SUPPORTING INFORMATION

Measurement of ligand-target residence times by ^{1}H relaxation dispersion NMR spectroscopy

Thomas Moschen, Sarina Grutsch, Michael A. Juen, Christoph H. Wunderlich, Christoph Kreutz* and Martin Tollinger*

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DETALS OF THE NMR PULSE SCHEME. All solid pulses in the pulse scheme to measure ^{1}H relaxation dispersion data (see Figure 1 of the main text) have flip angles of 90° (narrow) or 180° (wide), applied with phase x unless indicated otherwise. The two pulses flanking the last 180° pulse in the WATERGATE solvent suppression scheme scheme[1] are water selective 90° rectangular flip-back pulses with a field strength of 0.16 kHz (500 MHz spectrometer) and 0.18 kHz (600 MHz spectrometer). The phase cycling employed is $\phi1$ = [y, –y], $\phi2$ = [x, –x], $\phi3$ = [y, –y], $\phi4$ = [–y, y], $\phi5$ = [–y, y], $\phi6$ = [x, x, –x, –x], $\phi7$ = 4[x], 4[y], $\phi8$ = 4[–x], 4[–y] and ϕ_{rec} = 2[x], 4[–x], 2[x].[2] Gradient strengths in G/cm (durations in ms) are g1 = 5.0 (0.1), g2 = 8.0 (0.6), g3 = 12.0 (1.0). Relaxation dispersion data are recorded by varying the number of basic CPMG elements, n, within a fixed time, T_{relax} to sample different values of CPMG refocusing frequencies $\nu_{CPMG} = 1/(4\ \tau_{CPMG})$, where $2\ \tau_{CPMG}$ is the time between successive refocusing pulses, along with a reference experiment where the CPMG element is omitted, as described.[3]

