Molecular Architects Scoring Sheet ***Page 1***

Test Protein: Calmodulin

Susan Holtzman

Residue Number and Name		Crystallography			CASPAR			Score		
		Alpha	Beta	Buried	Alpha	Beta	Buried	Alpha	Beta	Buried
1	A									
2	D									
3	Q									
4	L									
5	T									
6	E									
7	E									
8	Q									
9	I				X		X			
10	A				X					
11	E				X					
12	F				X		X			
13	K				X					
14	E				X					
15	A				X		X			
16	F				X		X			
17	S				X					
18	L				X		X			
19	F				X		X			
20	* D									
21	* K									
22	* D									

(General)

STATE OF CALIFORNIA
COUNTY OF San Francisco } SS.

On February 19, 1987 before me, the undersigned, a Notary Public in and for said State, personally appeared Susan Holtzman

personally known to me (or proved to me on the basis of satisfactory evidence) to be the person whose name is subscribed to the within instrument and acknowledged that she executed the same.

WITNESS my hand and official seal.

Signature Helen Dumont

Helen Dumont
Name (Typed or Printed)

OFFICIAL SEAL
HELEN DUMONT
NOTARY PUBLIC-CALIFORNIA
PRINCIPAL OFFICE IN
CITY & CO OF SAN FRANCISCO
MY COMMISSION EXPIRES JUNE 15,1988

(This area for official notarial seal)

OFC-2056

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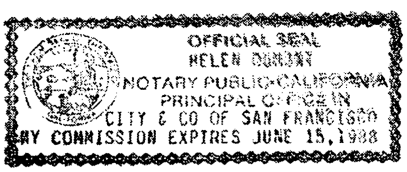
Test Protein: ***Calmodulin***

Susan Holtzman

Residue Number and Name		Crystallography			CASPAR			Score		
		Alpha	Beta	Buried	Alpha	Beta	Buried	Alpha	Beta	Buried
35	V				X		X			
36	M				X		X			
37	R				X					
38	S				X					
39	L				X		X			
40	G									
41	Q									
42	N									
43	P									
44	T									
45	E									
46	A									
47	E									
48	L				X		X			
49	Q				X					
50	D				X					
51	M				X		X			
52	I				X		X			
53	N				X					
54	E				X					
55	V				X		X			
56 *	D									
57 *	A									
58 *	D									
59 *	G									
60 *	N									
61 *	G									
62 *	T									
63 *	I									
64 *	D									
65 *	F									
66 *	P									
67 *	E				X					
68	F				X		X			

* Calcium binding region

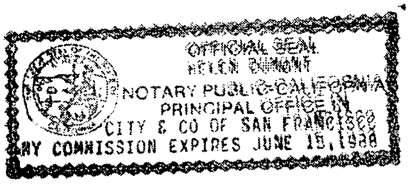


Test Protein: ***Calmodulin***

Susan Hilton

Residue Number and Name			Crystallography			CASPAR			Score		
			Alpha	Beta	Buried	Alpha	Beta	Buried	Alpha	Beta	Buried
69		L				X		X			
70		T				X					
71		M				X		X			
72		M				X		X			
73		A				X		X			
74		R				X					
75		K				X					
76		M				X		X			
77		K									
78	+	D									
79	+	T									
80	+	D									
81	+	S									
82	+	E									
83	+	E									
84	+	E									
85		I				X		X			
86		R				X					
87		E				X					
88		A				X		X			
89		F				X		X			
90		R				X					
91		V				X					
92		F				X		X			
93	*	D									
94	*	K									
95	*	D									
96	*	G									
97	*	N									
98	*	G									
99	*	Y									
100	*	I									
101	*	S									
102	*	A									

