# Abstract

Linked gramicidin-A channels (GA) have significantly (up to ~100-fold) larger lifetimes than do isolated channels. We show how nearby channels affect the activation barrier for splitting the GA dimer, considering a range of cluster sizes and using the smectic bilayer model to describe inclusion induced elastic deformation of the membrane. At the interface with the $i$-th channel of a cluster, the membrane's surface displacement $u_i$ fluctuates relative to $u_0$ prescribed by hydrophobic matching. Channel lifetimes are estimated using the Arrhenius description of activation for GA in a DMPC membrane. We calculate the probability, relative to that for an isolated channel, of a particular clustered channel reaching the transition state, all other channels remaining dimeric (with $u_i = u_0$). The unique channel's lifetime increases, an effect strongly dependent on the interchannel distance, $d$, and on the location of the channel in the cluster. For two channels ~ 1 nm apart in DMPC, the increase is almost 10-fold. It is even greater for inclusions with more nearest neighbors, reaching several orders of magnitude with 5 or 6 near neighbors. It practically disappears for $d > 3$–4 nm.

# Introduction

Membrane-spanning peptides in lipid bilayer membranes cause membrane deformations, which significantly influence both the *energetics of the insertion* and the *membrane-mediated interaction between peptides*. An ideal model system for studying such phenomena is the GA channel, a transmembrane dimer formed by head-to-head monomeric association.

Changes in GA lifetimes accurately report the energetics of membrane deformation associated with insertion. Membrane deformation imposes elastic forces on a channel, stressing its hydrogen bonding dimer linkage. For isolated channels, increased deformation facilitates monomer separation: the larger the deformation, the more easily the monomers separate and the shorter the channel lifetime.

Recently, interest has shifted towards the study of collective effects in channel kinetics. Specially designed GA monomers, coupled at their water termini by various macromolecular linkers, typically exhibit noticeably increased channel lifetimes [1-3].

We show that this mutual stabilization of the channels may be rationalized in terms of membrane-mediated interaction between the peptides [4,5].

# Basic Equations

In the simple model for GA insertion into a membrane, the elastic free energy is due to vertical displacement ($u_0$) of lipid molecules in immediate contact with a (cylindrical) insertion of radius $r_0$, defined by the "hydrophobic matching condition":

$$2u_0 = h_0 - l_H \tag{1}$$

where $h_0$ and $l_H$ are the unperturbed hydrophobic membrane thickness and the hydrophobic length of the peptide respectively.

Being perturbed at the interface, the membrane profile u(x,y) adjusts itself gradually to minimize the elastic energy, *F,* which, in the "smectic bilayer" model, is

$$F = \int [b(\Delta u)^2 + au^2]dxdy; \tag{2}$$

*a* and *b* are proportional to the membrane's stretching and bending elastic moduli respectively. The deformation profile is determined by

$$(a\Delta^2 + b)u(x, y) = 0. \tag{3}$$

## Boundary conditions

For finite clusters of inclusions both *u(**r**)* and $\nabla u(r)$ vanish at the external boundaries: $u(L_{ext}) = 0, \quad \nabla u(L_{ext}) = 0$. To study many-body effects we vary the monomer separation in channel *i*, $\delta_i$, and the corresponding interfacial displacement $u_i$

$$u_i = u_0 - \delta_i / 2, \tag{4}$$

and, consistent with the membrane's influence on channel lifetimes, use the "constrained boundary condition" for the slope,

$$s_i \equiv \nabla u(L_i) = 0. \tag{5}$$

Eqs. (2)-(5) are then solved numerically in a finite difference approach [**3**].

## Effective spring constants

Since Eqs. (2)-(5) are linear in u, F (Eq. 2) is quadratic in the boundary parameters $u_i$

$$F = \sum_{i=1}^{N} a_{ii} u_i^{\,2} + \sum_{i=1}^{N-1} \sum_{j=i}^{N} a_{ij} (u_i - u_j)^2 \tag{6}$$

where $a_{ij}$ are effective spring constants. The number (*m*) of independent spring constants depends on the cluster geometry and *N*.

