ESRF	**Experiment title:** Self-assembly of Filamentous Peptide-polymer conjugates in solution	**Experiment number**: MX-1610
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Names and affiliations of applicants (* indicates experimentalists):

Reidar Lund*, Dept. Chemistry, University of Oslo (NOR)

Line Trosterud,* Dept. Chemistry, University of Oslo (NOR)

Sebastian Geissler* Faculty of Dentistry, University of Oslo (NOR)

Hanna Tiainen*, Faculty of Dentistry, University of Oslo (NOR)

Report:

Unfolded, or structurally disordered, proline-rich regions of natural proteins such as collagen, amelogenin, and ameloblastin have recently been found to play an important role in biomineralisation. Short synthetic peptides that mimic the disordered regions of such proteins may therefore be bound onto biomaterial surfaces to stimulate bone formation and regeneration in vivo. Since the function and bioactivity of these peptides is likely to be strongly linked to their lack of well-defined three-dimensional structure, it is important to understand the behaviour of such biomolecules when dissolved in aqueous media. The structure of two dissolved synthetic proline-rich peptides (Table 1) that have previously shown potential in inducing bone mineralisation of both osteoblast-like cells and mesenchymal stem cells were examined.

The peptides were dissolved in either TRIS-buffered saline (pH 6-12) or sodium acetate buffer (pH 3-5.5) at 5 mg/ml concentration and their structure was analysed with X-ray scattering (BM29, bioSAXS beamline; ESRF, Grenoble). In order to evaluate the chain conformation, size and shape of the peptide aggregates, both model-independent (IFT, Guinier) analysis and geometrical body modelling were performed.

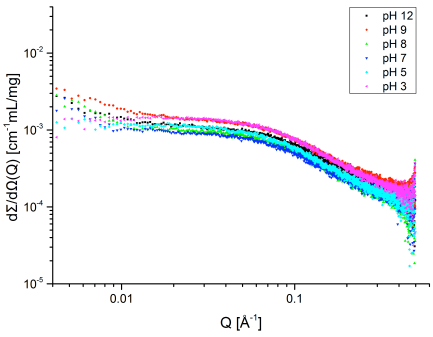
While P2 maintained its disordered random coil-like structure throughout the tested pH range (pH 3-12), P5 formed aggregates at high pH (pH 8-12). Radius of gyration nearly doubled and over tenfold increase in the calculated molecular weight was observed in this pH region, indicating that several peptide chains clustered together into nanoparticles whose scattering profile best fitted a triaxial ellipsoidal model. IFT analysis indicates a transition from single peptide chains with locally collapsed parts at pH 6-7 to peptide aggregates at pH 8-9.

The main difference between the two examined proline-rich polypeptides P2 and P5 is the presence of cysteine (C) in the amino acid sequence of P5. Cysteine being the only amino acid containing a thiol group (-SH), the observed self-assembly and aggregation at high pH may be explained by the formation of disulphide bonds (-S–S-) which link several polypeptide chains together as the thiol groups of the cysteines become

deprotonated with increasing pH (pKa_{thiol} = 8.3). Consequently, the disordered peptide chains may cross-link and assemble together forming aggregates that were observed as the characteristic increase in the scatter intensity. Self-assembly and aggregation of the polypeptide chains was not observed within physiological pH range, and it is therefore not expected to influence the bioactivity of the molecule. However, such pH dependent change in peptide characteristic must be taken into account when choosing the appropriate processing conditions for binding the disordered peptides onto biomaterial surfaces.

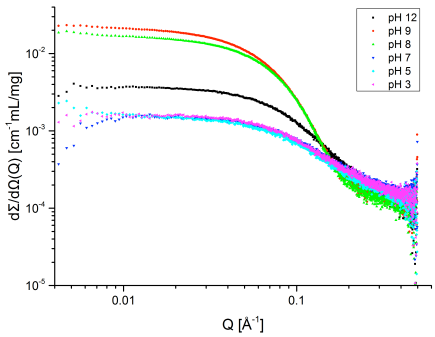
P2

PLVPSQPLVPSQPLVPSQPQPPLPP



P5

PLVPSSPLVPCCPLVPCCPSPPLPP



In the second part of the project we investigated the self-assembly of a series of antimicrobial peptides based on short sequences with a general formula of $WK_n(QL)_mK_n$. Robust beta-sheets nanofibers form when the ratio m/n reaches three. Interestingly, the fibrous morphology was not disrupted by conjugation with drugs and poly(ethylene glycol) (PEG). In the study we also investigated the effect of charge density by varying the terminal amino acids. The data and model fits, shown below, indicate well-defined nanosheets in all cases. However, the dimensions of the chains depend quite markedly on the charges at the rim. We also observe an internal correlation peak that can be attributed to the correlation along the long axis.