Competition between efficiency and selectivity in ring assembly

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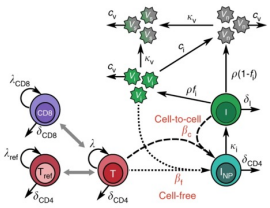
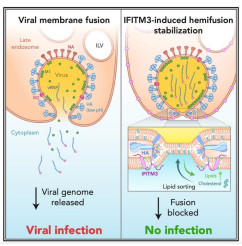
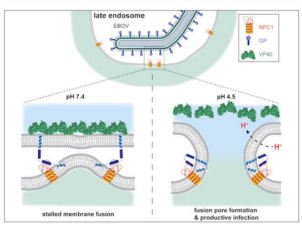
Recent work on viruses



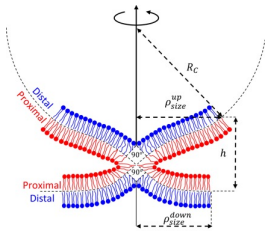
Imle et al., Environmental restrictions shape HIV-1 spread in 3D cultures, Nat Comms 2019



Klein et al., IFITM3 blocks influenza virus entry by sorting lipids and stabilizing hemifusion, Cell Host Microbe 2023



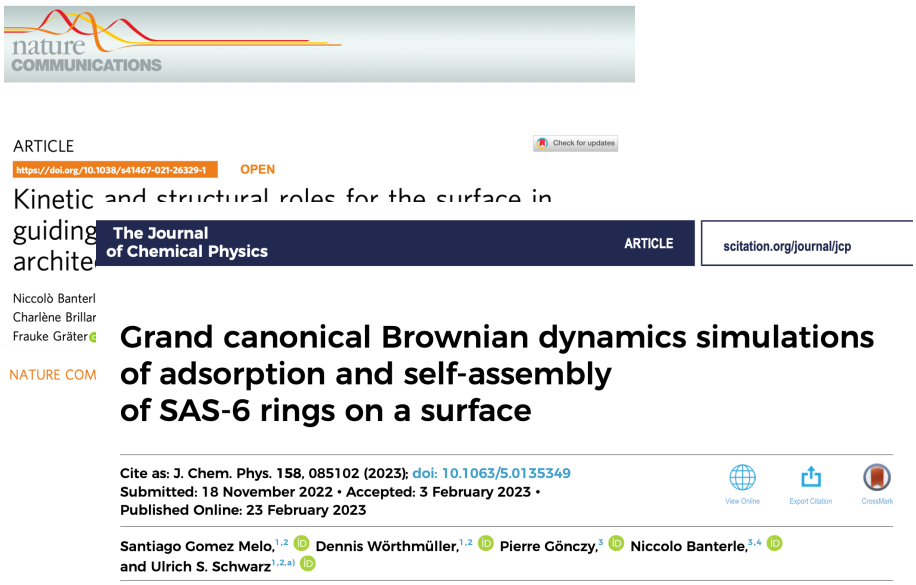
Winter et al., The Ebola virus VP40 matrix layer undergoes endosomal disassembly essential for membrane fusion, EMBO J 2023



Golani and Schwarz, High curvature promotes fusion of lipid membranes: Predictions from continuum elastic theory, Biophys J 2023

2

Recent work on SAS-6 rings



nature COMMUNICATIONS

ARTICLE

https://doi.org/10.1038/s41467-021-26329-1 OPEN

Kinetic and structural roles for the surface in guiding archite

Niccolò Banterl
Charlène Brillar
Frauke Gräter

NATURE COM

The Journal of Chemical Physics ARTICLE scitation.org/journal/jcp

Grand canonical Brownian dynamics simulations of adsorption and self-assembly of SAS-6 rings on a surface

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Collaborators on SAS-6 projects

- SAS-6: Pierre Gönczy, EPFL; Niccolo Banterle, EMBL Heidelberg; Paul Guichard & Virginie Hamel, Uni Geneva
- HS-AFM: Adrian Nievergelt and Georg Fanter, EPFL
- Molecular dynamics: Svenja de Buhr and Frauke Gräter, Heidelberg
- Own students: Heinrich Klein, Dennis Wörthmüller, Santiago Gomez-Melo

