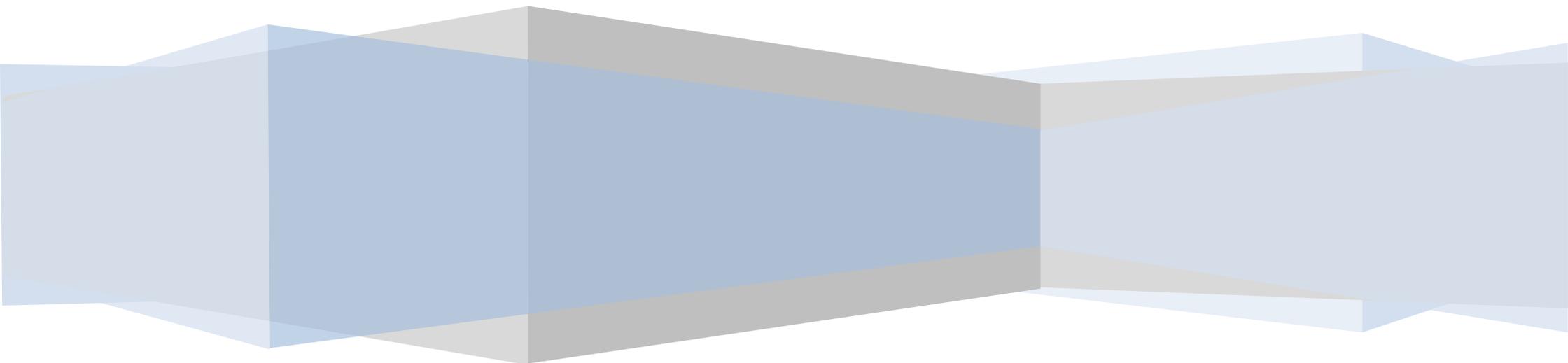


# **Self-consistent electrostatics of occupancy, conductivity and valence selectivity in Gramicidin- like ion channel**

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# 1. Outline

Electrostatic basis of conductivity and valence selectivity of biological ion channels has been discovered for Gramicidin, Sodium, Potassium and Calcium channels (B. Nadler 2003), (B. Corry 2005), (E. Spohr 2009). Dielectric Boundary Force (DBF) has been shown to play a crucial role in Gramicidin channel, it was also shown, that DBF doesn't depend on ion radius (B. Nadler 2003).

- Current presentation considers self-consistent purely-electrostatic model for single-site Gramicidin-like ion channel. The model allows to derive most of significant channel features from the analysis of solution for Poisson /Poisson-Boltzmann electrostatic equation with respect to the channel geometry and moving ions positions. Model is based on “primitive model” of hydrated ion (Dezso Boda 2011), Debye-Huckel limit law, and DBF. First-order model doesn't include explicit account of finite ion radius.
- DBF-related Self-potential barrier is the determinant electrostatic feature in ion channels. Self-potential barrier arises from DBF and corresponds to implicit model for hydration-dehydration of ions in such highly-polarised solvent as water and relates to ion-water interaction and water polarisation.
- Self-consistent 1D Brownian Dynamics (BD) simulations are used to check and confirm the results of electrostatics calculations.

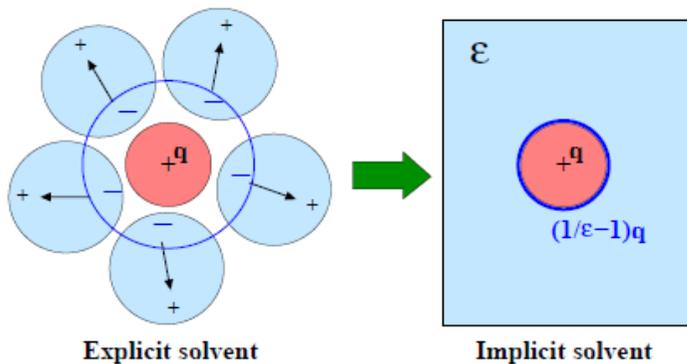
## 2. Ion channel features deriving from electrostatics

Typical ion channel represents the numerous electrostatics-defined features relevant to their biological functionality.

Feature	Description
<b>Valence selectivity</b>	Ability of ion channel to discriminate between ions with different sign and value of charge. Cationic ion channels doesn't conduct anions (Cl-) and typically selective between mono (Na+, K+) and divalent (Ca+) ions (B. Nadler 2003) (B. Corry 2005) (E. Spohr 2009).
<b>Strong binding to the binding site</b>	Electrostatic forces in ion channel geometry provide the formation of deep potential well. Ion captured by binding site can escape only due to thermal activation (Kramers escape).
<b>Occupied channel closure and limited occupancy mode</b>	Ion captured at the binding site neutralizes fixed charge making the channel closed for the entrance of new ions. Closure of occupied channel leads to single-occupancy (limited occupancy in general) permeation mode
<b>Axial ion movement and single-filing</b>	Ion inside channel is surrounded by low-polarized media and don't experienced significant radial forces comparable with axial forces.
<b>Saturation of current vs concentration</b>	Strong binding and single-occupancy mode provide fulfilment of reaction rate theory assumptions and thus explain observable saturation of ionic current as Michaelis-Menten (MM) saturation.
<b>Electrostatic amplification of shot noise of arrival ions</b>	Partial dehydration of ions inside narrow channel amplifies Coulomb repulsion between arrival and captured ions in comparison with bulk repulsion in water (D. G. Luchinsky 2009).

### 3. Implicit solvent models of electrolytes

Implicit solvent model is simple and accurate at low concentrations (Debye-Hückel limiting law) (Dezsó Boda 2011)

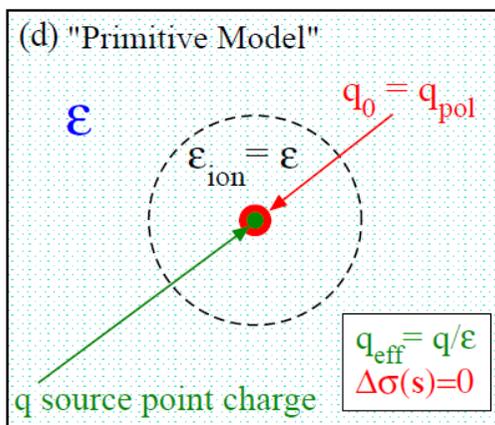


#### Implicit solvent model of electrolyte.

- Ions are modeled explicitly (charged hard spheres or Primitive Model (PM)) Solvent (water) is a continuum dielectric
- Water is strong solvent with high polarisability. Implicit solvent models account polarisation via macroscopic parameter – dielectric constant (relative permittivity)  $\epsilon$ . High values of  $\epsilon$  relates to high polarisability of solvent.
- Implicit model is simple and accurate at low concentrations (Debye-Hückel limiting law)

