Energetics of the open - closed transition in the RyR N-terminal region: importance for the CPVT phenotype

Andrea Faltinova

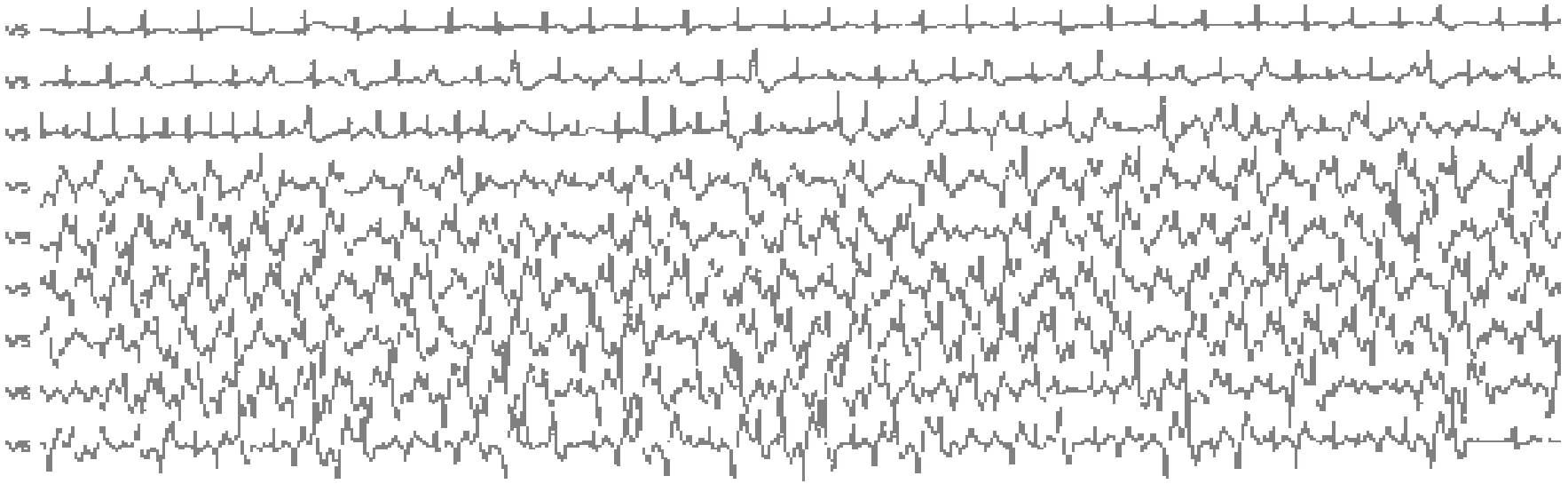
Jozef Ševčík

J. Sevcik[1], A. Faltinova[2], A. Zahradnikova[2,3]

[1] Institute of Molecular Biology, Slovak Academy of Sciences, Dúbravská cesta 21, 845 51 Bratislava, Slovakia

[2] Institute of Molecular Physiology and Genetics, Centre of Biosciences, Slovak Academy of Sciences, Dúbravská cesta 9, 840 05 Bratislava, Slovakia

[3] Institute of Experimental Endocrinology, Biomedical Research Centre, Slovak Academy of Sciences, Dúbravská cesta 9, 845 05 Bratislava, Slovakia

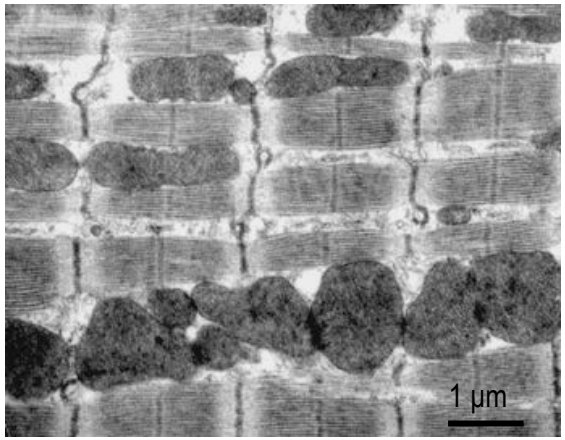


Regional Biophysics Conference 2018

19. 5. 2018

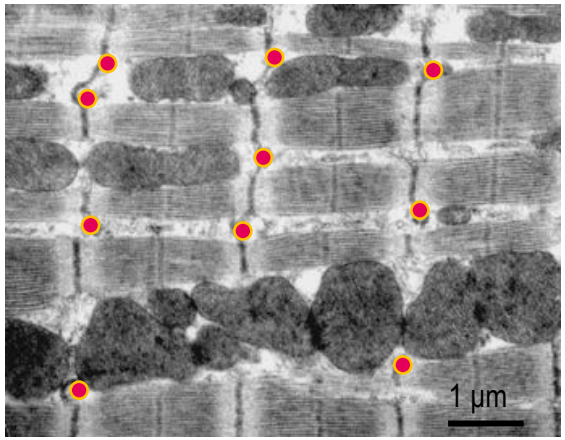
http://www.contica.eu/images/overview_fig1.gif

Excitation-contraction coupling



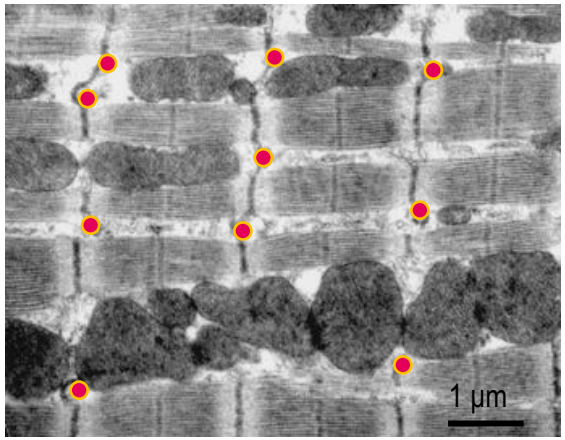
courtesy of M. Novotová, BMC SAS

Excitation-contraction coupling

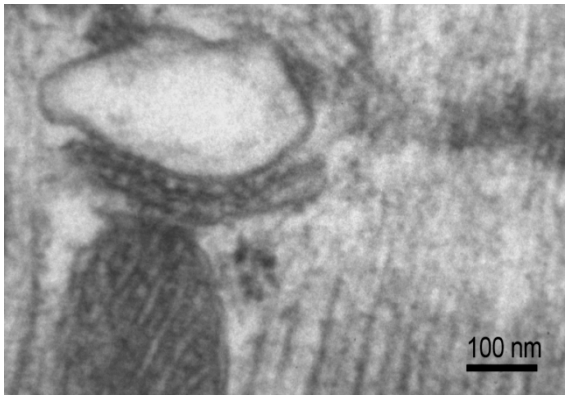


courtesy of M. Novotová, BMC SAS

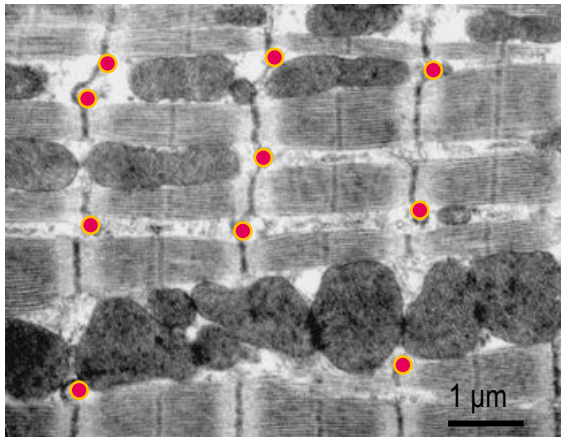
Excitation-contraction coupling



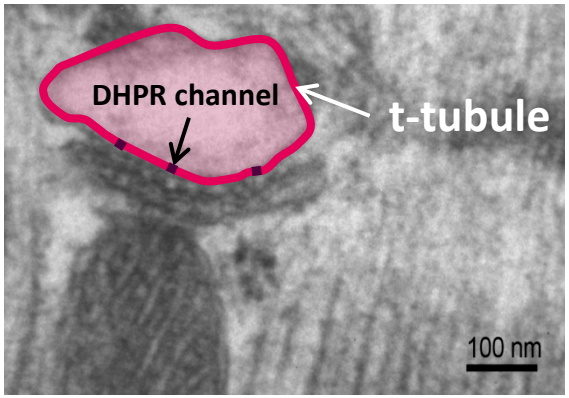
courtesy of M. Novotová, BMC SAS



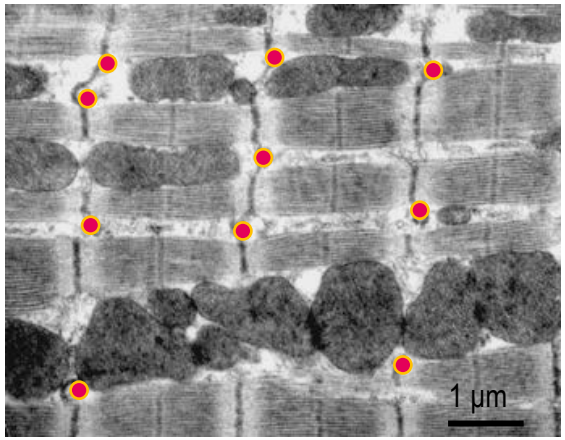
Excitation-contraction coupling



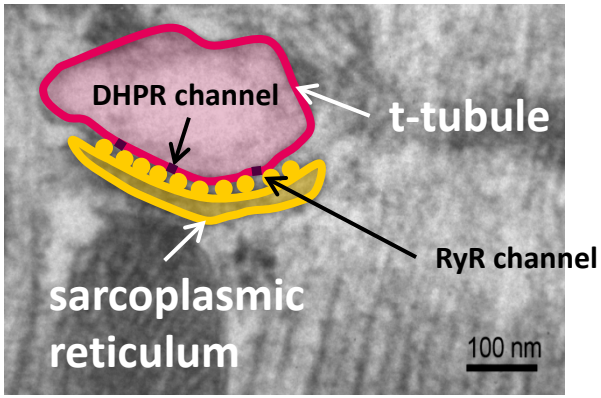
courtesy of M. Novotová, BMC SAS



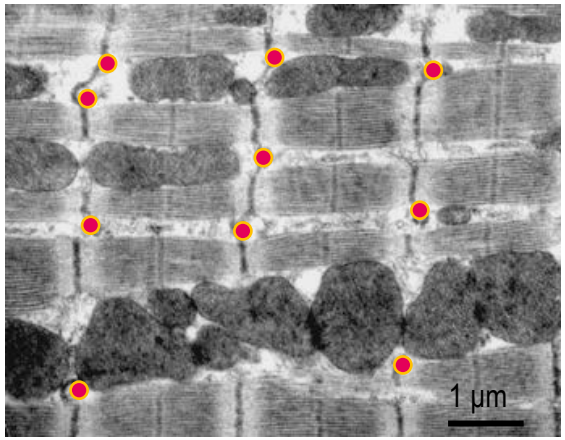
Excitation-contraction coupling



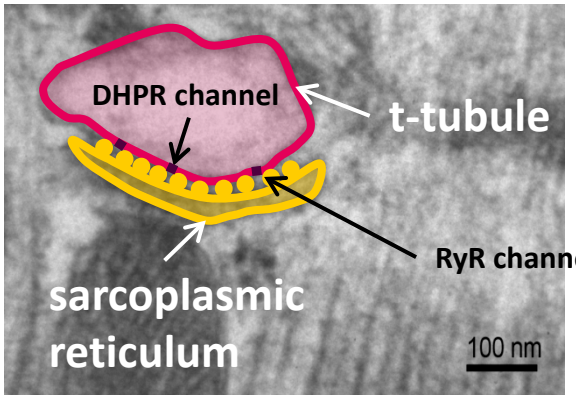
courtesy of M. Novotová, BMC SAS

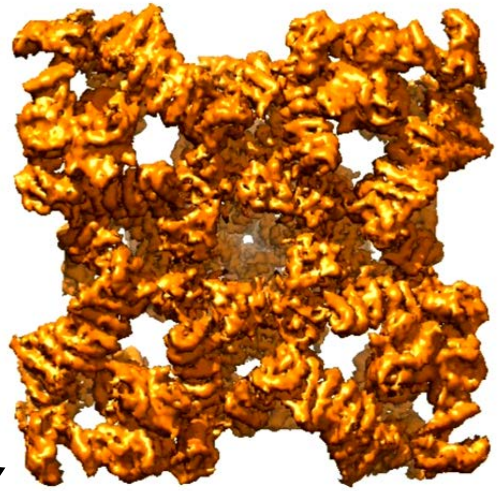


Excitation-contraction coupling



courtesy of M. Novotová, BMC SAS

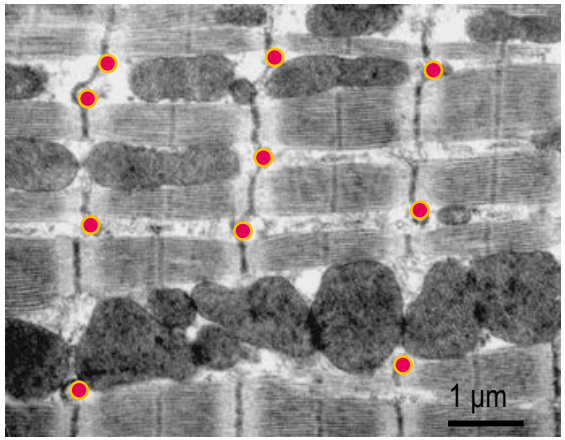




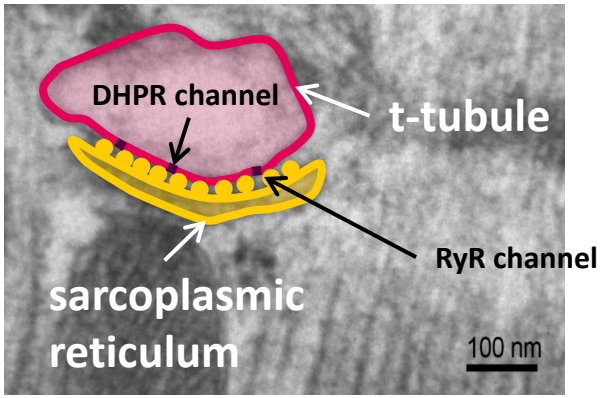
EMD-2807

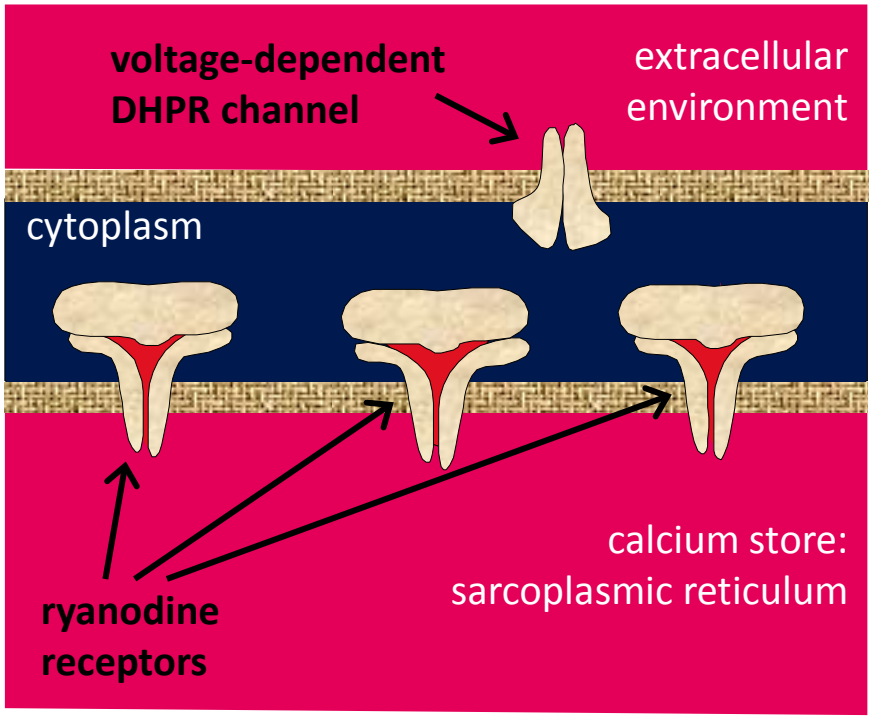
Yan *et al. Nature*. 517: 50-55, 2015 .