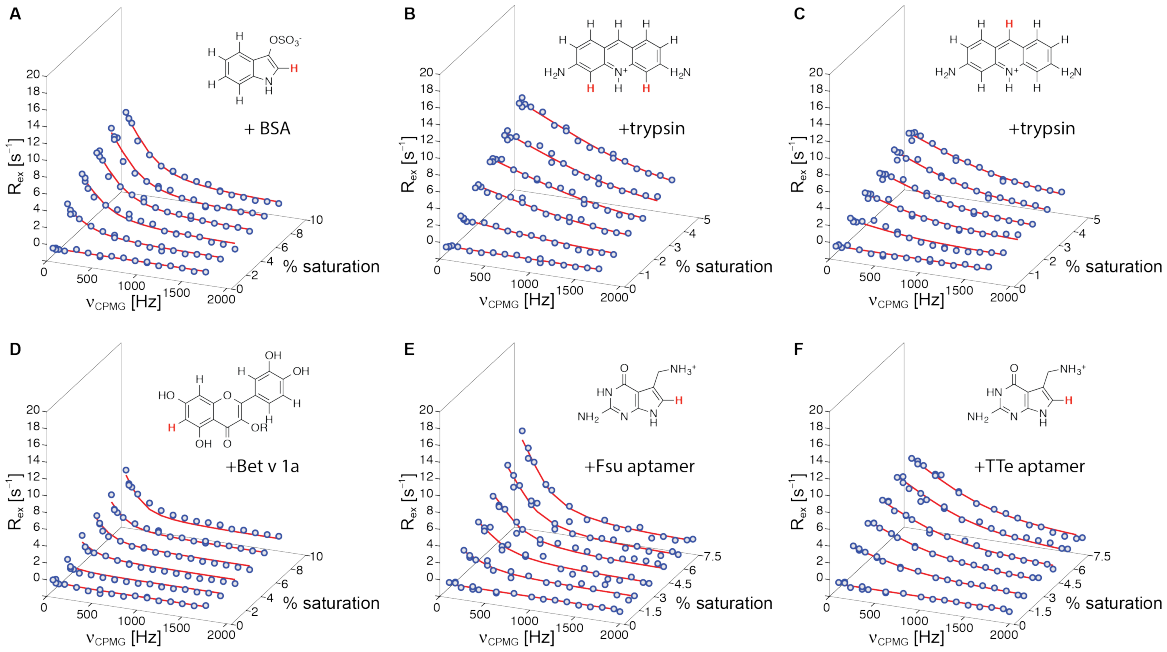

FIGURE S1. Ligand-detected ^{1}H-CPMG relaxation dispersion data, recorded on an Agilent DirectDrive2 500 MHz spectrometer equipped with a room-temperature probe, for (A) indoxyl sulfate binding to BSA, (B) and (C) proflavin binding to bovine trypsin, (D) isoquercetin (R=β-D-glucoside) binding to Bet v 1a, (E) and (F) preQ$_1$ binding to aptamers from *F. nucleatum* and *Th. tengcongensis*. Data are shown for the protons indicated in red in the ligand structures (indoxyl sulfate: ^{1}H-2, proflavin: ^{1}H-4/5 and ^{1}H-9, isoquercetin: ^{1}H-6, preQ$_1$: ^{1}H-8). The exchange contributions $R_{ex} = R_{2,eff} - R_{2,eff}(\nu_{CPMG} = \infty)$ to the relaxation dispersion profiles are shown. The solid lines are fits to the data.

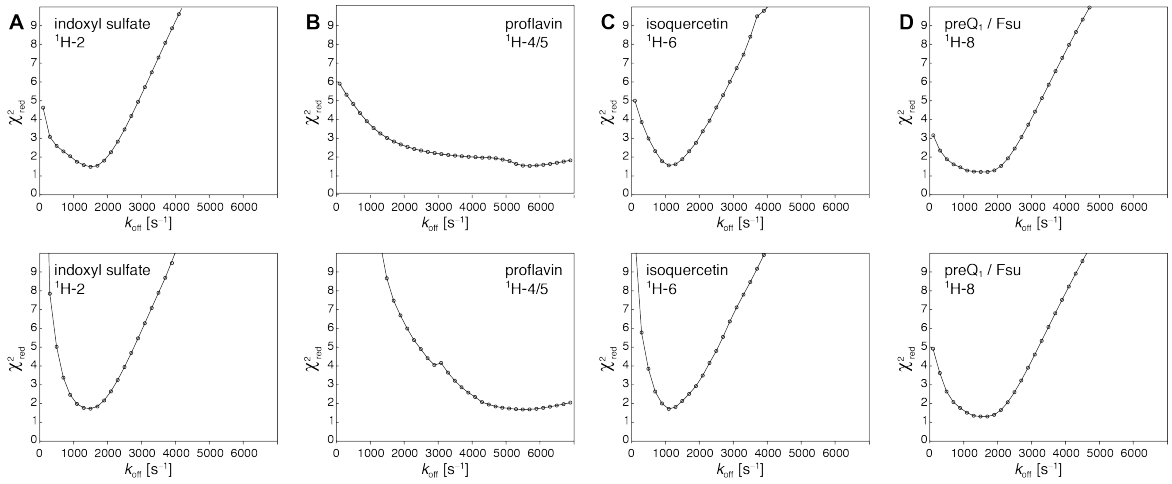

FIGURE S2. Variation of χ^2_{red} from fits of ^{1}H-CPMG relaxation dispersion as a function of off-rate, k_{off}. For calculation of χ^2_{red}, k_{off} was fixed at values between 100 s^{-1} and 6900 s^{-1} (in 200 s^{-1} intervals) and the two-site kinetic model described in the Experimental Section of the paper was fit to the experimental data. χ^2_{red} variations are shown for (A) indoxyl sulfate (^{1}H-2), (B) proflavin (^{1}H-4/5), (C) isoquercetin (^{1}H-6) and (D) preQ$_1$ (binding to the Fsu aptamer), using only the 600 MHz data (top) or both 500 MHz and 600 MHz data (bottom) for analysis. Well-defined minima in the χ^2_{red} *vs.* k_{off} profiles are found for (A), (C) and (D), while the broader minimum for faster off-rates in (B) is indicative for higher experimental uncertainties. In such cases, the performance of the experiment is enhanced by measurind RD data at two different magnetic field strengths.

