Supplemental Material for

THE V-*MYC*-INDUCED Q83 LIPOCALIN IS A SIDEROCALIN

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Supplemental Table S1. Primers used for site directed mutagenesis.

Supplemental Data S2. Doubly $^{15}N/^{13}C$-filtered ^{1}H-^{1}H NOESY experiment

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Supplemental Figure S4. Ionic strength dependency of Q83 affinity for $[Fe^{III}(Ent)]^{3-}$

Supplemental Table S1. List of primers used for site directed mutagenesis.

Mutation	Primers
K83A	Sense: 5'-CTCAGAGGAAGCCAAGAAAGCGGTGGAGGTGCTGGACACTGAC-3' Antisense: 5'-GTCAGTGTCCAGCACCTCCACCGCTTTCTTGGCTTCCTCTGAG-3'
R102A	Sense: 5'-CAGTAATCTATGCAACTGCGGTGAAGGACGGAAGGACCC-3' Antisense: 5'-GGGTCCTTCCGTCCTTCACCGCAGTTGCATAGATTACTG-3'
R113A	Sense: 5'-GCTGGACACTGACTTCAAGAGCTATGC-3' Antisense: 5'-GCTGTAGAGCGCCATCATGTGCAGGGTCC-3'

Supplemental Data S2. Doubly $^{15}N/^{13}C$-filtered ^{1}H-^{1}H NOESY experiment.

In order to measure intermolecular NOE correlation between Q83 and $[Ga^{III}(Ent)]^{3-}$, we designed a new doubly $^{15}N/^{13}C$-filtered ^{1}H-^{1}H NOESY experiment. This experiment is based on already described methodologies used for the simultaneous observation of intramolecular and intermolecular NOEs between an unlabeled ligand and a ^{13}C,^{15}N-labeled protein. These methods are 1) the use of an adiabatic ^{13}C inversion pulse optimized to selectively invert the protein protons (Zwahlen *at al.*, Eichmüller *et al.*); 2) the acquisition of 4 sub spectra and their subsequent addition/subtraction leading to 4 datasets containing distinct information (Slijper *et al.*). The experiment is detailed in Fig. S1-I.