Excitation-contraction coupling

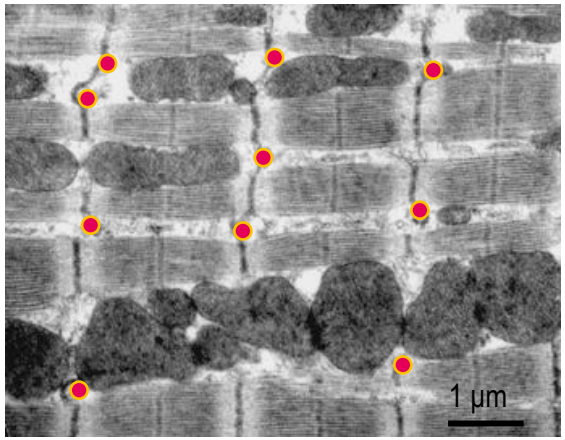


courtesy of M. Novotová, BMC SAS

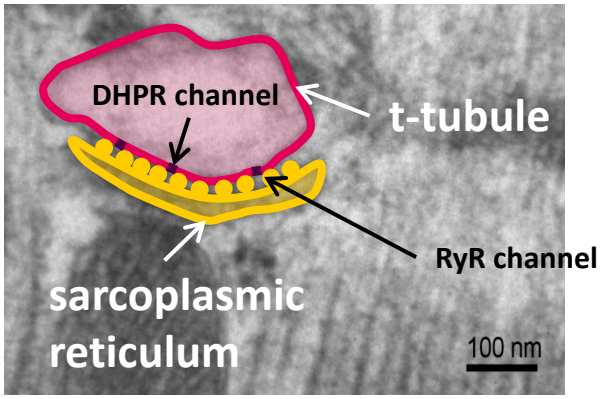


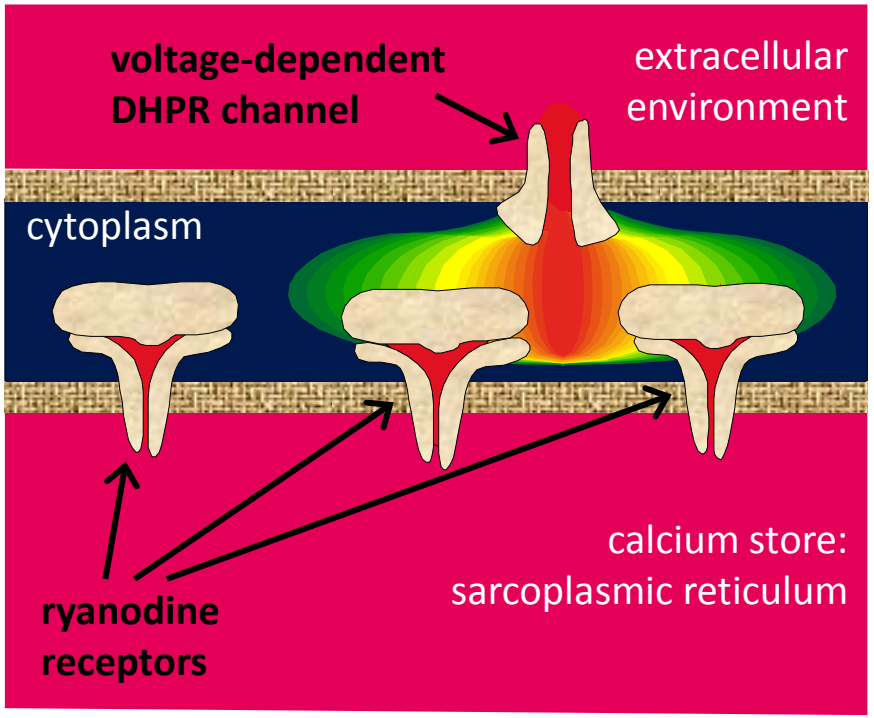


Excitation-contraction coupling

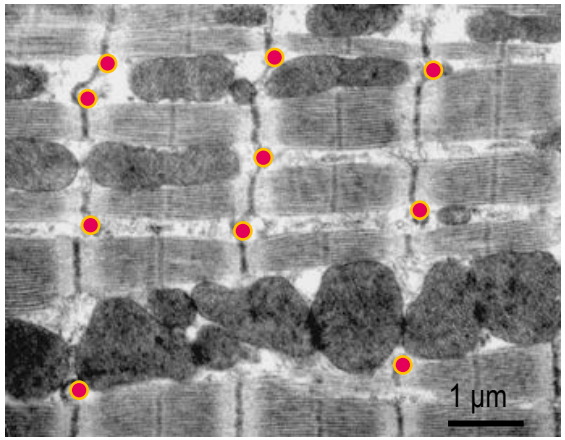


courtesy of M. Novotová, BMC SAS

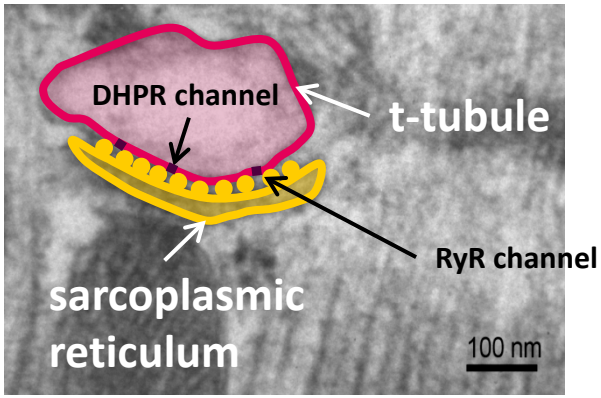


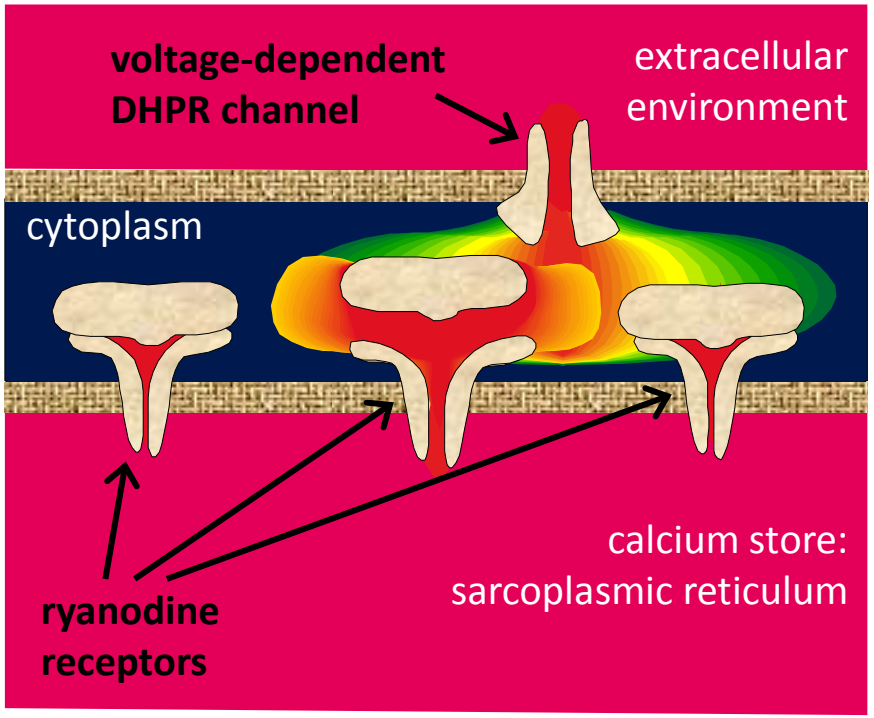


Excitation-contraction coupling



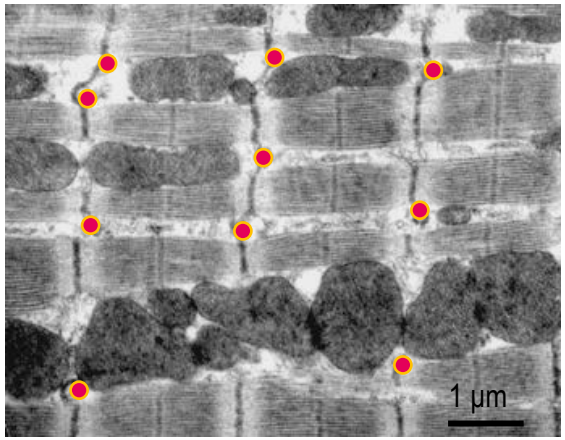
courtesy of M. Novotová, BMC SAS



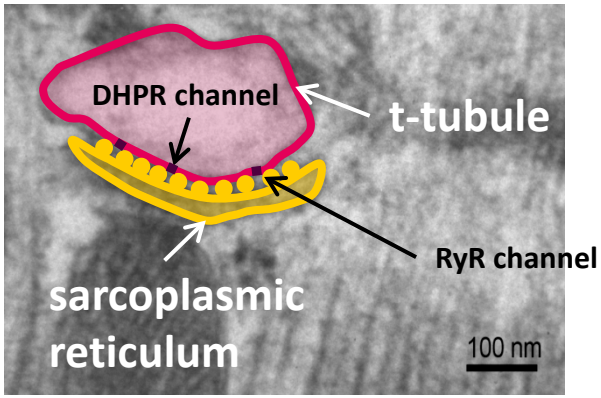


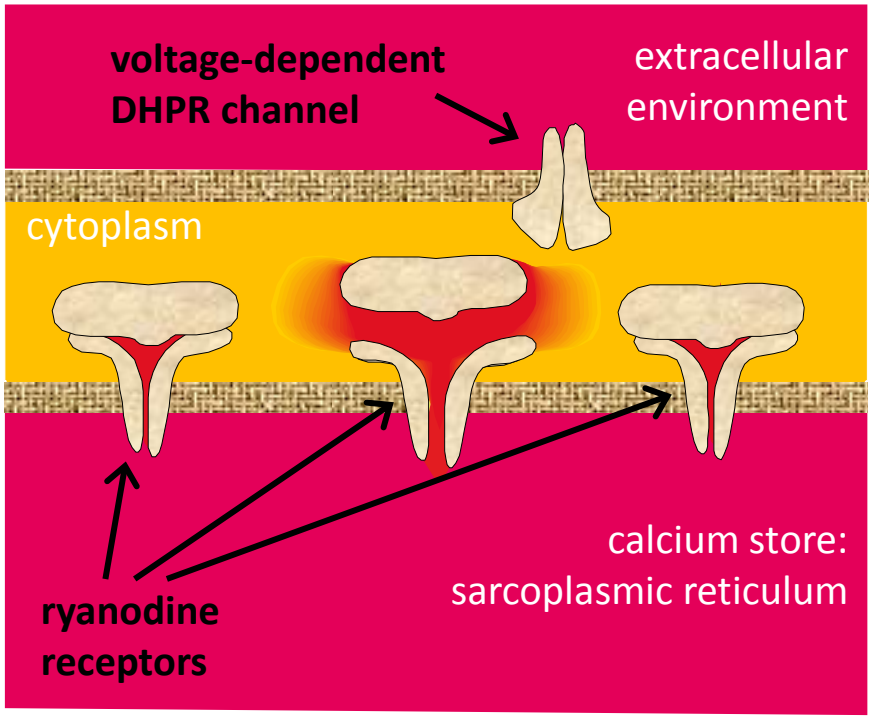
RyR2 supplies 60 – 70 % of calcium for heart contraction from calcium stores

Excitation-contraction coupling



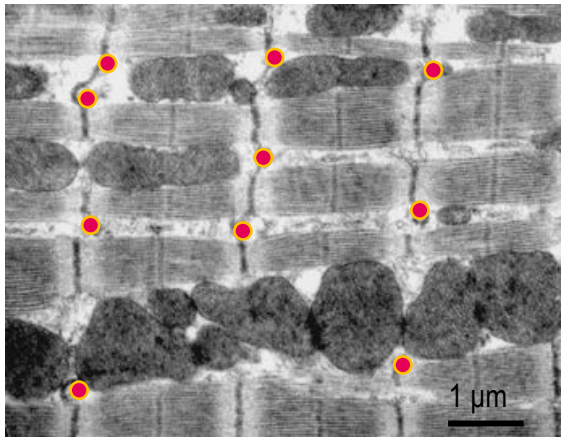
courtesy of M. Novotová, BMC SAS



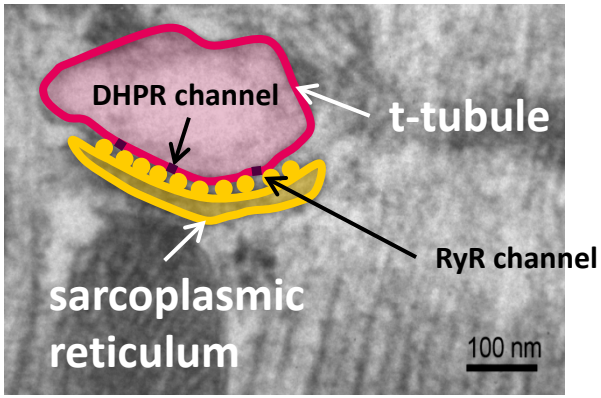


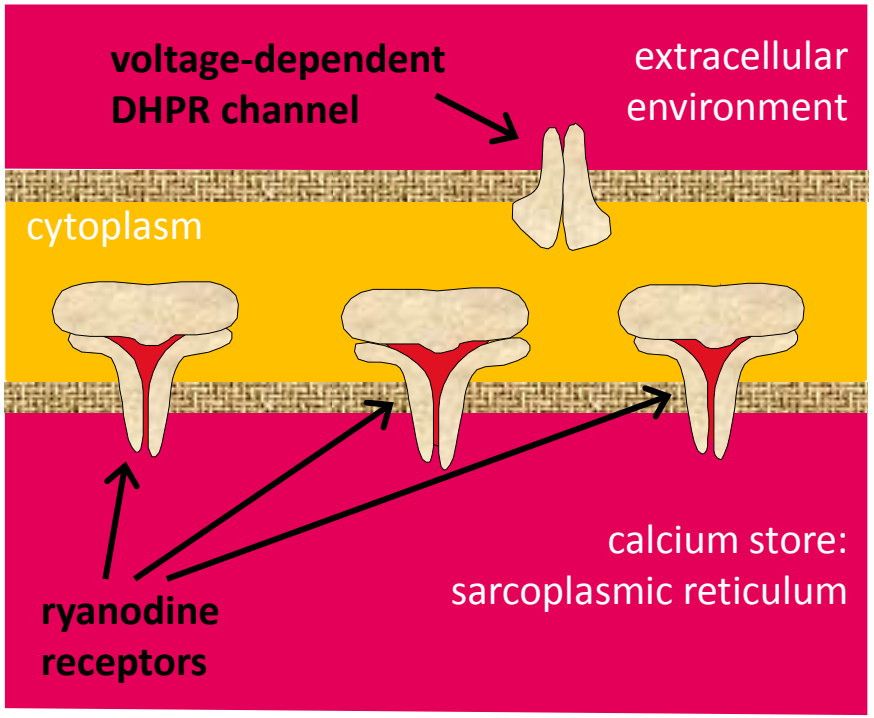
RyR2 supplies 60 – 70 % of calcium for heart contraction from calcium stores

Excitation-contraction coupling



courtesy of M. Novotová, BMC SAS

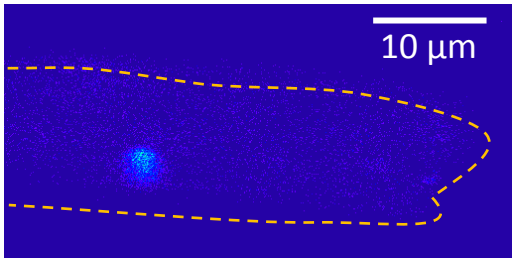




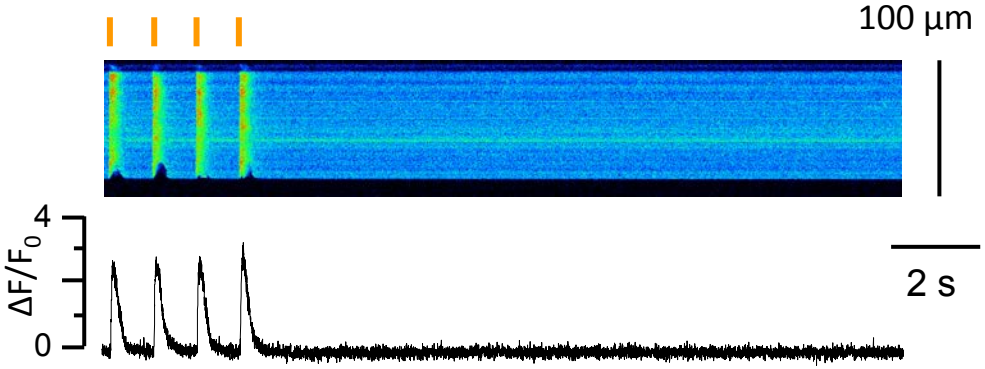
RyR2 supplies 60 – 70 % of calcium for heart contraction from calcium stores

Diastolic calcium release & arrhythmia

Healthy cardiac myocyte

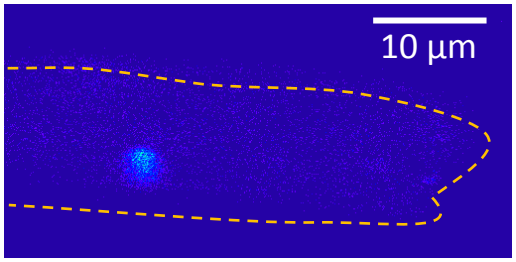


~ 20000 dyads / myocyte
~ 40 RyRs / dyad
$P_o \approx 0.0001$
~ 100 sparks / s / cell



