SUPPORTING INFORMATION

Insertion of a Calcium-Responsive Beta Roll Domain into a Thermostable Alcohol Dehydrogenase Enables Tunable Control over Cofactor Selectivity

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AdhD and β-AdhD amino acid sequences

AdhD

```
MGDYKDDDDK AKRVNAFNDL KRIGDDKVTA IGMGTWGIGG RETPDYSRDK ESIEAIRYGL    60
ELGMNLIDTA EFYGAGHAEE IVGEAIKEFE REDIFIVSKV WPTHFGYEEA KKAARASAKR   120
LGTYIDLYLL HWPVDDFKKI EETLHALEDL VDEGVIRYIG VSNFNLELLQ RSQEVMRKYE   180
IVANQVKYSV KDRWPETTGL LDYMKREGIA LMAYTPLEKG TLARNECLAK IGEKYGKTAA   240
QVALNYLIWE ENVVAIPKAS NKEHLKENFG AMGWRLSEED REMARRCVED PNSSSVD      297
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β-AdhD

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MGDYKDDDDK AKRVNAFNDL KRIGDDKVTA IGMGTWGIGG RETPDYSRDK ESIEAIRYGL    60
ELGMNLIDTA EFYGAGHAEE IVGEAIKEFE REDIFIVSKV WPTHFGYEEA KKAARASAKR   120
LGTYIDLYLL HWPGGSARDD VLIGDAGANV LNGLAGNDVL SGGAGDDVLL GDEGSDLLSG   180
DAGNDDLFGG QGDDTYLFGV GYGHDTIYES GGGHDTIRFD DFKKIEETLH ALEDLVDEGV   240
IRYIGVSNFN LELLQRSQEV MRKYEIVANQ VKYSVKDRWP ETTGLLDYMK REGIALMAYT   300
PLEKGTLARN ECLAKIGEKY GKTAAQVALN YLIWEENVVA IPKASNKEHL KENFGAMGWR   360
LSEEDREMAR RCVEDPNSSS VD                                             382
```

DNA sequences of AdhD and β-AdhD

AdhD

```
ATGGGGGACT ACAAAGACGA TGACGACAAG GCAAAACGCG TGAATGCATT TAACGACCTG     60
AAACGTATTG GTGATGACAA AGTAACCGCT ATCGGCATGG GTACTTGGGG CATCGGTGGT    120
CGTGAAACCC CGGATTACAG CCGCGACAAA GAGTCCATCG AGGCGATCCG TTATGGCCTG    180
GAGCTGGGTA TGAACCTGAT TGACACGGCG GAGTTTTATG GTGCCGGCCA CGCTGAAGAG    240
ATTGTCGGTG AAGCCATCAA AGAGTTCGAA CGCGAGGACA TCTTCATTGT TTCGAAGGTC    300
TGGCCGACCC ACTTTGGTTA TGAAGAGGCG AAGAAAGCTG CACGCGCCAG CGCGAAGCGT    360
CTGGGCACCT ACATTGATCT GTACCTGTTG CATTGGCCGG TCGACGACTT TAAAAAGATT    420
GAAGAAACCC TGCACGCACT CGAGGATTTG GTGGATGAGG GTGTCATTCG CTACATCGGC    480
GTTTCCAATT TCAATCTGGA GTTGCTGCAA CGTAGCCAGG AAGTGATGCG TAAGTACGAG    540
ATCGTGGCGA ACCAGGTCAA ATACAGCGTG AAGGACCGTT GGCCAGAAAC GACCGGCCTG    600
CTGGACTATA TGAAACGTGA GGGTATCGCG CTGATGGCCT ATACGCCTCT AGAAAAAGGT    660
ACCCTGGCGC GTAACGAGTG CCTGGCAAAG ATCGGTGAGA AGTACGGTAA GACGGCGGCA    720
CAAGTTGCCC TGAATTACCT GATTTGGGAA GAGAATGTTG TGGCGATTCC GAAGGCGAGC    780
AACAAAGAGC ATCTGAAAGA GAACTTCGGC GCGATGGGCT GGCGCCTGAG CGAAGAAGAT    840
CGTGAGATGG CGCGCCGGTG TGTTGAGGAT CCGAATTCGA GCTCCGTGGA C             891
```

β-AdhD

```
ATGGGGGACT ACAAAGACGA TGACGACAAG GCAAAACGCG TGAATGCATT TAACGACCTG     60
AAACGTATTG GTGATGACAA AGTAACCGCT ATCGGCATGG GTACTTGGGG CATCGGTGGT    120
CGTGAAACCC CGGATTACAG CCGCGACAAA GAGTCCATCG AGGCGATCCG TTATGGCCTG    180
GAGCTGGGTA TGAACCTGAT TGACACGGCG GAGTTTTATG GTGCCGGCCA CGCTGAAGAG    240
ATTGTCGGTG AAGCCATCAA AGAGTTCGAA CGCGAGGACA TCTTCATTGT TTCGAAGGTC    300
TGGCCGACCC ACTTTGGTTA TGAAGAGGCG AAGAAAGCTG CACGCGCCAG CGCGAAGCGT    360
CTGGGCACCT ACATTGATCT GTACCTGTTG CATTGGCCGG GGGGCAGCGC GCGTGATGAC    420
GTGCTGATCG GCGACGCAGG CGCCAACGTC CTCAATGGCC TGGCGGGCAA CGACGTGCTG    480
TCCGGCGGCG CTGGCGACGA TGTGCTGCTG GGCGACGAGG GCTCGGACCT GCTCAGCGGC    540
GATGCGGGCA ACGACGATCT GTTCGGCGGG CAGGGCGATG ATACTTATCT GTTCGGGGTC    600
GGGTACGGGC ACGACACGAT CTACGAATCG GGCGGCGGCC ATGACACCAT CCGCTTCGAC    660
GACTTTAAAA AGATTGAAGA AACCCTGCAC GCACTCGAGG ATTTGGTGGA TGAGGGTGTC    720
ATTCGCTACA TCGGCGTTTC CAATTTCAAT CTGGAGTTGC TGCAACGTAG CCAGGAAGTG    780
ATGCGTAAGT ACGAGATCGT GGCGAACCAG GTCAAATACA GCGTGAAGGA CCGTTGGCCA    840
GAAACGACCG GCCTGCTGGA CTATATGAAA CGTGAGGGTA TCGCGCTGAT GGCCTATACG    900
CCTCTAGAAA AAGGTACCCT GGCGCGTAAC GAGTGCCTGG CAAAGATCGG TGAGAAGTAC    960
GGTAAGACGG CGGCACAAGT TGCCCTGAAT TACCTGATTT GGGAAGAGAA TGTTGTGGCG   1020
ATTCCGAAGG CGAGCAACAA AGAGCATCTG AAAGAGAACT TCGGCGCGAT GGGCTGGCGC   1080
CTGAGCGAAG AAGATCGTGA GATGGCGCGC CGGTGTGTTG AGGATCCGAA TTCGAGCTCC   1140
GTGGAC                                                              1146
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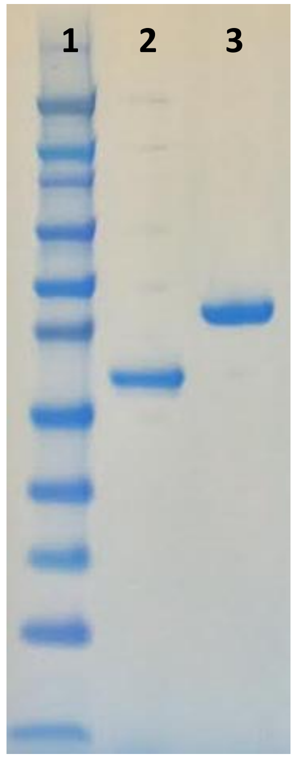


Figure S1. SDS-PAGE of AdhD and β-AdhD after gel filtration. Lane 1: molecular weight marker, lane 2: AdhD, lane 3: β-AdhD. Samples were lysed by heating for 1 h at 80°C prior to purification. A distinct band at approximately 34 kDa for AdhD and 42 kDa for β-AdhD is observed, consistent with the theoretical molecular masses.

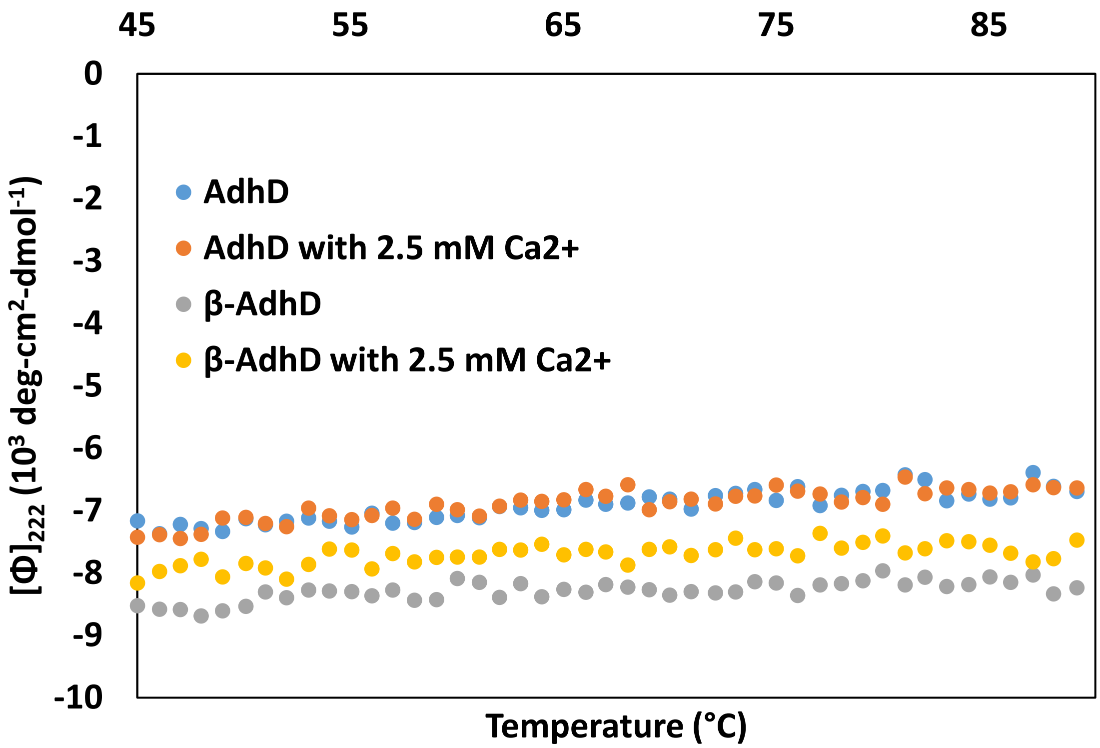


Figure S2. Thermal denaturation curves for AdhD and β-AdhD in the absence and presence of calcium as measured by the circular dichroic absorbance at 222 nm as temperature was varied from 25 to 90 °C. 1.25 µM samples were prepared in water and runs were performed in at least duplicate.

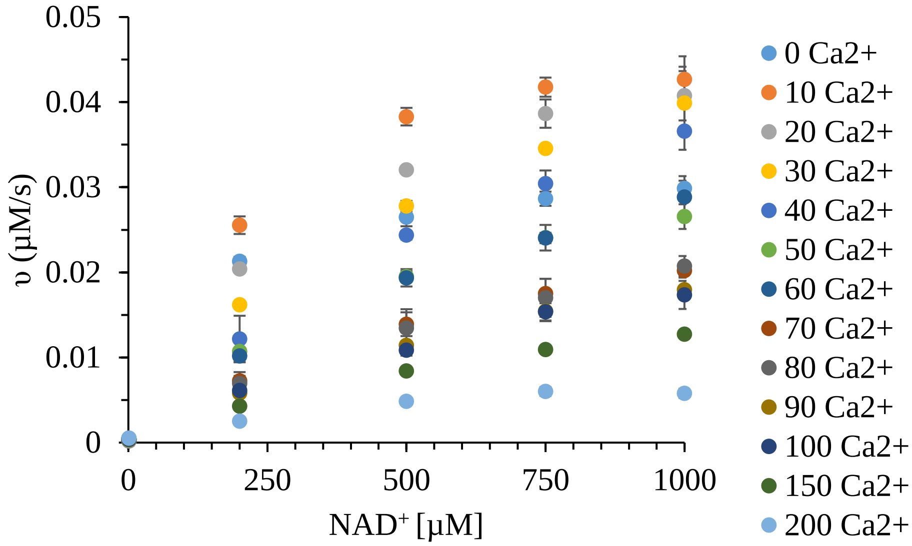


Figure S3. Rate versus NAD^+ at varying calcium concentrations for β-AdhD. Data were collected in at least triplicate.

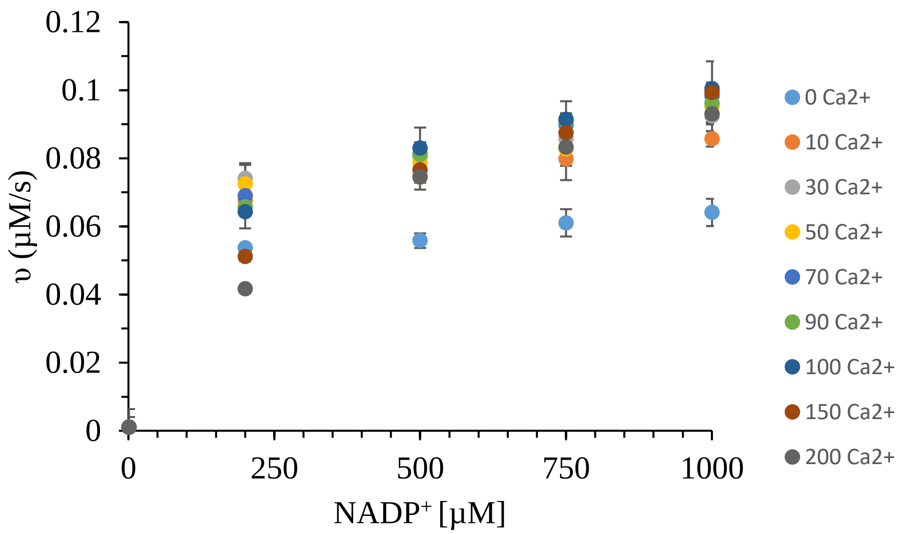
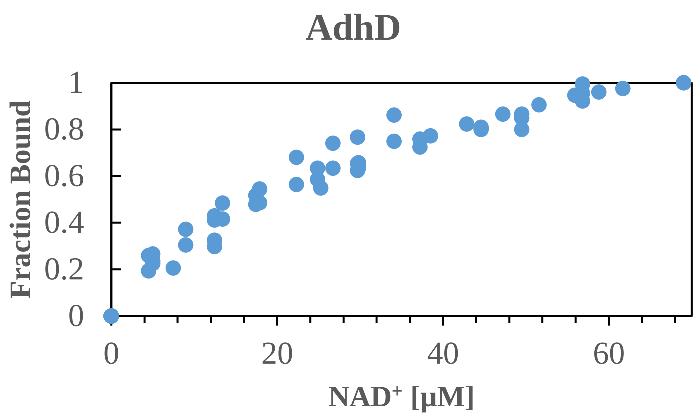
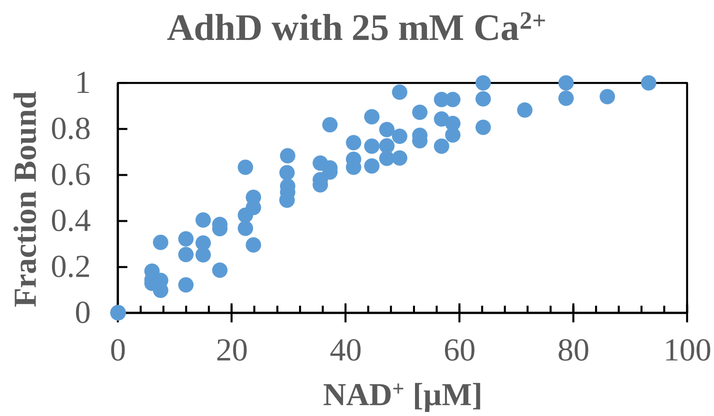
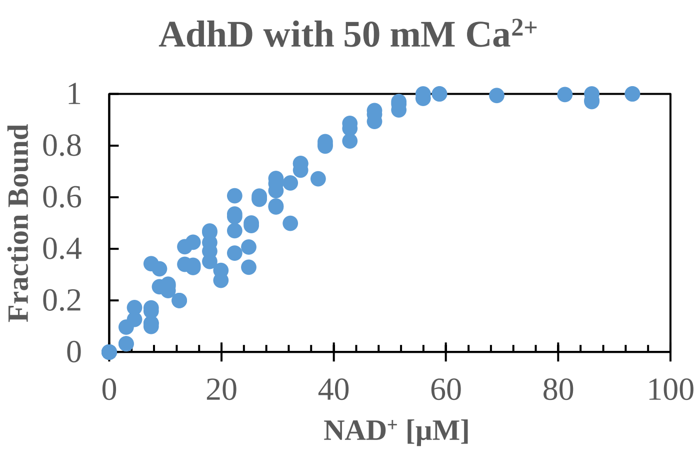
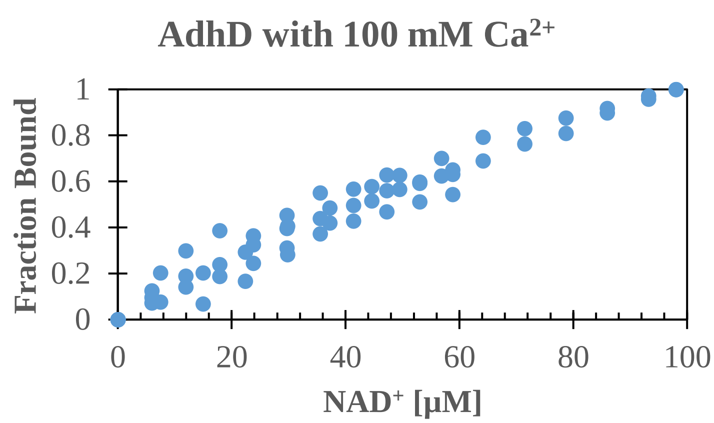


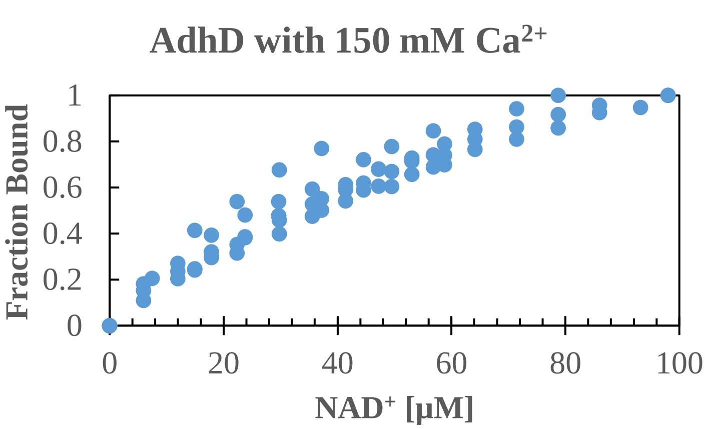
Figure S4. Rate versus $NADP^+$ at varying calcium concentrations for β-AdhD. Data were collected in at least triplicate.

