

**Strukturna biologija naravnih
imununskih odzivov v živalih in rastlinah**
*Structural biology of innate immunity signaling in
animals and plants*

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THE UNIVERSITY
OF QUEENSLAND
AUSTRALIA



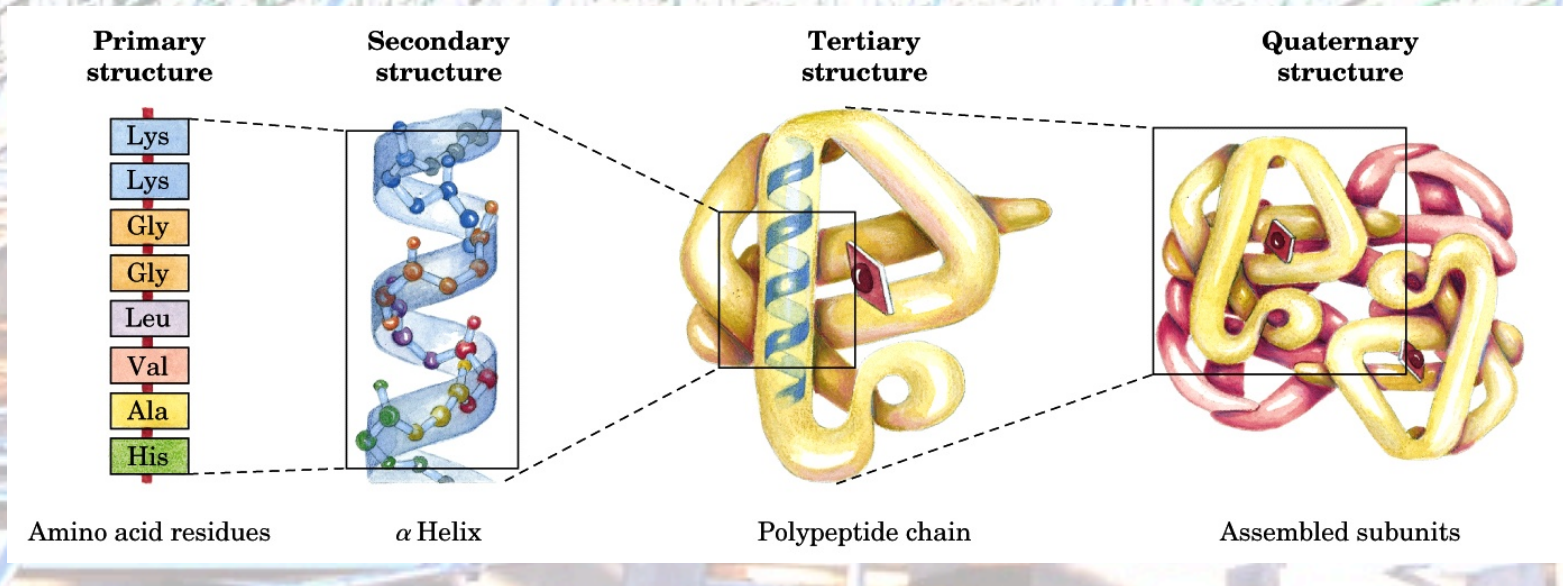
Metode za določanje 3D strukture makromolekul

- Rentgenska kristalografija
- Jedrska magnetna resonanca (NMR – nuclear magnetic resonance spectroscopy)
- (Krio)-elektronska mikroskopija (cryoEM)
- Komplementarne biofizikalne metode
- Predikcija strukture

“Protein Data Bank” trenutno vsebuje >160,000 struktur makromolekul

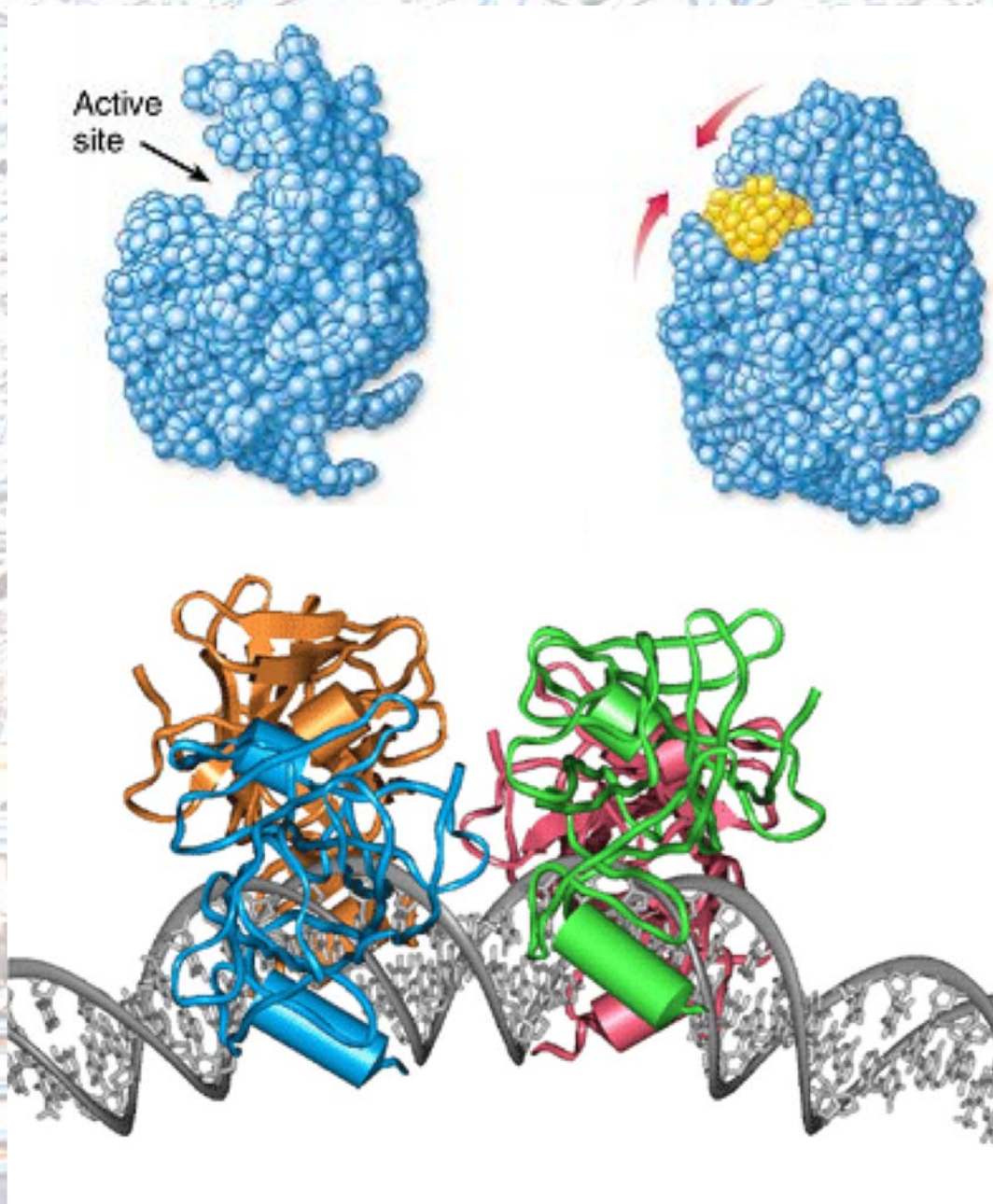
Zakaj je pomembno določiti 3D strukturo proteinov?

- Funkcija proteinov in drugih makromolekul temelji na njihovi 3D strukturi
 - Oblika, lastnosti in zato funkcija proteinov je odvisna od tega, kako se zaporedje amino-kislin zvije v prostoru
 - 3D-struktura nam omogoči razložiti, z atomsko ločljivostjo, kako molekula funkcionira



Primeri struktur, ki nam razložijo funkcijo

Encim veže substrat

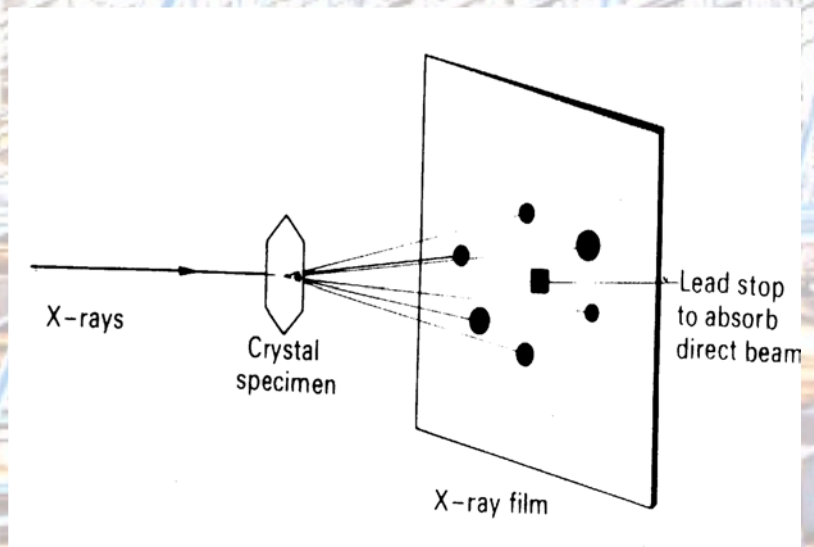
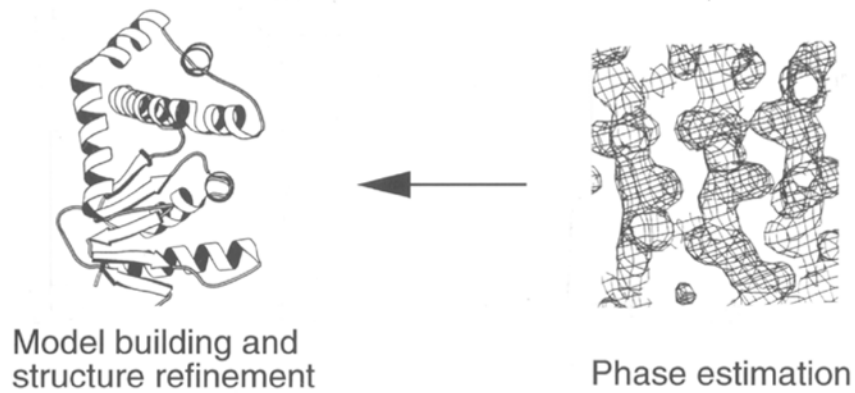
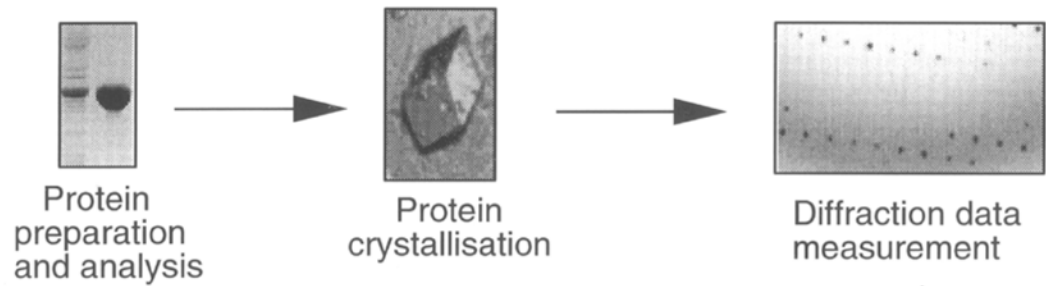
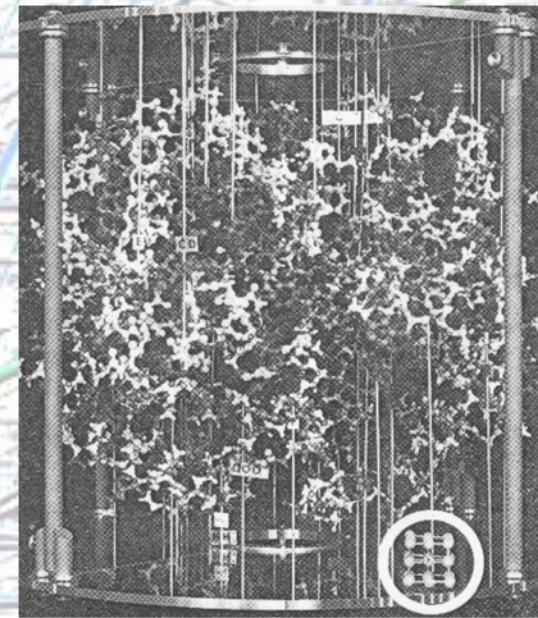


**Transkripcijski faktor
(p53) veže DNA**

Kakšna je vrednost poznanja 3D strukture makromolekul za medicino in biotehnologijo?

- Razumevanje funkcije
- Razvoj zdravil: “structure-based drug design”
- Razvoj vakcin
- Racionalne modifikacije proteinov: diagnostika, “humanizacija”, encimi za industrijo
- Razumevanje zvijanja (folding)
- Razumevanje stabilnosti in drugih fizikalnih lastnosti makromolekul

Rentgenska kristalografija



Diplomska naloga

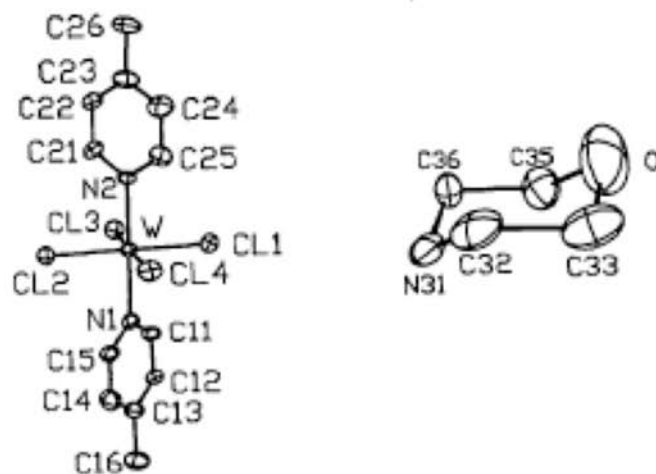


Fig. 1. Numbering schemes of the anion and cation.

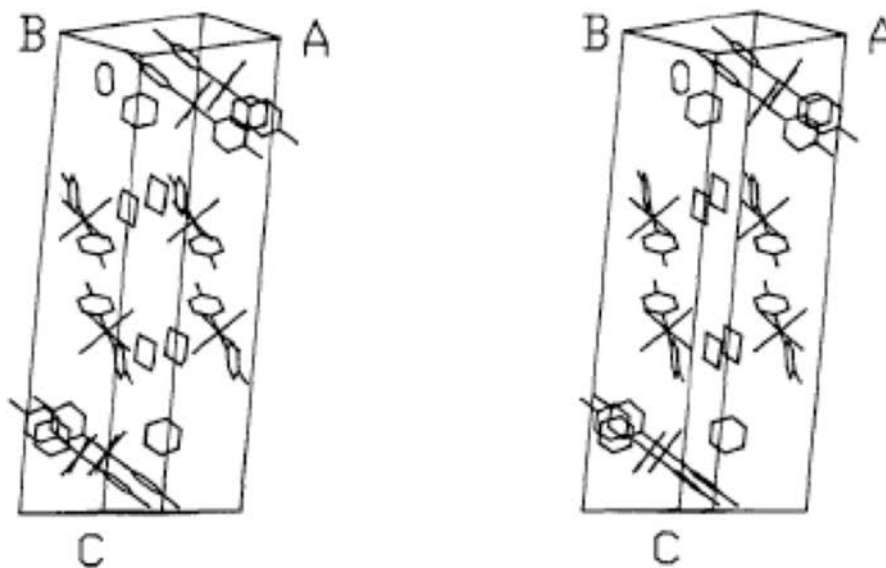


Fig. 2. Stereoscopic projection of the unit cell.

