

Supplementary Information

Electrostatic Fields Near the Active Site of Human

Aldose Reductase:

2. New Inhibitors and Complications due to Hydrogen Bonds

*Lin Xu, Aina E. Cohen and Steven G. Boxer**

1. Analysis of Stark Spectroscopy Data

The effect of an applied external electric field \vec{F} on the vibrational frequency shift $\Delta\bar{\nu}$ and the transition dipole M can be expressed as Taylor expansions:

$$\Delta\bar{\nu} = -\frac{1}{hc} \left(\Delta\vec{\mu} \cdot \vec{F} + \frac{\vec{F} \cdot \Delta\alpha \cdot \vec{F}}{2} + \dots \right); \quad (1)$$

$$M = M + A \cdot \vec{F} + \vec{F} \cdot B \cdot \vec{F} + \dots, \quad (2)$$

where h is Planck's constant, c is the speed of light, $\Delta\vec{\mu}$ is the difference dipole moment, also called the Stark tuning rate, $\Delta\alpha$ is the difference polarizability, A is the transition polarizability, and B is the transition hyperpolarizability. A Stark spectrum, $\Delta A_\chi(\bar{\nu})$, for an isotropic, immobilized sample is the absorption in the presence of a field, $A_\chi(\bar{\nu})$, probed with polarized light whose electric field vector makes an angle χ to the direction of the applied field, minus the absorption in the absence of the field:

$$\Delta A_\chi(\bar{\nu}) = |\vec{F}|^2 A_\chi A(\bar{\nu}) + \frac{|\vec{F}|^2}{15hc} B_\chi \bar{\nu} \frac{\partial}{\partial \bar{\nu}} \frac{A(\bar{\nu})}{\bar{\nu}} + \frac{|\vec{F}|^2}{30h^2c^2} C_\chi \bar{\nu} \frac{\partial^2}{\partial \bar{\nu}^2} \frac{A(\bar{\nu})}{\bar{\nu}} \quad (3)$$

where A_χ , B_χ , and C_χ are the zeroth, first and second derivative coefficients, respectively. These coefficients can be correlated with the Stark parameters $\Delta\vec{\mu}$, $\Delta\alpha$, A and B using expressions derived from Eqn. 1 and 2 (see ref. (1) for full details). With a series of simplifications for a one-dimensional system like a nitrile (1), three Stark parameters $|\Delta\vec{\mu}_\parallel|$, $\Delta\alpha_\parallel$, and A_\parallel can be determined from experimental data, where \parallel denotes the polarizability components that are parallel to the molecular axis.

The vibrational Stark spectrum of inhibitor **2** is shown in Figure 3A in the main paper. From the second derivative contribution, $|\Delta\mu_{CN}^\dagger|$ is found to be 0.032 ± 0.001 D (or expressed as a Stark tuning rate, $|\Delta\mu_{CN}^\dagger| = 0.53 \text{ cm}^{-1}/(\text{MV}/\text{cm})$), which implies a 0.53 cm^{-1} shift for molecules whose CN bond is aligned parallel with a 1 MV/cm electric field. Although there is an unusually large first and zeroth derivative contribution, $\Delta\alpha_\parallel$ is found to be small and slightly negative, with a value of $-1.01 \pm 0.19 \text{ \AA}^3$, which implies a 0.03 cm^{-1} shift to higher energy for molecules aligned parallel to a 1 MV/cm electric field. This result is on the same scale with previous data for aromatic nitriles (1). It indicates that the

large first derivative contribution observed in Figure 3A is due to a large transition polarizability (A_{\parallel}) and large difference dipole moment ($\Delta\vec{\mu}$), not to large difference polarizability ($\Delta\alpha_{\parallel}$). Therefore the large first and zeroth derivative contribution will not affect using $\Delta\vec{\mu}$, the linear Stark effect to interpret IR frequency shifts in terms of changes in local electrostatic fields (Eqn. 1 in the main paper).

2. Crystallographic Data

Table S1: X-ray crystallography data collection and refinement statistics of the WT *h*ALR2 / 2 / NADP⁺ complex. The PDB code is 3T42.

Number of crystals used	1
Space group	P1
Unit-cell parameters	
a, b, c (Å)	39.9, 47.0, 46.9
∠, β, γ (°)	76.3, 77.2, 67.8
Resolution range (Å)	22.28-1.28
Unique reflections	69094
Completeness (%)	88.44
Redundancy	3.9
$I/\sigma_{overall}$	18.4
Residues	316
Waters	141
Co-enzyme	1
Inhibitor	1
Other molecules	Citrate 1
R_{work} (%)	15.15
R_{free} (%)	17.85
RMSD bond (Å)	0.008
RMSD angle (°)	1.341
RMSD dihedral (°)	19.401

3. Temperature Dependent IR Data

Table S2: Measured nitrile vibrational frequency, full width at half maximum (FWHM) and area ratio of two peaks of inhibitor **2** in the specificity pocket of WT *h*ALR2 at various temperatures.

