Supporting Materials

D.K. Lee, D.J. Albershardt, and R.S. Cantor. "Exploring the mechanism of general anesthesia: Kinetic analysis of $GABA_A$ receptor electrophysiology"

Two Tables and three Figures are provided below. In addition, animation software is available for download that dynamically illustrates the time-dependent flow of probability among protein states in the kinetic scheme. It runs interactively, allowing the user to vary the three experimental parameters: c_{ag} (agonist concentration), c_{an} (anesthetic concentration), and τ (pulse duration). The user can select whether the anesthetic is coapplied with agonist during the pulse, or is continuously present. This file, "KineticSchemeAnimation.nb", requires either Mathematica™ or PlayerPro™ (Wolfram Research, Inc., Champaign, IL) to be run.

Table S1. Parameter set. The values of model parameters used in generating the predicted traces, chosen to reproduce the features of current traces (9, 11-13) for recombinant $\alpha_1\beta_2\gamma_{2L}$ $GABA_A$ receptors and the anesthetic isoflurane, over a broad range of agonist and isoflurane concentrations. A set of 28 independent parameters is indicated in **boldface** on the left; the parameters on the right are derived as indicated from this set. Of the 28, the 14 rate constants of agonist binding (a) and conformational transitions (b) depend only on the protein, i.e., they are independent of any effects of bilayer adsorption. In contrast, the bilayer adsorption/desorption rate constants (c) depend only on the solute (agonist or anesthetic) and the bilayer, i.e., they are independent of the protein. The sensitivities (d, e) depend both on the protein and on bilayer-mediated effects. Note that only three of the independent parameters $\{K_{d,R}, K_{ads,ag}, K_{ads,an}\}$ involve concentrations; the rest are either first-order rate constants or dimensionless. Transition rate constants of monoliganded protein are the geometric mean of the corresponding constants for unliganded and diliganded protein, i.e., $k_{A_1X\to A_1Y} = (k_{A_0X\to A_0Y}\, k_{A_2X\to A_2Y})^{1/2}$.

(a) Agonist binding/unbinding:

$\boldsymbol{k_{u,R}} = \mathbf{100\ s^{-1}}$	$\boldsymbol{K_{d,R}} = \mathbf{167\ \mu M}$	$k_{b,R} = k_{u,R} / K_{d,R} = 0.6\times10^6\ M^{-1}\ s^{-1}$
$\boldsymbol{k_{u,O}} = \mathbf{0.40\ s^{-1}}$		$K_{d,O} = K_{d,R}\,(K°_{open,2}/K°_{open,0})^{1/2} = 0.52\ \mu M$
		$k_{b,O} = k_{u,O} / K_{d,O} = 0.77\times10^6\ M^{-1}\ s^{-1}$
$\boldsymbol{k_{u,D}} = \mathbf{4.0\ s^{-1}}$		$K_{d,D} = K_{d,R}\,(K°_{desbr,2}/K°_{desbr,0})^{1/2} = 0.20\ \mu M$
		$k_{b,D} = k_{u,D} / K_{d,D} = 2.0\times10^6\ M^{-1}\ s^{-1}$

(b) Conformational transitions; standard rate and equilibrium constants:

Opening/closing:

$\boldsymbol{k°_{A_0R\rightarrow A_0O} = 8.1\times10^{-4}\ s^{-1}}$ $\boldsymbol{K°_{open,0} = 2.9\times10^{-4}}$ $k°_{A_0O\rightarrow A_0R} = k°_{A_0R\rightarrow A_0O}/K°_{open,0} = 2.8\ s^{-1}$

$\boldsymbol{k°_{A_2R\rightarrow A_2O} = 800\ s^{-1}}$ $\boldsymbol{K°_{open,2} = 30}$ $k°_{A_2O\rightarrow A_2R} = k°_{A_2R\rightarrow A_2O}/K°_{open,2} = 26.7\ s^{-1}$

Linear desensitization/resensitization:

$\boldsymbol{k°_{A_0O\rightarrow A_0D} = 0.84\ s^{-1}}$ $\boldsymbol{K°_{deslin,0} = 0.22}$ $k°_{A_0D\rightarrow A_0O} = k°_{A_0O\rightarrow A_0D}/K°_{deslin,0} = 3.82\ s^{-1}$