*To find all m $a_{ij}$ for any particular arrangement of inclusions, one should choose m linearly independent sets of $\{u_1, u_2, \ldots u_N\}$ for the rhs of Eq.6, determine the corresponding $F[\{u_1, u_2, \ldots u_N\}]$ from Eqs.1-5, put these instead of F in the lhs of Eq. 6, and solve the resulting system of m linear equations for m spring constants. After the $a_{ij}$ are determined, Eq. 6 determines F for all possible sets of $u_i$. In other words, for any arrangement of inclusions, the elastic problem must be solved only few times, which enormously simplifies the statistical-mechanical treatment of interacting inclusions.*

# Results and discussion

To study channel interaction, we consider a range of cluster sizes and symmetries for inclusions in *DMPC* membranes: $h_0 = 2.53\ nm, B = 50\ pN\ nm^{-2}, K = 40\ pN, u_0 = 0.165\ nm$. Three cases illustrate the general tendencies: two inclusions, three symmetric collinear inclusions and seven inclusions forming a regular, centered symmetric hexagon.

# Spring constants

(a) Two inclusions

There are two independent spring constants: $a_{11}(= a_{22})$ and $a_{12}(= a_{21})$. Results are presented in Fig. 1. The diagonal constant $a_{11}$ decreases only slightly as channels approach, while the coupling constant $a_{12}$, grows to ~1350 $kT/nm^2$! Thus splitting a GA dimer is harder if two channels are in close proximity. This is even more noticeable with several neighbors.