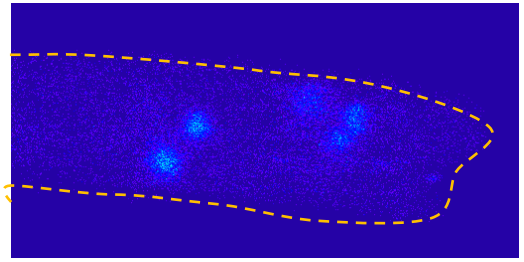
Diastolic calcium release & arrhythmia

Healthy cardiac myocyte

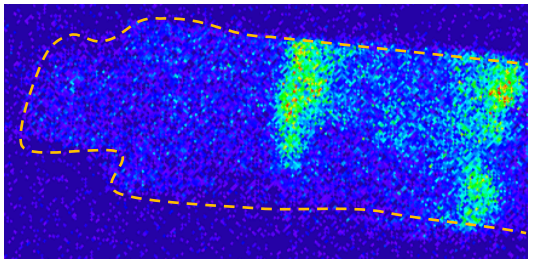


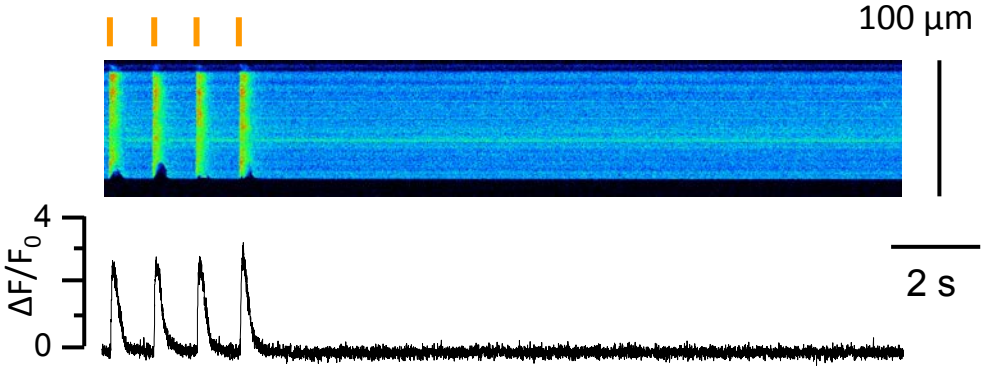
Diseased myocyte

minor dysfunction



arrhythmias





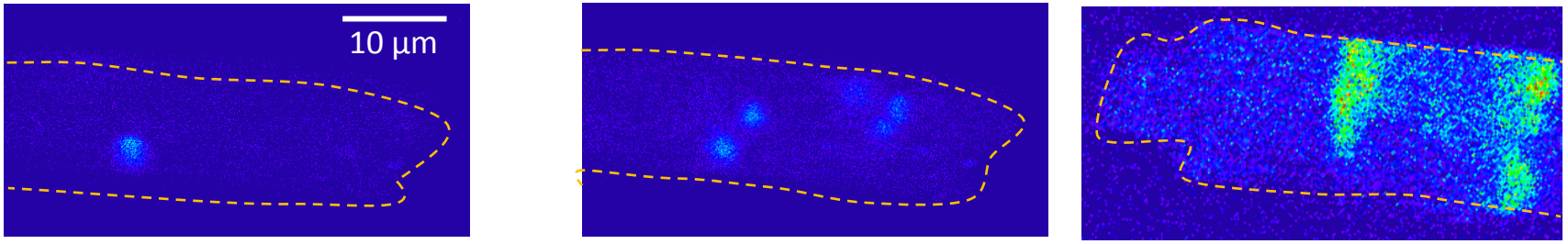
Diastolic calcium release & arrhythmia

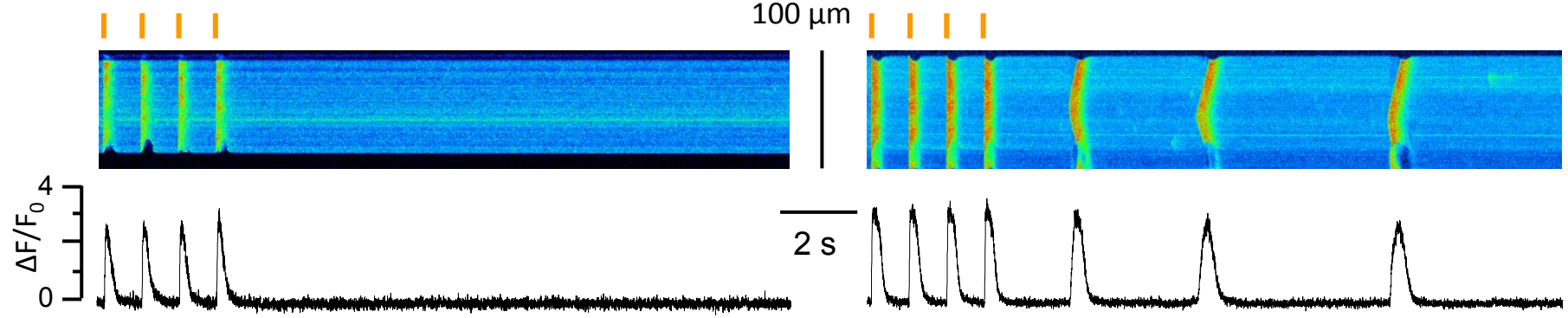
Healthy cardiac myocyte

Diseased myocyte

minor dysfunction

arrhythmias





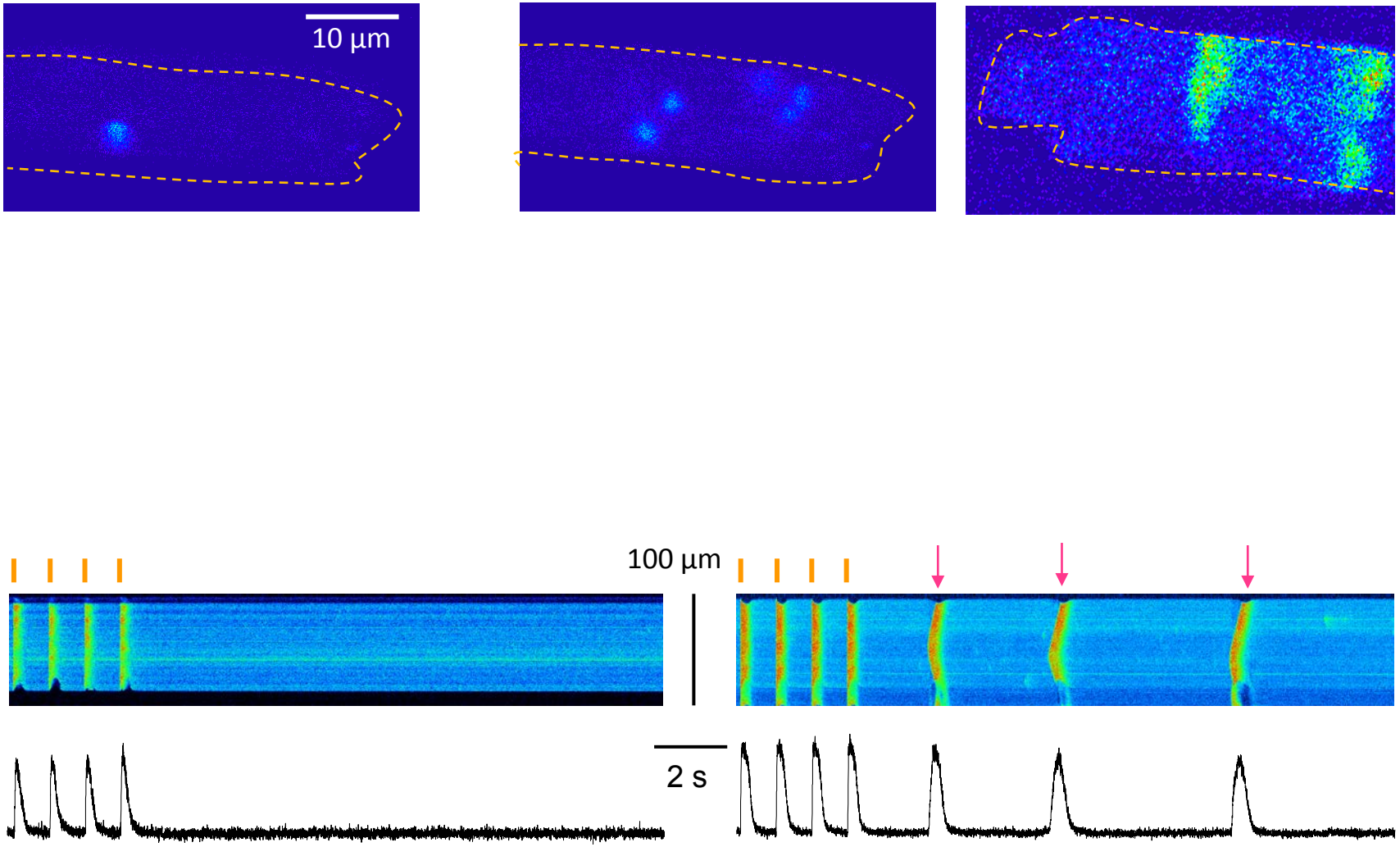
Diastolic calcium release & arrhythmia

Healthy cardiac myocyte

Diseased myocyte

minor dysfunction

arrhythmias



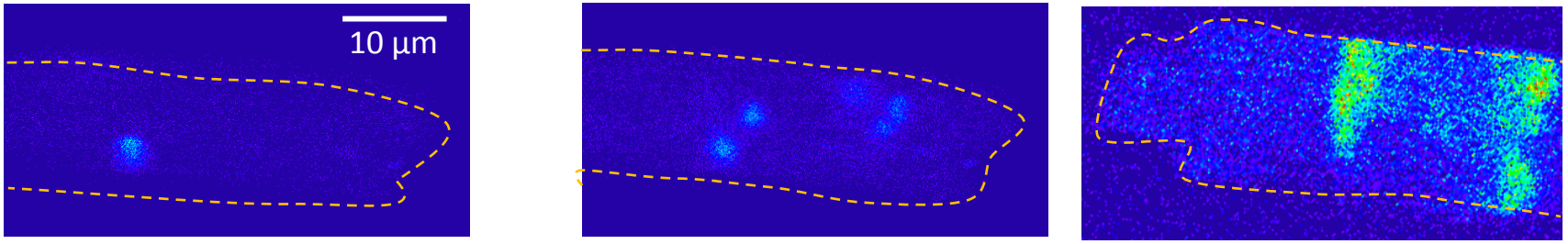
Diastolic calcium release & arrhythmia

Healthy cardiac myocyte

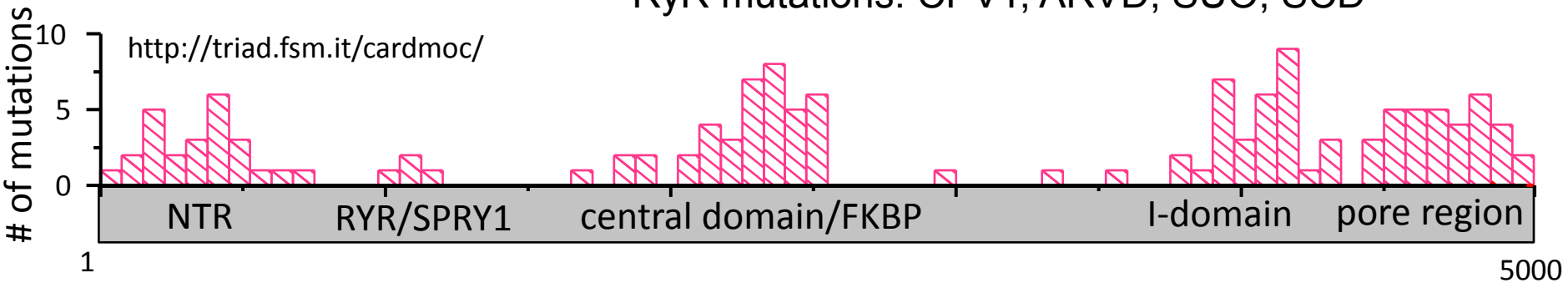
Diseased myocyte

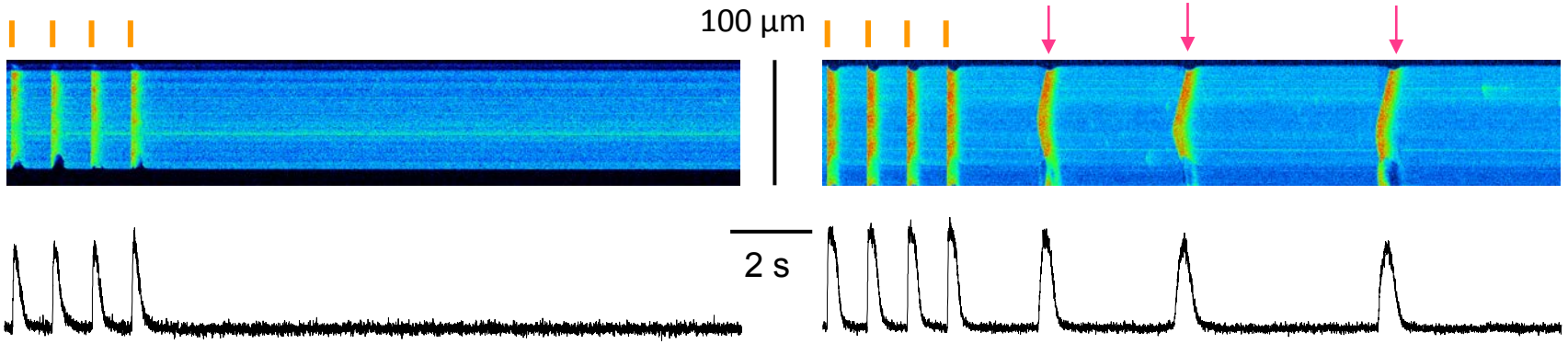
minor dysfunction

arrhythmias

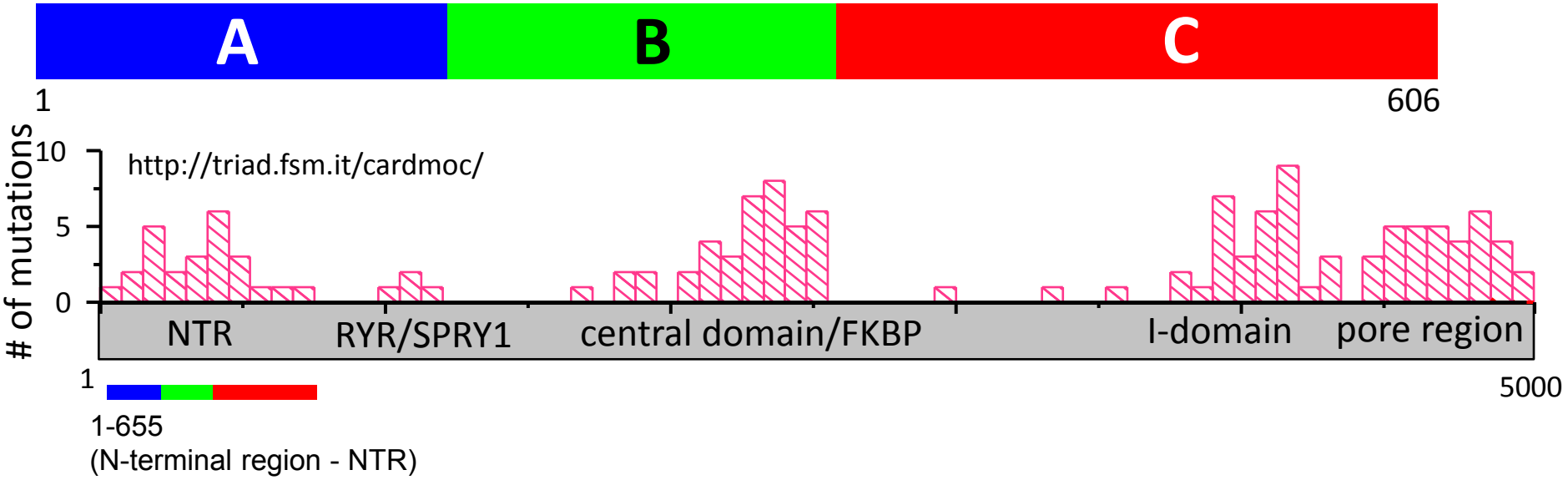


RyR mutations: CPVT, ARVD, SUO, SCD

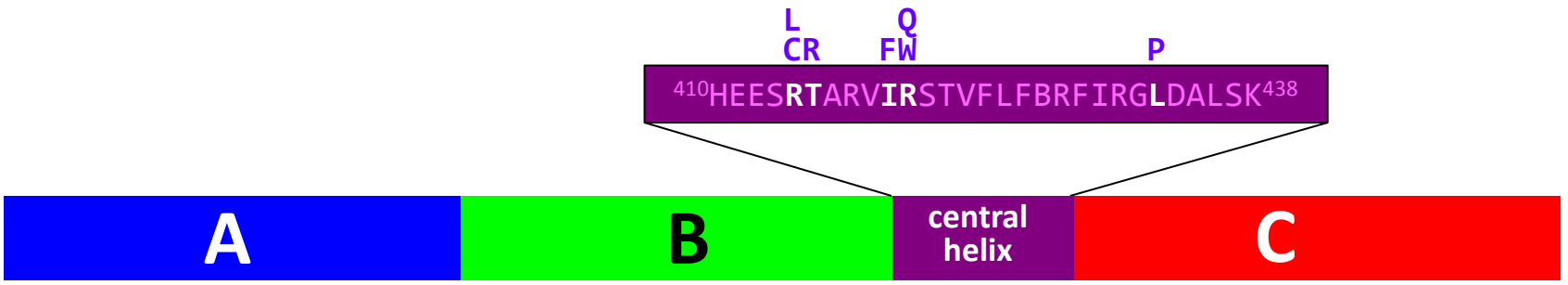


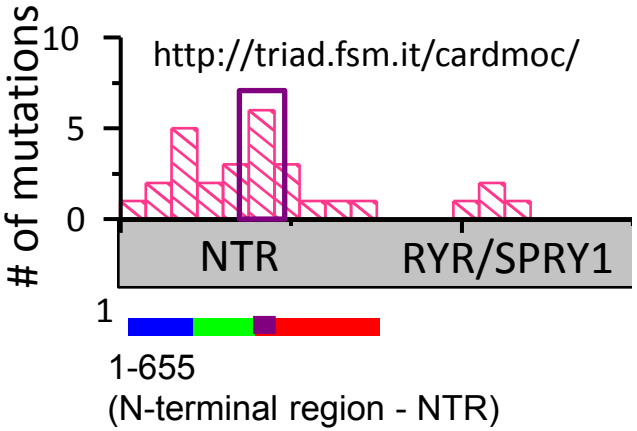


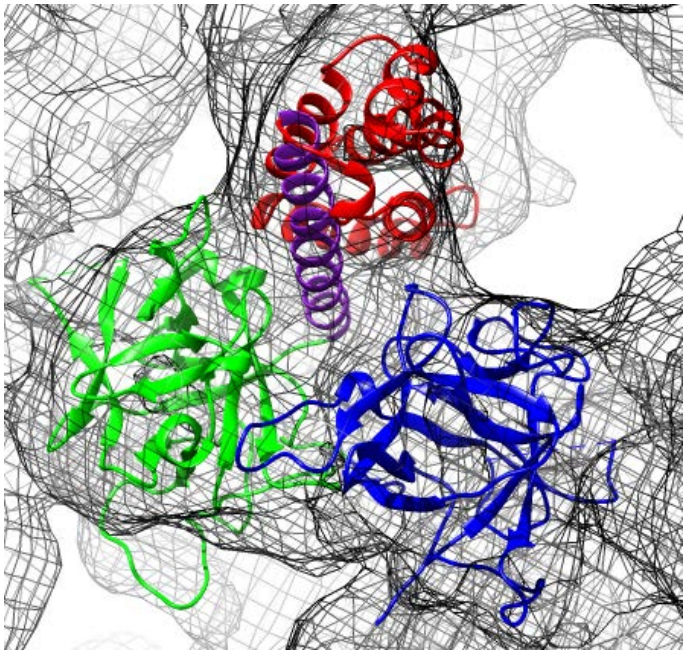
Diastolic calcium release & arrhythmia



Diastolic calcium release & arrhythmia



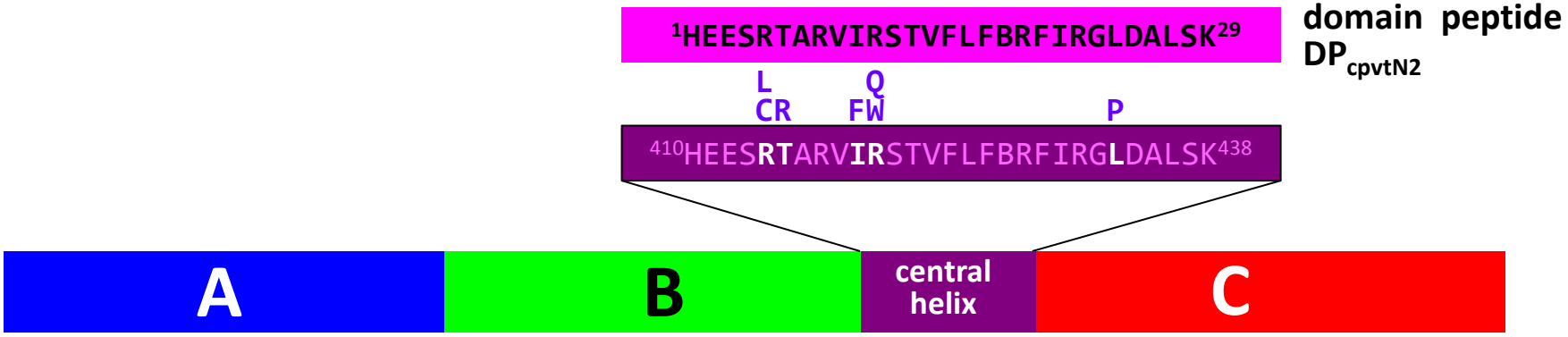


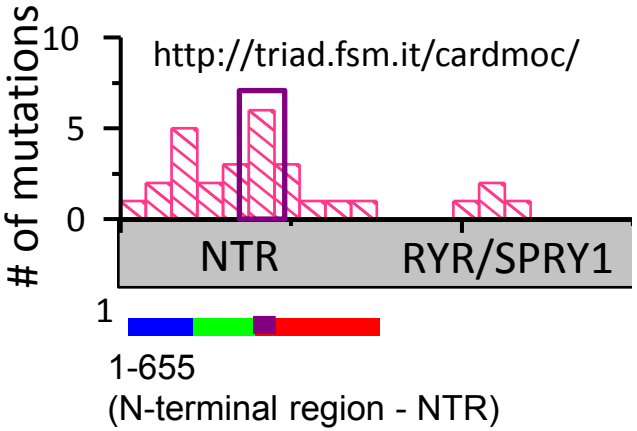


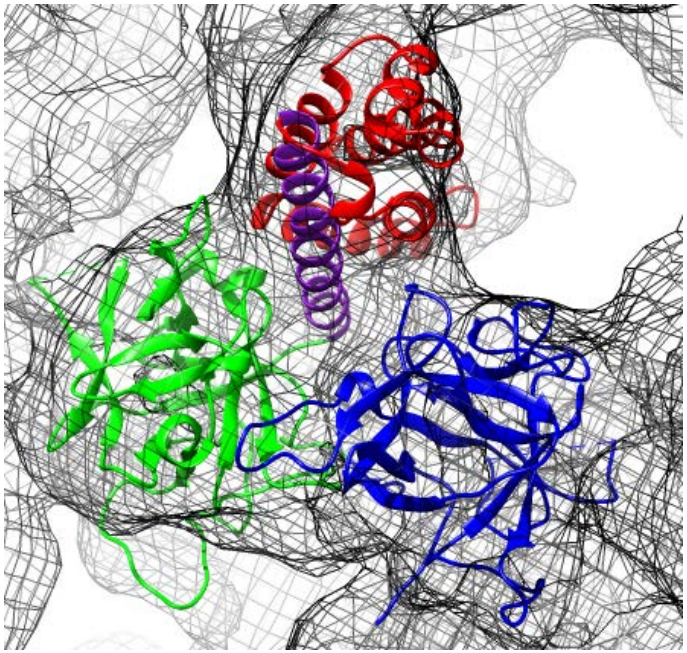
EMD 1606: Samso *et al.*, *PLoS Biol.* 7: e85, 2009

4JKQ: Borko *et al.*, *Acta Crystallogr D* 70: 2897-2912, 2014

Diastolic calcium release & arrhythmia



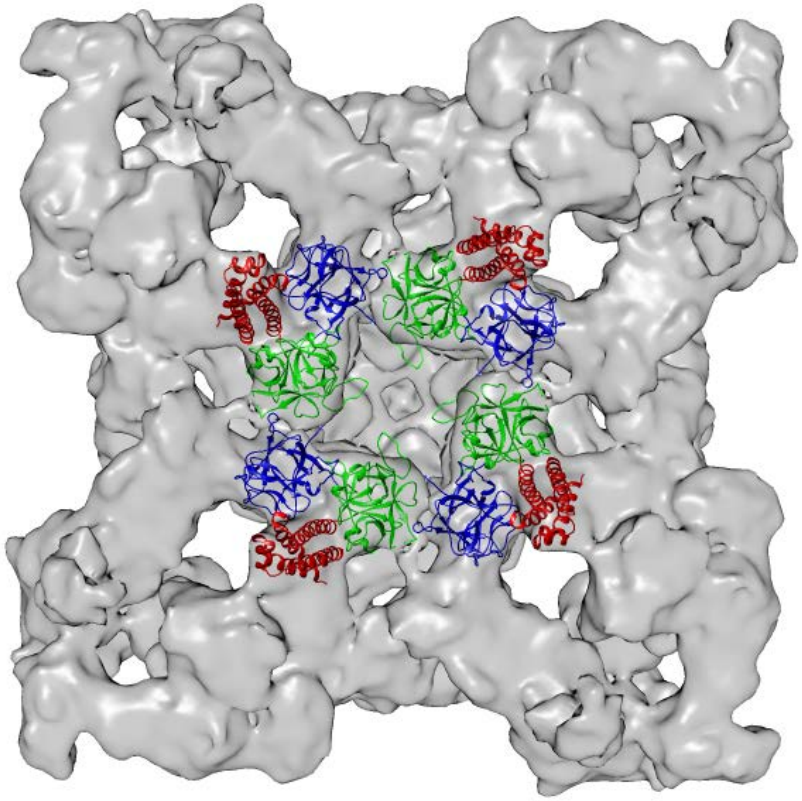




EMD 1606: Samso *et al.*, *PLoS Biol.* 7: e85, 2009

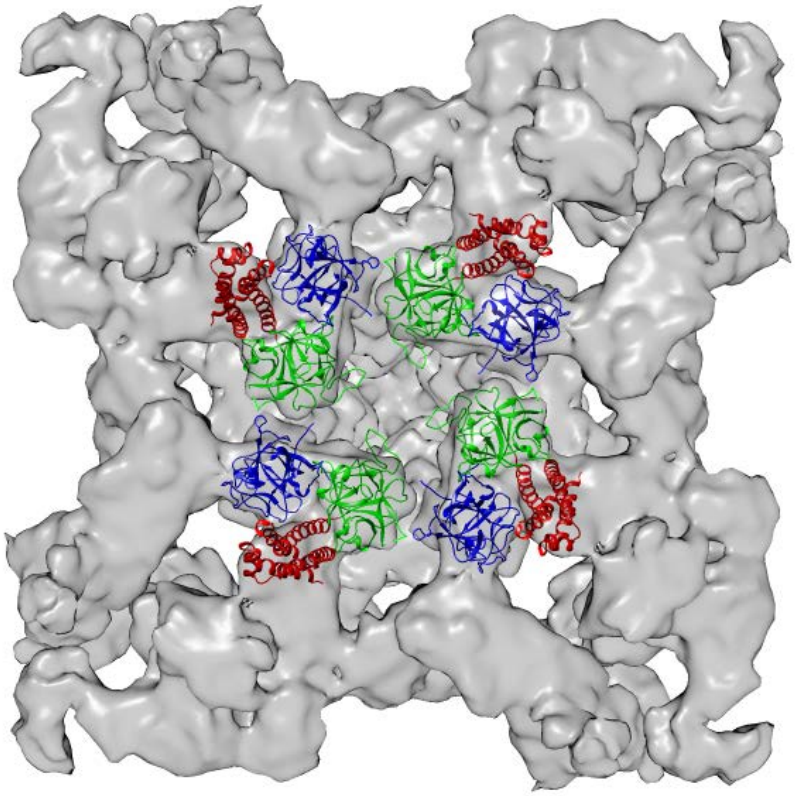
4JKQ: Borko *et al.*, *Acta Crystallogr D* 70: 2897-2912, 2014

