

# Critical Issues: The Caveolin Model

*Klaus Fiedler*

# Caveolin proteins

Homo sapiens

	<b>cav1N14</b>	
Caveolin-1	-MSGGKYVDSEGHLYTVPIREQGNIYKPNNKAMADELSEKQVYDAHTKEI	49
Caveolin-3	-MMAEEHTD-----LEAQIVKDIHCKEI	22
Caveolin-2	MGLETEKADVQLFMD-----DDSYSHHSGLEYADPEKFAD	35
	: . *                                                  * .	
	<b>cav1 57-77 / 'signature epitope'</b>	
Caveolin-1	DLVNRDPKHLNDDVVKIDFEDVIAEPEGTHSFDGIWKASFTTFTVTKYWF	99
Caveolin-3	DLVNRDPKNINEDIVKVD FEDVIAEPVGTYSFDGVWKVSYTTFVSKYWC	72
Caveolin-2	SDQDRDPHRLNS-HLKLGFEDVIAEPVTTHSFDKVVICS HALFEISKYVM	84
	. :***:..*: :*:***** *:*** :* *.: * :***	
	<b>membrane span</b>	
Caveolin-1	YLLSALFGIPMALIWGIYFAILSFLHIWA VVPCIKSFLIEIQCISRVYS	149
Caveolin-3	YLLSTLLGVPLALLWGF LFACISFCHIWA VVPCIKSYLIEIQCISHIYS	122
Caveolin-2	YKFLT VFLAIPLAFIAGILFATLSCLHIWILMPFVKTC LMVLPVQTIWK	134
	* :*: :*: :*: :*: :* :* :* ** :* ** :* :* : : . : : . :	
Caveolin-1	IYVHTVC DPLFEAVGKIFSNVRINLQKEI	178
Caveolin-3	LCIRTF CNPLFAALGQVCSSIKV VLRKEV	151
Caveolin-2	SVTDV I IAPLCTSVGRCFSSVSLQLS QD-	162
	.. ** :*: *.: : * ::	

**cholesterol  
binding**

Caveolin-1 binds to cholesterol

**A** -See A129V, Mutations implicated in breast cancer and SIDS

Murata et al. (1995)

# Sequence alignment: Caveolin - PITP $\alpha$

The caveolin model

## Possible hit in the database

Hit	Prob-ability	E-value	P-value
1 <a href="#">1kcm_A</a> PITP alpha	20.2	12	0.00064
2 <a href="#">2jwa_A</a> ERB-2	19.5	48	0.0025

Score	SS	Cols	Query	HMM
1 <a href="#">1kcm_A</a> PITP alpha	18.9	-0.3	160	1-178
2 <a href="#">2jwa_A</a> ERB-2	15.2	3.5	44	73-149

Template HMM  
77-269 (270)  
1- 44 (44)

The only good scoring full-length alignment

```

Q ss_pred          CCCCCECCCC-----CECCCC-----CCCCCCCCCCCCH--HHHHCCCCCCHHHHCCCCCCCCCCCCC
Q ss_conf          99873006776-----2741663-----66787669788022--22421011105554005664689886552303
Q caveolin-1      1 MSGGKYVDEG-----HLYTVPI-----REQGNIYKPNKAM--ADELSEKQVYDAHTKEIDLVNRDPKHLNDDVV   64
Q Consensus       1 ~~~~~~-----r~~~~kp~n~m-----id~dRDP~lN~h~l   64
                    -.+++++. .|||.|- .-++|+|+|+- +++|+..+.+----|.|+||-.-.|.
T Consensus       77 Pk~a~l~v~EkawNaYPy~T~yt~~~~~F~i~i~eT~h~d~g~eNv~L~~~~l~ReV~IDIan~d~v~-d-Y   154
T 1kcm_A          77 PEGALNIHEKAWNAYPYCRTVITNEYMKEDFLIKIETWHKPDLTGQENVHKLPEAWKHVEAIYIDIADRSQVLSK-D-Y   154
T ss_dssp         CTTTTEEEEEEETTEEEEEEETTGGGEEEEEEEESSSSCCSSTTCCCCHHHTTCEEEETTCGCGGSGCG-G-C
T ss_pred         CCCCCEEEEECCCCEEEEEECCCCCEEEEEEEEECCCCCCCCCCCCCHHHHCEEEEECCCCCCCCCCC-C-C
T ss_conf         9752288875205787048899767778704899988872798780666686967850634798423146667756-7-8
  