Brencic et al.
(1991). *Acta Cryst*
C47: 311-313

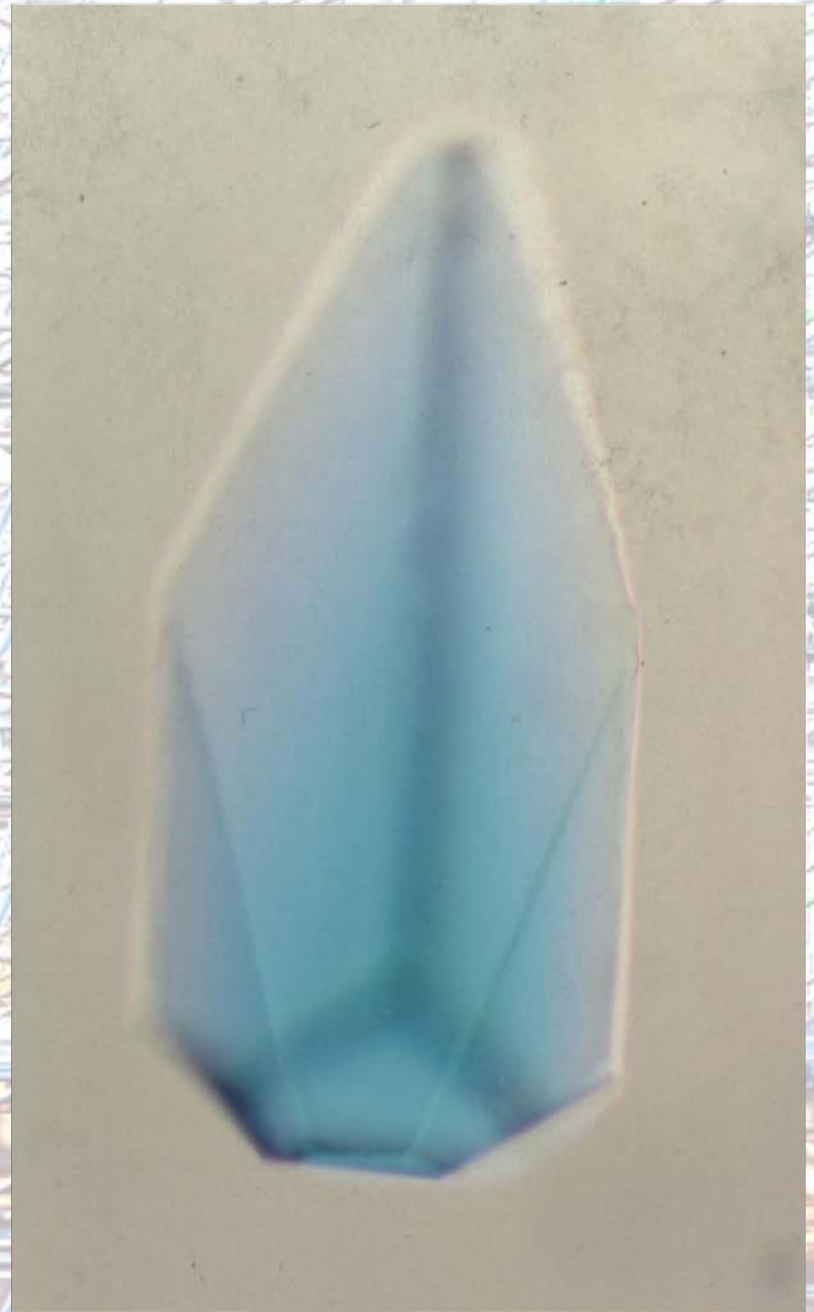
Inhibitor ribonukleaze (ribonuclease inhibitor) - zaporedje amino-kislin



A	5	10	15	20	25		
						M N L D I H C E Q L S D A R	1 - 14
A1	W T E L L P L L Q Q Y E V V R L D D C G L T E E H C K D I	15 - 43					
B1	G S A L R A N P S L T E - L C L R T N E L G D A G V H L V	44 - 71					
A2	L Q G L Q S P T C K I Q K L S L Q N C S L T E A G C G V L	72 - 100					
B2	P S T L R S L P T L R E - L H L S D N P L G D A G L R L L	101 - 128					
A3	C E G L L D P Q C H L E K L Q L E Y C R L T A A S C E P L	129 - 157					
B3	A S V L R A T R A L K E - L T V S N N D I G E A G A R V L	158 - 185					
A4	G Q G L A D S A C Q L E T L R L E N C G L T P A N C K D L	186 - 214					
B4	C G I V A S Q A S L R E - L D L G S N G L G D A G I A E L	215 - 242					
A5	C P G L L S P A S R L K T L W L W E C D I T A S G C R D L	243 - 271					
B5	C R V L Q A K E T L K E - L S L A G N K L G D E G A R L L	272 - 299					
A6	C E S L L Q P G C Q L E S L W V K S C S L T A A C C Q H V	300 - 328					
B6	S L M L T Q N K H L L E - L Q L S S N K L G D S G I Q E L	329 - 356					
A7	C Q A L S Q P G T T L R V L C L G D C E V T N S G C S S L	357 - 385					
B7	A S L L L A N R S L R E - L D L S N N C V G D P G V L Q L	386 - 413					
A8	L G S L E Q P G C A L E Q L V L Y D T Y W T E E V E D R L	414 - 442					
	Q A L E G S K P G L R V I S	443 - 456					

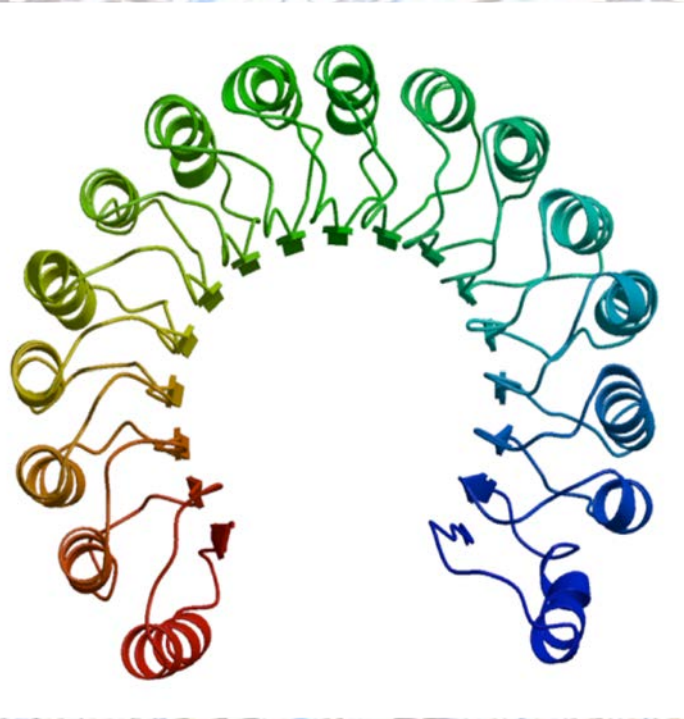


Dallas 1989



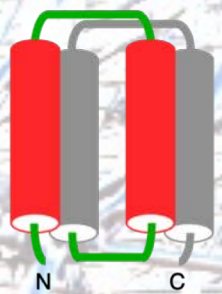
Dallas 1992

Kristalna struktura inhibitorja ribonukleaze (ribonuclease inhibitor)



Kobe & Deisenhofer (1993) Nature 366: 751-756
Kobe & Deisenhofer (1995) Nature 374: 183-186

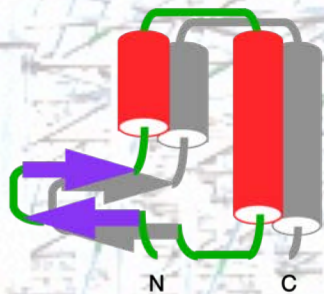
Solenoidni proteini



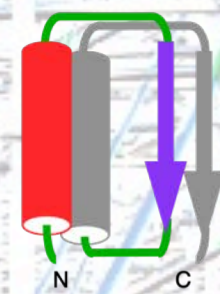
$\alpha/\alpha, \alpha/3_{10}$
Various



$\alpha/\alpha/\alpha$
Armadillo



$\beta/\beta/\alpha/\alpha$
Ankyrin



$\alpha/\beta, 3_{10}/\beta$
LRR

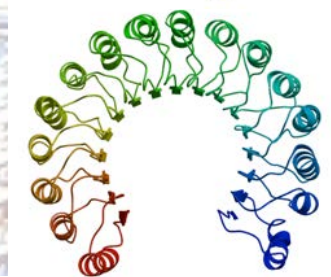
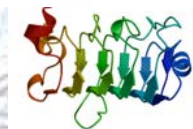
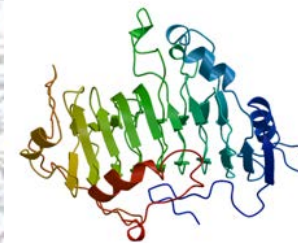
PeIC

IGF-1R L

U2A'

RI

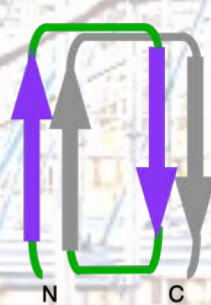
LRV



$\beta/\beta/\beta$
IGF-1R L



$\beta/\beta/\beta$
PeIC

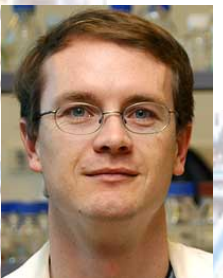
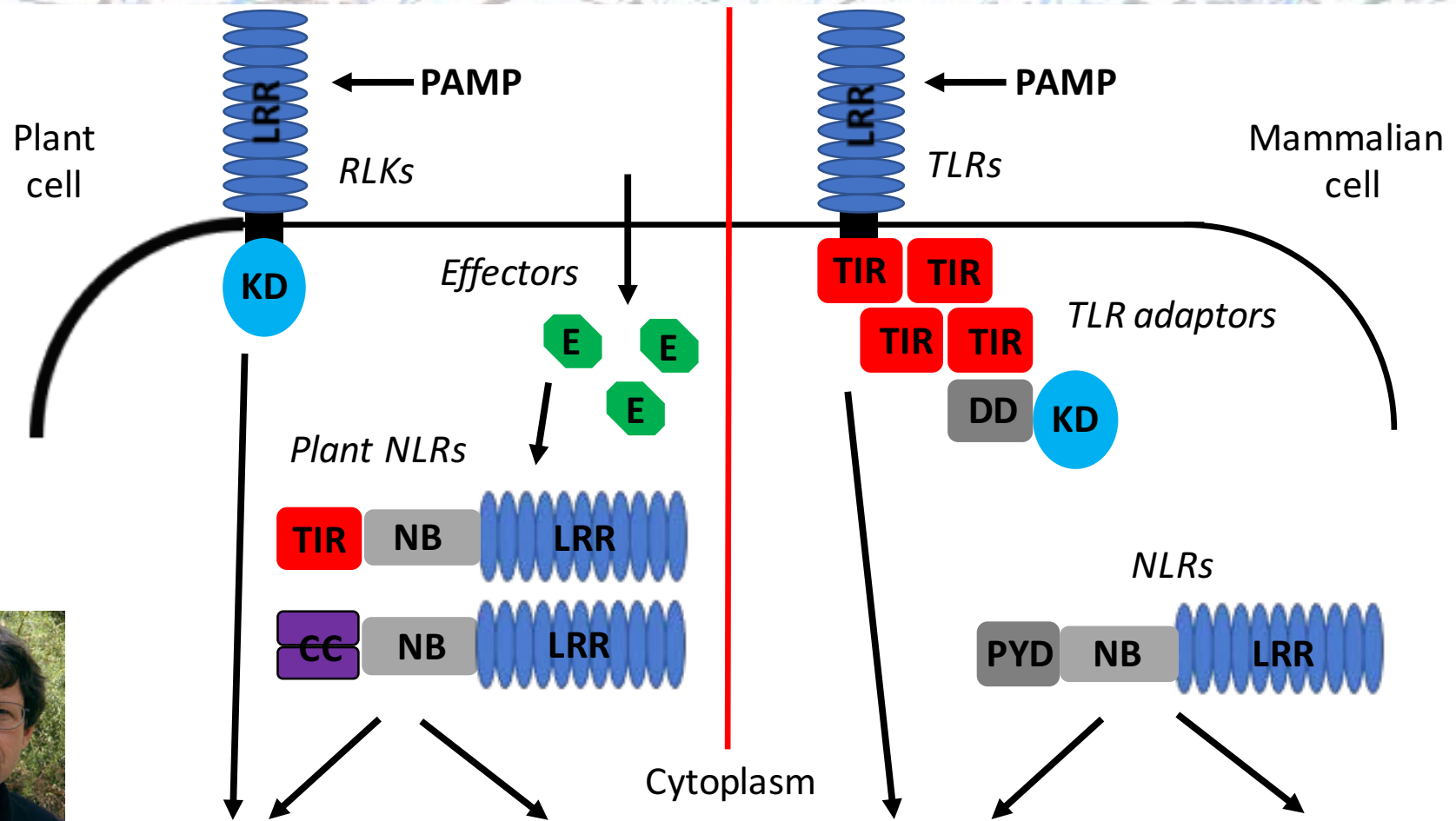


β/β
Serralysin



$\beta/\beta/\beta$
HPR

Naravni imunski odziv (innate immunity)