Large-scale movements upon opening



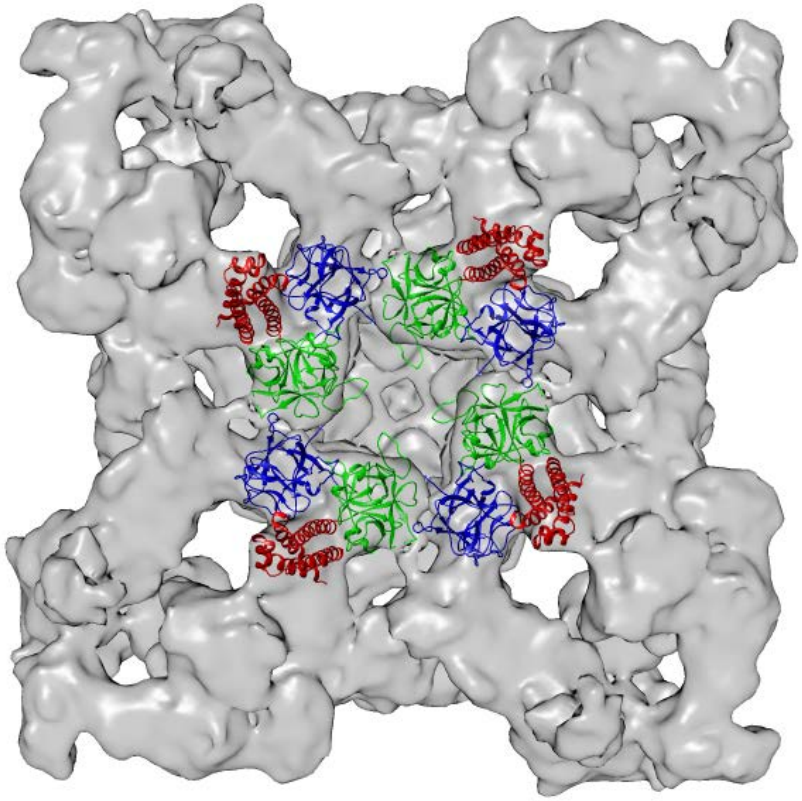
EMD 1606, 1607: Samso *et al.*, *PLoS Biol.* 7: e85, 2009
4JKQ: Borko *et al.*, *Acta Crystallogr D* 70: 2897-2912, 2014
5GUG, 5X9Z : Hamada *et al.*, *Proc. Natl. Acad. Sci. U.S.A.* 114: 4661-4666, 2017

Large-scale movements upon opening



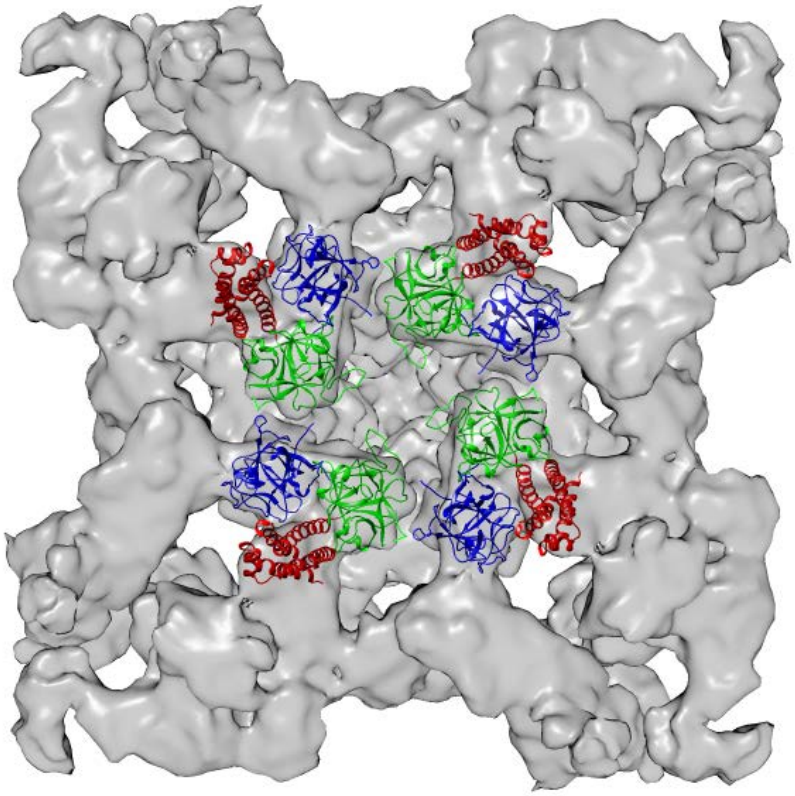
EMD 1606, 1607: Samso *et al.*, *PLoS Biol.* 7: e85, 2009
4JKQ: Borko *et al.*, *Acta Crystallogr D* 70: 2897-2912, 2014
5GUG, 5X9Z : Hamada *et al.*, *Proc. Natl. Acad. Sci. U.S.A.* 114: 4661-4666, 2017

Large-scale movements upon opening



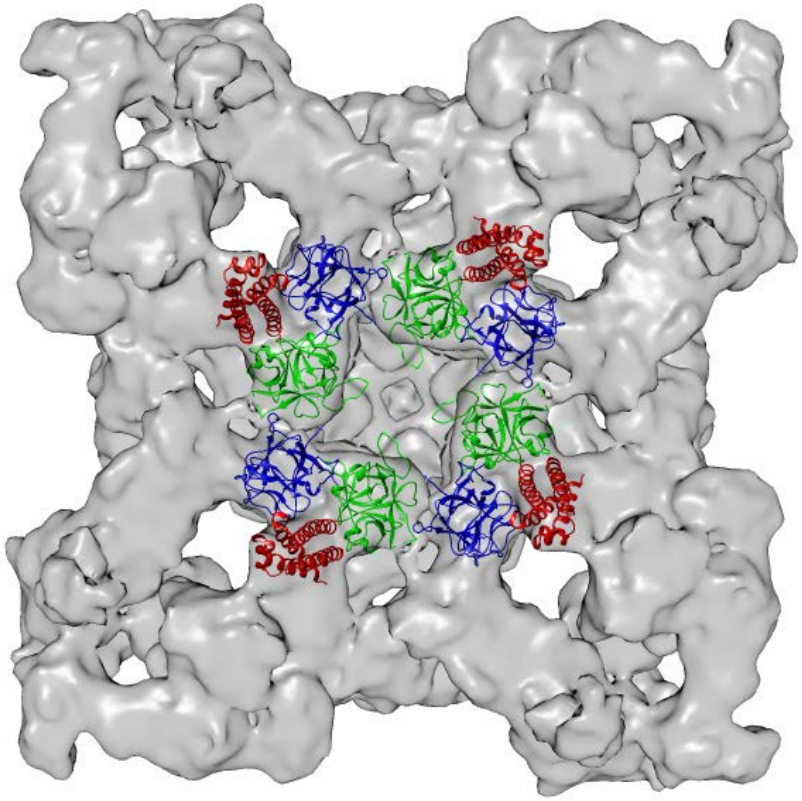
EMD 1606, 1607: Samso *et al.*, *PLoS Biol.* 7: e85, 2009
4JKQ: Borko *et al.*, *Acta Crystallogr D* 70: 2897-2912, 2014
5GUG, 5X9Z : Hamada *et al.*, *Proc. Natl. Acad. Sci. U.S.A.* 114: 4661-4666, 2017

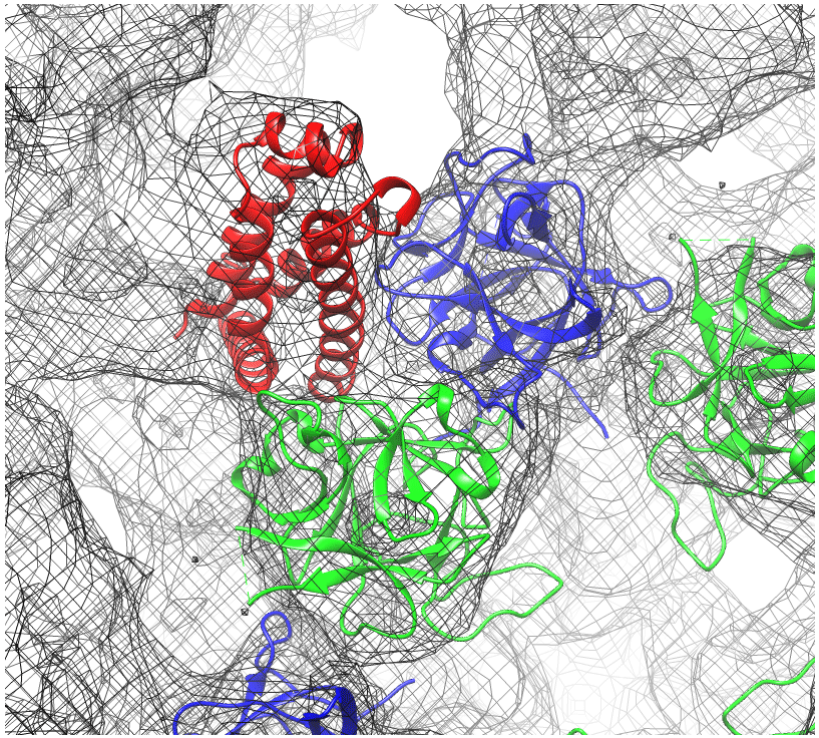
Large-scale movements upon opening



EMD 1606, 1607: Samso *et al.*, *PLoS Biol.* 7: e85, 2009
4JKQ: Borko *et al.*, *Acta Crystallogr D* 70: 2897-2912, 2014
5GUG, 5X9Z : Hamada *et al.*, *Proc. Natl. Acad. Sci. U.S.A.* 114: 4661-4666, 2017

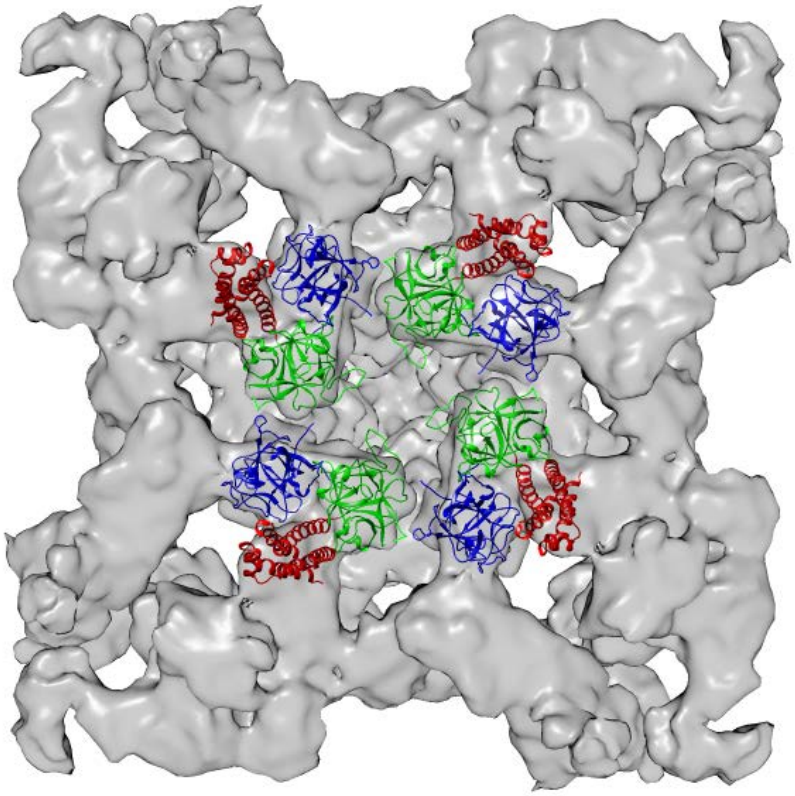
Large-scale movements upon opening

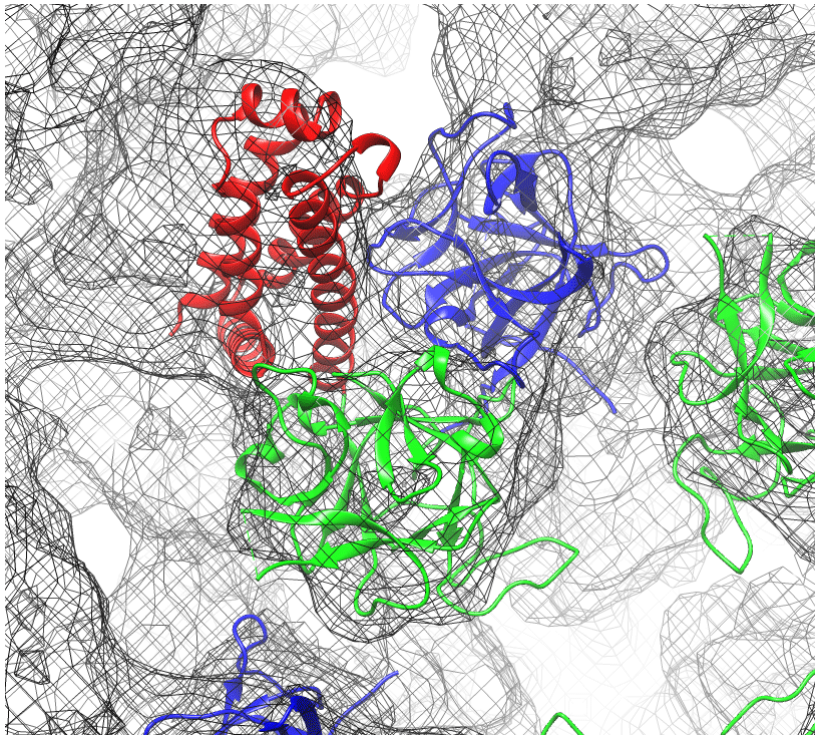




EMD 1606, 1607: Samso *et al.*, *PLoS Biol.* 7: e85, 2009
4JKQ: Borko *et al.*, *Acta Crystallogr D* 70: 2897-2912, 2014
5GUG, 5X9Z : Hamada *et al.*, *Proc. Natl. Acad. Sci. U.S.A.* 114: 4661-4666, 2017

Large-scale movements upon opening



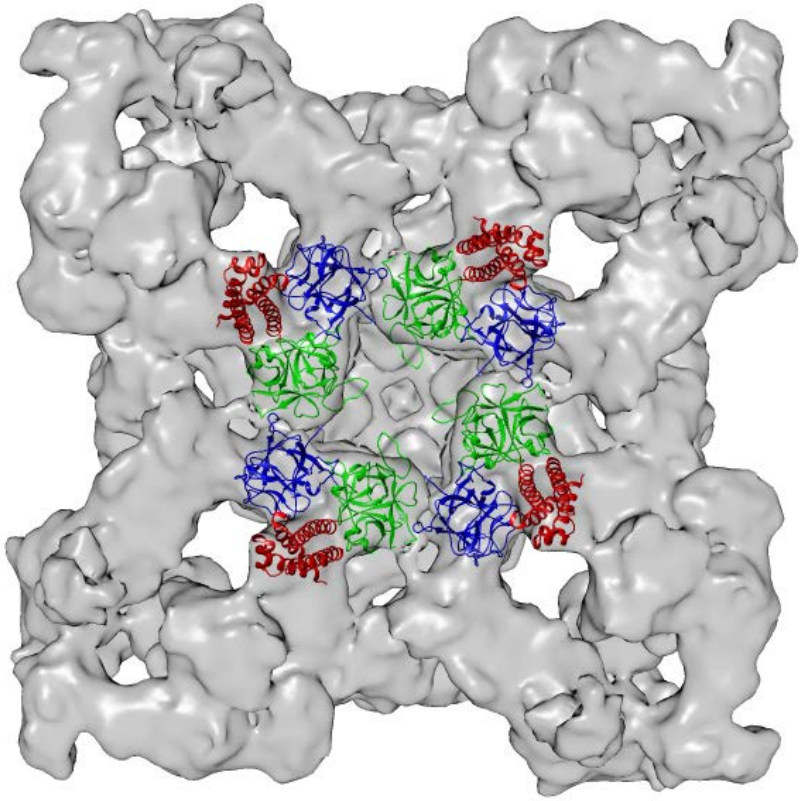


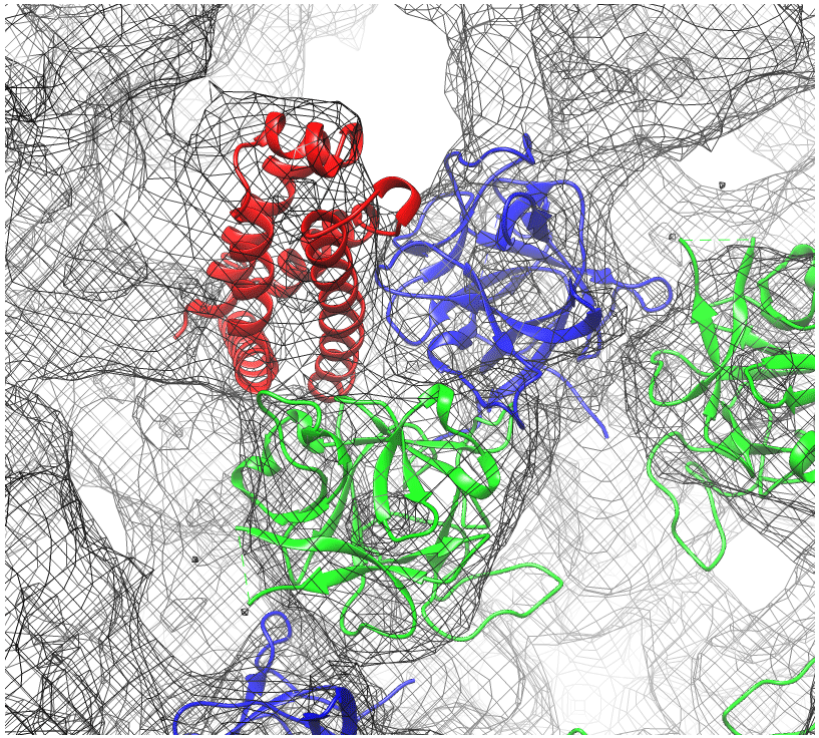
EMD 1606, 1607: Samso *et al.*, *PLoS Biol.* 7: e85, 2009

4JKQ: Borko *et al.*, *Acta Crystallogr D* 70: 2897-2912, 2014

5GUG, 5X9Z : Hamada *et al.*, *Proc. Natl. Acad. Sci. U.S.A.* 114: 4661-4666, 2017

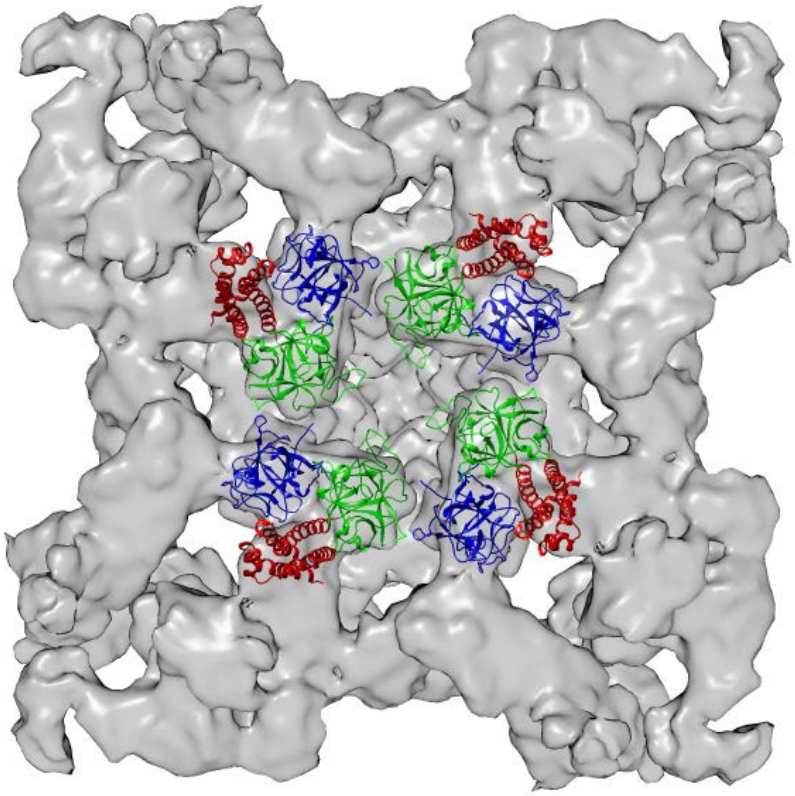
Large-scale movements upon opening

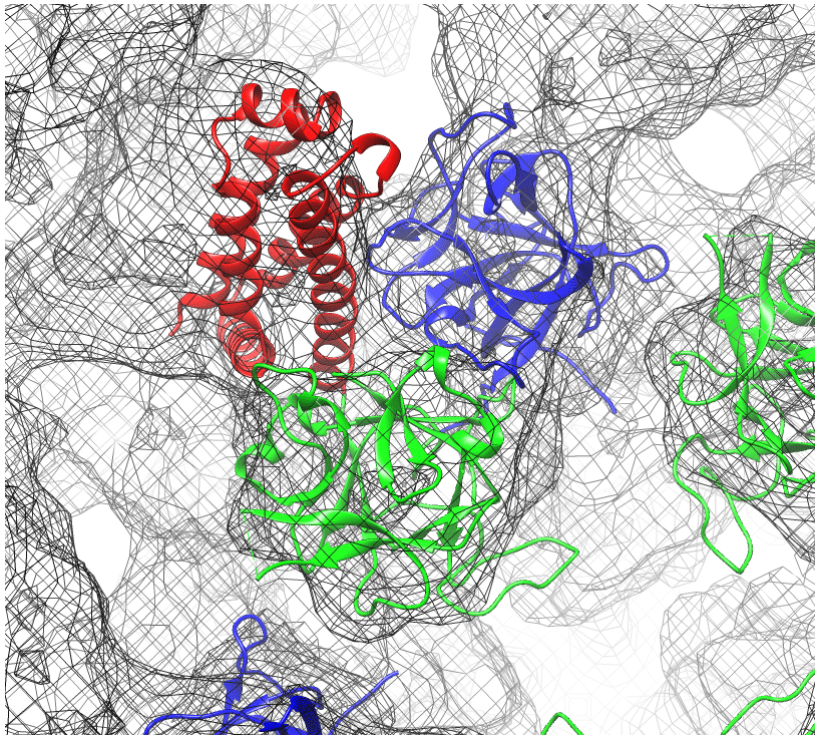




EMD 1606, 1607: Samso *et al.*, *PLoS Biol.* 7: e85, 2009
4JKQ: Borko *et al.*, *Acta Crystallogr D* 70: 2897-2912, 2014
5GUG, 5X9Z : Hamada *et al.*, *Proc. Natl. Acad. Sci. U.S.A.* 114: 4661-4666, 2017

