

# Exploratory study to design biomimetic particle chains for self-folding functional materials

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During my visit to the Laboratory for Supramolecular Materials in the Department of NanoBiotechnology at the BOKU - University of Natural Resources and Life Sciences-, Vienna from the first of May until the 11th of June 2011 I performed an exploratory study for the creation of a colloidal protein model and experimental setup for its investigation.

Amino acid peptide chains are a stimulating inspiration for materials scientists due to their ability to create structurally and functionally diverse materials through directed self-folding of chains of hetero-functional monomers. Properties of such materials derive from the 3-D structure favoured by the sequence of functional elements encoding the folding of a chain. This design and assembly approach is virtually experimentally unexplored for *de novo* designed man-made materials. Dr. Coluzza (University of Vienna) has developed the highly reductionist “caterpillar model”<sup>[1]</sup> for investigating design of folding peptide sequences *in silico*.

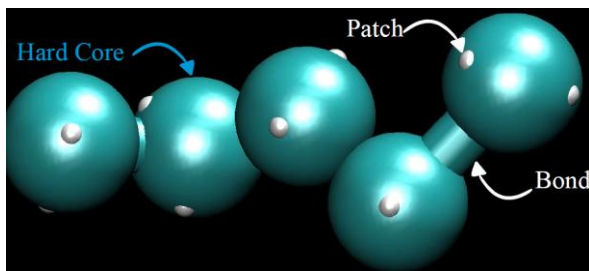


Figure 1. Schematic of the features of the particle chains to be experimentally realized.

The caterpillar model is attractive for the design of experimental models to explore self-folding inspired by, but not exactly mimicking, proteins. It has demonstrated that deterministic, structural complexity can be achieved starting from a chain of colloids with spherically symmetric interaction potentials to which directional bonds (patch interactions, see Figure 1) are added<sup>[2]</sup>.

The objective of the exchange was to conduct an exploratory study for a joint postdoc and PhD project on the design and study of chains of colloids with encoded symmetric and directional interactions along the chain that show directed self-folding analogous to the folding of proteins. The main goal of the visit of Dr. Peter van Oostrum to the DNBT was to explore the material choice and the experimental strategies to manipulate and study folding of chains of colloids. We worked on three main tasks:

1. Evaluating the synthesis of micron-thick polyelectrolyte brushes on ~1000 nm polystyrene colloids. Using living grafting-from methodology allows controlling the brush architecture like surface-grafting density, length and polydispersity of the polymer chains as well as the exact chemical composition<sup>[3]</sup>. Dr. Ronald Zirbs (DNBT) lead the study of the synthesis of the polymer-shell of the particles to (in order of priority) (a) adjust the charge-density (via molar-ratio of PMAA and PMMA during polymerization), (b) incorporation of crosslinking units to modify the stiffness of the polymeric shell and to link the backbone of the strings, and (c) coupling of specific hydrogen-bonding-groups enabling selective and exactly adjustable interactions to other particles<sup>[4]</sup>.

2. Preparations for the measurement of interaction potentials were made by Dr. van Oostrum as feedback to the synthesis effort. This was attempted by combining holographic microscopy<sup>[5]</sup> and the methods in reference [6] using dielectric manipulation focusing instead of optical tweezers.
3. Implementation of a microscope setup for holographic tracking of colloidal particles at the Imaging center of the BOKU located in the facilities of the DNBT<sup>[5]</sup>. The setup included induced dielectric dipole focusing to assemble chains of particles, a method recently developed in the van Blaaderen group<sup>[7]</sup>. Dipole focusing will also be used to control the particles distance for the measurements of the interaction potential.

During my visit in Vienna I had an extensive exchange of insights with Dr. Ivan Coluzza to find the experimentally simplest design of colloidal chains that can be expected to show directed self-assembly in their folding behavior. Instigated by these discussions Dr. Ivan Coluzza successfully adjusted his code to explore the folding behavior of strings of particles with only one attractive patch, perpendicular to the backbone of the string. He found that also strings of this type show very characteristic folded structures. Although these structures are generally different than those of natural proteins, these strings will show 'designability'. Similarly, simulations were performed in which the range of the interactions was limited to 1 radius of the core particles. Also this was found to not compromise the designability of the folding behavior, provided that at least two different types of differently interacting colloids were used. These less strict than the original requirements aided the design of a synthetic route to create the colloidal model system.

