

# Evidence of a New Type of Protein-Protein Interaction Demonstrated by Actomyosin

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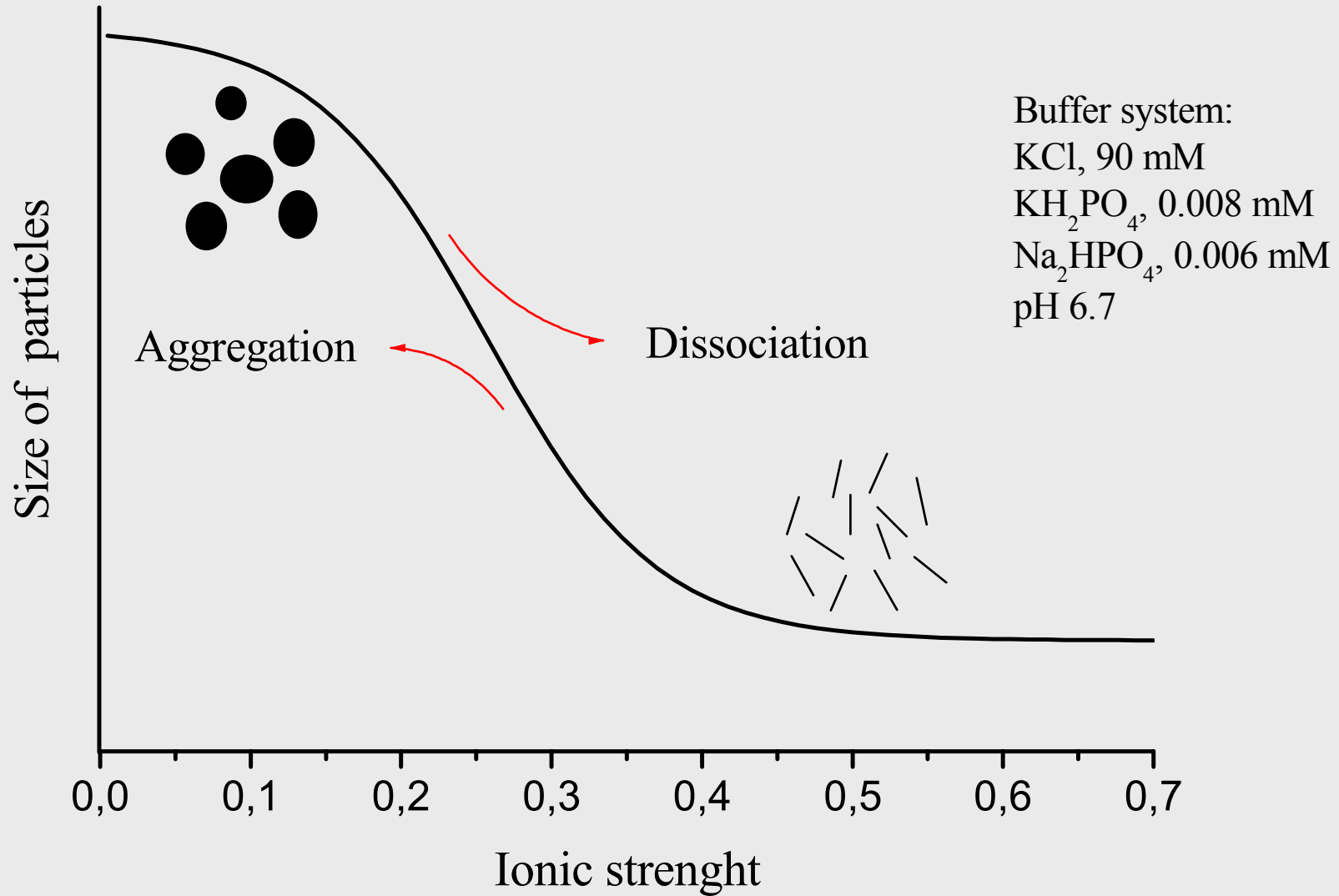
# The goal of this communication is to show that

(i) actomyosins with different calcium sensitivity interact with each other;

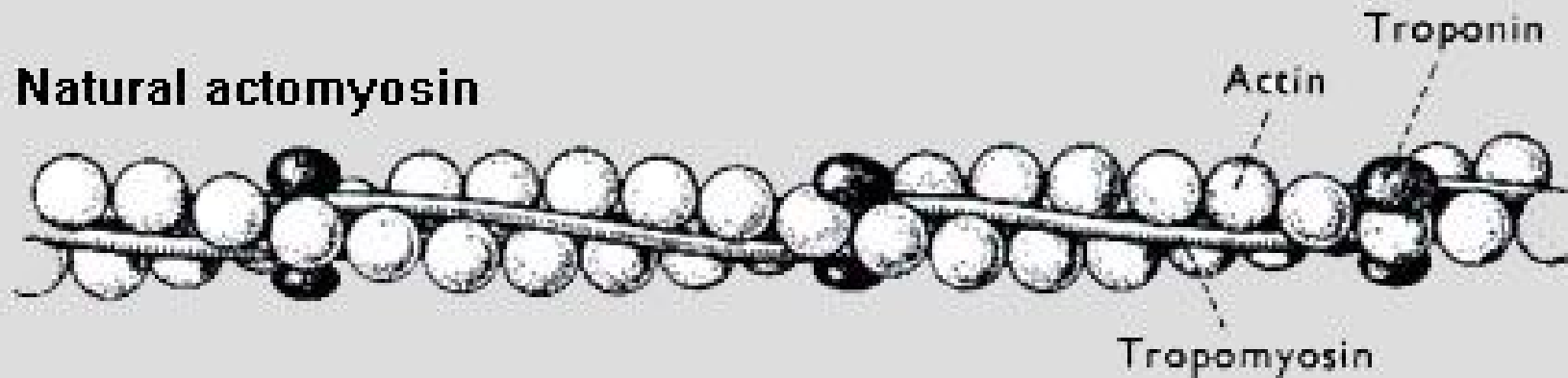
(ii) the desensitized actomyosin interacts with the natural actomyosin and modifies its calcium sensitive system. This modification consists both in a decrease of the natural actomyosin calcium sensitivity and in its complete disappearance. However, this disappearance is reversible;

(iii) the character of interaction of the desensitized actomyosin with natural actomyosin is unusual and is worth studying in detail.