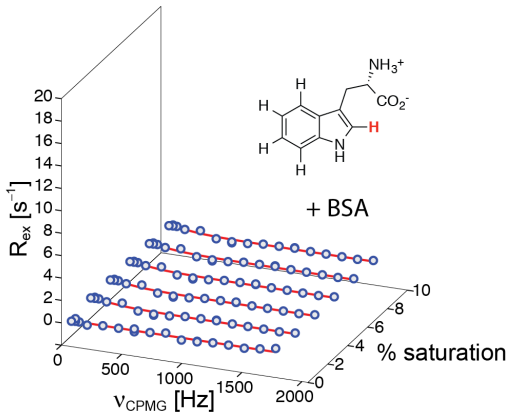

FIGURE S3. Ligand-detected ^{1}H-CPMG relaxation dispersion data, recorded at 500 MHz, for L-tryptophan binding to BSA. Data are shown for the indole ^{1}H-2 proton indicated in red. Experimental conditions: 50 mM potassium phosphate buffer (pH 5.8), 25 mM NaCl, 1 mM NaN_3, 100% D_2O, L-tryptophan: 2.0 mM, BSA: [0, 40, 80, 120, 160, 200 μM].

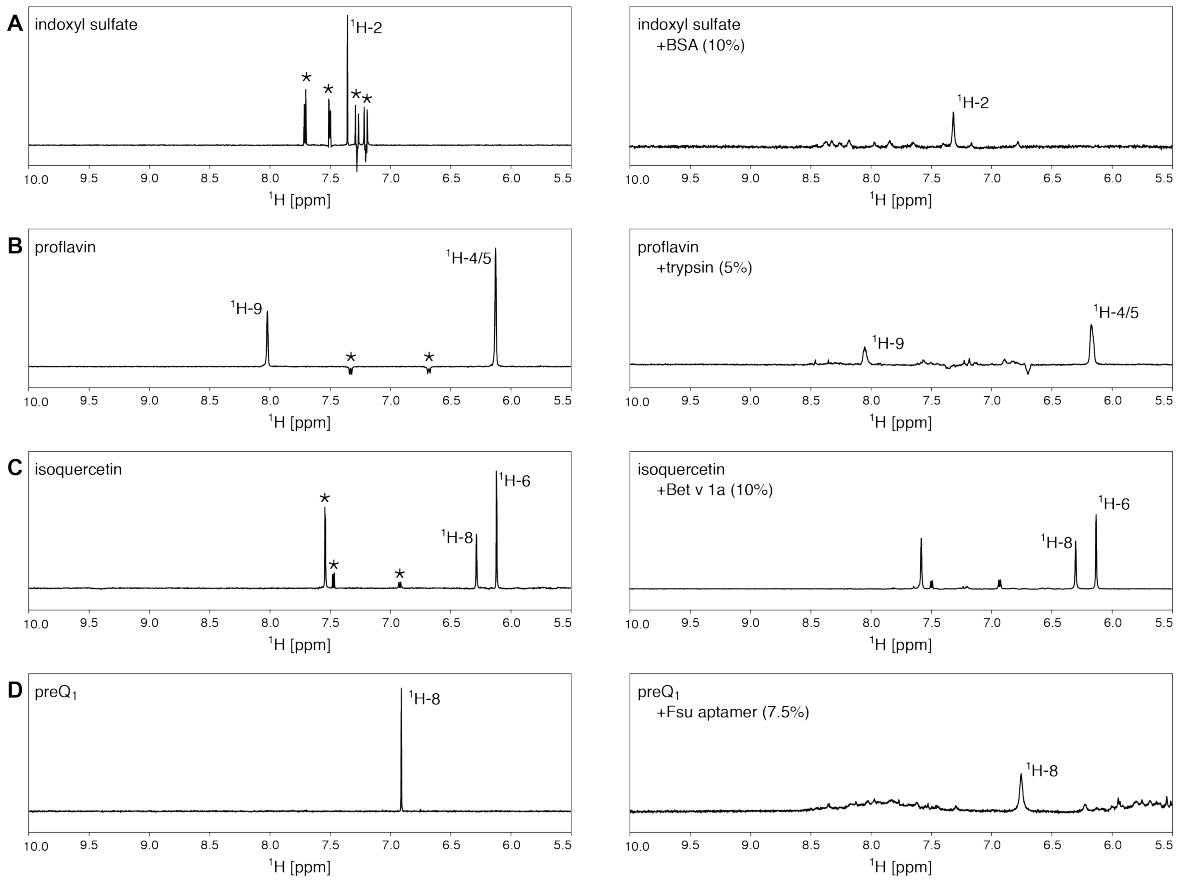

FIGURE S4. One-dimensional ^{1}H-CPMG relaxation dispersion spectra, recorded on a 600 MHz Bruker Avance II+ NMR spectrometer equipped with a prodigy cryogenic probe using the experimental scheme presented in this work. Low field portions (5.5 – 9.0 ppm) of spectra for the small-molecule ligands (A) indoxyl sulfate, (B) isoquercetin, (C) proflavin and (D) preQ$_1$ without receptor (left) and with the highest level of receptor saturation that was used (right) are shown. Assignments of ligand resonances that were used for ^{1}H-CPMG relaxation dispersion analysis are indicated; for isoquercetin and proflavin assignments were taken from the literature.[4, 5] T_{tfilt} was 60 ms for indoxyl sulfate, 80 ms for isoquercetin, 60 ms for proflavin and 40 ms for preQ$_1$. Resonances marked by asteriks have scalar couplings > 1.5 Hz and were not used for analysis. These resonances can be distorted in spectra with transverse relaxation filtering because of interconversion between in-phase and anti-phase magnetization during T_{tfilt}.

ligand	resonance	target	k_{off} [s^{-1}] a)	k_{off} [s^{-1}] b)	k_{off} [s^{-1}] c)
indoxyl sulfate	^{1}H-2	BSA	1500 ± 200	1500 ± 200	1500 ± 100
proflavin	^{1}H-4/5	trypsin	6000 ± 1000	5400 ± 700	5600 ± 600