```

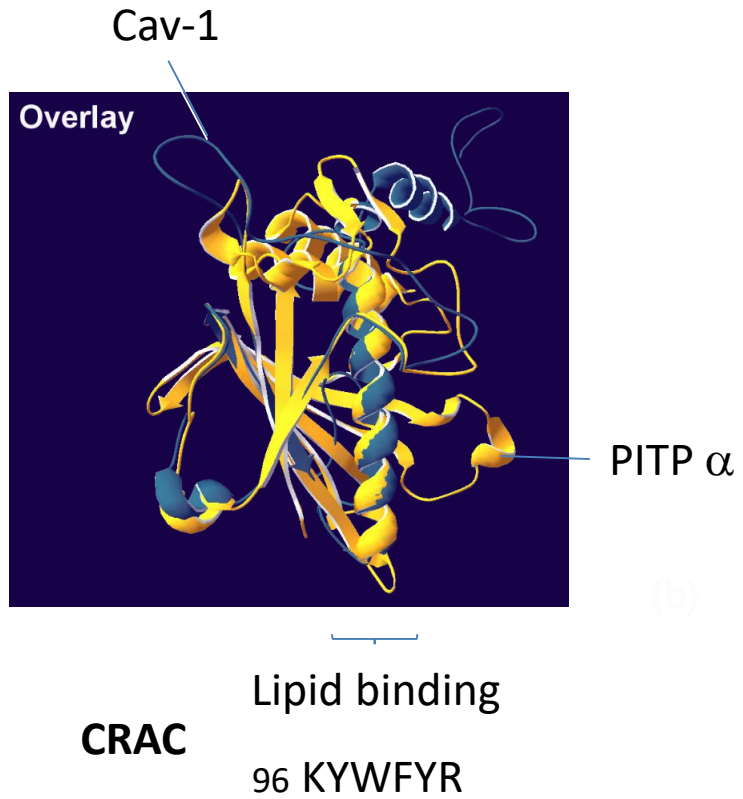
### CRAC

```

EECHHHHCCC--CCCCCEE-----EEEEEEEE-EEF-HHHHHHHHHHHHHHHHHHHHHHHHHHHHH
30678860688--7776865279-----9640899974-101--299999999999999999987543
KIDFEDVIAEP--EGTHSFHGIW-----KASFTTFTYTKYW--FYRLLSALFGIPMALIWGIYFAILSFLHI  127
KV~FeDViAEP~gthsfd~VW-----S~F~s~K~Yr~L~l~ai~P~A~Gi~FA~L~Sf~h~I   127
+..+..-. . -|---.+=| -|+|+|.+. .+| .-|+=+..-+.+.=-.+.+-+|=
155 ~eeDp~f~S~kTgRGPL~W~e~P~McaYKlv~v~f~wG~lq~v~E~f~I~r~H~Rq~fcW~De  234
155 KAEEDPAKFKSVKTGRGPLGPNWKQELVNQKDCPYMCAYKLVTKFKWGLQNKVENFTHKQEKRLFTNFHRQLFCWLDK  234
CGGGCTTCCCTTCCCCCTTHHHHTTSTTCCCCEEEEEEEECSSCHHHHHHHHHHHHHHHHHHHHHHHHHHH
CCCCCEEEEECCCCCCCCCCCCCCCCCCCCCEEEEEEEEEEECCCCCHHHHHHHHHHHHHHHHHHHHHEEECHH
8655860588865388988977410013489888899999999641014672789998668999988764241002
HHHHHHHHHHHHEEHHHHHHHHHHHHHHHHHHHHHHHHHHHHHHHCCEEEEEEECC
3333227471341899999999999999999999999999999999999999999999999998576089861569
WAVVPCIKSFLIEIQTSRVYSIYVHTVCDPLFEAVGKIFSNNRINLQKEI  178 (178)
W~v~PcIk~I~l~w~V~dP~f~S~Vg~f~S~I~r~v~k~e~  178 (178)
|. -|.+.+.+=.-. +.+.+.+=-+.+.+-
235 W-----gl~medIR~E~etq-----L~~~~g~  269 (270)
235 WV-----DLTMDDIRMEETK-----RQLDEMROKDPVKGMTAD  269 (270)
HT-----TCHHHHHHHHHHHHH-----HCCCC-----
HC-----CCHHHHHHHHHHHHH-----HHHHHHHHCCCCCCCC
12-----8888999999999999-----999999716732553257
  
```

Fiedler (2008)  
(see [www.klausfiedler.ch/cav1pitp.pdf](http://www.klausfiedler.ch/cav1pitp.pdf))

# Lipid exchange of Caveolin-1?



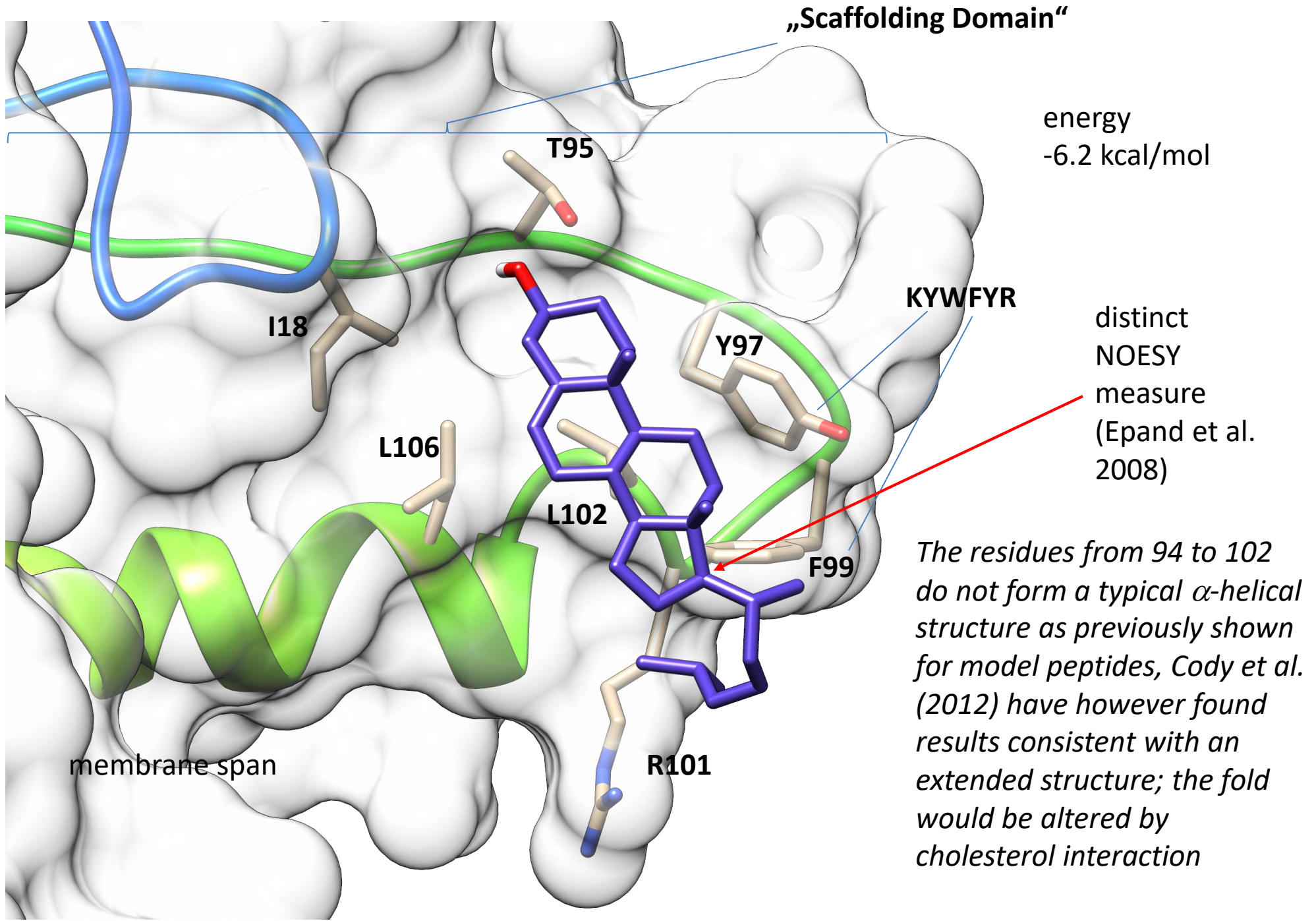
*Previous models [Samhan-Arias et al. (2012)] could not be compared due to a lack of statistical parameters*