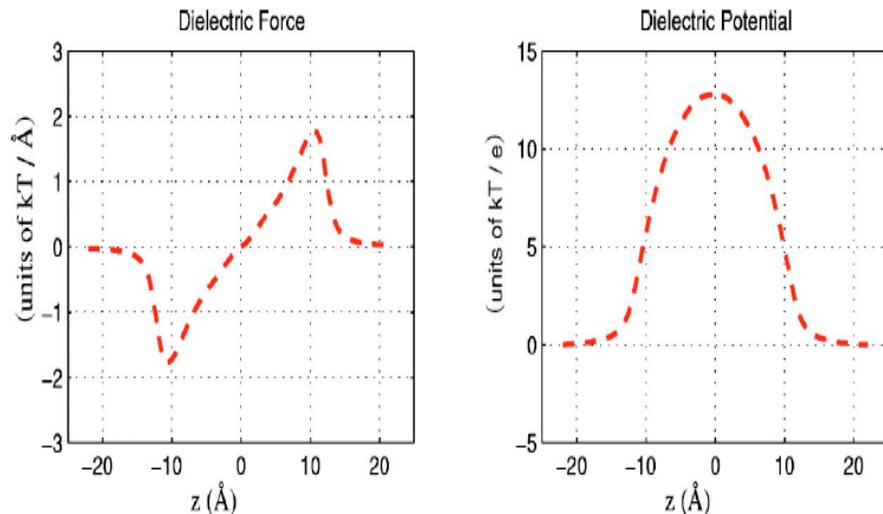
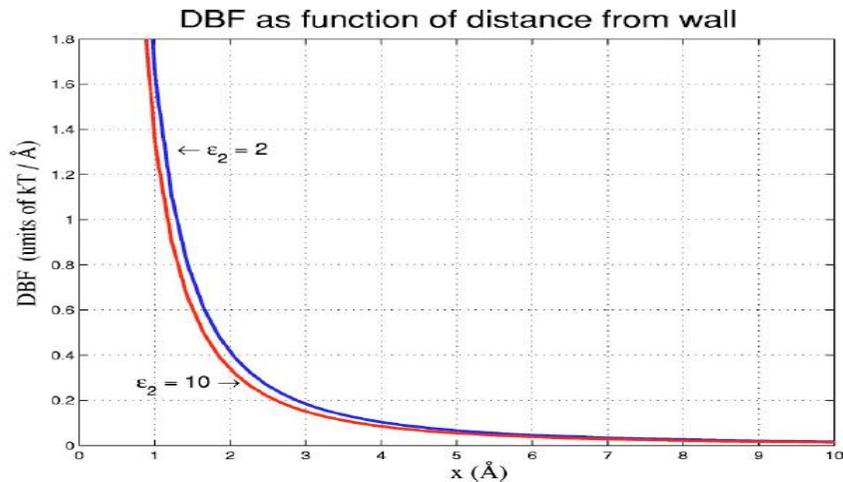
#### Primitive Model of ion hydration: effective charge

- $\epsilon_{\text{ion}} = \epsilon_{\text{water}}$
- $\sigma_s = 0, q_0 = q_{\text{pol}}$
- All the polarization charge is in the ion's center
- Effective charge:
- $q_{\text{eff}} = q + q_{\text{pol}} = q/\epsilon$
- We use primitive model of ion in electrostatics and BD simulations



## 4. Dielectric boundary force

In an electrostatic problem with nonuniform geometry, a charge  $Q$  in one region induces surface charges at boundaries between different dielectrics. The induced surface charges exert a force called dielectric boundary force (DBF) on the charge  $Q$  that induced them (B. Nadler 2003).



- The DBF is important when a charge  $Q$  is near dielectric interfaces. Self-potential barrier is calculating by integration of DBF
- The DBF plays a crucial role in the permeation of ions through the gramicidin channel.
- Due to a balance between DBF and Fixed Charge Force (FCF), a positive ion can permeate the channel, while a negative ion is excluded from it.
- In gramicidin, the balance between the FCF and DBF allows only singly charged positive ions to move into and through the channel.
- The DBF is not directly responsible for selectivity between the alkali metal ions (~e.g.,  $\text{Li}_i$ ,  $\text{Na}_i$ ,  $\text{K}_i$ ):
- **It is proved rigorously that the DBF on a mobile spherical ion is independent of the ion's radius (B. Nadler 2003).**

## 5. Scale of characteristics lengths in ion channel geometry

Ion radius plays extremely important role in all aspects of interaction of ions with other ions and with water molecules. Ion radius isn't the only length existing in ion channel model, and all these characteristic lengths represent the scale:

Debye screening length	~ 8Å for NaCl/KCl at M=150mmol
Bjerrum length (electrostatic energy == kT)	~ 7Å for water
Channel radius	~ 2Å for Gramicidin A
Water dipoles length	~ 1Å for OH length
Ion radius	~ 1Å for Na <sup>+</sup> and Ca <sup>++</sup>

Born hydration model uses explicit ion radius but for small ions Born radius (aka internal radius of first hydration shell) depends on water dipole length rather than on real radius of the ion.

DBF, dominated electrostatic force in Gramicidin channel doesn't depends on ion radius (B. Nadler 2003). Self-potential barrier arising as the result of DBF action also doesn't depend on ion radius. Other example of consistent physical model not accounting ion size is Debye-Hückel limit model. This model corresponds to using of Poisson-Boltzmann equation for calculation of electrostatic field.

Thus we can introduce self-consistent first-order electrostatic model based on Poisson-Boltzmann equation, primitive ion hydration model, relative permittivity approximation for water dipole strength and DBF for calculation of self-potential barrier. This model consistently describes valence selectivity, strong binding, single occupancy with MM saturation and some aspects of hydration-dehydration without explicit account of ion radius.

