Electronic Supplementary Material (ESI) for Physical Chemistry Chemical Physics

Supporting Information

Dynamic Nuclear Polarization of Spherical Nanoparticles

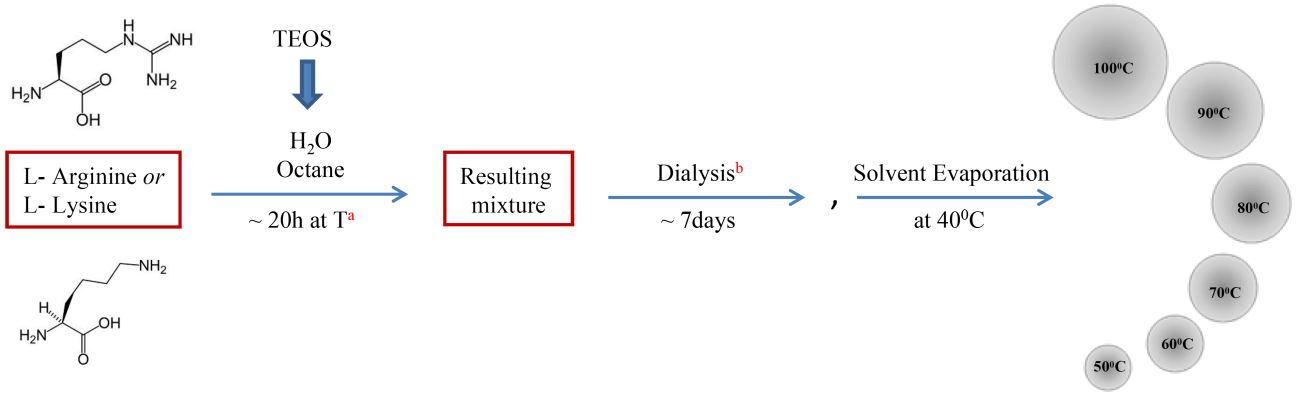
Ümit Akbey[1]*, Burcu Altin[2], Arne Linden[1], Serdar Özcelik[3], Michael Gradzielski[2], Hartmut Oschkinat[1]

[1] Leibniz-Institut für Molekulare Pharmakologie (FMP), NMR Supported Structural Biology, Robert-Rössle-Str. 10, 13125 Berlin, Germany. [2] Technische Universität Berlin, Institut für Chemie, Straße des 17. Juni 124, 10623 Berlin, Germany. [3] Izmir Institute of Technology, Department of Chemistry, 35430 Gülbahce, Urla, Izmir, Turkey.

Supplementary Figure 1.

1a.

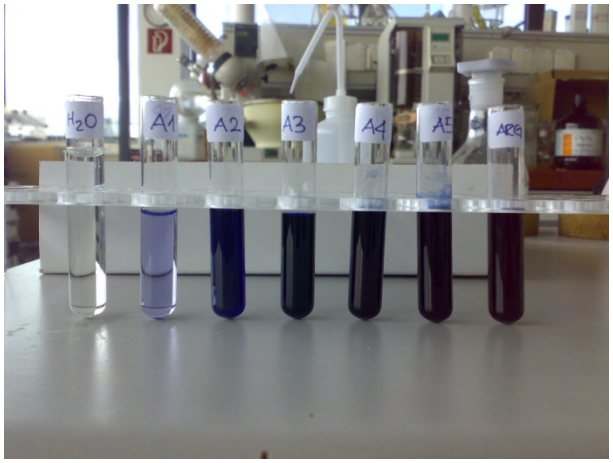
Silica nanoparticles were prepared by the sol-gel process on the basis of well-known Stöber method which is performed by the hydrolysis and condensation of tetraethyl orthosilicate (TEOS) as a silica source, in a mixture of ethanol/ water and with ammonia solution as a catalyst to initiate the reaction.[1-3] We produced both pure (no aminoacid added) and arginine/lysine functional silicon-oxide nanoparticles. We report the preparation of silica nanoparticles with different particle sizes which were carried out in the presence of basic amino acids (L-Lysine/ L-Arginine) as a catalyst, instead of ammonium hydroxide, and at different reaction temperatures.[1] The amino acids arginine/lysine incorporated into the silica nanoparticles (SiNPs) during the growth process that is catalyzed by their presence. The purification was done by dialyzing all the synthesized nanoparticles as silica dispersions, to remove unreacted reagents (L-Lysine/ L-Arginine, TEOS, by-products) using dialysis membranes with a molecular-weight cut-off at 14 kDa against water. Dialysis was carried for 1 week by refreshing Milli-Q water twice per day. The pure nanoparticle (without arginine/lysine: particle with the 100 nm particle size (N-100)) is prepared similar to the nanoparticles containing arginine/lysine as shown below, but in the absence of amino acids and catalyzed by ammonium hydroxide.



