<u>BACHELOR LEVEL COURSE / PROJECT</u>

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In 2009, Angela Belcher's research group published a paper on self-assembly at the nanoscale. They used M13 phages as a mean to fabricate biologic, genetically engineered, highpower lithium-ion batteries. They used what we consider to be a brute-force engineering approach. Their method consisted of subjectively manufacturing a phage library where the pIII or pVIII surface proteins have an insertion sequence, consisting of identical amino acids. Using a phage display she was able to select a single phage with the highest binding affinity towards various inorganic materials. To create "evolution" on her sample, she modified the insertion sequence by changing every other amino acid with a different, yet homologous one. Her research indicates that the biochemical proteins have the ability to bind any compound, as long as proteins are taught through evolution. Due to continuous, extensive artificial synthesis of new insertion sequences and the subjectivity of her method, we doubt if Belcher's strategy is the most efficient approach as she limit the degree of evolution due to the restricted variation– compared to the possible biological diversity.

An evolutionary strategy would be to create a random, objective protein variation followed by a selection in a cyclical process, until a sufficiently effective property is achieved. Sitedirected evolution, a synthetic method for DNA-variation in isolated regions has somewhat addressed the issue. However, any approach based on synthetic biology implies completely random mutations whereas real evolution converges towards an optimal solution.

Being able to apply a site-directed evolutionary step directly to a phage may prove a fast, cost-effective, and environmentally friendly method to optimize phage surface-proteins to bind to chosen compounds. We thus seek to introduce a new PCR strategy, the Variation of Isolated Regions PCR, able to introduce site directed evolution during PCR.

Our proposed method is further improvement of Belchers methods based on selfassembling biological proteins by combining this with a series of well-established biological reactions, several used for a different purpose than intended by established microbiological protocols. According to Lars Jelsbak Associate Professor, DTU Systems Biology, it is highly probable that the combination of steps will be successful, as each single-step of our 6-step method is experimentally confirmed. A proof-of-concept for the method will be undertaken in July 2012.

The method enables using phages, as well as other biological systems, for self-assembly of nano-scale materials. This creates enormous perspectives as it provides the opportunity for creating simpler, smaller, and better structures. Self-assembly simplifies the building process, reduces the labor required, and the risk of errors. The size of nano-scale structures reduces unnecessary use of materials, creating a product with the same function, but at an extremely reduced scale. The structures have also been experimentally verified to have amplified physical and chemical properties, for instance, phages assembled into nano-scale, dye-based solar cells are highly efficient, increasing efficiency by 30% compared to its normal sized, dye-based counterpart.

A further advantage of the evolutionary biotechnological approach in creating materials is that proteins can be adapted to non-covalently bind inorganic compounds. Several of the interactions created through Belcher's experiments were not known to ever have existed in nature. This enables the possibility of creating new enzymes and proteins that can be adapted to creating non-organic, value-added compounds in biorefining, thus leading to a move away the petroleum based refinery with its non-renewable or toxic chemicals.

In conclusion, our method is highly probable to be successful and serves as a platform for evolution-based and optimized, biological nano-materials for high-performance applications. The method has a minimal impact on the environment and is both fast and cost-effective. In addition, it can be applied to many areas of biotechnology for additional uses.