Journal Club
Tong Wang

3D Reconstructions of TRPV1 by Single-Particle Cryo-EM

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Nature **504**, 113-118 (2013)

TRP Ion Channels

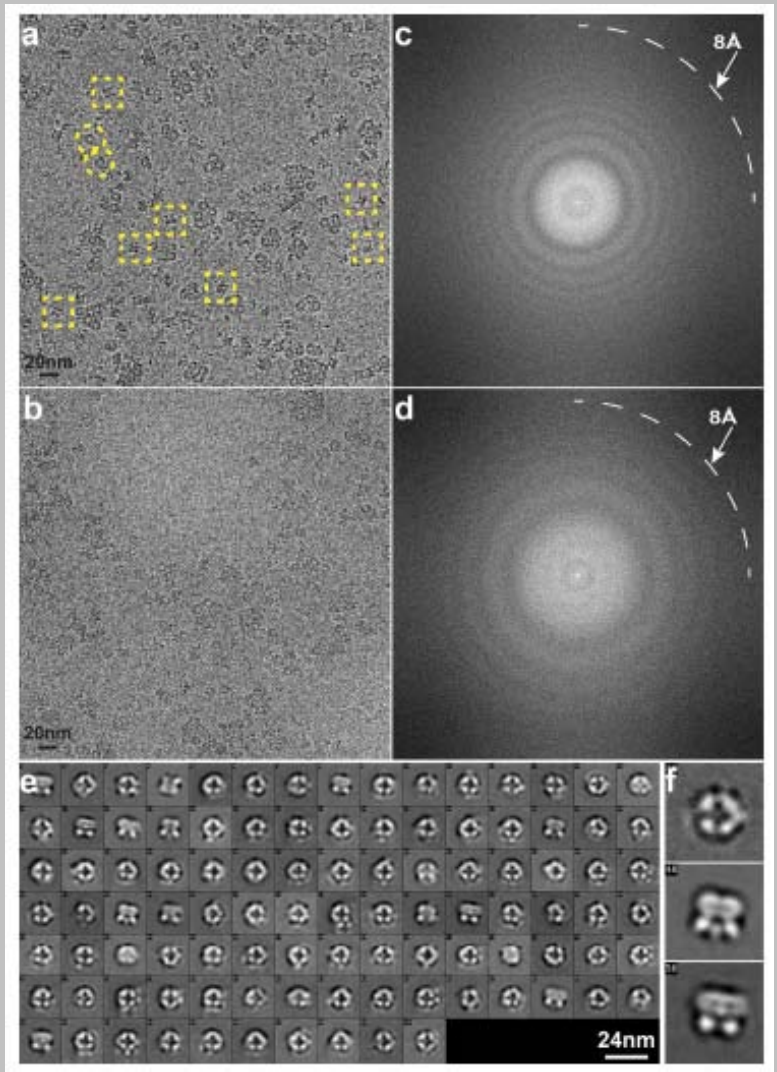
- TRP ion channels are members of family of non-selective cation channels. TRP channels are believed to resemble VGICs. However they share little in common with VGICs with regards to pharmacological and biophysical properties.

- No atomic structures have been available for any member of TRP family.

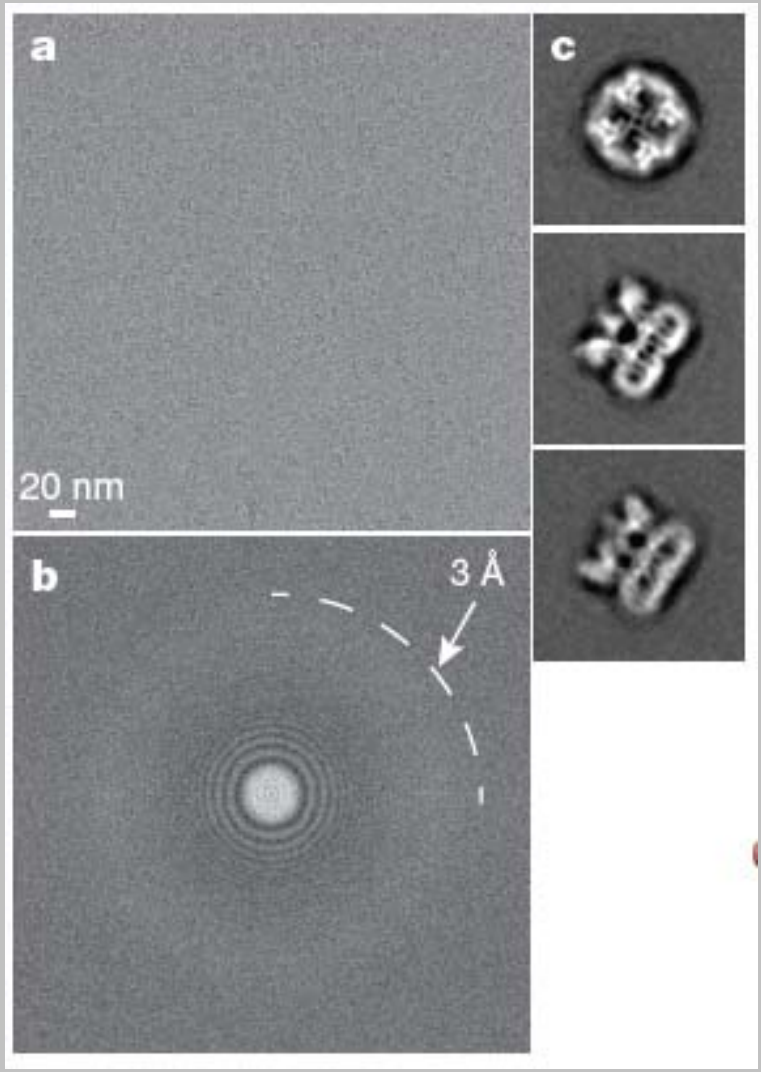
- Mammalian TRPV1 is heat-sensitive (thermo) ion channel and the receptor for capsaicin (in chili pepper) that elicits burning pain. It is also strongly activated by toxins. TRPV1 is arguably the best-characterized member of the vertebrate TRP family.

- TRPV1 (apo) at 3.4 Å; TRPV1-ligands (3.8 Å and 4.2 Å).