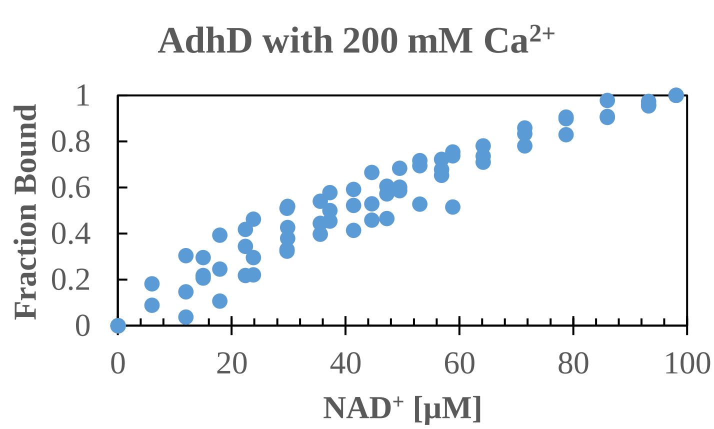

AdhD
Fraction Bound
NAD+ [μM]

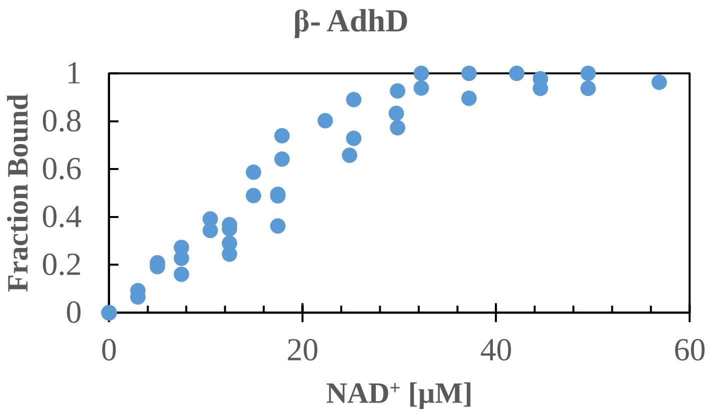

AdhD with 25 mM Ca2+
Fraction Bound
NAD+ [μM]

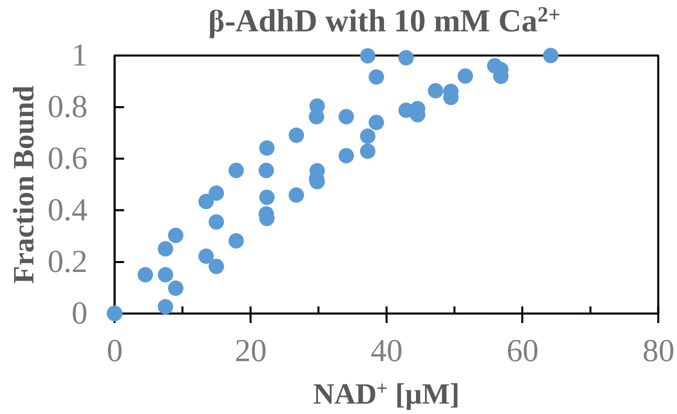

AdhD with 50 mM Ca2+
Fraction Bound
NAD+ [μM]

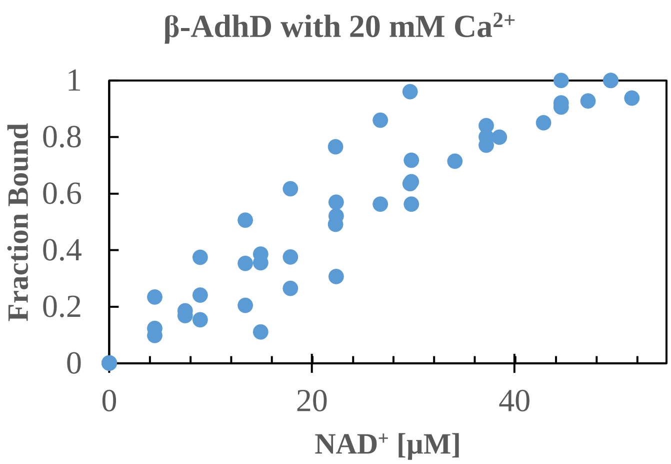

AdhD with 100 mM Ca2+
Fraction Bound
NAD+ [μM]

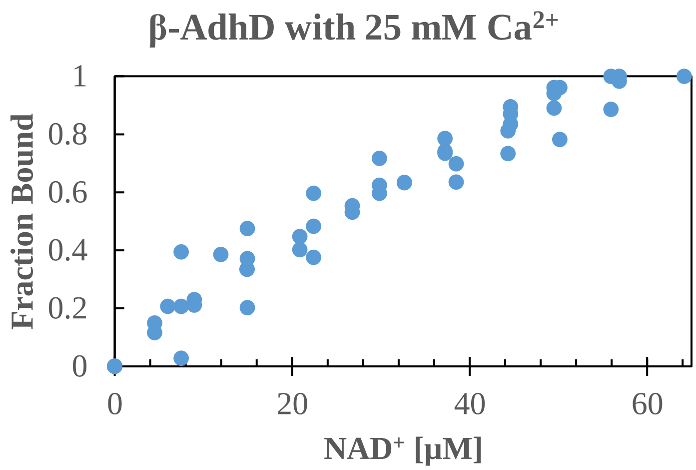

AdhD with 150 mM Ca2+
Fraction Bound
NAD+ [μM]

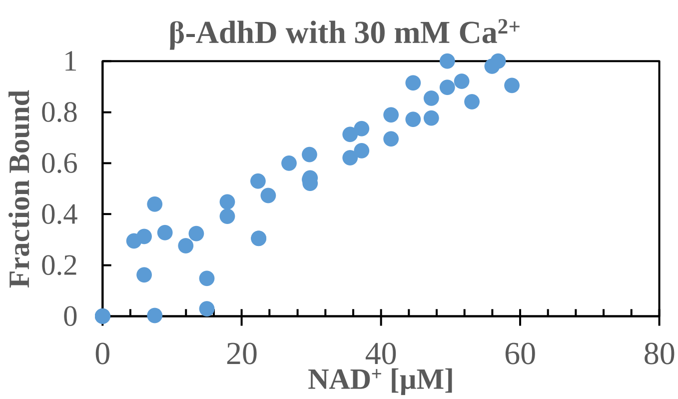

AdhD with 200 mM Ca2+
Fraction Bound
NAD+ [μM]

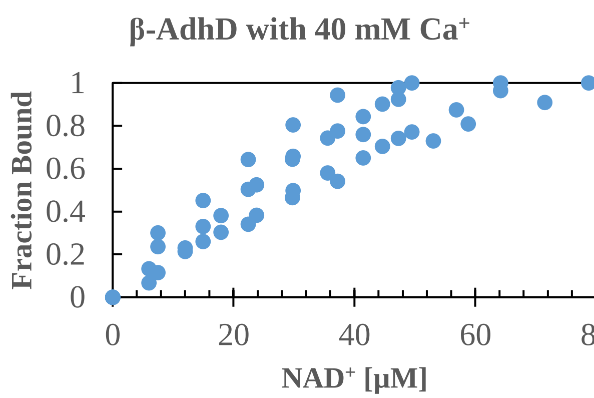

β- AdhD
Fraction Bound
1
0.8
0.6
0.4
0.2
0
0
20
40
60
NAD+ [μM]

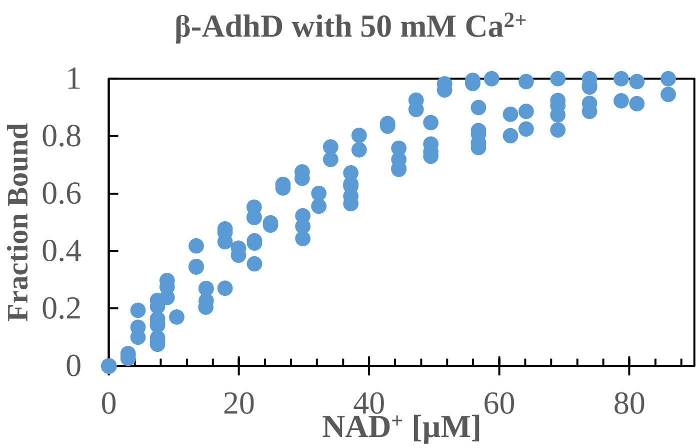

β-AdhD with 10 mM Ca2+
Fraction Bound
1
0.8
0.6
0.4
0.2
0
0
20
40
60
80
NAD+ [μM]

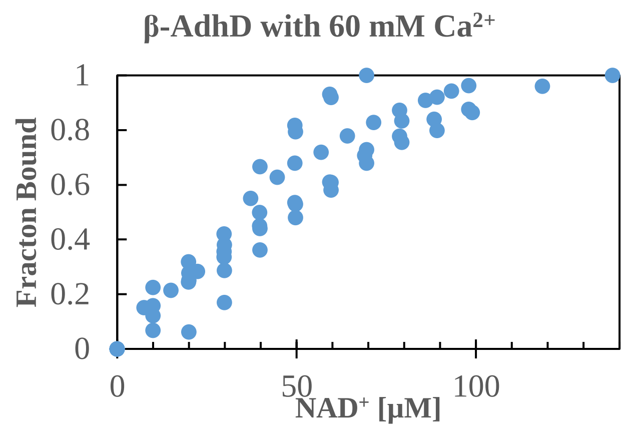

β-AdhD with 20 mM Ca2+
Fraction Bound
1
0.8
0.6
0.4
0.2
0
0
20
40
NAD+ [μM]

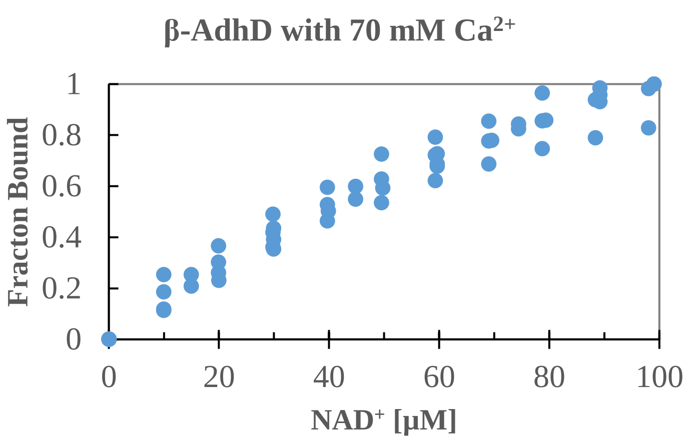

β-AdhD with 25 mM Ca2+
Fraction Bound
1
0.8
0.6
0.4
0.2
0
0
20
40
60
NAD+ [μM]

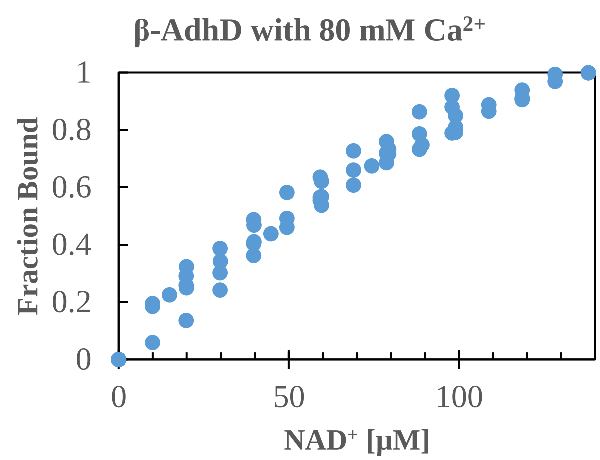

β-AdhD with 30 mM Ca2+
Fraction Bound
1
0.8
0.6
0.4
0.2
0
0
20
40
60
80
NAD+ [μM]

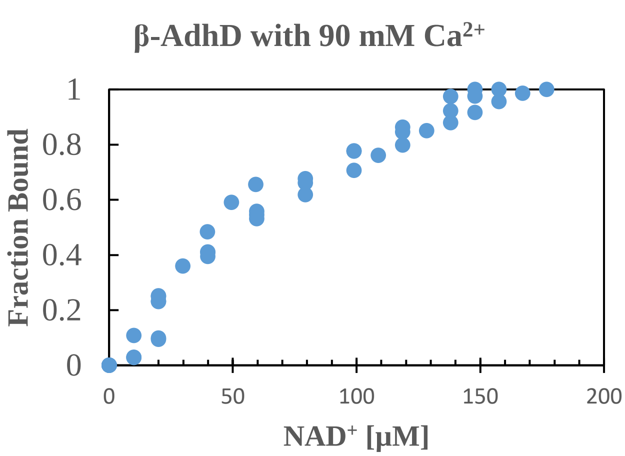

β-AdhD with 40 mM Ca+
Fraction Bound
1
0.8
0.6
0.4
0.2
0
0
20
40
60
80
NAD+ [μM]

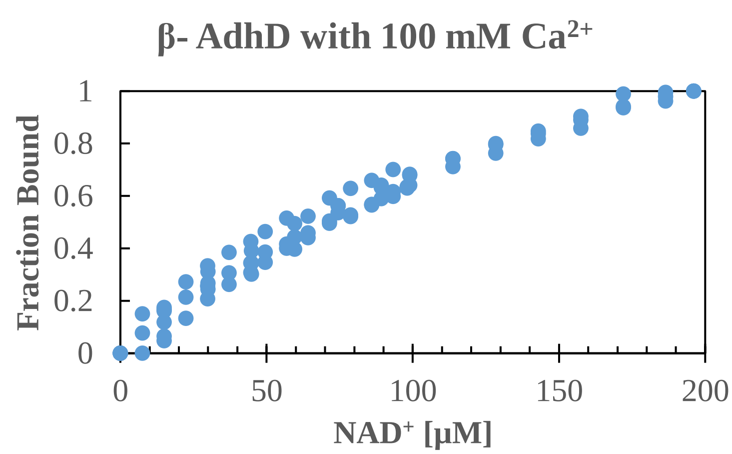

β-AdhD with 50 mM Ca2+
Fraction Bound
NAD+ [μM]


β-AdhD with 60 mM Ca2+
Fracton Bound
NAD+ [μM]


β-AdhD with 70 mM Ca2+
