Self-assembled protein nanostructures: Materials for improved biological targeting and recognition

Jason Varnell Literature Seminar October 16, 2014

Protein self-assembly presents a convenient and inexpensive method for creating functional nanomaterials for a variety of applications including use as biomaterials.[1] The self-assembly of proteins is primarily driven by the formation of hydrogen bonds between the polyamide backbone and by the interaction of protein side-chains. While this process can be carefully controlled by varying the protein structure and reaction conditions, directing the formation of specific structures remains difficult. In particular, controlling the assembly of multi-component structures with specific functionalization has proven challenging. Recent advances in this area have helped expand the applications of self-assembled protein nanomaterials.

In 1993 protein self-assembly was first observed when cyclic protein subunits were assembled in aqueous solution to form organic nanotubes.[2] It was found that protein rings with exposed N-containing functionalities stack together via the formation of hydrogen bonds with other cyclic subunits in an antiparallel β-sheet-like conformation to form nanotubes hundreds of nanometers long. Additional hydrogen bonding between the side-chains of assembled tubes led to the formation of large ordered arrays of nanotubes to form structures on the micron scale.[3] In 2003 the facile assembly of short aromatic peptides into nanotubes of greater than 1 nm in length was observed.[4] With properties resembling the β-amyloid structural motif it was believed the stacking of aromatic residues played an important role in the self-assembly of these materials.[5,6] This hypothesis was later confirmed using X-ray diffraction to observe the three-dimensional stacking arrangement of aromatic side-chain groups which hold together arrays of nanotubes in a crystalline structure.[7] This understanding of the interactions guiding self-assembly has led to the creation of new nanostructures from designer proteins for many applications.

In 2009 amyloid fibrils formed from the co-assembly of two protein monomers were used to increase the sensitivity of immunoassays for the detection of antigens and antibodies in blood serum.[8] In this approach β-sheet forming domains were linked to two different functional ligands and assembled to form a nanowire (**Fig. 1a**). One ligand is a protein which binds to a specific antigen while the other is an enzyme which catalyzes the degradation of substrate to generate a yellow byproduct with optical absorption at 405 nm.[9] Nanowires were formed by sonicating aggregated antibody protein monomers to form "seeds" and mixing them with the enzyme monomers to promote self-assembly. By measuring the optical density of the solution containing the substrate in the presence of the nanowires the concentration of the antigen can be determined. Compared to commercial ELISA tests the bifunctional nanowires achieve much higher sensitivity, especially at low concentration of antigen (**Fig. 1b**) due to the fact that there are many more enzyme units attached to each antibody in the nanowire generating more of the products used for detection. This technique can be used instead of the ELISA commonly used to

detect antibody concentrations in patients suffering from HIV/AIDS and other immunodifficiencies.[10]

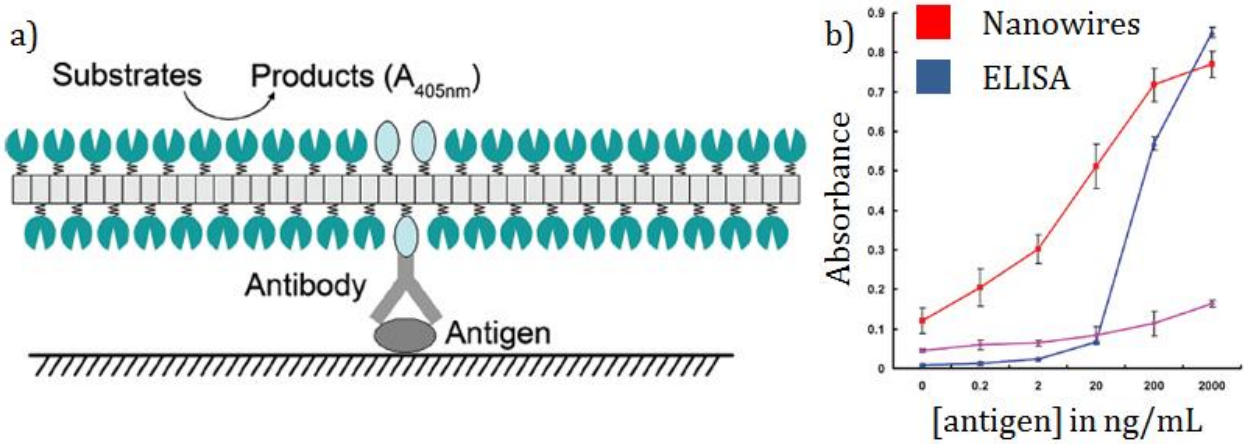

Figure 1. Nanowires for immunoassays **a)** depiction of nanowire binding to antigen and product generation **b)** comparison to commercial ELISA tests currently used for immunoassays.[8]

Another type of functional nanowire was created this year from three different functional monomers.[11] Precise control over the composition of the nanofibers was achieved by using a β-sheet fibrillizing peptide and functional protein ligands linked to β-sheet forming domains to promote self-assembly (**Fig. 2a)**.[12,13] The ratio of each monomer in the nanowire is controlled by the relative concentrations in solution as demonstrated by color matching experiments (**Fig. 2b)**.