[a] **T**, in the range between 100°C- 50°C

[b] The resulting particles were dialyzed one week.

The Ninhydrin-Test was used to test the presence of amino acids at the surfaces of prepared spherical nanoparticles. The text indicates the presence or absence of ammonia/amines in the test material. It can be used qualitatively (e.g. for chromatographic visualization) or quantitatively (e.g. for peptide sequencing). The qualitative determination of the aminoacids on the surfaces is sufficient for our studies. The α-amino acids typically give a blue-purple product, which indicates the presence of these amines. As it can be seen from the figure below, the Ninhydrin-Test done on the solutions of arginine containing nanoparticles resulted in blue-color. The first test-tube in the figure is water, and then A1-A5 indicates the solutions with silica nanoparticles containing arginine with increasing amounts. The last test-tube contains only a solution of pure arginine. The Ninhydrin-Test was done after an intensive dialysis of the particles for around a week, so that there should not be any remaining unreacted arginine in the solutions. As a result, the results are qualitative indication of the presence of the arginine amino acids on the surfaces on the nanoparticles.

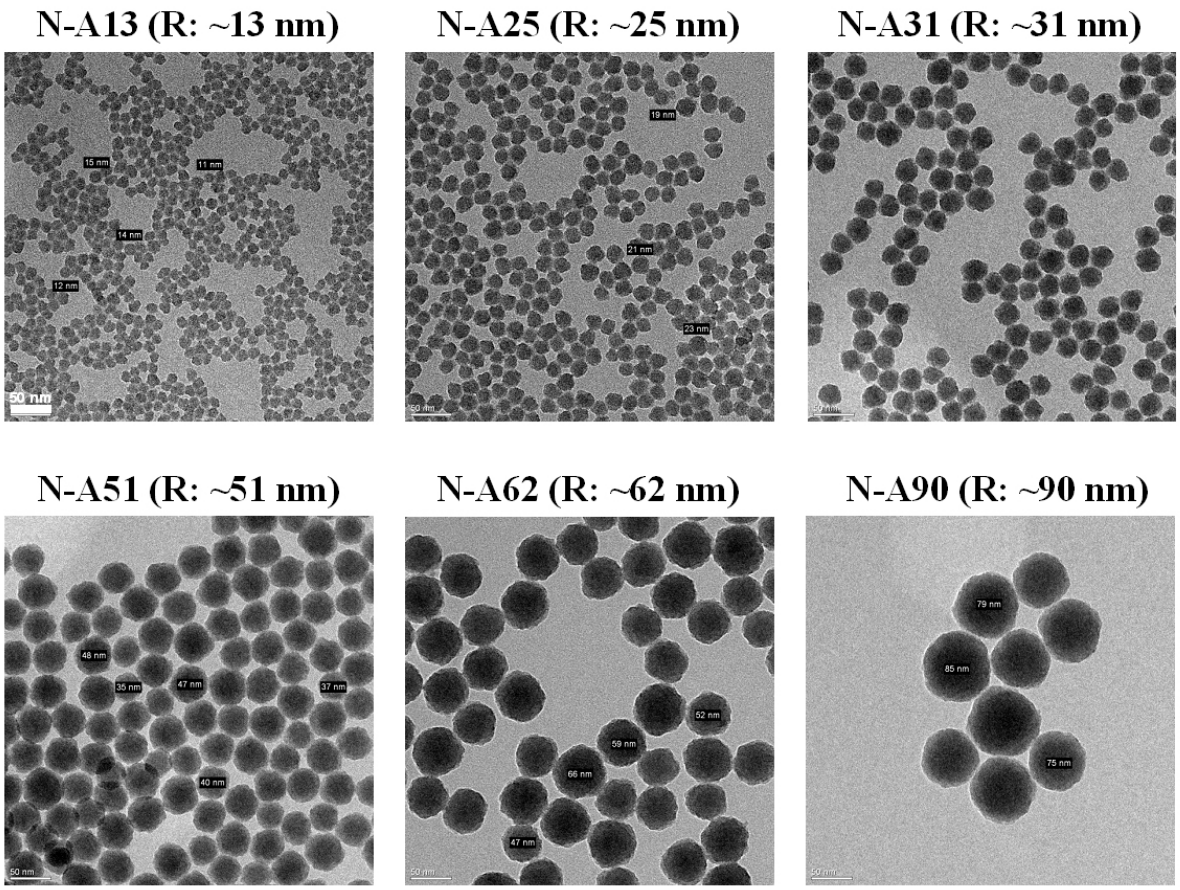


1b.
Nanoparticle sizes were determined by DLS measurements. The surface areas determined by BET measurements are as well listed below. The nomenclature of the samples is according to **Table 1**.

