UNIVERSITAS TARTUENSIS
1632

Application of baculovirus technology for Systems Biology studies of G Protein-Coupled receptors

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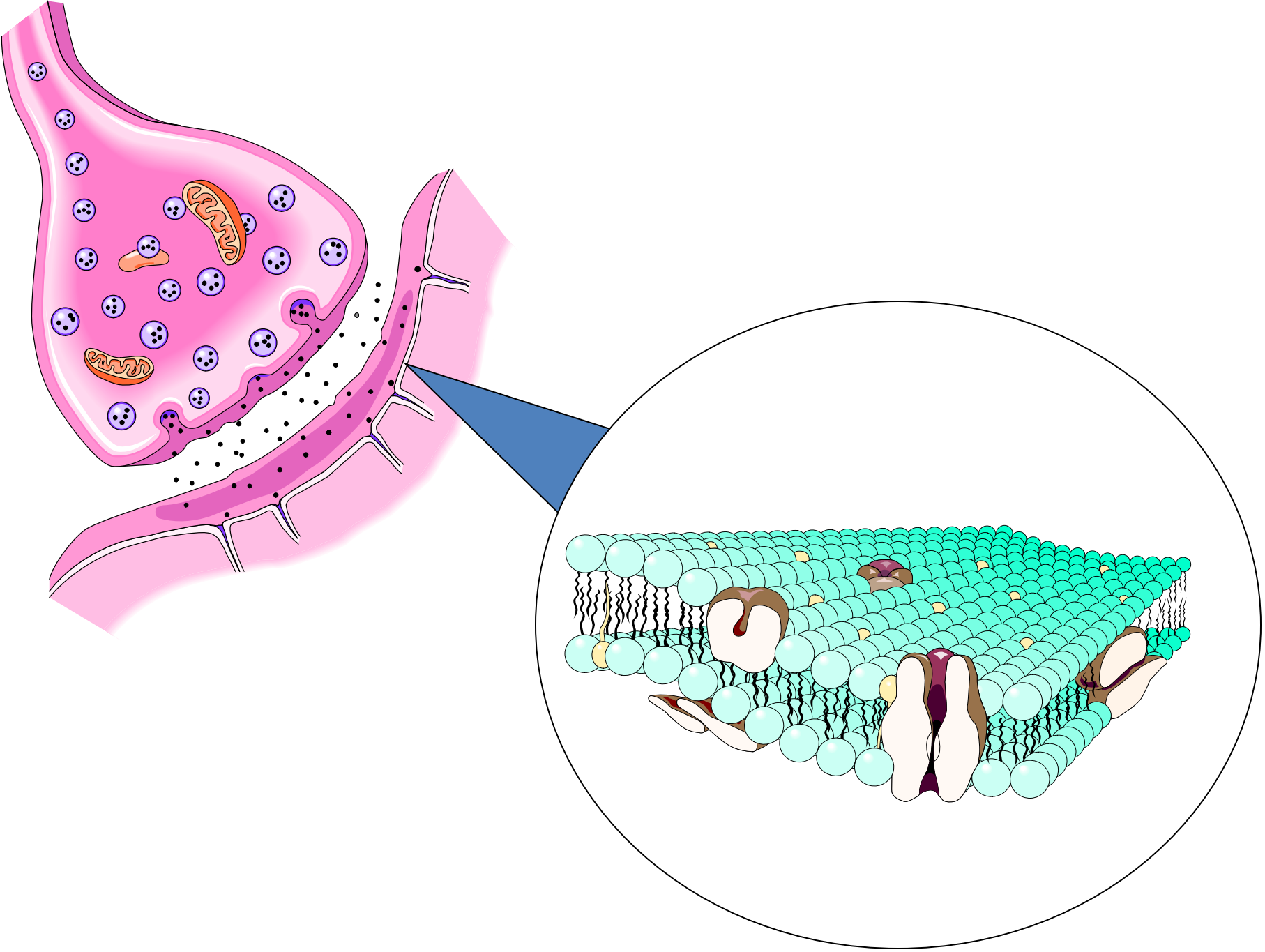
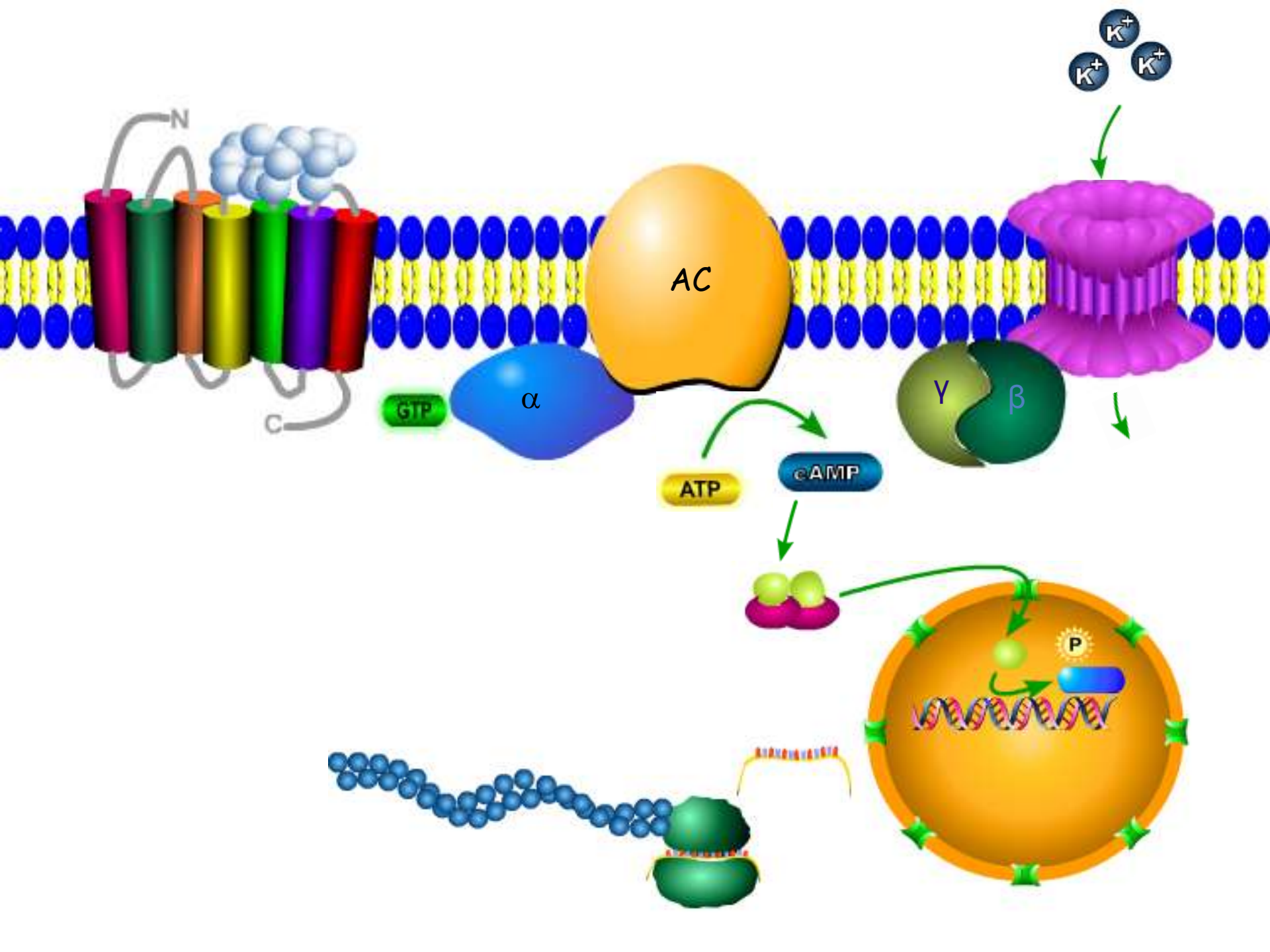
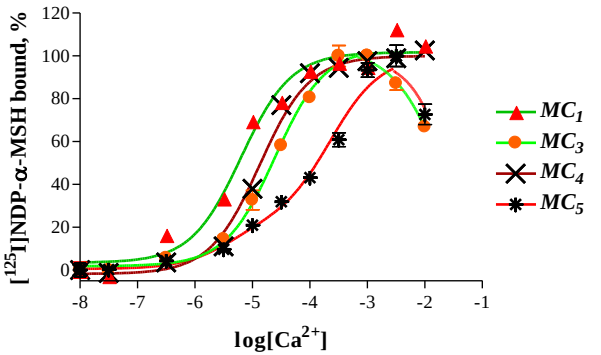
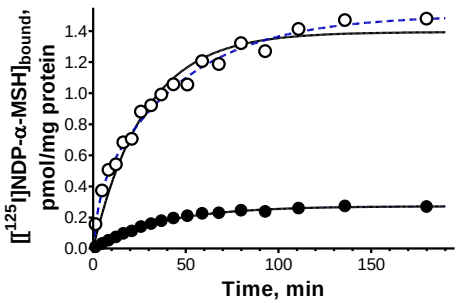
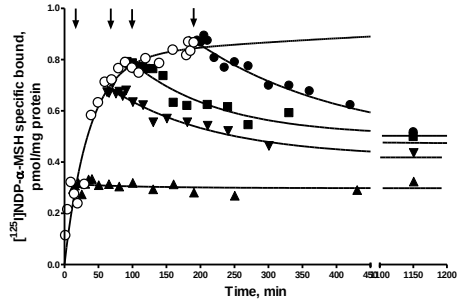


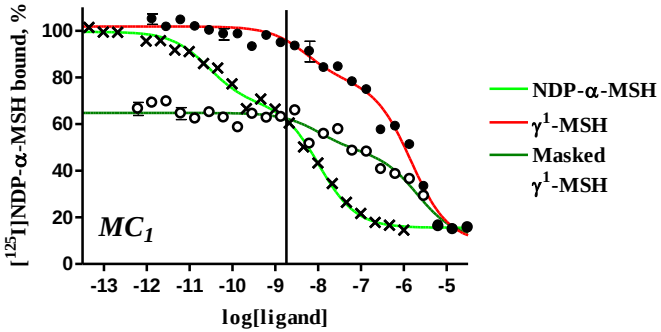

K+
K+
K+
N
C
AC
α
GTP
γ
β
ATP
cAMP
P

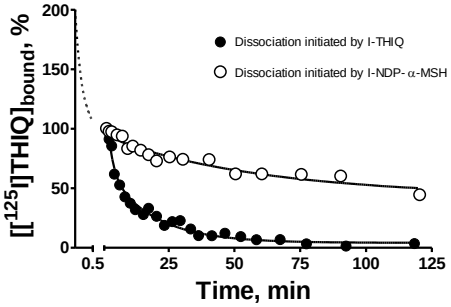
Binding assays with radioactivly labeled ligands to membranes of *Sf9* cells expressing the recombinant human Melanocortin (MC) receptor subtypes

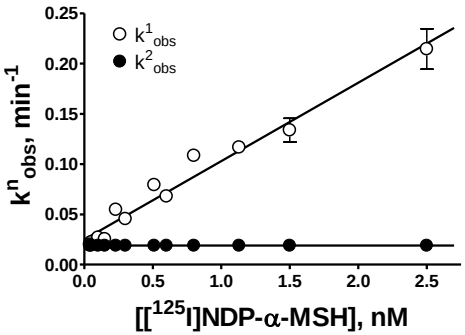


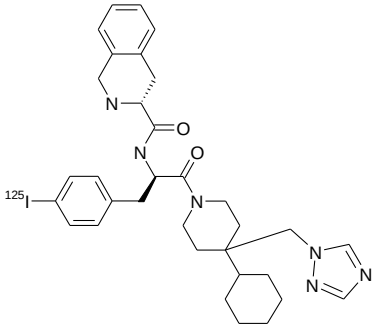












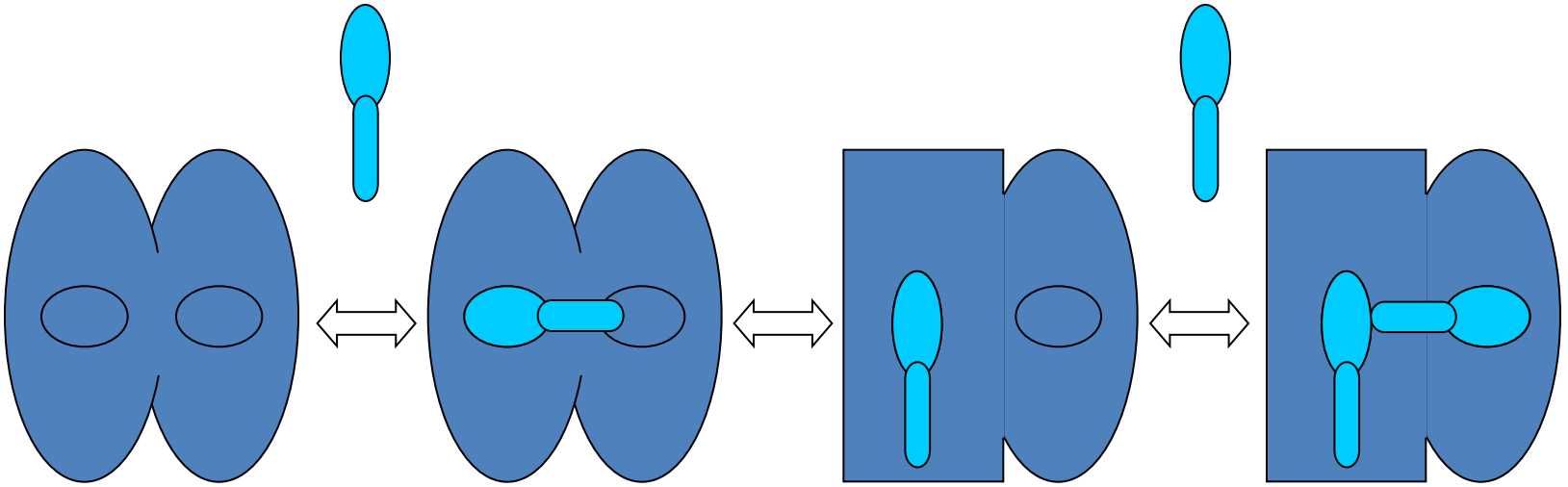
Kopanchuk S., *et al.*, 2006. Kinetic evidence for tandemly arranged ligand binding sites in melanocortin 4 receptor complexes, *Neurochem. Int.*