proflavin	^{1}H-9	trypsin	3700 ± 1000 d)	5100 ± 900	4400 ± 600
isoquercetin	^{1}H-6	Bet v 1a	1000 ± 200	1000 ± 100	1100 ± 100
$preQ_1$	^{1}H-8	Fsu aptamer	1400 ± 300	1600 ± 200	1500 ± 200
$preQ_1$	^{1}H-8	TTe aptamer	3900 ± 300	4400 ± 500	4000 ± 300

TABLE S1. Off-rates, k_{off}, of ligand-target complexes obtained by fitting a) 500 MHz data, b) 600 MHz data and c) 500 and 600 MHz data simultaneously. d) For the proflavin ^{1}H-9 resonance at 500 MHz exchange contributions are small (R_{ex} below 7 s^{-1} at 5% saturation with trypsin, Figure S1).

REFERENCES

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Certificate of Analysis

Product Name:
Bovine serum albumin – lyophilized powder, crystallized, ≥98.0% (GE)

Product Number:	**05470**
Batch Number:	**SLBK8105V**
Brand:	SIGMA
CAS Number:	9048-46-8
MDL Number:	MFCD00130384
Storage Temperature:	Store at 2 - 8 °C
Quality Release Date:	07 NOV 2014
Recommended Retest Date:	NOV 2019

Test	Specification	Result
Appearance (Color)	White to Off-White	Off-White
Appearance (Form)	Powder	Powder
Solubility (Color)	Faint Yellow to Yellow	Faint Yellow
Solubility (Turbidity) 50 mg/ml, H2O	Clear to Slightly Hazy	Slightly Hazy
Water (by Karl Fischer)	≤ 8 %	7 %
Agarose Electrophoresis	≥ 98 %	100 %
Zinc (Zn)	≤ 10 ppm	< 10 ppm
Copper (Cu)	≤ 20 ppm	9 ppm
Iron (Fe)	≤ 20 ppm	< 5 ppm
Nickel (Ni)	≤ 5 ppm	< 5 ppm
Cadmium (Cd)	≤ 5 ppm	< 5 ppm
Chromium (Cr)	≤ 100 ppm	< 5 ppm
Calcium (Ca)	≤ 500 ppm	< 100 ppm
Cobalt (Co)	≤ 5 ppm	< 5 ppm
Potassium (K)	≤ 50 ppm	28 ppm
PPM Magnesium	≤ 500	< 100
PPM Manganese	≤ 5	< 5
PPM Lead	≤ 5	< 5
Sulfate (SO4) < or = 1000 ppm	–	
Chloride (Cl) < or = 2000 ppm	–	