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SAS-6 rings in 3D

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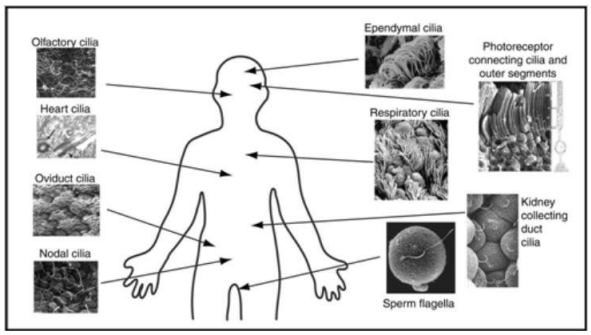
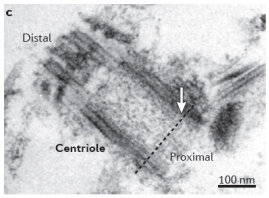
Cilia, flagella, centrioles, centrosomes, spindles

PhD thesis TB Warrington

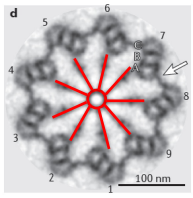


Cilia are cellular appendages that can beat. Our body is full of them. Moreover, most of our cells have a primary cilium.
Flagella have the same basic structure as motile cilia.

Centrioles organize the microtubules in our cells and have the same basic architecture as cilia. Two centrioles form the **centrosome** and the **spindle poles**.



SAS-6 ring



[P. Gönczy Nature Reviews 2012]

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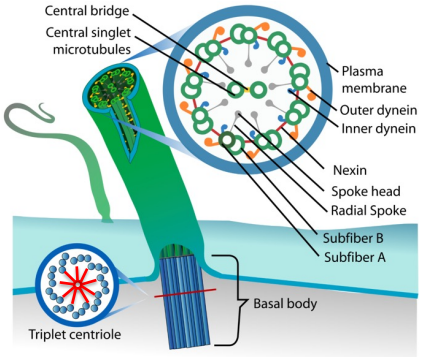
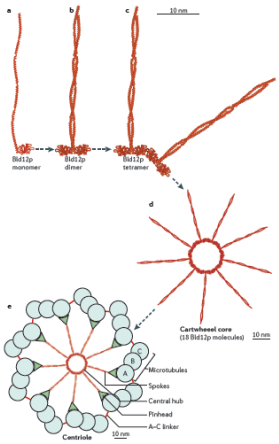
9-fold symmetry and the SAS-6 ring



Wikipedia

All cilia, flagella and centrioles have a 9-fold symmetry that results from the self-assembly of the Spindle assembly abnormal protein 6 (SAS-6).



[P. Gönczy Nature Reviews 2012]

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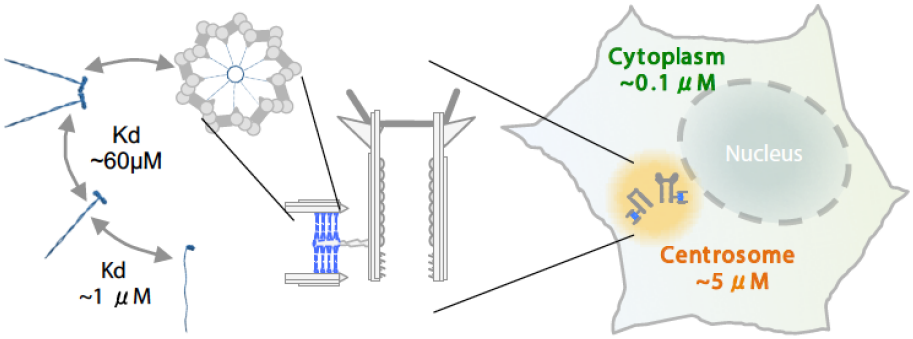
Relevant dissociation constants and concentrations



How can SAS-6 form rings ?

[Klein et al. Sci Reports 2016]

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Patchy particles computer simulations

SAS6 assembly

Heinrich C. R. Klein, Paul Guichard, Virginie Hamel
Pierre Gönczy and Ulrich S. Schwarz

Bulk assembly

with homodimer concentration 75μM

[Klein et al. Sci Reports 2016]

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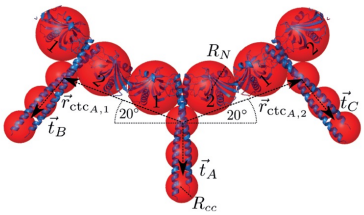
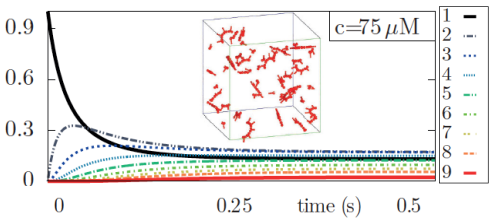
Simulation setup



A rigid patchy particle model for the SAS-6 homo-dimers was built from Protein Data Bank (PDB) structures 3Q0X and 3Q0Y.