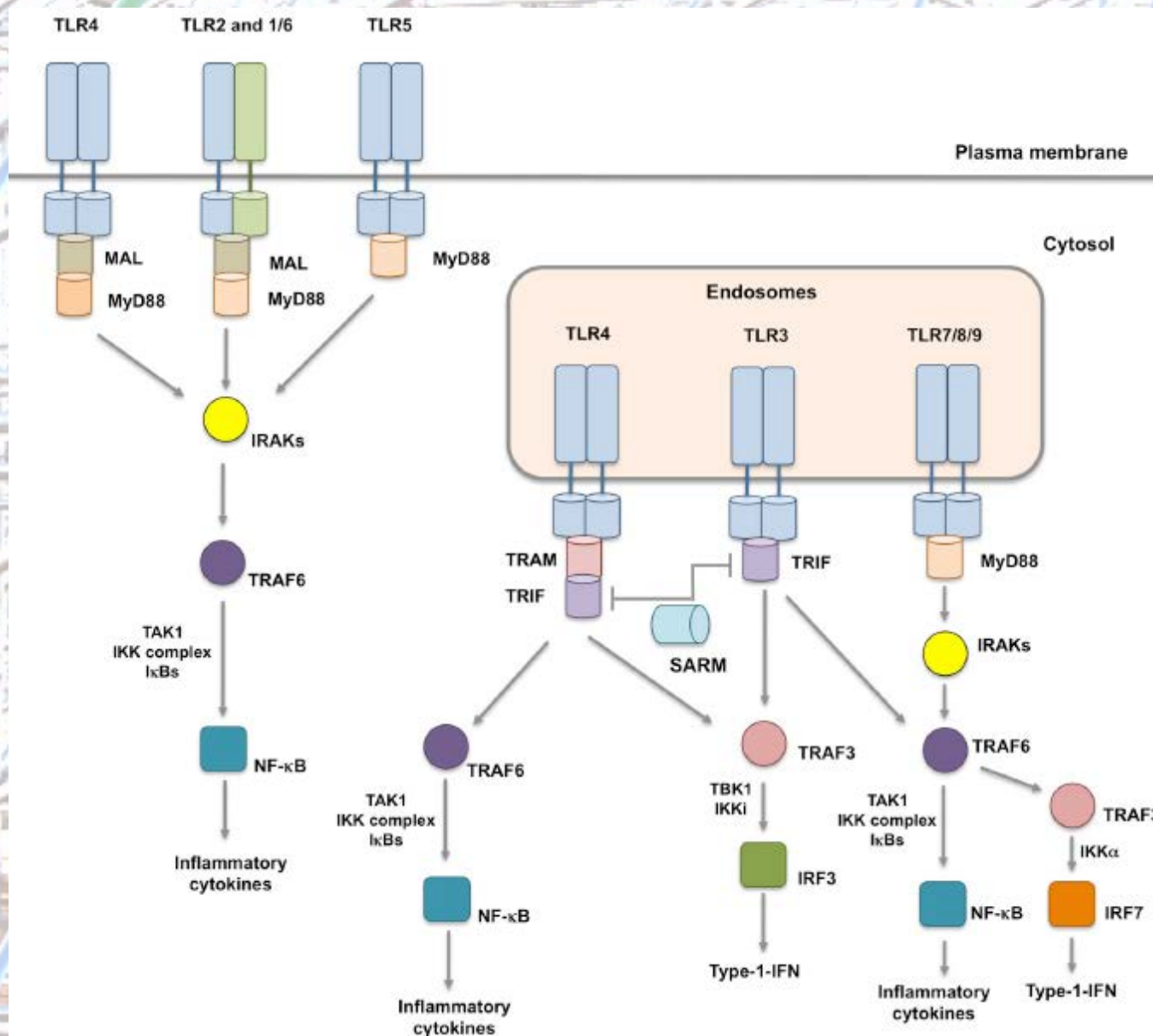


Rastline

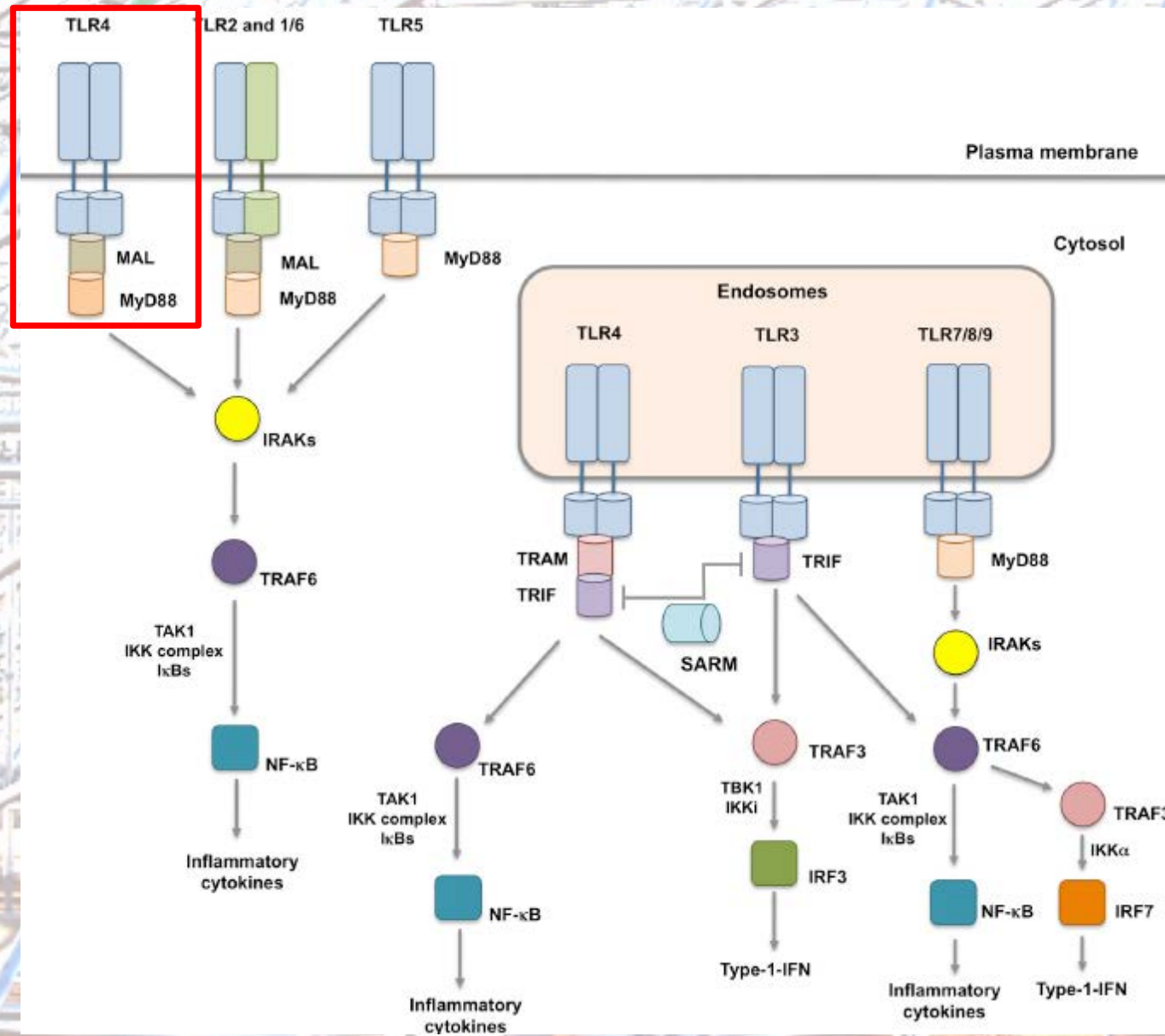
Sesalci



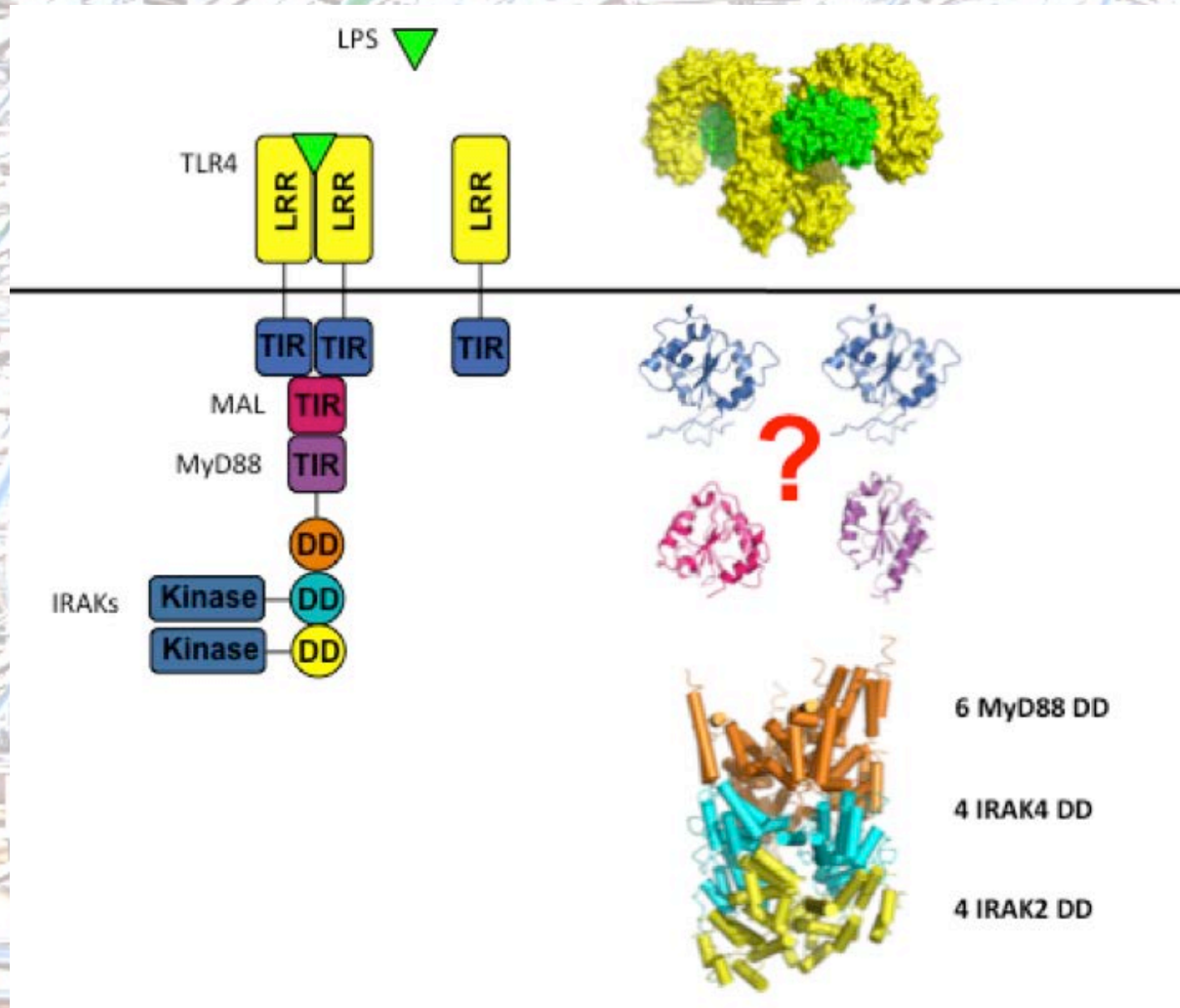
Toll-like receptors (TLRs)



Toll-like receptor 4 (TLR4)



Toll-like receptor 4



Kim et al (2007) Cell
130, 906–917

LRR = Leucine rich repeat
TIR = Toll/interleukin-1
receptor domain
DD = Death domain

6 MyD88 DD

4 IRAK4 DD

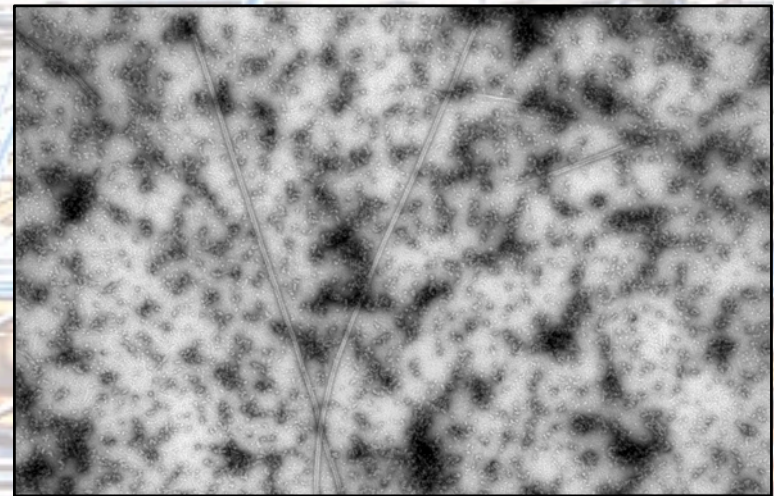
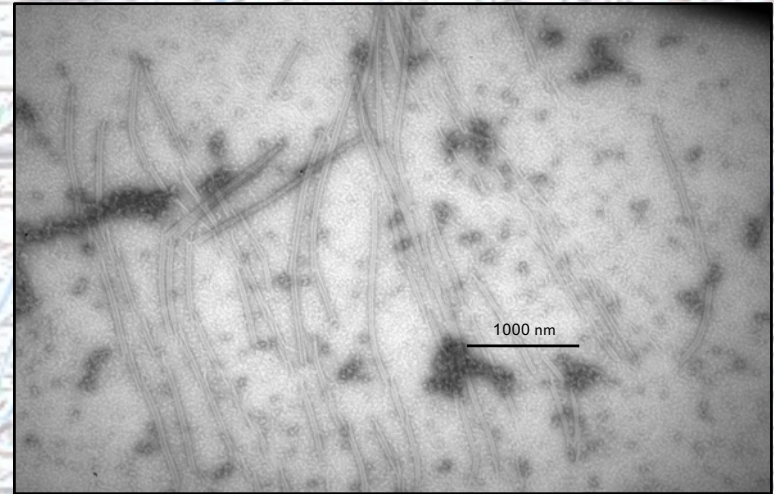
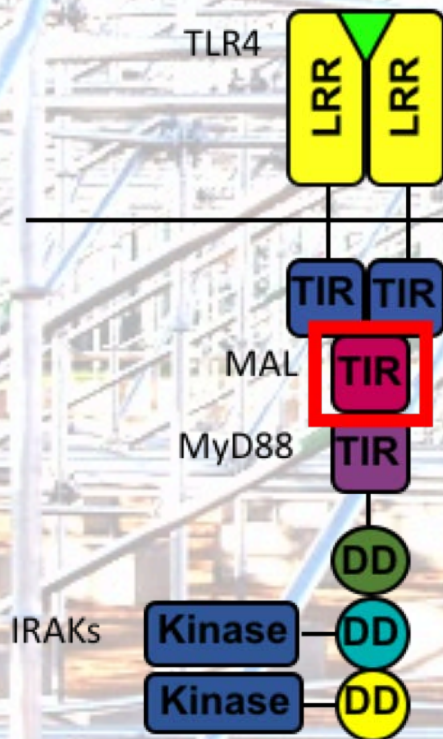
4 IRAK2 DD

Lin et al (2010)
Nature 465, 885-890

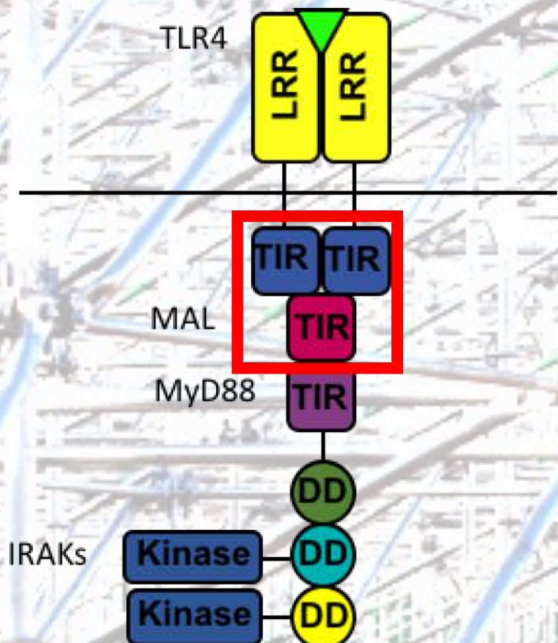
Reverzibilno obarjanje MAL^{TIR}



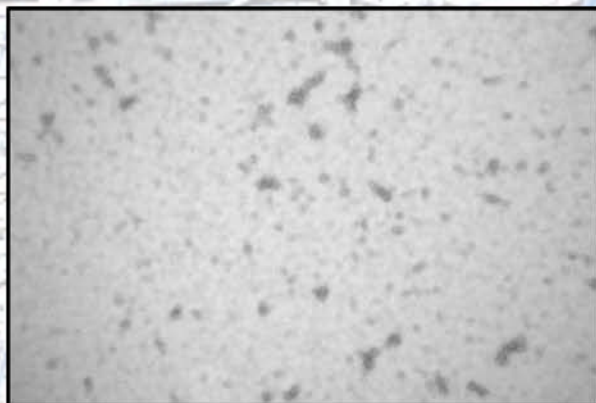
Adaptor MAL tvori filamente



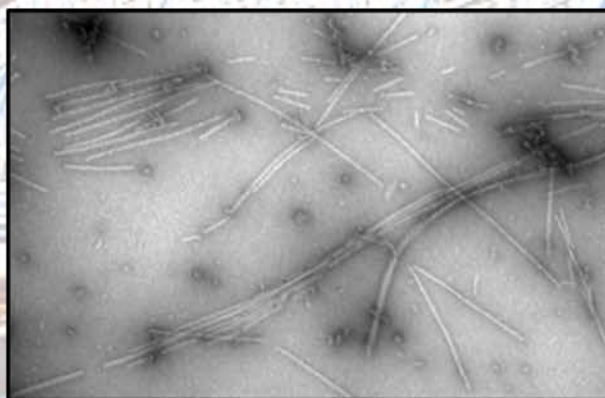
MAL^{TIR} and TLR4^{TIR} skupaj tvorita filamente



