
################ GB site: Center of mass
```

```
gbcrs     232    7.720     2.068    9.215     0.001     1.214     0.015
gbsite    232    0.000     0.000     0.000     1.0    0.0    0.0
################ EMP site 1
charge    231       0.000     1.315
mpsite    231       3.986443       0.421896       0.001667
################ EMP site 2
charge    233       0.000     1.315
dipole    233       0.991          -0.663             0.009
mpsite    233       0.000    0.000     0.000
#############################################################################
###    Cy7 (C31) rgbtype is 31
#############################################################################
###                              Mass          Ix          IyIz
crslm     31    "I31"         99.00     95.000    229.000    270.000
gblst     31    312
mplst     31    311    313
################ GB    Center of mass
gbcrs     312       6.687     2.800     3.305     0.001     0.913    0.715
gbsite    312       0.000     0.000     0.000     0.0    0.0    1.0
################ EMP site 1
charge    311       0.000     1.315
mpsite    311       0.246651       2.213083     -0.104018
################ EMP site 2
charge    313       0.000     1.315
dipole    313       0.021    -0.955       0.249
mpsite    313       0.000     0.000       0.000
#############################################################################
###    Cy7 (C32) rgbtype is 32
#############################################################################
###                              Mass          Ix          IyIz
crslm     32    "I32"         99.00     95.000    229.000    270.000
gblst     32    322
mplst     32    321    323
################ GB    Center of mass
gbcrs     322       6.982     4.050     2.281     3.245     0.702    5.845
gbsite    322       0.000     0.000     0.000     1.0    0.0    0.0
################ EMP site 1
charge    321       0.000     1.315
mpsite    321       1.078248       2.295007       0.644601
################ EMP site 2
charge    323       0.000     1.315
dipole    323       0.727          -1.353             0.362
mpsite    323       0.000     0.000     0.000
#############################################################################
```

```
###    Cy7 (C33) rgbtype is 33
################################################################################
###                              Mass          Ix          IyIz
crslm     33    "I33"        99.00     95.000    229.000    270.000
gblst     33    332
mplst     33    331    333
################ GB    Center of mass
gbcrs     332       6.982     4.050     2.281     3.245     0.702    5.845
gbsite    332       0.000     0.000     0.000     1.0    0.0    0.0
################ EMP site 1
charge    331       0.000     1.315
mpsite    331       0.247227       2.731796       0.333467
################ EMP site 2
charge    333       0.000     1.315
dipole    333       -0.505          0.856          -1.112
mpsite    333       0.000     0.000     0.000
################################################################################
###    DOSA (D41) rgbtype is 41
################################################################################
###                              Mass          Ix          IyIz
crslm     41    "D41"        83.000     68.000    200.000    200.000 Linear
gblst     41    412
mplst     41    411    413
################ GB    Center of mass
gbcrs     412       8.421     4.778     3.627     0.774     1.257    2.190
gbsite    412       0.000     0.000     0.000     0.0    0.0    1.0
################ EMP site@Center
charge    411       0.000    1.315
mpsite    411       6.816882       1.327703       0.091344
################ EMP site@Center
charge    413       0.000    1.315
dipole    413     -1.280    -0.803    -0.685
mpsite    413       0.000     0.000     0.000
################################################################################
###    Silipid (S51) rgbtype is 51
################################################################################
###                              Mass          Ix          IyIz
crslm     51    "S51"        155.000     154.408    585.922    627.056
gblst     51    512
mplst     51    511    513
################ GB    Center of mass
gbcrs     512       7.500     3.426     2.868     0.149     1.057    3.190
gbsite    512       0.000     0.000     0.000     1.0    0.0    0.0
################ EMP site@Center
```

```
charge    511     -0.000    1.315
dipole    511       0.039          -0.207           0.278
mpsite    511       1.671428       1.668688      -0.019266
################ EMP site@Center
charge    513     -0.000    1.315
dipole    513     -0.371           0.413          -0.164
mpsite    513     -2.272214     -1.361135     -0.003862
#############################################################################
###    Silipid (S52) rgbtype is 52
#############################################################################
###                              Mass          Ix          IyIz
crslm     52    "S52"         191.000       500.0      500.0      500.0    sphere
gblst     52    522
mplst     52    521
################ GB    Center of mass
gbcrs     522       4.717     4.717     1.427     1.000     1.057    1.000
gbsite    522       0.000     0.000     0.000     0.0    0.0    1.0
################ EMP site@Center
charge    521       0.000    1.315
dipole    521       0.268        0.275        -0.526
mpsite    521       0.000        0.000          0.000
################################ Bond terms ###################################
###FOL
crsbond     111     211          250.0       1.480
crsbond     214     221          250.0       1.480
crsbond     224     231          250.0       1.480
###Cy7
crsbond     111     311          250.0       1.480
crsbond     313     321          150.0       3.750
crsbond     313     331          150.0       3.750
##DOA
crsbond     111     411          250.0       1.750
##Silipid
crsbond     131     511          250.0       3.200
crsbond     511     121          250.0       4.400
crsbond     513     521          150.0       7.300
################################ Angle terms ##################################
###FOL
crsangle    112     111     211       100.0       150.0
crsangle    111     211     212       100.0       160.0
crsangle    213     214     221       100.0       130.0
crsangle    214     221     222       100.0       120.0
crsangle    223     224     231       100.0       120.0
crsangle    224     231     232       100.0       120.0
```

```
###Cy7
crsangle   112   111   311   100.0   140.0
crsangle   111   311   312   100.0   120.0
crsangle   311   312   321   100.0    90.0
crsangle   312   321   322   100.0   160.0
crsangle   311   312   331   100.0    80.0
crsangle   312   331   332   100.0   170.0
###DOA
crsangle   112   111   411   100.0   130.0
crsangle   111   411   412   100.0   110.0
##Silipid
crsangle   132   131   511   100.0   160.0
crsangle   131   511   512   100.0   120.0
crsangle   512   513   521   100.0   120.0
crsangle   512   511   121   100.0    70.0
crsangle   511   121   122   100.0   120.0
```

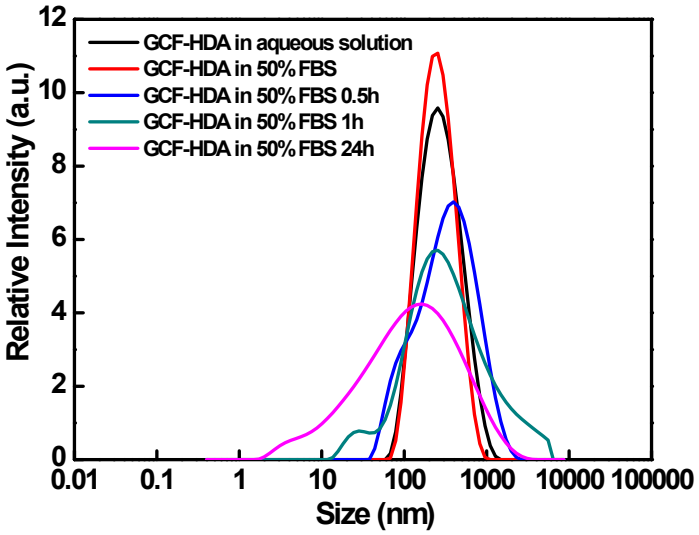


Fig. S10. Stability of liposome NPs in 50% fetal bovine serum (FBS) at different time.

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