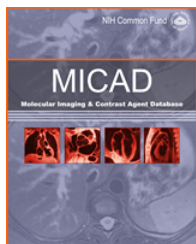


**NLM Citation:** Zhang H. [(Biotin-Ala-Ser-Lys-Lys-Pro-Lys-Arg-Asn-Ile-Lys-Ala)<sub>4</sub>-dendrimer]-streptavidin-[biotin-gadolinium 1,4,7-tris(carboxymethyl)-10-(2'-hydroxypropyl)-1,4,7,10-tetraazacyclododecane-loaded apoferritin] . 2007 Dec 27 [Updated 2008 Feb 4]. In: Molecular Imaging and Contrast Agent Database (MICAD) [Internet]. Bethesda (MD): National Center for Biotechnology Information (US); 2004-2013.

**Bookshelf URL:** <https://www.ncbi.nlm.nih.gov/books/>



## [(Biotin-Ala-Ser-Lys-Lys-Pro-Lys-Arg-Asn-Ile-Lys-Ala)<sub>4</sub>-dendrimer]-streptavidin-[biotin-gadolinium 1,4,7-tris(carboxymethyl)-10-(2'-hydroxypropyl)-1,4,7,10-tetraazacyclododecane-loaded apoferritin]

C3d-SA-GdAF

Huiming Zhang, PhD<sup>1</sup>

Created: December 27, 2007; Updated: February 4, 2008.

<b>Chemical name:</b>	[(Biotin-Ala-Ser-Lys-Lys-Pro-Lys-Arg-Asn-Ile-Lys-Ala) <sub>4</sub> -dendrimer]-streptavidin-[biotin-gadolinium 1,4,7-tris(carboxymethyl)-10-(2'-hydroxypropyl)-1,4,7,10-tetraazacyclododecane-loaded apoferritin]	
<b>Abbreviated name:</b>	C3d-SA-GdAF	
<b>Synonym:</b>		
<b>Agent category:</b>	Cage molecule	
<b>Target:</b>	Neural cell adhesion molecule (NCAM)	
<b>Target category:</b>	Adhesion molecule	
<b>Method of detection:</b>	Magnetic Resonance Imaging (MRI)	
<b>Source of signal/contrast:</b>	Gadolinium	
<b>Activation:</b>	No	
<b>Studies:</b>	<ul style="list-style-type: none"> <li><i>In vitro</i></li> <li>Rodents</li> </ul>	No structure is available in <a href="#">PubChem</a> .

## Background

[PubMed]

The neural cell adhesion molecule (NCAM) is a cell surface glycoprotein that belongs to the immunoglobulin (Ig) super-family of cell adhesion molecules (CAMs) (1). One of its critical functions in embryogenesis, neural plasticity, tumor progression, and oncogenesis is to mediate cell-cell adhesion through homophilic NCAM binding (2). The extracellular part of NCAM consists of five Ig homology modules and two fibronectin type III (F3) modules (3). Evidence demonstrates that the homophilic interaction is mediated by a double reciprocal

interaction between the IgI and IgII modules of two NCAM molecules (3). This homophilic NCAM–NCAM binding originates from a stretch of ~10 amino acids in the modules with a dissociation constant ( $K_d$ ) of 55  $\mu\text{M}$  (1, 4). Many short peptides have been synthesized to explore the mechanism of this homophilic binding. For instance, C3 peptide, which contains a sequence of 11 amino acids (Ala-Ser-Lys-Lys-Pro-Lys-Arg-Asn-Ile-Lys-Ala (ASKKPKRNIKA)), is an NCAM IgI ligand selected from a combinatorial library of synthetic peptides (4). C3d is a C3 peptide dendrimer formed by coupling four C3 peptides to a lysine dendrimer. As a multimeric ligand, C3d has an increased potency for binding to the NCAM IgI module ( $K_d \sim 10 \mu\text{M}$ ) compared to the monomer C3 peptide (4, 5). C3d can effectively disrupt NCAM-mediated cell adhesion, trigger intracellular signal cascades *in vitro*, and significantly modulate synaptic function *in vivo* (5).