Fracton Bound
NAD+ [μM]


β-AdhD with 80 mM Ca2+
Fraction Bound
NAD+ [μM]


β-AdhD with 90 mM Ca2+
Fraction Bound
NAD+ [μM]


β- AdhD with 100 mM Ca2+
Fraction Bound
NAD+ [μM]

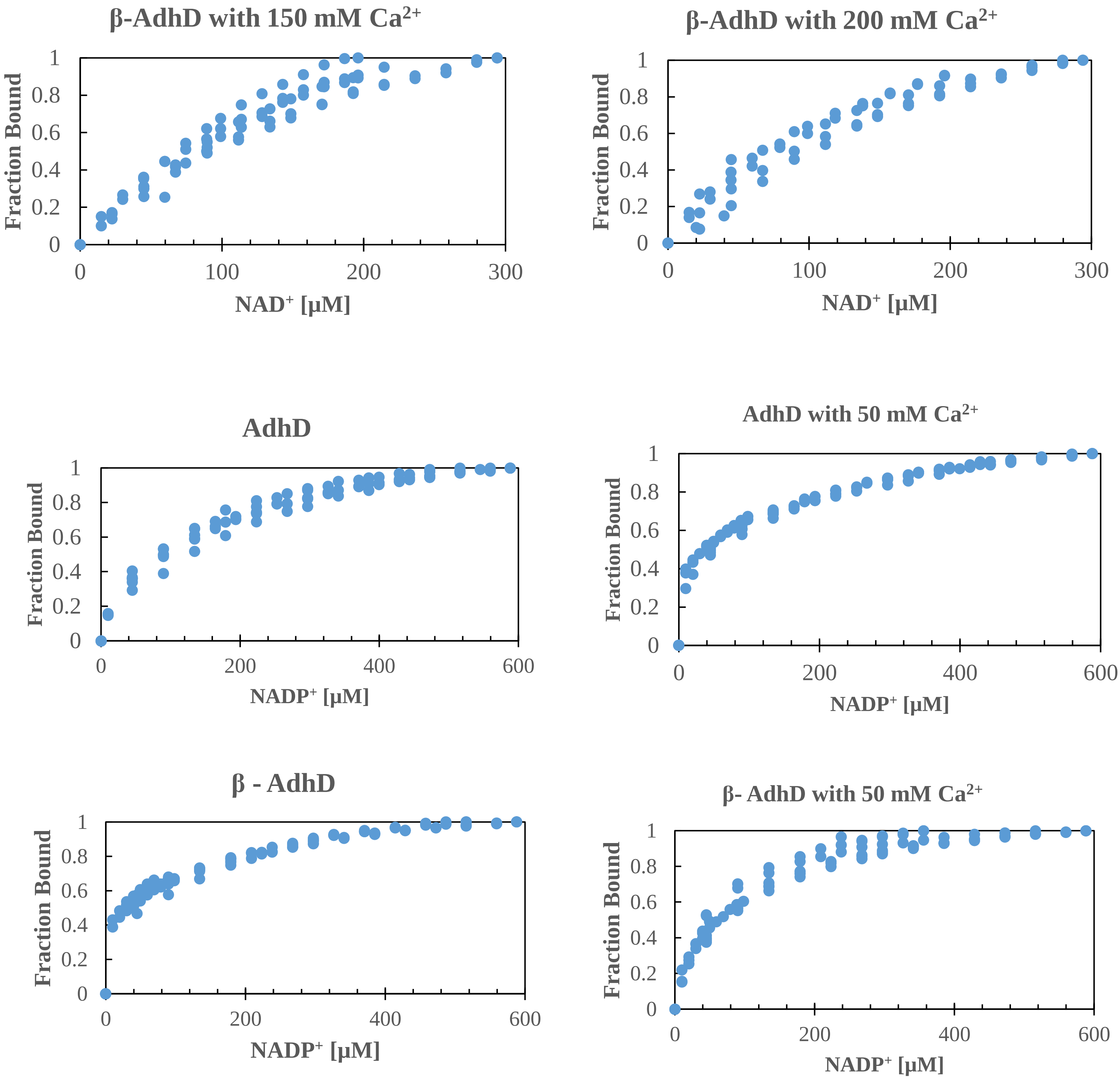


Figure S5. Fluorescence titrations for AdhD and β-AdhD from 0 to 200 mM calcium with NAD^+ and at zero and 50 mM calcium with $NADP^+$. Data were collected in at least triplicate.

Table S1. Dissociation constants for cofactor (NAD^+) for AdhD and β-AdhD at various calcium concentrations

Ca (mM)	AdhD K_{d,NAD^+} (μM)	β-AdhD K_{d,NAD^+} (μM)
0	12 ± 2	12 ± 3
10		14 ± 4
20		14 ± 4
25	21 ± 3	17 ± 3
30		19 ± 5
40		15 ± 4
50	18 ± 3	19 ± 2
60		29 ± 6
70		27 ± 4
80		36 ± 5
90		40 ± 7
100	36 ± 5	57 ± 6
150	24 ± 3	58 ± 7
200	31 ± 4	60 ± 8

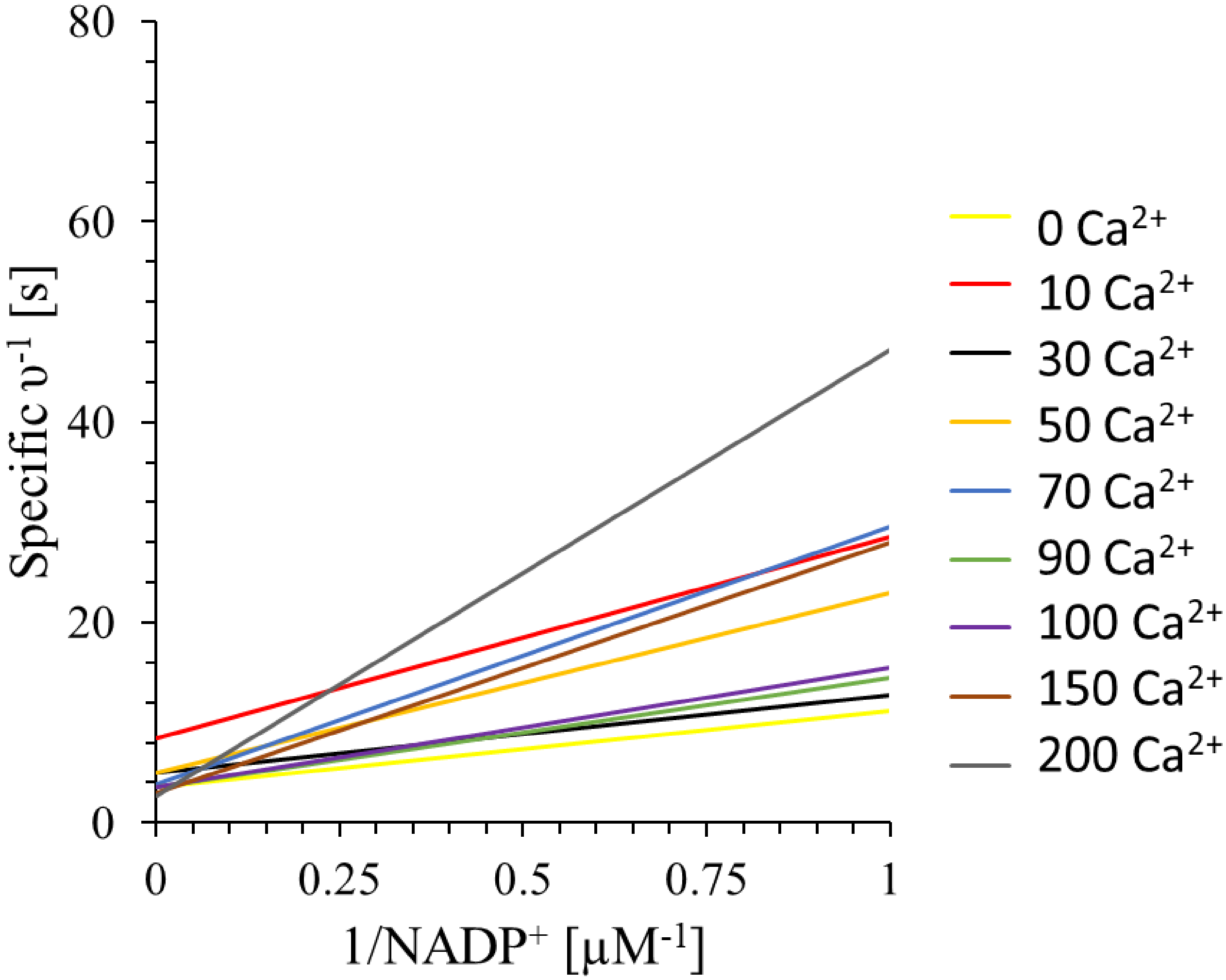


Figure S6. Double reciprocal plot of inverse specific activity for AdhD vs inverse $NADP^+$ with 100 mM 2,3-butanediol with increasing calcium concentrations. There is no clear trend as a function of increasing calcium concentration, indicating a mixed inhibition.

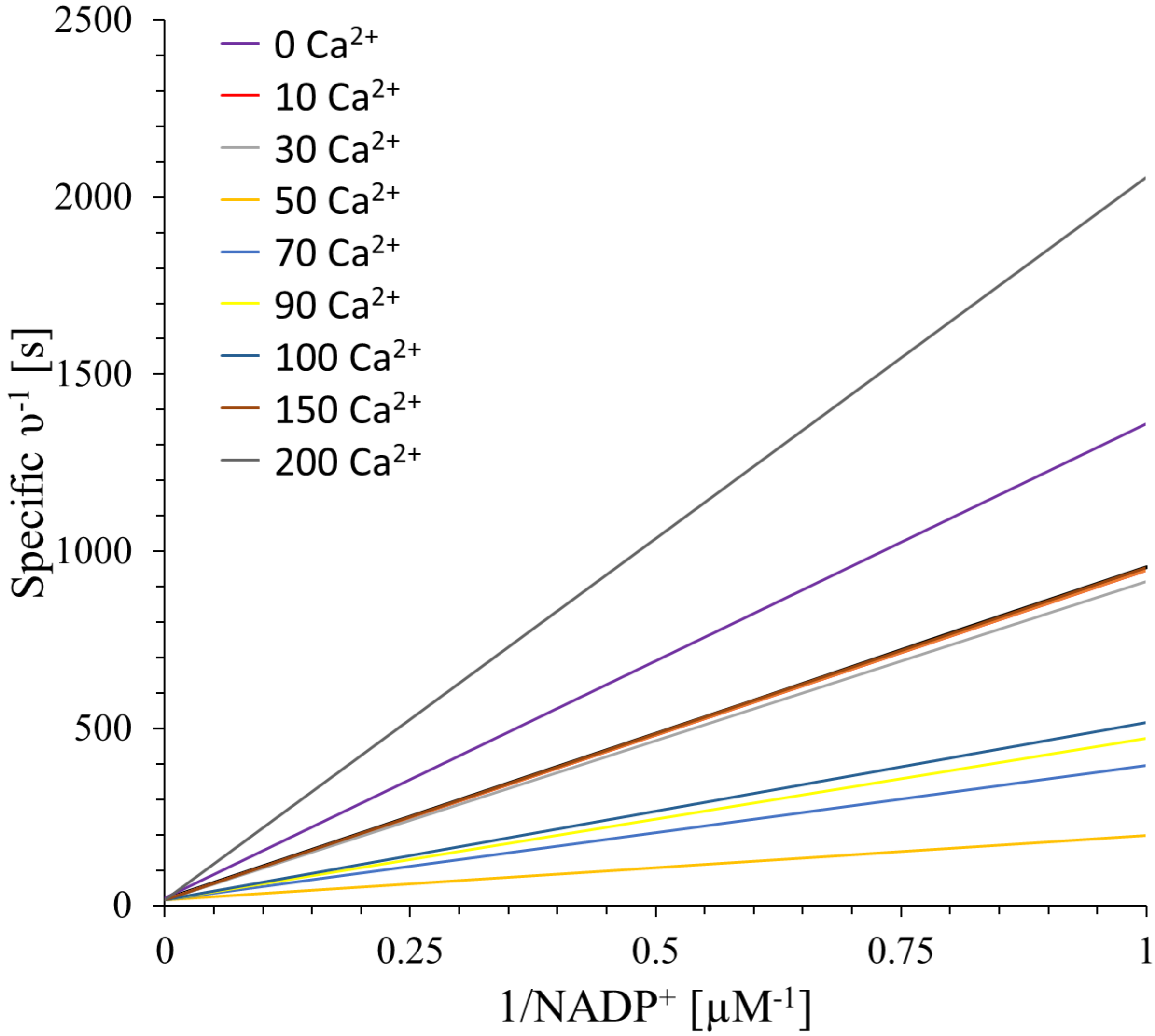
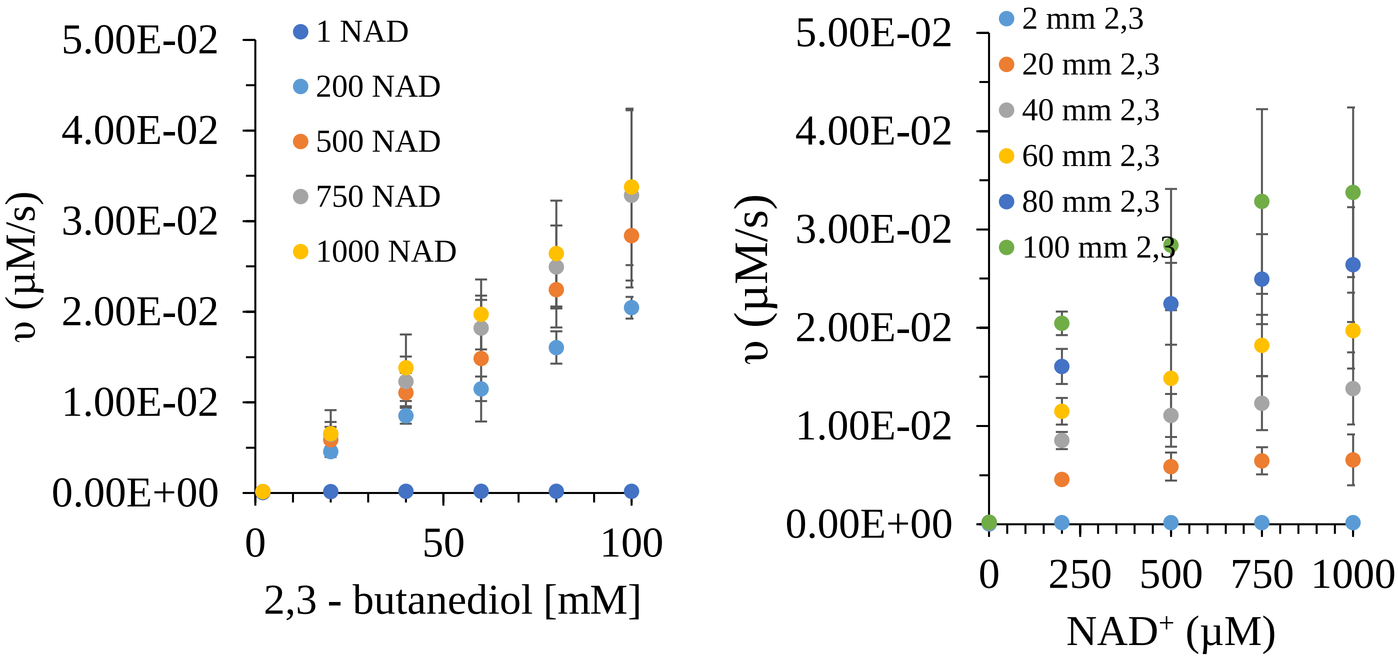


Figure S7. Double reciprocal plot of inverse specific activity for β-AdhD vs $NADP^+$ with 100 mM 2,3-butanediol with increasing calcium concentrations. There is no clear trend as a function of increasing calcium concentration, indicating a mixed inhibition.