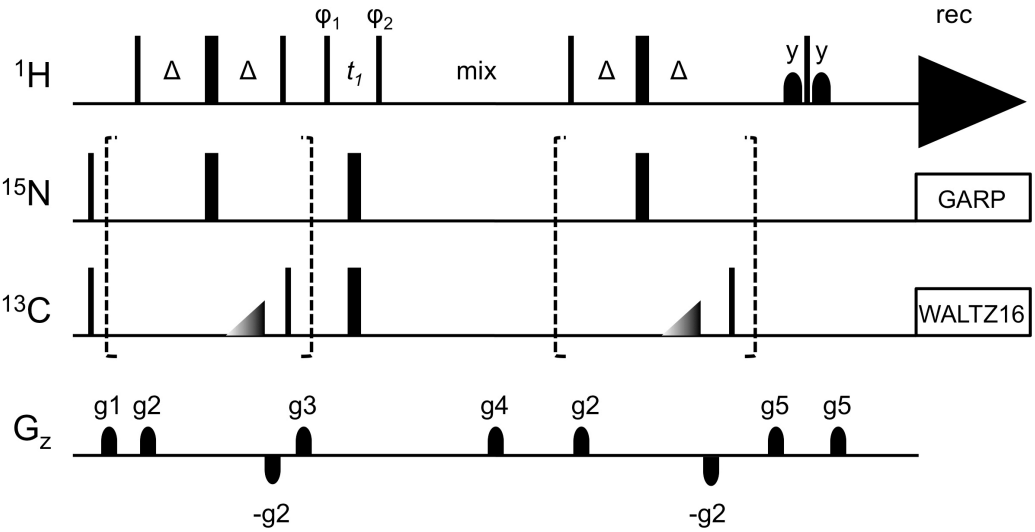


Figure S1-I. Pulse scheme for the simultaneous recording of intra-and intermolecular NOEs in protein ligand complexes. The half filters applied on the ^{13}C and ^{15}N are within brackets. Narrow and wide pulses indicate 90° and 180° pulses respectively. Unless indicated, all pulses are applied along the x-axis. Broadband ^{13}C inversion (during Δ) is achieved using a WURST adiabatic pulse with a peak γB_1 = 4.5 kHz and with apodization of the first and last 30 % using a sine function, a 60 kHz frequency sweep, and a duration of 2.8 ms. ^{13}C and ^{15}N decoupling during acquisition were achieved with WALTZ16 (γB_1 = 0.8 kHz) and GARP (γB_1 = 0.66kHz) decoupling scheme. The value for Δ was 5.2 ms and the NOE mixing time was set to 150 ms. Gradient levels were as follows: g1=1.0 ms, 2.6 Gcm^{-1}; g2=1.2 ms, 2 Gcm^{-1}; g3=2.4 ms, 7 Gcm^{-1}; g4=2.0 ms, 8 Gcm^{-1}; g5=1.0 ms, 8 Gcm^{-1}. The phase cycling was φ_1=(x,-x,-x,x,y,-y,-y,y); φ_2=(x,x,-x,-x,y,y,-y,-y) and receiver was (x,-x). Quadrature detection in F1 is achieved by States-TPPI of φ_1.

Four sub spectra are recorded depending if the half filters are applied ([+]) or not ([-]) :S1 : [-], [-]; S1 : [+], [-]; S1 : [-], [+]; S1 : [+], [+].

The linear combinations of this four sub spectra lead to four datasets. 2 datasets exhibiting either protein-protein (P→P) or ligand-ligand (L→L) intramolecular NOE, the 2 others carrying either protein-ligand (P→L) or ligand-protein (L→P) intermolecular NOE. The linear combinations are as follows:

S1	+ S2	+S3	+ S4 :	**L**	→	**L**	(intra)
S1	- S2	- S3	+ S4 :	**P**	→	**P**	(intra)
S1	- S2	+S3	- S4 :	**P**	→	**L**	(inter)
S1	+ S2	- S3	- S4 :	**L**	→	**P**	(inter)