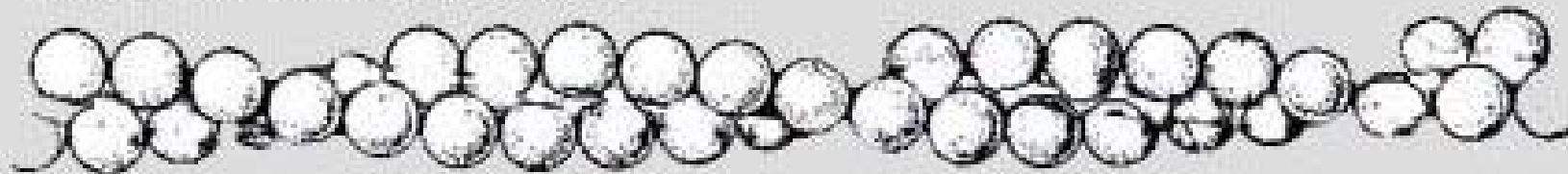
# Dependence of actomyosin particle size from ionic strenght (scheme)



# Removing of $\text{Ca}^{2+}$ -sensitive complex by washing



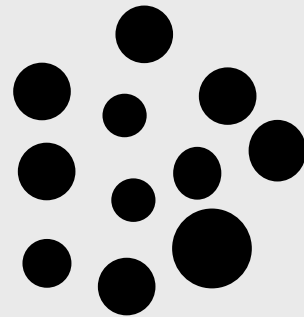
**Desensitized actomyosin**



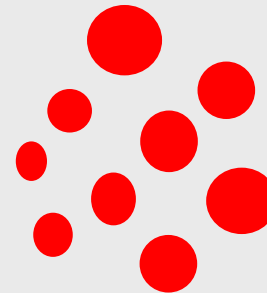
Preparation of desensitized actomyosin (*dAM*) was done by washing *nAM* at 4-6°C in pure water containing a minimal amount of Tris-buffer, pH 8.5-9.0 (Schaub, M.C. and Perry, S.V. 1969, *Biochem. J.* 115: 993)

