Critical Issues: The Caveolin Model

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Caveolin proteins

Homo sapiens

Caveolin-1 binds to cholesterol

Murata et al. (1995)

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

Sequence alignment: Caveolin - PITP α

The caveolin model

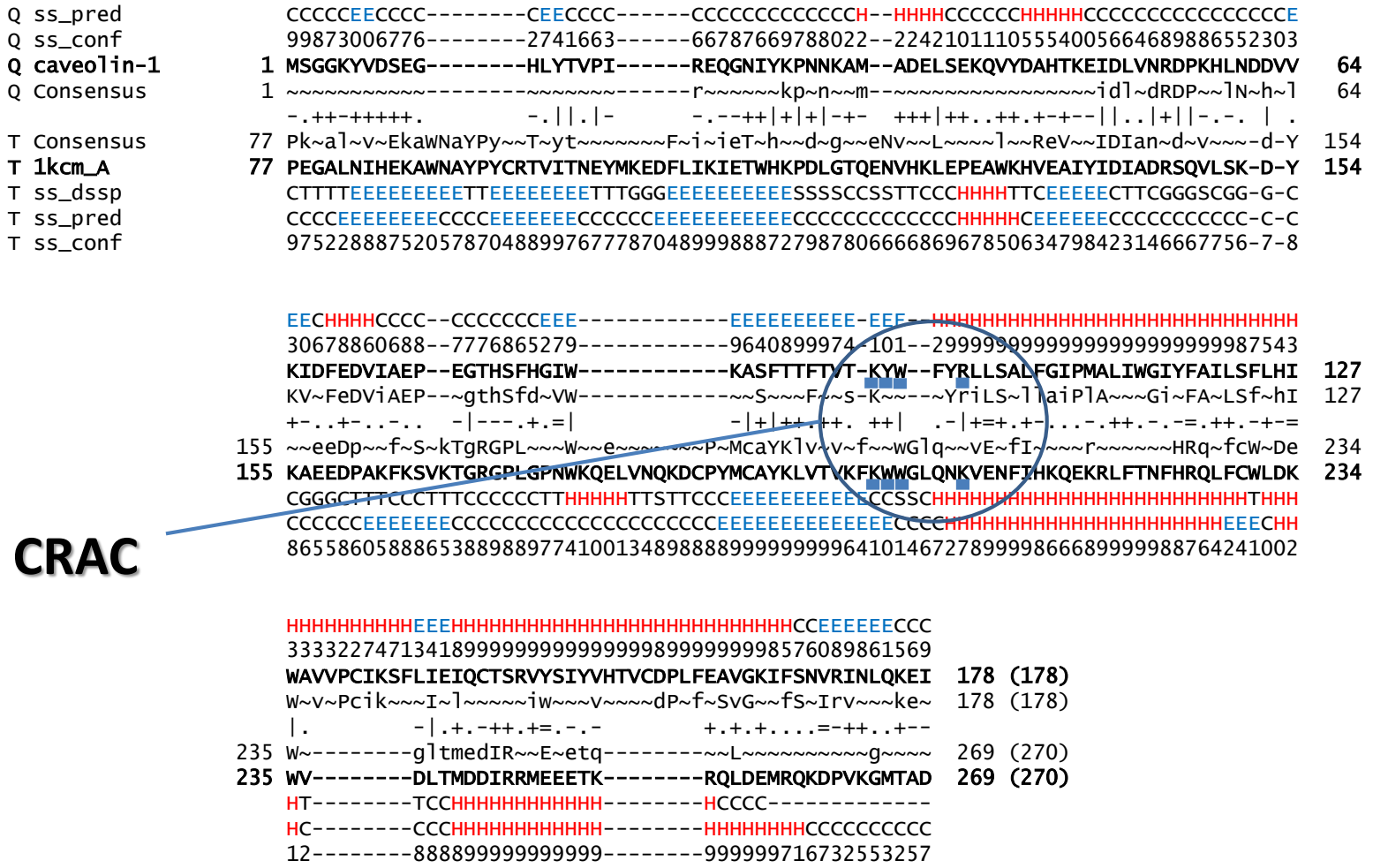
Possible hit in the database

	Hit		Prob-ability	E-value	P-value
1	1kcm_A	PITP alpha	20.2	12	0.00064
2	2jwa_A	ERB-2	19.5	48	0.0025

			Score	SS	Cols	Query HMM
1	1kcm_A	PITP alpha	18.9	-0.3	160	1-178
2	2jwa_A	ERB-2	15.2	3.5	44	73-149