B-AdhD, 0 Ca, NAD$^+$



B-AdhD, 50 Ca, NAD$^+$

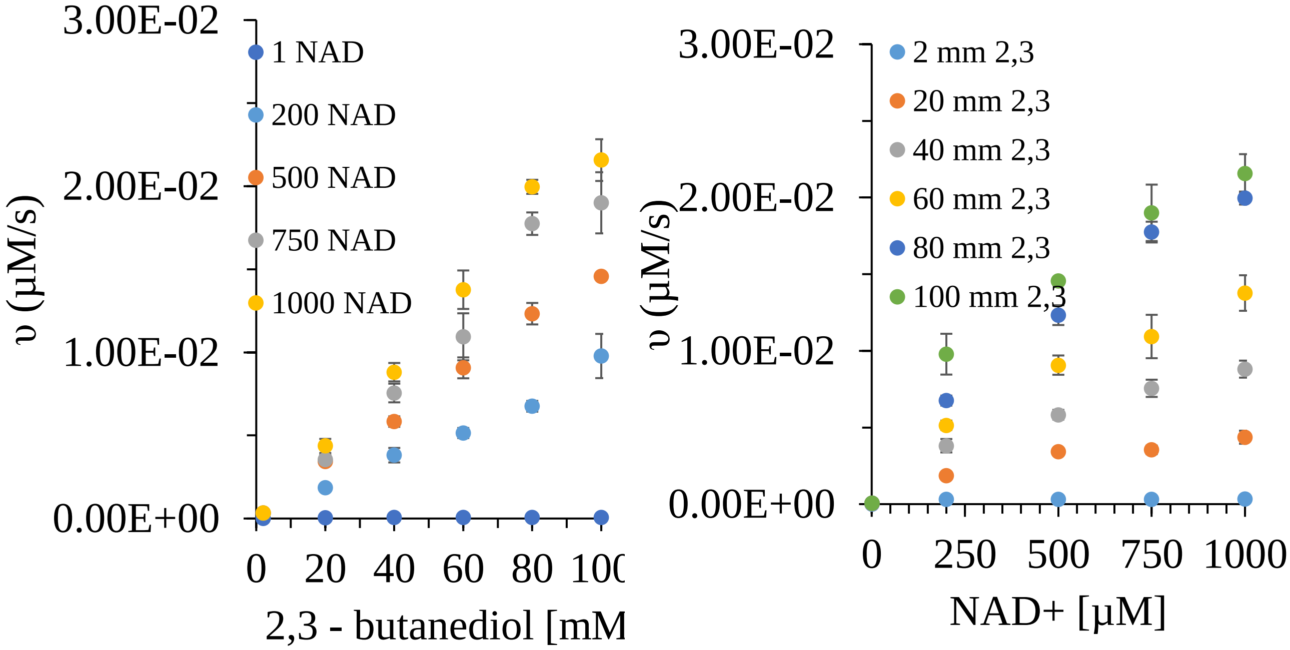
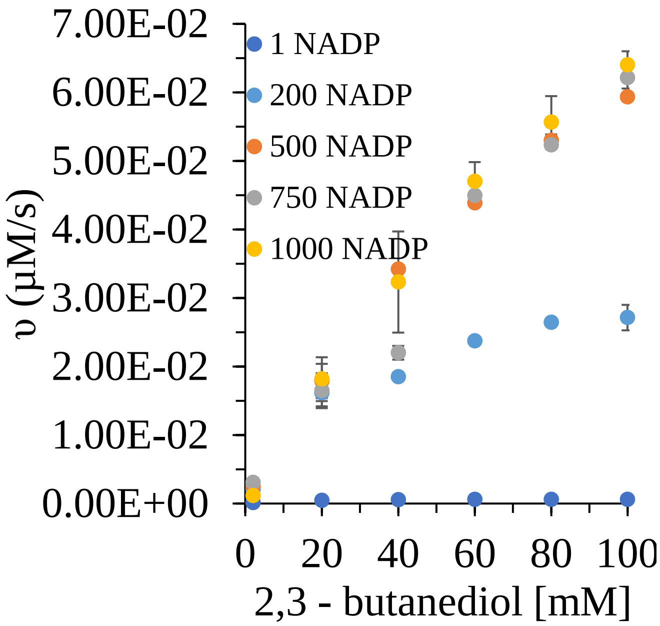
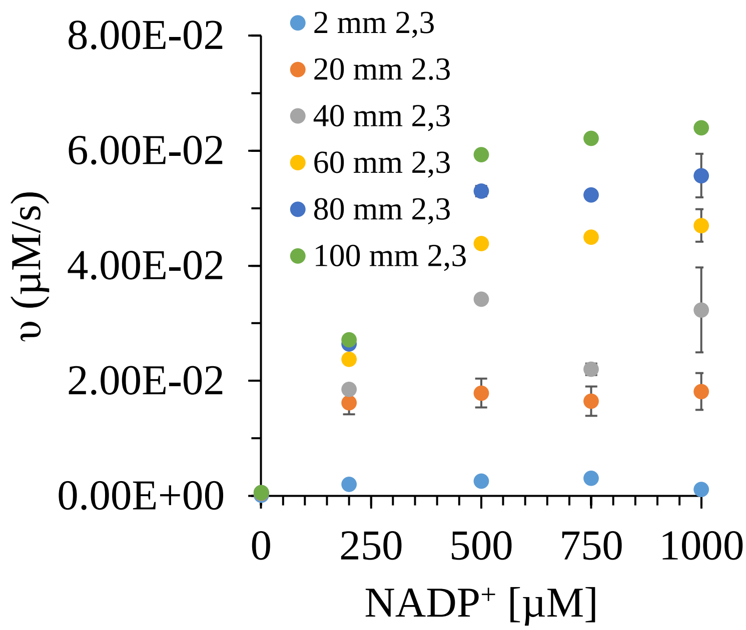


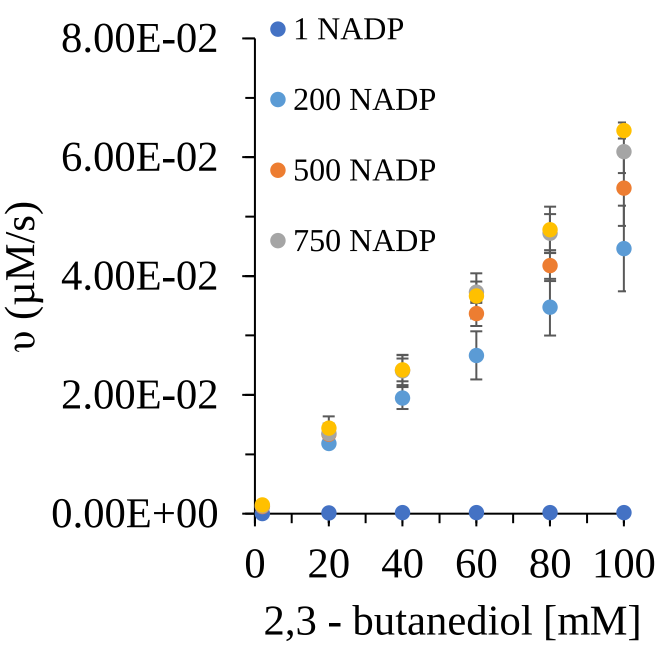
Figure S8. Steady state kinetic rate data for β-AdhD at 0 and 50 mM calcium with NAD$^+$. Data were collected in at least triplicate.

B-AdhD, 0 Ca, NADP⁺





B-AdhD, 50 Ca, NADP⁺



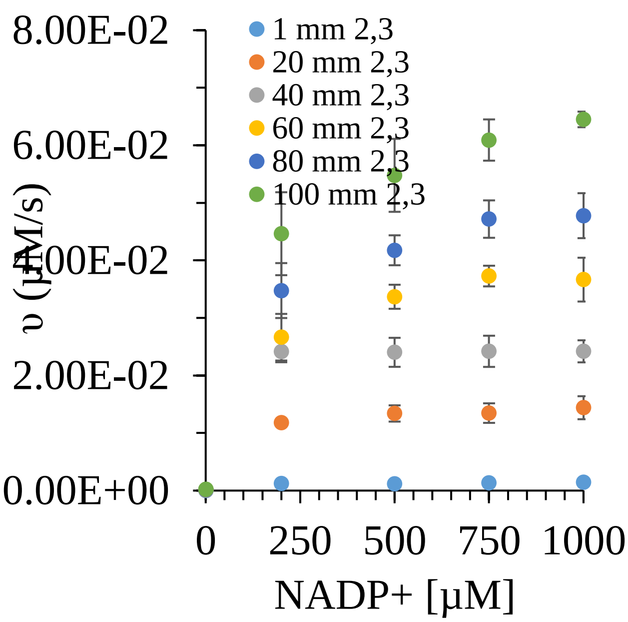


Figure S9. Steady state kinetic rate data for β-AdhD at 0 and 50 mm calcium with NADP⁺. Data were collected in at least triplicate.

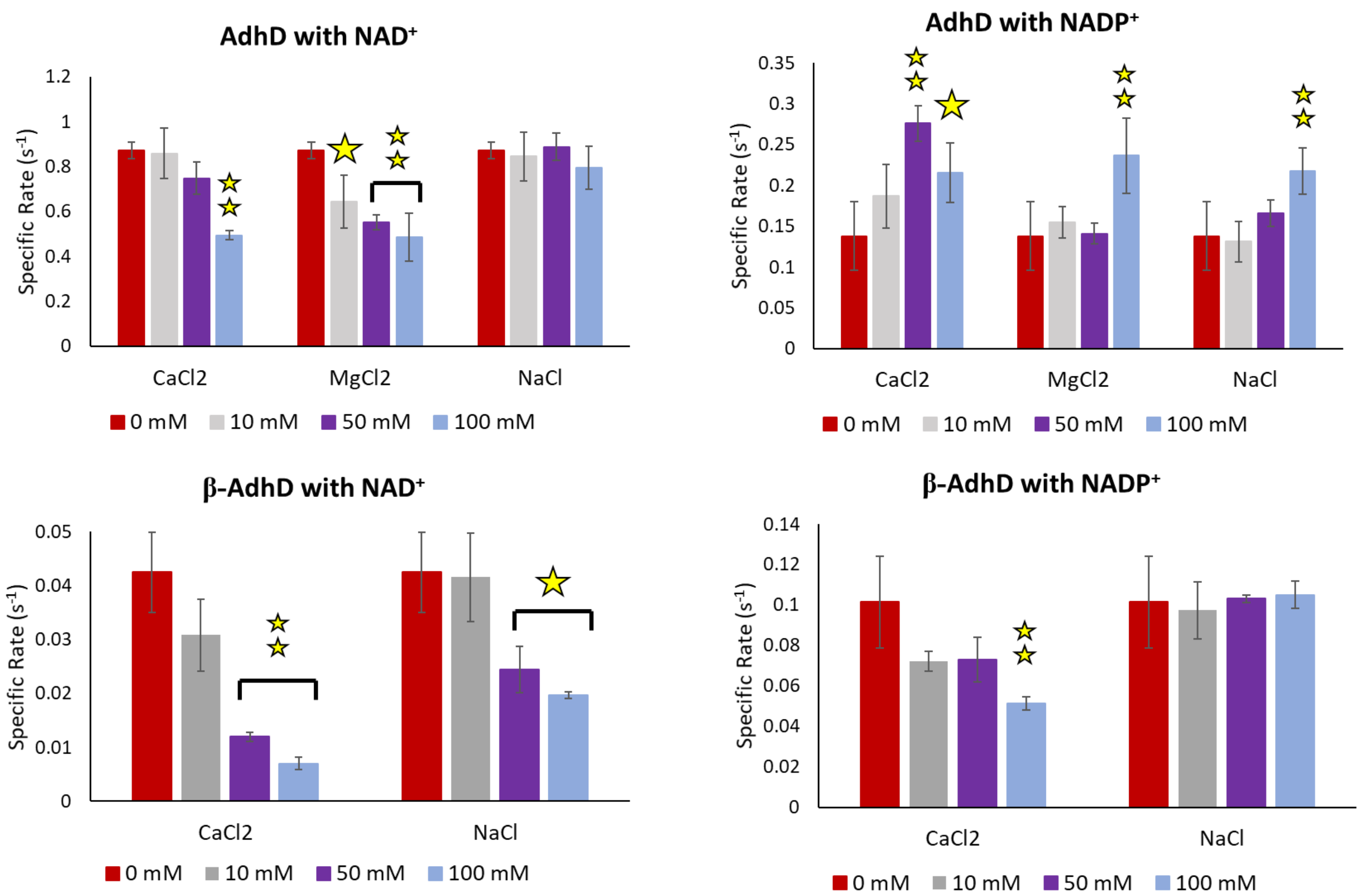


Figure S10. Specific rates for AdhD and β-AdhD with 0, 10, 50, and 100 mM salts with NAD^+ and $NADP^+$ as cofactors. Magnesium has a strong inhibitor effect on the activity of AdhD with NAD^+, and therefore was not an appropriate control. NaCl was used as a control to compare with $CaCl_2$ with β-AdhD. Stars indicate statistically significant rates (*p<0.05, **p<0.005) compared to the protein in the absence of the salt (red bars). Data were collected in at least triplicate.

Ordered Bi Bi Derivations

Case 1: varying [B]

V = Et * kcat * A * B / (Kia*Kb + Ka*B + A*Kb + AB)

Case 2: Constant [B]

- Ordered bi bi rate equation

V = Et * kcat * A * B / (Kia*Kb + Ka*B + A*Kb + AB)

- Constant B so can divide by B and Kb (both constants)

V = [(Et * kcat * A * B)/(Kb*B)] / (Kia*Kb/(Kb*B) + Ka*B/(B*Kb) + A*Kb/(B*Kb) + AB/(Kb*B)

V = (Et * (kcat *B/Kb + B) * A) / ((KiaKb + KaB)/(Kb + B) + A)

V = Et * kcat,app * A / (Ka,app + A)

** Same form as Michaelis-Menton Equation; used to determine apparent parameters

Case 3: Kb >> B

V = Et * kcat * A * B / (Kia*Kb + Ka*B + A*Kb + AB)

- Divide by Kb (Kb values exceed B)

V = (Et * kcat * A * B / Kb) / (Kia*Kb/Kb + Ka*B/Kb + A* Kb/Kb + AB/Kb)

V = (Et * kcat * A * B / Kb) / (Kia + 0 [kb >> B] + A + 0 [kb >> B])

V = (Et*kcat*A*B /kb) / (Kia + A)

Mixed competitive and non-competitive inhibition derivation for β-AdhD

- **The following script was entered into DynaFit**

```
reaction A + B  ---> P + Q
modifiers I, J

E + A  <==> EA       :    k1  k-1
EA + B <=> EAB       :    k2  k-2
EAB   <=> EPQ        :    kp  k-p
EPQ  <=> EQ + P      :    k3  k-3
```

EQ <=> E + Q : k4 k-4

E + I <=> EI : k5 k-5

E + J <=> EJ : k6 k-6

EJ + I <=> EJI : k7 k-7

EJ + A <=> EJA : k8 k-8

EJA + B <=> EJAB : k9 k-9

EJAB <=> EJPQ : k10 k-10

EJPQ <=> EJQ + P : k11 k-11

EJQ <=> EJ + Q : k12 k-12

Notes:

- Competitive inhibition, where calcium competes with the substrate, was previously shown to be true for AdhD
- Non-competitive inhibition, where calcium binds at a site other than the active site and decreases the affinity of the enzyme for the substrate, is assumed to represent the β-roll binding sites
- "I" represents calcium binding to AdhD
- "J" represents calcium binding to the β-roll
- There are no steps following the EJI complex because it is a dead end complex with calcium binding to the β-roll and the active site of AdhD. "I" is competing with cofactor so the calcium attachment is reversible. When calcium binds the β-roll it is not reversible. Therefore, EJI is used as opposed to EIJ.