Kopanchuk S., *et al.* 2005, Co-operative regulation of ligand binding to melanocortin receptor subtypes: Evidence for interacting binding sites, *Eur. J. Pharmcol.*

Mutulis F., et al., 2003. A non-peptide radioiodinated high affinity melanocortin-4 receptor ligand. *Journal of Labelled Compounds and Radiopharmaceuticals*

„Two-tandem binding“ model on constitutive dimers

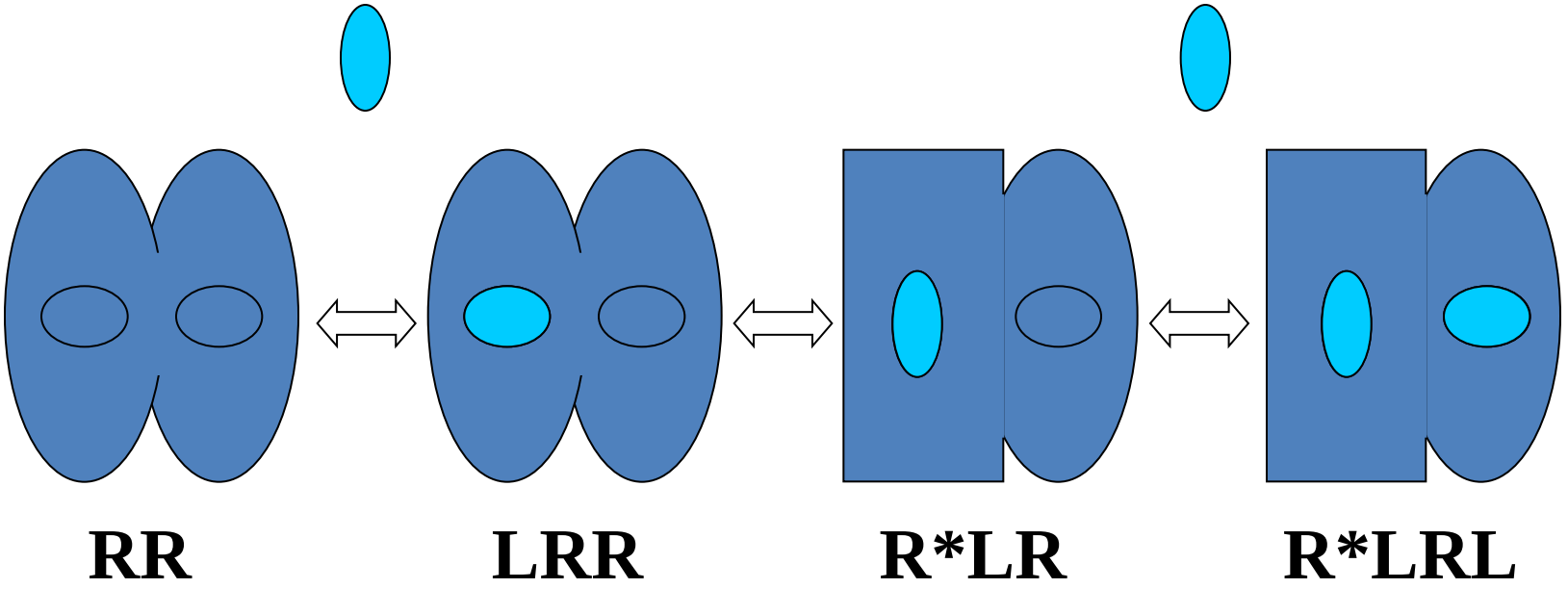
$$RR + L + L \underset{k_{-1}}{\overset{k_1}{\rightleftharpoons}} LRR + L \underset{k_{-2}}{\overset{k_2}{\rightleftharpoons}} R^*LR + L \underset{k_{-3}}{\overset{k_3}{\rightleftharpoons}} R^*LRL$$



RR LRR R*LR R*LRL

„Two-tandem binding“ model on constitutive dimers

$$RR + L + L \underset{k_{-1}}{\overset{k_1}{\rightleftharpoons}} LRR + L \underset{k_{-2}}{\overset{k_2}{\rightleftharpoons}} R^*LR + L \underset{k_{-3}}{\overset{k_3}{\rightleftharpoons}} R^*LRL$$



„Two-tandem binding“ model on constitutive dimers

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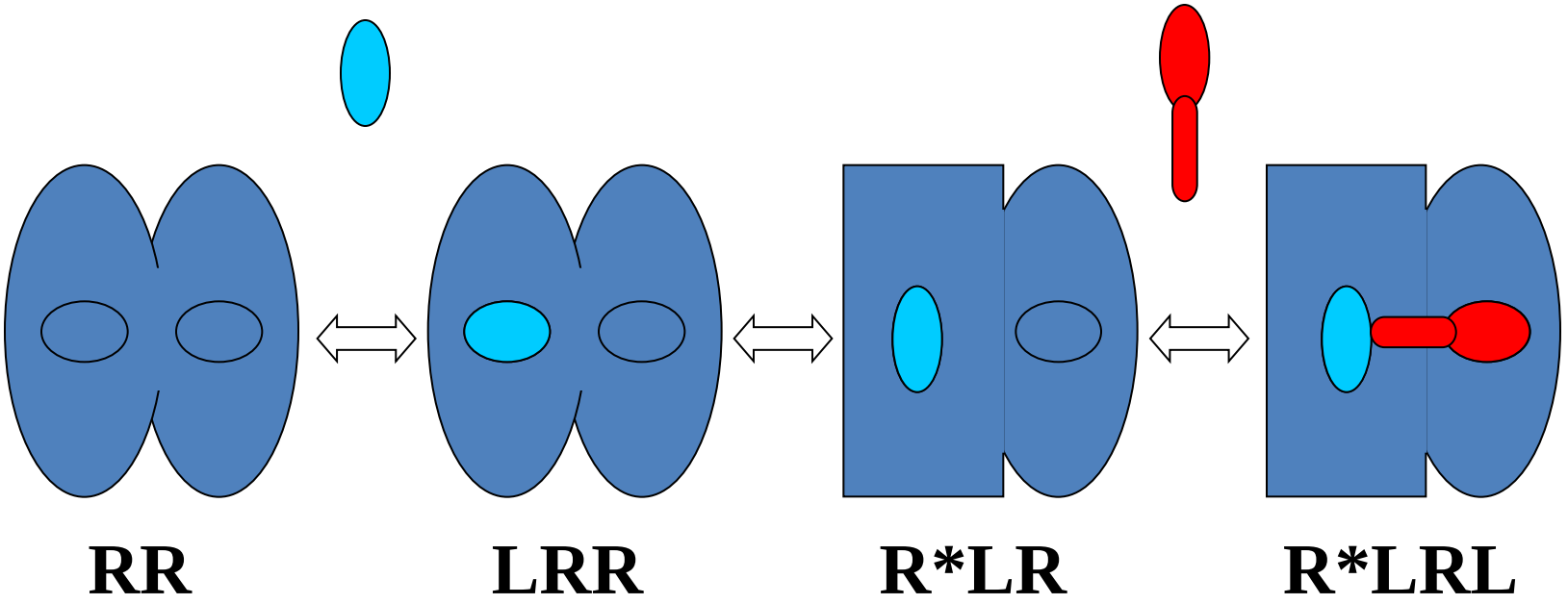


$$rl = \frac{r_o \cdot l \cdot K_2 \cdot K_3}{K_1 \cdot K_2 \cdot K_3 + K_3 \cdot (1 + K_2) \cdot l + l^2} \cdot \left(1 - e^{-\left(\frac{l \cdot (k_{-1} + k_2) \cdot K_2 \cdot K_3}{K_1 \cdot K_2 \cdot K_3 + K_3 \cdot l + l^2} + k_{-1} + k_2 \right) \cdot t} \right)$$

$$lr = \frac{r_o \cdot l \cdot K_3}{K_1 \cdot K_2 \cdot K_3 + K_3 \cdot (1 + K_2) \cdot l + l^2} \cdot \left(1 - e^{-\left(\frac{l \cdot (k_{-2} + k_3 \cdot l) \cdot K_3}{K_1 \cdot K_2 \cdot K_3 + K_2 \cdot K_3 \cdot l + l^2} + k_3 \cdot l + k_{-2} \right) \cdot t} \right)$$

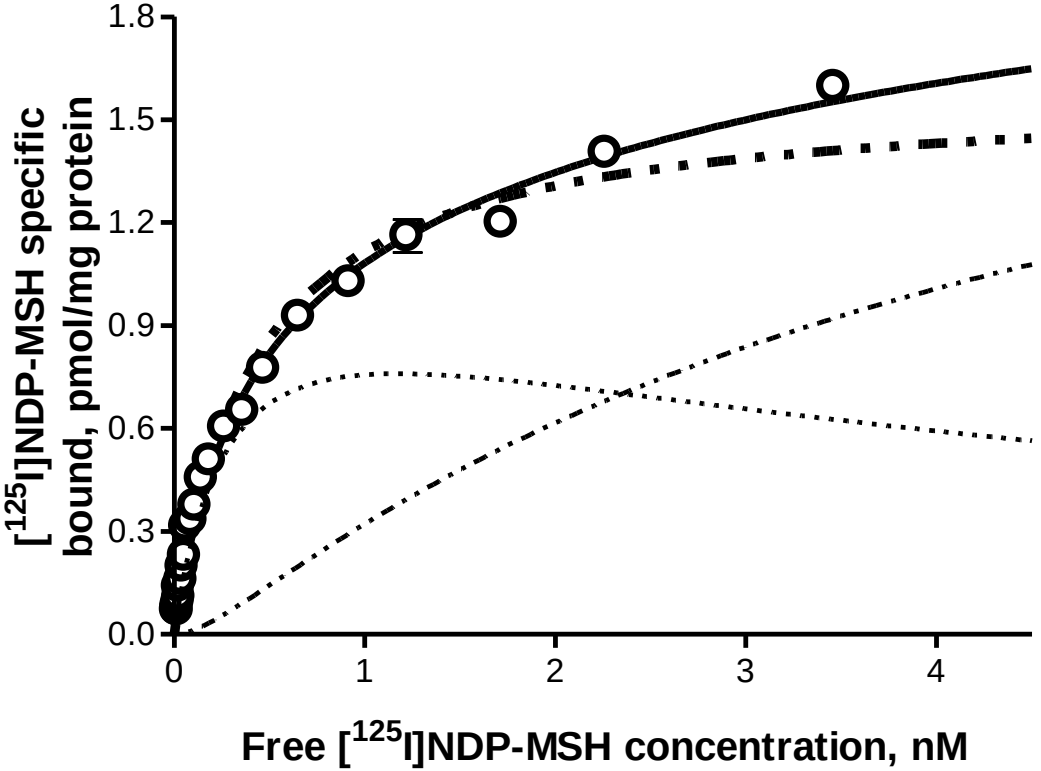
$$lrl = \frac{r_o \cdot l^2}{K_1 \cdot K_2 \cdot K_3 + K_3 \cdot (1 + K_2) \cdot l + l^2} \cdot \left(1 - e^{-\left(\frac{k_3 \cdot l^2}{K_1 \cdot K_2 + l \cdot (1 + K_2)} + k_{-3} \right) \cdot t} \right)$$

$$Bound = rl + lr + 2 \cdot lrl$$

$$RR + L + L \underset{}{\overset{K_1}{\rightleftharpoons}} LRR + L \overset{K_2}{\rightleftharpoons} R^*LR + L \overset{K_3}{\rightleftharpoons} R^*LRL$$

$$RR + L + L \underset{k_{-1}}{\overset{k_1}{\rightleftharpoons}} LRR + L \underset{k_{-2}}{\overset{k_2}{\rightleftharpoons}} R^*LR + L \underset{k_{-3}}{\overset{k_3}{\rightleftharpoons}} R^*LRL$$

$$t \to \infty$$



$$B = \frac{r_o \cdot l}{\frac{K_{all} - l^2}{K_{add} + 2 \cdot l} + l}$$

$$K_{all} = K_1 \cdot K_2 \cdot K_3$$

$$K_{add} = K_3 \left(1 + K_2\right)$$

$$RR+L+L \underset{}{\overset{K_1}{\rightleftharpoons}} LRR+L \overset{K_2}{\rightleftharpoons} R^*LR+L \overset{K_3}{\rightleftharpoons} R^*LRL$$

$$RR+L+L \underset{k_{-1}}{\overset{k_1}{\rightleftharpoons}} LRR+L \underset{k_{-2}}{\overset{k_2}{\rightleftharpoons}} R^*LR+L \underset{k_{-3}}{\overset{k_3}{\rightleftharpoons}} R^*LRL$$