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Product Number: **05470**
Batch Number: **SLBK8105V**

Test	Specification	Result
Sodium (ICP)	≤ 7000 ppm	< 7000 ppm

Rodney Burbach, Manager
Analytical Services
St. Louis, Missouri US

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Product Name:
Trypsin from bovine pancreas – Type XI, lyophilized powder, ≥6,000 BAEE units/mg protein

Product Number:	**T1005**
Batch Number:	**SLBK8241V**
Brand:	SIGMA
CAS Number:	9002-07-7
MDL Number:	MFCD00082094
Storage Temperature:	Store at -20 °C
Quality Release Date:	04 SEP 2014

Test	Specification	Result
% Protein (UV)	90 - 100	100
BAEE units/mg protein Unit Definition: One BAEE unit will produce a delta A253 of 0.001 per minute in a reaction volume of 3.2 ml at pH 7.6 at 25 deg C. (1 cm light path)	≥ 6000	8643
Chymotrypsin BTEE units/mg protein	≤ 0.1	0.0

Rodney Burbach, Manager
Analytical Services
St. Louis, Missouri US

3050 Spruce Street, Saint Louis, MO 63103 USA
Email USA: techserv@sial.com Outside USA: eurtechserv@sial.com

Certificate of Analysis

Product Name:	INDOXYL SULFATE POTASSIUM SALT -
Product Number:	I3875
Batch Number:	BCBN8349V
Brand:	Aldrich
CAS Number:	2642-37-7
Formula:	$C_8H_6NO_4SK$
Formula Weight:	251.30
Storage Temperature:	-20 C
Quality Release Date:	24 SEP 2014
Recommended Retest Date:	SEP 2016

TEST	SPECIFICATION	RESULT
APPEARANCE (COLOR)	WHITE TO YELLOW AND FAINT GREY TO GREY	WHITE
APPEARANCE (FORM)	POWDER OR CRYSTALS	CRYSTALS
PURITY (TLC AREA %)	≥98 %	100.0 %
SOLUBILITY (COLOR)	COLORLESS TO YELLOW-GREEN	LIGHT GREENISH-YELLOW
SOLUBILITY (TURBIDITY)	CLEAR TO HAZY	CLEAR (<3.5 NTU)
SOLUBILITY (METHOD)	50MG/ML IN WATER	50MG/ML IN WATER
WATER	REPORT RESULT	0.16 %
CARBON CONTENT	37.3 - 39.2 % (ANHYDROUS BASIS)	38.02 % (ANYHDROUS BASIS)
NITROGEN CONTENT	5.3 - 5.9 % (ANHYDROUS BASIS)	5.56 % (ANYHDROUS BASIS)
INFRARED SPECTRUM	CONFORMS TO STRUCTURE	CONFORMS

Dr. Claudia Geitner
Manager Quality Control
Buchs, Switzerland

Certificate of Analysis

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Product Name	3,6-Diaminoacridine hydrochloride, Dye content 95 %
Product Number	131105
Product Brand	SIAL
CAS Number	952-23-8
Molecular Formula	$C_{13}H_{11}N_3 \cdot HCl$
Molecular Weight	245.71

TEST	SPECIFICATION	LOT 16806CBV RESULTS
APPEARANCE	DARK ORANGE TO ORANGE-BROWN POWDER	DARK ORANGE POWDER
INFRARED SPECTRUM	CONFORMS TO STRUCTURE.	CONFORMS TO STRUCTURE.
UV-VISIBLE SPECTRUM	IN ACIDIFIED METHANOL	0.005G/L, MEOH
	E455(+/-3)NM = 44,000 (MINIMUM)	E457NM= 47,900
	E260(+/-3)NM = 50,000 (MINIMUM)	E260NM= 54,200
ELEMENTAL ANALYSIS	CARBON 59.7%-67.3%	CARBON 60.23%
	NITROGEN 15.9%-18.1%	NITROGEN 15.92%
TITRATION	REPORT % CL (WITH SILVER NITRATE)	107.7% (WITH SILVER NITRATE)
HIGH PRESSURE LIQUID CHROMATOGRAPHY	94.0% (MINIMUM)	96.3%
SOLUBILITY	1 MG/ML, H2O; CLEAR, ORANGE TO BROWN	1 MG/ML, H2O; CLEAR ORANGE-BROWN SOLUTION
QUALITY CONTROL ACCEPTANCE DATE		APRIL 2003