- The following model output was obtained (based on the King-Altman method):

Rate Equation

V = [E]0 N/D = d[P]/dt = + k3 [EPQ] - k-3 [EQ] [P] + k11 [EJPQ] - k-11 [P] [EJQ]

N = n1 [P][Q] + n2 [A][B] + n3 [P][Q][J] - n4 [P]2[Q] - n5 [B][P][Q] + n6 [A][B][J] + n7 [A][B][P] + n8 [A][B]2 - n9 [P]2[Q][J] - n10 [B][P][Q][J] + n11 [A][B][P][J] + n12 [A][B]2[J]

D = d1 + d2 [J] + d3 [I] + d4 [Q] + d5 [P] + d6 [B] + d7 [A] + d8 [I][J] + d9 [Q][J] + d10 [P][J] + d11 [P][I] + d12 [P][Q] + d13 [P]2 + d14 [B][J] + d15 [B][I] + d16 [B][Q] + d17 [B][P] + d18 [B]2 + d19 [A][J] + d20 [A][P] + d21 [A][B] + d22 [P][I][J] + d23 [P][Q][J] + d24 [P]2[J] + d25 [P]2[I] + d26 [P]2[Q] + d27 [B][I][J] + d28 [B][Q][J] + d29 [B][P][J] + d30 [B][P][I] + d31 [B][P][Q] + d32 [B]2[J] + d33 [B]2[I] + d34 [B]2[Q] + d35 [A][P][J] + d36 [A][P]2 + d37 [A][B][J] + d38 [A][B][P] + d39 [A][B]2 + d40 [P]2[I][J] + d41 [P]2[Q][J] + d42 [B][P][I][J] + d43 [B][P][Q][J] + d44 [B][P]2[Q] + d45 [B]2[I][J] + d46 [B]2[Q][J] + d47 [B]2[P][Q] + d48 [A][P]2[J] + d49 [A][B][P][J] + d50 [A][B][P]2 + d51 [A][B]2[J] + d52 [A][B]2[P] + d53 [B][P]2[Q][J] + d54 [B]2[P][Q][J] + d55 [A][B][P]2[J] + d56 [A][B]2[P][J]

Terms:

n1 = k-1 k-2 k-p k-3 k-4 k-5 k-6 k-7 k-8 k12 (- k-9 k-10 - k-9 k11 - k10 k11)

n2 = k1 k2 kp k3 k4 k-5 k-6 k-7 k-8 k12 (+ k-9 k-10 + k-9 k11 + k10 k11)

n3 = k-1 k4 k-5 k6 k-7 k-8 k-9 k-10 k-11 k-12 (- k-2 k-p - k-2 k3 - kp k3)

n4 = - k-1 k-2 k-p k-3 k-4 k-5 k-6 k-7 k-8 k-9 k-10 k-11

n5 = - k-1 k-2 k-p k-3 k-4 k-5 k-6 k-7 k9 k10 k11 k12

n6 = k-1 k4 k-5 k6 k-7 k8 k9 k10 k11 k12 (+ k-p k-2 + kp k3)

n7 = + k1 k2 kp k3 k4 k-5 k-6 k-7 k-8 k-9 k-10 k-11

n8 = + k1 k2 kp k3 k4 k-5 k-6 k-7 k9 k10 k11 k12

n9 = - k-1 k-2 k-p k-3 k-5 k6 k-7 k-8 k-9 k-10 k-11 k-12

n10 = - k2 kp k3 k4 k-5 k6 k-7 k-8 k-9 k-10 k-11 k-12

n11 = + k-1 k-2 k-p k-3 k-5 k6 k-7 k8 k9 k10 k11 k12

n12 = + k2 kp k3 k4 k-5 k6 k-7 k8 k9 k10 k11 k12

d1 = k-1 k4 k-5 k-6 k-7 k-8 k12 (k-2 k-p k-9 k-10 + k-2 k-p k-9 k11 + k-2 k-p k10 k11 + k-2 k3 k-9 k-10 + k-2 k3 k-9 k11 + k-2 k3 k10 k11 + kp k3 k-9 k-10 + kp k3 k-9 k11 + kp k3 k10 k11)

d2 = k-1 k4 k-5 k6 k-7 k-8 k12 (k-2 k-p k-9 k-10 + k-2 k-p k-9 k11 + k-2 k-p k10 k11 + k-2 k3 k-9 k-10 + k-2 k3 k-9 k11 + k-2 k3 k10 k11 + kp k3 k-9 k-10 + kp k3 k-9 k11 + kp k3 k10 k11)

d3 = k-1 k4 k5 k-6 k-7 k-8 k12 (k-2 k-p k-9 k-10 + k-2 k-p k-9 k11 + k-2 k-p k10 k11 + k-2 k3 k-9 k-10 + k-2 k3 k-9 k11 + k-2 k3 k10 k11 + kp k3 k-9 k-10 + kp k3 k-9 k11 + kp k3 k10 k11)

d4 = k-1 k-4 k-5 k-6 k-7 k-8 k12 (k-2 k-p k-9 k-10 + k-2 k-p k-9 k11 + k-2 k-p k10 k11 + k-2 k3 k-9 k-10 + k-2 k3 k-9 k11 + k-2 k3 k10 k11 + kp k3 k-9 k-10 + kp k3 k-9 k11 + kp k3 k10 k11)

d5 = k-1 k-5 k-6 k-7 k-8 (k-2 k-p k4 k-9 k-10 k-11 + k-2 k-p k-3 k-9 k-10 k12 + k-2 k-p k-3 k-9 k11 k12 + k-2 k-p k-3 k10 k11 k12 + k-2 k3 k4 k-9 k-10 k-11 + kp k3 k4 k-9 k-10 k-11)

d6 = k4 k-5 k-6 k-7 k12 (k-1 k-2 k-p k9 k10 k11 + k-1 k-2 k3 k9 k10 k11 + k-1 kp k3 k9 k10 k11 + k2 kp k3 k-8 k-9 k-10 + k2 kp k3 k-8 k-9 k11 + k2 kp k3 k-8 k10 k11)

d7 = k1 k4 k-5 k-6 k-7 k-8 k12 (k-2 k-p k-9 k-10 + k-2 k-p k-9 k11 + k-2 k-p k10 k11 + k-2 k3 k-9 k-10 + k-2 k3 k-9 k11 + k-2 k3 k10 k11 + kp k3 k-9 k-10 + kp k3 k-9 k11 + kp k3 k10 k11)

d8 = k-1 k4 k-5 k6 k7 k-8 k12 (k-2 k-p k-9 k-10 + k-2 k-p k-9 k11 + k-2 k-p k10 k11 + k-2 k3 k-9 k-10 + k-2 k3 k-9 k11 + k-2 k3 k10 k11 + kp k3 k-9 k-10 + kp k3 k-9 k11 + kp k3 k10 k11)

d9 = k-1 k4 k-5 k6 k-7 k-8 k-12 (k-2 k-p k-9 k-10 + k-2 k-p k-9 k11 + k-2 k-p k10 k11 + k-2 k3 k-9 k-10 + k-2 k3 k-9 k11 + k-2 k3 k10 k11 + kp k3 k-9 k-10 + kp k3 k-9 k11 + kp k3 k10 k11)

d10 = k-1 k-5 k6 k-7 k-8 (k-2 k-p k4 k-9 k-10 k-11 + k-2 k-p k-3 k-9 k-10 k12 + k-2 k-p k-3 k-9 k11 k12 + k-2 k-p k-3 k10 k11 k12 + k-2 k3 k4 k-9 k-10 k-11 + kp k3 k4 k-9 k-10 k-11)

d11 = k-1 k5 k-6 k-7 k-8 (k-2 k-p k4 k-9 k-10 k-11 + k-2 k-p k-3 k-9 k-10 k12 + k-2 k-p k-3 k-9 k11 k12 + k-2 k-p k-3 k10 k11 k12 + k-2 k3 k4 k-9 k-10 k-11 + kp k3 k4 k-9 k-10 k-11)

d12 = k-4 k-5 k-6 k-7 k-8 (k-2 k-p k-3 k-9 k-10 k12 + k-2 k-p k-3 k-9 k11 k12 + k-2 k-p k-3 k10 k11 k12 + k-1 k-p k-3 k-9 k-10 k12 + k-1 k-p k-3 k-9 k11 k12 + k-1 k-p k-3 k10 k11 k12 + k-1 k-2 k-3 k-9 k-10 k12 + k-1 k-2 k-3 k-9 k11 k12 + k-1 k-2 k-3 k10 k11 k12 + k-1 kp k-3 k-9 k-10 k12 + k-1 kp k-3 k-9 k11 k12 + k-1 kp k-3 k10 k11 k12 + k-1 k-2 k-p k-9 k-10 k-11 + k-1 k-2 k3 k-9 k-10 k-11 + k-1 kp k3 k-9 k-10 k-11)

d13 = k-1 k-2 k-p k-3 k-5 k-6 k-7 k-8 k-9 k-10 k-11

d14 = k4 k-5 k6 k-7 k12 (k-1 k-2 k-p k9 k10 k11 + k-1 k-2 k3 k9 k10 k11 + k-1 kp k3 k9 k10 k11 + k2 kp k3 k-8 k-9 k-10 + k2 kp k3 k-8 k-9 k11 + k2 kp k3 k-8 k10 k11)

d15 = k4 k5 k-6 k-7 k12 (k-1 k-2 k-p k9 k10 k11 + k-1 k-2 k3 k9 k10 k11 + k-1 kp k3 k9 k10 k11 + k2 kp k3 k-8 k-9 k-10 + k2 kp k3 k-8 k-9 k11 + k2 kp k3 k-8 k10 k11)

d16 = k-4 k-5 k-6 k-7 k12 (k-1 k-2 k-p k9 k10 k11 + k-1 k-2 k3 k9 k10 k11 + k-1 kp k3 k9 k10 k11 + k2 kp k3 k-8 k-9 k-10 + k2 kp k3 k-8 k-9 k11 + k2 kp k3 k-8 k10 k11)

d17 = k-5 k-6 k-7 (k-1 k-2 k-p k-3 k9 k10 k11 k12 + k2 kp k3 k4 k-8 k-9 k-10 k-11)

d18 = k2 kp k3 k4 k-5 k-6 k-7 k9 k10 k11 k12

d19 = k-1 k4 k-5 k6 k-7 k8 k12 (k-2 k-p k-9 k-10 + k-2 k-p k-9 k11 + k-2 k-p k10 k11 + k-2 k3 k-9 k-10 + k-2 k3 k-9 k11 + k-2 k3 k10 k11 + kp k3 k-9 k-10 + kp k3 k-9 k11 + kp k3 k10 k11)

d20 = k1 k-5 k-6 k-7 k-8 (k-2 k-p k4 k-9 k-10 k-11 + k-2 k-p k-3 k-9 k-10 k12 + k-2 k-p k-3 k-9 k11 k12 + k-2 k-p k-3 k10 k11 k12 + k-2 k3 k4 k-9 k-10 k-11 + kp k3 k4 k-9 k-10 k-11)

d21 = k1 k-5 k-6 k-7 k12 (k-2 k-p k4 k9 k10 k11 + k-2 k3 k4 k9 k10 k11 + kp k3 k4 k9 k10 k11 + k2 k-p k4 k-8 k-9 k-10 + k2 k-p k4 k-8 k-9 k11 + k2 k-p k4 k-8 k10 k11 + k2 k3 k4 k-8 k-9 k-10 + k2 k3 k4 k-8 k-9 k11 + k2 k3 k4 k-8 k10 k11 + k2 kp k4 k-8 k-9 k-10 + k2 kp k4 k-8 k-9 k11 + k2 kp k4 k-8 k10 k11 + k2 kp k3 k-8 k-9 k-10 + k2 kp k3 k-8 k-9 k11 + k2 kp k3 k-8 k10 k11)

d22 = k-1 k-5 k6 k7 k-8 (k-2 k-p k4 k-9 k-10 k-11 + k-2 k-p k-3 k-9 k-10 k12 + k-2 k-p k-3 k-9 k11 k12 + k-2 k-p k-3 k10 k11 k12 + k-2 k3 k4 k-9 k-10 k-11 + kp k3 k4 k-9 k-10 k-11)

d23 = k-1 k-5 k6 k-7 k-12 (k-2 k-p k4 k-9 k-10 k-11 + k-2 k3 k4 k-9 k-10 k-11 + kp k3 k4 k-9 k-10 k-11 + k-2 k-p k4 k-8 k-10 k-11 + k-2 k3 k4 k-8 k-10 k-11 + kp k3 k4 k-8 k-10 k-11 + k-2 k-p k4 k-8 k-9 k-11 + k-2 k-p k4 k-8 k10 k-11 + k-2 k3 k4 k-8 k-9 k-11 + k-2 k3 k4 k-8 k10 k-11 + kp k3 k4 k-8 k-9 k-11 + kp k3 k4 k-8 k10 k-11 + k-2 k-p k-3 k-8 k-9 k-10 + k-2 k-p k-3 k-8 k-9 k11 + k-2 k-p k-3 k-8 k10 k11)

d24 = k-1 k-2 k-p k-3 k-5 k6 k-7 k-8 k-9 k-10 k-11

d25 = k-1 k-2 k-p k-3 k5 k-6 k-7 k-8 k-9 k-10 k-11

d26 = k-3 k-4 k-5 k-6 k-7 k-8 k-9 k-10 k-11 (k-2 k-p + k-1 k-p + k-1 k-2 + k-1 kp)

d27 = k4 k-5 k6 k7 k12 (k-1 k-2 k-p k9 k10 k11 + k-1 k-2 k3 k9 k10 k11 + k-1 kp k3 k9 k10 k11 + k2 kp k3 k-8 k-9 k-10 + k2 kp k3 k-8 k-9 k11 + k2 kp k3 k-8 k10 k11)

d28 = k4 k-5 k6 k-7 k-12 (k-1 k-2 k-p k9 k10 k11 + k-1 k-2 k3 k9 k10 k11 + k-1 kp k3 k9 k10 k11 + k2 kp k3 k-8 k-9 k-10 + k2 kp k3 k-8 k-9 k11 + k2 kp k3 k-8 k10 k11)

d29 = k-5 k6 k-7 (k-1 k-2 k-p k-3 k9 k10 k11 k12 + k2 kp k3 k4 k-8 k-9 k-10 k-11)

d30 = k5 k-6 k-7 (k-1 k-2 k-p k-3 k9 k10 k11 k12 + k2 kp k3 k4 k-8 k-9 k-10 k-11)

d31 = k-4 k-5 k-6 k-7 (k-2 k-p k-3 k9 k10 k11 k12 + k-1 k-p k-3 k9 k10 k11 k12 + k2 k-p k-3 k-8 k-9 k-10 k12 + k2 k-p k-3 k-8 k-9 k11 k12 + k2 k-p k-3 k-8 k10 k11 k12 + k-1 k-2 k-3 k9 k10 k11 k12 + k-1 kp k-3 k9 k10 k11 k12 + k2 kp k-3 k-8 k-9 k-10 k12 + k2 kp k-3 k-8 k-9 k11 k12 + k2 kp k-3 k-8 k10 k11 k12 + k2 kp k3 k-8 k-9 k-10 k-11)

d32 = k2 kp k3 k4 k-5 k6 k-7 k9 k10 k11 k12

d33 = k2 kp k3 k4 k5 k-6 k-7 k9 k10 k11 k12

d34 = k2 kp k3 k-4 k-5 k-6 k-7 k9 k10 k11 k12

d35 = k-1 k-5 k6 k-7 k8 (k-2 k-p k4 k-9 k-10 k-11 + k-2 k-p k-3 k-9 k-10 k12 + k-2 k-p k-3 k-9 k11 k12 + k-2 k-p k-3 k10 k11 k12 + k-2 k3 k4 k-9 k-10 k-11 + kp k3 k4 k-9 k-10 k-11)

d36 = k1 k-2 k-p k-3 k-5 k-6 k-7 k-8 k-9 k-10 k-11

d37 = k4 k-5 k6 k-7 k8 (k2 kp k3 k-9 k-10 k12 + k2 kp k3 k-9 k11 k12 + k2 kp k3 k10 k11 k12 + k-1 k-2 k-p k9 k-10 k12 + k-1 k-2 k-p k9 k11 k12 + k-1 k-2 k3 k9 k-10 k12 + k-1 k-2 k3 k9 k11 k12 + k-1 kp k3 k9 k-10 k12 + k-1 kp k3 k9 k11 k12 + k-1 k-2 k-p k9 k10 k12 + k-1 k-2 k3 k9 k10 k12 + k-1 kp k3 k9 k10 k12 + k-1 k-2 k-p k9 k10 k11 + k-1 k-2 k3 k9 k10 k11 + k-1 kp k3 k9 k10 k11)

d38 = k1 k-5 k-6 k-7 (k-2 k-p k-3 k9 k10 k11 k12 + k2 k-p k4 k-8 k-9 k-10 k-11 + k2 k-p k-3 k-8 k-9 k-10 k12 + k2 k-p k-3 k-8 k-9 k11 k12 + k2 k-p k-3 k-8 k10 k11 k12 + k2 k3 k4 k-8 k-9 k-10 k-11 + k2 kp k4 k-8 k-9 k-10 k-11 + k2 kp k-3 k-8 k-9 k-10 k12 + k2 kp k-3 k-8 k-9 k11 k12 + k2 kp k-3 k-8 k10 k11 k12 + k2 kp k3 k-8 k-9 k-10 k-11)

d39 = k1 k2 k-5 k-6 k-7 k9 k10 k11 k12 (k-p k4 + k3 k4 + kp k4 + kp k3)

d40 = k-1 k-2 k-p k-3 k-5 k6 k7 k-8 k-9 k-10 k-11

d41 = k-1 k-2 k-p k-3 k-5 k6 k-7 k-11 k-12 (k-9 k-10 + k-8 k-10 + k-8 k-9 + k-8 k10)

d42 = k-5 k6 k7 (k-1 k-2 k-p k-3 k9 k10 k11 k12 + k2 kp k3 k4 k-8 k-9 k-10 k-11)

d43 = k-5 k6 k-7 k-12 (k2 kp k3 k4 k-9 k-10 k-11 + k-1 k-2 k-p k4 k9 k-10 k-11 + k-1 k-2 k3 k4 k9 k-10 k-11 + k-1 kp k3 k4 k9 k-10 k-11 + k2 kp k3 k4 k-8 k-10 k-11 + k-1 k-2 k-p k4 k9 k10 k-11 + k-1 k-2 k3 k4 k9 k10 k-11 + k-1 kp k3 k4 k9 k10 k-11 + k2 kp k3 k4 k-8 k-9 k-11 + k2 kp k3 k4 k-8 k10 k-11 + k-1 k-2 k-p k-3 k9 k10 k11)

d44 = k2 k-3 k-4 k-5 k-6 k-7 k-8 k-9 k-10 k-11 (k-p + kp)

d45 = k2 kp k3 k4 k-5 k6 k7 k9 k10 k11 k12

d46 = k2 kp k3 k4 k-5 k6 k-7 k9 k10 k11 k-12

d47 = k2 k-3 k-4 k-5 k-6 k-7 k9 k10 k11 k12 (k-p + kp)

d48 = k-1 k-2 k-p k-3 k-5 k6 k-7 k8 k-9 k-10 k-11

d49 = k-5 k6 k-7 k8 (k2 kp k3 k4 k-9 k-10 k-11 + k-1 k-2 k-p k4 k9 k-10 k-11 + k-1 k-2 k-p k-3 k9 k-10 k12 + k-1 k-2 k-p k-3 k9 k11 k12 + k-1 k-2 k3 k4 k9 k-10 k-11 + k-1 kp k3 k4 k9 k-10 k-11 + k-1 k-2 k-p k4 k9 k10 k-11 + k-1 k-2 k-p k-3 k9 k10 k12 + k-1 k-2 k3 k4 k9 k10 k-11 + k-1 kp k3 k4 k9 k10 k-11 + k-1 k-2 k-p k-3 k9 k10 k11)

d50 = k1 k2 k-3 k-5 k-6 k-7 k-8 k-9 k-10 k-11 (k-p + kp)

d51 = k2 kp k3 k4 k-5 k6 k-7 k8 k9 (k-10 k12 + k11 k12 + k10 k12 + k10 k11)

d52 = k1 k2 k-3 k-5 k-6 k-7 k9 k10 k11 k12 (k-p + kp)

d53 = k-1 k-2 k-p k-3 k-5 k6 k-7 k9 k-11 k-12 (k-10 + k10)

d54 = k2 kp k3 k4 k-5 k6 k-7 k9 k-11 k-12 (k-10 + k10)

d55 = k-1 k-2 k-p k-3 k-5 k6 k-7 k8 k9 k-11 (k-10 + k10)

d56 = k2 kp k3 k4 k-5 k6 k-7 k8 k9 k-11 (k-10 + k10)

<u>Simplification #1:</u>

- All terms with P and Q are cancelled as reactions were performed in the absence of products

N = n2 [A][B] + n6 [A][B][J] + n8 [A][B] + n12 [A][B]2[J]

D = d1 + d2 [J] + d3 [I] + d6 [B] + d7 [A] + d8 [I][J] + d14 [B][J] + d15 [B][I] + d18 [B]2 + d19 [A][J] + d21 [A][B] + d27 [B][I][J] + d32 [B]2[J] + d33 [B]2[I] + d37 [A][B][J] + d39 [A][B]2 + d45 [B]2[I][J] + d51 [A][B]2[J]