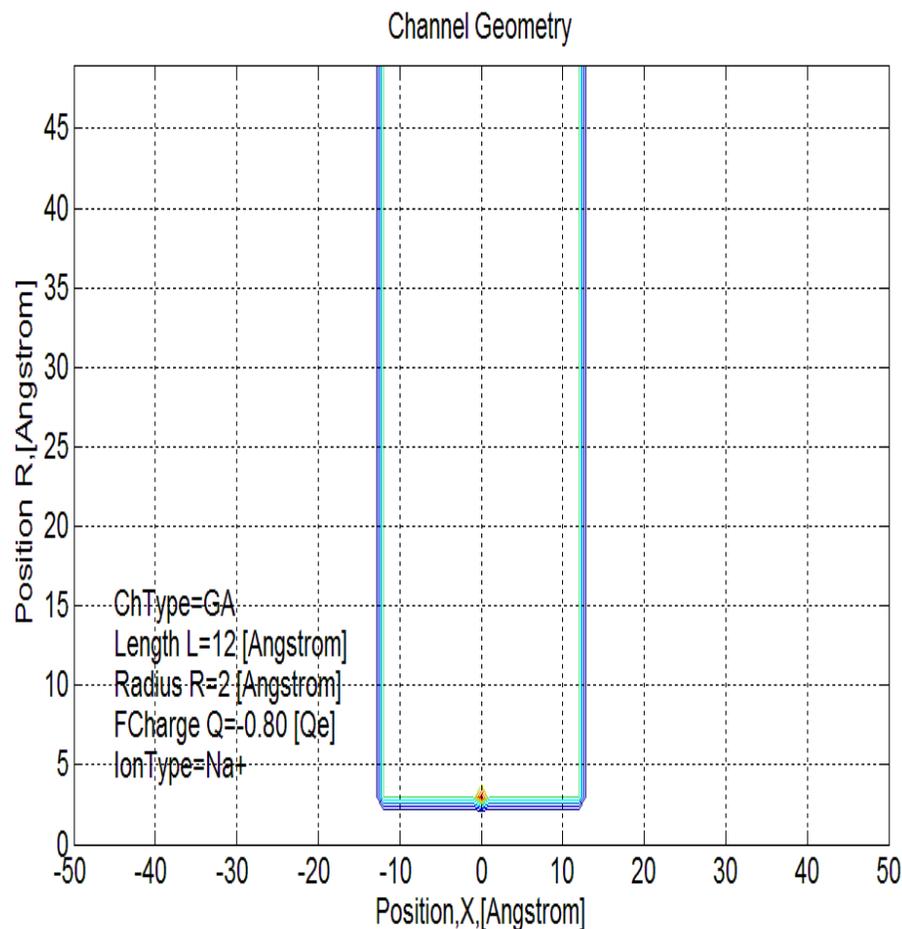
## 6. Reduced model of Gramicidin-like ion channel

In electrostatic approximation the reduced model of ion channel is described by only few parameters:

**Channel Geometry** Radius  $R_c$  and length  $L_c$  of channel, permittivity of water  $\epsilon_1$  and protein  $\epsilon_2$

**Binding site properties** Location and charge  $Q_f$  of fixed protein charge forming binding site.

**Moving ions properties** Ion charge (valence)  $Q_i$ , Diffusion coefficient  $D$  and ion size  $R_i$

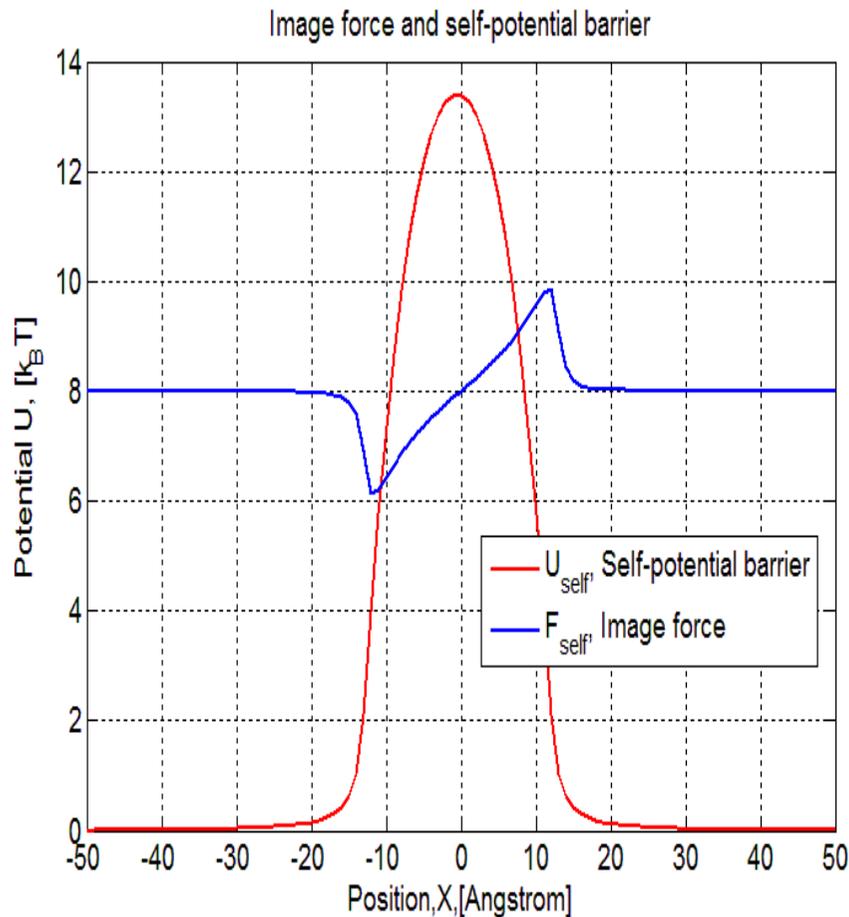


- **Geometry and computational domain for Gramicidin-like ion channel.**
- The reduced axis-symmetrical model consists of water-filled channel through the protein, with a single charged site. Model parameters are selected to match experimental data for Gramicidin A.
- Electrostatic potentials are defined from self-consistent numerical solution of Poisson electrostatic equation for axis symmetrical case.
- Lookup tables approach is used for simultaneous solution of Poisson equation at each simulation step.
- Dielectric permittivity of water and protein are taken as  $\epsilon_1=80$ ,  $\epsilon_2=2$

## 7. Self-potential barrier and dehydration inside the channel

Self-potential barrier arising in narrow water-filled channel is the most important and obvious feature of electrostatics of ion channel.

Self-potential barrier arises due to partial dehydration of ion at the channel mouth similarly to Born dehydration model.