$\boldsymbol{k°_{A_2O\rightarrow A_2D} = 1.1\ s^{-1}}$ $\boldsymbol{K°_{deslin,2} = 1.5}$ $k°_{A_2D\rightarrow A_2O} = k°_{A_2O\rightarrow A_2D}/K°_{deslin,2} = 0.73\ s^{-1}$

Branched desensitization/resensitization:

$\boldsymbol{k°_{A_0R\rightarrow A_0D} = 5.4\times10^{-3}\ s^{-1}}$ $K°_{desbr,0} = K°_{open,0}\ K°_{deslin,0} = 6.4\times10^{-5}$

$k°_{A_0D\rightarrow A_0R} = k°_{A_0R\rightarrow A_0D}/K°_{desbr,0} = 84.6\ s^{-1}$

$\boldsymbol{k°_{A_2R\rightarrow A_2D} = 1.0\ s^{-1}}$ $K°_{desbr,2} = K°_{open,2}\ K°_{deslin,2} = 45.0$

$k°_{A_2D\rightarrow A_2R} = k°_{A_2R\rightarrow A_2D}/K°_{desbr,2} = 0.022\ s^{-1}$

(c) Solute adsorption/desorption to bilayer (anesthetic = isoflurane):

$\boldsymbol{k_{off,an} = 320\ s^{-1}}$ $\boldsymbol{K_{ads,an} = (2\ mM)^{-1}}$ $k_{on,an} = k_{off,an}\ K_{ads,an} = 1.6\times10^{5}\ M^{-1}\ s^{-1}$

$\boldsymbol{k_{off,ag} = 40\ s^{-1}}$ $\boldsymbol{K_{ads,ag} = (10\ mM)^{-1}}$ $k_{on,ag} = k_{off,ag}\ K_{ads,ag} = 4.0\times10^{3}\ M^{-1}\ s^{-1}$

(d) Sensitivities to agonist (GABA):

$\boldsymbol{\alpha_{ag,R\rightarrow O} = 6.0}$ $\boldsymbol{\alpha_{ag,O\rightarrow R} = -1.0}$ $\alpha_{ag,open} = \alpha_{ag,R\rightarrow O} - \alpha_{ag,O\rightarrow R} = 7.0$

$\boldsymbol{\alpha_{ag,O\rightarrow D} = 0.0}$ $\boldsymbol{\alpha_{ag,D\rightarrow O} = 0.5}$ $\alpha_{ag,deslin} = \alpha_{ag,O\rightarrow D} - \alpha_{ag,D\rightarrow O} = -0.5$

$\boldsymbol{\alpha_{ag,R\rightarrow D} = 6.4}$ $\alpha_{ag,D\rightarrow R} = -0.1$ $\alpha_{ag,desbr} = \alpha_{ag,R\rightarrow D} - \alpha_{ag,D\rightarrow R} = 6.5$

(e) Sensitivities to anesthetic (isoflurane):

$\boldsymbol{\alpha_{an,R\rightarrow O} = -9.1}$ $\boldsymbol{\alpha_{an,O\rightarrow R} = -2.5}$ $\alpha_{an,open} = \alpha_{an,R\rightarrow O} - \alpha_{an,O\rightarrow R} = -6.6$

$\boldsymbol{\alpha_{an,O\rightarrow D} = -0.4}$ $\boldsymbol{\alpha_{an,D\rightarrow O} = 10.7}$ $\alpha_{an,deslin} = \alpha_{an,O\rightarrow D} - \alpha_{an,D\rightarrow O} = -11.1$

$\boldsymbol{\alpha_{an,R\rightarrow D} = -14.4}$ $\alpha_{an,D\rightarrow R} = 3.3$ $\alpha_{an,desbr} = \alpha_{an,R\rightarrow D} - \alpha_{an,D\rightarrow R} = -17.7$

