The extracellular side of the plasma membrane of many mammalian cells display as many as \(10^9\text{--}10^{10}\) protein thiol groups per single cell, the exact number being dependent upon the redox conditions of the extracellular milieu. Exofacial Protein Thiols (EPTs) are at a maximum under reducing conditions (such as hypoxia), whereas they are partially transformed into disulfide, S-thiolated, S-nitroso, or sulphenic forms under oxidizing conditions. Then, Exofacial Protein Thiols (EPTs) can be thought of as a target for imaging procedures aimed at visualizing the redox state of the extracellular microenvironment, which may be in turn indicative of patho-physiological alterations. For instance, malignant tumors are characterized by hypoxic conditions leading to a reducing extracellular microenvironment, that supports tumor growth. EPTs in the reduced form are quite reactive and can be chemically labeled by a suitably designed MRI contrast agent (compound GdL1A).

This contrast agent is composed of a GdDO3A based structure, a 2-pyridylidithio function for the recognition of EPTs and a flexible spacer connecting them. Compound GdL1A can react with cell EPTs to form a disulfide bridged adduct. Processing of this adduct by the cell machinery ultimately results into the internalization of substantial amounts of the GdDO3A complex. In vitro labelling experiments with human myeloid leukemia K562 cells and murine melanoma B16 cells showed high levels of EPTs-mediated gadolinium uptake. Moreover, the extent of Gd uptake is proportional to the concentration of free EPTs on the cell membrane, showing that the contrast agent is responsive to the redox state of EPTs. MR imaging of EPTs has been finally performed in an animal tumor model obtained by inoculating about 1 milion of B16 melanoma cells subcutaneously in B57Bl/6 mice. Mice injected with GdL1A showed a significant signal enhancement in the tumor region with respect to mice treated with control (unfunctionalized) GdDO3A. Wash-out kinetics of GdL1A from the tumor were consistent with the internalization of the contrast agent by tumor cells.