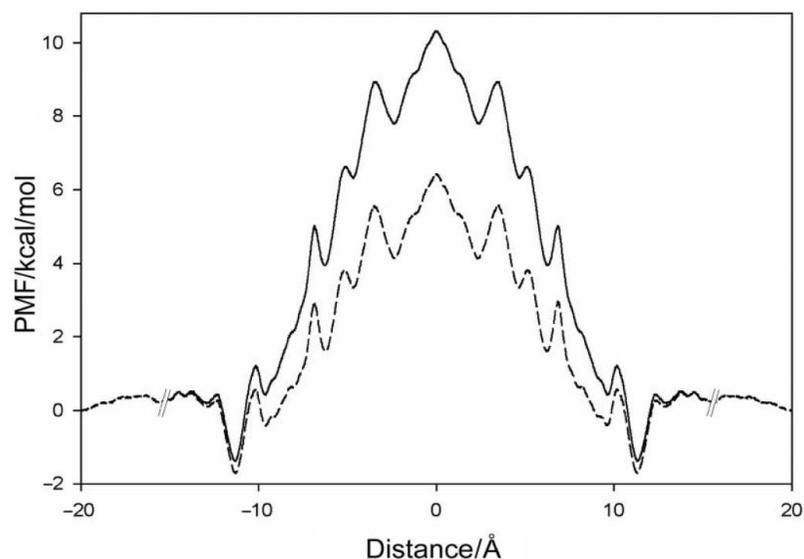
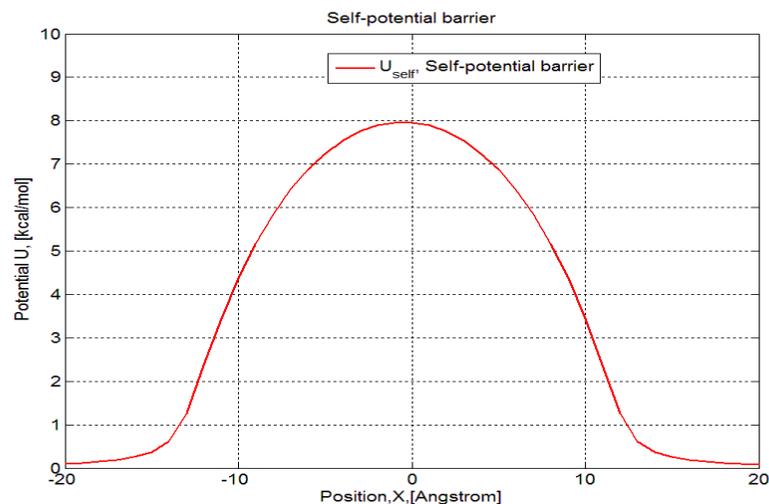


### Image force and self-potential barrier in Gramicidin-like channel geometry for $Na^+$ (monovalent) ion).

- Self-potential barrier accounts dehydration of ion inside the channel in electrostatic approximation, and it doesn't depend of sign of ion charge.
- Self-potential barrier height is proportional to  $Q^2$  and approximately proportional to  $1/Rc$ .
- Self-potential barrier arises from dielectric boundary forces and thus doesn't depend on ion radius.
- For Gramicidin A channel ( $Rc=2A$ ,  $2Lc=24A$ ) self-potential barrier for monovalent ion is high enough to prevent movement of any ion (both cation and anion) through the uncharged channel.

## 8. Self-potential barrier and Potential of Mean Force

Self-potential barrier in electrostatic model closely relates to Potential of mean force (PMF) calculated in the Molecular Dynamics (MD) models of ion channels.



**Image force and self-potential barrier in Gramicidin-like channel geometry for  $\text{Na}^+/\text{K}^+$  ion in electrostatic approximation.**

Barrier shape is calculated from numerical solution of Poisson electrostatic equation. Self-potential arises from dipole water molecules polarisation. Polarisation is accounting by introducing of dielectric phenomenological constant (relative permittivity)  $\epsilon$  into Maxwell equations.

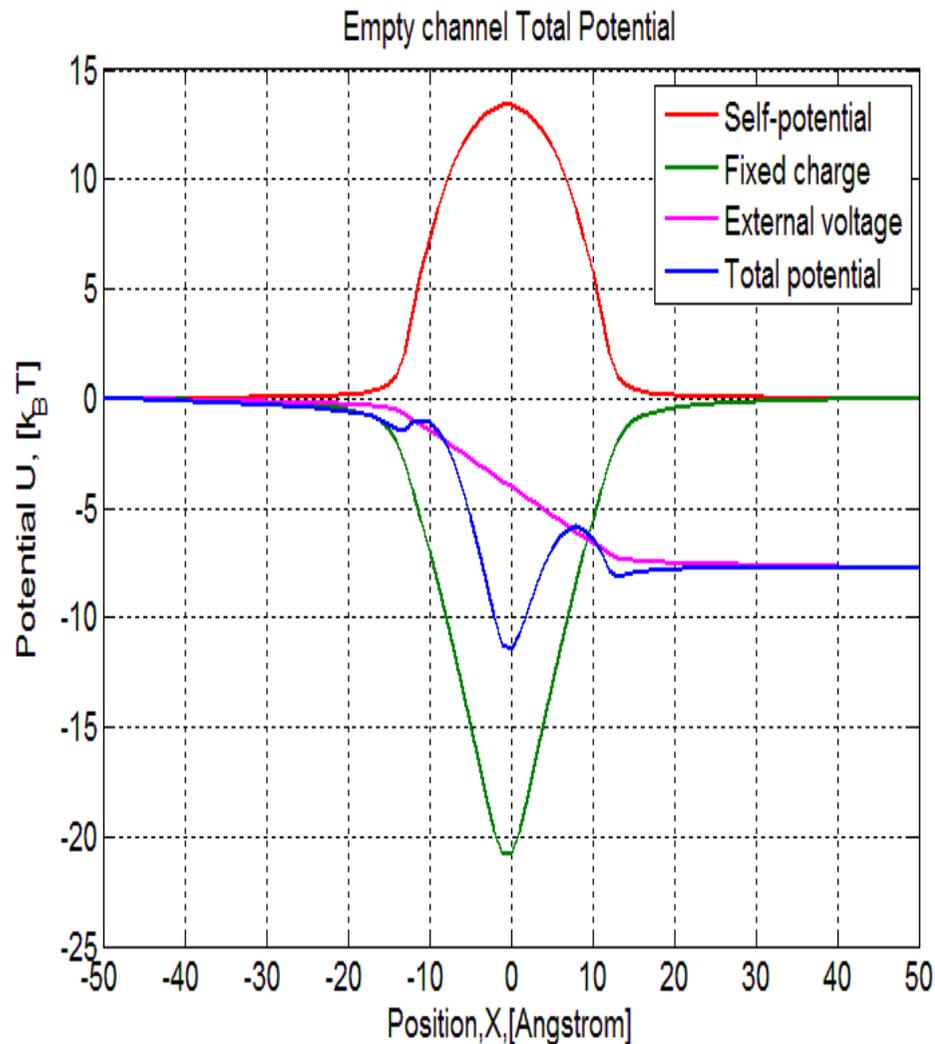
**One-dimensional PMF profile for Gramicidin channel calculated in MD (Roux 2004).**

MD is based on Maxwell equations for vacuum and polarisation and relative permittivity appears as result of calculation rather than a phenomenological constants.