In collaboration with Dr. Ronald Zirbs a literature study was performed on different polymerization techniques to create polymer shells which would fit the sought after colloidal model. To ensure a reproducible polymer-shell of definable thickness, living grafting-from methodologies were chosen to be used to attach copolymer chains onto the particle-surfaces. Furthermore, the reaction conditions were studied and tested for compatibility with the material and desired end result as described below.

Because of the necessary high level of control of the polymerizations, different polymerization techniques like ATRP<sup>[8]</sup>, RAFT<sup>[9]</sup> and RP<sup>[10,11]</sup> should be investigated during the continuation of the project. The major aspects are (a) very long polymer chains (high degree of livingness (absence of chain termination, chain transfer or side reactions) necessary to enable very long polymerization times), (b) the possibility of the co-polymerization of up to four different monomers (uncharged (PMMA or PS), charged (PMAA), cross linker for the branching of the polymer, reactive monomer for further modification and reactive cross linking for post-crosslinking of 2 particles) possibly both these tasks can be fulfilled by the same monomer, (c) the direct polymerization of a highly branched polymer to create a polymer shell with almost constant density profile and (d) the post-modification of the polymer with different specific groups for further reactions (crosslinking, hydrogen-bonding-systems, adjusting the polarity by binding of hydrophobic/hydrophilic groups onto the main-polymer-chain).

Depending on the exact composition of the reaction mixture, different co-polymers will be observed. Due to the complex monomer-mixture a strong focus will be devoted on the determination of the chemical composition and the physical behavior of the obtained core-shell-particles. To overcome some known limitations of single polymerization techniques (reactivity-differences of chemically unequal monomers), different initiation systems will be tested. The synthesis of these particles can be broken down into three major steps:

- I. Surface modification of the PS-particles with the matching initiator (examples shown in Figure 2).

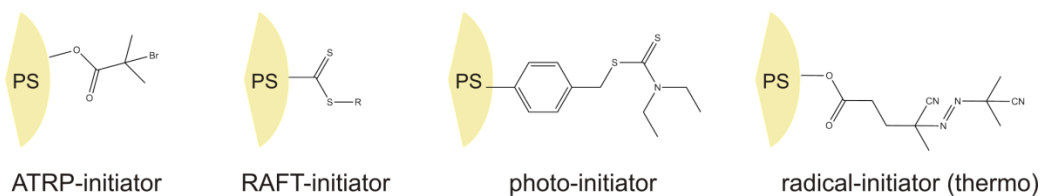


Figure 2. Depending on the planned polymerization technique different initiators (ATRP<sup>[8]</sup>; RAFT<sup>[9]</sup>; photo-initiation<sup>[10]</sup> and RP<sup>[11]</sup>) will be bound to the surface.

- II. Copolymerization of the Polymer brush. In Figure 3 we show an example of what such a brush might schematically look like. In order to be able to generate differently charged particles to form the alphabet for the folding experiments, protected PMAA monomers are incorporated that can be deprotected to increase the number of chargeable groups in the brush.

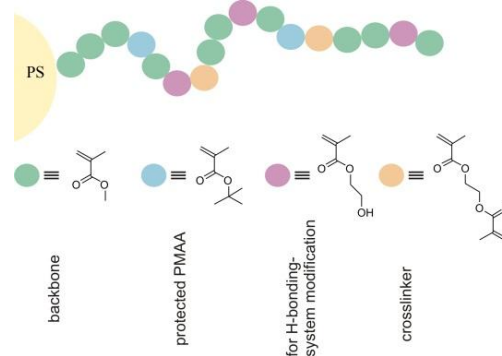


Figure 3. Polymer-brush grafted from a PS-Particle-surface using 4 different monomers (examples)

- III. Modification of specific reactive groups within the main -polymer- chain to create novel properties (H-bonding-systems, reactive groups for further crosslinking of 2 particles, and the creation of the “patches”). A nice technique that can be used to selectively modify only part of the brush relies on the partial protection of the brush by attaching the particles to an oil-water interface to the specifically modify the water exposed part of the brush. In order to prevent rotation of the particles

at the interface the oil phase could consist of wax that that can be melted and solidified<sup>[12]</sup>.