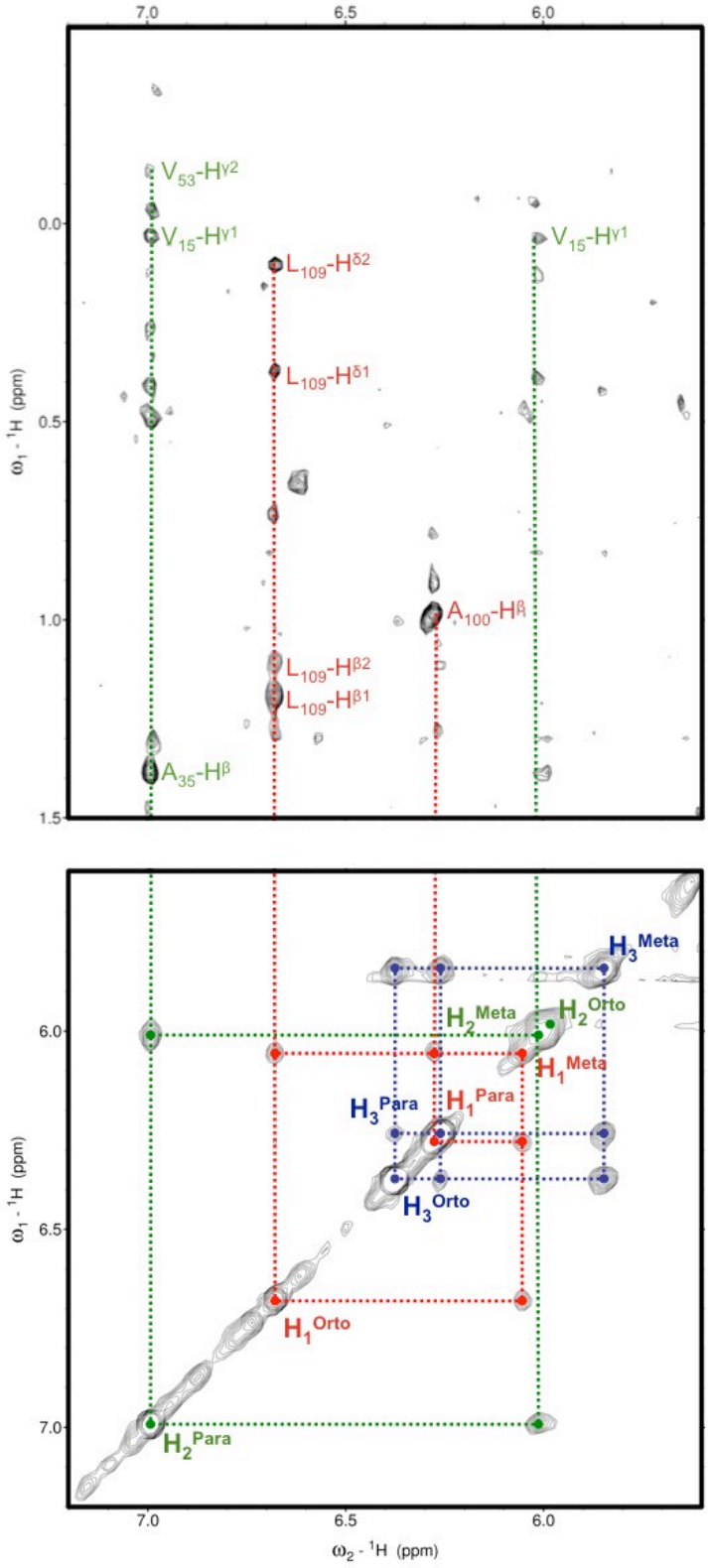
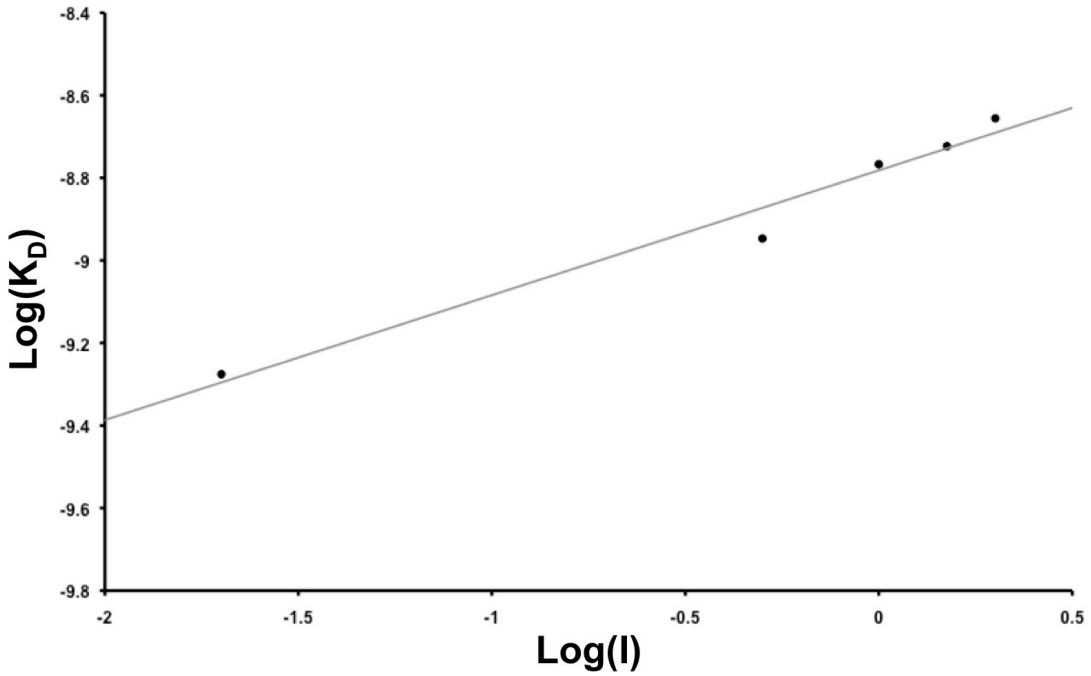