TLR4^{TIR}



TLR4^{TIR} + MAL^{TIR}



kDa

40

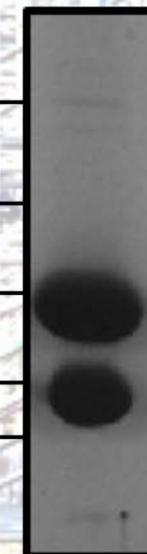
30

25

20

15

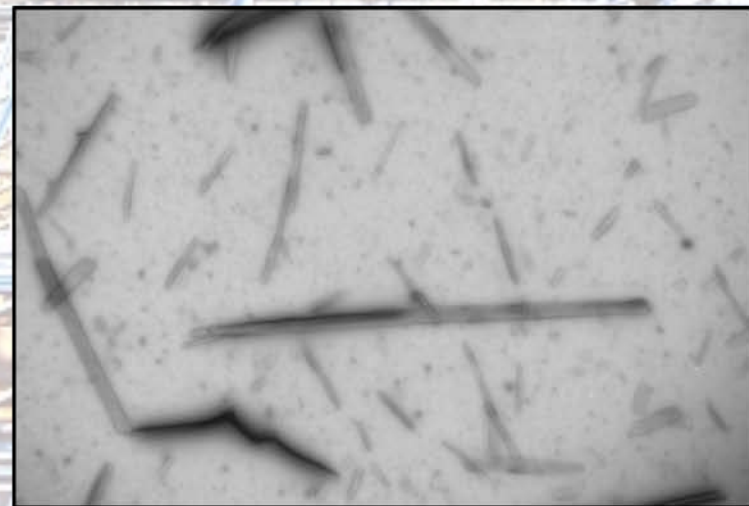
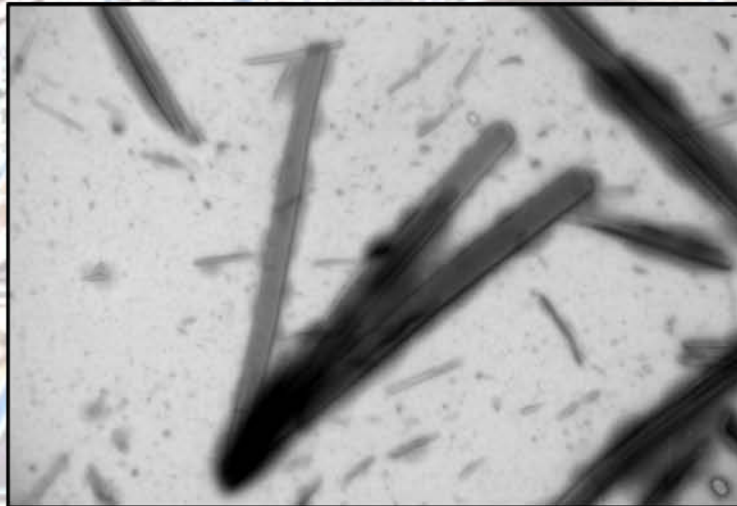
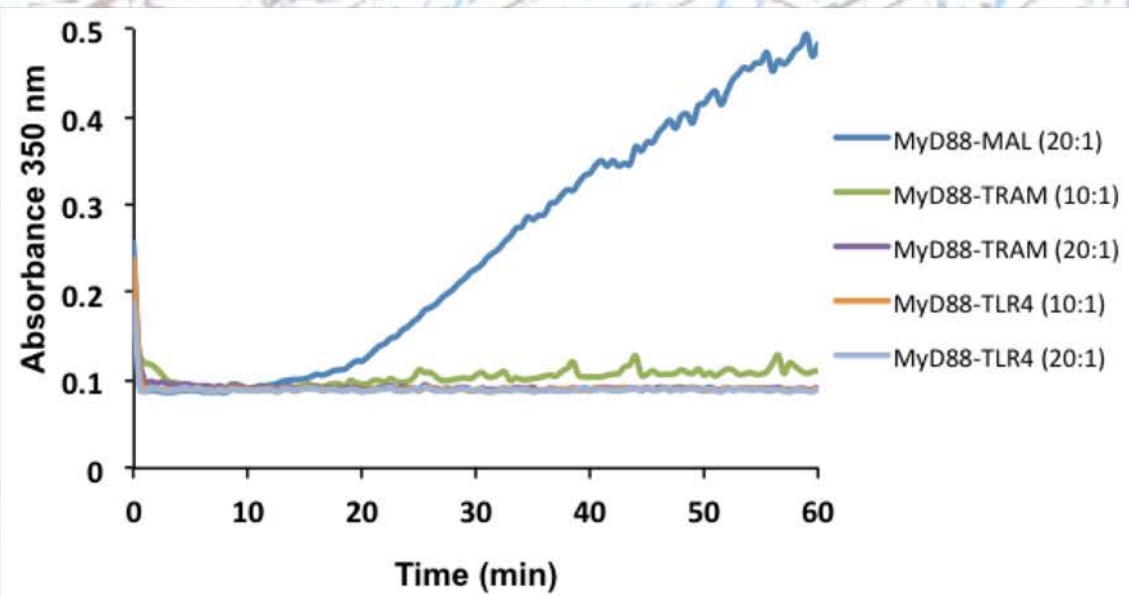
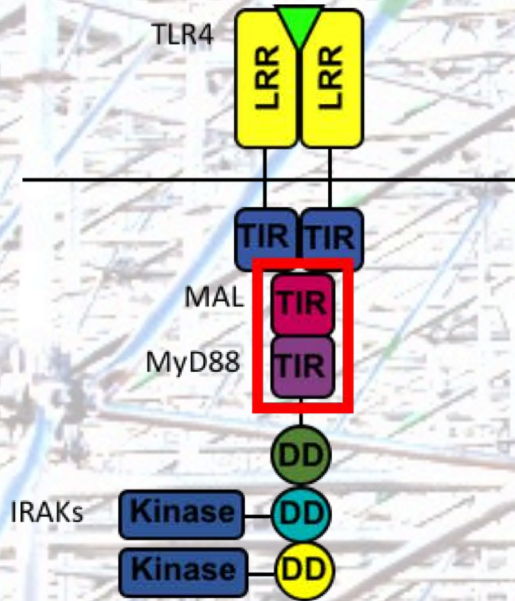
MAL^{TIR} + TLR4^{TIR}



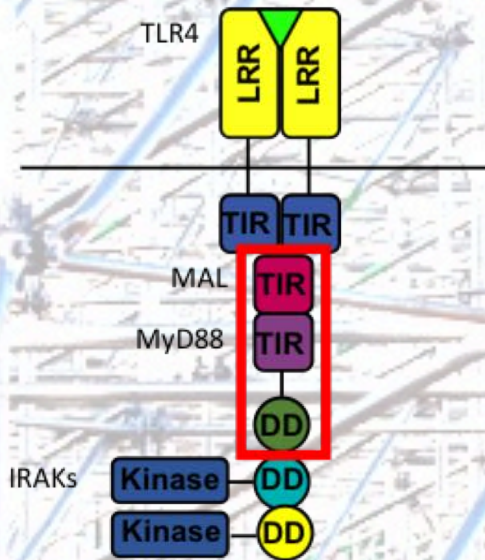
MAL^{TIR}

TLR4^{TIR}

MAL^{TIR} spodbudi sestavo kompleksov MyD88^{TIR}

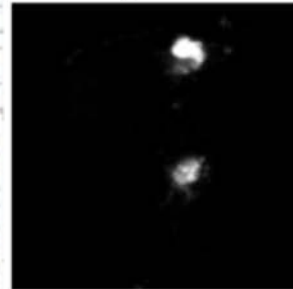


MAL spodbudi sestavo kompleksov MyD88 v celicah

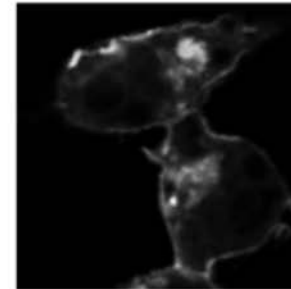


MyD88^{FL}
+ MAL^{FL}

MyD88

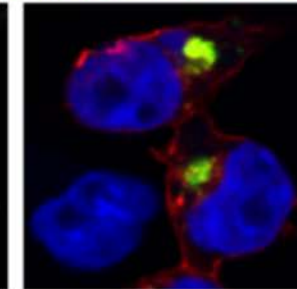


MAL

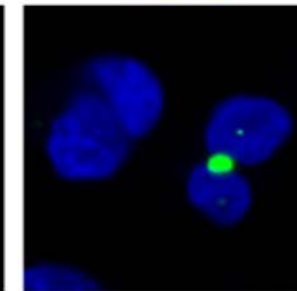
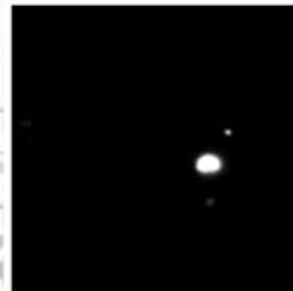