**Both approaches describe the same physical phenomena and show reasonable agreement.**

## 9. Fixed charge and formation of RR-type energy profile for binding site

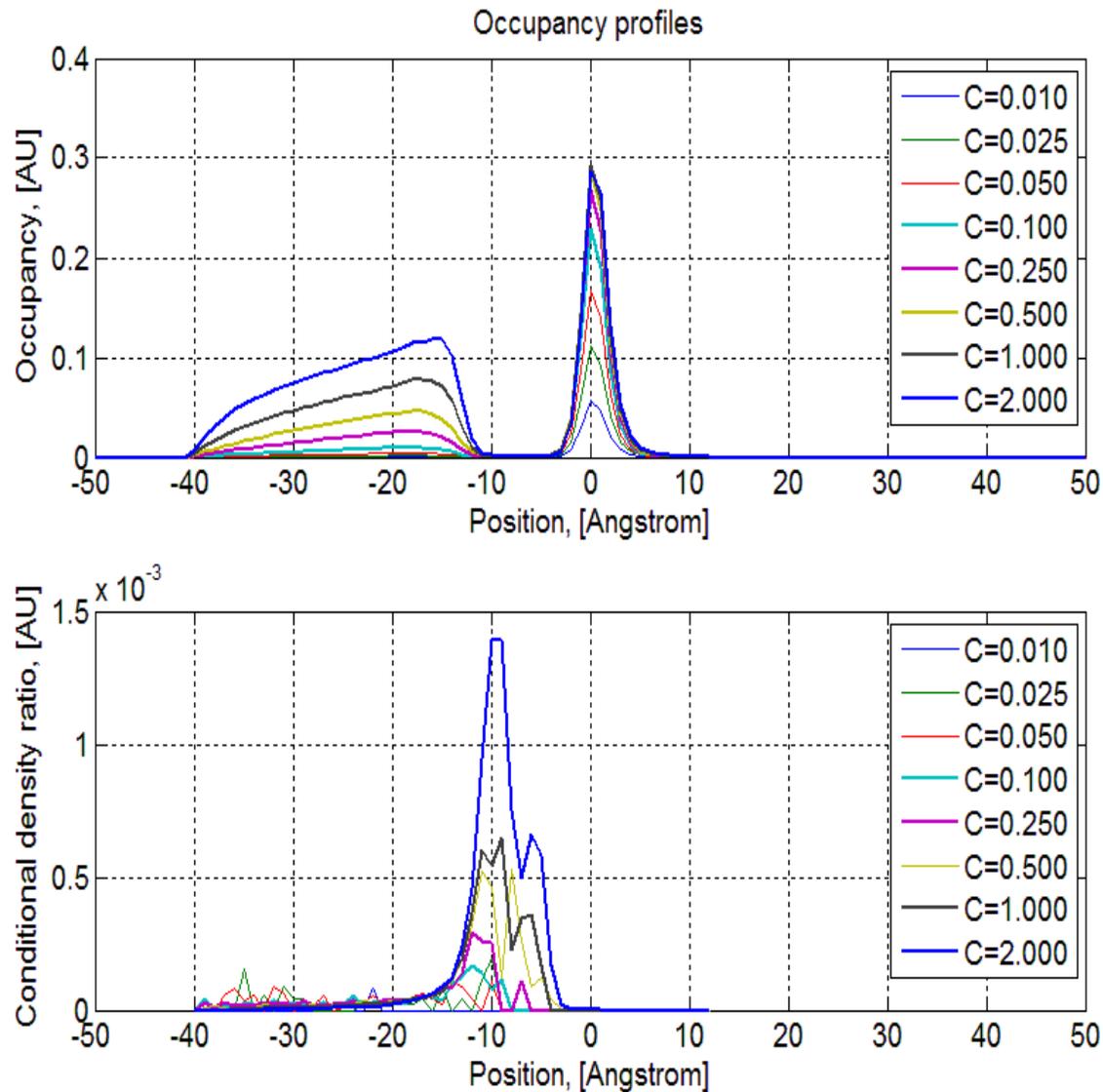
Binding site is formed as a result of joint neutralisation of self-potential barrier with potential well of fixed charge.



### Potential's components of binding site.

- Binding site is formed as a result of neutralisation of self-potential barrier and potential well of fixed charge. This neutralisation takes place for cations only
- Dehydration inside channel leads to decreasing of electric field attenuation by polarised water molecular dipoles and thus to observable amplification of electric field of fixed charge in comparison with the same field in water.
- **Resulting potential has a typical for Reaction Rate theory two-barrier-one-well shape. For strong binding site potential well is deep enough to obey to Kramers escape rule.**
- Anions are still strongly prohibited to permeation due to summing of self-potential barrier with fixed charge barrier