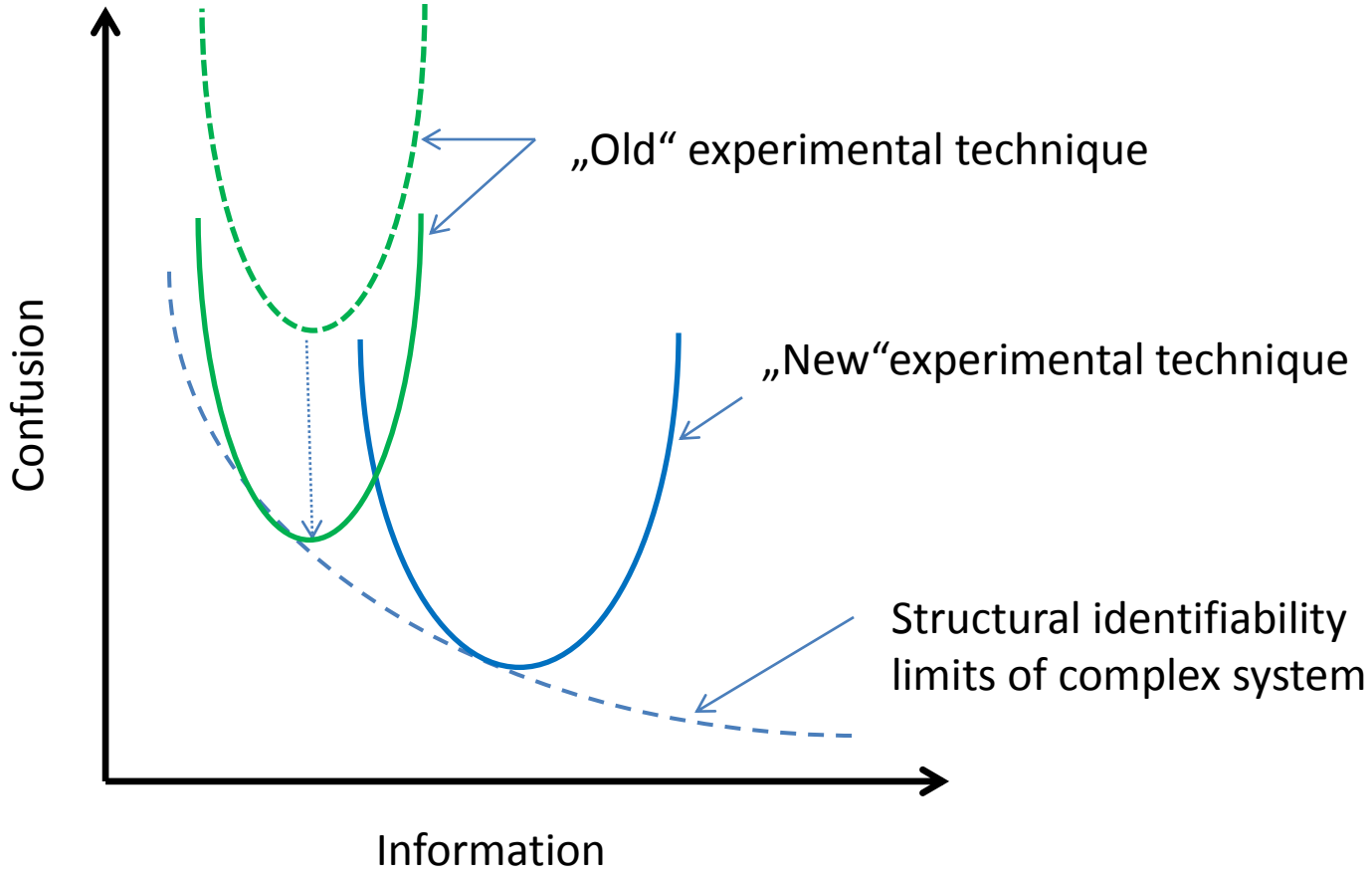
$$B = \frac{r_o \cdot l}{K(L) + l} \quad \longleftarrow \quad B = \frac{r_o \cdot l}{\frac{K_{all} - l^2}{K_{add} + 2 \cdot l} + l}$$

$$\frac{dK(L)}{dL} = \kappa$$

$$K_{all} = K_1 \cdot K_2 \cdot K_3$$

$$K_{add} = K_3 \left(1 + K_2\right)$$

Our confusion as function of available information with practical and structural identifibility limits



"To link or not to link: the "global" question"

$$Total\ Fluorescence\ Intensity\ (TFI) \qquad \sim I_x + I_y + I_z$$

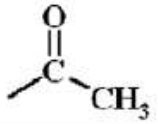
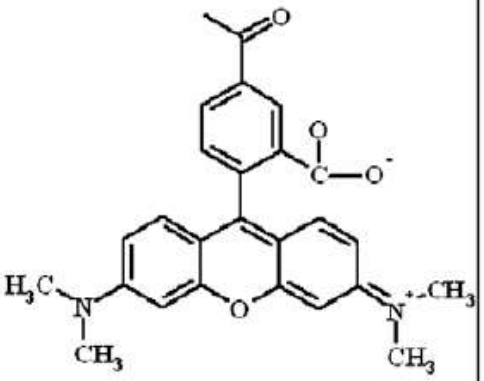
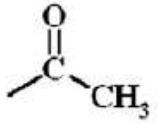
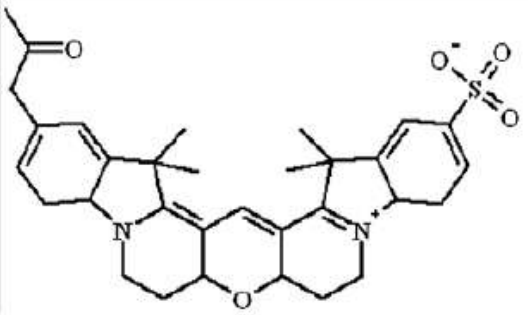
"sometimes" (*isotropic environment*): $I_x = I_y = I_{per}$ *and* $\neq I_z = I_{paral}$

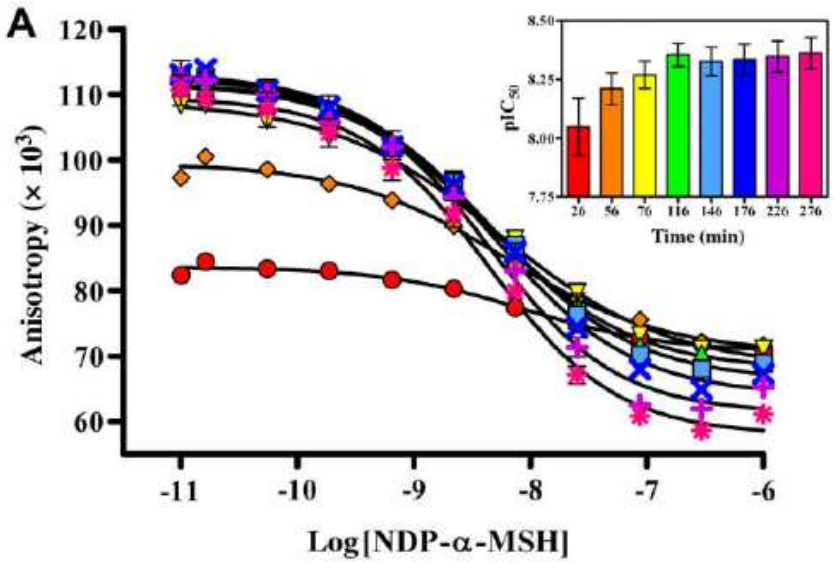
$$Detected\ Fluorescence\ Intensity\ (DFI) \quad \sim I_{paral} + I_{per} \quad \text{but} \quad TFI \sim I_{per} + 2 \cdot I_{paral}$$

$$Fluorescence\ Anisotropy\ (FA) \quad \boxed{r = \frac{I_{paral} - I_{per}}{I_{paral} + 2 \cdot I_{per}}} \qquad \boxed{TFI = \frac{3 \cdot DFI}{2 + r}}$$

R_1 R_2

Ser-Tyr-Ser-Nle-Glu-His-D-Phe-Arg-Trp-Gly-Lys-Pro-Val-NH_2

	R_1	R_2
NDP-α-MSH		– H
TAMRA-NDP-α-MSH		– H
Cy3B-NDP-α-MSH		



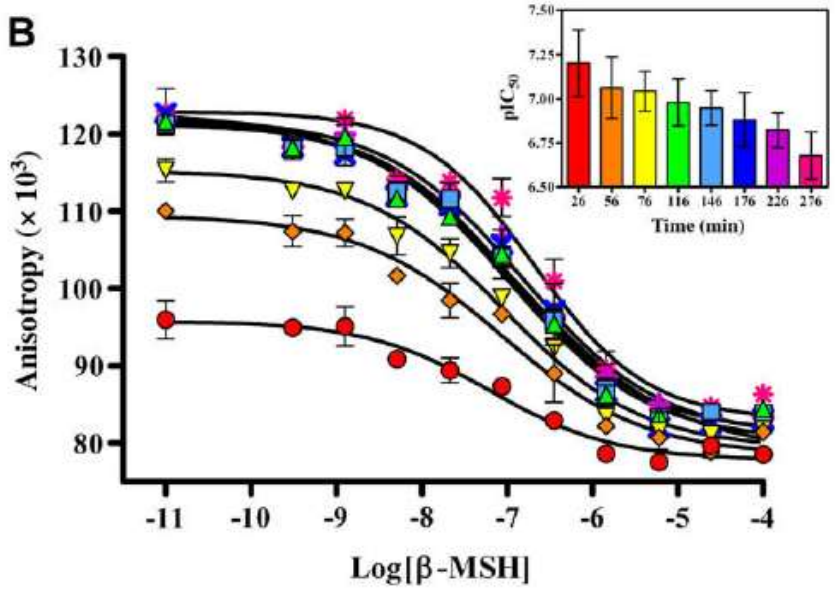
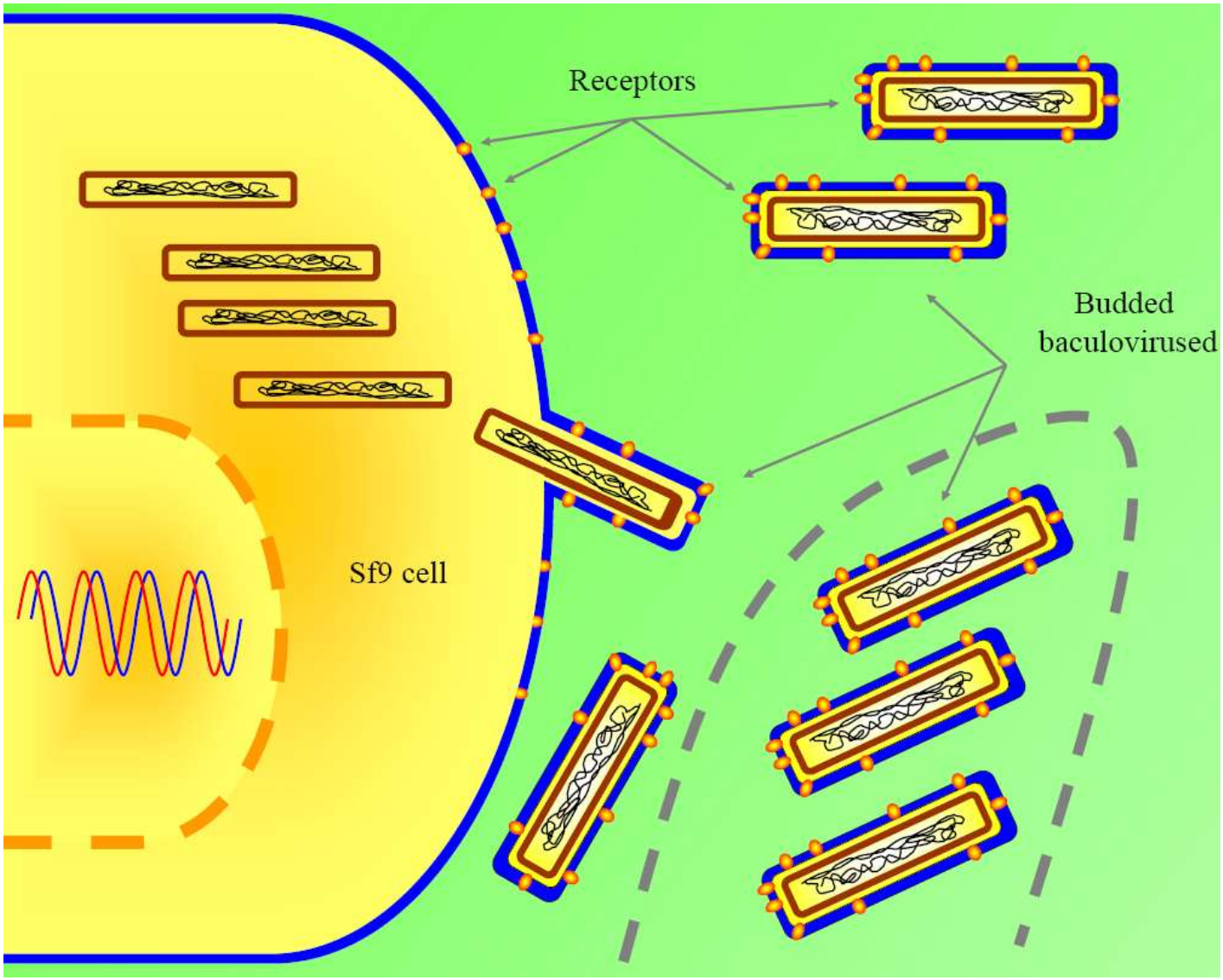


