Supporting Information

Nuclear singlet multimers (NUSIMERs) with long-lived singlet states

Philip Saul^[a,b], Salvatore Mamone^[a,b] and Stefan Glöggler*^[a,b]

Chemicals

The Boc protected tripeptide **1** was purchased from TCI chemicals and used as received. Generation 5 poly(amidoamid) dendrimer (G5-PAMAM) was purchased from SigmaAldrich as a methanol solution (5wt.%). The methanol was removed prior to the synthesis. GibcoTM phosphate buffered solution (pH=7.4 ,10x) was purchased from ThermoFisher Scientific.

Syntheses of (2) and (4)

Synthesis of G5-PAMAM-GGA-NH-Boc (2)

For the synthesis of **2** the following synthesis was conducted: G5-PAMAM was dissolved in a mixture of dimethylsulfoxide (DMSO) and dimethyl formamide (DMF) (4:1) and 256 equivalents of **1** was added with 256 equivalents of di-isopropyl-ethyl-amine (DIPEA) and 256 equivalents of *N*-(3-diaminopropyl)-*N'*- ethylcarbodiimide hydrochloride (EDC). The resulting solution was stirred at room temperature for 24 h and the solvent was removed. The obtained solid was taken up in H₂O and dialyzed against H₂O for one week. Afterwards the solvent was removed to obtain a colorless solid.

Synthesis of G5-PAMAM-GGA-NH₂ (4)

2 was dissolved in a mixture of Dichloromethane (DCM) and Trifluoroacetic acid (TFA) (1:1) for 30 minutes. Afterwards the solvent was removed and the obtained crude product was dissolved in H_2O and dialyzed against H_2O for one week. Afterwards the solvent was removed to obtain an amber highly viscous liquid.

NMR experiments

NMR experiments have been performed on a BRUKER AVIII 300 MHz spectrometer. The parameters for the respective experiments are shown in the following figures.

Estimation of the mean number of tripeptides per dendrimer unit

The mean derivatization number has been estimated by using pyrazine as an internal standard in quantitative NMR experiments. A solution of **2** and **4** has been measured with an internal standard of

50 mM pyrazine. The amount of **2** and **4** in the solutions was calculated to be 100 μ M assuming complete derivatization. Thus, the assumed molarity of tripeptide residues would be 12.8 mM. The actual molarity of tripeptide residues has been calculated by comparing the integral of the pyrazine signal with the integral of the methyl Protons of the Alanine. The actual derivatization is 0.69 for **2** and of 0.68 for **4**. This results in a mean coverage of about 90 tripeptides per NUSIMER.

On the accessibility of the singlet state in (2)

As mentioned in the main article the singlet state in the signal at 3.87 ppm was not accessible any more upon PBS addition. As shown in figure S1, measurements performed on a BRUKER 400 MHz machine in D_2O showed a difference in chemical shift in the signal at 3.80 ppm which was not visible in PBS solution anymore.



Figure S1:Expansion of the ¹H NMR spectrum of **2** showing from left to right the signal of the CH proton of alanine (quartet centered at 3.97ppm) and of the CH_2 protons of the two glycine residue at 3.87 ppm and 3.81 ppm, respectively . In the spectrum measured in D_2O (blue) a chemical shift difference is visible, whereas in PBS solution (red) the chemical shift difference can not be resolved anymore.

Overall, this region of the NMR spectrum seems to be highly influenced by the presence of PBS. In the same spectrum the signals arising from the methyl group of the alanine, as well as the signals of the Boc protection group, show a change in the other direction, showing higher resolution and the appearance of a splitting of the signals (figure S2).



Figure S2: Signals of the Boc protection group and the methyl group of the alanine in D_2O (blue) and in PBS solution (red). A splitting of the signal in the alanine (1.26 ppm) is clearly visible upon addition of PBS.

This observation leads us to assume a strong interaction of the NUSIMERs with the ions in the PBS solution. Further investigation of this phenomenon will be subject of our future research. Here, we speculate that in presence of PBS, the interaction leading to a chemical shift difference in the glycine signal at 3.81 ppm is influencing the signal at 3.87 ppm in the same way, making the singlet state inaccessible.

Singlet life-times have been investigated in D₂O for the signal at 3.81 ppm and a singlet relaxation time of T_s =2.51 ± 0.04 s. Removing the spin lock caused T_s to decrease significantly to T_s =0.73 ± 0.02 s. This reflects the observed change in chemical shift difference. For comparison T_s have been determined in H₂O (5 % D₂O) solutions without the addition of PBS for **2** as well. The singlet state in both signals has been accessible with T_s =1.08 s and T_s =3.46 s in the signals at 3.95 ppm and 4.02 ppm respectively, showing that the observed change in accessibility can be attributed to the PBS and is not dependent on the deuteration level of the solvent.