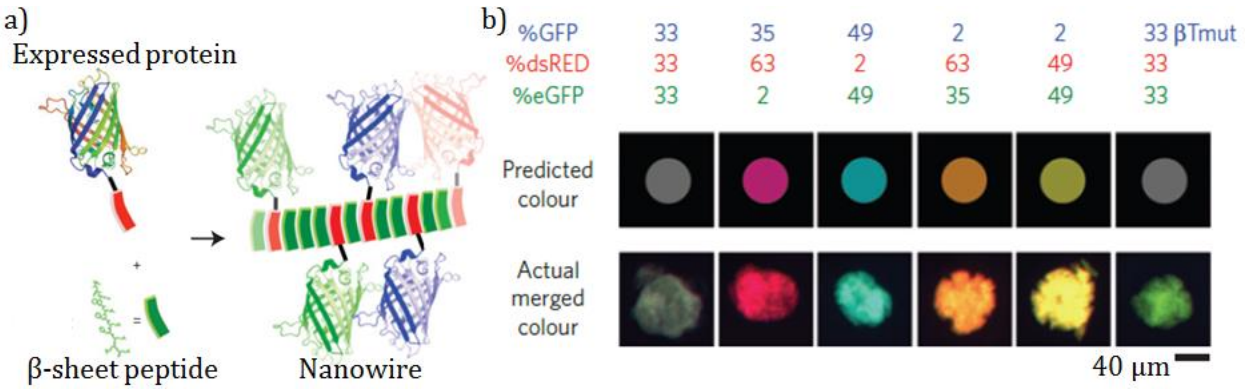

Figure 2. Functionalized nanowires **a)** assembly via β-sheet formation **b)** color matching results comparing predicted color using RGB pixel ratio to color of produced hydrogel.[11]

These functionalized nanowires can be used to increase antigen expression in vaccines by linking together many antigen units. This reduces the need for adjuvants which are currently used to increase immune response to vaccines and can cause unwanted side-effects.[14, 15] Nanowires prepared using the β-sheet fibrillizing approach have been shown to have low cytotoxicity in macrophages demonstrating the low side-effects that are expected from their use.[16] Further work in this area is required however, before these new materials can be approved for use in humans.

Through recent advances, a high degree of control over self-assembly has been achieved to create nanowires for specific functions. The biocompatibility of the prepared materials makes them ideal for applications in biology including immunoassays and vaccines. More work focusing on protein nanostructures can lead to the improvement of existing materials and the discovery of new materials for applications in biology and other fields. One area of particular interest in current and future work is the use of computational software to design new proteins and predict the structures that will be formed from self-assembly.[17] The use of computer simulation is a powerful method for discovering new self-assembled nanostructures.

1. Adler-Abramovich, L.; Gazit, G. *Chem. Soc. Rev.* **2014**, (Advance article DOI: 10.1039/C4CS00164H).
2. Ghadiri, M. R.; Granja, J. R.; Milligan, R. A.; McRee, D. E.; Khazanovich, N. *Nature* **1993**, 366, 324-327.
3. Hartgerink, J. D.; Granja, J. R.; Milligan, R. A.; Ghadiri, M. R. *J. Am. Chem. Soc.* **1996**, 118, 43-50.
4. Reches, M.; Gazit, E. *Science* **2003**, 300, 625-627.
5. Reches, M.; Gazit, E. *Nano Letters* **2004**, 4, 581-585.
6. Gazit, E. *Chem. Soc. Rev.* **2007**, 36, 1263-1269.
7. Gorbitz, C. H. *Chem. Commun.* **2006**, 2332-2334.
8. Men, D; Guo, Y.-C.; Zhang, Z.-P.; Wei, H.-P.; Zhou, Y.-F.; Ciu, Z.-Q.; Liang, X.-S.; Li, K.; Leng, Y.; You, X.-Y.; Zhang, X.-E. *Nano Letters* **2009**, 9, 2246-2250.
9. Yang, W.; Zhou, Y.-F.; Dai, H.P.; Bi, L.-J.; Zhang, Z.-P.; Zhang, X.-H.; Leng, Y.; Zhang, X.-E. *Anal. Bioanal. Chem.* **2008**, 390, 2133-2140.
10. Hornbeck, P. *Current Protocols in Immunology* **2001**, John Wiley & Sons, 2.1.1-2.1.22.
11. Hudalla, H. A.; Sun, T.; Gasiorowski, J. Z.; Han, H.; Tian, Y. F.; Chong, A. S.; Collier, J. H. *Nature Materials* **2014**, 13, 829-836.
12. Collier, J. H.; Messersmith, P. B. *Bioconjugate Chem.* **2003**, 14, 748-755.
13. Pagel, K.; Seri, T.; von Berlepsch, H.; Griebel, J.; Kirmse, R.; Bottcher, C.; Koksch, B. *ChemBioChem* **2008**, 9, 531-536.
14. Reed, S. G.; Bertholet, S.; Coler, R. N.; Friede, M. *Trends in Immunology* **2008**, 30, 23-32.
15. Aguilar, J. C.; Rodriguez, E. G. *Vaccine* **2007**, 25, 3752-3762.
16. Chen, J.; Pompano, R. R.; Santiago, F. W.; Maillat, L.; Sciammas, R.; Sun, T.; Han, H.; Topham, D. J.; Chong, A. S.; Collier, J. H. *Biomaterials* **2013**, 34, 8776-8785.
17. King, N. P.; Bale, J. B.; Sheffler, W.; McNamara, D. E.; Gonen, S.; Gonen, T.; Yeates, T. O.; Baker, D. *Nature* **2014**, 510, 103-108.