Imaging Agents

DOI: 10.1002/anie.200905731

Targeted LipoCEST Contrast Agents for Magnetic Resonance Imaging: Alignment of Aspherical Liposomes on a Capillary Surface**

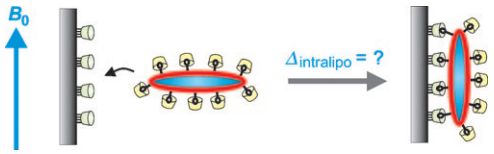
Dirk Burdinski, Jeroen A. Pikkemaat, Mustafa Emrullahoglu, Francesca Costantini, Willem Verboom, Sander Langereis, Holger Grüll, and Jurriaan Huskens*

Molecular imaging is likely to have a significant impact on healthcare through the early detection of disease on a cellular and molecular level. Among the clinical imaging modalities, magnetic resonance imaging (MRI) offers a unique combination of advantages including the recording of anatomical and contrast-enhanced images with a high spatial resolution, while avoiding the use of ionizing radiation. The use of MRI for imaging sparse molecular epitopes present on diseased cells is hampered by its low sensitivity, which can potentially be overcome with new contrast-amplifying nanocarriers.[1]

Liposomal chemical exchange saturation transfer (lipoCEST) contrast agents (CAs), which have reported detection limits in the picomolar range,[2] are particularly promising in this respect. LipoCEST CA detection is based on the selective saturation of the intraliposomal water signal with a selective radio frequency (RF) pulse. Water exchange across the liposomal membrane causes partial saturation of the bulk water signal and, as a consequence, negative contrast enhancement in the MR image.

To make lipoCEST CAs selectively addressable by the RF saturation pulse, a chemical shift agent (SA) is encapsulated in the aqueous core of the liposome, thus providing a pool of water protons with a chemical shift different from that of the bulk water protons. For in vivo applications, it is crucial to achieve large, well-defined intraliposomal chemical shifts ($\Delta_{intralipo}$), as larger shifts allow for better lipoCEST contrast enhancement, reduce the interference with background magnetization transfer effects, and allow for frequency-based multiplexing.[3]

Very large $\Delta_{intralipo}$ values are obtained upon aspherical deformation in response to osmotic shrinkage, and the additional incorporation of amphiphilic, paramagnetic lanthanide complexes within the liposomal phospholipid bilayer.[4] In this case, the direction of the chemical shift is governed by the alignment of the aspherical liposomes in the external magnetic field, which in turn is dictated by the sign of the magnetic anisotropy ($\Delta\chi$) of the incorporated amphiphilic lanthanide complex (Scheme 1).[3a,5]



Scheme 1. Reorientation with respect to an external magnetic field B_0 of aspherical (e.g., oblate) liposomes upon binding to a target surface.

The use of lipoCEST CAs in targeted probes for molecular MRI applications entails their specific binding and immobilization at the target site, for example, the surface of a biological structure or a cell. To maximize the attractive interactions, such aspherical liposomes will tend to align with the target structure. This enforced orientation may, however, be different from the magnetic alignment, which is dictated by $\Delta\chi$ (Scheme 1). As a consequence, the $\Delta_{intralipo}$ value of the bound CA may differ from that of the unbound CA. It is therefore essential to understand the interplay between the preferred magnetic and the enforced mechanical alignment of such aspherical lipoCEST CAs. Herein, we report the alignment change of such aspherical liposomes upon multivalent binding to a target surface, which was studied by using routine CEST MR methods.

The well-studied β-cyclodextrin(CD)–adamantane (Ad) model system for multivalent interactions was used to achieve the desired binding of aspherical liposomes to a glass capillary surface.[6] The inner surface of the glass capillary with an inner diameter of 100 μm was coated with a monolayer of a CD heptamine derivative. Such CD-functionalized surfaces are known to engage in specific hydrophobic interactions with multiple apolar Ad units, thus causing strong multivalent binding.[6]

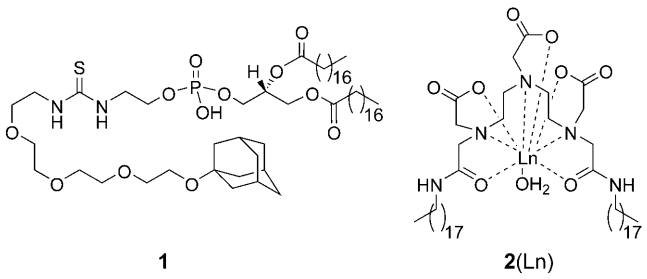
LipoCEST CAs consisting of the phospholipids distearoylphosphatidylcholine (DSPC, 40%), oleoylpalmitoylphosphatidylcholine (POPC, 20%), and an Ad-functionalized