Table 1
Apparent potencies of ligands to MC_4 receptors in Sf9 cell membranes determined in competition with Cy3B–NDP-α-MSH and TAMRA–NDP-α-MSH.

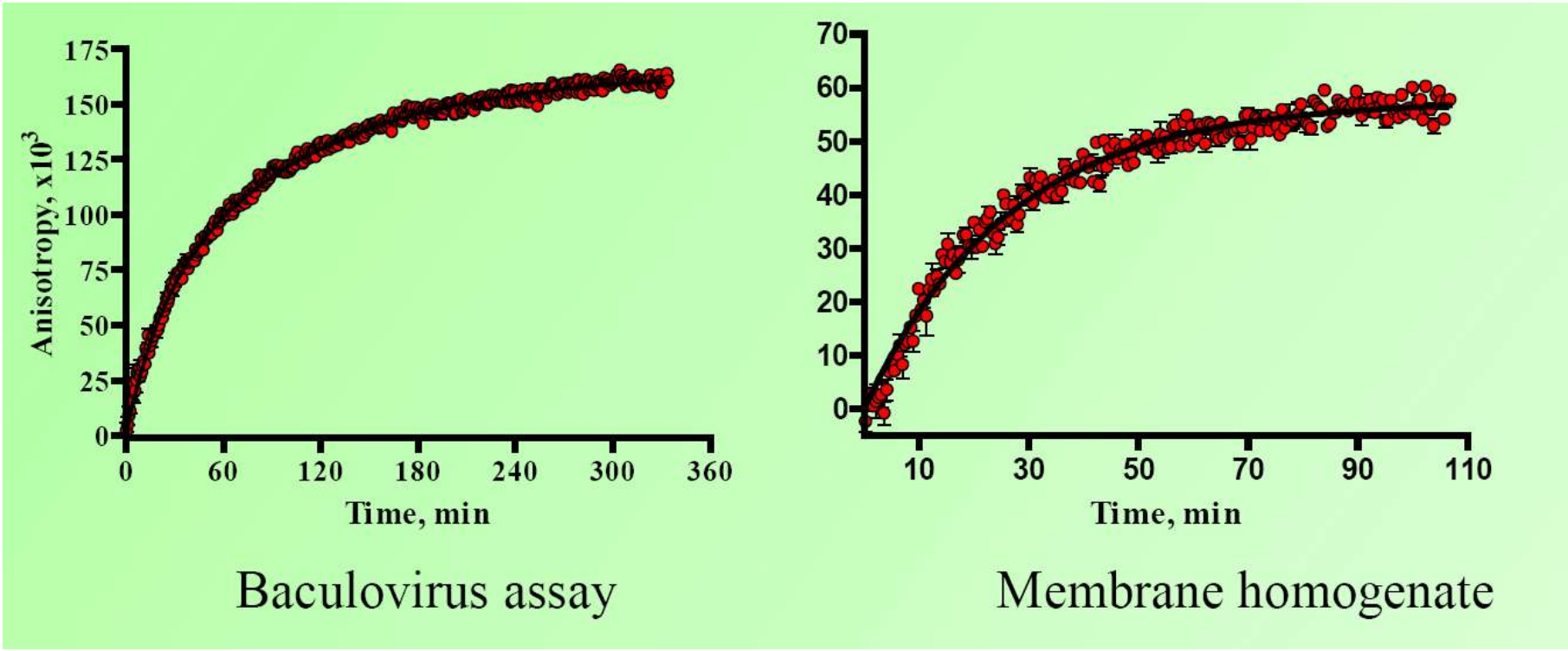
	Cy3B–NDP-α-MSH		TAMRA–NDP-α-MSH	
	pIC_{50}[a]	Hill slope	pIC_{50}[a]	Hill slope
NDP-α-MSH	8.35 ± 0.12	0.80 ± 0.18	8.14 ± 0.09	0.92 ± 0.15
β-MSH	6.55 ± 0.58	0.53 ± 0.08	6.14 ± 0.10	1.08 ± 0.21
HS-024	7.79 ± 0.12	0.97 ± 0.31	7.62 ± 0.10	1.08 ± 0.24
I-THIQ	7.59 ± 0.12	0.73 ± 0.15	7.69 ± 0.03	1.04 ± 0.08

Veiksina S., et al. 2010. Fluorescence anisotropy assays for pharmacological characterisation of ligand binding dynamics to melanocortin 4 receptors. *Analytical Biochemistry*

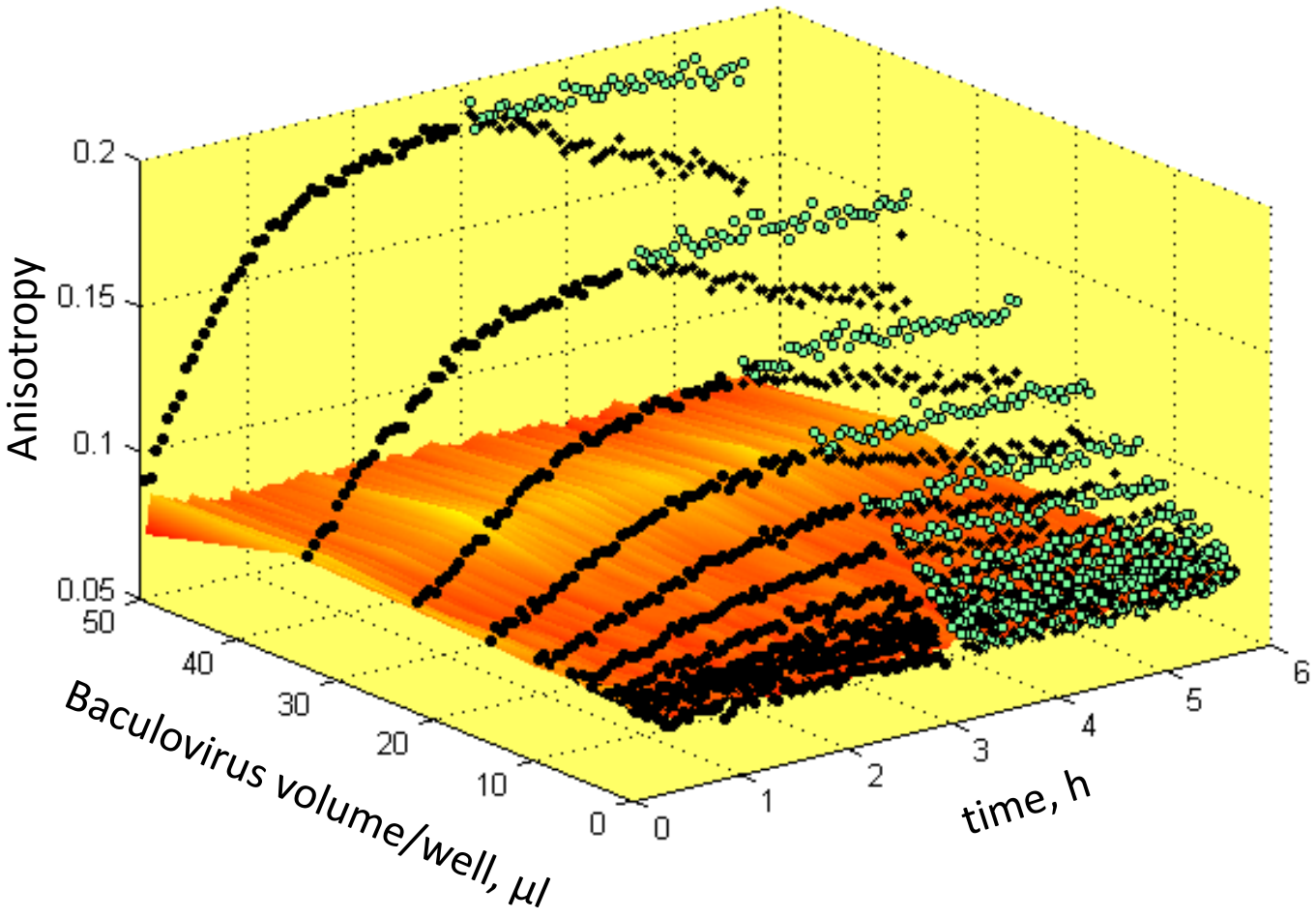
Baculovirus Surface Display System

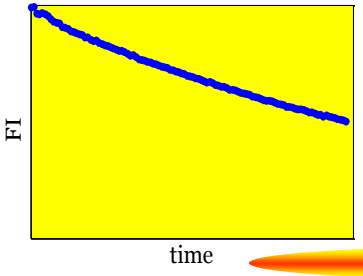


Time course of specific changes in fluorescence anisotropy caused by 1 nM Cy3B-NDP-α-MSH binding to MC_4 receptors

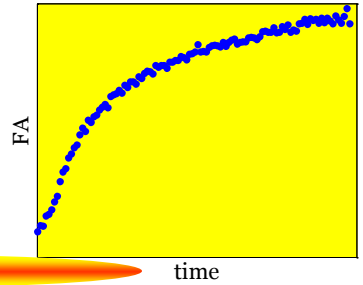


Time course of changes in fluorescence anisotropy caused by 1 nM Cy3B-NDP-α-MSH binding to MC_4 receptors





Multivariate Global Data Analysis (based on the mechanistic models)