3D Reconstruction of TRPV1 (apo) by Single-Particle Cryo-EM

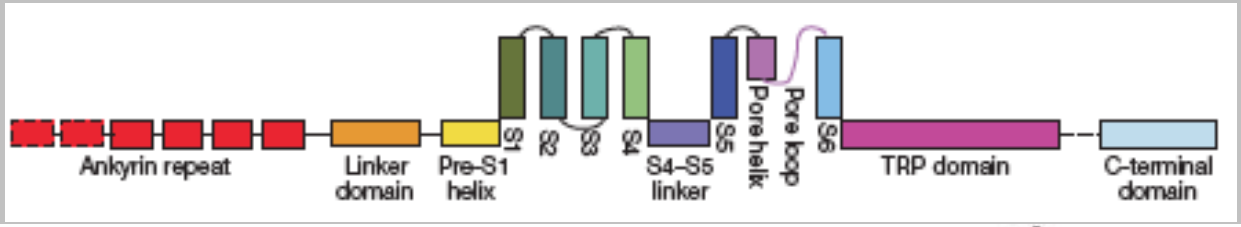


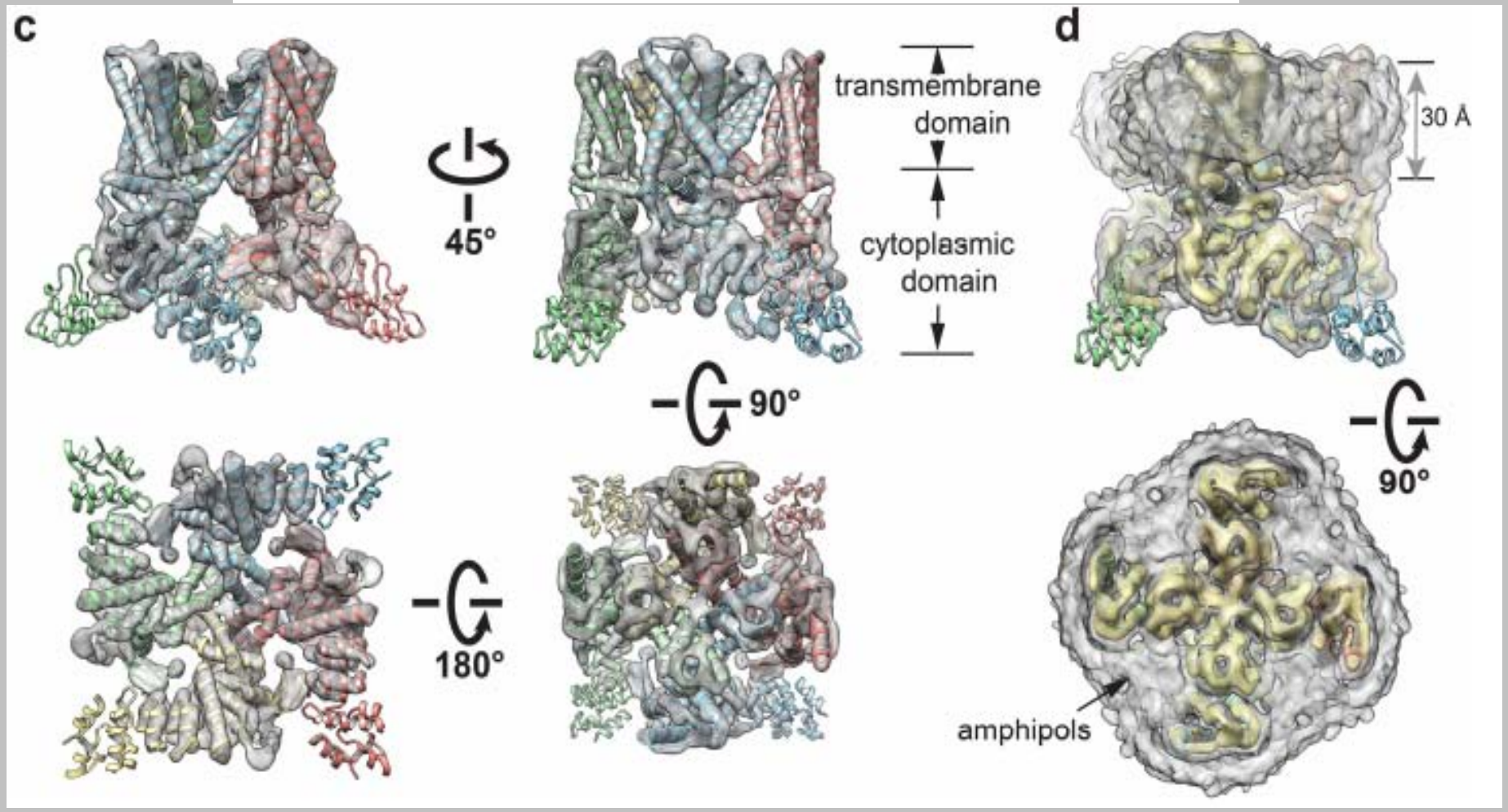
No motion correction
CMOS camera



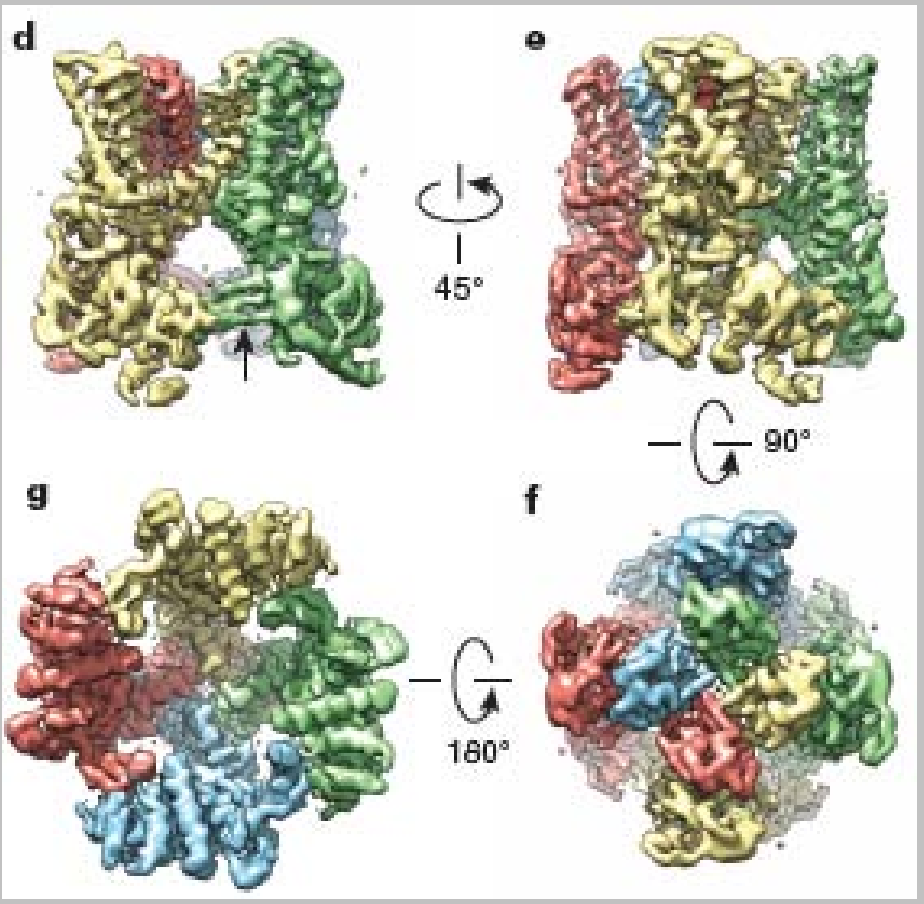
Motion correction
Direct electron detect camera