* Calcium binding region
\+ Binding site number 1

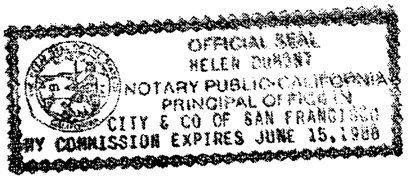


Test Protein: ***Calmodulin***

Susan Hattyman

Residue Number and Name			Crystallography			CASPAR			Score		
			Alpha	Beta	Buried	Alpha	Beta	Buried	Alpha	Beta	Buried
103	*	A									
104	*	E				X					
105		L				X					
106		R				X					
107		H				X					
108		V				X		X			
109		M				X		X			
110		T				X					
111		N				X					
112		L				X		X			
113		G									
114		E									
115	#	K									
116	#	L									
117	#	T									
118	#	D									
119	#	E									
120	#	E									
121	#	V				X					
122	#	D				X					
123	#	E				X					
124		M				X		X			
125		I				X		X			
126		R				X					
127		E				X					
128		A				X		X			
129	*	D									
130	*	I									
131	*	D									
132	*	G									
133	*	D									
134	*	G									
135	*	Q									
136	*	V									

* Calcium binding region
\# Binding site number 2



Calmodulin Feb. 19, 1987

		Kretsinger				Holtzman					
	AA	Alph Buried		Alpha	Buried	Buried Ca++		Alpha without Ca++			
1	A										
2	D										
3	Q										
4	L										
5	T										
6	E										
7	E										
8	Q	Y								+1	+1
9	I	Y		X	X		X	X		1	1
10	A	Y		X			X			1	1
11	E	Y		X			X			1	1
12	F	Y		X	X		X	X		1	1
13	K	Y		X			X			1	1
14	E	Y		X			X			1	1
15	A	Y		X	X		X	X		1	1
16	F	Y		X	X		X	X		1	1
17	S	Y		X			X			1	1
18	L	Y		X	X		X	X		1	1
19	F	Y		X	X		X	X		1	1
20	D	Y				X	X			+1	1
21	K					X					
22	D					X					
23	G					X					
24	D					X					
25	G					X					
26	T					X					
27	I		Y			X					
28	T					X					
29	T					X					
30	K	Y				X				+1	+1
31	E	Y				X				+1	+1
32	L	Y	Y				X	X		+1	1
33	G	Y					X			+1	1
34	T	Y					X			+1	1
35	V	Y		X	X		X	X		1	1
36	M	Y		X	X		X	X		1	1
37	R	Y		X			X			1	1
38	S	Y		X			X			1	1
39	L	Y		X	X		X	X		1	1
40	G	Y								+1	+1
41	Q										
42	N										

43	P												
44	T												
45	E												
46	A	Y										+1	+1
47	E	Y										+1	+1
48	L	Y		X	X			X	X			1	1
49	Q	Y		X				X				1	1
50	D	Y		X				X				1	1
51	M	Y		X	X			X	X			1	1
52	I	Y		X	X			X	X			1	1
53	N	Y		X				X				1	1
54	E	Y		X				X				1	1
55	V	Y		X	X			X	X			1	1
56	D	Y				X		X				+1	1
57	A					X							
58	D					X							
59	G					X							
60	N					X							
61	G					X							
62	T					X							
63	I		Y			X							
64	D					X							
65	F					X		X					-1
66	P	Y				X		X				+1	1
67	E	Y				X		X				+1	1
68	F	Y	Y	X	X			X	X			1	1
69	L	Y		X	X			X	X			1	1
70	T	Y		X				X				1	1
71	M	Y		X	X			X	X			1	1
72	M	Y		X	X			X	X			1	1
73	A	Y		X	X			X	X			1	1
74	R	Y		X				X				1	1
75	K	Y		X				X				1	1
76	M	Y		X	X			X	X			1	1
77	K	Y										+1	+1
78	D	Y		+				+				+1	+1
79	T	Y		+				+				+1	+1
80	D	Y		+				+				+1	+1
81	S	Y		+				+				+1	+1
82	E	Y		+				+				+1	+1
83	E	Y		+				+				+1	+1
84	E	Y		+				+				+1	+1
85	I	Y		X	X			X	X			1	1
86	R	Y		X				X				1	1
87	E	Y		X				X				1	1
88	A	Y		X	X			X	X			1	1
89	F	Y		X	X			X	X			1	1
90	R	Y		X				X				1	1
91	V	Y		X				X				1	1
92	F	Y		X	X			X	X			1	1
93	D	Y				X		X				+1	1
94	K					X							
95	D					X							