merge

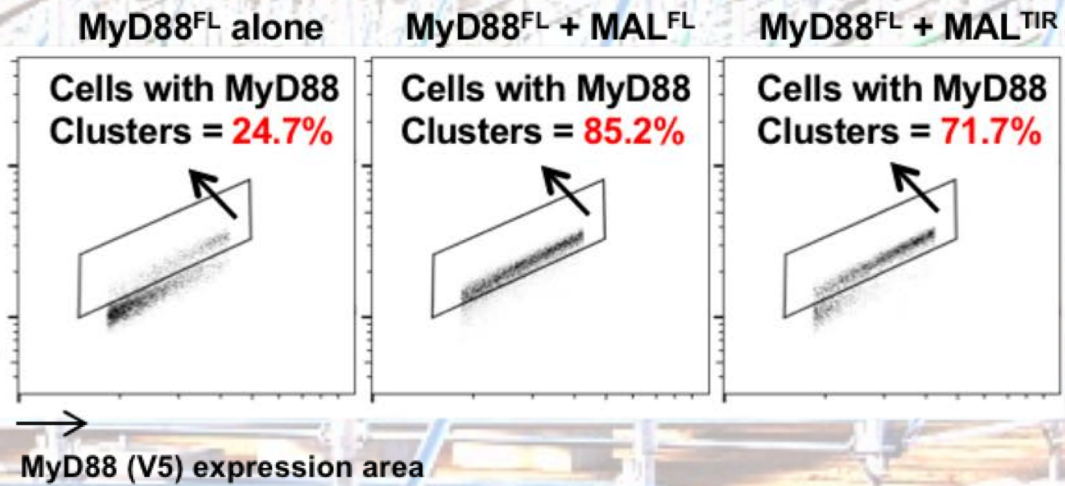
MyD88/MAL/DAPI



MyD88^{FL}
alone



MyD88 expression height



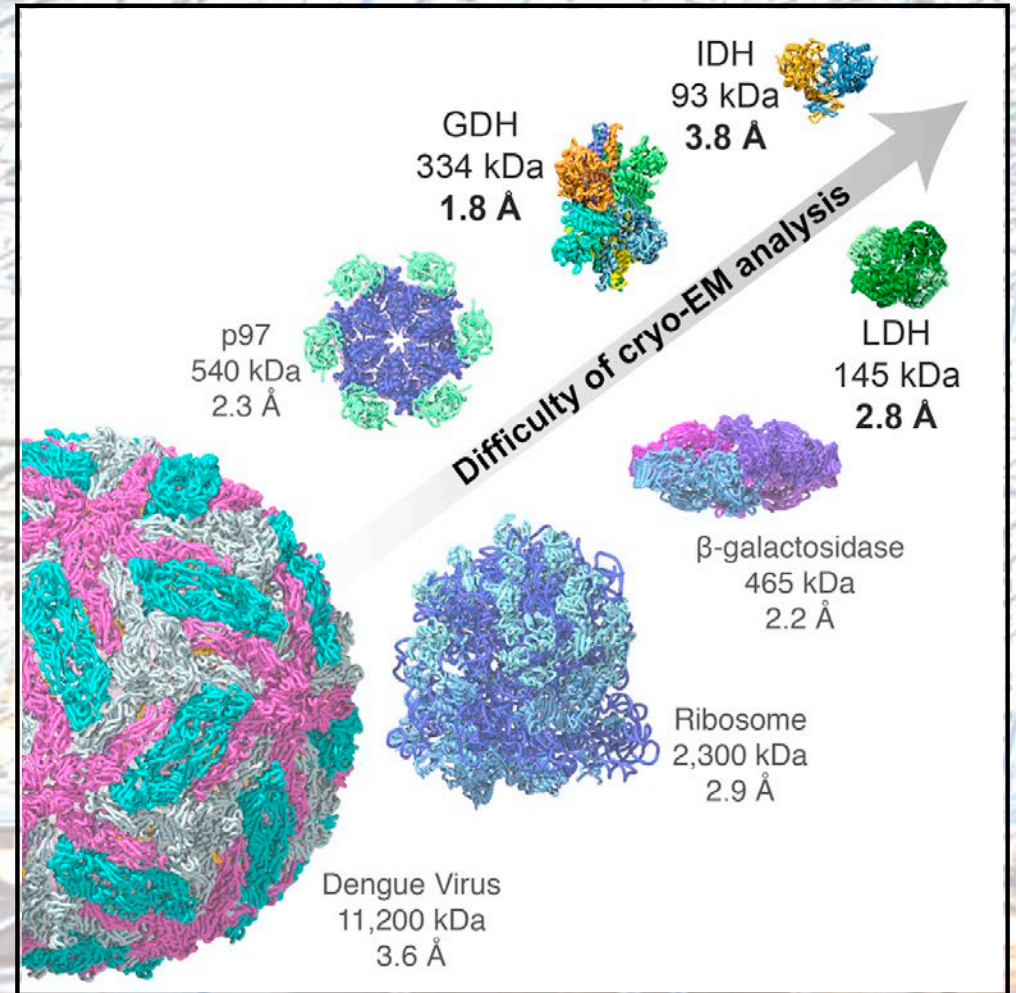
Krio-elektronska mikroskopija



University of Virginia

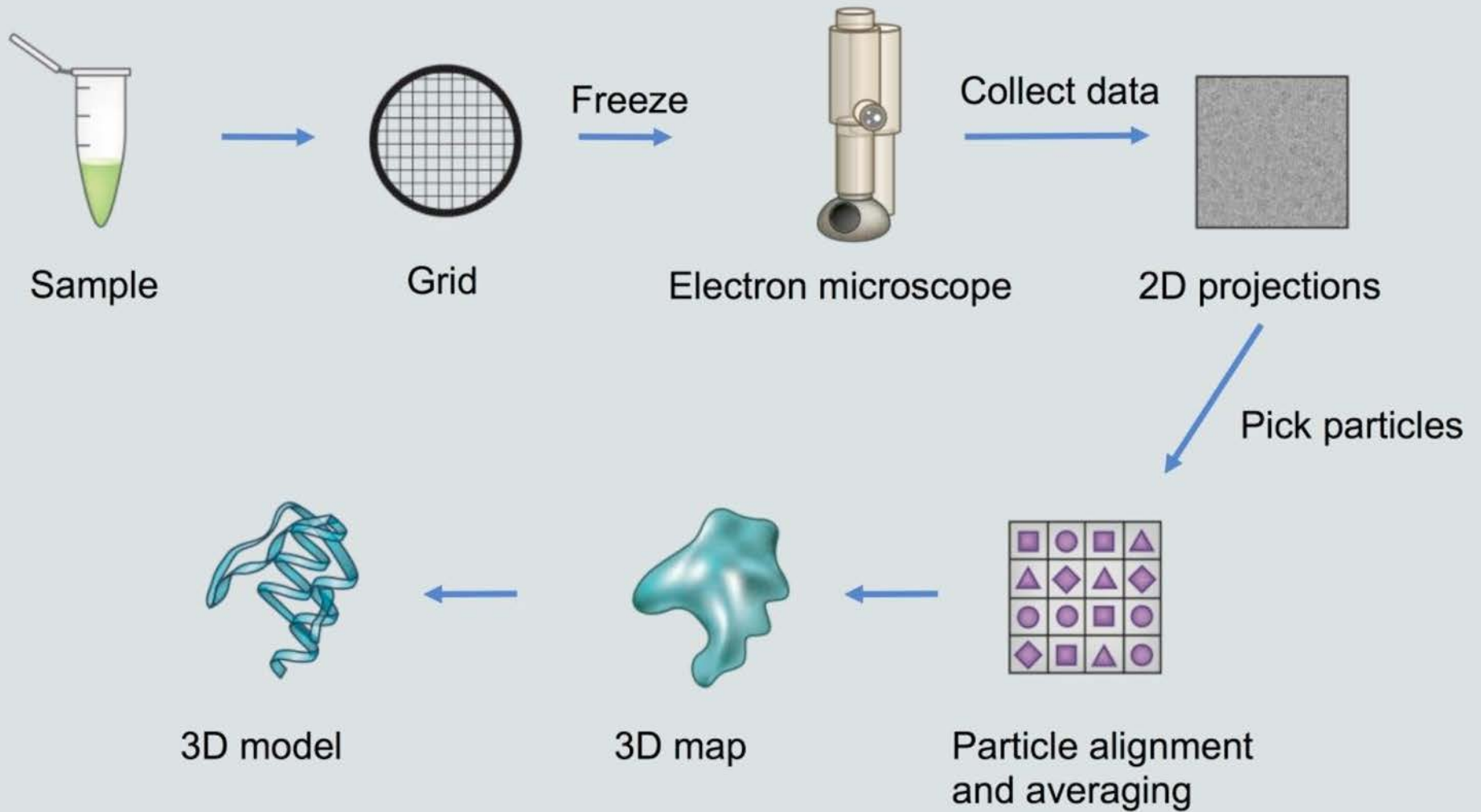


University of Queensland

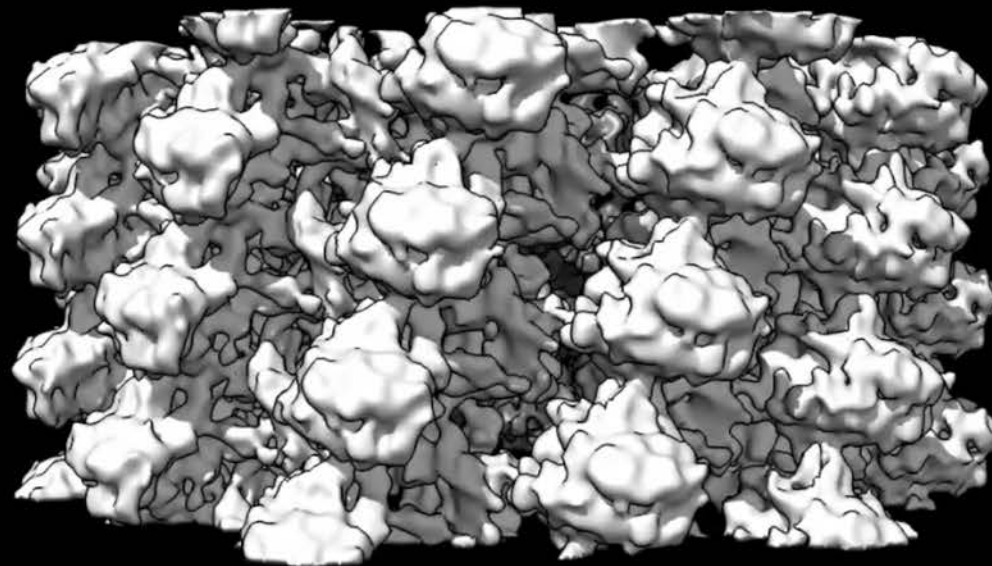


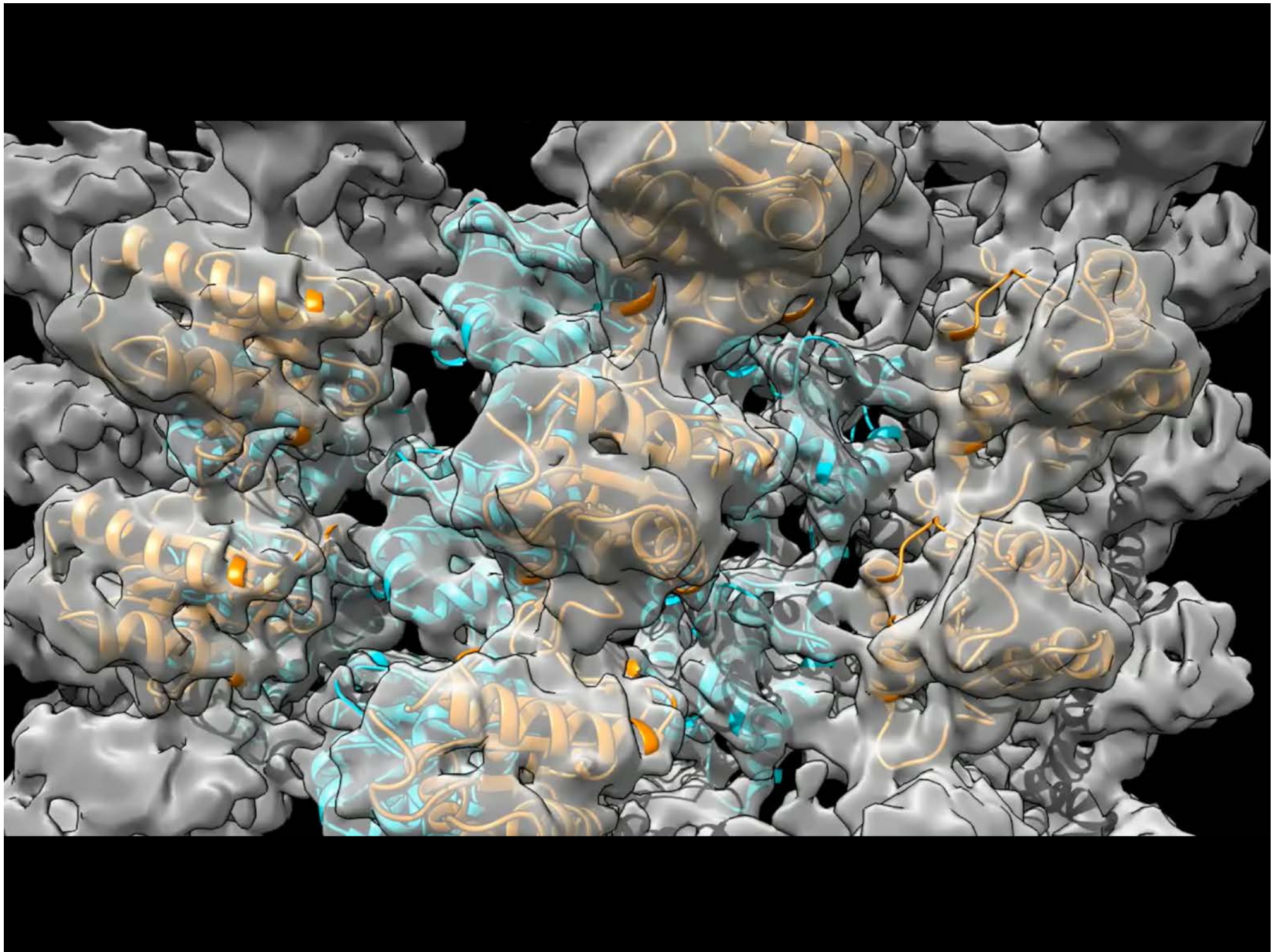
Merk et al
(2016) Cell
165, 1–10

Krio-elektronska microscopija

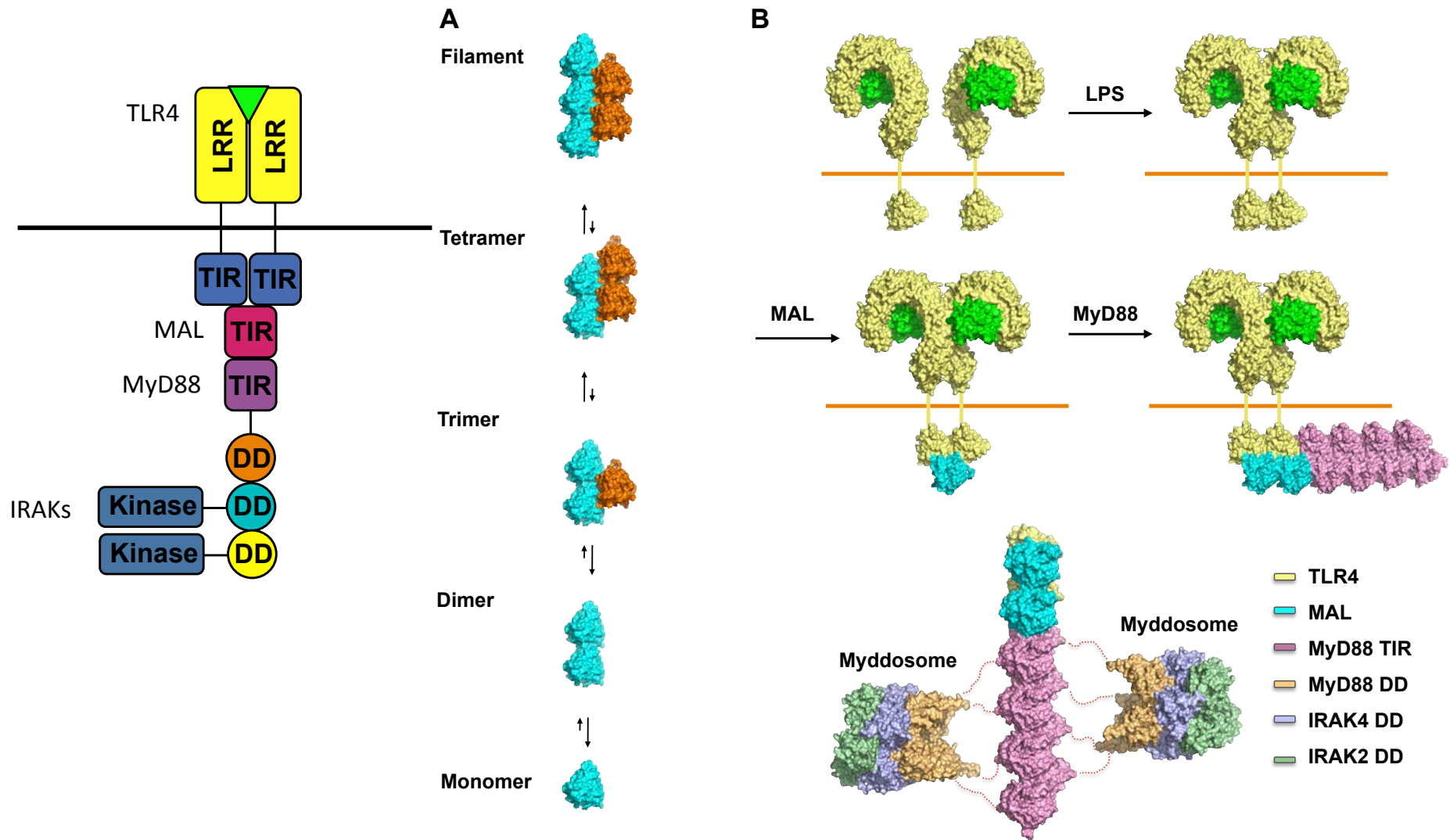


CryoEM struktura filamenta MAL^{TIR}

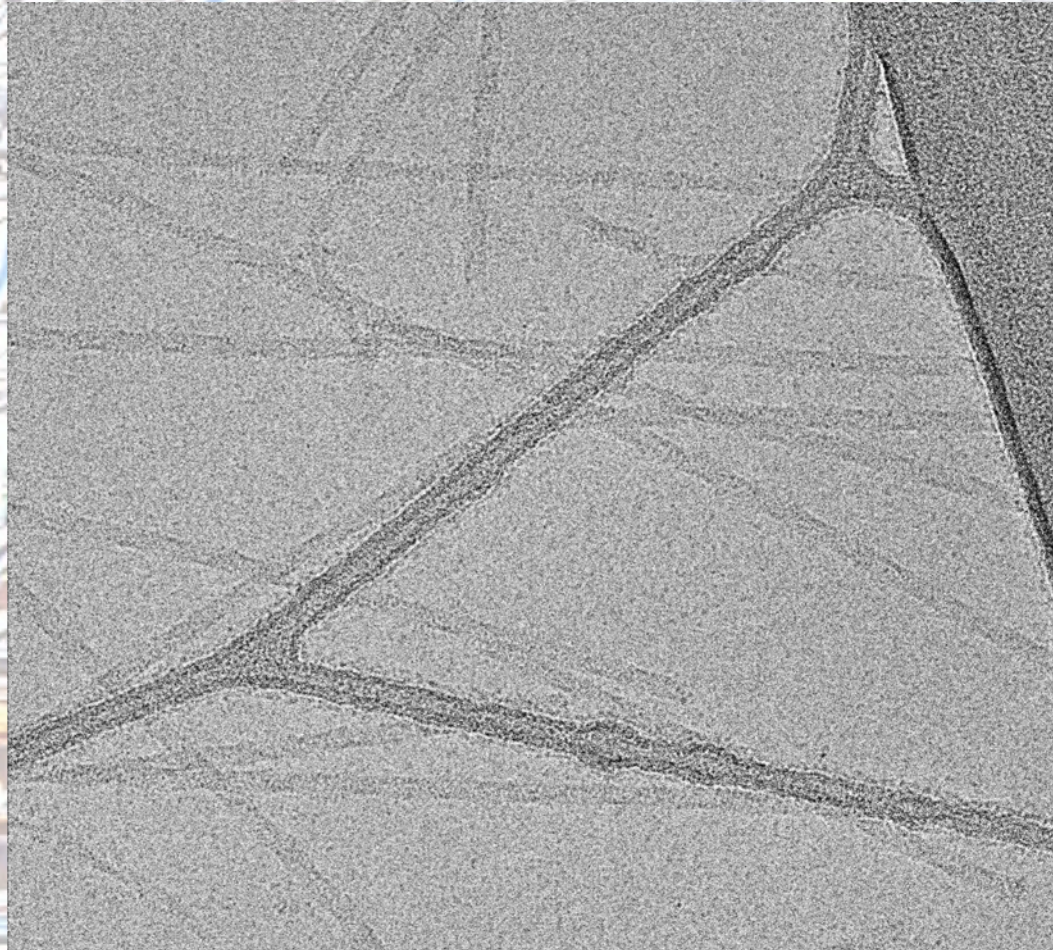




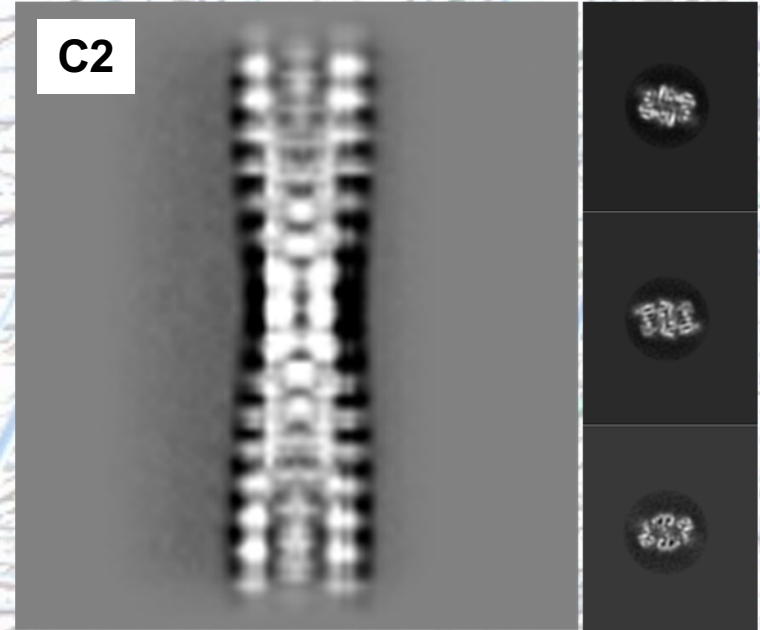
Model kooperativne formacije signalosoma TLR4



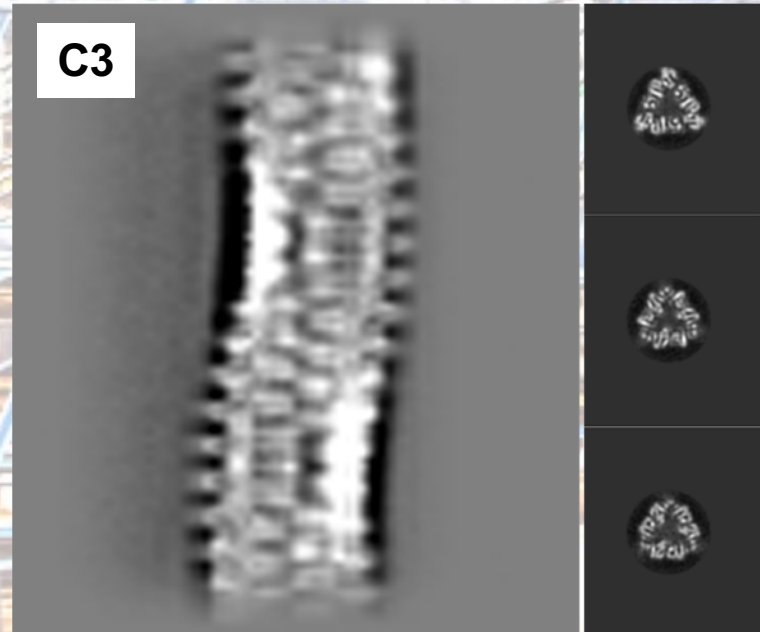
CryoEM struktura TLR4^{TIR}-MAL^{TIR} ko-filamenta



C2

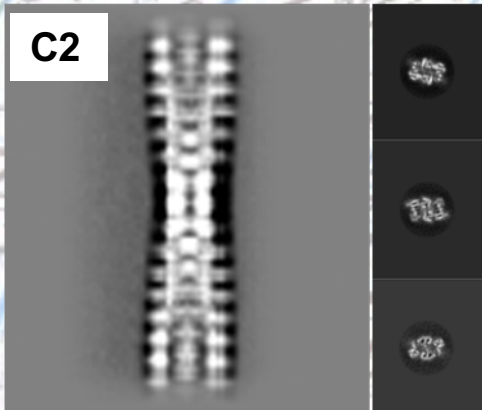


C3

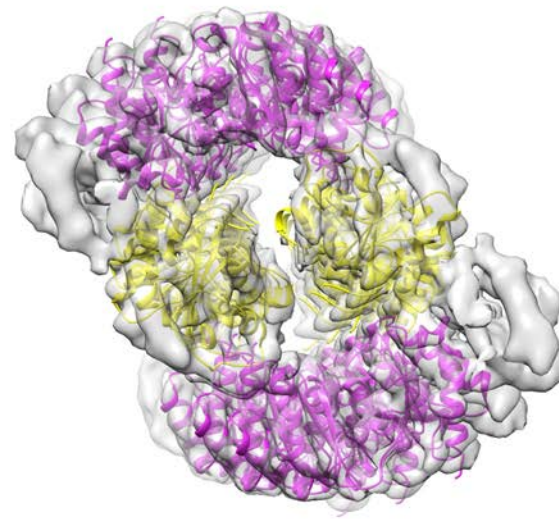




Jeff Nanson et al.