<u>Simplification #2:</u>

- All terms were simplified by canceling terms that contained k-2, k-p, k-9, and k-10 as we assume enzyme is irreversible

<u>Simplification #3:</u>

- Define dissociation constants and inhibition constants and simplify

  k-1/k1 = Kia
  k-5/k5 = KI
  k-6/k6 = KJ
  k-7/k7 = KJI
  k-8/k8 = Kiaj

<u>Simplification #4:</u>

- Kpk3k4 are part of kcat term in ordered bi bi derivation, analogously, k10k11k12 are part of kcatj. Divide all terms by kpk3k4k10k11k12
  k1 = kcat/Ka

k2 = kcat/Kb
k8 = kcat/Kaj
k9 = kcat/Kbj

Simplification #5:

- Re-write all terms into kinetic parameters

$$v = \frac{E_t \begin{pmatrix} k_{cat}K_{iaJ}K_{BJ}K_{I}K_JK_{JI}[A][B] + k_{cat}K_{AJ}K_IK_JK_{JI}[A][B]^2 + \\ k_{catJ}K_{ia}K_BK_IK_{JI}[A][B][J] + k_{catJ}K_AK_IK_{JI}[A][B]^2[J] \end{pmatrix}}{\begin{bmatrix} K_{ia}K_{iaJ}K_BK_{BJ}K_JK_IK_{JI} + K_{iaJ}K_BK_{BJ}K_JK_IK_{JI}[A] \\ +(K_{ia}K_{AJ}K_B + K_{iaJ}K_AK_{BJ})K_JK_IK_{JI}[B] + K_AK_{AJ}K_IK_JK_{JI}[B]^2 \\ + K_JK_IK_{JI}(K_BK_{AJ} + K_{iaJ}K_{BJ})[A][B] + (K_{AJ}K_JK_IK_{JI})[A][B]^2 \\ + K_{ia}K_BK_{BJ}K_IK_{JI}[A][J] + (K_{ia}K_{AJ}K_B + K_{iaJ}K_AK_{BJ})K_IK_{JI}[B][J] \\ +(K_{ia}K_{AJ}K_B + K_{iaJ}K_AK_{BJ})K_JK_{JI}[B][I] + \\ (K_{ia}K_{AJ}K_BK_I + K_{iaJ}K_AK_{BJ}K_I)[B][I][J] + (K_AK_{AJ}K_JK_{JI})[B]^2[I] + \\ K_AK_{AJ}K_IK_{JI}[B]^2[J] + K_IK_AK_{AJ}[B]^2[I][J] + K_IK_{JI}K_AK_{BJ}[A][B][J] + \\ K_IK_{JI}K_{ia}K_B[A][B][J] + K_IK_{JI}K_A[A][B]^2[J] + \\ K_{ia}K_{iaJ}K_BK_{BJ}K_JK_{JI}[I] + K_{ia}K_{iaJ}K_BK_{BJ}K_IK_{JI}[J] + K_{ia}K_{iaJ}K_BK_{BJ}K_I[I][J] \end{bmatrix}}$$

- **Note:** Setting I and J equal to zero and re-arranging the equation results in the ordered bi-bi rate equation

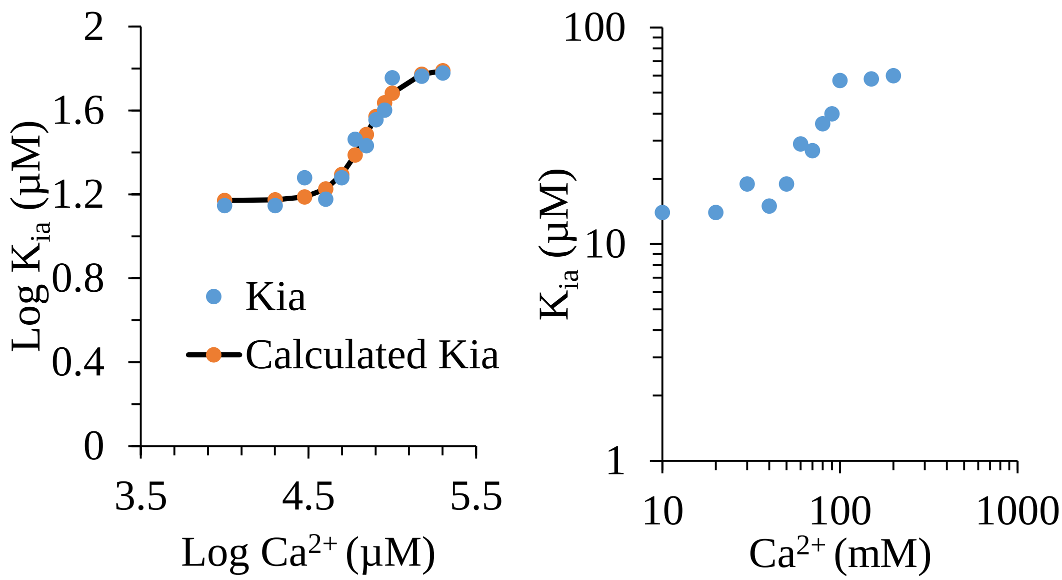


Figure S11. Sigmoidal relationship between calcium concentration and K_{ia} values for β-AdhD. The following values were obtained for the sigmoidal fit to the Log data:

$$y = base + \frac{max}{1 + \exp((x_{half} - x)/rate)}$$

base	= 1.2 ± 0.085
max	= 0.63 ± 0.16
x_{half}	= 4.8 ± 0.072
rate	= 0.10 ± 0.067

The concentration at the inflection point is 63±1 mM calcium.

Specific Initial Rate Kinetic Data

Table S2. Specific rate data for AdhD at varying NAD^+ and 2,3-butanediol concentration with zero calcium

AdhD, NAD, No Ca^{2+}

Rate (1/s)	NAD (µM)	2,3 Butanediol (µM)
0.228	200	2000
0.292	500	2000
0.496	750	2000
0.474	500	4000
0.488	750	4000
0.524001	1000	4000
1.223938	200	20000
1.676643	500	20000
1.946813	750	20000
1.23562	500	60000
1.310444	750	60000
1.456876	1000	60000
1.448604	750	80000
1.636246	1000	80000
1.713312	200	100000
1.727727	500	100000
1.818645	750	100000
1.849095	1000	100000
0.302	500	2000
0.592	750	2000
0.698	1000	2000
0.496	500	4000
0.508	750	4000
0.617999	1000	4000
1.766582	200	20000
1.830731	500	20000
2.071161	750	20000
1.683814	750	100000
1.917748	1000	100000
0.592759	200	20000
0.636689	500	20000
0.803461	500	40000
0.935139	750	40000
1.020002	200	60000
1.110001	500	60000

1.142002	750	60000
1.224537	200	80000
1.273342	500	80000
1.373617	750	80000
1.362036	200	100000
1.47894	500	100000
1.588096	750	100000
0.64492	500	40000
0.756061	750	40000
0.773461	1000	40000
0.99004	500	60000
1.00014	750	60000
1.033138	1000	60000
1.082943	500	80000
1.170837	750	80000
1.230739	1000	80000
1.419003	750	100000
1.43736	1000	100000
0.442706	750	20000
0.443198	1000	20000
0.729999	200	40000
0.776181	500	40000
0.788581	750	40000
1.008822	500	60000
1.028658	750	60000
1.086478	1000	60000
0.881679	200	80000
1.216183	500	80000
1.249922	750	80000
1.308541	1000	80000
1.413497	500	100000
1.474165	750	100000
1.531077	1000	100000
0.640921	200	40000
0.667481	500	40000
0.820001	200	60000
0.822	500	60000
0.998582	200	80000
1.013223	500	80000
0.13214	1	100000
0.87	20	100000
1.426005	200	100000
1.560002	500	100000
1.419003	750	100000
1.437205	1000	100000

0.195601	20	100000
1.465996	200	100000
1.413398	500	100000
1.474002	750	100000
1.529999	1000	100000
0.037948	20	100000
1.178002	200	100000
1.242321	500	100000
1.179997	750	100000
1.102317	1000	100000
0.652554	200	100000
0.860045	500	100000
0.925549	750	100000
0.953284	1000	100000
0.650656	200	100000
0.856788	500	100000
1.193766	750	100000
0.927007	1000	100000

Table S3. Specific rate data for AdhD at varying NAD^+ and 2,3-butanediol concentration with 50 mM calcium

AdhD, NAD, 50 mM Ca^{2+}

Rate (1/s)	NAD (µM)	2,3-Butanediol (µM)
0.50854	200	40000
0.5253	750	40000
0.76278	750	60000
0.84206	1000	60000
0.89546	200	100000
1.12294	1000	100000
0.36778	200	20000
0.420476	500	20000
0.55962	750	40000
0.70824	200	60000
0.7542	500	60000
0.77412	750	60000
0.79434	1000	60000
0.78166	200	80000
0.92974	500	80000
0.95674	1000	80000
0.82382	200	100000
0.986	750	100000
0.60926	500	20000
0.31936	200	40000
0.40178	500	40000
0.52294	750	40000
0.54642	1000	40000
0.5941	200	80000
0.92448	500	80000
0.97788	1000	80000
0.86766	500	100000
1.04338	750	100000
0.4427	750	20000
0.44352	1000	20000
0.73	200	40000
0.77618	500	40000
0.78858	750	40000
1.00882	500	60000
1.02866	750	60000
1.08648	1000	60000

Table S4. Specific rate data for β-AdhD at varying NAD^+ and 2,3-butanediol concentration with zero calcium

β-AdhD, NAD, No Ca^{2+}

Rate (1/s)	NAD (µM)	2,3-Butanediol (µM)
0.003551	200	20000
0.005748	500	20000
0.005706	750	20000
0.007289	200	40000
0.009838	500	40000
0.011242	750	40000
0.013043	1000	40000
0.016202	500	60000
0.016557	750	60000
0.018727	1000	60000
0.013388	200	80000
0.020021	500	80000
0.022265	750	80000
0.024566	1000	80000
0.016243	200	100000
0.028195	500	100000
0.036482	750	100000
0.017567	1000	100000
0.001316	1000	4000
0.003814	200	20000
0.005521	500	20000
0.00628	750	20000
0.006711	1000	20000
0.006007	200	40000
0.010515	500	40000
0.011997	750	40000
0.013062	1000	40000
0.016818	750	60000
0.016002	1000	60000
0.012743	200	80000
0.020559	500	80000
0.023007	750	80000
0.025038	1000	80000
0.036113	1000	100000
0.000926	750	4000
0.000787	1000	4000
0.003197	200	20000
0.003906	500	20000

0.004166	750	20000
0.002904	1000	20000
0.005436	200	40000
0.0067	500	40000
0.007611	750	40000
0.008042	1000	40000
0.007304	200	60000
0.01196	750	60000
0.012279	750	60000
0.009195	200	80000
0.013052	500	80000
0.015533	750	80000
0.015404	1000	80000
0.011577	200	80000
0.017643	500	100000
0.020965	750	100000
0.022091	1000	100000
0.003064	200	20000
0.003718	500	20000
0.004141	750	20000
0.006109	200	40000
0.007321	500	40000
0.008048	750	40000
0.010879	500	60000
0.011969	750	60000
0.012213	1000	60000
0.012069	200	80000
0.015293	500	80000
0.016579	750	80000
0.017172	1000	80000
0.014175	200	100000
0.020174	500	100000
0.021676	750	100000
0.022	1000	100000
0.003283	200	20000
0.003777	500	20000
0.004508	750	20000
0.004846	1000	20000
0.006692	200	40000
0.007362	500	40000
0.008042	750	40000
0.009131	1000	40000
0.009007	200	60000
0.011069	500	60000
0.012638	750	60000

0.013356	1000	60000
0.011827	200	80000
0.015403	500	80000
0.016776	750	80000
0.018456	1000	80000
0.014671	200	100000
0.019759	500	100000
0.021211	750	100000
0.023069	1000	100000
0.003195	200	20000
0.003231	500	20000
0.003687	750	20000
0.006048	200	40000
0.007091	500	40000
0.007404	750	40000
0.007546	1000	40000
0.009051	200	60000
0.011157	500	60000
0.011447	750	60000
0.012146	1000	60000
0.011857	200	80000
0.014639	500	80000
0.015882	750	80000
0.015938	1000	80000
0.01504	200	100000
0.018651	500	100000
0.020409	750	100000
0.020926	1000	100000
0.000971	1	100000
0.000971	1	100000
0.004243	20	100000
0.015191	200	100000
0.020147	500	100000
0.021618	750	100000
0.021985	1000	100000
0.00475	20	100000
0.015882	200	100000
0.019706	500	100000
0.021176	750	100000
0.023015	1000	100000
0.003235	20	100000
0.015882	200	100000
0.018603	500	100000
0.020409	750	100000
0.020926	1000	100000

0.002029	20	100000
0.007941	200	100000
0.009191	500	100000
0.009679	750	100000
0.010382	1000	100000
0.001809	20	100000
0.007721	200	100000
0.009117	500	100000
0.009835	1000	100000

Table S5. Specific rate data for β-AdhD at varying NAD^+ and 2,3-butanediol concentration with 50 mM calcium

β-AdhD, NAD, 50 mM Ca^{2+}

Rate (1/s)	NAD (µM)	2,3-Butanediol (µM)
0.001063	750	20000
0.00136	1000	20000
0.001959	200	40000
0.002225	500	40000
0.002742	750	40000
0.003213	1000	40000
0.003252	500	60000
0.004366	750	60000
0.004574	1000	60000
0.002464	200	80000
0.004871	500	80000
0.006048	750	80000
0.00714	1000	80000
0.003432	200	100000
0.005335	500	100000
0.006971	750	100000
0.00841	1000	100000
0.001121	750	20000
0.001317	1000	20000
0.001219	200	40000
0.002135	500	40000
0.002831	750	40000
0.003345	1000	40000
0.00202	200	60000
0.003429	500	60000
0.002273	750	60000
0.005646	1000	60000
0.002724	200	80000
0.004474	500	80000
0.006119	750	80000
0.006916	1000	80000
0.004925	200	100000
0.005505	500	100000
0.007375	750	100000
0.008517	1000	100000
0.001849	500	40000
0.00204	750	40000
0.002447	1000	40000

0.001563	200	60000
0.002577	500	60000
0.003232	750	60000
0.003635	1000	60000
0.002078	200	80000
0.003971	500	80000
0.0074	750	80000
0.002566	200	100000
0.005324	500	100000
0.005176	750	100000
0.006866	1000	100000
0.001367	750	20000
0.001571	1000	20000
0.001375	200	40000
0.001935	500	40000
0.003136	750	40000
0.003417	1000	40000
0.003175	500	60000
0.004657	750	60000
0.005201	1000	60000
0.002721	200	80000
0.004078	500	80000
0.006307	750	80000
0.007479	1000	80000
0.003461	200	100000
0.005269	500	100000
0.008423	750	100000
0.001263	500	20000
0.001554	750	20000
0.001684	1000	20000
0.001284	200	40000
0.002489	500	40000
0.003196	750	40000
0.003605	1000	40000
0.002034	200	60000
0.003946	500	60000
0.004785	750	60000
0.005288	1000	60000
0.002422	200	80000
0.005231	500	80000
0.006566	750	80000
0.007457	1000	80000
0.000682	200	20000
0.00126	500	20000
0.001408	750	20000

0.002095	1000	20000
0.001168	200	40000
0.002236	500	40000
0.002719	750	40000
0.0034	1000	40000
0.00195	200	60000
0.003624	500	60000
0.004809	750	60000
0.006033	1000	60000
0.002493	200	80000
0.004568	500	80000
0.006704	750	80000
0.007706	1000	80000

Table S6. Specific rate data for AdhD at varying $NADP^+$ and 2,3-butanediol concentration with zero calcium

AdhD, NADP, No Ca^{2+}

Rate (1/s)	NAD (μM)	2,3-Butanediol (μM)
0.020594	500	2000
0.024027	750	2000
0.022797	200	4000
0.026127	500	4000
0.030825	500	40000
0.034249	750	40000
0.033239	500	80000
0.034313	750	80000
0.038253	500	100000
0.036685	750	100000
0.0271	200	2000
0.027408	500	2000
0.028176	500	4000
0.029764	750	4000
0.033008	200	40000
0.034649	500	40000
0.030174	200	60000
0.033049	500	60000
0.034174	200	80000
0.034626	500	80000
0.034137	750	100000
0.038213	1000	100000
0.033043	500	20000
0.030072	750	20000
0.032028	750	40000
0.046321	1000	40000
0.039991	500	100000
