19 in the CHR significantly affected the aggregation rate. This is seen as an extended half-time and steeper concentration dependence suggesting a change in aggregation mechanism relative to the wild type protein. We also studied co-aggregation and cross-seeding between the kinetically faster Aβ42 and the slower Aβ40. The aggregation process starting from mixed monomers displays two transitions and our data imply that there is cross-reactivity between Aβ40 and Aβ42 at the level of primary nucleation only, while fibril elongation and surface-catalysed secondary nucleation are highly specific events. In contrast, co-aggregation of Aβ42 with the slower mutant F19L only displays a single conformational transition and the cross-seeding is as efficient as the self-seeding. The main reason for the discrimination in the different pathways is the length at the C-terminus rather than the difference in intrinsic aggregation rates.

To further investigate the relative role of intermolecular interactions we changed the temperature to provide information on energy barriers and their enthalpic and entropic components. With an analytically solved model we could do a global fitting to estimate the activation energy for Aβ42 for the primary nucleation, secondary nucleation and elongation.

3459-Pos Board B187
HIV-Tat Protein Enhances Amyloid Beta Peptide Aggregation
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We show that amyloid beta 1-40 peptide aggregation under physiological conditions in the presence of HIV-Tat protein results in significant structural modifications. Atomic force microscopy imaging shows that the predominant typical singular uniform amyloid fibrils that formed at 0.08 micromolar HIV-Tat protein was present and at higher Tat concentrations (0.4 to 1.8 micromolar) it turned into populations with more double twisted fibers when 0.08 micromolar HIV-Tat was present and at higher Tat concentrations. We have shown very recently that the full-length peptide undergoes enthalpy-entropy driven adsorption on the hydrophobic surface of high curvature (Biophys. J. 2012, 102, 1889; Phys. Chem. Chem. Phys. 2013, 15, 837). The process, initiated by the peptide’s central hydrophobic core (LI7VFFA21), is comprised of a dewetting transition followed by π-π stacking interactions with the surface. We report here the effect of the hydrophobic surface on the initial dimerization process of the full-length Aβ. While the interactions with the surface cannot out compete the strong monomer-monomer interactions, they are capable to a large extent in destabilizing pre-formed Aβ dimers. We further performed adaptive biasing force based free energy calculations to compare the Aβ trimerization process arising from addition of a monomeric unit to the surface adsorbed, weakened dimer complex, to that in pure solution. Our results indicate that presence of hydrophobic surfaces is likely to weaken the initial nucleation processes, and could eventually slow the self-assembly kinetics. These results should be of significance in the design of therapeutics aimed at countering the neurotoxic effects of Aβ oligomers.

3460-Pos Board B188
The Aggregation-Prone Mutant Huntington Protein in a Cellular Context - Approaches by Super-Resolution Imaging
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The identities of toxic aggregates species in Huntington’s disease (HD) pathogenesis remain ambiguous. While polyQ-expanded mutant huntingtin (Htt) is known to accumulate in compact inclusion bodies inside neurons, this is widely thought to be a protective coping response that sequesters misfolded conformations or aggregated states of the mutated protein. To define the spatial distributions of fluorescently-labeled Htt-exon1 species in the cell model PC12m (terminally differentiated into sympathetic-neuron-like cells with nerve growth factor), we employed highly sensitive single-molecule-based and stimulated emission depletion (STED) super-resolution fluorescence imaging modalities. In addition to inclusion bodies and the diffuse pool of monomers and oligomers, fibrillar aggregates ~100 nm in diameter and up to ~1-2 μm in length were observed for pathogenic polyQ tracts expression experiments with 46 and 97 repeats) after targeted photo-bleaching of the inclusion bodies. These short structures bear a striking resemblance to fibers described in vitro. We identified a sharp cut-off behavior of maximum fibril length and documented the ensuing bundling of these fibers into denser arrangements of varying complexity, both in the cytosolic space and inside the neuritic processes.

3461-Pos Board B189
Nanoscale Assembly of Proteins into Amyloid Oligomers, Pores and Fibris
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Protein amyloid aggregates are implicated in a variety of debilitating human disorders such as Alzheimer’s, Parkinson’s and prion diseases. The transition from a normal functional protein to an abnormal misfolded form involves a profound conformational change that serves as the key step in amyloid assembly leading to nanoscopic oligomers, pores and fibrils. Using Raman spectroscopy in combination with atomic force microscopy (Figure 1a,b), we have been able to delineate the key structural transitions during amyloid formation (Bhattacharya et al. J. Phys. Chem. Lett. 2013, 4, 480-485). Moreover, the underlying molecular mechanism by which amyloids are involved in inducing cellular toxicity remains elusive because the conventional optical microscopy does not allow one to monitor these processes directly at a high spatial resolution due to the diffraction-limit. Using near-field scanning fluorescence microscopy, we have been able to image the fibrils far beyond the diffraction-limit and interrogate individual fibrils by simultaneously monitoring both nanoscale optical and near-field bright surface-enhanced fluorescence brightness along the length of the fibrils (Figure 1c,d). Our nanoscale imaging results provide structural underpinnings of the supramolecular packing within the nanoscopic amyloids (Dalal & Bhattacharya et al. J. Phys. Chem. Lett. 2012, 3, 1783-1787).

3462-Pos Board B190
Atomic Simulations Reveal Mechanistic Insights into Plausible Ways of Perturbing the Nucleation Thermodynamics of the Full-Length Aβ Peptide
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Self-assembled forms of the 4 kDa amyloid beta (Aβ) peptide itself formed from proteolytic cleavage of the membrane embedded precursor, is strongly implicated in the onset of the neurodegenerative Alzheimer’s disease (AD). While amyloid aggregates found in patients’ brains are predominantly composed of the peptide’s fibrillar forms, its soluble oligomers have been reported to aggregate the peptide’s neurotoxicity. It is recognized, however, that all self-assembly pathways arise from the peptide’s initial nucleation, and therefore eliciting mechanistic details of this process as a function of physico-chemical conditions could unearth valuable strategies for disrupting the neurotoxicity. With a recent atomic molecular dynamics simulations, we have shown very recently that the full-length peptide undergoes enthalpy-driven adsorption on the hydrophobic surface of high curvature (Biophys. J. 2012, 102, 1889; Phys. Chem. Chem. Phys. 2013, 15, 837). The process, initiated by the peptide’s central hydrophobic core (LI7VFFA21), is comprised of a dewetting transition followed by π-π stacking interactions with the surface. We report here the effect of the hydrophobic surface on the initial dimerization process of the full-length Aβ. While the interactions with the surface cannot outcompete the strong monomer-monomer interactions, they are capable to a large extent in destabilizing pre-formed Aβ dimers. We further performed adaptive biasing force based free energy calculations to compare the Aβ trimerization process arising from addition of a monomeric unit to the surface adsorbed, weakened dimer complex, to that in pure solution. Our results indicate that presence of hydrophobic surfaces is likely to weaken the initial nucleation processes, and could eventually slow the self-assembly kinetics. These results should be of significance in the design of therapeutics aimed at countering the neurotoxic effects of Aβ oligomers.

3463-Pos Board B191
The Formation of Higher Order Structures by the Neuronal Protein Alpha-Synuclein: Self-Assembly Over Multiple Length Scales
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Alpha-synuclein (αS) is an intrinsically disordered neuronal protein that can self-assemble in vivo into amyloid fibrils. αS fibrils are chemically and mechanically very stable and once formed they tend to accumulate in the tissue. Such