[(Biotin-Ala-Ser-Lys-Lys-Pro-Lys-Arg-Asn-Ile-Lys-Ala)<sub>4</sub>-dendrimer]-streptavidin-[biotin-gadolinium 1,4,7-tris(carboxymethyl)-10-(2'-hydroxypropyl)-1,4,7,10-tetraazacyclododecane-loaded apoferritin] (C3d-SA-GdAF) is a magnetic resonance imaging (MRI) contrast agent used to image NCAM *in vivo* (6). C3d-SA-GdAF consists of three components, a biotinylated Gd-loaded apoferritin, a streptavidin, and a biotinylated C3d dendrimer (C3d-Bio). Streptavidin is tetrameric protein (molecular mass, 53 kDa) secreted from streptomyces bacteria and can provide four tightly noncovalent binding sites to biotin (molecular mass, 244 Da) with an extraordinarily high binding affinity ( $K_d \approx 10^{-15}$ – $10^{-16}$  M) (7). The bond formed between streptavidin and biotin is a rapid, irreversible process that is unaffected by most extremes of pH, organic solvents, and denaturing reagents (8). Because only the bicyclic ring of biotin is recognized by the streptavidin, the carboxyl group in the biotin side chain is modified chemically to obtain reactive biotinyl derivatives (9). Proteins are generally biotinylated *via* amino groups by using an N-hydroxysuccinimide ester (NHS-ester) of a biotin analog. The steric hindrance between the biotin and the protein is normally reduced by adding an extra spacer arm, as found in the biotinyl agent NHS-LC-biotin (10). In C3d-SA-GdAF, both the peptide C3d (as a ligand of NCAM) and the Gd-loaded apoferritin (as a MRI contrast enhancer) are biotinylated. Streptavidin serves as a linker between the two biotinylated molecules to generate a NCAM-specific MRI contrast agent (6).

The Gd-loaded apoferritin is obtained by the entrapment of small Gd chelates inside an apoferritin molecule (11). Gd-1,4,7-Tris(carboxymethyl)-10-(2'-hydroxypropyl)-1,4,7,10-tetraazacyclododecane (HPDO3A) is a neutral, highly hydrophilic contrast agent for clinical MRI. The coordination cage of Gd-HPDO3A is very tight ( $\log k_f = 23.8$ ) over a fairly large pH range and contains one structural water ( $q = 1$ ) (11, 12). Apoferritin is a spherical protein with a shell of 12.5 nm and an empty central cavity of 7–8 nm (13). There are 14 channels 3–4 Å in diameter that are formed at the intersections of 24 subunits. These channels connect the outside of the apoferritin with its interior and allow the external molecules to access the inner cavity (13). Molecules such as water or that have a diameter <7 Å can diffuse through the channels but not the Gd-HPDO3A chelates (11). Because apoferritin can be dissociated into subunits at low pH (2.0) and the subunits can be reconstituted back to the apoferritin at a high pH (8.5), Gd-HPDO3As are trapped in the apoferritin cavity *via* this dissociation/reconstitution process (11, 13). Once trapped in the apoferritin cavity, Gd-HPDO3A exhibits a 20-fold increase in  $T_1$  relaxivity compared to the untrapped Gd-HPDO3A (11). This is the highest relaxivity reported thus far, and it has been attributed to the reduction in the effective rotational correlation time of the chelates and the strong dipolar interactions with the exchangeable protons on the protein surface in the proximity of paramagnetic chelates (11). The inner cavity of apoferritin possesses a large surface that can provide amplified dipolar interactions. In addition, as an endogenous protein, apoferritin causes no immune response and provides high stability for easy biotinylation of surface amino groups (6).

## Synthesis

[PubMed]

C3d-SA-GdAF was obtained by mixing biotinylated Gd-loaded apoferritin, streptavidin, and biotinylated peptide dendrimer C3d (6). Aime et al. described the details of preparing Gd-loaded apoferritin (11).