At 75 □M, well above the centriolar concentration of 5 □M, steady state is reached within seconds, but only small oligomeres form, in agreement with *in vitro* experiments.

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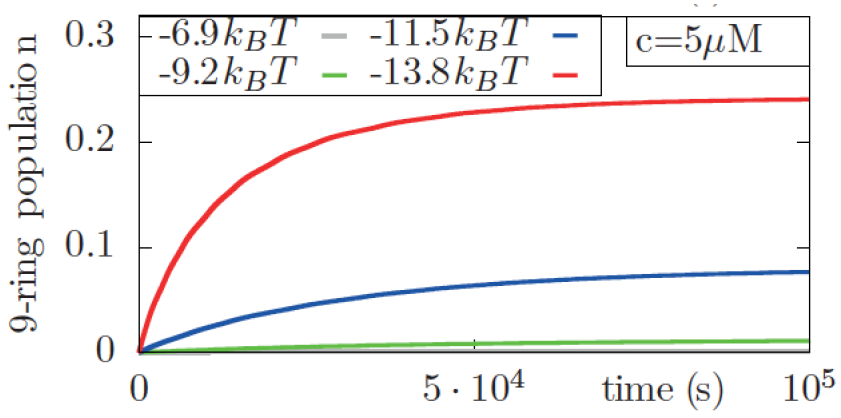
Cellular concentration



Only very strong stabilization of the last bond (different free energies given in the legend) leads to appreciable fractions of 9-rings. We conclude that standard conditions are **not** sufficient to get rings.

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Patchy particles computer simulations with half-spherical scaffold

SAS6 assembly

Heinrich C. R. Klein, Paul Guichard, Virginie Hamel
Pierre Gönczy and Ulrich S. Schwarz

Spherical scaffold
embedded in a planar surface

with homodimer concentration 5μM

[Klein et al. Sci Reports 2016]

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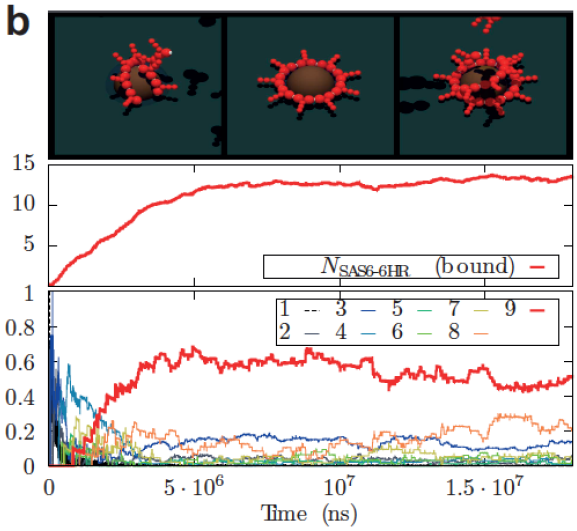
Potential role of a half-spherical scaffolds on a flat substrate



The half-spherical scaffold on a flat substrate allows for efficient ring formation; this would also explain why new centrioles form orthogonal to old ones

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SAS-6 rings on a surface

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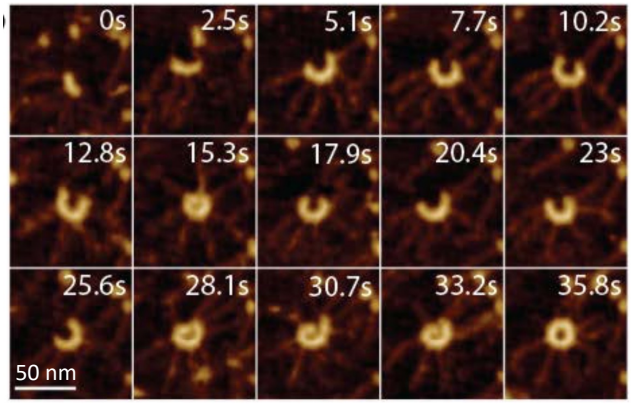
HS-AFM imaging with photothermal off-resonance tapping (PORT)



HS-PORT is sufficiently gentle such that SAS-6 ring assembly can be monitored with high temporal and spatial resolution.