Estimation of the recovered magnetization

The magnetization recovered after the end of the experiments have been estimated. For each of the signals the SLIC¹ pulse lengths and powers have been optimized and the singlet evolution time was set to 200 ms. For the protons of the glycine further away from the alanine residue we found that 5% of magnetization could be recovered (SLIC pulse length 310 ms, SLIC pulse power 6.6e⁻⁶ W). For the glycine protons closer to the alanine residue, 41% of the initial magnetization could be recovered (SLIC pulse length 50 ms, SLIC pulse power 8e⁻⁶ W). The theoretical maximum with the SLIC technique is 66% percent and our experiments show that optimization of the chemical shift difference between the two protons of interest can enhance the obtainable signal in the future.

Measurements of T_1

For the measurement of the T_1 relaxation times, an inversion recovery experiment has been conducted. The obtained plots are listed below. The values given in the main article are the mean values taken from the values obtained from two different samples.



Figure S3: Plot from the inversion recovery measurement of compound $\mathbf{1}$ in D₂O including the delays with the respective normalized integrals and the obtained T1 value.



Figure S4: Plot from the inversion recovery measurement of compound **1** in D₂O including the delays with the respective normalized integrals and the obtained T1 value.



Figure S5: Plot from the inversion recovery measurement of compound **1** in PBS including the delays with the respective normalized integrals and the obtained T1 value.



Figure S6: Plot from the inversion recovery measurement of compound **1** in PBS including the delays with the respective normalized integrals and the obtained T1 value.



Figure S7: Plot from the inversion recovery measurement of compound **1** in Agarose gel including the delays with the respective normalized integrals and the obtained T1 value.



Figure S8: Plot from the inversion recovery measurement of compound **1** in Agarose gel including the delays with the respective normalized integrals and the obtained T1 value.



Figure S9: Plot from the inversion recovery measurement of compound $\mathbf{2}$ in D₂O including the delays with the respective normalized integrals and the obtained T1 value.



Figure S10: Plot from the inversion recovery measurement of compound **2** in D₂O including the delays with the respective normalized integrals and the obtained T1 value.



Figure S11: Plot from the inversion recovery measurement of compound **2** in PBS including the delays with the respective normalized integrals and the obtained T1 value.



Figure S12: Plot from the inversion recovery measurement of compound **2** in PBS including the delays with the respective normalized integrals and the obtained T1 value.



Figure S13: Plot from the inversion recovery measurement of compound **2** in Agarose gel including the delays with the respective normalized integrals and the obtained T1 value.



Figure S14: Plot from the inversion recovery measurement of compound **3** in D₂O including the delays with the respective normalized integrals and the obtained T1 value.



Figure S15: Plot from the inversion recovery measurement of compound **3** in D₂O including the delays with the respective normalized integrals and the obtained T1 value.



Figure S16: Plot from the inversion recovery measurement of compound **3** in PBS including the delays with the respective normalized integrals and the obtained T1 value.



Figure S17: Plot from the inversion recovery measurement of compound **3** in PBS including the delays with the respective normalized integrals and the obtained T1 value.



Figure S18: Plot from the inversion recovery measurement of compound **3** in Agarose gel including the delays with the respective normalized integrals and the obtained T1 value.



Figure S19: Plot from the inversion recovery measurement of compound **3** in Agarose gel including the delays with the respective normalized integrals and the obtained T1 value.



Figure S20: Plot from the inversion recovery measurement of compound **4** in D₂O including the delays with the respective normalized integrals and the obtained T1 value.



Figure S21: Plot from the inversion recovery measurement of compound **4** in D₂O including the delays with the respective normalized integrals and the obtained T1 value.



Figure S22: Plot from the inversion recovery measurement of compound **4** in PBS including the delays with the respective normalized integrals and the obtained T1 value.



Figure S23: Plot from the inversion recovery measurement of compound **4** in PBS including the delays with the respective normalized integrals and the obtained T1 value.



Figure S24: Plot from the inversion recovery measurement of compound **4** in Agarose gel including the delays with the respective normalized integrals and the obtained T1 value.



Figure S25: Plot from the inversion recovery measurement of compound **4** in Agarose gel including the delays with the respective normalized integrals and the obtained T1 value.

T_s measurements

 $T_{\rm s}$ measurements have been performed using the SLIC sequence, as discussed in the main article. The obtained plots are listed below. The values given in the main article are the mean values taken from the values obtained from two different samples. The duration and power of the SLIC pulses where optimized for each sample. The singlet evolution time has been 200 ms in all cases.



Figure S26: Plot from the Ts measurement of compound $\mathbf{1}$ in D₂O including the delays with the respective normalized integrals and the obtained Ts value.



Figure S27: Plot from the Ts measurement of compound **1** in D₂O including the delays with the respective normalized integrals and the obtained Ts value.



Figure S28: Plot from the Ts measurement of compound **1** in PBS including the delays with the respective normalized integrals and the obtained Ts value.