(b) original model based on alignment

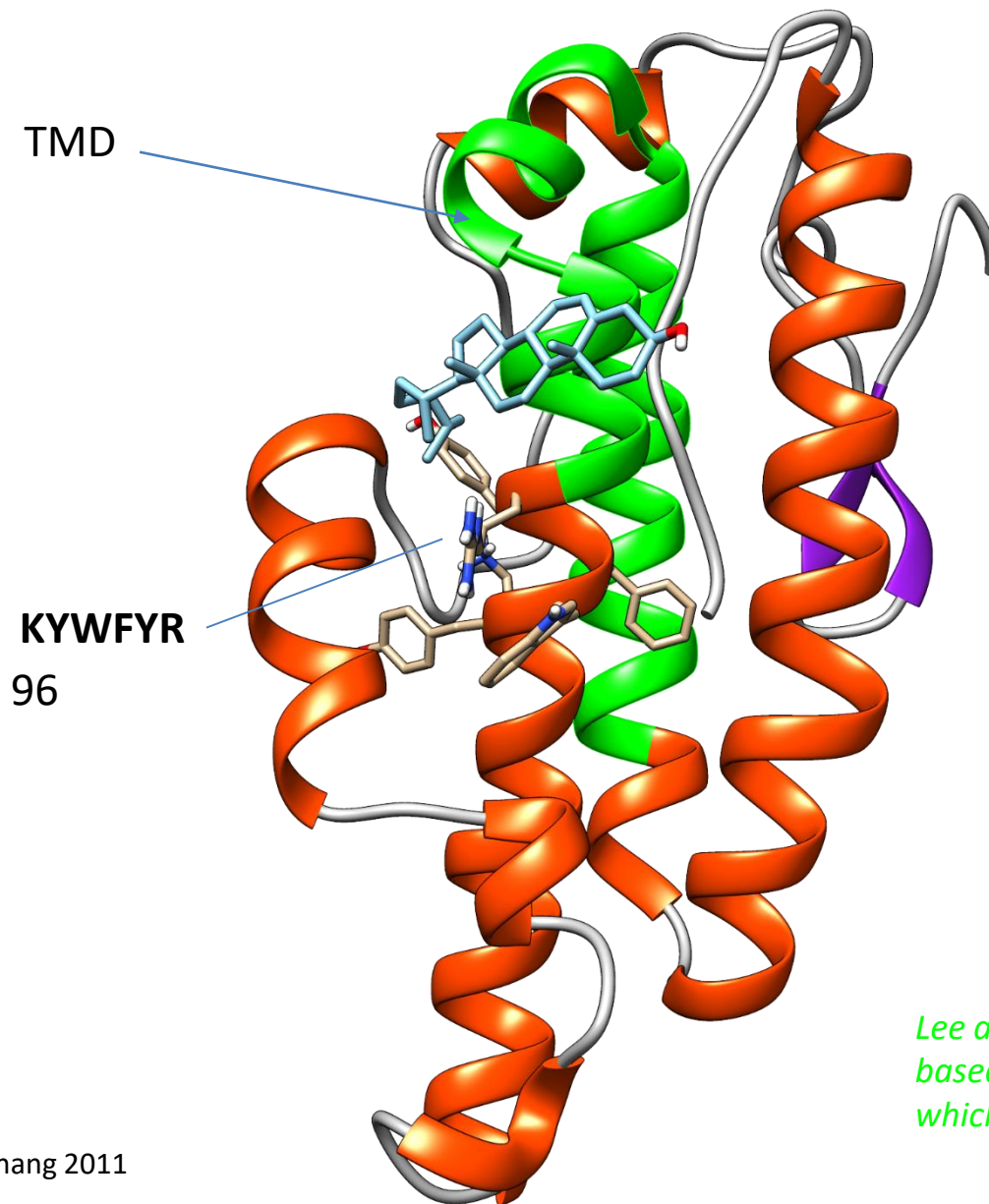
# CRAC binding

This model was generated with MODELLER and binding determined. Loop modeling was introduced to further optimize the structure (see Shen and Sali 2006) ([www.klausfiedler.ch/Modeling\\_of\\_Caveolin.htm](http://www.klausfiedler.ch/Modeling_of_Caveolin.htm)). The distinct structure probability has not been determined since abundant hydrophobic residues preclude the easy use of folding energy functions. Statistical potentials were used (DOPE).