Initial state values. Events. Parameter values

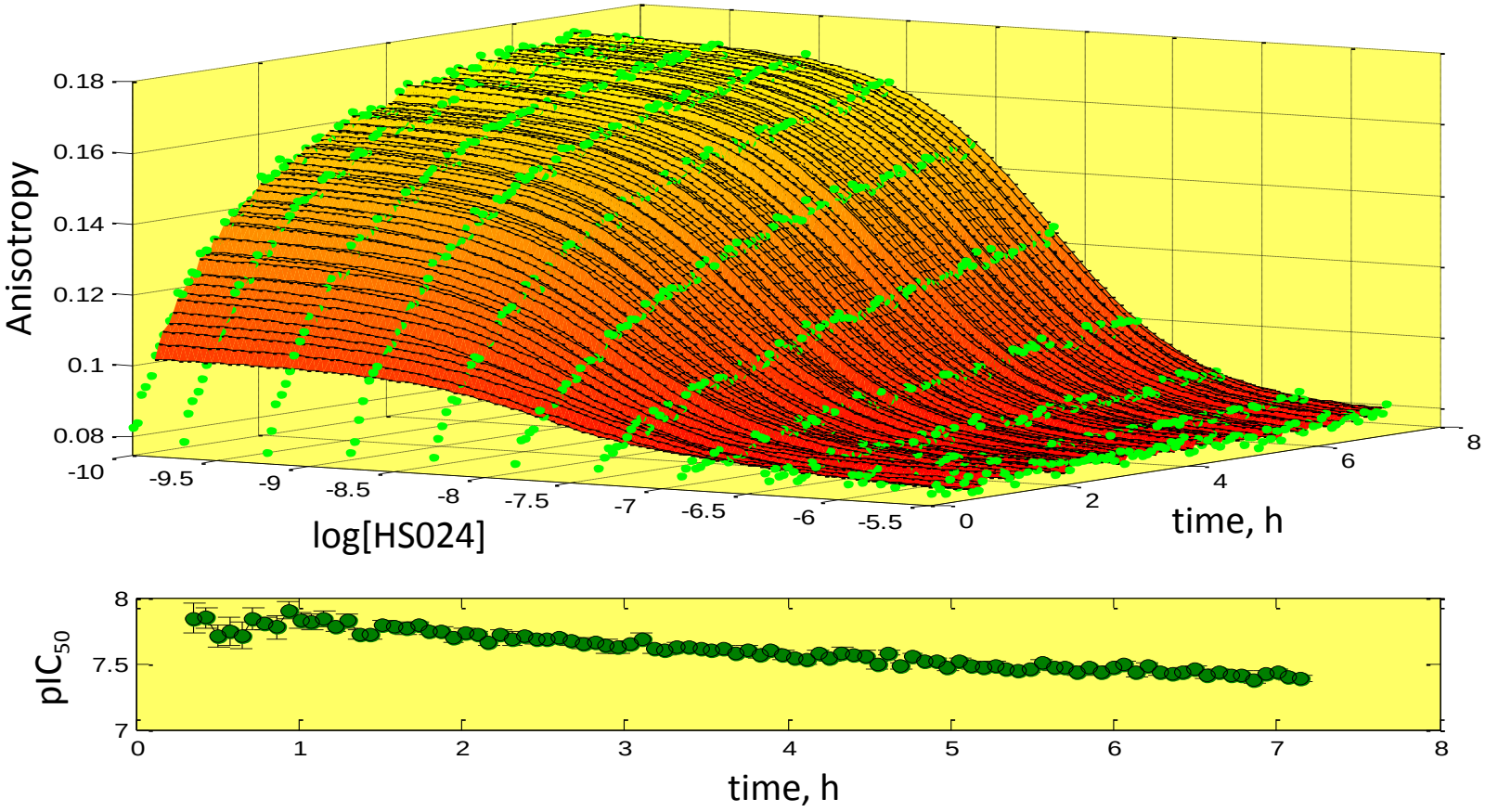
Fractions of each state are generated by numerical integrations with ODE solvers

Calculated *FI(t)*

Calculated *FA(t)*

Parameter estimation with global stochastic optimisation algorithms (Differential Evolution, Adaptive Particle Swarm, etc) and fitting (Nelder-Mead nonlinear simplex) for internally consistent solution

Time courses of changes in fluorescence anisotropy caused by Cy3B labeled NDP-α-MSH binding to MC_4 receptors exposed on baculovirus surface in the presence of different concentrations of HS024



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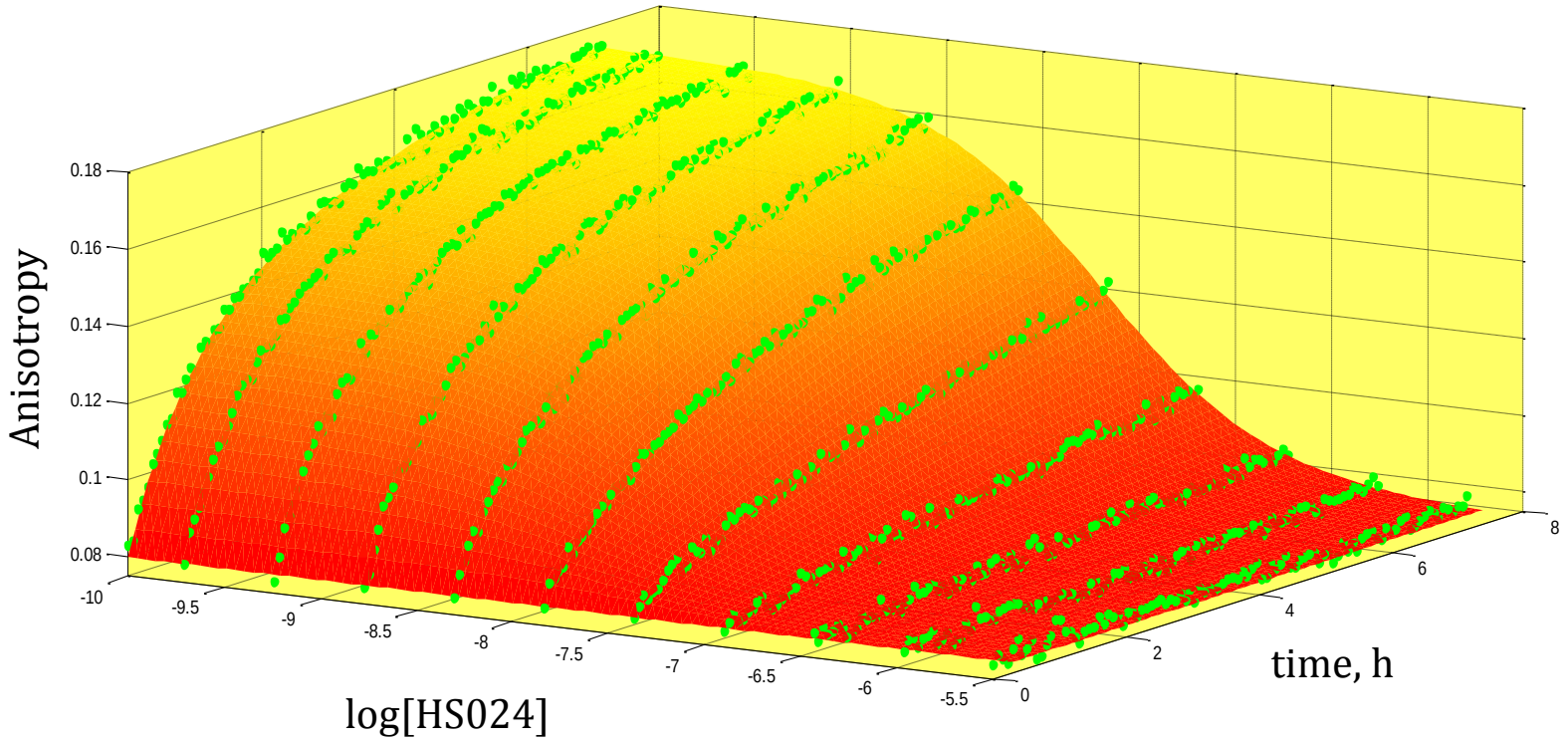
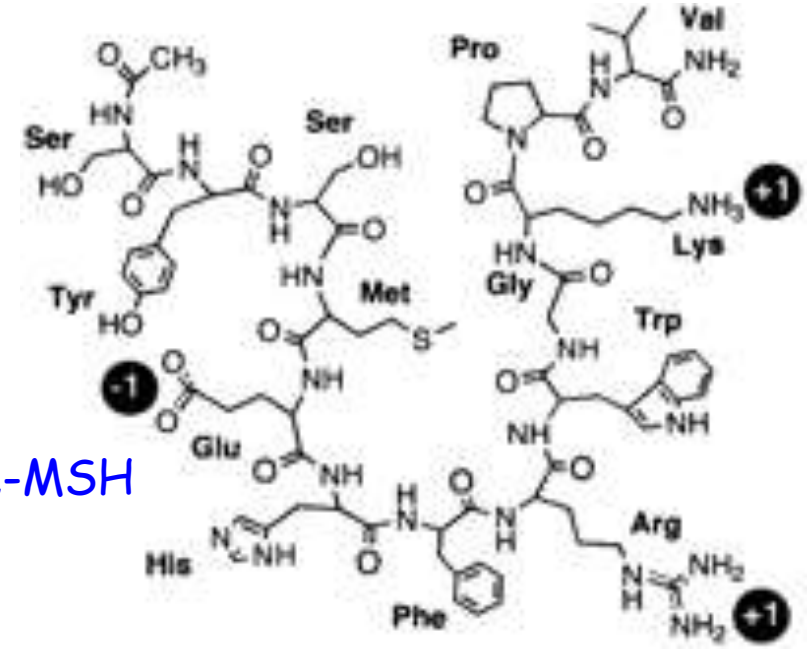
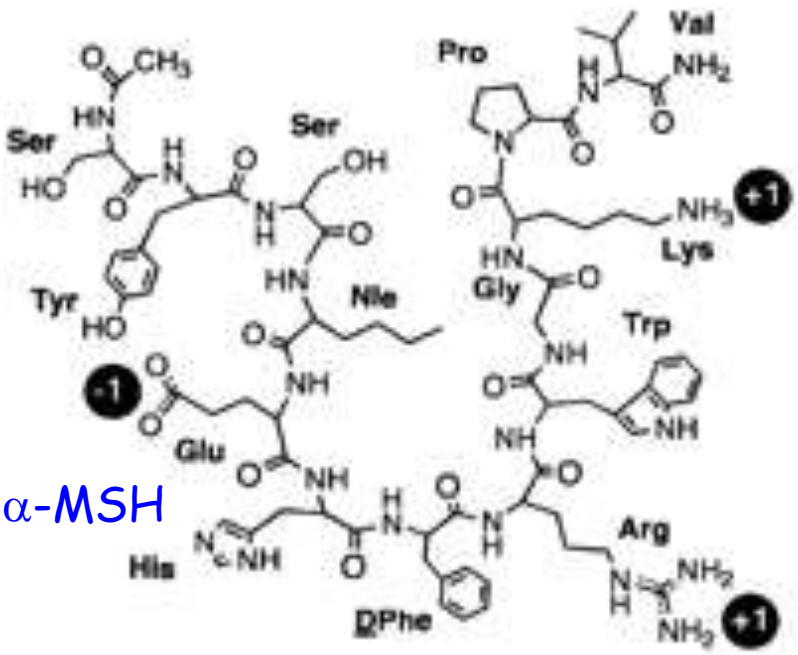