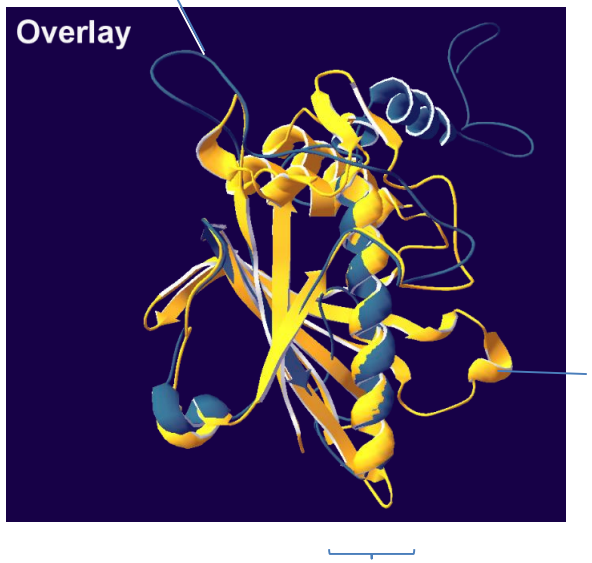
Template HMM
77-269 (270)
1- 44 (44)

The only good scoring full-length alignment



Fiedler (2008)
(see www.klausfiedler.ch/cav1pitp.pdf)

Lipid exchange of Caveolin-1?

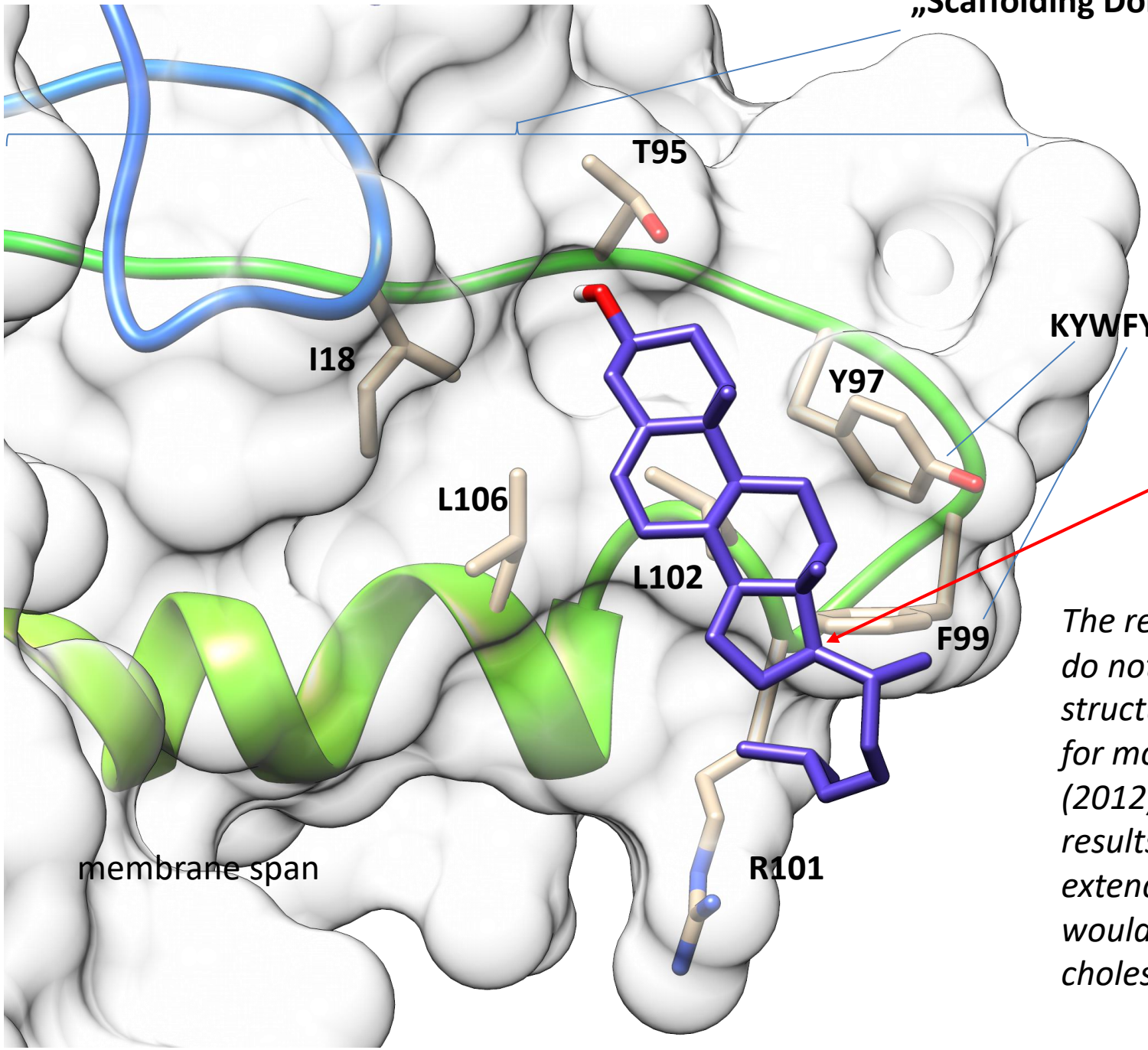


(b) original model based on alignment

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

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).
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).