Large-scale movements upon opening



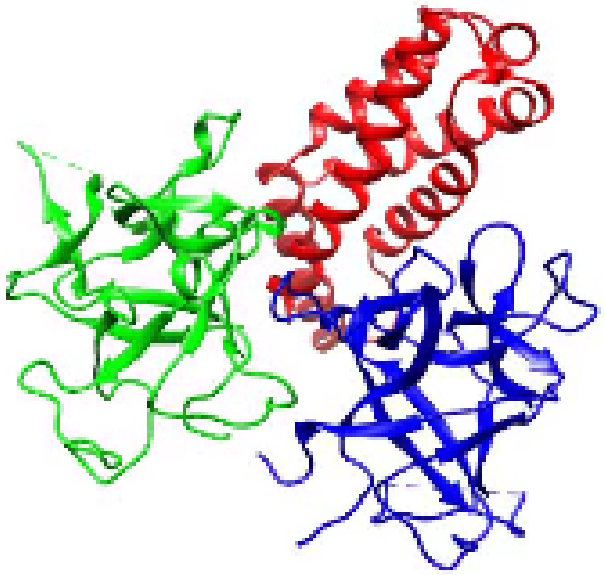


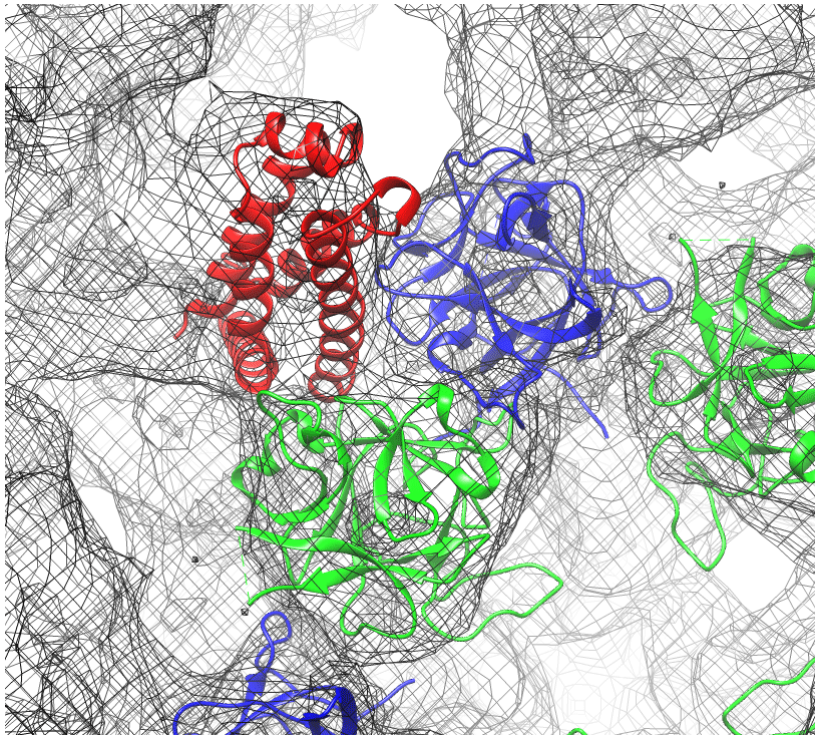
EMD 1606, 1607: Samso *et al.*, *PLoS Biol.* 7: e85, 2009

4JKQ: Borko *et al.*, *Acta Crystallogr D* 70: 2897-2912, 2014

5GUG, 5X9Z : Hamada *et al.*, *Proc. Natl. Acad. Sci. U.S.A.* 114: 4661-4666, 2017

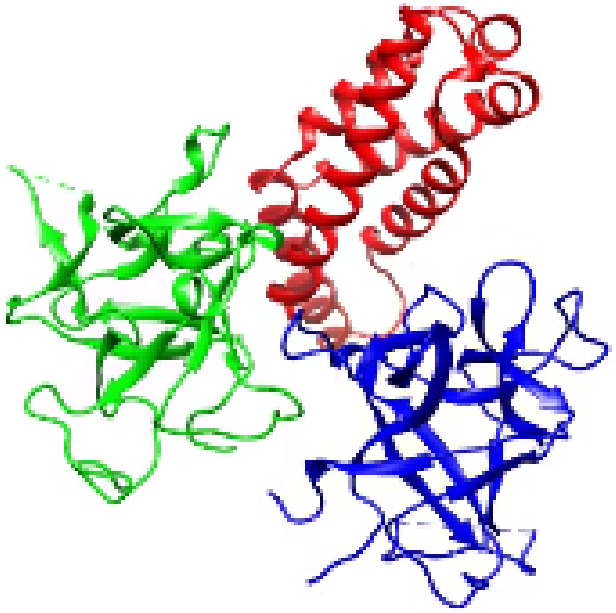
Large-scale movements upon opening

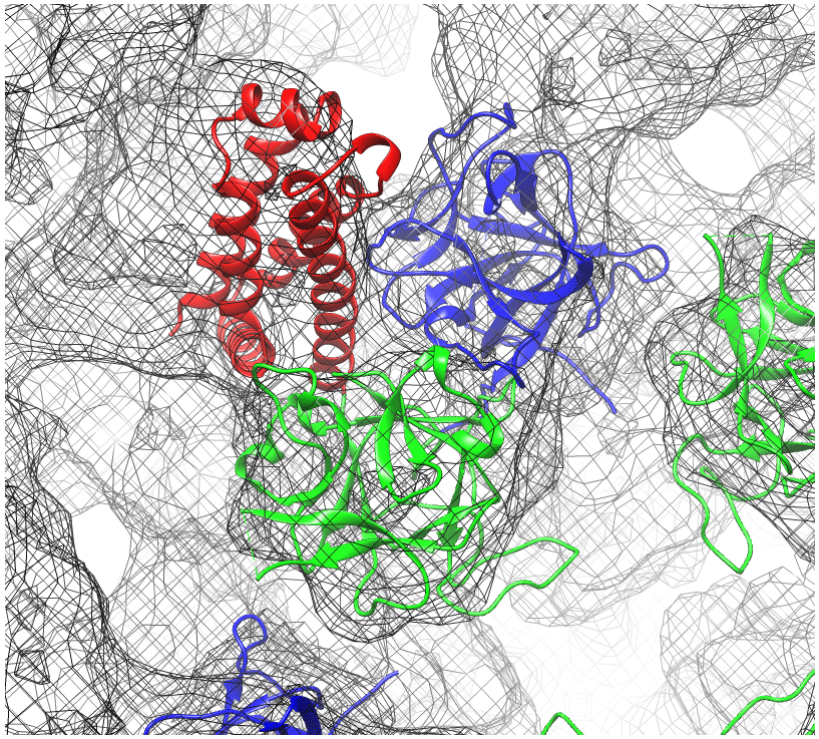




EMD 1606, 1607: Samso *et al.*, *PLoS Biol.* 7: e85, 2009
4JKQ: Borko *et al.*, *Acta Crystallogr D* 70: 2897-2912, 2014
5GUG, 5X9Z : Hamada *et al.*, *Proc. Natl. Acad. Sci. U.S.A.* 114: 4661-4666, 2017

Large-scale movements upon opening



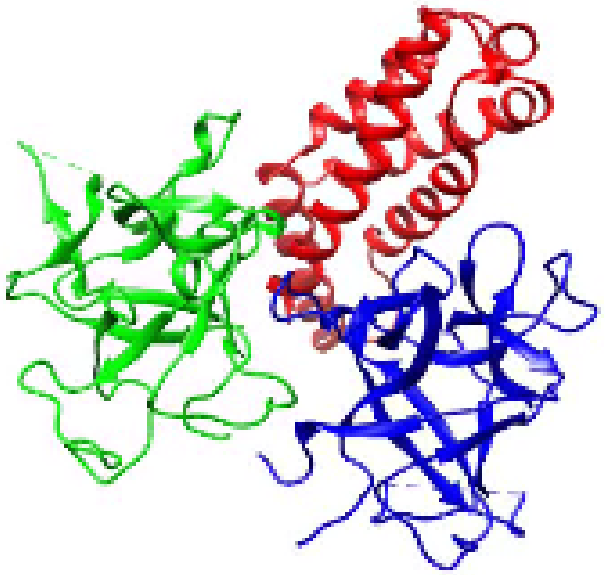


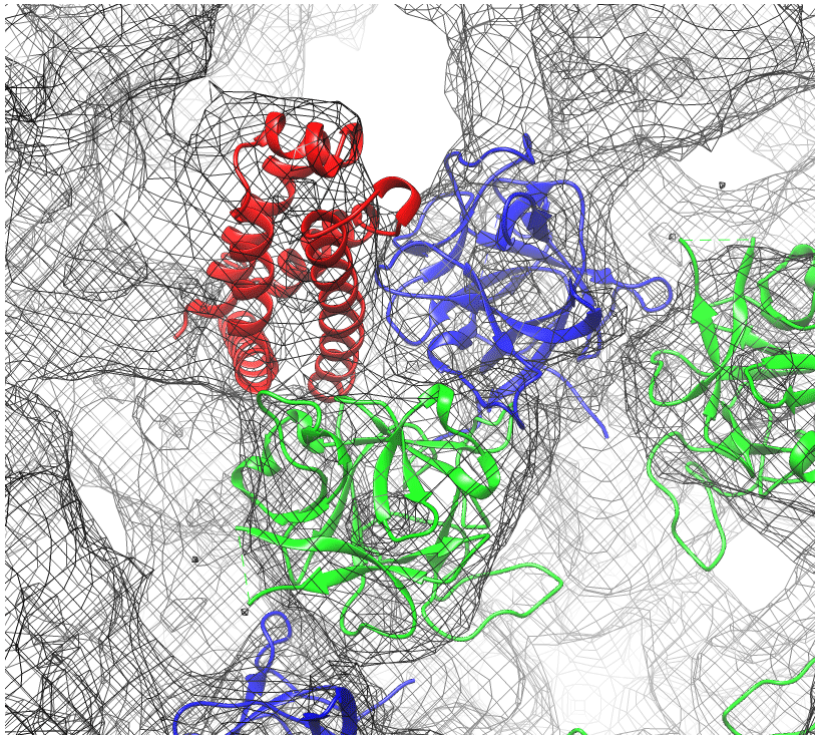
EMD 1606, 1607: Samso *et al.*, *PLoS Biol.* 7: e85, 2009

4JKQ: Borko *et al.*, *Acta Crystallogr D* 70: 2897-2912, 2014

5GUG, 5X9Z : Hamada *et al.*, *Proc. Natl. Acad. Sci. U.S.A.* 114: 4661-4666, 2017

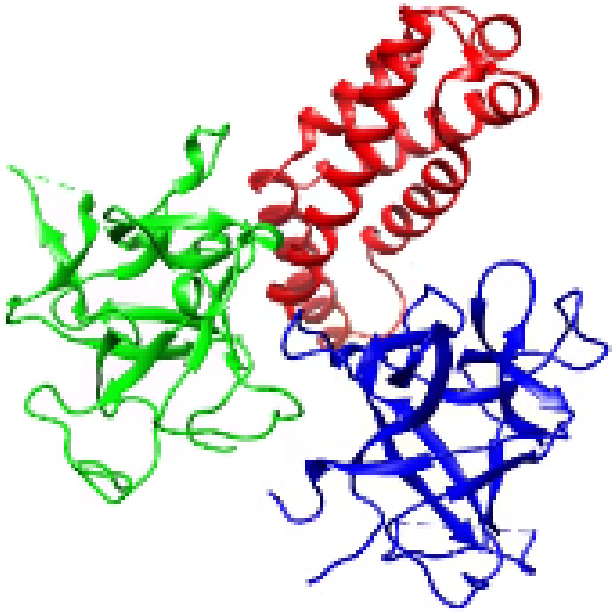
Large-scale movements upon opening

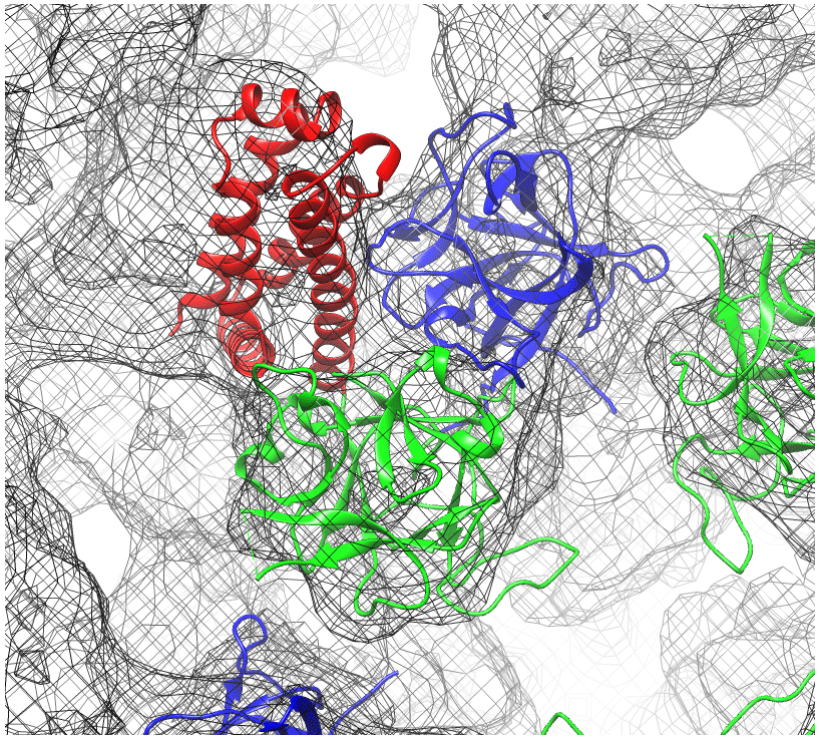




EMD 1606, 1607: Samso *et al.*, *PLoS Biol.* 7: e85, 2009
4JKQ: Borko *et al.*, *Acta Crystallogr D* 70: 2897-2912, 2014
5GUG, 5X9Z : Hamada *et al.*, *Proc. Natl. Acad. Sci. U.S.A.* 114: 4661-4666, 2017

Large-scale movements upon opening



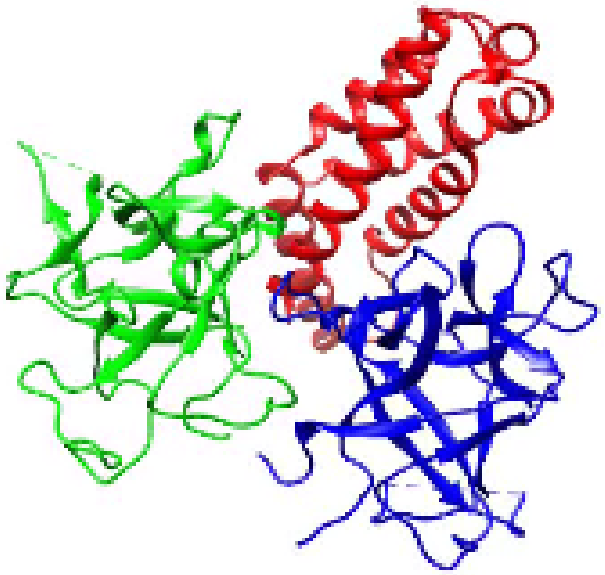


EMD 1606, 1607: Samso *et al.*, *PLoS Biol.* 7: e85, 2009

4JKQ: Borko *et al.*, *Acta Crystallogr D* 70: 2897-2912, 2014

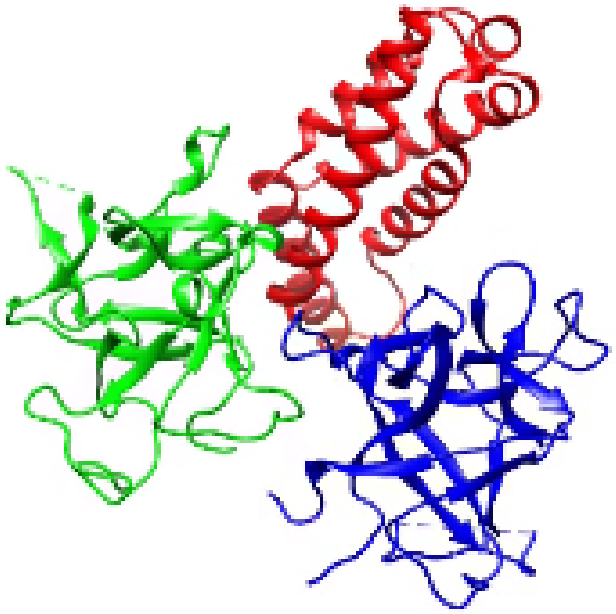
5GUG, 5X9Z : Hamada *et al.*, *Proc. Natl. Acad. Sci. U.S.A.* 114: 4661-4666, 2017

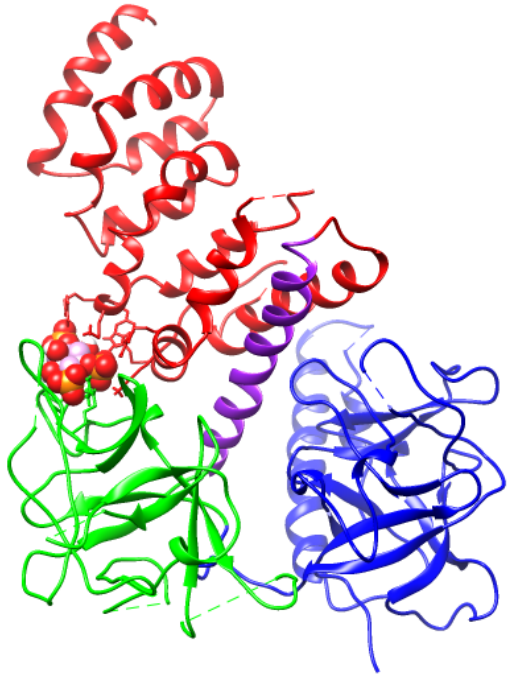
Large-scale movements upon opening



EMD 1606, 1607: Samso *et al.*, *PLoS Biol.* 7: e85, 2009
4JKQ: Borko *et al.*, *Acta Crystallogr D* 70: 2897-2912, 2014
5GUG, 5X9Z : Hamada *et al.*, *Proc. Natl. Acad. Sci. U.S.A.* 114: 4661-4666, 2017

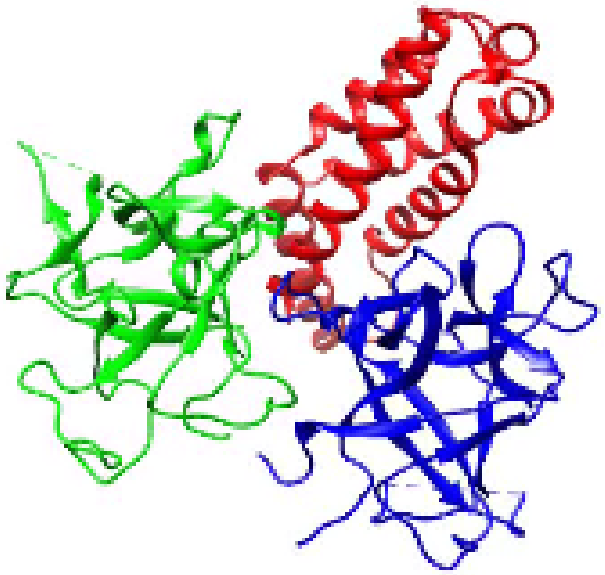
Large-scale movements upon opening





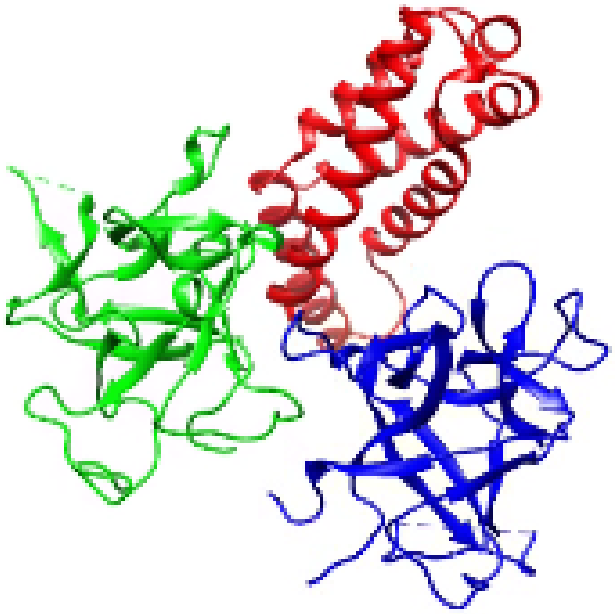
EMD 1606, 1607: Samso *et al.*, *PLoS Biol.* 7: e85, 2009
4JKQ: Borko *et al.*, *Acta Crystallogr D* 70: 2897-2912, 2014
5GUG, 5X9Z : Hamada *et al.*, *Proc. Natl. Acad. Sci. U.S.A.* 114: 4661-4666, 2017