Barbara Rajzer, Supervisor
Quality Control
Milwaukee, Wisconsin USA

3050 Spruce Street, Saint Louis, MO 63103 USA
Email USA: techserv@sial.com Outside USA: eurtechserv@sial.com

Certificate of Analysis

Product Name:	QUERCETIN 3-β-D-GLUCOSIDE >= 90 % HPLC
Product Number:	17793
Batch Number:	BCBP4835V
Brand:	Sigma
CAS Number:	482-35-9
Formula:	$C_{21}H_{20}O_{12}$
Formula Weight:	464.38
Storage Temperature:	-20 C
Quality Release Date:	05 FEB 2015

TEST	SPECIFICATION	RESULT
APPEARANCE (COLOR)	LIGHT YELLOW TO DEEP GREENISH--YELLOW	LIGHT GREENISH-YELLOW
APPEARANCE (FORM)	POWDER	POWDER
PURITY (HPLC AREA %)	≥ 90.0 %	92.1 %
MELTING POINT (DEC.)	215 - 219 C	219 C
INFRARED SPECTRUM	CONFORMS TO STRUCTURE	CONFORMS

Dr. Claudia Geitner
Manager Quality Control
Buchs, Switzerland

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Email USA: techserv@sial.com
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Certificate of Analysis

Product Name:
PreQ1 dihydrochloride – ≥98% (HPLC)

Product Number:	**SML0807**
Batch Number:	**123M4721V**
Brand:	SIGMA
CAS Number:	86694-45-3
Formula:	C7H9N5O Â· 2HCl
Formula Weight:	252.1 g/mol
Storage Temperature:	Store at 2 - 8 ℃
Quality Release Date:	25 FEB 2014

Test	Specification	Result
Appearance (Color)	White to Light Brown	Beige
Appearance (Form)	Powder	Powder
Elemental Composition C7H9N5O.2HCl	Pass	Pass
ELEMENTAL ANALYSIS X VALUE H2O (moles)	0.0 - 4.0	0.7
Purity (HPLC)	≥ 98 %	98 %
Identity	Confirmed	Confirmed

Brendan Nye, QC Supervisor
Quality Control
Natick, Massachusetts US

3050 Spruce Street, Saint Louis, MO 63103 USA
Email USA: techserv@sial.com Outside USA: eurtechserv@sial.com

Certificate of Analysis

Product Name:	L-TRYPTOPHAN reagent grade, >= 98 % TLC
Product Number:	T0254
Batch Number:	BCBL3276V
Brand:	Sigma-Aldrich
CAS Number:	73-22-3
Formula:	$C_{11}H_{12}N_2O_2$
Formula Weight:	204.23
Quality Release Date:	19 JUN 2013
Recommended Retest Date:	MAY 2017

TEST	SPECIFICATION	RESULT
APPEARANCE (COLOR)	WHITE TO OFF-WHITE	WHITE
APPEARANCE (FORM)	POWDER	POWDER
PURITY (TLC AREA %)	≥98 %	100.0 %
SPECIFIC ROTATION (20/D)	-29.4 TO -32.8 DEG	-31.2 DEGREES
CONCENTRATION	C=1 IN WATER	C=1 IN WATER
SOLUBILITY (COLOR)	COLORLESS TO YELLOW	ALMOST COLORLESS
SOLUBILITY (TURBIDITY)	CLEAR (< 3.5 NTU)	CLEAR (<3.5 NTU)
SOLUBILITY (METHOD)	50MG/ML IN 0.5M HYDROCHLORIC ACID	50MG/ML IN 0.5M HYDROCHLORIC ACID
CARBON CONTENT	63.2 TO 66.0 %	64.5 %
NITROGEN CONTENT	13.3 TO 14.1 %	13.7 %
INFRARED SPECTRUM	CONFORMS TO STRUCTURE	CONFORMS

Dr. Claudia Geitner
Manager Quality Control
Buchs, Switzerland