96	G					X							
97	N					X							
98	G					X							
99	Y					X							
100	I		Y			X							
101	S					X							
102	A					X							
103	A	Y				X					+1	+1	
104	E	Y				X					+1	+1	
105	L	Y	Y				X	X			+1		1
106	R	Y					X				+1		1
107	H	Y					X				+1		1
108	V	Y		X	X		X	X			1		1
109	M	Y		X	X		X	X			1		1
110	T	Y		X			X				1		1
111	N	Y		X			X				1		1
112	L	Y		X	X		X	X			1		1
113	G	Y									+1	+1	
114	E												
115	K			#			#						
116	L			#			#						
117	T			#			#						
118	D			#			#						
119	E	Y		#			#				+1	+1	
120	E	Y		#			#				+1	+1	
121	V	Y		#			X	X			+1		1
122	D	Y		#			X				+1		1
123	E	Y		#			X				+1		1
124	M	Y		X	X		X	X			1		1
125	I	Y		X	X		X	X			1		1
126	R	Y		X			X				1		1
127	E	Y		X			X				1		1
128	A	Y		X	X		X	X			1		1
129	D	Y				X	X				+1		1
130	I					X							
131	D					X							
132	G					X							
133	D					X							
134	G					X							
135	Q					X							
136	V		Y			X							
137	N					X	X						-1
138	Y					X	X						-1
139	E	Y				X	X				+1		1
140	E	Y				X	X				+1		1
141	F	Y	Y	X	X		X	X			1		1
142	V	Y		X	X		X	X			1		1
143	Q	Y		X			X				1		1
144	M	Y		X	X		X	X			1		1
145	M	Y		X	X		X	X			1		1
146	T	Y		X			X				1		1
147	A	Y		X			X				1		1
148	K	Y									+1	+1	

Scores:

Compare Ca++ predictions: 37 in helix not predicted; 58 predicted. No wrong helix predicted. 75% correct (111/148).

The Kaspar predictions without biasing for Ca++: 75 right, 20 under, and 3 over. 84% correct (123/148).

Walter Gilbert

March 14, 1987

Dear Bob,

Here are a few of Susan Holtzman's predictions for mutants of Calmodulin which should change the Calcium binding properties in non-obvious ways.

1) E87-->D87: This should destroy the -Ca++ relay. The long helix should be more stable in the absence of Ca++, and Ca++ should bind more tightly to site III.

2) E14-->D14: Ca++ should bind more tightly to site I.

3) D50-->E50: Weaker Ca++ binding at site II.

4) F19-->A19: This should stablize the Ca++ bound state at site I. Look in refinement for planar orintation of F12/F16 (and also F68/M71). In the presence of Ca++, F12/F16 are coplanar; in the absence of Ca++, F16/F19 are coplanar. F19-->A19 stalbilizes the Ca-bound state.

Wally

Dear Susan

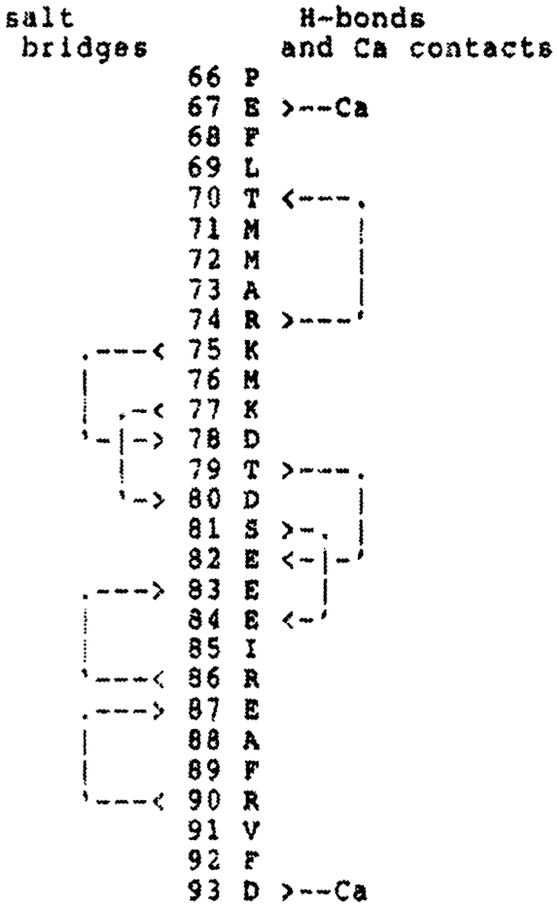
Here are the scoring sheets
+ a copy of the letter I sent
Best Greetings yesterday -