[*] Dr. D. Burdinski, Dr. S. Langereis, Prof. H. Grüll
Department of Biomolecular Engineering, Philips Research Europe
High Tech Campus 11, 5656 AE Eindhoven (The Netherlands)
Fax: (+31) 40-27-44906
E-mail: dirk.burdinski@philips.com
Homepage: http://www.research.philips.com
Prof. H. Grüll
Department of Biomolecular Engineering
Eindhoven University of Technology (The Netherlands)
Dr. J. A. Pikkemaat
Department of Materials Analysis
Philips Research Europe (The Netherlands)
M. Emrullahoglu, Dr. F. Costantini, Dr. W. Verboom, Prof. J. Huskens
Molecular Nanofabrication Group, University of Twente
Enschede (The Netherlands)

[**] We thank H. Keizer, H. Janssen (SyMO-Chem, The Netherlands), and B. Schmitt (Philips Research) for synthesis support and Marcel Verheijen (Philips Research) for cryo-TEM analyses. Financial support by the Dutch government (project code: BSIK 03033 "Molecular Imaging of Ischemic Heart Disease") is gratefully acknowledged. lipoCEST = liposomal chemical exchange saturation transfer.

Supporting information for this article is available on the WWW under http://dx.doi.org/10.1002/anie.200905731.

DSPE derivative (**1**, 20%; DSPE = distearoylphosphatidylethanolamine), were synthesized. The lipid bilayer further comprised an amphiphilic lanthanide complex **2**(Ln) (20%),



1 **2**(Ln)

where Ln is Dy^{3+} ($\Delta\chi < 0$) or Tm^{3+} ions ($\Delta\chi > 0$). Because of the opposing signs of the magnetic anisotropy values, aspherical lipoCEST CAs bearing **2**(Dy) were expected to exhibit an orthogonal magnetic alignment with respect to those bearing **2**(Tm).[4] The intraliposomal water phase further contained a SA **3**(Ln) ([Ln(hpdo3a)(H_2O)],[7] Ln = Dy or Tm, $c = 65$ mM). The 200 nm sized liposomes were aspherically deformed by dialysis against a HEPES/CD/NaCl buffer solution (300 mOsm, pH 7.4; HEPES = 4-(2-hydroxyethyl)-1-piperazineethanesulfonic acid) to yield predominantly oblate (lens-shaped) spheroids, as shown by cryo-TEM studies (Figure 1).

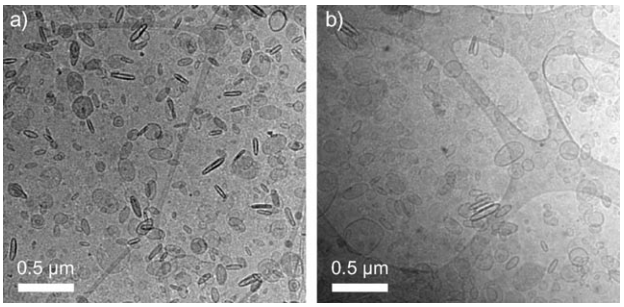


Figure 1. Cryo-TEM images of aspherically deformed lipoCEST CAs showing predominantly oblate (lens-shaped) spheroids: a) **2**(Dy)–**3**(Tm), b) **2**(Tm)–**3**(Dy).

A CD-modified capillary was filled with a solution of oblate **2**(Dy)–**3**(Tm) liposomes (see Figure S1 in the Supporting Information), mounted coaxially in a standard NMR tube, and aligned parallel to the B_0 field of a 7 T NMR spectrometer (A, Figure 2). The bulk water signal intensity was recorded as a function of the presaturation frequency (the Z spectrum). From those data, the amount of saturation transfer (% CEST) was calculated (see Equation S1 in the Supporting Information) and plotted as a function of the respective chemical shift values (Figure 3a, black squares). The appearance of a CEST peak in the positive chemical shift region (+11 ppm) was in accordance with reported data for comparable **2**(Dy)-based ($\Delta\chi < 0$) lipoCEST agents.[4] For agents in bulk solution, a parallel alignment of the longer particle axis with B_0 ($b_\parallel$) had been deduced by comparison

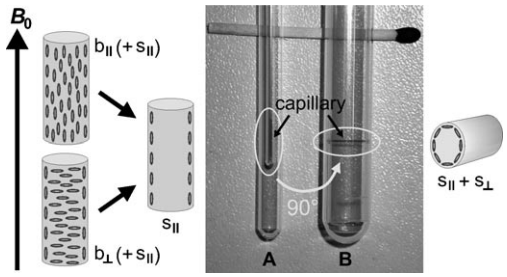


Figure 2. Parallel ($\parallel$) and perpendicular ($\perp$) alignment of oblate lipoCEST CAs with respect to the B_0 field in bulk solution (b) and at the capillary surface (s). Bulk liposomes were removed by flushing with buffer solution. Photo: capillaries in NMR tubes with fixed orientations $\parallel$ (A) and $\perp$ (B) with respect to B_0.