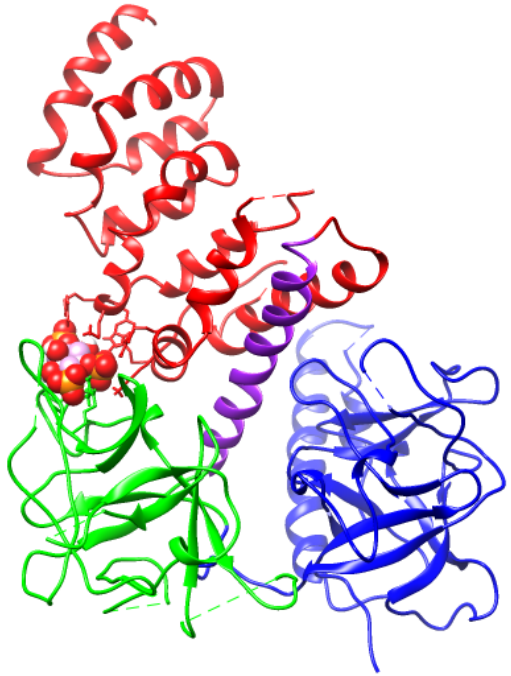
Large-scale movements upon opening



EMD 1606, 1607: Samso *et al.*, *PLoS Biol.* 7: e85, 2009
4JKQ: Borko *et al.*, *Acta Crystallogr D* 70: 2897-2912, 2014
5GUG, 5X9Z : Hamada *et al.*, *Proc. Natl. Acad. Sci. U.S.A.* 114: 4661-4666, 2017

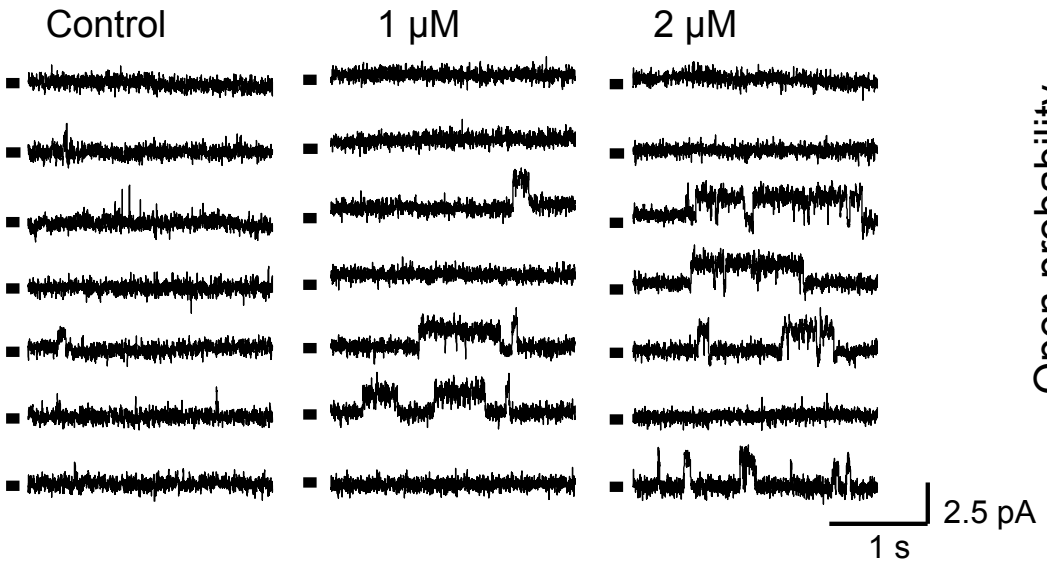
Large-scale movements upon opening

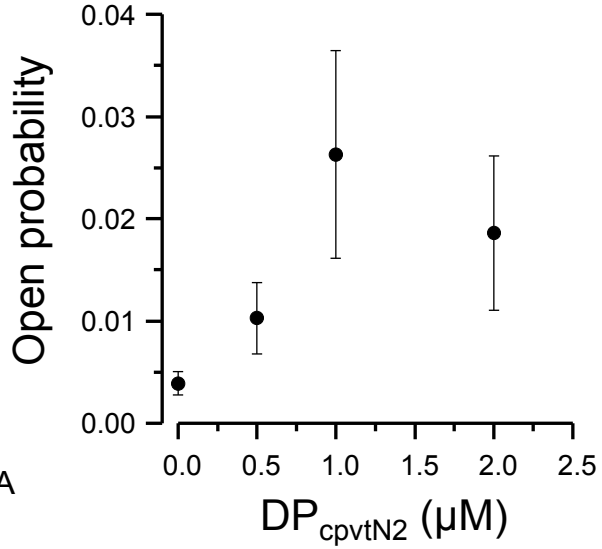




EMD 1606, 1607: Samso *et al.*, *PLoS Biol.* 7: e85, 2009
4JKQ: Borko *et al.*, *Acta Crystallogr D* 70: 2897-2912, 2014
5GUG, 5X9Z : Hamada *et al.*, *Proc. Natl. Acad. Sci. U.S.A.* 114: 4661-4666, 2017

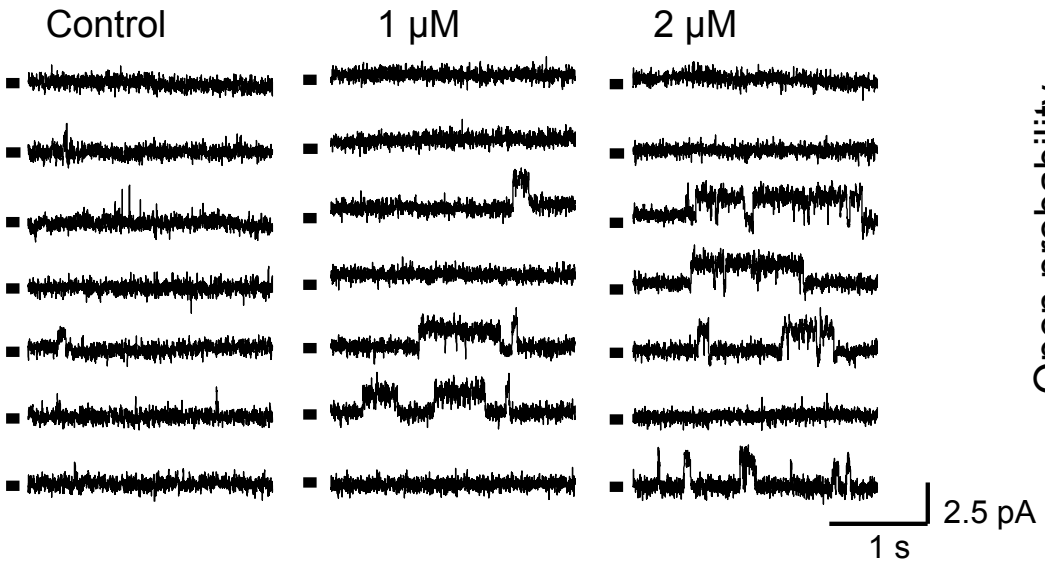
RyR2 activation by DP_{cpvtN2}

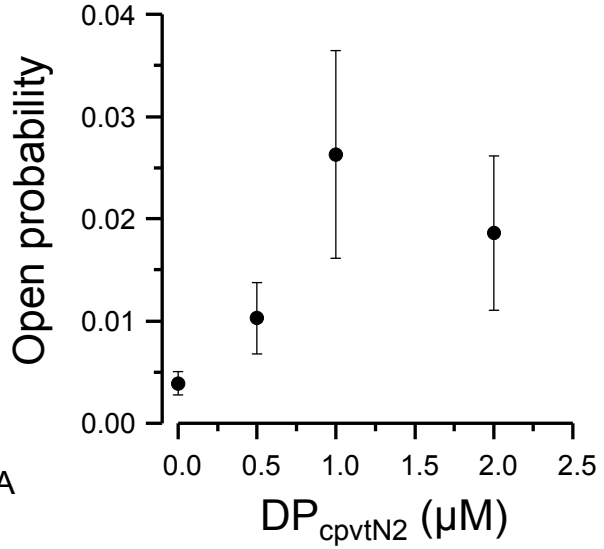


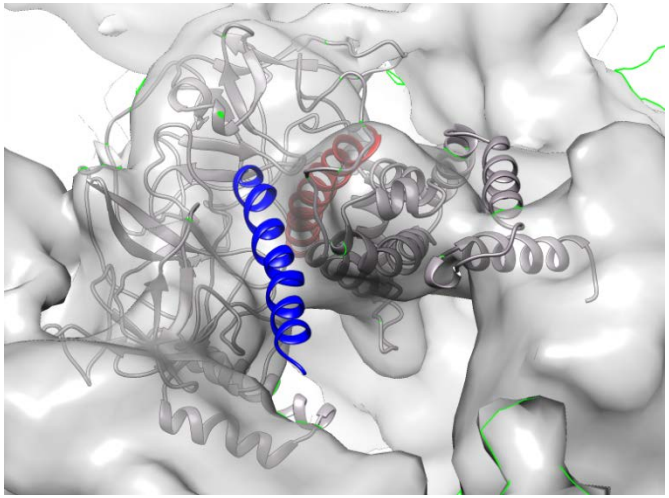


Faltinova *et al*., *Front Physiol.* 8: 443, 2017

RyR2 activation by DP_{cpvtN2}

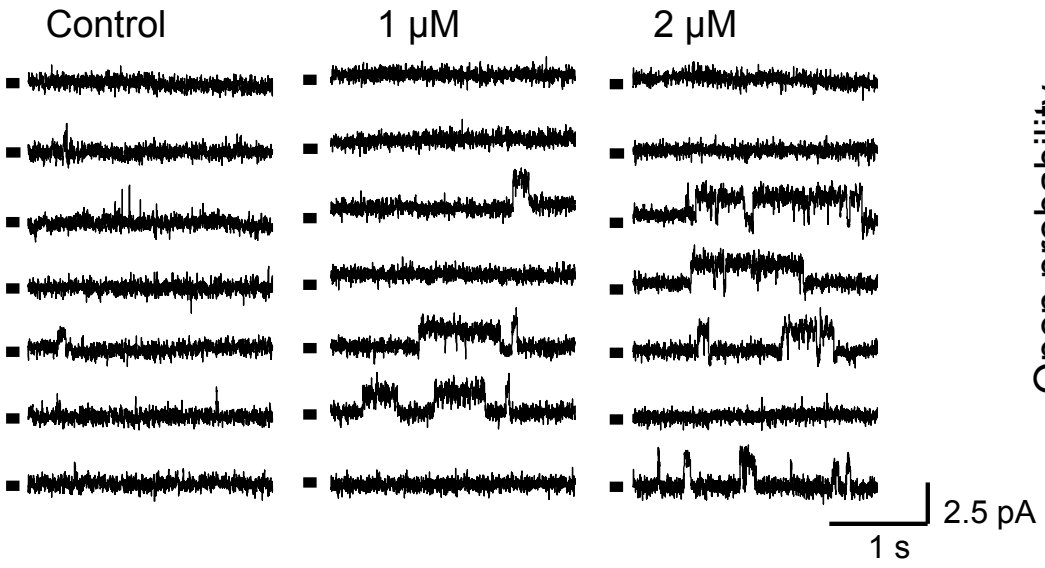


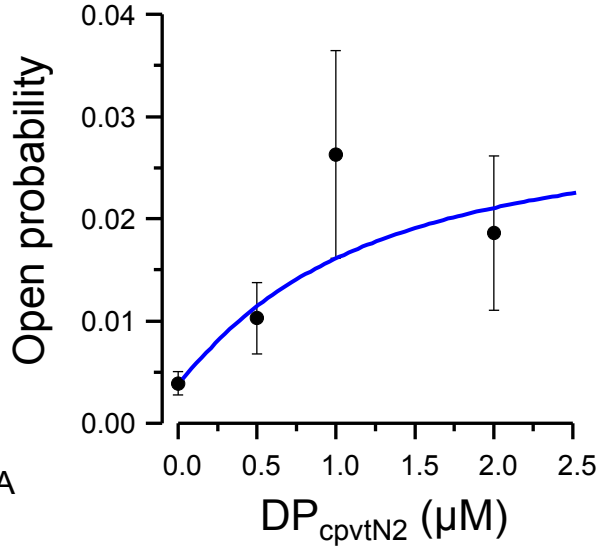




Faltinova *et al.*, *Front Physiol.* 8: 443, 2017

RyR2 activation by DP_{cpvtN2}

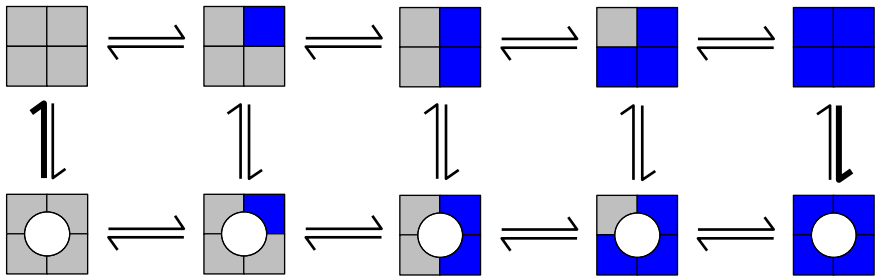


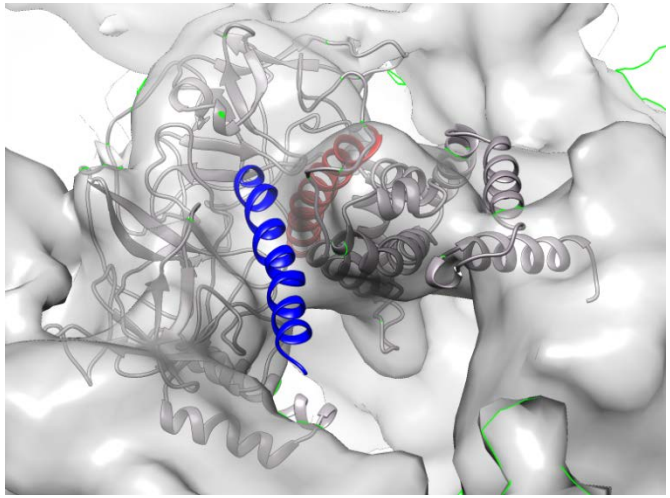


$P_O = (c_p + f_p K_p)^4/((c_p + f_p K_L)^4 + f_p^4(c_p + K_p)^4 (1/P_{O0} - 1))$

$K_L = 0.3$ μM; $f_L = 0.6$; $P_o^{max} = 0.03$

affinity increases upon opening or ligand binding

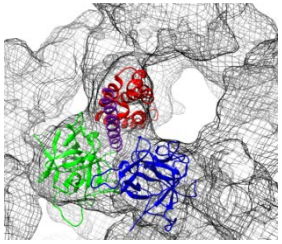




Faltinova *et al.*, *Front Physiol.* 8: 443, 2017

Construction of models

Chimera: visualization, interface to modelling software
SITUS: fitting structures into maps
Modeller: completion of loops
I-TASSER: construction of de novo models
GRAMM-X: construction of complexes
Molecular Modelling Toolkit: energy minimization

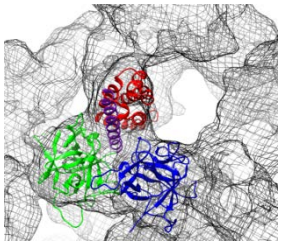


EMD-1606 4JKQ

X-ray structure

Construction of models

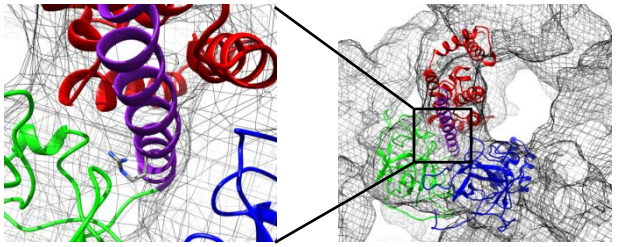
Chimera: visualization, interface to modelling software
SITUS: fitting structures into maps
Modeller: completion of loops
I-TASSER: construction of de novo models
GRAMM-X: construction of complexes
Molecular Modelling Toolkit: energy minimization



EMD-1606 4JKQ

X-ray structure

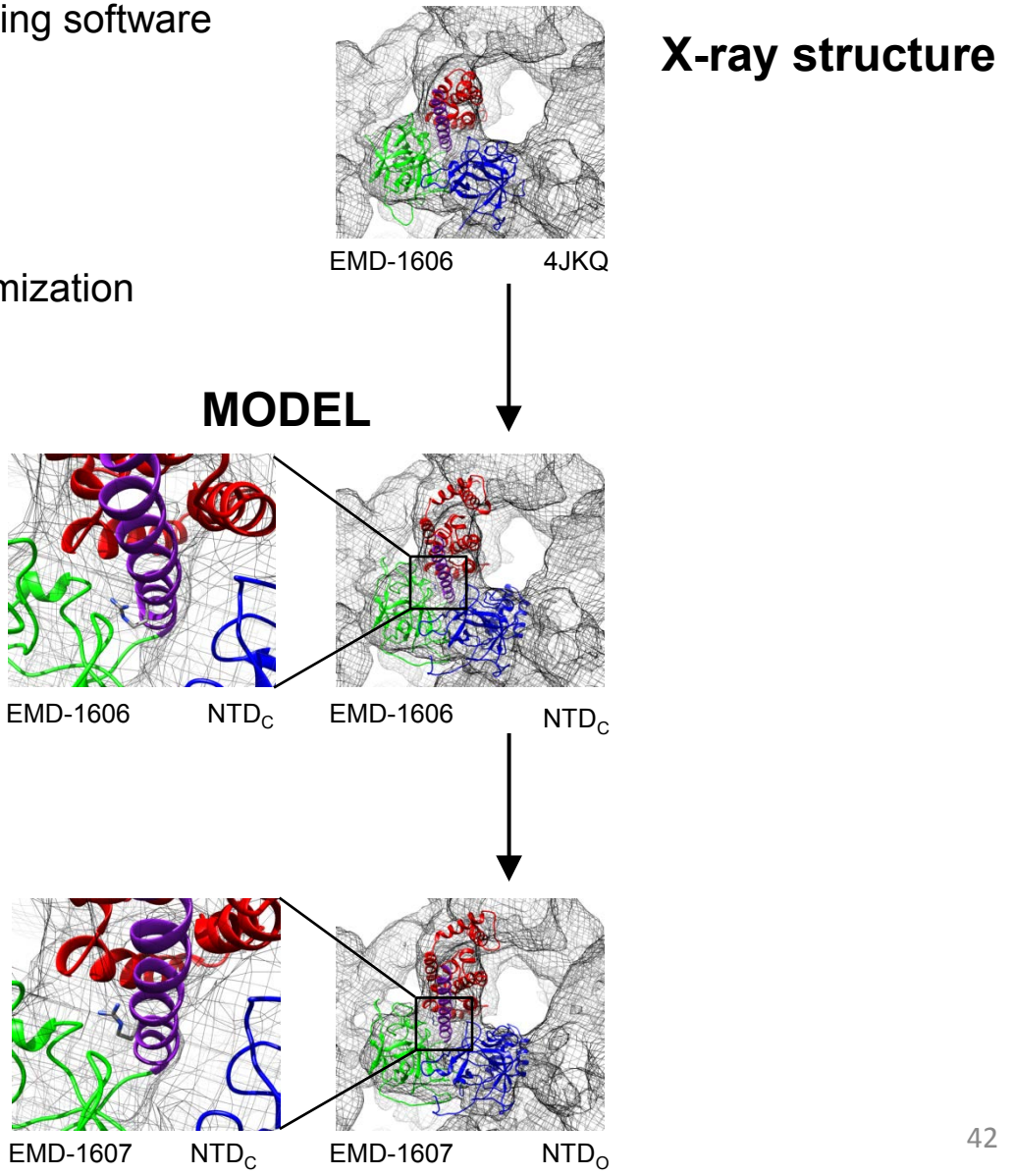
MODEL



EMD-1606 NTD_C EMD-1606 NTD_C

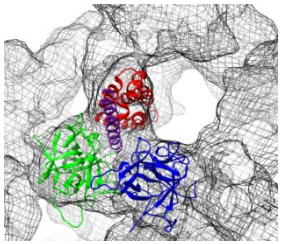
Construction of models

Chimera: visualization, interface to modelling software
SITUS: fitting structures into maps
Modeller: completion of loops
I-TASSER: construction of de novo models
GRAMM-X: construction of complexes
Molecular Modelling Toolkit: energy minimization



Construction of models

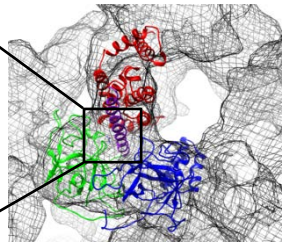
Chimera: visualization, interface to modelling software
SITUS: fitting structures into maps
Modeller: completion of loops
I-TASSER: construction of de novo models
GRAMM-X: construction of complexes
Molecular Modelling Toolkit: energy minimization