Ann Ashby

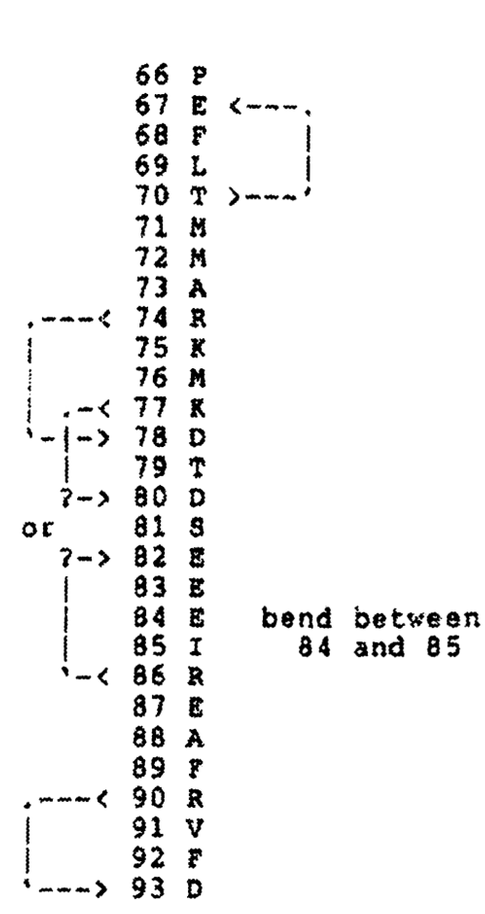
Dear Bob,

For the long helix in calmodulin:

Susan predicts these stabilizations in the presence of calcium:



And these without Ca: leading to a bend in the helix:



These salt bridges and H-bonds tie up all the other residues beyond those that her program identifies as nucleating alpha helix, thus the whole rod becomes rigid. When the Ca++ is removed, she predicts that R90 bridges to D93 and this shift leads to a bend between 84 and 85 at the end of a helix. Furthermore, T70 shifts to H-bond to E67, and the ensuing shifts further weaken the helix. She is doing a detailed analysis of the shifts around each calcium binding site and will send you a set of predictions about mutants.

Wally

Department of Biology
University of Virginia
Gilmer Hall
Charlottesville, Va. 22901

Dr. Susan Hoffmann
Molecular Architecture
231 S. Bemiston St.
Clayton MO 63105

revised

14 pages J.B.C. (May/Sept)

THE CENTRAL HELIX OF CALMODULIN FUNCTIONS AS A FLEXIBLE TETHER*

Anthony Persechini † and Robert H. Kretsinger

Department of Biology, Gilmer Hall, University of Virginia, Charlottesville, VA 22901

† Author to whom proofs are to be sent: telephone (804) 924-7039.

Sincerely

We look forward to hearing you.

Bob

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CENTRAL RESEARCH & DEVELOPMENT DEPARTMENT
EXPERIMENTAL STATION
(302) 695-1227

February 21, 1989

Dr. Susan Holtzman
Molecular Architects
231 South Bemiston
Clayton, MO 63105

Dear Dr. Holtzman,

At the Protein Society Meeting in San Diego last August you requested a preprint of our paper describing the photolabeling of calmodulin with peptides containing p-benzoylphenylalanine. It has taken us longer than anticipated to put the manuscript together, but we have finally submitted it for publication. Enclosed please find a preprint. Thank you for your interest.

Sincerely,

Karyn T. O'Neil

Karyn T. O'Neil

KTO:cf
Enclosure