# Preparation of two-component suspensions of actomyosin

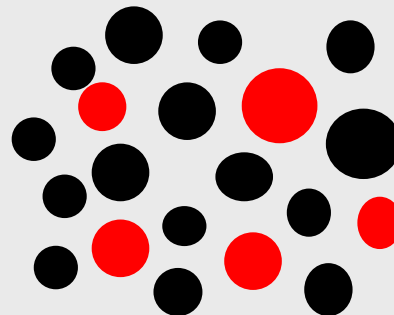
Natural AM



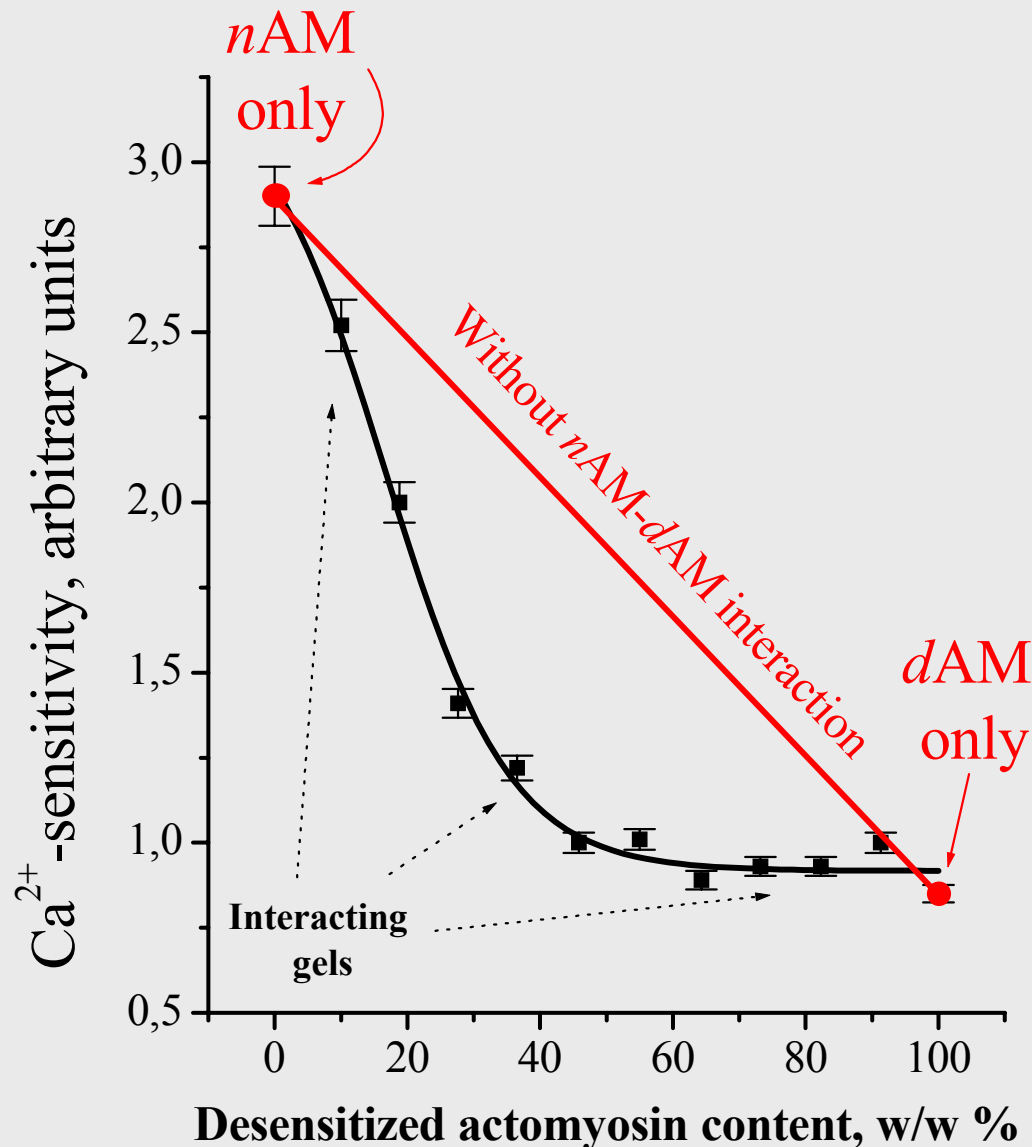
Desensitized AM



Two-component suspension



## Ca<sup>2+</sup>-sensitivity of two-component suspensions



**Superprecipitation** of *nAM*, *dAM*, and their mixtures was carried out at 20°C in phosphate buffer containing 0.5 mM Ca<sup>2+</sup> or 0.5 mM EGTA and 0.25 mM MgATP at a protein concentration of 0.1 mg/ml.

The reaction of superprecipitation was recorded by the method of **90°-angle light scattering at 450 nm**.

**Sensitivity to Ca<sup>2+</sup> (S<sub>ca</sub>)** was expressed quantitatively as a light scattering decrease (LSD):  $LSD = I_0 - I$ , in the presence both of 0.5 mM Ca<sup>2+</sup> (LSD<sub>ca</sub>) and of 0.5 mM EGTA (LSD<sub>egta</sub>), where  $I_0$  is the initial and  $I$ , the final level of the light scattering (in arbitrary units).

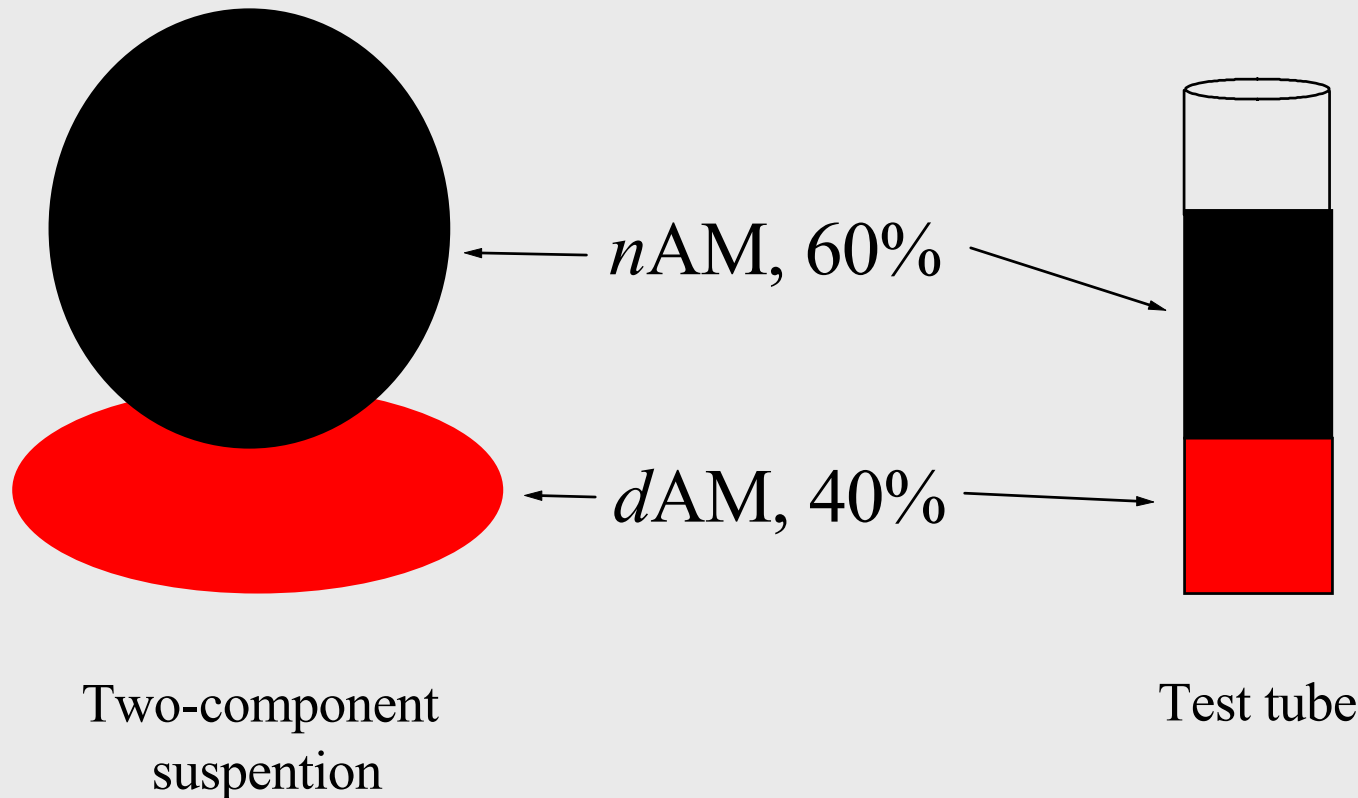
$$S_{ca} = LSD_{ca} / LSD_{egta}$$

As a rule, three measurements of S<sub>ca</sub> were done for each actomyosin sample.