Table S2. Rate constants. Values of rate constants of conformational transitions $k_{A_iX \rightarrow A_iY}$ at representative levels of bilayer adsorption (θ_{an}, θ_{ag}) and corresponding equilibrium aqueous concentrations (c_{an}, c_{ag}), calculated from

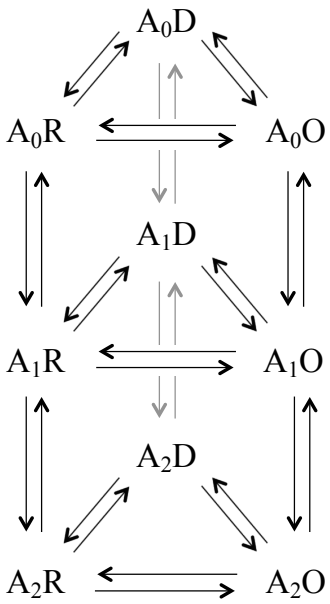
$$k_{A_iX \rightarrow A_iY} = k^{\circ}_{A_iX \rightarrow A_iY} \exp[-(\alpha_{ag,X \rightarrow Y}\, \theta_{ag} + \alpha_{an,X \rightarrow Y}\, \theta_{an})].$$

Rate constants are expressed in s^{-1} units; c_{an} and c_{ag} are expressed in mM units. Values corresponding to he free energy landscapes plotted in Fig. S2 are indicated in bold with corresponding color.

θ_{an}	c_{an}	θ_{ag}	c_{ag}	$k_{A_0R \rightarrow A_0O}$	$k_{A_0O \rightarrow A_0R}$	$k_{A_0O \rightarrow A_0D}$	$k_{A_0D \rightarrow A_0O}$	$k_{A_0R \rightarrow A_0D}$	$k_{A_0D \rightarrow A_0R}$
0	**0**	**0**	**0**	**0.00081**	**2.79**	**0.84**	**3.82**	**0.0054**	**84.6**
.2	.5	0	0	0.00500	4.61	0.91	0.45	0.096	43.7
.25	.67	0	0	0.00788	5.22	0.93	0.263	0.198	37.1
.33	1.0	0	0	0.0168	6.43	0.96	0.108	0.656	28.2
0.5	**2**	**0**	**0**	**0.0766**	**9.75**	**1.03**	**0.0181**	**7.23**	**16.3**
0.67	4	0	0	0.349	14.8	1.10	0.00305	79.7	9.38
0.75	6	0	0	0.746	18.2	1.13	0.00125	265	7.12
0.8	8	0	0	1.17	20.6	1.16	0.000732	544	6.04
0.89	16	0	0	2.64	25.8	1.20	0.000282	1956	4.50

θ_{an}	c_{an}	θ_{ag}	c_{ag}	$k_{A_2R \rightarrow A_2O}$	$k_{A_2O \rightarrow A_2R}$	$k_{A_2O \rightarrow A_2D}$	$k_{A_2D \rightarrow A_2O}$	$k_{A_2R \rightarrow A_2D}$	$k_{A_2D \rightarrow A_2R}$
0	**0**	**0**	**0**	**800**	**26.7**	**1.1**	**0.73**	**1.0**	**0.0222**
0.2	0.5	0	0	4940	45.0	1.19	0.086	17.8	0.0115
0.25	0.67	0	0	7780	49.8	1.22	0.0505	36.6	0.00974
0.33	1	0	0	16600	61.4	1.26	0.0207	122	0.00740
0.5	**2**	**0**	**0**	**75700**	**93.1**	**1.34**	**0.00348**	**1340**	**0.00427**
0.67	4	0	0	345000	141	1.44	0.000585	14760	0.00246
0.8	8	0	0	1160000	197	1.51	0.000140	100700	0.00159
0.89	16	0	0	2610000	246	1.57	0.0000543	362000	0.00118
0	0	0.2	2.5	241	32.6	1.1	0.664	0.278	0.0227
0	0	0.25	3.33	179	34.2	1.1	0.647	0.202	0.0228
0	0	0.33	5	108	37.2	1.1	0.621	0.118	0.0230
0	**0**	**0.5**	**10**	**39.8**	**44.0**	**1.1**	**0.571**	**0.0408**	**0.0233**
0	0	0.67	20	14.7	51.9	1.1	0.525	0.0140	0.0238
0	0	0.8	40	6.58	59.3	1.1	0.492	0.00598	0.0241
0	0	0.89	80	3.86	64.9	1.1	0.470	0.00338	0.0243

Figure S1. Kinetic scheme.