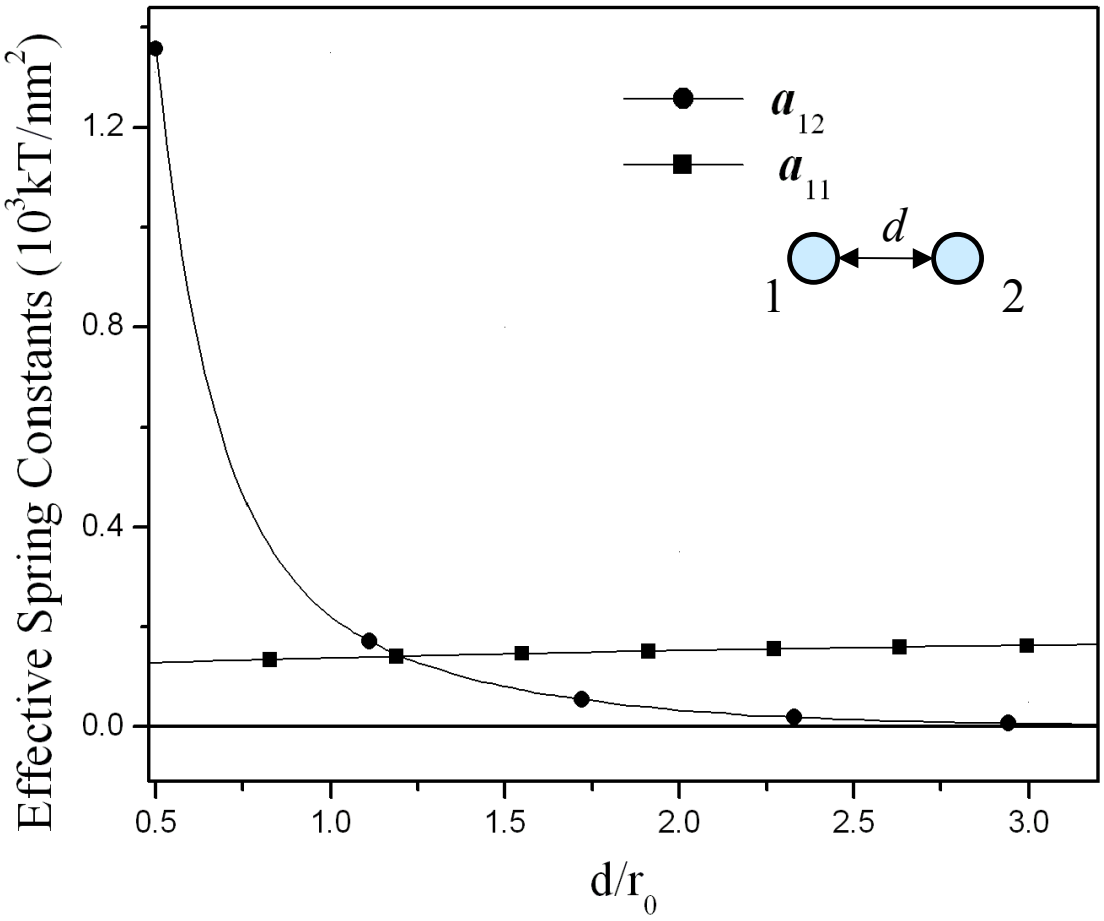


**Fig. 1**

(b) Three collinear inclusions

Four independent spring constants: $a_{11}(= a_{33})$, $a_{22}$, $a_{12}(= a_{32})$. and $a_{13}$. As in case (a), diagonal constants decrease slightly at short separations, with the effect more pronounced for the central insertion. The behavior of $a_{12}$, responsible for the center-edge coupling, is very similar to $a_{12}$ of case (a). Due to the presence of two nearest neighbors the total stabilizing effect is almost doubled. In addition, the edge-edge coupling is almost negligible for all separations.

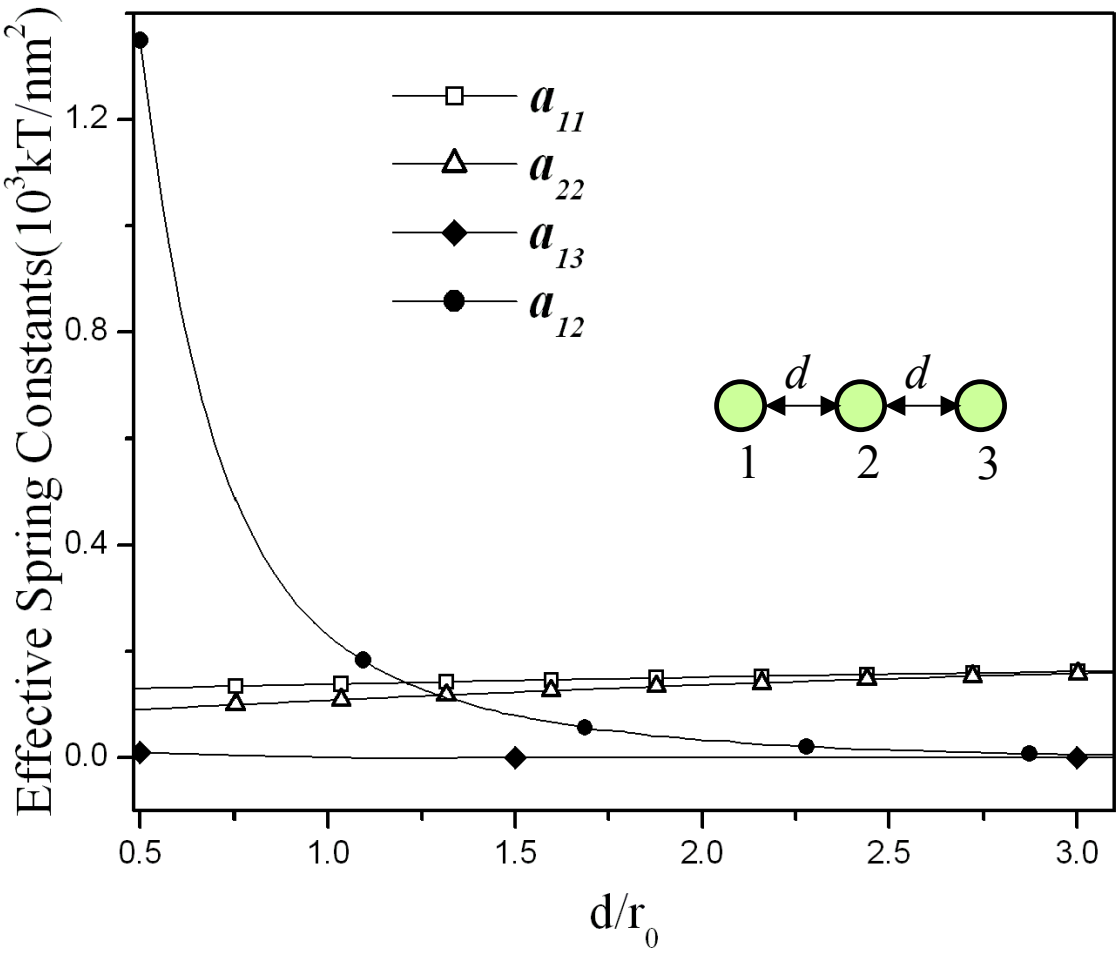


**Fig. 2**

(c) Regular centered hexagon.

