Heteroaggregation processes in colloidal particle and cell systems

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Zusammenfassung

Motiviert durch die selektive Adsorption von funktionalisierten Wirkstoffträgerpartikeln an bestimmte Zelltypen für medizinische Anwendungen werden in dieser Arbeit grundlegende Heteroaggregationsphänomene unter besonderer Berücksichtigung des dynamischen Verhaltens in physikalischen und biologischen Modellsystemen untersucht. Die Adsorption von Antikörpern als mögliche funktionelle Einheit an Rezeptoren auf Zelloberflächen stellt einen entscheidenden ersten Schritt in einer Reihe weiterer Transportbeschränkungen bei der zellulären Aufnahme funktionalisierter Wirkstoffträgerpartikel dar. Zur Etablierung geeigneter wissenschaftlicher Methoden für die Analyse von selektiven und kompetitiven Heteroaggregationsprozessen, wurden spezifische Interaktionen sowie die Heteroaggregation von mehreren unterschiedlichen kolloidalen Spezies zunächst in physikalischen Partikelsystemen untersucht. Die experimentellen Methoden umfassen vorrangig die Durchflusszytometrie sowie diverse mikroskopische Verfahren, während die Simulationen auf Populationsbilanzgleichungen basieren mit Kernen, die in klassischen kolloidchemischen Grundlagen wurzeln. Beide Ansätze wurden auf biologische Systeme angewendet, um eine quantitative Beschreibung der Dynamik und Effizienz von medizinischen Wirkstoffapplikationsprozessen zu erreichen. Dies könnte sich als wertvoll für künftige Optimierungsbestrebungen erweisen.

Zur Bestimmung der Aggregatzusammensetzung und ihrer Dynamik in Heteroaggregationsprozessen hat sich die Durchflusszytometrie als leistungsfähiges Messverfahren erwiesen. Sie ermöglicht eine unabhängige und sehr detaillierte Auflösung mehrdimensionaler Verteilungen durch eine zuverlässig automatisierte Einzelpartikelanalyse. Die Untersuchungen in binären und ternären Partikelgemischen fokussieren auf elektrostatische De- und Restabilisierungseffekte, die durch die Auswahl geeigneter Partikelspezies und deren Mischungsverhältnis maßgeblich gesteuert werden können. Die experimentellen Ergebnisse wurden mit mehrdimensionalen deterministischen Populationsbilanzen nachgestellt, in denen die internen Koordinaten die Partikelanzahl der jeweiligen Spezies in einem Aggregat abbilden. Der physikalisch diskrete Eigenschaftsraum wurde adaptiv mit einer semi-heuristischen Methode so reduziert, dass nur Eigenschaftskoordinaten mit hohen Partikelkonzentrationen im Modell berücksichtigt werden. Die verwendeten Aggregationskerne basieren auf deterministischen Modellen aus der Kolloidchemie, insbesondere der DLVO Theorie, und verknüpfen die Interaktionen auf der Einzelpartikelskala mit dem makroskopischen Verhalten mehrerer Partikelpopulationen. Die an Partikelsystemen entwickelten Methoden wurden erfolgreich für eine systematische, modellgestützte Aufklärung präferentieller Aggregationsprozesse in einem ternären System aus Antikörpern und zwei humanen Tumorzelllinien (KARPAS-299 und U-937) eingesetzt. Trotz angenommener instantanter Aggregation bei Rezeptor-Ligand-Kollisionen, verursacht die geringe Rezeptorkonzentration auf den Zelloberflächen einen ratenlimitierten Aggregationprozess (engl.: *rate limited cluster aggregation*, RLCA). Populationsbilanzsimulationen mit Kernen, die stark heterogene Oberflächenstukturen der aggregierenden Spezies berücksichtigen (*patchy particles*), bestätigen die experimentellen Befunde. Die zielgerichtete Verabreichung pharmazeutischer Wirkstoffe mittels funktionalisierten Trägerpartikeln an spezifische Zellen unter Minimierung nachteiliger Beeinflussung anderer Zelltypen (*targeted drug delivery*) stellt ein potentielles Anwendungsgebiet dieser Ergebnisse dar.