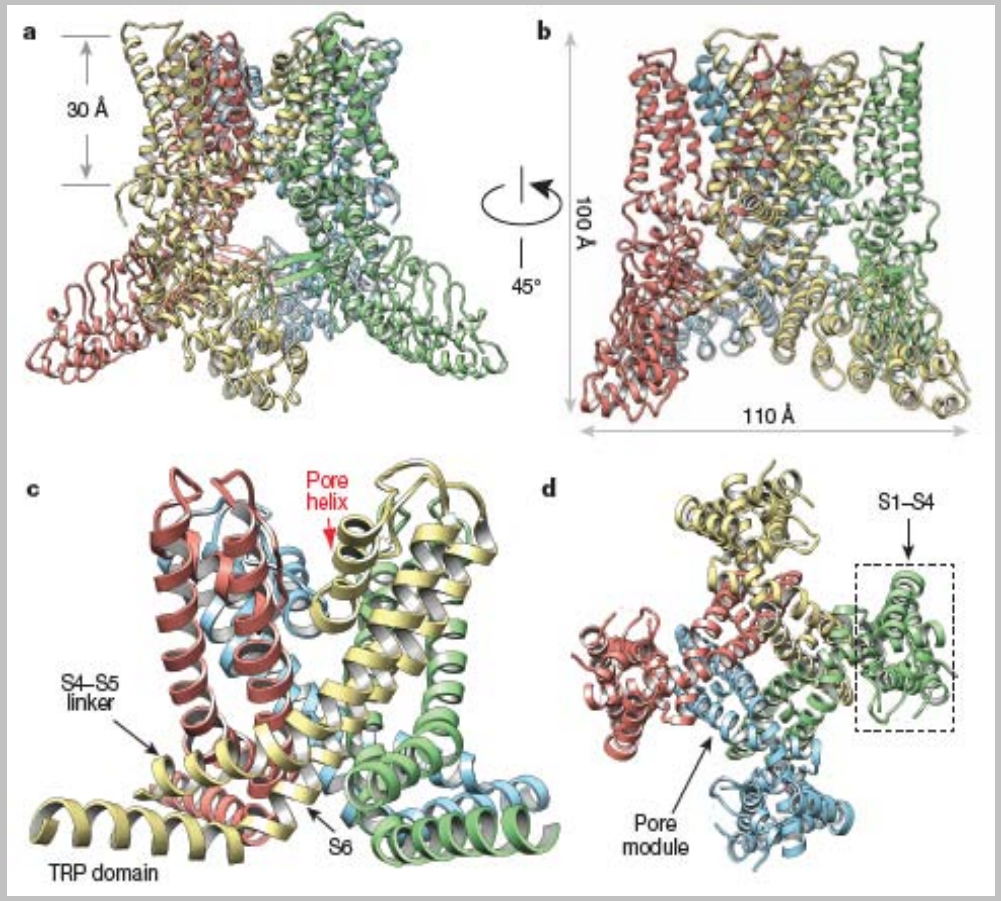
3D Density Map and Model Construction



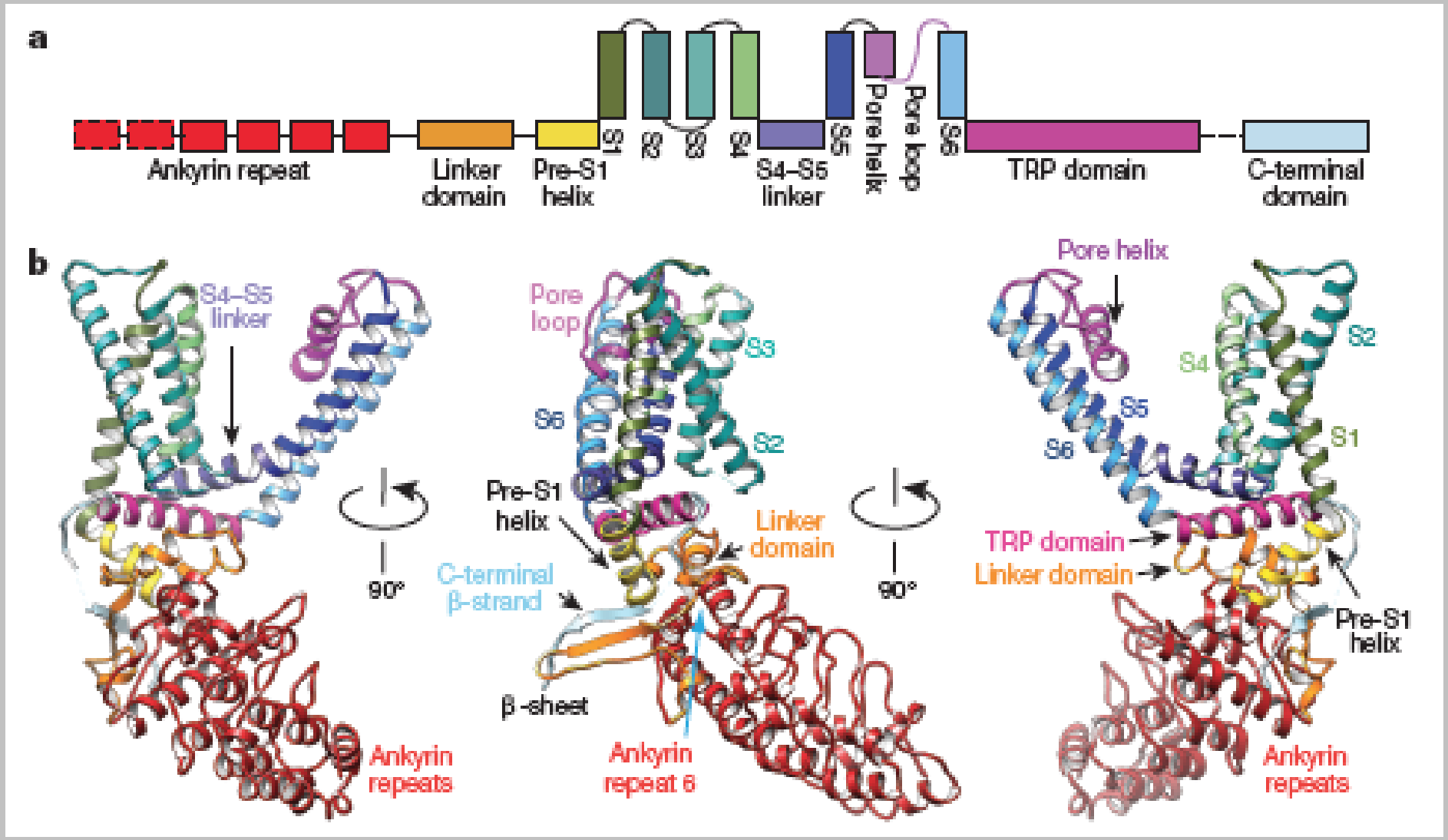


General Architecture of TRPV1

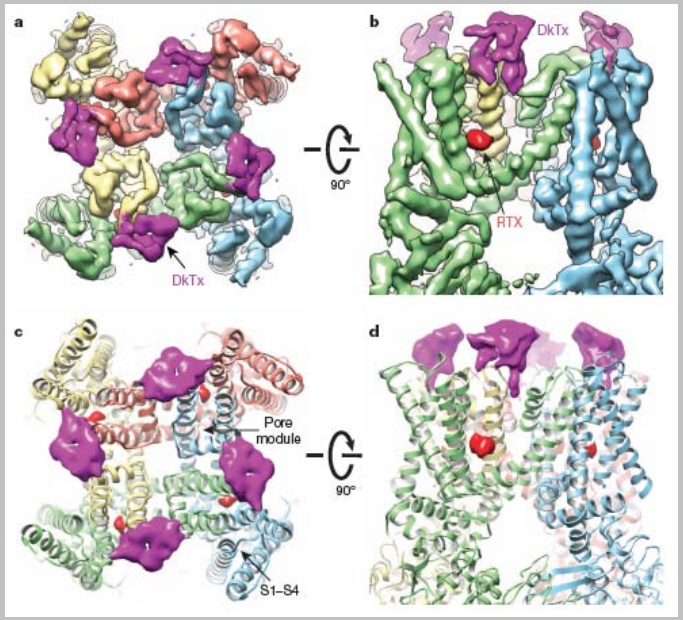


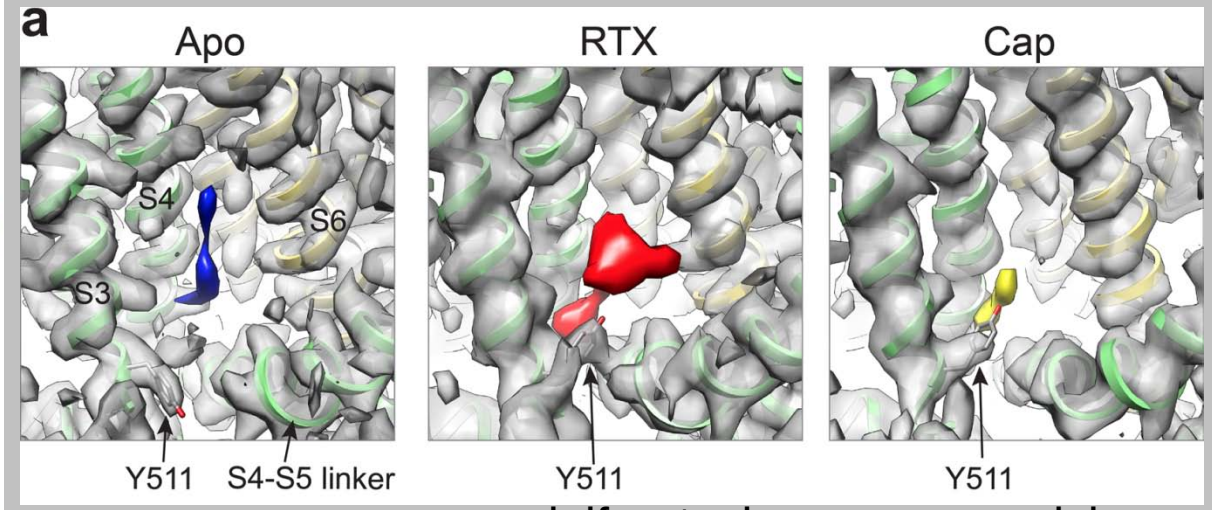


TRPV1 Subunit

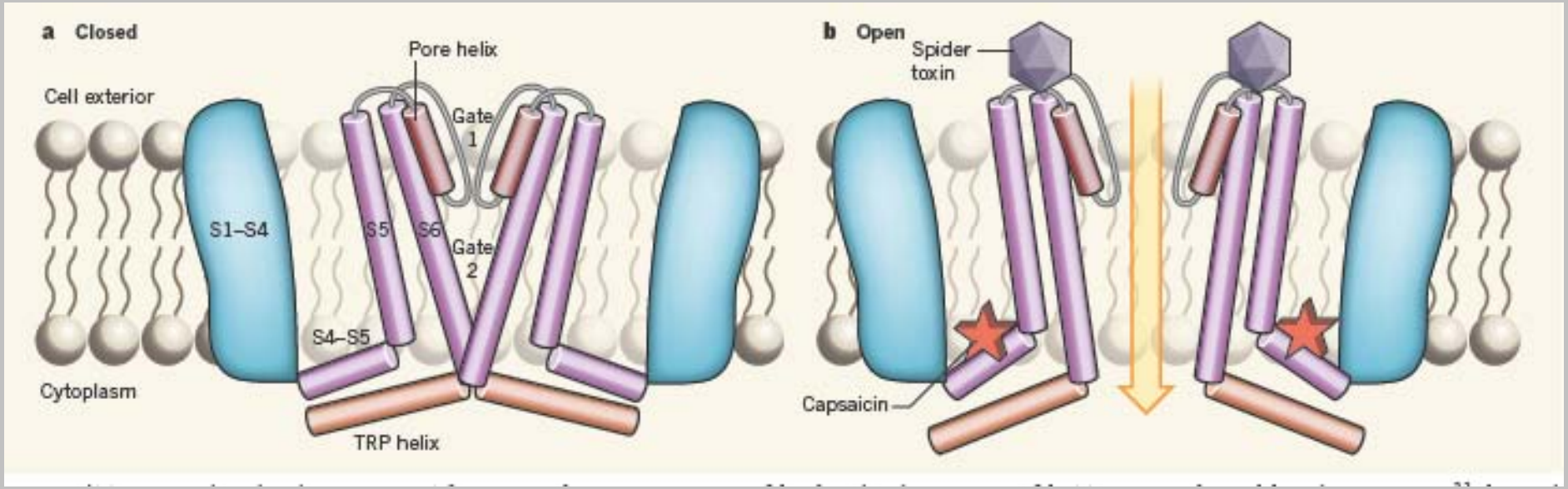


TRPV1-Ligands

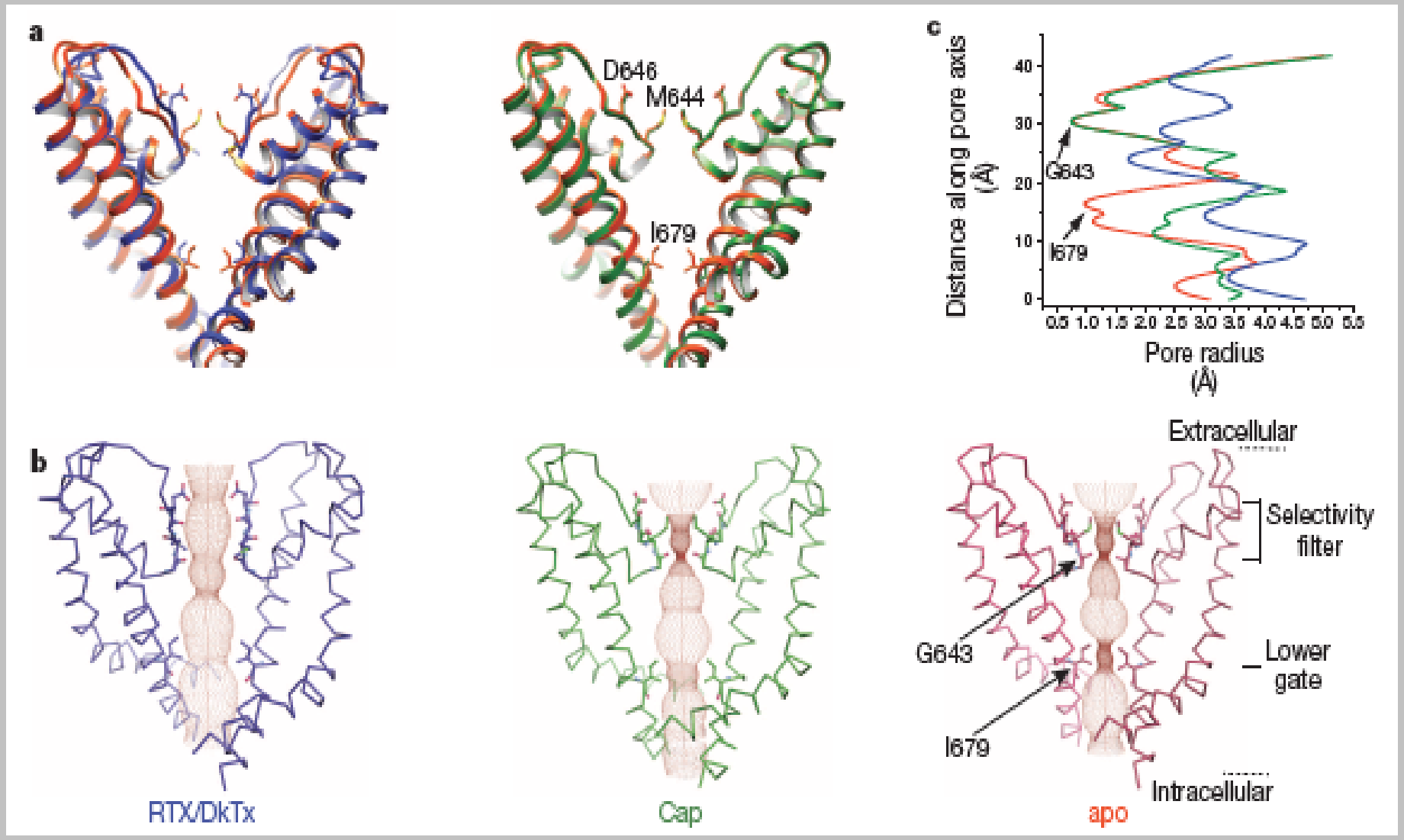




resiniferatoxin capsaicin



Ion Permeation Pathway



Dual Gates

Factors Contributing to High Resolution

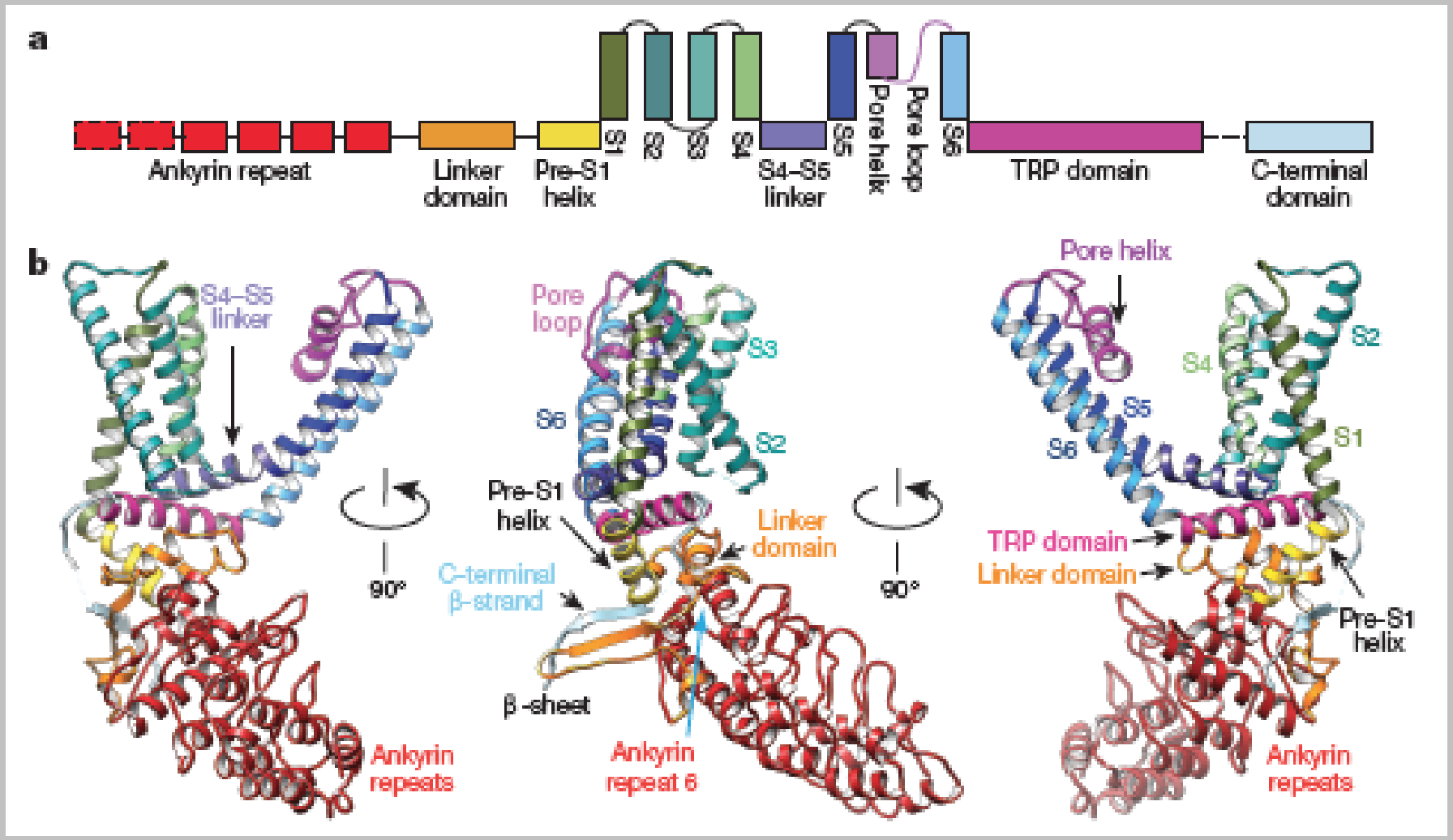