Figure S1-II. Doubly ^{15}N/^{13}C-filtered ^{1}H-^{1}H NOESY experiment recorded on a {^{13}C, ^{15}N}-Q83/[Ga^{III}(Ent)]$^{3-}$ sample. Top frame: close-up of the P → L intermolecular dataset. Bottom frame: close up on the L → L intramolecular dataset.

Supplement Table S3. List of the Q83-$[Ga^{III}(Ent)]^{3-}$ intermolecular contacts.

Pocket I	Pocket II	Pocket III
$Ala_{100}\ H^{\beta} \rightarrow H_1^{Para}$	$Val_{15}H^{\gamma1} \rightarrow H_2^{Para}$	$Trp_{62}\ H^{\varepsilon} \rightarrow H_3^{Meta}$
$Leu_{109}\ H^{N} \rightarrow H_1^{Meta}$	$Val_{15}H^{\gamma1} \rightarrow H_2^{Meta}$	$Trp_{62}\ H^{\zeta} \rightarrow H_3^{Meta}$
$Leu_{109}\ H^{N} \rightarrow H_1^{Orth}$	$Ala_{35}\ H^{\beta} \rightarrow H_2^{Para}$	$Ala_{81}\ H^{\beta} \rightarrow H_3^{Orth}$
$Leu_{109}H^{\beta1} \rightarrow H_1^{Orth}$	$Ala_{35}\ H^{\beta} \rightarrow H_2^{Meta}$	
$Leu_{109}H^{\beta2} \rightarrow H_1^{Orth}$	$Met_{36}H^{N} \rightarrow H_2^{Para}$	
$Leu_{109}H^{\delta1} \rightarrow H_1^{Orth}$	$Met_{36}H^{N} \rightarrow H_2^{Meta}$	
$Leu_{109}H^{\delta1} \rightarrow H_1^{Meta}$	$Ala_{52}\ H^{N} \rightarrow H_2^{Meta}$	
$Leu_{109}H^{\delta2} \rightarrow H_1^{Orth}$	$Val_{53}H^{\gamma2} \rightarrow H_2^{Para}$	
$Leu_{109}H^{\delta2} \rightarrow H_1^{Meta}$	$Val_{53}H^{\gamma2} \rightarrow H_2^{Meta}$	
$His_{110}\ H^{N} \rightarrow H_1^{Orth}$	$Val_{53}H^{\gamma2} \rightarrow H_2^{Orth}$	
$Met_{111}\ H^{N} \rightarrow H_1^{Meta}$		
$Met_{111}\ H^{N} \rightarrow H_1^{Orth}$		
$Met_{111}\ H^{\varepsilon} \rightarrow H_1^{Meta}$		
$Met_{111}\ H^{\varepsilon} \rightarrow H_1^{Orth}$		



Supplemental Figure S4. Ionic strength dependency of Q83 affinity for $[Fe^{III}(Ent)]^{3-}$. The affinity of Q83 for was measure by fluorescence quenching at different ionic strength (20 mM, 0.5 M, 1 M, 1.5 M and 2 M).

REFERENCES TO SUPPLEMENTAL MATERIAL

Zwahlen, C., Legault, P., Vincent, S., Greenblatt, J., Konrat, R., and Kay, L. E. (1997) *J. Am. Chem. Soc.* **119**, 6711-6721

Eichmuller, C., Schuler, W., Konrat, R., and Krautler, B. (2001) *J. Biomol. NMR* **21**, 107-116

Slijper, M., Kaptein, R., and Boelens, R. (1996) *J. Magn. Reson. B* **111**, 199-203