[Nievergelt, Banterle et al. Nature Nanotech 2018]

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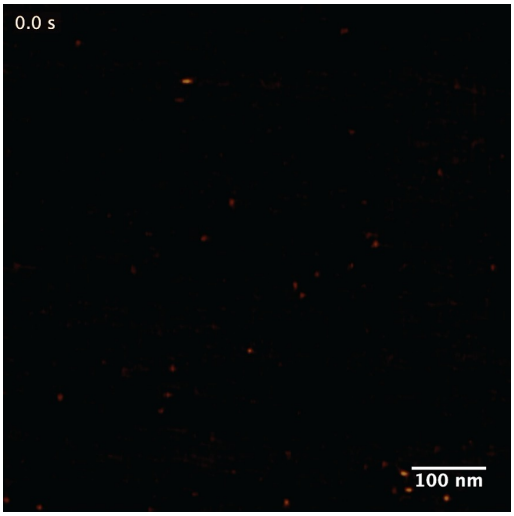
Dynamics of SAS-6 ring assembly



[Banterle et al. Nat Comms 2021]

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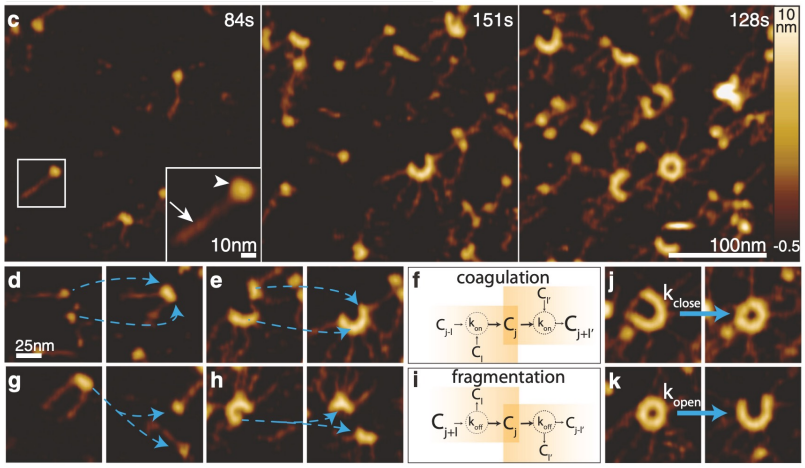
Oligomeres of different sizes interact



Both coagulation and fragmentation events are observed.

[Banterle et al. Nat Comms 2021]

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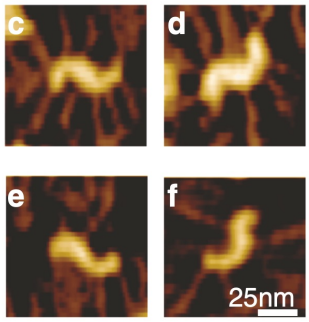
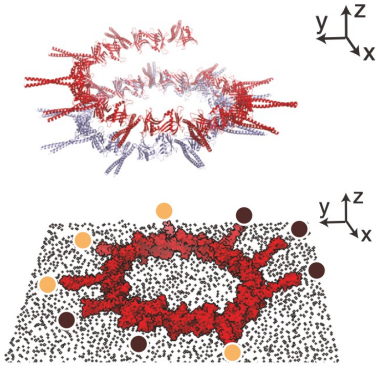
Malformed structures





The HS-AFM sometimes shows S-shaped structures that most likely are 3D spirals adsorbed to the 2D surface („squeelices“).

Molecular dynamics (MD) computer simulations confirm that SAS-6 in solution forms spirals that become strained rings on the surface.