- A minimal functional rat TRPV1 construct (110 to 603 and 627 to 764), ~ 300 kDa for tetramer.

- Detergent exchange from DDM to Amphipols that maintain the channels' stability and solubility in water.

- K2 summit direct electron detector camera in super-resolution counting mode that enables newly implemented procedures of dose fractionation and motion correction to eliminate motion-induced image blurring.

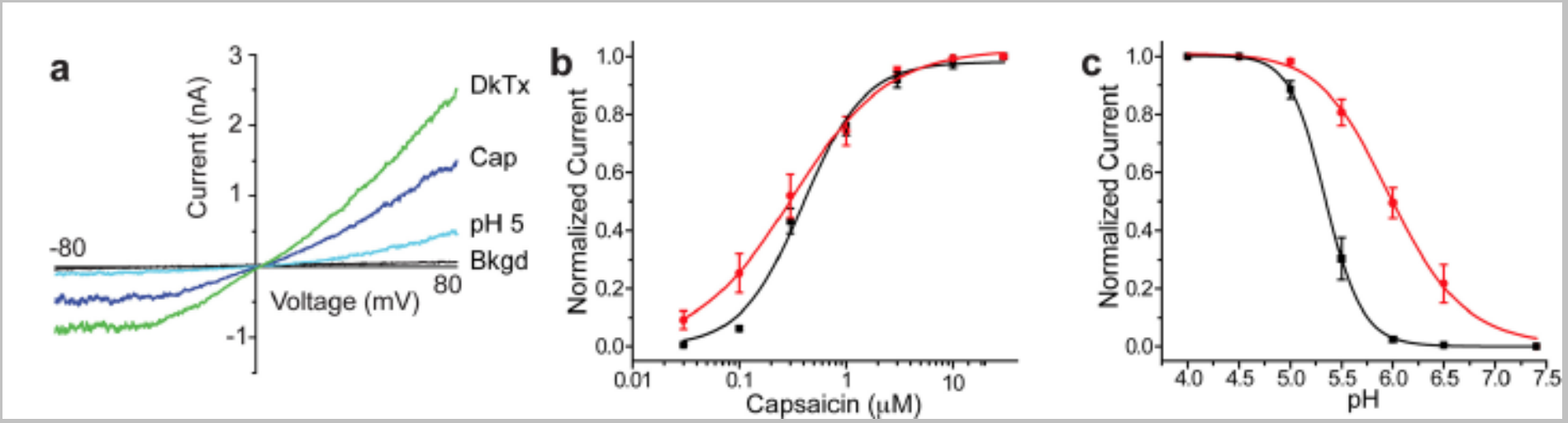
- State-of-the-art computer program that use statistical methods to deal with sample heterogeneity.