Opening/closing:

$$K_{\text{open,i}} = k_{A_iR \rightarrow A_iO} / k_{A_iO \rightarrow A_iR} \qquad i = \{0, 1, 2\}$$

Linear desensitization/resensitization:

$$K_{\text{deslin,i}} = k_{A_iO \rightarrow A_iD} / k_{A_iD \rightarrow A_iO} \qquad i = \{0, 1, 2\}$$

Branched desensitization/resensitization:

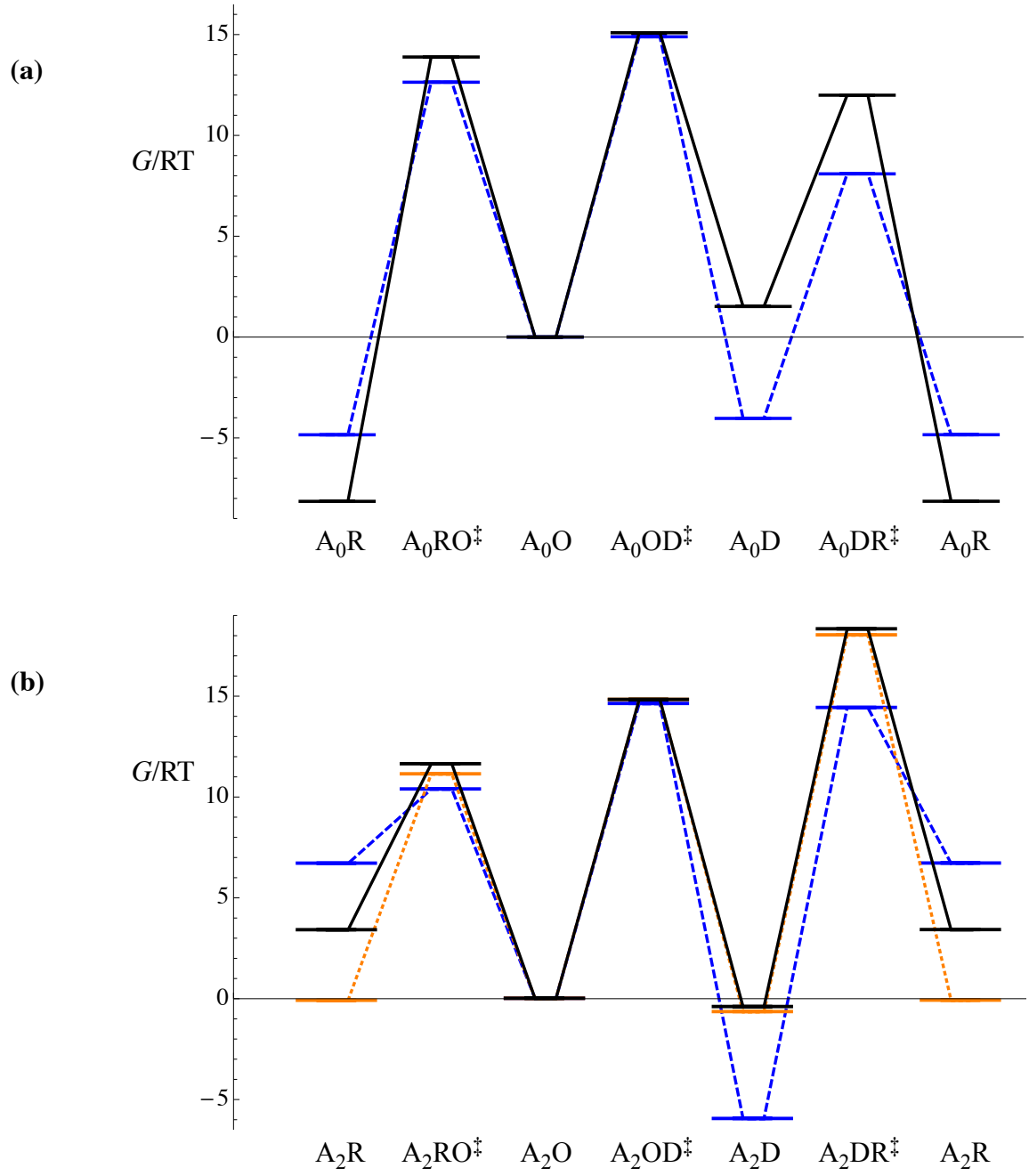
$$K_{\text{desbr,i}} = k_{A_iR \rightarrow A_iD} / k_{A_iD \rightarrow A_iR} \qquad i = \{0, 1, 2\}$$