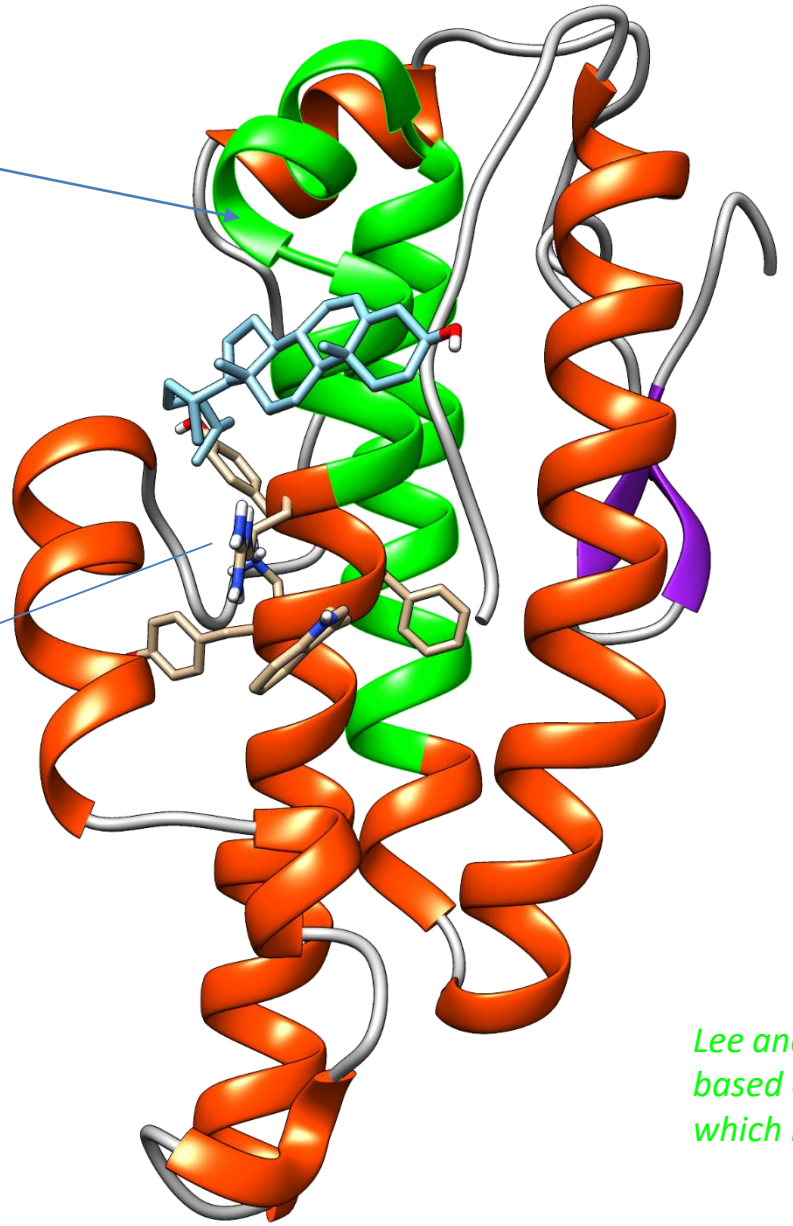
energy
-6.2 kcal/mol

distinct NOESY measure (Epand et al. 2008)

The residues from 94 to 102 do not form a typical α-helical structure as previously shown for model peptides, Cody et al. (2012) have however found results consistent with an extended structure; the fold would be altered by cholesterol interaction

Distances
< 4 Å

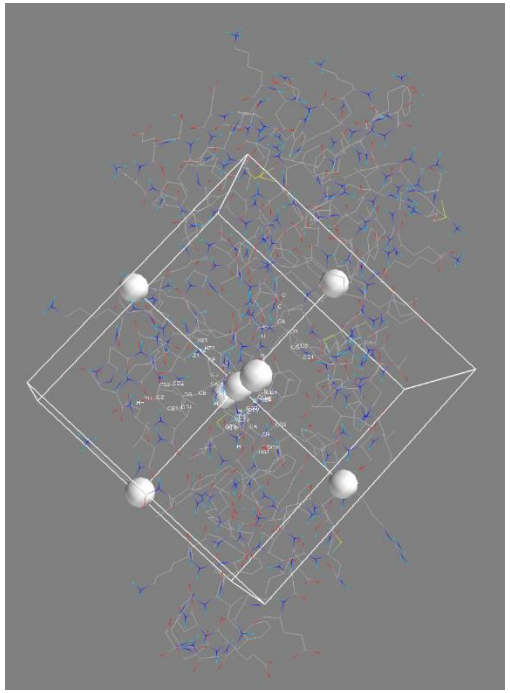
Ab initio predicted structures don't expose membrane residues