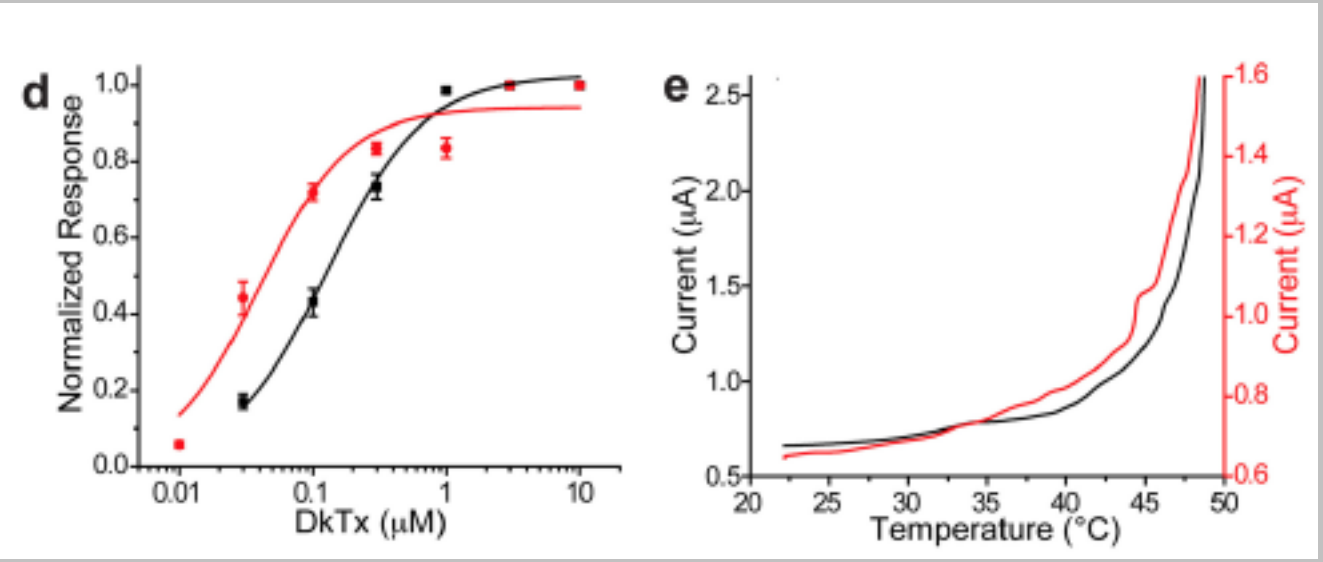
Minimal Functional Construct



Residues 110-764, excluding 604-626 (between S5 and Porehelix)

Minimal Functional Construct



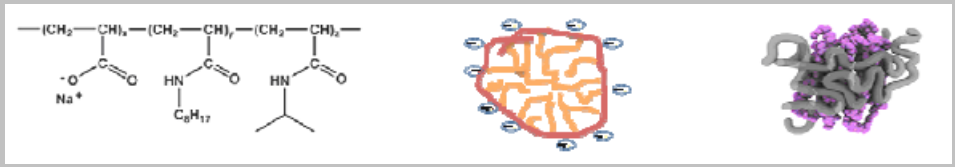


Detergent (Amphipols)

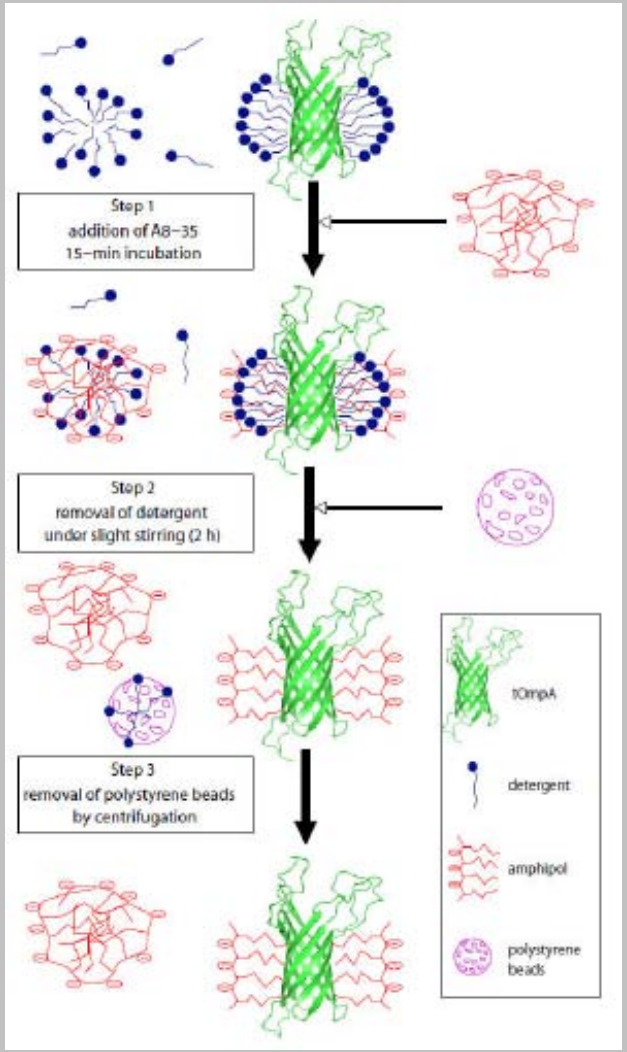
- The term amphipol (Apol) refers to “an amphipathic polymer that is able to keep individual MPs soluble under the form of small complexes”.
- Detergents may destabilize membrane proteins, possible because of lossing physical constrain and competitons of detergents with protein-protein and protein-lipid interaction.
- There are many types of Amphipols: polyacrylate-based Apols (A8–35),phosphorylcholine-based APols (PC-APols),lucose-based, nonionic APols (NAPols), sulfonated APols (SAPols), etc. A8-35 is the first and the most extensively studied APol to date.
- They self-assemble into well-defined particles comprising a few macromolecules, four molecules, on average, for 9- to 10-kDa A8–35, three for 25-kDa NAPols.
- The amount of APols required for efficient trapping is an important parameter that must be established early in any study. The ratio depends on the protein of interest and can be determined by carrying out quick trapping tests using a concentration range of APols, dilution below the CMC of the detergent, and ultracentrifugation.

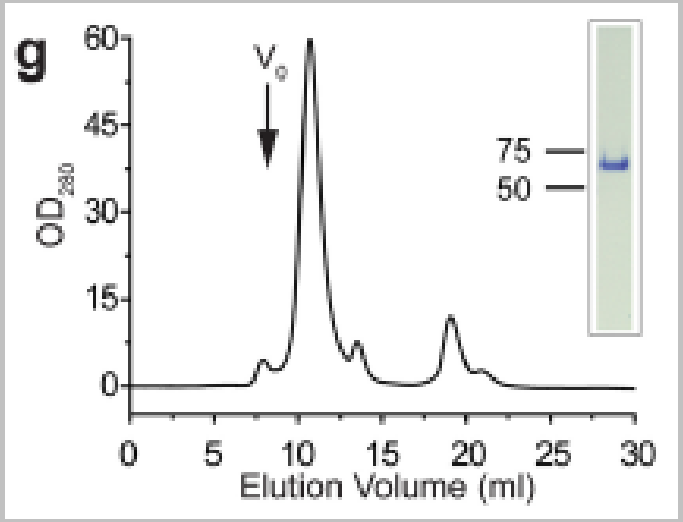
Annu. Rev. Biophys. 40, 379–408 (2011)

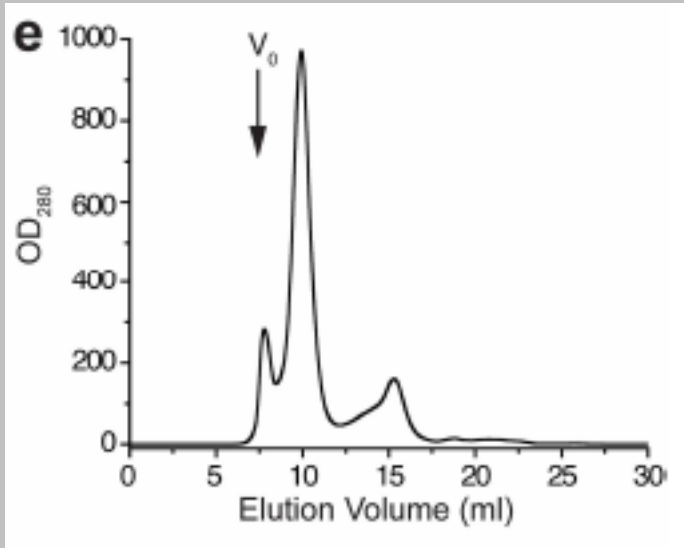
Detergent (Amphipols)

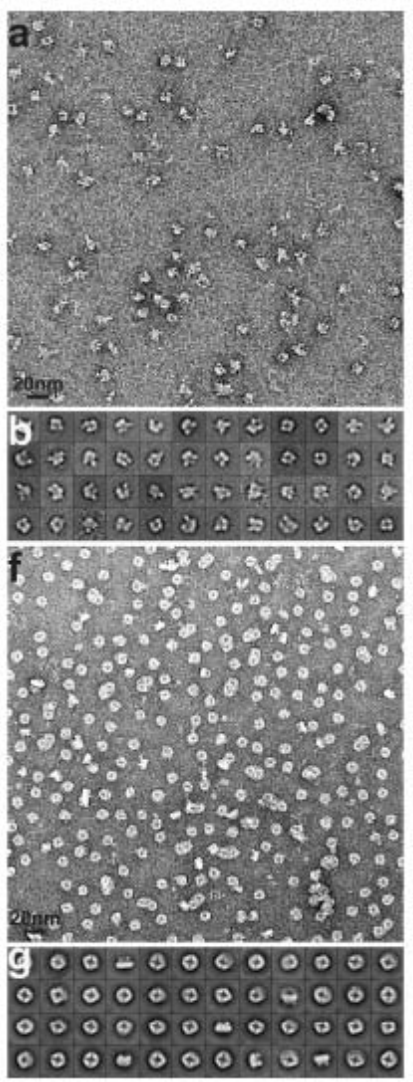


Reducing inactivation by the detergent and preserving membrane protein native structure in detergent-free solution.