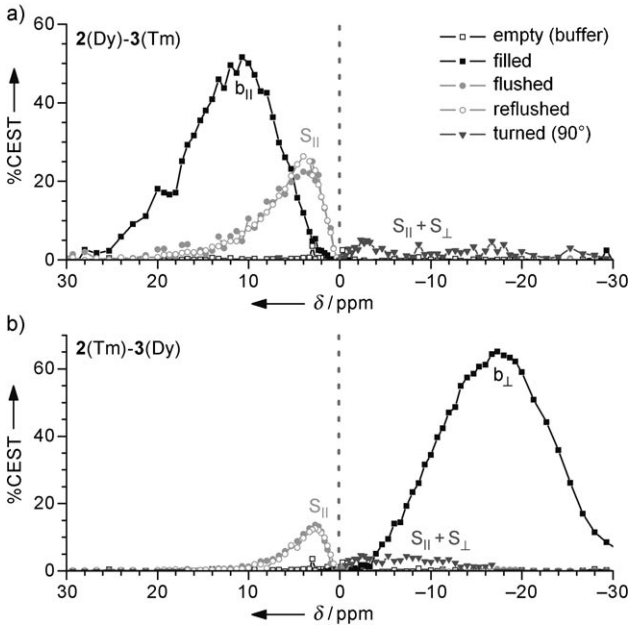


Figure 3. CEST MR spectra of capillaries loaded with two liposome formulations: a) **2**(Dy)–**3**(Tm) or b) **2**(Tm)–**3**(Dy). Capillaries (originally oriented parallel with B_0) were filled, flushed with buffer, and turned through 90°.

with respective **2**(Gd)-based ($\Delta\chi = 0$) liposomes, which are naturally aligned parallel with the external field.

To test the orientation of the surface-bound liposomes, the capillary was flushed with buffer solution to remove all non-surface-bound particles. In the flushed capillary, a CEST effect larger than 20% was still measured with $\delta > 0$ (Figure 3a, light gray dots), which remained stable even after another extensive flushing step (light gray circles). We ascribed this CEST effect to the presence of surface-bound liposomes, which were assumed to be aligned parallel with the capillary surface to maximize the number of attractive Ad–CD interactions.[6] Because the capillary surface was aligned parallel with B_0, the same orientation was to be assumed for the surface-bound liposomes ($s_\parallel$). Thus, the positive chemical shift of the observed CEST effect in both experiments indicated, as expected, that the liposomes at the surface, as well as those in the bulk, were aligned parallel with B_0.

We challenged this hypothesis by turning the capillary by 90° (B, Figure 2), thus orienting half of the capillary surface essentially perpendicular to B_0 ($s_\parallel + s_\perp$). The observed CEST effect vanished on the positive chemical shift side and a small overall CEST effect was observed at $\delta < 0$ spread over a large chemical shift range (Figure 3a, dark gray triangles). This effect can be understood by taking into account the circular capillary cross-section that effectively aligns parts of the capillary surface in all orientations between 0 and 90° with respect to B_0 (Figure 2), thus canceling out most of the CEST effect (calculated by Equation S1 in the Supporting Information).

In the next step, liposomes were prepared by exchanging the lanthanide ions Dy ($\Delta\chi < 0$) and Tm ($\Delta\chi > 0$) in **2** and **3**. The CEST effect of the bulk solution of these **2**(Tm)–**3**(Dy) liposomes in a CD-coated capillary was then observed on the chemical shift side opposite (negative) to **2**(Dy)–**3**(Tm) (Figure 3b, black squares), as the sign switch of the magnetic anisotropy of **2**(Ln) was expected to orient these liposomes perpendicular to those previously measured. Hence, **2**(Tm)–**3**(Dy) liposomes in bulk solution were hypothesized to be oriented perpendicular to B_0 ($b_\perp$). To prove this hypothesis, all bulk liposomes were removed by flushing the capillary with buffer, whereupon a residual stable CEST effect was observed at $\delta > 0$ (light gray dots and circles) in the A orientation, whereas the CEST signal vanished in the B orientation, thus giving rise to a small overall CEST effect at $\delta < 0$ (dark gray triangles).