CryoEM struktura TLR4^{TIR}-MAL^{TIR} ko-filamenta

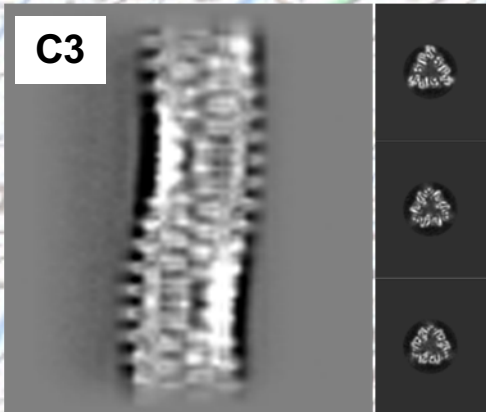


C2 symmetry
Twist = 11.6°
Rise = 31.5 \AA
~ 45,000 segments
Resolution = 4.4 \AA

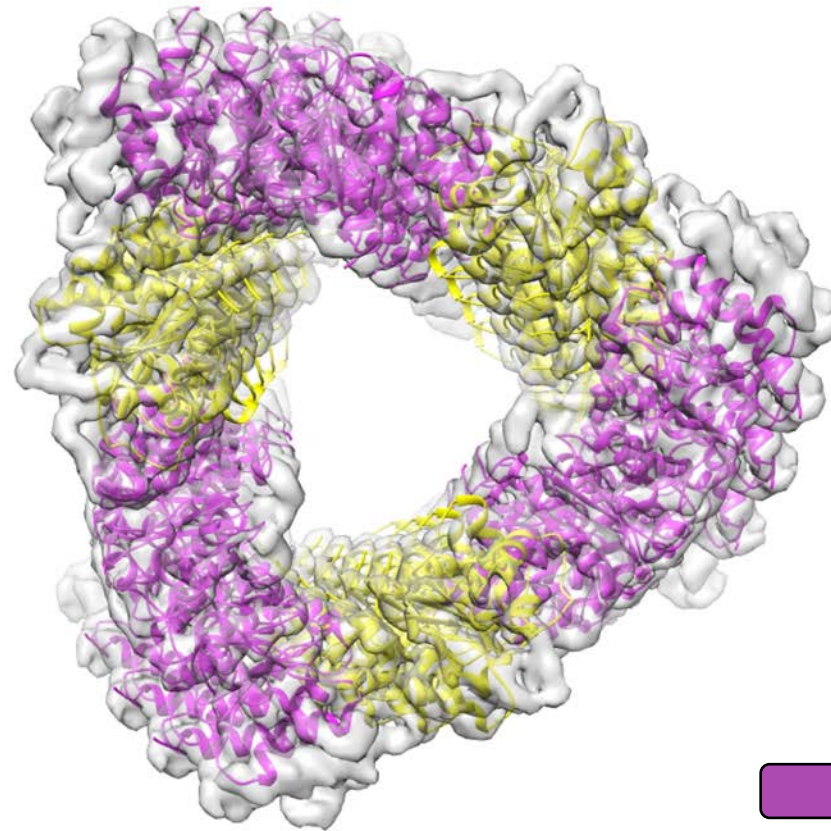


 MAL
 TLR4

CryoEM struktura TLR4^{TIR}-MAL^{TIR} ko-filamenta



C3 symmetry
Twist=9.2°
Rise=31.4Å
~70,000 segments
Resolution = 3.8 Å

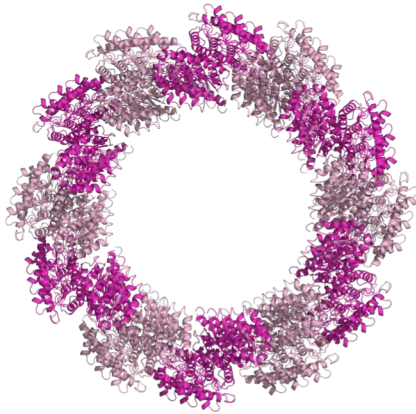


MAL
TLR4

CryoEM struktura TLR4^{TIR}-MAL^{TIR} ko-filamenta

MAL

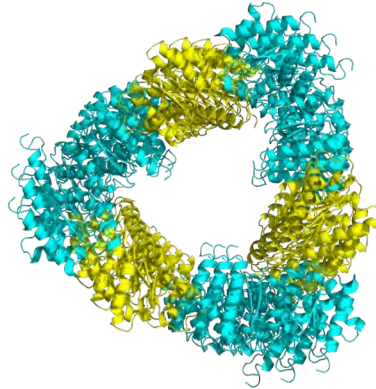
 MAL
 MAL



- 12 MAL protofilaments

C3 TLR4-MAL

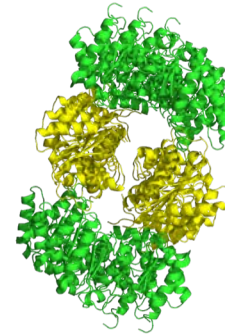
 MAL
 TLR4



- 3 MAL protofilaments
- 3 TLR4 strands

C2 TLR4-MAL

 MAL
 TLR4



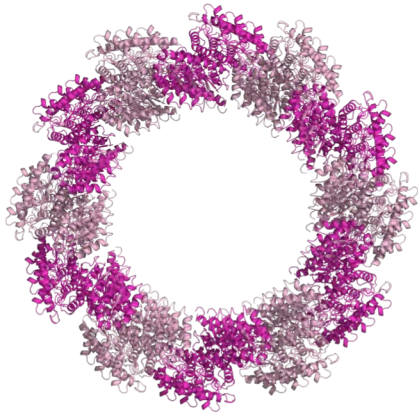
- 2 MAL protofilaments
- 2 TLR4 strands



CryoEM struktura TLR4^{TIR}-MAL^{TIR} ko-filamenta

MAL

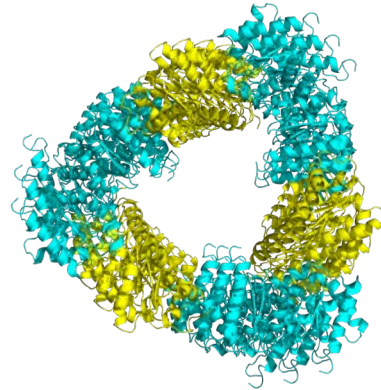
 MAL
 MAL



- 12 MAL protofilaments

C3 TLR4-MAL

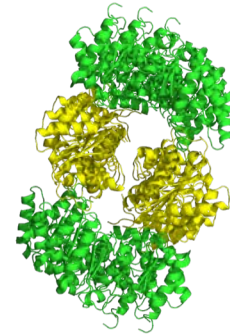
 MAL
 TLR4



- 3 MAL protofilaments
- 3 TLR4 strands

C2 TLR4-MAL

 MAL
 TLR4



- 2 MAL protofilaments
- 2 TLR4 strands



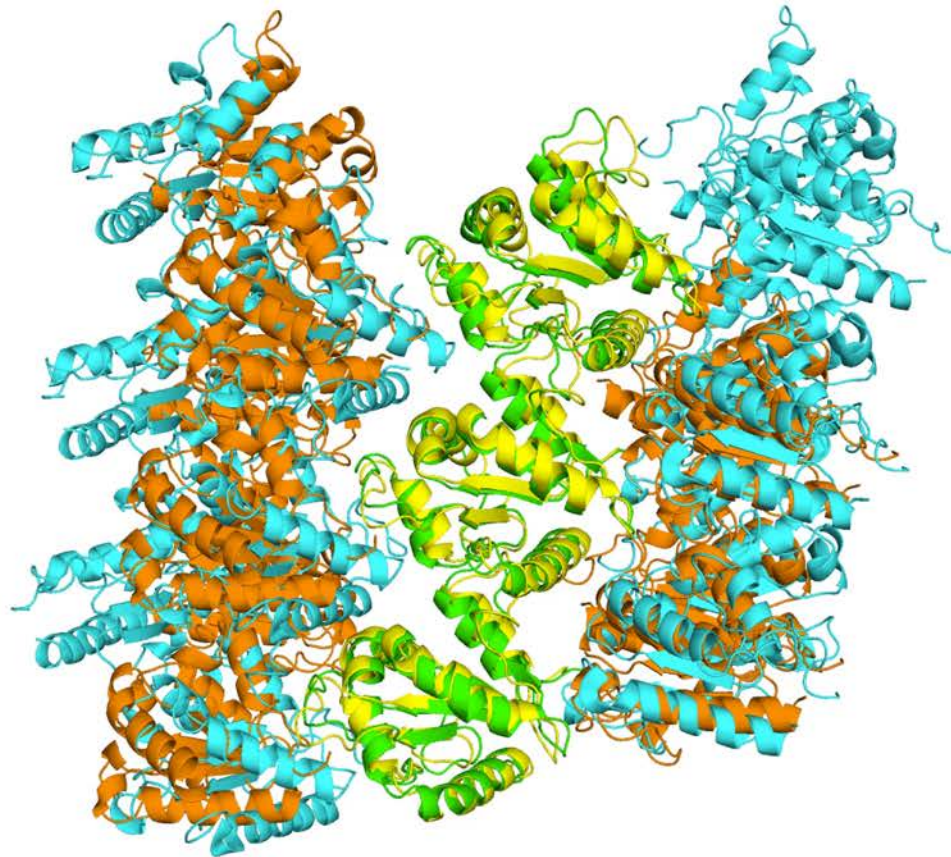
CryoEM struktura TLR4^{TIR}-MAL^{TIR} ko-filamenta

C3 TLR4-MAL

 MAL
 TLR4

C2 TLR4-MAL

 MAL
 TLR4



Interface
1

Interface
2

Interface 1:

TLR4: α C and α D helices, DD loop

MAL: α A and α E helices, AB loop

Interface 2:

TLR4: α E helix, BA loop

MAL: α D helix, AA and CC loops

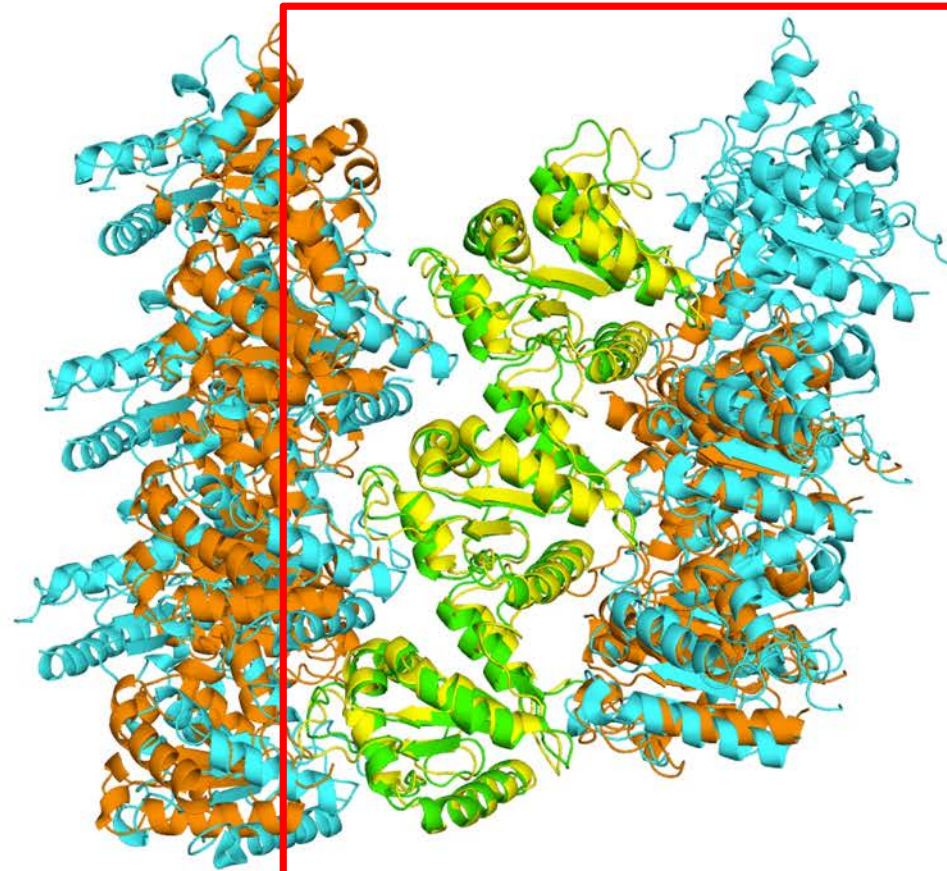
CryoEM struktura TLR4^{TIR}-MAL^{TIR} ko-filamenta

C3 TLR4-MAL

 MAL
 TLR4

C2 TLR4-MAL

 MAL
 TLR4



Interface
1

Interface
2

Interface 1:

TLR4: α C and α D helices, DD loop

MAL: α A and α E helices, AB loop

Interface 2:

TLR4: α E helix, BA loop

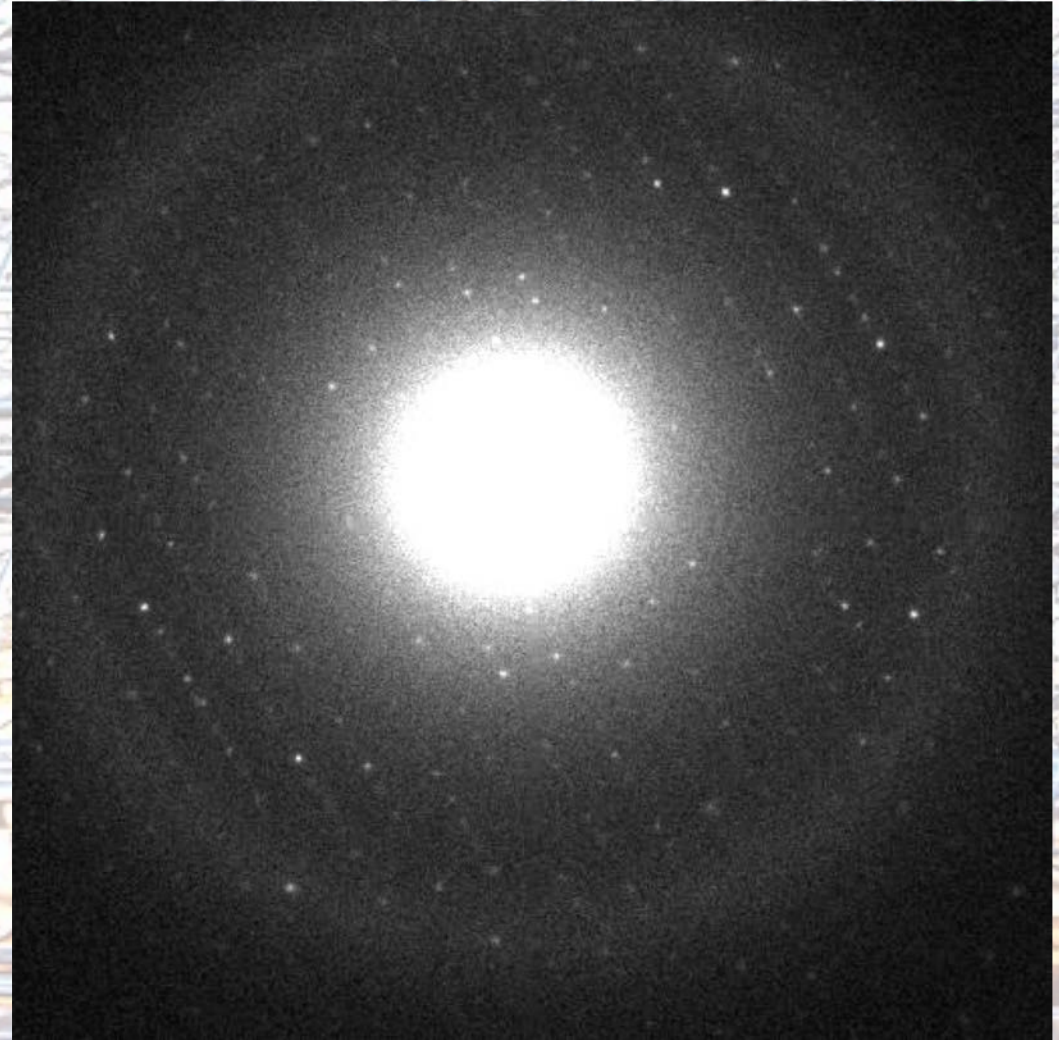
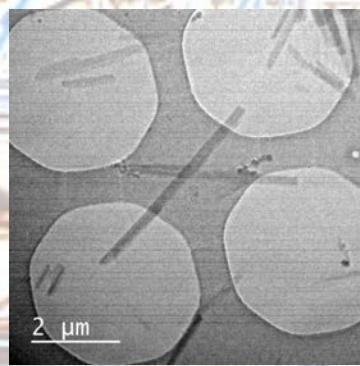
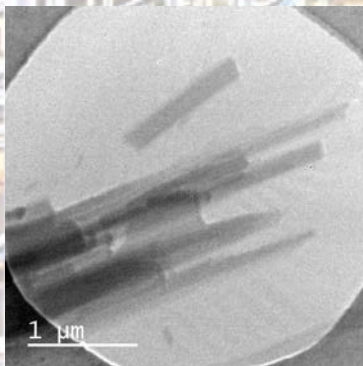
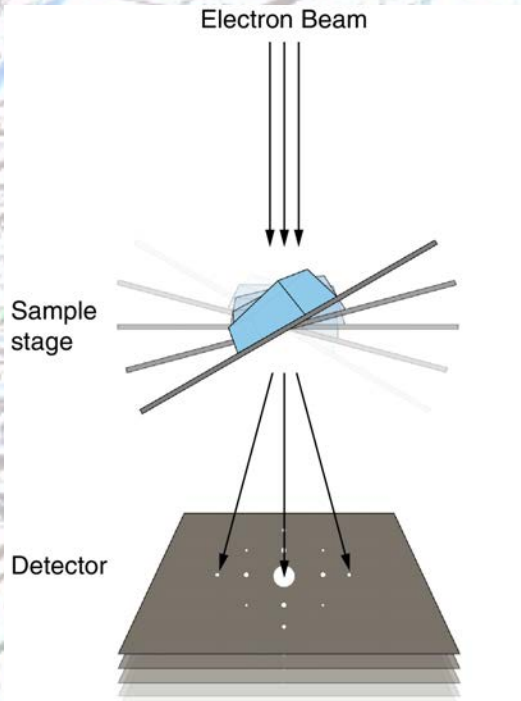
MAL: α D helix, AA and CC loops