Temperature (°C)	Frequency (cm ⁻¹)		FWHM (cm ⁻¹)		Area ratio ^a
	Peak 1 Non-hydrogen bonded -CN	Peak 2 Hydrogen bonded -CN	Peak 1 Non-hydrogen bonded -CN	Peak 2 Hydrogen bonded -CN	
35	2237.9±0.1	2252.2±0.1	12.0±0.2	9.8±0.2	1.2±0.1
25	2238.1±0.1	2252.8±0.1	12.9±0.6	9.6±0.2	1.3±0.1
15	2238.1±0.2	2253.3±0.1	14.4±0.4	9.4±0.2	1.4±0.1
5	2238.0±0.3	2253.7±0.1	14.8±0.5	9.4±0.1	1.7±0.2
-5	2237.9±0.3	2254.2±0.1	15.2±0.4	9.3±0.1	2.0±0.2
-15	2237.6±0.4	2254.6±0.1	15.5±1.5	9.3±0.1	2.4±0.4
-196	---	2258.1±0.2	---	9.1±0.2	---
-196 (T113A)	2240.9±0.1	---	6.3±0.3	---	---

^a Hydrogen bonded peak / Non-hydrogen bonded peak

4. Parameterization of Inhibitor 2

Figure S1: Atom names for **2**.

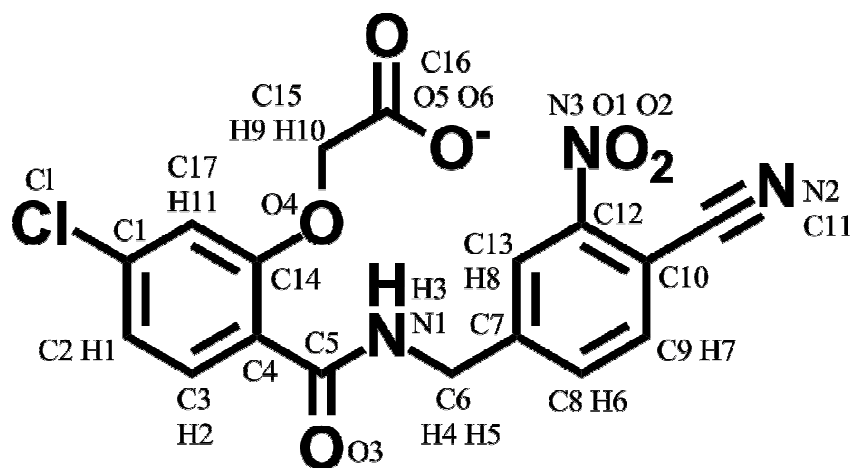


Table S3: Charges and nonbonded parameters of **2**.

Atom	Charge	Sigma (nm)	Epsilon (kJ mol ⁻¹)
O1	-0.37340	2.95992e-01	8.78640e-01
N3	0.61170	3.25000e-01	7.11280e-01
O2	-0.35650	2.95992e-01	8.78640e-01
C12	-0.15890	3.39967e-01	3.59824e-01
C13	-0.07600	3.39967e-01	3.59824e-01
H8	0.17030	2.59964e-01	6.27600e-02
C10	0.03090	3.39967e-01	3.59824e-01
C11	0.24030	3.39967e-01	3.59824e-01
N2	-0.35140	3.56400e-01	7.11280e-01
C9	-0.10510	3.39967e-01	3.59824e-01
H7	0.16280	2.59964e-01	6.27600e-02
C8	-0.06970	3.39967e-01	3.59824e-01
H6	0.23280	2.59964e-01	6.27600e-02
C7	-0.08740	3.39967e-01	3.59824e-01
C6	0.14240	3.39967e-01	4.57730e-01
H4	0.07920	2.47135e-01	6.56888e-02

H5	0.04920	2.47135e-01	6.56888e-02
N1	-0.55930	3.25000e-01	7.11280e-01
H3	0.38530	1.06908e-01	6.56888e-02
C5	0.68990	3.39967e-01	3.59824e-01
O3	-0.66230	2.95992e-01	8.78640e-01
C4	-0.16630	3.39967e-01	3.59824e-01
C3	-0.05460	3.39967e-01	3.59824e-01
H2	0.15130	2.59964e-01	6.27600e-02
C2	-0.18570	3.39967e-01	3.59824e-01
H1	0.13870	2.59964e-01	6.27600e-02
C1	0.06600	3.39967e-01	3.59824e-01
Cl	-0.12050	3.47094e-01	1.10876e+00
C17	-0.19810	3.39967e-01	3.59824e-01
H11	0.15920	2.59964e-01	6.27600e-02
C14	0.21100	3.39967e-01	3.59824e-01
O4	-0.33570	3.00001e-01	7.11280e-01
C15	0.00250	3.39967e-01	4.57730e-01
H9	0.03650	2.47135e-01	6.56888e-02
H10	0.06220	2.47135e-01	6.56888e-02
C16	0.90430	3.39967e-01	3.59824e-01
O5	-0.86830	2.95992e-01	8.78640e-01
O6	-0.79720	2.95992e-01	8.78640e-01

References:

(1) Andrews, S. S., and Boxer, S. G. (2000) Vibrational Stark Effects of Nitriles I. Methods and Experimental Results, *J. Phys. Chem. A* 104, 11853-11863.