Apoferitin at a concentration of 0.01 mM was dissociated into 24 subunits by lowering the pH to 2 and maintaining for 15 min. Then 0.1 M Gd-HPDO3A was added to the protein solution and the pH was adjusted to 7.4. The resulting solution was stirred at room temperature for ~2 h to allow for sufficient entrapment of Gd chelates. Then the solution was dialyzed to remove the untrapped Gd-HPDO3A. Crich et al. reported the details of the biotinylation step (6). The biotinylation of Gd-loaded apoferritin was accomplished with the use of [N-(+)-biotinyl-6-aminocaproic acid N-succinimidyl ester (NHS-LC-biotin) according to standard protein modification protocols. Each apoferritin contained  $5 \pm 1$  biotin residues as determined with the 2-(4'-hydroxyazobenzene) benzoic acid (HABA) colorimetric assay, as well as 8–10 molecules of Gd-HPDO3A as determined by inductively coupled plasma–mass spectrometer (ICP-MS). Crich et al. also detailed the synthesis of C3d-Bio (6). The peptide dendrimer C3d was obtained by standard Fmoc strategy with an Fmoc-Lys(Fmoc)-OH core and an Fmoc-PAL-PEG-PS resin. The biotinylation step was completed in solid phase using D-biotin and *N,N,N',N'*-tetramethyl-O-(7-azabenzotriazol-1-yl)uronium hexafluorophosphate (HTAU)/*N,N'*-diisopropylethylamine (DIPEA). The final product, C3d-Bio, was obtained with 60% yield. Four biotin residues per C3d dendrimeric peptide were found with HABA colorimetric assay.

## In Vitro Studies: Testing in Cells and Tissues

[PubMed]

The *in vitro* binding experiments for C3d-Bio were tested in tumor-derived endothelial cells (TEC), which is a NCAM-positive cell line (2). The C3d-Bio demonstrated a dose-dependent binding specificity on the basis of cytofluorimetric analysis of phycoerythrin (PE)-conjugated streptavidin. The *in vitro* binding experiments for C3d-SA-GdAF were conducted in TEC again (6). C3d-Bio peptide, streptavidin, and Gd-loaded apoferritin were sequentially added to TEC at a molar ratio of 20:1:1 at 20°C, with a 20-min time interval between the addition of each component. The amount of Gd bound to cells was measured by ICP-MS. The  $T_1$  relaxivity was found to be  $\sim 70 \pm 10 \text{ mM}^{-1} \cdot \text{s}^{-1}$  at 0.47 T and 25°C. In comparison, the  $T_1$  relaxivity for Gd-HPDO3A was  $3.7 \pm 0.1 \text{ mM}^{-1} \cdot \text{s}^{-1}$ . The Gd-loaded apoferritin exhibited a relaxivity hump at ~35 MHz in its  $T_1$  nuclear magnetic resonance dispersion (NMRD) profile, demonstrating slow tumbling of the Gd chelates. For the Gd-loaded apoferritin-bound TEC, the  $T_1$  relaxivity appeared to have the same bell shape as the unbound apoferritin in the NMRD profile. MR images of the Gd-loaded apoferritin-bound TEC were acquired with a 7-T imager, which appeared to be hyperintense compared to control cells.

## Animal Studies

### Rodents

[PubMed]

The *in vivo* study was conducted in severe combined immunodeficient (SCID) mice with implanted TEC (6). First, 20  $\mu\text{mol/kg}$  C3d-Bio was injected; 45 min later, 1  $\mu\text{mol/kg}$  streptavidin and 1  $\mu\text{mol/kg}$  Gd-loaded apoferritin were injected together. Each streptavidin molecule had three binding sites available for binding the biotin residues of C3d-Bio. Six days after implantation,  $T_1$ -weighted images were collected with a 7-T imager before and 10.0 min, 5 h, 24 h, and 48 h after the contrast administration. Signal intensity (SI) enhancement was found to be  $\geq 30\%$  in the tumor after 5 h, and this decreased to 20% after 24 h. The SI enhancements in the liver, kidneys, and muscle were 85%, 30%, and 3%, respectively, after 5 h, indicating that the Gd complex remained inside the apoferritin cavity. The modified apoferritin was absorbed by the hepatocytes as the native protein and followed the protein elimination pathways through the liver uptake. In comparison, Gd-HPDO3A was excreted only through the kidneys. The *in vivo* binding of C3d-SA-GdAF at TEC-neoformed vessels was confirmed by immunofluorescence imaging after *in vivo* administration of fluorescein isothiocyanate (FITC)-labeled C3d-SA-GdAF.