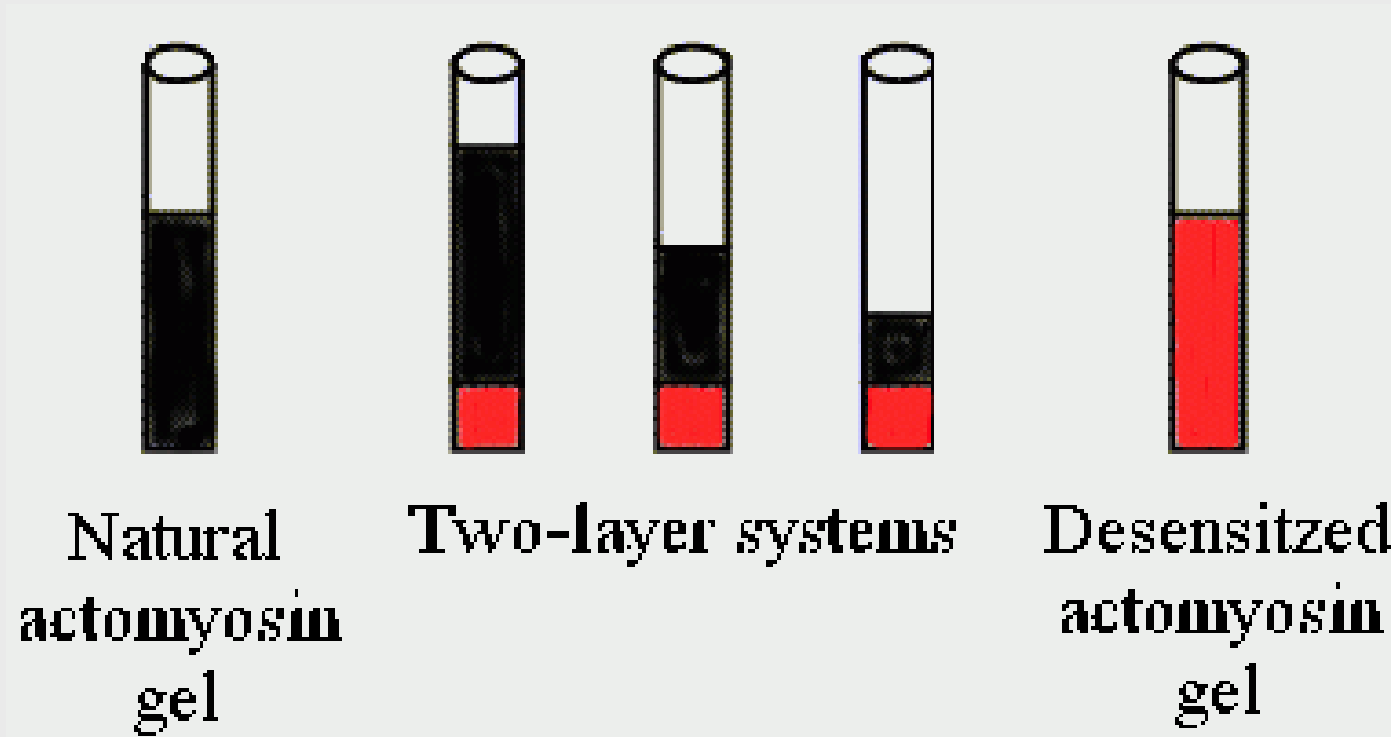
# Mechanical contact is needed to modify $\text{Ca}^{2+}$ -sensitivity of *n*AM