In conclusion, the orientation induced by the binding of aspherical liposomes to a target surface could be determined by using routine CEST MR methods. In bulk solution, oblate (lens-shaped) **2**(Dy)–**3**(Tm) and **2**(Tm)–**3**(Dy) liposomes had a $b_\parallel$ and a $b_\perp$ orientation, respectively, with respect to the external magnetic field B_0. Upon multivalent binding through their Ad head groups, however, both types of liposomes were aligned parallel with the CD-modified capillary surface and they maintained this local surface alignment independent of the capillary orientation in the B_0 field. Hence, in this strong binding situation, the enforced mechanical alignment of these lipoCEST CAs outweighed the preferred magnetic orientation in determining the MR properties of the liposomes.

Aspherical lipoCEST CAs therefore offer unique opportunities in molecular MRI applications as bound and unbound CAs may be discriminated based on their different CEST resonance frequencies, if multivalent binding occurs at suitably oriented target surfaces. It may prove to be of greater practical value, however, that the chemical shift of the intraliposomal water for target-bound multivalent agents becomes a direct measure for the orientation of the target surface. After removal of all unbound lipoCEST CAs by natural excretion, the observed CEST signal should therefore be sensitive to the orientation of target structures, such as membranes, vessel walls, or nerve fibers, with the potential for providing additional anatomical information.

Experimental Section

Glass capillaries (100 μm diameter; 10 cm long) were modified with a coating of a CD derivative as described earlier for microfluidic chips.[8] Experimental details of the modification, loading, and CEST MR studies of the glass capillaries along with synthetic procedures are given in the Supporting Information.

Received: October 12, 2009
Published online: February 12, 2010

Keywords: chemical exchange saturation transfer (CEST) · diagnostic agents · liposomes · multivalency · NMR imaging

[1] a) M. Atri, *J. Clin. Oncol.* **2006**, *24*, 3299–3308; b) W. J. M. Mulder, G. J. Strijkers, E. Vucic, D. P. Cormode, K. Nicolay, Z. A. Fayad, *Top. Magn. Reson. Imaging* **2007**, *18*, 409–417.

[2] a) D. Delli Castelli, E. Gianolio, S. Geninatti Crich, E. Terreno, S. Aime, *Coord. Chem. Rev.* **2008**, *252*, 2424–2443; b) A. D. Sherry, M. Woods, *Annu. Rev. Biomed. Eng.* **2008**, *10*, 391–411; c) S. Langereis, J. Keupp, J. L. J. van Velthoven, I. H. C. de Roos, D. Burdinski, J. A. Pikkemaat, H. Grüll, *J. Am. Chem. Soc.* **2009**, *131*, 1380–1381; d) S. Aime, D. Delli Castelli, S. Geninatti Crich, E. Gianolio, E. Terreno, *Acc. Chem. Res.* **2009**, *42*, 822–831; e) S. Aime, D. Delli Castelli, E. Terreno, *Angew. Chem.* **2005**, *117*, 5649–5651; *Angew. Chem. Int. Ed.* **2005**, *44*, 5513–5515.

[3] a) E. Terreno, C. Cabella, C. Carrera, D. Delli Castelli, R. Mazzon, S. Rollet, J. Stancanello, M. Visigalli, S. Aime, *Angew. Chem.* **2007**, *119*, 984–986; *Angew. Chem. Int. Ed.* **2007**, *46*, 966–968; b) E. Terreno, D. Delli Castelli, L. Milone, S. Rollet, J. Stancanello, E. Violante, S. Aime, *Contrast Media Mol. Imaging* **2008**, *3*, 38–43.

[4] D. Delli Castelli, E. Terreno, C. Carrera, G. B. Giovenzana, R. Mazzon, S. Rollet, M. Visigalli, S. Aime, *Inorg. Chem.* **2008**, *47*, 2928–2930.

[5] a) E. Terreno, D. Delli Castelli, E. Violante, H. M. H. F. Sanders, N. A. J. M. Sommerdijk, S. Aime, *Chem. Eur. J.* **2009**, *15*, 1440–1448; b) S. C.-K. Chu, Y. Xu, J. A. Balschi, C. S. Springer, Jr., *Magn. Reson. Med.* **1990**, *13*, 239–262.

[6] M. J. W. Ludden, D. N. Reinhoudt, J. Huskens, *Chem. Soc. Rev.* **2006**, *35*, 1122–1134.

[7] H_3hpdo3a = 10-(2-hydroxypropyl)-1,4,7,10-tetraazacyclododecane-1,4,7-triacetic acid.

[8] M. J. W. Ludden, X. Y. Ling, T. Gang, W. P. Bula, H. J. G. E. Gardeniers, D. N. Reinhoudt, J. Huskens, *Chem. Eur. J.* **2008**, *14*, 136–142.