[Banterle et al. Nat Comms 2021]

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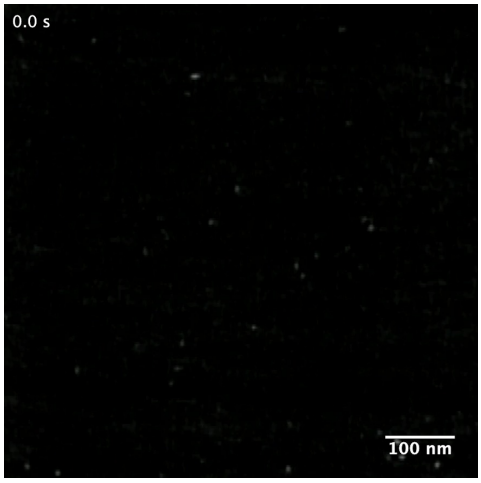
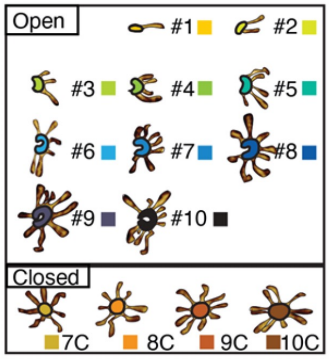
Intermediates can be classified by image processing (ilastik)





[Banterle et al. Nat Comms 2021]

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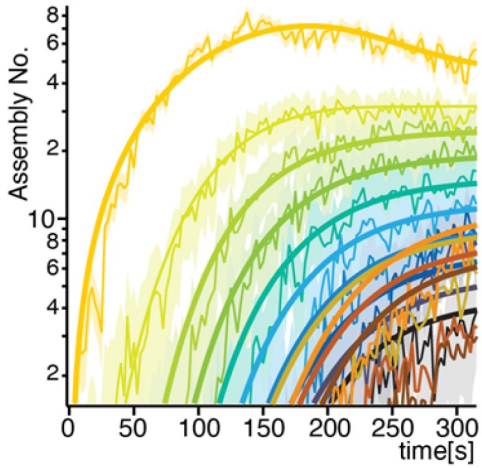
Quantitative analysis of ring assembly



- Monomer concentration first increases due to adsorption of SAS-6 homodimers onto mica surface
- Equilibrium not established in these traces, but relaxation towards equilibrium is visible
- Assembly curves can be fitted to coagulation-fragmentation model
- Surface dramatically improves self-assembly for kinetic and structural reasons

[Banterle et al. Nat Comms 2021]

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Theoretical analysis: coagulation-fragmentation (CF) equations

- Standard approach for reversible assembly of clusters and polymers
- Go back to Smoluchowski 1916, Becker and Döring 1935, Kolmogorov 1941
- Widely used not only in biophysics and soft matter physics, but also in astrophysics, environmental physics, geosciences, etc

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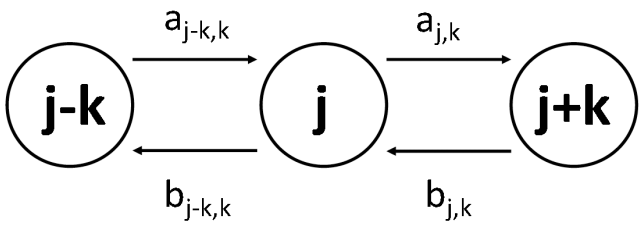
Reaction scheme for CF-equations



A cluster of size j can either
1. coagulate with a cluster of size k to become a cluster of size j+k
2. or fragment into two clusters of sizes j-k and k, respectively

The association and dissociation rates a and b are labeled with the cluster sizes.

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CF-equations for complexes of maximal size N

$$\dot{c}_j(t) = \frac{1}{2}\sum_{k=1}^{j-1} a_{j-k,k} c_{j-k} c_k - \sum_{k=1}^{N-j} a_{j,k} c_j c_k - \frac{1}{2}\sum_{k=1}^{j-1} b_{j-k,k} c_j + \sum_{k=1}^{N-j} b_{j,k} c_{j+k}$$

- System of ordinary differential equations for the concentrations $c_j(t)$ of the clusters of size j as a function of time t
- Mass action kinetics; because of the binary coagulation terms, the equations are non-linear
- Gain and loss terms have plus and minus signs, respectively
- The factors ½ avoid overcounting (1+4->5 is the same process as 4+1->5)
- In 3D association rates a are measured in 1/Ms or m^3/s; in 2D they are measured in m^2/s; dissociation rates b are measured in 1/s
- The CF-equations obey mass conservation: $\sum_{j=1} j\dot{c}_j(t) = 0$
- SAS-6 rings: N=9; for macroscopic systems, one often uses N->infinity.