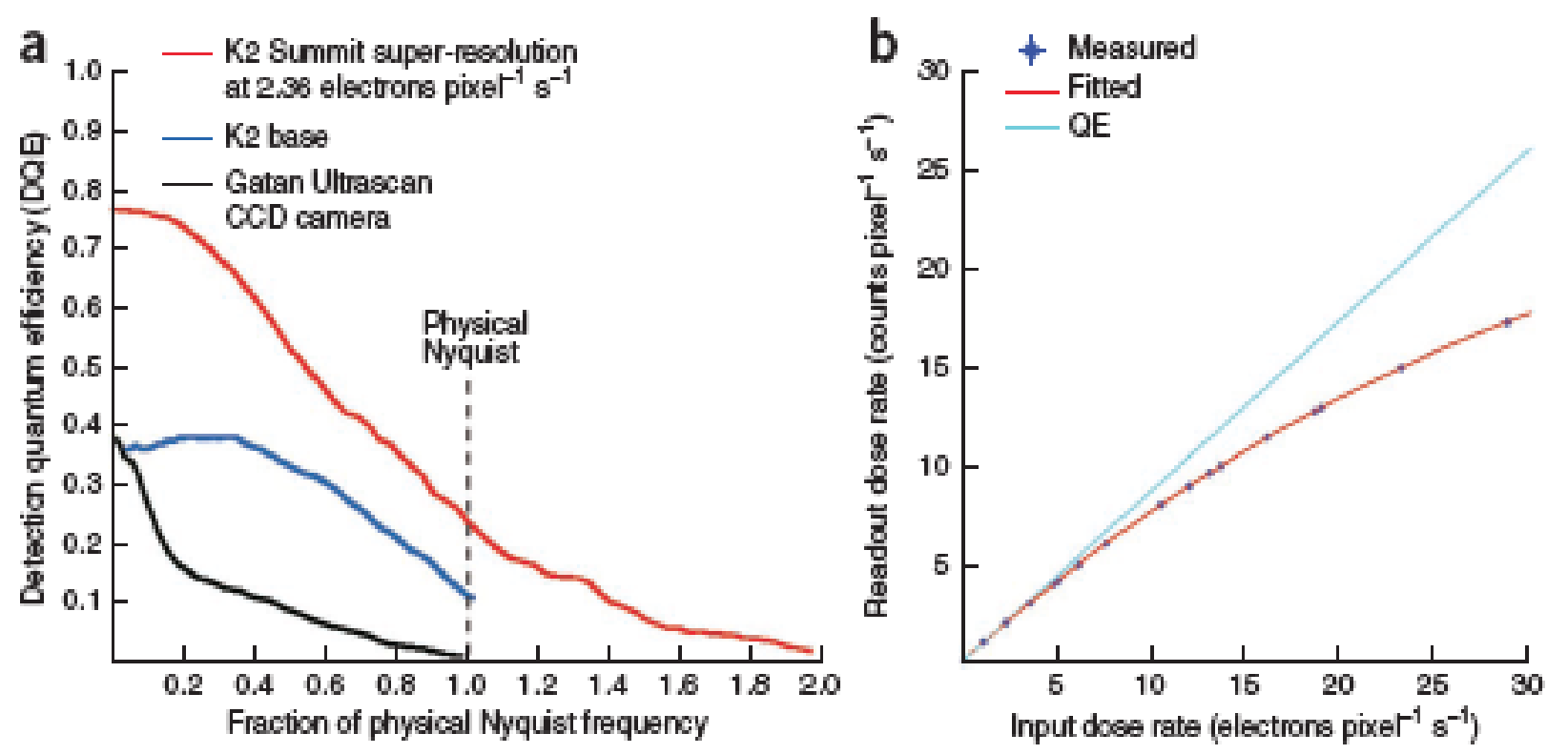






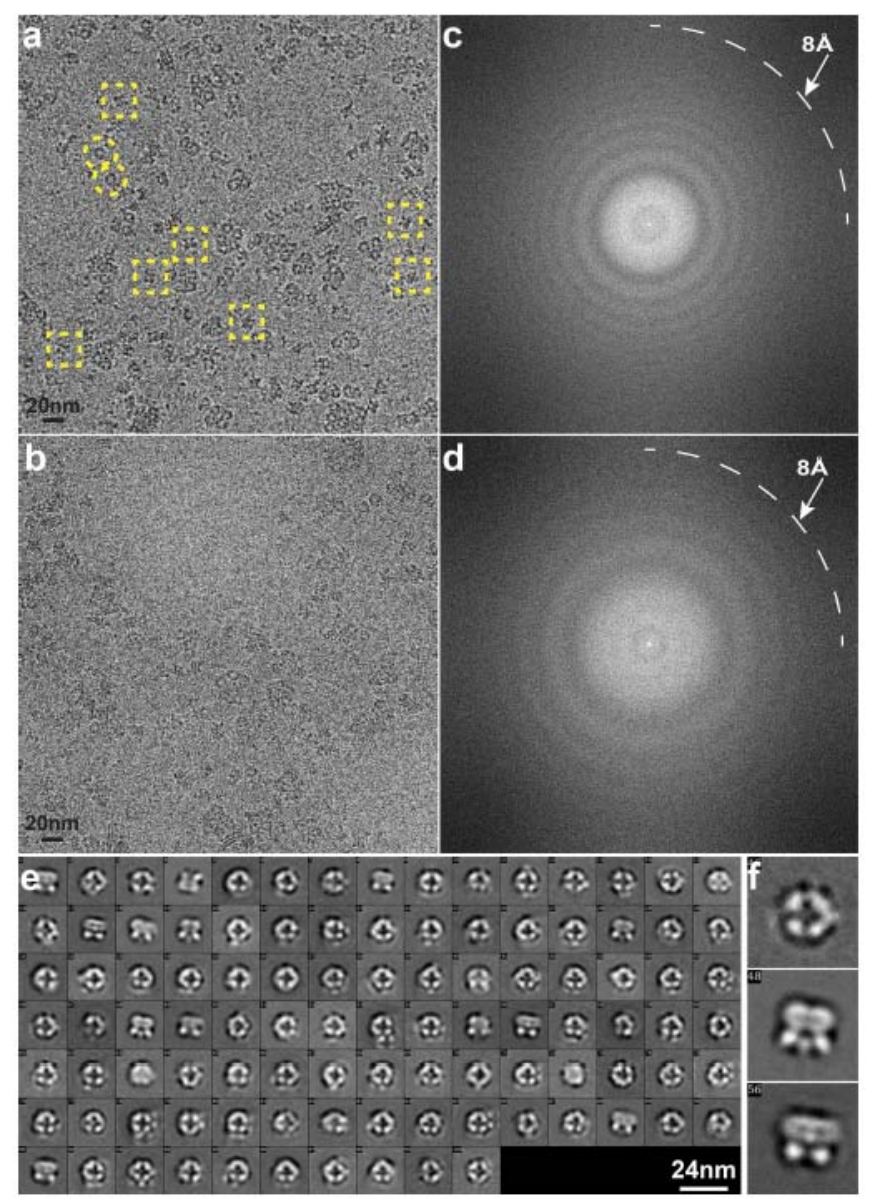
K2 Camera (Gatan)