Melanocortin peptides

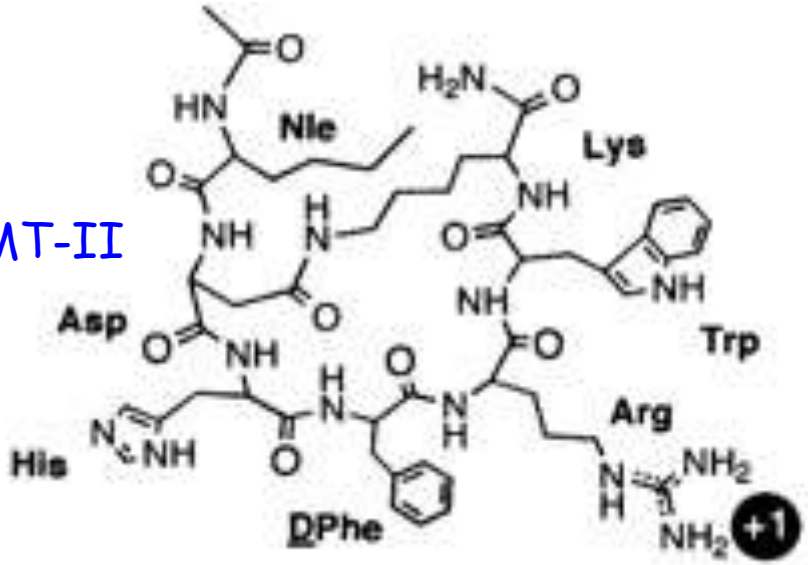
α-MSH



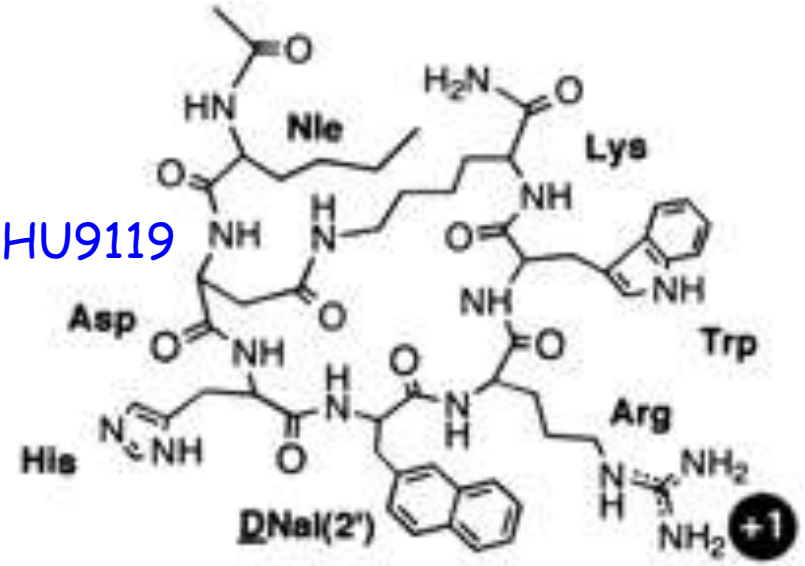
NDP-α-MSH



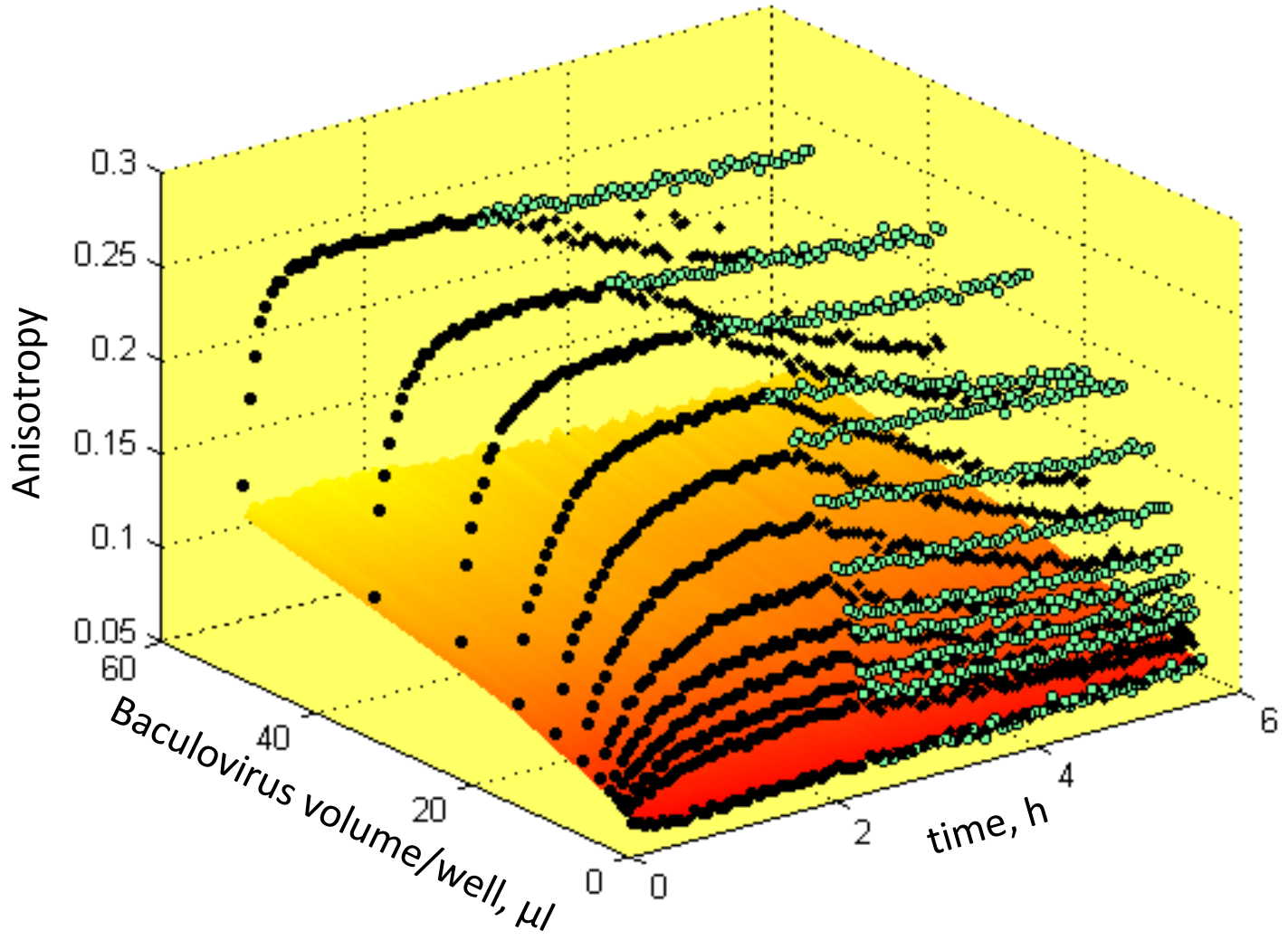
MT-II



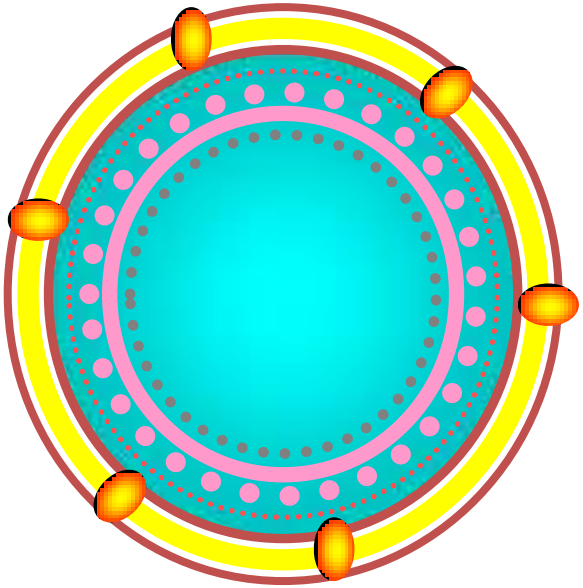
SHU9119

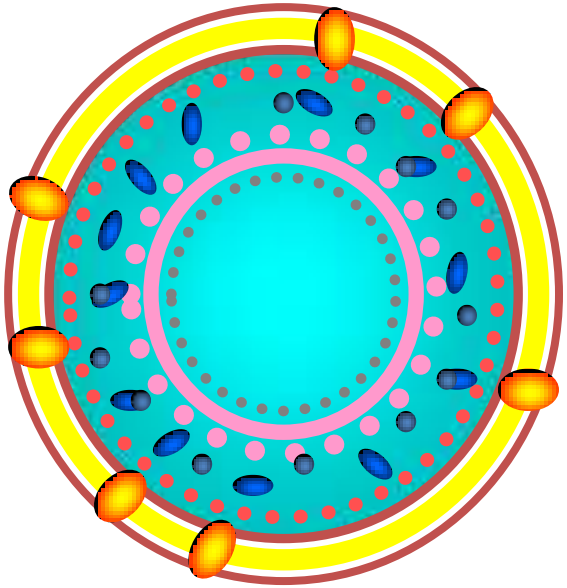


Time course of changes in fluorescence anisotropy caused by 1 nM Cy3B-Peptide_1 (A modification) binding to MC_4 receptors



Receptor displayed on the surface of retrovirus like particle (VLP) (*mammalian system*)

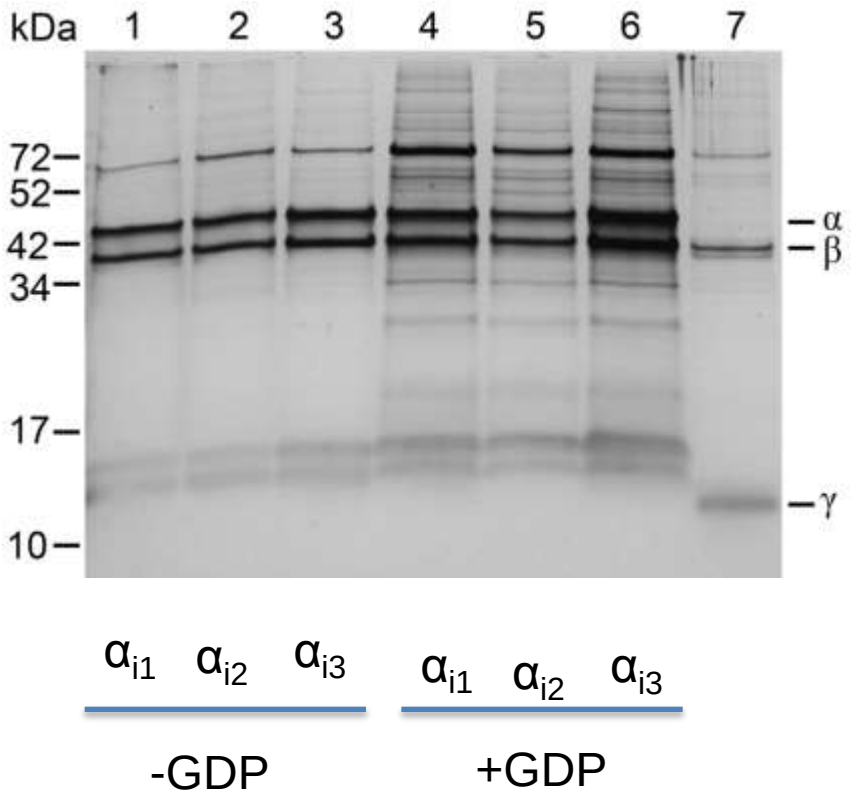




Receptors on VLPs

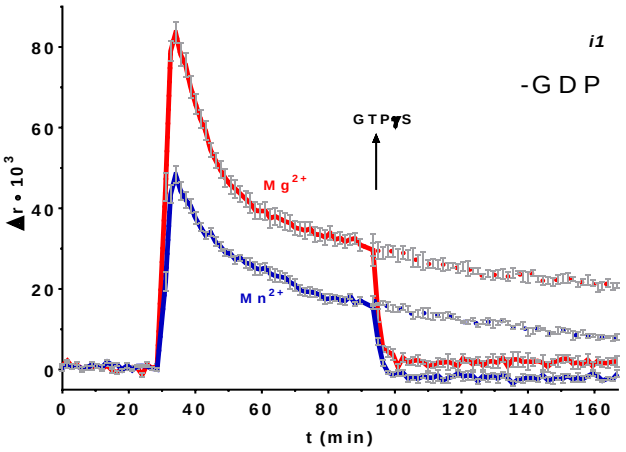
Receptors and other molecules of signaling mashinery

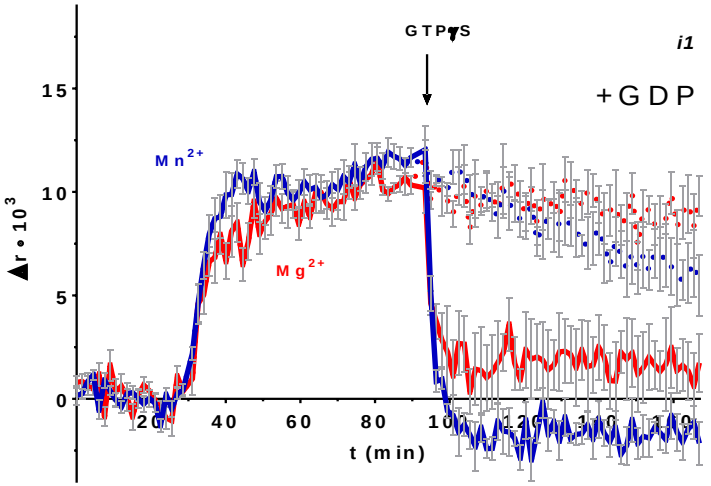
Recombinant heterotrimeric G-protein α_{i1}, α_{i2} and α_{i3} subunits were purified in GDP-depleting conditions by affinity chromatography using StrepII-tagged $\beta_1\gamma_2$ subunits



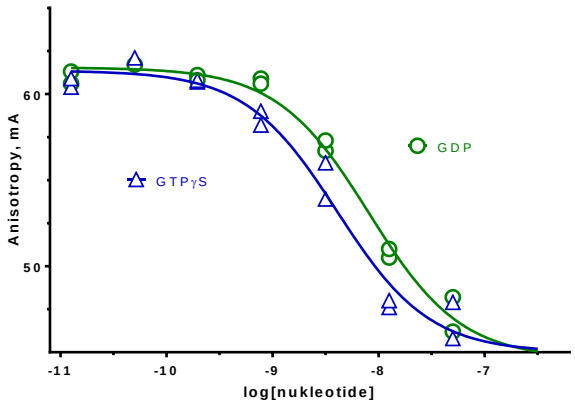
Tõnson L., et al. 2012. Characterization of heterotrimeric nucleotide-depleted Gαi-proteins by Bodipy-FL-GTPγS fluorescence anisotropy. *Archives of Biochemistry and Biophysics*