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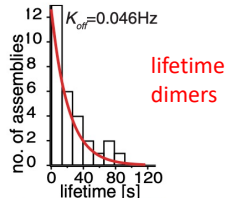
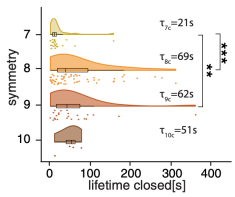
Fit of CA-model to SAS-6 assembly

- Average lifetime of dimers is 22 s
- Rings of 7-10 are observed; 9-rings have the largest lifetimes; 7-rings exist, but are unstable due to large strain
- Association time is estimate from overall fit to CA-model
- This leads to central result K_d = 79 dimers/□m^2
- Surface concentration c=500 dimers/□m^2
  ⇒ $R_{surf}=c/K_d=10$, assembly is possible
- Compare bulk: c=120 nM, K_d=60 uM ⇒ $R_{bulk}=c/K_d=10^{-3}$, assembly is not possible
- Surface shifts assembly kinetics by four orders of magnitude in this experiment





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Reaction versus diffusion limits

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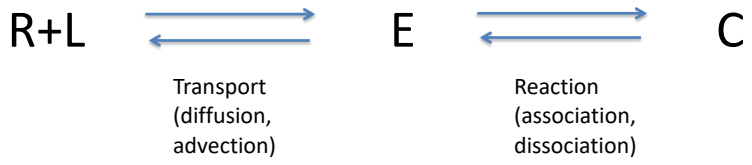
Protein-protein binding involves diffusion and reaction



This concept is helpful even when the encounter state is not well-defined.

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Binding as 2-step process

We introduce the encounter complex as intermediate step:

$$R + L \underset{k_-}{\overset{k_+}{\rightleftharpoons}} E \underset{k_{off}}{\overset{k_{on}}{\rightleftharpoons}} C, \; R + L \underset{k_r}{\overset{k_f}{\rightleftharpoons}} C$$

Steady state assumption for E:

$$k_f = \frac{k_+ k_{on}}{k_- + k_{on}}, \; k_r = \frac{k_- k_{off}}{k_- + k_{on}}$$

$k_- \ll k_{on}$ $\quad k_f = k_+, \; k_r = k_- \frac{k_{off}}{k_{on}}$ diffusion control

$k_- \gg k_{on}$ $\quad k_f = k_{on} \frac{k_+}{k_-}, \; k_r = k_{off}$ reaction control

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Binding as 2-step process - continued

A convenient way to rewrite our results:

$$\frac{1}{k_f} = \frac{1}{k_+} + \frac{1}{k_{on}} \frac{k_-}{k_+}, \; \frac{1}{k_r} = \frac{1}{k_-} \frac{k_{on}}{k_{off}} + \frac{1}{k_{off}}$$

Diffusion rates from solving the diffusion equation (Smoluchowski rates):

$$k_+ = 4\pi D s, \; k_- = \frac{3D}{s^2}$$

The diffusion constant D is universal due to the Stokes-Einstein relation (around $(10\ \text{um})^2/\text{s}$ with s=4 nm). A typical value for the Smoluchowski association rate therefore is 6×10^9 1/Ms. This is reduced by 4-5 orders of magnitude if one considers orientational degrees of freedom (Solc and Stockmayer).

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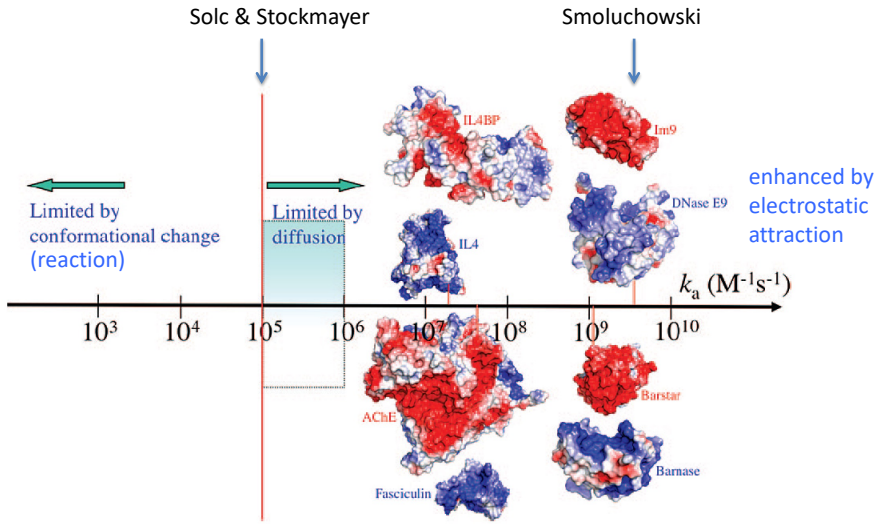
Protein-protein association