Several steps in the synthesis path take place in apolar solvents. Commercial particles were acquired and exposed to several apolar solvents to check if they were sufficiently cross-linked and for fluorescent particles if they could retain the dye in such solvents. Unfortunately, the results of these tests were negative. We have accordingly decided to synthesize our own polystyrene particles following a recipe similar to that in reference 13. The first batch of these particles was found to be resisting exposure to most apolar solvents. Moreover, a preliminary test in which an ATRP<sup>[1]</sup> brush of only polystyrene was grown was successfully conducted.

As stated above, the different types of interactions between the colloids are generated by having different charge densities on the polymer brushes on the particles. In order to be able to accurately predict the folding behavior of strings of these particles, also with the ambition of designing sequences to fold into particular structures in mind, it is important to know the exact interaction potential between the colloids.

The interaction between the particles can be deduced from the statistics of the trajectories of pairs or particles after they have somehow been released close to each other<sup>[6]</sup>. To be able to track the particles with sufficient accuracy we plan to use in-line Digital Holographic

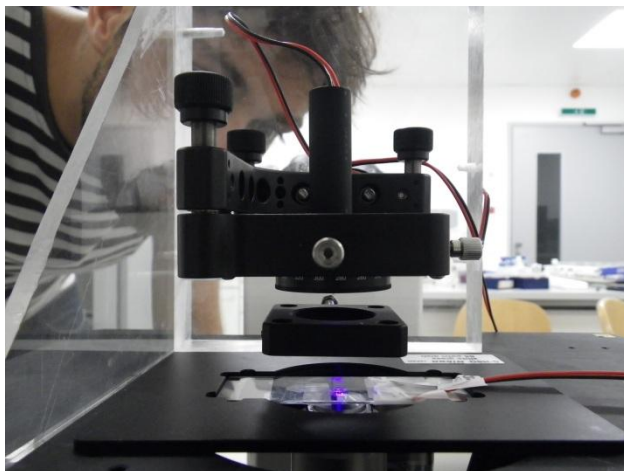


Figure 4. Portable holographic setup.

Microscopy<sup>[5]</sup>. During my stay in Vienna I devised a portable laser setup to be able to perform in-line holographic measurements on any inverted optical microscope that is equipped with a CCD camera. In Figure 4 the holographic setup is placed on the sample table of the fluorescence microscope at the DNBT.

The setup is made out of plexiglas and uses a 6-axis optical mount to mount an adapted laser pointer. The laser pointer has been opened to remove the batteries

that were replaced with a power supply and to fix it to a 1 inch metal ring for mounting. The mounting allows moving the laser in the plane and to tilt it slightly to assure that the laser shines into the inverted objective along its optical axis. The linearly polarized 410 nm laser can be rotated to attenuate the laser intensity by means of the polarizer plate underneath. 410 nm laser light was chosen because of the relative short wavelength that makes the scattering Mie particles show more minima in the scattering, allowing to characterize smaller particles more accurately<sup>[5,13]</sup>.

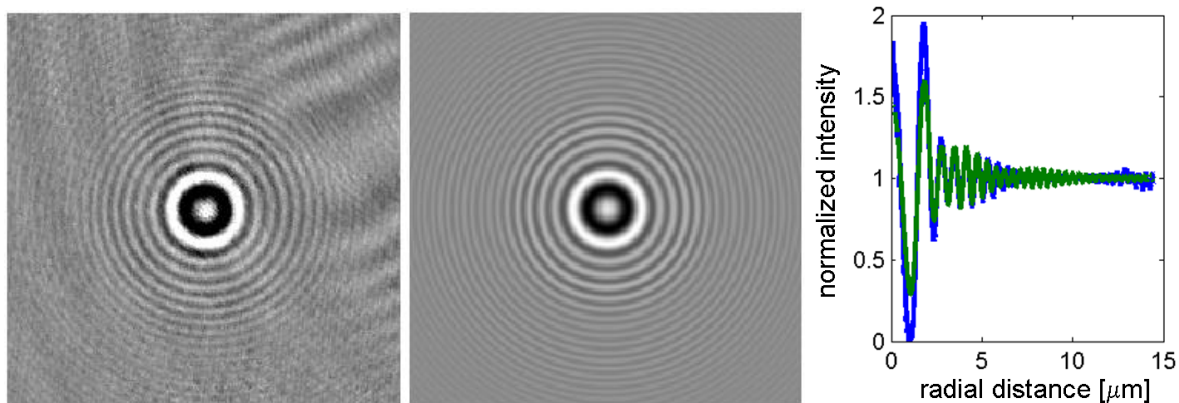


Figure 5. From the right to the left: experimental hologram of a 1  $\mu\text{m}$  polystyrene particle in water, fit of Mie theory to the experimental hologram and the radially averaged intensity in both the experimental hologram and fit, respectively blue and green. The holograms are 37 by 37  $\mu\text{m}$ .