Particles of *n*- and *d*AM  
came into collision

Two-layer system modeling  
the interaction of the particles

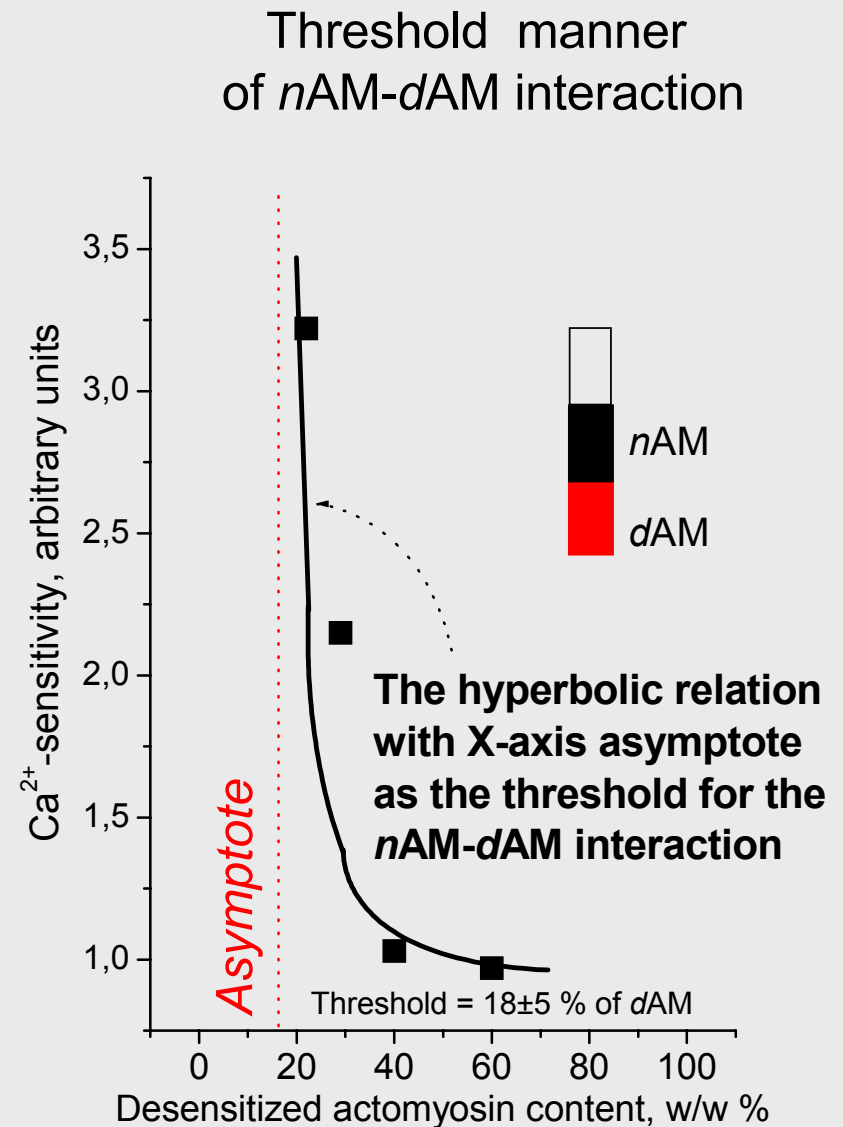
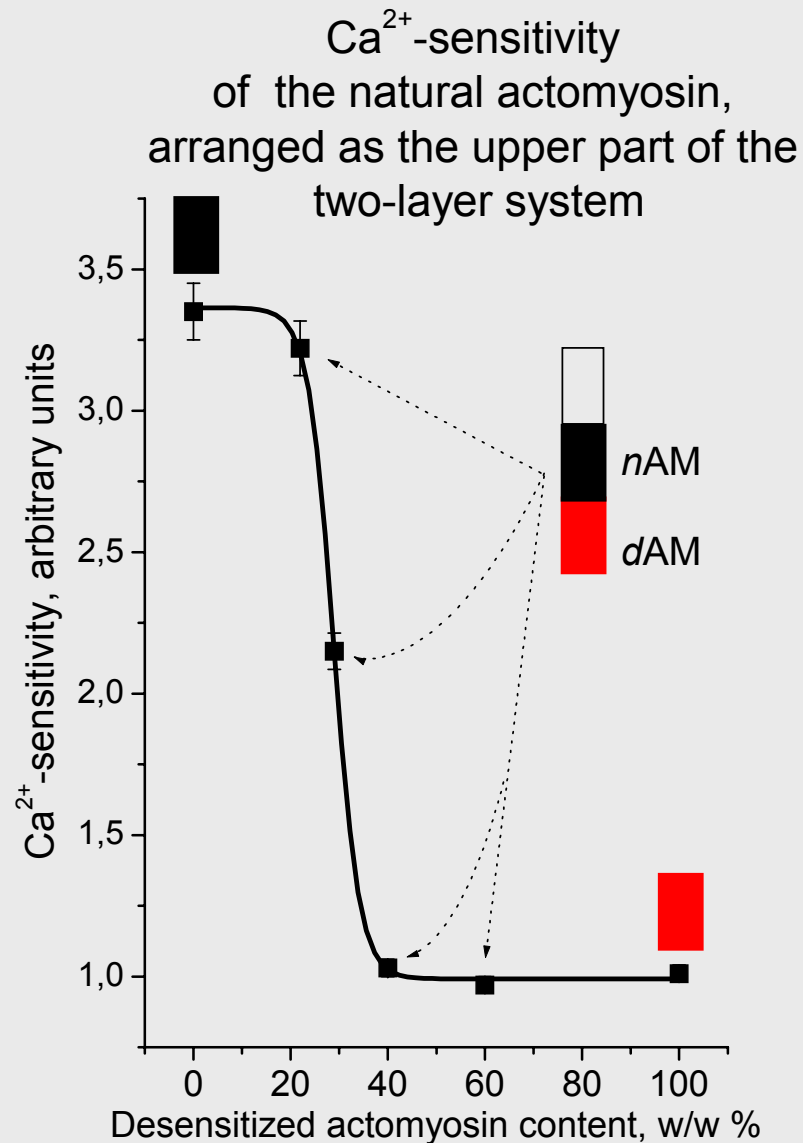


# Formation of two-layer gels

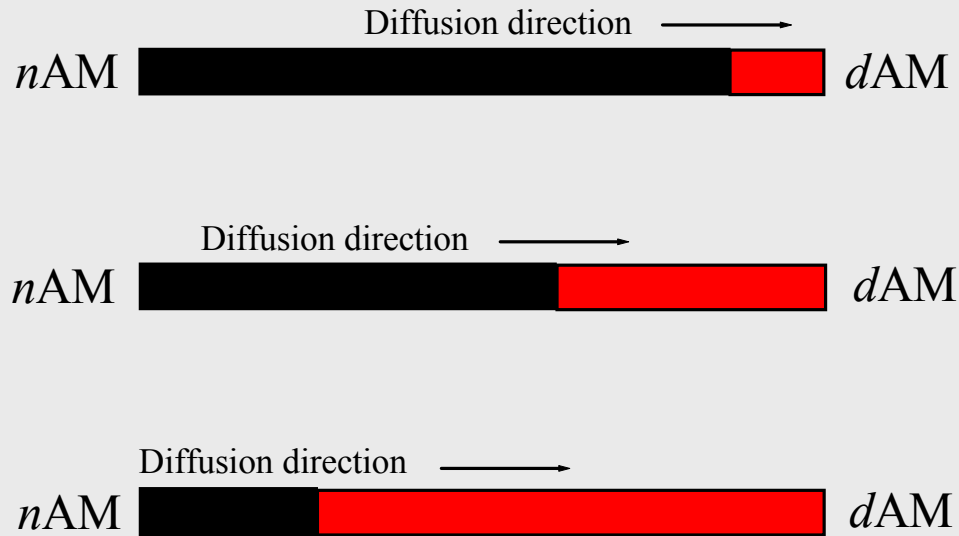


To form an actomyosin gel with non-uniform distribution of the TT-complex, a two-layer system was prepared in 10-ml centrifuge tubes, using the following steps. First, 30 mg of the *dAM* in suspension were placed in each tube and precipitated by centrifugation for 20 min at 10,000 g (with the final protein concentration of 30-40 mg/ml in the precipitate). Then the supernatant was removed; different amounts of the *nAM* suspension were added very carefully, and the centrifugation was repeated. The second centrifugation produced a two-layer system with the overlying supernatant that remained in the tubes during the entire experiment.