see
Xu and Zhang 2011

energy
≤-5.9 kcal/mol

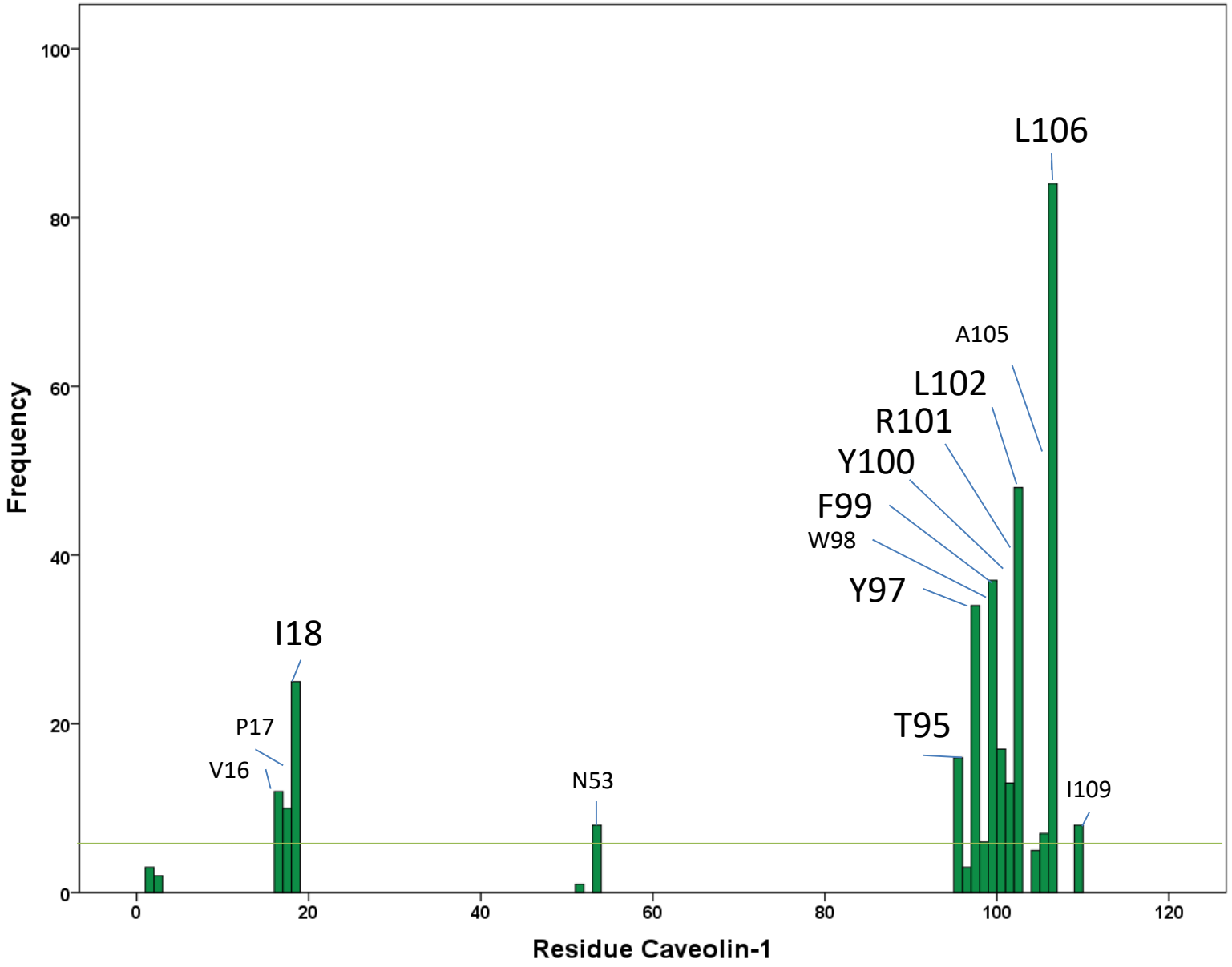
other stretches in the cav1 structural models may have higher affinity



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 α - homologue



Statistical evaluation of *CRAC* binding of

cholesterol

cut-off Van der Waals ≥ -0.4 Å (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¦	Ile114		RMD
Gly116Ser		Gly116	Yes	Breast Cancer
	Ala93Thr¦	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
Ty148rHis		Tyr148		Breast Cancer
	Ser141Arg	Ser168	Yes	LQTS

*Phe conserved
¦labeled differently in Kubisch et al. (2003)
table adapted from Gazzerro et al. (2010)