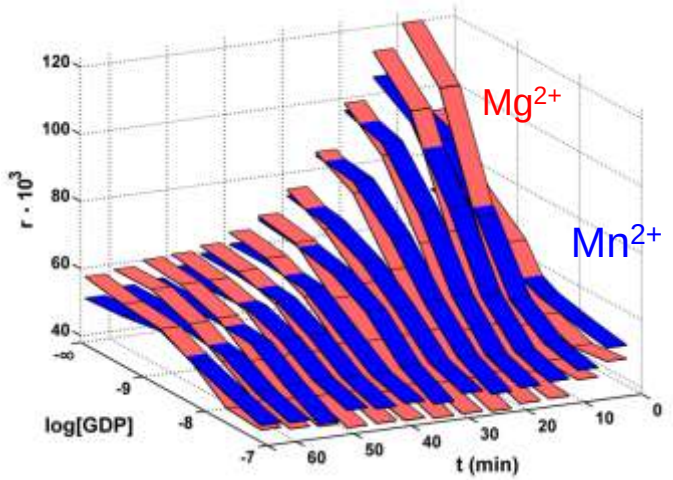
Association and dissociation kinetics of Bodipy-FL-GTPγS binding to G-protein heterotrimers



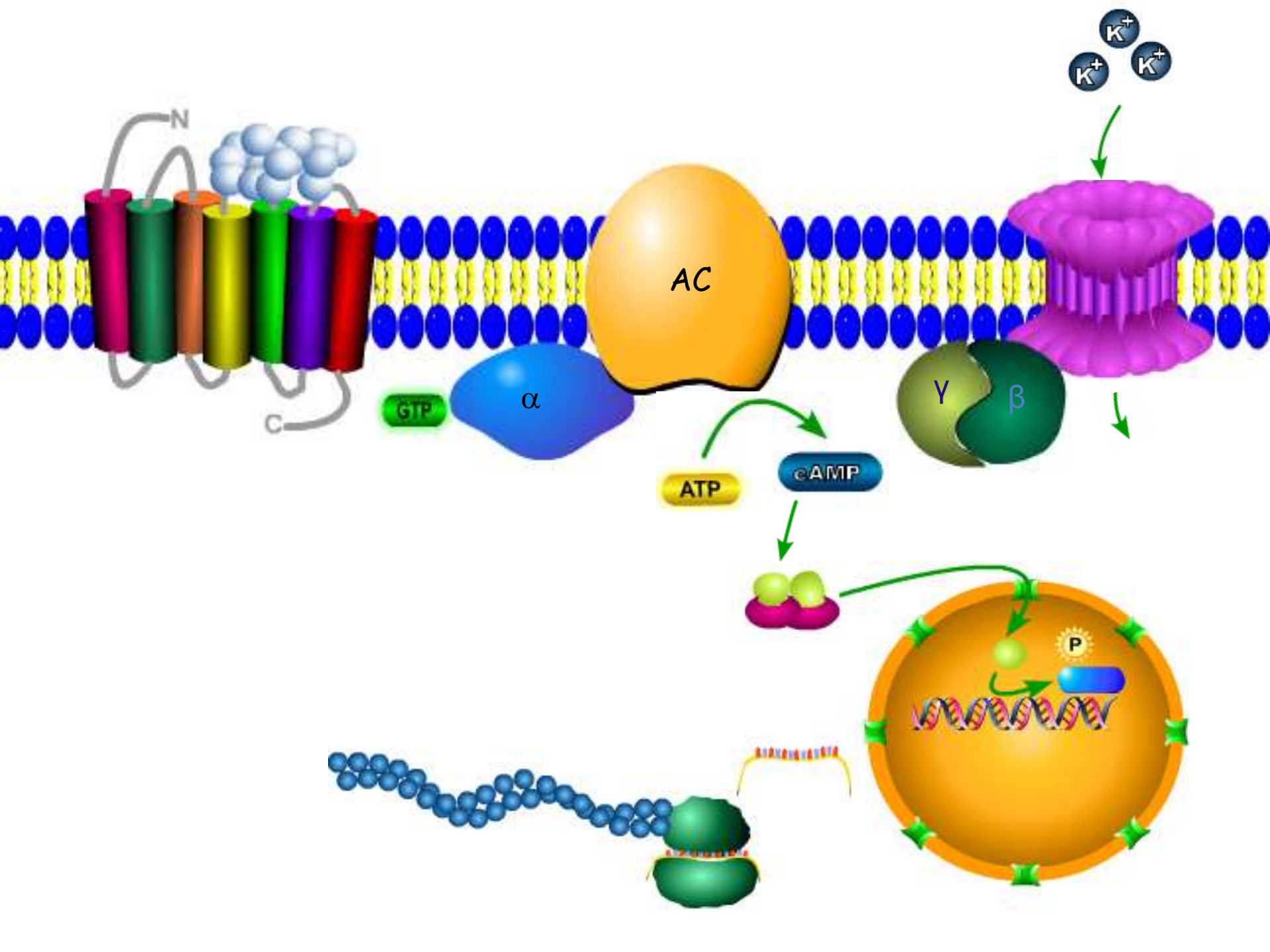


GDP depleted G protein



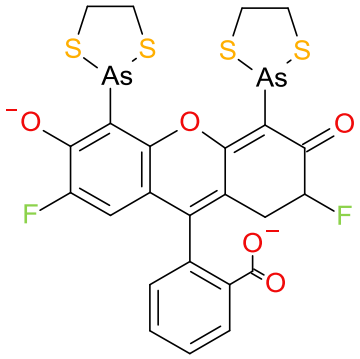


Tõnson L., et al. 2012. Characterization of heterotrimeric nucleotide-depleted Gαi-proteins by Bodipy-FL-GTPγS fluorescence anisotropy. *Archives of Biochemistry and Biophysics*

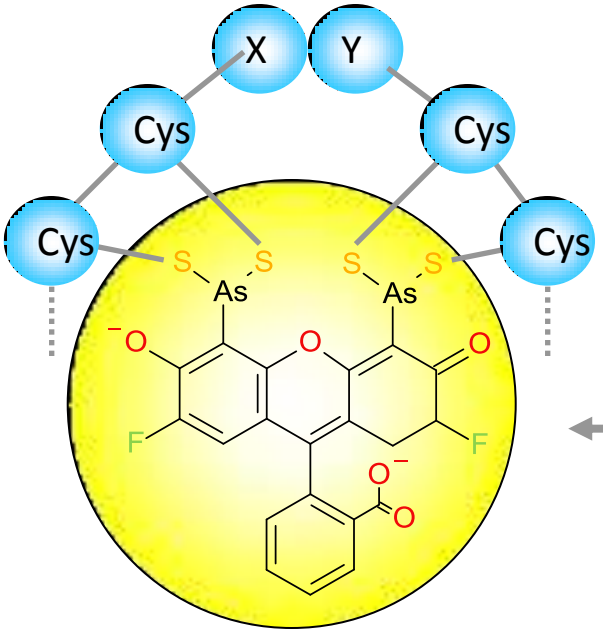

K+
K+
K+
N
C
AC
α
GTP
γ
β
ATP
cAMP
P

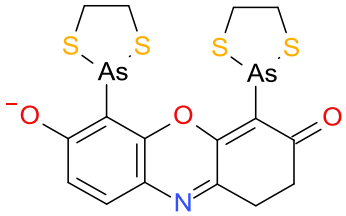
Fluorescently labeled G-proteins: α_s, α_{i2}, α_q, γ_1

X = Pro or Lys
Y = Gly or Ala



F2FlAsH





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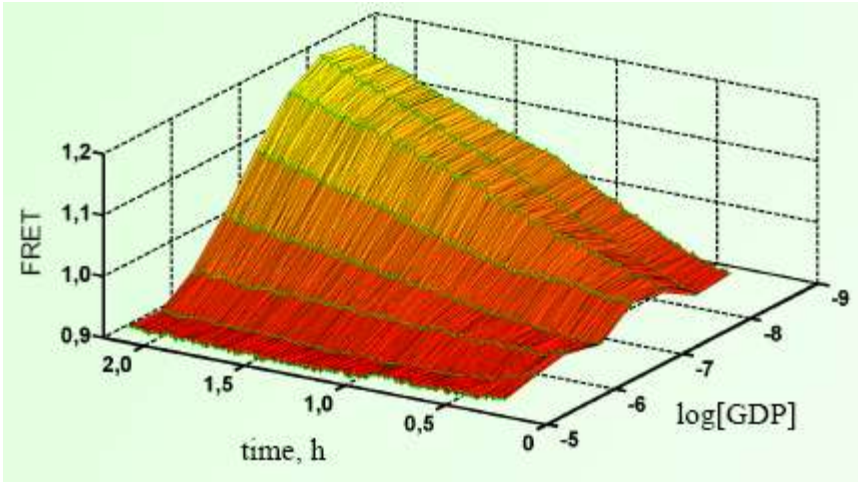
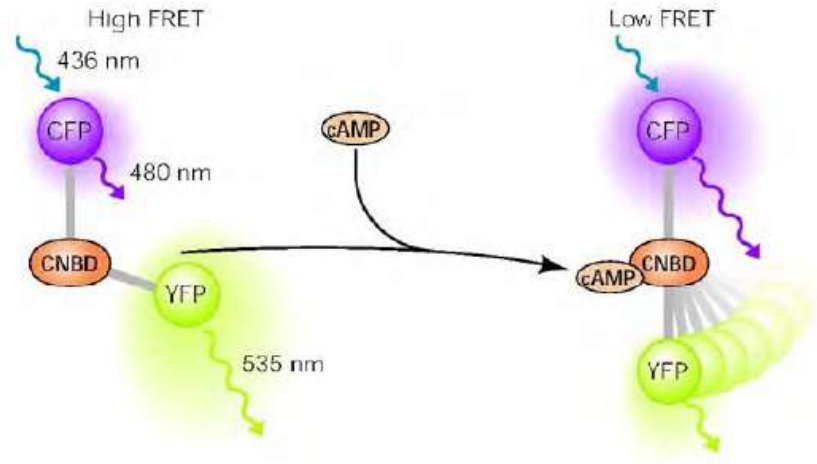
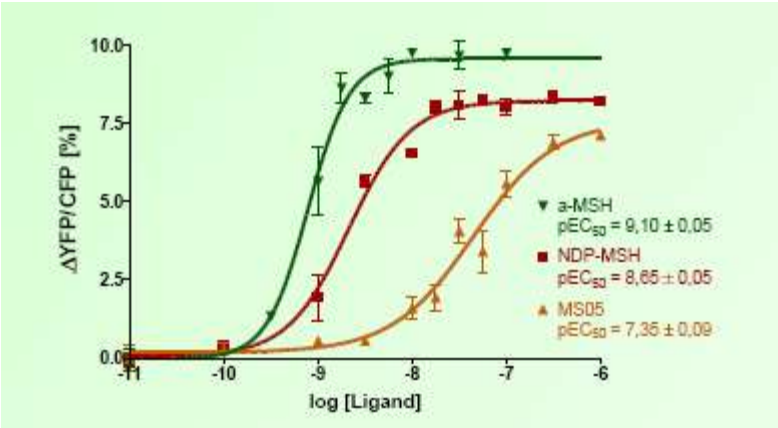


BacMam expression system is suitable for expression of cAMP-sensor protein (Epac-camps) in mammalian cells