Micro-electronsk difrakcija (microED)

MyD88^{TIR} nano-kristalov



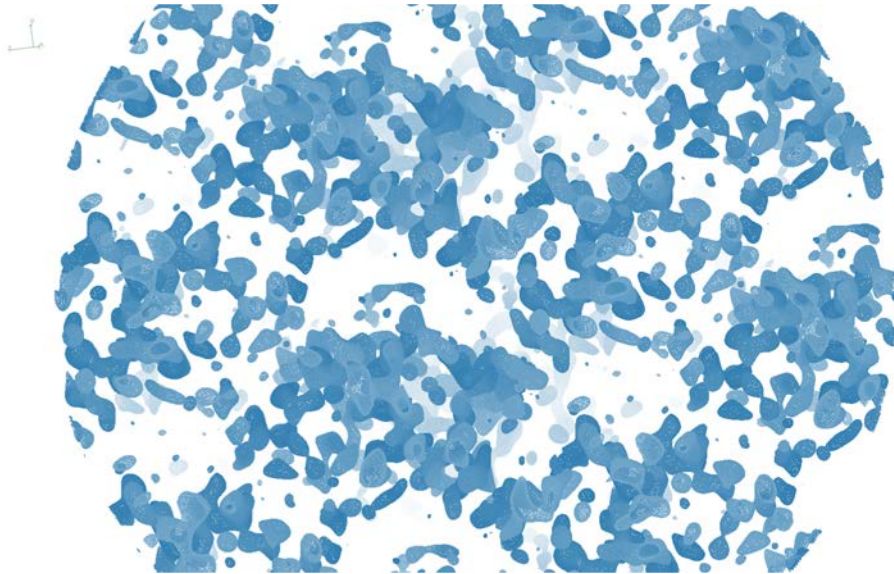
Nannenga and
Gonen (2014)
*Curr Opin
Struct Biol.*