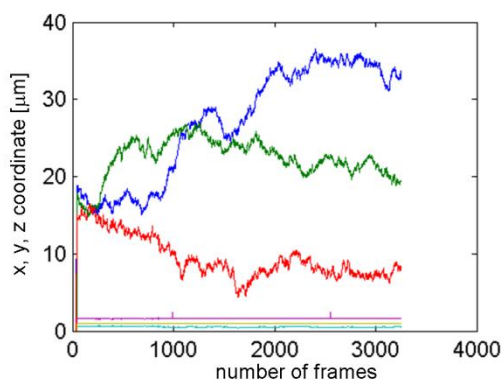
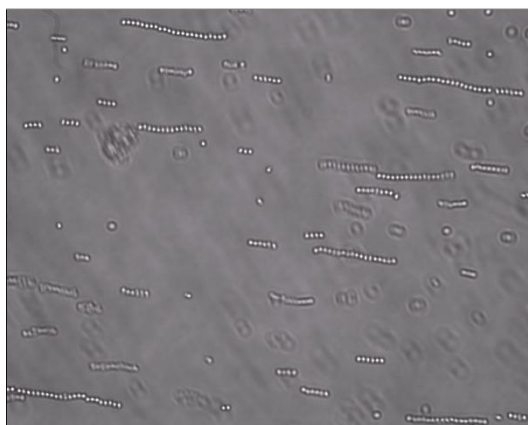


Figure 6. Fit values to the holographic movie of which Figure 5 represents a snapshot as a function of the frame number. The x, y and z coordinates with respect to the upper left corner of the camera frame are plotted in respectively blue, green and red. Violet, turquoise and yellow represent respectively the particle refractive index, its radius and the relative brightness of the frame.

In Figure 5 we show a typical hologram as recorded using the portable laser setup at one of the microscopes of the Imaging Center at the BOKU. The 1  $\mu\text{m}$  polystyrene particle from Polysciences was fitted to be at a distance of  $7.85 \pm 0.01 \mu\text{m}$ . In Figure 6 we plot the fit values for this 1  $\mu\text{m}$  polystyrene particle as it diffused and sedimented in water. The error estimates from the fitting algorithm agree with the error estimate from the offset in the mean squared displacement<sup>[5]</sup> and the mean fit-value of the radius of  $0.509 \pm 0.001 \mu\text{m}$  agrees with the specifications of the manufacturer. We thus conclude that the in-line holographic microscopy setup is working and can be used to measure colloidal interactions.

The sample on the stage in Figure 4 contains the same 1  $\mu\text{m}$  polystyrene particles, that can be polarized by applying an AC field on the two wires that have been inserted in the rectangular capillary (Vitrocom  $0.1 \times 2.0 \text{ mm}$ ). During my visit at the DNBT I also constructed the setup necessary to generate sufficiently strong electric fields. The signal for the alternating current is generated using a standard function generator (TTi TG101A) after which it is amplified by a broadband amplifier (Krohn-Hite 7602M). When the sample is exposed to high fields, 150 V over 2 mm at 1 MHz in this case, the particles gain an electric dipole moment that make them form strings<sup>[7]</sup>. In figure 7 we show a brightfield micrograph of the polystyrene particles while they are forming strings.

From the preparative work we have gained a better understanding of the possibilities to create a colloidal model that shows directed self-assembly that is inspired by the self-folding of proteins. There is a clearer plan for the synthesis of the particle brushes and both the holography and the dielectric focussing are now existing setups in Vienna. The project has therefore reached the first milestones and will continue in Vienna where funding of both the theoretical and experimental parts of the project have now been assured. I will take part in the continuation of the project and be among the authors of the resulting papers.



*Figure 7 Brightfield micrograph of 1  $\mu\text{m}$  PS spheres in water. The particles are polarized by a 1500 V/cm AC field at 1 MHz. Total frame is 118 by 96  $\mu\text{m}$ .*

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