Distances < 4 Å

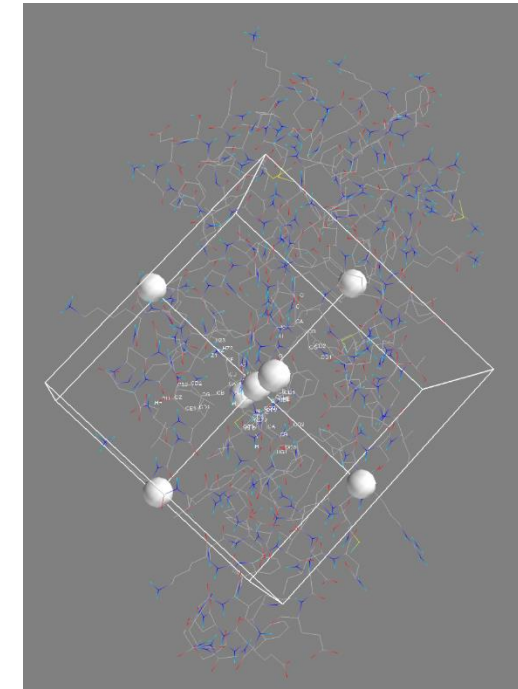


# Ab initio predicted structures don't expose membrane residues



energy  
 $\leq -5.9$  kcal/mol

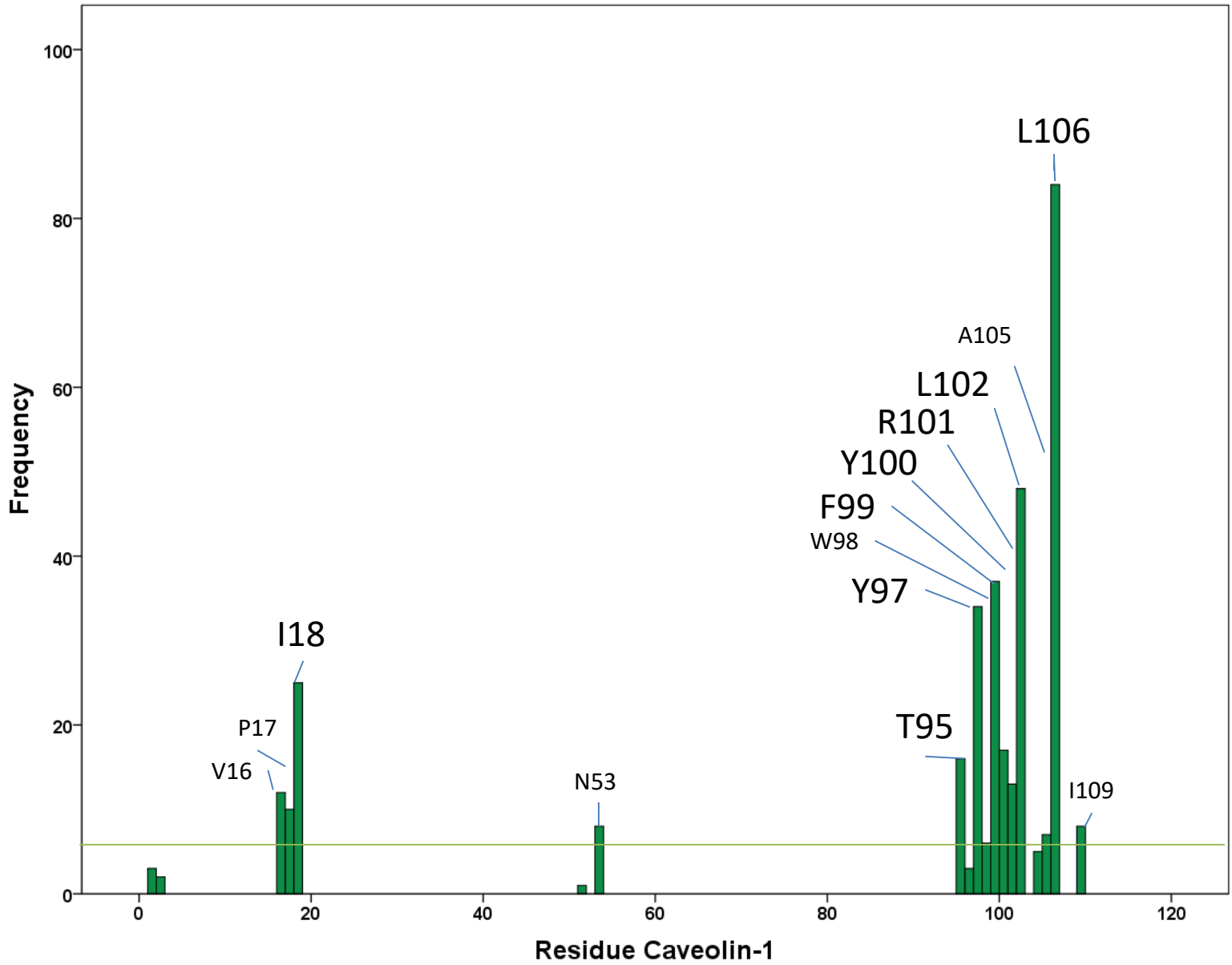
other stretches  
in the cav1  
structural  
models may  
have higher  
affinity



PyRx docking to the CRAC residues,  
cholesterol interacts distal to the CRAC  
residues

*Lee and Glover 2012 predicted a helical membrane span  
based on NMR analysis of a reconstituted cav1 96-136 peptide  
which in this structure is prevalently shielded from solvent*

# CRAC binding in the caveolin model: PITP $\alpha$ - homologue



Statistical evaluation of CRAC binding of cholesterol

cut-off Van der Waals  $\geq -0.4 \text{ \AA}$  (9 models)

The cavtratin effector stretch is located in region 89-95 of the CSD (cav1 scaffolding domain) (~82-101). The region distal to the effector stretch binds to cholesterol in the present model.