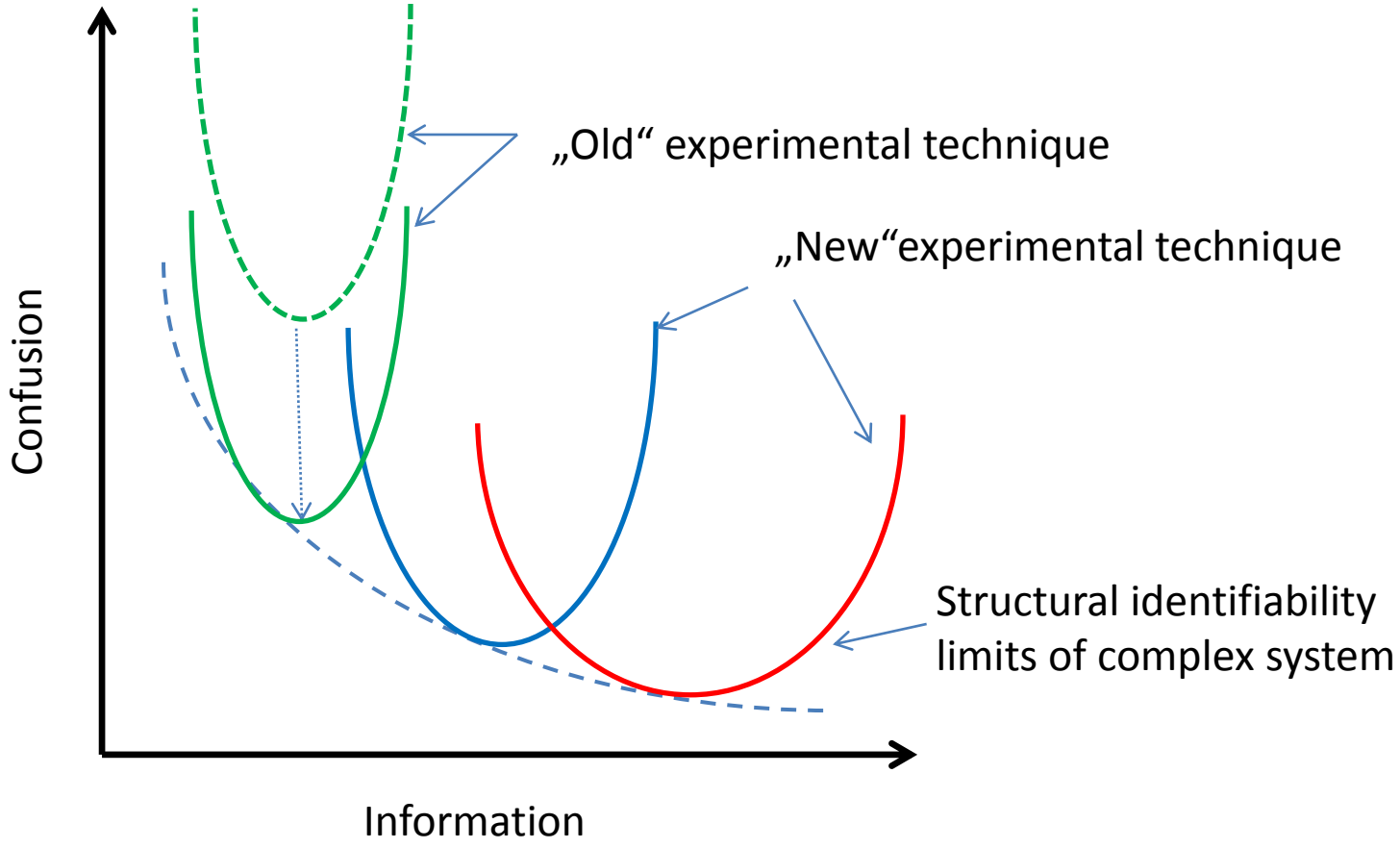


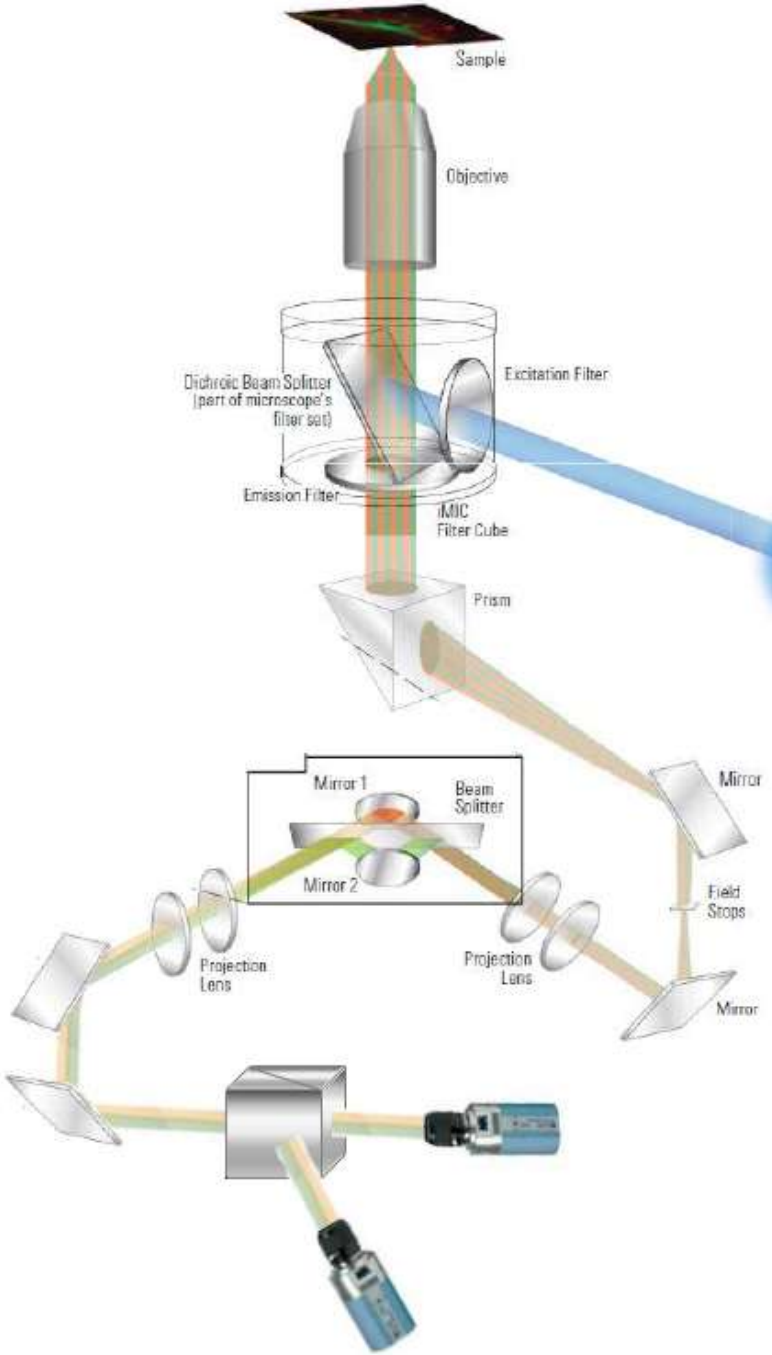
Nikolaev et al. (2004)



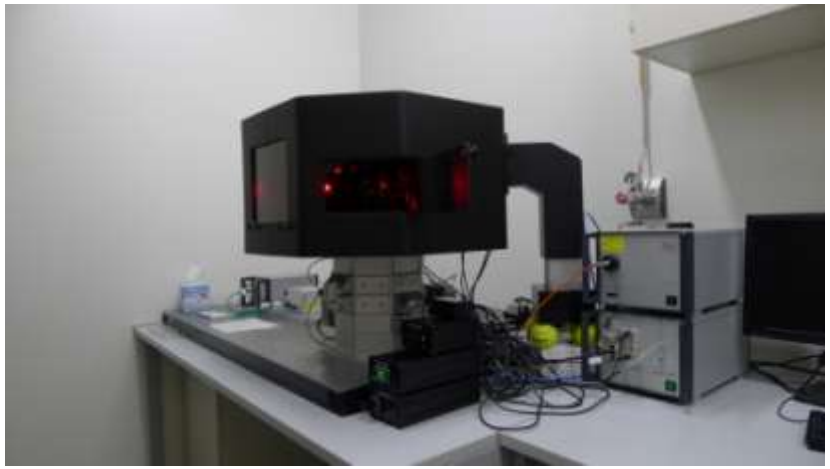
Mazina, O., et al. 2012. BacMam system for FRET based cAMP sensor expression in studies of melanocortin MC1 receptor activation. *Journal of Biomolecular Screening*

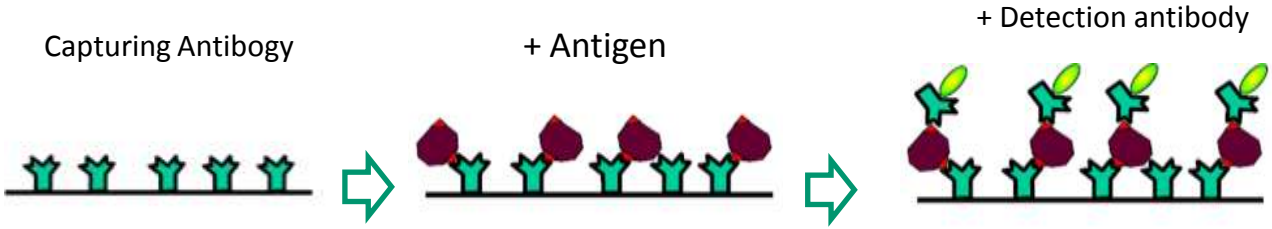
Our confusion as function of available information with practical and structural identifibility limits





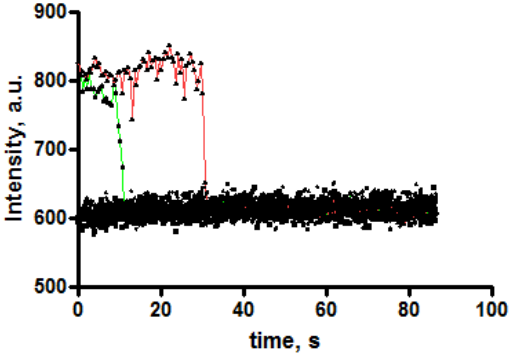
Custom made iMIC TIRF digital microscope from Till Photonics with Azzam polarimeter architecture in the emission path

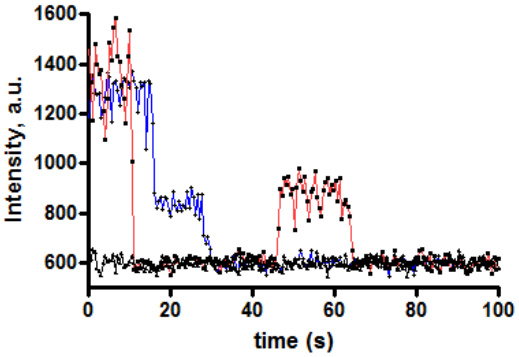


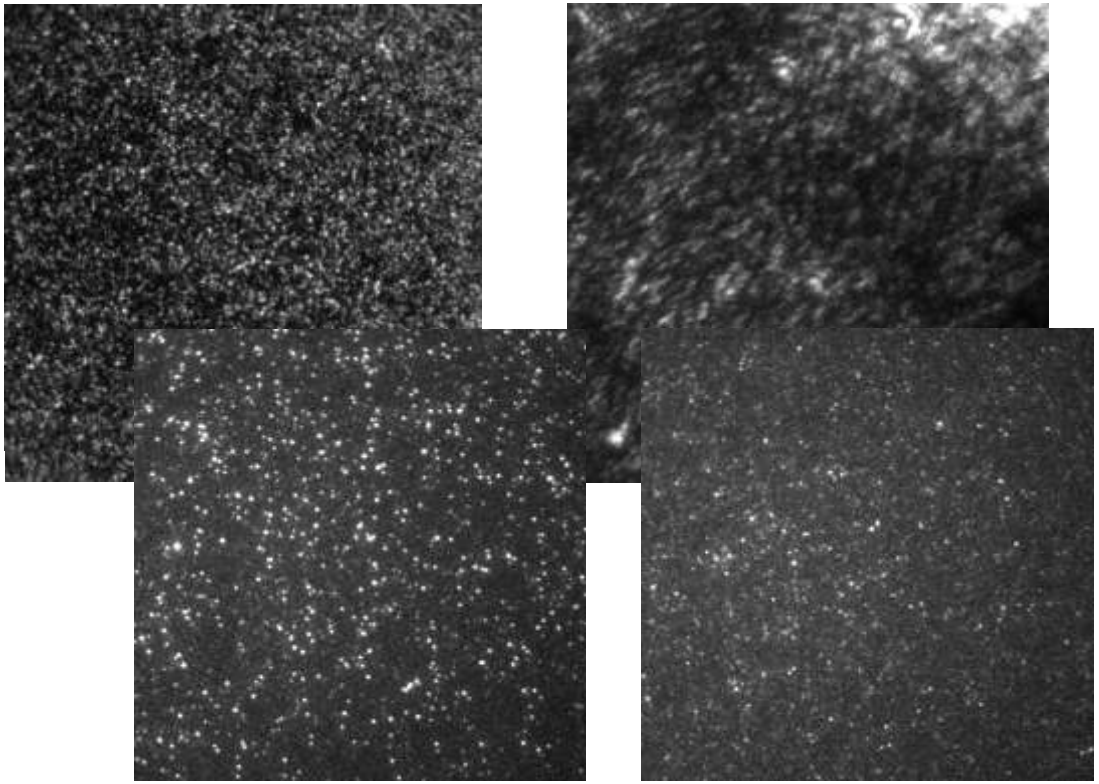


2D-TIRF

TIRF







1 pM Atto532-X

Control

Thank you for your attention!