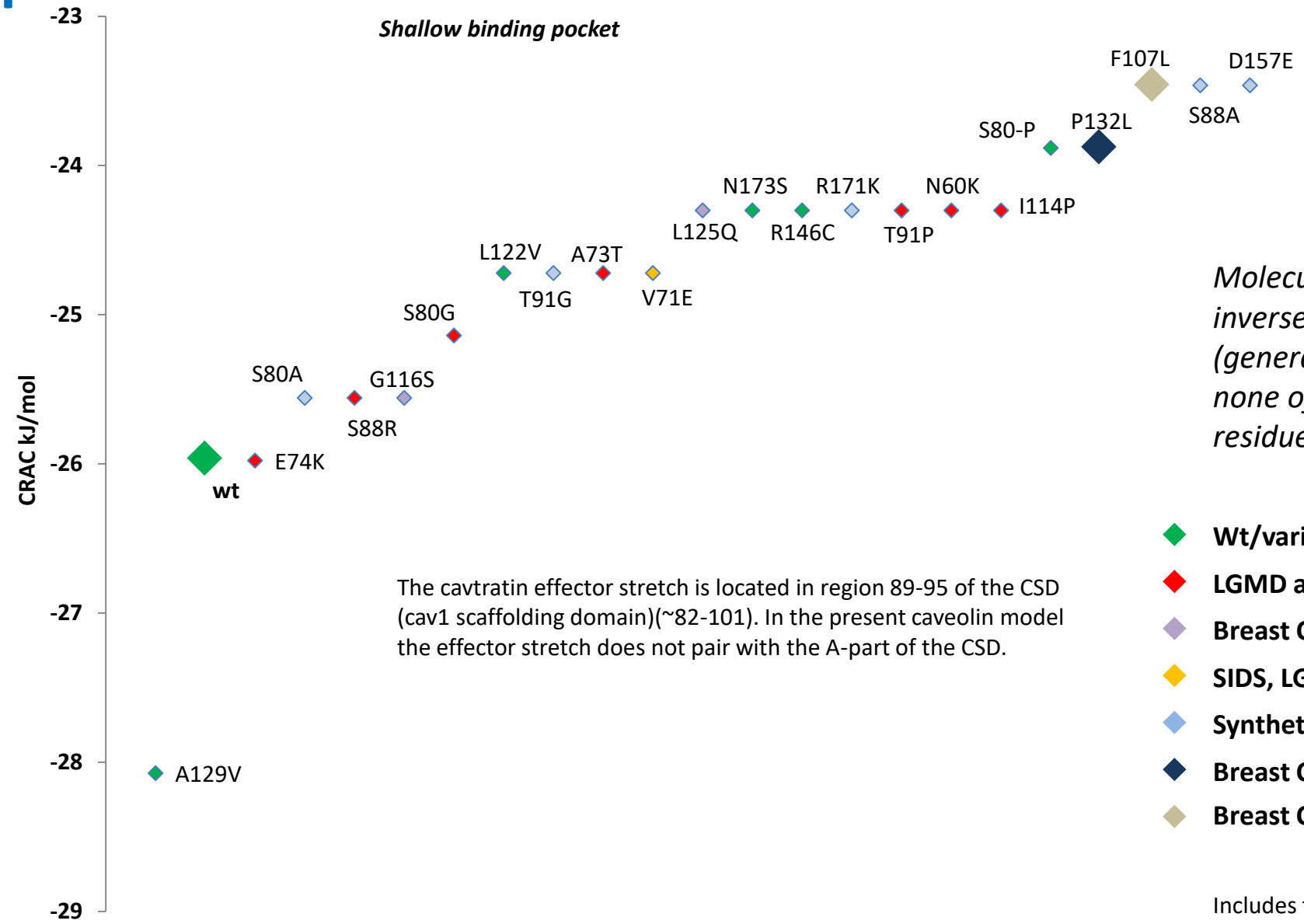
Cholesterol Interaction
CRAC
Putative Interaction

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



The cavtratin effector stretch is located in region 89-95 of the CSD (cav1 scaffolding domain)(~82-101). In the present caveolin model the effector stretch does not pair with the A-part of the CSD.

Molecular functions suggest inverse dependence on the GBSA (generalized Born/surface area): none of the actual binding site residues have been mutated