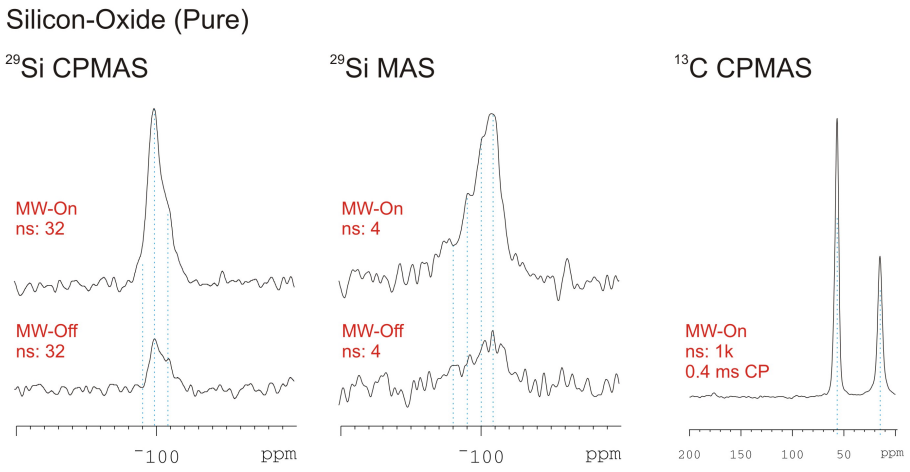
Samples	Particle Diameter (nm)	Surface Area (m^2/g)	Polydispersity Index (PDI)
N-A13	13	258	0.066
N-A25	25	197	0.093
N-A31	31	118	0.061
N-A51	51	86	0.035
N-A62	62	66	0.042
N-A90	90	50	0.046
N-100 (Pure NP, without aminoacid)	100	33	-

The Dynamic Light Scattering (DLS) experiments were performed by using ALV/LSE-5004 Correlator an ALV CGS-3 goniometer with a 22 mW light source (He-Ne laser) at 632.8 nm with a scattering angle of 90°. Five repeat measurements of the intensity autocorrelation function g^2 were performed on each sample at 25 °C. The electric field correlation function g^1 was obtained by using the Siegert relation ($g^2 - 1 = \beta[g^1]^2$ where β is a correction factor) and fitted with a second order cumulant fit to obtain the diffusion coefficient, D, and the polydispersity of the particle size distribution. The hydrodynamic diameter (D_h) was calculated by using the Stokes-Einstein relation $D_h = k*T/3*\pi*\eta*D$, where η is the solvent viscosity, and D the diffusion coefficient.

1c.
Transmission electron microscopy (TEM) images of the synthesized arginine functional nanoparticles with different sizes (diameter of ~13-~90 nm). TEM measurements were performed with FEI Tecnai G² 20 S-TWIN, operated at 200kV. Samples were prepared by dropping diluted water suspensions of silica nanoparticles onto a copper grid, followed by a solvent evaporation. The scales of the images are given left below as a white solid-line, which are 50 nm for all. The particle sizes given above the figures are obtained from DLS measurements.

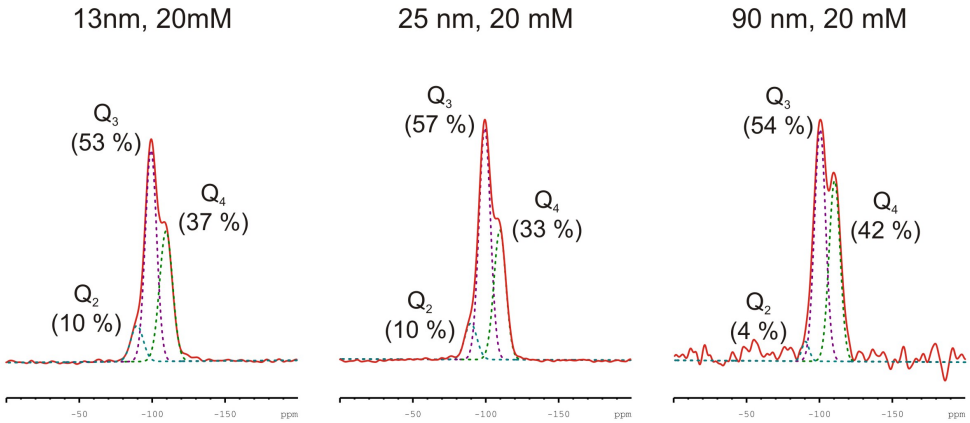


Supplementary Figure 2. The ^{29}Si CP and direct-excitation MAS NMR spectra, and ^{13}C CP MAS NMR spectra of the silica nanoparticle prepared without any surfactant (N-100) with particle size of ~100 nm.