0.042613	750	100000
0.042589	200	40000
0.039216	500	40000
0.038748	750	40000
0.032766	1000	40000
0.022442	200	60000
0.039449	500	60000
0.038206	750	60000
0.034613	500	80000
0.037133	750	80000
0.036543	500	100000

0.040179	750	100000
0.038322	200	20000
0.042783	500	20000
0.03626	750	40000
0.038465	1000	40000
0.037345	750	60000
0.039647	1000	60000
0.037691	750	100000
0.038607	1000	100000
0.033998	1000	40000
0.040369	500	60000

Table S7. Specific rate data for AdhD at varying $NADP^+$ and 2,3-butanediol concentration with 50 mM calcium

<u>AdhD, NADP, 50 mM Ca^{2+}</u>

Rate (1/s)	NAD (µM)	2,3-Butanediol (µM)
0.007223	200	2000
0.094775	500	2000
0.093238	750	2000
0.103996	1000	2000
0.030576	200	60000
0.236363	500	60000
0.224841	750	60000
0.21481	1000	60000
0.213012	200	80000
0.249488	500	80000
0.246737	750	80000
0.230379	1000	80000
0.023924	200	100000
0.258811	500	100000
0.265215	750	100000
0.24166	1000	100000
0.081967	200	2000
0.09375	500	2000
0.098873	750	2000
0.121414	200	4000
0.139344	500	4000
0.154713	750	4000
0.181865	200	20000
0.199621	500	20000
0.196834	750	20000
0.202049	1000	20000
0.244826	200	40000
0.236901	500	40000
0.226947	750	40000
0.232418	1000	40000
0.224027	200	60000
0.239933	500	60000
0.232121	750	60000
0.24772	1000	60000
0.237613	200	80000
0.250415	500	80000
0.247111	750	80000
0.256522	1000	80000

0.228586	200	100000
0.260963	500	100000
0.267587	750	100000
0.253519	1000	100000
0.049488	200	2000
0.078381	500	2000
0.095287	750	2000
0.13627	200	20000
0.018955	500	20000
0.019416	750	20000
0.194672	200	40000
0.214139	500	40000
0.145492	200	60000
0.222848	500	60000
0.230533	750	60000
0.194672	200	80000
0.215164	500	80000
0.220287	750	80000
0.189549	200	100000
0.240779	500	100000
0.306352	750	100000
0.056352	200	4000
0.079918	750	4000
0.060451	1000	4000
0.167008	200	20000
0.169057	500	20000
0.172643	750	20000
0.185963	500	40000
0.193135	750	40000
0.024744	200	60000
0.196209	500	60000
0.196721	750	60000
0.204918	500	80000
0.204918	750	80000
0.209529	200	100000
0.209529	500	100000
0.210041	750	100000
0.090164	200	4000
0.098361	500	4000
0.177254	200	20000
0.177766	500	20000
0.190574	750	40000
0.197234	1000	40000
0.197746	750	60000
0.207403	1000	60000

0.206455	200	100000
0.211066	750	100000
0.218238	1000	100000
0.082992	500	4000
0.085041	750	4000
0.162398	500	20000
0.165471	750	20000
0.179303	500	40000
0.189549	750	40000
0.186988	500	60000
0.196209	750	60000
0.204406	1000	60000
0.199795	500	80000
0.194672	750	80000
0.194672	200	100000
0.199795	500	100000
0.199795	750	100000

Table S8. Specific rate data for β-AdhD at varying $NADP^+$ and 2,3-butanediol concentration with zero calcium

β-AdhD, NADP, No Ca^{2+}

Rate (1/s)	NAD (µM)	2,3-Butanediol (µM)
0.001176	200	2000
0.002	500	2000
0.002221	750	2000
0.003176	200	4000
0.003449	500	4000
0.004588	750	4000
0.010882	750	20000
0.010882	1000	20000
0.013971	200	40000
0.016176	500	40000
0.016176	750	40000
0.004559	200	60000
0.019485	500	60000
0.019191	200	80000
0.022206	500	80000
0.020588	200	100000
0.023529	500	100000
0.024191	750	100000
0.003397	200	4000
0.00375	500	4000
0.004338	750	4000
0.010662	200	20000
0.011176	500	20000
0.011176	750	20000
0.012059	1000	20000
0.013529	200	40000
0.016471	500	40000
0.017647	200	60000
0.019118	500	60000
0.019485	750	60000
0.019265	200	80000
0.021471	750	80000
0.021471	1000	80000
0.018382	200	100000
0.024265	500	100000
0.024265	750	100000

0.024265	1000	100000
0.001471	200	2000
0.002279	750	2000
0.000846	1000	2000
0.002868	500	4000
0.003456	750	4000
0.010956	200	20000
0.01125	500	20000
0.013309	200	40000
0.015588	500	40000
0.015735	1000	40000
0.017279	200	60000
0.018603	500	60000
0.019853	200	80000
0.020882	500	80000
0.020882	200	100000
0.022279	500	100000
0.001684	200	2000
0.001838	500	2000
0.003559	200	4000
0.003618	500	4000
0.01375	500	20000
0.014265	750	20000
0.022059	200	40000
0.024779	500	40000
0.025368	1000	40000
0.004412	200	60000
0.031838	500	60000
0.033015	750	60000
0.033088	1000	60000
0.039706	500	80000
0.038235	750	80000
0.039706	1000	80000
0.041618	200	100000
0.044118	500	100000
0.044853	750	100000
0.047059	1000	100000
0.003632	200	4000
0.003662	500	4000
0.012059	200	20000
0.014338	500	20000
0.014265	1000	20000
0.022279	200	40000
0.025221	500	40000
0.026471	1000	40000

0.032353	500	60000
0.033088	750	60000
0.036029	1000	60000
0.038603	500	80000
0.038971	750	80000
0.044118	1000	80000
0.043382	500	100000
0.047059	750	100000
0.048529	1000	100000
0.001618	200	2000
0.001838	500	2000
0.003699	500	4000
0.003743	750	4000
0.004779	1000	4000
0.013897	200	20000
0.015221	500	20000
0.016176	1000	20000
0.023897	200	40000
0.025441	500	40000
0.0275	1000	40000
0.031765	200	60000
0.032574	500	60000
0.033088	750	60000
0.038529	500	80000
0.038235	750	80000
0.038971	1000	80000
0.040441	200	100000
0.043382	500	100000
0.045221	750	100000
0.045588	1000	100000

Table S9. Specific rate data for β-AdhD at varying $NADP^+$ and 2,3-butanediol concentration with 50 mM calcium

β-AdhD, NADP, 50 mM Ca^{2+}

Rate (1/s)	NAD (μM)	2,3-Butanediol (μM)
0.000632	500	2000
0.000882	750	2000
0.000904	1000	2000
0.001412	200	4000
0.003074	500	4000
0.008824	200	20000
0.010588	750	20000
0.014412	200	40000
0.019118	500	40000
0.020221	750	40000
0.025	500	60000
0.028676	750	60000
0.028676	1000	60000
0.026691	200	80000
0.031618	500	80000
0.036691	750	80000
0.036765	1000	80000
0.040441	500	100000
0.045956	750	100000
0.001029	200	2000
0.001397	200	4000
0.002353	500	4000
0.008603	200	20000
0.010588	500	20000
0.010662	750	20000
0.013897	200	40000
0.020074	500	40000
0.017794	200	60000
0.025368	500	60000
0.02875	750	60000
0.024926	200	80000
0.032132	500	80000
0.036471	750	80000
0.038235	200	100000
0.045588	500	100000
0.046471	1000	100000
0.001669	750	4000
0.002397	1000	4000

0.011838	200	40000
0.014706	500	40000
0.015441	750	40000
0.016176	1000	40000
0.016544	200	60000
0.021912	500	60000
0.022279	1000	60000
0.019779	200	80000
0.027353	500	80000
0.030809	750	80000
0.030882	1000	80000
0.024559	200	100000
0.031765	500	100000
0.040368	750	100000
0.048456	1000	100000
0.000824	200	2000
0.000949	500	2000
0.001074	750	2000
0.001809	500	4000
0.001772	750	4000
0.002743	1000	4000
0.015074	200	40000
0.017794	500	40000
0.024559	500	60000
0.025809	750	60000
0.026397	1000	60000
0.028529	200	80000
0.03125	500	80000
0.035294	750	80000
0.035882	1000	80000
0.033382	200	100000
0.040368	500	100000
0.045294	750	100000
0.047353	1000	100000
0.000853	200	2000
0.001037	500	2000
0.001397	750	2000
0.001838	500	4000
0.002404	750	4000
0.004044	1000	4000
0.008456	750	20000
0.011618	1000	20000
0.015	200	40000
0.016985	500	40000
0.017059	750	40000

0.01875	1000	40000
0.020956	200	60000
0.025294	500	60000
0.026397	750	60000
0.028676	1000	60000
0.027868	200	80000
0.031176	500	80000
0.034191	750	80000
0.036985	1000	80000
0.031544	200	100000
0.040441	500	100000
0.045	750	100000
0.001265	1000	2000
0.001478	200	4000
0.001735	500	4000
0.001882	1000	4000
0.008676	200	20000
0.009118	500	20000
0.000956	1000	20000
0.015735	200	40000
0.017941	500	40000
0.017941	750	40000
0.018456	1000	40000
0.023088	200	60000
0.026471	500	60000
0.027426	750	60000
0.02875	1000	60000
0.036397	200	100000
0.043088	500	100000
0.047353	750	100000

Table S10. Complete specific rate data for AdhD at varying NAD^+ and 2,3-butanediol concentration with varying calcium

<u>AdhD, varying calcium</u>

Rate (1/s)	NAD (µM)	2,3-Butandediol (µM)	Ca (µM)
0.228	200	2000	0
0.292	500	2000	0
0.496	750	2000	0
0.474	500	4000	0
0.488	750	4000	0
0.524	1000	4000	0
1.224	200	20000	0
1.6766	500	20000	0
1.9468	750	20000	0
1.2356	500	60000	0
1.3104	750	60000	0
1.4568	1000	60000	0
1.4486	750	80000	0
1.6362	1000	80000	0
1.7134	200	100000	0
1.7278	500	100000	0
1.8186	750	100000	0
1.849	1000	100000	0
0.302	500	2000	0
0.592	750	2000	0
0.698	1000	2000	0
0.496	500	4000	0
0.508	750	4000	0
0.618	1000	4000	0
1.7666	200	20000	0
1.8308	500	20000	0
2.0712	750	20000	0
1.6838	750	100000	0
1.9178	1000	100000	0
0.5928	200	20000	0
0.6366	500	20000	0
0.8034	500	40000	0
0.9352	750	40000	0
1.02	200	60000	0
1.11	500	60000	0
1.142	750	60000	0

1.2246	200	80000	0
1.2734	500	80000	0
1.3736	750	80000	0
1.362	200	100000	0
1.479	500	100000	0
1.588	750	100000	0
0.645	500	40000	0
0.756	750	40000	0
0.7734	1000	40000	0
0.99	500	60000	0
1.0002	750	60000	0
1.0332	1000	60000	0
1.083	500	80000	0
1.1708	750	80000	0
1.2308	1000	80000	0
1.419	750	100000	0
1.4374	1000	100000	0
0.4428	750	20000	0
0.4432	1000	20000	0
0.73	200	40000	0
0.7762	500	40000	0
0.7886	750	40000	0
1.0088	500	60000	0
1.0286	750	60000	0
1.0864	1000	60000	0
0.8816	200	80000	0
1.2162	500	80000	0
1.25	750	80000	0
1.3086	1000	80000	0
1.4134	500	100000	0
1.4742	750	100000	0
1.531	1000	100000	0
0.641	200	40000	0
0.6674	500	40000	0
0.82	200	60000	0
0.822	500	60000	0
0.9986	200	80000	0
1.0132	500	80000	0
0.43	20	100000	25000
1.042	200	100000	25000
1.206	500	100000	25000
1.238	750	100000	25000
1.24	1000	100000	25000
0.36	20	100000	25000
0.944	200	100000	25000

1.102	750	100000	25000
1.192	1000	100000	25000
0.358	20	100000	25000
0.958	200	100000	25000
1.136	500	100000	25000
1.1822	750	100000	25000
0.9726	20	100000	25000
1.226	500	100000	25000
1.2056	750	100000	25000
1.384	1000	100000	25000
0.366	20	100000	25000
0.932	200	100000	25000
1.19	500	100000	25000
1.252	750	100000	25000
1.405	1000	100000	25000
0.39	200	100000	50000
0.47	750	100000	50000
0.494	1000	100000	50000
1.4134	500	100000	50000
1.474	750	100000	50000
1.531	1000	100000	50000
0.2152	200	100000	50000
0.238	750	100000	50000
0.2266	1000	100000	50000
0.7722	200	100000	50000
1.16	500	100000	50000
1.1938	750	100000	50000
1.2094	1000	100000	50000
0.7722	200	100000	50000
1.0992	500	100000	50000
1.1858	750	100000	50000
1.223	1000	100000	50000
0.386	200	100000	100000
0.618	500	100000	100000
0.6634	750	100000	100000
0.7706	1000	100000	100000
0.378	200	100000	100000
0.624	500	100000	100000
0.722	750	100000	100000
0.746	1000	100000	100000
0.428	200	100000	100000
0.622	500	100000	100000
0.758	750	100000	100000
0.8332	1000	100000	100000
0.5854	200	100000	100000

0.962	500	100000	100000
1.1314	750	100000	100000
1.2248	1000	100000	100000
0.6176	200	100000	100000
0.9664	500	100000	100000
1.1548	750	100000	100000
1.216	1000	100000	100000
0.1906	200	100000	150000
0.342	500	100000	150000
0.466	750	100000	150000
0.596	1000	100000	150000
0.1034	20	100000	150000
0.174	200	100000	150000
0.304	500	100000	150000
0.348	750	100000	150000
0.478	1000	100000	150000
0.181	200	100000	150000
0.306	500	100000	150000
0.42	750	100000	150000
0.476	1000	100000	150000
0.8306	500	100000	150000
0.997	750	100000	150000
1.0802	1000	100000	150000
0.5008	200	100000	150000