# Mutants in caveolin proteins

Cav-1	Cav-3	Equivalent Cav-1	Conserved Cav1/2/3	Disease
	Val14Leu	Val41		SIDS
	Arg27Gln	Arg54	Yes	RMD/LGMD-1C/HyperCK
	Asp28Glu	Asp55	Yes	RMD/LGMD-1C
	Pro29Leu	Pro56	Yes	RMD/HyperCK/DM
	Asn33Lys	Asn60	Yes	LGMD-1C/HyperCK
	Lys38X	Lys65	Yes	LGMD-1C
	Val44Glu	Val71	Yes	LGMD-1C/SIDS
	Ala46Val/Thr	Ala73	Yes	LGMD-1C/RMD
	Glu47Ala/Lys	Glu74	Yes	RMD
	Ser53Gly	Ser80	Yes	RMD
	Gly56Ser	Gly83		LGMD-1C
	Val57Met	Ile84		HyperCKemia
	Ser61Arg	Ser88	Yes	LGMD-1C/HyperCK
	ΔThr64Phe65Thr66	91,92,93	*	LGMD-1C
	Thr64Pro	Thr91		LGMD-1C
	Trp71Ter	Trp98		RMD
	Cys72Trp	Phe99		LGMD-1C
	Thr78Met	Ala105		LQTS/SIDS/HyperCK/LGMD-1C
Phe107Leu	Leu79Arg	Phe107		SIDS/Breast Cancer
	Ala85Thr	Ala112	Yes	LQTS
	Leu87Pro <sup>†</sup>	Ile114		RMD
Gly116Ser		Gly116	Yes	Breast Cancer
	Ala93Thr <sup>†</sup>	Ala120	Yes	RMD/LGMD-1C
	ΔPhe97	Phe124		LGMD-1C/RMD/HyperCK
	Phe97Cys	Phe124		LQTS
Leu125Gln		Leu125		Breast Cancer
Pro132Leu	Pro105Leu	Pro132	Yes	LGMD-1C/Breast Cancer
Cys133Arg		Cys133		Breast Cancer
Ser136Cys		Ser136		Breast Cancer
Ile141Phe		Ile141		Breast Cancer
Tyr148rHis		Tyr148		Breast Cancer
	Ser141Arg	Ser168	Yes	LQTS

\*Phe conserved  
<sup>†</sup>labeled differently in Kubisch et al. (2003)  
table adapted from Gazzero et al. (2010)

Cholesterol Interaction  
CRAC  
Putative Interaction

4 Å

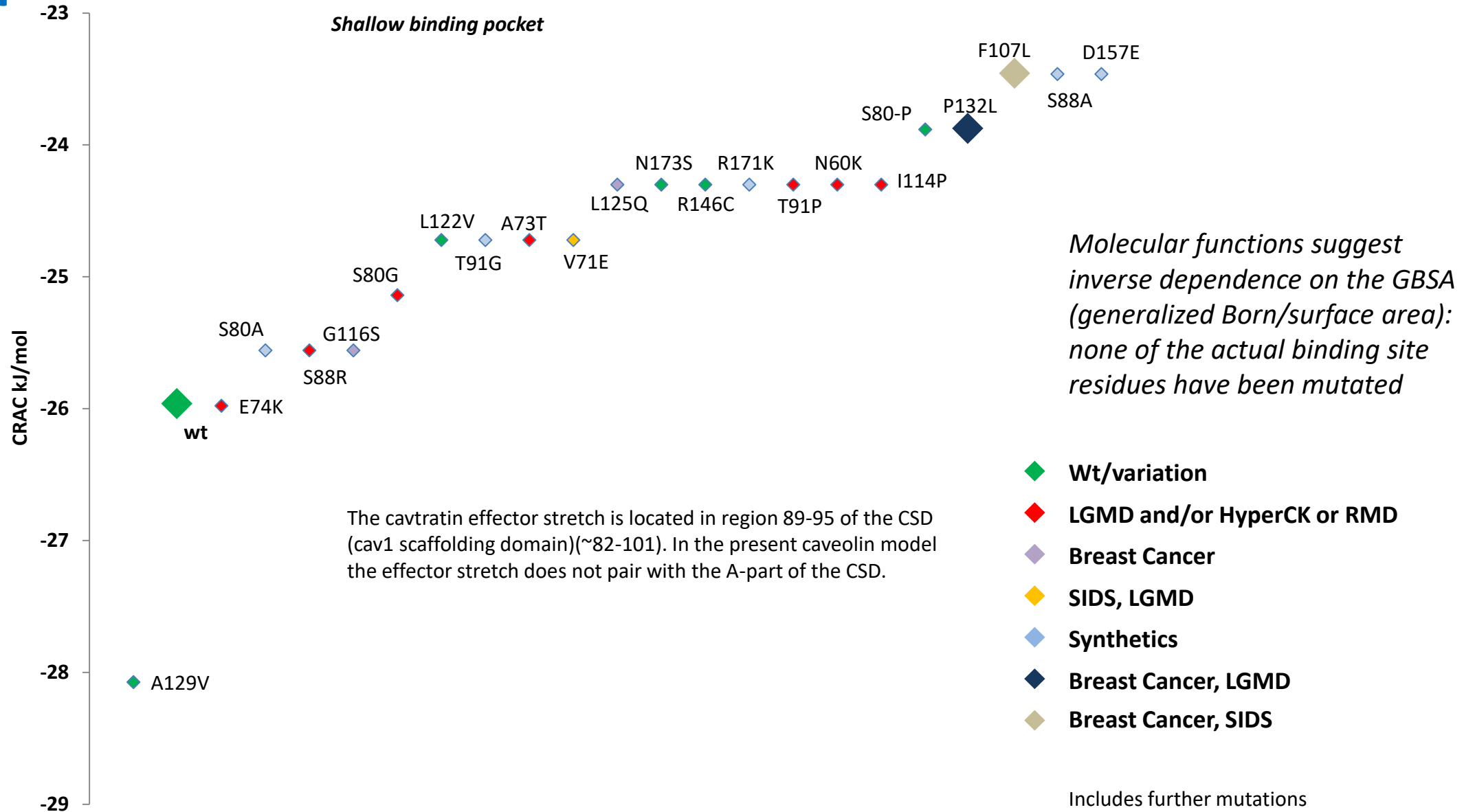
cavtratin-peptide  
(see Bernatchez et al. 2005)