Figure S29: Plot from the Ts measurement of compound **1** in PBS including the delays with the respective normalized integrals and the obtained Ts value.



Figure S30: Plot from the Ts measurement of compound **1** in Agarose gel including the delays with the respective normalized integrals and the obtained Ts value.



Figure S31: Plot from the Ts measurement of compound **1** in Agarose gel including the delays with the respective normalized integrals and the obtained Ts value.



Figure S32: Plot from the Ts measurement of compound ${\bf 2}$ in D₂O including the delays with the respective normalized integrals and the obtained Ts value.



Figure S33: Plot from the Ts measurement of compound **2** in D₂O including the delays with the respective normalized integrals and the obtained Ts value.



Figure S34: Plot from the Ts measurement of compound **3** in D_2O including the delays with the respective normalized integrals and the obtained Ts value.



Figure S35: Plot from the Ts measurement of compound **3** in D₂O including the delays with the respective normalized integrals and the obtained Ts value.



Figure S36: Plot from the Ts measurement of compound **3** in PBS including the delays with the respective normalized integrals and the obtained Ts value.



Figure S37: Plot from the Ts measurement of compound **3** in PBS including the delays with the respective normalized integrals and the obtained Ts value.



Figure S38: Plot from the Ts measurement of compound **3** in Agarose gel including the delays with the respective normalized integrals and the obtained Ts value.



Figure S39: Plot from the Ts measurement of compound **3** in Agarose gel including the delays with the respective normalized integrals and the obtained Ts value.



Figure S40: Plot from the Ts measurement of compound **4** in D_2O including the delays with the respective normalized integrals and the obtained Ts value.



Figure S41: Plot from the Ts measurement of compound $\mathbf{4}$ in D₂O without decoupling including the delays with the respective normalized integrals and the obtained Ts value.



Figure S42: Plot from the Ts measurement of compound **4** in D₂O including the delays with the respective normalized integrals and the obtained Ts value.



Figure S43: Plot from the Ts measurement of compound $\mathbf{4}$ in D₂O without decoupling including the delays with the respective normalized integrals and the obtained Ts value.



Figure S44: Plot from the Ts measurement of compound **4** in PBS including the delays with the respective normalized integrals and the obtained Ts value.



Figure S45: Plot from the Ts measurement of compound **4** in PBS including the delays with the respective normalized integrals and the obtained Ts value.



Figure S46: Plot from the Ts measurement of compound **4** in Agarose gel including the delays with the respective normalized integrals and the obtained Ts value.



Figure S47: Plot from the Ts measurement of compound **4** in Agarose gel including the delays with the respective normalized integrals and the obtained Ts value.

NMR spectra of (2) and (4)



Figure S48: Proton spectrum of a 100 μ M solution of **2** in D₂O with presaturation on the solvent signal at a magnetic field of 7.05 T. Two scans were run in this experiment with an inter-scan delay of *d1*=5 s. The 90° pulse was *p1*=16 μ s.



Figure S49: ¹³C spectrum of a concentrated solution of **2** in D₂O at a magnetic field of 7.05 T.. 8192 scans were run in this experiment with an inter-scan delay of d1=2 s. The 90° pulse was $p1=2.5 \mu$ s with a 30° flip angle.



Figure S50: Singlet state NMR spectrum of a 100 μ M solution of **2** in D2O at a magnetic field of 7.05 T. Four scans were run in this experiment with an inter-scan delay of *d1*=15 s. The 90° pulse was *p1*=16 μ s and the SLIC pulse length was *d21*=80 ms at 17 Hz nutation frequency.



Figure S51: Proton spectrum of a 100 μ M solution of **4** in D₂O with presaturation on the solvent signal at a magnetic field of 7.05 T. Two scans were run in this experiment with an inter-scan delay of *d*1=5 s. The 90° pulse was *p*1=15 μ s.



Figure S52: ¹³C spectrum of a concentrated solution of **4** in D₂O at a magnetic field of 7.05 T.. 4096 scans were run in this experiment with an inter-scan delay of d1=2 s. The 30° pulse was $p1=2.5 \ \mu$ s.



Figure S53: Singlet state NMR spectrum of a 100 μ M solution of **4** in D2O at a magnetic field of 7.05 T.. Eight scans were run in this experiment with a inter-scan delay of *d1*=30 s. The 90° pulse was *p1*=15 μ s and the SLIC pulse length was *d21*=310 ms at 17 Hz nutation frequency.

IR spectra of the starting material as well as of (2) and (4)



Figure S54: IR spectrum of the starting material G5-PAMAM-NH₂.







Figure S56: IR spectrum of 4.



Figure S57: IR spectrum of 1.



Figure S58: IR spectrum of 3.

References

1 S. J. DeVience, R. Walsworth and M. S. Rosen, *Phys. Rev. Lett.* **2013**, *111*, 173002.