[Schreiber, Haran & Zhou, Chem Rev 2009]

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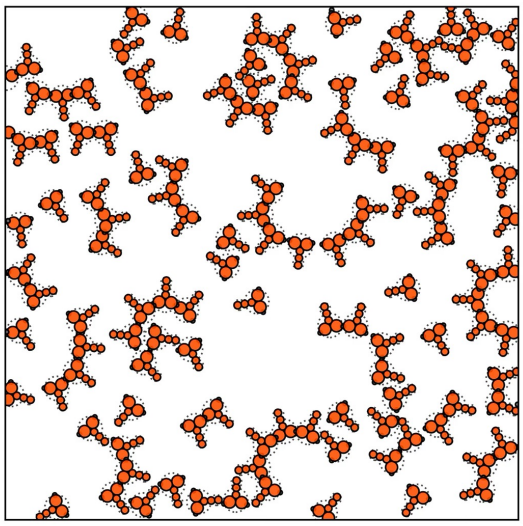
Limits of the CF-equations – comparison to Brownian Dynamics



Two main parameters:

1. Ratio of attractive versus repulsive energies $\varepsilon_a/\varepsilon_\rho$
2. Cut-off angle θ_c

[Gomez-Melo, Wörthmüller et al. JCP 2023]

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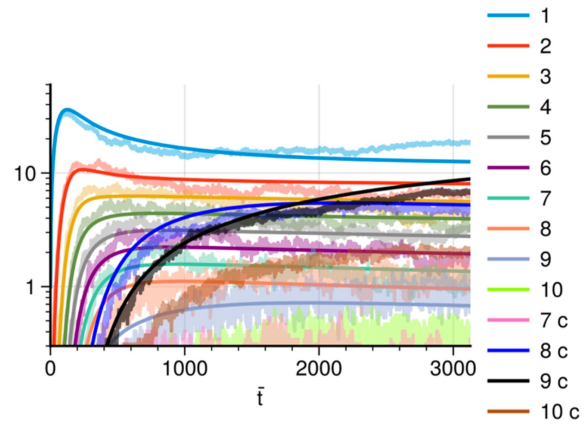
Reaction-limited case



$\varepsilon_a/\varepsilon_\rho$=2 (small), not every encounter leads to a reaction, reversible

very good agreement between Brownian Dynamics computer simulations and CF-equations

[Gomez-Melo, Wörthmüller et al. JCP 2023]

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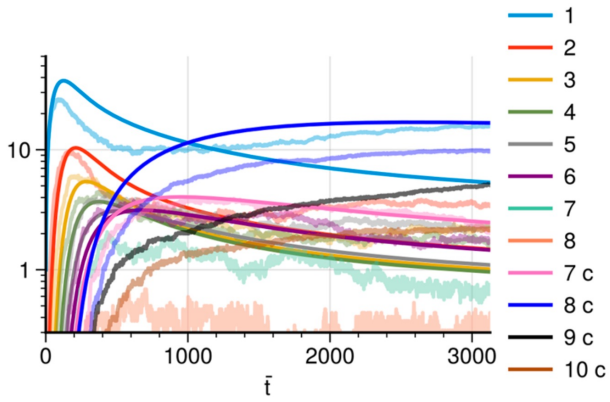
Diffusion-limited case



$\varepsilon_a/\varepsilon_\rho$=3 (large), every encounter leads to a reaction, irreversible

CF-equations fail because they do not represent the spatial aspects of the system

[Gomez-Melo, Wörthmüller et al. JCP 2023]

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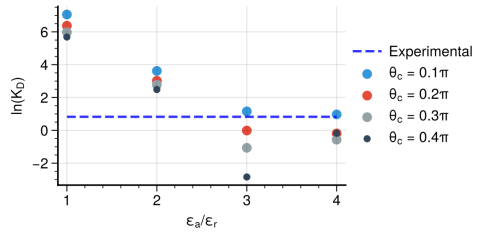
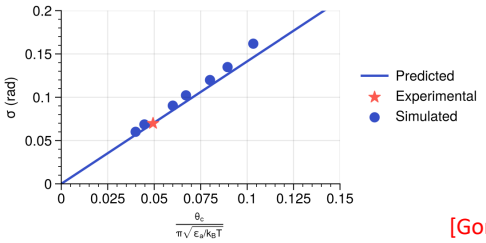
Comparison with experiments