Supplementary Figure 3. The results of the lineshape fits performed by DMFIT program. 2 and 10 second relaxation delays were used for the cross-polarization and direct-excitation ^{29}Si MAS DNP-NMR experiments, if otherwise stated in parenthesis. 20 mM TOTAPOL was used for different samples, except for the N-A25 (which was also prepared without any radical) and N-A90 (which was also prepared with 5 mM concentration).

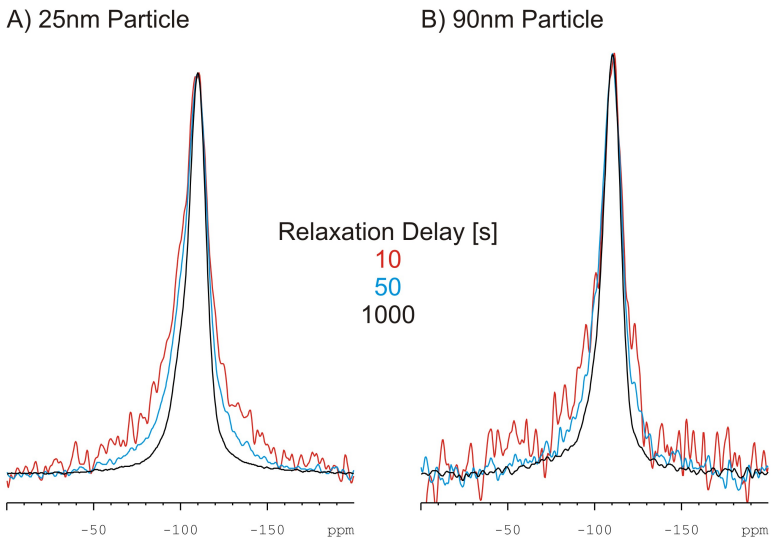
	Particle diameter (nm)	CP % Q_2	CP % Q_3	CP % Q_4	MAS % Q_2	MAS % Q_3	MAS % Q_4	MAS % Q_{4B}
N-A13 (20mM)	13	10	53	37	–	–	–	–
N-A25 (20mM)	25	10	57	33	–	–	–	–
N-A25 (No-Radical)	25	8	67	25	–	–	–	–
N-A90 (20mM)	90	4	54	42	~1	16	37	46
N-A90 (5mM)	90	12	50	38	~1	26	31	42
N-A90 (20mM) (D1: 50s for MAS)	90	–	–	–	4	6	49	41
N-A90 (20mM) (D1: 1000s for MAS)	90	–	–	–	3	7	63	27



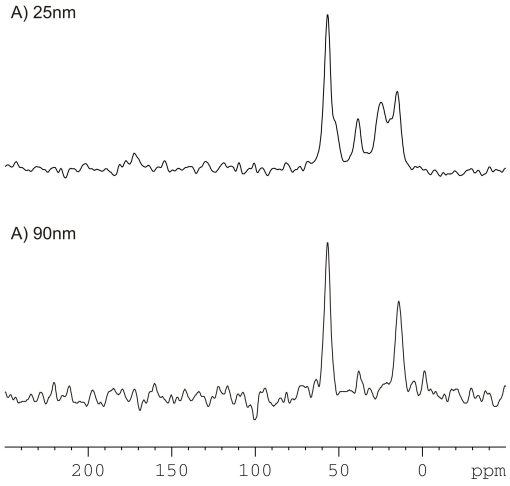
Supplementary Figure 4. Determination of the relative and absolute $^{1}H/^{29}Si$ DNP enhancements of a 11 nm arginine surface functional nanoparticle. 22 mg of dry powder sample was used for this determination. The sample was measured first at room temperature and then at ~100 K without radical. 22 µl of 20 mM TOTAPOL containing solvent was then added to the powder sample, and then the DNP enhancements were determined at ~100 K, by comparing µW on and off spectra.

A40 (11 nm) SiO2 Nanoparticle	DNP Enhancements	
22 mg dry powder sample	^{1}H DNP (via ^{29}Si CP)	^{29}Si DNP (via ^{29}Si MAS)
Compared to 100 K, with 22 µl TOTAPOL (20 mM), µW-Off	43	14
Compared to 100 K, no TOTAPOL, µW-Off	2	8
Compared to 280 K, no TOTAPOL, µW-Off	18	26

Supplementary Figure 5. The DNP enhanced ^{29}Si MAS NMR spectra recorded with different relaxation delays to represent the linewidth changes.