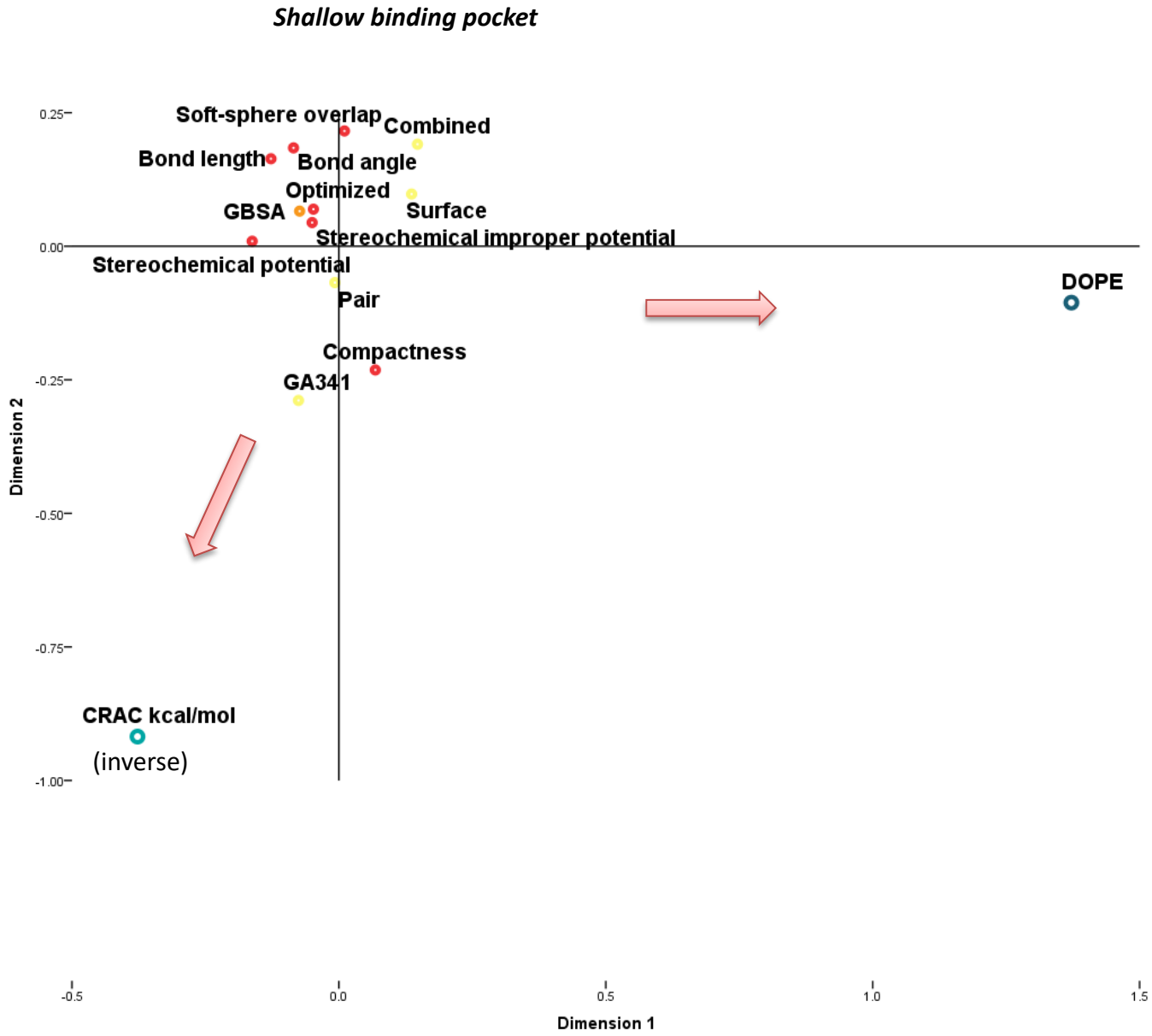
- **Wt/variation**
- **LGMD and/or HyperCK or RMD**
- **Breast Cancer**
- **SIDS, LGMD**
- **Synthetics**
- **Breast Cancer, LGMD**
- **Breast Cancer, SIDS**

Includes further mutations (Syeed et al. 2010)