cavtratin-peptide has  
been used for eNOS  
(endothelial nitric oxide  
synthase) inhibition  
studies

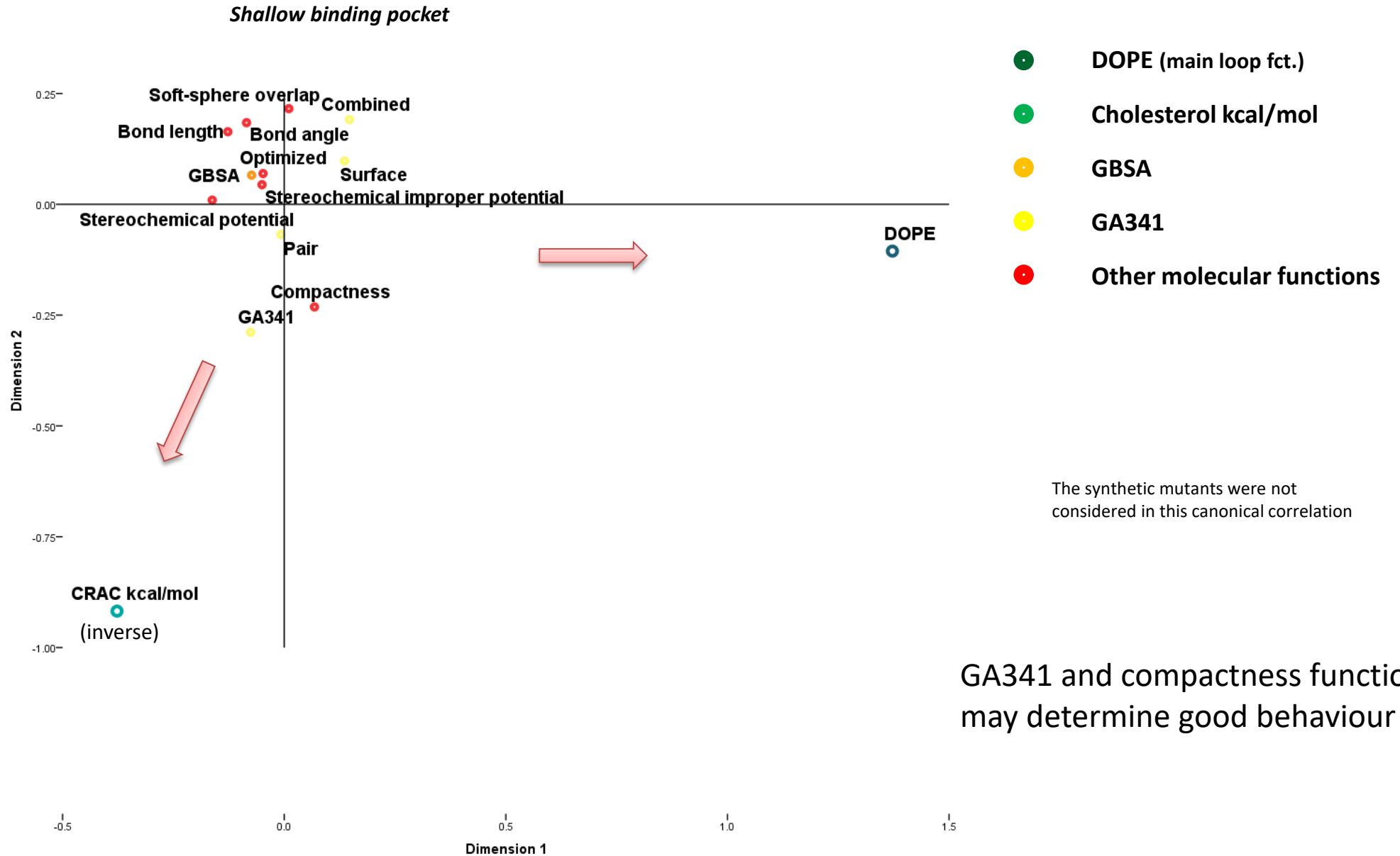


# Mutations implicated in breast cancer and SIDS: Putative structural model

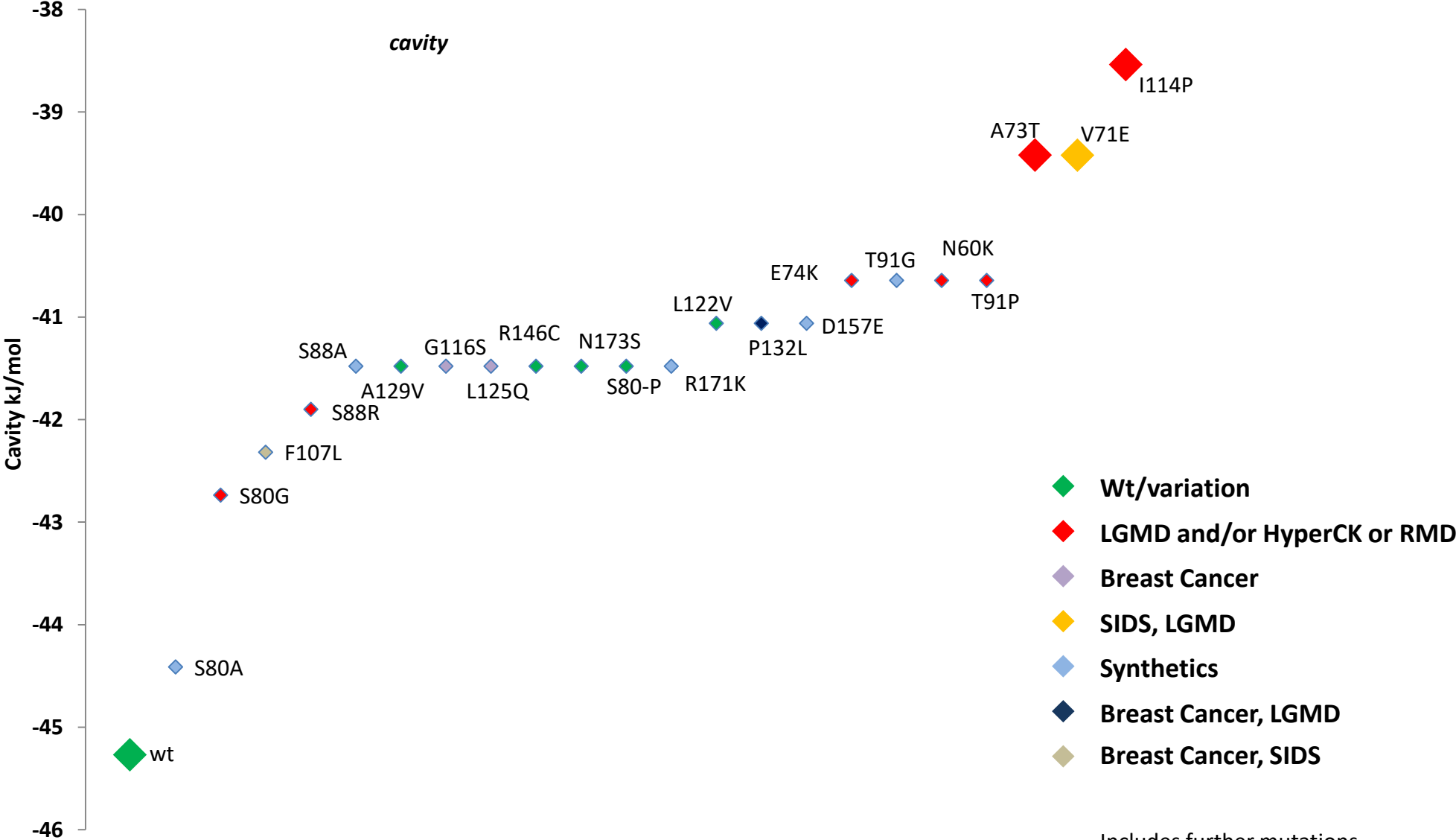


# Folding in this series of cholesterol docking mutants

Overalls canonical correlation



# Mutations implicated in LGMD, RMD and SIDS: Putative cavity structural model

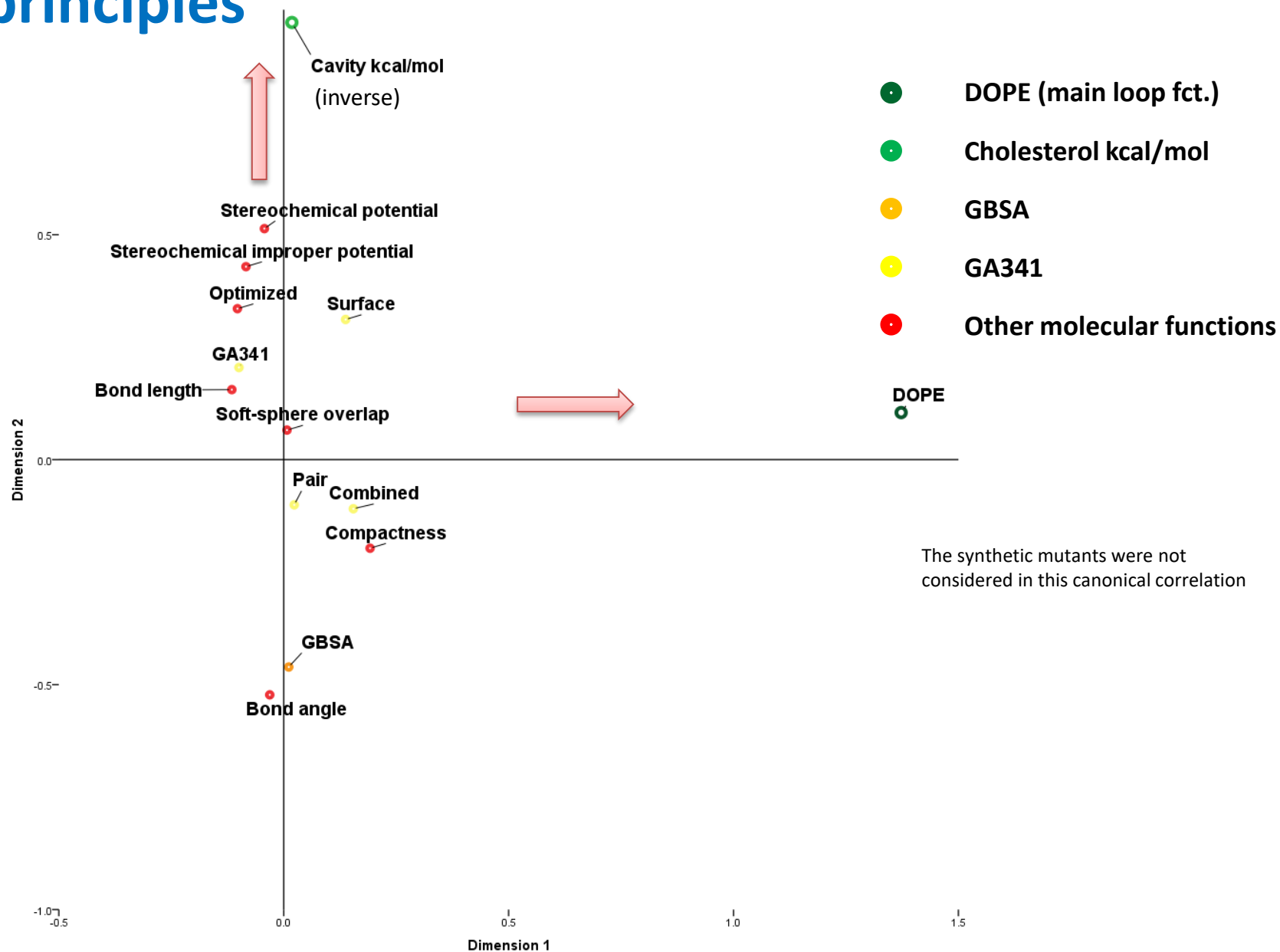