The independent spring constants are: $a_{11}, a_{12}, a_{13}. a_{14,}\, a_{17}$ and $a_{77}$. Softening of diagonal spring constants at small distances are now pronounced, especially for the central insertion. Coupling between nearest neighbors is similar to the previous cases (with differences < 10%). As in case (b), total stabilization is practically proportional to the number of nearest neighbors: three for the edges and six for the central inclusion. Coupling between the second and more distant neighbors (described by $a_{13}$ and $a_{14}$) is always negligible.

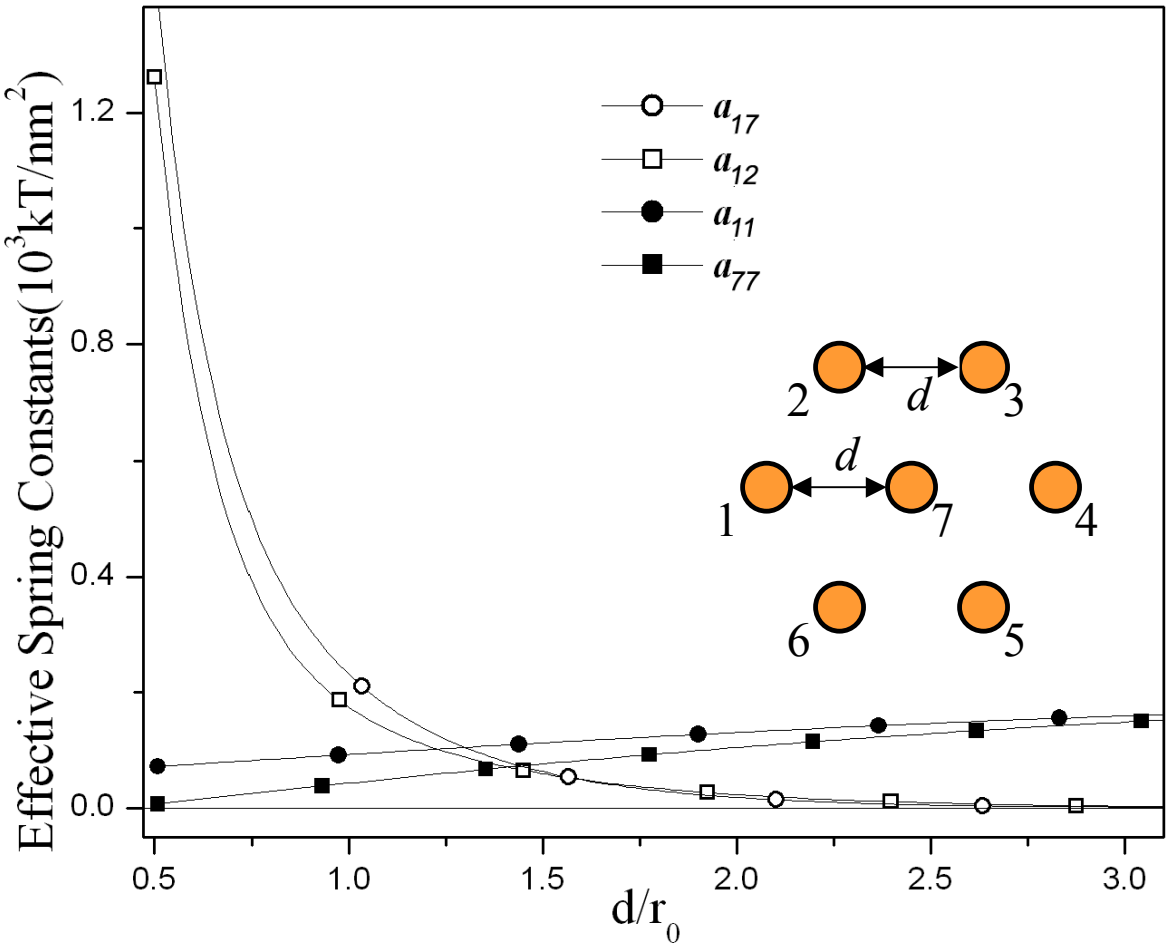


**Fig. 3**

## General features of the spring constants

Regardless of cluster type, the effective spring constants exhibit some commonality.