# Threshold manner of *nAM-dAM* interaction in the two-layer system

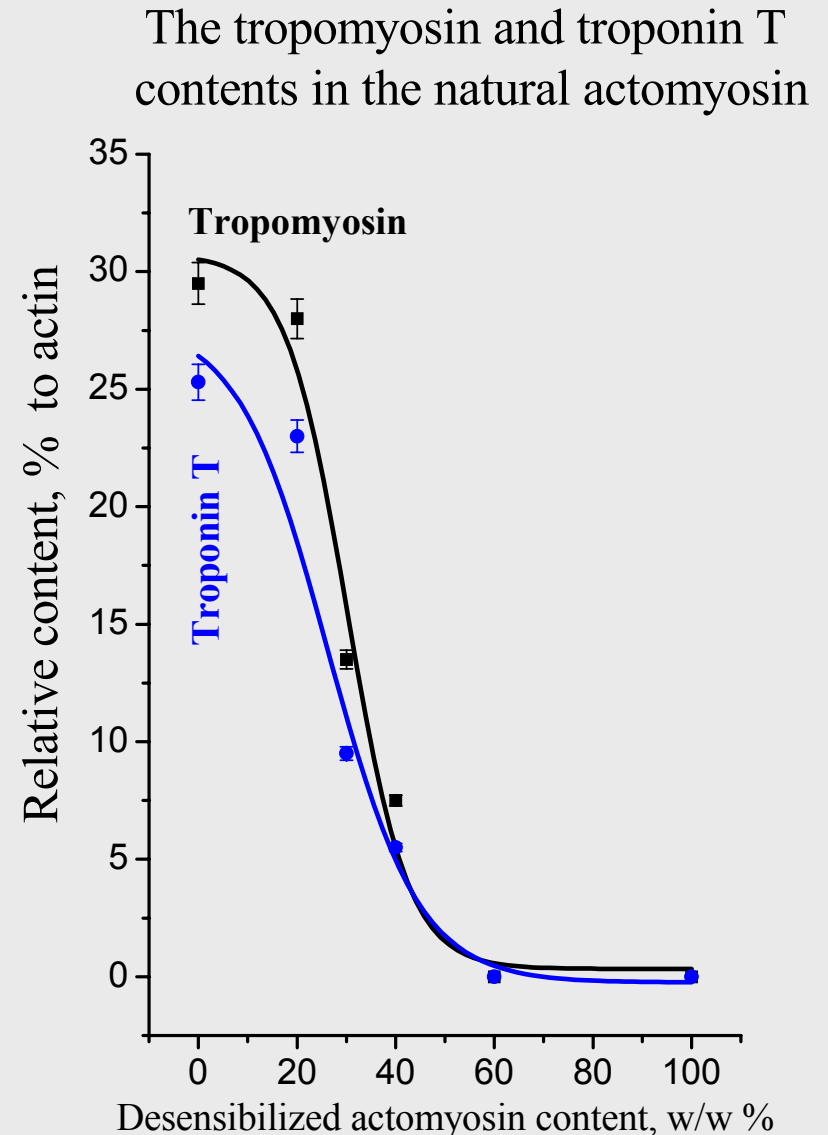


# Formation of two-tube systems for electrophoretical analysis of tropomyosin and troponin T distribution.

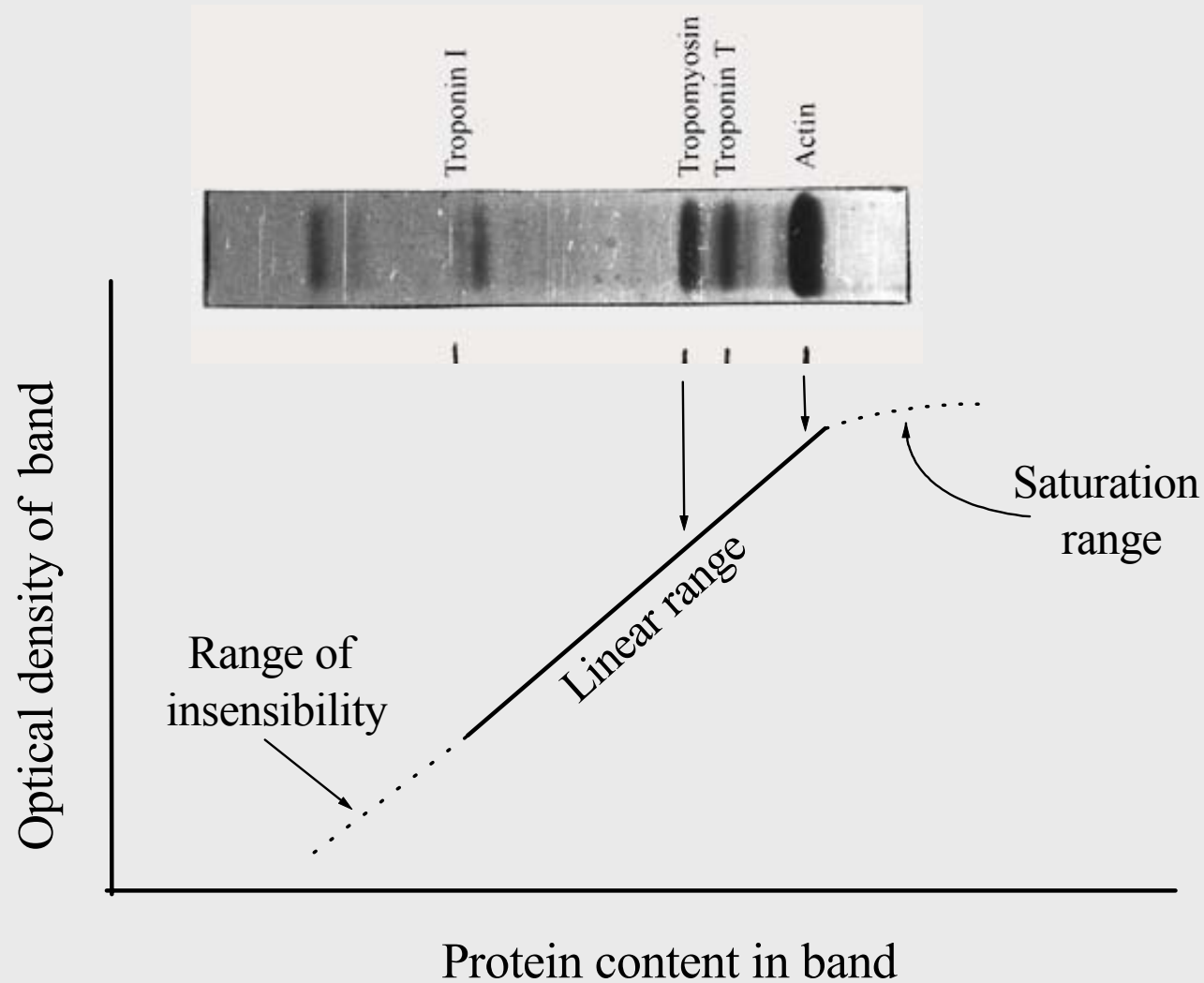


The apparent diffusion coefficient for the TT-complex in *dAM* gel is about  $(1-4) \cdot 10^{-4}$  cm<sup>2</sup>/sec, i.e. three