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

Folding in this series of cholesterol docking mutants

Overals canonical correlation

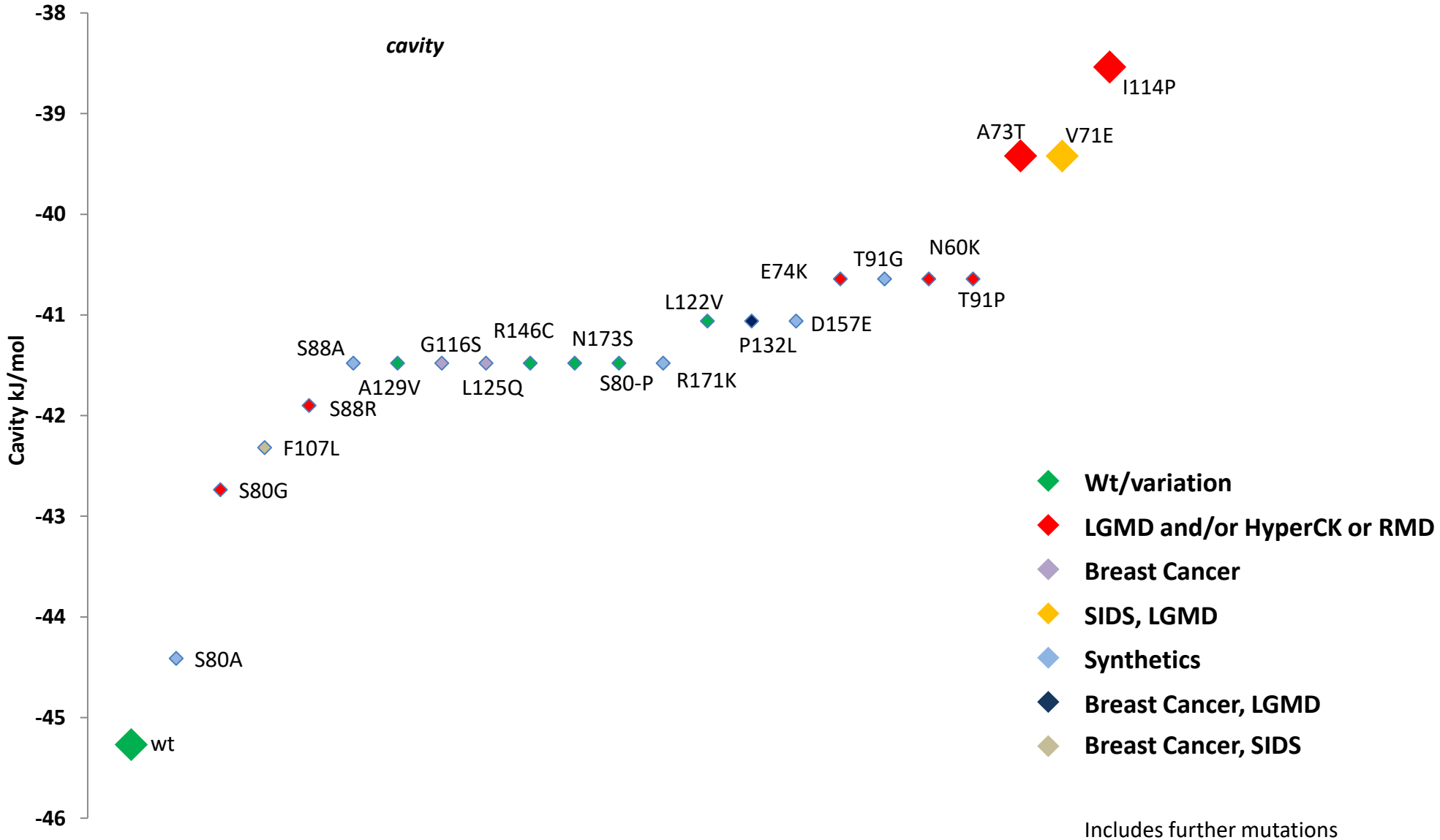


- DOPE (main loop fct.)
- Cholesterol kcal/mol
- GBSA
- GA341
- Other molecular functions