- For $d > 3.0$ nm, the diagonal constants, $a_{ii}$, approach those for independent insertions; non-diagonal constants $a_{ij}$, $\rightarrow 0$. Second and higher neighbor interaction is always negligible.
- The $a_{ii}$ soften at small d; this is enhanced almost additively with more nearest neighbors (i.e. for the central insertions: $a_{22}$ in Fig. 2, and $a_{77}$ in Fig. 3).
- The $a_{ij}$ increase as $d$ decreases. At separations $d_{ij} > 0.5$ nm, they are well described as $a_{i,j} \sim \alpha \, exp[(½ - d_{ij}/r_0)/\lambda]$ with the constants $\alpha$ and $\lambda$ essentially independent of the pair's position in the cluster.
- These features of the spring constants imply that at short separations inclusions are mutually stabilizing, an effect that is roughly proportional to the number of nearest neighbors. It thus follows that statistical analysis of assemblies of fluctuating channels can be greatly simplified by using the quite accurate exponential potential functions to describe membrane-mediated elastic interactions.
- Clustered inclusions adjust better to the collectively deformed membrane than an isolated insertion does to the unperturbed one. In effect the deformed membrane state is a new reference state for the elastic "springs" acting on channels.

## Cooperative influences on GA lifetime

We treat a simplified picture, emphasizing elastic coupling's effect on ion channel stability, assuming all but one channel is locked in the conductive dimer state, and determining the relative stability of this channel with respect to an isolated one.
This simplification is reasonable since $V_{\mathrm{int}\,r}$ the "intrinsic barrier" to separation due to the six inter-monomer hydrogen bonds, is very deep ($\sim 40kT$) and steep. Thus, a dimer resides predominantly near the potential minimum, ($u = u_0$).
The total cluster energy includes both intrinsic and elastic components

$$W(\mathrm{u_N}) = F(\mathrm{u_N}) + \sum_{i=1}^{N} V_{\mathrm{int}\,er}(u_i) \qquad (7)$$

We assume that the short-range intrinsic contribution is unaffected by the surrounding channels and proceed using the Arrhenius description of activation. From modeling and experiments with modified GA channels inter-monomer hydrogen bonds break easily when the separation δ reaches a "transition state" distance $\sim 0.16\ nm$ [6]. Following [6] we use this critical separation distance in estimating channel lifetimes. The relative probability of separation (i.e., attaining the transition state) for the preselected channel ($i$) in the cluster is

$$\frac{P^{i}_{\ cluster}}{P_{isolated}} \sim \exp[-(\Delta W^{i}(d) - \Delta W^{i}(\infty))/kT] \qquad (8)$$

Here $\Delta W^i(d)$ is the "transition barrier" for the *i*-th channel", the energy difference between the configurations with $u_i = u_0 - \delta/2$ (the *i-th* channel in its transition state) and $u_i = u_0$, with all other *N-1* channels "locked" in their dimer state ($u_j = u_0$ for $j \neq i$). The limit $d = \infty$ corresponds to an isolated channel. The intrinsic barrier cancels on the *rhs* of Eq. (8). Then for the lifetime it follows

$$2.3 \log(\tau^i{}_{cluster} / \tau_{isolated}) = (\Delta F^i(d) - \Delta F^i(\infty))/kT \qquad (9)$$

where $\Delta F^i(d)$ is a transition elastic barrier. Our results are presented for two limiting cases in Figs. 4 (two channels) and 5 (centered hexagon) .