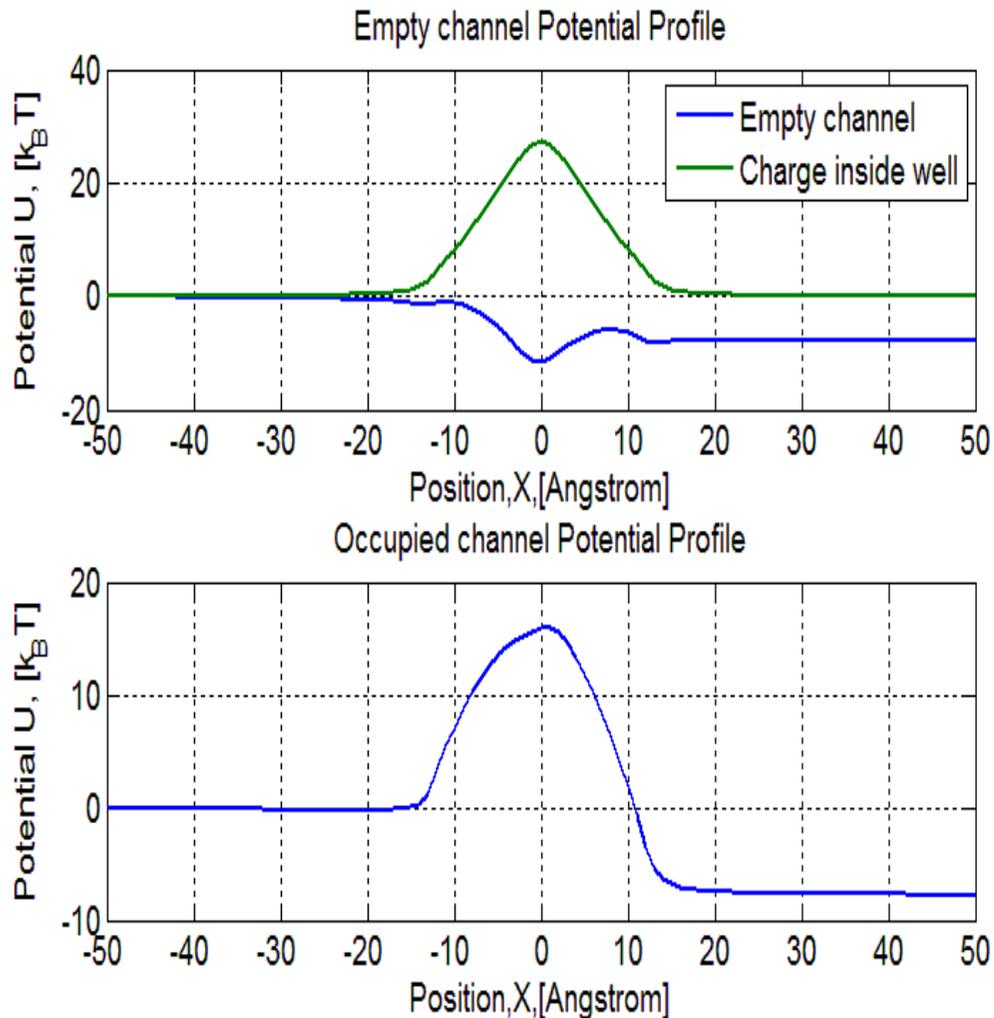
## 10. BD confirmation of strong binding of ion on the binding site



- BD-simulated occupancy profiles strongly concentrated near fixed charge confirming presence of strong affinity to the binding site
- Conditional probability profile –  $C_p$  reflects ions distribution at the time related to exit of ion from the channel, in comparison with common probability profile  $C_i$ .
- The ratio  $r(x)=C_p(x)/C_i(x)$  reflects statistical correlation between arrival of second ion to channel mouth and first ion exit.

# 11. Closure of occupied channel and formation of limited (single) occupancy mode

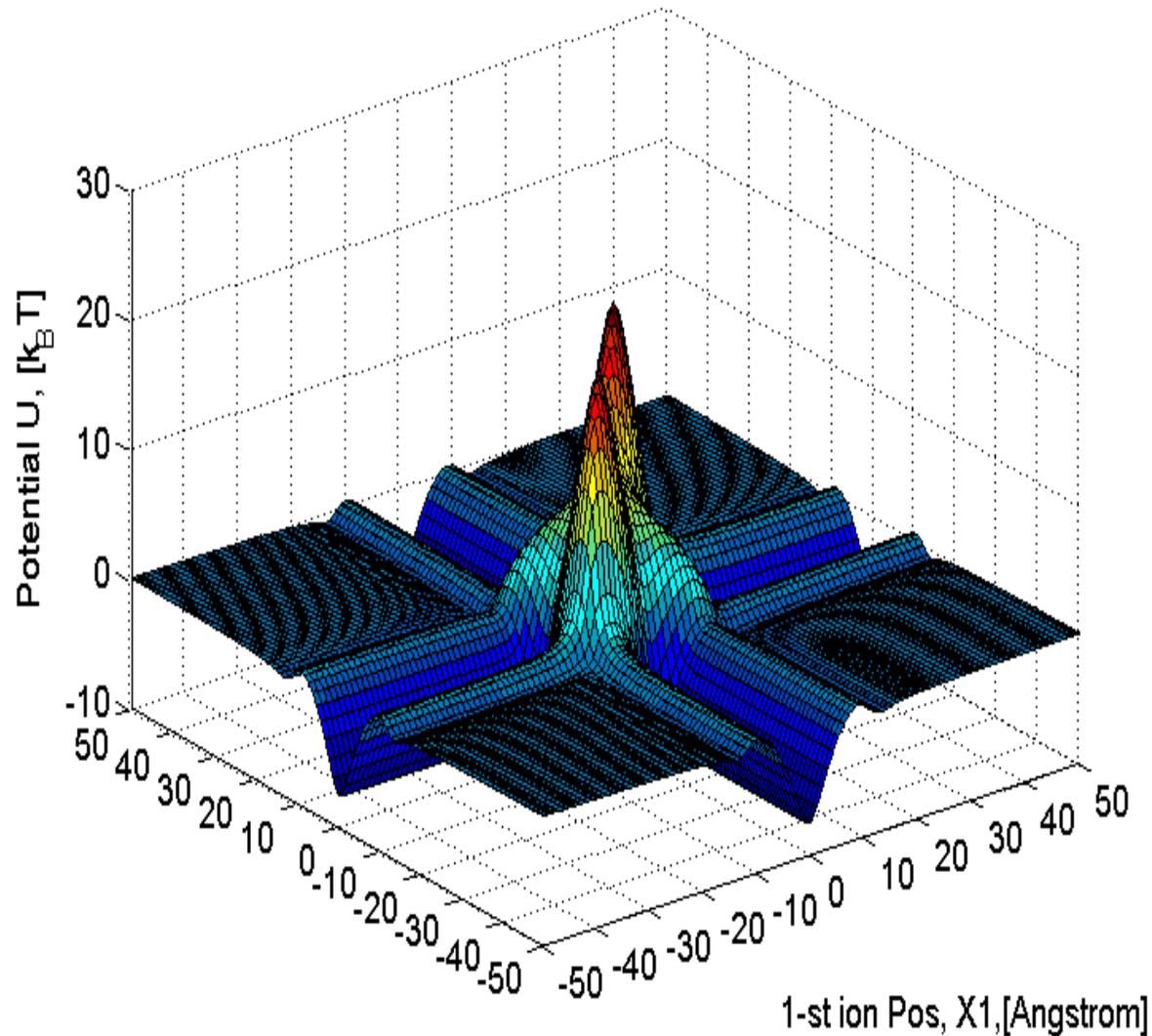
New ion is prohibited to enter occupied channel due to strong electrostatic repulsing between arrival and captured ions