orders higher than the same values for protein diffusion in water.

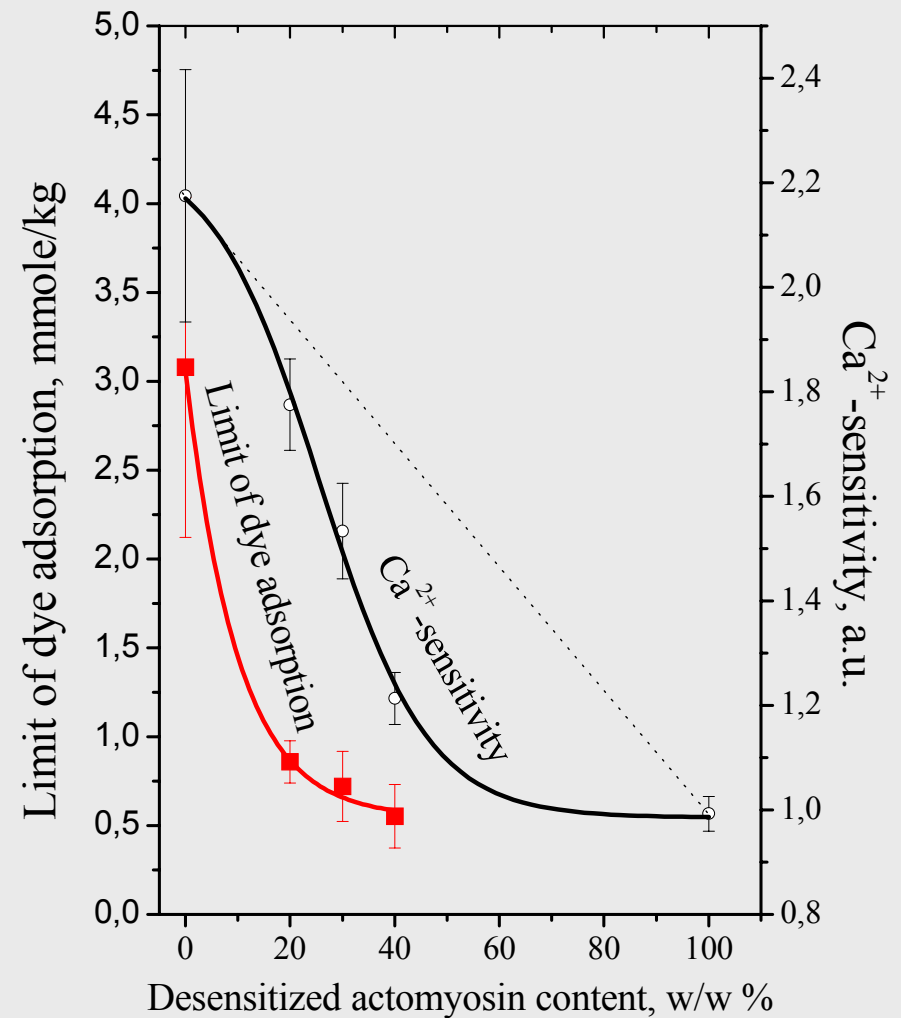
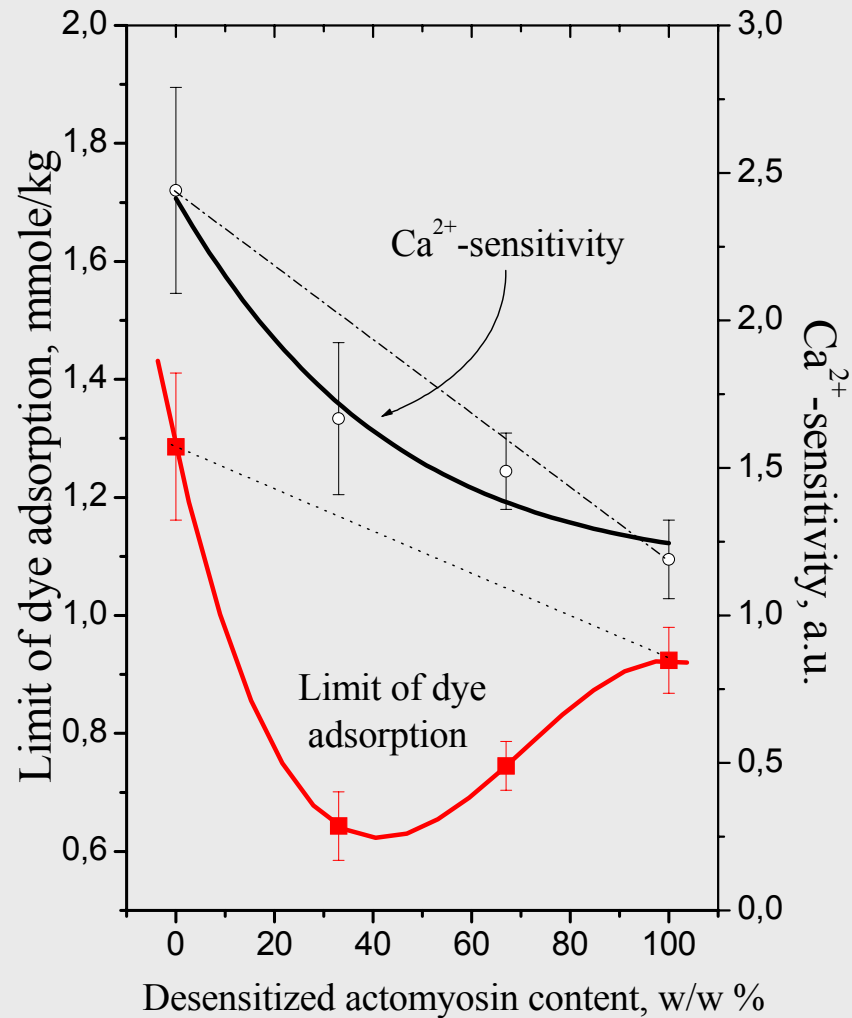
In our conditions, diffusion of tropomyosin-troponin complex began in 6 hour's time after creation of the two-tube systems and came to an end after 12 hours. So, as a whole the diffusion takes up about 6 hours.