Agonist unbinding/binding:

$$K_{d,X} = k_{u,X} / k_{b,X} \qquad X = \{R, O, D\}$$

The kinetic scheme includes all nine protein states and the elementary kinetic steps connecting pairs of states. Protein states, labeled A_iX, can exist in one of three conformational states {X = R, O, D}, each of which can be in one of three ligation states $i = \{0, 1, 2\}$, i.e., unliganded, monoliganded or diliganded. Vertical arrows represent agonist binding (down) and unbinding (up) steps; other arrows represent conformational transitions at fixed ligation. Relationships among thermodynamic equilibrium constants (K) and rate constants (k) are listed on the right.

Figure S2. Free energy landscapes.



The dimensionless free energy landscape G/RT is plotted for **(a)** unliganded and **(b)** diliganded protein. Black, solid lines: no adsorbed solutes: $\{\theta_{an} = 0, c_{an} = 0;\ \theta_{ag} = 0, c_{ag} = 0\}$. Blue, dashed lines: half-maximal adsorption of anesthetic $\{\theta_{an} = 0.5, c_{an} = K_{ads,an}^{-1} = 2$ mM; $\theta_{ag} = 0, c_{ag} = 0\}$. Orange, dotted lines: half-maximal adsorption of agonist $\{\theta_{an} = 0, c_{an} = 0;\ \theta_{ag} = 0.5, c_{ag} = K_{ads,ag}^{-1} = 10$ mM$\}$. Note that the resting state appears at both the left and right sides of the

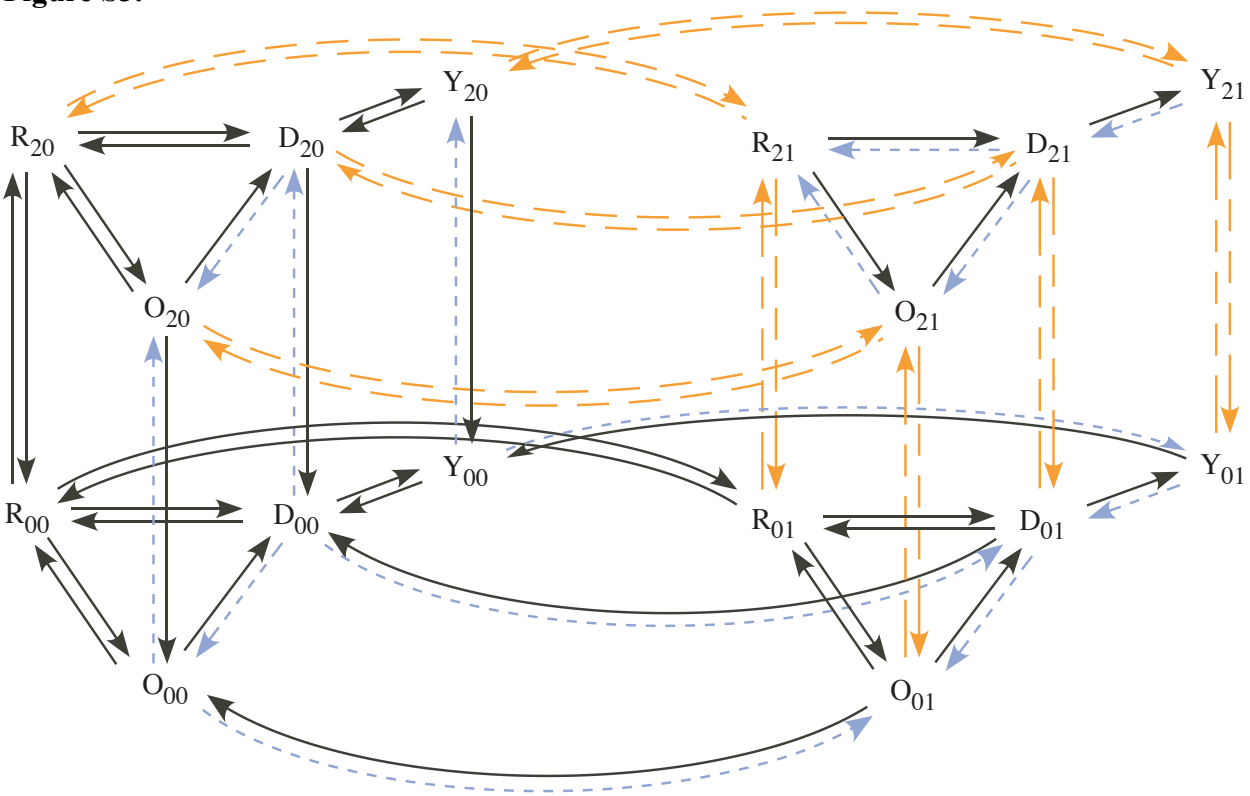
plot. The free energies of the conformational and transition states are indicated with horizontal lines; the lines connecting them serve only as a guide to the eye. Note that an additive constant (shift up or down) of any of the landscapes carries no physical significance; only free energy *differences* on a given landscape are meaningful. The relative vertical position of each landscape is thus arbitrary; the zero of free energy for each has arbitrarily been assigned to the open state, irrespective of solute adsorption or agonist ligation.