Abstract

Motivated by the selective adsorption of functionalised drug carrier particles to certain cell types for medical applications this thesis investigates fundamental heteroaggregation phenomena under special consideration of the dynamic behaviour in physical and biological model systems. The adsorption of antibodies as possible functional moieties to receptors on cell surfaces represents an essential first step in a series of further transport limitations for the cellular uptake of functionalised drug carrier particles. To establish suitable scientific methods for the analysis of selective and competitive heteroaggregation processes, the specific interaction and heteroaggregation of multiple colloid constituents was studied in physical particle systems first. Experimental methods primarily include flow cytometry and diverse microscopic techniques, while simulations are based on population balance equations with kernel models rooting in classical colloid science. Both approaches were transferred to biological systems to achieve a more rigorous description of drug delivery dynamics and efficiency. This could prove valuable for future optimisation efforts.

Flow cytometry was established as a very powerful and convenient tool to characterise cluster composition and its dynamics in heteroaggregation processes. It enables an independent and very detailed resolution of multidimensional distributions by a reliably automated single particle analysis. Investigations in binary and ternary particle mixtures focus on electrostatic de- and restabilisation phenomena, that can be tailored by the choice of suitable particle species and their mixing ratio. Experimental results were reconstructed by multivariate population balance simulations in which the internal coordinates represent the particle number of the respective species inside an aggregate. The physically discrete property state space was adaptively reduced by a semi-heuristic approach, so that only property coordinates featuring high aggregate concentrations were considered in the model. The applied aggregation kernels are based on deterministic models from colloid science, in particular DLVO theory, and connect interactions on the single-particle level with the macroscopic behaviour of multiple particle populations. The methods established for particle systems were successfully transferred to a systematic, model-based investigation of preferential aggregation processes in a ternary system of antibodies and two human tumour cell lines (KARPAS-299 and U-937). Despite the assumed instantaneous aggregation following receptor-ligand collisions, the low receptor expression on cellular surfaces causes a rate limited aggregation process (RLCA). Population balance simulations with kernels that consider the strong surface heterogeneities of the aggregating species (patchy particles) confirm the experimental results. The targeted administration of pharmaceutical compounds by functionalised carrier particles to specific cells under minimisation of adverse effects represents a potential area of application of these results.

Preface

The work presented within this thesis was conducted during my time as scientific employee at the Max Planck Institute for Dynamics of Complex Technical Systems in Magdeburg from May 2006 until April 2010.

My special thanks is directed to Prof. Dr. Kai Sundmacher for the interesting and challenging topic as well as for the granted trust and scientific freedom. I am very grateful for many fruitful and supportive discussions, the luxury to pursue what the topic demanded in a rather unconstrained fashion as well as making numerous participations at national and international conferences possible. Additional thanks I owe to Prof. Dr. Johannes Khinast (Technical University of Graz, Austria) for reviewing and evaluating my thesis.

In completing this work I have benefited from the help of many people. I would like to thank all of my collaborators, in particular Prof. Dr. Heiko Briesen, Prof. Dr. Uwe Lendeckel, Dr. Fabian Heitzeberg, Prof. Dr. Michael Naumann, Prof. Dr. Udo Reichl, Dr. Michael Kappl, Dr. Rüdiger Berger and Prof. Dr. Hans-Jürgen Butt for their scientific input and kind support. I address my sincere appreciation to Dr. Thomas Hempel for preparing numerous electron micrographs, some of which are presented in the thesis, and to the laboratory staff, especially to Bianka Stein for her boundless energy, refreshing creativity and jovial nature, but also to Markus Ikert for his patience in conducting the AFM measurements. I am further indebted to my students Jens Karschau, Britta Ebeling and Annika Willitzki who considerably supported my research activities as well as to Dipl.-Biotech. Josef Schulze Horsel for his support in flow cytometry and to Dr. Peter Heidebrecht and Dr. Björn Niemann for fruitful discussions.