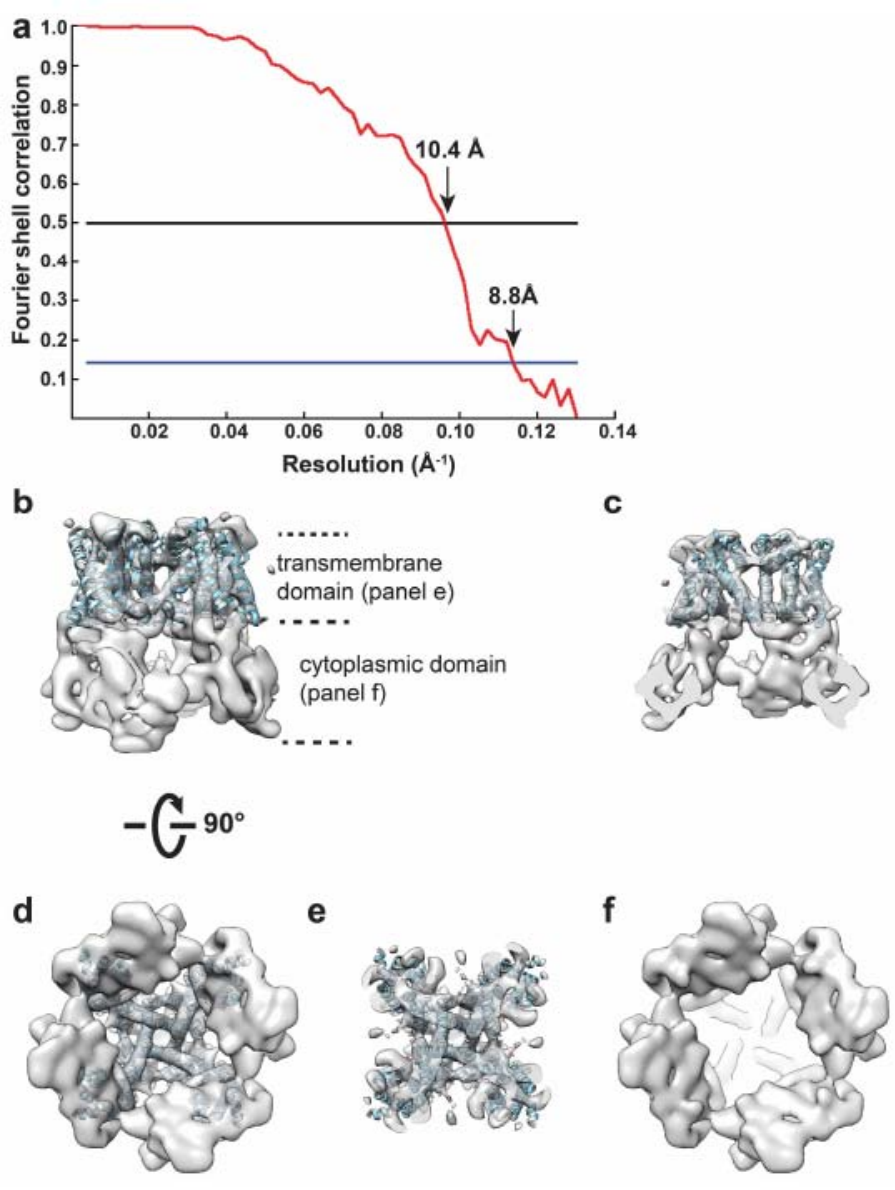
- The K2 camera has a fixed internal frame rate of 400 frames per second and the ability to find the centroid of the electron peak to subpixel accuracy (super-resolution mode).
- It minimizes noise of the detector, thereby dramatically improving the DQE.



Nature Methods 10, 584 (2013)

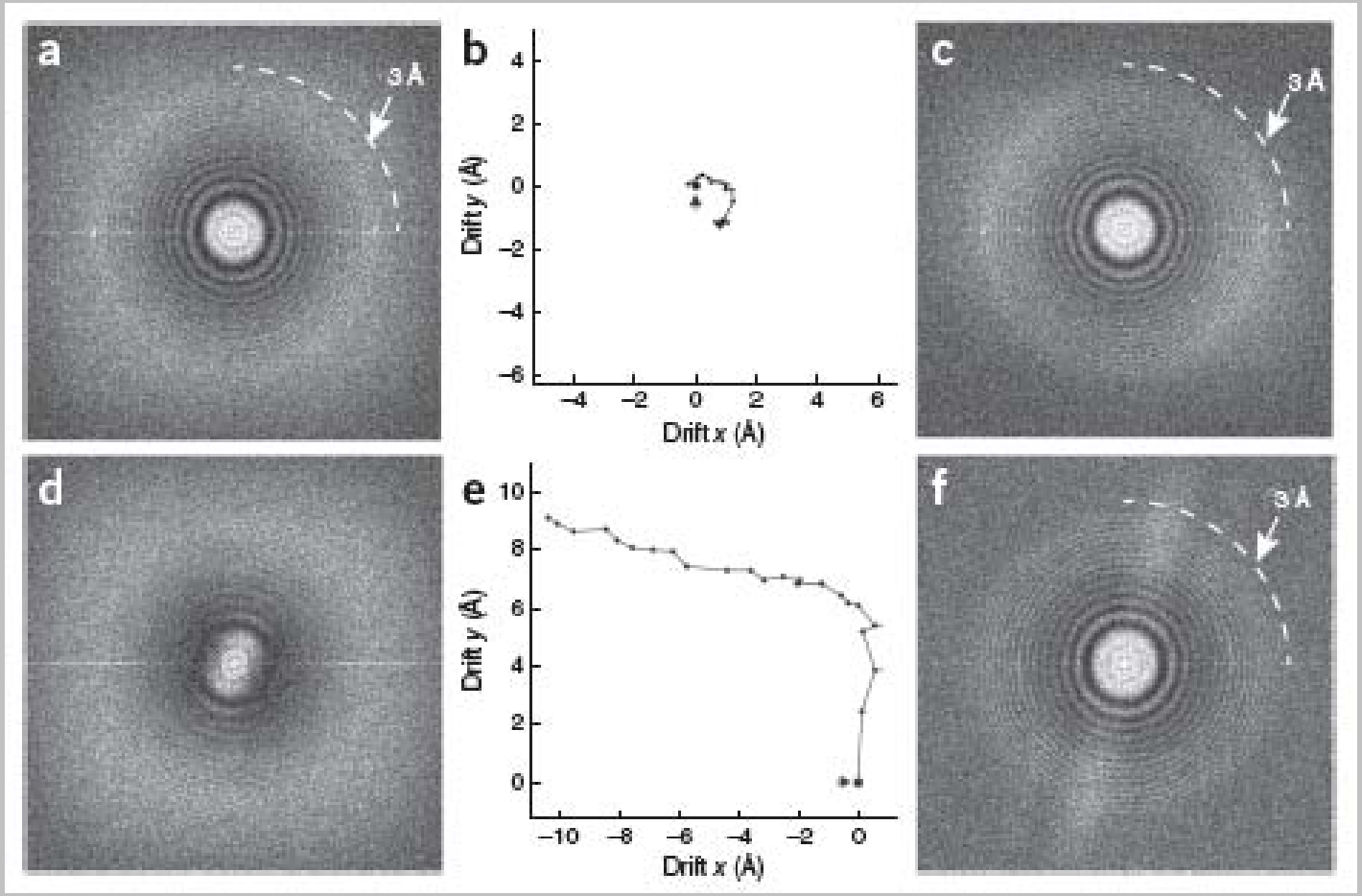
Cryo-EM of TRPV1 using CMOS Camera





Motion Correction with Dose-Fractionated Imaging

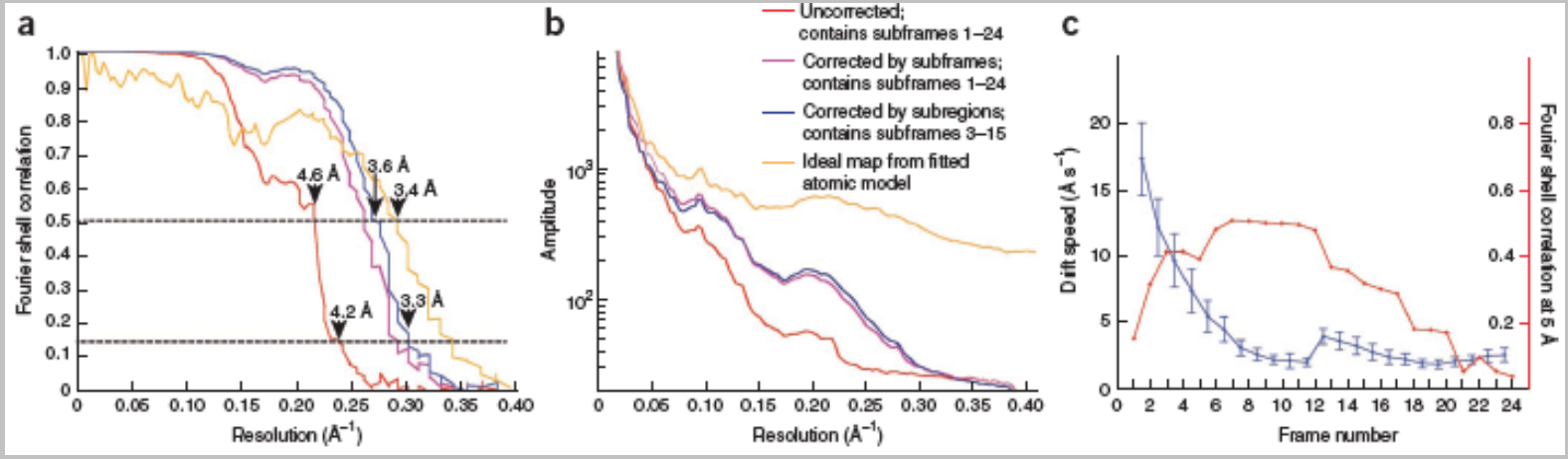
Broken the total exposure into a stack of subframes and then mitigated image blurring by aligning all subframes within one exposure.