K_d decreases with interaction energy and then comes flat (diffusion-limited). Experiments suggest $2 < \varepsilon_a/\varepsilon_r < 3$. From selectivity one estimates $0.1\ \pi < \theta_c < 0.2\ \pi$



Bond angle fluctuations are an Ornstein-Uhlenbeck process (straight line). Experiments with σ=4 deg suggest $\theta_c = 0.17\ \pi$ (31 deg)

[Gomez-Melo, Wörthmüller et al. JCP 2023]

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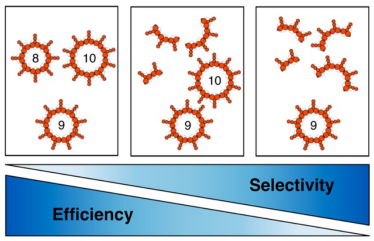
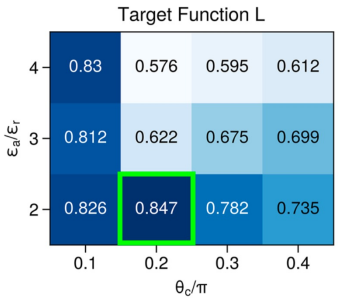
Competition between efficiency and selectivity





The SAS-6 system cannot achieve both efficiency (due to high reversibility avoiding kinetic trapping) and selectivity (selection of the 9-ring) at the same time, but has to balance these two targets (green frame). Additional molecular mechanisms for 9-ring selection must exist.

[Gomez-Melo, Wörthmüller et al. JCP 2023]

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Summary

- SAS-6 dimers forms 9-fold rings that can be observed with HS-AFM on mica surfaces
- The surface shifts the binding equilibrium up by four orders of magnitude compared to bulk, by concentrating the molecules
- This explains why daugther centrioles form on the surface of mother centrioles
- Due to the fast kinetics, the assembly curves can be fitted by the coagulation-fragmentation (CF) model
- Frequent dissociation avoids kinetic traps and leads to successful assembly, similar to the situation with viruses
- The comparison between reaction kinetics (CF-equations) and spatial simulations (Brownian Dynamics) showed the limits of the kinetic approach: spatial effects would be more important if interaction energies were higher
- Like for viruses, malformed structures can form (7-, 8-, 10-rings, squeelices)
- The simulations reveal the intrinsic challenge to balance efficiency and selectivity and suggests that additional molecular mechanisms must exist in the cell to select the 9-fold rings (similar to scaffolding proteins like P22 for viruses)

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Acknowledgements

MAX PLANCK SCHOOL matter to life

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POP PHYSICS OF PARASITISM

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Conference announcements

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March 13th – 15th, 2024 – Heidelberg, Germany

Prof. Dr. Job Boekhoven, TUM München
Session: Self-assembled functional materials

Prof. Dr. Rubén Costa, TUM
Session: Molecular biohybrid materials

Prof. Dr. Michael Dustin, University of Oxford
Session: Synthetic immunology

Prof. Dr. Alena Khmelinskaia, LMU
Session: Designing protein assemblies

Prof. Dr. Lene Oddershede, Novo Nordisk Foundation
Session: Nano-carriers for targeted therapeutics

Prof. Dr. Sai Reddy, BSSE Basel, ETH Zürich
Session: T-cell engineering and immunotherapy

Prof. Dr. Barbara Treutlein, ETH Zürich
Session: Organoids and single cell technologies

Prof. Dr. Dek Woolfson, University of Bristol
Session: De-novo design of biomolecular structures

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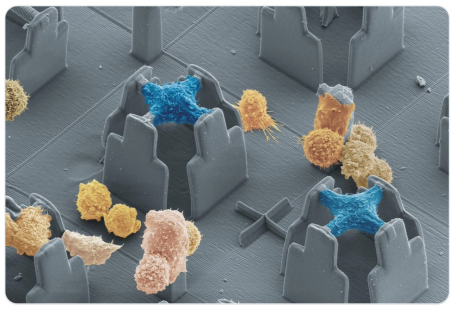

3DMM2O Cluster Conference
April 7-11, Kloster Schöntal

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