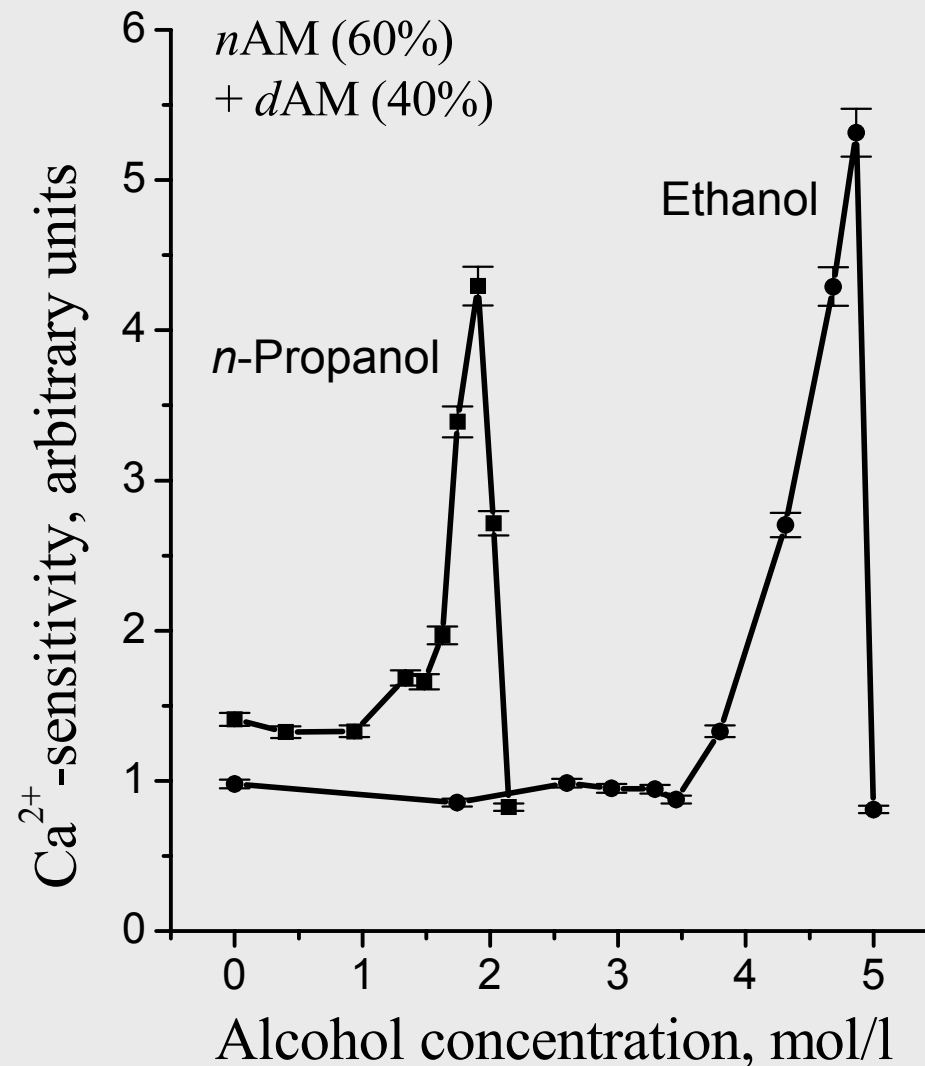
Keeping of actin band density in the linear part of the dependency is the main problem of the electrophoretical study



Structural changes in actomyosin gel as a result of interaction of *nAM* with *dAM*. Structural changes were tested by the dye, neutral red.



The  $\text{Ca}^{2+}$ -sensitivity of the suspension mixture containing 60% of *n*AM and 40% of *d*AM plotted as a function of *n*-propanol or ethanol concentration after 15-20 h incubation of the protein mixture with the alcohols.



# CONCLUSIONS

- The studied protein complexes interact in the gel state **as colloidal particles**.
- In this reason the interaction begins **at a surface** of interacting gel particles and extends over the entire gel volume.
- the interaction of actomyosin gels **reversibly** disturbs normal operation of the  $\text{Ca}^{2+}$ -sensitivity system and can change the troponin-tropomyosin complex **distribution** in the whole gel volume.
- The revealed peculiarities of the interaction allow claiming a new type of protein-protein interaction.

# Acknowledgements

The author wishes to thank sincerely

- Dr. A. A. Vereninov for a decisive support of this study.
- Dr. L. Z. Pevzner for useful criticism

The project home page:  
<http://actomyosin.narod.ru>