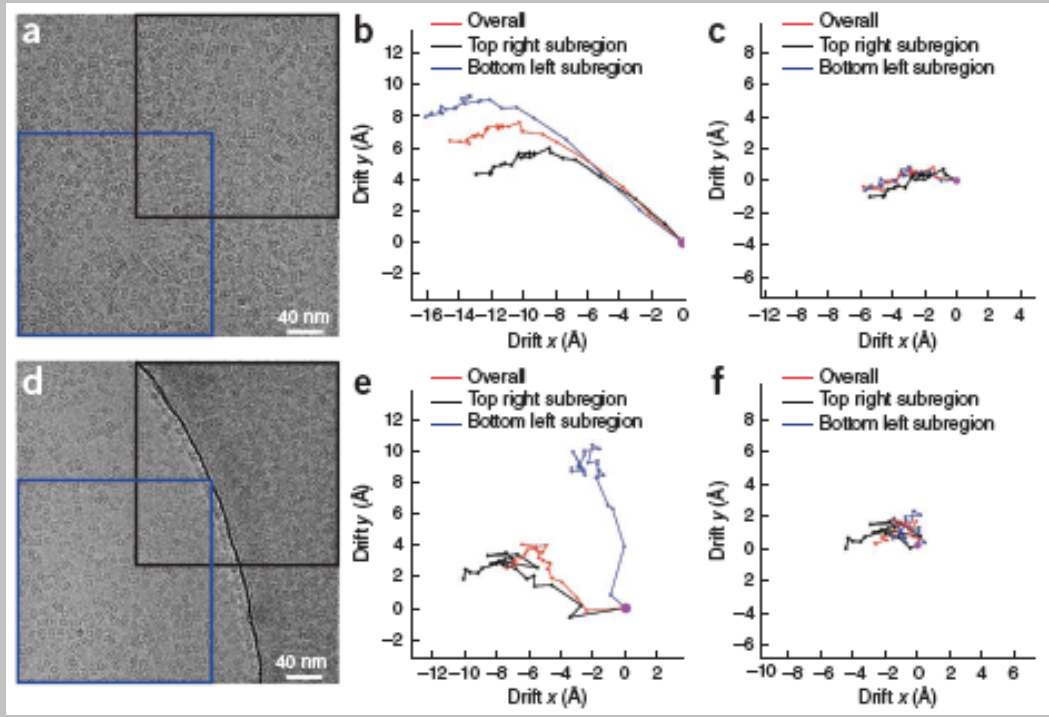


Each image was fractionated into a stack of 24 subframes, each of which was accumulated for 0.2s. Total specimen dose is ~ 35 electrons per $Å^2$.

Nature Methods 10, 584 (2013)

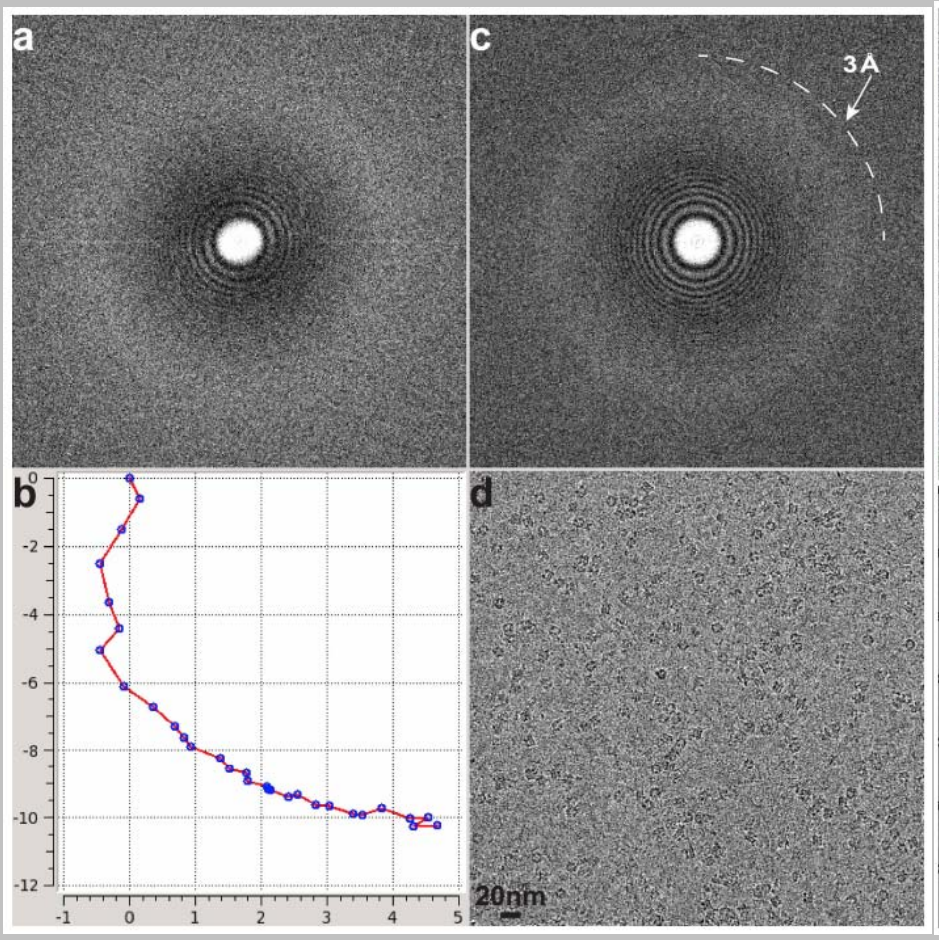
Motion-induced Image Blurring on Resolution of the 3D Reconstruction of 20S

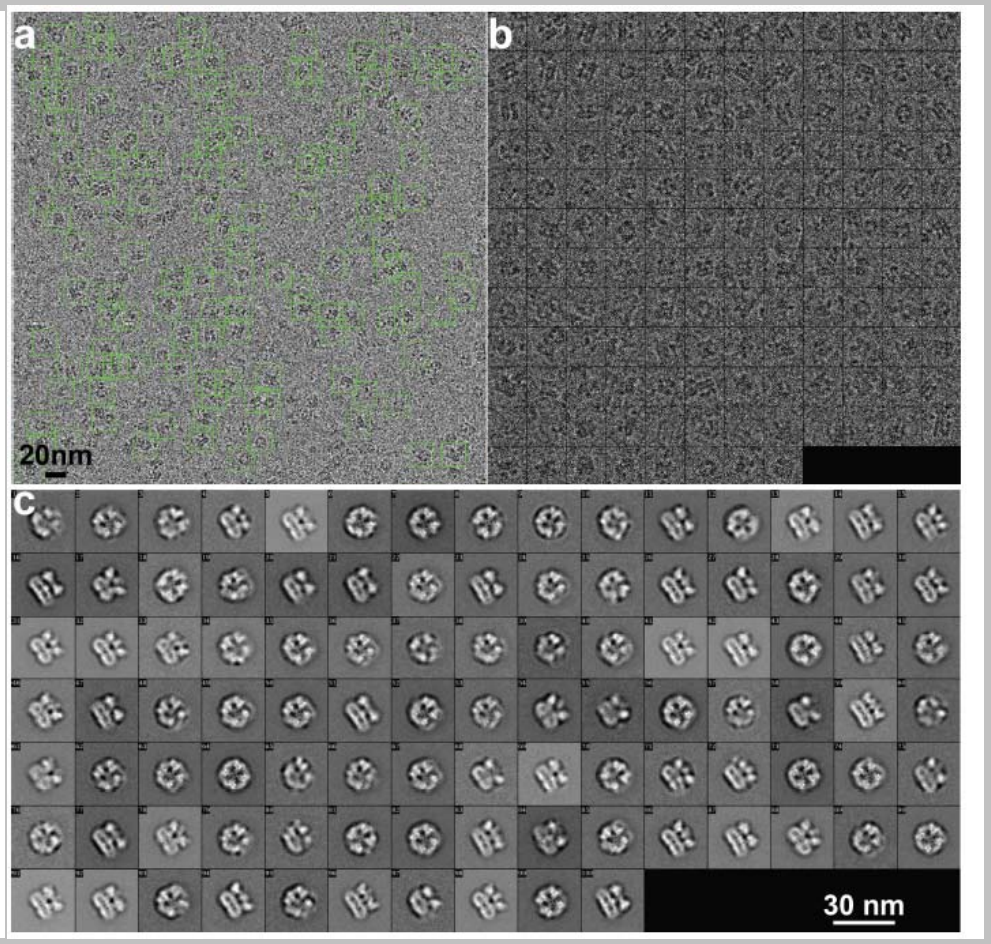




Nature Methods 10, 584 (2013)

Motion Correction Improves the Quality of TRPV1 Images





Each image was fractionated into a stack of 30 subframes, each of which was accumulated for 0.2s. Total specimen dose is ~ 41 electrons per Å$^{2.}$

Imaging Processing

Negative Stain and RCT 3D reconstruction in FREALIGN to provide initial model

Step (Cryo)	**CMOS camera**	**K2 Camera**
MRA-based semiautomatic particle picking and manual rejection	70585 (300)	97116 (946)
Reference-free 2D classification	45625	88915
3D classification in RELION (heterogeneity)	10357	35645
3D refinement in RELION (all subframes)	8.8 Å	3.6 Å
3D refinement in RELION (subframe 3-16)		3.4 Å

RELION: REgularized LIkelihood OptimizatioN

J. Struct. Biol. 180, 519–530 (2012)
J. Mol. Biol. 415, 406–418 (2012)