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Sascha Rollié Magdeburg, May 2010

Contents

List of symbols

Symbols that are only of local interest within the thesis will be explained where applied and are not included here.

Physical constants

e	Elementary charge	$1.6021 \cdot 10^{-19}$	[C]
ε_0	Dielectric static permittivity in vacuum	$8.8542 \cdot 10^{-12}$	$[\mathrm{A\,s\,V^{-1}\,m^{-1}}]$
h	Planck's constant	$6.6261 \cdot 10^{-34}$	[J s]
N_A	Avogardo's number	$6.0221415 \cdot 10^{23}$	$[\mathrm{mol^{-1}}]$
k_B	Boltzmann's constant	$1.3804 \cdot 10^{-23}$	$[\mathrm{J\,K^{-1}}]$
R	Universal gas constant	8.3143	$[\mathrm{J\,mol^{-1}K^{-1}}]$

Latin symbols

A	Hamaker constant	[J]
a	Surface-to-surface separation distance	[m]
B	Hydrodynamic correction factor	[−]
b	Hardness factor	[−]
C^m	Mean curvature	$[\mathrm{m^{-2}}]$
C	Electrical capacitance	[F]
$\mathcal{C}$	Fitting constant	[−]
c	Number concentration	$[\mathrm{m^{-3}}]$
c^M	Mass concentration	$[\mathrm{kg\,m^{-3}}]$
D	Diffusion coefficient	$[\mathrm{m^2\,s^{-1}}]$
d	Diameter	[m]
d_f	Fractal dimension	[−]
$\vec{e}$	Property coordinate vector	[a.u.]
F	Force	[N]
FL	Fluorescence intensity	[a.u.]
$FL^0_{/}$	Autofluorescence intensity	[a.u.]
FS	Forward scatter signal	[a.u.]
f	Number density function	$[\Pi_k^K\,[\mathrm{e_k}]^{-1}\ \mathrm{m^{-3}}]$
f_F	Probability function of formation	[−]
fl	Volume specific fluorescence intensity	$[\mathrm{a.u.\,m^{-3}}]$
g_i	Steric correction factor	[−]
i, j, k	Particle numbers	[−]
I	Ionic strength	$[\mathrm{mol\,m^{-3}}]$
J	Diffusive particle flux with superimposed drift	$[\mathrm{s^{-1}}]$
J_0	Diffusive particle flux	$[\mathrm{s^{-1}}]$
K	Cantilever spring constant	$[\mathrm{n\,m^{-1}}]$
k_f	Scaling factor	[−]

k^0	Extrapolated contact force	$[\mathrm{N\,m^{-1}}]$
l	Unreduced number of rows	$[-]$
$\overline{M}$	Molar mass	$[\mathrm{kg\,mol^{-1}}]$
$\mathcal{M}$	Mobility	$[\mathrm{m\,s^{-1}\,N^{-1}}]$
m	Mass	[kg]
N	Particle number	$[-]$
N_{11}	Number of collisions per encounter	$[-]$
N_i^{max}	Maximum number of surface receptors	$[-]$
n	Relative refractive index	$[-]$
P	Probability	$[-]$
P', P'', P	Particle or cluster	$[-]$
P^c	Probability of multiple collisions per encounter	$[-]$
$\mathcal{P}$	Property space	$[-]$
Q	Charge	[C]
r	Radius or radial coordinate	[m]
r^G	Radius of gyration	[m]
r_σ	Zero-potential separation	[m]
SS	Side scatter signal	[a.u.]
T	Temperature	[K]
t	Time	[s]
V	Potential energy	[J]
V_P	Particle volume	$[\mathrm{m^3}]$
$\dot{V}$	Flow rate	$[\mathrm{m^3\,s^{-1}}]$
W	Stability ratio	$[-]$
w_e	Velocity	$[[\mathrm{e_k}]\ \mathrm{s^{-1}}]$
w_x	Velocity	$[\mathrm{m\,s^{-1}}]$
x, y, z	Spatial coordinates	[m]
y	Property state of surrounding continuous phase	[a.u.]
z_i	Ion valency	$[-]$