0.835	500	100000	150000
0.9986	750	100000	150000
1.1402	1000	100000	150000
0.386	200	100000	200000
0.46	500	100000	200000
0.834	750	100000	200000
1.022	1000	100000	200000
0.165	200	100000	200000
0.272	500	100000	200000
0.4	750	100000	200000
0.658	1000	100000	200000
0.128	200	100000	200000
0.2722	500	100000	200000
0.3758	750	100000	200000
0.467	1000	100000	200000
0.3824	200	100000	200000
0.6876	500	100000	200000
0.857	750	100000	200000
0.949	1000	100000	200000
0.3474	200	100000	200000
0.708	500	100000	200000

0.8672	750	100000	200000
0.9782	1000	100000	200000
0.296	1000	80000	50000
0.5086	200	40000	50000
0.5252	750	40000	50000
0.7628	750	60000	50000
0.842	1000	60000	50000
0.8954	200	100000	50000
1.123	1000	100000	50000
0.3678	200	20000	50000
0.4204	500	20000	50000
0.3944	200	40000	50000
0.5596	750	40000	50000
0.7082	200	60000	50000
0.7542	500	60000	50000
0.7742	750	60000	50000
0.7944	1000	60000	50000
0.7816	200	80000	50000
0.9298	500	80000	50000
0.9568	1000	80000	50000
0.8238	200	100000	50000
0.986	750	100000	50000
1.2636	1000	100000	50000
0.3582	200	20000	50000
0.6092	500	20000	50000
0.3194	200	40000	50000
0.4018	500	40000	50000
0.523	750	40000	50000
0.5464	1000	40000	50000
0.594	200	80000	50000
0.9244	500	80000	50000
0.9778	1000	80000	50000
0.8676	500	100000	50000
1.0434	750	100000	50000
0.292	500	40000	50000
0.3096	500	60000	50000
0.3246	750	60000	50000
0.3408	200	80000	50000
0.382	500	80000	50000
0.3848	750	80000	50000
0.387	1000	80000	50000
0.3902	200	100000	50000
0.4716	750	100000	50000
0.4956	1000	100000	50000
0.4428	750	20000	50000

0.4436	1000	20000	50000
0.73	200	40000	50000
0.7762	500	40000	50000
0.7886	750	40000	50000
1.0088	500	60000	50000
1.0286	750	60000	50000
1.0864	1000	60000	50000
1.2078	200	80000	50000
1.2162	500	80000	50000
1.25	750	80000	50000
1.3086	1000	80000	50000
1.4674	200	100000	50000
1.4742	750	100000	50000
1.531	1000	100000	50000
0.1322	1	100000	0
0.87	20	100000	0
1.426	200	100000	0
1.56	500	100000	0
1.419	750	100000	0
1.4372	1000	100000	0
0.1956	20	100000	0
1.466	200	100000	0
1.4134	500	100000	0
1.474	750	100000	0
1.53	1000	100000	0
0.038	20	100000	0
1.178	200	100000	0
1.2424	500	100000	0
1.18	750	100000	0
1.1024	1000	100000	0
0.6526	200	100000	0
0.86	500	100000	0
0.9256	750	100000	0
0.9532	1000	100000	0
0.6506	200	100000	0
0.8568	500	100000	0
1.1938	750	100000	0
0.927	1000	100000	0

Table S11. Complete specific rate data for β-AdhD at varying NAD^+ and 2,3-butanediol concentration with varying calcium

β-AdhD, varying calcium

Rate (1/s)	NAD (μm)	2,3-Butanediol (μm)	Ca (μm)
0.000971	1	100000	0
0.004243	20	100000	0
0.015191	200	100000	0
0.020147	500	100000	0
0.021618	750	100000	0
0.021985	1000	100000	0
0.00475	20	100000	0
0.015882	200	100000	0
0.019706	500	100000	0
0.021176	750	100000	0
0.023015	1000	100000	0
0.003235	20	100000	0
0.015882	200	100000	0
0.018603	500	100000	0
0.020409	750	100000	0
0.020926	1000	100000	0
0.002029	20	100000	0
0.007941	200	100000	0
0.009191	500	100000	0
0.009679	750	100000	0
0.010382	1000	100000	0
0.001809	20	100000	0
0.007721	200	100000	0
0.009117	500	100000	0
0.009835	1000	100000	0
0.003551	200	20000	0
0.005748	500	20000	0
0.005706	750	20000	0
0.007289	200	40000	0
0.009838	500	40000	0
0.011242	750	40000	0
0.013043	1000	40000	0
0.016202	500	60000	0
0.016557	750	60000	0
0.018727	1000	60000	0
0.013388	200	80000	0
0.020021	500	80000	0

0.022265	750	80000	0
0.024566	1000	80000	0
0.016243	200	100000	0
0.028195	500	100000	0
0.036482	750	100000	0
0.001316	1000	4000	0
0.003814	200	20000	0
0.005521	500	20000	0
0.00628	750	20000	0
0.006711	1000	20000	0
0.006007	200	40000	0
0.010515	500	40000	0
0.011997	750	40000	0
0.013062	1000	40000	0
0.016818	750	60000	0
0.016002	1000	60000	0
0.012743	200	80000	0
0.020559	500	80000	0
0.023007	750	80000	0
0.025038	1000	80000	0
0.036113	1000	100000	0
0.000926	750	4000	0
0.000787	1000	4000	0
0.003197	200	20000	0
0.003906	500	20000	0
0.004166	750	20000	0
0.005436	200	40000	0
0.0067	500	40000	0
0.007611	750	40000	0
0.008042	1000	40000	0
0.007304	200	60000	0
0.01196	750	60000	0
0.012279	750	60000	0
0.009195	200	80000	0
0.013052	500	80000	0
0.015533	750	80000	0
0.015404	1000	80000	0
0.011577	200	80000	0
0.017643	500	100000	0
0.020965	750	100000	0
0.022091	1000	100000	0
0.003064	200	20000	0
0.003718	500	20000	0
0.004141	750	20000	0
0.006109	200	40000	0

0.007321	500	40000	0
0.008048	750	40000	0
0.010879	500	60000	0
0.011969	750	60000	0
0.012213	1000	60000	0
0.012069	200	80000	0
0.015293	500	80000	0
0.016579	750	80000	0
0.017172	1000	80000	0
0.014175	200	100000	0
0.020174	500	100000	0
0.021676	750	100000	0
0.022	1000	100000	0
0.003283	200	20000	0
0.003777	500	20000	0
0.004508	750	20000	0
0.004846	1000	20000	0
0.006692	200	40000	0
0.007362	500	40000	0
0.008042	750	40000	0
0.009131	1000	40000	0
0.009007	200	60000	0
0.011069	500	60000	0
0.012638	750	60000	0
0.013356	1000	60000	0
0.011827	200	80000	0
0.015403	500	80000	0
0.016776	750	80000	0
0.018456	1000	80000	0
0.014671	200	100000	0
0.019759	500	100000	0
0.021211	750	100000	0
0.023069	1000	100000	0
0.003195	200	20000	0
0.003231	500	20000	0
0.003687	750	20000	0
0.006048	200	40000	0
0.007091	500	40000	0
0.007404	750	40000	0
0.007546	1000	40000	0
0.009051	200	60000	0
0.011157	500	60000	0
0.011447	750	60000	0
0.012146	1000	60000	0
0.011857	200	80000	0

0.014639	500	80000	0
0.015882	750	80000	0
0.015938	1000	80000	0
0.01504	200	100000	0
0.018651	500	100000	0
0.020409	750	100000	0
0.020926	1000	100000	0
0.000324	1	100000	10000
0.007773	20	100000	10000
0.018483	200	100000	10000
0.029027	500	100000	10000
0.030228	750	100000	10000
0.030134	1000	100000	10000
0.007125	20	100000	10000
0.018218	200	100000	10000
0.02784	500	100000	10000
0.030228	750	100000	10000
0.031929	1000	100000	10000
0.005911	20	100000	10000
0.019648	200	100000	10000
0.02761	500	100000	10000
0.031659	750	100000	10000
0.032091	1000	100000	10000
0.005182	20	100000	20000
0.01483	200	100000	20000
0.023602	500	100000	20000
0.028407	750	100000	20000
0.030863	1000	100000	20000
0.005571	20	100000	20000
0.014813	200	100000	20000
0.023575	500	100000	20000
0.027206	750	100000	20000
0.031497	1000	100000	20000
0.000162	1	100000	20000
0.004426	20	100000	20000
0.015341	200	100000	20000
0.023548	500	100000	20000
0.029648	750	100000	20000
0.027529	1000	100000	20000
0.00384	20	100000	30000
0.012226	200	100000	30000
0.020944	500	100000	30000
0.025208	750	100000	30000
0.027448	1000	100000	30000
0.003644	20	100000	30000

0.011606	200	100000	30000
0.020094	500	100000	30000
0.025654	750	100000	30000
0.003401	20	100000	30000
0.011889	200	100000	30000
0.020256	500	100000	30000
0.02537	750	100000	30000
0.026585	1000	100000	30000
0.00332	20	100000	40000
0.017462	500	100000	40000
0.022725	750	100000	40000
0.022725	1000	100000	40000
0.003167	20	100000	40000
0.010108	200	100000	40000
0.017705	500	100000	40000
0.021727	750	100000	40000
0.026949	1000	100000	40000
0.003279	20	100000	40000
0.006626	200	100000	40000
0.018339	500	100000	40000
0.023683	750	100000	40000
0.026801	1000	100000	40000
7.2E-05	1	100000	50000
0.002785	20	100000	50000
0.008056	200	100000	50000
0.014682	500	100000	50000
0.018339	750	100000	50000
0.019932	1000	100000	50000
4.05E-05	1	100000	50000
0.002562	20	100000	50000
0.007935	200	100000	50000
0.014534	500	100000	50000
0.018339	750	100000	50000
0.020323	1000	100000	50000
4.22E-05	1	100000	50000
0.002321	20	100000	50000
0.007611	200	100000	50000
0.013832	500	100000	50000
0.016423	750	100000	50000
0.018312	1000	100000	50000
0.001965	20	100000	60000
0.006963	200	100000	60000
0.013427	500	100000	60000
0.017192	750	100000	60000
0.020013	1000	100000	60000

0.002321	20	100000	60000
0.007517	200	100000	60000
0.014372	500	100000	60000
0.017773	750	100000	60000
0.020823	1000	100000	60000
0.002402	20	100000	60000
0.007975	200	100000	60000
0.014912	500	100000	60000
0.01807	750	100000	60000
0.022779	1000	100000	60000
0.002143	20	100000	70000
0.006181	200	100000	70000
0.011228	500	100000	70000
0.014345	750	100000	70000
0.015357	1000	100000	70000
0.00471	200	100000	70000
0.009176	500	100000	70000
0.012051	750	100000	70000
0.014183	1000	100000	70000
0.001534	20	100000	70000
0.005101	200	100000	70000
0.010283	500	100000	70000
0.012213	750	100000	70000
0.015047	1000	100000	70000
0.001822	20	100000	80000
0.005762	200	100000	80000
0.011606	500	100000	80000
0.014331	750	100000	80000
0.016167	1000	100000	80000
0.001457	20	100000	80000
0.004494	200	100000	80000
0.008353	500	100000	80000
0.01116	750	100000	80000
0.015141	1000	100000	80000
0.001547	20	100000	80000
0.005115	200	100000	80000
0.009716	500	100000	80000
0.012037	750	100000	80000
0.014439	1000	100000	80000
0.001483	20	100000	80000
0.004966	200	100000	80000
0.010108	500	100000	80000
0.012577	750	100000	80000
0.015101	1000	100000	80000
0.001373	20	100000	90000

0.004129	200	100000	90000
0.008623	500	100000	90000
0.010364	750	100000	90000
0.01336	1000	100000	90000
0.001342	20	100000	90000
0.004318	200	100000	90000
0.008056	500	100000	90000
0.011754	750	100000	90000
0.012915	1000	100000	90000
0.00108	20	100000	90000
0.004386	200	100000	90000
0.008529	500	100000	90000
0.011916	750	100000	90000
0.013373	1000	100000	90000
0.001342	20	100000	90000
0.004683	200	100000	90000
0.009082	500	100000	90000
0.011525	750	100000	90000
0.013792	1000	100000	90000
0.001281	20	100000	90000
0.004642	200	100000	90000
0.008866	500	100000	90000
0.011619	750	100000	90000
0.014318	1000	100000	90000
0.001266	20	100000	100000
0.004278	200	100000	100000
0.007503	500	100000	100000
0.010634	750	100000	100000
0.011363	1000	100000	100000
0.0015	20	100000	100000
0.004615	200	100000	100000
0.008434	500	100000	100000
0.012105	750	100000	100000
0.013549	1000	100000	100000
0.000162	1	100000	100000
0.0015	20	100000	100000
0.004656	200	100000	100000
0.008002	500	100000	100000
0.011147	500	100000	100000
0.01336	1000	100000	100000
0.000864	20	100000	150000
0.003077	200	100000	150000
0.006167	500	100000	150000
0.008083	750	100000	150000
0.009406	1000	100000	150000

0.001012	20	100000	150000
0.003225	200	100000	150000
0.006086	500	100000	150000
0.008259	750	100000	150000
0.009109	1000	100000	150000
0.000958	20	100000	150000
0.003144	200	100000	150000
0.006302	500	100000	150000
0.007759	750	100000	150000
0.009622	1000	100000	150000
0.000621	20	100000	200000
0.001835	200	100000	200000
0.00332	500	100000	200000
0.004021	750	100000	200000
0.003981	1000	100000	200000
0.000526	20	100000	200000
0.001835	200	100000	200000
0.00363	500	100000	200000
0.00448	750	100000	200000
0.004534	1000	100000	200000
0.000513	20	100000	200000
0.001903	200	100000	200000
0.003738	500	100000	200000
0.004764	750	100000	200000