For a given landscape, the relative positions of the minima (conformational states) are unambiguously determined from the equilibrium constants. However, using the simple Arrenius-like expression from Eq. 2:

$$k_{A_iX \rightarrow A_iY} = A_{A_iX \rightarrow A_iY} \exp\{-\Delta G^{\ddagger}/RT\}, \quad \text{where} \quad \Delta G^{\ddagger} = G(A_iXY^{\ddagger}) - G(A_iX)$$

it is <u>not</u> possible to determine the free energies of the activated complexes, $G(A_iXY^{\ddagger})$, even assuming the pre-exponential factor A to have the same value for all transitions. For purposes of illustration of the effect of adsorption on the free energy of the activated complex, we have assumed a common value of A, one that is large enough to exceed the largest values of any of the rate constants in any of the plotted landscapes (and thus ensure that $\Delta G^{\ddagger} > 0$ for all transitions).

Figure S3.



Shown above is a generic kinetic scheme involving only *specific binding* of agonist and anesthetic, for the purpose of determining the minimum number of independent kinetic parameters; this can be compared with the number of parameters (28) of our kinetic model. It assumes a single anesthetic binding site and two equivalent agonist sites. A fourth conformational state "Y" is included, accessed from a single state, arbitrarily chosen to be D. States are labeled X_{ij}, where $i = \{0,1,2\}$ indicates agonist ligation and $j = \{0,1\}$ indicates anesthetic ligation. A complete set of independent parameters is indicated by solid lines for the corresponding binding and transition kinetic steps (the choice is not unique, but the number is fixed.) Dotted lines indicate processes whose rate constants are determined through thermodynamic constraints; dashed lines represent processes whose rate constants are identical to others in the diagram. The rate constant of each conformational transition of the monoliganded protein is assumed to be the geometric mean of the corresponding unliganded and diliganded transitions, and it is also assumed that anesthetic and agonist binding are independent, i.e., the rate constant of agonist binding to conformation X is independent of anesthetic ligation and vice versa. With these assumptions, for the purpose of determining the number of independent parameters, it is thus unnecessary to include the monoliganded states in the diagram (although they would be present and fully incorporated in the kinetics). For this case, with four conformational states, specification of 34 independent parameters would thus be required, the number increasing by a minimum of 8 for each additional conformational state.