- An empty channel is available for entering of new ion.
- Entering ion is captured by potential well and remains there for time  $1/k_2$ , where  $k_2$  – escape rate.
- Electrostatic field of captured ion neutralizes field of fixed charge and restores potential barrier closing the site.
- This behavior approves single-ion site binding model for generic ion channel

## 12. Double-ion potential energy surface for Gramicidin channel

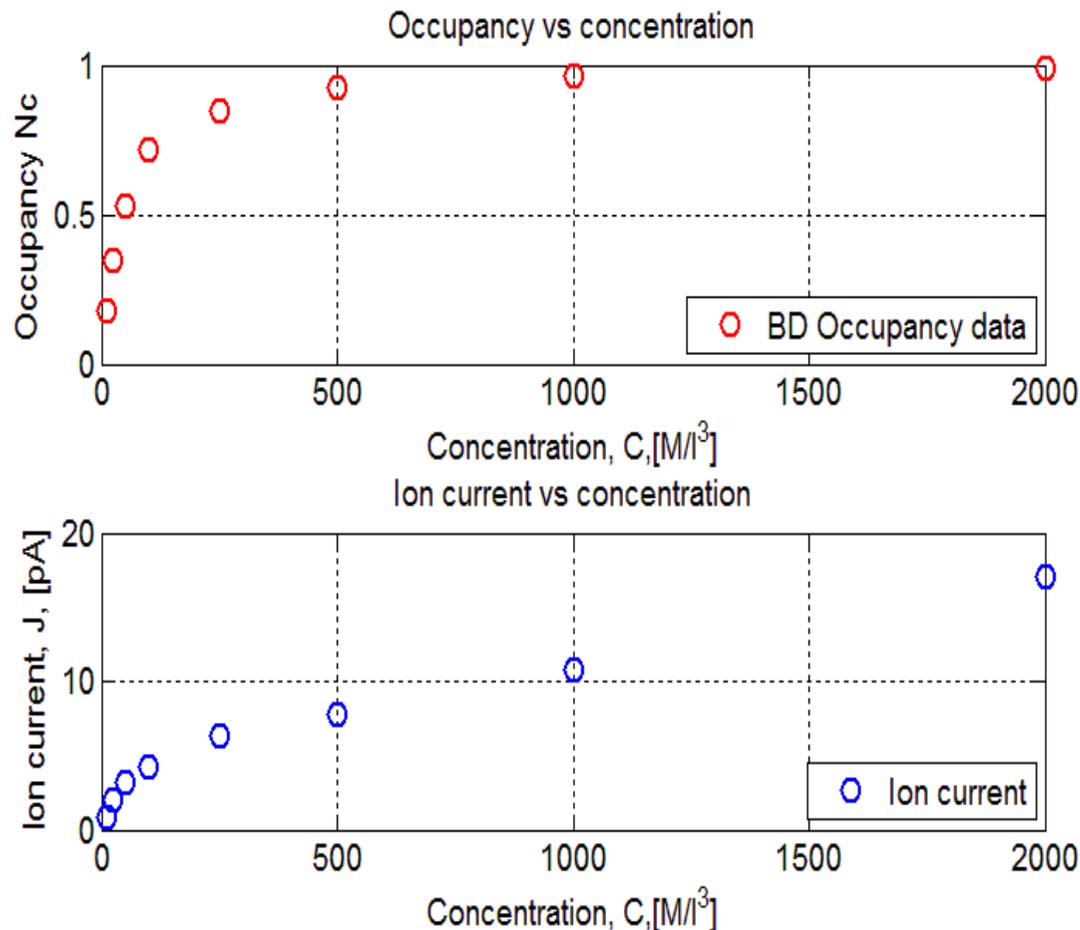
Double-ion potential energy surface



- Prohibition for entrance of second ion can be also clearly shown at double-ion Potential Energy Surface (PES). For singly-occupied channels central barrier prevents entrance of the next ion into the channel.
- For multiple occupied channels such as Calcium L-type channel PES demonstrates optimal path allowing energy lossless coordinated movement of coupled ions (V. N. Kharkyanen 2010)

### 13. Saturation of occupancy and current vs concentration

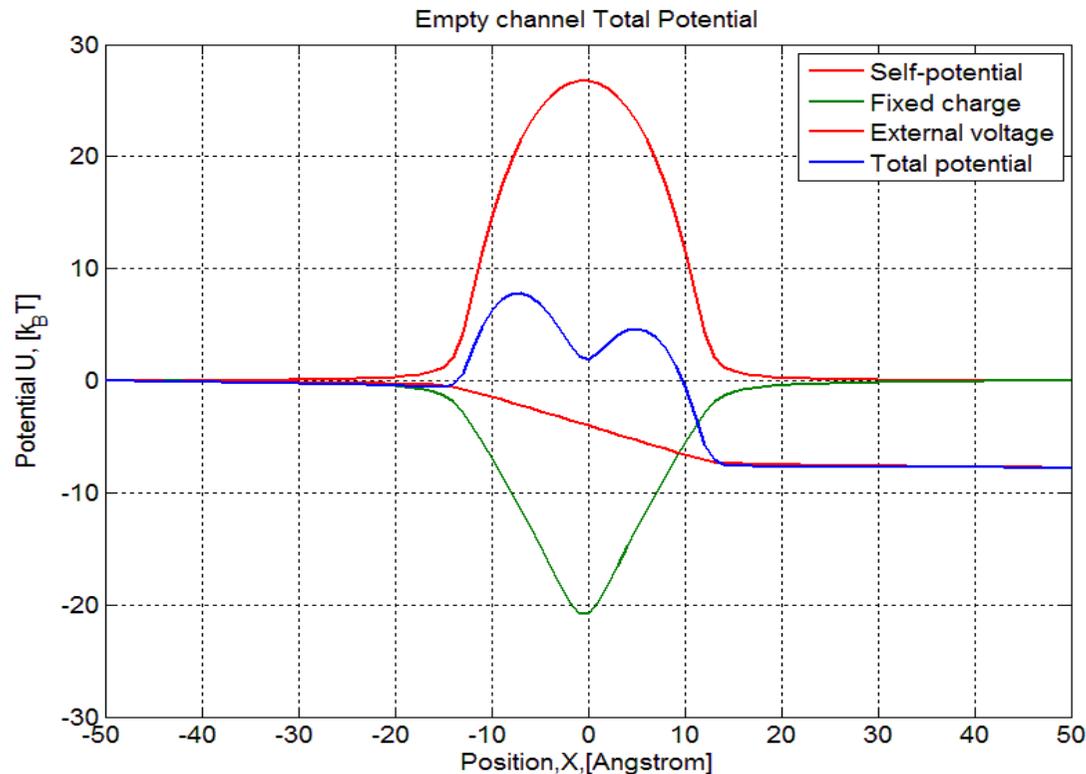
aturation of current vs concentration is a well-established experimental feature for the most of biological ion channels. Self-consistent electrostatics of Gramicidin-like ion channel provides single-occupancy permeation mode and Michaelis-Menten occupancy/current saturation for monovalent ions.