The synthetic mutants were not considered in this canonical correlation

GA341 and compactness functions may determine good behaviour

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

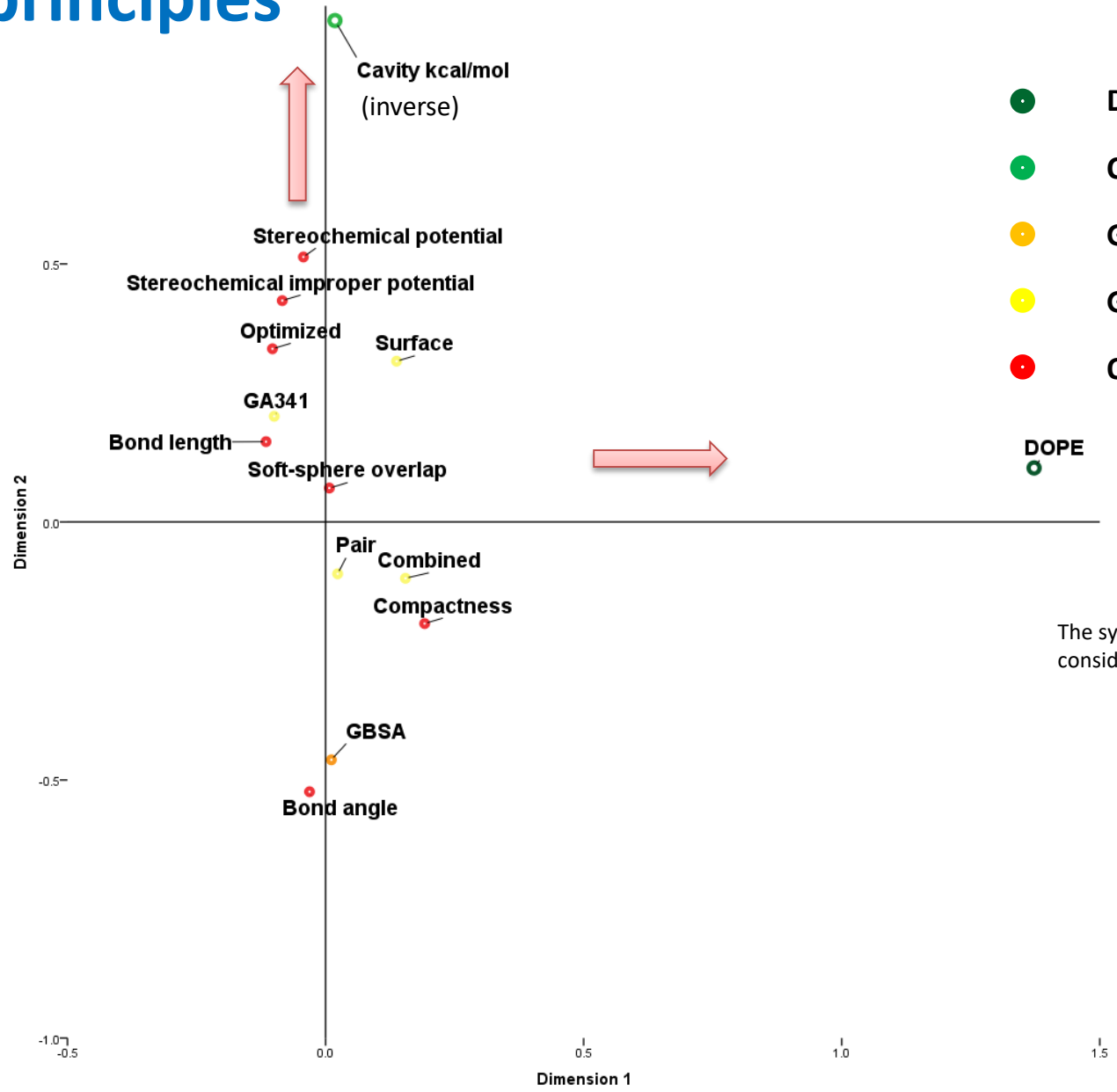


Includes further mutations (Syeed et al. 2010)

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

Hypothetical cavity interaction may be determined by different structural folding principles

Overals canonical correlation

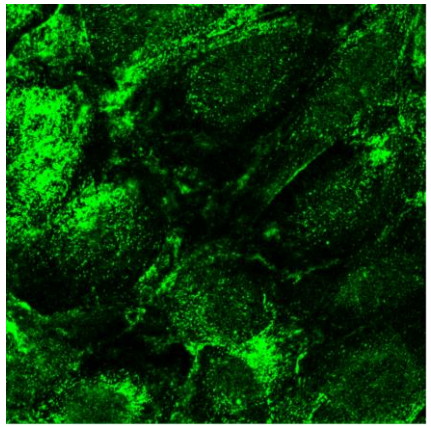


- DOPE (main loop fct.)
- Cholesterol kcal/mol
- GBSA
- GA341
- Other molecular functions

The synthetic mutants were not considered in this canonical correlation

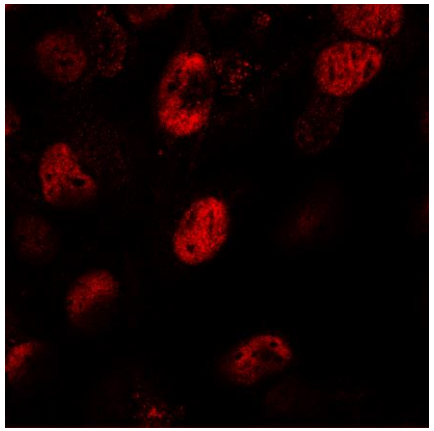
Nuclear caveolin-1 likely shows a particular conformer

Polyclonal antibodies (C13630)



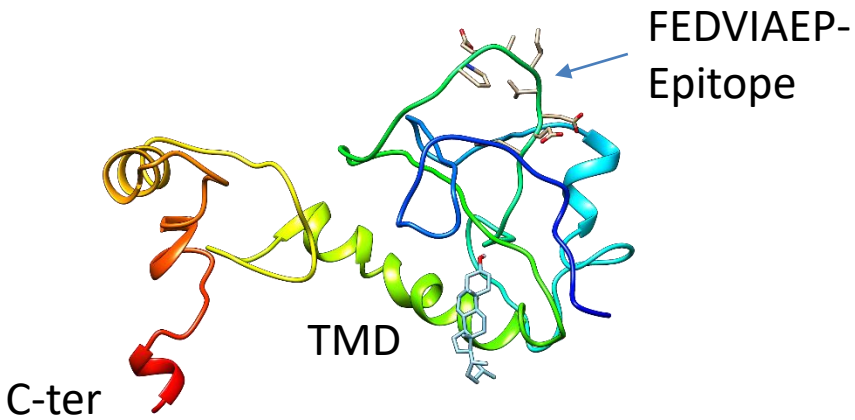
SVEC4-10

Polyclonal *Signature*-domain anti-bodies



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