LGMD, limb girdle muscular dystrophy; RMD, rippling muscle disease; SIDS, sudden infant death syndrome; HyperCK, high creatine kinase

Includes further mutations (Syeed et al. 2010)

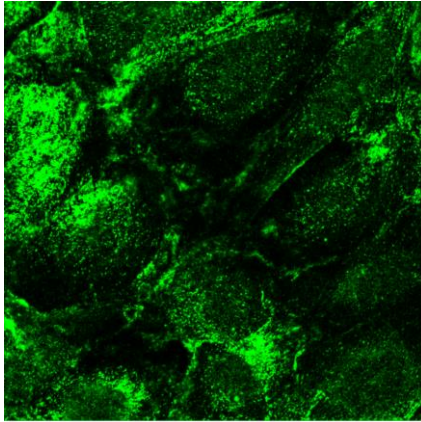
# Hypothetical cavity interaction may be determined by different structural folding principles

Overals canonical correlation



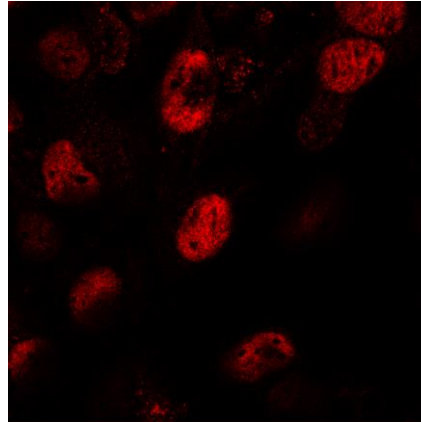
# Nuclear caveolin-1 likely shows a particular conformer

Polyclonal antibodies (C13630)



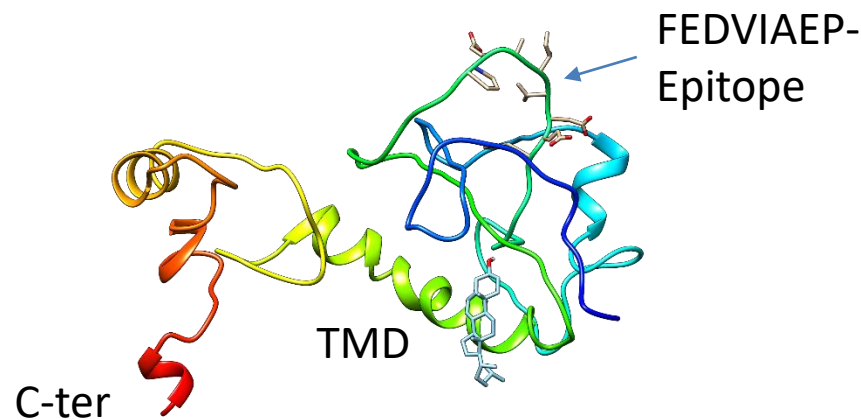
SVEC4-10

Polyclonal *Signature*-domain antibodies



SVEC4-10

The *folding* of caveolin may require the membrane bilayer and cholesterol and transport to the nucleus could, however, involve diffusion through the cellular cytoplasm wherein most hydrophobic residues are covered by amphipathic lipids and/or exposed to the cytoplasm as previously found for domains of intrinsically disordered proteins (Riback et al. 2017)



**Nuclear localization with cav-1 antibodies**

Chatenay-Rivauday et al. 2004