- BD simulations reproduce current-concentration dependence for Gramicidin A with realistic parameters values.
- **Gramicidin A-like ion channel works in strictly single-occupied mode.**
- Channel occupancy  $P$  demonstrates pronounced MM saturation and strictly single-occupied behaviour.
- Ion current demonstrates saturation with near MM kinetics.
-

## 14. Valence selectivity of Gramicidin-like channel: Na<sup>++</sup> vs Ca<sup>++</sup>

Valence selectivity features are defined by difference in properties of electrostatic attraction to the binding site and electrostatic self-repulsion from image charges (hydration-dehydration forces). Self-potential has the same sign for ions of any signs and proportional to  $Q^2$  whereas interaction with binding site is proportional to  $Q$  and depends on ion sign. This difference provides an appropriate difference in conductivity and permeation between cations and anions and between monovalent and divalent ions. The following is binding site potential scheme of Gramicidin-like channel for Ca<sup>++</sup> ion.

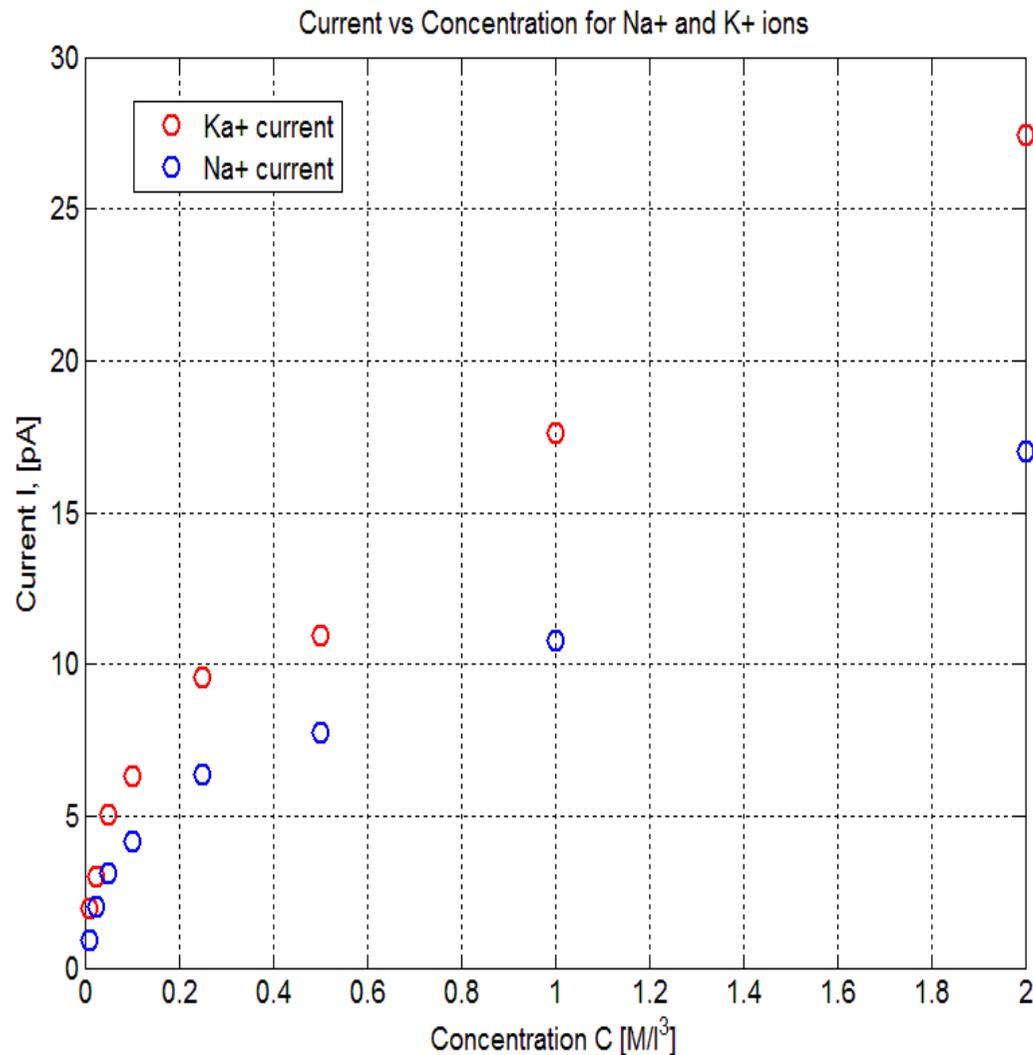


### Potential profiles for Ca<sup>++</sup> ion along axial position in Gramicidin-like ion channel.

- Self-potential barrier growth proportionally  $Q^2$  and provide almost perfect channel closure for Ca<sup>++</sup> ion.
- BD simulations don't show significant ion current as well.

## 15. Weak selectivity between alike ions

Electrostatic approximation explains typical for Gramicidin weak type of selectivity between  $\text{Na}^+$  and  $\text{K}^+$  ions via the difference in diffusion coefficient  $D$



### Ion current vs concentration graph for $\text{Na}^+$ and $\text{K}^+$ ions

- Electrostatic approximation explains typical for Gramicidin weak type of selectivity between  $\text{Na}^+$  and  $\text{K}^+$  ions via the difference in diffusion coefficient  $D$
- Selectivity between alike ions could be also included to model by introducing of effective channel cross-section and radius, in correspondence with ion radius.

## 16. Summary

Electrostatic approximation is based on Poisson/ Poisson –Boltzmann equation, implicit solvent model, primitive ion salvation model, Debye-Hückel limit model and dielectric boundary force. This approximation doesn't contain explicit dependence on ion radius but it still allows to describe some important observable features of gramicidin-like channels:

- Existence of self-potential barrier
- Valence selectivity
- Strong binding on the binding site
- Limited occupancy mode
- Current vs concentration saturation
- Electrostatic amplification of shot noise of arrival ions

Electrostatics could be also applied to explanation of features and conductivity and selectivity mechanisms of Calcium L-type channels (B. Corry 2005)

Finite ion radius effects could be explicitly introduced to the model through using of more advanced electrolyte models including Van der Waals forces and volume exclusion effects (Dezsó Boda 2011)

## 17. References

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