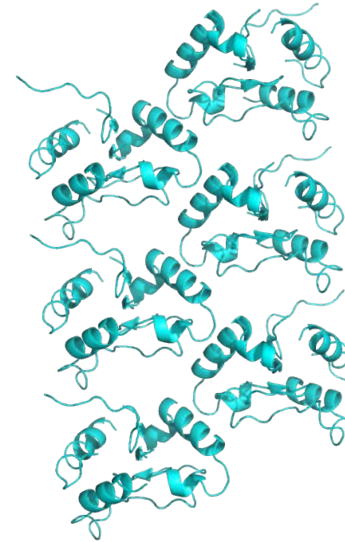
Hongyi Xu, Max Clabbers et al

MicroED MyD88^{TIR} nano-kristalov

Electrostatic potential map MyD88^{TIR}



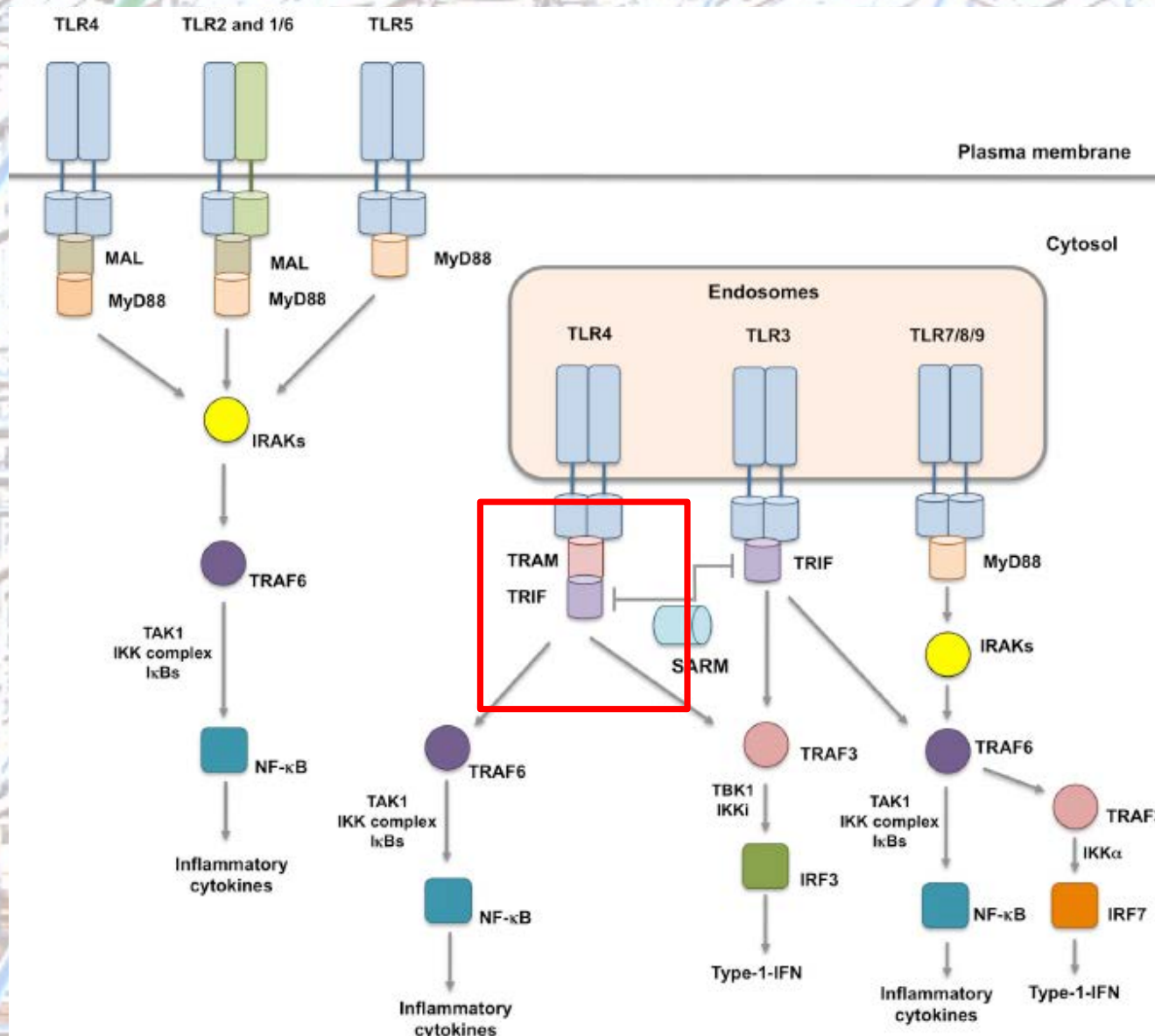
Packing of MyD88^{TIR}



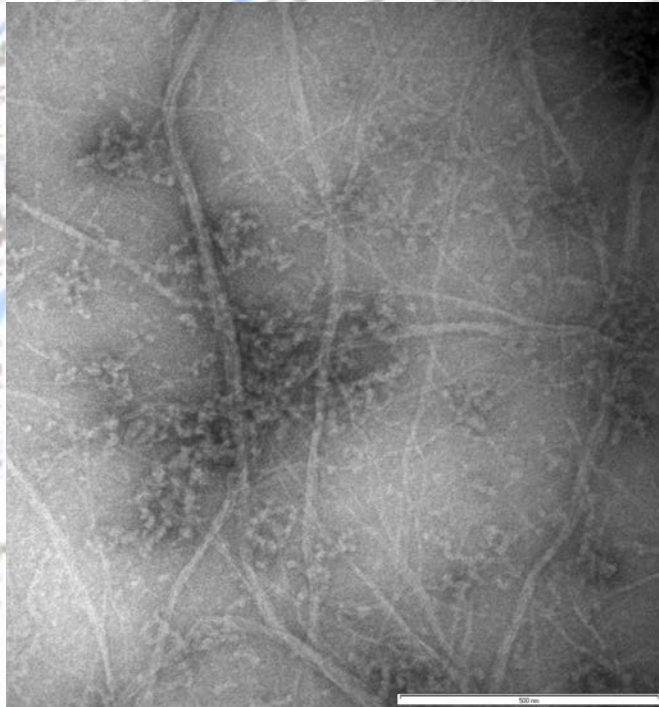
MAL^{TIR} filament



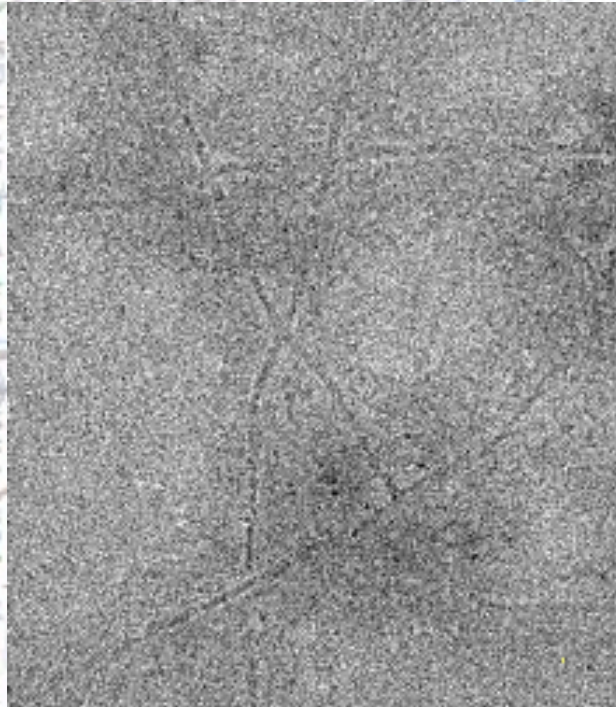
TRAM



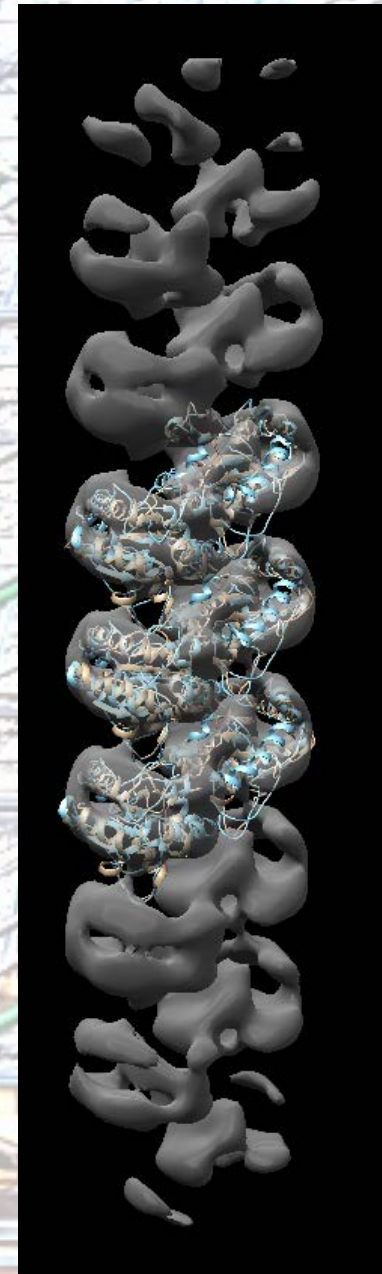
Filamenti TRAM^{TIR}



Negative-stain EM

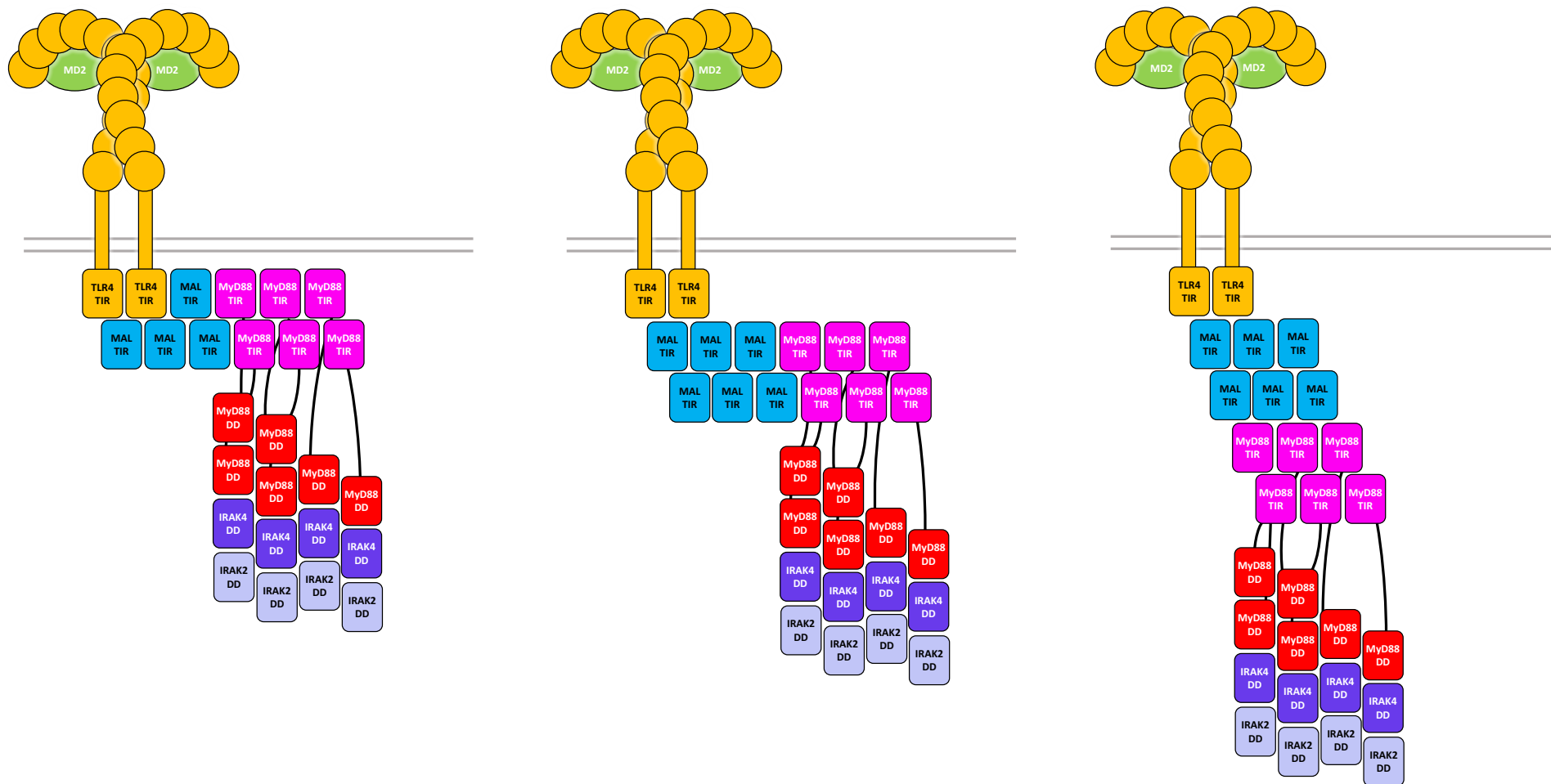


CryoEM

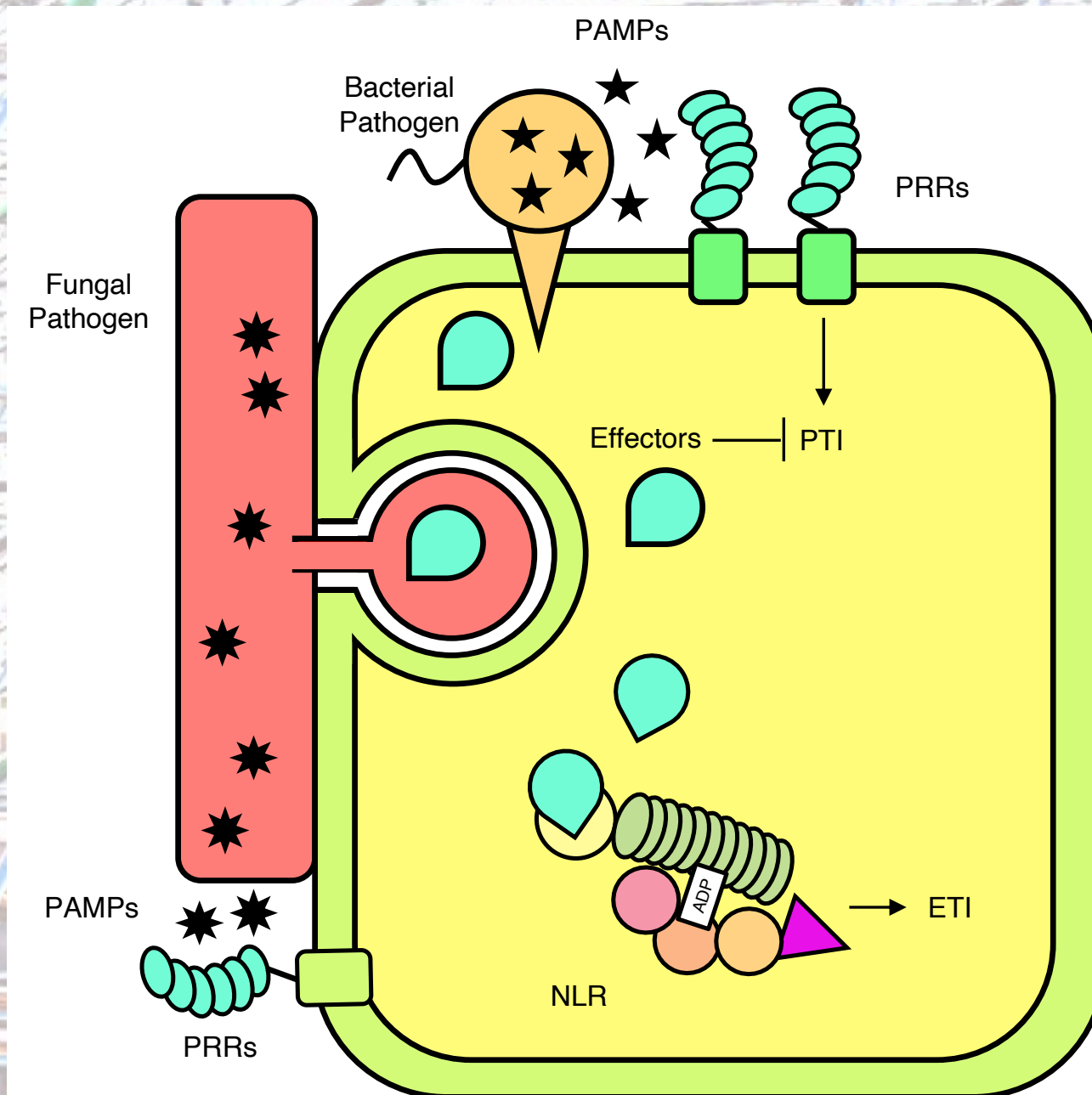


3D rekonstrukcija

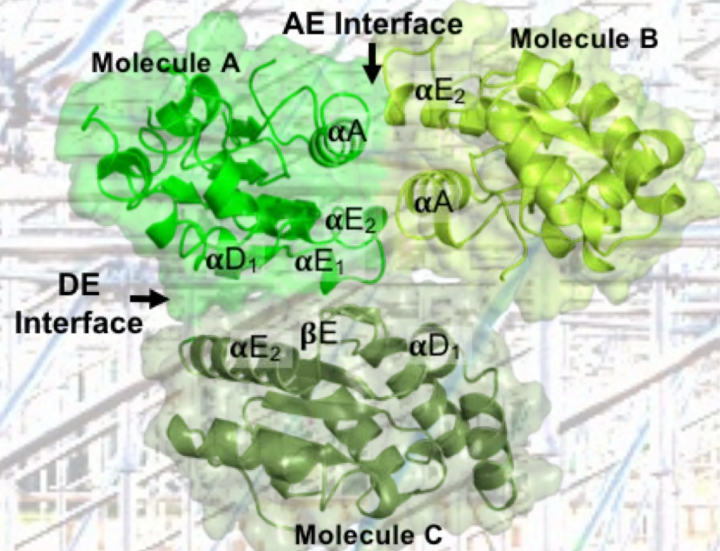
Model kooperativne formacije signalosoma TLR4



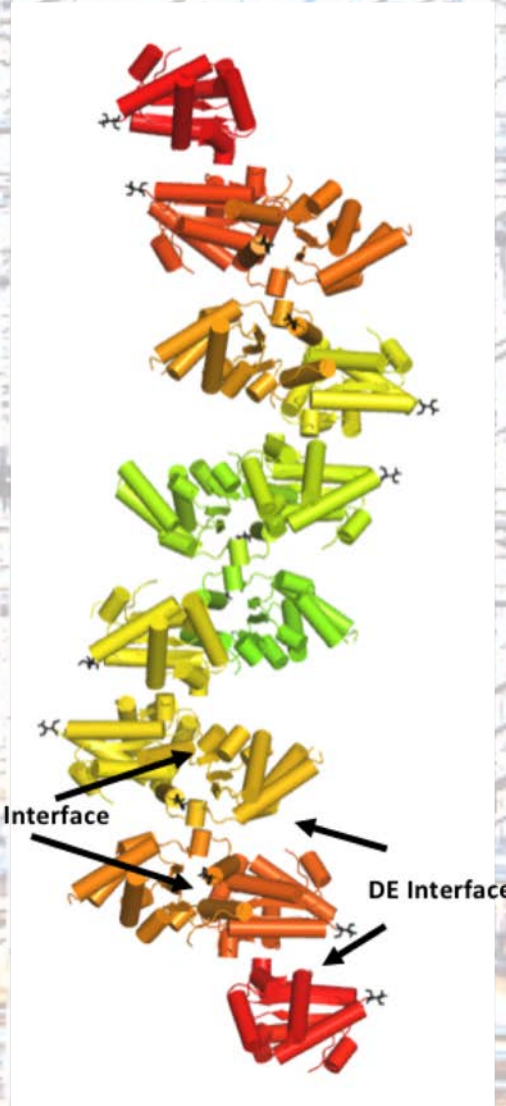
Imunski sistem v rastlinah



Neomejeni kompleksi TIR domen v rastlinskih NLR

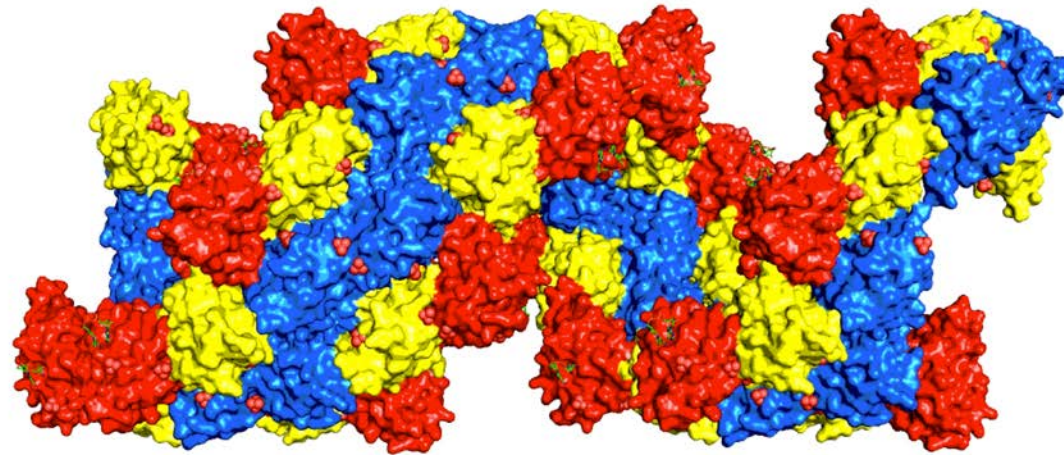


Kristalna struktura f SNC1^{TIR}

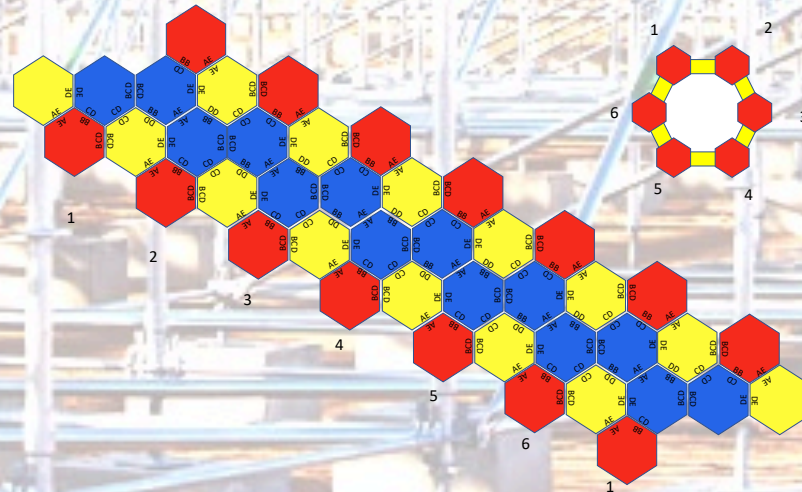


Zhang et al &
Kobe (2017)
PNAS 14, E2046-
E2052.

Neomejeni kompleksi TIR domen v rastlinskih NLR

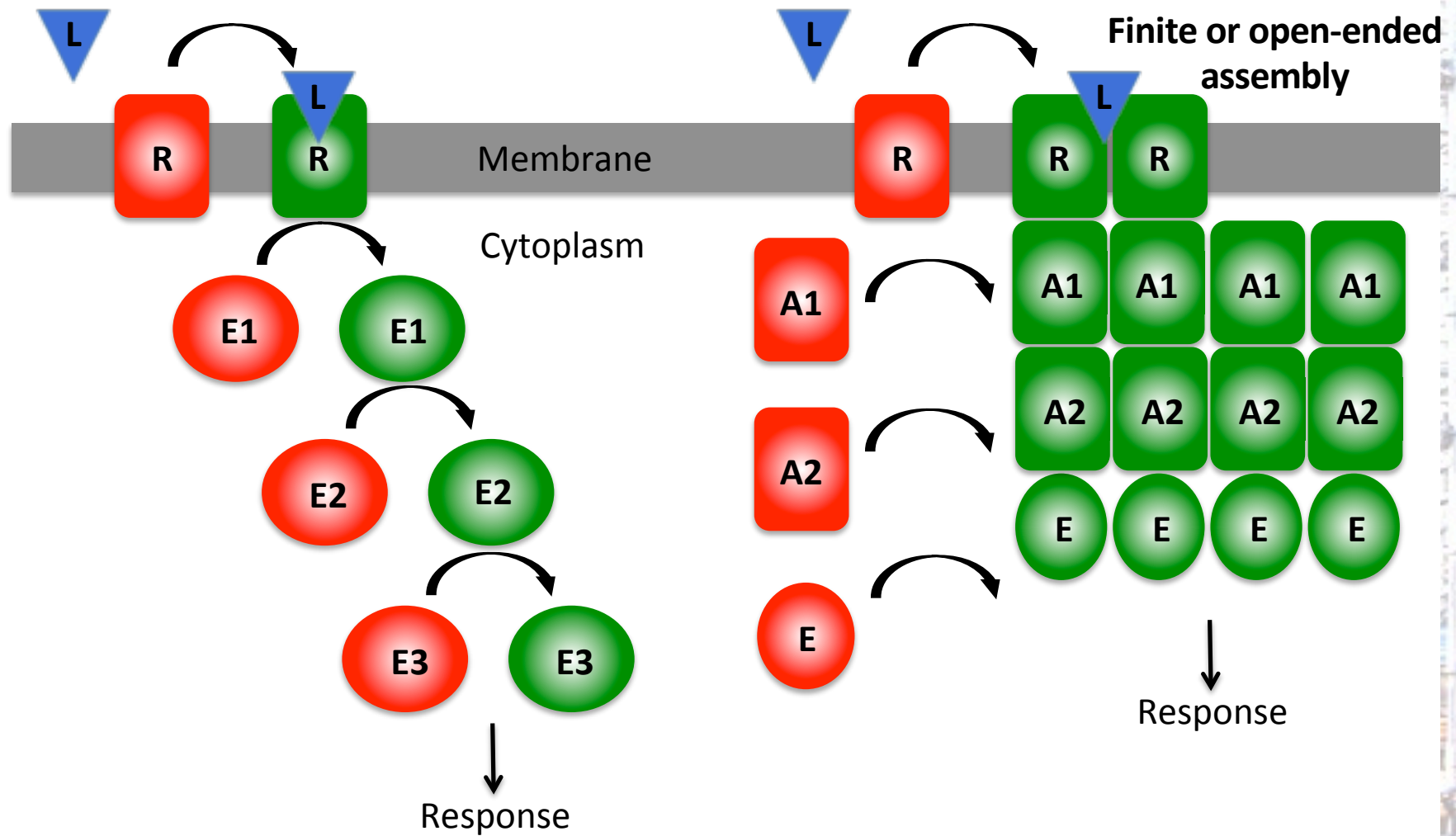


RUN1^{TIR}



Hayden Burdett et al

Signaling via cooperative assembly formation (SCAF)





Povzetek

- **Funkcija makromolekul temelji na njihovi 3D strukturi**
- **Strukturna biologija koristi terapiji in biotehnologiji**
 - **Poznavanje detajlov funkcije**
 - **Razumevanje učinka mutacij ki vodijo do bolezni**
 - **Razvoj zdravil**
 - **Inžinerstvo proteinov za biotehnološke aplikacije**
- **TIR domene v TLR tvorijo filamente; naravni imunski odziv na splosno temelji na SCAF**
- **TIR domene v SARM1 in rastlinskih NLR so encimi, ki cepijo NAD⁺ (Horsefield et al, Science (2019) 365: 793) – nova varianta SCAF**
- **Strukture bodo pomagale razviti zdravila proti kroničnim boleznim vnetja**

Sodelavci

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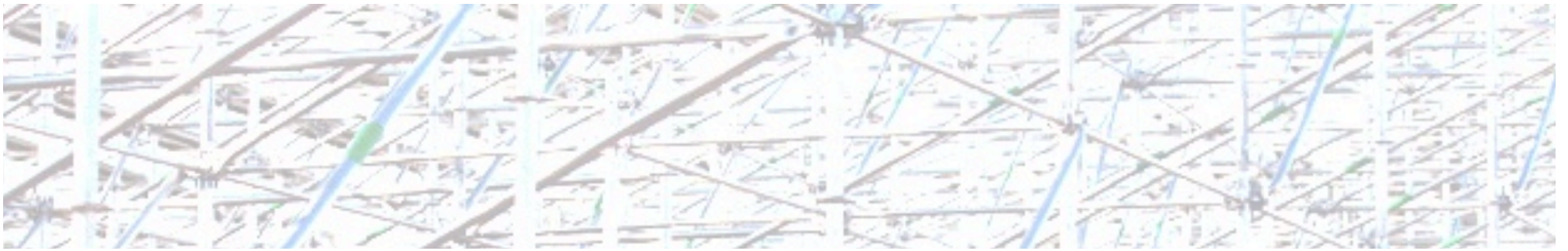
Financiranje



Australian Government
Australian Research Council



Australian Government
National Health and
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I Proteins