Supplementary Figure 6. The effect of nanoparticle size to the sensitivity of the DNP enhanced ^{13}C CPMAS NMR spectra. The spectra recorded with the 25 and 90 nm particle diameters are shown below. The intensity of the TEOS signals are normalized and different amount of surfactant (arginine) signal can immediately be realized due to different DNP enhancements.



Supplementary Figure 7. Details of the calculation of the total surface area per rotor, total TOTAPOL molecules per unit nanoparticle surface area, enhancements of $^{29}Si_{Q4}$, and the percentage of the polarizable part of the nanoparticle to the total particle volume.

Particle Size [nm]	Total Surface of Particles [m^2]	Molecules TOTAPOL per 100 nm^2 nanoparticle surface	Enhancement of Q_4 sites (MAS)	Percentage of the „shell“ (polarizable) volume per total nanoparticle volume of one particle by using a 5.7 nm penetration depth
100	1.1	53	3	30.4
90	1.3	48	7	33.3
62	1.8	33	9	45.5
51	2.2	27	10	53.1
31	3.7	16	17	74.6
25	4.5	13	20	83.8
13	8.7	7	21	99.8

The main difference of the various particle sizes for the DNP measurements is the different surface areas. As the total volume of all particles and the total amount of solid material in each rotor are same in all samples (same rotor volume of ~25 ul), the total surface area is increasing with a decreased particle size. The surface and volume of the particles were calculated by using a perfect spherical shape. Since the total mass of particles are similar for all the samples used in the study as well as the apparent density of Si-O-Si materials, the number of molecules per sample was estimated. This value multiplied by a single surface area resulted in the total surface area of the material in one rotor, shown in the above table.
Additionally, the number of TOTAPOL molecules per surface are is decreasing with decreasing particle size, due to the increased total surface are of the system. To illustrate this effect we calculated the values for a case where all the TOTAPOL molecules are attached to the particle surfaces (the results are given above in the table).
If the transfer of polarization is limited in length (meaning that there is a sizeable DNP penetration depth which is smaller than the particle radius), our measurements can be used to create a simplified model to calculate the size of this penetration depth (in other words spin-diffusion barrier). We divided each single particle in an outer shell (polarized part of the nanoparticle) which is fully polarized, and an inner core (non-polarized part of the nanoparticle) which is not polarized at all. Following this simplification, the ratio of the polarizable volume ($V_{polarized}$) to the total particle volume ($V_{nanoparticle}$) can be calculated which depends on the particle size (radius r) and the "DNP polarization depth" (a). From this formalism the value of the penetration depth can be calculated since we know the nanoparticle size dependent DNP enhancements (enhancement is proportional to ~ $V_{polarized}/V_{nanoparticle}$) by fitting the data shown in table above and in the text (**Table 1**).

$$\frac{V_{polarized}}{V_{nanoparticle}} = \frac{4/3\,\pi r^3 - 4/3\,\pi (r-a)^3}{4/3\,\pi r^3}$$

To compare this model with our measurements, we first defined the highest enhancement as 1 for the smallest particle size and fit the date to minimize the squared differences of the above model and the measured enhancements. The converged result represent an estimated DNP penetration depth, "**a**". By using the DNP enhancements measured with ^{29}Si direct-excitation NMR (representing mostly the Q_4 sites), the calculated penetration depth is ~5.7 nm, whereas, the same calculation based on the CP based DNP enhancements results a penetration depth of ~4.2 nm (represents mostly the $Q_{2\text{-}3}$ sites). For the first case the percentages of the polarized-part on the total particle volume are displayed in the table above. A shorter penetration depth is obtained from the data obtained by CP experiments, because in the studied nanoparticles more protons are at the surfaces than in the core of the particles ad as result the CP experiments monitors more the surface areas and can span less area compared to the direct-excitation experiment.

References:

1. Yokoi, T.; Sakamoto, Y.; Terasaki, O.; Kubota, Y.; Okubo, T.; Tatsumi, T. Periodic arrangement of silica nanospheres assisted by amino acids. *Journal of the American Chemical Society* **2006,** *128* (42), 13664-13665.
2. Altin, B. Synthesis an dcharacterization of monodisperse silica based functional nanoparticles for multi-purpose applications. Master of Science Izmir Institute of Technology, 2009.
3. W. Stober, A. Fink, E. Bohn, J. Colloid Interface Sci. 26 (1968) 62.