Greek symbols

α	Scaling parameter	$[-]$
Γ_N	Transport across control volume boundary	$[\mathrm{s^{-1}}]$
γ	Excluded surface area	$[-]$
δ_{ij}	Kronecker function	$[-]$
ε_r	Relative static dielectric perimittivity	$[-]$
ζ	Zeta-potential	[V]
η	Dynamic viscosity	[Pa s]
κ^{-1}	Debye length	[m]
λ	Decay length or wavelength	[m]
ν_P	Dimensionless particle volume	$[-]$
ξ	Threshold value	$[-]$
ρ	Density	[a.u.]
Σ_N	Source / sink of control volume	$[\mathrm{s^{-1}}]$
σ_N	Source / sink of control volume	$[\Pi_\mathrm{k}^\mathrm{K}\,[\mathrm{e_k}]^{-1}\ \mathrm{m^{-3}\,s^{-1}}]$
σ	Surface charge density	$[\mathrm{C\,m^{-2}}]$
σ^2	Variance	[a.u.]
υ	Daughter particle number	$[-]$

ϕ	Covered surface fraction	$[-]$
$\phi_{N,e}$	Transport density, internal coordinates	$[\Pi_k^{K-1}\,[e_k]^{-1}\ m^{-3}\,s^{-1}]$
$\phi_{N,x}$	Transport density, external coordinates	$[\Pi_k^{K}\,[e_k]^{-1}\ m^{-2}\,s^{-1}]$
Ψ	Interaction potential	$[V]$
ω	Transfer frequency	$[s^{-1}]$
ω_{Agg}	Aggregation rate	$[m^3\,s^{-1}]$

Subscripts

0	Surface
∞	Infinite distance from surface
AB	Antibody
Agg	Aggregation
Ar	Argon
Br	Breakage
$CD13$	Cell surface protein, aminopeptidase N
$CD33$	Cell surface protein, myeloid-associated marker
c	Cantilever
dl	Diffuse layer
e	Internal coordinates of property space
em	Emission
ex	Excitation
$FITC$	Fluorescein isothiocyanate
$HeNe$	Helium-Neon
$hydr$	Hydration
$IgG1$	Cell surface protein, Immunoglobulin G
i	Ion
$KARPAS-299$	Human tumour cells (anaplastic large cell lymphoma)
M	Molar
MF	Melamine formaldehyde
m	Monolayer
mol	Molecular
Nuc	Nucleation
P', P'', P	Particle or cluster
PBS	Phosphate buffered saline
PE	Phycoerythrin
PI	Propidium iodide
PS	Polystyrene
p	Piezoelement
RhB	Rhodamine B
RL	Receptor-ligand interaction
$RPMI$	Roswell Park Memorial Institute cell culture medium
Sol	Solvation
s	Stern layer
$U-937$	Human tumour cells (histiocytic lymphoma)
x	External coordinates of property space

Supercripts

0	Surface
$+$	Source
$-$	Sink
$\pm$	Source and sink
add	Additional particles
B	Brownian
Born	Born interaction
C	Charge
D	Diffusive
E	Collision efficiency
eff	Effective
el	Electrostatic interaction
eq	Equilibrium
exp	Experimental
F	Collision frequency
hydr	Hydration
m	Mean
max	Maximum
min	Minimum
N	Particle number
NaCl	Sodium chloride
R	Reduced
rec	Receptor
sim	Simulated
sol	Solvation interaction
spec	Specific
tot	Total
unspec	Unspecific
vdW	Van der Waals interaction