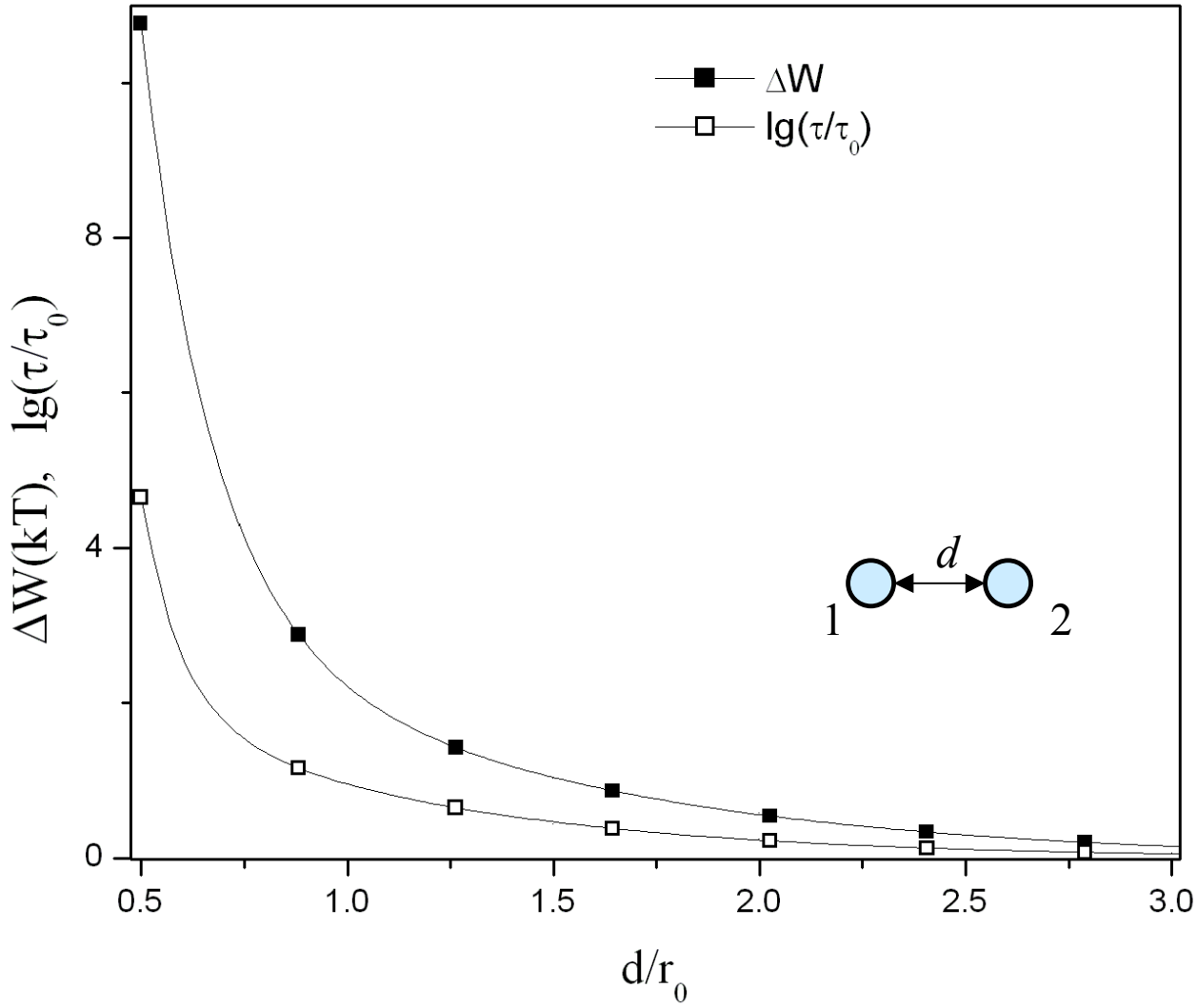


**Fig. 4**

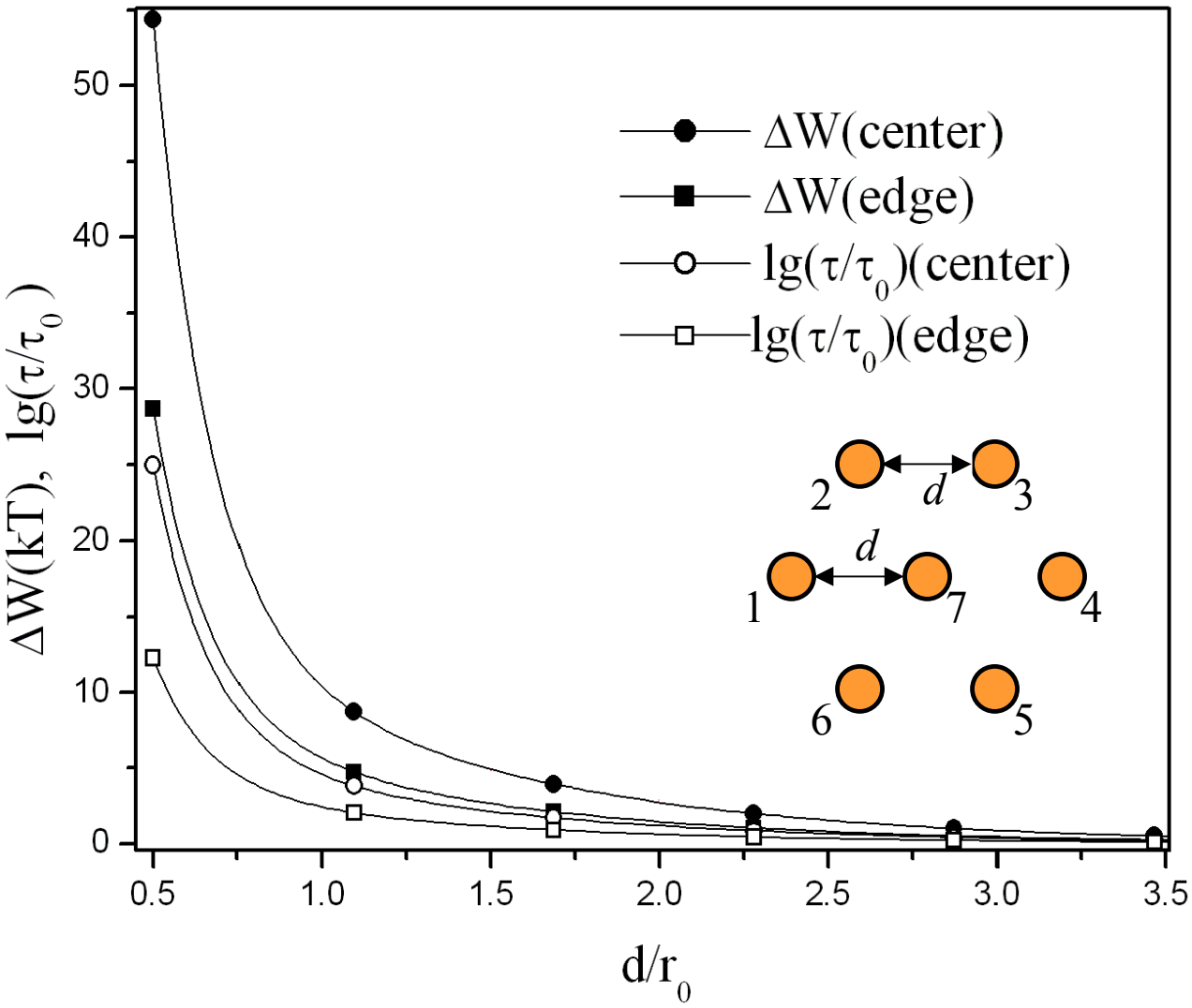
ΔW(center)
ΔW(edge)
lg(τ/τ0)(center)
lg(τ/τ0)(edge)
ΔW(kT), lg(τ/τ0)
d/r0
0
10
20
30
40
50
0.5
1.0
1.5
2.0
2.5
3.0
3.5
d
1
2
3
4
5
6
7

**Fig. 5**

# Conclusions

- Clustering increases channel lifetimes, and the effect is more pronounced for channels with more near neighbors. Thus, for two channels $d = 1$ nm apart interaction increases the lifetime 4-5 times, while for the channel at the center of a hexagon the effect reaches 3-4 orders of magnitude.
- This behavior is only representative. It is very sensitive to membrane specifics and especially to the mismatch. A mismatch change of only *20%* (assuming no change in any other parameters) would double the lifetime. Altogether it indicates that the elastic coupling can serve as an important mechanism of channel stabilization.
- In our view, additional stabilization can be caused by short-range interaction between the channels in a cluster. Such interaction would couple the GA monomers from neighboring channels, which can suppress the fluctuations responsible for breaking dimers.

# Acknowledgements

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