EMD-1606 4JKQ

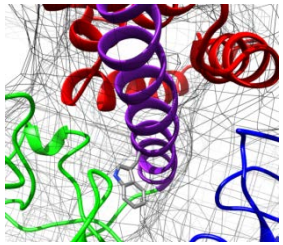
X-ray structure

MODEL

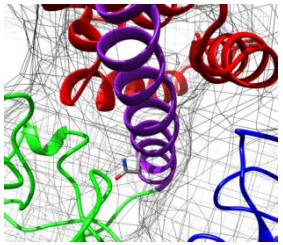


EMD-1606 NTD_C

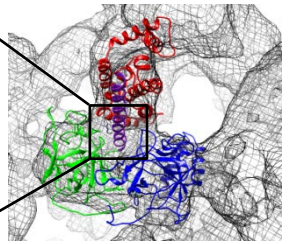
MUTATIONS



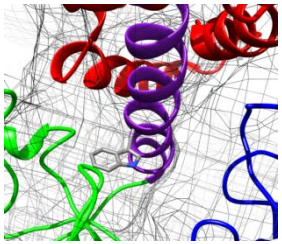
EMD-1606 $R420W_C$



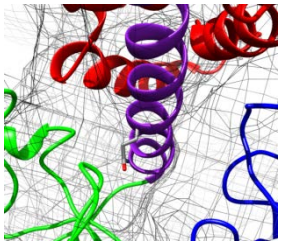
EMD-1606 $R420Q_C$



EMD-1607 NTD_O



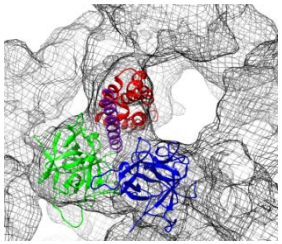
EMD-1607 $R420W_O$



EMD-1607 $R420Q_O$

Construction of models

Chimera: visualization, interface to modelling software
SITUS: fitting structures into maps
Modeller: completion of loops
I-TASSER: construction of de novo models
GRAMM-X: construction of complexes
Molecular Modelling Toolkit: energy minimization



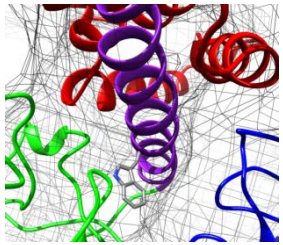
EMD-1606 4JKQ

X-ray structure

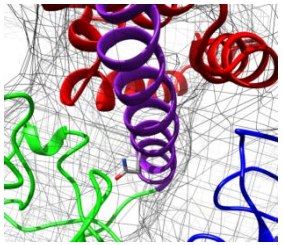
MUTATIONS

MODEL

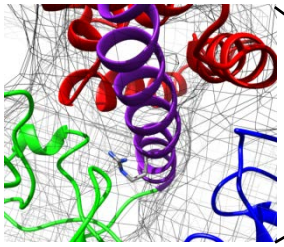
COMPLEX
MODEL + peptide



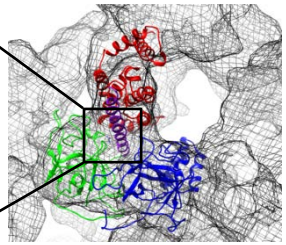
EMD-1606 $R420W_C$



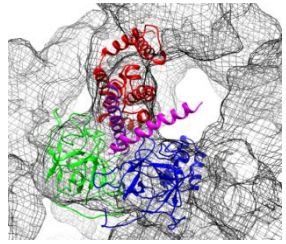
EMD-1606 $R420Q_C$



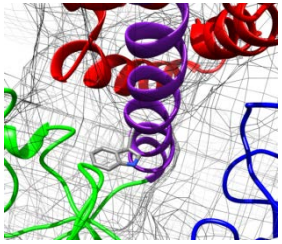
EMD-1606 NTD_C



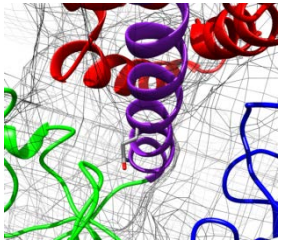
EMD-1606 NTD_C



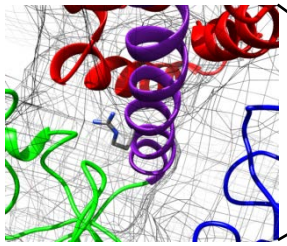
EMD-1606 $complex_C$



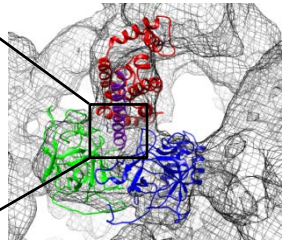
EMD-1607 $R420W_O$



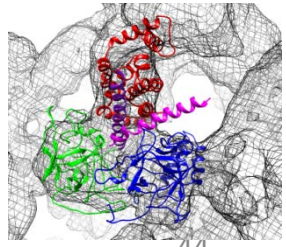
EMD-1607 $R420Q_O$



EMD-1607 NTD_C

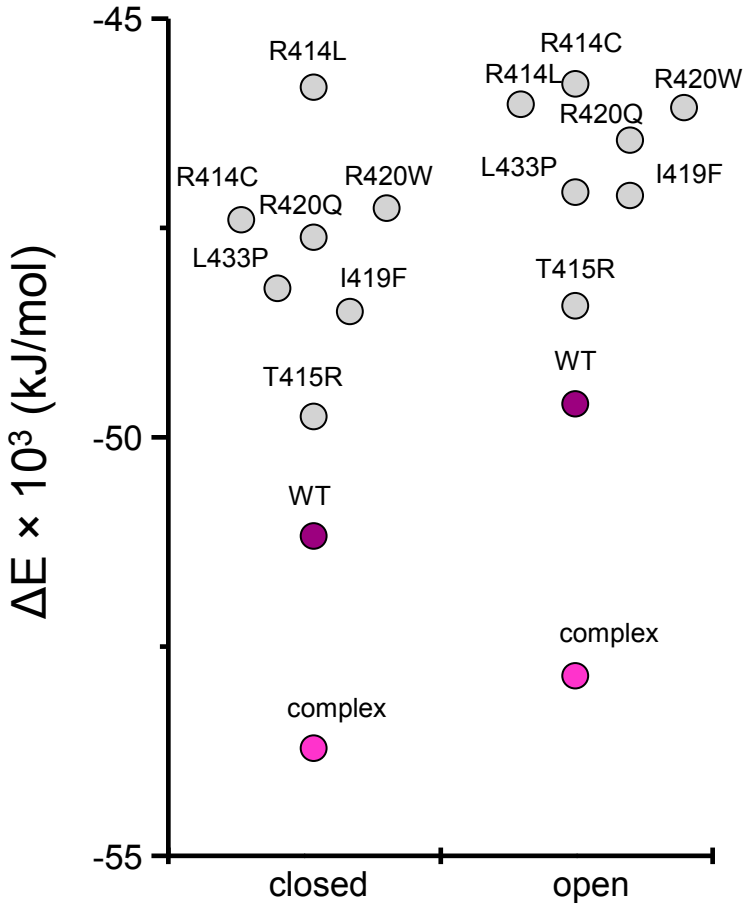


EMD-1607 NTD_O

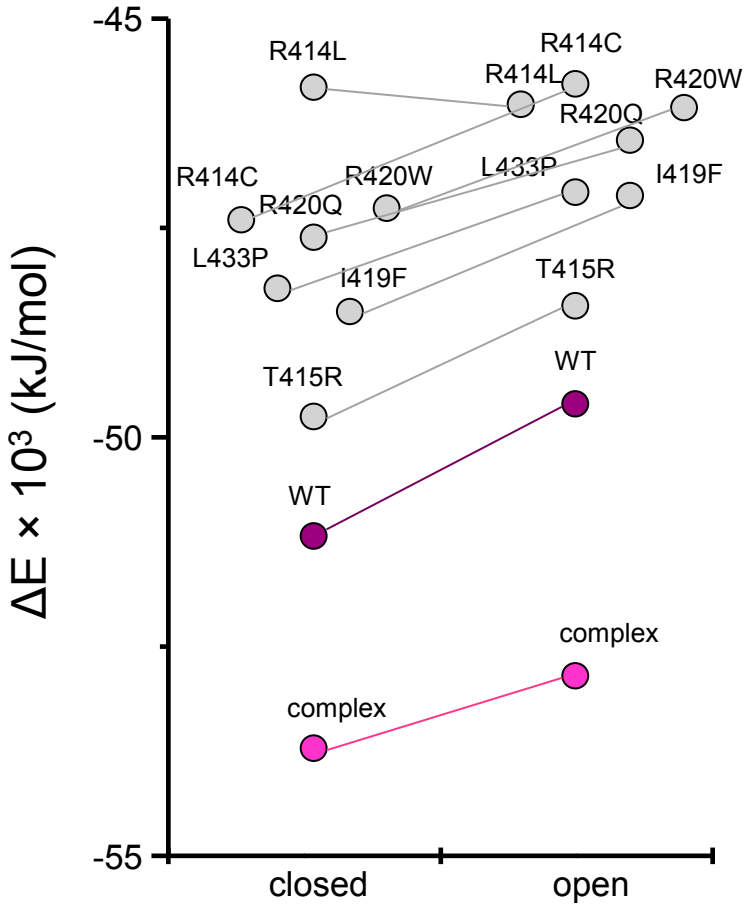


EMD-1607 $complex_O$

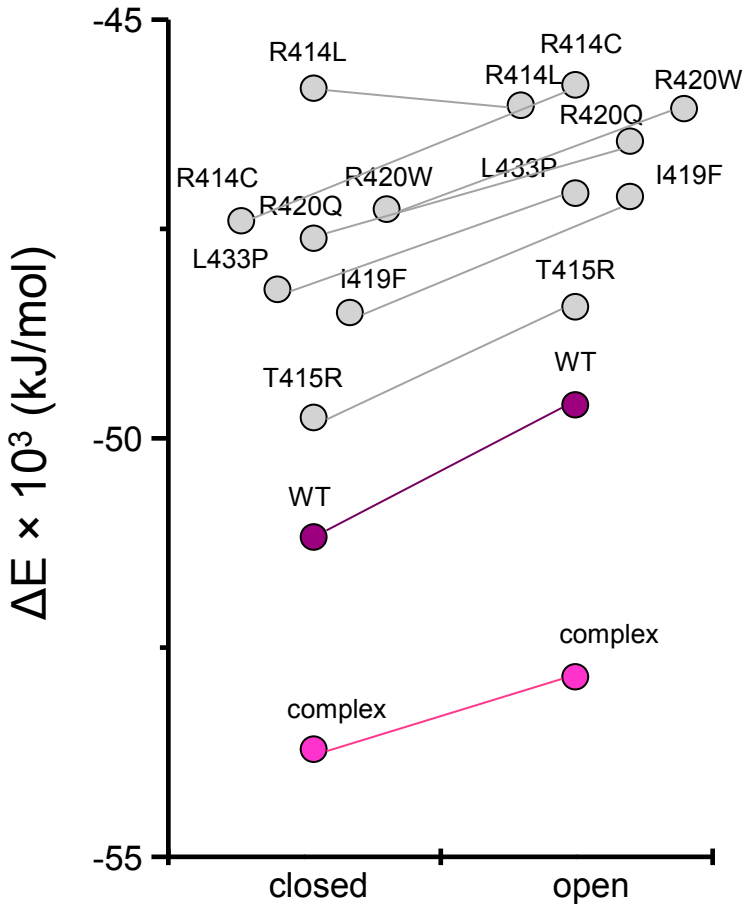
Comparison of complex and mutants

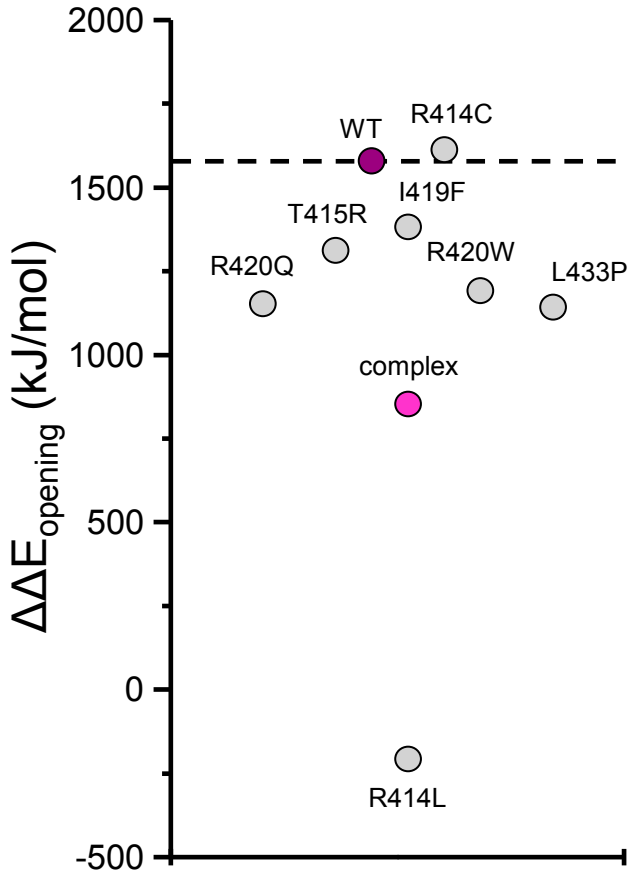


Comparison of complex and mutants



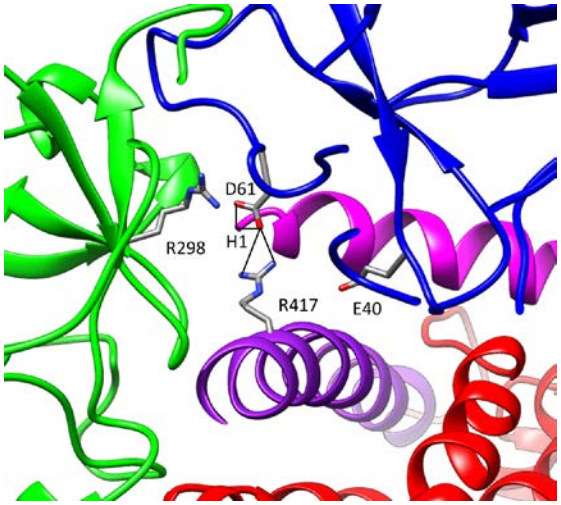
Comparison of complex and mutants



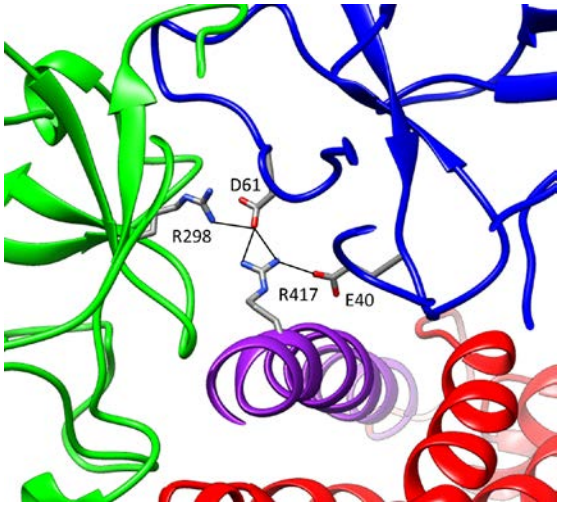


Comparison of complex and mutants

complex

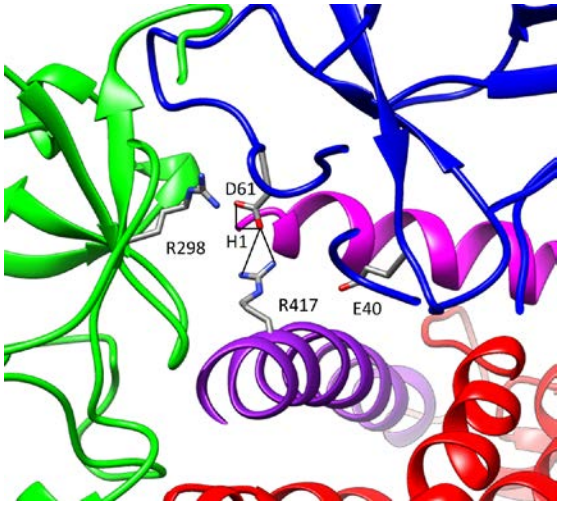


WT

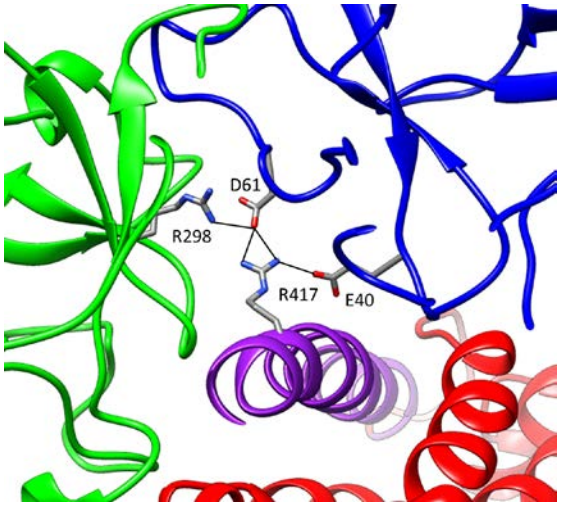


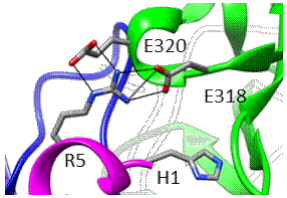
Comparison of complex and mutants

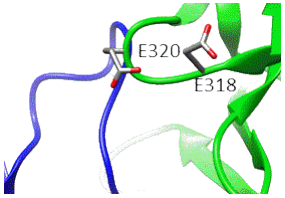
complex



WT

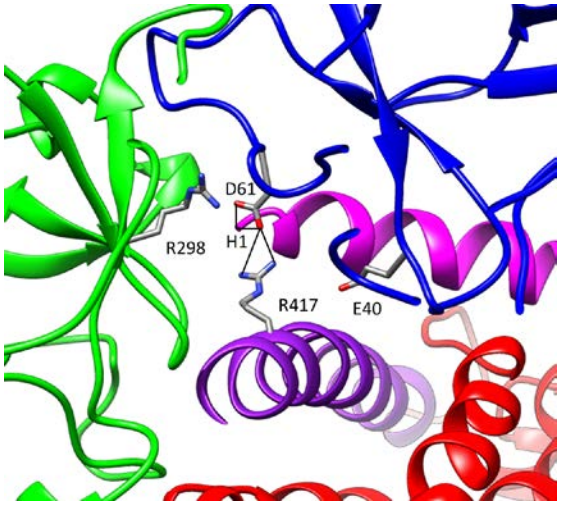




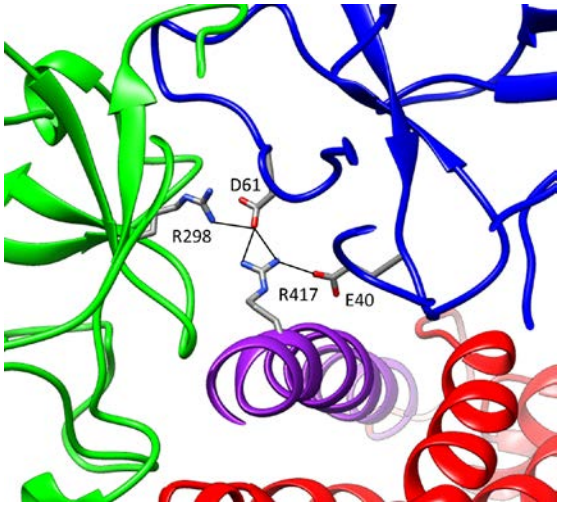


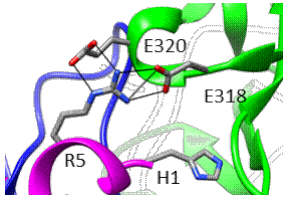
Comparison of complex and mutants

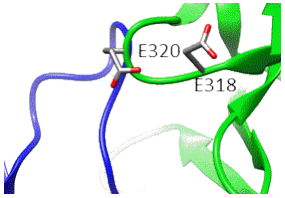
complex



WT







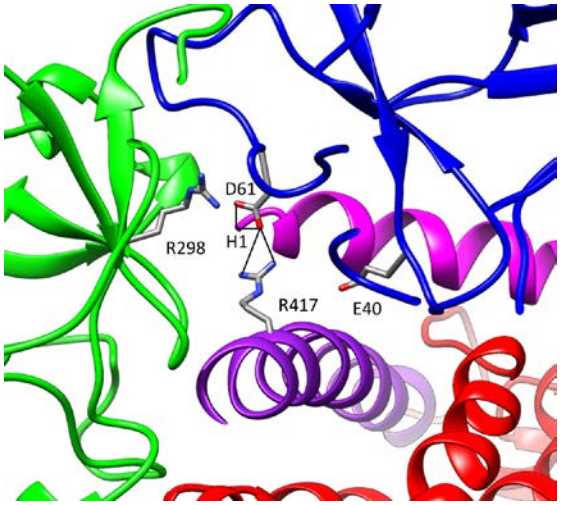
Effect of peptide binding:

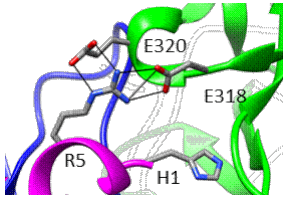
Disruption of ABC interaction network

Creation of new interaction network

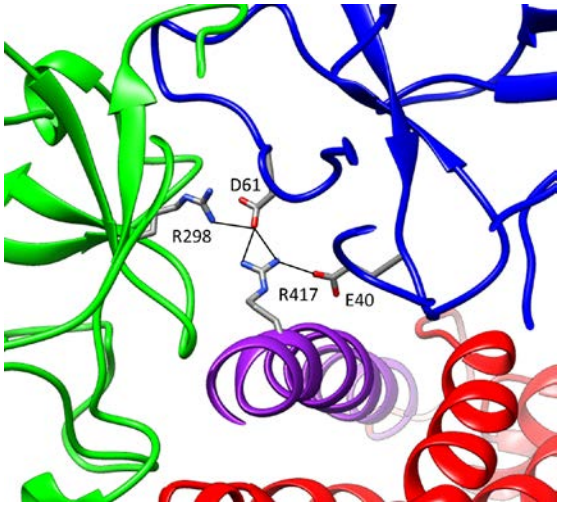
Comparison of complex and mutants

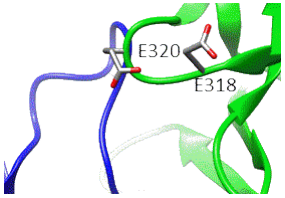
complex



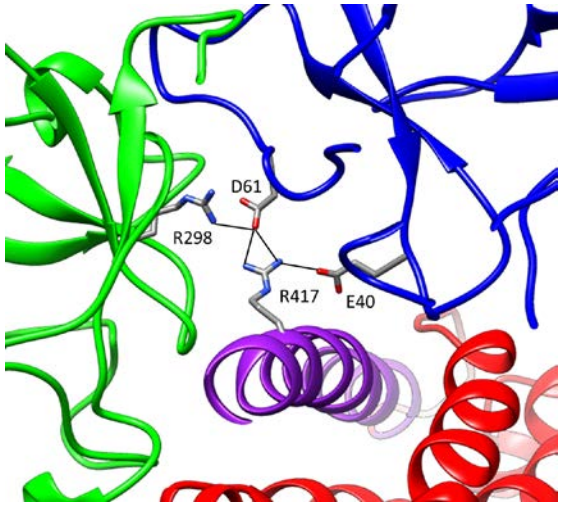


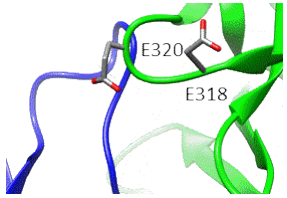
WT





mutations





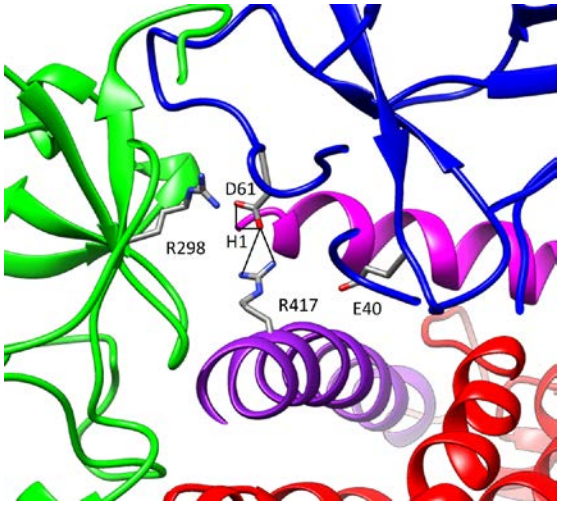
Effect of peptide binding:

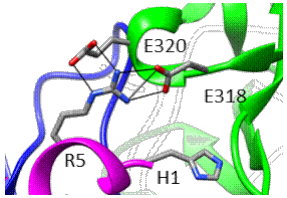
Disruption of ABC interaction network

Creation of new interaction network

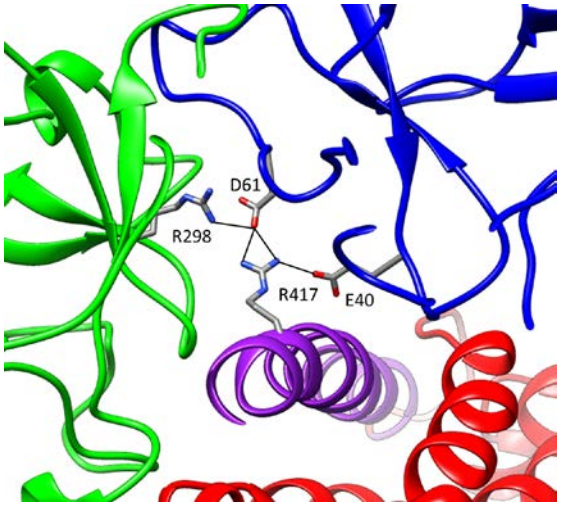
Comparison of complex and mutants

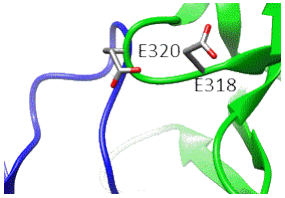
complex



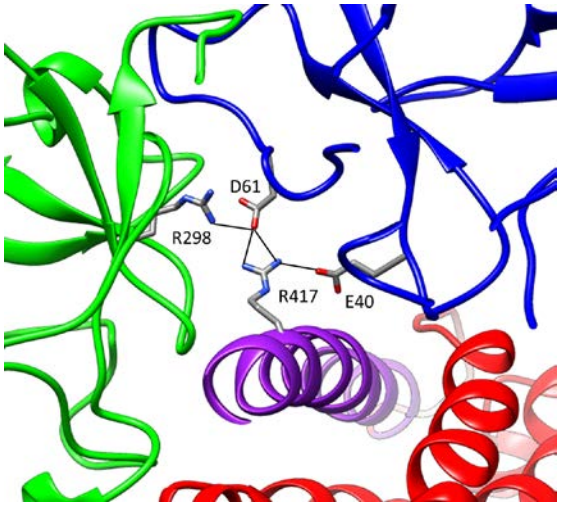


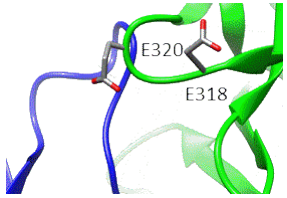
WT





mutations



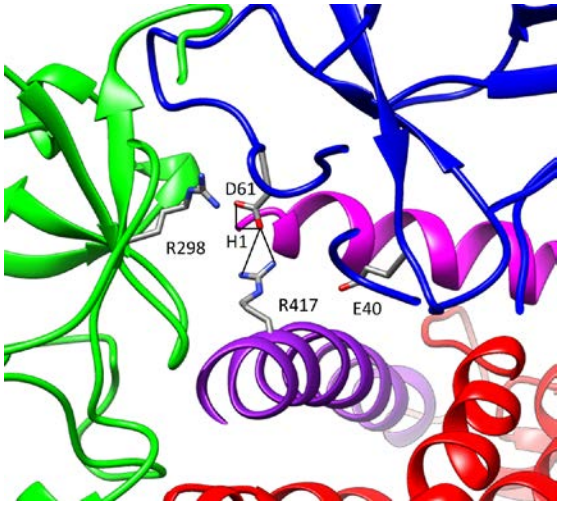


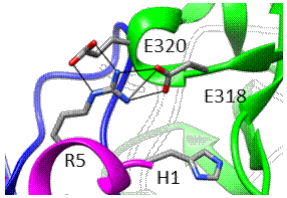
Effect of peptide binding:
Disruption of ABC interaction network
Creation of new interaction network

Effect of mutations:
No effect on ABC interaction network
No new interaction network

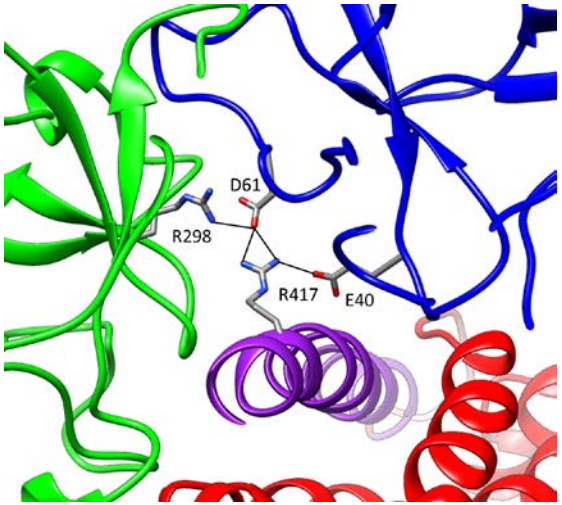
Comparison of complex and mutants

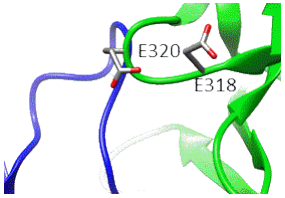
complex



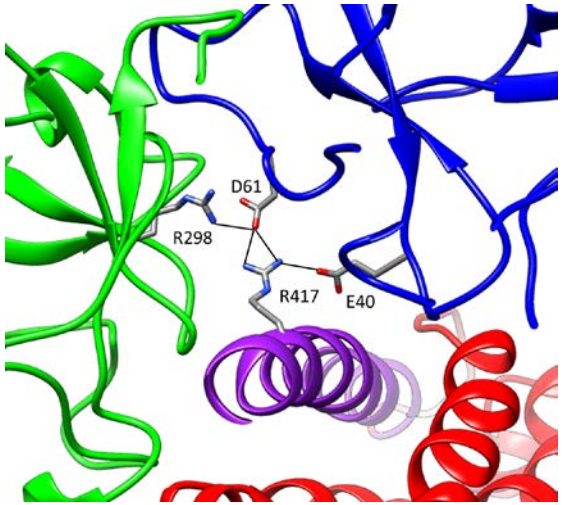


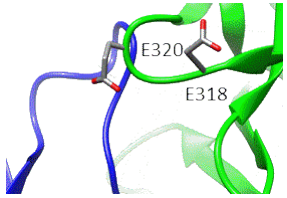
WT





mutations





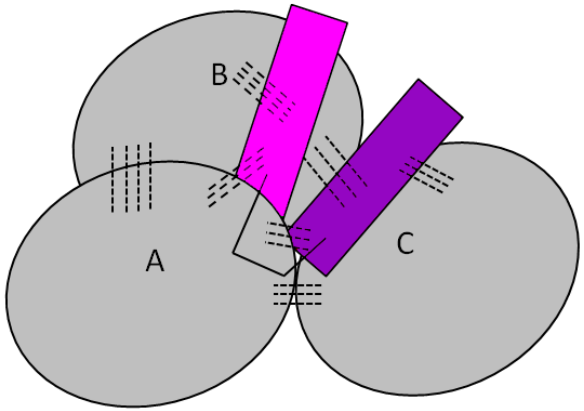
Effect of peptide binding:
Disruption of ABC interaction network
Creation of new interaction network

Effect of mutations:
No effect on ABC interaction network
No new interaction network

Closed: decreased N_{HB}^{SC} ($\Delta N_{HB}^{SC} = -21 \pm 2.7$, $P < 0.001$)
Open: decreased N_{HB}^{SC} ($\Delta N_{HB}^{SC} = -3 \pm 0.7$, $P < 0.01$)

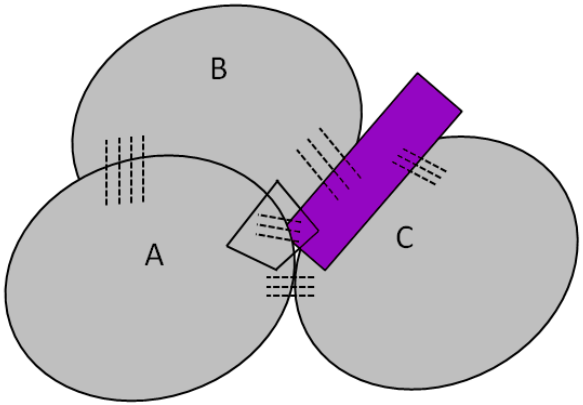
Conclusion

complex

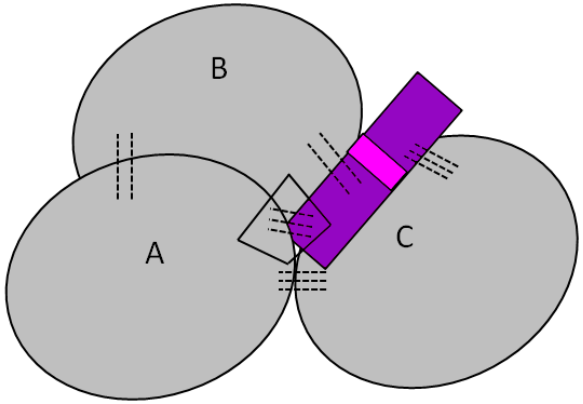


Increased stability:
New interaction network in both closed and open state

WT



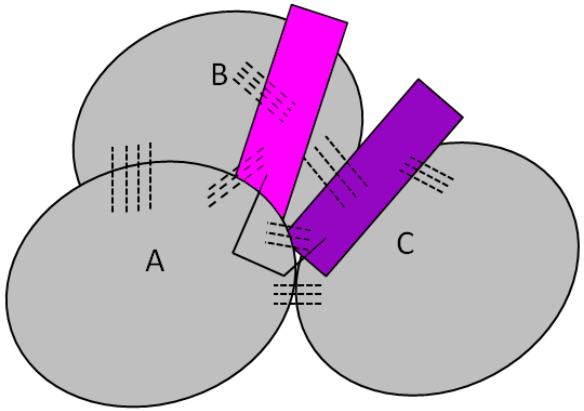
mutations



Decreased stability:
Disruption of H-bonds between AA sidechains outside interaction network

Conclusion

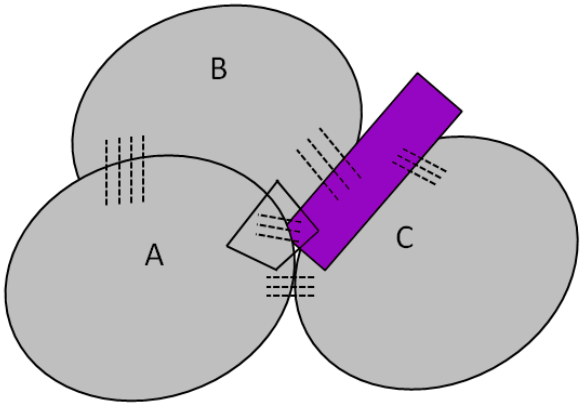
complex



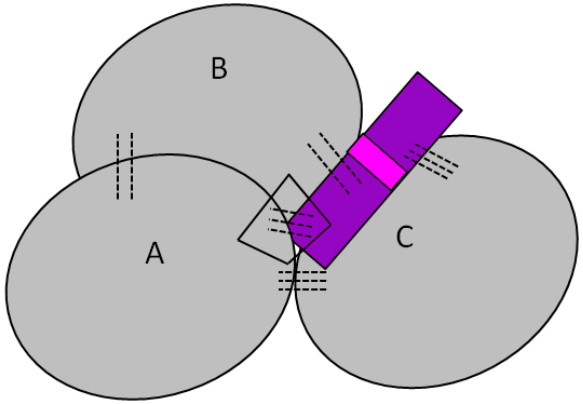
Increased stability:
New interaction network in both closed and open state

Facilitation of opening:
Less H-bonds formed in closed than in the open state

WT



mutations

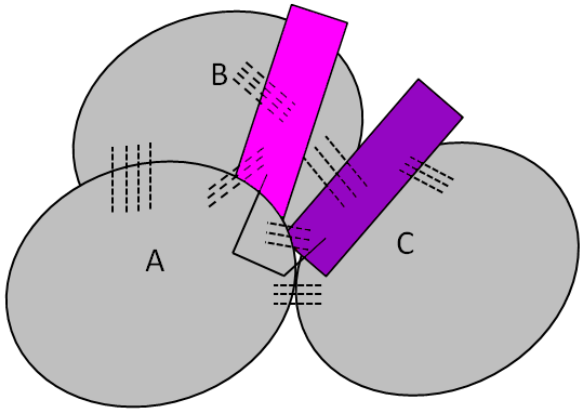


Decreased stability:
Disruption of H-bonds between AA sidechains outside interaction network

Facilitation of opening:
More H-bonds disrupted in closed than in the open state

Conclusion

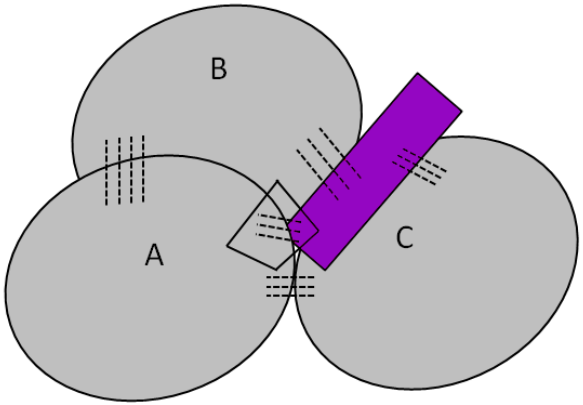
complex



Increased stability:
New interaction network in both closed and open state

Facilitation of opening:
Less H-bonds formed in closed than in the open state

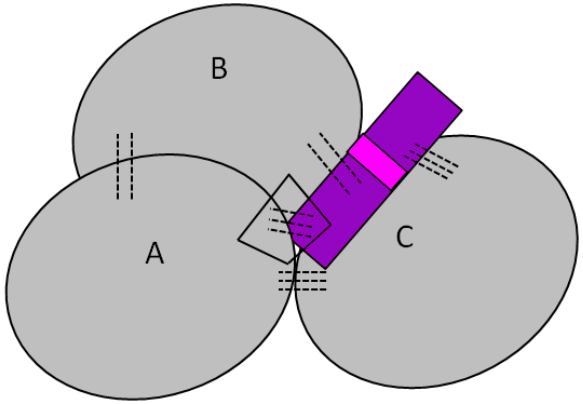
WT



Different mechanisms

Similar effects on $\Delta\Delta E_{opening}$

mutations

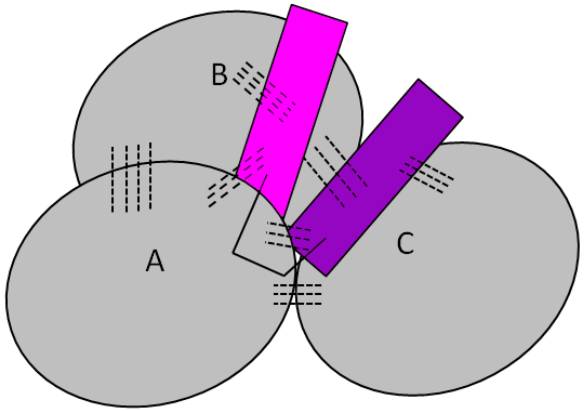


Decreased stability:
Disruption of H-bonds between AA sidechains outside interaction network

Facilitation of opening:
More H-bonds disrupted in closed than in the open state

Conclusion

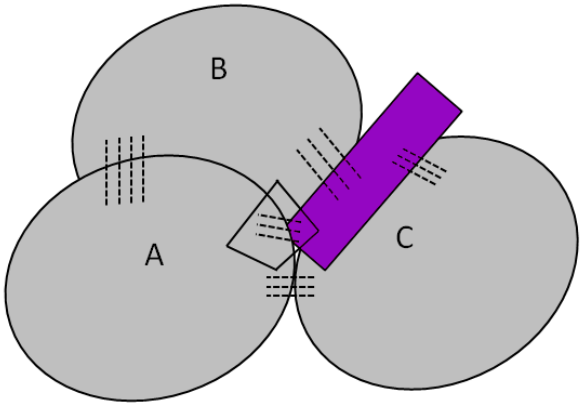
complex



Increased stability:
New interaction network in both closed and open state

Facilitation of opening:
Less H-bonds formed in closed than in the open state

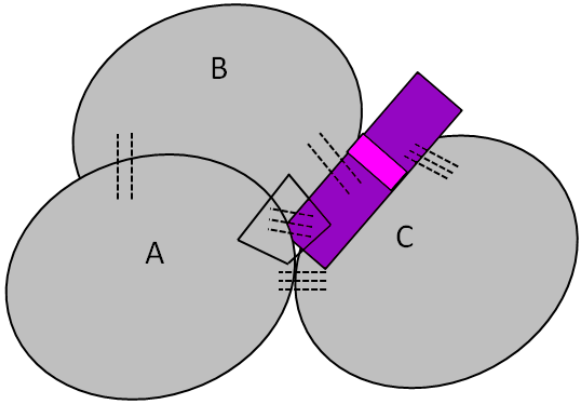
WT



Different mechanisms

Similar effects on $\Delta\Delta E_{opening}$

mutations



Decreased stability:
Disruption of H-bonds between AA sidechains outside interaction network

Facilitation of opening:
More H-bonds disrupted in closed than in the open state

Decrease of the ΔΔE between closed and open NTR conformation may contribute to CPVT phenotype

Acknowledgment

Grant support

Slovak Research and Development Agency
APVV-15-0302

Scientific grant agency of the Ministry of Education, Science, Research and Sport of the Slovak Republic and of Slovak Academy of Sciences
VEGA 2/0143/17

Acknowledgment

Grant support

Slovak Research and Development Agency
APVV-15-0302

Scientific grant agency of the Ministry of Education, Science, Research and Sport of the Slovak Republic and of Slovak Academy of Sciences
VEGA 2/0143/17

Open source software and public web servers

http://situs.biomachina.org/
https://salilab.org/modeller/
http://www.cgl.ucsf.edu/chimera/
https://zhanglab.ccmb.med.umich.edu/I-TASSER/
http://vakser.compbio.ku.edu/resources/gramm/grammx/
http://dirac.cnrs-orleans.fr/MMTK.html