High-spin complexes of Gd(III) and Mn(II) were introduced as polarizing agents (PAs) for solid-state dynamic nuclear polarization (DNP) in 2011. This dissertation was undertaken in 2013, with the intention of exploring these PAs further. Major goals of this work were to understand their DNP mechanism(s) and explore their application in biomolecular research. This cumulative thesis details the methods, advantages, and practical implications of using high-spin PAs for MAS DNP. Data from electron paramagnetic resonance (EPR) and NMR spectroscopy are discussed for a complete understanding of DNP mechanisms.

Out of the two main mechanisms − solid effect (SE) and cross effect (CE − active under experimental conditions of solid-state DNP, commonly used nitroxide PAs evoke CE owing to their broad EPR spectra. On the other hand, DNP mechanisms evoked by high-spin metal ions seem non-trivial due to additional features (originating from spin-orbit coupling or zero field splitting) in their EPR spectra. The features of the EPR signal generally influence the shape of enhancement profiles. Therefore, the metal ion with a simpler EPR signal i.e., Gd(III) , is chosen as the starting point for the investigation of DNP mechanisms. Varying concentrations (2, 10, 20 mM) of a water-soluble and stable complex Gd-DOTA was dissolved as the PA in a glycerol-water solution of ^{13}C,^{15}N - urea. Field profiles of DNP enhancement on each nuclear type (^{1}H, ^{13}C, and ^{15}N) establishes SE as the active DNP mechanism at the smallest PA concentration (2 mM). This confirms the theoretical predictions that narrow line width of the Gd(III) EPR signal arising from the central transition (CT, m_s = -1/2 → +1/2) allows for resolved SE DNP. However, that is no longer the case at higher PA concentrations of 10 and 20 mM. At higher Gd(III) concentrations, the CE mechanism contributes significantly and varies with nuclear Larmor frequency (ω_n) of the concerned nuclei. The enhancement maxima shifts towards the EPR resonance as the contribution from CE increases. This shift is evident in the field profiles of ^{15}N and ^{13}C, whereas that of ^{1}H is least influenced. This observation can be explained by combining theoretical estimates with the experimental data; the CE is evoked by increased dipolar coupling (D_{ee}) – a prerequisite for CE – between neighboring Gd(III) spins as the statistical inter-spin distance shortens at elevated concentrations. This finding is important because the knowledge of active DNP mechanisms is essential for accurate interpretation of results from DNP experiments.

From the experiments on Gd-DOTA it becomes clear that concentration, inter-spin distances, and hence induced D_{ee} are intertwined. In order to explicitly address the influence of inter-spin distances on DNP mechanisms we started a collaboration with the group of Adelheid Godt (Bielefeld). In this collaborative project, bis-complexes of the type Gd(III)-spacer-Gd(III) with variable spacer lengths were investigated. These PAs provided an excellent model system where the influence of only inter-spin distances can be determined for a fixed Gd(III) concentration. A small PA concentration of 4 mM is used to ensure absence of significant inter-molecular dipolar interactions. A mono-Gd complex of similar geometry and chemistry is taken as a reference for SE DNP.

The mono-Gd complex yields enhancements arising from SE as expected from negligible inter-molecular D_{ee}. The contribution of CE increases as the inter-spin distances between Gd(III) ions become shorter going from 3.4 nm → 2.1 nm → 1.4 nm → 1.2 nm due to corresponding increase in D_{ee}. The extent of CE on ω_n follows the same trend as for Gd-DOTA. Highest CE contribution is observed on nuclei with the smallest ω_n ^{15}N because smaller ω_n approaches the width of the EPR signal, this is an additional requirement for CE DNP.

The field position for maximum DNP enhancement corresponding to Gd-DOTA, is used for DNP experiments on Ubiquitin with an attached Gd-tag as PA. The success of DNP on this sample illustrates the possibility of site-directed DNP with metal ions tags as PAs. As a perspective Gd-

tags can be used to examine change in conformation of a protein that would give higher enhancements due to CE if two Gd(III) labeled domains are closer in space. In a separate project, Mn(II) (s=5/2) bound to the divalent site of a hammerhead ribozyme was used as a PA which resulted in the first demonstration of intra-complex DNP using an intrinsically bound metal ion PA.