## Other Non-Primate Mammals

[PubMed]

No publication is currently available.

## Non-Human Primates

[PubMed]

No publication is currently available.

## Human Studies

[PubMed]

No publication is currently available.

## References

1. Kiselyov V.V., Soroka V., Berezin V., Bock E. Structural biology of NCAM homophilic binding and activation of FGFR. *J Neurochem.* 2005;94(5):1169–79. PubMed PMID: 16045455.
2. Bussolati B., Grange C., Bruno S., Buttiglieri S., Deregibus M.C., Tei L., Aime S., Camussi G. Neural-cell adhesion molecule (NCAM) expression by immature and tumor-derived endothelial cells favors cell organization into capillary-like structures. *Exp Cell Res.* 2006;312(6):913–24. PubMed PMID: 16406048.
3. Ronn L.C., Olsen M., Soroka V. O.S. S, S. Dissing, F.M. Poulsen, A. Holm, V. Berezin, and E. Bock, Characterization of a novel NCAM ligand with a stimulatory effect on neurite outgrowth identified by screening a combinatorial peptide library. *Eur J Neurosci.* 2002;16(9):1720–30. PubMed PMID: 12431225.
4. Ronn L.C., Olsen M., Ostergaard S., Kiselyov V., Berezin V., Mortensen M.T., Lerche M.H., Jensen P.H., Soroka V., Saffell J.L., Doherty P., Poulsen F.M., Bock E., Holm A. Identification of a neuritogenic ligand of the neural cell adhesion molecule using a combinatorial library of synthetic peptides. *Nat Biotechnol.* 1999;17(10):1000–5. PubMed PMID: 10504702.
5. Kiryushko D., Kofoed T., Skladchikova G., Holm A., Berezin V., Bock E. A synthetic peptide ligand of neural cell adhesion molecule (NCAM), C3d, promotes neuritogenesis and synaptogenesis and modulates presynaptic function in primary cultures of rat hippocampal neurons. *J Biol Chem.* 2003;278(14):12325–34. PubMed PMID: 12502709.
6. Geninatti Crich S., Bussolati B., Tei L., Grange C., Esposito G., Lanzardo S., Camussi G., Aime S. Magnetic resonance visualization of tumor angiogenesis by targeting neural cell adhesion molecules with the highly sensitive gadolinium-loaded apoferritin probe. *Cancer Res.* 2006;66(18):9196–201. PubMed PMID: 16982763.
7. Laitinen O.H., Hytonen V.P., Nordlund H.R., Kulomaa M.S. Genetically engineered avidins and streptavidins. *Cell Mol Life Sci.* 2006;63(24):2992–3017. PubMed PMID: 17086379.
8. Laitinen O.H., Nordlund H.R., Hytonen V.P., Kulomaa M.S. Brave new (strept)avidins in biotechnology. *Trends Biotechnol.* 2007;25(6):269–77. PubMed PMID: 17433846.
9. Wilchek M., Bayer E.A. The avidin-biotin complex in bioanalytical applications. *Anal Biochem.* 1988;171(1):1–32. PubMed PMID: 3044183.
10. Diamandis E.P., Christopoulos T.K. The biotin-(strept)avidin system: principles and applications in biotechnology. *Clin Chem.* 1991;37(5):625–36. PubMed PMID: 2032315.
11. Aime S., Frullano L., Geninatti Crich S. Compartmentalization of a gadolinium complex in the apoferritin cavity: a route to obtain high relaxivity contrast agents for magnetic resonance imaging. *Angew Chem Int Ed Engl.* 2002;41(6):1017–9. PubMed PMID: 12491298.

12. Girard E., Chantalat L., Vicat J., Kahn R. Gd-HPDO3A, a complex to obtain high-phasing-power heavy-atom derivatives for SAD and MAD experiments: results with tetragonal hen egg-white lysozyme. *Acta Crystallogr D Biol Crystallogr.* 2002;58(Pt 1):1–9. PubMed PMID: 11752774.
13. Webb B., Frame J., Zhao Z., Lee M.L., Watt G.D. Molecular entrapment of small molecules within the interior of horse spleen ferritin. *Arch Biochem Biophys.* 1994;309(1):178–83. PubMed PMID: 8117106.